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Concurrent modulation of anxiety and memory in the infralimbic ventromedial prefrontal cortex

Advisor: Dr. Claude Messier

Thesis presented to the department of Psychology, University of Ottawa, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Psychology, Neuroscience Specialization.

Acceptance Form

Abstract

The present series of experiments systematically investigated the relative contributions of the infralimbic area of the orbitomedial prefrontal cortex to the concurrent modulation of anxiety-like behaviour, aversive learning and active working memory in CD-1 mice. Overlapping neural transmission was evaluated by testing the possibility that the behavioural effects of kappa opioid or muscarinic M1 drug infusions in the infralimbic orbitomedial prefrontal cortex could concurrently influence both cognitive/behavioural processes.

The behavioural results derived from Experiments 2a, 2b, 3, 4a, 4b, 4c and 5 provide direct evidence for orbitomedial prefrontal cortical involvement in the concurrent modulation of anxiety and associative working memory. In addition, the aggregate results support the hypotheses that (a) cholinergic muscarinic M1 receptor activation in the infralimbic cortex enhances anxiety, aversive learning and active working memory, (b) kappa opioid receptor activation in the infralimbic cortex reduces anxiety, disrupts aversive learning and enhances active working memory, (c) kappa opioid and muscarinic M1 receptors interact in the infralimbic cortex in the concurrent modulation of anxiety and memory, and (d) basal forebrain – orbitomedial prefrontal cholinergic terminal activation likely influences anxiety and working memory in the orbitomedial prefrontal cortex through effects on attentional processing. Results are discussed in light of theoretical perspectives that view anxiety disorders in the cognitive, rather than the emotional domain.

We think our systematic approach to the investigation of the role of the orbitomedial prefrontal cortex in the concurrent modulation of anxiety, working memory and attentional processing, is contributing to the understanding of the complex nature of orbitomedial prefrontal neural circuitry and the complex executive functions subserved by this associative cortical network.

Acknowledgements

I would first and foremost like to express my sincerest gratitude to my friend and advisor, Dr. Claude Messier, for allowing me to tackle such nebulous concepts as 'memory' and 'anxiety' and run with them. You stuck with me Claude regardless of how stubborn and contentious I could be, and for that I am grateful. Our common belief that great spirits and minds will always be held back by mediocre minds (Einstein) saw us through some rather heated arguments. Thankyou Claude.

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I would also like to express my sincerest love and gratitude to my wife Judy G. for her continued love and support during these past four years. Your patience and sacrifices will always be remembered in my heart and soul dearest Judy.

PM Wall would also like to acknowledge the invaluable professional help and care of our animal care technician Mme Sylvie Emond. Thankyou my friend.

PM Wall would also like to acknowledge the contributions of Dr. C Messier as co-author (editor) in all the manuscripts comprising the present thesis, as well as J Flinn in her experimental work and co-authorship in Experiments 4a and 4b.

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List of Abreviations

mPFC	medial prefrontal cortex
vmPFC	ventromedial prefrontal cortex
IL	infralimbic medial prefrontal cortex
PL	prelimbic medial prefrontal cortex
DA	dopamine
ACh	acetylcholine
GABA	gamma amino butyric acid
CCK	cholecystokinin
kappa	kappa opioid
BNI	nor-binaltorphamine, selective kappa receptor antagonist
PIR	pirenzepine, selective M1 antagonist
M1	acetylcholine muscarinic-1 receptor
OE	open arm entries
OTR	open arm time ratio
UHD	unprotected head dips
USA	unprotected stretch attends
PSA	protected stretch attends
PHD	protected head dips
PHDR	protected head dip ratio
PSAR	protected stretch attend ratio
CE	closed arm entries
CTR	closed arm time ratio
HTR	centre hub ratio
TVS	total vertical stretches (rears)
CVS	closed vertical stretches (rears)
MI	modification index
χ^2	goodness-of-fit statistic
$\Delta\chi^2$	goodness-of-fit improvement
R^2	proportion of indicator variance explained by its' factor
ϕ	correlation between pairs of factors
ξ	latent factor
λ	regression co-efficient (factor loading) of each indicator variable
δ	estimate error or error variance for each indicator
RMSEA	root mean square error of approximation
ECVI	expected cross validation index
CFI	comparative fit index

1.0. INTRODUCTION

A relationship between the neuromechanisms involved in memory and anxiety states has been recognized in laboratory animals and humans (Carli, Tatarczynska, Cervo, & Samanin, 1993; Colpaert, 1990; Frussa-Filho, Otoboni, Uema, & Sa-Rocha, 1991; Gargiulo, Viana, Graeff, Silva, & Tomaz, 1996; Graeff, Viana, & Tomaz, 1993; Lucki & Rickels, 1988; McNally, 1995, 1997; Scharf, Fletcher, & Graham, 1988; Viana, Tomaz, & Graeff, 1994; Withers & Deane, 1995). For example, the ventromedial prefrontal cortex (vmPFC) may function in conjunction with limbic forebrain structures as an emotional memory repository (LeDoux, 1993b). Moreover, other investigators propose that emotional memories could be stored in the orbitomedial PFC in the form of somatic engrams (associative neural representations) of autonomic, endocrine or visceral components of emotion (Damasio, 1998; Fuster, 1997). The motivational aspects of emotion have also been suggested to influence the functional dynamics of memory processing (Sandner et al., 1993). The notion that emotion influences memory not only implies that the psychological state of an animal in a given situation influences its relative memory processing capacity, but also implies that associative 'emotional' centres in the PFC influence current emotional state levels (sets). In this regard, Damasio suggested that the pairing of emotion and fact can be reactivated when a similar situation occurs, and such reactivation can occur as a visceral signal into consciousness (e.g., gut feeling) or as a 'nonconscious bias' (Damasio, 1998).

Recent interest has focused attention on the neural mechanisms of learning and memory involving the vmPFC in rodents (Ragozzino, Adams, & Kesner, 1998; Ragozzino, Detrick, & Kesner, 1999; Ragozzino & Kesner, 1998; Seamans, Floresco, & Phillips, 1998), non-human (Dias, Robbins, & Roberts, 1996a, 1996b; Parker & Gaffan, 1998) and human primates (Ackermann, Daum, Schugens, & Grodd, 1996; Buckner, Raichle, Miezin, & Petersen, 1996; Rugg, Fletcher,

Frith, Frackowiak, & Dolan, 1996). In addition to extensive research concerning the neurobiology and behavioural psychology of learning and memory processes in the vmPFC, some focus has also been aimed at the neurobiology and behavioural psychology of the induction and expression of anxiety-related behaviour from the orbitomedial PFC. For example, anxiety-related behavioural responsivity in the elevated plus-maze was altered following manipulations in the medial prefrontal cortex (mPFC) in rats (Espejo, 1997b) and mice (Wall, 1997). Moreover, some investigators have shown that in 2-trial procedures, central drug manipulations can concurrently influence aversive learning, associative memory and anxiety-like behaviour in the elevated plus-maze (File, 1993) or the T-maze (Viana et al., 1994). Taken together, these data suggest that some animal models of anxiety may be concomitantly sensitive to both anxiety and memory processing.

In addition to the sensitivity and utility of the elevated plus-maze and T-maze to tap into anxiety-like behaviour and some memory processes, several other animal models of anxiety combine aversively-motivated Pavlovian conditioning and appetitively-motivated operant conditioning to produce apparent conflict situations in animals (Dawson & Tricklebank, 1995; Shephard, 1986). Moreover, conditioned emotional responses to stressors or stressor-induced immobility have been employed as animal models of anticipatory anxiety (Nabeshima, Katoh, Wada, & Kameyama, 1992; Yamada & Nabeshima, 1995). In all cases, some form of learning takes place as a consequence of such conditioning procedures. In a related manner, positive or negative reinforcers such as food or footshock, respectively, are often employed in operant conditioning models of learning or memory impairment (Itoh, Takashima, Igano, & Inouye, 1989; Izquierdo & Medina, 1997; Messier, Wall, & Ethier, 1999). Conceivably, different anxiety-provoking situations such as novelty/conflict exposure, aversively-motivated conditioning, stressor manipulations or various combinations of such aversive-reinforcing events provoke neurochemical responses in the

vmPFC and certain sub-cortical and autonomic brainstem nuclei which have been suggested to overlap with learning related neurotransmission (Gabriel, 1990; LeDoux, 1993a).

The notion that memory and anxiety processes can co-exist in some brainsites is also supported by various demonstrations that many drugs appear to concomitantly modulate both cognitive-behavioural processes. In this regard, such drugs influence several neurotransmitter systems, including GABA (Davis, Rainnie, & Cassell, 1994; Mathis, Paul, & Crawley, 1994; Tohyama, Nabeshima, Ichihara, & Kameyama, 1991), acetylcholine (Arneric et al., 1994; Brioni et al., 1994), serotonin (Carli et al., 1993; Guimaraes, Del Bel, Padovan, Netto, & de Almeida, 1993; Scorza et al., 1996), dopamine (Jentsch, Tran, Le, Youngren, & Roth, 1997; Murphy, Arnsten, Jentsch, & Roth, 1996; Ukai, Shinkai, & Kameyama, 1998), cholecystokinin (Adamec, Shallow, & Budgell, 1997; Derrien, Dauge, Blommaert, & Roques, 1994) and opioid receptor ligands (Castellano, Introini-Collison, & McGaugh, 1993; Castellano, Introini-Collison, Pavone, & McGaugh, 1989; McDaniel, Mundy, & Tilson, 1990; Sandin et al., 1998), among others.

Important questions have been raised: 1) How do we know that the behavioural changes observed following anxiogenic or anxiolytic drug administration in animal models of anxiety are not subsequent to the effects such drugs could have on active learning and memory processes? 2) Is conscious perception (participation) indeed necessary for the induction or expression of anxiety-related behavioural states? The implications of these questions to the validity of anxiety paradigms in animal research are considerable, yet could lead to the development of more anxioselective paradigms. Moreover, implications to the human condition may lead to the development of more effective cognitive/emotional therapies which address, for example, central associative memory components of protracted anxiety-related symptomatology. At this point it should be mentioned that we are not suggesting that the neuromechanisms in the vmPFC underlying anxiety and memory are one and the same. Rather, we are suggesting that the psychological constructs of associative

working memory and anxiety may well be highly correlated, thus the neural mechanisms underlying these cognitive/emotional/behavioural constructs likely overlap in the orbitomedial PFC.

The following review first outlines the strikingly similar anatomical architecture of the orbitoventromedial PFC between rats and non-human primates. Although only mice are subjects in the present thesis experiments, it is important to note the architectonic similarities between rodents and primates in the orbitomedial PFC to hi-light the relevance and suitability of the current research to the human condition. Particular emphasis is focused on major reciprocated connections between the orbitomedial PFC and the hippocampal formation, cholinergic basal forebrain nuclei, as well as the autonomic and reticular brainstem. These central sites are all thought to be involved in the concurrent modulation of anxiety, associative working memory and attentional processing.

Current concepts of 'active' working memory, attention and anxiety are then reviewed. The concepts of active working memory, attention and representations of past/present experience were for the most part taken from our review article: Wall, PM & Messier, C (2001), The hippocampal formation–orbitomedial prefrontal cortex circuit in the attentional control of memory. Behav Brain Res (*in press*). Concepts of attention are reviewed along with working memory owing to the growing view that attention and associative working memory appear to be inextricably intertwined (Fuster, 1989; Fuster, 1995; Fuster, 1997; Fuster, 2000; Sarter & Bruno, 1997a, 1999, 2000; Sarter, Bruno, & Turchi, 1999).

The concepts of anxiety were for the most part taken from our review article: Wall, PM & Messier, C (2001), Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behaviour, Neurosci Biobehav Rev 25: 275-286. Problems associated with the measurement of animal anxiety are discussed in this section with a view to outline the strategy we used in Experiment 1 to address these issues.

Growing evidence that associative working memory, attention and anxiety can each seperately be influenced in the orbitoventromedial PFC is presented in Section 1.5. Some neurochemical correlates of working memory, attention and anxiety in the vmPFC are then reviewed in Section 1.6. Particular emphasis is placed on acetylcholine (ACh), kappa opioid (kappa) and dopamine (DA) systems. Although drug manipulations were restricted to muscarinic ACh (M1) and/or kappa receptor ligands in Experiments 2a, 2b, 3, 4a, 4b, 4c, and 5, DA influences are also reviewed owing to our interpretation that the present experimental results may have at least partially occurred through DA interactions with M1 and kappa receptors in the infralimbic (IL) area of the vmPFC.

Finally, hypothesized concepts regarding possible relationships between anxiety and working memory in the orbitoventromedial PFC and other discrete brain regions are reviewed in Section 1.7. Evidence is presented describing the orbitomedial PFC as an associative cortical network (in conjunction with the hippocampal formation and basal forebrain) involved in the attentional control of working memory and working set (Wall & Messier, 2001a). This section outlines growing data that together led us to the overall strategy of the present research.

1.1. Functional reciprocal connection patterns with the orbitomedial PFC

The primary aim of this section is to present an overview of the reciprocal connection patterns between the orbitomedial PFC and the hippocampal formation, basal forebrain nucleus basalis cholinergic nuclei, and the the visceral and reticular brainstem, in rats and primates (Figure 1).

Place Figure 1 about here

As will be discussed in later Sections of this review, we view (1) the dense reciprocal circuit between the orbitomedial PFC and the hippocampal formation as an associative working memory

network involved in the continuous adjustments of active unfolding cognitive, emotional, behavioural prospective working set activity; (2) the dense reciprocal connections between the orbitomedial PFC and the cholinergic basal forebrain and reticular brainstem nuclei as an associative attentional network in the attentional control of working memory (Sarter, Givens, & Bruno, 2001); and (3) the dense reciprocal connections between the orbitomedial PFC and visceral autonomic brainstem nuclei as an associative attentional network in the attentional control of working emotional set (Wall & Messier, 2001a). Figure 1 is a composite depiction meant to illustrate common connection patterns with the orbitomedial PFC in both rats and primates (Groenewegen & Uylings, 2000). It should be mentioned that the notion that the orbitomedial PFC serves homologous associative working memory functions in both species is relatively new. Traditionally, the dorsolateral PFC in primates has been widely believed to serve homologous working memory functions with the vmPFC in rats. However, based on recent tract-tracing, behavioural and pharmacological data in rats and primates (Carmichael & Price, 1995; Chiba, Kayahara, & Nakano, 2001; Conde, Audinat, Maire-Lepoivre, & Crepel, 1990; Groenewegen & Uylings, 2000; Takagishi & Chiba, 1991; Van Eden & Buijs, 2000), the orbitomedial PFC is now beginning to be considered functionally homologous in both species (De Bruin et al., 2000).

There is a great deal of similarity between primates and rats regarding the identification and organization of the afferent and efferent connectivity of the orbitomedial PFC (Groenewegen & Uylings, 2000). For the purposes of the present comparison between rat and primate neuroanatomy, the PFC can be divided into dorsolateral and orbitomedial 'limbic' subdivisions. The orbitomedial areas comprise the ventral anterior cingulate (vAC or Cg3), prelimbic (PL), infralimbic (IL) and medial orbital (MO) cortices in rats (Conde, Maire-Lepoivre, Audinat, & Crepel, 1995; Groenewegen & Uylings, 2000) (Figure 2). In primates, the ventromedial areas can be grouped with caudal orbitomedial areas to form the limbic cortex (Barbas, 1995). The primate orbitomedial

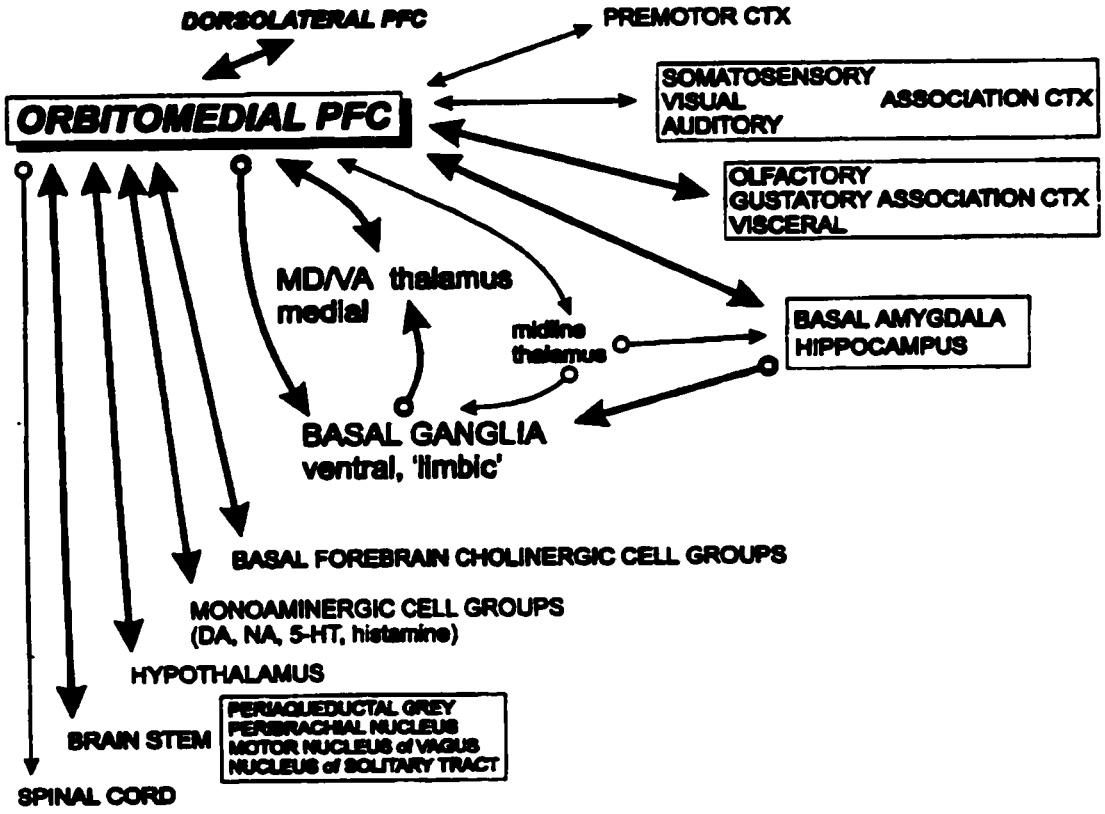

DORSOLATERAL PFC
PREMOTOR CTX
ORBITOMEDIAL PFC
SOMATOSENSORY
VISUAL
AUDITORY
ASSOCIATION CTX
OLFACTORY
GUSTATORY ASSOCIATION CTX
VISCERAL
MD/VA thalamus
medial
midline
thalamus
BASAL AMYGDALA
HIPPOCAMPUS
BASAL GANGLIA
ventral, 'limbic'
BASAL FOREBRAIN CHOLINERGIC CELL GROUPS
MONOAMINERGIC CELL GROUPS
(DA, NA, 5-HT, histamine)
HYPOTHALAMUS
BRAIN STEM
PERIAQUEDUCTAL GREY
PERIBRACHIAL NUCLEUS
MOTOR NUCLEUS of VAGUS
NUCLEUS of SOLITARY TRACT
SPINAL CORD

Figure 1

'limbic' PFC contains areas PAII, 13 and 25 on the caudal orbitomedial surface, and areas PAII, 24, 25 and 32 on the caudal ventromedial surface (Barbas, 2000; Groenewegen & Uylings, 2000) .

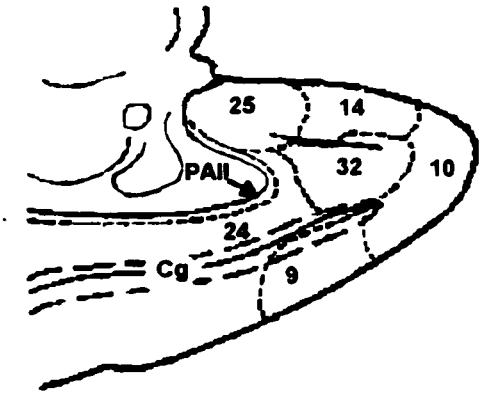
Place Figure 2 about here

The hippocampal formation and adjacent cortices are similarly connected with the orbitomedial PFC in both species (Insausti, Herrero, & Witter, 1997). The entorhinal cortex also reciprocates the orbitomedial PFC areas in both species (Conde et al., 1995; Groenewegen & Uylings, 2000), and the CAI/subiculum receives direct afferents from the orbitomedial PFC in both species (Conde et al., 1995; Groenewegen & Uylings, 2000). The perirhinal cortex reciprocates all unimodal sensorium cortices, the polymodal parietal perceptual cortex and the entorhinal cortex in both species (Barnes, McNaughton, Mizumori, Leonard, & Lin, 1990; Suzuki & Amaral, 1994; Suzuki, Miller, & Desimone, 1997). Finally, the entorhinal cortex appears to be the dominant cortical input/output source of hippocampal circuitry (Sybirska, Davachi, & Goldman-Rakic, 2000).

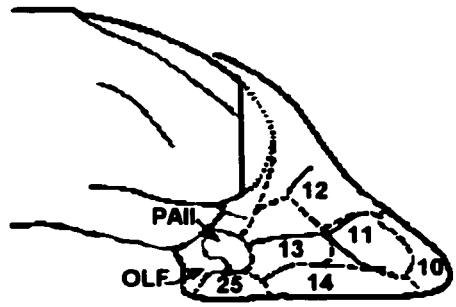
1.1.1. Rat

The medial prefrontal cortex in rats has been subdivided along a dorsal/ventral axis of the medial wall (Broersen, 2000; De Bruin et al., 2000; Sesack, Deutch, Roth, & Bunney, 1989; Takagishi & Chiba, 1991). Whereas dorsomedial wall areas are suggested to influence occulomotor, head and trunk orientation function (Groenewegen & Uylings, 2000; Neafsey, Hurley-Gius, & Arvanitis, 1986; Sesack et al., 1989), the medial orbital and ventromedial wall areas have been characterized as viscerosensory and visceromotor areas (Neafsey, 1990; Terreberry & Neafsey, 1983, 1987). The orbitoventromedial PFC reciprocates dense connections with the autonomic visceral (nucleus of the solitary tract, rostral ventrolateral medulla, central periaqueductal gray, perabrachial nuclei, and motor nucleus of the vagus), and reticular (pedunculopontine and

A.



B.



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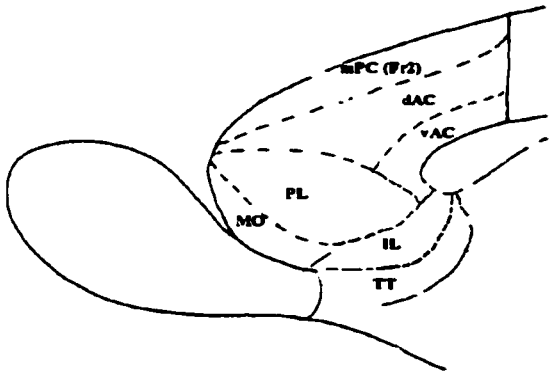

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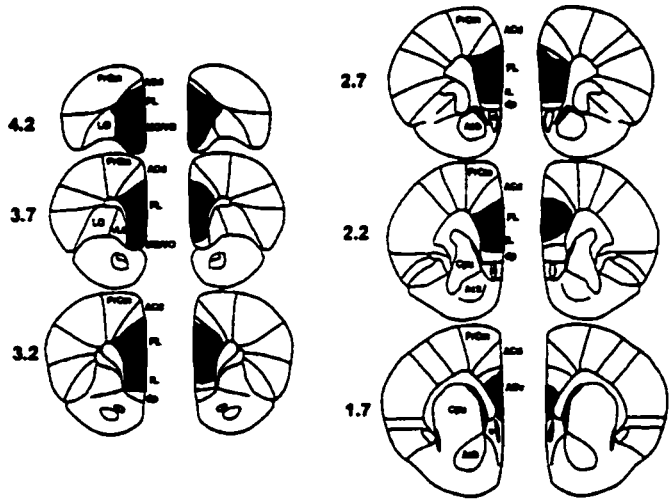

Figure 2

laterodorsal tegmentum, dorsal raphe, locus coeruleus and ventral tegmentum) brainstem (Conde et al., 1995; Sesack et al., 1989). Cholinergic projections from the laterodorsal and pedunculopontine tegmental nuclei also innervate the basal forebrain complex (Cornwall, Cooper, & Phillipson, 1990; Semba & Fibiger, 1992). In turn, the basal forebrain nucleus basalis (NBM) cholinergic pathway densely and reciprocally innervates the orbitomedial PFC (Groenewegen & Uylings, 2000).

The orbitomedial PFC also densely and reciprocally innervates the hippocampal formation (Jay, Glowinski, & Thierry, 1989; Jay & Witter, 1991; Swanson & Kohler, 1986). For the most part, the orbitomedial PFC projects to and receives afferents from the perirhinal and entorhinal cortices (Amaral, 1994; Conde et al., 1995; Sesack et al., 1989; Takagishi & Chiba, 1991), as well as from the ventral CA1-field and subiculum (Jay et al., 1989; Jay & Witter, 1991; Verwer, Meijer, Van Uum, & Witter, 1997). It should be noted that the entorhinal/perirhinal cortices receive exteroceptive sensory information from all polymodal association cortices (Mumby & Glenn, 2000), and interoceptive information from the associative orbitomedial PFC sensorium (Groenewegen & Uylings, 2000). Finally, ventral CA1/subiculum projections to the PL and IL cortex make dendritic and somatic synaptic contact with subsets of efferent neurones projecting to the nucleus of the solitary tract in the autonomic brainstem (Ruit & Neafsey, 1988, 1990).

1.1.2. Primate

The orbitomedial PFC in primates is extensively connected with medial temporal lobe structures (Carmichael & Price, 1995). The orbitomedial PFC receives relatively dense (reciprocal) projections from the parahippocampal gyrus (entorhinal and perirhinal cortices) of the inferomedial temporal cortex (Carmichael & Price, 1995; Fuster, 1995). The CA1-field and subiculum also similarly innervate the orbitomedial PFC (Barbas & Blatt, 1995; Barbas, Ghashghaei, Dombrowski, & Rempel-Clower, 1999). The orbitomedial PFC is also densely and reciprocally connected with the cholinergic basal forebrain and the laterodorsal and pedunculopontine tegmental ACh nuclei in

the reticular brainstem (Mesulam & Mufson, 1984; Mesulam, Mufson, Wainer, & Levey, 1983). Visceral brainstem nuclei including the nucleus tractus solitarius, rostral ventrolateral medulla, central periaqueductal gray, peribrachial nuclei, and the motor nucleus of the vagus are also densely and reciprocally connected with the orbitomedial PFC (Bandler & Keay, 1996; Chiba et al., 2001; Groenewegen & Uylings, 2000).

In summary, the reciprocal connection patterns between the orbitomedial PFC, reticular and visceral brainstem, the basal forebrain and hippocampal formation are strikingly similar in both rats and primates. These connective patterns position the orbitomedial PFC as an associative network for the integration of exteroceptive sensory and interoceptive visceral information in the concurrent modulation of working memory, attention and anxiety.

1.2. Concepts of working memory and representations of past/present experience

In this section, memory is discussed in relation to theoretical perspectives that take interactivity between the orbitomedial PFC and hippocampal formation into consideration. Interactivity between the orbitomedial PFC and the cholinergic NBM and reticular brainstem nuclei will be reviewed in Section 1.6. Two basic premises of memory are discussed first.

1.2.1. Two basic premises of memory

The first premise of the present thesis, that 'all *memory* is *associative*', is derived from Fuster (Fuster, 1995; Fuster, 1997). It is based on the assumption that a *memory* is basically a network of cortical neurons and the connections that link them (Fuster, 1995). A network is formed by experience resulting from the concurrent activation of neuronal ensembles that "……represent diverse aspects of the internal and external environment, and motor action" (Fuster, 1995). Any network (memory) is one of countless separate or interconnected networks that is continuously

subject to activation, change and attrition (Fuster, 1995). Critical to this view is that any network is therefore open-ended, thus modifiable by further experience.

A second basic premise concerning memory in the present Thesis follows from the notion that associative memory is not a system of memory, but a basic functional property of all brain systems. Fuster has emphasized the 'memory of *systems*' not the '*systems* of memory' (Fuster, 1995). In this perspective, memory cannot be ascribed exclusively to any one cortical area, thus premise 2: It is the temporal *state* of memory (i.e., active or inactive or somewhere in between) (Fuster, 1995) that in our view best describes the continuous cognitive representational process of memory as a function of dynamic activation-deactivation-reactivation neural activities throughout the cortex. This type of on-going memory processing can be termed 'active' associative working memory. It is also important to note that analogous delay-dependent working memory tasks are routinely used in animal and human research.

1.2.2. Long-term, short-term, delay-dependent and delay-independent working memory

Memory has also been conceptually categorized into short-term and long-term storage processes, where short-term memory is hypothesized to reside in the temporal lobes and subsequently transfered to relevant association cortex(ices) for long-term storage (McClelland & Goddard, 1996; McGeer, Eccles, & McGeer, 1978). A further conception of memory has given rise to the concept of working memory, which was essentially a reconception of short-term memory (Olton & Papas, 1979). In most delay-dependent animal models of working memory, an animal is required to hold newly acquired task-relevant information, gained during training, over a short (variable) delay until needed only in one subsequent trial demand (Pontecorvo, Sahgal, & Steckler, 1996). In contrast, it is useful to point out that 'reference' memory tasks require an animal to hold newly acquired information indefinitely for use in any number of subsequent test situations

(Pontecorvo et al., 1996). Thus delay-dependent working memory tasks require the appearance of time-dependent memory impairment.

Several authors have suggested that so-called 'delay-independent' disruptive effects on working memory result from attentional disturbances (rather than memory deficits) (Delatour & Gisquet-Verrier, 2000; Robinson, Scheff, Pierre-Louis, & Han, 2000). The issue here is the notion that delay-independent working memory deficits seemingly do not involve remembering. It could be suggested, however, that working memory continuously (actively) operates in an awake behaving animal, thus delay-independent disruptions likely reflect effects on associative executive functions such as prospective attention and working memory/set processes.

This hypothesis is somewhat supported by recent suggestions that working memory can be characterized as a workspace for holding items of information in mind as they are recalled, manipulated and/or associated with other ideas and/or incoming information (Goldman-Rakic, 1996c). Goldman-Rakic suggested that dysfunctions in working memory neurocircuitry could conceivably result in the inadequate maintenance or cessation of neural representations and thereby disturb 'on-line' working memory processes (Goldman-Rakic, 1996c). Thus, the on-going adequate and flexible updating of information is just as, if not more important than maintenance of information in an active state (Druzin, Kurzina, Malinina, & Kozlov, 2000).

1.2.3. Spontaneous 'active' working memory

The concept of spontaneous 'active' working memory is derived from the aforementioned notion that oftentimes working memory can be 'delay-independently' disrupted in animals (Floresco, Seamans, & Phillips, 1997; Seamans et al., 1998). For example, spontaneous alternation performance in mice in a T-maze conceivably stems from active mnemonic processing (Tako, Beracochea, & Jaffard, 1988). Moreover, it could be suggested that non-matching-to-sample tasks may tap into active working memory processes. In both cases, animals can perform such tasks by

utilizing relatively 'automatic' or active memory processing strategies (Granon, Poucet, Thinus-Blanc, Changeux, & Vidal, 1995; Tako et al., 1988). Spontaneous foraging in an 8-arm radial maze has also been suggested to be an active form of working memory (Floresco et al., 1997; Seamans et al., 1998). These investigators suggested that whereas in a delay-dependent working memory test in an 8-arm radial maze, rats engage in *prospective* working memory processing after a delay, but in a spontaneous foraging situation, rats engage in *retrospective* working memory processing (Floresco et al., 1997). Conceivably then, spontaneous foraging and spontaneous alternation memory performance are driven by the same (or similar) active working memory processes. We subsequently hypothesized that the cortical systems driving spontaneous working memory in animals and the type of implicit memory processing suggested by McNally (1995) to underlie anxiety induction in humans, may be functionally related.

In this respect, any information accessed or recalled unintentionally, unintentionally employed to alter existing associative neural networks or guide subsequent behaviours can be referred to as active associative working memory. Indeed, associative working memory processes may even directly mediate attitude formation in humans (Cacioppo, Priester, & Berntson, 1993), as well as encompass the psychological concepts of sensory orientation or directed attention, temporal ordering and orientation in space (Fuster, 1997). Taken together, the need for a more encompassing and integrative conceptual definition of active associative working memory was indicated. Therefore active associative working memory can be considered synonymous with spontaneous working memory in the present thesis, with the assumption that anxiety states in a given situation are in essence behavioural snapshots of active associative neuronally-encoded cognitive/behavioural output (set) processing.

1.2.4. The 'relational' memory hypothesis: The hippocampus as the seat of associative memory

Some investigators have argued that the hippocampus is essential for associative memory processing (Bunsey & Eichenbaum, 1993, 1995, 1996; Cohen & Eichenbaum, 1993). In this respect, Cohen & Eichenbaum proposed that the hippocampal formation is a functional memory system concerned with relational associations between widespread 'dedicated' cortical network representations in rats (Cohen & Eichenbaum, 1993). The hippocampus is supposedly necessary to encode an ongoing record of regularities, consistencies, and novelties that comprise unfolding structures of episodes, into 'declarative' memory (Shapiro & Eichenbaum, 1999).

1.2.5. The 'perceptuo – motor' network memory hypothesis

Fuster suggested that the post-Rolandic parietal association cortices serve as an associative 'perceptual' memory network, and the pre-Rolandic prefrontal cortices serve as an associative 'cognitive/emotional/motor set' active memory network, in non-human and human primates (Fuster, 1995; Fuster, 1997; Fuster, 2000). Moreover, the hippocampal formation is suggested to be involved in the formation of long-term network associations (memories) in each of the two widespread associative cortical networks, but likely not (or perhaps transiently) required for short-term associative working 'perceptual' memory or short-term working 'set' memory (Fuster, 1995; Fuster, 1997). Consequently, the associative sites thought to link perceptual representations with cognitive/emotional/motor 'set' output (i.e., the perception – action cycle) reside in the PFC (Fuster, 1995; Fuster, 1997; Fuster, 2000).

1.2.6. The 'associative' hippocampal – orbitomedial PFC memory network hypothesis

An alternative associative memory network hypothesis to explain the dynamic associative network activity of active working memory is here presented. Accordingly, a re-entrant circuit consisting of the hippocampal formation, orbitomedial PFC and the entorhinal cortex in rats, human and non-human primates may serve to bring perceptual and emotional representations of past

experience 'on-line' to influence on-going complex cognitive/emotional/behavioural output (set) processes (Groenewegen & Uylings, 2000). This hypothesis is in keeping with the 'somatic marker' hypothesis proposed by Damasio (Damasio, 1994, 1996, 2000), which suggests that viscero-emotional somatic responses exert profound influences on cognitive aspects of decision-making in complex socio-emotional behavioural settings (Damasio, 1996; Tranel, Bechara, & Damasio, 2000). The hippocampal – orbitomedial PFC circuit provides a simplified neural substrate for Damasio's somatic marker hypothesis that attempts to bridge the gap between divergent conscious and unconscious memory processing perspectives.

In summary, where the associative memory network hypothesis differs from Cohen & Eichenbaum's (Cohen & Eichenbaum, 1993) relational memory hypothesis, and Fuster's (Fuster, 1995; Fuster, 1997) perceptuo-motor memory hypothesis, lies in the role of the hippocampal formation. Whereas the relational theory *requires* the hippocampus to flexibly solidify relations between cortical representations into declarative memory, and the perceptuo-motor theory requires the hippocampus to consolidate and from time-to-time temporarily reactivate stored networks, the associative memory network hypothesis requires that each polymodal associative cortex 'solidify' its own respective associative memory network. The role of the hippocampal formation then would be to serve as an attentional monitor (or associative comparator) for polymodal cortical associative networks to compare present with immediate past representations. In this way, 'misfits' signal the continuous attentional modification of currently active prefrontal working 'set' activity. Hence, in the hippocampus – orbitomedial PFC circuit model, the hippocampus is not essential to store new memories, rather, we suggest that the hippocampus highlights 'novelty' or 'discrepencies' between current and past representations in the orbitomedial associative cortex. Thus active associative representations and currently unfolding cognitive/behavioural sets in the orbitomedial PFC are only

'altered' and perhaps transformed into memory engrams when signaled by the hippocampus in 'novel' or 'discrepent' situations.

1.3. Concepts of attention

Attention, like associative working memory, is a functional property of cortical systems (Fuster, 1995). For certain, the role of attention is inextricably tied to active associative memory processing, such that 'on-line' activation of any sort of information is a kind of attention directed to various internal representations (Fuster, 1997). Moreover, attentional mechanisms in the vmPFC can manipulate and monitor information and guide behavioural output on a moment-to-moment basis within working memory (Dunbar & Sussman, 1995; Seamans et al., 1998).

Baddeley argued that the central executive of working memory is primarily concerned with the *attentional* control of cognition and behaviour (Baddeley, 1992). Several attentional functions have been hypothesized: (a) retrospective *selective* attention of working memory, (b) prospective attention of working set, (c) *exclusionary* attention of inhibitory control (Fuster, 1997), and attentional monitoring (Banich et al., 2000; Coull, Frackowiak, & Frith, 1998; Gazzaniga, 2000). Importantly, whereas the resources of attention are thought to stem from the visceral (Brooks, 1981; Van Bockstaele & Aston-Jones, 1995) and reticular (Estevez-Gonzalez, Garcia-Sanchez, & Junque, 1997; Kinomura, Larsson, Gulyas, & Roland, 1996) brainstem, the neuromechanisms of attention are suggested to stem from the cholinergic basal forebrain (Sarter & Bruno, 1997a, 1999, 2000; Sarter et al., 1999).

1.3.1. Retrospective attentional control of associative working memory

In neural terms, working memory has been described as the sustained activation of network representations of various attributes of a sensory cue (including its behavioural significance, physical attributes and spatiotemporal context), that is determined by attention (Fuster, 1997). This

role has been ascribed to the dorsolateral and orbitomedial prefrontal cortices in primates (Goldman-Rakic, 1995a, 1995b, 1995c, 1996a; Rolls, 1996; Rolls, Critchley, Mason, & Wakeman, 1996), and the orbitomedial PFC in rats (Floresco et al., 1997; Gurden, Tassin, & Jay, 1999; Ragozzino & Kesner, 1998; Schoenbaum & Eichenbaum, 1995a, 1995b). The important point here is that active working memory requires the dynamic activation, monitoring and alteration of internal representations, and such attentional activity requires the exclusionary inhibitory control input from the orbitomedial PFC (Sarter et al., 2001).

1.3.2. Inhibitory attentional control of cognitive/emotional/behavioural working set

Just as retrospective attention sustains the relative activational status of a network, inhibitory attention may in a sense define the network. This notion has been proposed by Facoetti and Molteni, whereby visual selection of relevant information may occur through a mechanism that inhibits information from distractor locations (Facoetti & Molteni, 2000), Baddeley and Della Salla, whereby selective attention is the *simultaneous* capacity to focus attention to one stream of information while shutting out irrelevant material (Baddeley & Della Sala, 1996), and others. Fuster proposed that the inhibitory control of emotion, cognition, behaviour, distractions and interference stems from the orbitomedial PFC in human and non-human primates (Fuster, 1997).

1.3.3. Decision-making and cognitive/emotional/behavioural prospective 'set' attention

Whereas active working memory can be construed as retrospective attention directed to the activation of internal representations, working 'set' is attention directed to prospective cognitive or behavioural action. Viewed in this way, working memory and working set are suggested to be two sides of the same coin, the temporal integration of behaviour (Fuster, 1997). In essence, the temporary "on-line" active retention of information (sensory or otherwise) is a kind of retrospective attention to internal representations, the failure of which renders an individual unable to use experience of the recent past (learn from experience) (Fuster, 1997). By analogy, faulty prospective

memory (working set) renders an individual unable to plan for the future (Fuster, 1997). Now we can see that cognitive/emotional/behavioural working 'set' refers to attention directed to prospective action (i.e. speech, internal action, logical reasoning, emotional states, locomotor activity etc.) (Fuster, 1997). All internal sets require proper order across time and space, thus active working set can be viewed as a dynamic continuum, presumably reflecting preparation for an upcoming act, sequencing, monitoring and continuously updating the act (Fuster, 2000).

Fuster argued that the orbitomedial PFC regulates, integrates and initiates emotional behaviour. He further argued that emotions and motives enter into "prefrontal" decision making processes (Fuster, 1997). This notion is supported by Damasio and colleagues (Damasio, 2000; Damasio, Grabowski, Frank, Galaburda, & Damasio, 1994), who argued that emotional memory could be stored in frontal networks in the form of "somatic markers" or emotional engrams (set) of autonomic, endocrine and viscerel representations of emotion (Fuster, 1997). Thus, depending on the relative emotional set weight produced by prefrontal attentional 'decision-making' outcomes, emotional working set influences cognitive/motor working set and subsequent behaviour. These ideas together led us to hypothesize that the attentional control of active working memory and working emotional set are processes that can be concurrently modulated in the vmPFC in anxiety-provoking situations.

1.3.4. Attentional monitoring

The associative memory network hypothesis places the role of attentional monitoring in the hippocampal formation (including the rhinal and parahippocampal cortices). This notion is not a new one. The septohippocampal theory proposed by Gray posits that direct input to the subiculum from the entorhinal cortex describes the current state of the world, and input from the hippocampus to the subiculum determines whether such 'descriptions' are important (Gray, 1982; Gray, Feldon, Rawlins, Hemsley, & Smith, 1991). Others have suggested that the tri-synaptic hippocampal circuit

monitors for conditions of environmental uncertainty and novelty, with subsequent motivational influences on active cognitive and behavioural sequences (sets) (Shen, Specht, De Saint Ghislain, & Li, 1994). We suggest that by virtue of the reciprocal connections with the polymodal associative cortices, the hippocampal formation plays an essential role in the attentional monitoring and updating of associative working network activity in the orbitomedial PFC.

In summary, we have seen that various aspects of attention comprise the working machinery of associative working memory and working set. The mechanisms of attention comprise functions related to the internal/external monitor, (retrospective) activation, cognitive/emotional/ behavioural (prospective) set, and exclusionary (inhibitory control). Collectively, these attentional functions provide a sense of awareness and control of a spatially- and temporally-integrated environment.

1.4. Concepts of Anxiety

Concepts of generalized anxiety disorder (GAD) are reviewed in this section primarily to show the pervasiveness of the disorder in humans, thus the need for infrahuman experimental research.

Generalized anxiety disorder is typically characterized by unrealistic excessive apprehension and nonspecific worry about any person or unspecified past/present/future life event (Barlow & Lehman, 1996; Craig, Brown, & Baum, 1995; Yonkers, Warshaw, Massion, & Keller, 1996), symptoms frequently comorbid with motor tension (e.g., trembling, twitching, repetitive movements or restlessness) hypervigilance and scanning (e.g., exaggerated startle, difficulty concentrating, apprehension or irritability) (APA, 1987; Craig et al., 1995; Edwards, 1991; Rogers et al., 1999). The diagnostic criteria for GAD was modified in DSM IV (APA, 1994) to reduce the number of somatic symptoms from eighteen to six: restlessness, easily fatigued, difficulty concentrating, irritability, muscle tension and aches, and trouble sleeping (Rogers et al., 1999). The majority of people suffering with GAD report substantial interference with their lives, a high degree of professional help-seeking, and high medication use (Rogers et al., 1999; Wittchen, Zhao, Kessler, &

Eaton, 1994). Only recently, researchers have viewed GAD as a primary independent entity (Rogers et al., 1999) rather than as a residual or prodromal state (Roy-Byrne & Katon, 1997). Although the diagnosis of GAD has been thought difficult owing to frequent comorbidity with other psychopathologies and anxiety disorders in particular (Edwards, 1991), it can be differentiated from other anxiety disorders with a distinct set of somatic symptoms, overfocused worry on minor things, uncontrollability and pervasiveness (Barlow, Blanchard, Vermilyea, Vermilyea, & DiNardo, 1986; Brown, Barlow, & Liebowitz, 1994; Maxmen, 1986).

The use of diagnostic measurement devices (e.g., Beck Anxiety Inventory (Beck, Epstein, Brown, & Steer, 1988) typically including question and answer self-reports is axiomatic in psychiatric diagnostic interviews in humans. Equally important, however, is the careful observation of somatic symptom clusters along with (but seldom considered) overt behavioural symptoms (Troisi, 1999). The ongoing pervasive presence of GAD is therefore critical (APA, 1994). Lister (1990) suggested that anxiety investigation in an unselected general population of animals is implicitly assumed in many animal models, but only a significant minority from a random sample of the human population would be sufficiently disturbed to be diagnosed (Lister, 1990). Furthermore, Handley and McBlane suggested that comparatively little of human anxiety appears in overt behaviour (Handley & McBlane, 1993a), rather, human anxiety is psychophysiologically based (Edwards, Hampton, Ashby, Zhang, & Wang, 1996). In this sense, nearly all of the physiological data from a patient are measured to at least one decimal point in parametric scales, but patient behaviours are only given ordinal ratings (i.e., assessed primarily through indirect reports and measured on Likert-type scales) (Troisi, 1999). Such a profound lack of direct psychiatric observation of behaviour during diagnostic and/or assessment interviews, results in methodological and conceptual difficulties in the integration of human and animal data with respect to drug effects on pathological behaviour (Troisi, 1999).

The crux of the issue lies in the notion that not knowing how an animal feels or what it might be experiencing mentally necessitates inferences with respect to observable behavioural drug effects, but psychometric assessment is primarily relied on to evaluate drug effects on human behaviour (Troisi, 1999). Finally, Troisi suggested that such methodological differences between human and animal studies largely impedes the development of valid animal models of psychopathology (Troisi, 1999). The question of whether homologous behavioural manifestations of anxiety between humans and animals can be similarly addressed with anxiolytic drug treatment, is of considerable importance (Blanchard, Griebel, & Blanchard, 2001).

1.4.1. Primary vs. secondary anxiety

There is currently some consensus regarding the taxonomy of anxiety manifestations with respect to primary (e.g., nervousness and fear) and secondary (e.g., phobic avoidance) symptom clusters (Maxmen, 1986; Van Riezen & Segal, 1988). There remains, however, much debate regarding similarities (Rachman, 1991) or differences (Barlow, 1988) between fear and anxiety (Craig et al., 1995). It has been suggested that anxiety implies impending danger, therefore may serve as a 'priming' device for compensatory behaviour (Gray, 1982). Anxiety can also reflect generalized unfocused responsivity to unknown threats or interoceptive conflict, whereas fear may result from experienced or known exteroceptive aversive situations in the immediate environment, thus describes a focused response to a known object or experience (Craig et al., 1995; Lister, 1990). However, the expression of endogenous (trait) fear or relative degrees of conditioned (state) fear may reflect psychological manifestations of anticipatory anxiety (Green, 1991).

1.4.2. Trait vs. State anxiety in animals and humans

Can provoked anxiety-like behaviours in animals resemble analogous anxiety-related behavioural profiles in humans? The taxonomy of anxiety into 'trait' vs. 'state' dimensions somewhat addresses this question. Trait anxiety supposedly does not vary from moment to moment

and is thought to be an enduring feature of an individual (Lister, 1990). Moreover, an individual may experience trait anxiety in the normal range and in most circumstances a low state anxiety level, but in an anxiety-provoking situation, acute state anxiety will ensue (Lister, 1990). Taken from this perspective, individuals in most mammalian species likely maintain certain levels of trait anxiety and given appropriate circumstances, would experience acute rises in state anxiety. But these high (reactive) 'anxiety' levels quickly subside in normal non-anxious individuals. Most would agree that everyone experiences anxiety, but only some are impaired by it (Maxmen, 1986). The idea here is that individuals suffering with anxiety disorders conceivably experience protracted inappropriately high 'trait' anxiety levels.

The issue then is the nature of provoked state anxiety. Intuitively, most ethologically-derived animal models provoke some form of state anxiety (Belzung & Le Pape, 1994; Belzung, Pineau, Beuzen, & Misslin, 1994; Lister, 1990). But does such an acute rise in state anxiety reflect acutely raised levels of trait anxiety? Moreover, some investigators have suggested that provoked state anxiety simply reflects an enhanced fear state (File, Zangrossi, Viana, & Graeff, 1993; Gray, 1982; Gray & McNaughton, 1983; Mora, Netto, & Graeff, 1997). However, it would appear that this interpretation is over-simplified owing to growing data showing that provoked state anxiety can be distinguished from provoked fear states in some ethological animal models (Blanchard, Blanchard, & Hori, 1989; Blanchard, Blanchard, Rodgers, & Weiss, 1990; Blanchard, Griebel, Henrie, & Blanchard, 1997; Blanchard et al., 1998). Whereas the fear/defense test battery measures reactions to an approaching or contacting predator (flight, freezing, defensive threat and attack), the anxiety/defense test battery measures risk assessment activity and inhibition of non-defensive behaviours to a potential predator threat (Blanchard, Griebel, Henrie et al., 1997).

1.4.3. Provoked state anxiety in ethological animal models

Blanchard and colleagues suggested that risk assessment behaviours serve to a) gather information concerning potential threat, and b) permit a frightened animal to return to normal non-defensive behaviours following exposure to a threatening stimulus (Blanchard et al., 1990; Weiss, Wadsworth, Fletcher, & Dourish, 1998). More specifically, in situations where freezing is the prepotent response to the threat (scent) of predator exposure, anxiolytic drugs tend to increase risk assessment behaviour while decreasing avoidance behaviour (Blanchard, Griebel, Henrie et al., 1997). But when animals are placed in the proximity of a predator, risk assessment behaviours are generally reduced and lead to more intensive defensive behaviours (Blanchard, Griebel, Henrie et al., 1997). These authors also demonstrated that less intense threatening situations, such as novelty exposure, elicit similar behavioural patterns to those observed following exposure to potential danger, a predator scent (i.e., short initial avoidance, risk assessment activity and inhibition of non-defensive behaviours) (Blanchard, Griebel, Henrie et al., 1997). These observations, taken together, support the notion that some risk assessment behaviours in potentially threatening situations are sensitive measures of provoked state anxiety in animals (Blanchard et al., 1990).

The elicitation of risk assessment behaviours in response to novelty (Griebel, Perrault, & Sanger, 1998), defensive withdrawal in the open field (Quartermain, Stone, & Charbonneau, 1996; Stone, Rhee, & Quartermain, 1996), predator scent and/or exposure (Blanchard & Blanchard, 1989), defensive burying situations (Pinel, Mumby, Dastur, & Pinel, 1994), as well as in the elevated plus-maze (Cole & Rodgers, 1993) has prompted many investigators to view inappropriate levels of risk assessment activity in threatening environments as analogous to some aspects of generalized anxiety and phobic disorders in humans (Adamec, Kent, Anisman, Shallow, & Merali, 1998; Adamec & McKay, 1993; Blanchard et al., 2001; Blanchard et al., 1989; Blanchard, Griebel, Guardiola-Lemaitre et al., 1997). Conceivably, provoked state anxiety in animals and anxiety-

disordered humans, particularly in threatening situations, result in psychobehavioural changes reflecting phobic tendencies, hypervigilance, scanning and excessive worry. From this perspective, animals given one or more trials in the elevated plus-maze display analogous behavioural profiles.

1.4.4. Problems associated with the measurement of anxiety in the elevated plus-maze

Handley and Mithani originally evaluated the elevated plus-maze as a possible animal model of anxiety (Handley & Mithani, 1984). These investigators evaluated the effects of anxiolytic/ anxiogenic drugs on the ratio of open arm entries to total arm entries, an index thought to measure the relative aversiveness of the open unprotected arms (Handley & McBlane, 1993a). The use of the elevated plus-maze to study animal anxiety-like behaviour was subsequently pharmacologically validated for rats (Pellow, Chopin, File, & Briley, 1985) and mice (Lister, 1987). Since that time, several of the aforementioned defensive-related risk assessment behavioural indices and some spatiotemporal behavioural measures have been incorporated into the battery of elevated plus-maze measures in efforts to further develop and enhance the sensitivity of the apparatus to subtle changes in anxiety-related behavioural activity (Cole & Rodgers, 1993, 1995; Rodgers & Cole, 1993a, 1993b, 1994; Rodgers & Dalvi, 1997; Rodgers & Shepherd, 1993). A complex array of behavioural reactions to the apparatus consequently emerged, that in many ways resembled the risk assessment behavioural profiles in mice and rats characterized by Blanchard and Blanchard and colleagues as anxiety-related defensive postures (Blanchard & Blanchard, 1989). Although the incorporation of many ethological indices into the elevated plus-maze test battery certainly increased the sensitivity of the apparatus to anxiolytic drug effects (Cole & Rodgers, 1994; Rodgers, Johnson, Champion, & Mills, 1996; Rodgers, Nikulina, & Cole, 1994), the underlying behavioural structures subsequently became much more difficult to interpret. It became clear that the growing number of measured behavioural indices in the elevated plus-maze test battery should be reduced, and that some of the underlying constructs thought to drive behaviour in the apparatus were in need of re-evaluation.

These issues are addressed in Experiment 1. Clearly the numerous methodological, conceptual and complexity issues associated with the factorial validity of recently published ethologically-derived principal components analyses of elevated plus-maze behaviour presented us with the problem of identifying the behavioural indices (among some 32 reported measures) that would most parsimoniously measure underlying anxiety constructs. The utility of the confirmatory factor analyses conducted in our laboratory to test various structural hypotheses of anxiety-relevant elevated plus-maze behaviour, (the results of which we believe addressed most of these issues), are presented and discussed in Experiment 1.

1.5. Memory, attention and anxiety in the orbitomedial PFC

There can be no doubt that the orbitomedial PFC influences working memory, attention and emotion in rodents, non-human and human primates (Broersen, 2000). There is ample evidence suggesting that the orbitomedial PFC is involved in associative memory, inhibitory attention, behavioural flexibility, and orientation in space (Delatour & Gisquet-Verrier, 2000; Dias & Aggleton, 2000; Ragozzino et al., 1998; Ragozzino et al., 1999; Schnider, von Daniken, & Gutbrod, 1996). Moreover, the orbitomedial PFC in primates has been suggested to exert tonic feedback on all neocortical activity (Barbas, 2000; Sarter et al., 2001).

1.5.1. Recognition and working memory in the orbitomedial PFC

Ventromedial PFC lesions impaired object recognition (Bachevalier & Mishkin, 1986) and associative memory (Meunier, Bachevalier, & Mishkin, 1997) in primates. Moreover, vmPFC lesions impaired most delay, visual discrimination and reversal tasks (Broersen, 2000; De Bruin et al., 2000; Fuster, 1997; Iversen & Mishkin, 1970; Kowalska, Bachevalier, & Mishkin, 1991), as well as spatial and non-spatial working memory tasks (Dias & Aggleton, 2000; Dias et al., 1996a,

1996b; Dias, Robbins, & Roberts, 1997; Floresco et al., 1997; Parker & Gaffan, 1998; Ragozzino et al., 1998; Seamans, Floresco, & Phillips, 1995; Seamans et al., 1998), in rats and primates.

1.5.2. Prospective versus retrospective associative working memory in the orbitomedial PFC

In addition to the aforementioned concept of prospective set attention reviewed in Section 1.3, prospective memory can be understood as the ability to activate information in anticipation of its use in the immediate or near future (Rainer, Rao, & Miller, 1999). Although several electrophysiological recording studies have shown sustained cellular activation in dorsolateral PFC areas in primates during the delay period in working memory tasks (Fuster, 2000; Rainer et al., 1999), one cannot dissociate such neural activity, presumably encoding a recent representation of an object or spatial array, from contiguous activated neural activity presumably representing the prospective planning of a requisite motor response. Thus, although the retrospective sustained activation of an internal representation during the delay period in visual delayed-response tasks is likely mediated in the dorsolateral PFC (Fuster, 1997), it is not at all clear that the prospective activation of any preparatory action 'set' is also mediated by contiguous firing patterns in the dorsolateral PFC (Fuster, 2000). Hence, the contributions of the orbitomedial PFC to the inhibitory control of interference as an integral part of the sustained activation and unfolding of any action set must be taken into consideration (Wall & Messier, 2001a).

Indeed, recent human neuroimaging studies have shown involvement of the medial prefrontal cortical wall during working memory (Petit, Courtney, Ungerleider, & Haxby, 1998), attentional (Iidaka, Anderson, Kapur, Cabeza, & Craik, 2000) as well as anxiety-related processing (Fredrikson, Fischer, & Wik, 1997; Lane, Fink, Chau, & Dolan, 1997; Lane et al., 1998). For example, the posterior orbital, ventromedial prefrontal and anterior cingulate cortices were simultaneously activated with subjective feelings of anticipatory anxiety in healthy human subjects (Drevets, Videen, Snyder, Macleod, & Raichle, 1994). Moreover, the vmPFC in rats has been

consistently shown to be involved in behavioural coding, behavioural flexibility and selection (Delatour & Gisquet-Verrier, 2000; Ragozzino et al., 1999). It could be speculated that if the prospective planning (or choice) of a behavioural response (i.e., feeling, thought, speech, categorization, discrimination or motor act, among others) in any given moment depends on a critical balance between internal and external representations in prefrontal associative working memory networks, then the orbitomedial PFC inhibitory attentional control of interference likely in no small way shapes such prospective working memory activity. In this sense, there are numerous examples in the literature showing that the vmPFC influences most working memory tasks that require perceptual discrimination, prospective planning, behavioural selection and sustained attention (Burgess & Shallice, 1996; Delatour & Gisquet-Verrier, 2000; Goel & Grafman, 1995; Karnath, Wallesch, & Zimmermann, 1991; Mishkin & Manning, 1978; Ragozzino et al., 1999; Sarter & Bruno, 2000; Sarter et al., 2001; Stuss et al., 1982). Fuster suggested that planning or behavioural flexibility deficits evident among animals with orbitomedial PFC lesions can be understood as an inability to withstand proactive interference of previous experiences, previous learning or previous trials, and such learning deficits are revealed as an "...incapacity to change 'set' as readily as circumstances demand" (Fuster, 1997).

1.5.3. Attentional control of behaviour in the orbitomedial PFC

Under what circumstances should the orbitomedial PFC exert attentional control over well-established behavioural or even cognitive routines? The obvious answer to this question would be in situations when any particular routine is inappropriate at that particular moment. Conceivably, activating an alternative behavioural set (or altering a currently activated set) requires decision-making, and decision-making requires wide-spread access to associative network representations from gustatory, olfactory, somatosensory (external), autonomic visceral (internal), auditory and visual (external), and speech (internal) centres. The orbitomedial PFC enjoys such multi-modal

access in rats and primates (Figure 1) (Barbas, 1995, 2000; Carmichael, Clugnet, & Price, 1994; Carmichael & Price, 1995). Thus it is reasonable to suggest that attentional decision-making outcomes stemming from the visceral associative orbitomedial PFC are inextricably grounded in the internal emotional environment of an individual (Barbas, 2000; Damasio, 1996).

1.5.4. The orbitomedial PFC and anxiety-related memory and behaviour

Apart from discussing the mechanisms of orbitomedial decision-making or attentional control, one might ask how the orbitomedial PFC somehow inextricably links cognition with emotion, and subsequently influences behaviour? Conceivably, orbitomedial PFC connections with all sensory modalities, including subcortical and brainstem visceral centres, precipitate the integration of complex internal–external representations to provide appropriate contexts in behavioural settings (Barbas, 2000; Van Eden & Buijs, 2000). This places the orbitomedial PFC in a stronger position to integrate emotion and behaviour than its previously suggested role in merely suppressing emotional autonomous expressions (Van Eden & Buijs, 2000).

The orbitomedial PFC influences anxiety-related cognition, emotion and motivated behaviour in rodents, non-human and human primates (Gold & Chrousos, 1999; Gottschalk et al., 1991, 1992; Hollerman, Tremblay, & Schultz, 2000; Jinks & McGregor, 1997; Li et al., 2000; Morgan & LeDoux, 1995; Reivich, Gur, & Alavi, 1983; Wu et al., 1991). Conceivably, the reciprocated connections with the autonomic brainstem position the orbitomedial PFC as a key structure to mediate central and autonomic nervous system interactions (Damasio, 1994, 1996, 1998, 2000) (i.e., an associative emotional working 'set' network). In this respect, it has long been known that electrical stimulation in the orbitomedial PFC in rats and primates influences heart-rate, respiration and blood pressure (Anand & Dua, 1956; Frysztak & Neafsey, 1994; Hall, Livingston, & Bloor, 1977; Hardy & Holmes, 1988), along with profound effects on emotional behaviour (reviewed in (Fuster, 1997)). Moreover, monkeys with orbital lesions show heightened signs of agitation and

anxiety in aversive situations (Tanaka, 1974), heightened orality, perverse eating habits and distractibility (Butter & Snyder, 1972; Kling & Steklis, 1976; Ursin, Rosvold, & Vest, 1969). Finally, dense reciprocal connections with the nucleus of the solitary tract and the dorsal motor nucleus of the vagus, suggests that the orbitomedial PFC might profoundly influence (shape) humoral representations in associative emotional processing (Damasio, 1998). Together these data suggest that the orbitomedial PFC recruits visceral, humoral, cardiovascular and respiratory functions to actively shape and integrate emotion with cognition and subsequent behaviour.

1.6. Neurochemical correlates of memory, attention and anxiety in the ventromedial PFC

Various neurochemicals colocalized within the connective pathways with the orbitomedial PFC (Figure 1) that appear to be involved in memory, attention and anxiety include gamma aminobutyric acid (GABA) (Griebel, 1995; Handley, 1995; Handley & McBlane, 1993b; Inoue, Koyama, & Yamashita, 1993; Stanford, 1996; Taylor & Moon, 1991; Yamada et al., 1995), dopamine (DA) (Espejo, 1997b; File, 1996; Goldstein, Rasmusson, Bunney, & Roth, 1996; Kaneyuki et al., 1991; Rodgers, Johnson, Champion et al., 1996; Taylor et ai., 1982), acetylcholine (ACh) (Mark, Rada, & Shors, 1996) and opioids such as the κappa agonist dynorphin (DYN) (Nabeshima et al., 1992; Olson, Olson, & Kastin, 1993, 1995, 1996; Privette & Terrian, 1995), among others. These neurochemicals are variously co-released in the brainstem, hindbrain, midbrain, forebrain limbic areas and the vmPFC (Deutch & Roth, 1990; Inoue, 1993) following encounters with stressful environmental events and/or 'emotional' interoceptive stimuli. Of these several neurotransmitters, cholinergic terminals in the mPFC from the basal forebrain NBM appear to robustly influence attentional processing (Hasselmo, 1995; Muir, Dunnett, Robbins, & Everitt, 1992; Robbins, Everitt, Marston et al., 1989; Sarter & Bruno, 2000; Sarter et al., 2001) and working memory (Broersen, Heinsbroek, de Bruin, Uylings, & Olivier, 1995; Granon et al., 1995; Ragozzino et al., 1999; Ragozzino & Kesner, 1998), as well as interactivity between autonomic activation and anxiety

(Berntson, Hart, Ruland, & Sarter, 1996; Berntson, Hart, & Sarter, 1997; Berntson, Sarter, & Cacioppo, 1998). Dopamine terminals in the vmPFC from the ventral tegmental area also influence memory, attention and anxiety (Broersen et al., 2000; Broersen, Heinsbroek, de Bruin, & Olivier, 1996; Granon et al., 2000; Lacroix, Broersen, Feldon, & Weiner, 2000; Morrow, Roth, & Elsworth, 2000; Seamans et al., 1998). Moreover, kappa receptor systems have been shown to interact with DA systems in the mPFC to produce conditioned place learning in rats (Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993), interact with cholinergic systems to produce effects on memory (Hiramatsu, Murasawa, Mori, & Kameyama, 1998) and effects on cardiovascular responsivity (Wang & Ingenito, 1992). Finally, interactivity between ACh and DA in memory processing (Steele, Hodges, Levesque, Locke, & Sandage, 1996) in the mPFC could therefore conceivably be influenced by kappa receptors.

Central Dopamine Activity

It has been suggested that perturbations within the orbitomedial PFC DA system contribute to some cognitive aspects of psychopathology evident among individuals compromised with schizophrenia (Deutch, Clark, & Roth, 1990; Sesack & Bunney, 1989), attentional deficits (Broersen, 2000; Broersen, Feldon, & Weiner, 1999; Broersen et al., 1996; Ernst, Zametkin, Matochik, Jons, & Cohen, 1998; Russell, de Villiers, Sagvolden, Lamm, & Taljaard, 1995), depression (Anisman & Zacharko, 1990; Bertolucci-D'Angio, Serrano, & Scatton, 1990b; Brown & Gershon, 1993; Diehl & Gershon, 1992; Imperato, Angelucci, Casolini, Zocchi, & Puglisi-Allegra, 1992; Puglisi-Allegra, Imperato, Angelucci, & Cabib, 1991; Rossetti, Lai, Hmaidan, & Gessa, 1993; Tanda, Carboni, Frau, & Di Chiara, 1994; Zacharko & Anisman, 1991) or anxiety (Beck & Fibiger, 1995; Gruen, Wenberg, Elahi, & Friedhoff, 1995; Kaneyuki et al., 1991; Rodgers, Johnson, Champion et al., 1996; Simon, Dupuis, & Costentin, 1994; Taylor et al., 1982). In this regard, encounters with aversive life events have been associated with enhanced DA release and metabolic

activity in vmPFC terminal sites (Abercrombie, Keefe, DiFrischia, & Zigmond, 1989; Bertolucci-D'Angio, Serrano, & Scatton, 1990a; Carlson, Fitzgerald, Keller, & Glick, 1991; Deutch & Roth, 1990; Dunn & File, 1983; Herman et al., 1982). For example, acute restraint selectively increases DA release in the mPFC in rats (Deutch & Cameron, 1992). Conditioned cue exposure associated with footshock, as well as mild footshock itself also selectively enhanced DA metabolism in the mPFC in rats (Deutch, Tam, & Roth, 1985). Recent microdialysis studies have also shown that acute isolation, footshock, or immobilization selectively enhanced DA release in the mPFC in rats (Gresch, Sved, Zigmond, & Finlay, 1994). Interestingly, the experience of rats witnessing other rats receiving footshock also enhanced DA metabolic activity in the mPFC (Kaneyuki et al., 1991). These data suggest that mPFC DA activity in rats is selectively sensitive to aversive events.

Intrinsic ventral PL and IL interneurons and projection neurons in the mPFC are tonically inhibited by DA from the VTA, (Benes, Vincent, & Molloy, 1993; Deutch et al., 1990; Deutch & Roth, 1990; Godbout, Mantz, Pirot, Glowinski, & Thierry, 1991; Gresch et al., 1994; King & Finlay, 1995; Le Moal & Simon, 1991; Mantz, Thierry, & Glowinski, 1989; Pirot et al., 1992; Retaux, Besson, & Penit-Soria, 1991; Retaux, Trovero, & Besson, 1994; Seguela, Watkins, & Descarries, 1988; Sesack & Bunney, 1989; Sokolowski, McCullough, & Salamone, 1994; Verney, Alvarez, Geffard, & Berger, 1990; Vincent, Khan, & Benes, 1993). Accordingly, Mantz et al (1988) have demonstrated that electrical stimulation of the VTA inhibited the enhanced electrophysiological activity in the mPFC induced by tailshock in rats. Furthermore, the enhanced electrophysiological activity in the mPFC resulting from electrical stimulation in the dorsomedial nucleus of the thalamus, is also inhibited by electrical stimulation of the VTA (Mantz et al, 1989). Sesack and Bunney (1989) have demonstrated that iontophoretic application of DA in the ventral PL area of the mPFC hyperpolarized intrinsic GABA/CCK cells and glutamatergic (GLU) pyramidal cells, the effects of which were blocked by the D_2 receptor antagonist, sulpiride. These

data were supported by the demonstration that DA terminals inhibit intrinsic pyramidal and non-pyramidal cells in the mPFC both directly through D_2 receptors and indirectly through D_1 receptors on GABA interneurons (Benes et al., 1993; Vezina, Blanc, Glowinski, & Tassin, 1994). In addition, van der Kooy et al (1984) demonstrated that a subset of neurons in the ventral PL mPFC in rats that innervate the autonomic brainstem, receive overlapping dorsomedial thalamic GLU and ventral tegmental DA projections. It is conceivable that DA depletions in the vmPFC produced by protracted or severe aversive situations, or D_2 receptor blockade in this cortical site, could result in enhanced electrophysiological activity in neuronal subsets which project to brainstem and subcortical nuclei associated with anxiety-related behavioural and emotional changes.

Evidence in support of the notion that dopaminergic perturbations in the vmPFC might produce aversive internal states stems from the demonstration that the kappa opioid receptor agonist, U-50,488H, or the dynorphin derivative, E-2078, produced conditioned place aversion following microinfusion in the mPFC in rats, effects coincident with reduced DA levels (Bals-Kubik et al., 1993). These data coupled with the consistent demonstration that electrical stimulation of the vmPFC decreased blood pressure in rats through the activation of corticofugal brainstem nuclei (Hardy & Holmes, 1988; Verberne, 1996), renders the notion that acutely enhanced DA release in the vmPFC might serve to dampen such parasympathetic activation. Alternatively, it is also entirely possible that DA depletion following high doses of kappa receptor agonist infusions in the mPFC likely serves to enhance such parasympathetic activation, resulting in an aversive internal milieu.

From a functional point of view, although there are accumulating systemic data suggesting a role for DA in anxiety (File, 1996; Bartoszyk, 1998), there are sparse data showing direct effects of DA manipulations in the vmPFC on anxiety-like behaviour in animal models. According to some investigators, a reduction in DA transmission has been suggested to be involved in some aspects of anxiety-related behavioural symptoms (Taylor et al, 1982). This hypothesis is partially supported by

recent studies showing that systemic D2 agonist administration reduced anxiety in rats (Bartoszyk, 1998). The central sites mediating these D2 activating effects, however, can only be speculated on.

There are some recent data showing that non-specific DA receptor activation in the mPFC in rats following infusions of apomorphine produced anxiogenic behavioural effects (Broersen, Heinsbroek, de Bruin, Laan et al., 1995). Moreover, non-specifically blocking DA receptors following flupenthixol infusions in the mPFC produced anxiolytic effects in rats (Broersen, Heinsbroek, de Bruin, Laan et al., 1995). Broersen and colleagues also demonstrated that medial prefrontal dopamine is directly involved in the anxiogenic interoceptive effects of pentylenetetrazol (PTZ) administration in rats (Broersen et al., 2000). Finally, it will be recalled that DA depletion in the mPFC following 6-OHDA infusion in rats produced an anxiogenic behavioural profile in the elevated plus-maze (Espejo, 1997). These data suggest that appropriate DA levels are necessary in the mPFC to allow an animal to cope with stressful environments. Together with the aforementioned evidence showing that DA reductions in the mPFC render aversive states in rats (Bals-Kubik et al, 1993), it is conceivable that the sensitivity of DA levels in the vmPFC to exogenous stressful events likely play a role in associative visceral-emotional working memory functions in the orbitomedial PFC.

Indirect evidence in support of this hypothesis stems from the observation that briefly exposing rats to a predator odour enhanced DA metabolic activity in the mPFC and disrupted working memory in a non-matching to sample task (Morrow et al., 2000). Moreover, various aversive manipulations not only enhance mPFC DA activity, but also impair working memory in rats and primates (Arnsten & Goldman-Rakic, 1998; Murphy, Arnsten, Goldman-Rakic, & Roth, 1996; Murphy, Arnsten, Jentsch et al., 1996). These data render the suggestion that appropriate DA levels in the vmPFC are also particularly important for working memory performance in rats and primates.

Consistent with this hypothesis, spatial working memory in rats requires D1 receptor activation in the vmPFC, as well as D1 receptor modulation of hippocampal inputs to the vmPFC (Seamans et al., 1998). In contrast, however, microinfusion of the D1 agonist, SKF 81297, in the PFC impaired delayed alternation performance in rats, effects reversed by SCH-23390 (Zahrt, Taylor, Mathew, & Arnsten, 1997). Taken together, it could be suggested that the integrity of the DA projections to the hippocampus (Gasbarri, Sulli, Innocenzi, Pacitti, & Brioni, 1996) and the vmPFC (Seamans et al, 1998) is necessary for normal working memory function in rats (Robbins, 2000b). But there appears to be discrepent findings in the literature with respect to the role of medial prefrontal D1 receptors on working memory in rats. Such discrepencies might be due in part to the nature of the working memory task and the dose-range of the drugs used. For example, in the 8-arm radial maze working memory task of Seamans et al., the delay period was 30 min in duration, the animals were tested in repeated daily sessions under non-stressful conditions, and a D1 antagonist impaired performance (D1 agonist effects not evaluated) (Seamans et al., 1998). In the study of Zahrt et al., a delayed alternation task was used with varying intertrial delays (max. 30 s) and a D1 agonist impaired performance (Zahrt et al., 1997). It could be suggested that delayed alternation tasks may tap into active attentional mechanisms, rather than long delay-dependent short-term memory. Other laboratories have also consistently shown that enhanced DA transmission in the vmPFC is associated with impaired performance in delayed alternation procedures (Arnsten & Goldman-Rakic, 1998; Moore, Fadel, Sarter, & Bruno, 1999; Murphy, Arnsten, Goldman-Rakic et al., 1996; Murphy, Arnsten, Jentsch et al., 1996; Robbins, 2000a, 2000b). The distinguishing feature of DA receptor activation-induced working memory or attentional dysfunction might be the relationship between DA reactivity in the mPFC to stressful events (Moore, West, & Grace, 1999). Thus it would appear that concurrently stimulating DA receptor sub-types (D1 and D2) in the vmPFC likely

disrupts aspects of attention related to working memory, with subsequent influences on working emotional/behavioural set.

The observation that non-specifically stimulating DA receptors in the mPFC disrupts attentional processing in rats (Lacroix et al., 2000), lends support to this hypothesis. These investigators showed that intra-mPFC infusions of the non-specific DA agonist, apomorphine, disrupted attention in rats in a prepulse inhibition procedure (Lacroix et al., 2000). It should be mentioned, however, that 10 µg/side volume infusions were used in this study. This could be important since low-dose infusion of a D1 agonist in the vmPFC in rats improved, and infusions of the D1 antagonist, SCH-23390, impaired attentional performance in the 5-choice serial reaction time task (Granon et al., 2000). The intriquing aspects of these data clearly suggest that non-specifically hyperactivating DA receptors in the vmPFC in rats (i.e., mimicking the effects of aversive events) appears to be anxiogenic, and disrupts attentional processing.

In summary, a role for DA receptors in several aspects of executive function in the vmPFC has been demonstrated. Several lesion, pharmacological, immunohistochemical and behavioural studies have shown that aberrant DA neurotransmission in the orbitomedial PFC, particularly in response to stressful or anxiogenic situations, adversely impacts anxiety, attentional and working memory processing. Moreover, it would appear that these executive dysfunctions resulting from disrupted DA activity in the mPFC may occur through interactivity with the basal forebrain – mPFC cholinergic system (Moore, Fadel et al., 1999; Moore, West et al., 1999). Finally, it is entirely possible that disrupted DA transmission in the vmPFC likely (directly or indirectly) concurrently modulates these cognitive/emotional/behavioural functions.

1.2.1. Central cholinergic influences

There have been several recent hypotheses aimed at explaining the role of the basal forebrain NBM – medial PFC cholinergic projections. Aberrant ACh transmission in the PFC has been

associated with the positive signs of schizophrenia (Moore, Fadel et al., 1999; Muir, Everitt, & Robbins, 1996; Sarter & Bruno, 1997a, 1997b, 1998b), senile dementia (Everitt & Robbins, 1997; Perry, Walker, Grace, & Perry, 1999; Sarter & Bruno, 1997a), attentional deficits (Lawrence & Sahakian, 1995, 1998; McGaughy, Everitt, Robbins, & Sarter, 2000; Muir, Everitt, & Robbins, 1994), anxiety (Berntson et al., 1996; Berntson et al., 1998; Hart, Sarter, & Berntson, 1998, 1999), and working memory dysfunction (Broersen, Heinsbroek, de Bruin, Uylings et al., 1995; Granon et al., 1995; Ragozzino et al., 1999; Ragozzino & Kesner, 1998).

It should be noted that the septo-hippocampal cholinergic system has long been hypothesized to mediate anxiety (File, Kenny, & Cheeta, 2000; Gray, 1982; Gray, 1983, 1985a, 1985b; Gray et al., 1991; Gray & McNaughton, 1983, 1996; Pesold & Treit, 1992, 1996; Treit & Pesold, 1990), and working memory (Kesner, Farnsworth, & DiMattia, 1989; Olton, 1977, 1987; Olton & Papas, 1979; Olton & Werz, 1978; Olton, Wible, & Shapiro, 1986). More recently however, the septo-hippocampal cholinergic projections have been suggested to be more involved in attentional function (Baxter, Bucci, Holland, & Gallagher, 1999; Baxter, Holland, & Gallagher, 1997; Lawrence & Sahakian, 1995), rather than memory processing (Blokland, 1995). Moreover, some investigators have suggested that septo-hippocampal GABA projections influence memory processing, rather than cholinergic projections (Alreja et al., 2000; Wu, Shanabrough, Leranth, & Alreja, 2000). In any event, these data together add to the general hypothesis that basal forebrain cholinergic degeneration leads to executive dysfunction, particularly the loss of attentional control of executive function (Baxter, Bucci et al., 1999; Baxter et al., 1997; Robbins, 2000a, 2000b; Robbins & Everitt, 1995; Sarter & Bruno, 2000; Sarter et al., 1999).

The widely held view that basal forebrain cholinergic projections to the prefrontal cortex are generally concerned with cognitive function, and attentional processing in particular, has been extensively tested in animal and human research (Furey, Pietrini, Alexander, Schapiro, & Horwitz,

2000; McGaughy et al., 2000; Robbins, 2000b; Sarter & Bruno, 2000; Sarter et al., 2001). The most widely used test of attention in animal studies is the 5-choice serial reaction time task developed by Robbins and Everitt and colleagues (Inglis, Olmstead, & Robbins, 2001; Robbins, 1998). This model requires animals to monitor the location of a briefly presented light in 1 out of 5 spatially-arranged target areas. (Sarter et al., 2001). The task combines aspects of the continuous performance tasks commonly used in human studies (Mirsky & Rosvold, 1960; Wilkinson, 1963, 1990) and primarily tests sustained spatial attention (Sarter et al., 2001). Moreover, Inglis et al. suggest that the 5-choice serial reaction time procedure can provide independent measures of several different aspects of attention in rats: (a) the ability to detect random and unpredictable sensory stimuli, (b) to maintain readiness to respond to stimuli (sustained attention), (c) discriminate significant from insignificant stimulus presentations (selective attention), and (d) select and perform the appropriate motor response to the stimulus (executive control) (Inglis et al., 2001).

Sarter and colleagues have extensively used the 5-choice serial reaction time task to systematically evaluate the role of cortically-projecting basal forebrain cholinergic fibres in rats (Sarter et al., 2001), for review). A brief summary of their findings follows. (1) Immunotoxic ACh lesions in the basal forebrain NBM in rats disrupted the animal's signal detection ability (McGaughy, Kaiser, & Sarter, 1996). (2) Infusion of BDZs in the NBM reduced ACh activity in the mPFC and also impaired sustained attention in rats (Holley, Turchi, Apple, & Sarter, 1995). (3) Infusions of $GABA_A$ agonists in the NBM in rats transiently reduced cholinergic activity and also impaired sustained attention (Sarter & Bruno, 1994). (4) Infusion of $GABA_A$ inverse agonists in the NBM in rats transiently activated cortical ACh activity and disrupted attention (Holley et al., 1995; Sarter & Bruno, 1994). (5) Infusion of a NMDA antagonist in the NBM in rats mimicked the effects of cholinergic lesions or $GABA_A$ agonists on cortical ACh release and attentional performance (Fadel, Sarter, & Bruno, 2001). (6) Infusion of NMDA in the NBM in rats mimicked the effects of

$GABA_A$ inverse agonists on cortical ACh release and attentional performance (Gill, Sarter, & Givens, 2000). Finally, Gill et al. demonstrated that ACh release and modulation of cortical activity in the mPFC in rats was correlated with attentional demands (Gill et al., 2000). Collectively these data implicate the NBM – mPFC cholinergic projections in attentional processing.

There have also been different hypotheses as to how the basal forebrain NBM might become dysregulated to the extent that hyperactivated cholinergic activity in the PFC may underly the positive symptoms of schizophrenia and the etiology of anxiety, and hypoactive PFC cholinergic activity might result in declining executive functions, especially evident in disrupted attentional control of working memory (Moore, Fadel et al., 1999; Sarter & Bruno, 1999). First, there is some suggestion that hyperactivated ventral tegmental DA cell activity results in excessive DA release in the nucleus accumbens, with subsequent D2 receptor-mediated inhibition of local GABA cells. Inhibited GABA projections to the NBM results in chronically disinhibited cholinergic projections and hyper ACh release in the medial PFC (Sarter & Bruno, 1997a, 1998b, 1999, 2000).

A second hypothesis proposes that hyperactivated GLU terminals in the NBM from the dorsomedial thalamic nuclei, the central amygdaloid complex, or the hippocampus may serve to overactivate cortical cholinergic terminal activity (Sarter & Bruno, 1997a, 1997b; Sarter et al., 2001). A detailed inspection and discussion of this hypothesis will be found in the General Discussion section of the present Thesis.

A third hypothesis proposes that the projections from the cholinergic pedunculopontine tegmental nucleus in the reticular brainstem to the NBM may modulate cortical cholinergic activity (Inglis et al., 2001). In this regard, some investigators have shown that pedunculopontine terminals influence the basal neuronal firing patterns in the NBM (Bertorelli, Forloni, & Consolo, 1991). This hypothesis is indirectly supported by the demonstration that cholinergic immunotoxic pedunculopontine tegmental lesions disrupted sustained attention in rats (Inglis et al., 2001) in a

similar fashion to the attentional disruptive effects reported by McGaughy and colleagues following immunotoxic NBM lesions (McGaughy et al., 1996). Interestingly, Leri and Franklin showed that pedunculopontine lesions were also anxiogenic in the elevated plus-maze (Leri & Franklin, 1998). These authors suggested that the pedunculopontine nucleus was likely not involved in learning and memory (based on the observation that these lesions did not effect working memory in their study), but in the regulation of anxiety (Leri & Franklin, 1998). Additional evidence to support the notion that the pedunculopontine tegmental nucleus is not involved in working memory processes stems from the observation that bilateral radiofrequency lesions of this cholinergic reticular brainstem nucleus in rats did not produce accuracy deficits in a delayed non-matching to position task (Steckler, Inglis, Winn, & Sahgal, 1994). These data render the suggestion that ascending cholinergic fibres from the reticular brainstem influence attentional processes related to anxiety-like behavioural expression.

Finally, in a review of the possible roles for the pedunculopontine tegmental nucleus in attentional processing, data showing that bilateral lesions of this nucleus in rats produced a constellation of behavioural changes reminiscent of the 'frontal syndrome' (i.e., involving disinhibition of behaviour, the release of inappropriate behaviour, and the production of perseverative behaviour) that is observed in rats following mPFC lesions, was discussed (Winn, 1998). These authors suggested that the psychological changes underlying this syndrome are thought to involve executive functions, including (a) the prospective planning of sequences of actions, (b) attentional shifting, and (c) working memory.

Cholinergic fibres from the brainstem dorsolateral tegmental nucleus in rats also innervate the basal forebrain NBM (Jones & Cuello, 1989; Zaborszky & Cullinan, 1996; Zaborszky, Cullinan, & Braun, 1991) and the vmPFC (Sesack et al., 1989; Swerdlow & Koob, 1987). In this regard, several investigators have suggested that ascending cholinergic projections from the brainstem dorsolateral

tegmental nucleus are involved in fear conditioning, conditioned reinforcement, aversively-motivated discriminative avoidance, as well as cardiovascular conditioning (Berntson et al., 1996; Gabriel, Poremba, Ellison-Perrine, & Miller, 1990; Inglis, Dunbar, & Winn, 1994).

A fourth hypothesis proposes that cholinergic basal forebrain NBM projections to the medial PFC selectively mediates anxiety-associated changes in cardiovascular reactivity (Berntson et al., 1998). This hypothesis is supported by the observations that the cardiovascular reactive effects of $GABA_A$ inverse agonist infusion (FG-7142) in the NBM in rats were mediated by enhanced ACh release in the mPFC (Berntson et al., 1996; Berntson et al., 1998). Moreover, the sympathoexcitatory effects of FG-7142 were blocked by cholinergic immunotoxic lesions of the NBM (Berntson et al., 1998). Indeed, Berntson et al (1996) demonstrated that intraventricular infusion of the M1/M2/M3 agonist, carbachol, mimicked the cardioacceleratory response produced by intraventricular FG 7142 infusions in rats. Moreover, intraventricular infusion of the M1\M3 antagonist, atropine, blocked the carbachol-induced tachycardia in these rats (Berntson et al, 1996). Importantly, central muscarinic activation resulted in sympathetic arousal, thus mimicked effects reliably produced by a referent anxiogenic β-carboline compound in laboratory animals. Other laboratories have also shown that centrally-administered cholinergic agonists activated the sympathetic nervous system in rats (Brezenoff, 1972; Brezenoff & Giuliano, 1982). Ruggeriero et al (1990) also implicated the forebrain NBM in cardiovascular responsivity to stressors and defensive arousal in rats. Berntson and colleagues have subsequently suggested that exaggerated cardioacceleratory responses reflecting enhanced emotional reactivity to sensory stimuli (i.e., resulting in artificially-exaggerated stimulus intensities) may encode anxiogenic states, particularly in exteroceptive aversively-motivated situations (Berntson et al., 1998).

In summary, the interesting link between Berntson and colleagues' hypothesis that exaggerated cholinergic activity in the mPFC brought about by disinhibiting NBM cholinergic cortical

projections, rapidly enhanced the defensive anxiety-like cardioacceleratory responses in rats (Berntson et al., 1996; Quigley, Sarter, Hart, & Berntson, 1994), and Sarter and colleagues' hypothesis that similarly invoked cholinergic activation in the mPFC in rats enhances attentional processing, caught our attention. It is conceivable that cholinergic activation in the vmPFC should exert anxiogenic effects in the elevated plus-maze and enhance working memory and attentional processing in the Y-maze. This hypothesis was tested in Experiment 4 in the present thesis. Moreover, the fact that cholinergic influences on working memory, attention and anxiety in the vmPFC are influenced by DA (Kim & Levin, 1996) and kappa receptor (Hiramatsu, Murasawa, Mori et al., 1998; Hiramatsu, Murasawa, Nabeshima, & Kameyama, 1998; Itoh, Ukai, & Kameyama, 1993a) interactions, might explain why medial prefrontal DA and ACh receptor mechanisms may exert concurrent modulatory influences on associative working memory, attention and anxiety.

Central κappa opioid influences

The profound central effects of opioid peptides on emotional valence in response to environmental events is well documented in the literature (Agmo, Galvan, Heredia, & Morales, 1995; Brandao, Cardoso, Melo, Motta, & Coimbra, 1994; Nabeshima et al., 1992; Stein, Hiller, & Simon, 1992; Yamada & Nabeshima, 1995). Moreover, several investigators have suggested that kappa receptor agonists serve to regulate overactive DA systems in the brain, thus for example, exert dampening influences on the rewarding effects of abused drugs such as cocaine and morphine (Shippenberg, LeFevour, & Heidbreder, 1996; Shippenberg & Rea, 1997).

Opioid κappa$_1$ receptors have been identified in deep layers of the vmPFC, the central nucleus of the amygdala, the tri-synaptic hippocampal circuits, as well as in the nucleus accumbens in rats (Mansour, Fox, Akil, & Watson, 1995; Unterwald, Knapp, & Zukin, 1991). In addition, moderately high levels of the endogenous κappa agonist, dynorphin, are found in these mesocorticolimbic

terminal sites (Mansour, Khachaturian, Lewis, Akil, & Watson, 1988). Interestingly, dynorphin inhibits spontaneous and electrically-evoked DA release from terminal sites within these limbic forebrain structures (Olson et al., 1993).

The notion that opioids are involved in emotional responding to stressful environments is not a new one (e.g., (Stein et al., 1992)). Some investigators have suggested that opioid activation may be a necessary component of anxiolysis in animal models of anxiety. For example, Ågmo and colleagues demonstrated that systemic naloxone administration blocked the anxiolytic properties of systemic BDZ administration in the elevated plus maze in rats. It was suggested that the proconflict effects of naloxone could have been mediated through κappa receptor inhibition (Agmo et al., 1995). This hypothesis was supported by the demonstration that norBNI administration blocked the anxiolytic effects of the BDZ, chlordiazepoxide, in the elevated plus-maze in rats (Agmo & Belzung, 1998). Furthermore, various stressors consistently activate limbic opioid mechanisms thought to influence some emotional aspects of stress (Agmo et al., 1995; Brandao et al., 1994; Nabeshima et al., 1992; Stein et al., 1992; Yamada & Nabeshima, 1995).

There are some behavioural data showing direct effects of kappa agonist administration on anxiety-like behaviour, that appear to support this hypothesis. For example, systemic administration of the κappa receptor agonists, U-50,488H or U-69,593, was anxiolytic at low doses but augmented avoidance behaviour in the elevated plus-maze at doses $\geq$ 1 mg\kg in rats (Privette & Terrian, 1995). Likewise, systemic U-50,488H (high doses) induced ultrasonic vocalizations (distress calls) in rat pups reminiscent of those associated with maternal separation (Olson et al., 1993, 1995). U-69,593 or U-50,488H also enhanced conditioned avoidance behaviour following microinjection in the posterior nucleus accumbens in rats (Shippenberg, Bals-Kubik, & Herz, 1993), effects which were coincidental with decreased DA release in this accumbal site (Spanagel, Herz, & Shippenberg, 1992). Recall as well that U-50,488H or the dynorphin derivative, E-2078, infused in the mPFC also

produced conditioned avoidance behaviour in rats (Bals-Kubik et al., 1993). Moreover, U-69,593 and the kappa antagonist, norBNI, infusions in the vmPFC produced anxiolytic and anxiogenic behavioural profiles, respectively, in the elevated plus-maze in mice (Wall, 1997). These data suggest that under baseline conditions, sufficiently hi-dose kappa agonist infusions in the mPFC induce aversive effects (conditioned avoidance), but micromolar concentrations infused in the vmPFC prior to exposure to the elevated plus-maze, may have exerted a limiting control over provoked DA release to exert anxiolytic effects in our previous study (Wall, 1997).

Kappa receptor agonists have also been shown to decrease locomotor activity in rats under conditioned stressful situations (Yamada & Nabeshima, 1995). More specifically, systemic U-69,593 potentiated conditioned stress-induced immobility, while the κappa antagonist, norBNI, attenuated this fear-related behavioural response (Yamada & Nabeshima, 1995). These data prompted Yamada and Nabeshima to suggest that U-69,593 potentiated a conditioned emotional response thus exerted anxiogenic effects. Alternatively, it could be suggested that, since systemic kappa agonist administration at $\geq$ 1 mg/kg doses significantly decreased DA release in the ventral striatum and nucleus accumbens, along with largely reduced locomotor activity in rats (Di Chiara & Imperato, 1988b), then the locomotor disrupting effects of U-69593 observed by Yamada & Nabeshima likely would also have been observed in the absence of fear conditioning. This notion is supported by the demonstration that whereas systemically-administered kappa agonists at doses $\geq$ 1 mg/kg in rats profoundly disrupted locomotion in the elevated plus-maze, lower doses exerted anxiolytic effects (Privette & Terrian, 1995).

Memory-impairing effects of intra-hippocampal infusions of the endogenous κappa agonist, dynorphin, have been demonstrated. For example, bilateral dynorphin infusion in the hippocampus impaired spatial working memory in a radial maze win-stay task in rats (McDaniel et al., 1990). Furthermore, microinjection of dynorphin B in the CA3 region of the hippocampus impaired spatial

learning in the Morris water maze in rats, effects blocked by norBNI, following microinfusion in the same site (Sandin et al., 1998). In contrast, post-training intraventricular dynorphin infusions did not effect the retention of learned behaviours in rats (Tilson, McLamb, & Hong, 1986; Ukai, Itoh, Kobayashi, Shinkai, & Kameyama, 1997). However, intraventricular infusions of kappa agonists typically attenuate or reverse the memory impairments produced by various amnestic treatments (Hiramatsu & Kameyama, 1998; Hiramatsu, Murasawa, Nabeshima et al., 1998; Itoh, Ukai, & Kameyama, 1993b, 1994; Ukai et al., 1997; Ukai, Shinkai, & Kameyama, 1995). For example, dynorphin A reversed the amnestic effects of scopolamine (Itoh et al., 1993a), and U-50488H blocked the amnestic effects of the muscarinic agonist, carbachol (Hiramatsu, Hyodo, & Kameyama, 1997). Together, these data suggest that kappa agonist influences on memory are site-specific: Intra-hippocampal κappa agonist infusions disrupt memory, while more diffuse kappa agonist administration through the ventricular route produces no effects unless memory has previously been disrupted. Moreover, kappa opioids often interact with cholinergic mechanisms to exert these effects on memory.

At the time of writing, there were no studies reporting the effects of κappa receptor drug injections in the IL vmPFC on active learning and working memory parameters. However, based on the aforementioned evidence that κappa agonist administration in the mPFC can influence learning in rats (Bals-Kubik et al., 1993), as well as the demonstration that U-69,593 and norBNI infusions in the vmPFC produced an anxiolytic and anxiogenic behavioural profile, respectively, in the elevated plus maze in mice (Wall, 1997), it was hypothesized that κappa receptor drugs would likely concomitantly influence aversive learning, active working memory and anxiety-like behaviour following microinjection in the IL area of the vmPFC in CD-1 mice.

1.7. Some hypothesized relationships between anxiety and associative working memory

So far in this review, we have seen that the ventromedial PFC can influence associative working memory, attentional processing and anxiety-like behaviour in rodents and primates. Furthermore, these executive functions can be influenced by cholinergic, dopamine and kappa opioid receptor mechanisms in the vmPFC. The purpose of this section is to review various hypotheses that attempt to explain possible relationships between anxiety and associative working memory. Collectively, these proposals provided the necessary theoretical and empirical background to support the present experimental rationale.

The relationship between anxiety and memory is extremely complex, particularly as neither memory nor anxiety are unitary psychological constructs (Beuzen & Belzung, 1995; Clement & Chapouthier, 1998; McNally, 1997). It should be mentioned, however, that many psychological constructs are correlated, at least to some degree. Several researchers have for some time suggested that information-processing abnormalities may underlie the phenomenology of anxiety disorders (MacLeod & McLaughlin, 1995; Mathews, Mogg, Kentish, & Eysenck, 1995; Mathews, Mogg, May, & Eysenck, 1989; McNally, 1990, 1995, 1996, 1997; McNally, Hornig, Otto, & Pollack, 1997). The point here is that anxiety-disordered individuals often suffer from recurrent, unwarrented thoughts about personal harm (McNally, 1997). From a cognitive perspective, stored information about potentially dangerous situations might be potentiated by its ease of accessibility for people with anxiety disorders (McNally, 1997). McNally also suggested that enhanced access to such information would taint ambiguous situations as threatening and would likely exacerbate chronic anxiety proneness.

The suggestion that threat information is often processed outside of awareness partially addresses this issue. McNally suggested that the inability to terminate anxiety-related processing once it begins, may well be the hallmark of pathological anxiety (McNally, 1995). In our view, it is

also possible that once an exteroceptive event activates an anxiety-related cognitive/behavioural set, it may not be stoppable in anxiety prone individuals. In this regard, it is widely believed that anxiolytic drugs, such as benzodiazepine agonists (BDZs), may interfere with such associative working memory processing (Clement & Chapouthier, 1998). In this regard, some investigators have suggested that BDZs might better be termed 'acquisition-impairing' (Clement & Chapouthier, 1998), or 'attention deficit producing' (Berntson et al., 1998), rather than 'amnestic' agents. It could also be suggested that BDZs might impair prospective attentional mechanisms as well. Some research has focused on the notion that some anxiety sub-types (e.g., post-traumatic stress disorder, PTSD) might also be related to biased attentional processing (Ehlers & Breuer, 1996).

Although these ideas certainly address biased memory processes in the protracted expression of anxiety-related cognition in humans, the question remains as to how active associative working memory processing might relate to the protracted inappropriate behavioural expression of anxiety. In other words, is provoked state anxiety in animal models influenced by active associative memory processing? Some investigators suggest that it is (Beuzen & Belzung, 1995). In a behavioural correlation analysis of different mouse strains in differing anxiety-provoking situations, these investigators found that the more anxious an animal was (in the light/dark box for example), the better it performed in an emotional memory test (passive avoidance). Thus it was suggested that the anxiety 'state' of an animal was correlated with aversive learning and memory processing (Beuzen & Belzung, 1995). These data certainly indicate an obvious relationship between anxiety and aversive learning tasks (Clement & Chapouthier, 1998), but do not address relationships between active associative memory processing and anxiety in conflict/exploration situations.

File and colleagues were the first to investigate the relationship between learning and anxiety in a 2-trial elevated plus-maze procedure with rats (File, 1990, 1992, 1993; File et al., 2000; File et al., 1993). They demonstrated that in naive rats BDZs have clear anxiolytic effects in the elevated plus-

maze, but in rats with previous plus-maze experience benzodiazepines are for the most part ineffective (File, 1993). File suggested that naïve animals learn a type of phobic avoidance during trial-1 that shows up as noticeably reduced open arm exploration activity in trial-2 (File, Gonzalez, & Andrews, 1998; File et al., 2000). Thus associative aversive learning and memory processing that occurs during the first elevated plus-maze trial, may well be analagous to the type of implicit processing suggested by McNally (1995) to subserve anxiety induction in vulnerable humans. For these reasons we also decided to use a 2-trial elevated plus-maze procedure primarily to estimate drug effects on (and further characterize) the type of learning that presumably occurs during trial-1.

Accumulating data from several human and infrahuman pharmacological studies consistently show that some neurotransmitter systems in particular brain sites can influence learning, memory, attention and anxiety (reviewed in Section 1.6). It would appear, however, that the neurotransmitter systems that can influence both anxiety and memory, exert such dual influences in specific brain sites only. For example, 5-HT_{1A} agonists can exert anxiolytic effects as well as impair learning and memory in the hippocampus (Guimaraes et al., 1993). Cholecystokinin levels in the central nucleus of the amygdala or the nucleus accumbens also appear to influence anxiety, attention and memory processes in rats (Dauge, Derrien, Blanchard, & Roques, 1992; Dauge & Lena, 1998). These data, together with the previously reviewed effects of ACh, DA and kappa opioids on associative memory and anxiety in the vmPFC, led us to investigate the possibility that cholinergic and/or kappa opioid mechanisms could concurrently modulate anxiety and active working memory in the IL area of the ventromedial PFC.

2.0. EXPERIMENTATION

Despite the evidence suggesting an interactive role for kappa opioid and ACh transmitter systems in working memory processing, or perhaps even anxiety-related behavioural responses to stressful exteroceptive events, it is surprising to note the paucity of data describing the role of the vmPFC in anxiety-related behavioural responsivity in novelty/conflict paradigms such as the elevated plus-maze. In view of the reciprocal innervation patterns between the IL vmPFC and multiple brainstem sites presumed to influence the induction and behavioural expression of anxiety (Frysztak & Neafsey, 1991, 1994; Owens & Verberne, 1996), the following experiments will evaluate the role of the IL area of the vmPFC in behavioural responsivity related to anxiety in the elevated plus-maze following κappa or ACh receptor manipulations. In addition to the use of the elevated plus-maze, anxiety will also be evaluated in an open field defensive-withdrawal situation, and spontaneous working memory will be evaluated in the Y-maze following κappa and/or ACh muscarinic receptor manipulations *.

* Here it should be noted that transfer latencies (as an index of learning) in the elevated plus-maze and defensive/withdrawal latencies (as an index of anxiety) in the open field were only measured in Experiments 2a, 2b and 3. Preliminary measures in the muscarinic drug studies indicated minimal effects. In addition, we reasoned that the elevated plus-maze 2-trial procedures we used could adequately detect drug effects on aversive learning without measuring transfer latencies, and the nature of the elevated plus-maze test battery conceivably incorporated elements of defensive/withdrawal. For these reasons, both latency scores were not measured in Experiments 4a, 4b, 4c and 5.

Although the dorsal 5-HT and NE systems, as well as mesocortical DA/CCK systems, all of which innervate the vmPFC through the medial forebrain nerve bundle, are readily activated in response to threatening environmental stimuli (Goldstein et al., 1996), there is a paucity of data describing the influence of κappa or ACh receptor ligands on anxiety and spontaneous memory performance following intracerebral administration in the infralimbic cortex.

The present series of experiments systematically investigated the relative contributions of the IL vmPFC to the concurrent modulation of anxiety-like behaviour, aversive learning and active

working memory in CD-1 mice. Overlapping neurotransmission was evaluated by testing the possibility that the behavioural effects of kappa opioid or muscarinic M1 drug infusions in the IL vmPFC could concurrently influence both cognitive/behavioural processes. It should be noted that the kappa and muscarinic drugs used in these studies were used as tools only to uncover possible relations between anxiety induction and working memory processing in the orbitomedial PFC. Consequently, these studies do not address dose-response or dose-effect pharmacological issues. Therefore in each experiment, dose-related drug effects were only interpreted with a view to better understand prefrontal executive functions with respect to cognitive/behavioural relationships.

The following list outlines the sequential series of published and submitted manuscripts that in total make up the entire experimental bases of the present thesis.

1) An extensive ethological confirmatory factor analysis of elevated plus-maze behaviour in CD-1 mice. This study provides a detailed analysis of 10 dependent variables in an attempt to confer the most parsimonious elevated plus-maze model by testing the factorial validity of a three-factor, or competing two-factor anxiety model using the LISREL 8.0 statistics program. Moreover 2 trials were analyzed (n = 200), seperated by a 24-hr intertrial interval. Results from this study validated a two-factor model and conferred the most appropriate behavioural indices to measure in the remaining pharmacological experiments (Wall & Messier, 2000b).

2) The behavioural effects of the kappa-1 receptor agonist, U-69,593, in the elevated plus-maze, open field defensive/withdrawal and the Y-maze following microinfusion in the IL vmPFC in CD-1 mice (Wall & Messier, 2000c) *.

* Here it should be noted that this manuscript was submitted and published in Brain Research before the Experiment 1 confirmatory factor analysis was completed. Therefore the behavioural indices reported in the manuscript are somewhat different than those reported in Experiment 2 in the present Thesis. Experiments 3, 4 and 5 did however measure and report on the 7 indicator variables identified and validated in our confirmatory factor analysis 2-factor solution. For reasons of continuity, the same 7 indicator variables were remeasured and reported on in Experiments 2a and 2b.

3) The behavioural effects of the kappa antagonist, norBNI, in the elevated plus-maze, open field defensive/withdrawal and the Y-maze following microinfusion in the infralimbic mPFC in CD-1 mice. This study is a follow-up to Experiment 2 (Wall & Messier, 2000a).

4) The behavioural effects of IL infusions of the non-specific muscarinic antagonist, scopolamine, the specific M1 antagonist, pirenzepine, and the specific M1 agonist, McN-A-343, in the elevated plus-maze and the Y-maze in CD-1 mice (Wall, Flinn, & Messier, 2001).

5) The behavioural interactive effects of norBNI – pirenzepine mixed drug infusions on elevated plus-maze and Y-maze performance following microinfusion in the IL vmPFC in CD-1 mice (Wall & Messier, 2001c).

2.1. Experimental Rationale

The aforementioned experiments were designed to address the following questions:

1) What is the most parsimonious structure of animal anxiety in the elevated plus-maze?
2) Are the neurological bases of anxiety induction and working memory performance related?
3) Does the vmPFC concurrently modulate anxiety and working memory?
4) Does the kappa1 opioid receptor system influence active working memory processes in the IL vmPFC as it has been shown to influence anxiety-like behaviour?
5) Does the acetylcholine M1 receptor system influence anxiety-like behaviour in the IL vmPFC as it has been shown to influence some forms of working memory?
6) Can combined kappa opioid/M1 drug infusions in the IL vmPFC interactively influence the concurrent modulation of anxiety and working memory?

7) Is it possible to separate attentional from working memory processes following mixed drug infusions in the IL cortex?

Answers to these questions should provide further insight into the associative nature of the IL vmPFC in the concurrent modulation of active working memory and anxiety in challenging situations, thus provide further useful data for the development of treatment strategies that can address, for example, impaired prefrontal attentional control of working memory and working set in relation to anxiety disorders.

2.2. EXPERIMENT 1

Wall, P. M. & Messier, C. (2000). Ethological confirmatory factor analysis of anxiety-like behaviour in the murine elevated plus-maze. *Behavioural Brain Research* 114: 199-212.

2.2.1. Abstract

The elevated-plus maze has been used in animal research to measure anxiety since 1985 and is currently the most widely used animal model of anxiety. Since this paradigm has been the subject of several principal components analyses, it is well qualified for confirmatory factor analysis research. The current report builds on the substantial theoretical knowledge and empirical data obtained from these structural analyses with a view to obtain further progress in the evolution of our understanding of animal anxiety in the elevated plus-maze.

The purpose of the present report was twofold: (a) to test if the a piori imposition of a 3-factor model, or a competing 2-factor elevated plus-maze model, would fit our sample (n = 200 CD-1 mice) data in each of two trials within an inferential confirmatory factor analytic framework; (b) provide a well-fitting model that confers indicator variables that can most effectively and parsimoniously measure underlying constructs of elevated plus-maze behaviour.

Multiple model-fitting criteria were used, and issues related to data nonnormality, outliers, replicability of the model, sampling error and error of approximation in the estimation of final model fit were addressed. The final 2-factor model, with estimated error covariance between two different pairs of indicator variables, was a good fit on the trial-1 data, although it was necessary to allow unprotected stretch attends to nonsignificantly cross-load on factor-2. A 2-factor model also fit the trial-2 data from the present analysis, although it was necessary to allow closed arm time ratio to negatively cross-load on factor-1.

These results indicate that inferential hypothesis testing and model building procedures within a confirmatory factor analysis framework produces interpretable animal anxiety indices in the elevated plus-maze. Moreover, a 2-factor, rather than a 3-factor model, parsimoniously and unambiguously explained the underlying constructs of anxiety-like mouse behaviour in the elevated plus-maze in the present study. Taken together, a reduction in the growing number of behavioural indices reported in elevated plus-maze pharmacological studies is suggested.

2.2.2. Introduction

Novelty/exploration conflict models, such as the elevated plus-maze (elevated plus-maze), reportedly capitalize on the endogenous fear rodents exhibit for unprotected open spaces. The elevated plus-maze has been validated as an animal model of anxiety for both rats (Pellow et al., 1985; Pellow & File, 1986) and mice (Lister, 1987; Stephens, Meldrum, Weidmann, Schneider, & Grutzner, 1986), is currently one of the most widely used animal models in behavioural pharmacology (Dawson & Tricklebank, 1995; Handley & McBlane, 1993a; Hogg, 1996), and is routinely used for studying the effects of putative anxiolytic drugs, as well as the neurobiological mechanisms of anxiety (Cruz, Frei, & Graeff, 1994; Pellow & File, 1986; Trullas et al., 1991; Vasar, Lang, Harro, Bourin, & Bradwejn, 1994). It is noteworthy that elevated plus-maze test procedures do not inflict physical pain or constraints (i.e., foot-shock or physical restraint) on test

animals, therefore arguably address inherent unconditioned psychophysiological facets of anxiety. In this respect, rats and mice tend to naturally avoid the open arms and stay more in the protected areas of the maze (Dawson & Tricklebank, 1995; Handley, McBlane, Critchley, & Njung'e, 1993; Lister, 1987; Treit, Robinson, Rotzinger, & Pesold, 1993).

The behavioural indices usually analyzed in the elevated plus-maze, presumably related to anxiety and exploration/assessment activity, have been identified in several factor analytic studies for both mice and rats (Cruz et al., 1994; Fernandes & File, 1996; Holmes & Rodgers, 1998; Lister, 1987; Rodgers & Johnson, 1995; Trullas & Skolnick, 1993). Factor analytic techniques have been used in elevated plus-maze research for two principal reasons: a) to identify the relationships between specific observed variables and their underlying latent factors as they relate to facets of anxiety, and b) to assess whether different animal models measure the same subtypes of anxiety (Fernandes & File, 1996; Lister, 1987; Trullas & Skolnick, 1993). In the present analysis, we were concerned with the former reason, particularly since we believe that such anxiety relevant relationships are important for pharmacological research.

Although findings from reported studies consistently demonstrated that 'traditional' anxiety indices such as cumulative open arm entries and the ratio of open:total arm entries, total arm entries, open arm time and the ratio of open:total time generally load on the 'anxiety' factor (labeled factor-1 in all reported studies), some of these observed variables also ambiguously load on one or more of the other factors (i.e., factor-2 generally interpreted as a locomotor activity construct and factor-3 generally interpreted as an approach/avoidance conflict – decision-making construct, in most studies). This is particularly evident when 'ethologically-derived' indices are included with traditional measures in an analysis. Accordingly, results have been reported for two- (Fernandes & File, 1996; Lister, 1987; Rodgers & Johnson, 1995), three- (File, 1993; Rodgers & Johnson, 1995; Trullas & Skolnick, 1993), four- (Cruz et al., 1994; Fernandes & File, 1996; Fernandez Espejo,

1997; File, 1993), five- (Fernandes & File, 1996) and six-factor (Holmes & Rodgers, 1998; Rodgers & Johnson, 1995) solutions.

All of the aforementioned studies used principal components analysis with varimax component matrix rotation within an exploratory analytic approach. A major limitation of principal components analysis is that it does not lend itself to model hypothesis testing, thus component solution interpretations and generalizabilities are limited. More specifically, (a) principal components analysis may extract components that correlate with only one observed variable, thus should likely be viewed as 'unstable' and uninterpretable (Tabachnick & Fidell, 1996), (b) there are no critera against which to test the component solution (Bentler & Kano, 1990), thus principal components analysis is a descriptive data analytic technique only, where assumptions regarding distributions of observed variables are not applicable (Tabachnick & Fidell, 1996), (c) principal components analysis analyzes variance, whereby each observed variable contributes one unit of variance to the positive diagonal of the correlation matrix, thus total (unique and error) variance is distributed to the components (Tabachnick & Fidell, 1996), and (d) in this way, principal components analysis extracts maximum variance from a data set thus generates a unique mathematical solution (Tabachnick & Fidell, 1996). The difficulty with maximum variance extraction lies within principal components analysis procedures whereby component structures will always be biased and inflated since error variance is not taken into consideration, thus the sample correlation matrix will always reproduce its estimated population correlation matrix (Gorsuch, 1990; Snook & Gorsuch, 1989). Finally, when there is little or no a priori knowledge of unique correlations among observed variables, principal components techniques are useful as a method of dimension reduction (i.e., reducing the number of constructs to a meaningful level) (Bentler & Kano, 1990).

Confirmatory factor analysis in many respects differs from principal components analysis in that some a priori knowledge about latent structures is known (Byrne, 1998). First, based on

theoretical knowledge and empirical research, relations between the observed variables and the underlying factors are postulated a priori, and the goodness-of-fit between the hypothesized model and the sample data is statistically tested (Byrne, 1998). Differences between the model and sample data (i.e., residual or measurement error) are represented by the diagonal, and error correlations (covariances) among the observed variables are represented by the off-diagonal, in the variance/covariance matrix. Second, the confirmatory factor analysis model analyzes the resultant variance/covariance matrices. Hence, the key parameters to be estimated in a confirmatory factor analysis model are (a) the matrix of the regression coefficients (factor loadings) related to each factor, (b) the symmetrical variance/covariance matrix among the factors, and (c) the symmetrical variance/covariance matrix among the errors of measurement of the observed variables (Byrne, 1998). Third, confirmatory factor analysis procedures allow model hypothesis testing by constraining all covariances (correlated errors of estimate) among indicator variables to zero, while allowing variance between an indicator variable and itself to be estimated to determine the model variance/covariance matrix. In summary, confirmatory factor analysis is an inferential data analysis technique, analyzes covariance structures and allows for model hypothesis testing, compared with principal components analysis that is a descriptive data analysis technique only, analyzes correlation matrices and does not allow for hypothesis testing.

With this backdrop of information concerning some of the basic differences between principal components analysis and confirmatory factor analytic methods, inherent interpretation difficulties in most of the reported elevated plus-maze principal components analysis solutions have become apparent. Lister (Lister, 1987) originally identified and operationally-defined two latent components underlying a limited scope of elevated plus-maze behavioural indices with NIH Swiss mice (n = 91). Factor-1 was interpreted as an anxiety construct owing to the exclusive loadings of open entry ratio and open time ratio. Factor-2 was interpreted as a locomotor activity construct owing to the

exclusive loading of total entries (Lister, 1987). A similar two-component model emerged from a principal components analysis of a broader spectrum of observed indices among hooded Lister rats (n = 34), but closed arm time and total entries significantly cross-loaded on both factors (Fernandes & File, 1996). A 2-component elevated plus-maze principal components solution also emerged with DBA/2 mice (n = 90), except in this case, whereas closed entries and total entries exclusively loaded on factor-2, open entries cross-loaded on both factors (Rodgers & Johnson, 1995). Notably, when % hub time was added to the analysis, a third factor emerged (interpreted as descision-making – approach/avoidance conflict) with hub time ratio loading exclusively, but total entries cross-loaded on factors 1 and 2, and closed arm time ratio cross-loaded on factors 1 and 3 (Rodgers & Johnson, 1995).

Together, the aforementioned elevated plus-maze principal components analysis two-component (and one 3-component) solutions indicated that measuring total arm entries fails to convincingly differentiate between the constructs of locomotor activity and anxiety, therefore arguably cannot unambiguously measure either latent construct. Moreover, open entries and open entry ratio, as well as closed entries and closed entry ratio are highly correlated, as is open time with open time ratio, and closed time with closed time ratio. Thus the utility of including both variables of each highly correlated pair in a factor analysis seems redundant.

In light of such limitations, and in addition to the 'traditional' arm-related time and entry indicator variables most often measured in the elevated plus-maze, more 'ethologically-derived' behavioural indices have been included in some of the reported elevated plus-maze principal components analyses. The rationale for including such behaviours stems from several reports showing that mice and rats engage in considerable risk assessment activity in the elevated plus-maze (Fernandes & File, 1996; Fernandez Espejo, 1997; Holmes & Rodgers, 1998; Rodgers & Johnson, 1995; Rodgers, Johnson, Cole et al., 1996), behaviours previously characterized as

antipredator/defensive-related (Blanchard et al., 1990). However, the greater the number of indicators that have been included in some of the analyses, the greater the number of latent components have been extracted in the principal components solutions. Hence, interpretation difficulties arise as a result of increasing complexity associated with higher numbers of extracted components. In this respect, it has been suggested that different numbers of extracted components can be partially explained by the number and type of behavioural indices analyzed (Holmes & Rodgers, 1998).

Six-factor elevated plus-maze principal components solutions reported by Rodgers and Johnson (Rodgers & Johnson, 1995) with DBA/2 mice (N = 90), and Holmes & Rodgers (Holmes & Rodgers, 1998) with Swiss-Webster mice (N = 74) were examples of such interpretation difficulties. These investigators included a broad array of ethological indices along with the usual arm-related measures in their analyses. The extracted 6 components for DBA/2 mice were interpreted as (1) anxiety, (2) locomotor activity, (3) decision-making and/or approach/avoidance conflict, (4) related to approach/avoidance conflict, (5) vertical activity, and (6) exploration activity (Rodgers & Johnson, 1995). The six components differed somewhat with Swiss-Webster mice, (1) anxiety, (2) locomotor activity, (3) directed exploration, (4) vertical activity, (5) displacement, and (6) defensive ambulation (Holmes & Rodgers, 1998). Inspection of the factor loading matrix of the former study, however, revealed numerous cross-loadings (total entries, closed entries, hub time ratio and closed time ratio ambiguously measured more than one factor), thus factors 3, 4, 5 and 6 appeared unstable and ambiguous (Rodgers & Johnson, 1995). The latter study also showed significant cross-loading patterns for total entries, closed time ratio and hub time ratio, among other measures, thus indicating that factors 4, 5 and 6 were likely unstable and ambiguous (Holmes & Rodgers, 1998). Taken together, strain differences as well as the high number of measured indices contributed to the ambiguity of the aforementioned principal component solutions (Holmes & Rodgers, 1998).

Another ethological principal components analysis of elevated plus-maze behaviour with Wistar rats (n = 30) produced a four-component solution with open entries and total entries cross-loaded on factors 1 and 2, and protected risk assessment (including protected stretch attends, PSA) cross-loaded on factors 1, 3 and 4 (Cruz et al., 1994). The factor loading matrix in that particular study also indicated that factors 3 and 4 were unstable and therefore difficult to interpret. A more recent ethologically-derived principal components analysis of elevated plus-maze behaviour with Swiss albino mice (n = 30) also yielded a four-component solution (Fernandez Espejo, 1997). However, OE and CE cross-loaded on factors 1 and 2, sniffing ambiguously loaded on factors 1, 3 and 4, rearing (vertical stretches) cross-loaded on factors 2 and 4, and unprotected stretch attends (USA) cross-loaded on factors 3 and 4 (Fernandez Espejo, 1997). With such a degree of cross-loading, factors 2, 3 and 4 should be considered unstable, thus difficult to interpret. Finally, a principal components analysis of traditional elevated plus-maze indices, using combined data from sixteen inbred mouse strains (combined n = 319) yielded a 3-component solution (Trullas & Skolnick, 1993), but again, the pattern of factor loadings in this particular study indicated that factors 2 and 3 could be considered unstable, thus difficult to interpret. Since it has been suggested that numerous cross-loadings (i.e., loadings > .20 on more than 1 factor) produce complex structures that are difficult to interpret (Byrne & Baron, 1993), such cross-loading patterns are indicative of overfactored solutions (Gorsuch, 1983).

Many of the extracted component solutions in the aforementioned studies cannot, apparently, be meaningfully interpreted. Moreover, simply adding more indicator variables to an analysis did not correct the problem, but merely added to the ambiguity and complexity of the solution. Had there been larger sample sizes in each of the aforementioned analyses, perhaps some of the extracted components would have stabilized with two or more indicators uniquely loading on them. In this respect, since correlation coeficients are less reliable when estimated from small samples

(Tabachnick & Fidell, 1996), then solutions with several high loading indicators (i.e., loadings > .80) require a minimum of 150 subjects (Guadagnoli & Velicer, 1988), but usually a minimum of 200 subjects should be analyzed to produce a reasonably stable and interpretable solution (Comrey & Lee, 1992). Taken together, some of the elevated plus-maze principal components solutions, reported to date, could be considered ambiguous, unstable and difficult to interpret owing to (a) small sample sizes, (b) biased and inflated components, (c) numerous cross-loadings, (d) complexity due to far too many indicators, (e) lack of hypothesis testing, (f) ignoring variable normality assumptions, (g) unrealistically assuming that all observed variables contain no errors of estimate (Snook & Gorsuch, 1989), and (h) the tenet that mathematically calculated orthogonal principal components solutions (regardless of inflated loadings) are 'clear' therefore simple to interpret (Snook & Gorsuch, 1989).

Regardless of such shortcomings, however, it should be emphasized that the aforementioned analyses have provided the growing body of empirical data and extensive theoretical knowledge necessary to conduct the present confirmatory factor analyses. To this end, the purpose of the above review of some of the shortcomings inherent in reported principal components analyses was not to show that this analytic tool should be discarded in favour of more detailed analyses, but to underscore the readiness of the elevated plus-maze, through the work of such previously-conducted analyses, to be analyzed in a more rigid hypothesis-testing fashion. Both analytic methods can therefore be used in parallel for continued research concerning animal models of anxiety.

Since it is not clear that solutions with more than 3 latent factors add any value to the use of the elevated plus-maze as a meaningful measurement instrument of animal anxiety, the present study was designed to test the factorial validity of a 2- or 3-factor elevated plus-maze structure using a confirmatory factor analysis approach based on analyses of covariance structures (Byrne, 1992).

Ultimately, we sought to identify the most parsimonious model that can best describe underlying structures driving the behaviour of a large sample of CD-1 mice in the elevated plus-maze.

It made intuitive sense to initially conceptualize the elevated plus-maze as consisting of three distinct sectors (i.e. the unprotected open arms, the protected closed arms and centre platform) (Rodgers & Cole, 1993a, 1993b, 1994; Rodgers, Cole, Aboualfa, & Stephenson, 1995; Rodgers, Cole, & Davies, 1994; Rodgers & Johnson, 1995), to facilitate testing a 3-factor hypothesis. A 3-factor model is in keeping with reported 3-component solutions for mice (Rodgers & Johnson, 1995; Trullas & Skolnick, 1993) and rats (File, 1993; File et al., 1993). However, the differences between the aforementioned 3-component solutions and our 3-factor model lie in the hypothesis that the indices related to each sector of the maze (i.e., open, closed, centre) would load on a distinct sector-related factor. Whereas factor-1 has historically been interpreted as an anxiety factor (Lister, 1987; Pellow et al., 1985), it may be more appropriately interpreted as an anxiety/unprotected exploration factor with open arm time and entry measures loading with unprotected risk assessment measures (unprotected stretch attends and unprotected head dips). Moreover, whereas factor-2 has usually been interpreted as a locomotor activity measure (Cruz et al., 1994; Fernandez Espejo, 1997; Lister, 1987, 1990; Rodgers & Johnson, 1995), it could be interpreted as a protected exploration activity factor (Fernandes & File, 1996) with closed arm time and entry measures loading with closed arm activity (total vertical stretches, TVS). Finally, while the third factor related to the centre platform has been interpreted as an approach/avoidance – conflict/decision factor by several laboratories (Cruz et al., 1994; File et al., 1993; Rodgers & Johnson, 1995), we took it as an approach/avoidance conflict construct with hub time ratio (HTR) loading with protected risk assessment measures (protected stretch attends and protected head dips).

Alternatively, two factors may well explain elevated plus-maze behaviour (factor-1 – anxiety/unprotected exploration and factor-2 – protected exploration). This model eliminates the

hypothesized approach/avoidance conflict construct in the 3-factor model. Overall, the factorial validity of a 2- or 3-factor elevated plus-maze anxiety model has never been tested using confirmatory factor analysis hypothesis testing procedures.

2.2.3. Materials and Methods

Sample. 200 naïve, male CD-1 mice served as subjects . The mice were obtained from Charles River (St. Constant, Quebec) at approximately five weeks of age. All mice were initially housed in groups of four in polypropylene cages in a temperature controlled room (21 ± 1° C), and permitted ad libitum acccess to food and water. Mice were acclimatized for 6 to 8 weeks and acheived a weight range of 36-46 grams prior to the analysis. All mice were maintained on a 12 hour light/dark cycle (lights on at 7 a.m.).

Apparatus. The elevated plus-maze used in the present analysis was a modified version of a previously-described apparatus (Lister, 1987). The two opposing closed arm runways were 30 cm x 5 cm and enclosed by 15 cm high walls on each side and ends. The two opposing open arms were also 30 cm x 5 cm with a .25 cm high Plexiglas edge on either side and at the end of each runway. The center platform was 5 cm x 5 cm. The enclosed arm walls were constructed with urethane-coated wood and lined with Plexiglas. The floor of the maze was covered with a thin layer of vulcanized black rubber to facilitate inter-trial cleaning. The apparatus was positioned 40 cm from the floor on a wooden stand hidden from view and the floor under the stand was covered with black cloth to reduce height cues.

Procedure. Mice were transferred from the main animal holding area to the laboratory and left undisturbed for at least one hour prior to behavioural testing. The floor and Plexiglas walls of the maze were wiped between trials with a 70% alcohol/distilled water solution to prevent individual mice from following the scent of a previously tested animal. All testing was conducted during the morning phase of the light/dark cycle between 0700 and 1300 hours. All trials took place in a dimly

illuminated chamber and were recorded by a ceiling-mounted SONY video camera that was linked to a Panasonic video monitor and VCR, and an IBM-compatible computer positioned outside the test chamber. Videotapes were subsequently scored by one of us and a trained observer. Individual trials were five minutes in duration and commenced following placement of the animal in the central platform facing an open arm. A mouse was considered in an arm when all four paws were positioned within an arm runway, and considered in the centre hub when at least one paw was out of an arm. Trial-2 was conducted in the same manner following a 24-hr. interval.

The Elevated plus-maze as a Measurement Instrument. The range of behavioural indices were operationally-defined as follows: Open arm entries (OE), open arm time ratio (open arm time ÷ total time on the maze – OTR), closed arm time ratio (closed arm time ÷ 300 seconds – CTR), hub time ratio (total time in central hub ÷ 300 seconds – HTR), unprotected head dips (scan-like dipping of the animal's head and shoulders over the sides of the open arms – UHD, Fig. 1), protected head dips (head dipping from the centre hub around the edge of a closed arm wall – PHD), unprotected stretch attend postures (an animal stretches forward while in the open arms and retracts to its original position without locomoting forward or moving its hind paws – USA, Fig. 2) (Rodgers & Johnson, 1995), protected stretch attends (occuring from closed arm into centre hub or from centre hub into open arms – PSA), closed arm entries (CE), total vertical stretches (wall climbing/rearing – TVS).

Place Figures 3 and 4 about here

It should be noted that confirmatory factor analysis testing strategies were originally developed to test hypothesized underlying structures of various psychological measurement instruments in humans. Insofar as observed animal behavioural indices in aversive situations can emulate human responses in a psychological assessment such as a self-report questionnaire, confirmatory factor

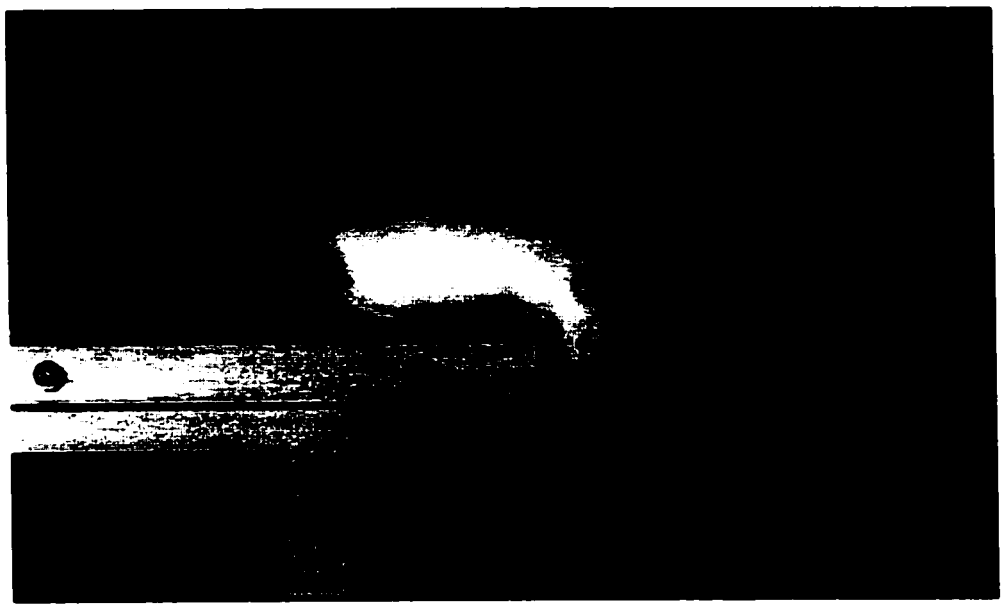

Figure 3

Figure 4

analysis procedures should similarly facilitate analysis of anxiety-like constructs in rodents employing the elevated plus-maze as a measurement instrument.

Model Testing Strategy

The data from 200 CD-1 mice were analyzed in four stages. First, the zero-order correlation matrix of 18 observed variables was analyzed and any variable pairs with correlations > .90 were flagged (i.e., OE and OER were highly correlated, thus OER was eliminated). The decision to eliminate, for example, OER and CER as redundant variables stems from the fact that entry ratios take both open and closed arm entry activity into consideration thus do not represent a distinct measure. The remaining 10 indicator variables (OE, OTR, CTR, HTR, USA, UHD, PHD, PSA, CE and TVS) were subsequently screened for outliers and distribution normality. Second, the goodness-of-fit of the hypothesized 3-factor model (anxiety/open assessment, closed assessment, approach/avoidance conflict) on the data from both elevated plus-maze trials was tested with confirmatory factor analysis maximum likelihood procedures using the LISREL 8.0 statistics program (Joreskog & Sorbom, 1993b). Third, given low goodness of fit statistics indicating a poor-fitting initially hypothesized model, as well as in the respecified nested 3-factor model (i.e., post-hoc model-building procedures were used to identify the causes of model misfit), exploratory factor analysis procedures (SPSS 8.0) were used to specifiy a statistically and substantively meaningful alternative model of factor structure (Byrne, 1992). Fourth, an alternative 2-factor model was then analyzed in a further exploratory factor analysis of the data from both trials. The indicator variables included in the 2-factor analyses were chosen based on a) the R^2 values from the final nested 3-factor confirmatory factor analysis model, b) the *t*-values (i.e., magnitude of parameter loadings divided by their standard errors to indicate whether each loading is significantly different from 0 where *t*-values > 1.96 are significant at $p < .05$) (Byrne & Baron, 1993), c) the substantive meaningfulness of each parameter to the overall model (MacCallum, 1986; Silvia & MacCallum,

1988), and d) theoretical and empirical knowledge (Byrne & Baron, 1993). Given evidence of reasonable Chi-square statistics, this 2-factor model was then tested for goodness-of-fit on the data from both trials in post-hoc confirmatory factor analysis model-fitting procedures. It should be noted that post hoc model-fitting procedures are only appropriate when model respecification is grounded in sound theoretical rationale (Byrne, 1994). We believe that model-fitting procedures were justified here.

Goodness-of-Fit Criteria

The evaluation of model fit was based on substantive, statistical and practical fit criteria: a) substantive meaningfulness of the model relative to existing empirical evidence (see introduction), b) the χ^2 likelihood ratio statistic, c) the comparative fit index (CFI), d) the root mean square error of approximation (RMSEA), and e) the expected cross-validation index (ECVI) along with the critical *N*. The χ^2 likelihood ratio statistic measures the closeness of fit between the sample covariance matrix and the fitted model covariance matrix, thus serves as an indicator of overall model fit (Byrne, 1994). The closer the χ^2 value is to its corresponding *df* value, as well as a χ^2 probability of $\geq .05$, the better the model fit is to the sample covariance matrix (Byrne, 1998). The CFI is an incremental fit index that is derived from the comparison of a restricted model (structure imposition on the data) with an independence (or null) model (all correlations among variables are zero) in the determination of goodness of fit (Byrne, 1994). Although a CFI value of .90 has been considered indicative of a good model fit, a value of at least .93 is expected for models to be well-fitted (Byrne, 1994). The RMSEA takes into account the error of approximation in the population (i.e., how well would the model, with unknown but optimally chosen parameter values, fit the population covariance matrix if it were available?) (Bollen & Long, 1993). A root mean square error of approximation value of .05 represents a good fit, and values as high as .08 are considered reasonable errors of approximation in the population (Browne & Cudeck, 1993). The ECVI

measures the discrepancy between the fitted covariance matrix on the sample, and the expected covariance matrix that would be obtained in another sample of equivalent size (Byrne, 1998), and the critical *N* indicates the sample size that would be necessary to replicate the fitted covariance matrix.

Initial Hypothesized Model to be Tested

The initial model hypothesized a priori that a) elevated plus-maze behaviour could be explained by three factors (anxiety/unprotected assessment, protected assessment, and approach/avoidance conflict), b) each indicator would have a non-zero loading on the elevated plus-maze factor it was designed to measure and zero loadings on all other factors, c) the three factors would be correlated, and d) measurement error terms would be uncorrelated (Byrne, 1994). The initial 3-factor model is depicted in Figure 5, a schematic diagram of the initial 3-factor model used in this analysis.

Place Figure 5 about here

The boxes represent measurements (indicator variables); the circles represent the constructs (latent variables) which underly the measurements. The straight arrows from the factors (circles) to their respective indicator variables (boxes) represent the regression co-efficient (λ), or factor loading, indicating that the factors precipitate the observed performance on the measures (Byrne, 1986). The three arrows (two straight and one curved) linking factors one and two, factors two and three, and factors one and three, respectively, represent the correlation (ϕ) between each pair of factors. The δ's pointing to each indicator (boxes) represent individual indicator measurement errors (Byrne, 1986).

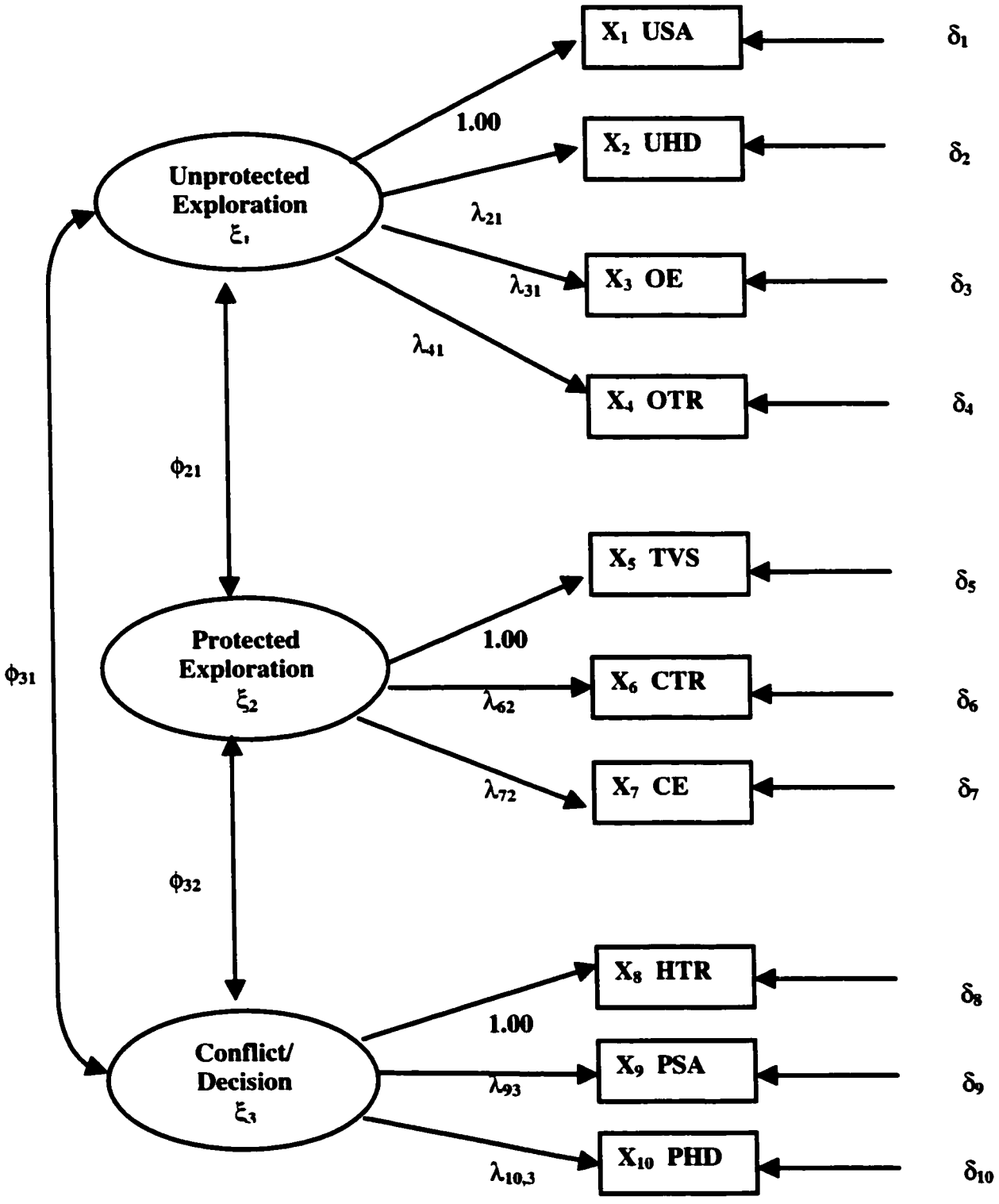
X1 USA
δ1
1.00
X2 UHD
δ2
Unprotected Exploration
ξ1
λ21
λ31
X3 OE
δ3
λ41
X4 OTR
δ4
ϕ21
X5 TVS
δ5
Protected Exploration
ξ2
1.00
ϕ31
λ62
X6 CTR
δ6
λ72
X7 CE
δ7
ϕ32
X8 HTR
δ8
1.00
Conflict/ Decision
ξ3
λ93
X9 PSA
δ9
λ10,3
X10 PHD
δ10

Figure 5

2.2.4. Results

Preliminary analyses of the 10 variables to be analyzed, using SPSS 8, identified 7 outliers in trial-1 and 5 outliers in trial-2. Deletion of these outlier cases resulted in sample sizes of 193 for trial-1 and 195 for trial-2. Analyses of the resultant data indicated univariate normality in the trial-1 data (range = -.062 to .893, M = .125) and in the trial-2 data (range = -.236 to 1.165, M = .372). Skewness values > 1 are considered significant, hence, UHD was significantly skewed to the right (1.165) and open entries approached significance (.957) in trial-2 (for skewness and kurtosis trial comparisons – Table 1).

Place Table 1 about here

Although there was some evidence of skewness in trial-2, it was expected that a significant number of subjects would display lower open arm entry and exploration activity in trial-2 (i.e., large cluster of subjects at or near zero). Moreover, there was also evidence of high positive and negative kurtosis in some of the distributions. In this regard, nonnormal data in some of the distributions from both trials may have led to downwardly biased standard errors of estimate with a resultant inflated number of significant parameters (Byrne, 1994; Muthen & Kaplan, 1985). This did not appear to be the case, however, in the present study.

Trial-1 Confirmatory Factor Analyses

Based on the poor fit of the hypothesized 3-factor model from a statistical (χ^2) and practical (CFI and ECVI) perspective, this model was rejected (Table 2). We then took an exploratory

Place Table 2 about here

Table 1

Experiment 1.

Skewness and Kurtosis Comparisons Between Trials 1 and 2

Indicator	Skewness Trial-1	Skewness Trial-2	Kurtosis Trial-1	Kurtosis Trial-2
OE	.368	.957	−.303	.466
CE	.028	−.223	−.557	−.191
OTR	.605	.803	.315	−.228
CTR	.194	.117	−.115	−.178
HTR	.062	.293	−.443	.109
UHD	.893	1.165 *	.326	.660
PHD	.167	.756	−.307	.969
USA	.281	.740	−.148	−.223
PSA	.515	.503	.149	.026
TVS	.261	−.236	.189	−.061
Mean	.125	.372	−.09	.133

Note. * = significant at .05.

Table 2

Experiment 1.

Goodness-of-Fit Statistics for Trial-1 3-Factor Models

Model	*df*	χ^2	$\Delta\chi^2$	Δdf	RMSEA[a]	ECVI[b]	CFI[c]	Crit. *N*[d]
Hypothesized	32	201.43			.17	1.3	.87	
Final with error covariance between PSA and PHD and OE X-loading	30	150.14	51.29	2	.14	1.05	.91	65.74

Note. [a] Root Mean Square Error of Approximation. [b] Expected Cross-Validation Index.

[c] Comparative Fit Index. [d] Critical *N* necessary to replicate final model-3 statistics.

approach within the confirmatory factor analysis framework in a model building process to respecify a final 3-factor nested model (Figure 6). Model-2 was then tested with error covariance between PSA and PHD free to be estimated. The justification to allow this correlated error estimate stemmed from (a) a modification index (MI) of 24.41, (b) a significant *t*-value of 4.49, $p < .05$, (c) a $\Delta\chi^2$ goodness-of-fit improvement of 25.5 ($df = 1$), and (d) the notion that correlated errors among items may result from a high degree of overlap in item content (i.e., both items measure much the same thing) (Byrne, 1998). The resulting model-2 goodness-of-fit statistics still indicated a poor fit, CFI = .89, RMSEA = .16 and ECVI = 1.17. We then turned to the model-2 MI tables to locate remaining misspecified items. The largest degree of misfit (MI = 23.73) indicated that allowing OE to cross-load on factor-2 would result in a much improved fit. This cross-loading was not surprising owing to observations that OE has often cross-loaded on the same factor that CE loaded on in several reported principal components analysis studies (Cruz et al., 1994; Fernandez Espejo, 1997; File, 1993; Rodgers & Johnson, 1995; Trullas & Skolnick, 1993). These data, coupled with a significant loading *t*-value (-4.56, $p < .05$) on factor-2, prompted us to test model-3 with OE free to cross-load.

Notwithstanding the respecified error covariance between PSA and PHD, as well as OE cross-loaded on factor-2, this final model was also statistically and practically rejected owing to a) a moderately acceptable CFI (.91), b) a high RMSEA (.14) and ECVI (1.05), c) substantial standardized correlation between factors two and three ($\phi_{32} = 1.02$), d) low loadings for TVS, PSA and PHD, and e) substantial measurement errors for TVS, HTR, PSA and PHD (Figure 6).

Place Figure 6 about here

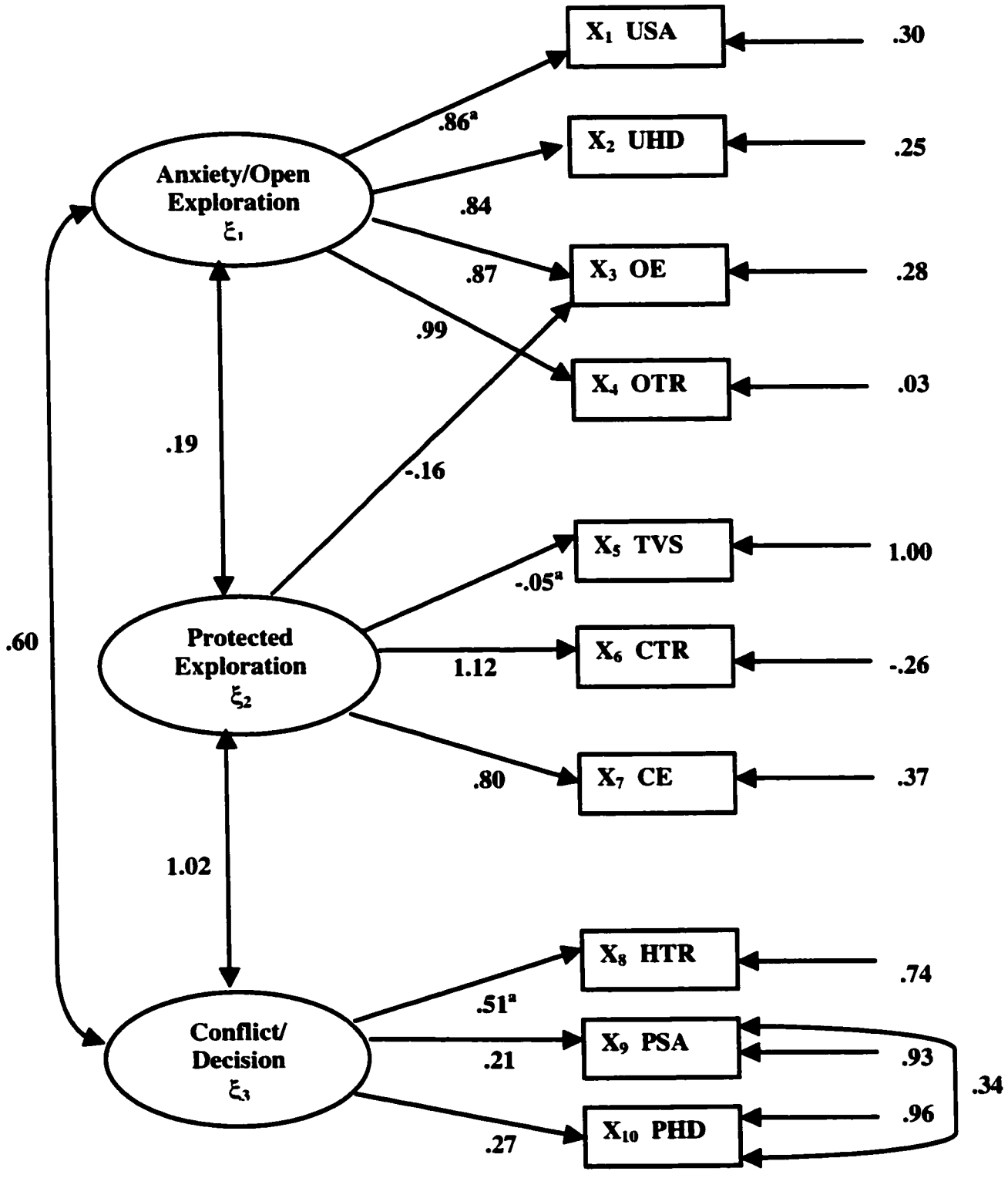
X1 USA
X2 UHD
X3 OE
X4 OTR
X5 TVS
X6 CTR
X7 CE
X8 HTR
X9 PSA
X10 PHD
Anxiety/Open Exploration ξ1
Protected Exploration ξ2
Conflict/ Decision ξ3
.30
.25
.28
.03
1.00
-.26
.37
.74
.93
.96
.34
.86a
.84
.87
.99
-.16
.19
-.05a
1.12
.80
1.02
.60
.51a
.21
.27

Figure 6

These results were indicative of either an over- or under-factored model (Byrne, 1994). Moreover, inspection of the R^2 values in this final 3-factor nested model indicated that factor-2 could not explain any of the TVS (R^2 = .00) variance, while factor-3 explained little of the PHD (R^2 = .04) and PSA (R^2 = .07) variance, and only a modest amount of the HTR variance. Taken together with the range of components in several reported principal components analysis studies, we subsequently evaluated whether the data from trial-1 could be better described by a 2- or 4-factor structure, while keeping the same 10 indicators. The data were subsequently reanalyzed using SPSS8 exploratory factor analysis procedures with maximum likelihood estimation and direct oblimin factor matrix rotation.

Trial-1 Exploratory Factor Analyses

Exploratory factor analyses were conducted using SPSS 8.0 to evaluate 2- and 4-factor structures with the same 10 indicator variables. Factor loadings of .35 or greater were considered meaningful (Gorsuch, 1983).

Four-factor Structure (10 indicators). The 4-factor structure was rejected for substantive and statistical reasons. Substantively, the third and fourth factors could not be meaningfully interpreted. Statistically, the fourth factor had an eigenvalue less than 1.0 and the χ^2 statistic was not generated. These data indicated that the confirmatory factor analysis 3-factor final nested model was not underfactored with 10 indicator variables.

Two-factor Structure (10 indicators). The 2-factor structure was also rejected for substantive and statistical reasons. Substantively, the second factor presented interpretation difficulties (HTR cross-loaded, PHD did not significantly load on either factor and PSA loaded on factor 1). Statistically, two factors only accounted for 67.49% of the variance and the χ^2 statistic was not generated. These data indicated that a) the 2-factor exploratory factor analysis solution measured with 10 indicators did not adequately describe the trial-1 data, and b) TVS, HTR, PSA and PHD

were clearly misplaced in a 2-factor structure as well as in the confirmatory factor analysis 3-factor model. In this regard, we decided to respecify a 2-factor structure with 6 indicator variables.

Respecified Trial-1 2-factor Structure (6 indicators). It made intuitive sense to respecify a 2-factor structure without TVS, HTR, PHD and PSA, as protected risk assessment activity loaded ambiquously in our sample with 10 measurement variables, and each of these four variables had poor R^2 values in the confirmatory factor analysis 3-factor final model. Thus OE, OTR, USA and UHD were expected to load on factor-1, open exploration, while CTR and CE were expected to load on factor-2, protected exploration.

Statistically, both factors accounted for 83.18% of the variance and produced a good fit ($\chi^2_{(df = 4)} = 25.2$). Substantively, no indicators meaningfully cross-loaded, thus both factors were straight forward to interpret. However, a confirmatory factor analysis was required to test the significance of the factor loadings and to ultimately test the respecified 2-factor model fit on the trial-1 data, particularly as confirmatory factor analysis procedures can a) yield unique factorial solutions, b) define a testable model, c) test the extent to which the hypothesized model fits the data, and d) suggest modifications for model improvement (Byrne, 1992).

Trial-1 Post Hoc Confirmatory Factor Analyses

The second hypothesized confirmatory factor analysis model to be tested is depicted in Figure 7. It should be noted that this confirmatory factor analysis test of the respecified exploratory factor

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Place Figure 7 about here

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analysis 2-factor model fit remains in a confirmatory factor analysis exploratory mode (Anderson & Gerbing, 1988). That is, OE, OER, USA and UHD were specified to load exclusively on factor-1,

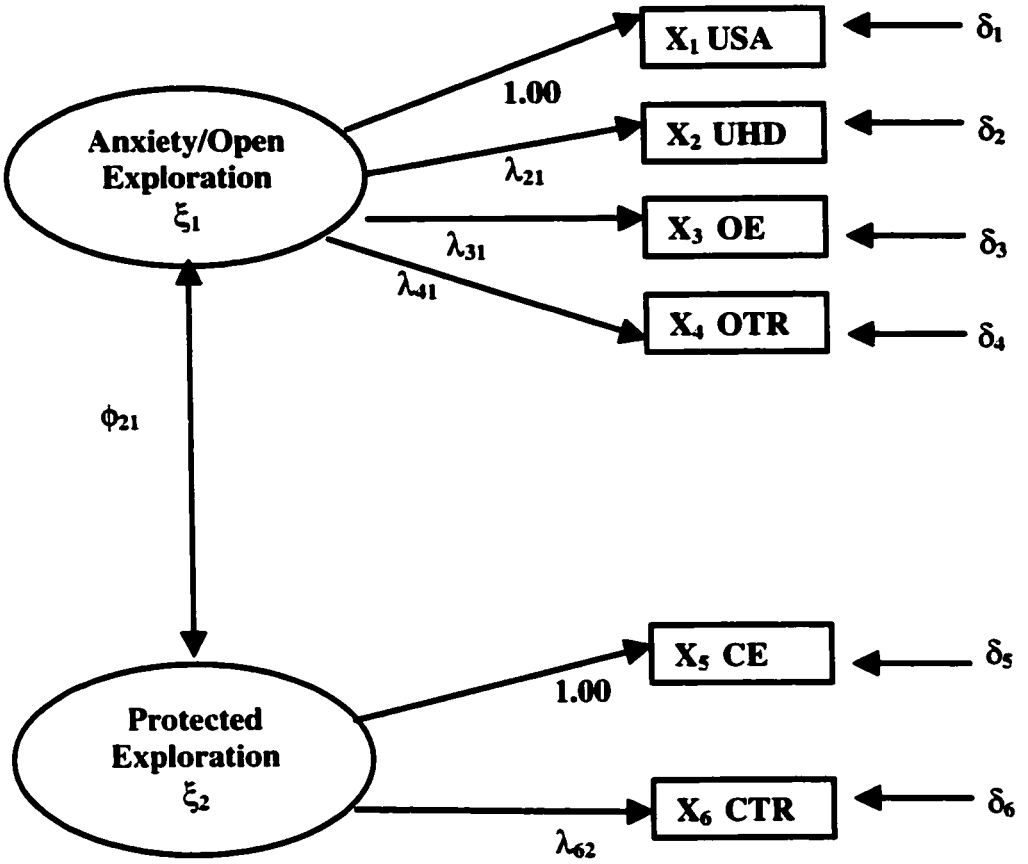

Anxiety/Open Exploration
ξ1
Protected Exploration
ξ2
1.00
λ21
λ31
λ41
1.00
λ62
ϕ21
X1 USA
X2 UHD
X3 OE
X4 OTR
X5 CE
X6 CTR
δ1
δ2
δ3
δ4
δ5
δ6

Figure 7

while CTR and CE were specified to load exclusively on factor-2. The goodness-of-fit statistics are presented in Table 3.

Place Table 3 about here

The initial 2-factor model with six indicator variables was a better fit than the initial 3-factor model with ten variables on the trial-1 data. However, inspection of the initial model MI tables indicated that allowing error covariance between OTR and CTR to be freely estimated (MI = 28.03) would result in a substantially better fitting model. Subsequently, the test of model-2 fit resulted in a significant correlated error parameter (t = 4.94, p < .05), as well as a $\Delta\chi^2$ goodness-of fit improvement of 27.79 (df = 1). A third nested model was also respecified allowing USA to cross-load on factor-2 (MI = 13.2, t-value = 4.12). Model-3 produced a total $\Delta\chi^2$ goodness-of fit improvement of 44.12 (df = 2). Finally, we considered that allowing error covariance between OE and USA to be estimated was reasonable based on the MI (9.19) and the significant t-value for this parameter (2.85). Accordingly, model-4 (nested in the initial 2-factor model) was our final 2-factor model owing to a) a significant goodness-of-fit improvement ($\Delta\chi^2_{(df=3)} = 53.3$, $p < .05$), b) the CFI (.99), c) the lowest ECVI (.24), d) significant R^2 values (USA = .71, UHD = .74, OE = .68, OTR = .99, CE = 1.00, CTR = .79), and e) a critical N of 205.1 subjects that would be needed to replicate these model-4 statistics with N = 193 subjects (Figure 8).

Place Figure 8 about here

One could argue that allowing the two error covariance estimates in the model building process merely to improve the model fit is not an acceptable practice (Byrne, 1998; Joreskog & Sorbom, 1993a), but we believe that, in addition to the statistical reasons, there was also sufficient

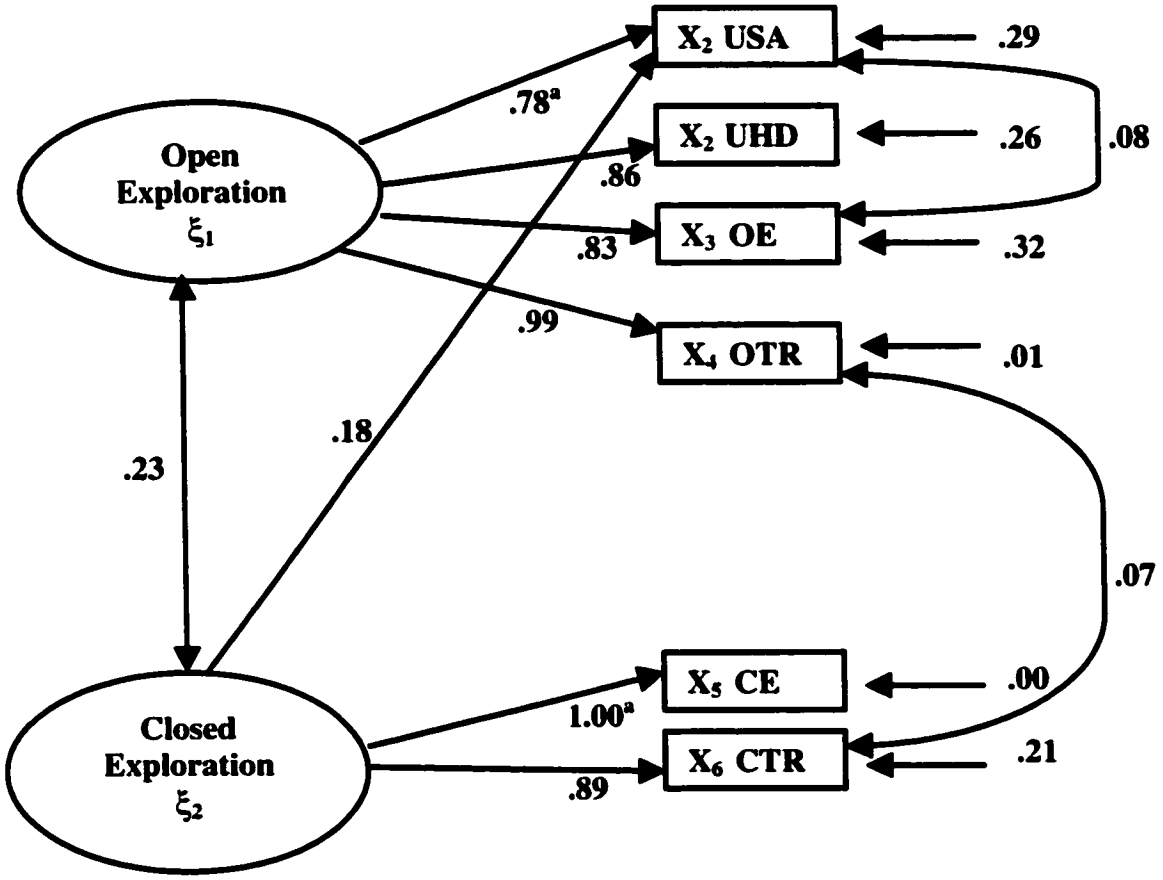

Open Exploration
ξ1
Closed Exploration
ξ2
X2 USA
X2 UHD
X3 OE
X4 OTR
X5 CE
X6 CTR
.78a
.86
.83
.99
1.00a
.89
.18
.23
.29
.26
.32
.01
.00
.21
.08
.07

Figure 8

substantive and empirical evidence to allow these correlated errors. In this regard, OTR and CTR are not mirror images of one another, rather, the ratio of time spent in the centre hub may well have forced a correlation between the former temporal measures. In addition, OE is clearly related to USA, since unprotected stretch attending could not occur without entering an open arm.

In summary, it would appear that the final 2-factor model, with ethological and traditional indices, best fits the trial-1 data in the present study. Not surprisingly, both central and peripheral anxiogenic or anxiolytic drug administration often influences these same six indices (Cole & Rodgers, 1994; Rodgers et al., 1995; Rodgers, Cole et al., 1994; Rodgers, Johnson, Champion et al., 1996; Wall, 1997).

Trial-2 Confirmatory Factor Analyses

The same 10 indicators were incorporated in the initial 3-factor model (same as in Figure 5). Although the trial-2 hypothesized 3-factor model was better fitting than the trial-1 initial 3-factor model, from a statistical (χ^2 value) and practical (RMSEA and ECVI values) perspective, this model was rejected and post-hoc exploratory model building confirmatory factor analysis procedures were undertaken to respecify a final 3-factor nested model (Table 4). Model-2, then,

insert Table 4 about here

allowed error covariance between TVS and CE based on a MI of 65.73. This estimated covariance parameter was significant (t = 7.01, $p < .05$), which resulted in a substantial $\Delta\chi^2$ goodness-of-fit improvement of 80.27 (df = 1). A third nested 3-factor model was then tested allowing PHD to cross-load on factor-1 (MI = 37.04). The total $\Delta\chi^2$ goodness-of-fit improvement was 121.41 (df = 2) with a CFI value of .96. The model-3 MI tables, however, clearly indicated that allowing error covariance between PHD and PSA to be estimated (MI = 19.87) in a fourth and final nested 3-factor

Table 3

Experiment 1.

Goodness-of-Fit Statistics for Trial-1 2-Factor Models

Model	*df*	χ^2	$\Delta\chi^2$	Δdf	RMSEA[a]	ECVI[b]	CFI[c]	Crit. *N*[d]
Hypothesized with items TVS, PHD, PSA, HTR deleted	8	67.42			.20	.49	.94	
Final model-4 with error covariance between USA and OE, OTR and CTR; USA X-loading	5	14.12	53.30	3	.098	.24	.99	205.1

Note. [a] Root Mean Square Error of Approximation. [b] Expected Cross-Validation Index.
[c] Comparative Fit Index. [d] Critical *N* necessary to replicate final model-4 statistics.

Table 4

Experiment 1.

Goodness-of-Fit Statistics for Trial-2 3-Factor Models

Model	*df*	χ^2	$\Delta\chi^2$	Δdf	RMSEA[a]	ECVI[b]	CFI[c]	Crit. *N*[d]
Hypothesized	32	238.68			.18	1.47	.91	
Final model-4 with error covariance between CE and TVS, PHD and PSA; PHD X-loading	29	96.29	3	142.39	.11	.76	.97	100.9

Note. [a] Root Mean Square Error of Approximation. [b] Expected Cross-Validation Index.
[c] Comparative Fit Index. [d] Critical *N* necessary to replicate final model-4 statistics.

model would produce a further substantial improvement in model fit (Figure 9). There was sufficient evidence to pull in this parameter based on a strikingly similar error covariance in the trial-1 3-factor model.

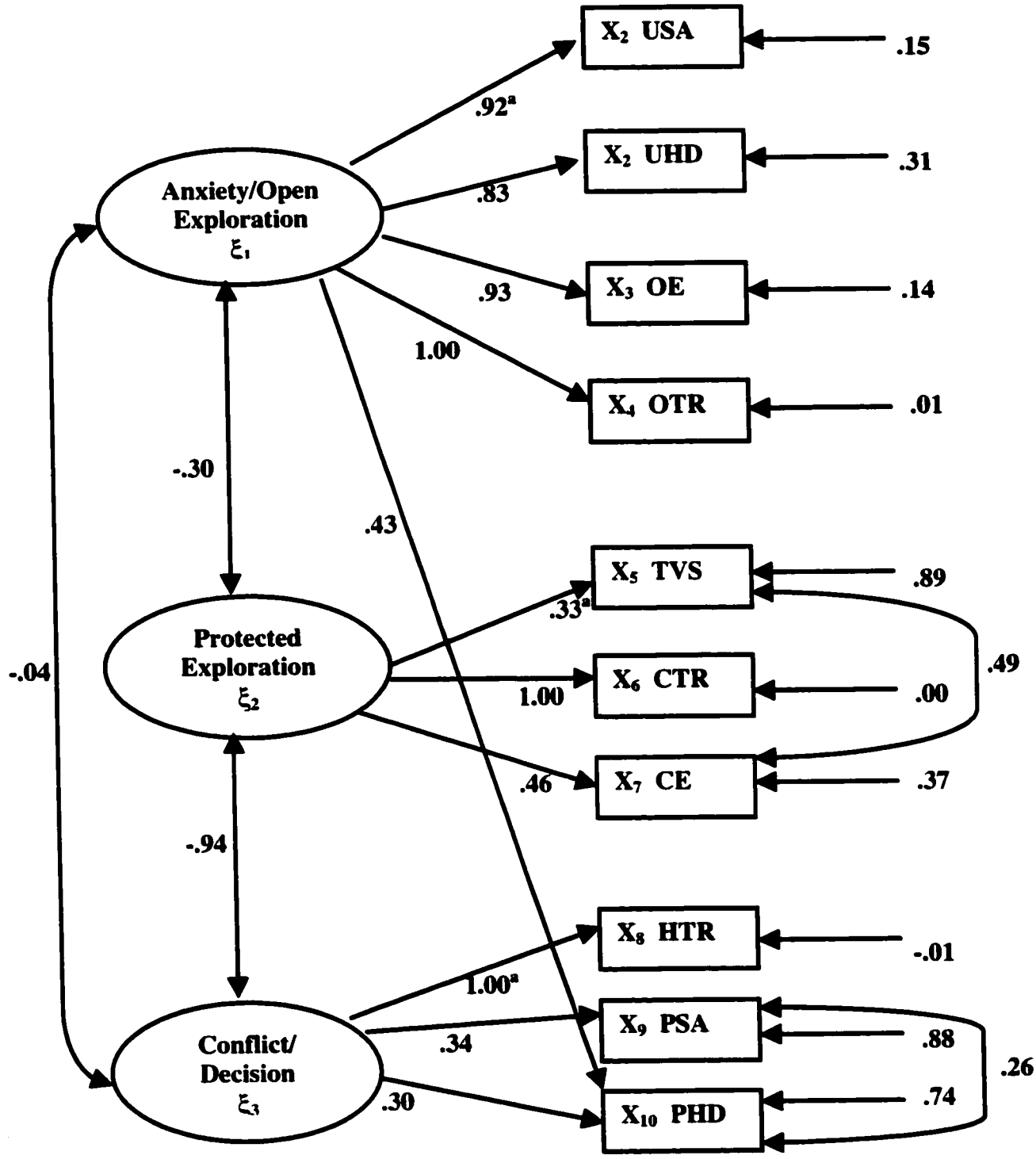
Place Figure 9 about here

One could argue that this final model, with the total $\Delta\chi^2$ goodness-of-fit improvement of 142.39 ($df = 3$), the CFI of .97, RMSEA of .11 and ECVI of .76, was a good fit on the trial-2 data. Nevertheless, there were still some notable missfitting parameters in the model, one of which could not be improved owing to the fact that when we tried to allow error covariance between HTR and CTR (MI = 22.39) in a fifth nested model, the LISREL 8.0 program would not converge on a solution. Furthermore, there were high measurement errors for TVS (.89), PSA (.88) and PHD (.74), as well as a substantial error covariance between TVS and CE (.49). There was also a high negative correlation between factors 2 and 3 (-.94). Taken together, there was sufficient evidence to reject model-4 and take an exploratory factor analysis approach to determine whether a 2- or 4-factor model could better desribe the trial-2 data.

Trial-2 Exploratory Factor Analyses

Exploratory factor analyses were conducted to evaluate 2- and 4-factor models with the same 10 indicator variables. Factor loadings of .35 or greater were considered meaningful (Gorsuch, 1983).

Four-factor Structure (10 indicators). In a similar fashion to trial-1, the 4-factor structure was rejected for substantive and statistical reasons. Substantively, the third and fourth factors could not be meaningfully interpreted. Statistically, the fourth factor had an eigenvalue less than 1.0 and the χ^2 statistic was not generated.


X2 USA
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Anxiety/Open Exploration
ξ1
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X3 OE
.14
1.00
X4 OTR
.01
-.30
.43
X5 TVS
.89
.33a
Protected Exploration
ξ2
-.04
1.00
X6 CTR
.00
.49
.46
X7 CE
.37
-.94
X8 HTR
-.01
1.00a
X9 PSA
.88
.34
Conflict/ Decision
ξ3
.26
.30
X10 PHD
.74

Figure 9

Two-factor Structure (10 indicators). The 2-factor model was also rejected for substantive and statistical reasons. Substantively, the second factor presented interpretation difficulties. Statistically, two factors only accounted for 67.8% of the variance and the χ^2 statistic was not generated.

Respecified Two-factor Structure (7 indicators). As in the trial-1 exploratory approach, it made intuitive sense to respecify a 2-factor model without HTR (cross-loaded in the 2-factor exploratory factor analysis), PHD and PSA (both loaded ambiguously), but keeping TVS as it had a robust loading on factor-2 in the exploratory factor analysis. Accordingly, OE, OTR, USA and UHD were expected to load on factor-1, anxiety/open exploration, while CTR, CE and TVS were expected to load on factor-2, protected exploration.

Statistically, both factors accounted for 81.2% of the variance and produced a good fit ($\chi^2_{(df = 8)}$ = 13.65). Substantively, no indicators significantly cross-loaded, thus both factors were straight forward to interpret. However, a confirmatory factor analysis was again necessary to test the significance of the factor loadings, as well as the respecified 2-factor model fit on the trial-2 data (same as in Figure 5, but with TVS included as a 7th indicator).

Trial-2 Post Hoc Confirmatory Factor Analyses

The initial 2-factor model reasonably fit the trial-2 data. The goodness-of-fit statistics are presented in Table 5. However, with good reason to allow CTR to negatively cross-load (-.39) on

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Place Table 5 about here

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factor-1 (MI = 29.67) in a nested model-2, this final model χ^2 goodness-of-fit of 23.29 (df = 12, p = .025) was a substantial improvement over the initial model fit ($\Delta\chi^2$ = 35.79, df = 1) (Fig. 10).

Place Figure 10 about here

Table 5

Experiment 1.
Goodness-of-Fit Statistics for Trial-2 2-Factor Model

Model	*df*	χ^2	$\Delta\chi^2$	Δdf	RMSEA[a]	ECVI[b]	CFI[c]	Crit. *N*[d]
Hypothesized with items PHD, PSA, HTR deleted 91.9	13	59.08			.14	.46	.96	
Final model with CTR X-loading	12	23.29	35.79	1	.07	.28	.99	219.4

Note. [a] Root Mean Square Error of Approximation. [b] Expected Cross-Validation Index. [c] Comparative Fit Index. [d] Critical *N* necessary to replicate final model-2 statistics.

Interestingly, the maximum MI from the final model (7.89) suggested that error covariance between CE and CTR would only slightly improve the model fit, but there was no justification to bring in this parameter, thus there were no error covariances as part of the model fit. Moreover, the R^2 values for USA (.87), UHD (.70), OTR (.97), OE (.88), CTR (.41) and CE (.71) indicated that a significant proportion of each indicator variable's variance was explained by its' respective underlying factor.

The main differences between the trial-1 2-factor final model and the trial-2 2-factor final model were the inclusion of TVS, higher measurement errors for CE and CTR, as well as CTR significantly cross-loading in trial-2. Reasons for such discrepancies are not readily known.

2.2.5. Discussion

This is the first study to test the factorial validity of 3- and 2-factor elevated plus-maze models of animal anxiety. Primarily, the present analysis adds to the acceptability of the use of the elevated plus-maze as a measurement instrument in the study of animal anxiety. Results from this confirmatory factor analysis indicate that a 2-factor model not only substantively fits the present data from both trials, but also is the most parsimonious model that can best describe underlying anxiety-related structures driving the behaviour of a large sample of CD-1 mice in the elevated plus-maze. These results clearly attest to the necessity of large sample sizes and rigorous testing procedures in the model building process that ultimately allows for straight forward interpretability. In this sense, often reported problematic items in the aforementioned principal components analyses, clearly in need of further investigation, were presently addressed.

The notion that each of the three sectors of the maze represents a distinct related factor was modeled after the factor structures of some psychological measurement instruments in human studies, where subscales of a measurement instrument are considered to represent related factors and all indices of a particular subscale are expected to load on its' related factor (Byrne, 1998). We

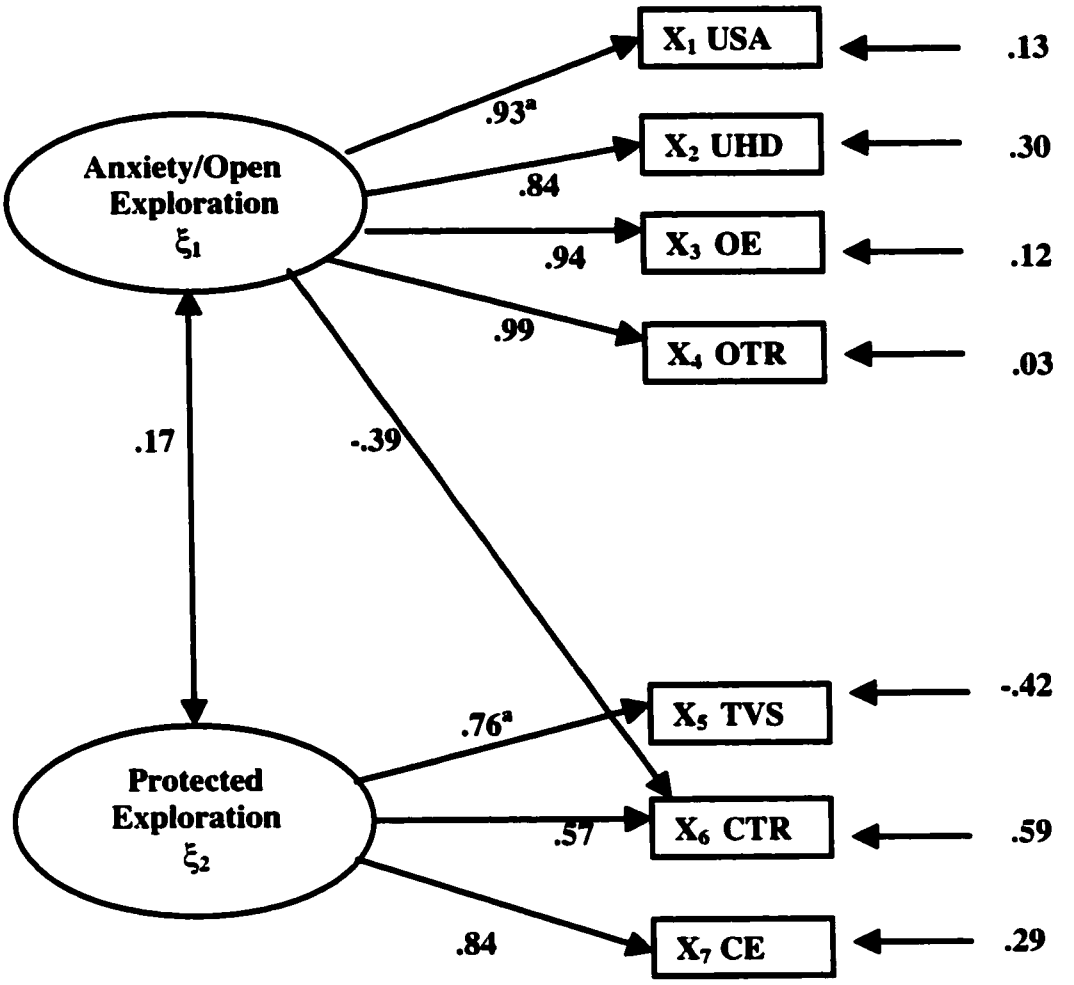
Anxiety/Open Exploration
ξ1
Protected Exploration
ξ2
X1 USA
X2 UHD
X3 OE
X4 OTR
X5 TVS
X6 CTR
X7 CE
.93a
.84
.94
.99
.76a
.57
.84
.17
-.39
.13
.30
.12
.03
-.42
.59
.29

Figure 10

likewise expected the appropriate time and activity indices related to each sector of the maze to load on the related factor.

The first issue addressed in the development of the initial 3-factor model required a reconceptualization of factor-2 (i.e., usually interpreted as locomotor activity) owing to the fact that there does not appear to be much substantive meaningfulness to a locomotor activity latent construct driving elevated plus-maze behaviour. In this respect, several investigators simply do not agree as to what indicator(s) is (are) 'pure' locomotor activity index(es). To elaborate, some investigators have reported total entries as a locomotor activity indicator (Fernandez Espejo, 1997; Lister, 1987; Pellow et al., 1985; Rodgers & Johnson, 1995), total entries as a mixed anxiety/locomotor activity indicator (Cruz et al., 1994; Elliott, Heal, & Marsden, 1992; Fernandes & File, 1996), closed entries as a pure locomotor activity indicator (Cruz et al., 1994; Elliott et al., 1992; Rodgers & Johnson, 1995), and closed entries as an index of protected exploration (Fernandes & File, 1996). Taken together, there does not appear to be any unambiguous indicators of 'locomotor activity', thus it does not appear to be a valid latent construct underlying behaviour in the elevated plus-maze. For these reasons, closed entries was taken as an indicator of a reconceptualized protected exploration second factor, as was first suggested by Fernandes and File (Fernandes & File, 1996), along with closed time ratio and total vertical stretches.

A second issue addressed in the present analysis relates to the problematic nature of an approach/avoidance or conflict/decision factor. Indeed, due to the frequency and extent to which time and activity indices related to the centre platform have ambiguously loaded in the present and other exploratory factor analyses (Cruz et al., 1994; Holmes & Rodgers, 1998; Rodgers & Johnson, 1995), these items were in need of re-evaluation. Moreover, the high measurement errors for these items, as well as substantial error covariance between items protected head dips and protected stretch attends in the trial-1 and trial-2 final 3-factor models, indicated that behaviours engaged in

from the centre hub likely do not measure a unique third factor. Rather, centre hub behaviours likely contain elements of the first two factors and perhaps a small hidden factor (Byrne, 1998). Indeed, putative anxiolytic drug treatments that consistently influence factor-1 indices, often do not influence the ratio of time in the centre hub (Cole & Rodgers, 1994; Rodgers, Cole et al., 1994; Rodgers, Johnson, Champion et al., 1996; Wall & Messier, 2000c). Taken together, there was ample evidence for us to conclude that factor-3 was, at best, a weak underlying construct driving CD-1 mouse behaviours in the present elevated plus-maze analyses.

The observation that there was little difference between the factor-1 (anxiety) indicator loading patterns of each trial was interesting given some reported anxiety factor loading differences between trials 1 and 2 in the elevated plus-maze in rats (File, 1993; File et al., 1993) and mice (Holmes & Rodgers, 1998). However, these differences were only found when arm time and entry data from both trials were factored together; trial-1 anxiety indicators loaded on factor-1 and trial-2 anxiety indicators loaded on factor-2 of a 3-factor structure in rats (File, 1993; File et al., 1993). It was suggested that rats extinguish 'state' anxiety during trial-1, but develop a type of phobic anxiety during trial-2 (File, 1993; File et al., 1993). Holmes & Rodgers (Holmes & Rodgers, 1998) found similar differential trial-1 to trial-2 anxiety factor loading patterns in Swiss-Webster mice. These authors suggested that the experience of trial-1 produced a qualitative shift in the emotional responses to trial-2 exposure (Holmes & Rodgers, 1998). In the present study, the anxiety indices (OE, OTR, USA, UHD) data were reduced by about 50% in trial-2. This would suggest that the animals experienced a different anxiety state during the second trial. However, consistent high loadings and low measurement errors for each of the four factor-1 anxiety indicators in the 2-factor final models of both trials (Figures 6 & 8) would indicate that the animals were not experiencing different states of anxiety, but perhaps a more intense level of the same emotional state during trial-2 exposure, in the present study.

A striking similarity between the study of Holmes & Rodgers (Holmes & Rodgers, 1998) and the present study lies in the factor-2 activity indicator levels. In both studies, CE levels were the same in trials 1 and 2, and CTR was about 50% higher in trial-2 than in trial-1 (trial-1 CTR was .271 in Holmes & Rodgers study and .269 in our study; trial-2 CTR was .391 in Holmes & Rodgers study and .391 in our study). Other laboratories have also reported consistent CE levels in trials 1 and 2 (Espejo, 1997a; Fernandes & File, 1996; File et al., 1993).

The only noteworthy difference between the trials-1 and 2 2-factor structures in the present study was in the item total vertical stretches. Whereas in trial-1, TVS non-significantly loaded on factor-2 in the 3-factor final model (t = -.85, NS) and was subsequently not included in the respecified 2-factor analysis, in trial-2, TVS significantly loaded on factor-2 in the 3-factor final model ($t = 4.81$, $p < .05$), thus was included in the trial-2 respecified 2-factor analysis. Total vertical stretches did, however, significantly load on factor-2 in the 2- and 4-structure exploratory factor analysis solutions in trial-1. The failure of TVS to significantly load on factor-2 in the trial-1 confirmatory factor analysis 3-factor final model was therefore puzzling, given its significant loading with CE in several reported principal components analysis studies in rats (Cruz et al., 1994; Fernandes & File, 1996) and mice (Fernandez Espejo, 1997; Rodgers & Johnson, 1995). Moreover, there were no differences in total vertical stretches means between trials-1 and 2 in the present or other studies (Espejo, 1997a; Fernandes & File, 1996; Holmes & Rodgers, 1998).

One explanation for this discrepancy may be that trial-2 closed arm activity was likely increased, therefore may have altered the TVS loading. This was not the case, however, since the trial-1 means +/- S.E.M. for CE (9.05 +/- .18) and TVS (22.5 +/- .5) were similar to the corresponding trial-2 CE (8.4 +/- .21) and TVS (19.7 +/- .5) statistics. Alternatively, whereas CE skewness (.028) was somewhat different than TVS skewness (.261) in trial-1, the striking similarity between CE (-.223) and TVS (-.236) skewness in trial-2 may have influenced the loading patterns

of the two indicators to be similar in the second trial. Another explanation could be that the significant TVS loadings evident in the trial-1 exploratory factor analyses did not reflect error variance. In any event, TVS was clearly functioning inappropriately in protected exploration activity measurement in trial-1. If factors 1 and 2 are to be considered representative of different corresponding facets of animal anxiety, then perhaps TVS does not adequately measure anxiety in mice in the elevated plus-maze.

In summary, a 2-factor model, that unambiguously described CD-1 anxiety-related behaviour in two separate trials in the elevated plus-maze, was validated in the present study. We concluded that confirmatory factor analysis hypothesis testing and model building procedures are useful tools to enhance the validity of the elevated plus-maze as a psychometric measurement instrument in the study of animal anxiety.

Addendum to Experiment 1 Discussion:

Following publication of Experiment 1, we attempted to find out why TVS was a weak protected exploration index in trial-1, but not trial-2. We reasoned that as in any other cumulative behavioural measure such as TE, THD and TSA, TVS was a composite measure from two different maze sectors, thus did not reflect vertical assessment strictly in the closed arms. The closed arm vertical stretches (CVS) were subsequently scored from each trial and replaced TVS in further exploratory factor analyses. The 2-factor exploratory rotated factor loading matrices for trials 1 and 2 using SPSS 9.0 (maximum likelihood extraction with varimax rotation) are shown in Tables 6 and 7, respectiviely. The 2-factor solutions accounted for 79.3% and 81.2% of the trials 1 and 2 variances, respectively.

Table 6

Experiment 1 (Addendum)

Trial-1 Rotated 2-Factor Matrix
(maximum likelihood with varimax rotation) including CVS

	Factor-1	Factor-2
OE	.870	-.053
OTR	.985	-.059
UHD	.859	-.128
USA	.891	.013
CE	-.001	.683
CTR	-.175	.766
CVS	.015	.749

Table 7

Experiment 1 (Addendum)

Trial-2 Rotated 2-Factor Matrix
(maximum likelihood with varimax rotation) including CVS

	Factor-1	Factor-2
OE	.937	.010
OTR	.984	.015
UHD	.848	.019
USA	.934	.029
CE	.054	.783
CTR	-.297	.615
CVS	.102	.831

These data appear in:

Wall, P. M. & Messier, C. Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behaviour, Neuroscience & Biobehavioural Reviews 25: 275-286.

Overall, the results from Experiment 1 and subsequent analyses provided us with an unambiguous 2-factor model, that described CD-1 anxiety-related behaviour in two separate trials in the elevated plus-maze. The seven indicator variables were therefore measured in all subsequent pharmacological experiments described in the remainder of the experimental section of the present Thesis.

2.3. EXPERIMENT 2

Wall, P. M. & Messier, C. (2000). U-69,593 microinjection in the infralimbic cortex reduces anxiety and enhances spontaneous alternation memory in mice. *Brain Research* 856: 259-280.

2.3.1. Abstract

The present report investigated the contributions of the ventromedial prefrontal cortex to the control of spontaneous alternation/working memory and anxiety-related behaviour. In Experiment 2a, we examined the effects of microinjections of the selective κappa$_1$ receptor agonist, U-69,593, in the infralimbic cortex (IL) of CD-1 mice on several ethologically-derived anxiety indices in the elevated plus-maze and defensive/withdrawal (D/W) anxiety in the open field, as well as on memory in the elevated plus-maze transfer-latency (T-L) test and implicit spontaneous alternation memory (SAP) in the Y-maze. In week 1, pretreatment with one injection of vehicle, 1, 10 or 25 nmol/1.0 μl U-69,593 in the IL dose-dependently prolonged T-L and produced a dose-dependent anxiolytic behavioural profile in the first elevated plus-maze trial. Following a 24-hr delay, the same mice were given a drug–free second trial in the elevated plus-maze tests of T-L memory and anxiety. Whereas T-L memory was not disturbed, small but detectable carry-over effects were

observed in trial-2 elevated plus-maze behaviour relative to vehicle-treated animals. In week 2, the same groups of mice were again pretreated with one injection of the same doses of U-69,593 in the IL and given a D\W test in an open field, followed immediately by an 8-minute SAP trial in the Y-maze. The smallest U-69,593 dose was anxiolytic in the D\W test, and SAP/working memory was dose-dependently enhanced in the Y-maze. In Exp. 2b, we evaluated whether 0.5 µl volume microinjections would produce comparable behavioural and carry-over effects in the IL of three new groups of CD-1 mice, in the event that the 1.0 µl volume injections used in Exp. 2a diffused beyond the IL and therefore may have confounded some effects. Experiment 2b procedures were carried out in the same manner as in Experiment 2a, except the animals were tested in reverse order. Thus in week 1, SAP memory was tested in the Y-maze followed by D/W anxiety in the open field for half of the animals in each group, and the other half was tested in reverse order. In week 2, T/L memory and anxiety were tested in the elevated plus-maze in 2 trials as described in Exp. 2a. Pretreatment with one injection of vehicle, 10 or 25 nmol/0.5 µl U-69,593 in the IL reduced D/W anxiety and enhanced SAP memory regardless of testing order in week 1. In week 2, the same groups of mice were again pretreated with one injection of the same doses of U-69,593 in 0.5 µl volumes in the IL and tested in the elevated plus-maze. In a similar fashion to Experiment 1, U-69,593 dose-dependently prolonged T/L and produced an anxiolytic behavioural profile in the first elevated plus-maze trial. Following a 24-hr delay, T/L recall memory was again not significantly influenced, but a robust anxiolytic behavioural profile was observed in the second drug-free anxiety trial in the elevated plus-maze relative to vehicle-treated animals. Results are discussed relative to a) injection volumes and testing order, b) the possible influence κappa receptors may exert on neurochemical responsivity to anxiety-provoking environments in the IL area of the mPFC, c) the possibility that κappa-mediated anxiolysis from the IL in CD-1 mice results from interactions with neurochemical systems involved in the blunting of incoming anxiety-provoking information, d)

evidence that SAP memory may be an implicit subtype of working memory, and e) the possibility that IL implicit working memory processes may modulate the induction and expression of anxiety-related behaviour.

2.3.2. Introduction

A relationship between the neuromechanisms involved in memory and anxiety states has been recognized in laboratory animals (Bhattacharya, Satyan, & Chakrabarti, 1997; Carli et al., 1993; Colpaert, 1990; Frussa-Filho et al., 1991; Gargiulo et al., 1996; Graeff et al., 1993; Lucki & Rickels, 1988; McNally, 1995, 1997; Scharf et al., 1988; Viana et al., 1994; Withers & Deane, 1995). Some brain pathways have been identified as putative emotional memory sites. For example, Ledoux and colleagues suggested that emotional memory involves the implicit learning and storage of information about the emotional significance of environmental events. They also suggested that emotional memory, behaviours and autonomic responsivity depend on pathways involving reciprocal connections between the amygdala, hippocampus, thalamus and prefrontal cortex (LeDoux, 1992, 1993a, 1993b). Indeed, the amygdala is proposed by some investigators to mediate the induction of emotional memories, particularly involving conditioned fear learning in rats (LeDoux, 1993b, 1995), stimulus-reinforcement associations in rats (Rolls, 1995), as well as to play a role in attention, learning and anticipatory anxiety (Davis et al., 1994; Schulkin, McEwen, & Gold, 1994). In addition, septo-hippocampal manipulations have resulted in anxiety (Gray, 1982; Gray, 1983; Gray & McNaughton, 1983; Pesold & Treit, 1992; Treit & Pesold, 1990) or memory (Markowska, Olton, & Givens, 1995; Messier, Wall et al., 1999; Ohno, Yamamoto, & Watanabe, 1994) alterations. The medial prefrontal cortex has also been shown to be involved in memory (Grafman, 1995; Granon et al., 1995; Granon, Vidal, Thinus-Blanc, Changeux, & Poucet, 1994; Kolb, Buhrmann, McDonald, & Sutherland, 1994) and anxiety (Espejo, 1997b; Wall, 1997) processes. Conceivably, different anxiety-provoking situations such as novelty/conflict exposure,

aversively-motivated conditioning, stressor manipulations or various combinations of such aversive-reinforcing events provoke neurochemical responses in the mPFC, hippocampus, amygdala and certain autonomic brainstem nuclei which have been suggested to overlap with learning-related neurotransmission (Gabriel, 1990; LeDoux, 1993b). The aforementioned hypotheses suggest that some functional interactivity exists between memory and anxiety processes.

There are accumulating data suggesting that the ventromedial prefrontal cortex (vmPFC) may be a convergent brain region that functions in conjunction with the aforementioned limbic structures, as well as the autonomic brainstem to influence memory-related emotions (Verberne & Owens, 1998). A review of some of the evidence in support of this hypothesis follows.

First, insofar as the autonomic brainstem is involved in anxiety induction and/or expression, there are some tract-tracing and electrophysiological data indicating functional anatomical connectivity between the ventral prelimbic/infralimbic (PL/IL) mPFC and visceral/autonomic brain regions involved in visceral/emotional responsivity. For example, some sub-regions in the IL area of the vmPFC innervate the nucleus of the solitary tract (Takagishi & Chiba, 1991), the rostral and caudal ventrolateral medulla (Dampney, Goodchild, Robertson, & Montgomery, 1982), the pedunculopontine tegmental nucleus in the autonomic brainstem in rats (Semba & Fibiger, 1992), the autonomic hypothalamus in Rhesus monkeys and rats (Rempel-Clower & Barbas, 1998; Takagishi & Chiba, 1991), the dorsal periaqueductal gray (Sesack et al., 1989), the entire amygdaloid complex (Takagishi & Chiba, 1991), and the CA1/subiculum hippocampal areas (Jay & Witter, 1991). Furthermore, the CA1/subiculum area of the hippocampus projects to the IL and makes dendritic and some somatic synaptic contact on a subset of efferent projecting neurons to the nucleus of the solitary tract in rats (Ruit & Neafsey, 1988, 1990). These studies show that there are numerous connections that position the vmPFC to modulate visceral/emotional responsivity and learning.

Accordingly, functional studies have shown that influencing vmPFC electrophysiological activity can produce autonomic and visceral changes. For example, electrical stimulation of the vmPFC influences blood pressure, heart rate, respiration, gastric motility, urination and regional blood flow in rats (Frysztak & Neafsey, 1994). These authors also demonstrated that the vmPFC is necessary for complete sympathetic activation of cardiovascular responses to both severe and mildly stressful stimuli in rats (Frysztak & Neafsey, 1994). Moreover, electrical stimulation of, or microinfusion of glutamate into, the ventral hippocampus in anesthetized or awake rats decreased heart rate and blood pressure concomitant with slower, deeper and more regular breathing (Ruit & Neafsey, 1988). Interestingly, the cardiovascular and respiratory responses to ventral hippocampal activation evident among these rats were blocked by bilateral vmPFC lesions. Taken together, it is conceivable that the vmPFC can modulate the expression of autonomic responsivity to emotional stimuli, as well as the expression of visceral emotional responsivity to conditioned stimuli (Owens & Verberne, 1996).

A second body of evidence indicates that the ventral PL-IL mPFC influences learning and some forms of working memory systems in laboratory animals (DeCoteau, Kesner, & Williams, 1997; Joel, Tarrasch, Feldon, & Weiner, 1997; Kesner, Hunt, Williams, & Long, 1996; Morgan, Romanski, & LeDoux, 1993; Ragozzino et al., 1998; Rempel-Clower & Barbas, 1998). For example, lesions of the mPFC impaired delayed alternation behaviour in a T-maze in rats (Wortwein, Mogensen, & Divac, 1993). Moreover, lesions of the ventral PL-IL produced working memory deficits in a delayed nonmatching-to-sample procedure in rats (Kesner et al., 1996). Furthermore, acetylcholine muscarinic receptor blockade in the vmPFC produced acquisition deficits in the water maze (Waite & Thal, 1996) and delayed matching-to-position or sample tasks in rats (Broersen et al., 1994; Broersen et al., 1996; Granon et al., 1994). Finally, microinjection of

the muscarinic antagonist, scopolamine, in the ventral PL-IL impaired spatial working memory in a 12 arm radial maze in rats (Ragozzino et al., 1998).

A third body of evidence indicates that the ventral PL-IL influences the induction of anxiety states, as well as the expression of anxiety-related behaviour (Espejo, 1997b; Jaskiw & Weinberger, 1990). For example, electrolytic lesions of the IL provoked anxiety in the open field, the elevated plus-maze and the step-down passive avoidance task in rats (Jinks & McGregor, 1997). Furthermore, ibotenic acid lesions of the mPFC potentiated the anxiogenic effects of the β-carboline, FG-7142, in a novel environment in rats (Jaskiw & Weinberger, 1990). Finally, dopamine depletion following 6-OHDA infusion in the mPFC also produced an anxiogenic profile in the elevated plus-maze in rats (Espejo, 1997b). These data lend support to the hypothesis that the IL can modulate anxiety in laboratory animals. Together, the aforementioned evidence gives rise to the suggestion that the vmPFC is a brain area capable of processing convergent information from several sub-cortical sites involved in mediating both memory and anxiety processes.

The notion that memory and anxiety processes can co-exist is also supported by various demonstrations that many drugs appear to concomitantly modulate memory and anxiety states. In this regard, such drugs influence several neurotransmitter systems, including GABA (Davis et al., 1994; Mathis et al., 1994; Tohyama et al., 1991), acetylcholine (Arneric et al., 1994; Brioni et al., 1994), serotonin (Carli et al., 1993; Guimaraes et al., 1993; Scorza et al., 1996), dopamine (Jentsch et al., 1997; Murphy, Arnsten, Jentsch et al., 1996; Ukai et al., 1998), cholecystokinin (Adamec et al., 1997; Derrien et al., 1994) and opioid receptor ligands (Castellano et al., 1993; Castellano et al., 1989; McDaniel et al., 1990; Sandin et al., 1998), among others.

Of these many neurotransmitter systems through which memory and anxiety appear to interact, kappa opioid receptor agonists counteract the memory-disrupting effects of the referent amnestic drug, scopolamine (Hiramatsu, Murasawa, Nabeshima et al., 1998), and kappa receptor antagonists

completely block the anxiety-reducing effects of a referent anxiolytic benzodiazepine, chlordiazepoxide (Agmo & Belzung, 1998). Moreover, kappa receptor systems have been shown to interact with the dopamine system in the mPFC to produce conditioned place learning in rats (Bals-Kubik et al., 1993), interact with cholinergic systems to produce effects on memory (Hiramatsu, Murasawa, Mori et al., 1998) and effects on cardiovascular responsivity (Wang & Ingenito, 1992). Finally, whereas systemic low-dose administration of the kappa$_1$ receptor agonists, U-69,593 or U-50,488H, produced an anxiolytic behavioural profile in the elevated plus-maze in rats (Privette & Terrian, 1995), U-69,593 microinfusion in the vmPFC produced an anxiolytic behavioural profile in the elevated plus-maze in CD-1 mice (Wall, 1997). Together, it is conceivable that the kappa opioid system may influence anxiety states through the modulation of implicit learning and memory processes. For these reasons we became particularly interested in evaluating whether kappa receptor ligands could influence anxiety in the elevated plus-maze, as well as spontaneous alternation performance memory, arguably related to working memory, in the Y-maze following U-69,593 microinjection in the IL in CD-1 mice. We hypothesized that kappa$_1$ receptor activation in the IL would serve to blunt incoming information regarding the aversiveness of either of the mazes, thus effectively truncate the disruptive effects of visceral input on implicit cognitive processes. In this way, implicit memory in the Y-maze would likely be enhanced. In other words, disrupting implicit aversive memory should lead to reduced anxiety and enhanced implicitly-driven exploration in the Y-maze.

The present series of experiments sought to determine whether U-69,593 could not only replicate the aforementioned anxiolytic effects observed among CD-1 mice from the vmPFC in the elevated plus-maze, but also influence associative learning in the elevated plus-maze transfer-latency test in trial-1 (drug condition). Following a 24-hr delay, the possibility that pretrial-1 U-69,593 could influence transfer-latency memory and memory of the aversiveness of the elevated

plus-maze in trial-2 (no drug condition) was evaluated. In week 2, we evaluated whether a second pretrial U-69,593 microinjection in the IL could influence defensive/withdrawal anxiety and spontaneous alternation performance memory in the Y-maze in the same CD-1 mice. In Exp. 2 we evaluated whether a 50% smaller drug injection volume (0.5 relative to 1.0 µl) in the IL cortex could comparably influence the aforementioned behaviours in 3 new groups of CD-1 mice, but in reverse order testing.

2.3.3. Experiment 2a – Materials and Methods

Subjects

Subjects were 49 naive male CD-1 mice, obtained from Charles River (St. Constant, Quebec) at approximately 5 weeks of age. All mice were initially housed in polypropylene cages (groups of 10 per cage) in a temperature controlled room (21 ± 1° C), with ad lib access to food and water. The mice were acclimatized for 6 to 8 weeks until they reached a weight range of 32-45 grams. All mice were maintained on a 12 hour light/dark cycle (lights on at 7 a.m.).

Surgery

Surgery was performed in accordance with the procedures of our laboratory elaborated elsewhere (Messier, Emond, & Ethier, 1999). Briefly, pre-operative analgesia was provided by adding an acetaminophen solution (80 mg/5 ml ; children's Tempra, Mead Johnson) to the drinking water bottles (1 ml of Tempra in 100 ml H_2O) for three days prior to surgery. Fifteen minutes before surgery, mice were administered 0.01 mg/kg subcutaneous (s.c.) glycopyrrolate (0.01 mg/mouse ; Robinul, Wyeth-Ayerst, Montreal, Canada) to prevent the accumulation of salivatory and bronchial secretions. Mice were then pre-medicated with sodium pentobarbitol (0.65 mg/ml) at doses ranging between 0.2 and 0.4 ml/100 g to provide a relaxed muscle tone. Five to ten minutes later, the mice

were anesthetized with methoxyflurane (Metofane, Janssen Pharmaceutica, North York, Ont.) and subsequently monitored until an anesthetic plane, suitable for surgery, was attained.

A 26 gauge stainless steel guide cannula, 0.80 cm in length, was unilaterally implanted in the infralimbic prefrontal cortex using a David Kopf micromanipulator. All cannulae were implanted in accordance with the following stereotaxic coordinates: A.P. +2.0 mm anterior to Bregma, L. +0.4 mm from the midsagital suture and V. -2.0 mm from a flat skull surface. The guide cannula was fixed in place with dental cement and jewelers' screws. Post-operatively, mice were individually housed and continued on an acetaminophen/drinking water regime for three days post-surgery. Animals were allowed a 2-wk recovery period in the main animal housing room prior to experimentation.

Drugs

U-69,593 and cyclodextrin were obtained from Research Biochemicals International (Natick, MA). U-69,593 was dissolved using a 45% cyclodextrin solution to concentrations of 1, 10 and 25 nmol/µl. Vehicle was a 45% cyclodextrin/distilled H_2O solution. Cyclodextrin is a cyclic sucrose array that increases drug solubility. Because of poor solubility in saline, U-69,593 is often injected in suspension. Cyclodextrin is inert and excreted rapidly (Pitha, Gerloczy, & Olivi, 1994). The use of cyclodextrin allowed for more precise microinjections and avoided cannula blockage.

Intracerebral Drug Administration

Following a 2-wk recovery period, mice were transferred from the main holding area to the laboratory and left undisturbed for 1 hour prior to drug administration (Rodgers et al., 1995). The mice were randomly assigned to one of a vehicle, 1, 10 or 25 nmol U-69,593 treatment condition. Each mouse was lightly restrained and a 30 gauge injection cannula was inserted into the guide cannula, the injection cannula connected using polyethylene tubing to a 5 µl Hamilton

microsyringe. Each drug concentration was infused in a 1 µl volume over a 30-second period. The injection cannula remained in place for an additional 30 seconds to allow for drug diffusion.

Immediately following drug infusion, each animal was returned to its home cage, brought into the testing room and left undisturbed for 5 minutes prior to behavioural evaluation. All testing was conducted during the morning phase of the light/dark cycle between the hours of 8 a.m. and 1 p.m. All trials took place in a dimly illuminated chamber and were recorded by a ceiling-mounted SONY video camera that was linked to a Panasonic video monitor and VCR, and an IBM-compatible computer positioned outside the test chamber.

Elevated plus-maze

The elevated plus-maze used in the present experiments was a modified version of a previously-described apparatus (Lister, 1987). The two opposing closed arm runways were 30 cm x 5 cm and enclosed by 15 cm high walls on each side and ends. The two opposing open arms were also 30 cm x 5 cm with a .25 cm high Plexiglas edge on either side and at the end of each runway. The center platform was 5 cm x 5 cm. The enclosed arm walls were constructed with urethane-coated wood and lined with Plexiglas. The floor of the maze was covered with a thin layer of vulcanized black rubber to facilitate inter-trial cleaning. The apparatus was positioned 40 cm from the floor on a wooden stand hidden from view and the floor under the stand was covered with black cloth to reduce height cues.

Y-maze

The Y-maze was a modified version of a previously described apparatus (Hiramatsu, Hyodo, & Kameyama, 1996; Hiramatsu, Sasaki, Nabeshima, & Kameyama, 1997; Sarter, Bodewitz, & Stephens, 1988). The 3 enclosed arm runways were 30 cm long x 5 cm wide and enclosed by 15 cm high walls on each side and ends. The arms converged on an equilateral triangular center platform

(5 cm x 5 cm x 5 cm). The materials used to construct, and the appearance of, the Y-maze were identical to the elevated plus-maze.

Open Field

The defensive/withdrawal apparatus consisted of an open field approximately 1 m x 1 m that was enclosed with 15 cm high walls all constructed with gray Plexiglas. A totally enclosed box (4 walls and ceiling) with a small entrance in one wall was positioned in the center of the open field. The box was constructed with corrugated cardboard and was 20 cm x 20 cm x 20 cm high. A weight was placed on the box to hold it in place during a trial.

Behavioural Testing Procedures

Week 1: (1) Drug infusion – 5 min wait. (2) transfer-latency measured (up to 90 sec) in the elevated plus-maze. (3) five min elevated plus-maze anxiety trial begins immediately, without removing the animal from the elevated plus-maze. (4) 24 hrs later (no drug) transfer-latency recall test followed immediately by a five min anxiety test. In all cases, the floor and Plexiglas walls of the maze were wiped between trials with a 70% alcohol solution to prevent a mouse from following the scent of a previously tested animal.

The transfer-latency memory test in the elevated plus-maze was a modified version of a previously described procedure (Itoh, Nabeshima, & Kameyama, 1991; Itoh et al., 1993b). In the first trial, 5 minutes after drug infusion, a mouse was placed at the end of an open arm runway facing away from the center platform, and the time taken (up to a 90-sec. limit) to transfer from the open arm into either of the two closed arms was recorded. The next day, a recall session was initiated in which the animal was again placed at the end of an open arm and the latency to enter a closed arm was measured (up to 90 sec). Memory for transfer-latency trial-1 learning was measured as trial-2 transfer-latency differences between drug- and vehicle-treated animals.

If an animal did not enter a closed arm within 90 seconds, he was gently nudged into one at that time. Upon entering a closed arm, a 5-min anxiety trial commenced immediately. Since an animal was placed at the end of an open arm to commence a transfer-latency trial, then open arm time and entries would have been biased if the transfer-latency and anxiety tests were both combined in a 300 second period. It was therefore decided that transfer-latency times would be added to, rather than part of a 5-min elevated plus-maze anxiety trial. In this way, a 5 minute anxiety trial would only commence following entry into a closed arm, thus precluding any open arm bias.

Individual trials in the elevated plus-maze anxiety test were 5 minutes in duration and commenced immediately following entry into a closed arm. A mouse was considered to have entered an arm when all 4 paws were positioned within an arm runway (Andrews & File, 1993; Derrien, Durieux, Dauge, & Roques, 1993; Lister, 1987), or in the center hub when at least 1 paw was out of an arm. After a 24-hr delay, a second transfer-latency and anxiety trial was conducted and recorded in the same manner as in trial-1, with the exception that no pretrial drugs were injected.

The anxiety-related behavioural indices in the elevated plus-maze, used in the present study, have been validated and identified in several principal components analysis studies for both mice and rats (Cruz et al., 1994; Fernandes & File, 1996; Rodgers & Johnson, 1995; Trullas & Skolnick, 1993; Wall & Messier, 2000b). Included measures are open arm entries (OE), open time ratio (open time ÷ total time on the maze x 100, OTR), closed arm entries (CE), closed time ratio (CTR), unprotected head dips (UHD), unprotected stretch attends (USA), and total closed vertical stretches (CVS). Unprotected head dips were operationally defined as peering over the edge of an open arm with head, neck and shoulders. Unprotected stretch attend postures were operationally defined as the animal stretching its whole body forward and retracting to its original position without moving its hind paws in an open arm. Increases in OE, OTR, UHD and USA were taken as anxiolytic

indices of drug treatment effects relative to vehicle-treated animals. Cumulative CE, CTR and CVS were taken as indices of protected exploration activity (Fernandes & File, 1996).

Week 2: (1) Drug infusion – 5 min wait. (2) defensive/withdrawal test in open field (up to 90 sec) followed immediately by (3) an 8 min spontaneous alternation performance trial in the Y-maze.

The same mice were used in week 2. Each animal was challenged with the same pretrial vehicle, 1, 10 or 25 nmol U-69,593 microinjection in the IL. A defensive/withdrawal test began by placing a mouse in the enclosed box and time started (up to a 90-sec limit) when a weight was placed on top of the box to hold it in place. The defensive/withdrawal anxiety index was assessed by recording the amount of time taken for a mouse to emerge from the enclosed box positioned in the middle of the open field. As soon as a mouse emerged with all 4 paws outside the door threshold, it was removed from the open field and placed immediately in the Y-maze.

Spontaneous alternation performance was assessed by visually recording the spontaneous alternation behaviour of each mouse individually in the Y-maze in a manner similar to the procedure described by Hiramatsu and colleagues (Hiramatsu, Sasaki et al., 1997). Immediately following the defensive/withdrawal task, each mouse participated in a single trial that commenced by placing the animal in the center hub of the Y-maze. A mouse was considered to have entered an arm when all 4 paws were positioned in the arm runway. Each mouse was allowed to freely explore the maze for eight minutes. Alternations were operationally-defined as successive entries into each of the three arms on overlapping triplet sets, and % spontaneous alternation performance (%SAP) was defined as the ratio of actual to possible alternations (total arm entries - 2) X 100. Total entries (YTE) in the Y-maze were also scored.

Histology

Mice were sacrificed with an overdose of sodium pentobarbitol and perfused intracardially with 10cc 0.9% saline followed by 10cc 10% formalin. Mice were decapitated, the head caps removed,

brains excised from the cranial cavity and stored in a 10% formalin/30% sucrose solution for at least one week prior to histological analyses. Individual brains were subsequently blocked and frozen on a cryostat. Coronal sections (30 μm) were taken through the rostral-caudal extent of the cannula placement. Brain sections were mounted, stained with cresyl violet and examined under a microscope. The distribution of cannulae placements in the IL were mapped on schematic representations employing the mouse brain atlas of Franklin and Paxinos (Franklin & Paxinos, 1997). Only those subjects with correct cannulae placements in the IL within the rostral-caudal range defined as +1.8 mm to +2.2 mm anterior to Bregma, the medial-lateral range defined as +.1 mm to +.7 mm lateral to the midsagital suture and the dorsal-ventral range defined as -2.5 mm to -3.25 mm ventral to a flat skull surface were included in the present analysis. Two mice from the vehicle, 3 from the 1 nmol, 4 from the 10 nmol and 3 from the 25 nmol groups were excluded due to placements outside this region. The cannulae placements included in the Exp. 2a analysis are depicted in Figure 11.

Place Figure 11 about here

Statistical Analyses

The behavioural data describing OE, OTR, USA, UHD, CE, CTR and CVS in the elevated plus-maze were analyzed by oneway analysis of variance (ANOVA) for trials 1 and 2 ($\alpha = .05$). Post hoc Bonferroni-corrected *t*-tests were conducted for significant treatment effects relative to control means ($\alpha = .05/3 = .017$). The transfer-latency, defensive/withdrawal and Y-maze arm entries (YTE) and % spontaneous alternation performance (% SAP) data were analyzed by oneway ANOVA ($\alpha = .05$). Post hoc Bonferroni-corrected *t*-tests were conducted for significant treatment effects relative to control means ($\alpha = .05/3 = .017$).

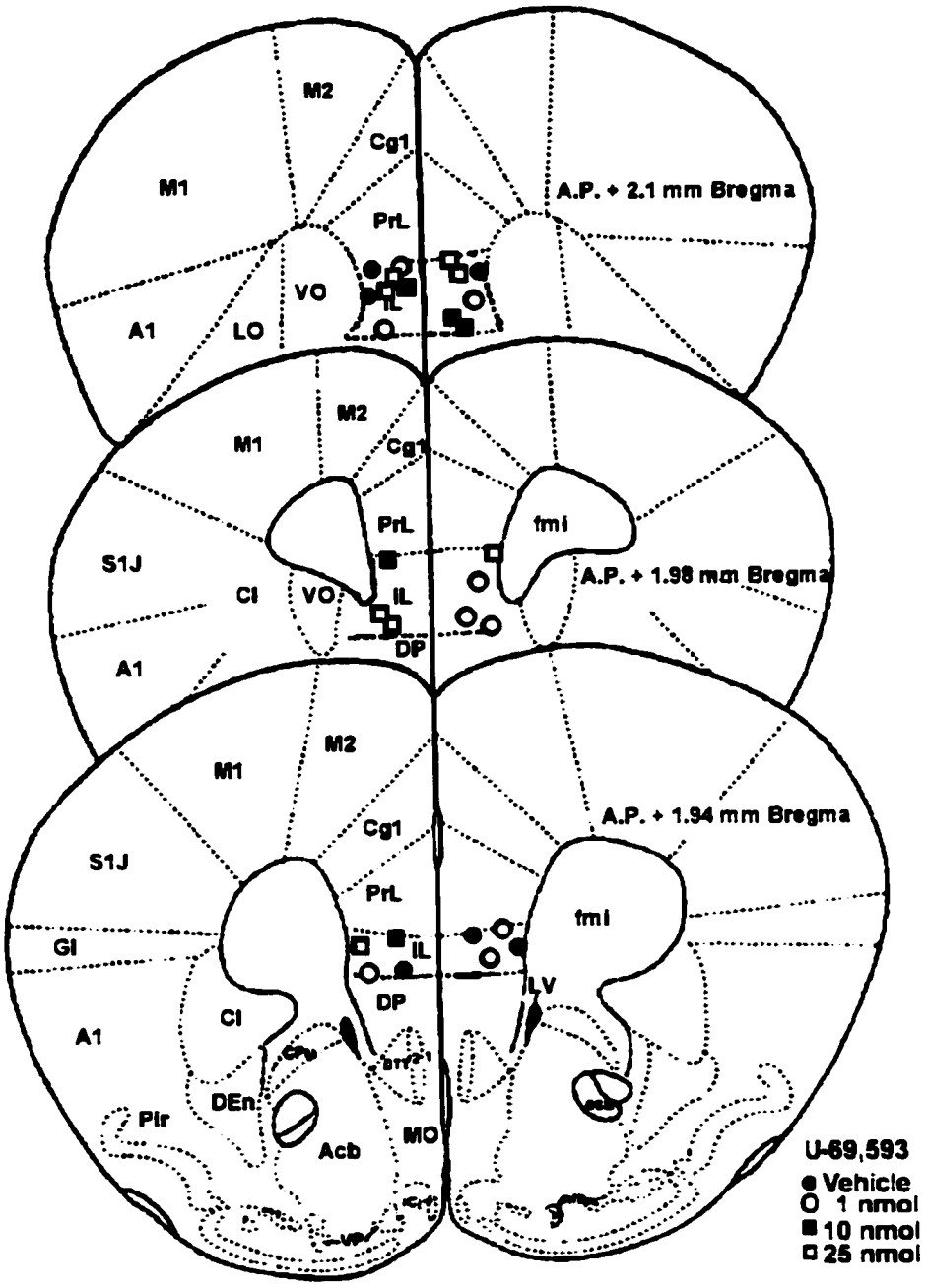
M2
Cg1
M1
A.P. + 2.1 mm Bregma
PrL
VO
IL
A1
LO
M2
M1
Cg1
PrL
fmi
S1J
A.P. + 1.98 mm Bregma
CI
VO
IL
DP
A1
M2
M1
A.P. + 1.94 mm Bregma
Cg1
S1J
PrL
fmi
GI
IL
LV
DP
CI
A1
DEn
Plr
Acb
MO
U-69,593
Vehicle
1 nmol
10 nmol
25 nmol

Figure 11

2.3.4. Results

Week-1:

Elevated plus-maze Transfer-Latency and Anxiety Tests Trial-1. Oneway ANOVA revealed that U-69,593 pretreatment prolonged transfer-latencies in the trial-1 drug condition ($F_{3,24} = 6.69$, $p < .002$). Post-hoc LSD tests revealed that the 25 nmol U-69,593 dose prolonged transfer-latencies in trial-1 ($p < .017$) relative to vehicle-treated animals (Figure 12a).

Place Figure 12 about here

Oneway between-subjects ANOVAs were performed on 7 dependent variables in the elevated plus-maze anxiety test: OE, OTR, USA, UHD, CE, CTR and CVS. The independent variable consisted of four treatment levels (vehicle and three U-69,593 doses). In addition to cannula placement exclusion criteria, two additional mice from the 25 nmol group were excluded due to extreme outlier behaviour in both mazes for the entire test durations. In all, 6 mice in the vehicle group, 9 in the 1 nmol, 8 in the 10 nmol and 5 in the 25 nmol groups (N = 28) were analyzed.

The data along with associated ANOVA's and post-hoc LSD tests for trial-1 elevated plus-maze are summarized in Table 8, and where applicable, some of the figures depict results

Place Table 8 about here

from both trials for comparison purposes. Oneway ANOVA revealed that there were main effects of U-69,593 treatment on OTR, UHD and USA. Post-hoc LSD tests revealed that the 10 and 25 nmol doses increased OTR ($p < .017$) and UHD ($p < .017$), while the 10 nmol drug dose increased USA ($p < .017$) relative to vehicle-treated mice (Figure 13). There were also main effects of U-69,593

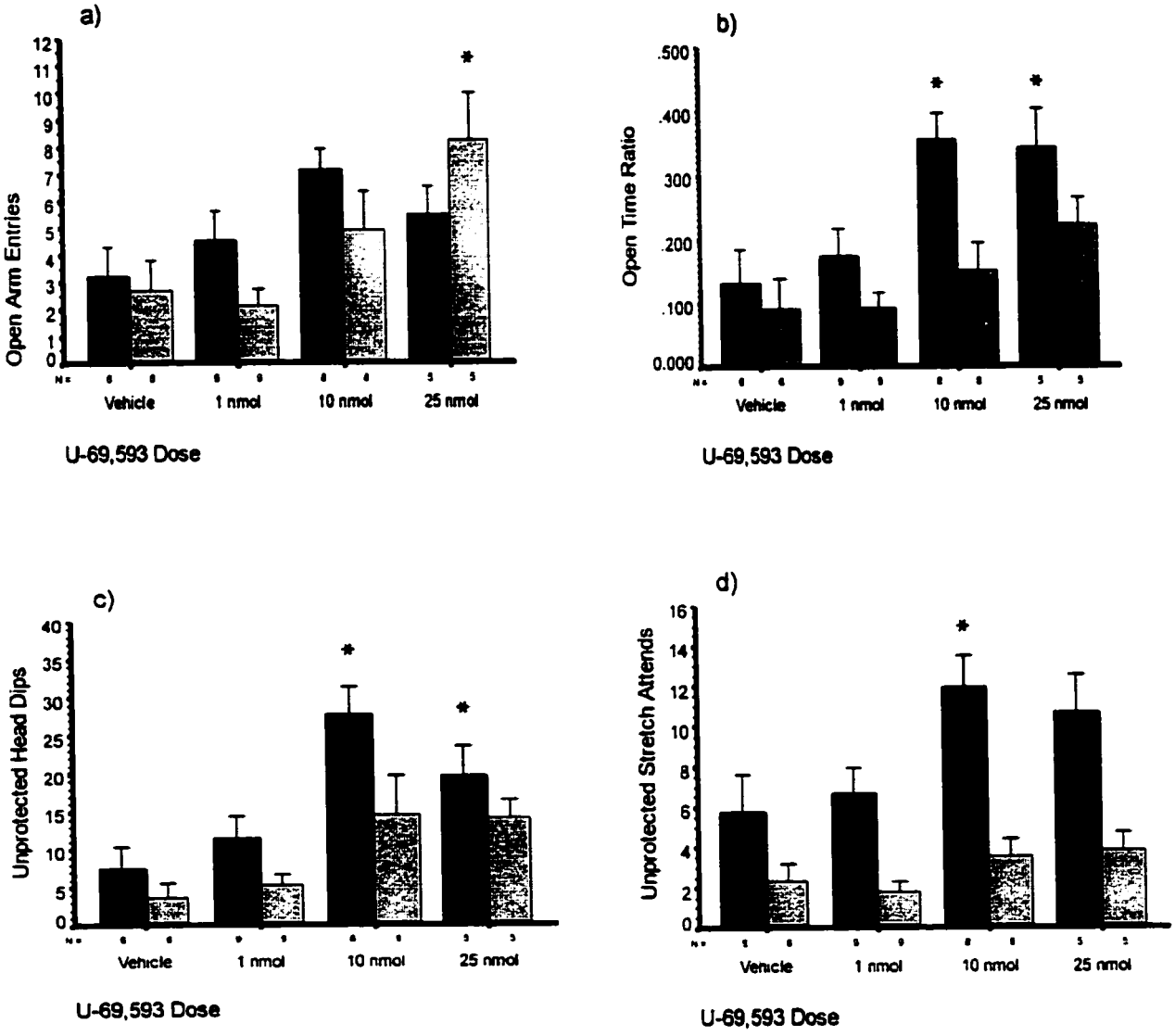
a)
Open Arm Entries
Vehicle
1 nmol
10 nmol
25 nmol
U-69,593 Dose
b)
Open Time Ratio
Vehicle
1 nmol
10 nmol
25 nmol
U-69,593 Dose
c)
Unprotected Head Dips
Vehicle
1 nmol
10 nmol
25 nmol
U-69,593 Dose
d)
Unprotected Stretch Attends
Vehicle
1 nmol
10 nmol
25 nmol
U-69,593 Dose

Figure 13

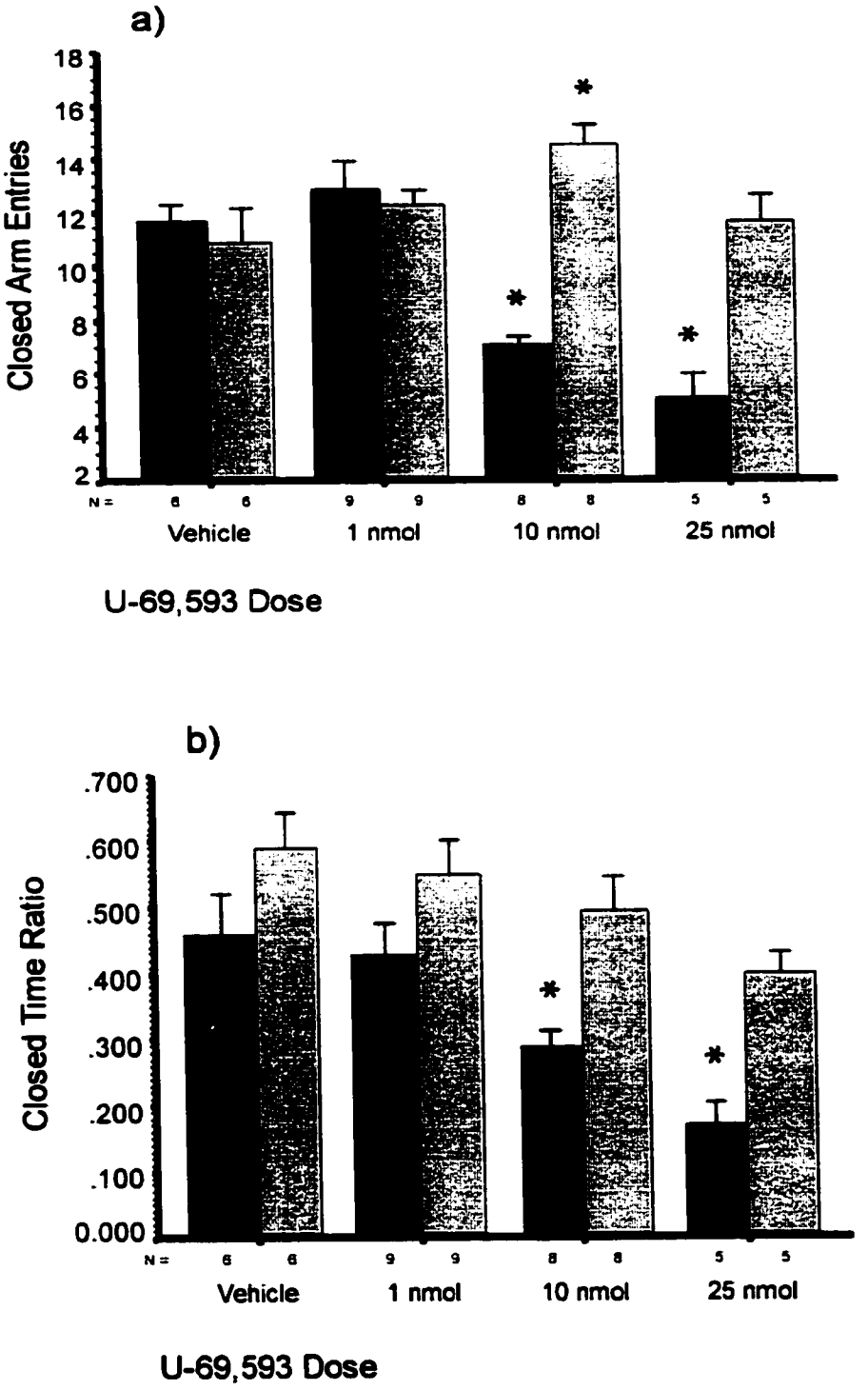
a)
Closed Arm Entries
18
16
14
12
10
8
6
4
2
N = 6 6 9 9 8 8 5 5
Vehicle
1 nmol
10 nmol
25 nmol
U-69,593 Dose
b)
Closed Time Ratio
.700
.600
.500
.400
.300
.200
.100
0.000
N = 6 6 9 9 8 8 5 5
Vehicle
1 nmol
10 nmol
25 nmol
U-69,593 Dose

Figure 14

PRETRIAL-1 1.0 µl U-69,593/ ELEVATED PLUS-MAZE TRIAL-1

Behav.	Vehicle	1 nmol	10 nmol	25 nmol	$F_{(3,24)}$	*P*
OE	3.17 ± 1.2	4.44 ± 1.2	7.13 ± .8	5.4 ± 1.1	2.37	NS
OTR	0.129 ± .05	0.172 ± .04	0.355 ± .04 *	0.340 ± .06 *	5.42	0.005
UHD	7.3 ± 3.1	11.3 ± 3.1	27.6 ± 3.7 *	19.6 ± 3.9 *	6.93	0.002
USA	5.7 ± 1.9	6.7 ± 1.3	11.9 ± 1.6 *	10.6 ± 1.9	3.42	0.034
CE	11.7 ± .6	12.8 ± 1.1	7.0 ± .4 *	5.0 ± 1.0 *	17.14	0.001
CTR	0 .456 ± .07	0.427 ± .05	0.285 ± .03 *	0.167 ± .04 *	6.88	0.002
CVS	18.7 ± 3.0	24.8 ± 3.1	20.1 ± 2.2	16.6 ± 6.2	1.04	NS

Table 8. Experiment 2a. Infralimbic U-69,593 effects on elevated plus-maze behaviour. Values represent means +/- SEM. Time ratios represent the amount of time (sec) in a section ÷ 300 sec. OE, open arm entries; OTR, open time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), sig. different than vehicle ($p < .017$).

(NO INJECTION) ELEVATED PLUS-MAZE TRIAL-2

Behav.	Vehicle	1 nmol	10 nmol	25 nmol	$F_{(3,24)}$	*P*
OE	2.7 ± 1.1	2.1 ± 0.6	4.9 ± 1.5	8.2 ± 1.8 *	4.41	0.013
OTR	0.089 ± 0.05	0.09 ± 0.03	0.149 ± 0.04	0.219 ± 0.04	1.97	NS
UHD	3.5 ± 2.1	5.2 ± 1.5	14.4 ± 5.2	14.0 ± 2.5	2.74	NS
USA	2.3 ± 0.8	1.8 ± 0.6	3.5 ± 0.9	3.8 ± 0.9	1.45	NS
CE	10.8 ± 1.4	12.2 ± 0.6	14.5 ± 0.8 *	11.6 ± 1.0	3.1	0.046
CTR	0.584 ± 0.06	0.547 ± 0.06	0.493 ± 0.06	0.398 ± 0.03	1.66	NS
CVS	19.3 ± 2.9	21.0 ± 2.8	25.3 ± 2.4	27.4 ± 3.9	1.43	NS

Table 9. Experiment 2a. Pretrial-1 infralimbic U-69,593 carry-over effects on elevated plus-maze behaviour. Values represent means +/- SEM. Time ratios represent the amount of time (sec) in a section ÷ 300 sec. OE, open arm entries; OTR, open time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), sig. different than vehicle ($p < .017$).

Place Figure 13 about here

treatment on CE and CTR. Subsequent post-hoc LSD tests revealed that the 10 and 25 nmol U-69,593 concentrations decreased CE and CTR ($p < .017$) relative to control mice (Figure 14).

Place Figure 14 about here

Elevated plus-maze Transfer-Latency and Anxiety Tests Trial-2. Pretrial-1 U-69,593 infusion in the IL did not influence transfer-latencies 24-hrs. later ($F_{3,24} = 1.15$, NS), in the trial-2 condition (Figure 12a). Oneway ANOVAs revealed significant carry-over effects of pretrial-1 U-69,593 treatment on OE and CE parameters (Table 9). Post-hoc LSD tests revealed that the 10 nmol dose

Place Table 9 about here

increased OE and CE ($p < .017$) relative to vehicle-treated animals (Figures 13 and 14). These results indicate that the initial injections of U-69,593 had no effect on transfer-latency recall and limited carry-over effects on elevated plus-maze behaviour in the no-injection trial 24 hrs. later.

Week-2:

Defensive/Withdrawal Anxiety. The defensive/withdrawal means +/- SEM are graphically depicted in Figure 12b. Oneway ANOVA revealed that pretrial U-69,593 treatment influenced defensive/withdrawal latencies ($F_{3,24} = 3.92$, $p < .025$). Subsequent post-hoc LSD tests revealed that the 1 nmol U-69,593 concentration reduced defensive/withdrawal latencies ($p < .017$) relative to vehicle-treated mice. These data indicate that the second 1.0 µl injection of the 10 and 25 nmol

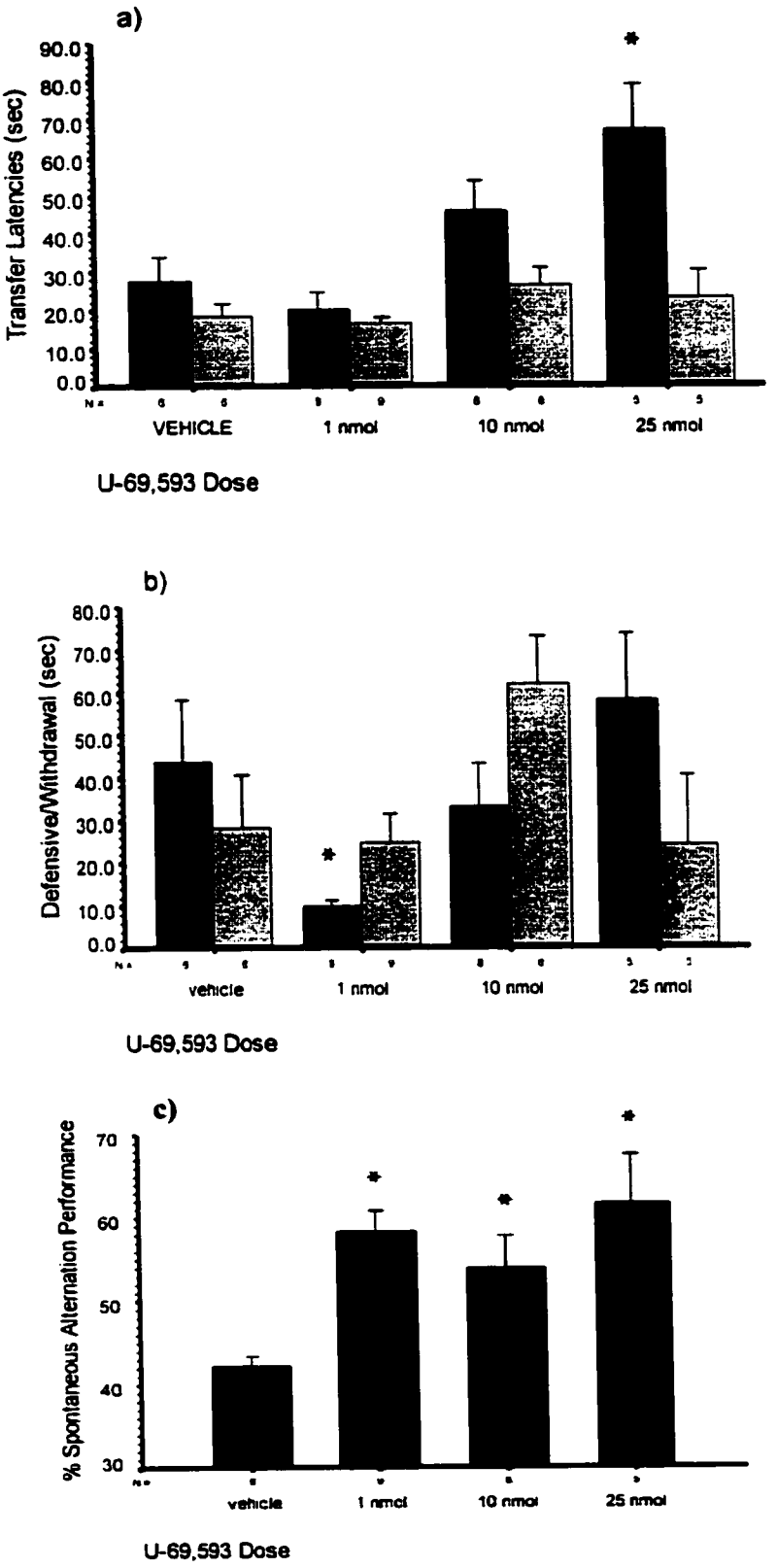
a)
Transfer Latencies (sec)
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
VEHICLE
1 nmol
10 nmol
25 nmol
U-69,593 Dose
b)
Defensive/Withdrawal (sec)
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
vehicle
1 nmol
10 nmol
25 nmol
U-69,593 Dose
c)
% Spontaneous Alternation Performance
70
60
50
40
30
vehicle
1 nmol
10 nmol
25 nmol
U-69,593 Dose

Figure 12

doses may have been of a sufficient magnitude to diffuse into the dorsal striatum or the nucleus accumbens to reduce ambulatory activity (Di Chiara & Imperato, 1988b), but the 1 nmol concentration mainly influenced IL-mediated behaviour.

Spontaneous Alternation Memory in the Y-Maze. Group means +/- SEM are graphically depicted in Fig. 13. Oneway ANOVA revealed that U-69,593 microinjections in the IL influenced YTE ($F_{3,24} = 4.32$, $p < .015$) and % SAP ($F_{3,24} = 4.32$, $p < .015$) in the Y-Maze. Post-hoc LSD tests revealed that YTE was not reduced, but the 1 and 25 nmol U-69,593 doses ($p < .017$) enhanced % SAP (Figure 12c).

2.3.5. Discussion

The present results provide behavioural evidence to support IL involvement in the regulation of behavioural responsivity to anxiety provocation in CD-1 mice. Although there is a substantial literature describing the sensitivity of mPFC neurochemical responsivity to stressful or anxiogenic manipulations (Abercrombie et al., 1989; Kaneyuki et al., 1991; Shanks, Zalcman, Zacharko, & Anisman, 1991; Yoshioka, Matsumoto, Togashi, & Saito, 1995; Zacharko & Anisman, 1991), most studies have not evaluated behavioural or cognitive alterations following putative anxiolytic drug administration in the IL. In the present study, pretrial U-69,593 challenge in the IL reduced transfer-latencies and anxiety indices in the elevated plus-maze in trial-1.

The present data also demonstrated that pretrial-1 U-69,593 infusion in the IL did not influence transfer-latency recall, but had a small carry-over effect on two of the behavioural indices in the drug-free elevated plus-maze trial 24-hrs. later. Infralimbic U-69,593 administration also reduced defensive/withdrawal anxiety and enhanced spontaneous alternation memory in the Y-maze in the present study. These results present the first evidence obtained from the same animals that a type of working memory (as measured in the spontaneous alternation task) and anxiety (as measured in the

defensive/withdrawal and elevated plus-maze tasks) can be modulated by the microinfusion of an opioid drug in the IL cortex. These data also indicate that the anxiolytic effects of U-69,593 were accompanied by improved spontaneous working memory performance.

The data from Exp. 2a, however, should be interpreted with some caution. First, week-1 elevated plus-maze testing always preceded week-2 defensive/withdrawal and Y-maze testing. Second, the defensive/withdrawal anxiety test always preceeded the test of spontaneous alternation memory. Third, each mouse received two (1 wk apart) 1.0 µl volume microinjections, thus drug diffusion beyond the target area, as well as pharmacological sensitization, may have occurred. It is possible that the 1.0 µl microinjection was of sufficient volume to diffuse into the striatum and subsequently disrupt the behaviour of some of the animals. In this regard, microdialysis studies have shown that κappa receptor agonists dose-dependently reduce DA release and metabolic activity in the striatum along with spontaneous locomotor activity in rats (Di Chiara & Imperato, 1988a). Such drug effects may explain the reduction in closed arm entries observed among the animals from the 10 and 25 nmol groups in Experiment 2a.

2.3.6. Experiment 2b – Materials and Methods

Experiment 2b was designed to address the concern that 1.0 µl volume injections used in Exp. 2a may have resulted in drug diffusion into the dorsal striatum or nucleus accumbens. If the results in Exp. 2a were partially due to such drug diffusion, we would expect that the effects of a much smaller injection volume would subsequently be smaller. Accordingly, we evaluated whether 0.5 µl volume U-69,593 injections in the IL in three new groups of CD-1 mice would produce comparable behavioural and carry-over effects. Furthermore, because of the possibility that pharmacological sensitization might explain some of the wk-2 results in Exp 2a, we randomized defensive/withdrawal – Y-maze tests (week-1), then conducted elevated plus-maze tests (week-2).

Subjects

Subjects were 29 naive male CD-1 mice, obtained from Charles River (St. Constant, Quebec) at approximately 5 weeks of age. All mice were initially housed in polypropylene cages (groups of 10 per cage) in a temperature controlled room (21 ± 1° C), with ad lib access to food and water. The mice were acclimatized for 6 to 8 weeks until they reached a weight range of 32-45 grams. All mice were maintained on a 12 hour light/dark cycle (lights on at 7 a.m.).

Drugs

U-69,593 and cyclodextrin were obtained from Research Biochemicals International (Natick, MA). U-69,593 was dissolved using a 45% cyclodextrin solution to concentrations of 10 and 25 nmol/0.5 µl. Vehicle was a 45% cyclodextrin/distilled H_2O solution.

Behavioural Testing Procedures

All mice were challenged with vehicle (n = 9), 10 nmol (n = 10) or 25 nmol (n = 10) U-69,593 in the IL before defensive/withdrawal and Y-maze testing in week-1, and before trial-one elevated plus-maze transfer-latency and anxiety testing in week-2.

Week-1: (1) One half of the animals from each group (vehicle n = 4, 10 nmol n = 5, 25 nmol n = 5) were tested for defensive/withdrawal anxiety followed immediately by spontaneous alternation performance testing in the Y-maze. (2) The remaining animals from each group (veh. n = 5, 10 nmol n = 5, 25 nmol n = 5) were tested in reverse order.

Week-2: The same mice were used in week 2. Each animal was challenged with the same pretrial vehicle, 10 or 25 nmol/0.5 µl U-69,593 microinjection in the IL. (1) (Drug condition) The transfer-latency and anxiety tests in the elevated plus-maze were conducted as described in Exp. 2a. (2) (No drug condition) After a 24-hr delay, a second transfer-latency and anxiety trial was conducted and recorded in the same manner as in Exp. 2a.

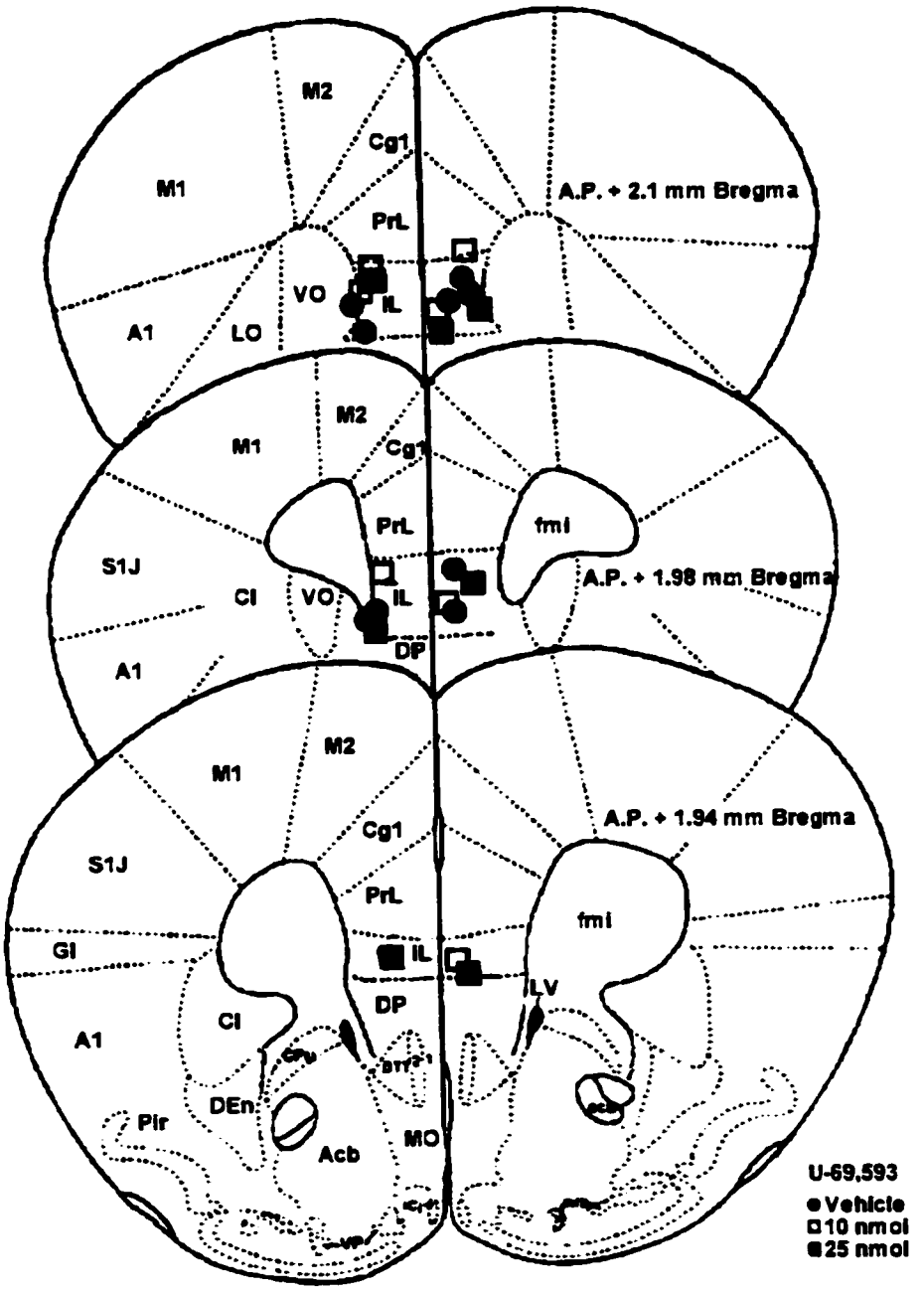
M2
Cg1
M1
A.P. + 2.1 mm Bregma
PrL
VO
IL
A1
LO
M2
M1
Cg1
fmi
PrL
S1J
A.P. + 1.98 mm Bregma
Cl
VO
IL
DP
A1
M2
M1
A.P. + 1.94 mm Bregma
Cg1
S1J
PrL
fmi
Gl
IL
LV
DP
Cl
A1
DEn
Pir
Acb
MO
U-69,593
Vehicle
10 nmol
25 nmol

Figure 15

Histology

The distribution of cannulae placements are depicted in Figure 15. Three mice from each of the 10 nmol and 25 nmol groups were rejected due to placements beyond the target area. In all, 9 mice in the vehicle group, 7 in the 10 nmol and 7 in the 25 nmol groups (N = 23) were analyzed.

Place Figure 15 about here

2.3.7. Results

Week One:

Defensive/Withdrawal Anxiety. Group means +/- SEM are graphically depicted in Figure 16.

Place Figure 16 about here

Oneway ANOVA revealed that U-69,593 treatment influenced defensive/withdrawal latencies ($F_{2,20} = 5.23$, $p < .02$). Subsequent post-hoc LSD tests revealed that the 10 nmol U-69,593 concentration reduced defensive/withdrawal latencies ($p < .025$) relative to vehicle-treated mice. It is interestiing that, whereas the 1 nmol dose reduced defensive/withdrawal anxiety in Exp. 2a, the 10 nmol dose reduced defensive/withdrawal anxiety in the present experiment. These data indicate that the 1 nmol dose in 1.0 µl likely exerted its effects from the IL in Exp. 2a, and since the 10 nmol dose in 0.5 µl was the first injection in Exp. 2b, then those effects were also likely confined to the IL.

Spontaneous Alternation Memory in the Y-Maze. Group means +/- SEM are graphically depicted in Figure 16. Oneway ANOVA revealed that U-69,593 microinjections in the IL influenced YTE ($F_{2,20} = 5.91$, $p < .015$) and % SAP ($F_{2,20} = 7.48$, $p < .005$) in the Y-Maze. Post-hoc LSD tests

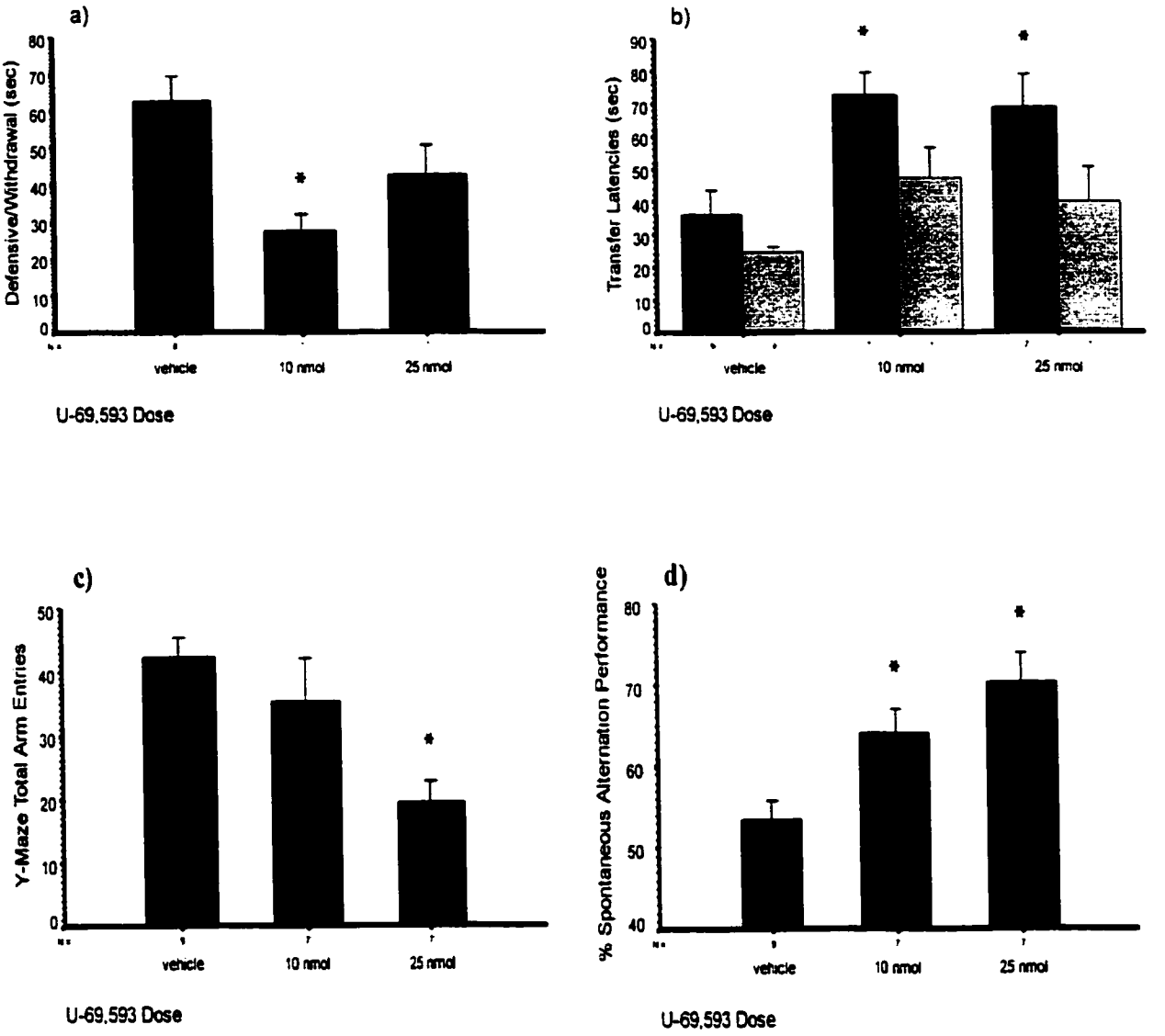
a)
Defensive/Withdrawal (sec)
vehicle
10 nmol
25 nmol
U-69,593 Dose
b)
Transfer Latencies (sec)
vehicle
10 nmol
25 nmol
U-69,593 Dose
c)
Y-Maze Total Arm Entries
vehicle
10 nmol
25 nmol
U-69,593 Dose
d)
% Spontaneous Alternation Performance
vehicle
10 nmol
25 nmol
U-69,593 Dose

Figure 16

revealed that the 25 nmol drug dose reduced YTE ($p < .025$), while the 10 and 25 nmol concentrations enhanced % SAP ($p < .025$) relative to vehicle controls Figure 16c and d).

It would appear from these data that testing order did not influence spontaneous alternation performance, owing to the demonstration that % SAP was similarly improved when Y-maze testing always followed defensive/withdrawal testing in week-2 of Exp. 2a, as well as when defensive/withdrawal and % SAP were randomly tested in week-1 of Exp. 2b.

However, testing order may have differentially effected the data in Experiments 2a and 2b YTE. In this regard, vehicle-treated mice entered Y-maze arms 24 times on average in Exp. 2a and 42 times in the present experiment. These data render the suggestion that elevated plus-maze exposure may have influenced Y-maze ambulatory activity in Exp. 2a.

Week Two:

Elevated plus-maze T-L and Anxiety Tests Trial-1. Oneway ANOVA revealed that U-69,593 pretreatment influenced transfer-latencies in the trial-1 drug condition ($F_{2,20} = 5.56$, $p < .015$). Post-hoc LSD tests revealed that the 10 and 25 nmol U-69,593 doses prolonged transfer-latencies ($p < .025$) relative to vehicle-treated animals (Figure 16b).

The data along with associated ANOVA's and post-hoc LSD tests for trial-1 elevated plus-maze anxiety are summarized in Table 10. Oneway ANOVA revealed that there were main effects

Place Table 10 about here

of U-69,593 treatment on OTR and UHD. Post-hoc LSD tests revealed that the 10 and 25 nmol doses increased both parameters ($p < .025$) relative to vehicle controls (Fig. 17). U-69,593 treatment

Place Figure 17 about here

PRETRIAL-1 0.5 µl U-69,593/ ELEVATED PLUS-MAZE TRIAL-1

Behav.	Vehicle	10 nmol	25 nmol	$F_{(2,20)}$	*P*
OE	3.1 ± 1.0	3.7 ± 0.4	3.9 ± 0.8	.26	NS
OTR	0.103 ± 0.03	0.321 ± 0.08 *	0.438 ± 0.08 *	5.94	0.008
UHD	3.3 ± 1.3	11.0 ± 2.4 *	10.8 ± 2.2 *	4.49	0.022
USA	4.6 ± 1.1	8.2 ± 1.3	7.4 ± 1.5	2.13	NS
CE	6.2 ± 1.0	4.9 ± 0.9	2.4 ± 0.6 *	5.6	0.01
CTR	0.446 ± 0.06	0.364 ± 0.07	0.284 ± 0.11	.89	NS
CVS	9.1 ± 2.3	9.7 ± 1.5	5.1 ± 2.0	1.7	NS

Table 10. Experiment 2b. Infralimbic U-69,593 effects on elevated plus-maze behaviour. Values represent means +/- SEM. Time ratios represent the amount of time (sec) in a section ÷ 300 sec. OE, open arm entries; OTR, open time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), sig. different than vehicle ($p < .025$).

(NO INJECTION) ELEVATED PLUS-MAZE TRIAL-2

Behaviour	Vehicle	10 nmol	25 nmol	$F_{(2,20)}$	*P*
OE	4.1 ± 0.6	7.0 ± 1.2	8.0 ± 1.2 *	3.43	0.048
OTR	0.130 ± 0.02	0.324 ± 0.05 *	0.281 ± 0.05 *	5.84	0.008
UHD	2.7 ± 0.6	17.2 ± 3.1 *	15.4 ± 4.1 *	6.57	0.005
USA	4.0 ± 0.5	11.4 ± 2.4 *	11.5 ± 2.2 *	5.01	0.015
CE	10.9 ± 1.4	12.0 ± 1.5	10.8 ± 1.1	0.26	NS
CTR	0.403 ± 0.06	0.314 ± 0.04	0.336 ± 0.03	1.16	NS
CVS	16.4 ± 3.2	29.4 ± 3.2 *	23.7 ± 3.2	4.02	0.031

Table 11. Experiment 2b. Pretrial-1 infralimbic U-69,593 carry-over effects on elevated plus-maze behaviour. Values represent means +/- SEM. Time ratios represent the amount of time (sec) in a section ÷ 300 sec. OE, open arm entries; OTR, open time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), sig. different than vehicle ($p < .025$).

also influenced CE. Subsequent post-hoc LSD tests revealed that the 25 nmol concentration reduced CE ($p < .025$), relative to control mice (Fig. 18).

Place Figure 18 about here

These behavioural patterns indicate that the 0.5 µl injection volume produced a similar anxiolytic profile suggesting that the U-69,593 effects were likely site-specific to the IL mPFC.

Moreover, the reverse of week-1 with week-2 as well as Y-maze with defensive/withdrawal testing orders did not produce noticeable changes in Exp. 2b which suggests that the trial-1 effects observed in Exp. 2a were not due to the order in which the tests were presented to the animals. These data render the hypothesis that pharmacological sensitization might explain the week-2 Y-maze and defensive/withdrawal results in Exp. 2a, less plausible.

Elevated plus-maze T-L and anxiety tests Trial-2. Oneway ANOVA revealed that pretrial-1 U-69,593 did not influence transfer-latencies in the trial-2 no drug condition ($F_{2,20} = 2.12$, NS).

The behavioural data along with associated ANOVA's and post-hoc LSD tests for trial-2 elevated plus-maze anxiety are summarized in Table 11. Oneway ANOVA revealed that there were

insert Table 11 about here

main carry-over effects of pretrial-1 U-69,593 treatment on trial-2 OE, OTR, UHD and USA. Post-hoc LSD tests revealed that the 10 and 25 nmol doses increased OTR, UHD and USA ($p < .025$), and the 25 nmol dose increased OE ($p < .025$) relative to vehicle controls (Fig. 17). Pre-trial-1 U-69,593 treatment also influenced CVS. Post-hoc LSD tests revealed that the 10 nmol dose increased CVS ($p < .025$) relative to control mice (Fig. 18).

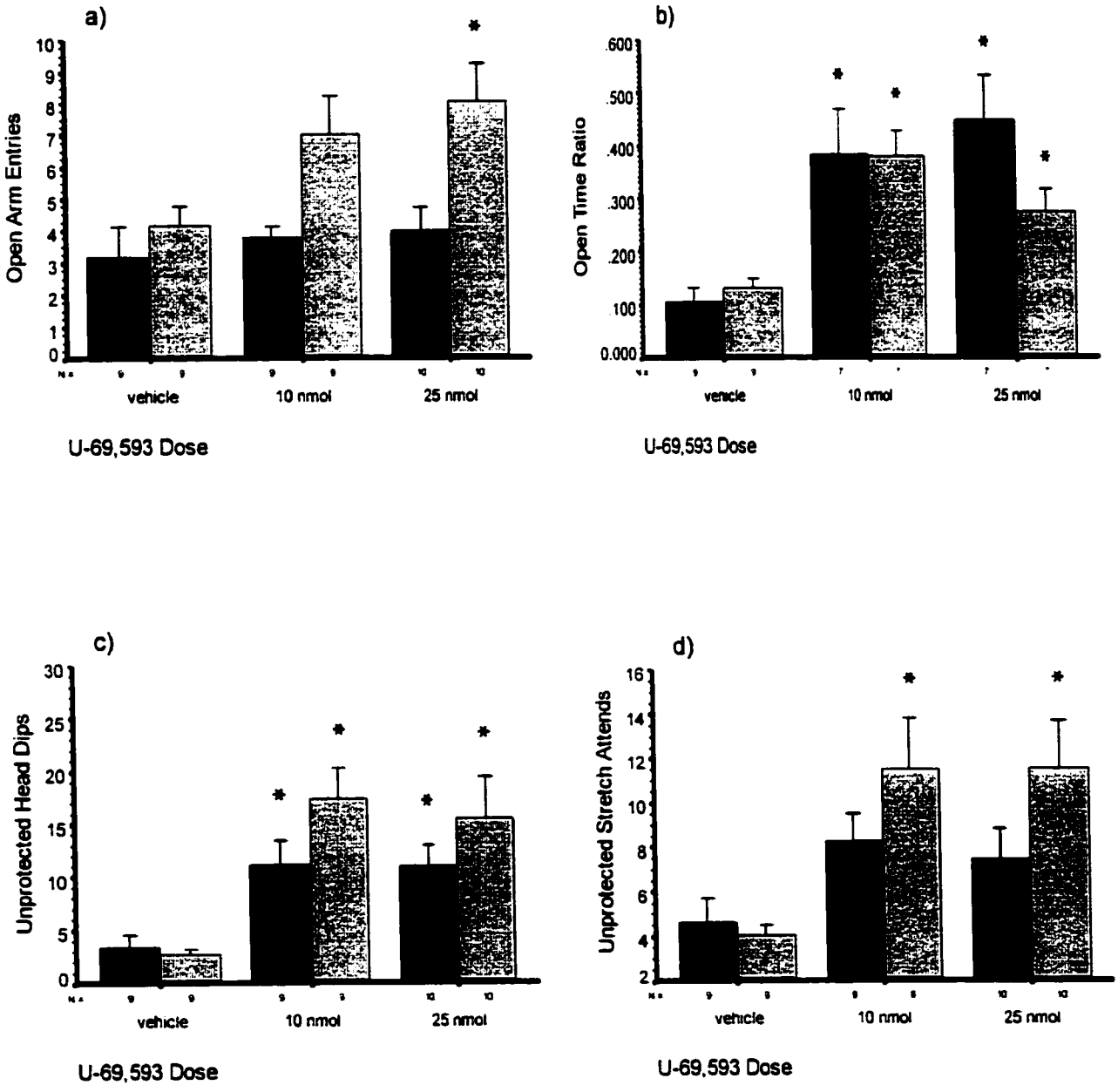
a)
Open Arm Entries
vehicle
10 nmol
25 nmol
U-69,593 Dose
b)
Open Time Ratio
vehicle
10 nmol
25 nmol
U-69,593 Dose
c)
Unprotected Head Dips
vehicle
10 nmol
25 nmol
U-69,593 Dose
d)
Unprotected Stretch Attends
vehicle
10 nmol
25 nmol
U-69,593 Dose

Figure 17

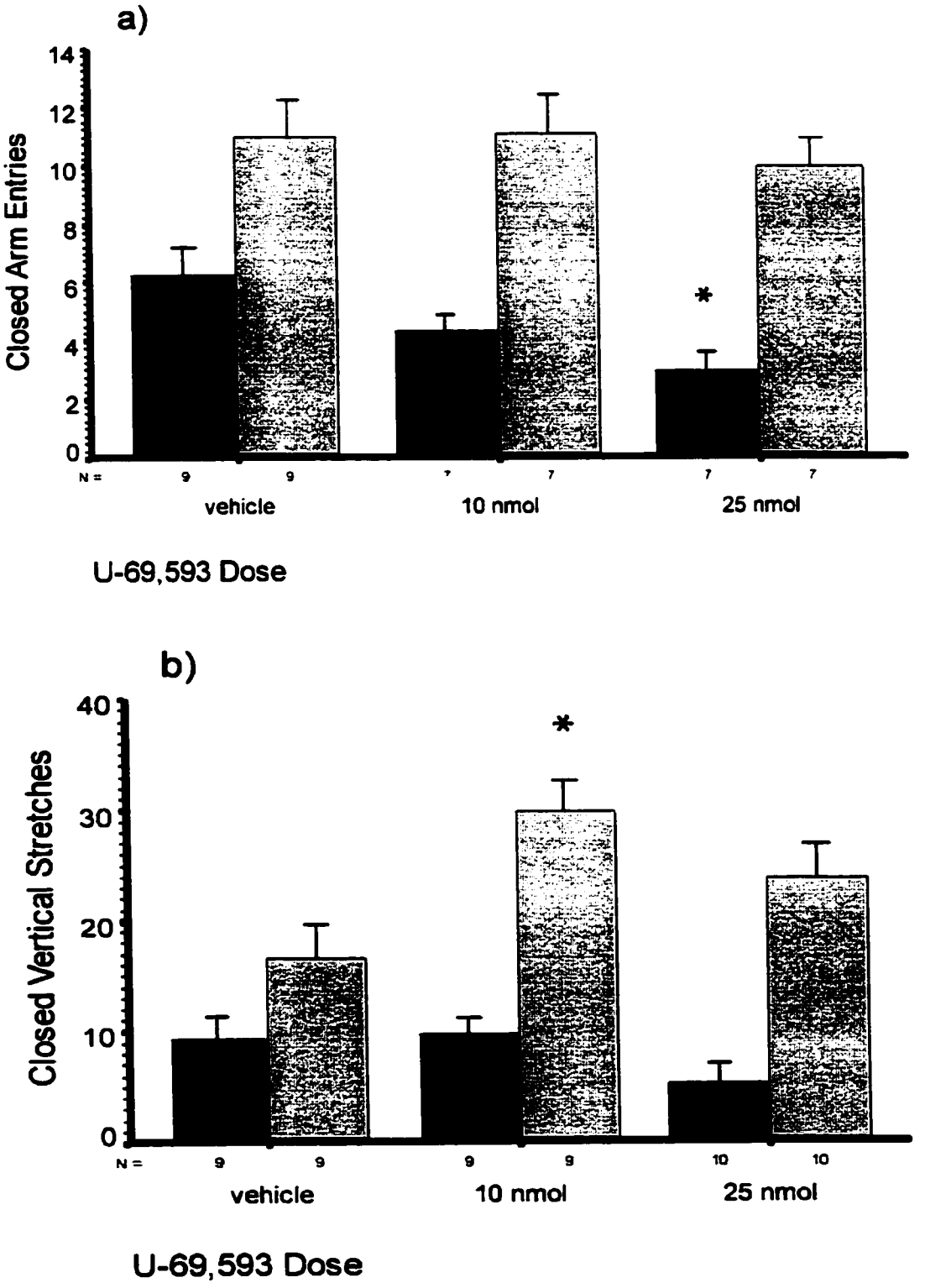
a)
Closed Arm Entries
14
12
10
8
6
4
2
0
N =
9
9
7
7
7
7
*
vehicle
10 nmol
25 nmol
U-69,593 Dose
b)
Closed Vertical Stretches
40
30
20
10
0
N =
9
9
9
9
10
10
*
vehicle
10 nmol
25 nmol
U-69,593 Dose

Figure 18

There were noticable differences between experiments 2a and 2b elevated plus-maze trial-2 behavioural profiles. It is conceivable that injection volume differences produced these trial-2 profile differences between experiments. Hence, the second pretrial 0.5 µl volume injections in week-2 may have exerted additive neurochemical effects with week-1 injections that were sufficient to impair aversive learning in the first elevated plus-maze trial. Moreover, insofar as the elevated plus-maze can be considered a model of approach/avoidance conflict, the aforementioned aversive (avoidance) learning impairments could also be interpreted as enhanced approach learning that was expressed as protracted trial-2 drug effects on approach memory and anxiety in Exp. 2b. Taken together, 0.5 µl volume injections in Exp. 2b appeared to only influence trial-2 behaviours in the elevated plus-maze.

2.3.8. General Discussion

The behavioural data derived from Exp. 2a showed that pretrial kappa$_1$ receptor activation in the IL cortex reduced transfer-latency and elevated plus-maze anxiety in week-1, did not influence transfer-latency recall but slightly influenced anxiety-related behaviour in the drug-free retest 24-hrs later, reduced defensive/withdrawal anxiety, and enhanced spontaneous alternation memory in week-2. In addition, the Exp. 2b results replicated the results from Exp.2a (except the elevated plus-maze trial-2 data), regardless of testing order and smaller injection volumes. The present behavioural data also replicated results from previous experiments showing anxiolytic behavioural profiles in the elevated plus-maze following U-69,593 microinfusion in the vmPFC in CD-1 mice (Wall, 1997). To our knowledge, this is the first study showing opioid effects on spontaneous alternation memory and anxiety in the same animals from the vmPFC. These preliminary data indicate that kappa$_1$ receptor activation in the IL area of the vmPFC can serve to reduce anxiety and enhance spontaneous on-line memory in anxiety-provoking situations.

A number of issues are raised, however, that need to be addressed. First, there is some suggestion that spontaneous alternation memory is related to working memory, therefore is a reasonable test of working memory (Kameyama, Ukai, & Shinkai, 1998; Maurice et al., 1994; Ukai et al., 1998). Indirect evidence in support of this hypothesis stems from several studies showing that central scopolamine administration impairs delay-dependent working memory tasks such as spatial alternation (Markowska et al., 1995), delayed matching- (Granon et al., 1995) and nonmatching-to-sample (Dunnett, 1990), or matching-to-position tasks in rats (Herremans, Hijzen, Welborn, Olivier, & Slangen, 1996), as well as disrupts spontaneous alternation memory in the Y-maze (Itoh et al., 1994; Parsons & Gold, 1992; Stone, Walser, Gold, & Gold, 1991; Ukai et al., 1997; Ukai, Shinkai, & Kameyama, 1996; Ukai et al., 1998; Ukai, Shinkai, Ohashi, & Kameyama, 1995; Walker, McGlynn, Grey, Ragozzino, & Gold, 1991). These data render the suggestion that immediate and delayed memory processing may involve similar working memory systems. However, the present spontaneous alternation memory data are preliminary only, thus further investigations into delay-dependent working memory are required to conclusively demonstrate kappa opioid modulation of working memory and anxiety from the IL cortex.

A second issue addresses the extent to which 'locomotor activity' indices can be meaningfully extracted from anxiety-related indices in the elevated plus-maze. There does not appear to be much substantive meaningfulness to such a latent construct driving elevated plus-maze behaviour. Moreover, several investigators simply do not agree as to what indicator(s) is (are) 'pure' locomotor activity index(es). To elaborate, some investigators have reported TE as a valid locomotor activity indicator (Fernandez Espejo, 1997; Lister, 1987; Pellow et al., 1985; Rodgers & Johnson, 1995), TE as a mixed anxiety/locomotor activity indicator (Cruz et al., 1994; Elliott et al., 1992; Fernandes & File, 1996), CE as a pure locomotor activity indicator (Cruz et al., 1994; Elliott et al., 1992; Rodgers et al., 1995), and CE as an index of protected exploration (Fernandes & File, 1996). It would appear

that ever since TE was originally interpreted as a locomotor activity indicator in Lister's PCA of mouse behaviour in the elevated plus-maze (regardless of the fact that OER, OTR and TE only were analyzed in that study (Lister, 1987), or that TE generally cross-loads in several reported PCA studies), many investigators continue to measure it as a substantive locomotor activity index. Fernandes and File (Fernandes & File, 1996) first suggested that CE could be interpreted as an index of protected area exploration. Taken together, there does not appear to be any unambiguous indicators of 'locomotor activity', thus locomotor activity does not appear to be a valid latent construct underlying behaviour in the elevated plus-maze. For these reasons, there is little justification to measure locomotor activity (or any indicator thought to assess locomotor activity, such as TE), thus was not analyzed or reported on in the present study. Rather, CE was taken as an indicator of protected exploration activity (Fernandes & File, 1996).

A third issue addresses the nature and extent to which memory and anxiety might interact in anxiety-provoking situations. There are some behavioural data that, to some extent, demonstrate a link between anxiety and memory. For example, Beuzen and Belzung (Beuzen & Belzung, 1995) demonstrated a link between emotional memory for inhibitory passive avoidance and state anxiety provoked in the light/dark box in a PCA of mouse behaviour that analyzed several parameters in 3 different paradigms together (i.e., lt/dark test, free novelty exploration and passive avoidance). These investigators tentatively suggested that the more anxious mice were in the light/dark test, the better their performance in the subsequent passive avoidance memory test. Clearly, inhibitory passive or active avoidance tests involve emotional learning and memory processing (Beuzen & Belzung, 1995; Viana et al., 1994). Thus, testing an animal in an anxiety-provoking situation first conceivably influenced subsequent memory performance in their study (Beuzen & Belzung, 1995). In the present study, however, we did not find that anxiety states provoked by defensive/withdrawal testing influenced subsequent spontaneous alternation memory in Exp. 1, or vice-versa in Exp. 2.

The elevated T-maze was developed as a method to test the extent to which discrete brain sites can simultaneously influence anxiety and memory in rats, as well as to test the amnestic potential of putative anxiolytic drugs (Gargiulo et al., 1996; Graeff, Viana, & Mora, 1996; Graeff et al., 1993; Scorza et al., 1996; Viana et al., 1994). One-way (transfer-latency) escape (likened to anticipatory anxiety) and inhibitory (defensive/withdrawal) avoidance (likened to innate fear) have been routinely evaluated (Gargiulo et al., 1996). Briefly, defensive/withdrawal anxiety and learning, as well as transfer-latency anxiety have been altered, but transfer-latency memory has not in 2 separate pharmaco-behavioural studies (Gargiulo et al., 1996; Viana et al., 1994). In the present report, defensive/withdrawal and trial-1 transfer-latency anxiety were altered, trial-2 transfer-latency recall memory was not altered, elevated plus-maze anxiety memory was somewhat affected in Experiment 2, but spontaneous alternation memory in the Y-maze was clearly enhanced. Together, it would appear that transfer-latency memory, in the T-maze or elevated plus-maze, is likely not a sensitive memory test when given in conjunction with anxiety testing procedures, thus more than one apparatus is likely necessary to concomitantly test for any effects of a single drug injection on anxiety and memory parameters.

A final question remains as to the mechanisms by which IL kappa receptor activation influenced anxiety and spontaneous memory in the present experiments. There are some autoradiographic, immunohistochemical and in situ hybridization data indicating that kappa$_1$ receptors are expressed in deep layer DA terminals in the mPFC (Mansour et al., 1995; Unterwald et al., 1991). In addition, moderately high levels of the endogenous kappa agonist, dynorphin, coexist in intrinsic GABA interneurons in the mPFC (Jones & Hendry, 1986). Functionally, dynorphin is thought to inhibit DA release from mesocortical terminal sites (Olson et al., 1993). Furthermore, some investigators have suggested that kappa receptors can inhibit all DA neuronal systems, but only after these systems have been activated (Manzanares, Lookingland, & Moore,

1990). Since it is also well known that various stressful or anxiety-provoking manipulations enhance mesocortical DA activity (Abercrombie et al., 1989; Bertolucci-D'Angio et al., 1990b; Carlson et al., 1991; Deutch & Roth, 1990; Dunn & File, 1983; Herman et al., 1982) and DA activity enhancement impairs spatial working memory in rats and monkeys (Murphy, Arnsten, Goldman-Rakic et al., 1996; Murphy, Arnsten, Jentsch et al., 1996), then the DA release-inhibiting effects of dynorphin may exert a limiting control over hyperactivated DA mechanisms in the mPFC (Bals-Kubik et al., 1993; Spanagel et al., 1992; Unterwald, Rubenfeld, & Kreek, 1994). Moreover, hyperactivated DA activity in anxiety-provoking situations may serve to take IL automatic memory processing off-line, allowing subcortically-driven behaviour to prevail.

Some investigators have suggested that abnormal dopamine regulation in the mPFC may contribute to the etiology and symptoms of schizophrenia (Goldman-Rakic & Selemon, 1997; Robbins, 1990), attention deficit disorder (Castellanos, 1997; Ernst et al., 1998; Russell et al., 1995) and cognitive aspects of Parkinson disease (Gotham, Brown, & Marsden, 1988) among others. Moreover, stress is known to precipitate or exacerbate these disorders (Carroll, Barrett, & American Psychopathological Association. Meeting, 1991; Murphy, Arnsten, Jentsch et al., 1996). In this regard, too much DA inhibition/inactivation or depletion in the mPFC induces conditioned place aversion (Bals-Kubik et al., 1993), hyperactivity (Bubser & Schmidt, 1990), anxiety in the elevated plus-maze (Espejo, 1997b), and working memory deficits (Brozoski, Brown, Rosvold, & Goldman, 1979; Sawaguchi & Goldman-Rakic, 1991, 1994; Seamans et al., 1998; Simon, Scatton, & Moal, 1980). Hence IL kappa agonist infusions may have improved spontaneous alternation memory through the inhibition of anxiety-provoked enhanced DA release, thus maintained homeostatic DA levels and thereby allowed the IL mPFC to remain on-line during maze performance. Moreover, the U-69,593 doses used in the present experiments were most-likely not sufficient to produce DA depletions, otherwise, anxiogenic behaviour (Espejo, 1997b),

hyperactivity (Jones & Robbins, 1992) and spontaneous alternation memory deficits would undoubtedly have been observed. It would appear that a need exists for therapeutic drugs that can attenuate stress-induced DA hyperactivity in the mPFC (Murphy, Arnsten, Jentsch et al., 1996). In this regard, further investigation is called for to evaluate the role of kappa opioids in the regulation of mPFC DA dysfunction associated with psychopathological disorders.

There are some data demonstrating that concurrently enhanced cholinergic activity in the IL area of the vmPFC (Ragozzino & Kesner, 1998) and the dorsal hippocampus (Ragozzino, Unick, & Gold, 1996) coincides with working memory processing, conditioned stimuli paired with footshock (Acquas, Wilson, & Fibiger, 1996) and inescapable restraint (Mark et al., 1996) in rats. Scopolamine has also been shown to simultaneously induce hyperactivity and anxiety in the elevated plus-maze in mice (Rodgers & Cole, 1995), as well as hyperactivity and spontaneous alternation memory impairments in the Y-maze in mice (Itoh et al., 1993a). In addition, IL mPFC DA and ACh levels increase during memory acquisition and recall in rats (Izaki, Hori, & Nomura, 1998). Moreover, dynorphin interacts with cholinergic systems in learning and memory (Hiramatsu, Murasawa, Mori et al., 1998), and DA interacts with ACh in memory processing (Steele et al., 1996). It has also been suggested that basal forebrain ACh activation underlies anxiety induction (Belotti & Galey, 1996). Taken together, U-69,593 infusion in the IL cortex may have directly or indirectly interfered with dopaminergic, cholinergic or DA/ACh interactions to produce the observed effects on anxiety and memory in the present experiments. Such neurochemical effects may have impacted the animal's attention processes related to the immediate orientation to, perception of, and subsequent memory for potentially dangerous situations.

In summary, the present data support the hypothesis that the IL influences anxiety states and spontaneous memory, in part, through kappa opioid receptor activation. In this regard, IL $kappa_1$ receptor activation may be a necessary (or facilitative) neurochemical component of anxiolysis,

whereby implicit memory processes may simultaneously regulate visceroemotional responsivity and guide exploratory activity in anxiety-provoking environments. Further studies are currently underway to determine whether (a) the kappa receptor antagonist, norBNI, will oppositely influence anxiety and spontaneous alternation memory from the IL mPFC, (b) both drugs can influence delay-dependent working memory in CD-1 mice in the eight arm radial maze, and (c) systemic norBNI injections can counteract the effects of U-69,593 observed in the present experiments.

2.4. EXPERIMENT 3

Wall, P. M. & Messier, C. (2000). Concurrent modulation of anxiety and memory. *Behavioural Brain Research*. 109: 229-241.

2.4.1. Abstract

We have previously shown that the ventromedial prefrontal cortex (vmPFC) is involved in spontaneous working memory and anxiety-related behaviour in CD-1 mice. Specifically, pretrial microinjection of the kappa$_1$ agonist, U-69,593, in the infralimbic (IL) area of the vmPFC produced a robust anxiolytic behavioural profile in the elevated plus-maze and enhanced spontaneous working memory in the Y-maze. In the present study we sought to determine whether these effects were specific to IL kappa receptors. We hypothesized that microinjection of the kappa antagonist, norBNI, in the IL cortex would influence anxiety and spontaneous memory in an opposite direction to the effects produced by the kappa$_1$ agonist. In week-1, transfer-latency reference memory and anxiety were tested in the elevated plus-maze in two separate trials with an intertrial interval of 24-hrs. In week-2, spontaneous working memory was tested in the Y-maze followed immediately by defensive/withdrawal anxiety in the open field for one half of the animals in each group, and the other half was tested in reverse order. Pretreatment with one injection of vehicle, 1, 5 or 10 nmol/0.5 µl norBNI in the IL cortex dose-dependently reduced transfer-latencies and produced an anxiogenic behavioural profile in the first elevated plus-maze trial. Following a 24-hr delay,

transfer-latency reference memory was not influenced, but a robust anxiogenic behavioural profile was observed in the second no-injection anxiety trial in the elevated plus-maze relative to control animals. In week-2, the same groups of mice were again pretreated with one injection of the same doses of norBNI in the IL cortex and tested in the open field and Y-maze. NorBNI pretreatment was anxiogenic in the defensive/withdrawal anxiety test and disrupted spontaneous working memory regardless of testing order. The present results show the influence of kappa receptor modulation on anxiety induction and spontaneous working memory. These results also support the hypothesis that immediate memory processing may modulate the induction of anxiety-related behaviours.

2.4.2. Introduction

There are growing data demonstrating that the ventral prelimbic (PL) and infralimbic (IL) vmPFC are involved in memory processing in mice (Wall & Messier, 2000c), rats (Fritts, Asbury, Horton, & Isaac, 1998; Jung, Qin, McNaughton, & Barnes, 1998; Poucet, 1997; Ragozzino et al., 1998; Ragozzino & Kesner, 1998), monkeys, (Bachevalier & Mishkin, 1986; Murphy, Arnsten, Goldman-Rakic et al., 1996; Murphy, Arnsten, Jentsch et al., 1996; Wilson, 1987) and humans (Goldman-Rakic, 1995c, 1996b, 1996c, 1998; Goldman-Rakic & Selemon, 1997; Maguire & Mummery, 1999). The ventral PL and IL cortices have also been shown to be involved in anxiety regulation in mice (Wall, 1997; Wall & Messier, 2000c), rats (Duncan, Knapp, & Breese, 1996; Goldstein, Rasmusson, Bunney, & Roth, 1994; Goldstein et al., 1996; Izumi, 1998; Jinks & McGregor, 1997; Morgan & LeDoux, 1995; Morgan et al., 1993; Petty, Jordan, Kramer, Zukas, & Wu, 1997) and humans (Gold & Chrousos, 1999). Whether the vmPFC regulates memory and anxiety simultaneously or separately is presently not known. There are, however, some data in support of the notion that spontaneous working memory and anxiety-like behaviour can be concurrently regulated in the IL cortex. For example, a single microinjection of the selective $\kappa appa_1$ receptor agonist, U-69,593, in the IL cortex reduced defensive/withdrawal open field avoidance and

enhanced working memory in the Y-maze in CD-1 mice, regardless of testing order (Wall & Messier, 2000c). The question remained, however, as to what mechanisms were disrupted by an exaggerated κappa$_1$ agonist presence, and why were such cognitive/behavioural effects so prominent in the vmPFC considering data showing a light density of κappa$_1$ receptors expressed in deep layers of this cortical site (Mansour et al., 1995).

Some autoradiographic, immunohistochemical and in situ hybridization data indicate that kappa$_1$ receptors are expressed in deep layer dopamine (DA) terminals in the vmPFC (Mansour et al., 1995; Unterwald et al., 1991). In addition, moderately high levels of the endogenous kappa agonist, dynorphin, coexist in intrinsic GABA interneurons in the vmPFC (Jones & Hendry, 1986), and dynorphin is thought to functionally inhibit DA release from mesocortical terminal sites (Olson et al., 1993). In this respect, kappa receptor agonists have been shown to truncate electrically-evoked and drug-induced DA release in mesolimbic, nigrostriatal, mesocortical and tuberinfundibular terminal fields (Donzanti, Althaus, Payson, & Von Voigtlander, 1992; Manzanares, Lookingland, & Moore, 1991; Olson et al., 1993, 1995; Shippenberg et al., 1993; Werling, Frattali, Portoghese, Takemori, & Cox, 1988). Moreover, drug-induced activation of some dopaminergic systems serves to activate corresponding dynorphin systems (You et al., 1994).

Since it is also well known that various stressful or anxiety-provoking manipulations enhance mesocortical DA activity (Abercrombie et al., 1989; Bertolucci-D'Angio et al., 1990b; Carlson et al., 1991; Deutch & Roth, 1990; Dunn & File, 1983; Herman et al., 1982), and DA activity enhancement impairs spatial working memory in rats and monkeys (Murphy, Arnsten, Goldman-Rakic et al., 1996; Murphy, Arnsten, Jentsch et al., 1996), then the DA release-inhibiting effects of dynorphin may exert a limiting control over hyperactivated DA mechanisms in the mPFC (Bals-Kubik et al., 1993; Spanagel et al., 1992; Unterwald et al., 1994). Taken together, these data prompted us to suggest that IL U-69,593 infusions may have improved spontaneous working

memory through the inhibition of anxiety-provoked DA release, thus possibly optimized DA levels in the IL mPFC to improve Y-maze performance in our previous study (Wall & Messier, 2000c).

In contrast to the hypothesis that improved spontaneous working memory and reduced anxiety were a consequence of kappa receptor agonist influences on IL DA activity in challenging situations, some investigators have suggested that stressor-enhanced DA release in the mPFC may serve to take the mPFC 'off-line' to allow more automatic subcortical mechanisms to temporarily regulate behaviour (Zahrt et al., 1997). In this regard, intraventricular norBNI infusions have been shown to activate some DA systems in rats (Manzanares, Lookingland, LaVigne, & Moore, 1991). These data prompted us to suggest that ventral PL/IL kappa receptor inhibition may serve to enhance an already overactivated DA system in anxiety-provoking situations and subsequently enhance anxiety-like states in the elevated plus-maze and impair spontaneous working memory in the Y-maze.

Nor-binaltorphamine (norBNI) is a selective κappa opioid receptor antagonist in mice (Portoghese, Garzon-Aburbeh, Nagase, Lin, & Takemori, 1991; Portoghese, Lipkowski, & Takemori, 1987; Portoghese, Nagase, Lipkowski, Larson, & Takemori, 1988; Portoghese, Nagase, & Takemori, 1988). Data in support of the hypothesis that norBNI should be anxiogenic and impair spontaneous working memory (i.e., exert opposite effects to U-69,593) stem from the demonstration that norBNI administration blocked the anxiolytic effects of chlordiazepoxide in the elevated plus-maze (Agmo & Belzung, 1998). Moreover, we have previously shown that norBNI microinjection in the vmPFC produced a dose-related anxiogenic-like behavioural profile in the elevated plus-maze in CD-1 mice (Wall, 1997). Finally, intraventricular norBNI infusion blocked the improvement of the μ agonist DAMGO-induced impairment of spontaneous alternation memory in the Y-maze by dynorphin-A (Itoh et al., 1994). To our knowledge, direct effects of IL norBNI infusion on spontaneous working memory have not been demonstrated.

In the present series of experiments, we evaluated the possibility that norBNI infusion in the IL cortex in CD-1 mice would produce effects on anxiety and spontaneous working memory in an opposite direction to the previously-demonstrated anxiolytic and memory improvement effects of IL U-69,593 microinjections. Specifically, it was expected that norBNI would exert anxiogenic effects in the first trial, as well as in the second no-injection trial 24-hrs later in the elevated plus-maze. We also evaluated whether a single IL norBNI infusion would concurrently prolong defensive/withdrawal open field avoidance and impair spontaneous working memory in the Y-maze, regardless of testing order.

2.4.3. Materials and Methods

Subjects

Subjects were 40 naive male CD-1 mice, obtained from Charles River (St. Constant, Quebec) at approximately 5 weeks of age. All mice were initially housed in polypropylene cages (groups of 10 per cage) in a temperature controlled room (21 ± 1° C), with ad lib access to food and water. The mice were acclimatized for 6 to 8 weeks until they reached a weight range of 32-45 grams. All mice were maintained on a 12 hour light/dark cycle (lights on at 7 a.m.).

Surgery

Surgery was performed in the same manner as described in Experiment 2.

Drugs

NorBNI was obtained from Research Biochemicals International (Natick, MA). NorBNI was dissolved in artificial CSF to concentrations of 1, 5 and 10 nmol/0.5 µl. Artificial CSF was the vehicle.

Intracerebral Drug Administration

Following a 2-wk recovery period, mice were transferred from the main holding area to the laboratory and left undisturbed for 1 hour prior to drug administration (Rodgers et al., 1995). The mice were randomly assigned to one of a vehicle, 1, 5 or 10 nmol norBNI treatment condition. Each mouse was lightly restrained and a 32 gauge injection cannula was inserted into the guide cannula, the injection cannula connected using polyethylene tubing to a 5 µl Hamilton microsyringe. Each drug concentration was infused in a 0.5 µl volume over a 60-second period. The injection cannula remained in place for an additional 30 seconds to allow for drug diffusion.

Immediately following drug infusion, each animal was returned to its home cage, brought into the testing room and left undisturbed for 5 minutes prior to behavioural evaluation. All testing was conducted during the morning phase of the light/dark cycle between the hours of 8 a.m. and 1 p.m. All trials took place in a dimly illuminated chamber and were recorded by a ceiling-mounted SONY video camera that was linked to a Panasonic video monitor and VCR, and an IBM-compatible computer positioned outside the test chamber.

Apparati

The elevated plus-maze, Y-maze and open field used in Experiment 3 were the same as described in Experiment 2.

Behavioural Testing Procedures

The behavioural testing procedures were conducted in the same manner as described in Experiment 2. The anxiety-related behavioural indices in the elevated plus-maze, used in the present study, have been extensively validated and identified in several principal components analyses with rats and mice (Cruz et al., 1994; Fernandes & File, 1996; Rodgers & Johnson, 1995; Trullas & Skolnick, 1993), as well as in a recent extensive confirmatory factor analysis of mouse behaviour in the elevated plus-maze conducted in our laboratory (Wall & Messier, 2000b). We found that a 2-

factor model with six indicator variables was the most parsimonious model that could unambiguously explain unique underlying constructs of plus-maze behaviour. Importantly, four anxiety/open exploration indices (i.e., including 2 traditional open arm time and entry measures OE and OTR, and 2 ethologically-derived risk assessment measures USA and UHD) were shown to uniquely and robustly measure facets of animal anxiety related to unprotected exploration in our confirmatory factor analysis. In addition, CE and CTR were shown to be uniquely and robustly measure facets of animal anxiety related to protected exploration. Accordingly, the same six indices were analyzed in the present study: Open entries (OE), open time ratio (open time ÷ total time on the maze x 100, OTR), unprotected head dips (UHD), unprotected stretch attends (USA), closed arm entries (CE) and closed time ratio (CTR). Unprotected head dips were operationally defined as peering over the edge of an open arm with head, neck and shoulders, while out on an open arm (Cole & Rodgers, 1994). Unprotected stretch attends were operationally defined as the animal stretching its whole body forward while in an open arm and retracting to its original position without moving its hind paws (Cole & Rodgers, 1994). Decreases in OE, OTR, USA and UHD were interpreted as anxiogenic-like indices of drug treatment relative to vehicle-treated animals, and decreases in CE, CTR and CVS were interpreted as reduced protected exploration activity.

Histology

The distribution of cannulae placements in the IL were mapped on schematic representations employing the mouse brain atlas of Franklin and Paxinos (Franklin & Paxinos, 1997). One mouse from the vehicle, 3 mice from the 1 nmol, 2 from the 5 nmol and 4 from the 10 nmol groups were excluded due to placements outside this region. The cannulae placements included in the analysis are depicted in Figure 19.

Place Figure 19 about here

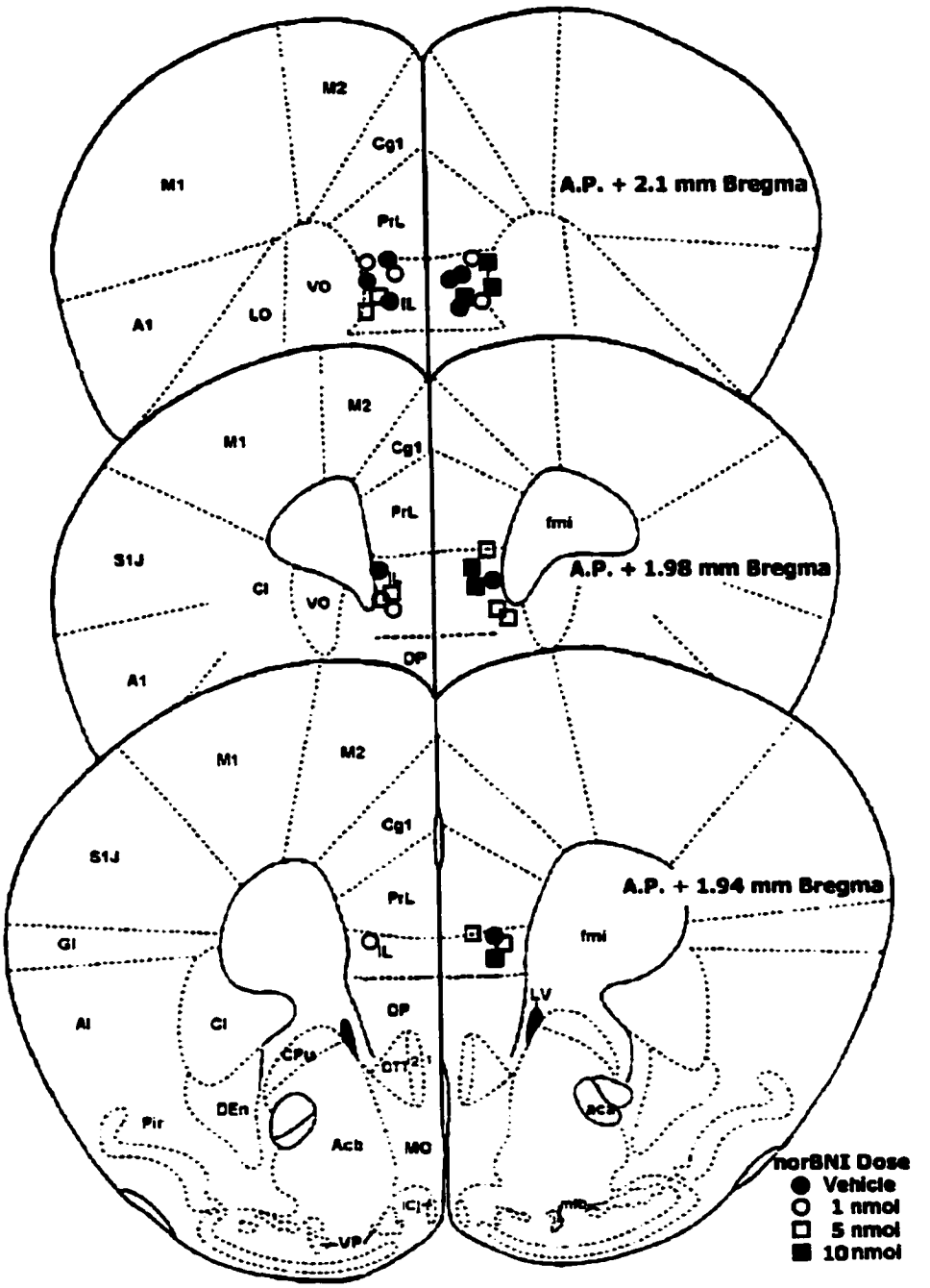
M2
Cg1
M1
A.P. + 2.1 mm Bregma
PrL
VO
IL
LO
A1
M2
M1
Cg1
PrL
fmi
S1J
A.P. + 1.98 mm Bregma
CI
VO
IL
DP
A1
M1
M2
Cg1
S1J
A.P. + 1.94 mm Bregma
PrL
GI
IL
fmi
LV
DP
AI
CI
CPu
DTT
DEn
Pir
Acb
MO
ICj
VP
norBNI Dose
Vehicle
1 nmol
5 nmol
10 nmol

Figure 19

Statistical Analyses

The data describing OE, OTR, UHD, USA, CE, CTR and CVS in the elevated plus-maze were analyzed by oneway analysis of variance (ANOVA) for trials 1 and 2 (α = .05), using SPSS 9.0 statistics package. Post-hoc LSD tests were conducted for significant treatment effects relative to control means (α Bonferroni-corrected, α = .05/3 = .017).

The transfer-latency, defensive/withdrawal, YTE and %SAP data were analyzed by oneway ANOVA (α = .05). Post hoc LSD tests were conducted to detect significant treatment effects relative to control means (α = .05/3 = .017).

2.4.4. Results

Week-1: ***Elevated plus-maze Transfer-Latency and Anxiety Tests Trial-1.*** Oneway ANOVA revealed that norBNI pretreatment reduced transfer-latencies in the trial-1 drug condition ($F_{3,26}$ = 3.69, $p < .025$). Post-hoc LSD tests revealed that the 10 nmol norBNI dose reduced transfer-latencies in trial-1 ($p < .017$) relative to vehicle-treated animals (Figure 20).

Place Figure 20 about here

Oneway between-subjects ANOVAs were performed on 7 dependent variables in the elevated plus-maze anxiety test: OE, OTR, USA, UHD, CE, CTR and CVS. The independent variable consisted of four treatment levels (vehicle and three norBNI doses). In all, 9 mice in the vehicle, 7 in the 1 nmol, 8 in the 5 nmol and 6 in the 10 nmol groups (N = 30) were analyzed.

The behavioural data along with associated ANOVA's and post-hoc LSD tests for trial-1 behavioural indices are summarized in Table 12, and where applicable, some of the figures depict

Place Table 12 about here

PRETRIAL-1 0.5 µl norBNI/ ELEVATED PLUS-MAZE TRIAL-1

Behav.	Vehicle	1 nmol	5 nmol	10 nmol	$F_{(3,26)}$	*P*
OE	4.2 ± 0.6	4.3 ± 0.8	2.3 ± 0.6	1.5 ± 0.7 *	4.46	0.012
OTR	0.178 ± 0.03	0.146 ± 0.03	0.083 ± 0.02 *	0.047 ± 0.02 *	6.27	0.002
UHD	8.1 ± 1.9	6.3 ± 2.4	1.9 ± 0.7 *	0.8 ± 0.5 *	4.45	0.012
USA	8.3 ± 1.3	4.1 ± 1.2 *	3.4 ± 0.9 *	1.0 ± 0.5 *	8.43	0.001
CE	12.1 ± 1.0	9.6 ± 0.5	12.3 ± 1.1	13.3 ± 1.4	2.21	NS
CTR	0.393 ± 0.04	0.375 ± 0.03	0.510 ± 0.04	0.452 ± 0.02	2.84	NS

Table 12. Experiment 3. Infralimbic norBNI effects on elevated plus-maze behaviour.Values represent means +/- SEM. Time ratios represent the amount of time (sec) in a section ÷ 300 sec. OE, open arm entries; OTR, open time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio. (*), significantly different than vehicle ($p < .017$).

(NO INJECTION) ELEVATED PLUS-MAZE TRIAL-2

Behav.	Vehicle	1 nmol	5 nmol	10 nmol	$F_{(3,26)}$	*P*
OE	5.9 ± 1.1	3.4 ± 0.5	2.6 ± 0.7 *	1.2 ± 0.7 *	5.78	0.004
OTR	0.224 ± 0.04	0.113 ± 0.02	0.087 ± 0.02 *	0.042 ± 0.02 *	6.24	0.002
UHD	10.4 ± 3.4	4.7 ± 2.4	1.0 ± 0.4 *	1.3 ± 1.0 *	3.71	0.024
USA	10.6 ± 1.6	4.5 ± 1.0 *	4.3 ± 2.0 *	1.8 ± 1.0 *	8.92	0.000
CE	11.0 ± 1.4	10.4 ± 1.1	12.1 ± 1.6	11.7 ± 1.2	.28	NS
CTR	0.371 ± 0.06	0.397 ± 0.05	0.477 ± .05	0.581 ± 0.04	2.62	NS

Table 13. Experiment 3. Pretrial-1 infralimbic norBNI carry-over effects on elevated plus-maze behaviour. Values represent means +/- SEM. Time ratios represent the amount of time (sec) in a section ÷ 300 sec. OE, open arm entries; OTR, open time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio. (*), significantly different than vehicle ($p < .017$).

results from both trials for comparison purposes. Oneway ANOVA revealed that there were main effects of norBNI treatment on OE, OTR, UHD and USA. Post-hoc LSD tests revealed that the 5 and 10 nmol drug dose reduced OE, OTR and UHD ($p < .017$), while the 1, 5 and 10 nmol concentrations reduced USA ($p < .017$) relative to vehicle-treated mice (Figure 21).

Place Figure 21 about here

Finally, norBNI did not influence CE, CTR or CVS. These results show that norBNI injections in the IL cortex produced robust and immediate anxiogenic-like behavioural response patterns in the elevated plus-maze.

Elevated plus-maze Transfer-Latency and Anxiety Tests Trial-2. Pretrial-1 IL norBNI infusion did not influence transfer-latencies 24-hrs. later ($F_{3,26} = .238$, NS) in trial-2 (no-injection).

Oneway between subjects ANOVAs revealed significant carry-over effects of pretrial-1 norBNI treatment on trial-2 OE, OTR, UHD and USA (Table 13). Post-hoc LSD tests revealed

insert Table 13 about here

that while the 5 and 10 nmol norBNI concentrations decreased UHD ($p < .017$), the 1, 5 and 10 nmol drug doses reduced OE, OTR and USA ($p < .017$) relative to control mice (Fig. 21). In a similar fashion to trial-1, neither CE, CTR nor CVS were affected by norBNI treatment, in trial-2.

Week-2:

Defensive/Withdrawal Anxiety. The defensive/withdrawal means +/- SEM are graphically depicted in Figure 20. Oneway ANOVA revealed that IL norBNI injections influenced defensive/withdrawal latencies ($F_{3,26} = 3.54$, $p < .05$). Subsequent post-hoc LSD tests revealed that the 5 nmol norBNI concentration prolonged open field avoidance relative to vehicle-treated mice ($p < .017$). These data

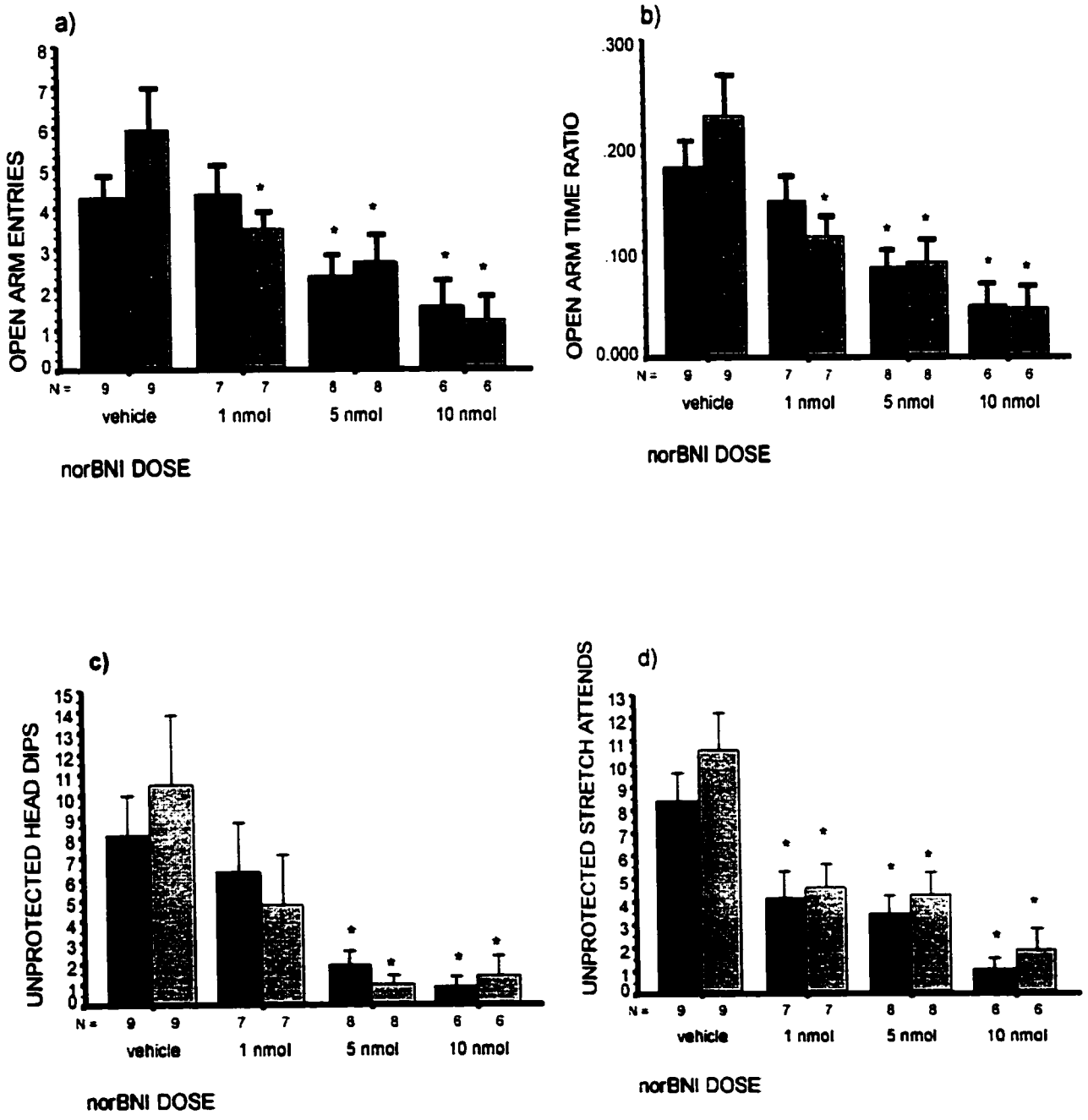
a)
OPEN ARM ENTRIES
N= 9 9 7 7 8 8 6 6
vehicle 1 nmol 5 nmol 10 nmol
norBNI DOSE
b)
OPEN ARM TIME RATIO
.300
.200
.100
0.000
N= 9 9 7 7 8 8 6 6
vehicle 1 nmol 5 nmol 10 nmol
norBNI DOSE
c)
UNPROTECTED HEAD DIPS
N= 9 9 7 7 8 8 6 6
vehicle 1 nmol 5 nmol 10 nmol
norBNI DOSE
d)
UNPROTECTED STRETCH ATTENDS
N= 9 9 7 7 8 8 6 6
vehicle 1 nmol 5 nmol 10 nmol
norBNI DOSE

Figure 21

indicate that the second 5 nmol norBNI injection influenced IL-mediated behaviour in a second animal model of anxiety.

Spontaneous Working Memory in the Y-Maze. Group means +/- SEM are graphically depicted in Figure 20. Oneway ANOVA revealed that norBNI microinjections in the IL cortex did not influence YTE ($F_{3,26} = 2.69$, NS), but disrupted % SAP ($F_{3,26} = 8.84$, $p < .001$) in the Y-Maze. Post-hoc LSD tests revealed that the 1 nmol ($p < .001$), 5 nmol ($p < .001$)and 10 nmol ($p < .01$) norBNI doses disrupted % SAP (Figure 20).

2.4.5. Discussion

The present results provide additional evidence to support IL involvement in the regulation of behavioural responsivity to anxiety provocation in CD-1 mice. In an opposite fashion to the anxiolytic and memory enhancing effects of IL U-69,593 infusions (Wall & Messier, 2000c), the present results clearly demonstrated that pretrial norBNI challenge in the IL cortex reduced transfer-latencies and was anxiogenic on four anxiety-related behavioural indices in the elevated plus-maze in trial-1. The present data also demonstrated that pretrial-1 norBNI infusion in the IL cortex did not influence transfer-latency reference memory, but exerted salient anxiogenic carry-over effects on the same four behavioural indices in the no-injection 2nd elevated plus-maze trial 24-hrs. later. Infralimbic norBNI was also anxiogenic in defensive/withdrawal avoidance latencies and disrupted spontaneous alternation memory in the Y-maze in the present study. These results present additional evidence to support the notion that a non-delay-dependant type of working memory (as measured in the spontaneous alternation task) and differing anxiety-related behavioural indices (as measured in the defensive/withdrawal and elevated plus-maze tasks) can be concurrently influenced by kappa receptor manipulations in the IL cortex.

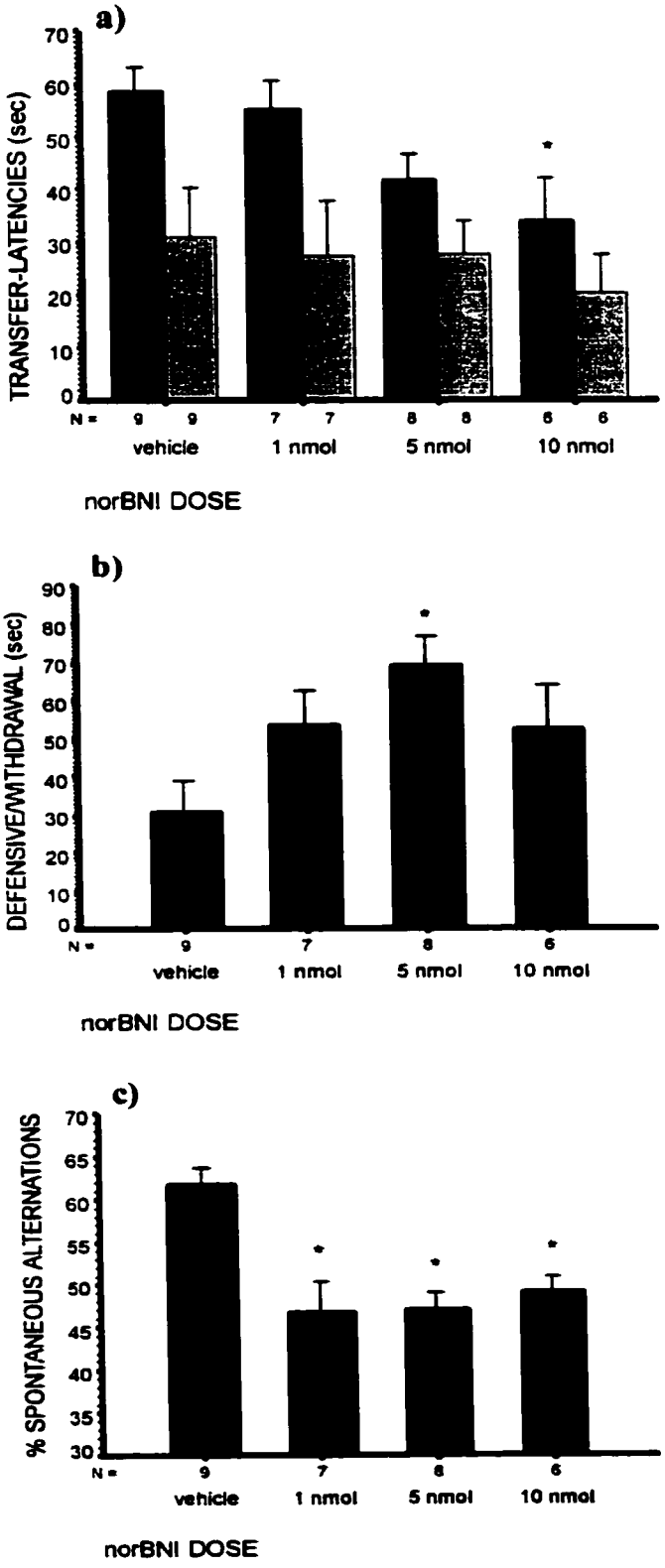
a)
TRANSFER-LATENCIES (sec)
70
60
50
40
30
20
10
0
N = 9 9 7 7 8 8 6 6
vehicle
1 nmol
5 nmol
10 nmol
norBNI DOSE
b)
DEFENSIVE/WITHDRAWAL (sec)
90
80
70
60
50
40
30
20
10
0
N = 9 7 8 6
vehicle
1 nmol
5 nmol
10 nmol
norBNI DOSE
c)
% SPONTANEOUS ALTERNATIONS
70
65
60
55
50
45
40
35
30
N = 9 7 8 6
vehicle
1 nmol
5 nmol
10 nmol
norBNI DOSE

Figure 20

Several investigators have reported that prior experience of a single undrugged trial in the elevated plus-maze fundamentally alters behavioural response patterns in a second drug-naïve trial (Almeida, Garcia, & de Oliveira, 1993; Dawson, Crawford, Stanhope, Iversen, & Tricklebank, 1994; Espejo, 1997a; Fernandes & File, 1996; File, 1993; Gonzalez & File, 1997; Rodgers, Johnson, Carr, & Hodgson, 1997; Rodgers, Lee, & Shepherd, 1992; Rodgers & Shepherd, 1993; Treit, Menard, & Royan, 1993). Similarly, in our recent confirmatory factor analysis of CD-1 mouse behaviour in the elevated plus-maze, OE, OTR, USA and UHD were reduced by about 50% in trial-2. In contrast to these between trial behavioural response differences, presumably resulting from some form of learning during trial-1 (Espejo, 1997a; File, 1993; File et al., 1993; Holmes & Rodgers, 1998), there were striking similarities between the trial-1 (drug injection) and trial-2 (no drug injection) anxiety measures in the present study. It is conceivable that norBNI exerted carry-over effects on trial-2 behaviour owing to data showing protracted effects of central norBNI administration in other situations (Endoh, Koike, Matsumuura, & Nagase, 1990; Horan, Taylor, Yamamura, & Porreca, 1992; Jones & Holtzman, 1992). Moreover, the trial-2 anxiety-related behavioural data from our confirmatory factor analysis closely resembled the corresponding trial-1 anxiety data from the norBNI treated groups in the present study. Infralimbic U-69,593 administration also produced similar behavioural profiles in both elevated plus-maze trials in our previous study (Wall & Messier, 2000c). Taken together, kappa agonist or antagonist administration in the IL cortex does not produce behavioural pattern changes from one trial to the next in the elevated plus-maze that are normally observed in drug-naïve animals.

Data from several reports indicate that the mPFC primarily processes implicit forms of memory such as temporal order learning (Chiba, Kesner, & Reynolds, 1994; Dietrich & Allen, 1998; Kesner, 1990), the sequential arrangement of behavioural components (Jung et al., 1998; Mogensen & Holm, 1994), directing attention to task relevant behaviour (Herremans et al., 1996), response

selection (Granon et al., 1994), spatial and delayed working memory (Fritts et al., 1998; Herremans et al., 1996; Penna et al., 1998; Ragozzino & Kesner, 1998; Sanchez-Santed, de Bruin, Heinsbroek, & Verwer, 1997), short-term memory (Dunnett, 1990), preference judgements (Elliott & Dolan, 1998), formation of affective memory (Liang, Hu, & Chang, 1996), processing emotional memory (Bremner et al., 1999) and novelty encoding (Tulving, Markowitsch, Kapur, Habib, & Houle, 1994), among others. There are additional data from human studies that suggest a link between implicit memory processing and anxiety states (Lang & Craske, 1997; McNally, 1997; Sawchuk, Lohr, Lee, & Tolin, 1999). For example, Peretti (1998) suggested that state anxiety produces working memory deficits (Peretti, 1998). Moreover, some behavioural data indicate that patients with anxiety disorders generally display implicit memory and attention biases (Lang & Craske, 1997; MacLeod & McLaughlin, 1995; Mathews et al., 1995; Mathews et al., 1989; McNally, 1997). Finally, recent PET imaging in healthy human subjects revealed that the posterior orbital, ventromedial prefrontal and anterior cingulate cortices were simultaneously activated with subjective feelings of anticipatory anxiety, thus the suggestion that these prefrontal cortical areas may process the experience or expression of anxiety (Drevets et al., 1994). These data raise the question as to the nature and extent to which implicit working memory and anxiety might interact in the vmPFC in challenging situations.

Although the results of the present study indicate that the observed effects on automatic memory processing and anxiety states in our previous study (Wall & Messier, 2000c) were mediated by kappa receptors, the mechanisms by which IL kappa receptor activation or blockade might exert such effects remain in question. We have previously suggested that IL kappa receptor activation may have truncated anxiety-provoked DA release, thus favourably influenced DA levels in the IL mPFC to improve maze performance in our previous study.

We have also suggested possible kappa receptor interactivity with cholinergic (ACh) mechanisms to exert such effects. For example, intraventricular norBNI infusion in mice blocked the reversal of scopolamine-induced working memory impairment in the Y-maze by intraventricular dynorphin-A infusion (Itoh et al., 1993a). Similarly, learning and memory impairments along with reduced ACh release, produced by the muscarinic agonist, carbachol, and the nictotinic antagonist, mecamylamine, were reversed by dynorphin administration. Finally, scopolamine-induced memory impairment and increased ACh release were blocked by intraventricular norBNI administration (Hiramatsu, Murasawa, Mori et al., 1998; Hiramatsu, Murasawa, Nabeshima et al., 1998). These results indicate that learning and memory impairments and reduced ACh release, which may be associated with presynaptic cholinergic dysfunction, can be modulated by kappa-opioid receptors (Hiramatsu, Murasawa, Mori et al., 1998).

Cholinergic fibres from the brainstem dorsolateral tegmental nucleus in rats innervate the basal forebrain (Jones & Cuello, 1989; Zaborszky & Cullinan, 1996; Zaborszky et al., 1991) and the vmPFC (Sesack et al., 1989; Swerdlow & Koob, 1987). In this regard, several investigators have suggested that cholinergic neurons from the brainstem dorsolateral tegmental nucleus are involved in fear conditioning, conditioned reinforcement, aversively-motivated discriminative avoidance, as well as cardiovascular conditioning in rats (Berntson et al., 1996; Berntson et al., 1998; Gabriel, 1990; Inglis et al., 1994). Furthermore, the pedunculopontine and dorsolateral tegmental ACh systems have been implicated in attentional processes (Inglis et al., 1994; Robbins & Everitt, 1995), as have the basal forebrain nucleus basalis (NBM) cholinergic systems (Hasselmo, 1995; Muir et al., 1992; Robbins, Everitt, Marston et al., 1989) that also project to the vmPFC. These connection patterns position the vmPFC to modulate afferent visceral information from the cholinergic brainstem.

Conceivably, cholinergic projections from the pedunculopontine tegmental nucleus to the vmPFC may be a source of anxiety-relevant information, and cholinergic projections from the basal forebrain may be an implicit memory-processing system in the vmPFC. Indirect evidence in support of this hypothesis stems from studies showing that immunotoxic lesions in the basal forebrain resulting in ACh reductions in the vmPFC or muscarinic receptor blockade in the vmPFC produced acquisition deficits in the water maze (Waite & Thal, 1996) and delayed matching-to-position or sample tasks in rats (Broersen, Heinsbroek, de Bruin, Uylings et al., 1995; Granon et al., 1995). These data support the notion that the basal forebrain NBM cholinergic system may be primarily concerned with learning and working memory processing.

Although bilateral N-methyl-d-aspartate lesions of the peduculopontine tegmental nucleus blocked the acquisition of a delayed working memory task, pretest diazepam administration reversed the acquisition impairments. Moreover, since the same pedunculopontine lesions were anxiogenic in the elevated plus-maze, these authors suggested that the pedunculopontine nucleus was likely not involved in learning and memory, but in the regulation of anxiety (Leri & Franklin, 1998). Additional evidence to support the notion that the pedunculopontine tegmental nucleus is not involved in working memory processes stems from the observation that bilateral radiofrequency lesions of this brainstem nucleus in rats did not produce accuracy deficits in a delayed non-matching to position task. These authors also concluded that the involvement of the pedunculopontine tegmental nucleus in memory processing was unlikely (Steckler et al., 1994).

Finally, in a review of the possible roles for the pedunculopontine tegmental nucleus, data showing that bilateral lesions of this nucleus in rats produced a constellation of behavioural changes reminiscent of the 'frontal syndrome' (i.e., involving disinhibition of behaviour, the release of inappropriate behaviour, and the production of perseverative behaviour) that is observed in rats following mPFC lesions, was discussed (Winn, 1998). These authors suggested that the

psychological changes underlying this syndrome are thought to involve executive functions, including (a) the prospective planning of sequences of actions, (b) attentional shifting, and (c) working memory.

Taken together, it is conceivable that simultaneous cholinergic afferent input to the mPFC from the brainstem and basal forebrain may have concurrently influenced implicit memory processing and anxiety induction in the present study. In this regard, IL kappa receptor activation or blockade may have directly (or indirectly through DA mechanisms) interfered with afferent IL ACh neurotransmission to produce our previous and present results.

To summarize, anxiety and memory were conjointly modulated following kappa receptor blockade in the IL cortex. Together with our previous report, IL kappa$_1$ receptor activation may be a necessary (or facilitative) neurochemical component of anxiolysis, whereby automatic implicit memory processing may simultaneously regulate visceroemotional responsivity and guide exploratory activity in anxiety-provoking environments.

2.5. EXPERIMENT 4

Wall, P.M., Flinn, J. & Messier, C. Infralimbic muscarinic M1 receptors modulate anxiety-like behaviour and spontaneous working memory in mice. *Psychpharmacology*. 155(1): 58-68.

2.5.1. Abstract

Spontaneous working memory and anxiety-like behaviour can be concurrently influenced following kappa opioid agonist or antagonist infusions in the infralimbic (IL) area of the ventromedial prefrontal cortex (vmPFC) in CD-1 mice. The present study sought to evaluate whether acetylcholine (ACh) muscarinic (M) receptor drugs can similarly influence these cognitive-behavioural processes in the IL cortex. Anxiety was evaluated in the elevated plus-maze and spontaneous working memory was evaluated in the Y-maze following scopolamine, pirenzepine or

McN-A-343 infusion in the IL cortex. In experiment 4a, the non-specific M receptor antagonist, scopolamine, was anxiogenic in trial-1 (5, 10 and 20 nmol), but did not influence behaviour in trial-2 (no-injection) in the elevated plus-maze 24 h later. In week-2, scopolamine only disrupted spontaneous working memory in the Y-maze at the highest dose (20 nmol). In experiment 4b, pretreatment with the M1 antagonist, pirenzepine, was anxiolytic in trial-1 (5 and 10 nmol), as well as in trial-2 (no-injection) in the elevated plus-maze 24 h later (0.25, 1.25, 2.5, 5 and 10 nmol). In week-2, pirenzepine disrupted spontaneous working memory in the Y-maze (2.5, 5 and 10 nmol). In experiment 4c, pretreatment with the M1 agonist, McN-A-343, was anxiogenic in trial-1 (2.5, 5, 10 and 20 nmol), as well as in trial-2 (no-injection) in the elevated plus-maze 24 h later (2.5, 5, 10 and 20 nmol). In week-2, McN-A-343 enhanced spontaneous working memory in the Y-maze (2.5, 5, 10 and 20 nmol). These data suggest that 1) Enhanced ACh transmission in the vmPFC induces anxiety in challenging environments, and enhances spontaneous working memory performance, 2) blocking or activating post-synaptic M1 receptors in the vmPFC may truncate or exaggerate, respectively, incoming anxiety-relevant information, and 3) IL pirenzepine and McN-A-343 exert long-term opposite effects on aversive learning during trial-1 in the elevated plus-maze.

2.5.2. Introduction

There are growing data demonstrating that the vmPFC is involved in memory processing in mice (Wall & Messier, 2000a, 2000c) and rats (Fritts et al., 1998; Jung et al., 1998; Poucet, 1997; Ragozzino & Kesner, 1998). The ventral prelimbic (PL) and IL cortices have also been shown to be involved in anxiety regulation in mice (Wall & Messier, 2000a, 2000c) and rats (Duncan et al., 1996; Goldstein et al., 1996; Morgan & LeDoux, 1995; Morgan et al., 1993; Petty et al., 1997). Whether the vmPFC regulates specific memory subtypes and anxiety-related behaviour simultaneously or separately is only now beginning to be understood.

We have previously shown that spontaneous working memory and anxiety-like behaviour can be concurrently regulated in the IL cortex. Whereas the selective κappa$_1$ receptor agonist, U-69,593, enhanced spontaneous working memory in the Y-maze and was anxiolytic in trials 1 and 2 in the elevated plus-maze (Wall & Messier, 2000c), the κappa receptor antagonist, norBNI, disrupted spontaneous working memory in the Y-maze and was anxiogenic in trials 1 and 2 in the elevated plus-maze (Wall & Messier, 2000a). It is noteworthy that kappa receptors interact with acetylcholine (ACh) muscarinic receptor systems in learning and memory (Hiramatsu, Murasawa, Mori et al., 1998), thus kappa drug infusions in the IL cortex may have directly or indirectly interfered with ACh mechanisms to produce the observed effects on anxiety and memory in our previous studies. Such neurochemical effects may have impacted the animal's attentional processes related to the immediate orientation to, perception of, memory for and immediate behavioural responses in potentially dangerous situations.

Cholinergic afferents to the IL cortex stem, in part, from the basal forebrain nucleus basalis (Gaykema, van Weeghel, Hersh, & Luiten, 1991). The nucleus basalis cholinergic system has been implicated in attentional (Hasselmo, 1995; Muir et al., 1992; Robbins, Everitt, Ryan et al., 1989), memory (Beninger, Ingles, Mackenzie, Jhamandas, & Boegman, 1992; DeSousa, Beninger, Jhamandas, & Boegman, 1994) and anxiety induction processes (Belotti & Galey, 1996; Berntson et al., 1998). Moreover, some investigators have suggested that an overactive cholinergic system in the mPFC in rats serves to bias the processing of fear and anxiety-related information (Berntson et al., 1998; Hart et al., 1999; Sarter & Bruno, 1999). Sarter and Bruno (1999) also suggested that overactive cholinergic terminals in the mPFC serve to disrupt some attentional mechanisms such that the ability to detect, select, discriminate and process important stimuli is compromised resulting in an inability to filter out irrelevant stimuli, memory dysfunction and anxiety symptomatology.

Conceivably, basal forebrain-vmPFC cholinergic terminals may normally serve to carry anxiety-related information in potentially dangerous situations such as exposure to the elevated plus-maze.

Non-specifically blocking cholinergic neurotransmission in the vmPFC produces amnestic effects on some types of working memory in rats. For example, scopolamine infusion in the vmPFC impaired both matching- and non-matching-to sample tasks (Granon et al., 1995), choice accuracy in a delayed matching-to-position task (Broersen, Heinsbroek, de Bruin, Uylings et al., 1995; Dunnett, 1990), as well as working memory for spatial locations (Ragozzino & Kesner, 1998), in a delay-dependent manner. In contrast, scopolamine infusions in the vmPFC delay-independently disrupted working memory in delayed non-matching-to-position (Broersen, Heinsbroek, de Bruin, Uylings et al., 1995; Dunnett, 1990) and matching-to-position tasks (Herremans et al., 1996) in rats, effects suggested to result from attentional mechanism, rather than working memory processing, disruption, *per se*.

Spontaneous alternation performance and non-matching-to-sample tasks may tap into a delay-independent form of working memory. In both cases animals can perform such tasks by utilyzing relatively 'automatic' memory processing systems (Granon et al., 1995; Tako et al., 1988). It is therefore puzzling that the effects of M1 receptor blockade in the vmPFC on working memory processing have not yet been studied, particularly since intraventricular administration of the MI antagonist, pirenzepine, disrupted spontaneous working memory in the Y-maze in mice, (Ukai, Shinkai, & Kameyama, 1995b), and intra-hippocampal pirenzepine injections disrupted some working memory parameters in rats (Ohno, Kikusui, Yoshimatsu, Yamamoto, & Watanabe, 1994; Ohno, Yamamoto et al., 1994). Thus, in addition to anxiety-related information processing (Hart et al., 1999), different forms of working memory may be mediated, in part, through cholinergic transmission at post-synaptic muscarinic receptor sites in the vmPFC (Broersen, Heinsbroek, de

Bruin, Uylings et al., 1995; Granon & Poucet, 1995; Herremans et al., 1996; Ragozzino & Kesner, 1998).

In the present study, Experiment 1 evaluated whether scopolamine infusion in the IL cortex would disrupt spontaneous working memory (as it has been shown to disrupt working memory in the vmPFC in rats) and influence anxiety in CD-1 mice. Experiment 2 evaluated whether the M1 antagonist, pirenzepine, would exert anxiolytic effects and disrupt aversive learning and working memory. Experiment 3 evaluated whether the M1 agonist, McN-A-343, would exert anxiogenic effects, as well as enhance aversive learning and spontaneous working memory.

2.5.3. General Materials and Methods

Subjects

Subjects were 185 male CD-1 naive mice, obtained from Charles River, St. Constant, Quebec. All mice were initially housed in polypropylene cages (groups of 10 per cage) in a temperature controlled room (21 ± 1° C), with ad lib access to food and water. The mice were acclimatized for 6 to 8 weeks until they reached a weight range of 32-45 grams. All mice were maintained on a 12 h light/dark cycle (lights on at 07:00 h).

Surgery

All surgical procedures were performed in the same manner as described in Experiment 2.

Drugs

Scopolamine, pirenzepine and McN-A-343 were obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in artificial CSF (Scopolamine to concentrations of 5, 10 and 20 nmol/0.5 µl, Pirenzepine to concentrations of 0.25, 1.25, 2.5, 5 and 10 nmol/0.5 µl, and McN-A-343 to concentrations of 2.5, 5, 10 and 20 nmol/0.5 µl). Artificial CSF was the vehicle in all experiments.

Intracerebral Drug Administration

Following a 2-wk recovery period, mice were transferred from the main holding area to the testing laboratory and left undisturbed for 1 h prior to drug administration. The mice were randomly assigned to one of a vehicle or specific nmol drug dose treatment condition. Each mouse was lightly restrained and a 32 gauge injection cannula (connected using polyethylene tubing to a 10 µl Hamilton microsyringe) was inserted into the guide cannula. Each drug concentration (0.5 µl volume) was infused over a 60-second period. The injection cannula remained in place for an additional 30 seconds to allow for maximum drug diffusion.

Immediately following drug infusion, each animal was returned to its home cage, brought into the testing room and left undisturbed for 5 minutes prior to behavioural evaluation. All testing was conducted during the morning phase of the light/dark cycle between the hours of 08:00 and 13:00 h. All trials took place in a dimly illuminated chamber and were recorded by a ceiling-mounted SONY video camera that was linked to a Panasonic video monitor and VCR, and an IBM-compatible computer positioned outside the test chamber.

Apparati

The elevated plus-maze and Y-maze used in Experiment 4 were the same as described in Experiment 2.

Behavioural Testing Procedures

For each experiment, half of the animals from each group were tested in the first elevated plus-maze trial after a single injection followed by a no-injection trial 24 h later in week-1, then tested in the Y-maze after a second microinjection, in week-2. The remaining animals were tested in reverse order (Y-maze in week-1 and elevated plus-maze trials 1 and 2 in week-2).

1) *Elevated plus-maze anxiety test.* Each animal was challenged with one of a pre-trial vehicle or nmol drug dose /0.5 µl microinjection in the IL cortex. Individual trials in the elevated plus-maze were 5 minutes in duration and commenced by placing the animal in the centre hub, facing an open arm. A mouse was considered to have entered an arm when all four paws were positioned within an arm runway, or exited an arm (i.e., in the centre hub) when at least one paw was outside an arm threshold. After a 24-h delay, a second trial was conducted and recorded in the same manner as in trial-1, except there were no pre-trial drug injections. In this way, possible carry-over drug effects on aversive learning during trial-1 were evaluated in the second elevated plus-maze trial.

The anxiety-related behavioural indices in the elevated plus-maze, used in the present study, have been extensively validated and identified in several principal components analyses with rats and mice (Cruz et al., 1994; Fernandes & File, 1996; Rodgers & Johnson, 1995; Trullas & Skolnick, 1993), as well as in a recent extensive confirmatory factor analysis of mouse behaviour in the elevated plus-maze conducted in our laboratory (Wall & Messier, 2000b). We found that a 2-factor model with 7 indicator variables was the most parsimonious model that could unambiguously explain unique underlying constructs of plus-maze behaviour in 2 trials seperated by a 24 h interval. Importantly, 4 anxiety/open exploration indices (i.e., including 2 traditional open arm time and entry measures, cumulative open arm entries and open arm time ratio, and 2 ethologically-derived risk assessment measures, unprotected stretch attends and unprotected head dips (Rodgers & Johnson, 1995) were shown to uniquely and robustly measure facets of animal anxiety related to unprotected exploration in our confirmatory factor analysis. In addition, cumulative closed arm entries, closed arm time ratio and closed arm vertical stretches were shown to uniquely and robustly measure facets of animal anxiety related to protected exploration (Fernandes & File, 1996). Accordingly, the same 7 indices were analyzed in the present study. Unprotected head dips were operationally-defined as peering over the edge of an open arm with head, neck and shoulders, while

out on an open arm runway (Cole & Rodgers, 1994). Unprotected stretch attends were operationally-defined as the animal stretching its whole body forward while in an open arm and retracting to its original position without moving its hind paws (Cole & Rodgers, 1994). Decreases in open entries, open time ratio, unprotected stretch attends and unprotected head dips were interpreted as anxiogenic indices of drug treatment relative to vehicle-treated animals, and decreases in closed entries, closed time ratio and closed vertical stretches were interpreted as reduced protected exploration activity.

2) *Y-maze spontaneous alternation memory test.* Each animal was challenged with one of a pre-trial vehicle, or drug dose/0.5 µl injection in the IL cortex. Spontaneous alternation performance was assessed by visually recording the spontaneous alternation behaviour of each mouse individually in the Y-maze in the same manner as previously described (Hiramatsu, Sasaki et al., 1997; Itoh et al., 1994; Sarter et al., 1988). A mouse was considered to have entered an arm when all 4 paws were positioned in the arm runway. Each mouse was allowed to freely explore the maze for 8 minutes. Alternations were operationally-defined as successive entries into each of the 3 arms on overlapping triplet sets, and % spontaneous alternation performance (%SAP) was defined as the ratio of actual (total alternations) to possible alternations (total arm entries - 2) X 100. Total entries (YTE) were also scored as an index of ambulatory activity in the Y-maze (Hiramatsu, Sasaki et al., 1997).

Histology

Mice were sacrificed with an overdose of sodium pentobarbital and perfused intracardially with 10cc 0.9% saline, followed by 10cc 10% formalin. Mice were decapitated, head caps removed, brains excised from the cranial cavity and stored in a 10% formalin/30% sucrose solution for at least one week before histological analysis. Mouse brains were individually blocked and frozen on a cryostat. Coronal sections (30 µm) were taken through the rostral-caudal extent of each cannula placement. Brain sections were mounted, stained with cresyl violet and examined under a

microscope. The distribution of cannulae tip placements in the IL cortex were mapped on schematic representations employing the mouse brain atlas of Franklin and Paxinos (Franklin & Paxinos, 1997). Only those subjects with correct cannulae tip placements in the IL cortex within the rostral-caudal range defined as +1.94 mm to +2.1 mm anterior to Bregma, the medial-lateral range defined as +.1 mm to +.7 mm lateral to the midsagital suture and the dorsal-ventral range defined as –2.5 mm to –3.25 mm ventral to a flat skull surface were included in the present analyses. The cannulae placements included in the Experiments 4a, 4b and 4c analyses are similar to the placements depicted in previous reports (Wall & Messier, 2000a, 2000c). Five mice from the vehicle, 4 from the 5 nmol, 4 from the 10 nmol and 4 from the 20 nmol groups were excluded due to placements outside this region in Experiment 4a. In Experiment 4b, 2 mice from the vehicle, 2 from the 0.25 nmol, 4 from the 1.25 nmol, 3 from the 2.5 nmol and 2 from the 10 nmol groups were excluded due to unacceptable placements. In Experiment 4c, 4 mice from the vehicle, 3 from the 2.5 nmol, 3 from the 5 nmol, 2 from the 10 nmol and 1 from the 20 nmol groups were also excluded.

Statistical Analyses

For each experiment, the elevated plus-maze data were analyzed by oneway ANOVA for trials-1 and -2 (α = .05). Post-hoc LSD tests were conducted (Bonferroni-corrected) for significant treatment effects relative to control means (Exp 4a, α = .017; 4b, α = .01; 4c, α = .013). Y-maze total entries and spontaneous working memory were also analyzed by oneway ANOVA (α = .05). Post hoc LSD tests were conducted as above (α Bonferroni-corrected).

2.5.4. Experiment 4a – Results:

Elevated plus-maze Anxiety Test Trial-1. The behavioural data along with associated ANOVA's and post-hoc LSD tests for trial-1 are summarized in Table 14. Figure 22 depicts results from both trials for comparison purposes.

PRETRIAL-1 0.5 µl SCOPOLAMINE / ELEVATED PLUS-MAZE TRIAL-1

Behav.	Vehicle	5 nmol	10 nmol	20 nmol	$F_{(3,35)}$	*P*
OE	7.11 ± 1.1	4.1 ± 0.9	4.6 ± 1.1	3.2 ± 1.0	2.56	NS
OTR	0.273 ± 0.04	0.174 ± 0.04	0.170 ± 0.04	0.105 ± 0.03 *	3.48	0.026
UHD	16.3 ± 3.6	7.7 ± 2.0 *	8.3 ± 1.9 *	5.2 ± 2.1 *	3.72	0.02
USA	16.1 ± 2.3	10.4 ± 2.5	10.2 ± 2.4	5.7 ± 1.8 *	3.45	0.027
CE	10.2 ± 0.7	11.7 ± 0.7	10.5 ± 1.7	14.5 ± 1.1 *	3.03	0.042
CTR	0.270 ± 0.02	0.364 ± 0.03	0.305 ± 0.06	0.347 ± 0.05	0.95	NS
CVS	14.7 ± 1.7	16.0 ± 2.3	12.1 ± 2.5	13.4 ± 2.3	0.58	NS

Table 14. Experiment 4a. Infralimbic scopolamine effects on elevated plus-maze behaviour. Values represent means +/- SEM. Time ratios represent the amount of time (sec) in a section ÷ 300 sec. OE, open arm entries; OTR, open time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), sig. different than vehicle ($p < .017$).

Elevated plus-maze Trial-2. Infralimbic pretrial-1 scopolamine infusion did not influence any parameters relative to vehicle mice in trial-2 (no injection) 24 h. later.

Place Figure 22 about here

Y-Maze Spontaneous Working Memory Test. Oneway between subjects ANOVA revealed that scopolamine treatment was without effect on total arm entries in the Y-maze ($F_{(3,35)} = 1.60$, NS). Percent spontaneous working memory was affected by scopolamine ($F_{(3,35)} = 5.14$, $p < .01$). Post-hoc LSD tests revealed that percent spontaneous alternations was reduced in the 20 nmol group ($p < 0.017$) relative to vehicle-treated control mice (Figure 23a).

2.5.5. Discussion

The behavioural results from Experiment 1 indicate that cholinergic transmission can influence anxiety-like behaviour and spontaneous working memory in the IL cortex in CD-1 mice. Scopolamine conceivably exerted these anxiogenic behavioural effects by enhancing ACh release through presynaptic M2 receptor blockade, as well as competitively blocking some of the post-synaptic M1 receptors to begin to disrupt spontaneous working memory in the Y-maze at the highest dose administered. The M1 receptor blocking effects of the lower doses of scopolamine were, however, likely not sufficient to overcome the enhanced availability of synaptic ACh to activate post-synaptic M1 receptors. Moreover, the 20 nmol dose of scopolamine increased closed arm entries, an effect which might render the open arm-related anxiogenic trial-1 data somewhat equivocal. Thus, although the observed anxiogenic effects of IL scopolamine on open arm activity may have been at least partially due to some non-specific muscarinic receptor blocking effects on closed arm entry activity, given the observed anxiogenic effect of lower doses of scopolamine on unprotected head dips, the present data do provide some support for the hypothesis that post-

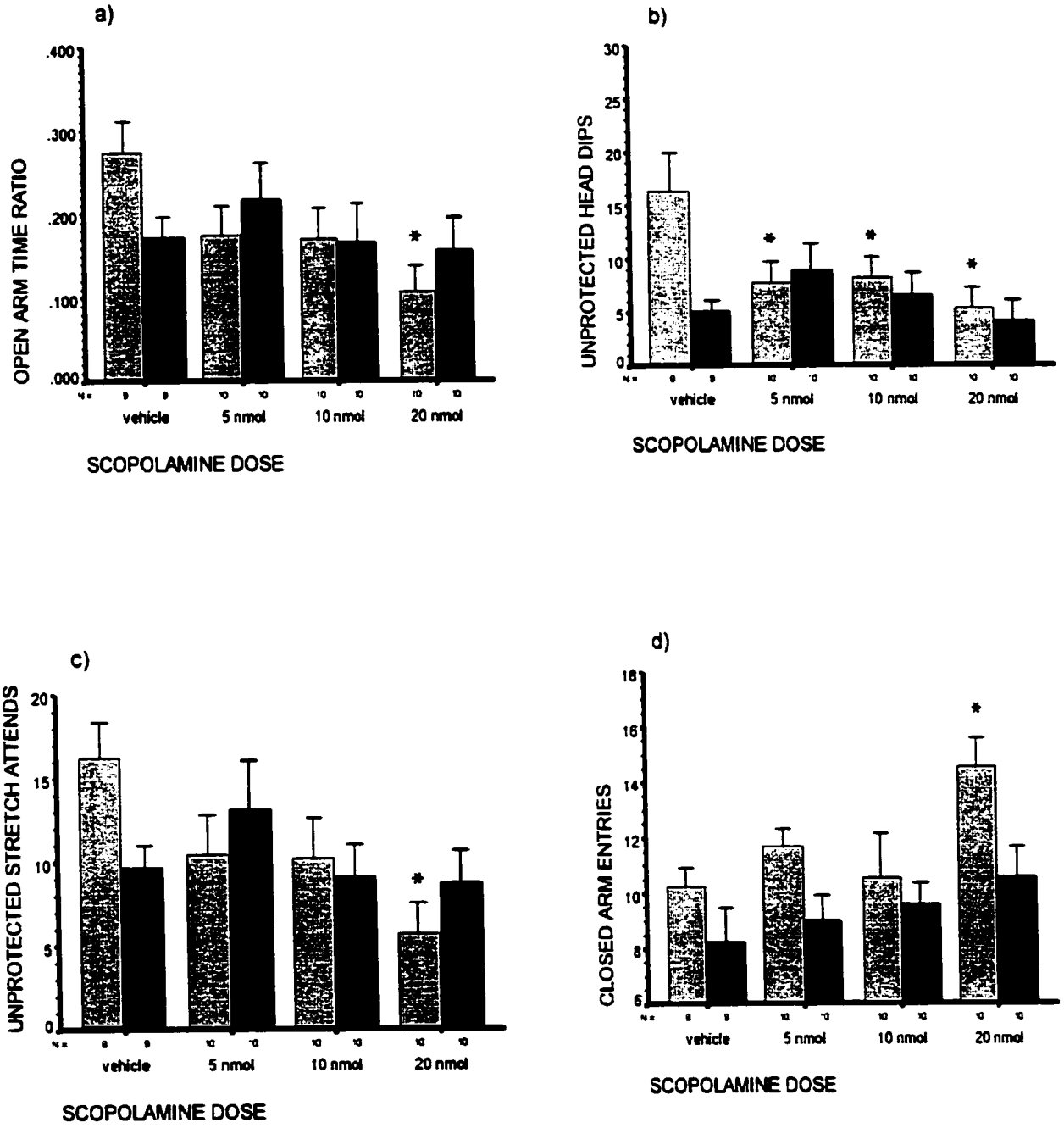
a)
OPEN ARM TIME RATIO
.400
.300
.200
.100
.000
vehicle
5 nmol
10 nmol
20 nmol
SCOPOLAMINE DOSE
b)
UNPROTECTED HEAD DIPS
30
25
20
15
10
5
0
vehicle
5 nmol
10 nmol
20 nmol
SCOPOLAMINE DOSE
c)
UNPROTECTED STRETCH ATTENDS
20
15
10
5
0
vehicle
5 nmol
10 nmol
20 nmol
SCOPOLAMINE DOSE
d)
CLOSED ARM ENTRIES
18
16
14
12
10
8
6
vehicle
5 nmol
10 nmol
20 nmol
SCOPOLAMINE DOSE

Figure 22

synaptic M1 receptor activation, through low-dose scopolamine-enhanced synaptic ACh availability in the vmPFC, is anxiogenic in the elevated plus-maze.

Experiment 4b evaluated the possibility that IL pirenzepine should therefore disrupt aversive learning thus reduce anxiety during trial-1 in the elevated plus-maze, along with a subsequent reduction in anxiety-like behaviour in trial-2 24 h later. Infralimbic pirenzepine should also disrupt spontaneous working memory in the Y-maze.

2.5.6. Experiment-4b – Results:

Elevated plus-maze Anxiety Test Trial-1. The behavioural data along with associated ANOVA's and post-hoc LSD tests for trial-1 are summarized in Table 15. Figure 24 depicts results from both trials for comparison purposes.

Place Table 15 about here

Elevated plus-maze Trial-2. The trial-2 (no injection) behavioural data, along with associated ANOVA's and post-hoc LSD tests are summarized in Table 16.

Place Table 16 about here

Place Figure 24 about here

Y-Maze Spontaneous Working Memory Test. Oneway between subjects ANOVA showed that IL pirenzepine was without effect on total entries in the Y-maze ($F_{(5,53)} = 1.421$, NS). Spontaneous working memory performance was significantly affected by IL pirenzepine infusion ($F_{(5,53)} = 4.028$,

PRETRIAL-1 0.5 µl PIRENZEPINE / ELEVATED PLUS-MAZE TRIAL-1

Behav.	Vehicle	0.25 nmol	1.25 nmol	2.5 nmol	5 nmol	10 nmol	$F_{(5,56)}$	*P*
OE	4.9 ± 1.0	2.8 ± 1.0	4.8 ± 1.3	7.8 ± 1.8	8.7 ± 1.2	10.5 ± 2.1 *	4.05	.003
OTR	0.19 ± 0.03	0.10 ± 0.03	0.17 ± 0.04	0.25 ± 0.05	0.28 ± 0.04	0.30 ± 0.04*	3.87	.005
UHD	9.3 ± 2.3	4.0 ± 1.3	6.6 ± 1.7	14.0 ± 4.5	13.1 ± 2.5	20.3 ± 4.1 *	3.85	.005
USA	6.8 ± 1.5	5.8 ± 1.9	6.5 ± 2.5	9.1 ± 1.8	5.8 ± 1.7 *	15.7 ± 2.5 *	4.08	.003
CE	9.4 ± 0.9	10.6 ± 1.3	10.0 ± 1.2	10.9 ± 1.4	12.3 ± 1.2	10.2 ± 1.0	0.76	NS
CTR	0.35 ± 0.06	0.36 ± 0.06	0.36 ± 0.06	0.24 ± 0.03	0.32 ± 0.03	0.27 ± 0.03	1.15	NS
CVS	14.0 ± 1.4	14.3 ± 1.6	15.5 ± 3.2	14.6 ± 2.7	16.0 ± 2.2	15.8 ± 2.4	0.15	NS

Table 15. Experiment 4b. Infralimbic pirenzepine effects on elevated plus-maze behaviour. Values represent means ± SEM. Time ratios represent the amount of time (sec) in a section/ 300 sec. OE, open arm entries; OTR, open arm time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), sig. different than vehicle ($p < .01$).

(NO INJECTION) ELEVATED PLUS-MAZE TRIAL-2

Behav.	Vehicle	0.25 nmol	1.25 nmol	2.5 nmol	5 nmol	10 nmol	$F_{(5,56)}$	*P*
OE	2.9 ± 0.7	5.9 ± 1.2 *	8.8 ± 1.4 *	7.0 ± 0.9	5.2 ± 1.0	8.4 ± 1.0 *	4.23	.003
OTR	0.09 ± 0.02	0.20 ± 0.03*	0.28 ± 0.04*	0.22 ± 0.03*	0.18 ± 0.04	0.24 ± 0.03*	3.58	.007
UHD	2.9 ± 1.1	8.9 ± 1.7	16.6 ± 4.2 *	11.1 ± 2.8 *	8.2 ± 2.8	12.7 ± 1.9 *	3.23	.013
USA	3.9 ± 0.8	8.8 ± 1.9 *	11.9 ± 1.6 *	8.4 ± 1.0	9.8 ± 2.2 *	11.1 ± 1.5 *	2.83	.024
CE	8.8 ± 1.3	9.9 ± 0.8	12.4 ± 1.6	10.1 ± 1.5	11.0 ± 1.0	11.3 ± 1.0	1.03	NS
CTR	0.38 ± 0.07	0.33 ± 0.03	0.37 ± 0.03	0.32 ± 0.05	0.39 ± 0.04	0.38 ± 0.03	0.47	NS
CVS	14.9 ± 3.1	17.4 ± 2.5	23.1 ± 3.2	14.9 ± 3.2	15.4 ± 1.9	22.0 ± 2.0	1.91	NS

Table 16. Experiment 4b. Pretrial-1 infralimbic pirenzepine carry-over effects on elevated plus-maze behaviour. Values represent means ± SEM. Time ratios represent the amount of time (sec) in a section/ 300 sec. OE, open arm entries; OTR, open arm time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), sig. different than vehicle ($p < .01$).

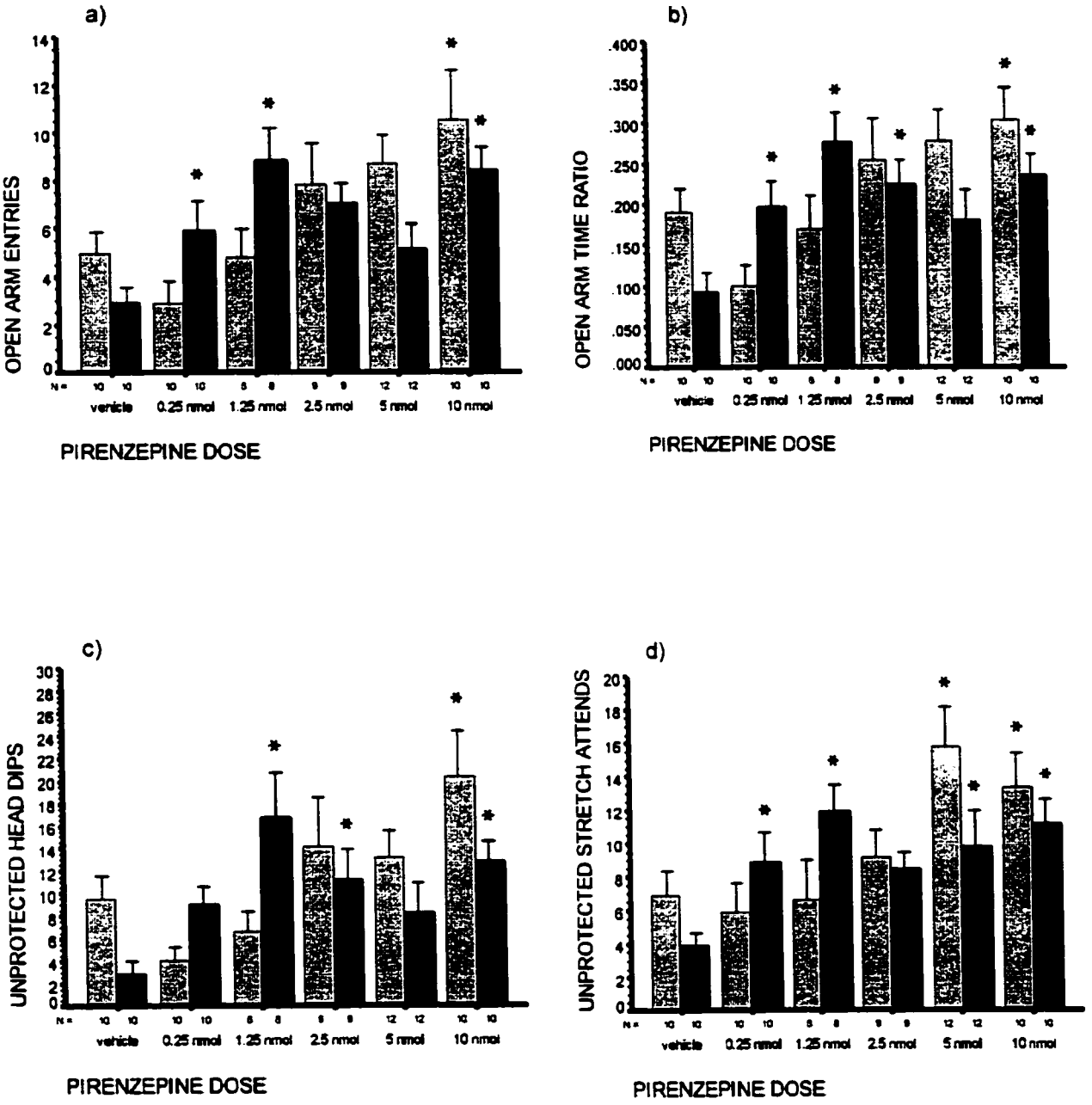
a)
OPEN ARM ENTRIES
vehicle
0.25 nmol
1.25 nmol
2.5 nmol
5 nmol
10 nmol
PIRENZEPINE DOSE
b)
OPEN ARM TIME RATIO
vehicle
0.25 nmol
1.25 nmol
2.5 nmol
5 nmol
10 nmol
PIRENZEPINE DOSE
c)
UNPROTECTED HEAD DIPS
vehicle
0.25 nmol
1.25 nmol
2.5 nmol
5 nmol
10 nmol
PIRENZEPINE DOSE
d)
UNPROTECTED STRETCH ATTENDS
vehicle
0.25 nmol
1.25 nmol
2.5 nmol
5 nmol
10 nmol
PIRENZEPINE DOSE

Figure 24

$p < 0.01$). Post-hoc LSD tests revealed that percent spontaneous alternations was reduced in the 2.5, 5 and 10 nmol drug-dose groups ($p < 0.01$), relative to vehicle-treated animals (Figure 23b).

Place Figure 23 about here

2.5.7. Discussion

The behavioural data from Experiment 4b lend support to the hypothesis that enhanced cholinergic neurotransmission in the IL cortex mediates anxiety induction and spontaneous working memory performance in challenging environments through post-synaptic M1 receptor activation. Experiment 4c was designed to directly test the hypothesis that McN-A-343 microinjection in the IL cortex should enhance anxiety-like behaviour in the elevated plus-maze and improve spontaneous working memory in the Y-maze.

2.5.8. Experiment 4c – Results

Elevated plus-maze Anxiety Test Trial-1. The behavioural data along with ANOVA's and post-hoc LSD tests for trial-1 are summarized in Table 17. Figure 25 depicts results from both trials for comparison purposes.

Place Table 17 about here

Elevated plus-maze Anxiety Test Trial-2. The trial-2 (no injection) behavioural data, along with associated ANOVA's and post-hoc LSD tests are summarized in Table 18.

Place Table 18 about here

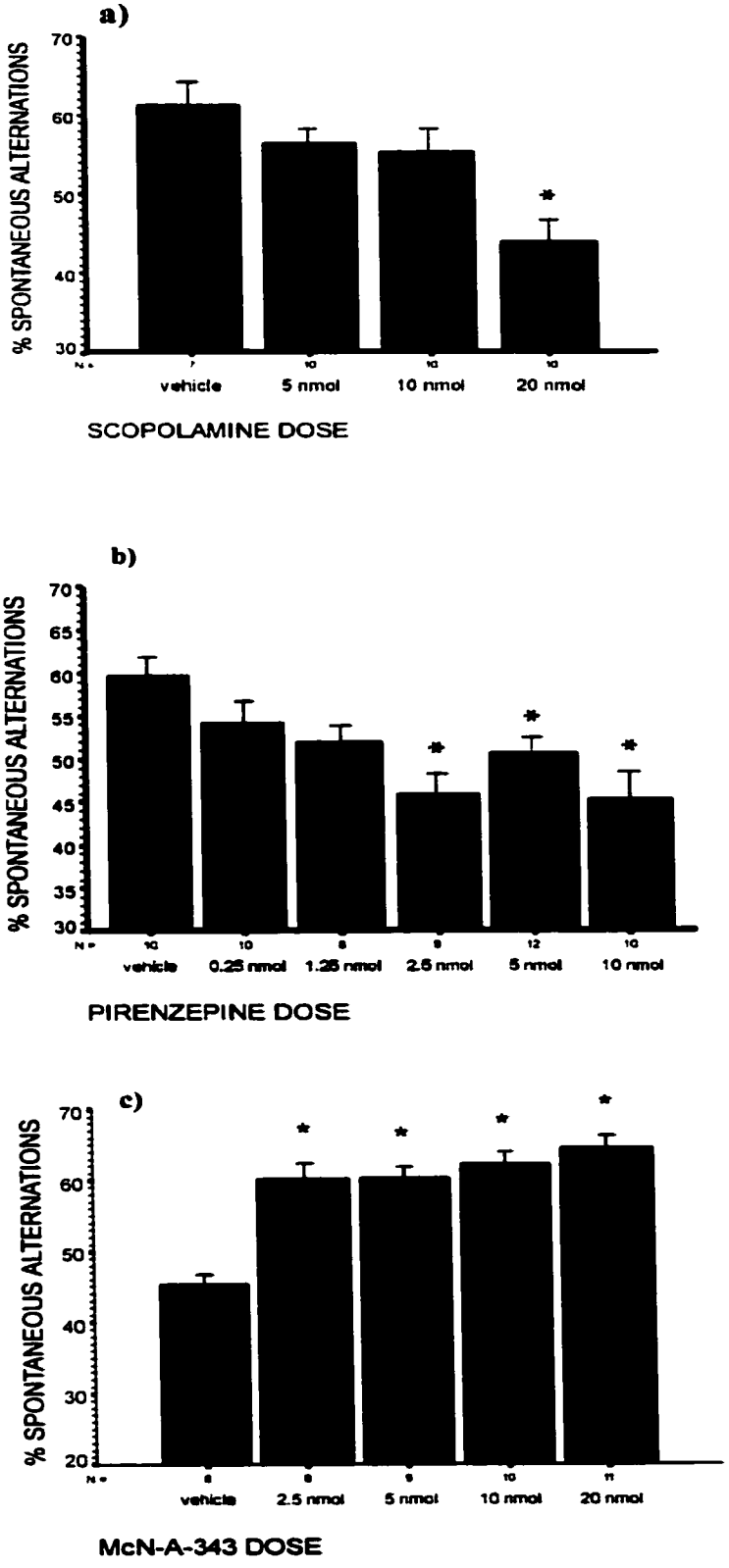
a)
% SPONTANEOUS ALTERNATIONS
70
60
50
40
30
vehicle
5 nmol
10 nmol
20 nmol
SCOPOLAMINE DOSE
b)
% SPONTANEOUS ALTERNATIONS
70
65
60
55
50
45
40
35
30
vehicle
0.25 nmol
1.25 nmol
2.5 nmol
5 nmol
10 nmol
PIRENZEPINE DOSE
c)
% SPONTANEOUS ALTERNATIONS
70
60
50
40
30
20
vehicle
2.5 nmol
5 nmol
10 nmol
20 nmol
McN-A-343 DOSE

Figure 23

PRETRIAL-1 0.5 µl McN-A-343/ ELEVATED PLUS-MAZE TRIAL-1

Behav.	Vehicle	2.5 nmol	5 nmol	10 nmol	20 nmol	$F_{(4,41)}$	*P*
OE	8.5 ± 2.3	3.4 ± 0.8 *	2.3 ± 0.6 *	3.4 ± 0.8 *	3.2 ± 0.9 *	4.21	.006
OTR	0.29 ± 0.06	0.14 ± 0.04*	0.09 ± 0.02*	0.13 ± 0.03*	0.10 ± 0.03*	4.48	.004
UHD	15.8 ± 5.1	4.1 ± 1.7 *	3.1 ± 1.1 *	3.2 ± 0.7 *	4.5 ± 1.3 *	5.15	.002
USA	7.8 ± 1.3	5.1 ± 1.8	4.2 ± 1.4	4.3 ± 1.1	3.7 ± 0.8	1.52	NS
CE	10.1 ± 0.8	8.6 ± 1.2	9.4 ± 1.4	12.0 ± 1.0	12.1 ± 1.2	1.84	NS
CTR	0.30 ± 0.04	0.29 ± 0.05	0.33 ± 0.05	0.30 ± 0.04	0.34 ± 0.04	0.27	NS
CVS	15.5 ± 2.3	11.1 ± 2.2	8.9 ± 1.6	16.1 ± 2.4	15.7 ± 2.8	1.85	NS

Table 17. Experiment 4c. Pretrial infralimbic McN-A-343 effects on elevated plus-maze behavior. Values represent means ± SEM. Time ratios represent the amount of time (sec) in a section ÷ 300 sec. OE, open arm entries; OTR, open arm time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), significantly different than vehicle ($p < .013$).

(NO INJECTION) ELEVATED PLUS-MAZE TRIAL-2

Behav.	Vehicle	2.5 nmol	5 nmol	10 nmol	20 nmol	$F_{(4,41)}$	*P*
OE	8.4 ± 2.3	3.4 ± 0.9 *	2.7 ± 0.5 *	3.7 ± 0.7 *	3.7 ± 0.7 *	3.82	.01
OTR	0.28 ± 0.07	0.11 ± 0.03*	0.09 ± 0.03*	0.12 ± 0.03*	0.12 ± 0.02*	3.68	.012
UHD	13.5 ± 5.0	2.4 ± 1.2 *	1.2 ± 0.6 *	1.5 ± 0.5 *	2.7 ± 0.8 *	5.76	.001
USA	7.3 ± 1.6	3.0 ± 1.0 *	2.8 ± 0.9 *	3.4 ± 0.6 *	3.7 ± 0.7 *	3.31	.019
CE	10.6 ± 2.0	9.6 ± 1.5	10.8 ± 0.9	11.9 ± 0.9	12.3 ± 0.8	0.80	NS
CTR	0.37 ± 0.07	0.36 ± 0.04	0.37 ± 0.01	0.36 ± 0.03	0.42 ± 0.04	0.50	NS
CVS	16.9 ± 2.8	18.9 ± 3.6	14.8 ± 2.5	18.5 ± 2.2	19.8 ± 3.5	0.45	NS

Table 18. Experiment 4c. Pretrial-1 infralimbic McN-A-343 carry-over effects on elevated plus-maze behavior. Values represent means ± SEM. Time ratios represent the amount of time (sec) in a section ÷ 300 sec. OE, open arm entries; OTR, open arm time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), significantly different than vehicle ($p < .013$).

Place Figure 25 about here

Y-Maze Spontaneous Working Memory Test. Oneway between subjects ANOVA showed that IL McN-A-343 was without effect on total entries in the Y-maze ($F_{(4,41)} = 0.17$, NS). Spontaneous working memory performance was affected by IL McN-A-343 infusion ($F_{(4,41)} = 11.32$, $p < 0.001$). Post-hoc LSD tests revealed that percent spontaneous alternations was enhanced in the 2.5, 5, 10 and 20 nmol drug-dose groups ($p < 0.01$), relative to vehicle-treated animals (Figure 23c).

2.5.9. General Discussion

The behavioural results from the present experiments support the involvement of post-synaptic M1 receptors in the infralimbic (IL) vmPFC in the regulation of spontaneous working memory and anxiety induction in CD-1 mice. Scopolamine infusion in the IL cortex was anxiogenic in the elevated plus-maze in trial-1 and disrupted working memory in the Y-maze. Infralimbic pirenzepine was anxiolytic in trials-1 and -2 in the elevated plus-maze, and also disrupted spontaneous working memory in the Y-maze. Infralimbic McN-A-343 was anxiogenic in trials-1 and -2 in the elevated plus-maze, and enhanced spontaneous working memory in the Y-maze. These data lend support to the hypotheses that a) enhanced basal forebrain – mPFC cholinergic activity may underly the cognitive/behavioural symptoms of anxiety (Sarter & Bruno, 1999; Sarter et al., 1999) and b) post-synaptic M1 receptors modulate working memory, aversive learning and anxiety-like behaviour.

It is interesting that only the highest doses of vmPFC scopolamine produced salient working memory deficits in rats in previously-reported studies (Broersen, Heinsbroek, de Bruin, Uylings et al., 1995; Granon et al., 1995; Herremans et al., 1996), as well as in Experiment 1. Thus, although

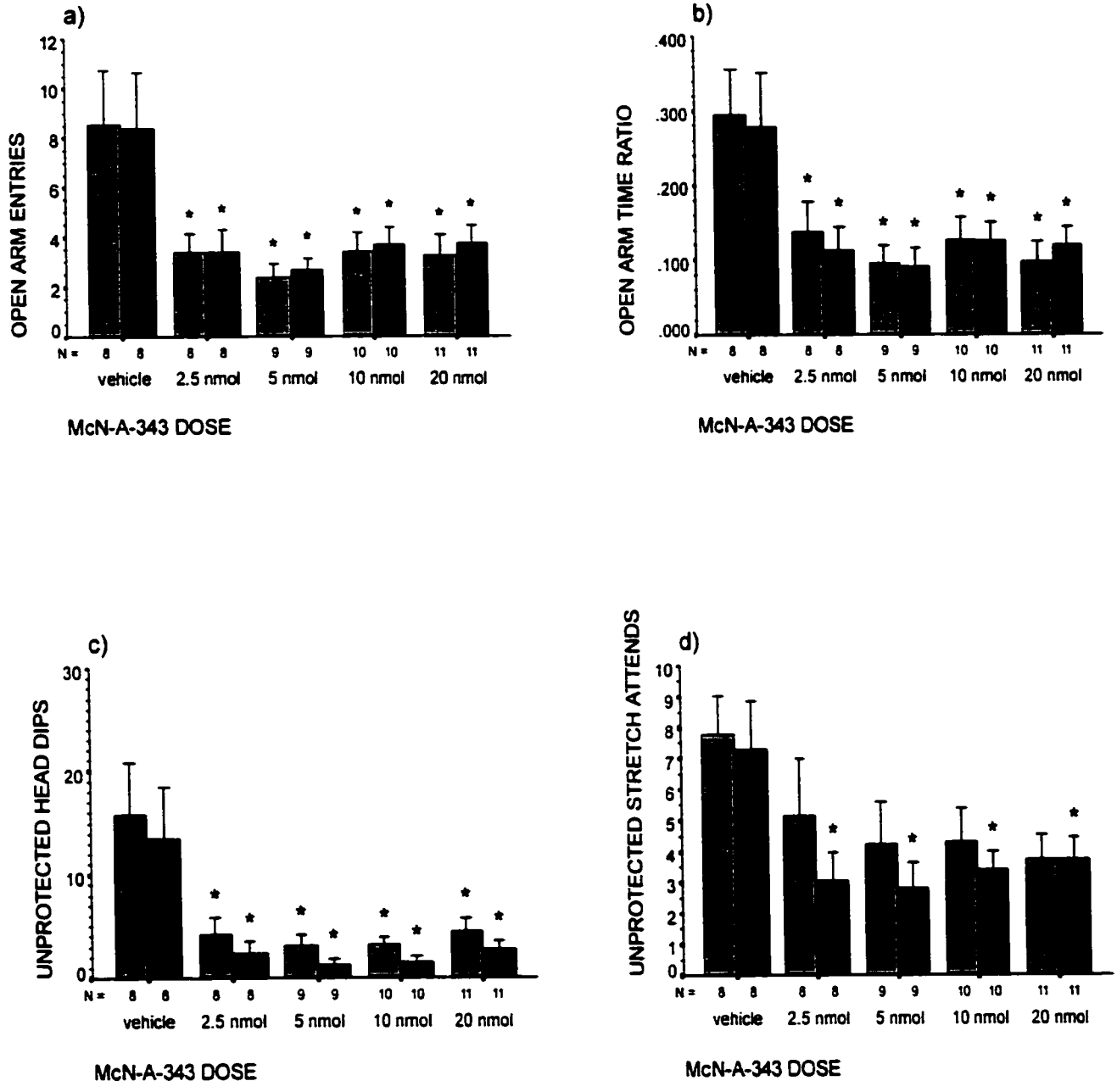
a)
OPEN ARM ENTRIES
12
10
8
6
4
2
0
N = 8 8 8 8 9 9 10 10 11 11
vehicle 2.5 nmol 5 nmol 10 nmol 20 nmol
McN-A-343 DOSE
b)
OPEN ARM TIME RATIO
.400
.300
.200
.100
.000
N = 8 8 8 8 9 9 10 10 11 11
vehicle 2.5 nmol 5 nmol 10 nmol 20 nmol
McN-A-343 DOSE
c)
UNPROTECTED HEAD DIPS
30
20
10
0
N = 8 8 8 8 9 9 10 10 11 11
vehicle 2.5 nmol 5 nmol 10 nmol 20 nmol
McN-A-343 DOSE
d)
UNPROTECTED STRETCH ATTENDS
10
9
8
7
6
5
4
3
2
1
0
N = 8 8 8 8 9 9 10 10 11 11
vehicle 2.5 nmol 5 nmol 10 nmol 20 nmol
McN-A-343 DOSE

Figure 25

the 20 nmol scopolamine dose disrupted spontaneous working memory in the Y-maze, memory for the aversiveness of the elevated plus-maze did not appear to be significantly affected relative to control mice in the second no-injection trial 24 h later. These results were surprising given the previously reported robust carry-over anxiogenic effects of pretrial-1 norBNI infusion in the IL cortex in the second no-injection elevated plus-maze trial (Wall & Messier, 2000a). Moreover, it is unlikely that the drop in the vehicle group trial-2 activity levels were due to a habituation effect, rather, it is likely that these animals learned a type of phobic avoidance during trial-1 (File et al., 1998; File et al., 2000). Indeed, in our confirmatory factor analysis study of naïve CD-1 mouse behaviour in the elevated plus maze (N = 200), open entries, open time ratio, unprotected head dips and unprotected stretch attends were reduced by approximately 50% in trial-2 (Wall & Messier, 2000b). In contrast to these between trial behavioural response differences, indicating some form of learning during trial-1 (Espejo, 1997a; File et al., 1998; File, Gonzalez, & Gallant, 1999; File et al., 2000; Holmes & Rodgers, 1998; Kenny, Cheeta, & File, 2000; Ouagazzal, Kenny, & File, 1999), there were some noticeable increases between the trial-1 (drug injection) and trial-2 (no drug injection) unprotected exploration measures in Experiment 4a. Whereas the vehicle group showed lower trial-2 unprotected exploration activity levels, the 5 and 20 nmol groups actually showed some higher trial-2 unprotected exploration activity levels (Figure 22). This was not the case in our norBNI study. Thus in Experiment 4a, although pretrial-1 scopolamine injections failed to significantly influence trial-2 unprotected behaviours relative to (trial-2) vehicle controls, it could be suggested that the 20 nmol dose was sufficient to marginally increase unprotected exploration activity in trial-2 (relative to trial-1 20 nmol levels). It would have been interesting to have evaluated whether higher scopolamine doses might have further influenced some aspects of aversive learning during trial-1, as the post-synaptic competitive M1 antagonist activity would begin to out-weigh the increased availability of synaptic ACh by the antagonistic actions on pre-

synaptic M2 autoreceptors (Messier, Wall et al., 1999). Conceivably, low-dose IL scopolamine competitively blocks presynaptic M2 receptors first, then higher doses begin to competitively block post-synaptic M1 receptors.

In contrast to these apparent anxiogenic effects of trial-1 IL scopolamine microinjections, pretrial-1 IL pirenzepine infusions not only exerted anxiolytic effects on all 4 anxiety/unprotected exploration indices in trial-1, but also produced a robust anxiolytic profile at the full dose range in the no-injection second trial 24 h later in Experiment 4b. Such carry-over anxiolytic effects of pretrial-1 IL pirenzepine resembled the previously reported anxiolytic carry-over effects of pretrial-1 IL U-69,593 infusions (Wall & Messier, 2000c). Pirenzepine also dose-dependently disrupted spontaneous working memory in the three highest dose groups in Experiment 4b. These data contrast the effects of kappa1 agonist infusion in the IL cortex on Y-maze performance. Whereas IL U-69,593 injections reduced anxiety and *enhanced* spontaneous working memory (Wall & Messier, 2000c), IL pirenzepine injections reduced anxiety and *disrupted* spontaneous working memory. Moreover, in Experiment 4c, IL McN-A-343 was anxiogenic in trial-1, enhanced aversive learning during trial-1 to produce subsequent anxiogenic effects in trial-2 in the elevated plus-maze, and enhanced spontaneous working memory performance in the Y-maze. Thus, whereas IL norBNI increased anxiety and *disrupted* spontaneous working memory in our previous study (Wall & Messier, 2000a), IL McN-A-343 increased anxiety and *enhanced* spontaneous working memory in Experiment 4c. Conceivably, the kappa1 and M1 receptor systems in the IL cortex differentially influence working memory related to spontaneous exploration. Of interest to the present investigation, however, is the fact that both receptor systems markedly influenced both cognitive/behavioural processes.

In a similar fashion to the Experiment 4b Y-maze results, pirenzepine infusions in the dorsal hippocampus impaired working memory in rats (Ohno, Yamamoto et al., 1994). In contrast to the

Experiments 4b and 4c results however, pirenzepine was anxiogenic and McN-A-343 was anxiolytic in the elevated plus-maze in rats following microinfusion in the dorsal hippocampus (File et al., 1998; File et al., 2000). Whereas File et al. (2000) suggested that enhanced ACh transmission in the dorsal hippocampus exerts some anxiolytic influences, results from the present investigation render the suggestion that enhanced ACh transmission in the vmPFC exerts anxiogenic effects, in the elevated plus-maze. These contrasting effects of M1 drugs in the dorsal hippocampus versus the vmPFC suggest interactivity between the two brain sites in the regulation of anxiety-like behaviour. Investigations into possible interactivity between the hippocampus and vmPFC in the concurrent modulation of working memory and anxiety is warranted.

In conclusion, results from the present study indicate that enhanced ACh transmission in the IL cortex may be important in the expression of anxiety-like behaviour. These data provide some support for an overactive basal forebrain-mPFC cholinergic theory of anxiety (Hart et al., 1999; Sarter & Bruno, 1999). Conceivably, the immediate learning and memory of the aversiveness of a situation and working memory may be partially modulated by the same post-synaptic ACh M1 receptor mechanisms in the IL cortex. Given the observed differences between IL M1 and kappa1 drug effects on anxiety and memory in the present and previous studies, investigations into possible interactivity between IL kappa1 and M1 receptor sytems in the concurrent regulation of anxiety-like behaviour and spontaneous working memory processing are also warranted.

2.6. EXPERIMENT 5

Wall, P. M. & Messier, C. (2001). Ventromedial prefrontal kappa and muscarinic M1 interactions in the concurrent modulation of anxiety and memory. *Psychopharmacology*. (Submitted).

2.6.1. Abstract

Spontaneous working memory and anxiety-like behaviour can be concurrently influenced following kappa opioid or muscarinic M1 antagonist infusions in the infralimbic (IL) area of the ventromedial prefrontal cortex (vmPFC) in CD-1 mice. The present study sought to evaluate (a) whether our previously reported anxiogenic and amnestic effects of norBNI and anxiolytic and amnestic effects of pirenzepine data could be replicated, and (b) whether IL kappa and M1 receptor mechanisms interactively influence these cognitive-behavioural processes. Anxiety was evaluated in the elevated plus-maze and spontaneous working memory was evaluated in the Y-maze following pirenzepine (PIR), norBNI (BNI) or 2 levels of BNI/PIR drug mix infusions in the IL cortex. Pretreatment with the M1 antagonist, PIR, was anxiolytic in trial-1 (10 nmol), as well as in trial-2 (no-injection) in the elevated plus-maze 24 h later. PIR also disrupted active working memory in the Y-maze. Pretreatment with the kappa antagonist, BNI, was anxiogenic in trial-1 (10 nmol), as well as in trial-2 (no-injection) in the elevated plus-maze 24 h later. BNI also disrupted spontaneous working memory in the Y-maze. The BNI-10 nmol/PIR-10 nmol mix was somewhat anxiogenic in trial-1 but had no carry-over effects in trial-2 in the elevated plus-maze. The BNI-10 nmol/PIR-10 nmol drug mix was disruptive in the Y-maze. The BNI-5 nmol/PIR-10 nmol drug mix had no effect on trials 1 or 2 anxiety measures in the elevated plus-maze in week-1, yet also disrupted Y-maze performance. 1) The effects of IL infusions of BNI or PIR on elevated plus-maze behaviour appear robust. 2) M1 and kappa receptors counteract each others' effects on anxiety-related behaviour in the elevated plus-maze. 3) Simultaneous M1 and kappa receptor blockade synergistically produces profound spontaneous working memory deficits.

2.6.2. Introduction

There are growing data demonstrating that the ventromedial PFC is involved in working memory processing in mice (Wall et al., 2001; Wall & Messier, 2000a, 2000c) and rats (Fritts et al., 1998; Jung et al., 1998; Poucet, 1997; Ragozzino & Kesner, 1998). The ventral prelimbic (PL) and IL cortices have also been shown to be involved in anxiety regulation in mice (Wall et al., 2001; Wall & Messier, 2000a, 2000c) and rats (Duncan et al., 1996; Goldstein et al., 1996; Morgan & LeDoux, 1995; Morgan et al., 1993; Petty et al., 1997). It is now becoming clear that the ventromedial PFC concurrently regulates spontaneous working memory and anxiety-related behaviour.

We have previously shown that spontaneous working memory and anxiety-like behaviour can be concurrently regulated in the IL cortex. Whereas the selective κappa$_1$ receptor agonist, U-69593, enhanced spontaneous working memory in the Y-maze and was anxiolytic in trials 1 and 2 in the elevated plus-maze (Wall & Messier, 2000c), the κappa receptor antagonist, norBNI (BNI), disrupted spontaneous working memory in the Y-maze and was anxiogenic in trials 1 and 2 in the elevated plus-maze (Wall & Messier, 2000a). In addition to the observed kappa receptor drug effects in the IL cortex, the selective muscarinic M1 agonist, McN-A-343, was anxiogenic in trials 1 and 2 in the elevated plus-maze and enhanced spontaneous working memory in the y-maze, and the selective M1 antagonist, pirenzepine (PIR), was anxiolytic in trials 1 and 2 in the elevated plus-maze and disrupted Y-maze performance (Wall et al., 2001). The fact that PIR and BNI exerted opposite effects on anxiety in the elevated plus-maze and similar disruptive effects on spontaneous working memory in the Y-maze was intrigueing. These data suggested to us that both receptor mechanisms may interact in the IL cortex in the concurrent regulation of anxiety and memory.

It is noteworthy that kappa receptors have been shown to interact with muscarinic receptor systems in learning and memory (Hiramatsu, Murasawa, Mori et al., 1998), thus kappa drug

infusions in the IL cortex may well have directly or indirectly interfered with cholinergic mechanisms to produce the observed effects on anxiety and memory in our previous studies. Such kappa–cholinergic interactivity may have impacted the animal's attentional processes related to the immediate orientation to, perception of, memory for and immediate behavioural responses in potentially dangerous situations.

The basal forebrain nucleus basalis cholinergic system has been implicated in attentional (Hasselmo, 1995; Muir et al., 1992; Robbins, Everitt, Ryan et al., 1989), memory (Beninger, Ingles, Mackenzie, Jhamandas, & Boegman, 1992; DeSousa, Beninger, Jhamandas, & Boegman, 1994) and anxiety induction processes (Belotti & Galey, 1996; Berntson et al., 1998). Moreover, some investigators have suggested that an overactive cholinergic system in the mPFC in rats serves to bias the processing of fear and anxiety-related information (Berntson et al., 1998; Hart et al., 1999; Sarter & Bruno, 1999). Sarter and Bruno (1999) also suggested that disrupted cholinergic terminal activity in the PFC serves to disrupt some attentional mechanisms such that the ability to detect, select, discriminate and process important stimuli is compromised resulting in an inability to filter out irrelevant stimuli, attentional and memory dysfunction. Conceivably, basal forebrain-vmPFC cholinergic terminals may normally serve to carry anxiety-related information in potentially dangerous situations such as exposure to the elevated plus-maze (Wall et al., 2001), as well as serve to activate attentional mechanisms related to spontaneous exploration activity.

The observed anxiogenic effects of IL infusions of the M1 agonist, McN-A-343 in our previous study (Wall et al., 2001) supports the notion that activated medial PFC cholinergic terminals produce anxiogenic effects (Berntson et al., 1998; Hart et al., 1999; Sarter & Bruno, 1999). Moreover, given the observation that spontaneous memory was improved in the Y-maze following IL M1 activation, the notion that cholinergic terminals in the medial PFC might modulate attentional mechanisms related to memory processes was also supported. Infralimbic PIR not only,

in our view, truncated anxiety-provoking information carried by activated cholinergic terminal activity during trial 1 in the elevated plus-maze, but also disrupted experience-dependent aversive learning in trial-1 such that the usual increase in anxiety-related behaviour during trial-2 did not occur.

Given the growing data in support of the idea that cholinergic terminals in the medial PFC are crucial for attentional mechanisms that may very well determine the formation of memory and on-going retrieval of information (Sarter & Bruno, 1999), it is possible that the previously observed working memory deficits following PIR infusion in the IL cortex may in fact have encompassed attentional deficits as well. We hypothesize that spontaneous exploration activity places attentional monitoring demands on cholinergic neurotransmission in the ventromedial PFC. This attentional monitoring activity is crucial to the unfolding and smooth operation of exploratory behavioural set. Conceivably, PIR infusion in the IL cortex truncated aversive learning in the elevated plus-maze and disrupted attentional monitoring mechanisms in the Y-maze (Wall et al., 2001). Moreover, BNI enhanced aversive learning in the elevated plus-maze but also may have disrupted attentional monitoring mechanisms related to spontaneous exploration in the Y-maze (Wall & Messier, 2000a). These data render the possibility that simultaneous BNI-PIR drug infusions in the IL cortex may very well severely disrupt attentional mechanisms related to spontaneous working memory.

In the present study, we primarily sought to evaluate whether concomitant kappa opioid and M1 receptor blockade in the IL cortex would exert interactive effects in the concurrent regulation of anxiety and -working memory in CD-1 mice.

2.6.3. Materials and Methods

Subjects

Subjects were 60 male CD-1 naive mice, obtained from Charles River, St. Constant, Quebec. All mice were initially housed in polypropylene cages (groups of 10 per cage) in a temperature

controlled room (21 ± 1° C), with ad lib access to food and water. The mice were acclimatized for 6 to 8 weeks until they reached a weight range of 32-45 grams. All mice were maintained on a 12 h light/dark cycle (lights on at 07:00 h).

Surgery

The surgical procedures used in Experiment 5 were conducted in the same manner as described in Experiment 2 and in (Messier, Emond et al., 1999).

Drugs

NorBNI and PIR were obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in artificial CSF each to a concentration of 10 nmol/0.5 µl. There were also two drug combinations: 1) BNI-10 nmol/PIR-10 nmol mix/0.5 µl, and 2) BNI-5 nmol/PIR-10 nmol mix/ 0.5 µl. Artificial CSF was the vehicle.

Intracerebral Drug Administration

Drug infusion procedures were conducted in the same manner as described in Experiment 4.

Apparati

The elevated plus-maze and Y-maze used in Experiment 5 were the same as described in Experiment 2.

Behavioural testing procedures

For each experiment, half of the animals from each group were tested in the first elevated plus-maze trial after a single injection followed by a no-injection trial 24 h later in week-1, then tested in the Y-maze after a second microinjection, in week-2. The remaining animals were tested in reverse order (Y-maze in week-1 and elevated plus-maze trials 1 and 2 in week-2).

1) Elevated plus-maze anxiety test. The elevated plus-maze procedures were conducted in the same manner as described in Experiment 4.

2) Y-maze spontaneous alternation memory tests. Each animal was challenged with one of a pre-trial vehicle, or drug dose/0.5 µl injection in the IL cortex. Spontaneous alternation performance was assessed by visually recording the spontaneous alternation behaviour of each mouse individually in the Y-maze in the same manner as previously described (Hiramatsu, Sasaki et al., 1997; Itoh et al., 1994; Sarter et al., 1988). A mouse was considered to have entered an arm when all 4 paws were positioned in the arm runway. Each mouse was allowed to freely explore the maze for 8 minutes. Alternations were operationally-defined as successive entries into each of the 3 arms on overlapping triplet sets, and % spontaneous alternation performance (%SAP) was defined as the ratio of actual (total alternations) to possible alternations (total arm entries - 2) X 100. Total entries (YTE) were also scored as an index of ambulatory activity (Hiramatsu, Sasaki et al., 1997).

In an effort to measure attentional mechanisms within spontaneous working memory, we scored cumulative arm returns for each animal. That is, if an animal went, for example, from arm A to arm B and back to arm A, 1 arm return was recorded. In addition, because some animals entered far fewer arms than others, we reasoned that a cumulative arm return score would be biased. Thus, cumulative arm returns were expressed as a ratio of cumulative arm returns ÷ total arm entries x 100. It should be mentioned that 'same' arm returns were not included in this measure. Because the cumulative arm return ratio is a novel score, we thought that same arm returns would contaminate the measure. We did however score same arm returns separately also as a ratio over total arm entries x 100. We reasoned that this measure might further tap into the degree of attentional deficits the animal might be experiencing. The cumulative arm return and same arm return ratios may together reflect some degree of attentional difficulties the animals are experiencing in the Y-maze.

Histology

Mice were sacrificed with an overdose of sodium pentobarbital and perfused intracardially with 10cc 0.9% saline, followed by 10cc 10% formalin. Mice were decapitated, head caps removed,

brains excised from the cranial cavity and stored in a 10% formalin/30% sucrose solution for at least one week before histological analysis. Mouse brains were individually blocked and frozen on a cryostat. Coronal sections (30 μm) were taken through the rostral-caudal extent of each cannula placement. Brain sections were mounted, stained with cresyl violet and examined under a microscope. The distribution of cannulae tip placements in the IL cortex were mapped on schematic representations employing the mouse brain atlas of Franklin and Paxinos (Franklin & Paxinos, 1997). Only those subjects with correct cannulae tip placements in the IL cortex within the rostral-caudal range defined as +1.94 mm to +2.1 mm anterior to Bregma, the medial-lateral range defined as +.1 mm to +.7 mm lateral to the midsagital suture and the dorsal-ventral range defined as –2.5 mm to –3.25 mm ventral to a flat skull surface were included in the present analyses. The included cannulae placements are similar to the placements depicted in previous reports (Wall & Messier, 2000a, 2000c). Two mice from the vehicle, 3 from the PIR-10 nmol, 2 from the BNI-10 nmol, and 2 from the BNI-10/PIR-10 nmol mix groups were excluded due to placements outside this region.

Statistical Analyses

The behavioural data from the elevated plus-maze were analyzed by oneway analysis of variance (ANOVA) for trials-1 and -2 ($\alpha = .05$). Post-hoc LSD tests (Bonferroni-corrected) were conducted for significant treatment effects relative to control means ($\alpha = .05/4 = .013$). Y-maze total entries, spontaneous working memory and the 'attentional' scores were also analyzed by oneway ANOVA ($\alpha = .05$). Post hoc LSD tests were conducted as above ($\alpha = .05/4 = .013$).

2.6.4. Results

Elevated plus-maze Anxiety Test. The behavioural data along with associated ANOVA's and post-hoc LSD tests for trials-1 and -2 are summarized in Tables 19 and 20, respectively. Figures 26 and 27 depict results from both elevated plus-maze trials for comparison purposes.

Place Tables 19 and 20 about here

Place Figures 26 and 27 about here

Y-Maze Spontaneous Working Memory Test and 'attentional' scores.

One-way between-subjects ANOVA showed that no drug treatment influenced Y-maze total arm entries ($F(4,46) = 0.4$, NS). Drug treatment did, however, influence spontaneous working memory in the Y-maze ($F(4,46) = 18.6$, $P<0.001$). Post hoc LSD tests revealed that spontaneous working memory was disturbed by all 4 drug treatment levels ($P<0.013$) relative to vehicle treated control mice (Figure 28a).

Place Figure 28 about here

Drug treatment also influenced the 'attentional' scores, % Cumulative arm returns ($F(4,46) = 8.3$, $P<0.001$), % same arm returns ($F(4,46) = 2.9$, $P<0.05$). Post hoc LSD tests revealed that all 4 drug treatments increased % cumulative arm returns ($P<0.05$) relative to control mice, and the BNI-10/PIR-10 infusions increased % same arm returns ($P<0.05$) relative to vehicle treated mice (Figure 28). These latter data lend some support to the notion that the BNI-10/PIR-10 mix treated animals not only experienced spontaneous working memory problems, but also may well have been experiencing attentional difficulties related to orderly spontaneous exploration activity.

PRETRIAL-1 0.5 µl MIX DRUG INJECTION / ELEVATED PLUS-MAZE TRIAL-1

Behav.	Vehicle	PIR 10 nmol	BNI 10 nmol	BNI 10/PIR 10	BNI 5/PIR 10	$F_{(4,46)}$	*P*
OE	4.4 ± 1.0	5.7 ± 0.8	1.7 ± 0.4*	1.8 ± 0.4*	3.3 ± 0.6	5.8	0.001
OTR	0.149 ± 0.031	0.243 ± 0.029*	0.063 ± 0.013*	0.076 ± 0.021*	0.111 ± 0.021	8.7	0.000
UHD	5.7 ± 1.0	8.4 ± 1.5	0.8 ± 0.3*	2.4 ± 0.8*	3.2 ± 0.9	9.0	0.000
USA	7.3 ± 1.3	11.0 ± 1.8*	3.2 ± 0.8*	3.8 ± 1.1	6.1 ± 1.1	5.9	0.001
CE	11.5 ± 1.5	11.9 ± 1.5	12.9 ± 1.3	12.5 ± 1.2	12.7 ± 1.0	0.2	NS
CTR	0.389 ± 0.036	0.422 ± 0.037	0.396 ± 0.043	0.405 ± 0.046	0.412 ± 0.023	0.1	NS
CVS	16.8 ± 2.1	22.9 ± 3.8	18.5 ± 2.6	17.0 ± 2.4	16.5 ± 1.6	1.1	NS

Table 19. Experiment 5. Pretrial-1 infralimbic mix drug effects on elevated plus-maze behavior. Values represent means +/- SEM. Time ratios represent the amount of time (sec) in a section + 300 sec. OE, open arm entries; OTR, open time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), significantly different than vehicle ($p < .013$).

(NO INJECTION) ELEVATED PLUS-MAZE TRIAL-2

Behav.	Vehicle	PIR 10 nmol	BNI 10 nmol	BNI 10/PIR 10	BNI 5/PIR 10	$F_{(4,46)}$	*P*
OE	3.7 ± 0.8	6.1 ± 0.4*	2.2 ± 0.5	3.4 ± 0.7	3.1 ± 0.4	6.2	0.000
OTR	0.126 ± 0.027	0.254 ± 0.024*	0.067 ± 0.015	0.143 ± 0.028	0.118 ± 0.018	8.5	0.000
UHD	4.1 ± 0.9	8.1 ± 1.6*	0.9 ± 0.4*	4.5 ± 1.2	1.9 ± 0.7	7.8	0.000
USA	6.5 ± 1.3	10.3 ± 0.8*	3.1 ± 0.6*	8.4 ± 1.8	5.8 ± 0.9	5.2	0.002
CE	8.5 ± 0.9	13.7 ± 1.2*	14.3 ± 0.7*	11.4 ± 1.2*	12.3 ± 1.0*	5.1	0.002
CTR	0.329 ± 0.032	0.418 ± 0.032	0.482 ± 0.028*	0.404 ± 0.048	0.435 ± 0.027*	2.6	0.045
CVS	13.6 ± 1.5	23.4 ± 2.3*	25.8 ± 1.6*	18.2 ± 2.3	22.0 ± 1.5*	6.7	0.000

Table 20. Experiment 5. Pretrial-1 infralimbic mix drug carry-over effects on trial-2 elevated plus-maze behavior 24 h later. Values represent means +/- SEM. Time ratios represent the amount of time (sec) in a section + 300 sec. OE, open arm entries; OTR, open time ratio; UHD, unprotected head dips; USA, unprotected stretch attends; CE, closed arm entries; CTR, closed time ratio; CVS, closed vertical stretches. (*), significantly different than vehicle ($p < .013$).

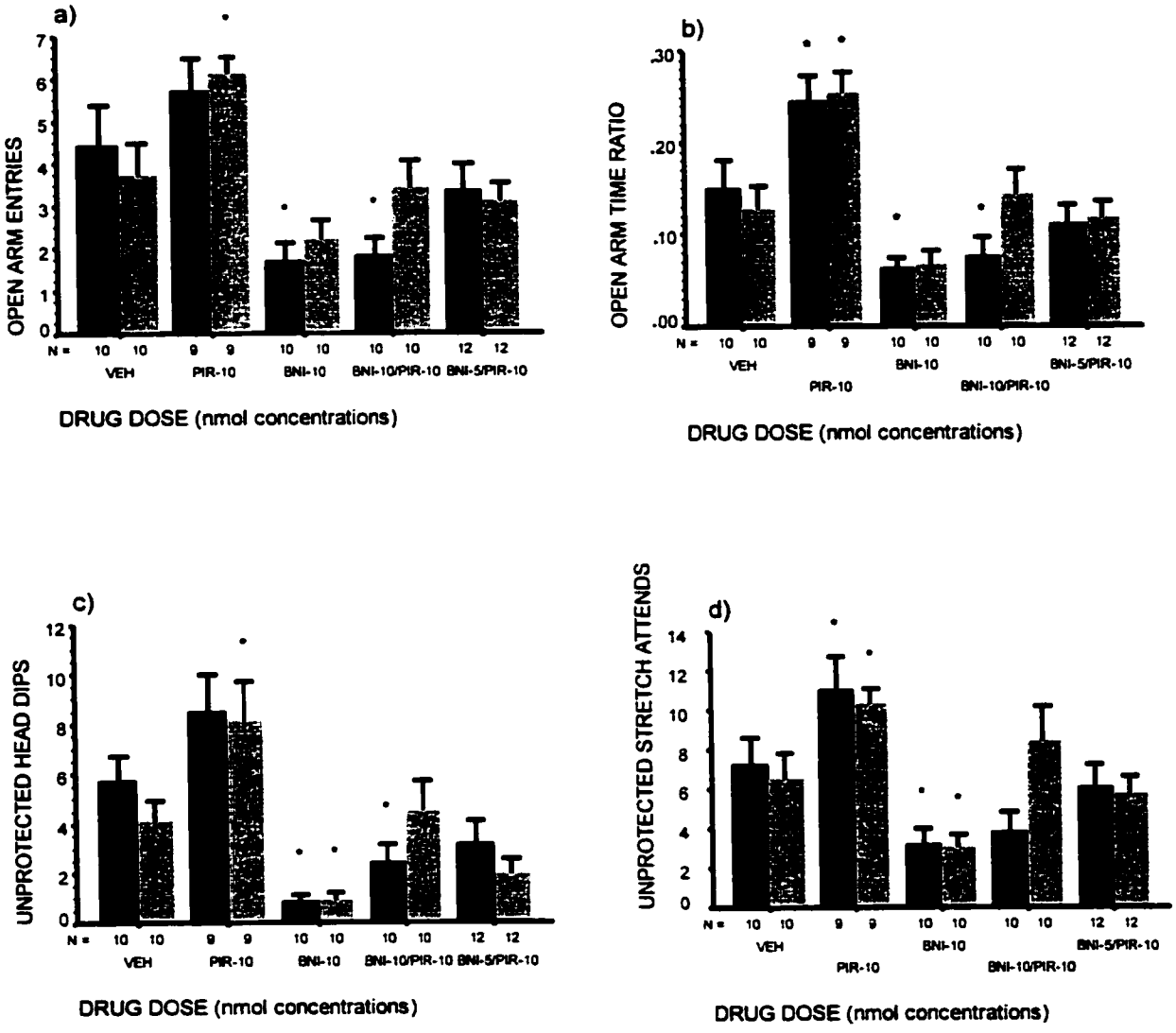
a)
OPEN ARM ENTRIES
N = 10 10 9 9 10 10 10 10 12 12
VEH PIR-10 BNI-10 BNI-10/PIR-10 BNI-5/PIR-10
DRUG DOSE (nmol concentrations)
b)
OPEN ARM TIME RATIO
N = 10 10 9 9 10 10 10 10 12 12
VEH PIR-10 BNI-10 BNI-10/PIR-10 BNI-5/PIR-10
DRUG DOSE (nmol concentrations)
c)
UNPROTECTED HEAD DIPS
N = 10 10 9 9 10 10 10 10 12 12
VEH PIR-10 BNI-10 BNI-10/PIR-10 BNI-5/PIR-10
DRUG DOSE (nmol concentrations)
d)
UNPROTECTED STRETCH ATTENDS
N = 10 10 9 9 10 10 10 10 12 12
VEH PIR-10 BNI-10 BNI-10/PIR-10 BNI-5/PIR-10
DRUG DOSE (nmol concentrations)

Figure 26

2.6.5. Discussion

The behavioural results from the present investigation support the involvement of infralimbic post-synaptic M1 and kappa receptor interactivity in the regulation of spontaneous working memory and anxiety induction in CD-1 mice. Moreover, The PIR-10 and BNI-10 nmol data replicated results from previous experiments (Wall et al., 2001; Wall & Messier, 2000a). Whereas PIR (10 nmol) was anxiolytic in trials 1 and 2 in the elevated plus-maze and disrupted spontaneous working memory in the Y-maze, BNI (10 nmol) was anxiogenic in both elevated plus-maze trials and also disrupted spontaneous working memory performance in the Y-maze, in the present study. The notion that pretrial-1 IL PIR administration disrupts aversive learning during trial-1 thus exerts long-term anxiolytic effects on trial-2 elevated plus-maze behaviours (Wall et al., 2001) appears a robust phenomena. In addition, the observation that norBNI enhanced aversive learning during trial-1, thus exerted anxiogenic carry-over effects on unprotected exploratory activity during trial-2 in the present study, also appears to be a robust phenomena. The interesting fact is that both receptor systems markedly influenced both cognitive/behavioural processes.

Our findings can now be summarized as follows: Whereas IL infusions of the kappa1 agonist, U-69593, reduced anxiety and *enhanced* spontaneous working memory (Wall & Messier, 2000c), IL PIR injections reduced anxiety and *disrupted* spontaneous working memory [(Wall et al., 2001), present study]. Moreover, whereas IL BNI was anxiogenic and *disrupted* spontaneous working memory [(Wall & Messier, 2000a), present study], IL infusions of the M1 agonist, McN-A-343, was anxiogenic and *enhanced* spontaneous working memory performance (Wall et al., 2001). Conceivably, the kappa1 and M1 receptor systems in the IL cortex differentially influenced attentional mechanisms related to spontaneous exploration.

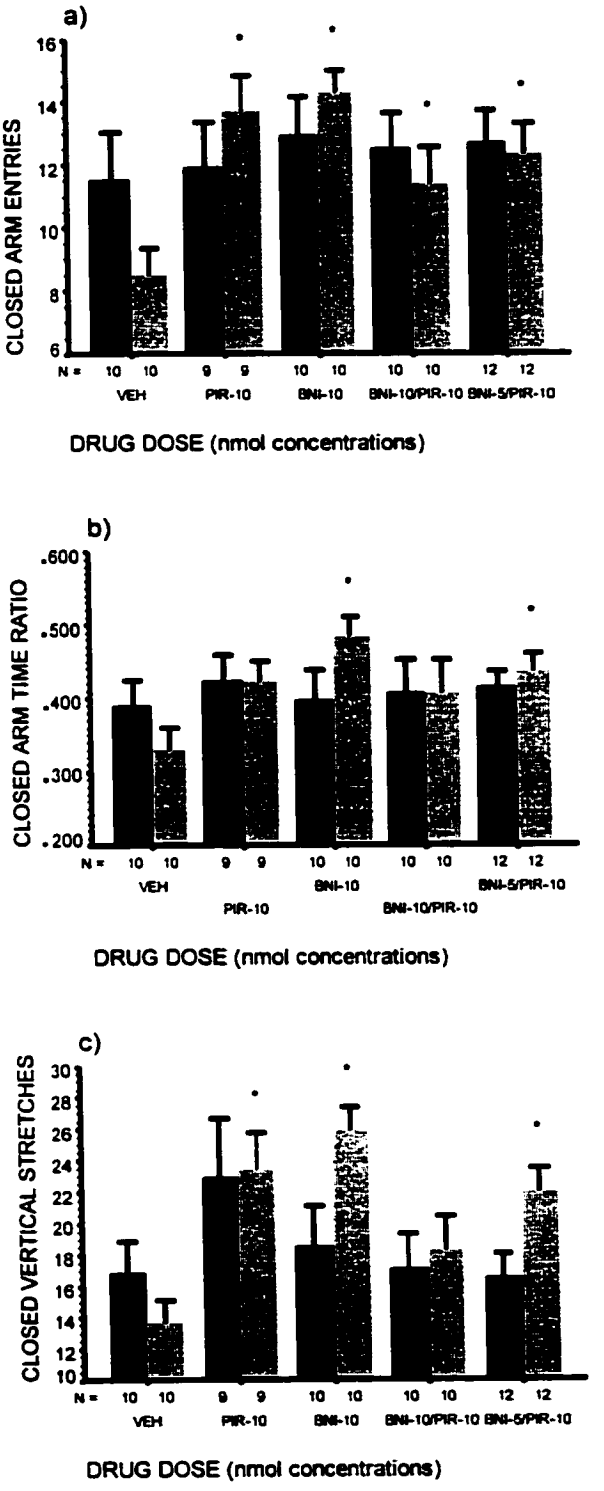
a)
CLOSED ARM ENTRIES
16
14
12
10
8
6
N = 10 10 9 9 10 10 10 10 12 12
VEH
PIR-10
BNI-10
BNI-10/PIR-10
BNI-5/PIR-10
DRUG DOSE (nmol concentrations)
b)
CLOSED ARM TIME RATIO
.600
.500
.400
.300
.200
N = 10 10 9 9 10 10 10 10 12 12
VEH
PIR-10
BNI-10
BNI-10/PIR-10
BNI-5/PIR-10
DRUG DOSE (nmol concentrations)
c)
CLOSED VERTICAL STRETCHES
30
28
26
24
22
20
18
16
14
12
10
N = 10 10 9 9 10 10 10 10 12 12
VEH
PIR-10
BNI-10
BNI-10/PIR-10
BNI-5/PIR-10
DRUG DOSE (nmol concentrations)

Figure 27

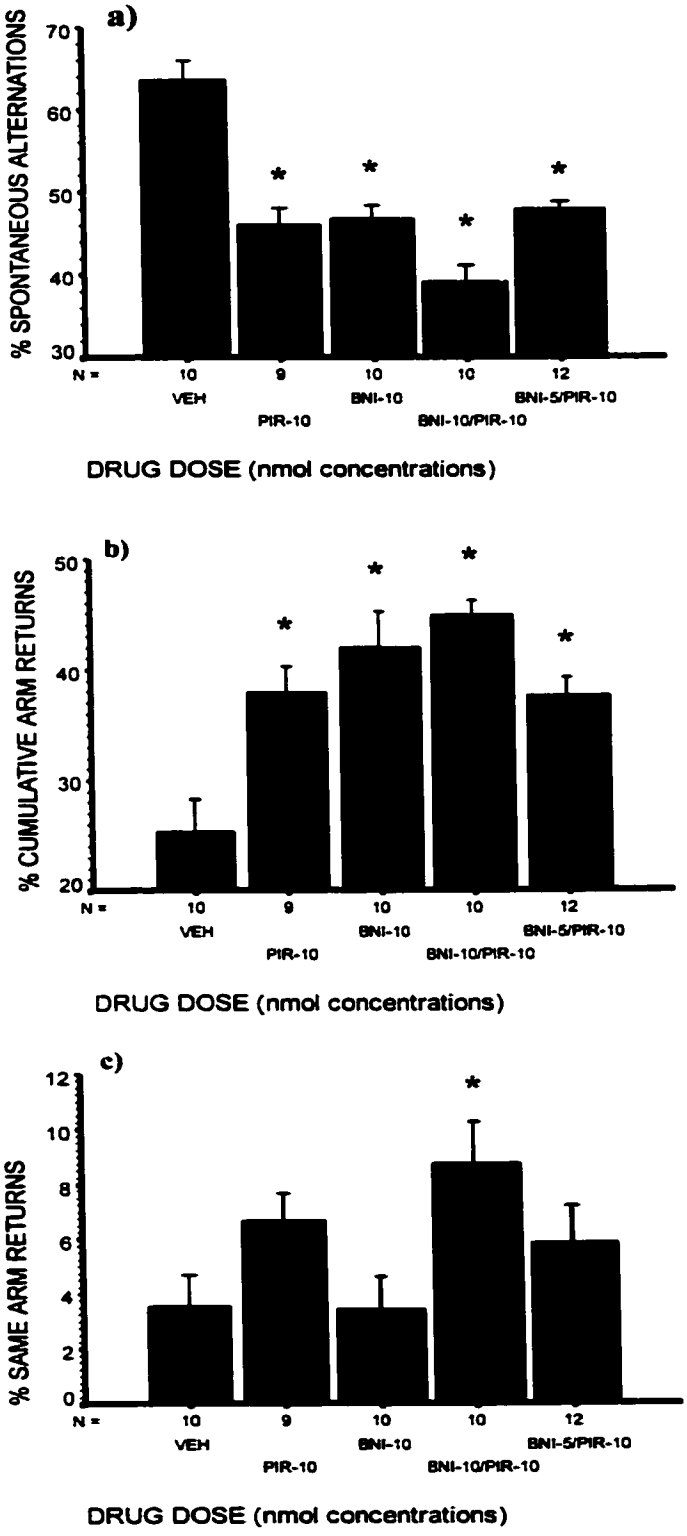
a)
% SPONTANEOUS ALTERNATIONS
70
60
50
40
30
N =
10
9
10
10
12
VEH
PIR-10
BNI-10
BNI-10/PIR-10
BNI-5/PIR-10
DRUG DOSE (nmol concentrations)
b)
% CUMULATIVE ARM RETURNS
50
40
30
20
N =
10
9
10
10
12
VEH
PIR-10
BNI-10
BNI-10/PIR-10
BNI-5/PIR-10
DRUG DOSE (nmol concentrations)
c)
% SAME ARM RETURNS
12
10
8
6
4
2
0
N =
10
9
10
10
12
VEH
PIR-10
BNI-10
BNI-10/PIR-10
BNI-5/PIR-10
DRUG DOSE (nmol concentrations)

Figure 28

These data lend further support to the hypotheses that (a) enhanced basal forebrain – medial PFC cholinergic activity may underly the cognitive/behavioural symptoms of anxiety (Sarter & Bruno, 1999; Sarter et al., 1999), (b) post-synaptic M1 receptors in the IL cortex concurrently modulate working memory, aversive learning and anxiety-like behaviour, and (c) infralimbic kappa opioid and M1 receptors may interact in the concurrent modulation of anxiety and memory.

The mixed drug infusion results lend some support to the latter hypothesis. The PIR-10 nmol/BNI-10 nmol drug mix appeared to exert similar anxiogenic effects in trial-1 of the elevated plus-maze as 10 nmol BNI alone. This was surprisingly not the case in trial-2, however. Whereas it is usually the case that naïve animals generally decrease unprotected exploration activity during trial-2 in the elevated plus-maze (Wall & Messier, 2000b), the BNI-10/PIR-10 group actually showed some increased unprotected exploration activity during trial-2 relative to controls in the present study. These data suggest that the anxiolytic carryover effects of 10 nmol PIR counteracted by the anxiogenic carryover effects of 10 nmol BNI.

This interpretation is further supported by the BNI-5 nmol/PIR-10 nmol drug mix results. There were no effects of the IL BNI-5/PIR-10 drug mix infusions on any trial-1 or trial-2 elevated plus-maze anxiety measure. Protected exploration activity was enhanced in trial-2, however, relative to trial-2 vehicle control animals. We interpret these data to mean that the animals were equally active in the protected areas of the maze in trials 1 and 2, but trial-2 protected activity was somewhat reduced in the vehicle group. Taken together, the anxiogenic effects of BNI were cancelled out by the anxiolytic effects of PIR, thus aversive learning was neither disrupted nor enhanced in the elevated plus-maze.

In contrast to these counteractive effects of the mixed drug infusions on elevated plus-maze behaviour, the mixed drug injections profoundly influenced Y-maze active working memory. In fact, the IL BNI-10/PIR-10 infusions appeared to disrupt attentional mechanisms associated with

spontaneous exploratory activity in the Y-maze. Although the mice in the BNI-10/PIR-10 group did not appear any more active than vehicle controls, *per se* (i.e., no difference in total arm entries) in the Y-maze, these animals showed what appeared to resemble attentional deficits. Although it is certainly the case that attentional mechanisms are inextricably intertwined with working memory processing, the notion that so-called 'delay-independent' disruptive effects on working memory result from attentional disturbances (rather than memory deficits) (Delatour & Gisquet-Verrier, 2000) begins to make sense since attentional control mechanisms in the orbitomedial PFC may very well be the essence of 'active' working memory processing such that activation of any sort of information is attention directed to internal representations (Fuster, 1997). Moreover, the on-going adequate and flexible updating of information is just as, if not more important than maintenance of information in an active state (Druzin et al., 2000). In this way, attentional mechanisms in the ventromedial PFC can manipulate and monitor information and guide behavioural output on a moment-to-moment basis within working memory (Dunbar & Sussman, 1995; Seamans et al., 1998; Wall & Messier, 2001a). We suggest that the BNI-10/PIR-10 drug infusions not only disturbed spontaneous working memory, but also disturbed attentional mechanisms.

To assess the possibility that disrupted spontaneous working memory may encompass disrupted attentional mechanisms, we included 2 'attentional' measures in our Y-maze test battery. The cumulative arm return ratio measured the number of times an animal alternated from 1 arm to a second and back to the first again, and was expressed as a ratio over total arm entries. We hypothesized that this score would likely estimate measures of spontaneous working memory performance and attentional monitoring systems. Moreover, the ratio of same arm returns over total entries should further characterize the degree of attentional deficits an animal may be experiencing.

Clearly, the BNI-10/PIR-10 drug mix infusions in the IL cortex profoundly influenced both 'attentional' measures. These animals had the lowest spontaneous alternation score, the highest

cumulative arm return ratio, and were the only animals with a higher same arm return ratio than control animals. Moreover, close inspection of Figure 3 reveals that 10 nmol PIR infusions alone disrupted spontaneous working memory, increased cumulative arm returns and somewhat increased (not significant) same arm returns. In contrast, 10 nmol BNI infusions disrupted spontaneous working memory, enhanced cumulative arm returns, but did not influence same arm returns. Only the BNI-10/PIR-10 infusion containing PIR enhanced same arm returns. These data render the suggestion that the same arm return ratio may be tapping into attentional mechanisms within working memory, and add to the growing body of evidence showing that disrupting cholinergic neurotransmission in the PFC leads to attentional deficits in laboratory animals (Dunnett, Everitt, & Robbins, 1991; Muir et al., 1992; Sarter & Bruno, 1999, 2000; Sarter et al., 1999; Voytko et al., 1994). The interesting point in the present study is that kappa receptor blockade synergized with M1 blockade to more severely impact attentional processes than M1 or kappa receptor inhibition alone.

It is interesting that the cognitive aspects of Alzheimer's, schizophrenia and attention deficits are strongly linked to dopaminergic and cholinergic dysfunction in the medial PFC (Bymaster et al., 1999; Cosford et al., 2000; Ernst et al., 1998; Faraone et al., 2000; Goldman-Rakic, Muly, & Williams, 2000; Knable & Weinberger, 1997; Lawrence & Sahakian, 1998; Okubo et al., 1997). These data underscore the need to not only understand the link between attention deficits and working memory in the ventromedial PFC, but also how cholinergic, peptidergic and dopaminergic sytems interact to link the cognitive/behavioural aspects of anxiety and working memory.

In conclusion, results from the present study indicate that concomitant IL M1 and kappa receptor blockade produces counteractive effects on the regulation of anxiety-like behaviour, and synergistic effects to profoundly disrupt 'attentional' mechanisms related to spontaneous working memory. These data suggest that concurrent kappa and M1 receptor blockade in the IL area of the ventromedial prefrontal cortex may represent an interesting animal model of attentional deficits.

2.7. Summary of Experimental Results

The behavioural results derived from Experiments 2a, 2b, 3, 4a, 4b, 4c and 5 provide direct evidence for orbitomedial PFC involvement in the concurrent modulation of anxiety and associative working memory. In addition, the aggregate results support the hypotheses that (a) ACh receptor activation in the IL vmPFC enhances anxiety (Berntson et al., 1996; Berntson et al., 1998), aversive learning and active working memory, (b) kappa receptor activation in the IL vmPFC reduces anxiety, disrupts aversive learning and enhances active working memory, (c) kappa opioid and muscarinic receptors interact in the IL vmPFC in the concurrent modulation of anxiety and memory, and (d) NBM – vmPFC cholinergic terminal activation likely influences anxiety and memory in the orbitomedial PFC through effects on attentional processing (Sarter et al., 1999).

3.0. GENERAL DISCUSSION

Two primary questions were addressed in the present series of experiments: (1) Can anxiety-like behaviour and spontaneous working memory be concurrently modulated, and (2) does such concurrent modulation occur in the infralimbic (IL) area of the ventromedial prefrontal cortex (vmPFC)? Five secondary questions were also addessed: (1) Can the use of confirmatory factor analysis hypothesis-testing and model building procedures yield a valid and parsimonious factorial construct of anxiety-like behaviour in the elevated plus-maze? (2) Do kappa opioid receptors concurrently modulate anxiety and working memory in the IL vmPFC? (3) Do muscarinic M1 receptors concurrently influence anxiety and working memory in the IL vmPFC? (4) Do infralimbic M1 and kappa receptors interact in the concurrent modulation of anxiety and working memory in the IL vmPFC? (5) Can attentional mechanisms be teased apart from working memory mechanisms in the concurrent modulation of anxiety and working memory in the IL vmPFC? The treatment of these seven questions together comprised the experimental research thrust of the present Thesis. The ensuing discussion addresses both primary questions in conjunction with a detailed and orderly discussion of each of the five secondary questions.

The methodological and conceptual issues in the measure of animal anxiety in the elevated plus-maze (addressed in Experiment 1), are discussed first. Next, based on the aggregate results of Experiments 2, 3, 4 and 5, possible neurochemical receptor interactivity that may underly the concurrent modulation of anxiety, working memory and attention in the IL vmPFC, is discussed. On the whole, it is argued that the neurobiological substrates of anxiety and associative working memory are under the attentional control of the orbitomedial PFC – hippocampus circuit described in the Introduction. Other testable hypotheses are then considered, followed by a brief discussion on the relevance of the present findings to future research into the neurocircuitry underlying dysfunctional cognitive-behavioural relationships.

In the next section, the rationale to conduct the extensive confirmatory factor analysis in Experiment 1, and the validity and utility of the resultant 2-factor solutions, are discussed. We argue that the elevated plus-maze is a useful animal model of anxiety because it incorporates many different facets of animal anxiety, thus circumvents the need for the use of 3 or 4 different apparati in pharmacological/behavioural experiments. In addition, the rationale behind our 2-trial testing strategy in the elevated plus-maze will also be discussed.

3.1. Utility of the elevated plus-maze in the measure of animal anxiety and aversive learning

The arguments presented in this section appear for the most part in:

Wall, P. M. & Messier, C. (2001). Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement of animal anxiety-like behaviour. *Neuroscience and Biobehavioural Reviews*. 25: 275-286.

In Experiment 1 we used rigorous inferential hypothesis-testing and model building statistical procedures to address the numerous methodological and conceptual issues inherent in the use of the elevated plus-maze in measuring animal anxiety-like behaviour. Our main objective was to confer a meaningful, parsimonious and testable behavioural model of anxiety-like behaviour. A brief history of the development of the elevated plus-maze (and subsequent accompanying interpretation difficulties), that provided the rationale to conduct such an extensive analysis, follows.

Montgomery originally characterized the conflictive nature of an elevated Y-maze as the drive to approach and explore an open arm (alley) in conflict with fear and subsequent avoidance of the unprotected alley(s) (Montgomery, 1955). Handley and Mithani later showed that a modified plus shaped version of the maze with two opposing open arms perpendicular to two opposing closed arms could detect anxiety-influencing properties of putative anxiolytic drugs by measuring the ratio of open:total arm entries (OER, a measure of the relative aversiveness of the open arms) (Handley

& Mithani, 1984). Pellow et al. later added the ratios of time spent on each arm type (open time ratio - OTR, closed time ratio - CTR), but changed OER to reflect open:closed arm entries rather than open:total arm entries (Pellow et al., 1985). Total arm entries (TE) also began to be measured as a supposed index of locomotor activity in pharmacological validation studies in rats (Pellow et al., 1985) and mice (Lister, 1987). In this way, drug-induced increases in OTR and OER, without alterations in locomotor activity (TE), could supposedly be strictly attributed to anxiolytic drug effects (Weiss et al., 1998).

The original 2-factor structural model of elevated plus-maze behaviour. Conceptual difficulties first arose following the divergence of different interpretations of Montgomery's original description of a single construct, represented by two behavioural dimensionsin an elevated Y-maze (Montgomery, 1955). The fear drive (avoidance) and the exploration drive (approach) were the two dimensions, taken together described the psychological conflictive nature of the apparatus. Thus the ratio of open:closed alley entries was the index of this construct (Montgomery, 1955). For unknown reasons, the exploration (approach) drive was re-interpreted by some investigators as an activity or a locomotor construct, thus equivocally labeled as a locomotor activity factor (Lister, 1987; Pellow et al., 1985). A 2-factor structure of elevated plus-maze behaviour consequently emerged which a) eliminated the 'approach' side of the original approach/avoidance conflict structural model, and b) tried to partial out so-called locomotor activity from an avoidance (anxiety) construct.

Conceptual links between approach drive, exploration activity (or motivation to explore) and locomotor activity are slight, at best. The notion that, when studying drug effects on plus-maze became untenable (Weiss et al., 1998), primarily because that is the same thing as saying that the appraoch drive, or the exploration drive, was unaltered. Moreover, a number of principal components analyses clearly showed that TE often-times ambiguously loaded on the 'anxiety' and 'locomotor activity' components (Table 21, cross-loadings in bold-face type). These data led

Table 21

Reported structural models of elevated plus-maze behaviour (pca solutions). Sig. cross-loadings in bold-face type. Adopted from (Wall & Messier, 2001b).

	Factor-1	Factor-2	Factor-3	Factor-4	Factor-5	Factor-6
Lister, 1987	**Anxiety** OER, OTR	**Locomotor Activity** TE				
Fernandes & File, 1996	**Anxiety** OE, OT, **CT, TE,** OER, OTR	**Locomotor Activity** CE, **CT, TE'**, HT				
File, 1993 (trial-1 data only)	**Anxiety** OE, **OT, OER, OTR,** TE	**Locomotor Activity** **OE,** CE, **TE**				
Rodgers & Johnson, 1995	**Anxiety** **OE,** OER, OTR, CTR	**Locomotor Activity** **OE,** TE, CE				
Rodgers & Johnson, 1995	**Anxiety** **TE,** OE, OER, OTR, **CTR**	**Locomotor Activity** **TE,** CE	**Decision-Making Approach-Avoidance Conflict** **CTR,** HTR			
File, 1993 (trials 1 & 2 factored together)	**Anxiety-1** **OE-1,** OT-1, OER-1, OTR-1, **TE-1**	**Locomotor Activity** OE-1, **CE-1,** TE-1,**CE-2,** TE-2	**Anxiety-2** OE-2, OT-2, OER-2, OTR-2, **TE-2**			
Trullas & Skolnick, 1993	**Fear Index** OTR, OER, OT, **CT, OE, CE**	**General Activity** **OE, CE,** TE	**Decision-Making** **CT,** HT			
Cruz, Frei & Graeff, 1994	**Anxiety** **OE, TE, PSA,** OER, OT, CT, OTR, UHD	**General Motor Activity** **OE, TE,** CE, TR	**Waiting Capacity/ Decision-Making** HT, **PSA**	**Displacement** **PSA,** Gr		
Espejo, 1997	**Anxiety** **OE, CE, SN,** PSA, PHD, UHD	**Motor Activity** **OE, CE, Sn, TR,** TE	**Approach-Avoidance Conflict** CR, **USA**	**Displacement** **Sn, TR,** Gr, **USA**		
File et al, 1993 (Trials 1 & 2 factored together)	**Anxiety-1** OE-1, OT-1, CT-1	**Anxiety-2** OE-2, OT-2, CT-2, **HT-2,**	**General Activity** CE-1, CE-2	**Decision Time** HT-1, **HT-2**		
Fernandes & File, 1996 (no edges open arms)	**Anxiety** OE, OT, OT-d, OE-d, UHD, UHD-t, USA-t	**Decision (Height)** **HT,** PHD, PHD-t	**Activity** TR	**Decision(Open)** CE, **HT,** PSA-t, FBA-t		
Fernandes & File, 1996 (edges open arms)	**Anxiety** OE, OT, OT-d, OE-d, USA-t	**Decision (Open)** HT, PSA-t, FBA-t	**Height Assessment** UHD, UHD-t	**Decision (Height)** PHD, PHD-t	**Activity** CE, TR	
Rodgers & Johnson, 1995 (DBA-2 mice)	**Anxiety** **TE,** OE, **CR,** OER, OTR, **HTR, CTR,** PHDR, PSAR, PSn, FBAR	**Locomotor Activity** **TE, CE,** FBA-t	**Risk Assessment/Decision -Making** **TSA,** SN-t	**Decision-Making & Approach-Avoidance Conflict** **CR,** Gr-t, **HTR, CTR**	**Vertical Activty** **TE, CE,** TR, **Gr-t**	**Exploration** THD, **TSA**
Holmes & Rodgers, 1998 (Swiss-Webster mice)	**Anxiety** **TE,** OE, OER, OTR, **HTR,** PHDR, PSAR, PSnR, **CR, FBAR**	**Locomotor Activity** **TE,** CE, **CTR,** TSA, **CR**	**Directed Exploration** THD, Sn-t	**Vertical Activity** **CTR,** TR, TR-t	**Displacement** **CTR, HTR,** Gr-t	**Defensive Ambulation** FBA-t, **FBAR**

several investigators to suggest that, since CE often uniquely loaded on a 'locomotor activity' component in some analyses (Cruz et al., 1994; Fernandes & File, 1996; File, 1993; File et al., 1993; Rodgers & Johnson, 1995), then it could best partial out drug effects on locomotor activity from drug effects on anxiety (Holmes & Rodgers, 1999; Rodgers & Johnson, 1995). This interpretation also appears no longer tenable, particularly since CE has similarly been shown to co-load on more than one component in other studies (Fernandez Espejo, 1997; Rodgers & Johnson, 1995; Trullas & Skolnick, 1993). Finally, Weiss et al. suggested that the use of principal components analysis should be restricted to the analysis of relationships between indices, since such data analysis techniques are of questionable value for interpreting functional significance of any behavioural measure (Weiss et al., 1998). Clearly, drug-induced reductions in CE and drug-induced reductions in exploration activity or approach drive should not have been similarly interpreted.

The 3-factor model emerged reflecting open/centre/closed spatiotemporal measures. Some investigators attempted to re-incorporate an approach/avoidance conflict construct back into the analysis of elevated plus-maze behaviour (Lee & Rodgers, 1990). These investigators were the first to reason that the centre hub was a 'decision' point for approach/avoidance conflict re open arm exploration, so may reflect elements of anxiety (Lee & Rodgers, 1990, 1991; Rodgers et al., 1992).

The idea of reconceptualizing an approach/avoidance (decision-making) behavioural construct made intuitive sense, but in a similar fashion to a locomotor activity construct, does not appear to hold much substantive meaningfulness (Wall & Messier, 2001b). Aside from these conceptual issues that rendered structure interpretations difficult, there were numerous methodological issues concerning current practices in the use of the elevated plus-maze to delineate behavioural structures. It was clear that the greater the number of indicators that were included in recent analyses, the greater the number of latent components were extracted in the principal components solutions. Hence, interpretation difficulties arose as a result of increasing complexity associated with higher

numbers of extracted components. For example, Fernandes & File reported a 5-component solution extracted from *fifteen* indicator variables (Table 1) (Fernandes & File, 1996). In this particular study, a full *twenty-five* indicator variables were analyzed but only those *fifteen* variables with loading coefficients of ≥ .60 were reported in the factor loading matrix (Fernandes & File, 1996) (Table 1 for factor labels). In addition, 6-factor principal components solutions reported by Rodgers & Johnson (Rodgers & Johnson, 1995) and Holmes & Rodgers (Holmes & Rodgers, 1998) presented similar interpretation difficulties. These investigators also included a broad array of ethological indices along with the usual arm-related spatiotemporal measures in their analyses. The extracted six components from *nineteen* indicator variables in the former study were interpreted as 1) anxiety, 2) locomotor activity, 3) decision-making and/or approach/avoidance conflict, 4) related to approach/avoidance conflict, 5) vertical activity, and 6) exploration activity (Rodgers & Johnson, 1995). The extracted six components from *nineteen* indicator variables differed somewhat in the latter study 1) anxiety, 2) locomotor activity, 3) directed exploration, 4) vertical activity, 5) displacement, and 6) defensive ambulation (Holmes & Rodgers, 1998) (Table 1). Inspection of the factor loading matrix of the former study, however, revealed numerous cross-loadings (TE, CE, HTR and CTR ambiguously measured more than one component), thus factors 3, 4, 5 and 6 appeared unstable and ambiguous (Rodgers & Johnson, 1995). The latter study also showed significant cross-loading patterns for TE, CTR and HTR, among other measures (Table 21), thus indicating that factors 4, 5 and 6 were likely unstable and ambiguous (Holmes & Rodgers, 1998). Clearly 6 component solutions are difficult, if not impossible to interpret, thus underscore the conceptual and methodological issues surrounding such structural solutions and are therefore of questionable value in further understanding animal anxiety-like behaviour in the elevated plus-maze (Wall & Messier, 2000b; Weiss et al., 1998).

The results from Experiment 1 clearly demonstrated the utility of confirmatory factor analysis techniques to address the aforementioned issues. In addition to the utility of our 2-factor model to parsimoniously describe underlying structures driving anxiety-related behaviour in the elevated plus-maze, the four factor-1 anxiety indices incorporate traditional open arm spatiotemporal, as well as ethologically-derived unprotected risk assessment measures. Moreover, the four factor-1 anxiety indices were reliably influenced by the kappa$_1$ agonist, U-69,593 (Wall & Messier, 2000c) and antagonist, norBNI (Wall & Messier, 2000a), the muscarinic1 antagonist, pirenzepine, and agonist, McN-A-343 (Wall et al., 2001), all from the infralimbic area of the vmPFC in CD-1 mice (Experiments 2, 3, 4 and 5). These pharmacological data attest to the sensitivity of these anxiety-related measures identified in Experiment 1.

In summary, understanding the underlying anxiety-related psychological constructs driving behaviour in the elevated plus-mze is essential to further develop and validate the apparatus as a psychological measurement in the study of animal anxiety. Rigorous hypothesis-testing and model-building procedures, to this end, are clearly necessary steps.

3.1.1. *The 2-trial elevated plus-maze procedure and aversive learning*

Using a 2-trial procedure in Experiment 1 primarily provided an opportunity to not only measure the degree of aversive learning that presumably occurs during trial 1 in the elevated plus-maze in drug-naïve mice, but also to estimate the memory for such learning in trial-2 24 h. later. Moreover, the large number of subjects used in Experiment 1 (N = 200) provided useful estimates of baseline behavioural indices. The 2-trial procedure also permitted us to test the hypothesis that anxiolytic drugs should disrupt aversive learning and anxiogenic drugs should facilitate aversive learning in our pharmacological studies.

It should be mentioned that so far there have been two different 2-trial procedures used in intracerebral pharmacological studies in the elevated plus-maze (File et al., 2000; Wall & Messier, 2000c). In the procedure of File and colleagues, drug-naïve animals are given trial-1 with no injection, followed by a pretrial-2 intracerebral drug injection 24 h. later. In our studies, animals are given a pretrial-1 intracerebral drug injection, followed by a no-injection trial-2 24 h. later. The rationale behind the use of either of the two 2-trial procedural strategies depends on (a) whether the experimenter wishes to investigate drug effects on aversive learning – with subsequent long-term effects on memory 24 h. later, or (b) investigate drug effects on aversive memory 24 h. after aversive learning presumably occurred during a no-drug trial-1.

File and colleagues' strategy developed after consistent observations that referent anxiolytic BDZs were not anxiolytic in rats with previous maze experience (1 no-drug trial 24 h. earlier) (File, 1992, 1993; File, Andrews, Wu, Zharkovsky, & Zangrossi, 1992; File et al., 1993). Other investigators have also observed this phenomena in mice (Holmes & Rodgers, 1999), (Lister, 1987; Rodgers et al., 1992; Rodgers & Shepherd, 1993). Moreover, File and colleagues also demonstrated that systemic pretrial-1 BDZ injections exerted anxiolytic effects, but were mostly void of any anxiolytic effects when injected prior to trial-2 24 h. later (File, 1990; File, Mabbutt, & Hitchcott, 1990). This '1-trial tolerance' (File, 1990) to the anxiolytic effects of BDZs was shown to be independent of drug state in trial-1, the texture of the material used to construct the maze, or the inter-trial interval duration (Espejo, 1997a; File, 1990; File et al., 1990; Holmes & Rodgers, 1999; Lister, 1987; Rodgers et al., 1992). In addition, several investigators have reported that drug-naïve animals consistently engage in noticeably reduced unprotected exploration activity during trial 2 (Dawson et al., 1994; Espejo, 1997a; Fernandes & File, 1996; Holmes & Rodgers, 1998; Schmitt & Hiemke, 1998; Treit, Menard et al., 1993). These data prompted the suggestion that animals learn some sort of phobic response to the aversive nature of the open arms during trial-1, that shows up as

reduced unprotected exploration activity during trial-2 (Almeida et al., 1993; Dawson et al., 1994; Espejo, 1997a; File et al., 1993; Gonzalez & File, 1997; Rodgers et al., 1997; Rodgers et al., 1992; Rodgers & Shepherd, 1993; Treit, Menard et al., 1993).

File and colleagues began to investigate this phenomena further by using principle components analysis to evaluate the possibility that trial-1 behavioural profiles might fundamentally differ from trial-2 profiles. They indeed reported anxiety factor loading differences between trials 1 and 2 in the elevated plus-maze in rats (File, 1993; File et al., 1993). However, these differences were only found when traditional spatiotemporal indices from both trials were factored together; trial-1 anxiety indicators loaded on factor-1 and trial-2 anxiety indicators loaded on factor-2 of a 3-factor structure in rats (File, 1993; File et al., 1993). These data further supported their suggestion that rats extinguish 'state' anxiety during trial-1, but develop a type of phobic anxiety during trial-2 (File, 1993; File et al., 1993). Holmes & Rodgers found similar differential trial-1 to trial-2 anxiety factor loading patterns in Swiss-Webster mice (Holmes & Rodgers, 1998). These authors suggested that the experience of trial-1 produced a qualitative shift in the emotional responses to trial-2 exposure.

The observation that there was little difference between the factor-1 (anxiety) indicator loading patterns of trials 1 and 2 in Experiment 1, was interesting given the aforementioned anxiety factor loading differences between trials 1 and 2 in the elevated plus-maze in rats (File, 1993; File et al., 1993) and mice (Holmes & Rodgers, 1998). Moreover, in Experiment 1, the anxiety indices (OE, OTR, USA, UHD) data were reduced by about 50% in trial-2. This suggested to us that the animals might have experienced a different anxiety state during the second trial. However, consistent high loadings and low measurement errors for each of the four factor-1 anxiety indicators in the 2-factor final models of both trials (Figures 8 & 10) would indicate that the animals were not experiencing a different type of anxiety, but perhaps a more intense level of the same anxiety state during trial-2 exposure. Thus, we agreed with other investigators that animals experience aversive learning during

trial-1, but this learning is reflected in trial-2 as a potentiation of the same anxiety state. This hypothesis was also corroborated by the consistent observation that vehicle-treated animals from Experiments 2, 3, 4 and 5 showed enhanced anxiogenic profiles in trial-2. Moreover, there were striking similarities between the trial-2 enhanced anxiety-related behavioural data from Experiment 1, and the enhanced trial-1 anxiety-related measures in the norBNI treated groups in Experiment 3, and the McN-A-343 treated groups in Experiment 4c.

The fact that associative learning during trial-1 potentiated anxiogenic responses in animals re-exposed to the elevated plus-maze 24 h. later, indicated close relationships between associative learning and memory and anxiety induction. These relationships also indicated to us that the neural mechanisms underlying both phenomena were also closely related. Consequently, we decided to use the pretrial-1 drug infusion 2-trial testing strategy, primarily because we could evaluate drug effects on trial-1 aversive learning, thus further investigate possible relationships between active working memory, attentional processing and anxiety induction.

In summary, several laboratories have demonstrated that trial-2 anxiety-related behavioural profiles in the elevated plus-maze are (a) not amenable to anxiolytic effects of referent BDZs, (b) reflect enhanced anxiety states relative to trial-1, and (c) suggest a close relationship between associative working memory, attentional processing, and anxiety induction. These observations led us to adopt the 2-trial testing strategy used in Experiments 2, 3, 4 and 5.

3.2. The concurrent modulation of anxiety and memory in the infralimbic vmPFC

The results from Experiments 2 and 3 clearly demonstrate that aversive learning and memory and anxiety can be concurrently modulated in the elevated plus-maze following kappa receptor drug infusions in the IL vmPFC. The Experiments 2 and 3 data were only suggestive, however, with respect to effects on active working memory performance in the Y-maze, owing to the variability

between control groups in both experiments (Figures 12c, 16d, and 20c). There were nevertheless opposite effects on spontaneous alternation performance between both drugs. Moreover, the anxiolytic effects of U-69593, and anxiogenic effects of norBNI in the elevated plus-maze appear to be robust phenomena (i.e., anxiolytic effects of U-69593 were observed in 3 separate experiments: (Wall, 1997), Experiments 2a and 2b; as were the anxiogenic effects of norBNI (Wall, 1997), Experiments 3 and 5). It would therefore appear reasonable to suggest that (a) endogenous kappa opioids in the orbitomedial PFC function to reduce anxiety-related behavioural responses in aversive situations, and (b) these anxiety-reducing actions result from concurrent (interactive) effects on associative working memory and attentional processing. Furthermore, it could be suggested that kappa opioids in the orbitomedial PFC may influence autonomic responsivity in aversive situations, which could also influence attentional mechanisms and anxiogenic responding.

The question remains as to the neuromechanisms by which IL kappa receptor drugs might have influenced anxiety and active working memory in Experiments 2 and 3. In the Experiment 2 Discussion, we suggested that since it is also well known that various stressful or anxiety-provoking manipulations enhance DA activity in the vmPFC (Abercrombie et al., 1989; Bertolucci-D'Angio et al., 1990b; Carlson et al., 1991; Deutch & Roth, 1990; Dunn & File, 1983; Herman et al., 1982), and DA activity enhancement impairs spatial working memory in rats and primates (Murphy, Arnsten, Goldman-Rakic et al., 1996; Murphy, Arnsten, Jentsch et al., 1996), then the DA release-inhibiting effects of U-69593 may have exerted a limiting control over hyperactivated DA mechanisms in the mPFC (Bals-Kubik et al., 1993; Spanagel et al., 1992; Unterwald et al., 1994) in anxiety-provoking situations, to improve Y-maze performance (Wall & Messier, 2000c). This hypothesis makes intuitive sense given the consistent observations that (a) intrinsic ventral prelimbic and infralimbic interneurons and projection neurons in the vmPFC are tonically inhibited by DA (Benes et al., 1993; Deutch et al., 1990; Deutch & Roth, 1990; Godbout et al., 1991; Gresch

et al., 1994; King & Finlay, 1995; Le Moal & Simon, 1991; Mantz et al., 1989; Pirot et al., 1992; Retaux et al., 1991; Retaux et al., 1994; Seguela et al., 1988; Sesack & Bunney, 1989; Sokolowski et al., 1994; Verney et al., 1990; Vincent et al., 1993), and (b) evoked DA release in the mPFC dampens the activated electrophysiological activity produced by evoked GLU release (Mantz, Godbout, Pirot, Glowinski, & Thierry, 1992; Mantz, Milla, Glowinski, & Thierry, 1988). These data are consistent with the notion that stressor provoked DA release in the vmPFC serves to dampen excitatory projections to the autonomic brainstem, which, without such DA regulatory activity, would have enhanced the parasympathetic tone and reduced sympathetic tone.

In the Experiment 3 Discussion, we suggested that norBNI produced anxiogenic effects in the IL vmPFC by enhancing an already provoked DA release, and such enhanced DA release likely also perturbed working memory performanance in the Y-maze (Wall & Messier, 2000a). We also suggested possible kappa receptor interactivity with cholinergic mechanisms to exert such effects. For example, intraventricular norBNI infusion in mice blocked the reversal of scopolamine-induced working memory impairment in the Y-maze by intraventricular dynorphin-A infusion (Itoh et al., 1993a). Similarly, learning and memory impairments produced by the muscarinic agonist, carbachol, and the nictotinic antagonist, mecamylamine, were reversed by dynorphin administration. Finally, scopolamine-induced memory impairment and increased ACh release were blocked by intraventricular norBNI administration (Hiramatsu, Murasawa, Mori et al., 1998; Hiramatsu, Murasawa, Nabeshima et al., 1998). Hence, the data from Experiments 2 and 3 suggest that IL kappa receptor activation or blockade may have directly (or indirectly through DA mechanisms) interfered with afferent IL ACh neurotransmission from the reticular brainstem and/or basal forebrain NBM, to concurrently influence anxiety and working memory.

Before we could test this hypothesis, however, it was necessary to evaluate the effects of muscarinic receptor activation or blockade in the IL vmPFC on anxiety in the elevated plus-maxe

and active working memory in the Y-maze. Although the behavioural data from Experiments 4b and 4c clearly demonstrated that muscarinic M1 receptors in the IL cortex concurrently influence anxiety, aversive learning and memory, as in Experiments 2 and 3, the effects on associative working memory were not so straightforward. Again, this interpretation is due to the variability between control groups in spontaneous alternation performance in the Y-maze in Experiment 4 (Figure 23a, b and c). Thus, some of the intriguing differences between the concurrent effects of kappa drugs and M1 drugs on anxiety and active working memory might be explained by control group differences in alternation performance. However, in the absence of further data replicating the current Y-maze results, we can nevertheless address these differential relationships. Whereas the kappa antagonist was *anxiogenic* and disrupted working memory (Wall & Messier, 2000a), the M1 antagonist was *anxiolytic* and disrupted working memory (Wall et al., 2001). On the other side of the coin, whereas the kappa agonist was *anxiolytic* and enhanced working memory, the M1 agonist was *anxiogenic* and enhanced working memory. The sobering fact that the kappa *agonist* anxiolytic effects were coupled with memory *enhancement*, and the M1 *antagonist* anxiolytic effects were coupled with memory *impairment*, caught our attention.

We realized that no simple relational 'rules' between anxiety and working memory could be readily conceptualized. For example, in the case of M1 drug effects, we could suggest that M1 activation leads to enhanced anxiety associated with enhanced aversive learning and enhanced working memory performance. This rule roughly corresponds with Beuzen and Belzung's suggestion that enhanced anxiety is associated with enhanced aversive learning and memory (Beuzen & Belzung, 1995), as well as Berntson and colleagues' suggestion that activated cholinergic transmission in the vmPFC is associated with enahanced anxiety-like defensive reactions and enhanced memory performance (Berntson et al., 1998). Furthermore, the opposite rule can apply to our observed M1 antagonist effects: M1 blockade leads to reduced anxiety associated

with disrupted aversive learning and perturbed working memory performance. This rule corresponds to Berntson and colleagues' suggestion that disrupting cholinergic activity in the vmPFC is associated with reduced anxiety-like defensive reactions and perturbed memory performance. However, in the case of kappa drug effects, the rules remain the same with respect to the correspondence between anxiety and aversive learning, but change with respect the correspondence between anxiety and working memory performance. So for example, kappa activation reduced anxiety associated with reduced aversive learning and enhanced working memory performance, and kappa blockade enhanced anxiety associated with enhanced aversive learning and disrupted working memory. Explanations for these peculiar discrepencies are not readily available (see below). We did, however, hypothesize that since kappa and M1 receptor blockade produced opposite effects on anxiety and similarly disrupted working memory in Experiments 3 and 4b, then perhaps simultaneous antagonist drug infusions in the IL vmPFC would interact to concurrently modulate anxiety and working memory in CD-1 mice.

The behavioural results from Experiment 5 suggested that kappa and M1 receptors do indeed interact in the IL vmPFC to concurrently modulate anxiety and working memory in potentially dangerous situations. Moreover, in the mixed drug condition, the anxiogenic aversive learning enhancement effects of norBNI were counteracted by the anxiolytic and aversive learning disruption effects of pirenzepine in the the elevated plus-maze. More striking however, were the synergistic effects of the mixed drug infusions on spontaneous working memory performance in the Y-maze. In the Experiment 5 Discussion, we suggested that the profound disruptive effects of the mixed drug infusions on working memory performance likely also reflected disruptive effects on some attentional mechanisms related to active working memory. To assess that possibility, we conceptualized two 'attentional' measures in the Y-maze: same arm returns and cumulative arm returns (see Experiment 5 Discussion). The mixed drug infusions also exacerbated these measures,

which suggested to us that attentional mechanisms could be assessed independent of spontaneous working memory performance.

Although the Experiment 5 data certainly suggest interactivity between kappa opioid and muscarinic receptors in the IL vmPFC in the concurrent modulation of anxiety and spontaneous working memory, we can now only speculate as to the neuromechanisms involved. In addition, the Experiment 5 data do not offer any additional clues to explain the aforementioned discrepencies between the two (separate) receptor drug effects on the direction of the correspondence between anxiety and working memory (see above). We can now, however, offer a tentative explanation. Perhaps the kappa drug effects observed in Experiments 2 and 3 were mediated primarily through interactivity with DA mechanisms in the IL vmPFC. Furthermore, acute DA activation in the vmPFC has been primarily associated with aversive events and anxiety-like responses (Broersen, Heinsbroek, de Bruin, Laan et al., 1995), and working memory disruption (Arnsten & Goldman-Rakic, 1998; Murphy, Arnsten, Goldman-Rakic et al., 1996; Murphy, Arnsten, Jentsch et al., 1996). Thus it appears reasonable to suggest that DA receptors influence associative working memory in aversive situations. In this way, kappa – DA receptor interactions might primarily influence anxiety and associative working memory.

In addition to these possibilities, we can suggest that M1 receptors might primarily influence prospective attentional processing associated with working emotional set and anxiety. This hypothesis is in keeping with Sarter and Bruno's proposal that prolonged disruption of cholinergic transmission in the PFC results in attentional deficits (Sarter & Bruno, 1999). Moreover, oftentimes working memory disrupting effects of muscarinic antagonist infusions in the vmPFC have been reported as disruptive effects on attention or response inhibition (selection) (Broersen, Heinsbroek, de Bruin, Uylings et al., 1995; Dunnett, 1990; Herremans, Hijzen, & Olivier, 1997; Herremans et al., 1996), especially in delayed discrimination tasks. These data, to some degree at least, support

the notion that muscarinic receptors in the IL vmPFC may have much more widespread influences on attentional processing, than previously suggested. In this respect, norBNI infusions alone likely interacted with DA mechanisms to exert concurrent influences of anxiety and working memory, and pirenzepine infusions alone likely disrupted attentional processing related to working emotional set and anxiety (i.e., perhaps precluded attentional biases to threat signal, which would also show up as perturbed working memory). But the 10 nmol norBNI/10 nmol pirenzepine drug mix infusions likely concurrently involved DA interactions (see above) to produce the observed counteractive effects on anxiety, as well as synergistic effects on working memory (norBNI primarily disrupted *retrospective* attentiontional processing related to associative working memory) (Fuster, 1997), and working emotional set (pirenzepine primarily disrupted *prospective* attentional processing related to working set) (Fuster, 1997). These hypotheses are speculative, however, thus require further study for verification (see below).

A final question remains as to how the drug manipulations in Experiments 2, 3, 4 and especially 5, may have impacted on our hypothesized hippocampus – orbitomedial PFC – entorhinal cortex re-entrant circuit. Before discussing some possibilities, however, relationships between attention, working memory and anxiety, and the neurocircuits that might subserve these functions, warrant further discussion.

3.3. Another look at associative working memory/set, attentional processing and anxiety

It should be mentioned that throughout the present Discussion (and in the Experimental Section), there are some terms used, from time to time, to describe certain phenomena. Given the unpalatable nature of some descriptive terms to some research disciplines (e.g. 'automatic' processing), where appropriate, such terms are duly noted and accompanied with explanatory captions. During the course of our research endeavors, we became increasingly aware that the

concepts of associative working memory, attentional processing and anxiety were undergoing rapid change. Of particular relevance is the continuing development of the 'umbrella' concept – 'executive function'. Executive function is a neuropsychological concept that originally evolved from the cognitive dysfunctional syndrome observed among individuals with frontal lobe damage (Denckla, 1993; Fuster, 1997; Glosser & Goodglass, 1990; Lhermitte, Pillon, & Serdaru, 1986; Shallice, 1982; Stuss & Gow, 1992). There is, however, on-going debate as to the nature and definition of executive functions. Eslinger (1996) reported that a working committee on executive function strongly agreed that executive functions do not refer to basic cognitive processes such as sensation, perception, *motor activation*, *attention*, or *memory*, but do refer to psychological processes such as self-regulation, behavioural sequencing, flexibility of thought or responding, response inhibition, planning, and organization of behaviour (Eslinger, 1996; Tannock, 1998). Nevertheless, executive "dysfunction" remains commonly refered to as a neuropsychological weakness (hypothesized to result from dysfunction of the frontal lobes and/or its interconnected regions) that involves difficulties with selective and sustained *attention*, verbal and non-verbal response inhibition, strategic memorization, the organization, self-monitoring, planning and sequencing of complex behaviours, as well as spatio-temporal orientation and management (Baddeley, 1995, 1996, 1998a, 1998c; Baddeley & Della Sala, 1996; Barkley, 1997; Bronowski, 1977; Denckla, 1991, 1993; Fuster, 1989, 1997; Knights, Grabowecky, & Scabini, 1995). In other words, the attentional control of associative working memory and working set. The important point here is that dysfunctional neuronal systems in the prefrontal areas of the brain result in several cognitive deficits that primarily centre around attentional deficits.

We also became aware that, whereas the concepts of executive function were once squarely planted within the realm of cognitive psychology and neuropsychology in human research, these concepts are now also firmly embedded in neuroscience and animal research. Moreover, many

researchers have suggested that attentional control appears to be the central core of executive functions (Baddeley, 1995, 1998a, 1998b, 1998c; Baddeley, Cocchini, Della Sala, Logie, & Spinnler, 1999; Baddeley & Della Sala, 1996; Fuster, 1997), particularly, as we have seen, with respect to associative working memory and working emotional set (Fuster, 1997; van den Hout & Barlow, 2000; Wall & Messier, 2001a). This hypothesis has far reaching implications, particularly with respect to the on-going debate over distinctions between automatic (outside of awareness, unconscious or implicit) and effortful (controlled) attentional processing (Pashler, 1998). *

* It is obvious that most information processing occurs outside of our awareness, e.g., we are not 'conscious' of the motor set program that directs our tennis swing, nor of the innumerable computations that organize the simplest visual percept (Lang, Davis, & Ohman, 2000). The term 'automatic' in the sense that we have used it in this Discussion and in the accompanying manuscripts really has nothing to do with the term 'automaticity' as conceptualized by cognitive psychologists. Researchers have had such a problem with the term 'automatic' that new terms have been coined in efforts to describe the type of attentional processes that occur 'unconsciously' (or in other words – beyond conscious control), such as 'preattentive or preconscious processing'. It would appear that the debate is endless, because it merely argues semantics. Consider for a moment the term 'controlled attentional processing' – this is surely a misnomer – processing occurs *outside of our awareness* – thus how is it possible that we ever came to the understanding that we 'control' processing? Another thorny issue to the proponents of controlled attentional processing is the notion that attentional processing lies at the heart of executive control functions (Baddeley, 1995; Baddeley & Della Sala, 1996; Fuster, 1997). It would be quite obviously difficult to conceptualize something like 'controlled executive function processing'. Yet another issue lies in the term 'inhibitory attentional control'. It would be near impossible to conceive of the term 'controlled inhibitory attention'. Finally, we suggest that cognition can be viewed as the psychological act of thinking, but thinking, for reasons stated above, should not be viewed as processing. Certainly, controlled thinking means that we can control what we think about, and controlled attention means we can control (under normal circumstances) what we attend to, but the mechanisms of processing within the brain's complex associative network circuitry are quite frankly – beyond control. For those readers interested in a more in depth and provokative discussion of these issues, refer to (Pashler, 1998), pp. 357-398.

We are not at this point advocating the notion that attentional processing, working memory and anxiety induction are one and the same concept. We are, however, suggesting that disorders of attention (i.e., in the context of executive dysfunction) likely lie at the core of many psychopathological disorders (Sarter & Bruno, 1999). In the next section, three hypothesized neural circuits are discussed that have been proposed to underly possible relations between parasympathetically-mediated autonomic tone, attention and anxiety. These brain circuits are discussed in relation to our hypothesized orbitomedial PFC – hippocampal re-entrant circuit (outlined in the Introduction), Sarter and colleagues' basal forebrain NBM – cortical cholinergic

attentional circuit (Sarter & Bruno, 1999; Sarter et al., 2001), Berntson and colleagues' NBM – medial PFC cholinergic autonomic/anxiety circuit, as well as McNaughton and Gray's revised septo-hippocampal anxiety circuit (Gray & McNaughton, 2000; McNaughton, 1997; McNaughton & Gray, 2000). Our intention is to provide a comprehensive analysis, drawn from diverse sources, of possible afferent influences on the orbitomedial PFC – hippocampus re-entrant circuit in the attentional control of working memory and anxiety.

3.3.1. Hypothesized brain circuits in the attentional control of working memory and anxiety

Our knowledge of the possible neuromechanisms underlying the attentional control of associative working memory and anxiety stems mainly from experimental work with animals, in which pharmacological, neuroanatomical, and neurosurgical tools are used to define the neural structures (and connective pathways) that are crucial to these executive functions (Lang et al., 2000; Lang & Maser, 2000). Recently, however, converging experimental evidence from human neuroimaging and neuropsychological studies with infrahuman studies from a wide range of neuroscience disciplines, have led to the identity of three strikingly similar (common) brain circuits that are thought to subserve executive, social, attentional, emotional (anxiety-related) and motivated behaviour in humans and animals (Damasio, 1998; Devinsky, Morrell, & Vogt, 1995; Masterman & Cummings, 1997; Posner & Petersen, 1990; Spyer, 1989). First, the 'central autonomic network' is proposed to be an integrated circuit of an internal regulation system through which the brain controls visceromotor, neuroendocrine, and behavioural responses critical to goal-directed behaviour and adaptability (Benarroch, 1993, 1997). The central autonomic network comprises the anterior cingulate, insular and ventromedial PFC, autonomic sub-cortical nuclei (the central nucleus of the amygdala, the paraventricular and related hypothalamic nuclei, periaquaductal gray), and autonomic brainstem nuclei (Benarroch, 1993, 1997; Thayer & Lane, 2000), for review). Second, the 'anterior executive region' is hypothesized to assess the motivational content of external/internal

stimuli (representations) and regulate context-dependent behaviours (Devinsky et al., 1995). The anterior executive region (sometimes termed 'the rostral limbic system') comprises the anterior, insular and orbitomedial PFC, the amygdala, periaquaductal gray and ventral striatum (including nucleus accumbens), and autonomic brainstem nuclei (Devinsky et al., 1995). Third, Damasio's 'emotion circuit' consists of the anterior cingulate, insular and orbitoventromedial PFC, the amygdala, periaquaductal gray and basal ganglia, and autonomic brainstem nuclei (Damasio, 1998).

These hypothesized brain circuits are also proposed to exert functional links between the central and autonomic nervous systems, particularly in the facilitation of executive functions associated with response organization and selection (prospective planning), and the modulation of psychophysiological resources in attention and emotion (Friedman & Thayer, 1998a, 1998b; Thayer & Lane, 2000). The key common feature among them is the reciprocal influences between the cardiovascular control centres in the brainstem, and the orbitoventromedial PFC associative network (described in the Introduction). Moreover, several investigators have suggested that a relative reduction in the parasympathetically (vagal brainstem)-mediated cardiovascular tone is consistent with the cardiac symptoms of panic anxiety, as well as the psychological symptoms of attentional dysfunction, poor emotional control and behavioural inflexibility (Friedman & Thayer, 1998b; Thayer & Lane, 2000).

In a recent study of possible relationships between worry in GAD patients and dysregulated parasympathetic vagal tone, Thayer and colleagues demonstrated that a reduced vagal tone in GAD patients during worry represented a breakdown of the inhibitory influences (mediated by their proposed 'autonomic' circuit) that allow for efficient self-regulation, such as the shifting of attentional focus (Thayer, Friedman, & Borkovec, 1996). It will be recalled that GAD is characterized by persistent and excessive unrealistic worry, and these dysfunctional states are supported by dysregulated attentional mechanisms, such as hypervigilance and scanning, as well as

attentional bias for threat information (van den Hout & Barlow, 2000). This hypothesis rests on the assumption that relative sympathetic arousal levels, modulated by parasympathetic vagal tone on cardiovascular function, are related to relative attentional biases in anxiety-provoking situations. Put in another way, a reduced parasympathetic tone results in a disinhibited sympathetic tone, actions presumably leading to heightened arousal and loss of attentional control (e.g. hyperfocused on behaviourally relevant threat cues). Given these hypothesized relationships between dysfunctional attentional bias, disinhibited sympathetic arousal, and anxiety (excessive worry and decline in executive function), it is not clear why these proposed 'autonomic' brain circuits ignore the influences of ascending cholinergic systems to attentional processing.

Two salient features of the aforementioned hypothesized autonomic brain circuits emerged: (1) all three circuits strikingly overlap in the orbitomedial and anterior cingulate PFC, as well as in subcortical and brainstem autonomic nuclei, and (2) all three circuits remarkably omit the basal forebrain and reticular cholinergic nuclei, and the hippocampal formation. Figure 29 depicts Brodmann's map of the relevant orbitoventromedial areas of the PFC in the human brain (Fuster, 1997). The orbitomedial PFC in humans comprises mainly the ventral Figure 2. In addition, a review of Figure 1 shows the dense reciprocal connection patterns between the orbitomedial PFC and the visceral brainstem, as well as with autonomic sub-cortical nuclei (hypothalamus and amygdala), all hypothesized to comprise the aforementioned autonomic brain circuits. Moreover, the dense reciprocal connection patterns between the orbitomedial PFC and the basal forebrain ACh nuclei, as well as with the hippocampal formation (Figure 1), has prompted other investigators to propose an alternative brain circuit that not only includes basal forebrain ACh nuclei, but is also thought to mediate interactions between autonomic arousal, and the cognitive aspects of anxiety (Berntson et al., 1998).

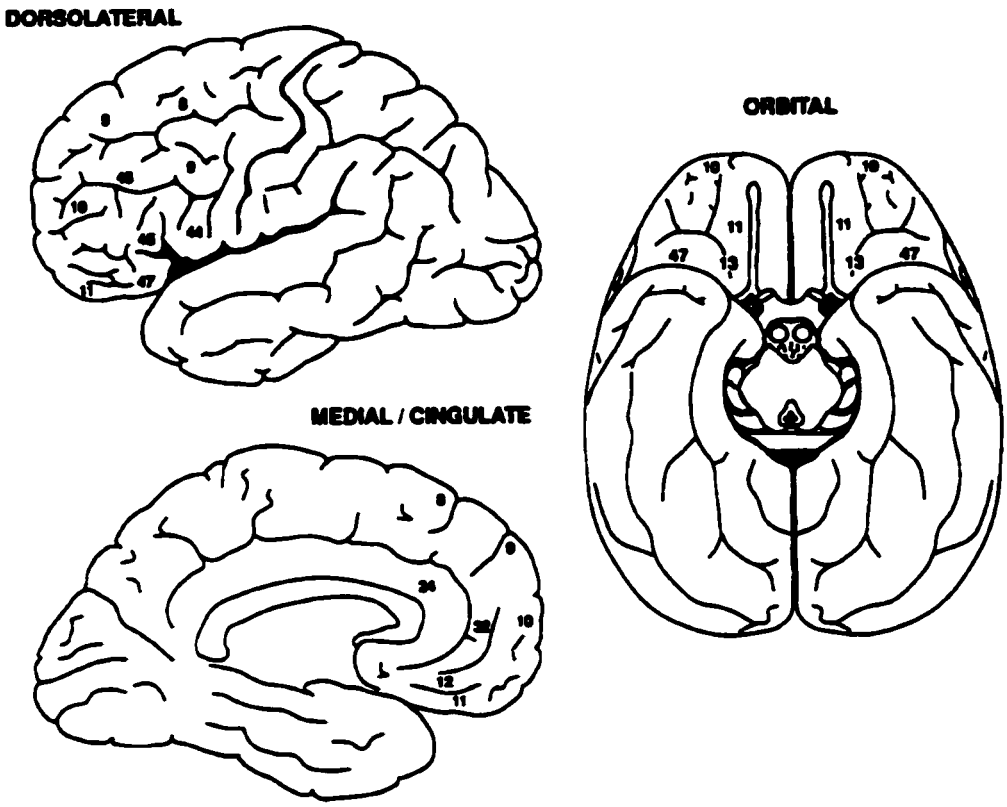

Figure 29 Lateral, ventral (orbital), and medial views of PFC. #'s are Brodmann's cytoarchitectonic labels in PFC. (adopted from (Fuster, 1997) p. 173).

The first of the cholinergic circuits proposed to underly interactivity between autonomic arousal and anxiety induction consists of reciprocal NBM – vmPFC cholinergic projections (Berntson et al., 1998). Berntson and colleagues proposed that viscero-emotional autonomic reactivy is mediated by this ACh pathway, such that acutely hyperactived ACh activity in the vmPFC in rats, resulted in heightened cardiovascular defensive reactions that were associated with anxiety-like behaviour (Berntson et al., 1996; Berntson et al., 1998). This hypothesis is also importantly based on the assumption that the autonomically-regulated cardiovascular tone is tightly bound to ascending attentional influences and anxiety. If we take Berntson and colleagues' hypothesis together with Sarter and colleagues' consistent demonstration that the NBM – PFC cholinergic pathway is primarily concerned with attentional processing (Sarter & Bruno, 1997a, 1999, 2000; Sarter et al., 1999; Sarter et al., 2001), then we can begin to appreciate the impact that the muscarinic drug manipulations might have had on the NBM – vmPFC neurocircuitry to produce the observed behavioural results in Experiments 4 and 5.

Gray's (1982) septo-hippocampal theory of anxiety represents an alternative basal forebrain cholinergic circuit that was proposed to mediate the induction and expression of fear and anxiety (Gray, 1982). Although the original and current versions of Gray's hypothesis do not include the term 'cholinergic' in the description of the proposed circuit (Gray, 1982; Gray & McNaughton, 2000; McNaughton & Gray, 2000), most investigators refer to the septo-hippocampal pathway as the septo-hippocampal cholinergic system. It is interesting that at the time Gray originally introduced his septo-hippocampal theory of anxiety, the belief that the septo-hippocampal system (and particularly the hippocampus) was primarily concerned with memory formation was firmly entrenched in the research community. In light of this, Gray's argument that the hippocampus was more concerned with anxiety modulation, rather than memory consolidation, was a radical proposal (McNaughton & Gray, 2000).

Briefly, Gray's hippocampal theory of anxiety primarily proposes that (a) the septo-hippocampal circuit can be understood as a behavioural inhibition system (BIS), (b) the behavioural effects of anxiolytic drugs are best described as impairment of the BIS, (c) the most important common neural actions of anxiolytic drugs are to impair the control of 'theta' electrical activity in the septo-hippocampal system, and (d) changes in septo-hippocampal function (particularly theta activity) can underly both normal and pathological changes in anxiety (McNaughton & Gray, 2000). Most importantly, Gray and Mchaughton continue to assert that the function of the hippocampus is not solely to control anxiety, rather, the septo-hippocampal system is proposed to act as a simple comparator of imputs (McNaughton & Gray, 2000). These inputs presumably code for simple events, predictions about expected events, and importantly, predictions for upcoming steps in a currently executing motor program (motor set monitor), and for future steps (plans) (McNaughton & Gray, 2000). Discrepencies between currently executing set activity and current input, produce immediate 'inhibitory' effects on behaviour (within the BIS) to resolve such conflicts, but when hyperarousal (presumed to result from hyperactivated ascending reticular monoamine projections) accompanies serious conflicts, 'hypermnesia', attentional disruption and dysfunctional anxiety often follows. Thus persistent hippocampal hyperactiviy has been proposed by McNaughton to be a possible neurobiological cause of anxiety disorder (McNaughton, 1997). Moreover, it should be noted that Gray and McNaughton's septo-hippocampal theory of anxiety conceptualizes anxiety as more of a cognitive (executive) disorder, rather than as a disorder of emotion (McNaughton, 1997).

We have also proposed that the hippocampus serves as an attentional monitor (or associative comparator), as an integral part of our hypothesized orbitomedial PFC – hippocampal – entorhinal cortex re-entral circuit (Wall & Messier, 2001a). At this point in the discussion, it seems reasonable to suggest that the observed behavioural effects of muscarinic drug infusions in the IL vmPFC, in Experiments 4 and 5 likely stemmed from disruptions in the hippocampus – vmPFC circuit.

Evidence for functional connectivity in the orbitomedial PFC – hippocampus circuit in relation to influences from the basal forebrain, as well as to influences on autonomic responses, operant learning and working memory, is discussed next.

3.3.2. Evidence for functional connectivity in the orbitomedial PFC – hippocampus circuit

So far we have seen that the orbitomedial PFC and the hippocampal formation can seperately influence autonomic responsivity (Introduction section of Experiment 2), working memory, attentional processing and anxiety-related behaviour in rodents and primates (Wall & Messier, 2001a). The principal aims of this section are to provide an overview of studies that have examined the effects of concomitant manipulations (or conducted simultaneous or comparative electrophysiological recordings) in both brain sites on autonomic responses and memory performance in rats.

Functional interactivity in autonomic regulation. We have seen that electrical (or chemical) stimulation of the ventral hippocampus in rats depresses heart-rate, blood pressure and respiration (Ruit & Neafsey, 1988). Recall that pyramidal projections from the ventral CA1-field and subiculum project to the vmPFC in rats (Ruit & Neafsey, 1990). Moreover, a subset of projection neurons in the IL area of the vmPFC, which project to the autonomic brainstem, are innervated by hippocampal pyramidal projections (Neafsey et al., 1986; Ruit & Neafsey, 1990). It is interesting that electrical stimulation of the IL vmPFC also typically elicits a bradycardic and vasodepressor response in rats (Hardy & Holmes, 1988; Owens & Verberne, 1996; Verberne, 1996; Verberne & Owens, 1998). With this in mind, Ruit & Neafsey demonstrated that IL PFC lesions blocked the cardiovascular depressor effects of ventral hippocampal activation (Ruit & Neafsey, 1988). These data demonstrate functional interactivity between both brainsites in the hippocampus – vmPFC circuit in the regulation of autonomic responsivity, by specifically enhancing parasympathetic cardiovascular tone. Moreover, the hypothesis that the autonomic brainstem is intimately connected

with the orbitomedial PFC in the parasympathetically-mediated tonic control of cardiovascular responsivity to threat, attentional processing and anxiety induction, begins to make sense in light of the aforementioned proposed 'autonomic' brain circuits.

Behavioural and pharmacological evidence. Co-operativity between the hippocampal formation and the orbitomedial PFC in memory, arousal, emotional and attentional processing has long been suspected (Becker & Olton, 1980; Becker, Olton, Anderson, & Breitinger, 1981; Becker, Walker, & Olton, 1980; Buzsaki et al., 1988; Day, Damsma, & Fibiger, 1991; Gray, 1982; Gray, 1983; Inglis & Fibiger, 1995; Metherate, Cox, & Ashe, 1992; Vanderwolf, Buzsaki, Cain, Cooley, & Robertson, 1988). In fact, the broad range of stimuli that concomitantly enhance orbitomedial PFC and hippocampal ACh release has prompted the suggestion that basal forebrain cholinergic nuclei regulate and co-ordinate different aspects of attention in both brain sites (Acquas et al., 1996). Moreover, basal forebrain cholinergic nuclei (NBM and medial septum) have also been shown to regulate attentional aspects of associative working memory in rats and primates (Baxter, Bucci et al., 1999; Baxter, Frick et al., 1999; Baxter, Gallagher, & Holland, 1999; Baxter et al., 1997; Himmelheber, Sarter, & Bruno, 1997; Robbins & Everitt, 1995; Sarter & Bruno, 1997a, 1998a, 1999, 2000; Sarter et al., 1999). Data from some animal studies that appear to lend support to our hypothesis that the hippocampal formation may serve as a comparator mechanism in the orbitomedial PFC associative memory network, are presented next.

Working memory. Spatially mediated working memory in the 8-arm radial maze appears to at least partially depend on the ventral hippocampus – vmPFC circuit in rats (Floresco et al., 1997; Seamans et al., 1998). Consistent with our hypothesis that the hippocampal formation may serve as an attentional comparator/monitor in association with the orbitomedial PFC associative memory network, Floresco et al. demonstrated that asymmetric disconnection of the ventromedial PFC – ventral hippocampus circuit in rats disrupted a spatial delayed response task in the 8-arm radial

maze (Floresco et al., 1997). These investigators hypothesised that the hippocampus – ventromedial PFC cirtcuit is responsible for holding (sustained activation) and subsequently using information acquired before a delay, to locate food on the radial arm maze after the delay (Floresco et al., 1997). It was suggested that following a 30 minute delay, associative representations of locations might be accessed from the hippocampus and maintained in the vmPFC through re-entrant circuits between the hippocampus – vmPFC – entorhinal cortex (Floresco et al., 1997).

Seamans, Floresco and Phillips (1998) extended these findings by showing that unilateral lidocaine injections in the ventral hippocampus combined with microinjection of the dopamine D1 receptor antagonist, SCH-23390, in the contralateral vmPFC, also severely disrupted performance in their spatial working memory task in the 8-arm radial maze. These data provided some behavioural evidence that ventral tegmental DA mechanisms directly influence the hypothesized ventral hippocampal – ventromedial PFC – entorhinal cortex re-entrant circuit during working memory and working 'set' associative processing (Wall & Messier, 2001a).

Recognition memory. Ramus and Eichenbaum (Ramus & Eichenbaum, 2000) and Young et al. (Young, Otto, Fox, & Eichenbaum, 1997) demonstrated that the electrophysiological cellular activity in the orbital PFC and parahippocampal cortex (perirhinal, entrorhinal, postrhinal), respectively, is correlated with performance in a continuous odor-guided delayed non-matching to sample working memory task in rats. In fact the electrophysiological neuronal activity in both cortical areas reflected odor-selective responses, stimulus-selective match enhancement and match supression, as well as odor-selective firing during the delay interval (thus all indentifiable trial events in the odor recognition memory task) (Ramus & Eichenbaum, 2000; Young et al., 1997). There were some important differences between the perirhinal/entorhinal and orbital PFC cellular responses however. For example, whereas half as many orbital PFC cells showed odor-selective responses than entorhinal cortical cells, approximately twice the proportion of orbital PFC odor-

selective cells appeared to code the match/nonmatch contingency compared to the odor-selective perirhinal and entorhinal cells (Ramus & Eichenbaum, 2000). Moreover, Otto and Eichenbaum (Otto & Eichenbaum, 1992) had previously shown that whereas orbital PFC lesions impaired the acquisition and performance of (under conditions of high inter-item interference) the odor recognition task, combined perirhinal and entorhinal cortical lesions did not impair the acquisition but disrupted performance at longer delays across all levels of interference, in rats. These data taken together suggest that the entorhinal cortex and orbital PFC play complimentary roles in odor recognition memory in rats. It is, however, tempting to suggest that the reciprocal olfactory cortex connections with the associative orbitomedial PFC positions the hippocampus – orbitomedial PFC – entorhinal cortex re-entrant circuit to influence odor recognition memory.

Operant learning. In a similar fashion to the asymmetric disconnection method used by (Floresco et al., 1997), Izaki et al. demonstrated that asymmetric disconnection of the ventral hippocampus – orbitomedial PFC circuit disrupted operant bar-press learning in rats (Izaki, Hori, & Nomura, 2000). It is interesting that Doyere et al. reported that LTP in the ventral hippocampus – ventromedial PFC circuit is also associated with associative learning in rats (Doyere, Burette, Negro, & Laroche, 1993). These data prompted Izaki et al. to suggest that non-spatial information is relayed from the hippocampus to the ventromedial PFC to facilitate learning new associations and events (Izaki et al., 2000). This hypothesis is consistent with the notion that the hippocampus relays information related to novelty and discrepency detection to the orbitomedial PFC (Knight & Nakada, 1998; Sybirska et al., 2000).

In summary, it is now possible to suggest that our behavioural results from Experiments 2, 3, 4 and 5 resulted from drug effects on the hippocampus – orbitomedial PFC circuit, that were mediated by manipulating NBM – orbitomedial PFC cholinergic influences.

3.4. Concurrent modulation of anxiety, memory and attention in the orbitomedial PFC – hippocampus circuit

We have argued that the hippocampus serves as an attentional monitor (associative comparator) in the orbitomedial PFC – hippocampus associative working memory circuit (Wall & Messier, 2001a). Although there are growing data to support this hypothesized associative working memory/set model, it is still, nevertheless, speculative. In this section we discuss possible ways by which the kappa and/or M1 receptor drug infusions may have impacted this 'associative working memory circuit' to produce the observed effects in Experiments 2-5.

It will be recalled that, in Gray and McNaughton's hippocampal model of anxiety, the central function of the hippocampus is to serve as an information input comparator (i.e., monitors for discrepencies between currently unfolding behavioural sets and current incoming viscero-sensory information, as well as for expectancy disconformations) (Gray & McNaughton, 2000). Central to their theory are two important assumptions: (a) the hippocampus (within the BIS) also serves as an effector to alter currently unfolding working cognitive/emotional/motor sets to resolve conflicts or discrepencies, and (b) the hippocampus also recruits autonomic resources (arousal), particularly in threatening situations to effect alterations in currently operating emotional-behavioural set activity (McNaughton & Gray, 2000). However, although the septo-hippocampal theory of anxiety places the hippocampus as an imformation monitor/discrepency detector, it does not offer any plausible mechanisms (except of course, the BIS) by which the hippocampus might effect set alterations. Moreover, perhaps (as we suggest) the hippocampus does not effect set alterations at all, but signals the need for such alterations in the associative orbitomedial PFC network (Wall & Messier, 2001a).

Given the aforementioned evidence showing functional connectivity in the orbitomedial PFC – hippocampus circuit with respect to autonomic influences (Hardy & Holmes, 1988; Owens & Verberne, 1996; Verberne, 1996; Verberne & Owens, 1998), conditioned learning (Izaki et al.,

2000) and working memory (Floresco et al., 1997; Seamans et al., 1998), we suggest that the hippocampus monitors for discrepencies between immediate past and current representations in the orbitomedial PFC associative network, as well as between currently active working emotional/behavioural sets in the orbitomedial PFC and incoming information from the entorhinal cortex (Wall & Messier, 2001a). This model, of course, rests on the asumption that the hippocampus and orbitomedial PFC are in constant communication with one another.

Ventral hippocampal/subicular projections to the orbitomedial PFC are primarily glutamatergic excitatory inputs (Jay, Glowinski, & Thierry, 1995). These investigators have shown that electrical stimulation of the ventral hippocampus enhances GLU and elctrophysiological activity in the vmPFC (Jay, Glowinski et al., 1995). Moreover, these excitatory effects in the vmPFC could be blocked by either DA agonist or NMDA antagonist infusions (Jay, Burette, & Laroche, 1995; Jay, Glowinski et al., 1995). If we take these data together with data showing that electrical stimulation in the ventral hippocampus enhanced parasympathetic tone, and these effects were blocked by IL vmPFC lesions (Hardy & Holmes, 1988; Owens & Verberne, 1996; Verberne, 1996; Verberne & Owens, 1998), we can make the tentative suggestion that excitatory GLU input to the vmPFC from the hippocampus serves to signal the need for modifying currently active associative working emotional set activity.

Further indirect evidence in support of this hypothesis stems from in vivo microdialysis data showing that D1 receptor activation in the vmPFC reduced GLU release in rats (Abekawa, Ohmori, Ito, & Koyama, 2000). These investigators suggested that prolonged dopaminergic hyperactivity in the vmPFC may lead to prefrontal GLU hypofunction in rats (Abekawa et al., 2000). Moreover, M1 receptor activation following McN-A-343 infusions in the vmPFC enhanced GLU concentrations in rats, and these GLU-releasing effects were blocked by pirenzepine infusions (Sanz, Exposito, & Mora, 1997). It is therefore tempting to suggest that M1 activation in the vmPFC may have exerted

anxiogenic effects in Experiment 4c through its effects on hippocampal afferent GLU release. This hypothesis is highly speculative, however, owing to data showing that GLU afferents to the vmPFC stem from the hippocampus (Jay, Glowinski et al., 1995), the dorsomedial thalamus (Gigg, Tan, & Finch, 1994), and other cortical areas (Salt, Meier, Seno, Krucker, & Herrling, 1995).

In Summary, it is conceivable that the differential correspondence between working memory and anxiety following kappa and M1 receptor drug infusions (see above) may have occurred through bi-directional interactivity with hippocampal GLU afferents in the orbitomedial PFC.

3.5. Future directions: Testable hypotheses

The aforementioned electrophysiological, pharmacological and behavioral experimental data not only lend some support to the functional connectivity of the hippocampal – orbitomedial PFC pathway, but also to our hypothesis that the behavioural data from Experiments 2-5, resulted from drug effects on the integrity of the orbitomedial PFC – hippocampus associative memory circuit. There are a number of testable hypotheses amenable to examining this proposal. First, we speculate that no new (or alterations of existing) working representations/sets are formed or altered in the orbitomedial PFC unless signaled by the hippocampus. This implies that the orbitomedial PFC and hippocampus are in constant communication in an awake behaving animal. Second, we speculate that the basal forebrain ACh and VTA DA systems profoundly influence the associative working set dynamics within the hippocampal – orbitomedial PFC circuit, particularly with respect to anxiety-related 'emotional' behavior and memory.

DA-M1 interactions in the concurrent modulation of anxiety and memory. Contralateral cannulae placements modeling the asymmetric disconnection procedure reported by Floresco et al. (Floresco et al., 1997) can be used to test possible roles of the hippocampal – orbitomedial PFC circuit in the concurrent modulation of anxiety and active working memory. Primarily, the possibility that DA

and M1 receptor mechanisms might interact in such regulatory activity can be directly tested in the paradigm currently used in our laboratory. Accordingly, one can investigate the question of whether mesocortical DA mechanisms in the orbitomedial PFC can counteract M1 drug effects on anxiety and active working memory in the ventral hippocampus, or vice-versa.

In summary, DA – ACh, as well as cortical site interactions can be tested in a number of behavioural paradigms using bilateral asymmetric microinfusion techniques in the hippocampal – orbitomedial PFC – entorhinal cortex re-entrant circuit.

3.6. Conclusions and Relevance to the Human Condition

Several investigators from diverse neuroscience and psychological disciplines emphasize the need for valid animal models of psychopathology to develop a better understanding of the relationships between dysfunctional behavioural patterns and aberrant underlying neural mechanisms. Animal models attempt to capture various features of the human condition, from behavioural and physiological changes that are indicative of emotional states (e.g., defensive behaviours) to similarities in etiology, and effects of various pharmacological and behavioural interventions (Kavaliers & Ossenkopp, 2001; Troisi, 1999). The recent development of ethological animal models to study defense-related behaviour has contributed a great deal to our understanding of the relationship between aberrant defensive behaviours and psychopathology in humans (Blanchard et al., 2001; Lang & Maser, 2000).

To this end, detailed behavioural analysis in one species and paradigm has also been stressed (Blanchard et al., 2001). Blanchard et al. recently reiterated two long-standing cardinal rules that should govern the development of animal models for human behaviour: (1) *meaningful comparisons between species are based not upon the formal characteristics of behaviour, but upon its causal mechanisms and functional outcomes.....*, (2) *validity of interspecific generalization*

cannot exceed the reliability of intraspecific analysis. Significant comparisons of a particular type of behaviour in two different species is impossible unless and until the behaviour has been adequately analyzed in each species by itself (Blanchard et al., 2001). In keeping with these general principles, we have extensively studied mouse behavioural patterns in the elevated plus-maze. All the while however, we kept in mind the general principle that it is not the apparatus that is so important to understand, but the elicited behaviours in mice placed in the appararatus, and the outcome of those behaviours. In our view, once the behavioural patterns are understood, and before trying to generalize to other species, the tools of neuroscience can be used to explore the neuromechanisms underlying the behavioural patterns under investigation.

It is true that we and others have argued for the homologous nature of risk-assement defense-related behaviour in animals and humans (Blanchard et al., 2001; Lang et al., 2000; Wall & Messier, 2001b). This is because risk assessment behaviours have been extensively studied in a variety of different species in different situations, and have been found to correlate well with defense-related autonomic arousal and anxiety (see (Lang et al., 2000) for review). We and others have also argued for common neural systems underlying relations between memory, attention, arousal and anxiety (Berntson et al., 1998; Clement & Chapouthier, 1998; McNaughton, 1997; McNaughton & Gray, 2000; Thayer & Lane, 2000; van den Hout & Barlow, 2000). The development of these various theoretical perspectives would not have progressed without the use of well-understood behavioural assays.

We think our systematic approach to the investigation of the role of the orbitomedial PFC in the concurrent modulation of anxiety, working memory and attentional processing is contributing to the understanding of the complex nature of prefrontal neural circuitry and the complex executive functions subserved by this associative cortical network.

4.0 REFERENCES

Abekawa, T., Ohmori, T., Ito, K., & Koyama, T. (2000). D1 dopamine receptor activation reduces extracellular glutamate and GABA concentrations in the medial prefrontal cortex. *Brain Res, 867*(1-2), 250-254.

Abercrombie, E. D., Keefe, K. A., DiFrischia, D. S., & Zigmond, M. J. (1989). Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J Neurochem, 52*(5), 1655-1658.

Ackermann, H., Daum, I., Schugens, M. M., & Grodd, W. (1996). Impaired procedural learning after damage to the left supplementary motor area (SMA). *J Neurol Neurosurg Psychiatry, 60*(1), 94-97.

Acquas, E., Wilson, C., & Fibiger, H. C. (1996). Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: effects of novelty, habituation, and fear. *J Neurosci, 16*(9), 3089-3096.

Adamec, R. E., Kent, P., Anisman, H., Shallow, T., & Merali, Z. (1998). Neural plasticity, neuropeptides and anxiety in animals -- implications for understanding and treating affective disorder following traumatic stress in humans. *Neuroscience & Biobehavioral Reviews, 23*, 301-318.

Adamec, R. E., & McKay, D. (1993). Amygdala kindling, anxiety, and corticotrophin releasing factor (CRF). *Physiol Behav, 54*(3), 423-431.

Adamec, R. E., Shallow, T., & Budgell, J. (1997). Blockade of CCK(B) but not CCK(A) receptors before and after the stress of predator exposure prevents lasting increases in anxiety-like behavior: implications for anxiety associated with posttraumatic stress disorder. *Behav Neurosci, 111*(2), 435-449.

Agmo, A., & Belzung, C. (1998). The role of subtypes of the opioid receptor in the anxiolytic action of chlordiazepoxide. *Neuropharmacology, 37*(2), 223-232.

Agmo, A., Galvan, A., Heredia, A., & Morales, M. (1995). Naloxone blocks the antianxiety but not the motor effects of benzodiazepines and pentobarbital: experimental studies and literature review. *Psychopharmacology (Berl), 120*(2), 186-194.

Almeida, S. S., Garcia, R. A., & de Oliveira, L. M. (1993). Effects of early protein malnutrition and repeated testing upon locomotor and exploratory behaviors in the elevated plus-maze. *Physiol Behav, 54*(4), 749-752.

Alreja, M., Wu, M., Liu, W., Atkins, J. B., Leranth, C., & Shanabrough, M. (2000). Muscarinic tone sustains impulse flow in the septohippocampal GABA but not cholinergic pathway: implications for learning and memory. *J Neurosci, 20*(21), 8103-8110.

Amaral, D. G. (1994). Hippocampal formation. In G. Paxinos (Ed.), *The rat nervous system, 2nd Edition* (2nd ed., pp. 443-494). San Diego: Academic Press.

Anand, B. K., & Dua, S. (1956). Circulatory and respiratory changes induced by electrical stimulation of the limbic system (visceral brain). *J Neurophysiol, 19*, 393-400.

Anderson, J. C., & Gerbing, D. W. (1988). Structural equation modeling in practice: A review and recommended two-step approach. *Psychological Bulletin, 103*(3), 411-423.

Andrews, N., & File, S. E. (1993). Handling history of rats modifies behavioural effects of drugs in the elevated plus-maze test of anxiety. *Eur J Pharmacol, 235*(1), 109-112.

Anisman, H., & Zacharko, R. M. (1990). Multiple neurochemical and behavioral consequences of stressors: implications for depression. *Pharmacol Ther, 46*(1), 119-136.

APA. (1987). *Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, Revised.* Washington, DC: American Psychiatric Association.

APA. (1994). *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition.* Washington, DC: American Psychiatric Association.

Arneric, S. P., Sullivan, J. P., Briggs, C. A., Donnelly-Roberts, D., Anderson, D. J., Raszkiewicz, J. L., Hughes, M. L., Cadman, E. D., Adams, P., Garvey, D. S., & et al. (1994). (S)-3-methyl-5-(1-methyl-2-pyrrolidinyl) isoxazole (ABT 418): a novel cholinergic ligand with cognition-enhancing and anxiolytic activities: I. In vitro characterization. *J Pharmacol Exp Ther, 270*(1), 310-318.

Arnsten, A. F., & Goldman-Rakic, P. S. (1998). Noise stress impairs prefrontal cortical cognitive function in monkeys: evidence for a hyperdopaminergic mechanism. *Arch Gen Psychiatry, 55*(4), 362-368.

Bachevalier, J., & Mishkin, M. (1986). Visual recognition impairment follows ventromedial but not dorsolateral prefrontal lesions in monkeys. *Behav Brain Res, 20*(3), 249-261.

Baddeley, A. (1992). Working memory. *Science, 255*(5044), 556-559.

Baddeley, A. (1995). Working Memory. In M. S. Gazzaniga (Ed.), *The Cognitive Neurosciences* (pp. 755-764). Cambridge, Massachusetts: Bradford, The MIT Press.

Baddeley, A. (1996). The fractionation of working memory. *Proc Natl Acad Sci U S A, 93*(24), 13468-13472.

Baddeley, A. (1998a). The central executive: a concept and some misconceptions. *J Int Neuropsychol Soc, 4*(5), 523-526.

Baddeley, A. (1998b). Recent developments in working memory. *Curr Opin Neurobiol, 8*(2), 234-238.

Baddeley, A. (1998c). Working memory. *C R Acad Sci III, 321*(2-3), 167-173.

Baddeley, A., Cocchini, G., Della Sala, S., Logie, R. H., & Spinnler, H. (1999). Working memory and vigilance: evidence from normal aging and Alzheimer's disease. *Brain Cogn, 41*(1), 87-108.

Baddeley, A., & Della Sala, S. (1996). Working memory and executive control. *Philos Trans R Soc Lond B Biol Sci, 351*(1346), 1397-1403; discussion 1403-1394.

Bals-Kubik, R., Ableitner, A., Herz, A., & Shippenberg, T. S. (1993). Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. *J Pharmacol Exp Ther, 264*(1), 489-495.

Bandler, R., & Keay, K. A. (1996). Columnar organization in the midbrain periaqueductal gray and the integration of emotional expression. *Prog Brain Res, 107*, 285-300.

Banich, M. T., Milham, M. P., Atchley, R. A., Cohen, N. J., Webb, A., Wszalek, T., Kramer, A. F., Liang, Z., Barad, V., Gullett, D., Shah, C., & Brown, C. (2000). Prefrontal regions play a predominant role in imposing an attentional 'set': evidence from fMRI [In Process Citation]. *Brain Res Cogn Brain Res, 10*(1-2), 1-9.

Barbas, H. (1995). Anatomic basis of cognitive-emotional interactions in the primate prefrontal cortex. *Neurosci Biobehav Rev, 19*(3), 499-510.

Barbas, H. (2000). Connections underlying the synthesis of cognition, memory, and emotion in primate prefrontal cortices. *Brain Res Bull, 52*(5), 319-330.

Barbas, H., & Blatt, G. J. (1995). Topographically specific hippocampal projections target functionally distinct prefrontal areas in the rhesus monkey. *Hippocampus, 5*(6), 511-533.

Barbas, H., Ghashghaei, H., Dombrowski, S. M., & Rempel-Clower, N. L. (1999). Medial prefrontal cortices are unified by common connections with superior temporal cortices and distinguished by input from memory- related areas in the rhesus monkey. *J Comp Neurol, 410*(3), 343-367.

Barkley, R. A. (1997). Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychol Bull, 121*(1), 65-94.

Barlow, D. H. (1988). *Anxiety and its Disorders: The nature and treatment of anxiety and panic.* New York: Guilford.

Barlow, D. H., Blanchard, E. B., Vermilyea, J. A., Vermilyea, B. B., & DiNardo, P. A. (1986). Generalized anxiety and generalized anxiety disorder: description and reconceptualization. *Am J Psychiatry, 143*(1), 40-44.

Barlow, D. H., & Lehman, C. L. (1996). Advances in the psychosocial treatment of anxiety disorders. Implications for national health care. *Arch Gen Psychiatry, 53*(8), 727-735.

Barnes, C. A., McNaughton, B. L., Mizumori, S. J., Leonard, B. W., & Lin, L. H. (1990). Comparison of spatial and temporal characteristics of neuronal activity in sequential stages of hippocampal processing. *Prog Brain Res, 83*, 287-300.

Bartoszyk, G. D. (1998). Anxiolytic effects of dopamine receptor ligands: I. Involvement of dopamine autoreceptors. *Life Sci, 62*(7), 649-663.

Baxter, M. G., Bucci, D. J., Holland, P. C., & Gallagher, M. (1999). Impairments in conditioned stimulus processing and conditioned responding after combined selective removal of hippocampal and neocortical cholinergic input. *Behav Neurosci, 113*(3), 486-495.

Baxter, M. G., Frick, K. M., Price, D. L., Breckler, S. J., Markowska, A. L., & Gorman, L. K. (1999). Presynaptic markers of cholinergic function in the rat brain: relationship with age and cognitive status [In Process Citation]. *Neuroscience, 89*(3), 771-779.

Baxter, M. G., Gallagher, M., & Holland, P. C. (1999). Blocking can occur without losses in attention in rats with selective removal of hippocampal cholinergic input. *Behav Neurosci, 113*(5), 881-890.

Baxter, M. G., Holland, P. C., & Gallagher, M. (1997). Disruption of decrements in conditioned stimulus processing by selective removal of hippocampal cholinergic input. *J Neurosci, 17*(13), 5230-5236.

Beck, A. T., Epstein, N., Brown, G., & Steer, R. A. (1988). An inventory for measuring clinical anxiety: psychometric properties. *J Consult Clin Psychol, 56*(6), 893-897.

Beck, C. H., & Fibiger, H. C. (1995). Chronic desipramine alters stress-induced behaviors and regional expression of the immediate early gene, c-fos. *Pharmacol Biochem Behav, 51*(2-3), 331-338.

Becker, J. T., & Olton, D. S. (1980). Object discrimination by rats: the role of frontal and hippocampal systems in retention and reversal. *Physiol Behav, 24*(1), 33-38.

Becker, J. T., Olton, D. S., Anderson, C. A., & Breitinger, E. R. (1981). Cognitive mapping in rats: the role of the hippocampal and frontal system in retention and reversal. *Behav Brain Res, 3*(1), 1-22.

Becker, J. T., Walker, J. A., & Olton, D. S. (1980). Neuroanatomical bases of spatial memory. *Brain Res, 200*(2), 307-320.

Belotti, M., & Galey, D. (1996). Consequences of selective blockade of septal noradrenergic afferents on anxiety and spatial working memory performance in mice. *Pharmacol Biochem Behav, 53*(3), 541-547.

Belzung, C., & Le Pape, G. (1994). Comparison of different behavioral test situations used in psychopharmacology for measurement of anxiety. *Physiol Behav, 56*(3), 623-628.

Belzung, C., Pineau, N., Beuzen, A., & Misslin, R. (1994). PD135158, a CCK-B antagonist, reduces "state," but not "trait" anxiety in mice. *Pharmacol Biochem Behav, 49*(2), 433-436.

Benarroch, E. E. (1993). The central autonomic network: functional organization, dysfunction and perspective. *Mayo Clin Proc, 68*, 988-1001.

Benarroch, E. E. (1997). The central autonomic network. In P. A. Low (Ed.), *Clinical Autonomic Disorders* (2nd ed., pp. 17-23). Philadelphia: Lippincott-Raven.

Benes, F. M., Vincent, S. L., & Molloy, R. (1993). Dopamine-immunoreactive axon varicosities form nonrandom contacts with GABA-immunoreactive neurons of rat medial prefrontal cortex. *Synapse, 15*(4), 285-295.

Beninger, R. J., Ingles, J. L., Mackenzie, P. J., Jhamandas, K., & Boegman, R. J. (1992). Muscimol injections into the nucleus basalis magnocellularis of rats: selective impairment of working memory in the double Y-maze. *Brain Res, 597*(1), 66-73.

Bentler, P. M., & Kano, Y. (1990). On the equivalence of factors and components. *Multivariate Behavioral Research, 25*(1), 67-74.

Berntson, G. G., Hart, S., Ruland, S., & Sarter, M. (1996). A central cholinergic link in the cardiovascular effects of the benzodiazepine receptor partial inverse agonist FG 7142. *Behav Brain Res, 74*(1-2), 91-103.

Berntson, G. G., Hart, S., & Sarter, M. (1997). The cardiovascular startle response: anxiety and the benzodiazepine receptor complex. *Psychophysiology, 34*(3), 348-357.

Berntson, G. G., Sarter, M., & Cacioppo, J. T. (1998). Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link. *Behav Brain Res, 94*(2), 225-248.

Bertolucci-D'Angio, M., Serrano, A., & Scatton, B. (1990a). Differential effects of forced locomotion, tail-pinch, immobilization, and methyl-beta-carboline carboxylate on extracellular 3,4- dihydroxyphenylacetic acid levels in the rat striatum, nucleus accumbens, and prefrontal cortex: an in vivo voltammetric study. *J Neurochem, 55*(4), 1208-1215.

Bertolucci-D'Angio, M., Serrano, A., & Scatton, B. (1990b). Mesocorticolimbic dopaminergic systems and emotional states. *J Neurosci Methods, 34*(1-3), 135-142.

Bertorelli, R., Forloni, G., & Consolo, S. (1991). Modulation of cortical in vivo acetylcholine release by the basal nuclear complex: role of the pontomesencephalic tegmental area. *Brain Res, 563*(1-2), 353-356.

Beuzen, A., & Belzung, C. (1995). Link between emotional memory and anxiety states: a study by principal component analysis. *Physiol Behav, 58*(1), 111-118.

Bhattacharya, S. K., Satyan, K. S., & Chakrabarti, A. (1997). Anxiogenic action of caffeine: an experimental study in rats. *J Psychopharmacol, 11*(3), 219-224.

Blanchard, D. C., Griebel, G., & Blanchard, R. J. (2001). Mouse defense behaviours: pharmacological and behavioural assays for anxiety and panic. *Neurosci Biobehav Rev, 25*, 205-218.

Blanchard, R. J., & Blanchard, D. C. (1989). Attack and defense in rodents as ethoexperimental models for the study of emotion. *Prog Neuropsychopharmacol Biol Psychiatry, 13*(Suppl), S3-14.

Blanchard, R. J., Blanchard, D. C., & Hori, K. (1989). An ethoexperimental approach to the study of defense. In P. F. B. D. C. B. S. P. Robert J. Blanchard (Ed.), *Ethoexperimental approaches to the study of behavior. NATO Advanced Science Institutes series. Series D: Behavioural and social sciences, Vol. 48.* (pp. 114-136): Kluwer Academic Publishers, Dordrecht, Netherlands.

Blanchard, R. J., Blanchard, D. C., Rodgers, J., & Weiss, S. M. (1990). The characterization and modelling of antipredator defensive behavior. *Neurosci Biobehav Rev, 14*(4), 463-472.

Blanchard, R. J., Griebel, G., Guardiola-Lemaitre, B., Brush, M. M., Lee, J., & Blanchard, D. C. (1997). An ethopharmacological analysis of selective activation of 5-HT1A receptors: the mouse 5-HT1A syndrome. *Pharmacol Biochem Behav, 57*(4), 897-908.

Blanchard, R. J., Griebel, G., Henrie, J. A., & Blanchard, D. C. (1997). Differentiation of anxiolytic and panicolytic drugs by effects on rat and mouse defense test batteries. *Neurosci Biobehav Rev, 21*(6), 783-789.

Blanchard, R. J., Hebert, M. A., Ferrari, P., Palanza, P., Figueira, R., Blanchard, D. C., & Parmigiani, S. (1998). Defensive behaviors in wild and laboratory (Swiss) mice: the mouse defense test battery. *Physiol Behav, 65*(2), 201-209.

Blokland, A. (1995). Acetylcholine: a neurotransmitter for learning and memory? *Brain Res Brain Res Rev, 21*(3), 285-300.

Bollen, K. A., & Long, J. S. (1993). *Testing structural equation models*. Newbury Park: Sage Publications.

Brandao, M. L., Cardoso, S. H., Melo, L. L., Motta, V., & Coimbra, N. C. (1994). Neural substrate of defensive behavior in the midbrain tectum. *Neurosci Biobehav Rev, 18*(3), 339-346.

Bremner, J. D., Staib, L. H., Kaloupek, D., Southwick, S. M., Soufer, R., & Charney, D. S. (1999). Neural correlates of exposure to traumatic pictures and sound in Vietnam combat veterans with and without posttraumatic stress disorder: a positron emission tomography study. *Biol Psychiatry, 45*(7), 806-816.

Brezenoff, H. E. (1972). Cardiovascular responses to intrahypothalamic injections of carbachol and certain cholinesterase inhibitors. *Neuropharmacology, 11*(5), 637-644.

Brezenoff, H. E., & Giuliano, R. (1982). Cardiovascular control by cholinergic mechanisms in the central nervous system. *Annu Rev Pharmacol Toxicol, 22*, 341-381.

Brioni, J. D., O'Neill, A. B., Kim, D. J., Buckley, M. J., Decker, M. W., & Arneric, S. P. (1994). Anxiolytic-like effects of the novel cholinergic channel activator ABT- 418. *J Pharmacol Exp Ther, 271*(1), 353-361.

Broersen, L. M. (2000). Attentional processes and learning and memory in rats: the prefrontal cortex and hippocampus compared [In Process Citation]. *Prog Brain Res, 126*, 79-94.

Broersen, L. M., Abbate, F., Feenstra, M. G., de Bruin, J. P., Heinsbroek, R. P., & Olivier, B. (2000). Prefrontal dopamine is directly involved in the anxiogenic interoceptive cue of pentylenetetrazol but not in the interoceptive cue of chlordiazepoxide in the rat. *Psychopharmacology (Berl), 149*(4), 366-376.

Broersen, L. M., Feldon, J., & Weiner, I. (1999). Dissociative effects of apomorphine infusions into the medial prefrontal cortex of rats on latent inhibition, prepulse inhibition and amphetamine-induced locomotion. *Neuroscience, 94*(1), 39-46.

Broersen, L. M., Heinsbroek, R. P., de Bruin, J. P., Joosten, R. N., van Hest, A., & Olivier, B. (1994). Effects of local application of dopaminergic drugs into the dorsal part of the medial prefrontal cortex of rats in a delayed matching to position task: comparison with local cholinergic blockade. *Brain Res, 645*(1-2), 113-122.

Broersen, L. M., Heinsbroek, R. P., de Bruin, J. P., Laan, J. B., Joosten, R. N., & Olivier, B. (1995). Local pharmacological manipulations of prefrontal dopamine affect conflict behaviour in rats. *Behav Pharmacol, 6*(4), 395-404.

Broersen, L. M., Heinsbroek, R. P., de Bruin, J. P., & Olivier, B. (1996). Effects of local application of dopaminergic drugs into the medial prefrontal cortex of rats on latent inhibition. *Biol Psychiatry, 40*(11), 1083-1090.

Broersen, L. M., Heinsbroek, R. P., de Bruin, J. P., Uylings, H. B., & Olivier, B. (1995). The role of the medial prefrontal cortex of rats in short-term memory functioning: further support for involvement of cholinergic, rather than dopaminergic mechanisms. *Brain Res, 674*(2), 221-229.

Bronowski, J. (1977). Human and animal languages. In J. Bronowski (Ed.), *A sense of the future* (pp. 104-131). Cambrigde, MA: MIT Press.

Brooks, C. M. (1981). The autonomic nervous system, molder and integrator of function. Review of a concept. *Braz J Med Biol Res, 14*(2-3), 151-160.

Brown, A. S., & Gershon, S. (1993). Dopamine and depression. *J Neural Transm Gen Sect, 91*(2-3), 75-109.

Brown, T. A., Barlow, D. H., & Liebowitz, M. R. (1994). The empirical basis of generalized anxiety disorder. *Am J Psychiatry, 151*(9), 1272-1280.

Browne, M. W., & Cudeck, R. (1993). *Alternative ways of assessing model fit*. Newbury Park, CA: Sage.

Brozoski, T. J., Brown, R. M., Rosvold, H. E., & Goldman, P. S. (1979). Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. *Science, 205*(4409), 929-932.

Bubser, M., & Schmidt, W. J. (1990). 6-Hydroxydopamine lesion of the rat prefrontal cortex increases locomotor activity, impairs acquisition of delayed alternation tasks, but does not affect uninterrupted tasks in the radial maze. *Behav Brain Res, 37*(2), 157-168.

Buckner, R. L., Raichle, M. E., Miezin, F. M., & Petersen, S. E. (1996). Functional anatomic studies of memory retrieval for auditory words and visual pictures. *J Neurosci, 16*(19), 6219-6235.

Bunsey, M., & Eichenbaum, H. (1993). Critical role of the parahippocampal region for paired-associate learning in rats. *Behav Neurosci, 107*(5), 740-747.

Bunsey, M., & Eichenbaum, H. (1995). Selective damage to the hippocampal region blocks long-term retention of a natural and nonspatial stimulus-stimulus association. *Hippocampus, 5*(6), 546-556.

Bunsey, M., & Eichenbaum, H. (1996). Conservation of hippocampal memory function in rats and humans. *Nature, 379*(6562), 255-257.

Burgess, P. W., & Shallice, T. (1996). Response suppression, initiation and strategy use following frontal lobe lesions. *Neuropsychologia, 34*(4), 263-272.

Butter, C. M., & Snyder, D. R. (1972). Alterations in aversive and aggressive behaviors following orbital frontal lesions in rhesus monkeys. *Acta Neurobiol Exp, 32*(2), 525-565.

Buzsaki, G., Bickford, R. G., Ponomareff, G., Thal, L. J., Mandel, R., & Gage, F. H. (1988). Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. *J Neurosci, 8*(11), 4007-4026.

Bymaster, F. P., Shannon, H. E., Rasmussen, K., DeLapp, N. W., Ward, J. S., Calligaro, D. O., Mitch, C. H., Whitesitt, C., Ludvigsen, T. S., Sheardown, M., Swedberg, M., Rasmussen, T., Olesen, P. H., Jeppesen, L., Sauerberg, P., & Fink-Jensen, A. (1999). Potential role of muscarinic receptors in schizophrenia. *Life Sci, 64*(6-7), 527-534.

Byrne, B. M. (1986). Self-concept/academic achievement relations: An investigation of dimensionality, stability, and causality. *Canadian Journal of Behavioural Science, 18*(2), 173-186.

Byrne, B. M. (1992). The Maslach Burnout Inventory: Validating factorial structure and invariance across intermediate, secondary, and university educators. *Multivariate Behavioral Research, 26*(4), 583-605.

Byrne, B. M. (1994). Testing for the factorial validity, replication, and invariance of a measuring instrument: A paradigmatic application based on the Maslach Burnout Inventory. *Multivariate Behavioral Research, 29*(3), 289-311.

Byrne, B. M. (1998). *Structural equation modeling with LISREL, PRELIS, and SIMPLIS : basic concepts, applications, and programming*. Mahwah, N.J.: L. Erlbaum Associates.

Byrne, B. M., & Baron, P. (1993). The Beck Depression Inventory: Testing and cross-validating a hierarchical factor structure for nonclinical adolescents. *Measurement & Evaluation in Counseling & Development, 26*(3), 164-178.

Cacioppo, J. T., Priester, J. R., & Berntson, G. G. (1993). Rudimentary determinants of attitudes. II: Arm flexion and extension have differential effects on attitudes. *J Pers Soc Psychol, 65*(1), 5-17.

Carli, M., Tatarczynska, E., Cervo, L., & Samanin, R. (1993). Stimulation of hippocampal 5-HT1A receptors causes amnesia and anxiolytic-like but not antidepressant-like effects in the rat. *Eur J Pharmacol, 234*(2-3), 215-221.

Carlson, J. N., Fitzgerald, L. W., Keller, R. W., Jr., & Glick, S. D. (1991). Side and region dependent changes in dopamine activation with various durations of restraint stress. *Brain Res, 550*(2), 313-318.

Carmichael, S. T., Clugnet, M. C., & Price, J. L. (1994). Central olfactory connections in the macaque monkey. *J Comp Neurol, 346*(3), 403-434.

Carmichael, S. T., & Price, J. L. (1995). Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. *J Comp Neurol, 363*(4), 615-641.

Carroll, B. J., Barrett, J. E., & American Psychopathological Association. Meeting. (1991). *Psychopathology and the brain*. New York: Raven Press.

Castellano, C., Introini-Collison, I. B., & McGaugh, J. L. (1993). Interaction of beta-endorphin and GABAergic drugs in the regulation of memory storage. *Behav Neural Biol, 60*(2), 123-128.

Castellano, C., Introini-Collison, I. B., Pavone, F., & McGaugh, J. L. (1989). Effects of naloxone and naltrexone on memory consolidation in CD1 mice: involvement of GABAergic mechanisms. *Pharmacol Biochem Behav, 32*(2), 563-567.

Castellanos, F. X. (1997). Toward a pathophysiology of attention-deficit/hyperactivity disorder. *Clin Pediatr (Phila), 36*(7), 381-393.

Chiba, T., Kayahara, T., & Nakano, K. (2001). Efferent projections of infralimbic and prelimbic areas of the medial prefrontal cortex in the Japanese monkey, Macaca fuscata. *Brain Res, 888*(1), 83-101.

Clement, Y., & Chapouthier, G. (1998). Biological bases of anxiety. *Neurosci Biobehav Rev, 22*(5), 623-633.

Cohen, N. J., & Eichenbaum, H. (1993). *Memory, amnesia, and the hippocampal system*. Cambridge, Mass.: MIT Press.

Cole, J. C., & Rodgers, R. J. (1993). An ethological analysis of the effects of chlordiazepoxide and bretazenil (Ro 16-6028) in the murine elevated plus-maze. *Behavioural Pharmacology, 4*(6), 573-580.

Cole, J. C., & Rodgers, R. J. (1994). Ethological evaluation of the effects of acute and chronic buspirone treatment in the murine elevated plus-maze test: comparison with haloperidol. *Psychopharmacology (Berl), 114*(2), 288-296.

Cole, J. C., & Rodgers, R. J. (1995). Ethological comparison of the effects of diazepam and acute/chronic imipramine on the behaviour of mice in the elevated plus-maze. *Pharmacol Biochem Behav, 52*(3), 473-478.

Colpaert, F. C. (1990). Amnesic trace locked into the benzodiazepine state of memory. *Psychopharmacology, 102*(1), 28-36.

Comrey, A. L., & Lee, H. B. (1992). *A first course in factor analysis* (2nd ed.). Hillsdale, N.J.: L. Erlbaum Associates.

Conde, F., Audinat, E., Maire-Lepoivre, E., & Crepel, F. (1990). Afferent connections of the medial frontal cortex of the rat. A study using retrograde transport of fluorescent dyes. I. Thalamic afferents. *Brain Res Bull, 24*(3), 341-354.

Conde, F., Maire-Lepoivre, E., Audinat, E., & Crepel, F. (1995). Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. *J Comp Neurol, 352*(4), 567-593.

Cornwall, J., Cooper, J. D., & Phillipson, O. T. (1990). Afferent and efferent connections of the laterodorsal tegmental nucleus in the rat. *Brain Res Bull, 25*(2), 271-284.

Cosford, N. D., Bleicher, L., Vernier, J. M., Chavez-Noriega, L., Rao, T. S., Siegel, R. S., Suto, C., Washburn, M., Lloyd, G. K., & McDonald, I. A. (2000). Recombinant human receptors and functional assays in the discovery of altinicline (SIB-1508Y), a novel acetylcholine-gated ion channel (nAChR) agonist. *Pharm Acta Helv, 74*(2-3), 125-130.

Coull, J. T., Frackowiak, R. S., & Frith, C. D. (1998). Monitoring for target objects: activation of right frontal and parietal cortices with increasing time on task. *Neuropsychologia, 36*(12), 1325-1334.

Craig, K. J., Brown, K. J., & Baum, A. (1995). Environmental factors in the etiology of anxiety. In F. E. Bloom & D. F. Kupfer (Eds.), *Psychopharmacology: The fourth generation of progress: An official publication of the American College of Neuropsychopharmacology* (pp. xliii, 2002). New York, NY, US: Raven Press.

Cruz, A. P., Frei, F., & Graeff, F. G. (1994). Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacol Biochem Behav, 49*(1), 171-176.

Damasio, A. R. (1994). Descartes' error and the future of human life. *Sci Am, 271*(4), 144.

Damasio, A. R. (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex. *Philos Trans R Soc Lond B Biol Sci, 351*(1346), 1413-1420.

Damasio, A. R. (1998). Emotion in the perspective of an integrated nervous system. *Brain Res Brain Res Rev, 26*(2-3), 83-86.

Damasio, A. R. (2000). The fabric of the mind: A neurobiological perspective. In H. B. Uylings & C. G. Van Eden & J. P. de Bruin & M. G. Feenstra & C. M. Pennartz (Eds.), *Cognition, Emotion and Autonomic Responses: The Integrative Role of the Prefrontal Cortex and Limbic Structures* (Vol. Progress in Brain Research. 126, pp. 457-467). Amsterdam: Elsevier.

Damasio, H., Grabowski, T., Frank, R., Galaburda, A. M., & Damasio, A. R. (1994). The return of Phineas Gage: clues about the brain from the skull of a famous patient [published erratum appears in Science 1994 Aug 26;265(5176):1159]. *Science, 264*(5162), 1102-1105.

Dampney, R. A., Goodchild, A. K., Robertson, L. G., & Montgomery, W. (1982). Role of ventrolateral medulla in vasomotor regulation: a correlative anatomical and physiological study. *Brain Res, 249*(2), 223-235.

Dauge, V., Derrien, M., Blanchard, J. C., & Roques, B. P. (1992). The selective CCK-B agonist, BC 264 injected in the antero-lateral part of the nucleus accumbens, reduces the spontaneous alternation behaviour of rats. *Neuropharmacology, 31*(1), 67-75.

Dauge, V., & Lena, I. (1998). CCK in anxiety and cognitive processes. *Neurosci Biobehav Rev, 22*(6), 815-825.

Davis, M., Rainnie, D., & Cassell, M. (1994). Neurotransmission in the rat amygdala related to fear and anxiety. *Trends Neurosci, 17*(5), 208-214.

Dawson, G. R., Crawford, S. P., Stanhope, K. J., Iversen, S. D., & Tricklebank, M. D. (1994). One-trial tolerance to the effects of chlordiazepoxide on the elevated plus maze may be due to locomotor habituation, not repeated drug exposure. *Psychopharmacology (Berl), 113*(3-4), 570-572.

Dawson, G. R., & Tricklebank, M. D. (1995). Use of the elevated plus maze in the search for novel anxiolytic agents [see comments]. *Trends Pharmacol Sci, 16*(2), 33-36.

Day, J., Damsma, G., & Fibiger, H. C. (1991). Cholinergic activity in the rat hippocampus, cortex and striatum correlates with locomotor activity: an in vivo microdialysis study. *Pharmacol Biochem Behav, 38*(4), 723-729.

De Bruin, J. P., Feenstra, M. G., Broersen, L. M., Van Leeuwen, M., Arens, C., De Vries, S., & Joosten, R. N. (2000). Role of the prefrontal cortex of the rat in learning and decision making: effects of transient inactivation. *Prog Brain Res, 126*, 103-113.

DeCoteau, W. E., Kesner, R. P., & Williams, J. M. (1997). Short-term memory for food reward magnitude: the role of the prefrontal cortex. *Behav Brain Res, 88*(2), 239-249.

Delatour, B., & Gisquet-Verrier, P. (2000). Functional role of rat prelimbic-infralimbic cortices in spatial memory: evidence for their involvement in attention and behavioural flexibility. *Behav Brain Res, 109*(1), 113-128.

Denckla, M. B. (1991). Attention deficit hyperactivity disorder-residual type. *J Child Neurol, 6*(Suppl), S44-50.

Denckla, M. B. (1993). The child with developmental disabilities grown up: Adult residua of childhood disorders. *Behav Neurol, 11*(1), 105-124.

Derrien, M., Dauge, V., Blommaert, A., & Roques, B. P. (1994). The selective CCK-B agonist, BC 264, impairs socially reinforced memory in the three-panel runway test in rats. *Behav Brain Res, 65*(2), 139-146.

Derrien, M., Durieux, C., Dauge, V., & Roques, B. P. (1993). Involvement of D2 dopaminergic receptors in the emotional and motivational responses induced by injection of CCK-8 in the posterior part of the rat nucleus accumbens. *Brain Res, 617*(2), 181-188.

DeSousa, N. J., Beninger, R. J., Jhamandas, K., & Boegman, R. J. (1994). Stimulation of GABAB receptors in the basal forebrain selectively impairs working memory of rats in the double Y-maze. *Brain Res, 641*(1), 29-38.

Deutch, A. Y., & Cameron, D. S. (1992). Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. *Neuroscience, 46*(1), 49-56.

Deutch, A. Y., Clark, W. A., & Roth, R. H. (1990). Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. *Brain Res, 521*(1-2), 311-315.

Deutch, A. Y., & Roth, R. H. (1990). The determinants of stress-induced activation of the prefrontal cortical dopamine system. *Prog Brain Res, 85*, 367-402.

Deutch, A. Y., Tam, S. Y., & Roth, R. H. (1985). Footshock and conditioned stress increase 3,4-dihydroxyphenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. *Brain Res, 333*(1), 143-146.

Devinsky, O., Morrell, M. J., & Vogt, B. A. (1995). Contributions of anterior cingulate cortex to behaviour. *Brain, 118*(Pt 1), 279-306.

Di Chiara, G., & Imperato, A. (1988a). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A, 85*(14), 5274-5278.

Di Chiara, G., & Imperato, A. (1988b). Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. *J Pharmacol Exp Ther, 244*(3), 1067-1080.

Dias, R., & Aggleton, J. P. (2000). Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: differential involvement of the prelimbic-infralimbic and anterior cingulate cortices in providing behavioural flexibility [In Process Citation]. *Eur J Neurosci, 12*(12), 4457-4466.

Dias, R., Robbins, T. W., & Roberts, A. C. (1996a). Dissociation in prefrontal cortex of affective and attentional shifts. *Nature, 380*(6569), 69-72.

Dias, R., Robbins, T. W., & Roberts, A. C. (1996b). Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. *Behav Neurosci, 110*(5), 872-886.

Dias, R., Robbins, T. W., & Roberts, A. C. (1997). Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from "on-line" processing. *J Neurosci, 17*(23), 9285-9297.

Diehl, D. J., & Gershon, S. (1992). The role of dopamine in mood disorders [see comments]. *Compr Psychiatry, 33*(2), 115-120.

Donzanti, B. A., Althaus, J. S., Payson, M. M., & Von Voigtlander, P. F. (1992). Kappa agonist-induced reduction in dopamine release: site of action and tolerance. *Res Commun Chem Pathol Pharmacol, 78*(2), 193-210.

Doyere, V., Burette, F., Negro, C. R., & Laroche, S. (1993). Long-term potentiation of hippocampal afferents and efferents to prefrontal cortex: implications for associative learning. *Neuropsychologia, 31*(10), 1031-1053.

Drevets, W. C., Videen, T. O., Snyder, A. Z., Macleod, A. K., & Raichle, M. E. (1994). Regional cerebral blood flow changes during anticipatory anxiety. *Society for Neuroscience Abstracts, 20:pp. 368.*

Druzin, M. Y., Kurzina, N. P., Malinina, E. P., & Kozlov, A. P. (2000). The effects of local application of D2 selective dopaminergic drugs into the medial prefrontal cortex of rats in a delayed spatial choice task. *Behav Brain Res, 109*(1), 99-111.

Dunbar, K., & Sussman, D. (1995). Toward a cognitive account of frontal lobe function: simulating frontal lobe deficits in normal subjects. *Ann N Y Acad Sci, 769*, 289-304.

Duncan, G. E., Knapp, D. J., & Breese, G. R. (1996). Neuroanatomical characterization of Fos induction in rat behavioral models of anxiety. *Brain Res, 713*(1-2), 79-91.

Dunn, A. J., & File, S. E. (1983). Cold restraint alters dopamine metabolism in frontal cortex, nucleus accumbens and neostriatum. *Physiol Behav, 31*(4), 511-513.

Dunnett, S. B. (1990). Role of prefrontal cortex and striatal output systems in short-term memory deficits associated with ageing, basal forebrain lesions, and cholinergic-rich grafts. *Can J Psychol, 44*(2), 210-232.

Dunnett, S. B., Everitt, B. J., & Robbins, T. W. (1991). The basal forebrain-cortical cholinergic system: interpreting the functional consequences of excitotoxic lesions. *Trends Neurosci, 14*(11), 494-501.

Edwards, E., Hampton, E., Ashby, C. R., Zhang, J., & Wang, R. Y. (1996). 5-HT3-like receptors in the rat medial prefrontal cortex: further pharmacological characterization. *Brain Res, 733*(1), 21-30.

Edwards, J. G. (1991). Clinical anxiety and its treatment. *Neuropeptides, 19 Suppl*, 1-10.

Ehlers, A., & Breuer, P. (1996). How good are patients with panic disorder at perceiving their heartbeats? *Biol Psychol, 42*(1-2), 165-182.

Elliott, J. M., Heal, D. J., & Marsden, C. A. (1992). *Experimental approaches to anxiety and depression*. Chichester ; New York: John Wiley.

Elliott, R., & Dolan, R. J. (1998). Neural response during preference and memory judgments for subliminally presented stimuli: a functional neuroimaging study. *J Neurosci, 18*(12), 4697-4704.

Endoh, T., Koike, H., Matsumuura, H., & Nagase, H. (1990). *nor-Binaltorphamine (nor-BNI); a potent and selective kappa opioid receptor antagonist with ultra-long-lasting activity in vivo.* Amsterdam, The Netherlands: Elsevier Science Publishers.

Ernst, M., Zametkin, A. J., Matochik, J. A., Jons, P. H., & Cohen, R. M. (1998). DOPA decarboxylase activity in attention deficit hyperactivity disorder adults. A [fluorine-18]fluorodopa positron emission tomographic study. *J Neurosci, 18*(15), 5901-5907.

Eslinger, P. J. (1996). Conceptualizing, describing, and measuring components of executive function: A summary. In G. R. Lyon & N. A. Krasnegor (Eds.), *Attention, memory, and executive function*. Baltimore, MD: Paul H. Brookes Publishing Co.

Espejo, E. F. (1997a). Effects of weekly or daily exposure to the elevated plus-maze in male mice. *Behav Brain Res, 87*(2), 233-238.

Espejo, E. F. (1997b). Selective dopamine depletion within the medial prefrontal cortex induces anxiogenic-like effects in rats placed on the elevated plus maze. *Brain Res, 762*(1-2), 281-284.

Estevez-Gonzalez, A., Garcia-Sanchez, C., & Junque, C. (1997). [Attention: a complex cerebral function]. *Rev Neurol, 25*(148), 1989-1997.

Everitt, B. J., & Robbins, T. W. (1997). Central cholinergic systems and cognition. *Annu Rev Psychol, 48*, 649-684.

Facoetti, A., & Molteni, M. (2000). Is attentional focusing an inhibitory process at distractor location? *Brain Res Cogn Brain Res, 10*(1-2), 185-188.

Fadel, J., Sarter, M., & Bruno, J. P. (2001). Basal forebrain glutamatergic modulation of cortical acetylcholine release. *Synapse, 39*(3), 201-212.

Faraone, S. V., Biederman, J., Spencer, T., Wilens, T., Seidman, L. J., Mick, E., & Doyle, A. E. (2000). Attention-deficit/hyperactivity disorder in adults: an overview [In Process Citation]. *Biol Psychiatry, 48*(1), 9-20.

Fernandes, C., & File, S. E. (1996). The influence of open arm ledges and maze experience in the elevated plus-maze. *Pharmacol Biochem Behav, 54*(1), 31-40.

Fernandez Espejo, E. (1997). Structure of the mouse behaviour on the elevated plus-maze test of anxiety. *Behav Brain Res, 86*(1), 105-112.

File, S. E. (1990). One-trial tolerance to the anxiolytic effects of chlordiazepoxide in the plus-maze. *Psychopharmacology, 100*(2), 281-282.

File, S. E. (1992). Benzodiazepines and memory. *Clin Neuropharmacol, 15*(Suppl 1 Pt A), 331A.

File, S. E. (1993). The interplay of learning and anxiety in the elevated plus-maze. *Behav Brain Res, 58*(1-2), 199-202.

File, S. E. (1996). Recent developments in anxiety, stress, and depression. *Pharmacol Biochem Behav, 54*(1), 3-12.

File, S. E., Andrews, N., Wu, P. Y., Zharkovsky, A., & Zangrossi, H., Jr. (1992). Modification of chlordiazepoxide's behavioural and neurochemical effects by handling and plus-maze experience. *Eur J Pharmacol, 218*(1), 9-14.

File, S. E., Gonzalez, L. E., & Andrews, N. (1998). Endogenous acetylcholine in the dorsal hippocampus reduces anxiety through actions on nicotinic and muscarinic1 receptors. *Behav Neurosci, 112*(2), 352-359.

File, S. E., Gonzalez, L. E., & Gallant, R. (1999). Role of the dorsomedial hypothalamus in mediating the response to benzodiazepines on trial 2 in the elevated plus-maze test of anxiety. *Neuropsychopharmacology, 21*(2), 312-320.

File, S. E., Kenny, P. J., & Cheeta, S. (2000). The role of the dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety. *Pharmacol Biochem Behav, 66*(1), 65-72.

File, S. E., Mabbutt, P. S., & Hitchcott, P. K. (1990). Characterisation of the phenomenon of "one-trial tolerance" to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze. *Psychopharmacology, 102*(1), 98-101.

File, S. E., Zangrossi, H., Jr., Viana, M., & Graeff, F. G. (1993). Trial 2 in the elevated plus-maze: a different form of fear? *Psychopharmacology, 111*(4), 491-494.

Floresco, S. B., Seamans, J. K., & Phillips, A. G. (1997). Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. *J Neurosci, 17*(5), 1880-1890.

Franklin, K. B. J., & Paxinos, G. (1997). *The Mouse Brain: In stereotaxic coordinates.*: Academic Press.

Fredrikson, M., Fischer, H., & Wik, G. (1997). Cerebral blood flow during anxiety provocation. *J Clin Psychiatry, 58*(Suppl 16), 16-21.

Friedman, B. H., & Thayer, J. F. (1998a). Anxiety and autonomic flexibility: a cardiovascular approach. *Biol Psychol, 49*(3), 303-323.

Friedman, B. H., & Thayer, J. F. (1998b). Autonomic balance revisited: panic anxiety and heart rate variability. *J Psychosom Res, 44*(1), 133-151.

Fritts, M. E., Asbury, E. T., Horton, J. E., & Isaac, W. L. (1998). Medial prefrontal lesion deficits involving or sparing the prelimbic area in the rat. *Physiol Behav, 64*(3), 373-380.

Frussa-Filho, R., Otoboni, J. R., Uema, F. T., & Sa-Rocha, L. C. (1991). Evaluation of memory and anxiety in rats observed in the elevated plus- maze: effects of age and isolation. *Braz J Med Biol Res, 24*(7), 725-728.

Frysztak, R. J., & Neafsey, E. J. (1991). The effect of medial frontal cortex lesions on respiration, "freezing," and ultrasonic vocalizations during conditioned emotional responses in rats. *Cereb Cortex, 1*(5), 418-425.

Frysztak, R. J., & Neafsey, E. J. (1994). The effect of medial frontal cortex lesions on cardiovascular conditioned emotional responses in the rat. *Brain Res, 643*(1-2), 181-193.

Furey, M. L., Pietrini, P., Alexander, G. E., Schapiro, M. B., & Horwitz, B. (2000). Cholinergic enhancement improves performance on working memory by modulating the functional activity in distinct brain regions: a positron emission tomography regional cerebral blood flow study in healthy humans. *Brain Res Bull, 51*(3), 213-218.

Fuster, J. M. (1989). *The prefrontal cortex : anatomy, physiology, and neuropsychology of the frontal lobe* (2nd -- ed.). New York: Raven Press.

Fuster, J. M. (1995). *Memory in the cerebral cortex: An empirical approach to neural networks in the human and the non-human primate*. Cambridge, MA: MIT Press.

Fuster, J. M. (1997). *The prefrontal cortex : anatomy, physiology, and neuropsychology of the frontal lobe* (3rd ed.). Philadelphia: Lippincott-Raven.

Fuster, J. M. (2000). Prefrontal neurons in networks of executive memory. *Brain Res Bull, 52*(5), 331-336.

Gabriel, M. (1990). Functions of anterior and posterior cingulate cortex during avoidance learning in rabbits. *Prog Brain Res, 85*, 467-482.

Gabriel, M., Poremba, A. L., Ellison-Perrine, C., & Miller, J. D. (1990). Brainstem mediation of learning and memory. In W. R. Klemm & R. P. Vertes (Eds.), *Brainstem mechanisms of behavior* (pp. 269-313). New York: J. Wiley & Sons.

Gargiulo, P. A., Viana, M. B., Graeff, F. G., Silva, M. A., & Tomaz, C. (1996). Effects of anxiety and memory of systemic and intra-amygdala injection of 5-HT3 receptor antagonist BRL 46470A. *Neuropsychobiology, 33*(4), 189-195.

Gasbarri, A., Sulli, A., Innocenzi, R., Pacitti, C., & Brioni, J. D. (1996). Spatial memory impairment induced by lesion of the mesohippocampal dopaminergic system in the rat. *Neuroscience, 74*(4), 1037-1044.

Gaykema, R. P., van Weeghel, R., Hersh, L. B., & Luiten, P. G. (1991). Prefrontal cortical projections to the cholinergic neurons in the basal forebrain. *J Comp Neurol, 303*(4), 563-583.

Gazzaniga, M. S. (2000). Cerebral specialization and interhemispheric communication: does the corpus callosum enable the human condition? *Brain, 123*(Pt 7), 1293-1326.

Gigg, J., Tan, A. M., & Finch, D. M. (1994). Glutamatergic hippocampal formation projections to prefrontal cortex in the rat are regulated by GABAergic inhibition and show convergence with glutamatergic projections from the limbic thalamus. *Hippocampus, 4*(2), 189-198.

Gill, T. M., Sarter, M., & Givens, B. (2000). Sustained visual attention performance-associated prefrontal neuronal activity: evidence for cholinergic modulation. *J Neurosci, 20*(12), 4745-4757.

Glosser, G., & Goodglass, H. (1990). Disorders in executive control functions among aphasic and other brain- damaged patients. *J Clin Exp Neuropsychol, 12*(4), 485-501.

Godbout, R., Mantz, J., Pirot, S., Glowinski, J., & Thierry, A. M. (1991). Inhibitory influence of the mesocortical dopaminergic neurons on their target cells: electrophysiological and pharmacological characterization. *J Pharmacol Exp Ther, 258*(2), 728-738.

Goel, V., & Grafman, J. (1995). Are the frontal lobes implicated in "planning" functions? Interpreting data from the Tower of Hanoi. *Neuropsychologia, 33*(5), 623-642.

Gold, P. W., & Chrousos, G. P. (1999). The endocrinology of melancholic and atypical depression: relation to neurocircuitry and somatic consequences. *Proc Assoc Am Physicians, 111*(1), 22-34.

Goldman-Rakic, P. S. (1995a). Anatomical and functional circuits in prefrontal cortex of nonhuman primates. Relevance to epilepsy. *Adv Neurol, 66*, 51-63.

Goldman-Rakic, P. S. (1995b). Architecture of the prefrontal cortex and the central executive. *Ann N Y Acad Sci, 769*, 71-83.

Goldman-Rakic, P. S. (1995c). Cellular basis of working memory. *Neuron, 14*(3), 477-485.

Goldman-Rakic, P. S. (1996a). Memory: recording experience in cells and circuits: diversity in memory research. *Proc Natl Acad Sci U S A, 93*(24), 13435-13437.

Goldman-Rakic, P. S. (1996b). The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. *Philos Trans R Soc Lond B Biol Sci, 351*(1346), 1445-1453.

Goldman-Rakic, P. S. (1996c). Regional and cellular fractionation of working memory. *Proc Natl Acad Sci U S A, 93*(24), 13473-13480.

Goldman-Rakic, P. S. (1998). The cortical dopamine system: role in memory and cognition. *Adv Pharmacol, 42*, 707-711.

Goldman-Rakic, P. S., Muly, E. C., 3rd, & Williams, G. V. (2000). D(1) receptors in prefrontal cells and circuits. *Brain Res Brain Res Rev, 31*(2-3), 295-301.

Goldman-Rakic, P. S., & Selemon, L. D. (1997). Functional and anatomical aspects of prefrontal pathology in schizophrenia [see comments]. *Schizophr Bull, 23*(3), 437-458.

Goldstein, L. E., Rasmusson, A. M., Bunney, B. S., & Roth, R. H. (1994). The NMDA glycine site antagonist (+)-HA-966 selectively regulates conditioned stress-induced metabolic activation of the mesoprefrontal cortical dopamine but not serotonin systems: a behavioral, neuroendocrine, and neurochemical study in the rat. *J Neurosci, 14*(8), 4937-4950.

Goldstein, L. E., Rasmusson, A. M., Bunney, B. S., & Roth, R. H. (1996). Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *J Neurosci, 16*(15), 4787-4798.

Gonzalez, L. E., & File, S. E. (1997). A five minute experience in the elevated plus-maze alters the state of the benzodiazepine receptor in the dorsal raphe nucleus. *J Neurosci, 17*(4), 1505-1511.

Gorsuch, R. L. (1983). *Factor analysis* (2nd ed.). Hillsdale, N.J.: L. Erlbaum Associates.

Gorsuch, R. L. (1990). Common factor analysis versus component analysis: Some well and little known facts. *Multivariate Behavioral Research, 25*(1), 33-39.

Gotham, A. M., Brown, R. G., & Marsden, C. D. (1988). 'Frontal' cognitive function in patients with Parkinson's disease 'on' and 'off' levodopa. *Brain, 111*(Pt 2), 299-321.

Gottschalk, L. A., Buchsbaum, M. S., Gillin, J. C., Wu, J., Reynolds, C. A., & Herrera, D. B. (1991). Positron-emission tomographic studies of the relationship of cerebral glucose metabolism and the magnitude of anxiety and hostility experienced during dreaming and waking. *J Neuropsychiatry Clin Neurosci, 3*(2), 131-142.

Gottschalk, L. A., Buchsbaum, M. S., Gillin, J. C., Wu, J., Reynolds, C. A., & Herrera, D. B. (1992). The effect of anxiety and hostility in silent mentation on localized cerebral glucose metabolism. *Compr Psychiatry, 33*(1), 52-59.

Graeff, F. G., Viana, M. B., & Mora, P. O. (1996). Opposed regulation by dorsal raphe nucleus 5-HT pathways of two types of fear in the elevated T-maze. *Pharmacol Biochem Behav, 53*(1), 171-177.

Graeff, F. G., Viana, M. B., & Tomaz, C. (1993). The elevated T maze, a new experimental model of anxiety and memory: effect of diazepam. *Braz J Med Biol Res, 26*(1), 67-70.

Grafman, J. (1995). Similarities and distinctions among current models of prefrontal cortical functions. *Ann N Y Acad Sci, 769*, 337-368.

Granon, S., Passetti, F., Thomas, K. L., Dalley, J. W., Everitt, B. J., & Robbins, T. W. (2000). Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. *J Neurosci, 20*(3), 1208-1215.

Granon, S., & Poucet, B. (1995). Medial prefrontal lesions in the rat and spatial navigation: evidence for impaired planning. *Behav Neurosci, 109*(3), 474-484.

Granon, S., Poucet, B., Thinus-Blanc, C., Changeux, J. P., & Vidal, C. (1995). Nicotinic and muscarinic receptors in the rat prefrontal cortex: differential roles in working memory, response selection and effortful processing. *Psychopharmacology (Berl), 119*(2), 139-144.

Granon, S., Vidal, C., Thinus-Blanc, C., Changeux, J. P., & Poucet, B. (1994). Working memory, response selection, and effortful processing in rats with medial prefrontal lesions. *Behav Neurosci, 108*(5), 883-891.

Gray, J. A. (1982). *The neuropsychology of anxiety : an enquiry into the functions of the septo-hippocampal system*. Oxford: Clarendon Press.

Gray, J. A. (1983). A theory of anxiety: the role of the limbic system. *Encephale, 9*(4), 161B-166B.

Gray, J. A. (1985a). Emotional behaviour and the limbic system. *Adv Psychosom Med, 13*, 1-25.

Gray, J. A. (1985b). The neuropsychology of anxiety. *Issues Ment Health Nurs, 7*(1-4), 201-228.

Gray, J. A., Feldon, J., Rawlins, J. N., Hemsley, D. R., & Smith, A. D. (1991). The neuropsychology of schizophrenia. *Behav Brain Sci, 14*, 1-84.

Gray, J. A., & McNaughton, N. (1983). Comparison between the behavioural effects of septal and hippocampal lesions: a review. *Neurosci Biobehav Rev, 7*(2), 119-188.

Gray, J. A., & McNaughton, N. (1996). The neuropsychology of anxiety: reprise. *Nebr Symp Motiv, 43*, 61-134.

Gray, J. A., & McNaughton, N. (2000). *The Neuropsychology of Anxiety: an Inquiry into the Functions of the Septo-hippocampal system* (2nd ed.). Oxford: Oxford University Press.

Green, S. (1991). Benzodiazepines, putative anxiolytics and animal models of anxiety. *Trends in the Neurosciences, 14*, 101-104.

Gresch, P. J., Sved, A. F., Zigmond, M. J., & Finlay, J. M. (1994). Stress-induced sensitization of dopamine and norepinephrine efflux in medial prefrontal cortex of the rat. *J Neurochem, 63*(2), 575-583.

Griebel, G. (1995). 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacol Ther, 65*(3), 319-395.

Griebel, G., Perrault, G., & Sanger, D. J. (1998). Characterization of the behavioral profile of the non-peptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone [In Process Citation]. *Psychopharmacology (Berl), 138*(1), 55-66.

Groenewegen, H. J., & Uylings, H. B. M. (2000). The prefrontal cortex and the integration of sensory, limbic and autonomic information. *Prog Brain Res, 126*, 3-28.

Gruen, R. J., Wenberg, K., Elahi, R., & Friedhoff, A. J. (1995). Alterations in GABAA receptor binding in the prefrontal cortex following exposure to chronic stress. *Brain Res, 684*(1), 112-114.

Guadagnoli, E., & Velicer, W. F. (1988). Relation of sample size to the stability of component patterns. *Psychol Bull, 103*(2), 265-275.

Guimaraes, F. S., Del Bel, E. A., Padovan, C. M., Netto, S. M., & de Almeida, R. T. (1993). Hippocampal 5-HT receptors and consolidation of stressful memories. *Behav Brain Res, 58*(1-2), 133-139.

Gurden, H., Tassin, J. P., & Jay, T. M. (1999). Integrity of the mesocortical dopaminergic system is necessary for complete expression of in vivo hippocampal-prefrontal cortex long-term potentiation. *Neuroscience, 94*(4), 1019-1027.

Hall, R. E., Livingston, R. B., & Bloor, C. M. (1977). Orbital cortical influences on cardiovascular dynamics and myocardial structure in conscious monkeys. *J Neurosurg, 46*, 638-647.

Handley, S. L. (1995). 5-Hydroxytryptamine pathways in anxiety and its treatment. *Pharmacol Ther, 66*(1), 103-148.

Handley, S. L., & McBlane, J. W. (1993a). An assessment of the elevated X-maze for studying anxiety and anxiety- modulating drugs. *J Pharmacol Toxicol Methods, 29*(3), 129-138.

Handley, S. L., & McBlane, J. W. (1993b). Serotonin mechanisms in animal models of anxiety. *Braz J Med Biol Res, 26*(1), 1-13.

Handley, S. L., McBlane, J. W., Critchley, M. A., & Njung'e, K. (1993). Multiple serotonin mechanisms in animal models of anxiety: environmental, emotional and cognitive factors. *Behav Brain Res, 58*(1-2), 203-210.

Handley, S. L., & Mithani, S. (1984). Effects of alpha-adrenoceptor agonists and antagonists in a maze- exploration model of 'fear'-motivated behaviour. *Naunyn Schmiedebergs Arch Pharmacol, 327*(1), 1-5.

Hardy, S. G., & Holmes, D. E. (1988). Prefrontal stimulus-produced hypotension in rat. *Exp Brain Res, 73*(2), 249-255.

Hart, S., Sarter, M., & Berntson, G. G. (1998). Cardiovascular and somatic startle and defense: concordant and discordant actions of benzodiazepine receptor agonists and inverse agonists. *Behav Brain Res, 90*(2), 175-186.

Hart, S., Sarter, M., & Berntson, G. G. (1999). Cholinergic inputs to the rat medial prefrontal cortex mediate potentiation of the cardiovascular defensive response by the anxiogenic benzodiazephine receptor partial inverse agonist FG 7142. *Neuroscience, 94*(4), 1029-1038.

Hasselmo, M. E. (1995). Neuromodulation and cortical function: modeling the physiological basis of behavior. *Behav Brain Res, 67*(1), 1-27.

Herman, J. P., Guillonneau, D., Dantzer, R., Scatton, B., Semerdjian-Rouquier, L., & Le Moal, M. (1982). Differential effects of inescapable footshocks and of stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat. *Life Sci, 30*(25), 2207-2214.

Herremans, A. H., Hijzen, T. H., & Olivier, B. (1997). Effects of cholinergic drug infusions into the dorsal part of the medial prefrontal cortex on delayed conditional discrimination performance in the rat. *Behav Brain Res, 84*(1-2), 291-299.

Herremans, A. H., Hijzen, T. H., Welborn, P. F., Olivier, B., & Slangen, J. L. (1996). Effects of infusion of cholinergic drugs into the prefrontal cortex area on delayed matching to position performance in the rat. *Brain Res, 711*(1-2), 102-111.

Himmelheber, A. M., Sarter, M., & Bruno, J. P. (1997). Operant performance and cortical acetylcholine release: role of response rate, reward density, and non-contingent stimuli. *Brain Res Cogn Brain Res, 6*(1), 23-36.

Hiramatsu, M., Hyodo, T., & Kameyama, T. (1996). U-50488H, a selective kappa-opioid receptor agonist, improves carbon monoxide-induced delayed amnesia in mice. *Eur J Pharmacol, 315*(2), 119-125.

Hiramatsu, M., Hyodo, T., & Kameyama, T. (1997). U-50,488H, a selective kappa opioid receptor agonist, ameliorates memory impairments induced by muscarinic autoreceptor agonist, carbachol in mice. *Neurosci Lett, 236*(1), 45-48.

Hiramatsu, M., & Kameyama, T. (1998). Roles of kappa-opioid receptor agonists in learning and memory impairment in animal models. *Methods Find Exp Clin Pharmacol, 20*(7), 595-599.

Hiramatsu, M., Murasawa, H., Mori, H., & Kameyama, T. (1998). Reversion of muscarinic autoreceptor agonist-induced acetylcholine decrease and learning impairment by dynorphin A (1-13), an endogenous kappa-opioid receptor agonist. *Br J Pharmacol, 123*(5), 920-926.

Hiramatsu, M., Murasawa, H., Nabeshima, T., & Kameyama, T. (1998). Effects of U-50,488H on scopolamine-, mecamylamine- and dizocilpine- induced learning and memory impairment in rats. *J Pharmacol Exp Ther, 284*(3), 858-867.

Hiramatsu, M., Sasaki, M., Nabeshima, T., & Kameyama, T. (1997). Effects of dynorphin A (1-13) on carbon monoxide-induced delayed amnesia in mice. *Pharmacol Biochem Behav, 56*(1), 73-79.

Hogg, S. (1996). A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol Biochem Behav, 54*(1), 21-30.

Hollerman, J. R., Tremblay, L., & Schultz, W. (2000). Involvement of basal ganglia and orbitofrontal cortex in goal-directed behavior. *Prog Brain Res, 126*, 193-215.

Holley, L. A., Turchi, J., Apple, C., & Sarter, M. (1995). Dissociation between the attentional effects of infusions of a benzodiazepine receptor agonist and an inverse agonist into the basal forebrain. *Psychopharmacology (Berl), 120*(1), 99-108.

Holmes, A., & Rodgers, R. J. (1998). Responses of Swiss-Webster mice to repeated plus-maze experience: further evidence for a qualitative shift in emotional state? *Pharmacol Biochem Behav, 60*(2), 473-488.

Holmes, A., & Rodgers, R. J. (1999). Influence of spatial and temporal manipulations on the anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated plus-maze. *Neuroscience & Biobehavioral Reviews, 23*, 971-980.

Horan, P., Taylor, J., Yamamura, H. I., & Porreca, F. (1992). Extremely long-lasting antagonistic actions of nor-binaltorphimine (nor- BNI) in the mouse tail-flick test. *J Pharmacol Exp Ther, 260*(3), 1237-1243.

Iidaka, T., Anderson, N. D., Kapur, S., Cabeza, R., & Craik, F. I. (2000). The effect of divided attention on encoding and retrieval in episodic memory revealed by positron emission tomography. *J Cogn Neurosci, 12*(2), 267-280.

Imperato, A., Angelucci, L., Casolini, P., Zocchi, A., & Puglisi-Allegra, S. (1992). Repeated stressful experiences differently affect limbic dopamine release during and following stress. *Brain Res, 577*(2), 194-199.

Inglis, F. M., & Fibiger, H. C. (1995). Increases in hippocampal and frontal cortical acetylcholine release associated with presentation of sensory stimuli. *Neuroscience, 66*(1), 81-86.

Inglis, W. L., Dunbar, J. S., & Winn, P. (1994). Outflow from the nucleus accumbens to the pedunculopontine tegmental nucleus: a dissociation between locomotor activity and the acquisition of responding for conditioned reinforcement stimulated by d-amphetamine. *Neuroscience, 62*(1), 51-64.

Inglis, W. L., Olmstead, M. C., & Robbins, T. W. (2001). Selective deficits in attentional performance on the 5-choice serial reaction time task following pedunculopontine tegmental nucleus lesions. *Behav Brain Res, 123*, 117-131.

Inoue, T. (1993). [Effects of conditioned fear stress on monoaminergic systems in the rat brain]. *Hokkaido Igaku Zasshi, 68*(3), 377-390.

Inoue, T., Koyama, T., & Yamashita, I. (1993). Effect of conditioned fear stress on serotonin metabolism in the rat brain. *Pharmacol Biochem Behav, 44*(2), 371-374.

Insausti, R., Herrero, M. T., & Witter, M. P. (1997). Entorhinal cortex of the rat: cytoarchitectonic subdivisions and the origin and distribution of cortical efferents. *Hippocampus, 7*(2), 146-183.

Itoh, J., Nabeshima, T., & Kameyama, T. (1991). Utility of an elevated plus-maze for dissociation of amnesic and behavioral effects of drugs in mice. *Eur J Pharmacol, 194*(1), 71-76.

Itoh, J., Ukai, M., & Kameyama, T. (1993a). Dynorphin A-(1-13) markedly improves scopolamine-induced impairment of spontaneous alternation performance in mice. *Eur J Pharmacol, 236*(3), 341-345.

Itoh, J., Ukai, M., & Kameyama, T. (1993b). U-50,488H, a kappa-opioid receptor agonist, markedly prevents memory dysfunctions induced by transient cerebral ischemia in mice. *Brain Res, 619*(1-2), 223-228.

Itoh, J., Ukai, M., & Kameyama, T. (1994). Dynorphin A-(1-13) potently improves the impairment of spontaneous alternation performance induced by the mu-selective opioid receptor agonist DAMGO in mice. *J Pharmacol Exp Ther, 269*(1), 15-21.

Itoh, S., Takashima, A., Igano, K., & Inouye, K. (1989). Memory effect of caerulein and its analogs in active and passive avoidance responses in the rat. *Peptides, 10*(4), 843-848.

Iversen, S. D., & Mishkin, M. (1970). Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity. *Exp Brain Res, 11*(4), 376-386.

Izaki, Y., Hori, K., & Nomura, M. (1998). Dopamine and acetylcholine elevation on lever-press acquisition in rat prefrontal cortex. *Neurosci Lett, 258*(1), 33-36.

Izaki, Y., Hori, K., & Nomura, M. (2000). Disturbance of rat lever-press learning by hippocampo-prefrontal disconnection. *Brain Res, 860*(1-2), 199-202.

Izquierdo, I., & Medina, J. H. (1997). Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiol Learn Mem, 68*(3), 285-316.

Izumi, T. (1998). [Behavioral and neurochemical study on the role of the brain cholecystokinin system in anxiety]. *Hokkaido Igaku Zasshi, 73*(5), 463-473.

Jaskiw, G. E., & Weinberger, D. R. (1990). Ibotenic acid lesions of the medial prefrontal cortex potentiate FG- 7142-induced attenuation of exploratory activity in the rat. *Pharmacol Biochem Behav, 36*(3), 695-697.

Jay, T. M., Burette, F., & Laroche, S. (1995). NMDA receptor-dependent long-term potentiation in the hippocampal afferent fibre system to the prefrontal cortex in the rat. *Eur J Neurosci, 7*(2), 247-250.

Jay, T. M., Glowinski, J., & Thierry, A. M. (1989). Selectivity of the hippocampal projection to the prelimbic area of the prefrontal cortex in the rat. *Brain Res, 505*(2), 337-340.

Jay, T. M., Glowinski, J., & Thierry, A. M. (1995). Inhibition of hippocampoprefrontal cortex excitatory responses by the mesocortical DA system. *Neuroreport, 6*(14), 1845-1848.

Jay, T. M., & Witter, M. P. (1991). Distribution of hippocampal CA1 and subicular efferents in the prefrontal cortex of the rat studied by means of anterograde transport of Phaseolus vulgaris-leucoagglutinin. *J Comp Neurol, 313*(4), 574-586.

Jentsch, J. D., Tran, A., Le, D., Youngren, K. D., & Roth, R. H. (1997). Subchronic phencyclidine administration reduces mesoprefrontal dopamine utilization and impairs prefrontal cortical-dependent cognition in the rat. *Neuropsychopharmacology, 17*(2), 92-99.

Jinks, A. L., & McGregor, I. S. (1997). Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat. *Brain Res, 772*(1-2), 181-190.

Joel, D., Tarrasch, R., Feldon, J., & Weiner, I. (1997). Effects of electrolytic lesions of the medial prefrontal cortex or its subfields on 4-arm baited, 8-arm radial maze, two-way active avoidance and conditioned fear tasks in the rat. *Brain Res, 765*(1), 37-50.

Jones, B. E., & Cuello, A. C. (1989). Afferents to the basal forebrain cholinergic cell area from pontomesencephalic--catecholamine, serotonin, and acetylcholine-- neurons. *Neuroscience, 31*(1), 37-61.

Jones, D. N., & Holtzman, S. G. (1992). Long term kappa-opioid receptor blockade following nor-binaltorphimine. *Eur J Pharmacol, 215*(2-3), 345-348.

Jones, E. G., & Hendry, S. H. (1986). Peptide-containing neurons of the primate cerebral cortex. *Res Publ Assoc Res Nerv Ment Dis, 64*, 163-178.

Jones, G. H., & Robbins, T. W. (1992). Differential effects of mesocortical, mesolimbic, and mesostriatal dopamine depletion on spontaneous, conditioned, and drug-induced locomotor activity. *Pharmacol Biochem Behav, 43*(3), 887-895.

Joreskog, K. G., & Sorbom, D. (1993a). *LISREL 8: Structural equation modeling with the SIMPLIS command language*. Chicago, IL, US: Scientific Software International, Inc.

Joreskog, K. G., & Sorbom, D. (1993b). *New features in LISREL 8.* Chicago: Scientific Software.

Jung, M. W., Qin, Y., McNaughton, B. L., & Barnes, C. A. (1998). Firing characteristics of deep layer neurons in prefrontal cortex in rats performing spatial working memory tasks. *Cereb Cortex, 8*(5), 437-450.

Kameyama, T., Ukai, M., & Shinkai, N. (1998). Ameliorative effects of tachykinins on scopolamine-induced impairment of spontaneous alternation performance in mice. *Methods Find Exp Clin Pharmacol, 20*(7), 555-560.

Kaneyuki, H., Yokoo, H., Tsuda, A., Yoshida, M., Mizuki, Y., Yamada, M., & Tanaka, M. (1991). Psychological stress increases dopamine turnover selectively in mesoprefrontal dopamine neurons of rats: reversal by diazepam. *Brain Res, 557*(1-2), 154-161.

Karnath, H. O., Wallesch, C. W., & Zimmermann, P. (1991). Mental planning and anticipatory processes with acute and chronic frontal lobe lesions: a comparison of maze performance in routine and non-routine situations. *Neuropsychologia, 29*(4), 271-290.

Kavaliers, M., & Ossenkopp, K. (2001). Measuring anxiety in animal models: an introduction to the symposium. *Neurosci Biobehav Rev, 25*(3), 203-204.

Kenny, P. J., Cheeta, S., & File, S. E. (2000). Anxiogenic effects of nicotine in the dorsal hippocampus are mediated by 5-HT_{1A} and not by muscarinic M_1 receptors. *Neuropharmacology, 39*, 300-307.

Kesner, R. P., Farnsworth, G., & DiMattia, B. V. (1989). Double dissociation of egocentric and allocentric space following medial prefrontal and parietal cortex lesions in the rat. *Behav Neurosci, 103*(5), 956-961.

Kesner, R. P., Hunt, M. E., Williams, J. M., & Long, J. M. (1996). Prefrontal cortex and working memory for spatial response, spatial location, and visual object information in the rat. *Cereb Cortex, 6*(2), 311-318.

Kim, J. S., & Levin, E. D. (1996). Nicotinic, muscarinic and dopaminergic actions in the ventral hippocampus and the nucleus accumbens: effects on spatial working memory in rats. *Brain Res, 725*(2), 231-240.

King, D., & Finlay, J. M. (1995). Effects of selective dopamine depletion in medial prefrontal cortex on basal and evoked extracellular dopamine in neostriatum. *Brain Res, 685*(1-2), 117-128.

Kinomura, S., Larsson, J., Gulyas, B., & Roland, P. E. (1996). Activation by attention of the human reticular formation and thalamic intralaminar nuclei. *Science, 271*(5248), 512-515.

Kling, A., & Steklis, H. D. (1976). A neural substrate for affiliative behavior in nonhuman primates. *Brain Behav Evol, 13*(2-3), 216-238.

Knable, M. B., & Weinberger, D. R. (1997). Dopamine, the prefrontal cortex and schizophrenia. *J Psychopharmacol, 11*(2), 123-131.

Knight, R. T., & Nakada, T. (1998). Cortico-limbic circuits and novelty: a review of EEG and blood flow data. *Rev Neurosci, 9*(1), 57-70.

Knights, R. T., Grabowecky, A., & Scabini, D. (1995). Role of human prefrontal cortex in attention control. In H. H. Jasper & S. Riggio & P. S. Goldman-Rakic (Eds.), *Epilepsy and the functional anatomy of the frontal lobe* (pp. 21-34). New York: Raven Press.

Kolb, B., Buhrmann, K., McDonald, R., & Sutherland, R. J. (1994). Dissociation of the medial prefrontal, posterior parietal, and posterior temporal cortex for spatial navigation and recognition memory in the rat. *Cereb Cortex, 4*(6), 664-680.

Kowalska, D. M., Bachevalier, J., & Mishkin, M. (1991). The role of the inferior prefrontal convexity in performance of delayed nonmatching-to-sample. *Neuropsychologia, 29*(6), 583-600.

Lacroix, L., Broersen, L. M., Feldon, J., & Weiner, I. (2000). Effects of local infusions of dopaminergic drugs into the medial prefrontal cortex of rats on latent inhibition, prepulse inhibition and amphetamine induced activity. *Behav Brain Res, 107*(1-2), 111-121.

Lane, R. D., Fink, G. R., Chau, P. M., & Dolan, R. J. (1997). Neural activation during selective attention to subjective emotional responses. *Neuroreport, 8*(18), 3969-3972.

Lane, R. D., Reiman, E. M., Axelrod, B., Yun, L. S., Holmes, A., & Schwartz, G. E. (1998). Neural correlates of levels of emotional awareness. Evidence of an interaction between emotion and attention in the anterior cingulate cortex. *J Cogn Neurosci, 10*(4), 525-535.

Lang, A. J., & Craske, M. G. (1997). Information processing in anxiety and depression. *Behav Res Ther, 35*(5), 451-455.

Lang, P. J., Davis, M., & Ohman, A. (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *J Affect Disord, 61*(3), 137-159.

Lang, P. J., & Maser, J. D. (2000). Editorial. *J Affect Disord, 61*(3), 133-135.

Lawrence, A. D., & Sahakian, B. J. (1995). Alzheimer disease, attention and the cholinergic system. *Alzheimer Dis Assoc Disord, 9*(Suppl 2), 43-49.

Lawrence, A. D., & Sahakian, B. J. (1998). The cognitive psychopharmacology of Alzheimer's disease: focus on cholinergic systems. *Neurochem Res, 23*(5), 787-794.

Le Moal, M., & Simon, H. (1991). Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol Rev, 71*(1), 155-234.

LeDoux, J. E. (1992). Brain mechanisms of emotion and emotional learning. *Curr Opin Neurobiol, 2*(2), 191-197.

LeDoux, J. E. (1993a). Emotional memory: in search of systems and synapses. *Ann N Y Acad Sci, 702*, 149-157.

LeDoux, J. E. (1993b). Emotional memory systems in the brain. *Behav Brain Res, 58*(1-2), 69-79.

LeDoux, J. E. (1995). Emotion: clues from the brain. *Annu Rev Psychol, 46*, 209-235.

Lee, C., & Rodgers, R. J. (1990). Antinociceptive effects of elevated plus-maze exposure: influence of opiate receptor manipulations. *Psychopharmacology, 102*(4), 507-513.

Lee, C., & Rodgers, R. J. (1991). Effects of benzodiazepine receptor antagonist, flumazenil, on antinociceptive and behavioural responses to the elevated plus-maze in mice. *Neuropharmacology, 30*(12A), 1263-1267.

Leri, F., & Franklin, K. B. (1998). Learning impairments caused by lesions to the pedunculopontine tegmental nucleus: an artifact of anxiety? *Brain Res, 807*(1-2), 187-192.

Lhermitte, F., Pillon, B., & Serdaru, M. (1986). Human autonomy and the frontal lobes. Part I: Imitation and utilization behavior: a neuropsychological study of 75 patients. *Ann Neurol, 19*(4), 326-334.

Li, C. S., Chen, M. C., Yang, Y. Y., Chang, H. L., Liu, C. Y., Shen, S., & Chen, C. Y. (2000). Perceptual alternation in obsessive compulsive disorder--implications for a role of the cortico-striatal circuitry in mediating awareness. *Behav Brain Res, 111*(1-2), 61-69.

Liang, K. C., Hu, S. J., & Chang, S. C. (1996). Formation and retrieval of inhibitory avoidance memory: differential roles of glutamate receptors in the amygdala and medial prefrontal cortex. *Chin J Physiol, 39*(3), 155-166.

Lister, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology, 92*(2), 180-185.

Lister, R. G. (1990). Ethologically-based animal models of anxiety disorders. *Pharmacol Ther, 46*(3), 321-340.

Lucki, I., & Rickels, K. (1988). The effect of anxiolytic drugs on memory in anxious subjects. *Psychopharmacol Ser, 6*, 128-139.

MacCallum, R. (1986). Specification searches in covariance structure modeling. *Psychological Bulletin, 100*(1), 107-120.

MacLeod, C., & McLaughlin, K. (1995). Implicit and explicit memory bias in anxiety: a conceptual replication. *Behav Res Ther, 33*(1), 1-14.

Maguire, E. A., & Mummery, C. J. (1999). Differential modulation of a common memory retrieval network revealed by positron emission tomography [In Process Citation]. *Hippocampus, 9*(1), 54-61.

Mansour, A., Fox, C. A., Akil, H., & Watson, S. J. (1995). Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends Neurosci, 18*(1), 22-29.

Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H., & Watson, S. J. (1988). Anatomy of CNS opioid receptors. *Trends Neurosci, 11*(7), 308-314.

Mantz, J., Godbout, R., Pirot, S., Glowinski, J., & Thierry, A. M. (1992). Inhibitory effects of mesocortical dopaminergic neurons on their target cells: electrophysiological and pharmacological characterization. *Neurochem Int, 20 Suppl*, 251S-254S.

Mantz, J., Milla, C., Glowinski, J., & Thierry, A. M. (1988). Differential effects of ascending neurons containing dopamine and noradrenaline in the control of spontaneous activity and of evoked responses in the rat prefrontal cortex. *Neuroscience, 27*, 517-526.

Mantz, J., Thierry, A. M., & Glowinski, J. (1989). Effect of noxious tail pinch on the discharge rate of mesocortical and mesolimbic dopamine neurons: selective activation of the mesocortical system. *Brain Res, 476*(2), 377-381.

Manzanares, J., Lookingland, K. J., LaVigne, S. D., & Moore, K. E. (1991). Activation of tuberohypophysial dopamine neurons following intracerebroventricular administration of the selective kappa opioid receptor antagonist NOR-binaltorphimine. *Life Sci, 48*(12), 1143-1149.

Manzanares, J., Lookingland, K. J., & Moore, K. E. (1990). Kappa-opioid-receptor-mediated regulation of alpha-melanocyte- stimulating hormone secretion and tuberohypophyseal dopaminergic neuronal activity. *Neuroendocrinology, 52*(2), 200-205.

Manzanares, J., Lookingland, K. J., & Moore, K. E. (1991). Kappa opioid receptor-mediated regulation of dopaminergic neurons in the rat brain. *J Pharmacol Exp Ther, 256*(2), 500-505.

Mark, G. P., Rada, P. V., & Shors, T. J. (1996). Inescapable stress enhances extracellular acetylcholine in the rat hippocampus and prefrontal cortex but not the nucleus accumbens or amygdala. *Neuroscience, 74*(3), 767-774.

Markowska, A. L., Olton, D. S., & Givens, B. (1995). Cholinergic manipulations in the medial septal area: age-related effects on working memory and hippocampal electrophysiology. *J Neurosci, 15*(3 Pt 1), 2063-2073.

Masterman, D. L., & Cummings, J. L. (1997). Frontal-subcortical circuits: the anatomic basis of executive, social and motivated behaviors. *J Psychopharmacol, 11*(2), 107-114.

Mathews, A., Mogg, K., Kentish, J., & Eysenck, M. (1995). Effect of psychological treatment on cognitive bias in generalized anxiety disorder. *Behav Res Ther, 33*(3), 293-303.

Mathews, A., Mogg, K., May, J., & Eysenck, M. (1989). Implicit and explicit memory bias in anxiety. *J Abnorm Psychol, 98*(3), 236-240.

Mathis, C., Paul, S. M., & Crawley, J. N. (1994). Characterization of benzodiazepine-sensitive behaviors in the A/J and C57BL/6J inbred strains of mice. *Behav Genet, 24*(2), 171-180.

Maurice, T., Hiramatsu, M., Itoh, J., Kameyama, T., Hasegawa, T., & Nabeshima, T. (1994). Behavioral evidence for a modulating role of sigma ligands in memory processes. I. Attenuation of dizocilpine (MK-801)-induced amnesia. *Brain Res, 647*(1), 44-56.

Maxmen, J. S. (1986). *Essential Psychopathology*. New York: W. W. Norton & Company.

McClelland, J. L., & Goddard, N. H. (1996). Considerations arising from a complementary learning systems perspective on hippocampus and neocortex. *Hippocampus, 6*(6), 654-665.

McDaniel, K. L., Mundy, W. R., & Tilson, H. A. (1990). Microinjection of dynorphin into the hippocampus impairs spatial learning in rats. *Pharmacol Biochem Behav, 35*(2), 429-435.

McGaughy, J., Everitt, B. J., Robbins, T. W., & Sarter, M. (2000). The role of cortical cholinergic afferent projections in cognition: impact of new selective immunotoxins. *Behav Brain Res, 115*(2), 251-263.

McGaughy, J., Kaiser, T., & Sarter, M. (1996). Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behav Neurosci, 110*(2), 247-265.

McGeer, P. L., Eccles, J. C., & McGeer, E. G. (1978). *Molecular Neurobiology of the Mammalian Brain*. New York: Plenum.

McNally, R. J. (1990). Psychological approaches to panic disorder: a review. *Psychol Bull, 108*(3), 403-419.

McNally, R. J. (1995). Automaticity and the anxiety disorders. *Behav Res Ther, 33*(7), 747-754.

McNally, R. J. (1996). Cognitive bias in the anxiety disorders. *Nebr Symp Motiv, 43*, 211-250.

McNally, R. J. (1997). Memory and anxiety disorders. *Philos Trans R Soc Lond B Biol Sci, 352*(1362), 1755-1759.

McNally, R. J., Hornig, C. D., Otto, M. W., & Pollack, M. H. (1997). Selective encoding of threat in panic disorder: application of a dual priming paradigm. *Behav Res Ther, 35*(6), 543-549.

McNaughton, N. (1997). Cognitive dysfunction resulting from hippocampal hyperactivity--a possible cause of anxiety disorder? *Pharmacol Biochem Behav, 56*(4), 603-611.

McNaughton, N., & Gray, J. A. (2000). Anxiolytic action on the behavioural inhibition system implies multiple types of arousal contribute to anxiety. *J Affect Disord, 61*(3), 161-176.

Messier, C., Emond, S., & Ethier, K. (1999). New techniques in stereotaxic surgery and anesthesia in the mouse. *Pharmacol Biochem Behav, 63*(2), 313-318.

Messier, C., Wall, P. M., & Ethier, K. (1999). Contribution of cholinergic and gabaergic functions to memory processes in BALB/cANnCrlBR mice. *Brain Res, 818*(2), 583-592.

Mesulam, M. M., & Mufson, E. J. (1984). Neural inputs to the nucleus basalis of the substantia innominata (Ch. 4) in the rhesus monkey. *Brain, 104*, 253-274.

Mesulam, M. M., Mufson, E. J., Wainer, B. H., & Levey, A. I. (1983). Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience, 10*(4), 1185-1201.

Metherate, R., Cox, C. L., & Ashe, J. H. (1992). Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J Neurosci, 12*(12), 4701-4711.

Meunier, M., Bachevalier, J., & Mishkin, M. (1997). Effects of orbital frontal and anterior cingulate lesions on object and spatial memory in rhesus monkeys. *Neuropsychologia, 35*(7), 999-1015.

Mirsky, A. F., & Rosvold, H. E. (1960). The use of psychoactive drug as a neuropsychological tool in studies of attention in man. In H. Uhr & J. G. Miller (Eds.), *Drugs and Behaviour* (pp. 375-392). New York: Wiley.

Mishkin, M., & Manning, F. J. (1978). Non-spatial memory after selective prefrontal lesions in monkeys. *Brain Res, 143*(2), 313-323.

Mogensen, J., & Holm, S. (1994). The prefrontal cortex and variants of sequential behaviour indications of functional differentiation between subdivisions of the rat's prefrontal cortex. *Behav Brain Res, 63*(1), 89-100.

Montgomery, K. C. (1955). The relation between fear induced by novel stimulation and exploratory behaviour. *J. Comp. Physiol. Psychol., 48*, 264-280.

Moore, H., Fadel, J., Sarter, M., & Bruno, J. P. (1999). Role of accumbens and cortical dopamine receptors in the regulation of cortical acetylcholine release. *Neuroscience, 88*(3), 811-822.

Moore, H., West, A. R., & Grace, A. A. (1999). The regulation of forebrain dopamine transmission: relevance to the pathophysiology and psychopathology of schizophrenia. *Biol Psychiatry, 46*(1), 40-55.

Mora, P. O., Netto, C. F., & Graeff, F. G. (1997). Role of 5-HT2A and 5-HT2C receptor subtypes in the two types of fear generated by the elevated T-maze. *Pharmacol Biochem Behav, 58*(4), 1051-1057.

Morgan, M. A., & LeDoux, J. E. (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behav Neurosci, 109*(4), 681-688.

Morgan, M. A., Romanski, L. M., & LeDoux, J. E. (1993). Extinction of emotional learning: contribution of medial prefrontal cortex. *Neurosci Lett, 163*(1), 109-113.

Morrow, B. A., Roth, R. H., & Elsworth, J. D. (2000). TMT, a predator odor, elevates mesoprefrontal dopamine metabolic activity and disrupts short-term working memory in the rat. *Brain Res Bull, 52*(6), 519-523.

Muir, J. L., Dunnett, S. B., Robbins, T. W., & Everitt, B. J. (1992). Attentional functions of the forebrain cholinergic systems: effects of intraventricular hemicholinium, physostigmine, basal forebrain lesions and intracortical grafts on a multiple-choice serial reaction time task. *Exp Brain Res, 89*(3), 611-622.

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1994). AMPA-induced excitotoxic lesions of the basal forebrain: a significant role for the cortical cholinergic system in attentional function. *J Neurosci, 14*(4), 2313-2326.

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1996). The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. *Cereb Cortex, 6*(3), 470-481.

Mumby, D. G., & Glenn, M. J. (2000). Anterograde and retrograde memory for object discriminations and places in rats with perirhinal cortex lesions. *Behav Brain Res, 114*(1-2), 119-134.

Murphy, B. L., Arnsten, A. F., Goldman-Rakic, P. S., & Roth, R. H. (1996). Increased dopamine turnover in the prefrontal cortex impairs spatial working memory performance in rats and monkeys. *Proc Natl Acad Sci U S A, 93*(3), 1325-1329.

Murphy, B. L., Arnsten, A. F., Jentsch, J. D., & Roth, R. H. (1996). Dopamine and spatial working memory in rats and monkeys: pharmacological reversal of stress-induced impairment. *J Neurosci, 16*(23), 7768-7775.

Muthen, B., & Kaplan, D. (1985). A comparison of some methodologies for the factor analysis of non-normal Likert variables. *British Journal of Mathematical & Statistical Psychology, 38*(2), 171-189.

Nabeshima, T., Katoh, A., Wada, M., & Kameyama, T. (1992). Stress-induced changes in brain Met-enkephalin, Leu-enkephalin and dynorphin concentrations. *Life Sci, 51*(3), 211-217.

Neafsey, E. J. (1990). Prefrontal cortical control of the autonomic nervous system: anatomical and physiological observations. *Prog Brain Res, 85*, 147-165.

Neafsey, E. J., Hurley-Gius, K. M., & Arvanitis, D. (1986). The topographical organization of neurons in the rat medial frontal, insular and olfactory cortex projecting to the solitary nucleus, olfactory bulb, periaqueductal gray and superior colliculus. *Brain Res, 377*(2), 561-570.

Ohno, M., Kikusui, M., Yoshimatsu, A., Yamamoto, T., & Watanabe, S. (1994). Somatostatin alleviates impairment of working memory induced by hippocampal muscarinic M1 receptor blockade in rats. *Eur J Pharmacol, 271*(2-3), 557-560.

Ohno, M., Yamamoto, T., & Watanabe, S. (1994). Blockade of hippocampal M1 muscarinic receptors impairs working memory performance of rats. *Brain Res, 650*(2), 260-266.

Okubo, Y., Suhara, T., Suzuki, K., Kobayashi, K., Inoue, O., Terasaki, O., Someya, Y., Sassa, T., Sudo, Y., Matsushima, E., Iyo, M., Tateno, Y., & Toru, M. (1997). Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET [see comments]. *Nature, 385*(6617), 634-636.

Olson, G. A., Olson, R. D., & Kastin, A. J. (1993). Endogenous opiates: 1992. *Peptides, 14*(6), 1339-1378.

Olson, G. A., Olson, R. D., & Kastin, A. J. (1995). Endogenous opiates: 1994. *Peptides, 16*(8), 1517-1555.

Olson, G. A., Olson, R. D., & Kastin, A. J. (1996). Endogenous opiates: 1995. *Peptides, 17*(8), 1421-1466.

Olton, D. S. (1977). The function of septo-hippocampal connections in spatially organized behaviour. *Ciba Found Symp, 58*, 327-349.

Olton, D. S. (1987). The radial arm maze as a tool in behavioral pharmacology. *Physiol Behav, 40*(6), 793-797.

Olton, D. S., & Papas, B. C. (1979). Spatial memory and hippocampal function. *Neuropsychologia, 17*(6), 669-682.

Olton, D. S., & Werz, M. A. (1978). Hippocampal function and behavior: spatial discrimination and response inhibition. *Physiol Behav, 20*(5), 597-605.

Olton, D. S., Wible, C. G., & Shapiro, M. L. (1986). Mnemonic theories of hippocampal function. *Behav Neurosci, 100*(6), 852-855.

Otto, T., & Eichenbaum, H. (1992). Complementary roles of the orbital prefrontal cortex and the perirhinal- entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task. *Behav Neurosci, 106*(5), 762-775.

Ouagazzal, A. M., Kenny, P. J., & File, S. E. (1999). Modulation of behaviour on trials 1 and 2 in the elevated plus-maze test of anxiety after systemic and hippocampal administration of nicotine. *Psychopharmacology, 144*(1), 54-60.

Owens, N. C., & Verberne, A. J. (1996). An electrophysiological study of the medial prefrontal cortical projection to the nucleus of the solitary tract in rat. *Exp Brain Res, 110*(1), 55-61.

Parker, A., & Gaffan, D. (1998). Memory after frontal/temporal disconnection in monkeys: conditional and non-conditional tasks, unilateral and bilateral frontal lesions. *Neuropsychologia, 36*(3), 259-271.

Parsons, M. W., & Gold, P. E. (1992). Scopolamine-induced deficits in spontaneous alternation performance: attenuation with lateral ventricle injections of glucose. *Behav Neural Biol, 57*(1), 90-92.

Pashler, H. E. (1998). *The Psychology of Attention*. Cambridge, MA: Bradford/MIT Press.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods, 14*(3), 149-167.

Pellow, S., & File, S. E. (1986). Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. *Pharmacol Biochem Behav, 24*(3), 525-529.

Penna, A. M., Lee, S. Y., Scheidegger da Silva, L., Oliveira, R. W., de Freitas Gomes, C., & Nakamura Palacios, E. M. (1998). Behavioral and cognitive effects produced by electrical stimulation in the medial prefrontal cortex: an experimental model for high cortical activation. *Neuropsychobiology, 38*(4), 241-250.

Peretti, C. S. (1998). [Anxiety and cognition disorders]. *Encephale, 24*(3), 256-259.

Perry, E., Walker, M., Grace, J., & Perry, R. (1999). Acetylcholine in mind: a neurotransmitter correlate of consciousness? [see comments]. *Trends Neurosci, 22*(6), 273-280.

Pesold, C., & Treit, D. (1992). Excitotoxic lesions of the septum produce anxiolytic effects in the elevated plus-maze and the shock-probe burying tests. *Physiol Behav, 52*(1), 37-47.

Pesold, C., & Treit, D. (1996). The neuroanatomical specificity of the anxiolytic effects of intra-septal infusions of midazolam. *Brain Res, 710*(1-2), 161-168.

Petit, L., Courtney, S. M., Ungerleider, L. G., & Haxby, J. V. (1998). Sustained activity in the medial wall during working memory delays. *J Neurosci, 18*(22), 9429-9437.

Petty, F., Jordan, S., Kramer, G. L., Zukas, P. K., & Wu, J. (1997). Benzodiazepine prevention of swim stress-induced sensitization of cortical biogenic amines: an in vivo microdialysis study. *Neurochem Res, 22*(9), 1101-1104.

Pinel, J. P., Mumby, D. G., Dastur, F. N., & Pinel, J. G. (1994). Rat (Rattus norvegicus) defensive behavior in total darkness: risk- assessment function of defensive burying. *J Comp Psychol, 108*(2), 140-147.

Pirot, S., Godbout, R., Mantz, J., Tassin, J. P., Glowinski, J., & Thierry, A. M. (1992). Inhibitory effects of ventral tegmental area stimulation on the activity of prefrontal cortical neurons: evidence for the involvement of both dopaminergic and GABAergic components. *Neuroscience, 49*(4), 857-865.

Pitha, J., Gerloczy, A., & Olivi, A. (1994). Parenteral hydroxypropyl cyclodextrins: Intravenous and intracerebral administration of lipophiles. *J Pharm Sci, 83*, 833-837.

Pontecorvo, M. J., Sahgal, A., & Steckler, T. (1996). Further developments in the measurement of working memory in rodents. *Brain Res Cogn Brain Res, 3*(3-4), 205-213.

Portoghese, P. S., Garzon-Aburbeh, A., Nagase, H., Lin, C. E., & Takemori, A. E. (1991). Role of the spacer in conferring kappa opioid receptor selectivity to bivalent ligands related to norbinaltorphimine. *J Med Chem, 34*(4), 1292-1296.

Portoghese, P. S., Lipkowski, A. W., & Takemori, A. E. (1987). Binaltorphimine and nor-binaltorphimine, potent and selective kappa- opioid receptor antagonists. *Life Sci, 40*(13), 1287-1292.

Portoghese, P. S., Nagase, H., Lipkowski, A. W., Larson, D. L., & Takemori, A. E. (1988). Binaltorphimine-related bivalent ligands and their kappa opioid receptor antagonist selectivity [published erratum appears in J Med Chem 1988 Oct;31(10):2056]. *J Med Chem, 31*(4), 836-841.

Portoghese, P. S., Nagase, H., & Takemori, A. E. (1988). Only one pharmacophore is required for the kappa opioid antagonist selectivity of norbinaltorphimine. *J Med Chem, 31*(7), 1344-1347.

Posner, M. I., & Petersen, S. E. (1990). The attention system of the human brain. *Annu Rev Neurosci, 13*, 25-42.

Poucet, B. (1997). Searching for spatial unit firing in the prelimbic area of the rat medial prefrontal cortex. *Behav Brain Res, 84*(1-2), 151-159.

Privette, T. H., & Terrian, D. M. (1995). Kappa opioid agonists produce anxiolytic-like behavior on the elevated plus-maze. *Psychopharmacology (Berl), 118*(4), 444-450.

Puglisi-Allegra, S., Imperato, A., Angelucci, L., & Cabib, S. (1991). Acute stress induces time-dependent responses in dopamine mesolimbic system. *Brain Res, 554*(1-2), 217-222.

Quartermain, D., Stone, E. A., & Charbonneau, G. (1996). Acute stress disrupts risk assessment behavior in mice. *Physiol Behav, 59*(4-5), 937-940.

Quigley, K. S., Sarter, M. F., Hart, S. L., & Berntson, G. G. (1994). Cardiovascular effects of the benzodiazepine receptor partial inverse agonist FG 7142 in rats. *Behav Brain Res, 62*(1), 11-20.

Rachman, S. J. (1991). Disorders of emotion: Causes and consequences. *Psychological Inquiry, 2*, 86-87.

Ragozzino, M. E., Adams, S., & Kesner, R. P. (1998). Differential involvement of the dorsal anterior cingulate and prelimbic- infralimbic areas of the rodent prefrontal cortex in spatial working memory. *Behav Neurosci, 112*(2), 293-303.

Ragozzino, M. E., Detrick, S., & Kesner, R. P. (1999). Involvement of the prelimbic--infralimbic areas of the rodent prefrontal cortex in behavioural flexibility for place and response learning. *J Neurosci, 19*(11), 4585-4594.

Ragozzino, M. E., & Kesner, R. P. (1998). The effects of muscarinic cholinergic receptor blockade in the rat anterior cingulate and Prelimbic/Infralimbic cortices on spatial working memory. *Neurobiol Learn Mem, 69*(3), 241-257.

Ragozzino, M. E., Unick, K. E., & Gold, P. E. (1996). Hippocampal acetylcholine release during memory testing in rats: augmentation by glucose. *Proc Natl Acad Sci U S A, 93*(10), 4693-4698.

Rainer, G., Rao, S. C., & Miller, E. K. (1999). Prospective coding for objects in primate prefrontal cortex. *J Neurosci, 19*(13), 5493-5505.

Ramus, S. J., & Eichenbaum, H. (2000). Neural correlates of olfactory recognition memory in the rat orbitofrontal cortex. *J Neurosci, 20*(21), 8199-8208.

Reivich, M., Gur, R., & Alavi, A. (1983). Positron emission tomographic studies of sensory stimuli, cognitive processes and anxiety. *Hum Neurobiol, 2*(1), 25-33.

Rempel-Clower, N. L., & Barbas, H. (1998). Topographic organization of connections between the hypothalamus and prefrontal cortex in the rhesus monkey. *J Comp Neurol, 398*(3), 393-419.

Retaux, S., Besson, M. J., & Penit-Soria, J. (1991). Opposing effects of dopamine D2 receptor stimulation on the spontaneous and the electrically evoked release of [3H]GABA on rat prefrontal cortex slices. *Neuroscience, 42*(1), 61-71.

Retaux, S., Trovero, F., & Besson, M. J. (1994). Role of dopamine in the plasticity of glutamic acid decarboxylase messenger RNA in the rat frontal cortex and the nucleus accumbens. *Eur J Neurosci, 6*(12), 1782-1791.

Robbins, T. W. (1990). The case of frontostriatal dysfunction in schizophrenia. *Schizophr Bull, 16*(3), 391-402.

Robbins, T. W. (1998). Arousal and attention: psychopharmacological and neuropsychological studies in experimental animals. In R. Parasuraman (Ed.), *The Attentive Brain* (pp. 189-220). Cambridge, MASS: MIT Press.

Robbins, T. W. (2000a). Chemical neuromodulation of frontal-executive functions in humans and other animals. *Exp Brain Res, 133*(1), 130-138.

Robbins, T. W. (2000b). From arousal to cognition: the integrative position of the prefrontal cortex. *Prog Brain Res, 126*, 469-483.

Robbins, T. W., & Everitt, B. J. (1995). Arousal systems and attention. In M. I. Posner (Ed.), *The Cognitive Neurosciences* (pp. 703-720). Cambridge, Mass.: MIT Press.

Robbins, T. W., Everitt, B. J., Marston, H. M., Wilkinson, J., Jones, G. H., & Page, K. J. (1989). Comparative effects of ibotenic acid- and quisqualic acid-induced lesions of the substantia innominata on attentional function in the rat: further implications for the role of the cholinergic neurons of the nucleus basalis in cognitive processes. *Behav Brain Res, 35*(3), 221-240.

Robbins, T. W., Everitt, B. J., Ryan, C. N., Marston, H. M., Jones, G. H., & Page, K. J. (1989). Comparative effects of quisqualic and ibotenic acid-induced lesions of the substantia innominata and globus pallidus on the acquisition of a conditional visual discrimination: differential effects on cholinergic mechanisms. *Neuroscience, 28*(2), 337-352.

Robinson, J. K., Scheff, A., Pierre-Louis, J., & Han, C. J. (2000). Specificity of memory measures in an adjusting delayed nonmatching-to- position task for rats. *Behav Brain Res, 111*(1-2), 107-113.

Rodgers, R. J., & Cole, J. C. (1993a). Anxiety enhancement in the murine elevated plus maze by immediate prior exposure to social stressors. *Physiol Behav, 53*(2), 383-388.

Rodgers, R. J., & Cole, J. C. (1993b). Influence of social isolation, gender, strain, and prior novelty on plus-maze behaviour in mice. *Physiol Behav, 54*(4), 729-736.

Rodgers, R. J., & Cole, J. C. (1994). Anxiolytic-like effect of (S)-WAY 100135, a 5-HT1A receptor antagonist, in the murine elevated plus-maze test. *Eur J Pharmacol, 261*(3), 321-325.

Rodgers, R. J., & Cole, J. C. (1995). Effects of scopolamine and its quaternary analogue in the murine elevated plus-maze test of anxiety. *Behavioural Pharmacology, 6*(3), 283-289.

Rodgers, R. J., Cole, J. C., Aboualfa, K., & Stephenson, L. H. (1995). Ethopharmacological analysis of the effects of putative 'anxiogenic' agents in the mouse elevated plus-maze. *Pharmacol Biochem Behav, 52*(4), 805-813.

Rodgers, R. J., Cole, J. C., & Davies, A. (1994). Antianxiety and behavioral suppressant actions of the novel 5-HT1A receptor agonist, flesinoxan. *Pharmacol Biochem Behav, 48*(4), 959-963.

Rodgers, R. J., & Dalvi, A. (1997). Anxiety, defence and the elevated plus-maze. *Neurosci Biobehav Rev, 21*(6), 801-810.

Rodgers, R. J., & Johnson, N. J. (1995). Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacol Biochem Behav, 52*(2), 297-303.

Rodgers, R. J., Johnson, N. J., Carr, J., & Hodgson, T. P. (1997). Resistance of experientially-induced changes in murine plus-maze behaviour to altered retest conditions. *Behav Brain Res, 86*(1), 71-77.

Rodgers, R. J., Johnson, N. J., Champion, A. J., & Mills, S. (1996). Modulation of plus-maze behaviour in mice by the preferential D3- receptor agonist 7-OH-DPAT. *Pharmacol Biochem Behav, 54*(1), 79-84.

Rodgers, R. J., Johnson, N. J., Cole, J. C., Dewar, C. V., Kidd, G. R., & Kimpson, P. H. (1996). Plus-maze retest profile in mice: importance of initial stages of trail 1 and response to post-trail cholinergic receptor blockade. *Pharmacol Biochem Behav, 54*(1), 41-50.

Rodgers, R. J., Lee, C., & Shepherd, J. K. (1992). Effects of diazepam on behavioural and antinociceptive responses to the elevated plus-maze in male mice depend upon treatment regimen and prior maze experience. *Psychopharmacology, 106*(1), 102-110.

Rodgers, R. J., Nikulina, E. M., & Cole, J. C. (1994). Dopamine D1 and D2 receptor ligands modulate the behaviour of mice in the elevated plus-maze. *Pharmacol Biochem Behav, 49*(4), 985-995.

Rodgers, R. J., & Shepherd, J. K. (1993). Influence of prior maze experience on behaviour and response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice. *Psychopharmacology, 113*(2), 237-242.

Rogers, M. P., Warshaw, M. G., Goisman, R. M., Goldenberg, I., Rodriguez-Villa, F., Mallya, G., Freeman, S. A., & Keller, M. B. (1999). Comparing primary and secondary generalized anxiety disorder in a long- term naturalistic study of anxiety disorders. *Depress Anxiety, 10*(1), 1-7.

Rolls, E. T. (1995). A theory of emotion and consciousness, and its application to understanding the neural basis of emotion. In S. G. Michael (Ed.), *The cognitive neurosciences.* (pp. 1091-1106): MIT Press, Cambridge, MA, US.

Rolls, E. T. (1996). The orbitofrontal cortex. *Philos Trans R Soc Lond B Biol Sci, 351*(1346), 1433-1443; discussion 1443-1434.

Rolls, E. T., Critchley, H. D., Mason, R., & Wakeman, E. A. (1996). Orbitofrontal cortex neurons: role in olfactory and visual association learning. *J Neurophysiol, 75*(5), 1970-1981.

Rossetti, Z. L., Lai, M., Hmaidan, Y., & Gessa, G. L. (1993). Depletion of mesolimbic dopamine during behavioral despair: partial reversal by chronic imipramine. *Eur J Pharmacol, 242*(3), 313-315.

Roy-Byrne, P. P., & Katon, W. (1997). Generalized anxiety disorder in primary care: the precursor/modifier pathway to increased health care utilization. *J Clin Psychiatry, 58*(Suppl 3), 34-38; discussion 39-40.

Rugg, M. D., Fletcher, P. C., Frith, C. D., Frackowiak, R. S., & Dolan, R. J. (1996). Differential activation of the prefrontal cortex in successful and unsuccessful memory retrieval. *Brain, 119*(Pt 6), 2073-2083.

Ruit, K. G., & Neafsey, E. J. (1988). Cardiovascular and respiratory responses to electrical and chemical stimulation of the hippocampus in anesthetized and awake rats. *Brain Res, 457*(2), 310-321.

Ruit, K. G., & Neafsey, E. J. (1990). Hippocampal input to a "visceral motor" corticobulbar pathway: an anatomical and electrophysiological study in the rat. *Exp Brain Res, 82*(3), 606-616.

Russell, V., de Villiers, A., Sagvolden, T., Lamm, M., & Taljaard, J. (1995). Altered dopaminergic function in the prefrontal cortex, nucleus accumbens and caudate-putamen of an animal model of attention-deficit hyperactivity disorder--the spontaneously hypertensive rat. *Brain Res, 676*(2), 343-351.

Salt, T. E., Meier, C. L., Seno, N., Krucker, T., & Herrling, P. L. (1995). Thalamocortical and corticocortical excitatory postsynaptic potentials mediated by excitatory amino acid receptors in the cat motor cortex in vivo. *Neuroscience, 64*(2), 433-442.

Sanchez-Santed, F., de Bruin, J. P., Heinsbroek, R. P., & Verwer, R. W. (1997). Spatial delayed alternation of rats in a T-maze: effects of neurotoxic lesions of the medial prefrontal cortex and of T-maze rotations. *Behav Brain Res, 84*(1-2), 73-79.

Sandin, J., Nylander, I., Georgieva, J., Schott, P. A., Ogren, S. O., & Terenius, L. (1998). Hippocampal dynorphin B injections impair spatial learning in rats: a kappa-opioid receptor-mediated effect [In Process Citation]. *Neuroscience, 85*(2), 375-382.

Sanz, B., Exposito, I., & Mora, F. (1997). M1 acetylcholine receptor stimulation increases the extracellular concentrations of glutamate and GABA in the medial prefrontal cortex of the rat. *Neurochem Res, 22*(3), 281-286.

Sarter, M., Bodewitz, G., & Stephens, D. N. (1988). Attenuation of scopolamine-induced impairment of spontaneous alteration behaviour by antagonist but not inverse agonist and agonist beta- carbolines. *Psychopharmacology, 94*(4), 491-495.

Sarter, M., & Bruno, J. P. (1997a). Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. *Brain Res Brain Res Rev, 23*(1-2), 28-46.

Sarter, M., & Bruno, J. P. (1997b). Trans-synaptic stimulation of cortical acetylcholine and enhancement of attentional functions: a rational approach for the development of cognition enhancers. *Behav Brain Res, 83*(1-2), 7-14.

Sarter, M., & Bruno, J. P. (1998a). Age-related changes in rodent cortical acetylcholine and cognition: main effects of age versus age as an intervening variable. *Brain Res Brain Res Rev, 27*(2), 143-156.

Sarter, M., & Bruno, J. P. (1998b). Cortical acetylcholine, reality distortion, schizophrenia, and Lewy Body Dementia: too much or too little cortical acetylcholine? *Brain Cogn, 38*(3), 297-316.

Sarter, M., & Bruno, J. P. (1999). Abnormal regulation of corticopetal cholinergic neurons and impaired information processing in neuropsychiatric disorders. *Trends Neurosci, 22*(2), 67-74.

Sarter, M., & Bruno, J. P. (2000). Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. *Neuroscience, 95*(4), 933-952.

Sarter, M., Bruno, J. P., & Turchi, J. (1999). Basal forebrain afferent projections modulating cortical acetylcholine, attention, and implications for neuropsychiatric disorders. *Ann N Y Acad Sci, 877*, 368-382.

Sarter, M., Givens, B., & Bruno, J. P. (2001). The cognitive neuroscience of sustained attention: where top-down meets bottom-up. *Brain Res Rev, 35*, 146-160.

Sarter, M. F., & Bruno, J. P. (1994). Cognitive functions of cortical ACh: lessons from studies on trans- synaptic modulation of activated efflux. *Trends Neurosci, 17*(6), 217-221.

Sawaguchi, T., & Goldman-Rakic, P. S. (1991). D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science, 251*(4996), 947-950.

Sawaguchi, T., & Goldman-Rakic, P. S. (1994). The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J Neurophysiol, 71*(2), 515-528.

Sawchuk, C. N., Lohr, J. M., Lee, T. C., & Tolin, D. F. (1999). Exposure to disgust-evoking imagery and information processing biases in blood-injection-injury phobia. *Behav Res Ther, 37*(3), 249-257.

Scharf, M. B., Fletcher, K., & Graham, J. P. (1988). Comparative amnestic effects of benzodiazepine hypnotic agents. *J Clin Psychiatry, 49*(4), 134-137.

Schmitt, U., & Hiemke, C. (1998). Strain differences in open-field and elevated plus-maze behavior of rats without and with pretest handling. *Pharmacol Biochem Behav, 59*(4), 807-811.

Schnider, A., von Daniken, C., & Gutbrod, K. (1996). Disorientation in amnesia: A confusion of memory traces. *Brain, 119*, 1627-1632.

Schoenbaum, G., & Eichenbaum, H. (1995a). Information coding in the rodent prefrontal cortex. I. Single-neuron activity in orbitofrontal cortex compared with that in pyriform cortex. *J Neurophysiol, 74*(2), 733-750.

Schoenbaum, G., & Eichenbaum, H. (1995b). Information coding in the rodent prefrontal cortex. II. Ensemble activity in orbitofrontal cortex. *J Neurophysiol, 74*(2), 751-762.

Schulkin, J., McEwen, B. S., & Gold, P. W. (1994). Allostasis, amygdala, and anticipatory angst. *Neurosci Biobehav Rev, 18*(3), 385-396.

Scorza, M. C., Reyes-Parada, M., Silveira, R., Viola, H., Medina, J. H., Viana, M. B., Zangrossi, H., Jr., & Graeff, F. G. (1996). Behavioral effects of the putative anxiolytic (+/-)-1-(2,5-dimethoxy-4- ethylthiophenyl)-2-aminopropane (ALEPH-2) in rats and mice. *Pharmacol Biochem Behav, 54*(2), 355-361.

Seamans, J. K., Floresco, S. B., & Phillips, A. G. (1995). Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. *Behav Neurosci, 109*(6), 1063-1073.

Seamans, J. K., Floresco, S. B., & Phillips, A. G. (1998). D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J Neurosci, 18*(4), 1613-1621.

Seguela, P., Watkins, K. C., & Descarries, L. (1988). Ultrastructural features of dopamine axon terminals in the anteromedial and the suprarhinal cortex of adult rat. *Brain Res, 442*(1), 11-22.

Semba, K., & Fibiger, H. C. (1992). Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: a retro- and antero-grade transport and immunohistochemical study. *J Comp Neurol, 323*(3), 387-410.

Sesack, S. R., & Bunney, B. S. (1989). Pharmacological characterization of the receptor mediating electrophysiological responses to dopamine in the rat medial prefrontal cortex: a microiontophoretic study. *J Pharmacol Exp Ther, 248*(3), 1323-1333.

Sesack, S. R., Deutch, A. Y., Roth, R. H., & Bunney, B. S. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J Comp Neurol, 290*(2), 213-242.

Shallice, T. (1982). Specific impairments of planning. *Philos Trans R Soc Lond B Biol Sci, 298*(1089), 199-209.

Shanks, N., Zalcman, S., Zacharko, R. M., & Anisman, H. (1991). Alterations of central norepinephrine, dopamine and serotonin in several strains of mice following acute stressor exposure. *Pharmacol Biochem Behav, 38*(1), 69-75.

Shapiro, M. L., & Eichenbaum, H. (1999). Hippocampus as a memory map: synaptic plasticity and memory encoding by hippocampal neurons. *Hippocampus, 9*(4), 365-384.

Shen, Y., Specht, S. M., De Saint Ghislain, I., & Li, R. (1994). The hippocampus: a biological model for studying learning and memory. *Prog Neurobiol, 44*(5), 485-496.

Shephard, R. A. (1986). Neurotransmitters, anxiety and benzodiazepines: a behavioral review. *Neurosci Biobehav Rev, 10*(4), 449-461.

Shippenberg, T. S., Bals-Kubik, R., & Herz, A. (1993). Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. *J Pharmacol Exp Ther, 265*(1), 53-59.

Shippenberg, T. S., LeFevour, A., & Heidbreder, C. (1996). kappa-Opioid receptor agonists prevent sensitization to the conditioned rewarding effects of cocaine. *J Pharmacol Exp Ther, 276*(2), 545-554.

Shippenberg, T. S., & Rea, W. (1997). Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol Biochem Behav, 57*(3), 449-455.

Silvia, E. S., & MacCallum, R. C. (1988). Some factors affecting the success of specification searches in covariance structure modeling. *Multivariate Behavioral Research, 23*(3), 297-326.

Simon, H., Scatton, B., & Moal, M. L. (1980). Dopaminergic A10 neurones are involved in cognitive functions. *Nature, 286*(5769), 150-151.

Simon, P., Dupuis, R., & Costentin, J. (1994). Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. *Behav Brain Res, 61*(1), 59-64.

Snook, S. C., & Gorsuch, R. L. (1989). Component analysis versus common factor analysis: A Monte Carlo study. *Psychological Bulletin, 106*(1), 148-154.

Sokolowski, J. D., McCullough, L. D., & Salamone, J. D. (1994). Effects of dopamine depletions in the medial prefrontal cortex on active avoidance and escape in the rat. *Brain Res, 651*(1-2), 293-299.

Spanagel, R., Herz, A., & Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc Natl Acad Sci U S A, 89*(6), 2046-2050.

Spyer, K. M. (1989). Neural mechanisms involved in cardiovascular control during affective behaviour. *Trends Neurosci, 12*(12), 506-513.

Stanford, S. C. (1996). Prozac: panacea or puzzle? *Trends Pharmacol Sci, 17*(4), 150-154.

Steckler, T., Inglis, W., Winn, P., & Sahgal, A. (1994). The pedunculopontine tegmental nucleus: a role in cognitive processes? *Brain Res Brain Res Rev, 19*(3), 298-318.

Steele, T. D., Hodges, D. B., Jr., Levesque, T. R., Locke, K. W., & Sandage, B. W., Jr. (1996). The D1 agonist dihydrexidine releases acetylcholine and improves cognition in rats. *Ann N Y Acad Sci, 777*, 427-430.

Stein, E. A., Hiller, J. M., & Simon, E. J. (1992). Effects of stress on opioid receptor binding in the rat central nervous system. *Neuroscience, 51*(3), 683-690.

Stephens, D. N., Meldrum, B. S., Weidmann, R., Schneider, C., & Grutzner, M. (1986). Does the excitatory amino acid receptor antagonist 2-APH exhibit anxiolytic activity? *Psychopharmacology, 90*(2), 166-169.

Stone, E. A., Rhee, J., & Quartermain, D. (1996). Blockade of effect of stress on risk assessment behavior in mice by a beta-1 adrenoceptor antagonist. *Pharmacol Biochem Behav, 55*(2), 215-217.

Stone, W. S., Walser, B., Gold, S. D., & Gold, P. E. (1991). Scopolamine- and morphine-induced impairments of spontaneous alternation performance in mice: reversal with glucose and with cholinergic and adrenergic agonists. *Behav Neurosci, 105*(2), 264-271.

Stuss, D. T., & Gow, C. A. (1992). "Frontal dysfunction" after traumatic brain injury. *Neuropsychiatry Neuropsychol Behav Neurol, 5*, 272-282.

Stuss, D. T., Kaplan, E. F., Benson, D. F., Weir, W. S., Chiulli, S., & Sarazin, F. F. (1982). Evidence for the involvement of orbitofrontal cortex in memory functions: an interference effect. *J Comp Physiol Psychol, 96*(6), 913-925.

Suzuki, W. A., & Amaral, D. G. (1994). Perirhinal and parahippocampal cortices of the macaque monkey: cortical afferents. *J Comp Neurol, 350*(4), 497-533.

Suzuki, W. A., Miller, E. K., & Desimone, R. (1997). Object and place memory in the macaque entorhinal cortex. *J Neurophysiol, 78*(2), 1062-1081.

Swanson, L. W., & Kohler, C. (1986). Anatomical evidence for direct projections from the entorhinal area to the entire cortical mantle in the rat. *J Neurosci, 6*(10), 3010-3023.

Swerdlow, N. R., & Koob, G. F. (1987). Lesions of the dorsomedial nucleus of the thalamus, medial prefrontal cortex and pedunculopontine nucleus: effects on locomotor activity mediated by nucleus accumbens-ventral pallidal circuitry. *Brain Res, 412*(2), 233-243.

Sybirska, E., Davachi, L., & Goldman-Rakic, P. S. (2000). Prominence of direct entorhinal-CA1 pathway activation in sensorimotor and cognitive tasks revealed by 2-DG functional mapping in nonhuman primate. *J Neurosci, 20*(15), 5827-5834.

Tabachnick, B. G., & Fidell, L. S. (1996). *Using multivariate statistics* (3rd ed.). New York, NY: HarperCollins College Publishers.

Takagishi, M., & Chiba, T. (1991). Efferent projections of the infralimbic (area 25) region of the medial prefrontal cortex in the rat: an anterograde tracer PHA-L study. *Brain Res, 566*(1-2), 26-39.

Tako, A. N., Beracochea, D. J., & Jaffard, R. (1988). Accelerated rate of forgetting of spatial information following mammillary-body lesions in mice: Effects of context change on retention-test performance. *Psychobiology, 16*(1), 45-53.

Tanaka, D. (1974). Sparing of an escape response following serial prefrontal decortication in the monkey. *Brain Res, 65*, 195-201.

Tanda, G., Carboni, E., Frau, R., & Di Chiara, G. (1994). Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacology (Berl), 115*(1-2), 285-288.

Tannock, R. (1998). Attention Deficit Hyperactivity Disorder: Advances in cognitive. neurobiological, and genetic research. *J Child Psychol & Psychiatry, 39*(1), 65-99.

Taylor, D. P., & Moon, S. L. (1991). Buspirone and related compounds as alternative anxiolytics. *Neuropeptides, 19 Suppl*, 15-19.

Taylor, D. P., Riblet, L. A., Stanton, H. C., Eison, A. S., Eison, M. S., & Temple, D. L., Jr. (1982). Dopamine and antianxiety activity. *Pharmacol Biochem Behav, 17*(Suppl 1), 25-35.

Terreberry, R. R., & Neafsey, E. J. (1983). Rat medial frontal cortex: a visceral motor region with a direct projection to the solitary nucleus. *Brain Res, 278*(1-2), 245-249.

Terreberry, R. R., & Neafsey, E. J. (1987). The rat medial frontal cortex projects directly to autonomic regions of the brainstem. *Brain Res Bull, 19*(6), 639-649.

Thayer, J. F., Friedman, B. H., & Borkovec, T. D. (1996). Autonomic characteristics of generalized anxiety disorder and worry. *Biol Psychiatry, 39*(4), 255-266.

Thayer, J. F., & Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J Affect Disord, 61*(3), 201-216.

Tilson, H., McLamb, R., & Hong, J. (1986). Behavioral effects of centrally administered dynorphin and [D-ala2-D- leu] enkephalin (DADLE) in rats. *Neuropeptides, 8*(3), 193-206.

Tohyama, K., Nabeshima, T., Ichihara, K., & Kameyama, T. (1991). Involvement of GABAergic systems in benzodiazepine-induced impairment of passive avoidance learning in mice. *Psychopharmacology, 105*(1), 22-26.

Tranel, D., Bechara, A., & Damasio, A. R. (2000). Decision making and the somatic marker hypothesis. In M. S. Gazzaniga (Ed.), *The New Cognitive Neurosciences* (2nd ed., pp. 1047-1061). Cambridge, MA: MIT Press.

Treit, D., Menard, J., & Royan, C. (1993). Anxiogenic stimuli in the elevated plus-maze. *Pharmacol Biochem Behav, 44*(2), 463-469.

Treit, D., & Pesold, C. (1990). Septal lesions inhibit fear reactions in two animal models of anxiolytic drug action. *Physiol Behav, 47*(2), 365-371.

Treit, D., Robinson, A., Rotzinger, S., & Pesold, C. (1993). Anxiolytic effects of serotonergic interventions in the shock-probe burying test and the elevated plus-maze test. *Behav Brain Res, 54*(1), 23-34.

Troisi, A. (1999). Ethological research in clinical psychiatry: the study of nonverbal behavior during interviews. *Neurosci Biobehav Rev, 23*(7), 905-913.

Trullas, R., Folio, T., Young, A., Miller, R., Boje, K., & Skolnick, P. (1991). 1-aminocyclopropanecarboxylates exhibit antidepressant and anxiolytic actions in animal models. *Eur J Pharmacol, 203*(3), 379-385.

Trullas, R., & Skolnick, P. (1993). Differences in fear motivated behaviors among inbred mouse strains. *Psychopharmacology, 111*(3), 323-331.

Tulving, E., Markowitsch, H. J., Kapur, S., Habib, R., & Houle, S. (1994). Novelty encoding networks in the human brain: positron emission tomography data. *Neuroreport, 5*(18), 2525-2528.

Ukai, M., Itoh, J., Kobayashi, T., Shinkai, N., & Kameyama, T. (1997). Effects of the kappa-opioid dynorphin A(1-13) on learning and memory in mice. *Behav Brain Res, 83*(1-2), 169-172.

Ukai, M., Shinkai, N., & Kameyama, T. (1995). kappa-Opioid receptor agonists improve pirenzepine-induced disturbance of spontaneous alternation performance in the mouse. *Eur J Pharmacol, 281*(2), 173-178.

Ukai, M., Shinkai, N., & Kameyama, T. (1996). Neurokinin A and senktide attenuate scopolamine-induced impairment of spontaneous alternation performance in mice. *Nihon Shinkei Seishin Yakurigaku Zasshi, 16*(3), 97-101.

Ukai, M., Shinkai, N., & Kameyama, T. (1998). Involvement of dopamine receptors in beneficial effects of tachykinins on scopolamine-induced impairment of alternation performance in mice. *Eur J Pharmacol, 350*(1), 39-45.

Ukai, M., Shinkai, N., Ohashi, K., & Kameyama, T. (1995). Substance P markedly ameliorates scopolamine-induced impairment of spontaneous alternation performance in the mouse. *Brain Res, 673*(2), 335-338.

Unterwald, E. M., Knapp, C., & Zukin, R. S. (1991). Neuroanatomical localization of kappa 1 and kappa 2 opioid receptors in rat and guinea pig brain. *Brain Res, 562*(1), 57-65.

Unterwald, E. M., Rubenfeld, J. M., & Kreek, M. J. (1994). Repeated cocaine administration upregulates kappa and mu, but not delta, opioid receptors. *Neuroreport, 5*(13), 1613-1616.

Ursin, H., Rosvold, H. E., & Vest, B. (1969). Food preference in brain lesioned monkeys. *Physiol Behav, 4*, 609-612.

Van Bockstaele, E. J., & Aston-Jones, G. (1995). Integration in the ventral medulla and coordination of sympathetic, pain and arousal functions. *Clin Exp Hypertens, 17*(1-2), 153-165.

van den Hout, M., & Barlow, D. (2000). Attention, arousal and expectancies in anxiety and sexual disorders. *J Affect Disord, 61*(3), 241-256.

Van Eden, C. G., & Buijs, R. M. (2000). Functional neuroanatomy of the prefrontal cortex: autonomic interactions. *Prog Brain Res, 126*, 49-62.

Van Riezen, H., & Segal, M. (1988). Introduction to the evaluation of anxiety and related disorders, *Comparative Evaluation of Rating Scales for Clinical Psychopharmacology* (pp. 225-228). New York: Elsevier Science.

Vanderwolf, C. H., Buzsaki, G., Cain, D. P., Cooley, R. K., & Robertson, B. (1988). Neocortical and hippocampal electrical activity following decapitation in the rat. *Brain Res, 451*(1-2), 340-344.

Vasar, E., Lang, A., Harro, J., Bourin, M., & Bradwejn, J. (1994). Evidence for potentiation by CCK antagonists of the effect of cholecystokinin octapeptide in the elevated plus-maze. *Neuropharmacology, 33*(6), 729-735.

Verberne, A. J. (1996). Medullary sympathoexcitatory neurons are inhibited by activation of the medial prefrontal cortex in the rat. *Am J Physiol, 270*(4 Pt 2), R713-719.

Verberne, A. J., & Owens, N. C. (1998). Cortical modulation of the cardiovascular system. *Prog Neurobiol, 54*(2), 149-168.

Verney, C., Alvarez, C., Geffard, M., & Berger, B. (1990). Ultrastructural double-labelling study of dopamine terminals and GABA-containing neurons in rat anteromedial cerebral cortex. *Eur J Neurosci, 2*, 960-972.

Verwer, R. W., Meijer, R. J., Van Uum, H. F., & Witter, M. P. (1997). Collateral projections from the rat hippocampal formation to the lateral and medial prefrontal cortex. *Hippocampus, 7*(4), 397-402.

Vezina, P., Blanc, G., Glowinski, J., & Tassin, J. P. (1994). Blockade of D-1 dopamine receptors in the medial prefrontal cortex produces delayed effects on pre- and postsynaptic indices of dopamine function in the nucleus accumbens. *Synapse, 16*(2), 104-112.

Viana, M. B., Tomaz, C., & Graeff, F. G. (1994). The elevated T-maze: a new animal model of anxiety and memory. *Pharmacol Biochem Behav, 49*(3), 549-554.

Vincent, S. L., Khan, Y., & Benes, F. M. (1993). Cellular distribution of dopamine D1 and D2 receptors in rat medial prefrontal cortex. *J Neurosci, 13*(6), 2551-2564.

Voytko, M. L., Olton, D. S., Richardson, R. T., Gorman, L. K., Tobin, J. R., & Price, D. L. (1994). Basal forebrain lesions in monkeys disrupt attention but not learning and memory [published erratum appears in J Neurosci 1995 Mar;15(3 Pt 2):following table of contents]. *J Neurosci, 14*(1), 167-186.

Waite, J. J., & Thal, L. J. (1996). Lesions of the cholinergic nuclei in the rat basal forebrain: excitotoxins vs. an immunotoxin. *Life Sci, 58*(22), 1947-1953.

Walker, D. L., McGlynn, T., Grey, C., Ragozzino, M., & Gold, P. E. (1991). Naloxone modulates the behavioral effects of cholinergic agonists and antagonists. *Psychopharmacology, 105*(1), 57-62.

Wall, P. M. (1997). *Intracerebral Administration of U-69,593 and norBNI in the Medial Prefrontal Cortex and the Ventral Tegmental Area of CD-1 Mice: Behavioural Evaluation in the Elevated Plus Maze.*, Graduate Thesis, Carleton University, Ottawa, Canada.

Wall, P. M., Flinn, J., & Messier, C. (2001). Infralimbic muscarinic M1 receptors modulate anxiety-like behaviour and spontaneous working memory in mice. *Psychopharmacology, 155*, 58-68.

Wall, P. M., & Messier, C. (2000a). Concurrent modulation of anxiety and memory. *Behav Brain Res, 109*, 229-241.

Wall, P. M., & Messier, C. (2000b). Ethological confirmatory factor analysis of anxiety-like behaviour in the murine elevated plus-maze. *Behav Brain Res, 114*(1-2), 199-212.

Wall, P. M., & Messier, C. (2000c). U-69,593 microinjection in the infralimbic cortex reduces anxiety and enhances spontaneous alternation memory in mice. *Brain Res, 856*, 259-280.

Wall, P. M., & Messier, C. (2001a). The hippocampal formation-orbitomedial prefrontal cortex circuit in the attentional control of active memory. *Behav Brain Res, (in press).*

Wall, P. M., & Messier, C. (2001b). Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behaviour. *Neurosci Biobehav Rev, 25*, 275-286.

Wall, P. M., & Messier, C. (2001c). Ventromedial prefrontal kappa1 and muscarinic M1 interactions in the concurrent modulation of anxiety and memory. *Psychopharmacology, (submitted).*

Wang, J. Q., & Ingenito, A. J. (1992). Centrally mediated cardiovascular actions of dynorphin A(1-8) on rat hippocampal formation. *J Pharmacol Exp Ther, 261*(2), 678-685.

Weiss, S. M., Wadsworth, G., Fletcher, A., & Dourish, C. T. (1998). Utility of ethological analysis to overcome locomotor confounds in elevated maze models of anxiety. *Neurosci Biobehav Rev, 23*(2), 265-271.

Werling, L. L., Frattali, A., Portoghese, P. S., Takemori, A. E., & Cox, B. M. (1988). Kappa receptor regulation of dopamine release from striatum and cortex of rats and guinea pigs. *J Pharmacol Exp Ther, 246*(1), 282-286.

Wilkinson, R. T. (1963). Interaction of noise with knowledge of results and sleep deprivation. *J Exp Psychol, 66*, 332-337.

Wilkinson, R. T. (1990). Response-stimulus interval in choice serial reaction time: interaction with sleep deprivation, choice, and practice. *Q J Exp Psychol [A], 42*(2), 401-423.

Wilson, F. A. (1987). Cortical and subcortical structures involved in recognition memory: neurophysiological and anatomical studies. *Int J Neurol, 22*, 204-217.

Winn, P. (1998). Frontal syndrome as a consequence of lesions in the pedunculopontine tegmental nucleus: a short theoretical review [In Process Citation]. *Brain Res Bull, 47*(6), 551-563.

Withers, R. D., & Deane, F. P. (1995). Origins of common fears: effects on severity, anxiety responses and memories of onset. *Behav Res Ther, 33*(8), 903-915.

Wittchen, H. U., Zhao, S., Kessler, R. C., & Eaton, W. W. (1994). DSM-III-R generalized anxiety disorder in the National Comorbidity Survey. *Arch Gen Psychiatry, 51*(5), 355-364.

Wortwein, G., Mogensen, J., & Divac, I. (1993). Retention and relearning of spatial delayed alternation in rats after combined or sequential lesions of the prefrontal and parietal cortex. *Acta Neurobiol Exp, 53*(2), 357-366.

Wu, J. C., Buchsbaum, M. S., Hershey, T. G., Hazlett, E., Sicotte, N., & Johnson, J. C. (1991). PET in generalized anxiety disorder. *Biol Psychiatry, 29*(12), 1181-1199.

Wu, M., Shanabrough, M., Leranth, C., & Alreja, M. (2000). Cholinergic excitation of septohippocampal GABA but not cholinergic neurons: implications for learning and memory. *J Neurosci, 20*(10), 3900-3908.

Yamada, K., & Nabeshima, T. (1995). Stress-induced behavioral responses and multiple opioid systems in the brain. *Behav Brain Res, 67*(2), 133-145.

Yamada, K., Noda, Y., Nakayama, S., Komori, Y., Sugihara, H., Hasegawa, T., & Nabeshima, T. (1995). Role of nitric oxide in learning and memory and in monoamine metabolism in the rat brain. *Br J Pharmacol, 115*(5), 852-858.

Yonkers, K. A., Warshaw, M. G., Massion, A. O., & Keller, M. B. (1996). Phenomenology and course of generalised anxiety disorder. *Br J Psychiatry, 168*(3), 308-313.

Yoshioka, M., Matsumoto, M., Togashi, H., & Saito, H. (1995). Effects of conditioned fear stress on 5-HT release in the rat prefrontal cortex. *Pharmacol Biochem Behav, 51*(2-3), 515-519.

You, Z. B., Herrera-Marschitz, M., Nylander, I., Goiny, M., O'Connor, W. T., Ungerstedt, U., & Terenius, L. (1994). The striatonigral dynorphin pathway of the rat studied with in vivo microdialysis--II. Effects of dopamine D1 and D2 receptor agonists. *Neuroscience, 63*(2), 427-434.

Young, B. J., Otto, T., Fox, G. D., & Eichenbaum, H. (1997). Memory representation within the parahippocampal region. *J Neurosci, 17*(13), 5183-5195.

Zaborszky, L., & Cullinan, W. E. (1996). Direct catecholaminergic-cholinergic interactions in the basal forebrain. I. Dopamine-beta-hydroxylase- and tyrosine hydroxylase input to cholinergic neurons. *J Comp Neurol, 374*(4), 535-554.

Zaborszky, L., Cullinan, W. E., & Braun, A. (1991). Afferents to basal forebrain cholinergic projection neurons: an update. *Adv Exp Med Biol, 295*, 43-100.

Zacharko, R. M., & Anisman, H. (1991). Stressor-induced anhedonia in the mesocorticolimbic system. *Neurosci Biobehav Rev, 15*(3), 391-405.

Zahrt, J., Taylor, J. R., Mathew, R. G., & Arnsten, A. F. (1997). Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J Neurosci, 17*(21), 8528-8535.