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THE MEDIAL FOREBRAIN BUNDLE: EVIDENCE FOR MULTIPLE SYSTEMS OF REWARD

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DEDICATION

For my Mother and Father,

". . . you must have done something right"

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Of all the sections in this thesis, it is this one in particular that gives me the most pleasure to write. I must first thank my Mom and my Dad for instilling in me the notion that I could achieve anything I set my mind to (except sing perhaps!). I have the most special family in the world. Jay, I told you marriage wouldn't interfere with my thesis. Nice try though. Cathy and Pete, I could write a whole page of thank-you's just to you for all of your support over the years. Tara and Joey are lucky children. Lise, you are so much more than a sister to me, you've been a matchless friend and confidante, not to mention editor. Never lose your unique way of looking at things. Mike, what can I say, your interest in my experiments has always been beyond the call of brotherly duty. Thank you for helping me keep things in perspective. And finally, my darling Glen, you are the light at the end of my day. Here's to our future.

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ABSTRACT

In order to determine if multiple reward systems exist within the medial forebrain bundle, lateral hypothalamic (LH; anterior medial forebrain bundle) and ventral tegmental (VTA; posterior medial forebrain bundle) pulse frequencies that sustained the same proportion of the maximum self-stimulation rate (equipotent or threshold frequencies) were compared in a twin-lever psychophysical preference test. In a first experiment, the capacity of rats to discriminate between rewarding pulse frequencies delivered to the same reward site was determined. Results showed that rats equally preferred identical rewarding stimuli within 0.009 logfrequency units. Secondly, they discriminated between competing rewarding stimuli that differed by only 0.016 logfrequency units. Based on these criteria, it was hypothesized that if rats failed to equally prefer LH and VTA threshold frequencies, then each site may convey qualitatively different rewarding signals. Results of Experiment Two showed that LH and VTA threshold frequencies were equally preferred at one third of the pairs of sites tested. These data suggest that the medial forebrain bundle conveys equally rewarding signals at some electrode placements. However, at another third of the pairs of sites tested, rats failed to equally prefer anterior and posterior medial forebrain bundle threshold frequencies. These results suggest that this pathway may convey qualitatively different reward signals at other electrode placements. Mixed results were obtained at the remaining pairs of sites, where rats neither consistently chose one stimulus over the other, nor equally preferred both stimuli. A collision test was conducted in Experiment Three between all pairs of sites tested in the previous experiment in order to determine if the number of common reward fibres between electrodes was correlated with preference behavior. We rationalized that if the LH and VTA electrodes stimulated a substantial proportion of common rewarding fibres (as evidenced by a strong collision effect), then rats should equally prefer threshold frequencies. Unfortunately, a substantial collision effect was not noted in any of the subjects. Nevertheless, no correlation existed between modest collision effects or summation and preference behavior. Experiment Four examined the hypothesis that a reward-irrelevant factor, namely the co-activation of aversive neurons, contributed to the lack of equal preference observed at some pairs of sites. Results

showed that concomitant aversive nucleus reticularis gigantocellularis (Gi) pulses did indeed initially increase the VTA self-stimulation threshold frequency. However, the aversive pulses progressively lost their capacity to do so with repeated testing. Furthermore, the preference test conducted in two rats showed that the animals did not prefer VTA over combined VTA+Gi stimulation. In sum, a modest degree of anatomical linkage between electrodes could not predict animals' preference behavior. Secondly, the co-activation of aversive fibres could not account for the lack of equal preference observed between some medial forebrain bundle sites. In conclusion, this series of experiments supports the notion of multiple reward systems within the medial forebrain bundle.

Chapter One

THE MEDIAL FOREBRAIN BUNDLE: A CASE FOR MULTIPLE SYSTEMS OF REWARD

To explain all nature is too difficult a task for any one man or even for any age. 'Tis much better to do a little with certainty, and leave the rest for others that come after you, than to explain all things.

Newton (1642-1727)

INTRODUCTION

The Discovery of Brain Stimulation Reward

In 1954, James Olds and Peter Milner discovered that electrical stimulation of specific areas in the brain had an unprecedented reinforcing effect on a rat's behavior. This effect was so powerful that the rat could easily be guided, and shaped of a wide range of behaviors. In fact, the rat immediately learned to self-stimulate persistently by pressing a lever. It appeared as though the stimulating electrode had penetrated the brain's "pleasure centre". It seemed unlikely that a specific phenomenon such as brain stimulation reward would evolve that did not somehow participate in natural rewarding sensations. Thus, countless investigations of the neural bases of motivation and reward were launched in species ranging from the aplysia to humans.

Chapter Objective

It is now recognized that self-stimulation can be elicited in every major subdivision of the rat brain, from the telencephalon to the myelencephalon. Yet, it is not clear whether all of these reward neurons ultimately converge to form a single anatomical substrate, or if multiple, independent systems exist. A review of the evidence supporting multiple systems of reward in the entire brain is beyond the scope of this thesis, but is provided in Phillips (1984). The literature review is therefore generally confined to putative systems within the medial forebrain bundle, particularly the section between the lateral hypothalamus (LH) and the ventral tegmental area (VTA). The medial forebrain bundle is a catecholamine fibre bundle connecting the brainstem reticular formation with diencephalic and telencephalic regions. It is comprised of at least 50 distinct pathways. In the study of brain stimulation reward, this bundle has by far received the greatest amount of attention, as it supports vigorous self-stimulate for long periods of time, with little fatigue. In addition, the self-stimulation is minimally interrupted by interfering behaviors, such as grooming, freezing, seizing, sleeping, etc.

Historically, researchers have attempted to identify multiple systems of reward by (1) comparing different self-stimulation sites to naturally rewarding events (2) examining how lesions at one site affect the rewarding impact at another site (3) analyzing how pharmacological manipulations differentially affect various rewarding areas, and (4) comparing the various electrophysiological characteristics across sites. The goal of Chapter One is to review the evidence supporting the notion that the medial forebrain bundle consists of multiple systems of reward within each of these aforementioned domains. As will become apparent, most of the evidence gathered to date merely suggests, but does not prove, that the reward systems of this pathway are heterogenous.

Brain Stimulation Reward and Natural Functions

Lateral hypothalamic self-stimulation and feeding reward

Immediately after the discovery of the self-stimulation phenomenon, researchers began to explore the hypothesis that rewarding electrical stimulation of the brain activated similar substrates that naturally rewarding events activate. Evidence supporting this hypothesis was quickly gathered, and soon researchers began to dissociate brain reward systems based upon which natural function they subserved. The following are just some examples of studies that led towards establishing a relation between LH self-stimulation and feeding reward: (1) Food deprivation increased LH self-stimulation (Margules & Olds, 1962; Olds, 1962; Wilkinson & Peele, 1962; Blundell & Herberg, 1968; Olds, 1977), and overfeeding decreased it (Hoebel & Teitelbaum, 1962; Wilkinson & Peele, 1962; Hoebel, 1968). (2) LH lesions temporarily eliminated feeding and LH self-stimulation (Hernandez & Hoebel, 1989), whereas ventromedial hypothalamic (the brain's "satiety centre") lesions increased both (Hoebel, 1969; Hernandez & Hoebel, 1989). (3) Injections of pancreatic hormones such as glucose, insulin or phenylpropanolamine (an amphetamine analog; Hernandez & Hoebel, 1978) and the appetite suppressant d-fenfluramine decreased both feeding and LH self-stimulation (Balagura & Hoebel, 1967; Blundell & Leshem, 1973; Hoebel & Leibowitz, 1981; McClelland, Sarfaty, Hernandez & Hoebel, 1989). (4) Stimulation-induced feeding and LH

reward neurons were shown to have similar excitability characteristics (Wise, 1982; Hawkins, Roll, Puerto & Yeomans, 1983; Gratton & Wise, 1988). (5) In an electrophysiological study in freely moving rats, most LH neurons that were inhibited during feeding were also inhibited by rewarding LH stimulation (Sasaki, Ono, Muramoto, Nishino & Fukuda, 1984). (6) Electrical stimulation of the LH had the same inhibitory or excitatory effect on a given gustatory (nucleus tractus solitarius) neuron as stimulation of the tongue did (Murzi, Hernandez & Baptista, 1986). (7) Both feeding and LH self-stimulation increased dopamine turnover in the nucleus accumbens (Hernandez & Hoebel, 1988). (8) In a choice experiment, rats measured and evaluated gustatory and LH stimulation via a common qualitative process (Conover & Shizgal, 1994; Conover, Woodside & Shizgal, 1994). Specifically, rats distributed their choice as a function of the relative magnitudes between sucrose or sodium and LH reward. In other words, subjects preferred a standard sucrose or saline reward to a low number of LH pulses, but this preference reversed itself as the LH frequency was increased.

Furthermore, the link between self-stimulation and feeding appeared to be specific to the LH. For example, while LH self-stimulation was affected by metabolic manipulations, that of the medial hypothalamus (Olds, 1958), the septum (Hernandez & Briese, 1971; Hoebel, 1968), the VTA (Miliaressis & Cardo, 1973), the nucleus accumbens (Rolls, Burton & Mora, 1980; Sasaki, Ono, Muramoto, Nishino & Fukuda, 1984) and the posterior hypothalamus (Hoebel, 1968; Hernandez & Briese, 1971; Hernandez & Hoebel, 1978) was not. Secondly, changing a rat's state from food to water deprivation led to shifts in preference of one LH site over another (Gallistel & Beagley, 1971). In other words, overfeeding decreased reward at some LH sites, but not others. In sum, this anatomical specificity between LH self-stimulation and feeding supports the notion that multiple systems of reward exist not only within the brain, but within the medial forebrain bundle itself.

However, the correlation between LH self-stimulation and feeding reward is not perfect. For example, high-dose injections of amphetamine increased LH self-stimulation (Phillips & Fibiger, 1973; Phillips, Brooke & Fibiger, 1975; Franklin & Robertson, 1980; Gallistel & Karras,

1984; Gallistel & Freyd, 1987; Colle & Wise, 1988b), but decreased the probability of eating (Colle & Wise, 1988a). Another basic difference between LH reward and food is that the former does not accumulate in the gut. This observation suggests that postingestive feedback may differentially affect brain stimulation and feeding rewards. Indeed, Conover and Shizgal (1994b) showed that postingestive feedback attenuated a sucrose, but not LH stimulation reward. Specifically, they offered food-deprived rats a choice between a train of rewarding LH stimulation and a compound reward consisting of an identical stimulation train and an intraoral infusion of sucrose. In some feeding sessions (sham), the gastric cannula was open, and in others (real), the cannula was closed. During the sham sessions, rats maintained a clear preference for the compound (greater) reward. During the real sessions, the compound reward was preferred only at the beginning of each session. As the session progressed, preference for the compound reward either disappeared or reversed itself in favour of the LH-only reward. In other words, the gustatory and brain stimulation rewards were affected differentially with postingestive feedback. Thus, in certain instances, the neural systems involved in feeding and LH reward can be dissociated.

Posterior hypothalamic self-stimulation and sexual reward

Most research comparing self-stimulation to natural rewarding events has concentrated on the LH and feeding. However, reasonable evidence exists linking posterior hypothalamic self-stimulation to sexual reward (Caggiula & Hoebel, 1966). For example: (1) Sexually experienced male rats copulated repeatedly when their posterior hypothalamus was being stimulated, and seldomly when it was not (Caggiula & Hoebel, 1966). Even after the rats ejaculated, they remained sexually excited. (2) Posterior hypothalamic self-stimulation was sometimes accompanied by sperm discharge (Caggiula & Hoebel, 1966). (3) Castration decreased significantly self-stimulation rates, whereas injections of testosterone increased them (Caggiula & Hoebel, 1966). (4) Excitotoxic lesions just lateral to the posterior hypothalamus (LH, zona incerta and lateral tegmentum) attenuated, and in some cases eliminated, male sexual behavior (Maillard &

Edwards, 1991). These rats did not show any noticeable deficits in locomotion, gait, posture or somatosensory orientation. In brief, these observations are suggestive of a link between posterior hypothalamic self-stimulation and sexual reward.

Dorsolateral hypothalamic self-stimulation and memory facilitation

The idea that memory is improved by rewarding events is not new. However, it seems as though self-stimulation at some sites, but not others, facilitates the process of memory consolidation. For example, Major and White (1978) showed that water-deprived rats given a session of dorsolateral hypothalamic or substantia nigral self-stimulation immediately following training of an appetitive learning task (finding a drinking tube) showed significantly improved retention of the task 24 hours later. If the self-stimulation session was delayed for one hour after the training trial, there was a greatly diminished effect on retention 24 hours later. However, memory facilitation did not occur with medial LH or lateral preoptic area self-stimulation (Major & White, 1978). Although the latter experiment implied that reward was not a sufficient explanation for memory facilitation, it did show that self-stimulation of distinct areas in the brain had differential effects on memory consolidation. In conclusion, evidence exists linking LH, posterior hypothalamic and dorsolateral self-stimulation to feeding, copulation and memory facilitation, respectively. Together, this set of observations supports the notion that the medial forebrain bundle consists of multiple systems of reward, each subserving a different natural function.

Unfortunately, most of the early studies leading to these correlations relied on self-stimulation rates to measure changes in reward. Today, rates are considered to be an arbitrary measure assumed to reflect the contribution of reward-irrelevant variables such as training, priming, task difficulty, interfering motor reactions, illness, sluggishness, etc. (Hodos & Valenstein, 1964; Edmonds & Gallistel, 1974; Miliaressis, Rompré & Durivage, 1982; Miliaressis, Rompré, Laviolette, Philippe & Coulombe, 1986; Miliaressis & Rompré, 1987). Therefore, in order to properly assess the relation between self-stimulation and natural functions, it is critical to update

these experiments using frequency thresholds, defined as the number of pulses required to maintain self-stimulation at a particular proportion, say 50%, of the maximal performance (Miliaressis et al., 1982). Threshold changes are assumed to reflect differences in the actual magnitude of reward, irrespective of performance ability. For example, although food deprivation may increase LH rates(Margules & Olds, 1962; Olds, 1962; Wilkinson & Peele, 1962; Blundell & Herberg, 1968; Olds, 1977), it does not affect thresholds (Giovono & Wise, 1986; Frutiger, 1989). Thus, more evidence is required before we accept unequivocally that the medial forebrain bundle consists of multiple, independent reward systems, each subserving different natural functions.

Lesion Studies

Since 1954, researchers have also tried to unveil the anatomical organization of reward neurons with the use of lesions. During the pioneering days of brain stimulation reward, it was thought that if damage to one self-stimulation site did not affect the rewarding impact of a second site, then the two clusters might be independent of one another, thus supporting the notion of multiple reward systems within the brain. However, this argument failed to consider the direction of reward neurons, or converging or redundant systems. Alternatively, a neural model consisting of a single reward substrate would predict that critical damage to the principal source would abolish all self-stimulation. Interestingly, this prediction has not yet been borne out, despite extensive lesioning (Ward, 1960; Huston, 1982; Stellar, Illes & Mills, 1982; Huston, Ornstein & Lehrer, 1982; Pritzel, Huston & Buscher, 1983; Huston, Grimm & Ornstein, 1983). Not even the favoured LH appears to be the critical source of reward neurons, as LH microinjections of kainic, ibotenic acid (Sprick, Munoz & Huston, 1985) or N-methyl-D-aspartic acid (Stellar, Hall and Waraczynski, 1991) resulted in only small, temporary deficits in medial forebrain bundle self-stimulation. In brief, that self-stimulation persists in some areas, even after extensive lesioning, suggests: (a) That the critical source that gives rise to self-stimulation has not yet been identified;

(b) considerable redundancy or plasticity exists in the neural substrate of reward; (c) That there are independent systems of reward within the brain; or (d) Both b and c.

Numerous more circumscribed lesion studies concentrating on the section of the medial forebrain bundle between the LH and VTA have also been conducted. Unfortunately, they have been plagued by contradictory results. Originally, researchers thought that lesions anterior to the self-stimulation site were generally ineffective at reducing self-stimulation (Morgane, 1962; Lorens, 1966; Unemoto, 1968; Olds & Olds, 1969) whereas posterior lesions were more effective (Olds & Olds, 1969; Stellar & Neely, 1982). However, the following are but some of the studies suggesting that this interpretation may be too simple. For example:

(1) Although Olds and Olds (1969) found large decrements in anterior medial forebrain bundle rates following posterior medial forebrain bundle lesions, Lorens (1966) found no change following a similar protocol. (2) Although Schiff (1964) found large reductions in septal rates after lesioning the VTA, Valenstein and Campbell (1966) did not. (3) In a series of more sophisticated experiments, Janas and Stellar (1987), Glimcher & Gallistel, 1989; Murray and Shizgal (1991), Leon and Gallistel (1992b) and Arvanitogiannis and Shizgal (1993) found robust LH and/or VTA threshold increases after ipsilateral anterior medial forebrain bundle lesions. However, Stellar and Neeley (1982), Waraczynski (1988), Waraczynski, Ton and Shizgal (1990), Waraczynski, Conover and Shizgal (1992) and Sim, Lim and Gallistel (1993) did not find any systematic increases in medial forebrain bundle self-stimulation frequency thresholds with lesions anterior to the electrode. Only cuts in the lateral preoptic area or in the medial forebrain bundle just anterior to the stimulating electrode decreased rewarding efficacy in one study (Waraczynski, 1988). Yet these effects were temporary and only occurred if considerable concomitant rostrocaudal tissue damage was apparent around the k[illegible] cut. Although other examples could be given, it remains that lesion studies only suggest, but do not prove, that multiple reward systems exist within the medial forebrain bundle.

Many factors may have contributed to inconsistent lesion results. For example, as mentioned earlier, the undue reliance on self-stimulation rates as a measure of rewarding efficacy

in early studies may have led to erroneous conclusions. Secondly, electrolytic lesions (also used in early studies) are now known to unintendedly damage areas adjacent to the target, interrupt the fibres of passage and disrupt vasculature (Jarrard, 1991), thus making control very difficult. Other uncontrollable factors inherent to lesion studies include partial recovery, edema, functional reorganization and compensation for loss. Another critical factor includes the length of the period between lesion and test, which also varied from study to study. These observations, added to the fact that threshold increases are not always permanent, make very arduous the task of unveiling reward circuitry using lesions.

Pharmacological Studies

At about the same time that researchers were looking for a critical anatomical source giving rise to the substrate of reward, others were searching for a critical neurotransmitter. Prior to 1970, norepinephrine was thought to have that honour, based on the presence of ascending norepinephric axons located within the medial forebrain bundle (Stein, 1968). In accord with this hypothesis, Stein and Wise (1969) showed that norepinephrine was released in the hypothalamus and amygdala during self-stimulation. However, this candidate was cast aside when it became evident that near complete cortical depletions of norepinephrine affected only minimally hypothalamic or locus ceruleus self-stimulation (Lippa, Antelman, Fisher & Canfield, 1973; Cooper, Cott & Breese, 1974; Clavier, Fibiger & Phillips 1976; Corbett, Skelton & Wise, 1979). Similarly, in a brainstem mapping study, Corbett and Wise (1979) did not find any consistent correlation between the quality and presence of self-stimulation and the degree of norepinephric fibre density or cellular aggregation, nor did they find any correspondence between the boundaries of the reward and norepinephric systems.

Recently, however, clonidine, an alpha2 agonist, and yohimbine, an alpha2 antagonist, were shown to significantly decrease posterior medial forebrain bundle rewarding efficacy (Gallistel and Freyd, 1987). Notwithstanding, Gallistel and Freyd felt that these drugs did not necessarily alter the magnitude of the rewarding signal by acting directly on postsynaptic receptors

in the reward pathway. If they did, then the observed decreases in rewarding efficacy would be analogous to that obtained when current intensity is lowered (Gallistel & Freyd, 1987), in other words, when neural density is decreased. However, the maximum change in rewarding efficacy that could be reliably produced by these two drugs was a reduction by a factor of 2, even though lowering the current could have induced 25-30-fold changes. The authors suggested instead that these drugs may modulate some physiological homeostatic variable relevant to the proper operation of the reward pathway.

Comparatively few studies have been conducted investigating the effects of serotonin, acetylcholine and GABA manipulations on medial forebrain bundle self-stimulation, and will therefore not be reviewed in this context. On the other hand, dopamine has received a great amount of attention. Interest in dopamine was based mainly on the fact that, generally, its antagonists, such as pimozide, reliably decreased reward in a dose-dependent manner (Clavier et al., 1976; Wise, 1976; Gallistel & Karras, 1984; Miliaressis, Malette & Coulombe, 1987; Gallistel & Freyd, 1987), and its agonists, such as amphetamine, reliably increased it (Phillips & Fibiger, 1973; Phillips, Brooke & Fibiger, 1975; Franklin & Robertson, 1980; Gallistel & Karras, 1984; Gallistel & Freyd, 1987). However, first-stage self-stimulation neurons in the medial forebrain bundle were soon realized to be incongruent with dopaminergic ones for the following reasons: (1) The refractory period of neurons directly involved in self-stimulation ranged from 0.4-1.2 msec, (Yeomans, 1975), whereas that of dopamine ranged from 1.2-2.2 msec (Guyenet & Aghajanian, 1978; German, Dalsass & Kiser, 1980). However, Yeomans (1989) has recently suggested that dopamine refractory periods obtained using small electrode tips and large currents may contribute to the slower estimates obtained with self-stimulation neurons. (2) Dopamine neurons were shown to conduct at a speed of 0.3-0.9 m/sec (Feltz & Albe-Fessard, 1972; Guyenet & Aghajanian, 1978; Yim & Mogenson, 1980; German, Dalsass & Kiser, 1980), whereas self-stimulation fibres were shown to conduct at a speed of 1.0-8.3 m/sec (Bielajew & Shizgal, 1982; Shizgal, 1989). Yet, Murray and Shizgal (1994) have also recently identified a slower population of neurons, raising the possibility that dopaminergic neurons may play a role in the later phase of

recovery of self-stimulation fibres. (3) In a series of moveable electrode mapping studies, Gratton and Wise (1983) and Blander and Wise (1989) did not find any correlation between the boundaries of reward and dopamine fibre systems. (4) Increases in self-stimulation frequency thresholds following pimozide administration were also obtained in areas of the brain containing only a sparse population of dopaminergic neurons, such as the central grey (Miliaressis, Malette and Coulombe, 1986). (5) Although Cooper, Cott and Briese (1974) found a decrease in hypothalamic self-stimulation rates following 6-hydroxydopamine lesions, Colle and Wise (1987) only engendered a temporary 30% increase in hypothalamic thresholds. (6) If the rewarding signal did travel exclusively or in part along the meso-accumbens dopamine fibres, then equipotent VTA self-stimulation stimuli would yield a constant dopaminergic output in the nucleus accumbens (Miliaressis, Emond & Merali, 1991). However, this constancy was not met, suggesting the medial forebrain bundle rewarding signal is not relayed by the meso-accumbens dopamine cells. These experiments intimate that dopaminergic neurons may be involved in a homeostatic regulation or gate-like synaptic arrangement which modulates the flow of the reward pathway (Miliaressis et al., 1986, 1990; Gallistel & Freyd, 1987). It appears therefore, that while dopamine neurons may not carry the reward signal, they may play a modulatory role.

In sum, first-stage self-stimulation fibres have not yet been identified neurochemically. Alternatively, multiple neurotransmitters and neuropeptides may be involved in brain stimulation reward. With this hypothesis in mind, researchers began to chart differential reactions across self-stimulation sites to pharmacological manipulation. The following are just some examples of neurochemical dissociations of reward systems: (1) Carey, Goodall and Lorens (1975) and Goodall and Carey (1975) found that amphetamine injections increased LH rates, but not cortical rates, thus suggesting that dopamine played a role at the former, but not the latter site. (2) Hand and Franklin (1983) not only confirmed Goodall's results, but showed that rats shifted from an equal preference for LH and medial prefrontal cortical stimulation to a preference for the LH after amphetamine injections. Thus, LH and cortical reward neurons responded differentially to this dopamine agonist. (3) Corbett (1990) showed a marked difference in sensitivity between medial

forebrain bundle and medial prefrontal cortical self-stimulation to cis-flupenthixol challenge. He showed that low to moderate doses of this dopamine antagonist consistently increased frequency thresholds in the medial forebrain bundle, as compared to little or no effect on medial prefrontal cortical self-stimulation. Corbett (1990) took this experiment to indicate that medial forebrain bundle self-stimulation is much more dependent on dopamine than that of the prefrontal cortex. (4) According to Phillips, Carter and Fibiger (1976b), a dissociation between LH and caudate putamen reward systems exists, based on the fact that para-Chlorophenylalanine, a serotonin antagonist, facilitated self-stimulation rates in the former structure, while impeded rates in the latter. (5) Robertson and Mogenson (1978) showed that injections of spiroperidol, a dopamine antagonist, into the nucleus accumbens and the prefrontal cortex attenuated self-stimulation rates in the former, but not the latter site. These results suggested to the authors that the nucleus accumbens and the prefrontal cortex were mediated by separate reward systems. (6) Amphetamine injected into the caudal, but not the rostral nucleus accumbens, significantly decreased VTA self-stimulation (Ranaldi & Beninger, 1994).

Lesion experiments using selective neurotoxins have also intimated that multiple reward substrates exist within the brain. For example, in 1978, Phillips and Fibiger imposed a 6-hydroxydopamine lesion between the VTA and the nucleus accumbens or medial forebrain bundle. They found that these lesions abolished self-stimulation in the VTA, but only partially attenuated it in the nucleus accumbens and medial prefrontal cortex. Based on these results, the authors concluded that the telencephalic reward system was divergent from that of the mesencephalon. Secondly, Phillips, LePiane and Fibiger (1982) showed that dorsal striatum kainic acid lesions had no effect on VTA current intensity thresholds, but significantly increased those of the substantia nigra, suggesting a neurochemical dissociation between these two reward sites. However, varying current intensity changes the size of the stimulation field, thus eliminating the possibility of controlling for the density of reward fibres (Wise & Rompré, 1989).

Thus far, most evidence suggests that the LH and the VTA are part of a common neurochemical system of reward based upon their similar reactions to various pharmacological

manipulations. However, Gratton & Wise (1985) and Miliaressis and Rompré (1987) have identified two parallel or nonoverlapping subsystems of reward within the hypothalamus itself: one consisting of homogenous fast fibres with refractory periods of 0.4 msec., and a second consisting of heterogenous slower fibres, with refractory periods of 0.6 msec. According to Gratton and Wise (1985), the fast fibres may be mediated by a muscarinic synapse located in the VTA. They based this interpretation on the fact that cholinergic receptor blockade using atropine eliminated the contribution of the subset of fast fibres only, whereas dopaminergic blockade merely reduced responding without significantly influencing the directly activated fibre population. These neurochemical dissociations reveal the remarkable complexity of brain stimulation reward neurochemistry, a finding consistent with the existence of at least 50 neural pathways in the medial forebrain bundle alone.

As with many early experiments investigating the functions or anatomical organization of reward neurons, most older pharmacological studies relied on rates as a measure of reward value. Secondly, it is incorrect to necessarily assume that the differential reactions across self-stimulation sites to pharmacological manipulation imply the existence of independent reward systems. This line of thinking fails to consider the possibility that efferents from a single system can, theoretically, be mediated by more than one neurotransmitter. Thus, pharmacological studies merely intimate at the possibility of multiple independent brain reward systems, not prove so.

Electrophysiological Studies

Electrophysiological techniques have greatly elucidated the excitability properties of brain stimulation reward circuitry. Gathering refractory period estimates, for example, has allowed us to infer psychophysically the physiological and morphological characteristics of reward-related cells according to the time required for an axon to recover after firing an action potential. In brief, refractory periods are a functional-morphological tool of identification, classification, and distinction from other neurons concurrently activated by the stimulation. The estimates are gathered by stimulating a brain region with pairs of pulses while varying the interpulse interval.

The duration of the refractory periods is determined by the physical characteristics of the axons such as size and myelination, thus making it possible to make predictions about the morphology of the excited cells. For example, it has been determined that reward neurons of the medial forebrain bundle consist of small (0.3 - 2.5 mm in diameter), myelinated, non-catecholaminergic fibres with refractory periods ranging from 0.4 msec. to 1.2 msec. (Yeomans, 1975; Shizgal, Bielajew, Corbett, Skelton & Yeomans, 1980; Gallistel, Shizgal & Yeomans, 1981; Bielajew & Shizgal, 1982; Shizgal, 1989). In comparison, medial prefrontal cortex and caudate putamen fibres show slower recovery (20% recovery by 1.59 msec.; Schenck & Shizgal, 1982; Trczinska & Bielajew, 1990; Trczinska & Bielajew, 1992). In line with this finding, rheobase values (or the lowest intensity required to excite the neuron) suggest that the medial prefrontal cortex has less excitable, higher threshold, smaller fibres than those of the medial forebrain bundle (Schenk & Shizgal, 1985). Some researchers have used these data to suggest that different fibres subserve the rewarding consequences of medial prefrontal cortex and medial forebrain bundle stimulation. However, we must not fail to consider the possibility that these variations are normal within a homogenous population of neurons.

A stronger argument that the medial forebrain bundle and medial prefrontal cortex belong to separate reward substrates is made when one amalgamates the lesion and pharmacological data with refractory period estimates. Identifying the substrate lying between these two systems may further help unveil brain reward circuitry. Refractory periods suggest that the basal forebrain (amongst the origins of the descending component of the medial forebrain bundle) forms the intermediate link between the caudal medial forebrain bundle and the medial prefrontal cortex (Fouriezos, Walker, Rick & Bielajew, 1987). Basal forebrain recovery curves are known to begin rising at interpulse intervals between 0.6 and 0.8 msec., and do not level off until 5.0 msec. In 1987, Fouriezos and colleagues set out to determine if this gradual recovery from refractoriness was due to the activation of several subpopulations of reward neurons with different absolute refractory periods, or of a homogeneous population with a long relative refractory period. To accomplish this goal, they used the unequal pulse procedure, which involves increasing the current

of the second pulse. In theory, this procedure should accelerate recovery if basal forebrain self-stimulation is mediated by a homogeneous population of neurons with long relative refractory periods. However, no such acceleration was observed, indicating that basal forebrain self-stimulation is mediated by several populations of neurons with different absolute refractory periods and insignificant relative refractory period contributions. This finding suggests that basal forebrain structures lie in a zone that shares some reward fibres with the posterior medial forebrain bundle and others with the medial and sulcal prefrontal cortices (Fouriezos et al., 1987). Notwithstanding the fact that refractory periods within a homogenous population of neurons vary somewhat, the aforementioned estimates may be suggestive of morphologically distinct reward neurons within the brain.

Multiple Medial Forebrain Bundle Reward Systems: Summary

Identifying multiple reward systems along the medial forebrain bundle has proven quite challenging. The exclusive links between LH, posterior hypothalamic and dorsolateral hypothalamic self-stimulation and feeding, copulation and memory facilitation, respectively, suggest that medial forebrain bundle reward systems may be dissociated functionally. However, many of the older studies leading up to these correlations relied on rates, which may have fostered erroneous interpretations of rewarding efficacy. Secondly, lesion studies have consistently failed to unveil a critical anatomical source for brain reward. Furthermore, lesion studies concentrating specifically on the medial forebrain bundle have been plagued by inconsistent results, making the identification of multiple reward systems difficult. Thirdly, first-stage self-stimulation fibres have not yet been identified neurochemically. Although, in theory, a single reward substrate could be mediated by several neurotransmitters and neuropeptides, pharmacological studies are not inconsistent with the notion of multiple medial forebrain bundle reward systems. Finally, notwithstanding normal excitability variations in a homogenous population of neurons, the variety of refractory period estimates within the brain may be suggestive of multiple reward systems.

In sum, most of the evidence suggestive of multiple medial forebrain bundle reward systems is inconclusive or indirect, in that the dissociations are based on different morphological/physiological properties of neurons or on processes that do not go beyond the electrode tip. In other words, these experiments fail to penetrate the internal process that integrates stimulation of the first-stage neurons into reward. Consequently, it is difficult to truly assess whether the net rewarding experience is different at each reward site. However, psychophysical preference tests may offer a solution to this problem.

Preference Tests

Preference tests allow subjects to choose between two competing reward values by pressing one of two levers. In order to make this choice, it is assumed that subjects discriminate by evaluating the quantitative and qualitative properties of these competing stimuli. This technique rests upon the same assumptions as the preferential looking method used to study discrimination abilities in nonverbal subjects such as newborns (Fantz, 1961). In brief, psychophysical preference tests are unique in that they capture an animal's internal decision-making and discrimination abilities. For example, preference tests have revealed that the rewarding value of pulse frequencies continues to grow even after barpressing rates asymptote (Miliaressis & Malette, 1987). This conclusion was based on the observation that rats almost always preferred the highest of two pulse frequencies eliciting maximum rates, whether the electrode was implanted in the LH or the central grey. In sum, the frequency at which reward saturated was considerably higher than that at which rates levelled off. Miliaressis and Malette (1987) also found one interesting difference between the two sites: Namely, that reward saturation in the LH occurred 0.65-0.75 logfrequency units higher than the logfrequency required to sustain self-stimulation; yet in the central grey, reward saturation was never observed, even with a frequency eight times larger (0.9 logfrequency units) than the frequency at which the rates reached their maximum. (Unfortunately, these authors were unable to test higher frequencies due to technical limits). According to Miliaressis and Malette (1987),

this discrepancy may indicate that the rewarding signals from these two sites do not converge on the same integrator, thus offering support for the multiple brain reward systems hypothesis.

THESIS OBJECTIVES

The primary objective of this thesis is to determine if distinct reward systems exist within the medial forebrain bundle. Using the twin-lever psychophysical preference test, Experiment One will consist of determining the capacity of rats to discriminate between pulse frequencies delivered to the same reward system. In Experiment Two, rats will choose between LH and VTA frequencies that sustain the same proportion of the maximum self-stimulation rate (equipotent or threshold frequencies). Based on the results obtained in the first experiment, it is hypothesized that if rats fail to equally prefer LH and VTA threshold frequencies, then the medial forebrain bundle may convey qualitatively different rewarding signals.

Collision tests have revealed that LH and VTA share a good proportion of common rewarding fibres with certain electrode alignments (Shizgal, Bielajew, Corbett, Skelton & Yeomans, 1982; Durivage & Miliaressis, 1987; Murray & Shizgal, 1994). Therefore, Experiment Three will consist of conducting this paired-pulse test of anatomical linkage in each subject tested in the previous experiment. Although the collision test was conducted after the preference test, we predicted that rats should equally prefer threshold frequencies when the LH and VTA electrodes were stimulating a common axonal bundle (as evidenced by a substantial collision effect).

Finally, subjects can be expected to prefer a "pure" to a "mixed" rewarding signal. In other words, reward-irrelevant variables could affect a rat's preference, despite common circuitry. Therefore, Experiment Four will consist of comparing subjects' preference for a "pure" frequency threshold to one mixed with aversion.

In conclusion, it is hoped that this series of experiments will help to determine if multiple reward systems exist within the medial forebrain bundle.

Chapter Two

THE JUST NOTICEABLE DIFFERENCE OF MEDIAL FOREBRAIN BUNDLE STIMULATION REWARD

There is no conception in man's mind which hath not at first, totally or by parts, been begotten upon the organs of sense.

Thomas Hobbes (1651)

EXPERIMENT ONE

Introduction

As we have seen, early brain stimulation reward experiments were often plagued with inadequate behavioral measures and oversimplified interpretations of results. However, a more quantitative and precise era in self-stimulation came into being with the introduction of psychophysical studies of reward.

Psychophysics

Psychophysics is the study of the relation between stimulus and sensation. In 1834, Weber discovered that the more intense a stimulus is to begin with, the larger the change must be for a subject to notice it. This observation led to the formation of Weber's law, which states that the change in stimulus intensity that can just be discriminated is a constant fraction of the starting intensity of the stimulus. From Weber's law, Fechner derived the relation that the perceived magnitude of a stimulus is proportional to the logarithm of its physical intensity. Fechner's law, as this relation later became known, laid the foundation for the measurement of the sensitivity limits of sense organs (in Gesheider, 1985).

At the heart of psychophysics is the concept of absolute threshold, defined as the smallest amount of stimulus energy necessary to produce a sensation. Absolute threshold is a measure of an organ's sensitivity. Just as there must be a certain minimum stimulus that can be reliably discriminated from no stimulus at all, there must also be a difference threshold (DT). The DT is defined as the critical amount of change in a stimulus to produce a just noticeable difference (JND) in the sensation. It is a measure of discrimination between two stimuli.

The specific procedure followed for measuring DT's usually involves a psychophysical choice test. This test consists of presenting a subject with a pair of stimuli and asking them which

stimulus, if any, produces a sensation of greater magnitude. According to this method of limits, one of the stimuli remains fixed throughout the experiment, and is called the standard stimulus (STD). The other stimulus, or comparison stimulus (COMP), varies systematically from trial to trial in ascending, then descending order (Gesheider, 1985).

The upper threshold (Tu) represents the physical value of the COMP where "greater" responses change to "equal" responses. Similarly, the lower threshold (Tl) is the value where the "less" responses change to "equal" responses. The range over which a subject cannot perceive a difference between the STD and COMP stimuli is called the interval of uncertainty. This estimate is computed by subtracting the mean Tl from the mean Tu. The best estimate of the DT is half the length of the interval of uncertainty (Gesheider, 1985). The value of the COMP that is perceived subjectively as equal to the STD is called the point of subjective equality (PSE). The PSE is obtained by finding the value of the COMP that corresponds to the midpoint of the interval of uncertainty. Due to space and temporal errors, this point rarely corresponds exactly to the physical value of the STD (Gesheider, 1985). If the stimuli are presented simultaneously, space errors may be committed if the subject has a side preference. For this reason, the position of the two stimuli are often counterbalanced. On the other hand, temporal errors can occur when the STD and COMP are presented successively, a procedure conducive to memory decay (Gesheider, 1985). Furthermore, judgment can be affected by nonsensory factors such as motivational and attentional state and overall organ sensitivity. Finally, the difference between the STD and the PSE is called the constant error.

Psychophysical preference tests and brain stimulation reward As mentioned in Chapter One, the primary goal of this thesis is to determine if multiple systems of reward exist within the medial forebrain bundle. To accomplish this goal, a twin-lever psychophysical preference test will be conducted in Experiment Two that compares LH and VTA self-stimulation pulse frequencies sustaining the same proportion of the maximum self-stimulation rate (equipotent or threshold frequencies).

Before proceeding to this experiment however, we must determine rats' discrimination capabilities for electrical stimulation of the brain within a single reward substrate. Therefore, the objective of Experiment One was to establish a control preference profile by which to judge the results of Experiment Two. Specifically, the rats' JND and constant error were determined by combining the method of limits with a preference test. One lever delivered a fixed pulse frequency (STD), whereas the other lever delivered a COMP frequency (COMP) that varied systematically from trial to trial. The lever at which the rat spent more time barpressing was judged to deliver the preferred frequency. The Tu was defined as the minimum COMP frequency that was always preferred to the STD frequency. The Tl was defined as the maximum COMP frequency that was never preferred to the STD frequency. The interval of uncertainty, or the range over which the rat showed no consistent distinction between the two stimuli, was defined as the difference between these two thresholds. The DT was defined as half the length of this range. The frequency that corresponded to the midpoint of this range was the PSE. Figure 1 provides a graphical representation of these definitions for a single preference test. Finally, the constant error was defined as the difference between the STD and the PSE.

Figure 1: A single preference test. Each curve shows the time spent barpressing for the standard (STD) or comparison (COMP) stimulus as a function of the number of COMP pulses/train. Tu: upper threshold; Tl: lower threshold; IU: interval of uncertainty; DT: difference threshold; PSE: point of subjective equality.

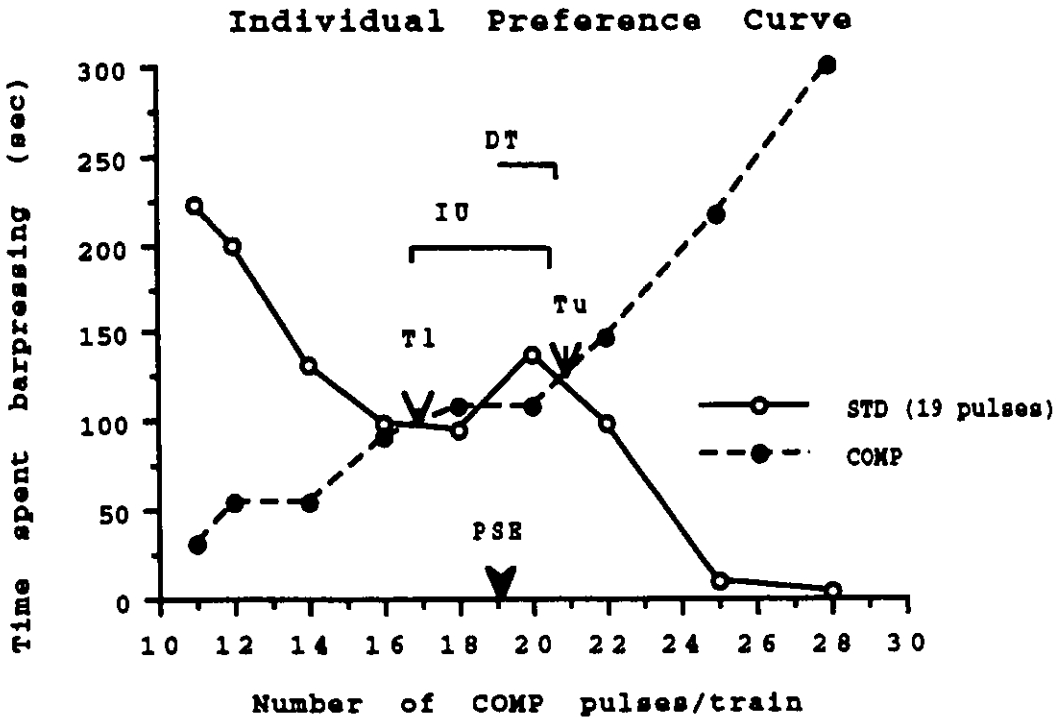
Individual Preference Curve
Time spent barpressing (sec)
300
250
200
150
100
50
0
DT
IU
T1
Tu
PSE
STD (19 pulses)
COMP
10 12 14 16 18 20 22 24 26 28 30
Number of COMP pulses/train

Fig. 1

In brief, the goal of Experiment One was to provide a profile of the quantitative characteristics of rats' discrimination process and abilities. This baseline will also serve as the criterion by which to judge the results of the upcoming LH-VTA preference tests.

Materials and Methods

Subjects and surgery

Three male Sprague-Dawley rats obtained from the Charles River Laboratory were housed individually in wire-mesh cages and maintained on a 12-hour light/12-hour dark cycle. Their monitored diet consisted of 6 Purina rat chow pellets per day, and water was provided ad libitum. Weighing approximately 300 g at the time of surgery, rats were first injected with 0.05 mg/kg of atropine sulfate to reduce bronchial secretions, followed a half an hour later by 0.65 mg/kg of sodium pentobarbitol. Supplemental injections of this anesthetic were administered as required.

With the skull held horizontal between bregma and lambda, each subject was stereotaxically implanted with two monopolar moveable electrodes aimed at the LH and VTA. Each rat was implanted with two moveable electrodes so that more than one site could be potentially tested per rat, thus minimizing the amount of subjects required. Furthermore, each subject was to be tested in Experiments Two and Three, which are two electrode experiments. The electrodes consisted of a 0.25 mm stainless-steel wire, insulated to its tip. For more detail on electrode construction, please consult Miliaressis, 1981. An Amphenol miniature plug, which was soldered to a stainless-steel wire wrapped around four miniature cranial screws, served as the anode. Electrodes and screws were rigidly attached to the skull with dental acrylic. Stereotaxic coordinates for the LH electrode were 1.8 mm posterior to bregma, 2.0 mm lateral to the sagittal suture and 7.5 mm below the cranial surface, and 5.3 mm, 0.7 mm and 8.1 mm, respectively, for the VTA electrode.

Apparatus and stimulation parameters

Subjects were tested in a single-lever operant chamber (29 x 33 x 30 cm) made of transparent acrylic. Stimulation parameters were programmed into a computer (software: *Self-Stimulation Experiments* by Madan Makasare; hardware: Tandy 3000 Personal Computer HL) connected to a constant current generator. Each barpress triggered the constant current generator to deliver a 0.3 sec train of cathodal rectangular pulses of fixed duration (0.1 msec) and variable frequency and intensity. A lock-out interval of 0.4 sec was imposed between the beginning of two consecutive pulse trains. Current intensity was monitored on an oscilloscope. Barpresses were recorded by the computer.

Shaping for self-stimulation

After at least five days of post-operative recovery, subjects were autoshaped to self-stimulate at one or both sites. If the subject failed to self-stimulate persistently at a current less than 800 mA and a frequency less than 35 pulses/train, the electrode was lowered by 0.16 mm, and shaping was resumed shortly thereafter. Once persistent self-stimulation was obtained, the electrodes and currents remained fixed for all subjects (except for subject #212, whose LH electrode was lowered by 0.32 mm to repeat the testing procedure at a more ventral site).

The rate-frequency function

A rate-frequency function (relating the rate of barpresses to the number of pulses/train1) was gathered for both electrode sites. The session consisted of gathering a series of 1-min self-stimulation trials separated by 30-sec period during which no stimulation was available. Between trials, the frequency was varied systematically in descending then ascending order twice, so as to capture the entire range of self-stimulation performance. These curves were replicated daily until

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Pulse frequency and number of pulses/train are used synonymously in the next four experiments.

they did not shift laterally for three consecutive days by more than 0.05 logfrequency units. The mean of the three curves plus the standard error of the mean were calculated for each electrode. Finally, the threshold frequency (corresponding to 38-97% of the maximum self-stimulation rate, depending on the subject) was determined from each mean rate-frequency function.

The preference test

In the second phase of this experiment, another identical lever was added 12 cm from the first lever on the same wall of the operant chamber. At this point, the first lever was disengaged, and another rate-frequency curve was gathered for each electrode, using the new lever. This replication ensured the stability of the threshold on the new lever. Then, both levers were engaged for the preference test.

Rats were first trained to sample each lever. The training session consisted of setting up the new lever to deliver a high frequency, and the original lever to deliver a low frequency via the same electrode. (The second electrode was disconnected). The value of these frequencies was inferred from the mean rate-frequency function. Rats were primed with the higher frequency. If the rat first sampled the lever with the higher frequency, or spontaneously alternated to the higher frequency, the lever to stimulus connections were reversed after five minutes and the trial was repeated. If the rat first sampled the lower frequency, and failed to spontaneously alternate, the first lever was blocked. At this point, the rat was primed with the higher frequency until he began to barpress at the second lever. The values of the competing stimuli were gradually brought into closer proximity until the rats spontaneously sampled both levers at the beginning of each trial. Simultaneous barpressing was prevented by the addition of a transparent acrylic partition between the levers.

Once the rats were sufficiently trained, the preference test was begun. Barpresses to one lever delivered a STD frequency, corresponding to the threshold frequency previously defined in the single-lever test. The second lever delivered a COMP frequency that was either higher, lower, or equal to the STD frequency. A testing session consisted of a series of 5-min trials separated by

a 60-sec interval during which no stimulation was available. The trial began once the rat spontaneously sampled each lever. Both the barpressing rate and time spent barpressing for each stimulus were recorded as a function of the number of COMP pulses/train. However, since the two data sets showed very similar profiles, only time measures are shown, to remain consistent with Experiment Two. Following each trial, the lever-to-stimulus connections were reversed so as to counterbalance a side preference. The COMP frequency was varied systematically between trials in ascending and then descending order. Each session was replicated three or four times, depending on the subject. The preference test was performed at five sites in all, three in the LH and two in the VTA.

Data analysis

The Tu, defined as the minimum COMP frequency (- 0.5 pulses to capture the real limit; Gesheider, 1985) that was always preferred to the STD frequency, was inferred from each individual pair of preference curves. The Tl, defined as the maximum COMP stimulus (+ 0.5 pulses) that was never preferred to the
STD frequency, was also inferred. The mean logarithmic interval of uncertainty (LIU) for each site tested was computed according to the following formula:

$$LIU = LTu - LTl$$

where LTu is the logarithmic mean Tu and LTl is the logarithmic mean Tl. The logarithmic values were used for this calculation simply to respect Weber's and Fechner's laws.

The logarithmic difference threshold (LDT) was calculated according to the following formula:

$$LDT = \tfrac{1}{2}LIU$$

To determine the logarithmic point of subjective equality (LPSE) the following formula was used:

$$LPSE = \frac{1}{2} (LTu + LTl)$$

The logarithmic constant error (LCE) was obtained from the following formula:

$$LCE = LPSE - LSTD$$

where LSTD is the logarithm of the STD stimulus.

Given that this same group of rats was subjected to Experiments Two and Three, the histology is provided at the end of Chapter Four.

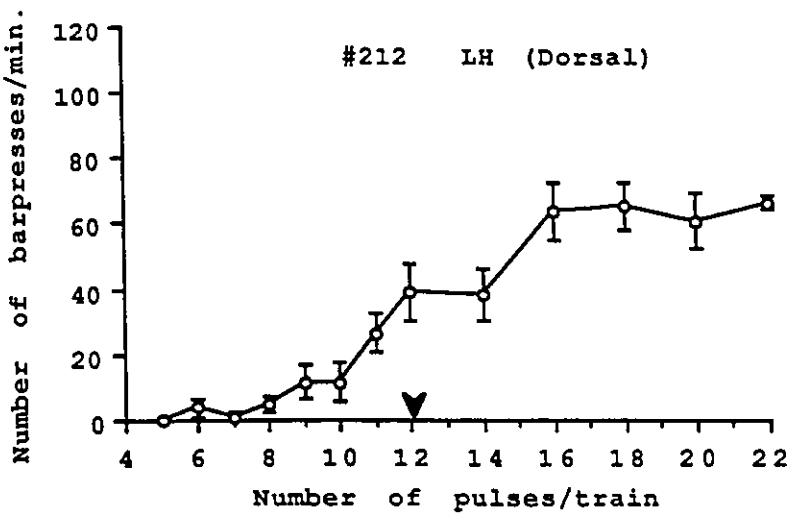
Results

Figures 2-6 represent data for each site tested. For each case, the upper graph shows the mean rate-frequency curve which plots the number of barpresses per min. as a function of the number of pulses per train (obtained in the single-lever operant box). The label above each upper graph identifies the subject number and site tested. The filled arrow intersecting the abscissa points to the threshold frequency used as the STD stimulus in the preference test.

The lower panel shows the preference curves, which plot the mean time spent barpressing for the STD or COMP stimuli as a function of the number of COMP pulses/train. The site tested and the value of the STD are given in brackets. The arrow intersecting the abscissa points to the value of the COMP stimulus that corresponds to the PSE (for calculations, see Data Analysis section). Open and closed circles refer to LH and VTA data, respectively. Solid and stippled lines refer to barpresses for the STD and COMP frequencies, respectively. In both graphs, standard errors of the mean are shown as vertical bars.

Figures 2-6: The upper graph represents the rate-frequency curve, which plots the mean number of barpresses per min. as a function of the number of pulses per train. The subject number and site tested are indicated at the top of this graph. The filled arrow intersecting the abscissa points to the threshold frequency that was used as the standard stimulus (STD) in the preference test.

The lower graph represents the preference curves, which plot the mean time spent barpressing for the STD or COMP frequency, as a function of the number of COMP pulses/train. The site tested and value of the STD is given in brackets. The arrow intersecting the abscissa points to the value of the COMP frequency that corresponds to the point of subjective equality (for calculation, see Data Analysis section). Open and closed circles refer to LH and VTA data, respectively. Solid and stippled lines refer to barpressing for the STD and COMP frequency, respectively. In both graphs, standard errors of the mean are shown as vertical bars.


#212 LH (Dorsal)
Number of barpresses/min.
120
100
80
60
40
20
0
4 6 8 10 12 14 16 18 20 22
Number of pulses/train

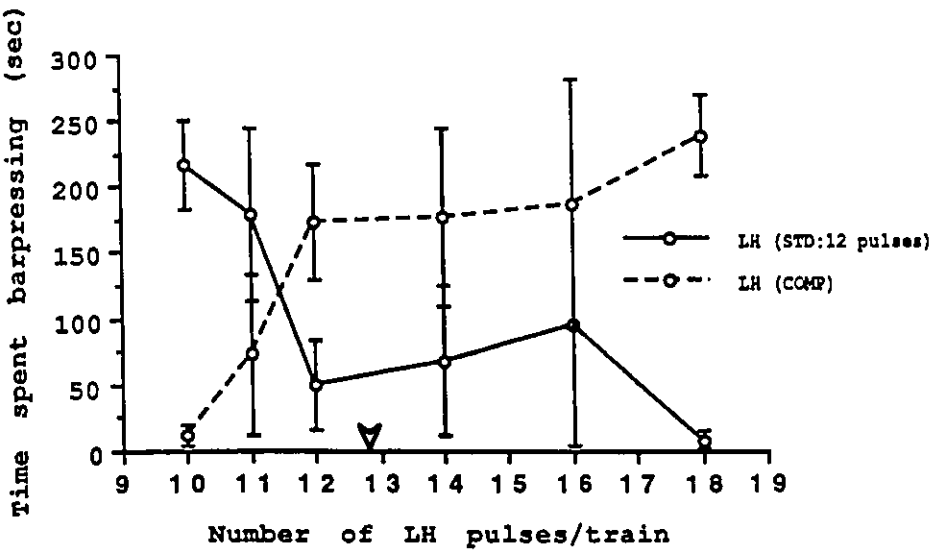
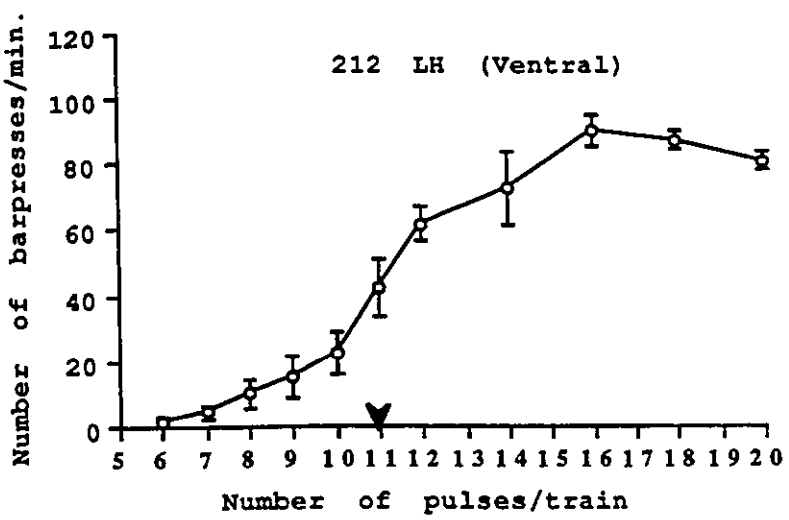

Time spent barpressing (sec)
300
250
200
150
100
50
0
9 10 11 12 13 14 15 16 17 18 19
Number of LH pulses/train
LH (STD:12 pulses)
LH (COMP)

Fig. 2


212 LH (Ventral)
Number of barpresses/min.
120
100
80
60
40
20
0
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Number of pulses/train

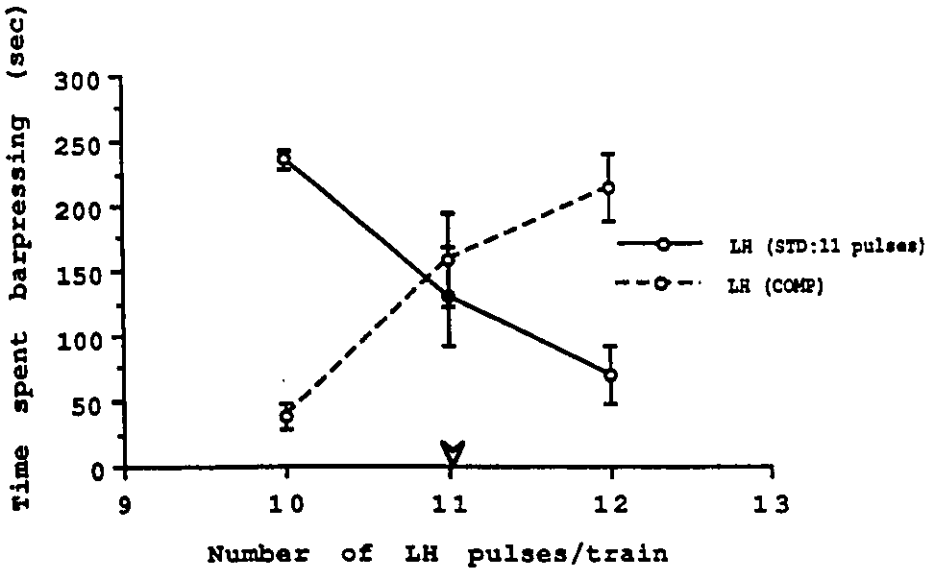
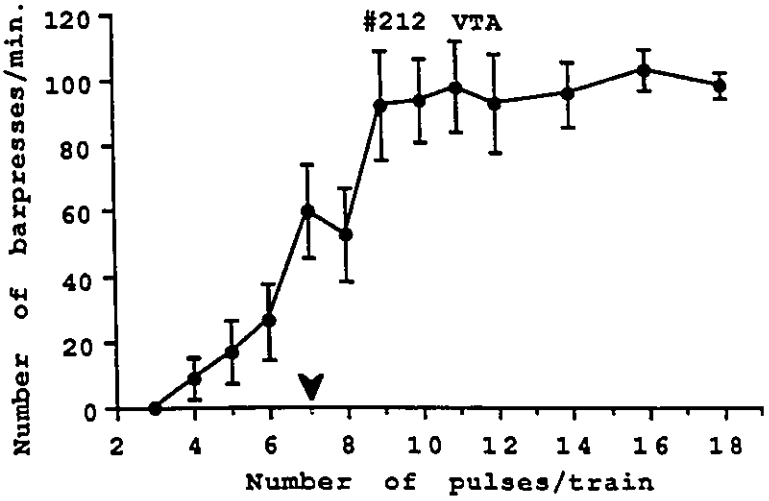

Time spent barpressing (sec)
300
250
200
150
100
50
0
9
10
11
12
13
Number of LH pulses/train
LH (STD:11 pulses)
LH (COMP)

Fig. 3


#212 VTA
Number of barpresses/min.
120
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
Number of pulses/train

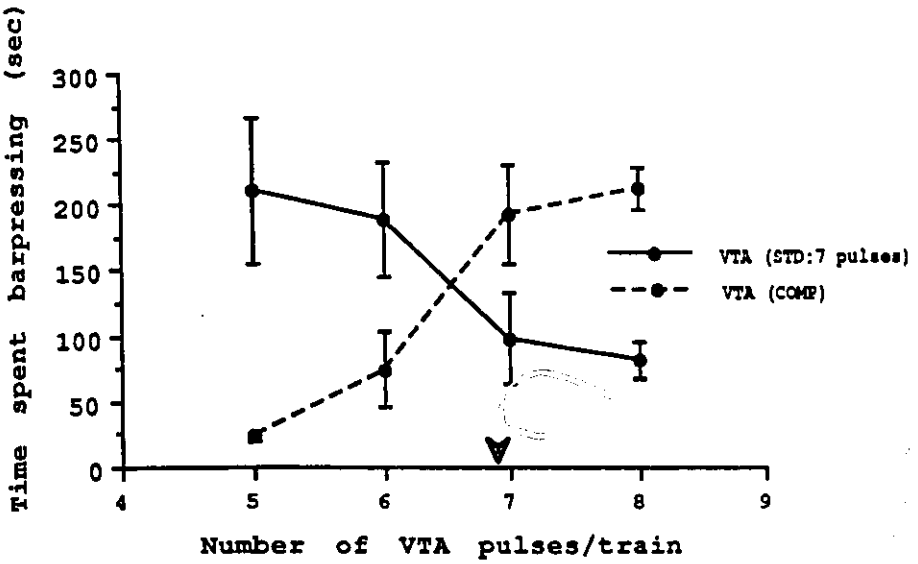
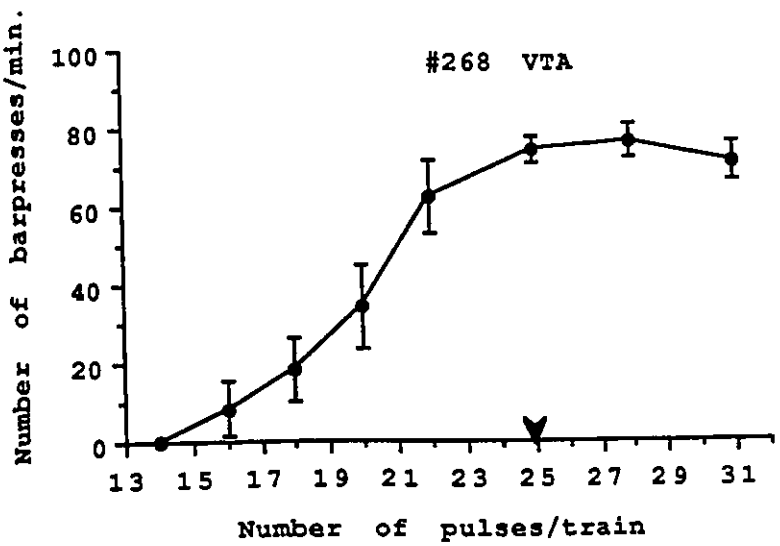

Time spent barpressing (sec)
300
250
200
150
100
50
0
4
5
6
7
8
9
Number of VTA pulses/train
VTA (STD:7 pulses)
VTA (COMP)

Fig. 4


#268 VTA
Number of barpresses/min.
100
80
60
40
20
0
13 15 17 19 21 23 25 27 29 31
Number of pulses/train

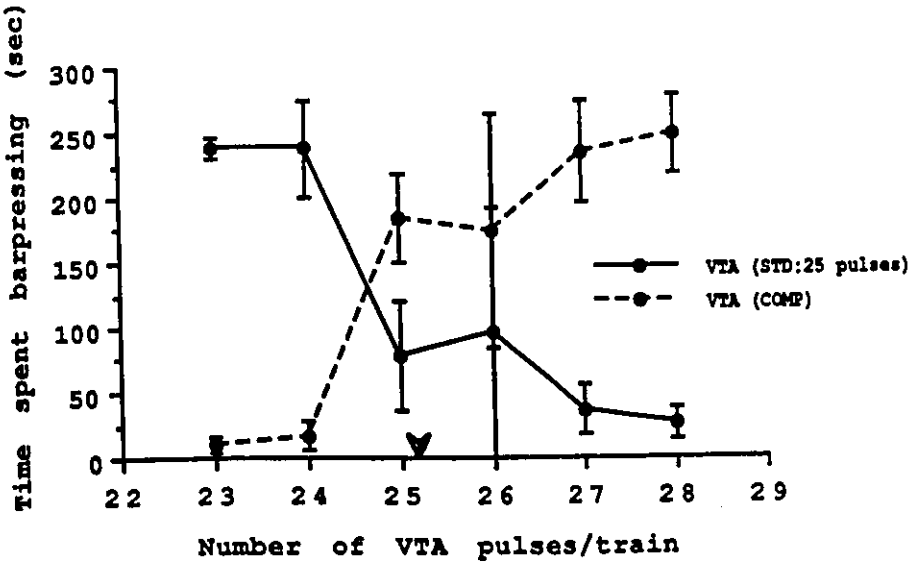
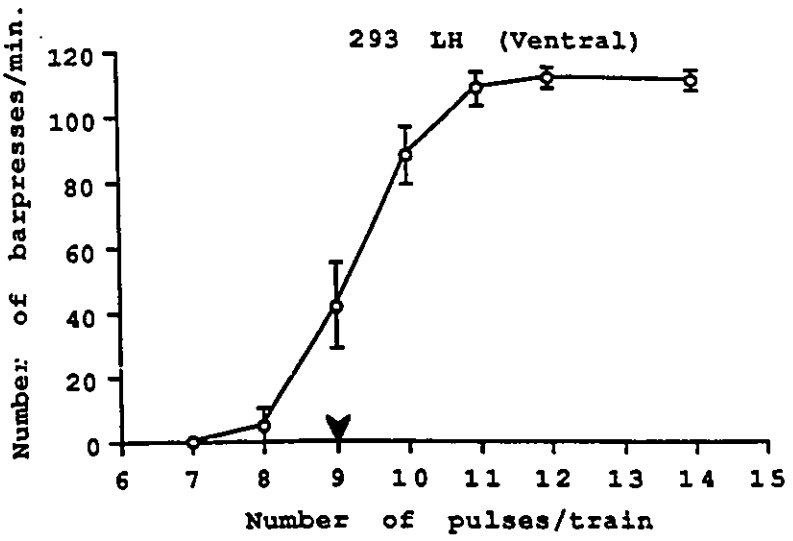

Time spent barpressing (sec)
300
250
200
150
100
50
0
22 23 24 25 26 27 28 29
Number of VTA pulses/train
VTA (STD:25 pulses)
VTA (COMP)

Fig. 5


293 LH (Ventral)
Number of barpresses/min.
120
100
80
60
40
20
0
6 7 8 9 10 11 12 13 14 15
Number of pulses/train

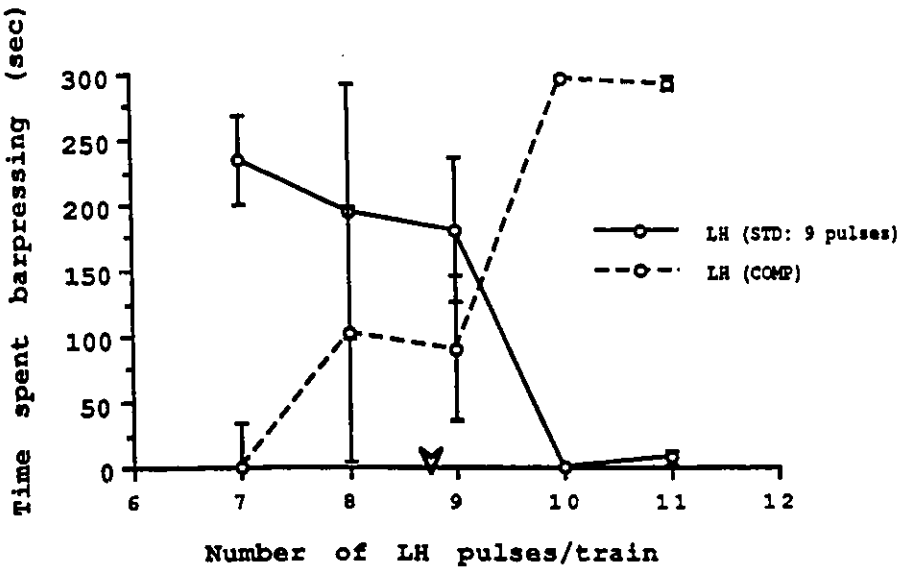

Time spent barpressing (sec)
300
250
200
150
100
50
0
6 7 8 9 10 11 12
Number of LH pulses/train
LH (STD: 9 pulses)
LH (COMP)

Fig. 6

Table 1 gives the LIU, LDT, LPSE, LSTD and the LCE for each subject. At the bottom of each column, the mean (X) and standard error of the mean (S.E.M.) are provided, where applicable.

Table 1: The logarithmic interval of uncertainty (LIU), differential threshold (LDT), point of subjective equality (LPSE), standard stimulus (LSTD) and constant error (LCE) are provided. At the bottom of each column, the mean (X) and standard error of the mean (S.E.M.) are provided, where applicable.

CHAPTER TWO

Table 1

SUBJECT #	LIU	LDT	LPSE	LSTD	LCE
212 LH (d)	0.103	0.052	1.103	1.079	0.024
212 LH (v)	0.013	0.007	1.041	1.041	0.000
212 VTA	0.000	0.000	0.834	0.845	-0.011
268	0.012	0.006	1.401	1.398	0.003
293	0.033	0.016	0.946	0.954	-0.008
X+/-S.E.M.	0.032+/- 0.018	0.016+/- 0.010			0.009+/- 0.004

For competing stimuli within the same reward substrate, the mean LIU was 0.032 logfrequency units (LIU = LTu - LTl). Thus, the mean LDT was 0.016 logfrequency units. The mean absolute LCE was 0.009 logfrequency units.

Discussion

The JND of brain stimulation reward and the rats' constant error values were determined according to the commonly used psychophysical method of limits. Results show that the critical amount of change required to produce a JND in a single rewarding substrate is 0.016 logfrequency units, which translates to approximately half of a pulse at the range of frequencies tested. These results suggest that rats are theoretically able to discriminate well between two rewarding stimuli that differ in magnitude by a single pulse. The interval of uncertainty, or the range over which rats cannot perceive a difference between the STD and COMP stimuli is therefore 0.032 logfrequency units, or just over a single pulse. Furthermore, the low constant error (0.009 logfrequency units, or one fifth of a pulse) shows that there is very little difference between the PSE and the STD stimulus.

According to Weber's law, the more intense a stimulus is to begin with, the larger the change must be for the subject to notice it. In other words, the JND is a constant fraction of stimulus intensity. Thus, one limitation of this study is the small range of frequencies tested. In order to properly assess the relation between stimulus strength and JND, a greater range of frequencies should have been tested. However, the thresholds tested in this experiment were meant to be consistent with those tested in the LH-VTA preference test (Experiment Two). Notwithstanding, we tested higher STD stimuli in four cases not shown here (25 pulses/train in 3 cases and 50 pulses/train in 1 case). In accordance with Weber's Law, the LDT was greater when higher STD stimuli were used.

In conclusion, we have established a profile of rats' discrimination abilities within a single substrate of reward. This baseline, will in turn serve as the criterion by which to judge the upcoming two-electrode preference test in Experiment Two.

Chapter Three

SUBJECTIVE EVALUATION OF BRAIN STIMULATION REWARD WITHIN THE MEDIAL FOREBRAIN BUNDLE

A man is too apt to forget that in his world he cannot have everything. A choice is all that is left him.

H. Mathews

EXPERIMENT TWO

Introduction

Since 1954, researchers have been mapping brain reward circuitry. These experiments have revealed that self-stimulation can be elicited in every major subdivision of the brain, from the telencephalon to the myelencephalon. Characterization of the functional, anatomical, chemical and electrophysiological properties of the relevant neurons support the notion that multiple distinct systems underlie the phenomenon of brain stimulation reward. However, most of the available evidence is indirect in that experimental procedures fail to penetrate the internal process that integrates stimulation of the first-stage fibres into reward sensation. Psychophysical preference tests overcome this problem by allowing rats to simply "tell" us if two rewarding stimuli differ. For example, in Experiment One, rats "told" us that they perceived a difference between STD and COMP frequencies that vary by only 0.016 logfrequency units, as they consistently spent more time barpressing for the higher of the two.

The medial forebrain bundle

As mentioned in Chapter One, the medial forebrain bundle has received a great amount of attention in studies of brain stimulation reward. Along this pathway, rats vigorously and persistently self-stimulate, with few interfering behaviors. Consequently, much has been learned about this reward pathway. For example, we know that first-stage medial forebrain bundle self-stimulation axons have absolute refractory periods in the range of 0.4-1.2 msec (Yeomans, 1975). They are long, thin and myelinated with conduction velocities of 2-8 m/sec (Shizgal et al., 1980). Collision tests, a behavioral method for inferring anatomical linkage, have revealed that the LH (anterior medial forebrain bundle) and the VTA (posterior medial forebrain bundle) share a good portion of rewarding fibres (Shizgal et al., 1980; Durivage & Miliaressis, 1987; Murray & Shizgal, 1994). In addition, Hand and Franklin (1983) believe that this common system may be dopamine-

related, as amphetamine caused a simultaneous equivalent increase in reward at both of these sites, an effect that did not affect baseline preference for either stimulus. In other words, this drug elevated the rewarding value of LH and VTA stimulation to a similar degree.

Recently, however, Leon and Gallistel (1992) suggested that the medial forebrain bundle may terminate in multiple reward-integrator systems. They came to this conclusion based on the observation that the function relating the subjective magnitude of brain stimulation reward to the current was shown to differ across electrode sites along this pathway within the same rat. In other words, there were dramatic differences in the rate at which reward grows within the medial forebrain bundle as the strength of the stimulation was increased; differences that could not be accounted for by electrode placement or fibre density. According to these authors, these multiple integrators may ultimately converge on a common group of cell bodies, which scale the to-be-recorded magnitude of the reward differently depending on which axons the signals arrive. A second possibility is that a distinct group of cell bodies exists at various anatomical sites which scale reward for action potentials received from different groups of reward-relevant axons coursing through the medial forebrain bundle.

Combining psychophysical preference tests with the curve-shift paradigm may help determine if distinct reward systems exist within the medial forebrain bundle. In the current experiment, rats compared LH and VTA frequencies that sustained the same proportion of the maximum self-stimulation rate (equipotent or threshold frequencies) in a twin-lever test similar to that described in Experiment One. For a review of the advantages of using threshold frequencies to estimate stimulation effectiveness rather than a constant number of barpresses, see Miliaressis, Rompré and Durivage (1982). The threshold value was inferred from the function relating self-stimulation rate to the frequency of pulses. According to the curve-shift paradigm, threshold frequencies that are obtained under various experimental conditions are assumed to elicit the same magnitude of reward. In 1990, Malette and Miliaressis provided evidence in support of this assumption using a psychophysical preference test. They first obtained two LH threshold frequencies by manipulating current intensity. Given a choice

between the two thresholds, rats distributed their time equally among both, suggesting to the authors that the stimuli were equally rewarding. This experiment showed that intensity-frequency trade-offs that generated a constant proportion of behavior gave rise to equally rewarding events.

In sum, comparing LH and VTA threshold frequencies allows us to keep reward magnitude constant. Therefore, if LH and VTA threshold frequencies are not equally preferred, then the medial forebrain bundle may convey qualitatively different rewarding signals. In theory, rats may show a preference if one stimulus conveys a more advantageous or desired "natural" rewarding sensation. For example, an animal may seek a sexual and a food reward with the same vigour if presented individually (as in the single-lever condition), but may show a clear preference for one of them if given a choice. Alternatively, rats may be predisposed by their evolutionary history to prioritize two rewards in a type of hierarchy, as a survival mechanism. Stated another way, it may be more adaptive to seek one reward than a variety of rewards simultaneously. In brief, preference for rewarding stimuli may vary according to physiological state and/or neural wiring.

Experiment One revealed how rats behave when rewarding stimuli from the same reward site are in competition. In brief, barpressing for each stimulus changed as a function of the difference between the physical magnitude of the two stimuli, a fact that resulted in the observed X-shaped profile. Secondly, they equally preferred identical STD and COMP frequencies within 0.009 logfrequency units. Finally, rats could discriminate between STD and COMP stimuli that differed by only 0.016 logfrequency units. If the LH and VTA are indeed part of a single system of reward, then the preference profile in the following experiment should not differ significantly from those of Experiment One.

Materials and Method

Subjects and surgery

In addition to the three rats tested in Experiment One, seven more were obtained from the Charles River Laboratory. They were housed and fed as those in the first experiment. All rats were implanted with an LH and VTA electrode using the same coordinates, surgical apparatuses and procedures as in Experiment One.

Shaping for self-stimulation

After five days of post-operative recovery, subjects were shaped to self-stimulate according to the same stimulation parameters as described in the first experiment. Once stable stimulation was obtained at both sites, the electrodes and currents remained fixed for all subjects. Please note however, that one or both of #91, #212, #268 and #293's electrodes were lowered by 0.32 mm following all testing to repeat the experiment at more ventral sites.

The rate-frequency function

A rate-frequency function was collected for both sites as in Experiment One. These curves were replicated daily until they did not shift for three consecutive days by more than 0.05 logfrequency units. The mean of the three curves plus the standard error of the mean (s.e.m.) were calculated for each electrode. The LH and VTA pulse intensities were adjusted so that the two functions were as close as possible on the frequency axis. Thereafter, the intensities were held constant for all subsequent testing, including the preference test. Frequency thresholds corresponding from 33-100% (depending on the subject) of the maximal self-stimulation rate were inferred from the mean rate-frequency curve.

The preference test: shaping

In this phase of the experiment, a second lever was added 12 cm from the first, on the same wall of the operant chamber. Another rate-frequency curve was collected at each site

using the new lever, to ensure stability of the threshold frequencies. Then, both levers were engaged, and subjects were primed and trained according to a procedure similar to that of Experiment One, with the following exceptions: Barpresses at the new lever triggered a high LH frequency, whereas barpresses at the other lever triggered a low VTA frequency (obtained from the rate-frequency curve). The lever-to-stimulus connections were then reversed, and the values of the competing stimuli were gradually brought into closer proximity until the rats spontaneously sampled both levers at the beginning of each 5-minute trial. At this point, they were considered to be sufficiently trained to begin the preference test.

The preference test: data collection

In a first session, the LH frequency was held fixed at one lever (the STD stimulus). This frequency, previously selected from within the linear segment of the rate-frequency function, corresponded to 33-100% of the maximum barpressing rate, depending on the subject. However, the STD frequency used for #91(d) corresponded to the point of reward saturation (obtained from data not shown here). The second lever delivered a variable (COMP) VTA frequency. The COMP frequency was changed between trials in ascending then descending order, as per the method of limits. The range of COMP frequencies generally corresponded to that tested in the rate-frequency function. A testing session consisted of a series of 5-minute trials separated by a 60-second interval during which the stimulation was not available. After each trial, the lever-to-stimulus connections were reversed so as to counterbalance a side preference. In the following session, the VTA frequency threshold became the STD, and the LH became the COMP. Each session was replicated three to six times, depending on the subject. Self-stimulation rates and time spent barpressing for each stimulus were plotted as a function of the COMP stimulus. Although these two data sets generally showed similar preference profiles, differences in asymptotic rates between the two sites (predetermined during the rate-frequency phase) suggested that the latter was a more justified dependent variable. For example, some rats distributed their time equally among

threshold frequencies, yet barpressed at unequal rates due to differential performance variables. The preference test was conducted between 15 pairs of sites in all.

Data analysis

The LPSE and LCE were calculated for each pair of sites, according to the formulae employed in Experiment One. It is important to note that in the LCE formula, the LSTD frequency was replaced with the frequency corresponding to the logarithm of the predicted point of equality (LPPE), which is defined as the equipotent (or threshold) frequency of the COMP site. The reason for this change is as follows: When two rate-frequency curves are gathered from different reward sites, thresholds do not necessarily correspond to the same frequency as in Experiment One.

Histology

Histological details will be provided in Chapter Four, as each of the subjects included here were also tested in Experiment Three.

Results

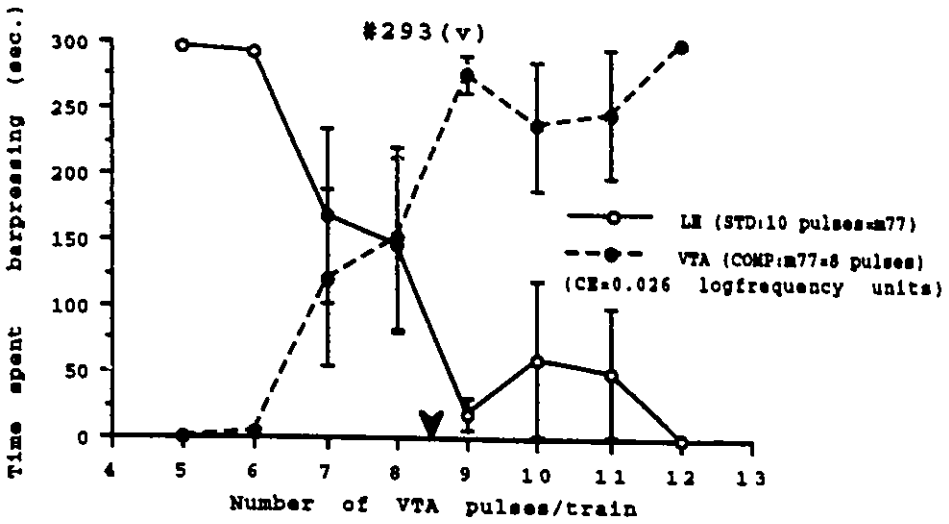
Figure 1 shows representative preference data for a subject [#293(v; ventral site)] that spent approximately the same amount of time barpressing for LH and VTA threshold frequencies. The upper and middle graphs show the time spent barpressing for the STD and COMP stimuli, as a function of the COMP stimulus. Open and closed circles represent time spent barpressing for the LH and VTA, respectively. Solid and stippled lines correspond to barpressing for the STD or COMP stimulus, respectively. The legend within each graph indicates the brain structure and pulse frequency for the STD stimulus, and the percent of maximum self-stimulation rate elicited by this stimulus in the single-lever box (for example, m77 = 77% of the maximum rate). The number beside the COMP stimulus indicates the pulse frequency (or predicted point of equality) which, in the single-lever box, elicited the same

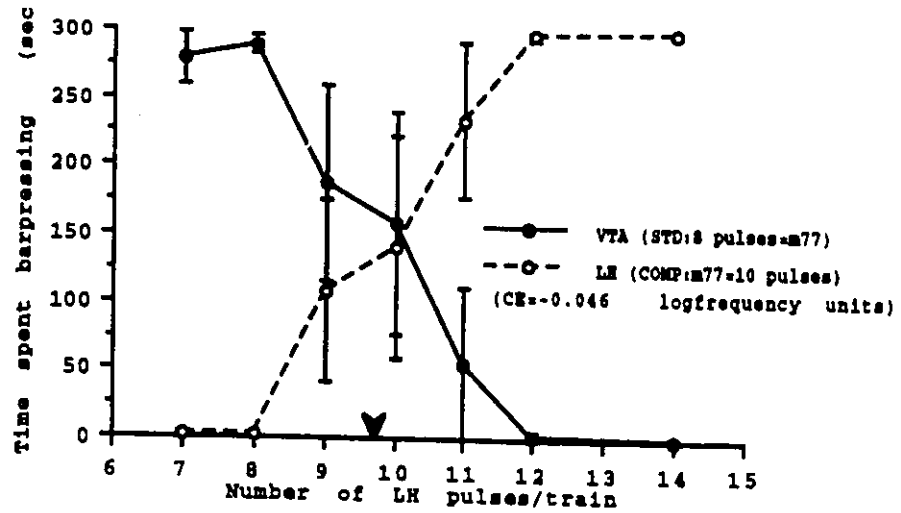
percent of the maximum rate. Filled and open arrows point to the VTA and LH points of subjective equality [calculated from the individual preference curves (not shown) as in Experiment One], respectively. The number in brackets refers to the LCE, or the difference between the LPPE and LPSE.

The lower graph shows the mean rate-frequency curve for each site. This curve plots the number of barpresses per minute as a function of the number of pulses per train, obtained in the single-lever operant box. The frequencies that were equally preferred by the animal are indicated by arrows. Open and filled arrows refer to the upper and middle preference tests, respectively. In all three panels, vertical bars represent the s.e.m.

Figure 1-2: The upper and middle graphs show the time spent barpressing for both the lateral hypothalamic (LH) and ventral tegmental (VTA) stimuli as a function of the comparison (COMP) stimulus. The label above each upper graph identifies the subject. Open and closed circles represent time spent barpressing for the LH and VTA, respectively. Solid and stippled lines correspond to barpressing for the standard (STD) or COMP stimulus, respectively. The legend within each graph indicates the brain structure and pulse frequency for the STD stimulus, and the percent of maximum self-stimulation rate elicited by this stimulus in the single-lever box. The number beside the COMP stimulus indicates the pulse frequency (or predicted point of equality;) which, in the single-lever box, elicited the same percent of the maximum rate. Filled and open arrows point to the VTA and LH points of subjective equality, respectively. The number in the square brackets refers to the LCE, or the difference between the predicted and subjective points of equality.

The lower graph shows the mean rate-frequency curve for each site. This curve plots the number of barpresses per minute as a function of the number of pulses per train, obtained in the single-lever operant box. Open and filled arrows refer to equally preferred frequencies in the upper and middle preference tests, respectively. In all three panels, vertical bars represent the s.e.m.


#293(v)
Time spent barpressing (sec.)
300
250
200
150
100
50
0
4 5 6 7 8 9 10 11 12 13
Number of VTA pulses/train
LH (STD:10 pulses=m77)
VTA (COMP:m77=8 pulses)
(CE=0.026 logfrequency units)


Time spent barpressing (sec
300
250
200
150
100
50
0
6 7 8 9 10 11 12 13 14 15
Number of LH pulses/train
VTA (STD:8 pulses=m77)
LH (COMP:m77=10 pulses)
(CE=-0.046 logfrequency units)

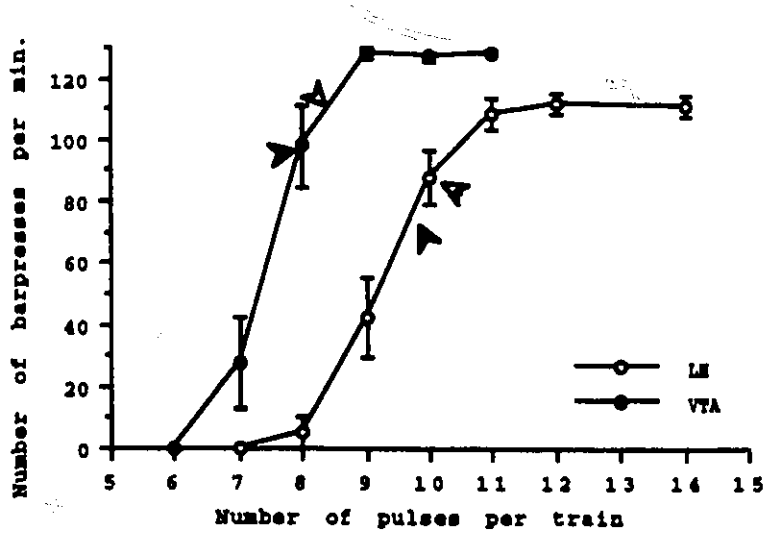
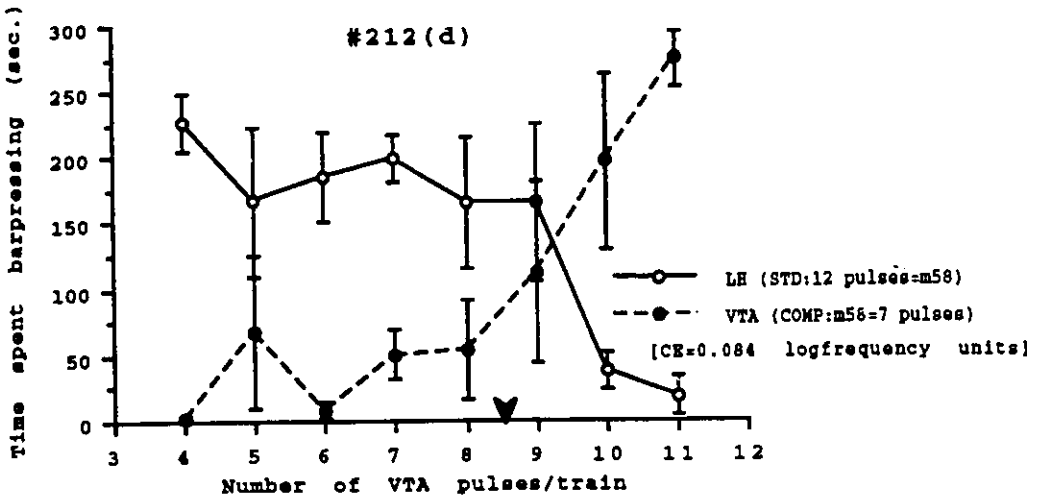
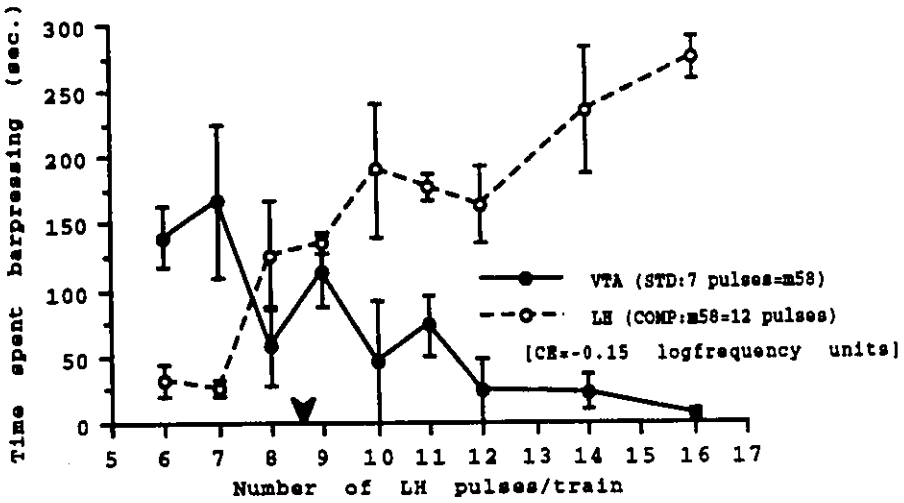

Number of barpresses per min.
120
100
80
60
40
20
0
5 6 7 8 9 10 11 12 13 14 15
Number of pulses per train
LH
VTA

Fig. 1


#212(d)
Time spent barpressing (sec.)
300
250
200
150
100
50
0
3 4 5 6 7 8 9 10 11 12
Number of VTA pulses/train
LH (STD:12 pulses=m58)
VTA (COMP:m58=7 pulses)
[CE=0.084 logfrequency units]


Time spent barpressing (sec.)
300
250
200
150
100
50
0
5 6 7 8 9 10 11 12 13 14 15 16 17
Number of LH pulses/train
VTA (STD:7 pulses=m58)
LH (COMP:m58=12 pulses)
[CE=-0.15 logfrequency units]

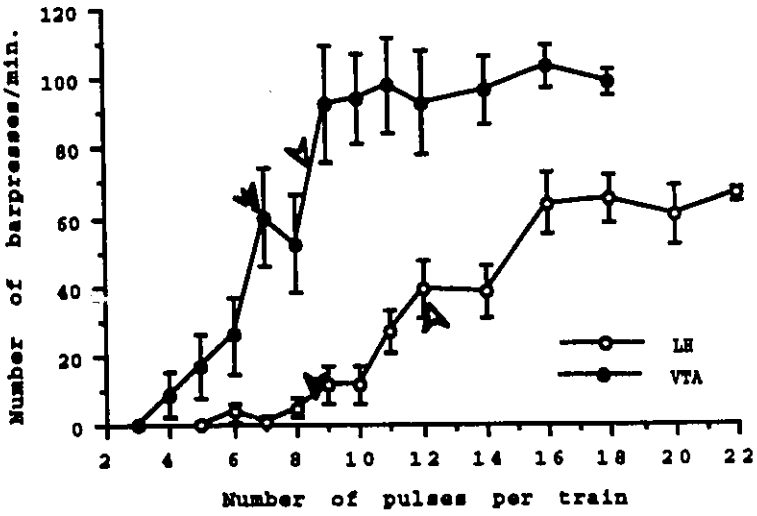

Number of barpresses/min.
120
100
80
60
40
20
0
2 4 6 8 10 12 14 16 18 20 22
Number of pulses per train
LH
VTA

Fig. 2

These data reveal that subject 293(v) equally preferred 10 LH and 8 VTA pulses, previously found to elicit similar proportions of the maximum self-stimulation rate (77%), regardless of the brain site used as the STD stimulus.

Figure 2 shows preference data for a representative subject 212 (d) that equally preferred pulse frequencies that sustained substantially different amounts of performance, regardless of what structure was held constant. The fact that this animal equally preferred 12 LH (m58) and 8.5 (m95) VTA pulses when the LH was STD, and 7 VTA (m58) and 8.5 LH (m8) pulses when the VTA was STD, demonstrates a marked preference for the LH stimulation.

Figures 3-14 show preference results for the remaining subjects. The label above each graph identifies the subject. Open and filled circles represent the number of barpresses for LH and VTA stimuli, respectively. Open arrows refer to equally preferred frequencies when the LH was held STD, whereas closed arrows refer to equally preferred frequencies when the VTA was held STD. The LCE for each preference test is provided in brackets. Vertical bars represent the s.e.m.

Individualized results

Note that data are not shown for subject #90, as no point of subjective equality was obtained for either STD stimulus. In other words, the two preference curves never converged, as this rat always preferred the VTA, regardless of the LH frequency. Second, the STD LH frequency for subject #91 (d) corresponded to the point of reward saturation (obtained from data not shown; 45 pulses per train), a frequency too high to show on the graph. Consequently the open arrows were not included in Figure 3. Third, subject #151 lost his electrode assembly before completion of the second preference test, consequently, the filled arrows were omitted. Finally, when the VTA was held STD for subject #177, the two preference curves did not converge, as this rat also always preferred the VTA stimulus, regardless of LH frequency. Consequently, the filled arrows were omitted.

Figures 3-14: The mean rate-frequency curves are plotted for each pair of sites tested. The label above each graph identifies the subject. Open and filled circles represent the number of barpresses for lateral hypothalamic (LH) and ventral tegmental (VTA) stimuli, respectively. Open arrows refer to equally preferred frequencies when the LH was held standard (STD), whereas closed arrows refer to equally preferred frequencies when the VTA was held STD. The logarithmic constant error (LCE) for each preference test is provided in brackets. Vertical bars represent the s.e.m.

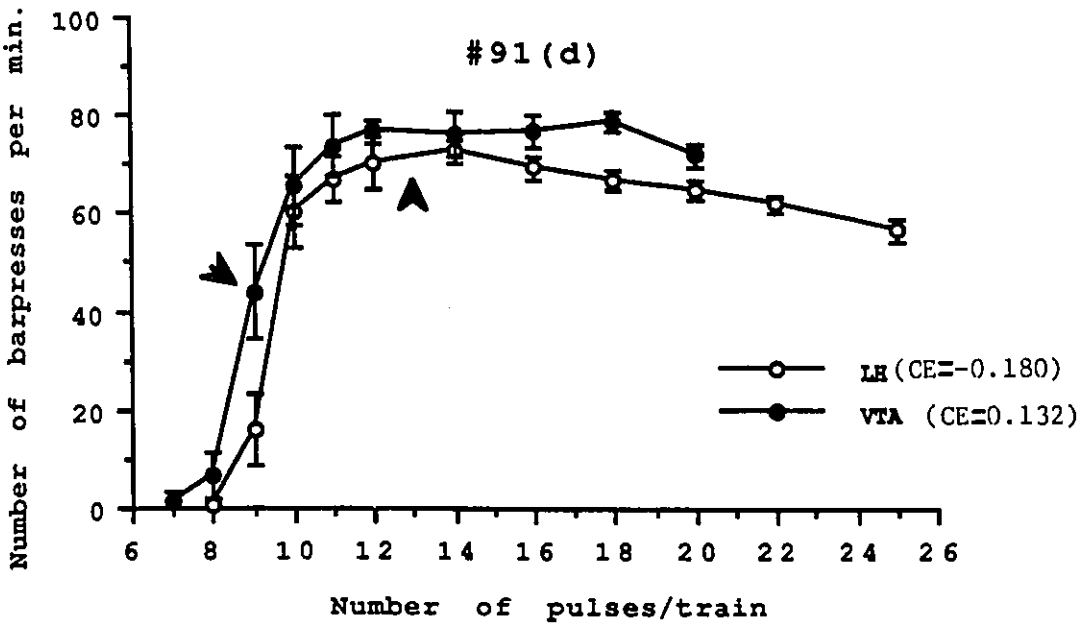

#91(d)
Number of barpresses per min.
100
80
60
40
20
0
6
8
10
12
14
16
18
20
22
24
26
Number of pulses/train
LH (CE=-0.180)
VTA (CE=0.132)

Fig. 3

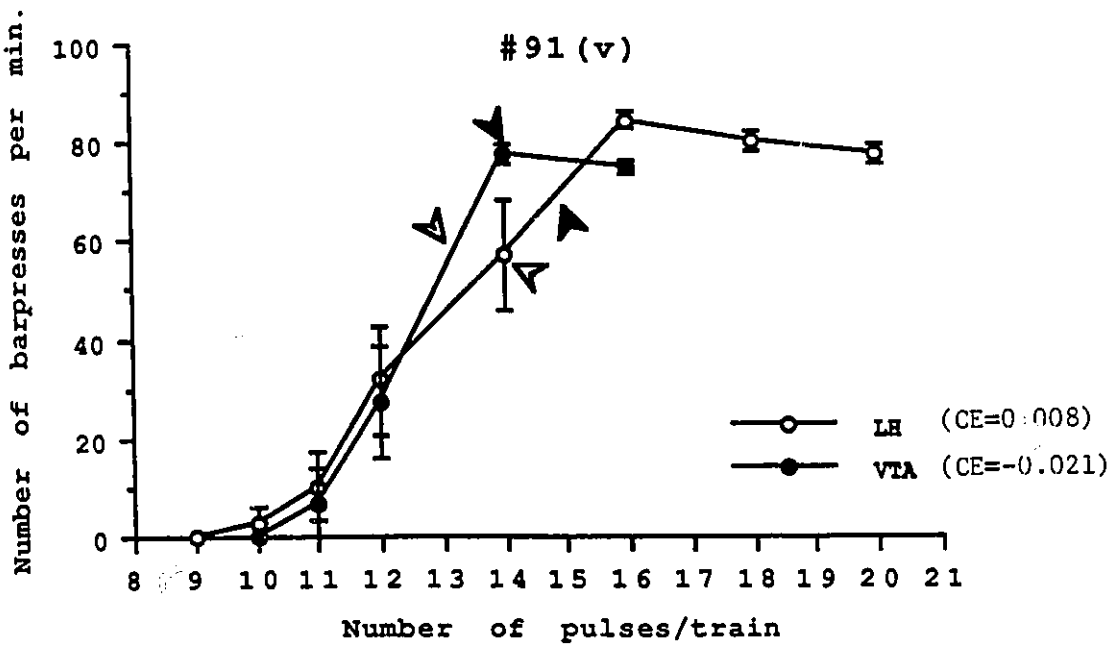

#91 (v)
Number of barpresses per min.
100
80
60
40
20
0
8 9 10 11 12 13 14 15 16 17 18 19 20 21
Number of pulses/train
LH (CE=0.008)
VTA (CE=-0.021)

Fig. 4

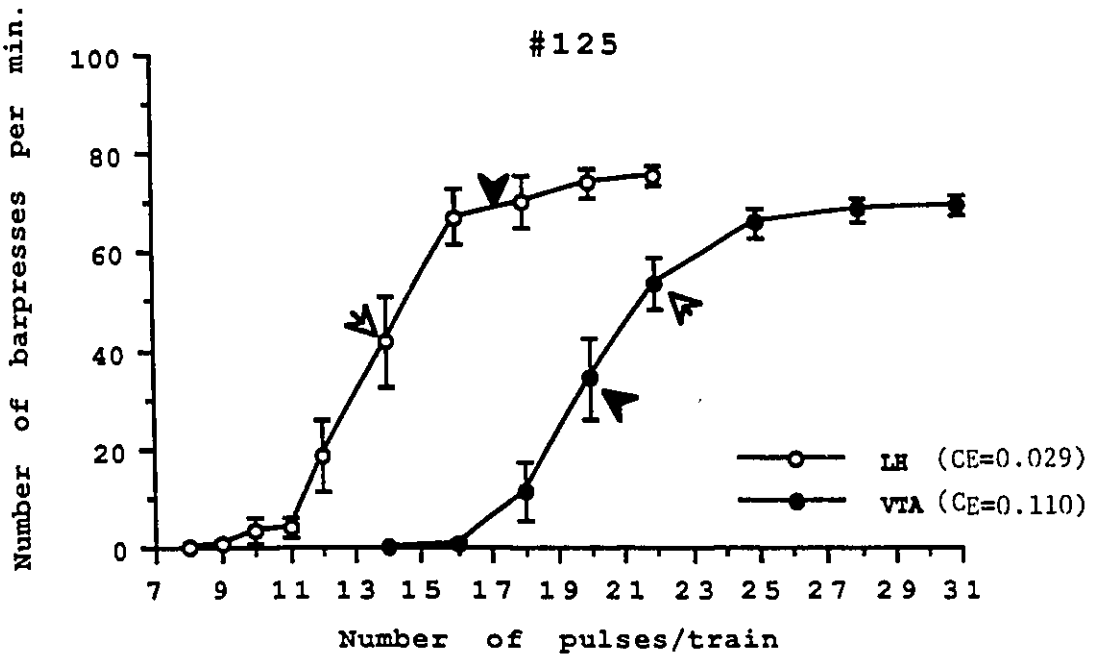

#125
Number of barpresses per min.
100
80
60
40
20
0
7 9 11 13 15 17 19 21 23 25 27 29 31
Number of pulses/train
LH (CE=0.029)
VTA (CE=0.110)

Fig. 5

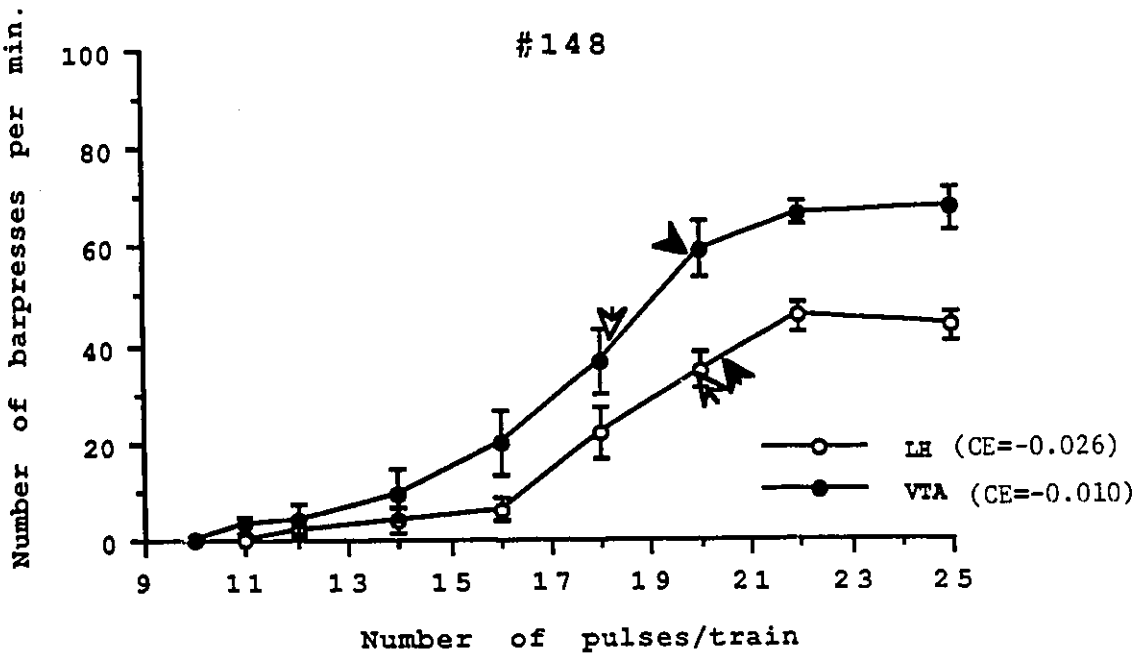

#148
Number of barpresses per min.
100
80
60
40
20
0
9
11
13
15
17
19
21
23
25
Number of pulses/train
LH (CE=-0.026)
VTA (CE=-0.010)

Fig. 6

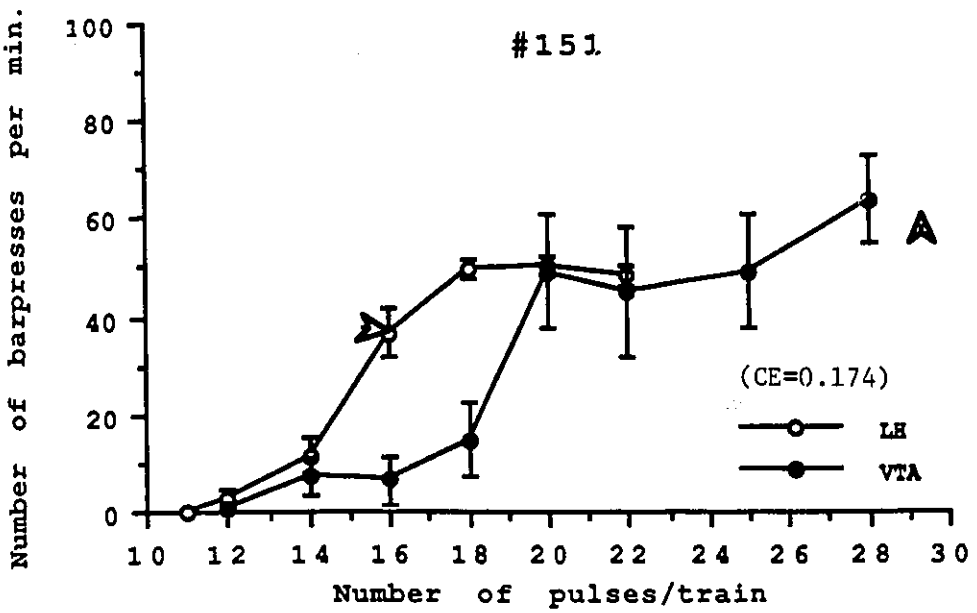

#151
Number of barpresses per min.
100
80
60
40
20
0
10 12 14 16 18 20 22 24 26 28 30
Number of pulses/train
(CE=0.174)
LH
VTA
A

Fig. 7

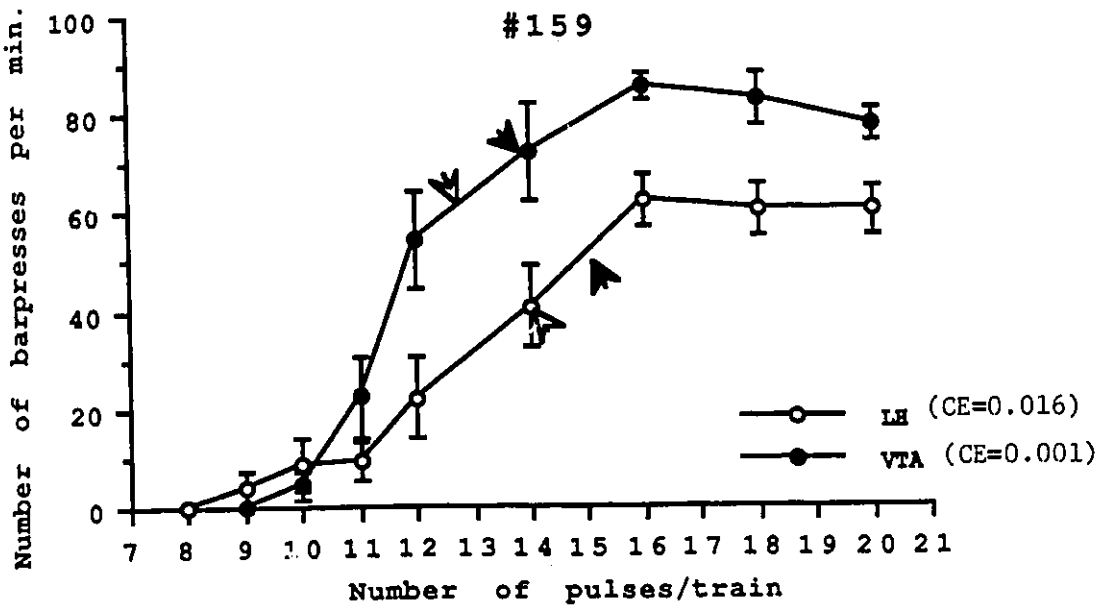

#159
Number of barpresses per min.
100
80
60
40
20
0
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Number of pulses/train
LH (CE=0.016)
VTA (CE=0.001)

Fig. 8

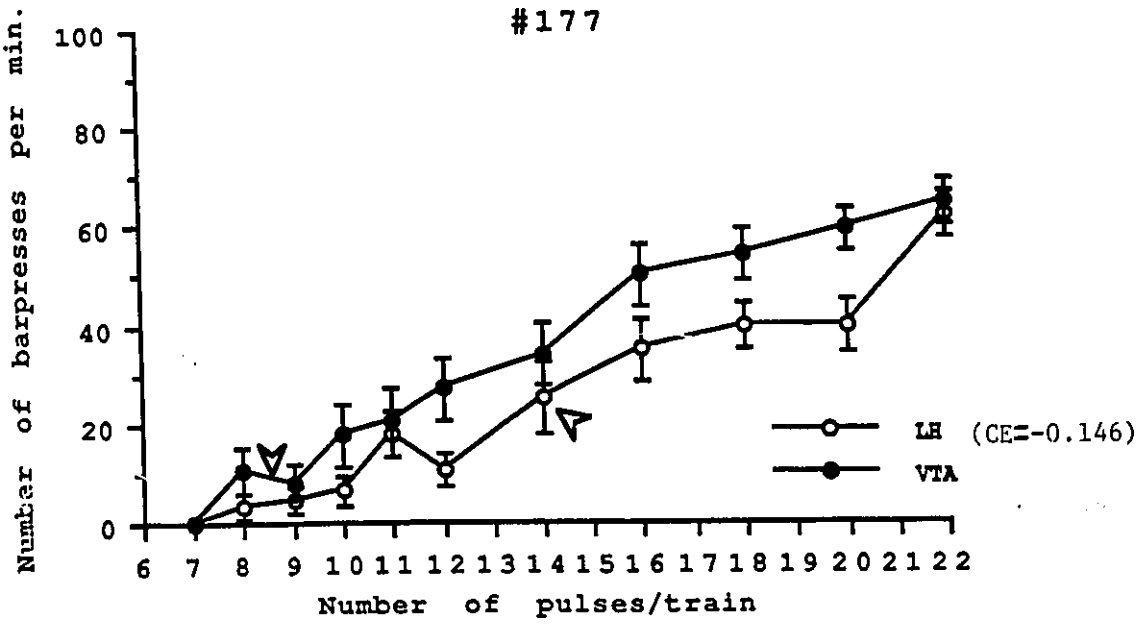

#177
Number of barpresses per min.
100
80
60
40
20
0
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
Number of pulses/train
LH (CE=-0.146)
VTA

Fig. 9

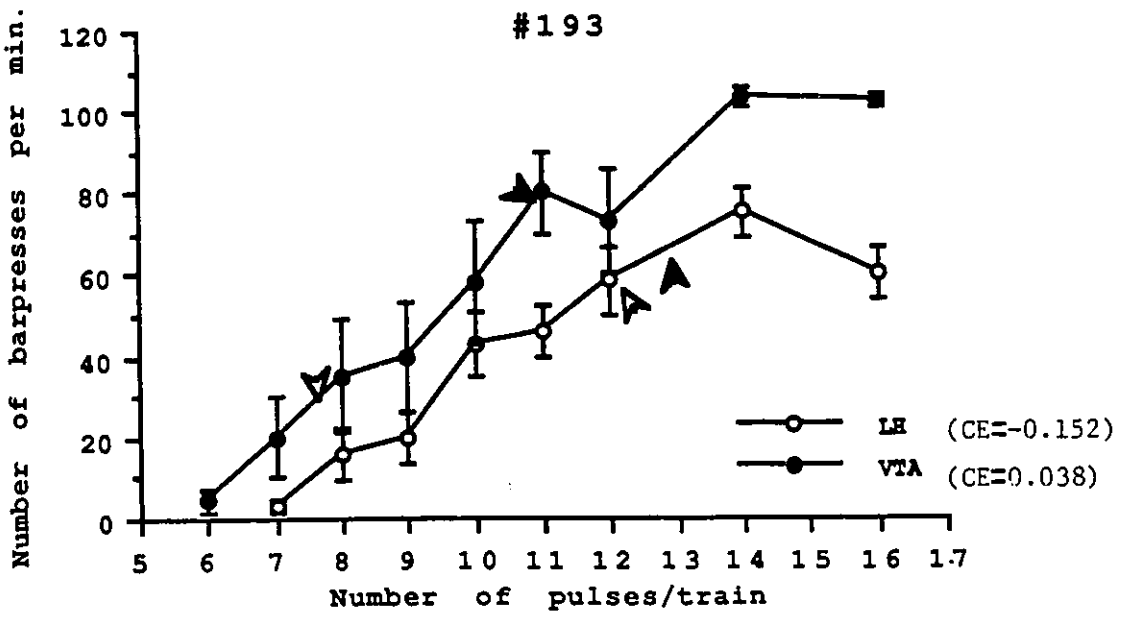

#193
Number of barpresses per min.
120
100
80
60
40
20
0
5 6 7 8 9 10 11 12 13 14 15 16 17
Number of pulses/train
LH (CE=-0.152)
VTA (CE=0.038)

Fig. 10

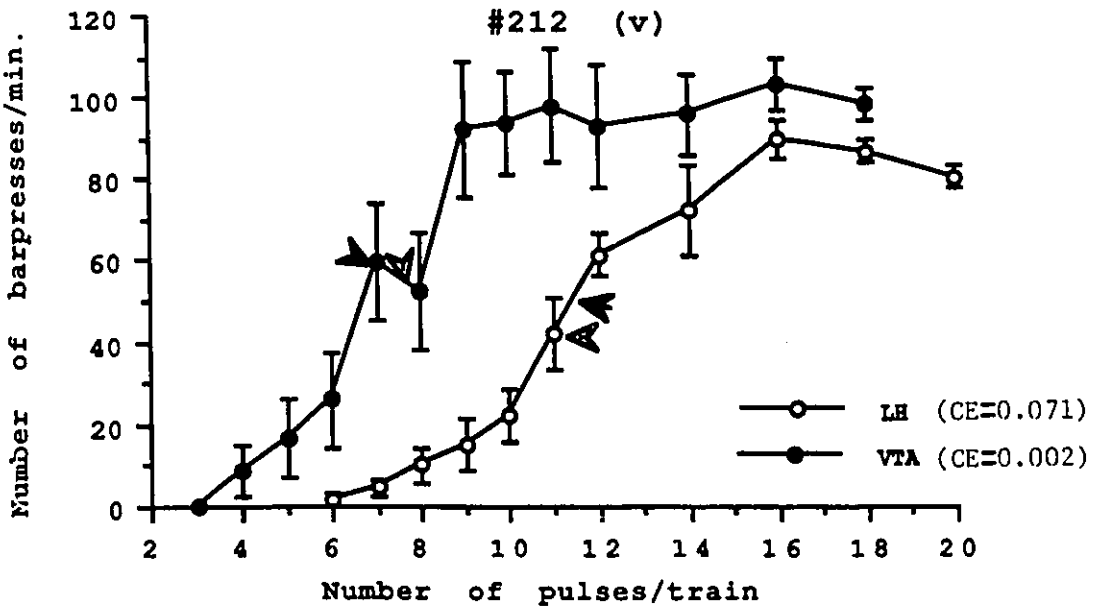

#212 (v)
Number of barpresses/min.
120
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
Number of pulses/train
LH (CE=0.071)
VTA (CE=0.002)

Fig.11

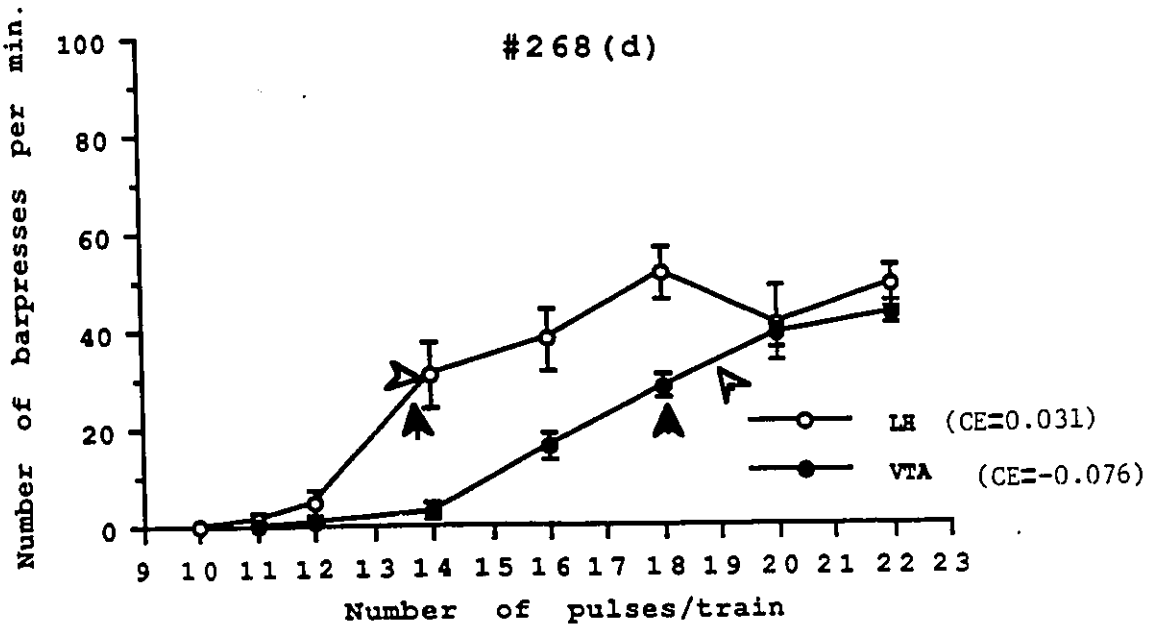

#268(d)
Number of barpresses per min.
100
80
60
40
20
0
9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Number of pulses/train
LH (CE=0.031)
VTA (CE=-0.076)

Fig. 12

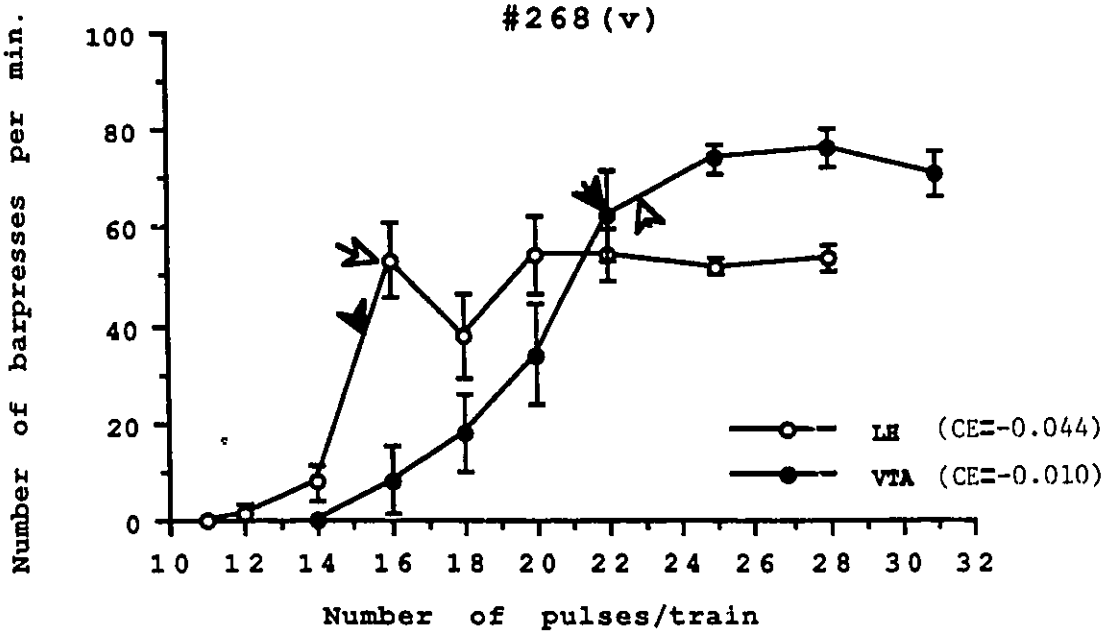

#268(v)
Number of barpresses per min.
100
80
60
40
20
0
10 12 14 16 18 20 22 24 26 28 30 32
Number of pulses/train
LH (CE=-0.044)
VTA (CE=-0.010)

Fig. 13

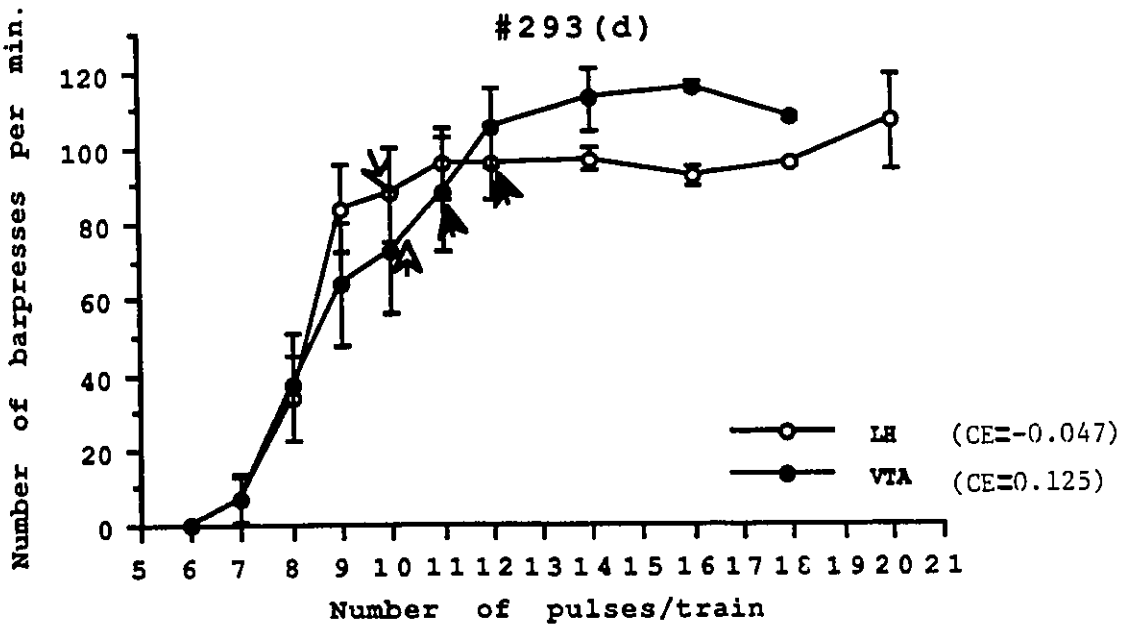

#293(d)
Number of barpresses per min.
120
100
80
60
40
20
0
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Number of pulses/train
LH (CE=-0.047)
VTA (CE=0.125)

Fig. 14

Table 1 gives the LPSE, the LPPE and the LCE for each pair of sites tested. The subject # and the STD (along with its frequency value obtained from the rate-frequency functions depicted in Figures 1-14) are provided in columns 1 and 2, respectively. This table should be used in conjunction with the preference data in Figures 1-14. The table can be read as follows: According to row 1, subject #91 (d) should, on average, distribute his time equally for 45 LH pulses or 1.65 logfrequency units (LSTD) and 1.491 VTA (COMP) logfrequency units (LPPE), if the qualitative and quantitative attributes of these stimuli belong to the same reward system. However, according to the obtained LPSE, the rat actually equally preferred 1.65 LH and 1.312 VTA logfrequency units. The difference between the LPPE and LPSE is 0.180 logfrequency units (LCE). The negative sign simply denotes a preference for the COMP stimulus. A positive sign would have denoted preference for the STD, as seen in row 2.

The mean (X) LCE and s.e.m. are provided at the bottom of column 5. The mean LCE was 0.068 logfrequency units.

Table 1: The logarithmic point of subjective equality (LPSE), point of predicted equality (LPPE) and constant error (LCE) for each pair of sites tested are provided in columns 3, 4 and 5, respectively. The subject # and the STD (along with its frequency value obtained from the rate-frequency functions depicted in Figures 1-14) are provided in columns 1 and 2, respectively. The mean (X) LCE and s.e.m. are provided at the bottom of column 5.

CHAPTER THREE

Table 1

Subject	STD	LPSE	LPPE	LCE
#91 (d)	LH: 45 Sat. pt.	1.312	1.491	-0.180
	VTA: 9 m56	1.114	0.982	0.132
#91 (v)	LH: 14 m68	1.122	1.114	0.008
	VTA: 14 m100	1.183	1.204	-0.021
#125	LH: 14 m57	1.342	1.314	0.029
	VTA: 20 m49	1.240	1.130	0.110
#148	LH: 20 m76	1.259	1.286	-0.026
	VTA: 20 m88	1.312	1.322	-0.010
#151	LH: 16 m75	1.475	1.301	0.174
#159	LH: 14 m66	1.106	1.090	0.016
	VTA: 14 m85	1.186	1.185	0.001
#177	LH: 14 m40	0.929	1.076	-0.146
#193	LH: 12 m78	0.889	1.041	-0.152
	VTA: 11 m78	1.117	1.079	0.038
#212 (d)	LH: 12 m58	0.929	0.845	0.084
	VTA: 7 m58	0.929	1.079	-0.150

SUBJECTIVE EVALUATION OF BRAIN STIMULATION REWARD

#212 (v)	LH: 11 m47	0.894	0.823	0.071
	VTA: 7 m58	1.062	1.061	0.002
#268 (d)	LH: 14 m60	1.279	1.248	0.031
	VTA: 18 m77	1.136	1.212	-0.076
#268 (v)	LH: 16 m98	1.354	1.398	-0.044
	VTA: 22 m82	1.186	1.196	-0.010
#293 (d)	LH:10 m83	1.014	1.061	-0.047
	VTA: 11 m75	1.079	0.954	0.125
#293 (v)	LH: 10 m77	0.929	0.903	0.026
	VTA: 8 m77	0.984	1.000	-0.046
X+/- s.e.m.				0.068+/- .012

Figure 15 plots the LCE between the PPE and PSE for the pair of sites tested. By convention, positive values represent preference for the VTA, and negative values represent preference for the LH. The stippled lines represent the confidence limits. Although these limits could have been defined from the mean LCE and s.e.m. of the control data in Experiment One (0.009+/-0.004), they were not, based on the following grounds: As stated in the Rate-frequency section of the Method, VTA or LH thresholds were considered stable if they did not vary more than 0.05 logfrequency units. This deviation is already greater than the upper confidence limit that would have been obtained from the data in Experiment One. The confidence limits were thus widened to accommodate this allowable deviation.

From this graph, it appears that subjects #91(v), 148, 159, 268(v) and 293(v) fall unequivocally within these limits. In other words, LH and VTA threshold frequencies were equally preferred. Subjects #90, 91(d), 151, 177 and 212(d) fall outside of these lines, indicating a clear preference for one brain site over the other. Subjects #125, 193, 212(v), 268(d) and 293(d) have one point within and one point outside these lines, indicating that subjects neither consistently equally preferred or favoured one stimulus over the other.

In sum, five rats showed equal preference for LH and VTA threshold frequencies, three preferred posterior stimulation and two preferred anterior stimulation. The five remaining subjects had one point within the confidence limits, and one point without.

Figure 15: The logarithmic constant error (LCE)is plotted for each pair of sites tested. The numbers along the X-axis identify the subjects. Positive values represent preference for the ventral tegmental area (VTA), and negative values represent preference for the lateral hypothalamus (LH). The stippled lines represent the confidence limits.

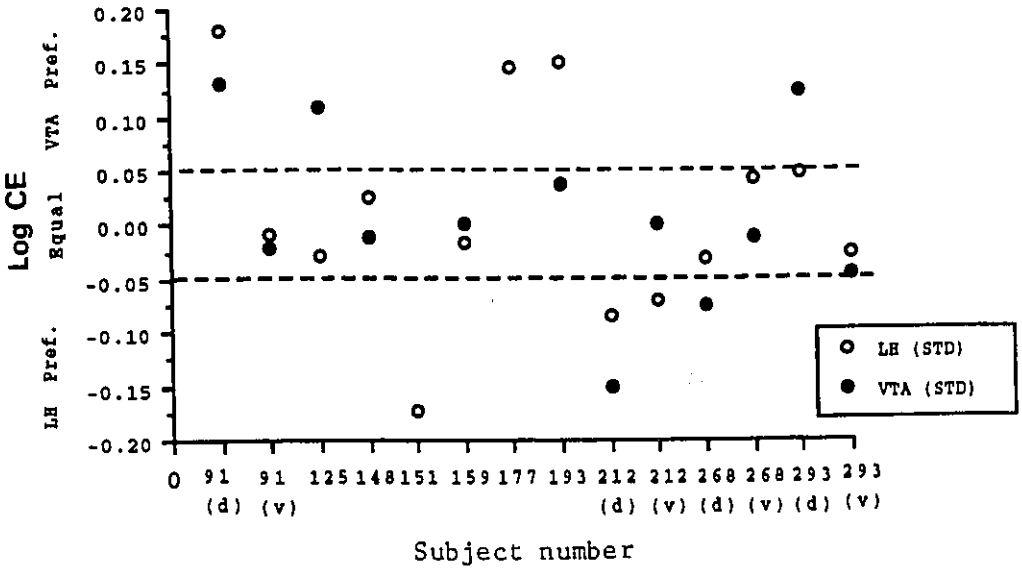

Log CE
VTA Pref.
Equal
LH Pref.
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0
91 (d)
91 (v)
125
148
151
159
177
193
212 (d)
212 (v)
268 (d)
268 (v)
293 (d)
293 (v)
LH (STD)
VTA (STD)
Subject number

Fig. 15

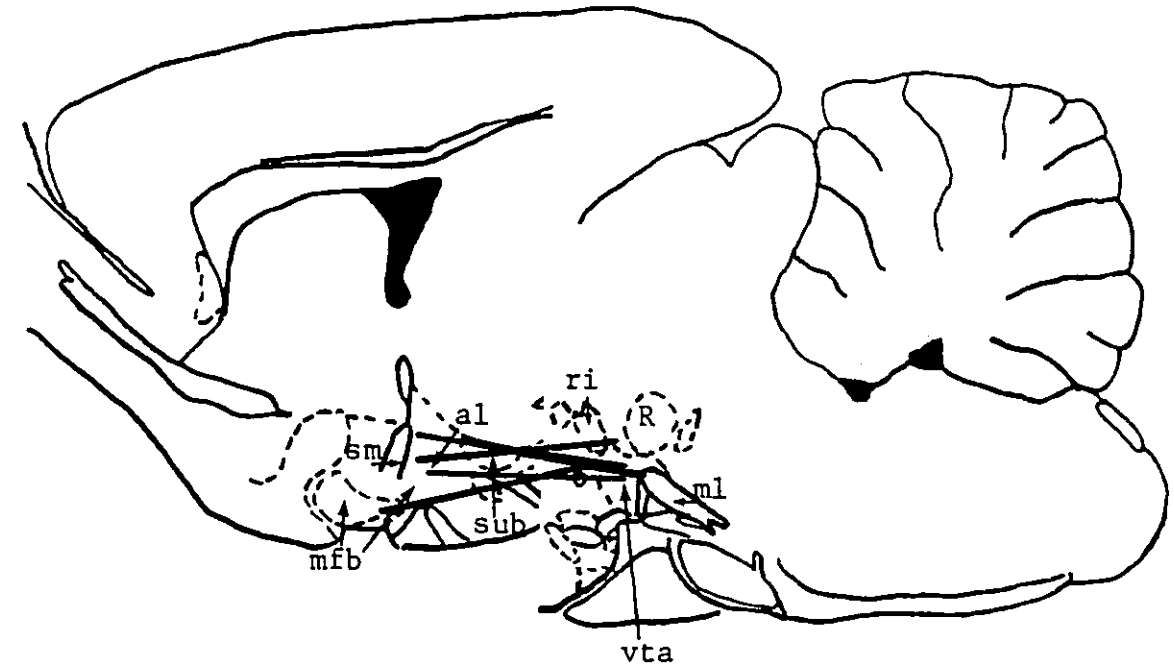
Individualized results II

Please note that subjects #90 or #177 (when the VTA was held STD) were not included in Table 1 or Figure 15, as no upper or lower thresholds were observed in the raw data. In other words, for these subjects, no crossover point was obtained, as the VTA was always preferred, regardless of the value of the LH stimulus. Thus, the raw preference profiles were not X-shaped in these two cases only. For subject #90, this irregular profile may have occurred due to an "indifference" in the LH STD stimulus even when the second lever delivered low COMP frequencies. This "indifference" manifested itself in a short amount of time spent barpressing (maximum 40%) for the LH stimulus across all COMP frequencies. However, the amount spent barpressing was in fact consistent with the threshold, which corresponded to a frequency eliciting 33% of the maximum barpressing rate in the single-lever condition. On the other hand, "indifference" was not noted when the VTA was held STD, as this subject barpressed close to 100% of the time for either stimulus across all COMP frequencies. Still, no upper threshold could be calculated with any of the raw curves. Even if the LCE was calculated with the point of convergence closest to the PPE (log of 8 pulses - log of 6 pulses = 0.125 logfrequency units) when the LH was the STD; and log of 12.6 pulses - log of 18 pulses = -0.155 logfrequency units) when the VTA was the STD, it would still fall outside the confidence limits. Consequently, this rat was classified as preferring the VTA threshold over that of the LH. The same line of reasoning was applied to #177 (when the VTA was held STD), who was also classified as preferring the VTA.

Subjects #91(v) and 148 (when the VTA was held STD) also did not spend the full 5 minutes barpressing for either stimulus (at low COMP frequencies), even though the STD frequencies elicited high proportions of the maximum self-stimulation rate in the single-lever condition. Nevertheless, upper and lower thresholds could be calculated, even though the X-shaped profile was not as pronounced as the other subjects. Consequently, after determining the LCE for these subjects, they were classified as equally preferring both the LH and VTA thresholds.

Figure 16 shows two LH and VTA sagittal sections (1.9 and 0.9 mm lateral to the sagittal suture, respectively) fused for illustration purposes. The solid lines on the top graph join LH and VTA sites where threshold frequencies were equally preferred. Generally, these electrodes fell within the LH or VTA, or along their border. The stippled lines on the bottom graph join anterior and posterior sites where thresholds were not equally preferred. Here, three of five anterior electrodes fell within the entopeduncular nucleus/internal capsule boundaries, and as many posterior electrodes fell within the boundaries of the prerubral/red nucleus field. See the histology in Chapter Four for more details.

Figure 16: LH and VTA sagittal sections (1.9 and 0.9 mm lateral to the sagittal suture, respectively) have been fused together for illustration purposes. Solid lines join LH and VTA sites where threshold frequencies were equally preferred. Stippled lines join anterior and posterior sites where thresholds were not equally preferred. al: ansa leticularis; mfb: medial forebrain bundle; ml: medial lemniscus; R: red nucleus; ri: rostral interstitial nucleus; sm: stria medullaris; subI: subincertal nucleus.



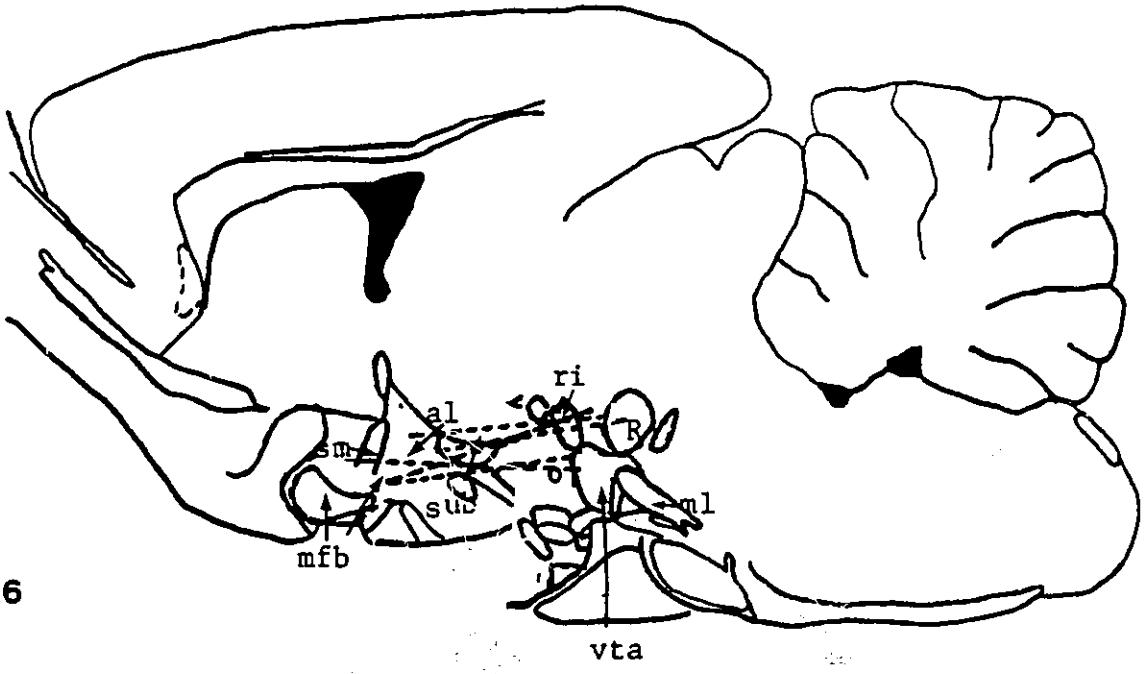


Fig.16

Discussion

In 1990, Malette and Miliaressis showed that equipotent (threshold) frequencies obtained from various experimental manipulations, but delivered to the same reward site, were equally rewarding. In our study, LH and VTA threshold frequencies were equally preferred, and thus equally rewarding, at five pairs of sites. These cases are consistent with Wise and Bozarth's (1984) hypothesis that the LH and VTA are part of a common reward circuit connected in series. However, we cannot exclude the possibility that these rats may have assigned an equivalent weight to equipotent frequencies delivered to independent reward systems. Regardless, histological verification confirms that the electrodes were indeed in the LH or VTA, or along their border.

In contrast, equal preference was not observed between five other pairs of sites. In three cases, the posterior site was preferred, whereas in two cases, the anterior site was preferred. In these cases, the anterior electrodes fell within the internal capsule region, whereas the posterior electrodes fell within the prerubral/red nucleus field. These results support the notion of multiple systems of reward within the medial forebrain bundle.

In sum, whether or not equal preference is observed along the medial forebrain bundle appears to be site dependent. The fact that a slight lowering of #91, 212, 268 and 293's electrode shifted the rats' preference profile supports this argument. These data are consistent with Leon and Gallistel's (1992) hypothesis that different groups of reward-relevant axons course through the medial forebrain bundle. However, we cannot exclude the possibility that reward-irrelevant variables, such as site-dependent motoric movements, dizziness or kindling (to name but a few), contributed the observed lack of equal preference.

As mentioned earlier, the failure to observe equal preference between equipotent frequencies at some sites can be expected if two rewarding neural signals that belong to different biological functions are differentially ranked on the animal's decisional scale. Alternatively, rats may be "programmed" to choose between two sources of identical rewards as a survival mechanism. If this were the case however, there is no strong reason to expect that rats would

systematically prefer one site over the other, as observed between some pairs of sites. Secondly, the fact that equal preference was obtained in some subjects argues against the alternative hypothesis.

The precise organization of the reward systems revealed here may help to explain why lesion experiments focusing on the anterior and posterior medial forebrain bundle have been plagued by such contradictory findings. Specifically, our experiment suggests that slight electrode displacements may penetrate a new reward system. Thus, anatomical imprecision could dramatically affect lesion results.

Finally, the results of this experiment suggest that brain reward circuitry is less redundant than lesion experiments previously led us to believe. On an evolutionary scale, having independent reward systems is adaptive, as this implies that some reward-related functions could be spared in the event of a focal lesion.

We cannot exclude the possibility that the activation of one reward site in the twin-lever box decreased the threshold of an otherwise identical second reward, thus resulting in the observed lack of equal preference. However, the presence of a sensitization effect has not yet been evidenced in rewarding medial forebrain bundle stimulation.

Alternatively, animals may prefer a "pure" reward sensation, over one mixed with aversion, despite the fact that the two signals are pursued with the same vigour in the single-lever paradigm. Aversive reactions are sometimes observed in self-stimulating rats. This possibility is discussed in Chapter Five.

Finally, the hint of anatomical specificity discussed earlier suggests that rats' preference may depend on the proportion of common neurons activated by both electrodes. The possibility that the electrodes of those rats showing equal preference were stimulating a common axonal bundle is explored in the next chapter.

Chapter Four

THE INFLUENCE OF ANATOMICAL LINKAGE ON SELF-STIMULATION PREFERENCE WITHIN THE MEDIAL FOREBRAIN BUNDLE

EXPERIMENT THREE

Introduction

Chapter three revealed that self-stimulating rats equally preferred anterior and posterior medial forebrain bundle threshold frequencies when the electrodes were placed within, or on the border of, the LH and VTA. This finding is not surprising given that collision tests have shown that the LH and VTA share a good proportion of rewarding fibres (Shizgal, Bielajew, Corbett, Skelton & Yeomans, 1980; Bielajew & Shizgal, 1982; Durivage & Miliaressis, 1987; Murray & Shizgal, 1994). The collision test rests on the neural property that when an axon is electrically stimulated via an electrode, an antidromic action potential is released in the direction of the soma, and an orthodromic action potential is simultaneously released toward the synapse. In 1913, Lucas showed that when two electrodes stimulate a nerve simultaneously or at short intervals, the orthodromic action potential triggered by the electrode closest to the soma collides with the antidromic action potential of the electrode furthest from the soma. Thus, neither of these action potentials conducts past the point of collision. By convention, the first pulse administered is referred to as the conditioning pulse (C pulse) and the second is referred to as the test pulse (T pulse). When the C-T interval is increased beyond the sum of the interelectrode conduction time and the refractory period at the site where the T pulse is delivered (collision interval), collision no longer occurs and impulses from both electrodes conduct to the synapse.

In the behavioral version of the collision test, two electrodes are implanted in the brain to determine if they are stimulating a common axonal bundle. Rats are first trained to self-stimulate, and threshold frequencies are determined for both sites. The single pair of C and T pulses are replaced with trains of such pairs. The C pulses are administered to one site, whereas the T pulses are administered to the second site. The C-T interval is varied, and the threshold frequencies for the paired pulse condition are compared to those of the single pulse condition. A collision effect is characterized by an increase in the paired pulse threshold at short C-T intervals, followed by a

progressive decrease in the threshold at longer C-T intervals. The magnitude of threshold increase at short C-T intervals reflects the proportion of fibres stimulated by both electrodes.

The failure to observe a collision effect does not necessarily imply that the two brain structures share no axons, as the electrodes may simply be misaligned. If misalignment is suspected, a sufficient increase in current, and hence an increase in stimulation area, or a slight move of one electrode, should result in a collision effect.

A third possible profile exists if two different axonal bundles ultimately converge at some further common point. In this situation, no collision effect is observed, but efficient temporal summation (or substantial decrease in the frequency threshold) occurs across all C-T intervals.1 For example, a high degree of summation was noted between the LH and the periaqueductal gray (Bielajew, Jordan, Fermé-Enright & Shizgal, 1981). In contrast, a low degree of summation across all C-T intervals was observed between the LH and the medial prefrontal cortex (Schenk & Shizgal, 1982; Shizgal & Murray, 1987) and between the LH and the amygdala (Kane, Coulombe & Miliaressis, 1991).

The collision test was conducted between all 15 pairs of sites tested in Chapter Three. We predicted post priori that if the anterior and posterior electrodes were stimulating a common axonal bundle in Experiment Two, (as evidenced by a perfect collision effect), then LH and VTA threshold frequencies should be equally preferred unless site-dependent reward-irrelevant variables induced a preference for one site. Note however, that no prediction could be made if collision was

---

The phenomenon of temporal summation is thought to be due to the release of neural transmitter at synapses more rapidly than it can be disposed of, yielding a summation of excitatory postsynaptic potentials toward firing threshold. Summation may be homosynaptic, characterized by impulses summating upon a postsynaptic neuron by repeated firing of the same presynaptic neuron, or heterosynaptic, characterized by summation upon the postsynaptic neuron from separate presynaptic neurons firing within a brief period of time. Unlike the former, the latter is not constrained by refractory period limitations given that the separate synapses which converge on the postsynaptic neuron are not activated by same fibres (Ungerleider & Coons, 1970).

not observed, as the electrodes may simply have been misaligned within the same functional system.

Materials and Method

Subjects

A collision test was performed at each pair of sites tested in Experiment Two.

Apparatus and stimulation parameters

All collision tests were performed in the single-lever operant chamber. Stimulation parameters were identical to those used in Experiment Two. Note however, that due to a slight threshold change following the preference test, the LH and VTA current intensities for #90 and #159 respectively, were lowered so that the threshold frequencies were as close as possible on the X-axis.

The collision test

At the beginning, middle and end of each collision test, the threshold frequency was determined for each electrode (single pulse condition). The threshold, which was defined as that frequency which elicited 50% of the maximal self-stimulation rate, was interpolated from the rate-frequency curves gathered in Experiment Two. These single pulse threshold frequencies were required for future calculation of paired pulse effectiveness. Before each self-stimulation trial, rats were primed a maximum of 10 times using the parameters destined for that trial. These thresholds were considered stable if they did not vary by more than 0.1 logfrequency units (instead of 0.05 logfrequency units), as this test is much longer than those presented in Chapters Two and Three, and rats are more subjected to fatigue effects. If this stability criterion was not met, the data was discarded and the test was attempted the next day.

Paired pulse threshold frequencies were determined by administering a C pulse to the LH followed by a T pulse to the VTA (LH-VTA condition). C-T intervals ranging from 0.2 - 5 msec

were tested. Double-frequency measures were also obtained at the beginning, middle and end of each test by setting the T pulse midway between two successive C pulses. The C-T order was then counterbalanced (VTA-LH condition). Each test was replicated 3-5 times (except for subject #148, who lost his electrode assembly before replications could be made). For each C-T interval, the threshold frequency was also interpolated as above for the calculation of paired pulse effectiveness.

Data analysis

In order to determine the overall paired-pulse effectiveness at any given C-T interval, Shizgal and colleagues' (1980) formula was employed:

$$E = (FSP1/FC\text{-}T - 1) \cdot FSP1/FSP2$$

where E is the paired pulse effectiveness, FSP1 and FSP2 are the frequency thresholds for the electrode showing the lower and higher threshold, respectively, and FC-T is the frequency threshold for the paired pulses corresponding to a given C-T interval. This formula yields a value ranging from the least paired pulse effectiveness score of 0 to the most effective score of 1. To determine if there was a significant rise in paired pulse effectiveness, the difference between the mean of the shorter (less than 0.7 msec) and the higher (greater than 1.5 msec) C-T intervals was calculated using the correlated groups t-test.

Histology

The animals were given a lethal dose of pentobarbital. An electrolytic lesion was formed at the tip of each electrode using a 6V battery. Rats were then perfused intracardially with a 0.9% saline solution followed by a 10% formalin solution which contained 3 g of potassium ferrocyanide, 3 g of potassium ferricyanide and 0.5 g of trichloracetic acid. This solution marked

in blue the lesioned site. For rats that lost their electrode assembly, an intracardial perfusion was performed using only 10% formalin. Each brain was removed and stored in 10% formalin for at least 72 hours. Following fixation at -15° C, 30 mm sections of brain tissue were sliced on a cryostat-microtome. Slices were then placed on gelatin-coated histological slides and stained using thionine. Slices were subsequently examined under a microscope to localize electrode traces using the atlas of Paxinos and Watson (1986).

Results

Figure 1 shows the location of self-stimulation sites on plates borrowed from Paxinos and Watson (1986). Filled circles represent the electrode tips. The number pointing to each filled circle identifies the subject. The number on each individual plate refers to the millimetric distance behind bregma. Note for subjects #91, 212, 268 and 293, two pairs of sites were tested. The letters "d" and "v" in brackets beside these subject numbers signify dorsal and ventral, respectively.

The anterior stimulation sites were located 1.3 to 2.56 mm behind bregma, 12 in the LH or on its border, 1 in the internal capsule, 1 in the substantia innominata. The posterior stimulation sites were located 4.8 to 6.04 mm behind bregma, 7 in the VTA or on its border, 2 in the prerubral field, 2 in the red nucleus and 1 in the medial lemniscus.

Figure 1: The location of each electrode tip is depicted by a filled circle on the corresponding plate of Paxinos and Watson's (1986) stereotaxic atlas. The number pointing to each filled circle identifies the subject. The number on each individual plate indicates the millimetric distance behind bregma. Note that for subject #91, #212, #268 and #293, a second pair of sites was tested. bn: bed nucleus of the stria medullaris; ep: entopeduncular nucleus; ic: internal capsule; if: interfascicular nucleus; ipr: rostral interpeduncular nucleus; lh: lateral hypothalamus; ml: medial lemniscus; mm: medial mammillary nucleus; pn: paranigral nucleus; pr: prerubral field; rpc: red nucleus, parvocellular; si: substantia innominata; sm: nucleus of the stria medullaris; sx: supramammillary decussation; vta: ventral tegmental area.

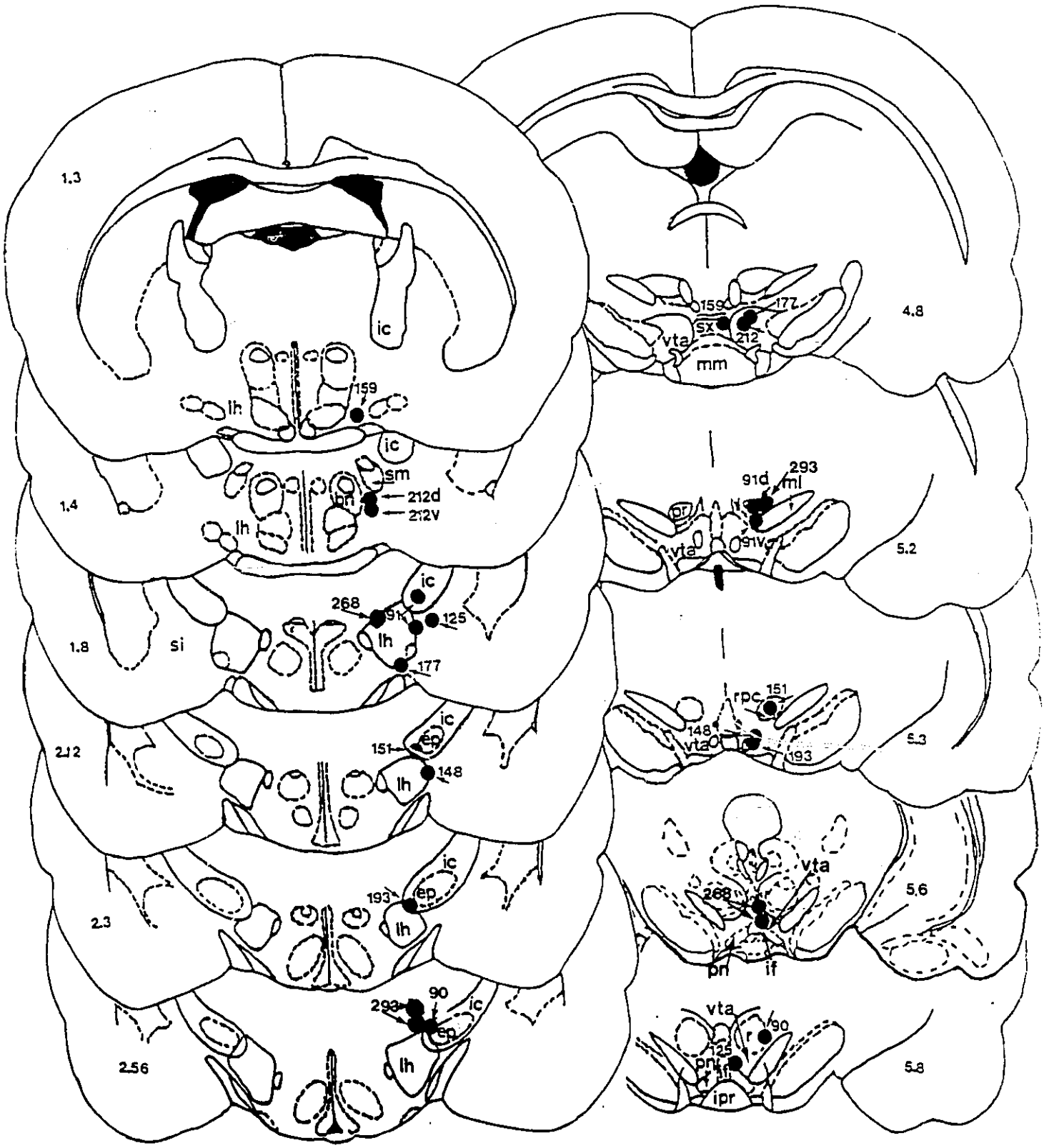

1.3
ic
159
lh
ic
sm
1.4
ph
212d
212v
lh
ic
268
91
125
1.8
si
lh
177
ic
ep
151
2.12
148
lh
ic
193
ep
2.3
lh
90
293
ic
ep
2.56
lh
159
177
sx
212
4.8
vta
mm
293
91d
ml
pr
vta
91v
5.2
rpc
151
148
vta
193
5.3
vta
268
5.6
pn
if
vta
90
125
pn
if
ipr
5.8

Fig.1

Figures 2-16 show the effectiveness of paired pulses delivered through ipsilateral anterior and posterior electrodes, as a function of the C-T interval. The number at the top of each graph identifies the subject. Please note that the collision test was performed at two pairs of sites for subjects #91, 212, 268, and 293. Open circles denote that the C and T pulses were delivered to the anterior and posterior sites, respectively; whereas the reverse is true for closed circles. A paired pulse effectiveness of 0 indicates that the combined anterior and posterior stimulation was not better than single stimulation. A value of 1.0 indicates that the rewarding effects of the two pulses summated perfectly.

Figures 2-16: The effectiveness of paired pulses delivered through ipsilateral anterior and posterior electrodes are plotted as a function of the C-T interval for all subjects. The number at the top of each graph identifies the subject. Open circles denote that the C and T pulses were delivered to the anterior and posterior sites, respectively. Closed circles show the reverse order. Please note that the collision test was performed at two pairs of sites for subject #91, 212, 268, and 293. Vertical bars denote the standard errors of the mean.

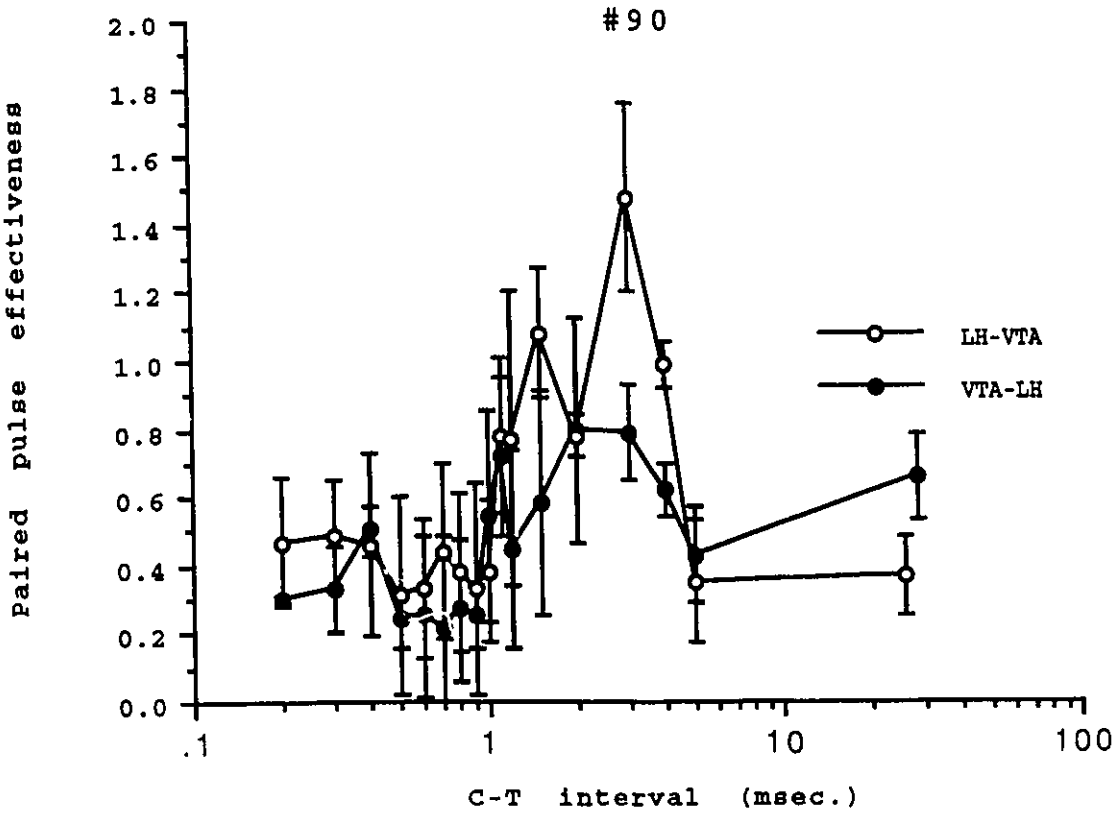

#90
Paired pulse effectiveness
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 2

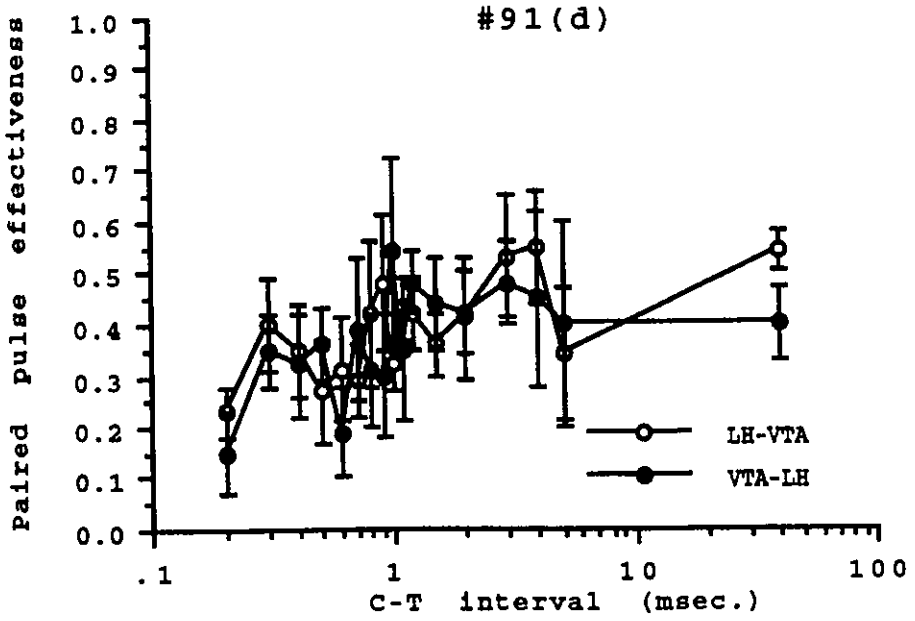

#91(d)
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 3

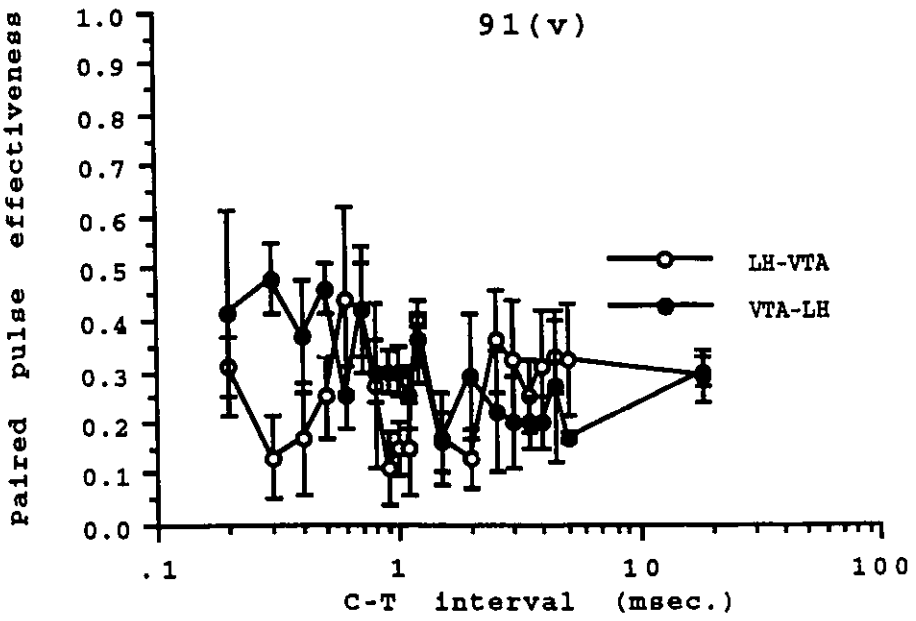

91(v)
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 4

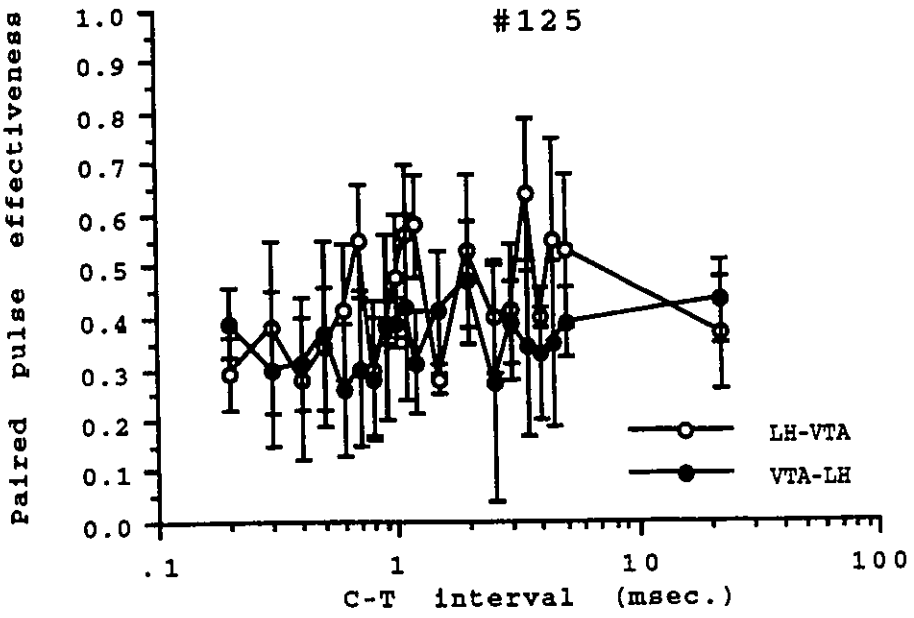

#125
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 5

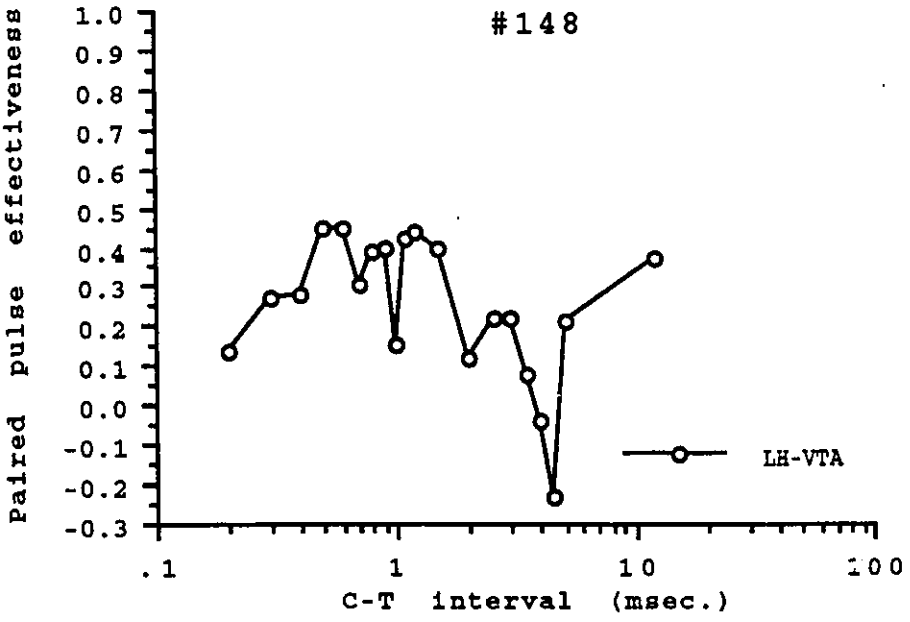

#148
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
.1
1
10
100
C-T interval (msec.)
LH-VTA

Fig. 6

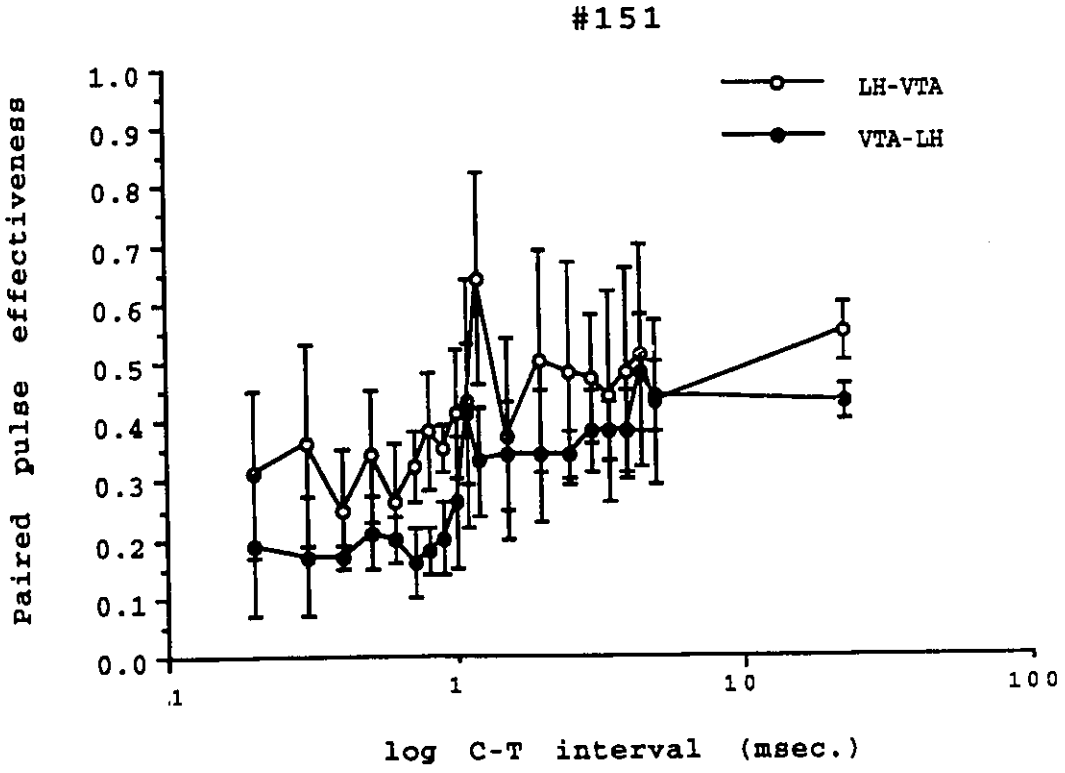

#151
LH-VTA
VTA-LH
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
log C-T interval (msec.)

Fig. 7

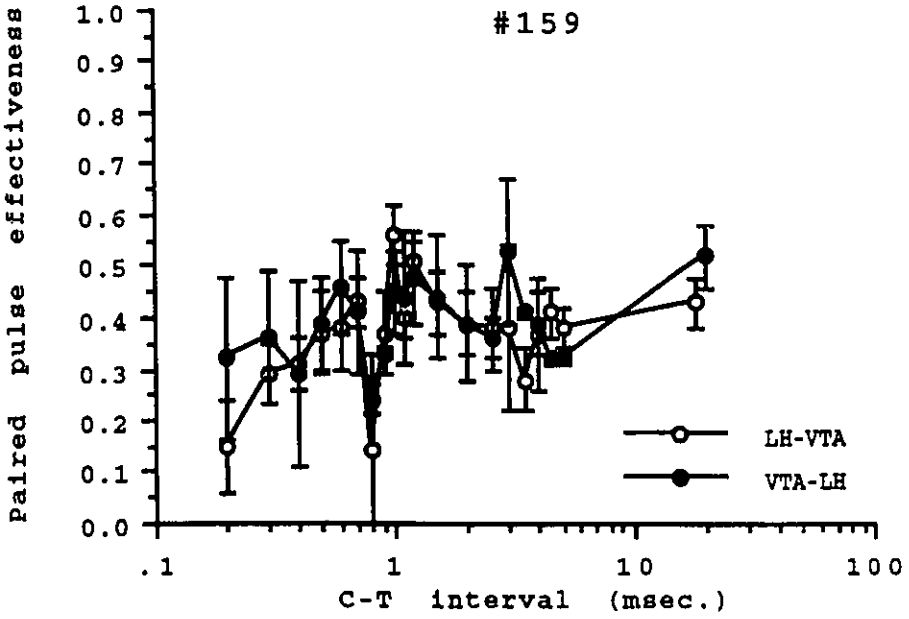

#159
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 8

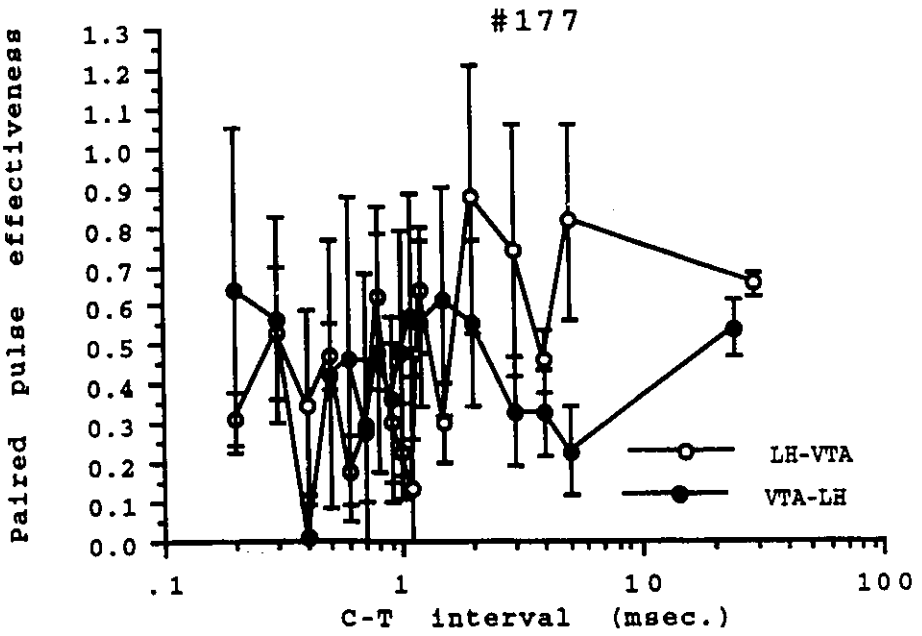

#177
Paired pulse effectiveness
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 9

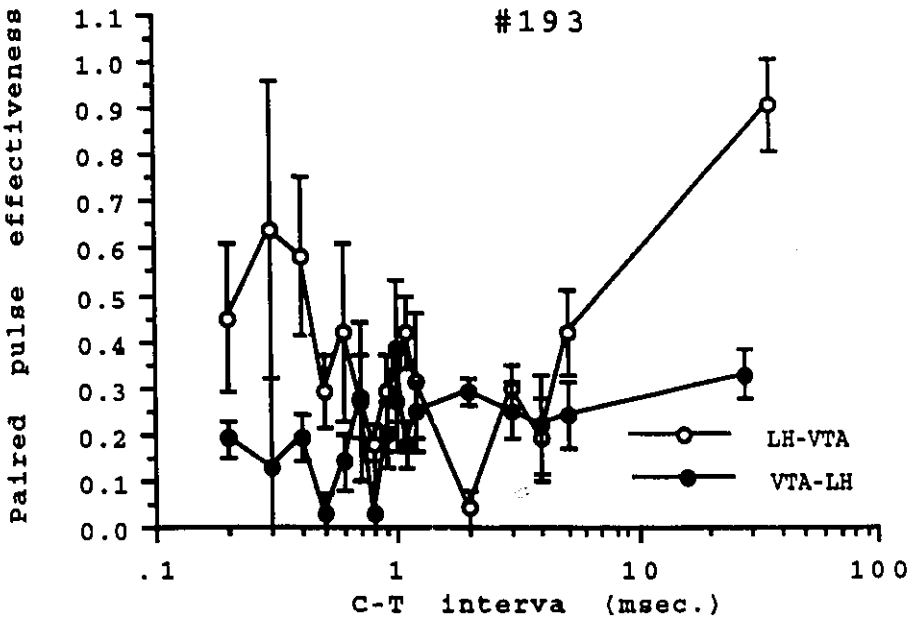

#193
Paired pulse effectiveness
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
C-T interva (msec.)
LH-VTA
VTA-LH

Fig. 10

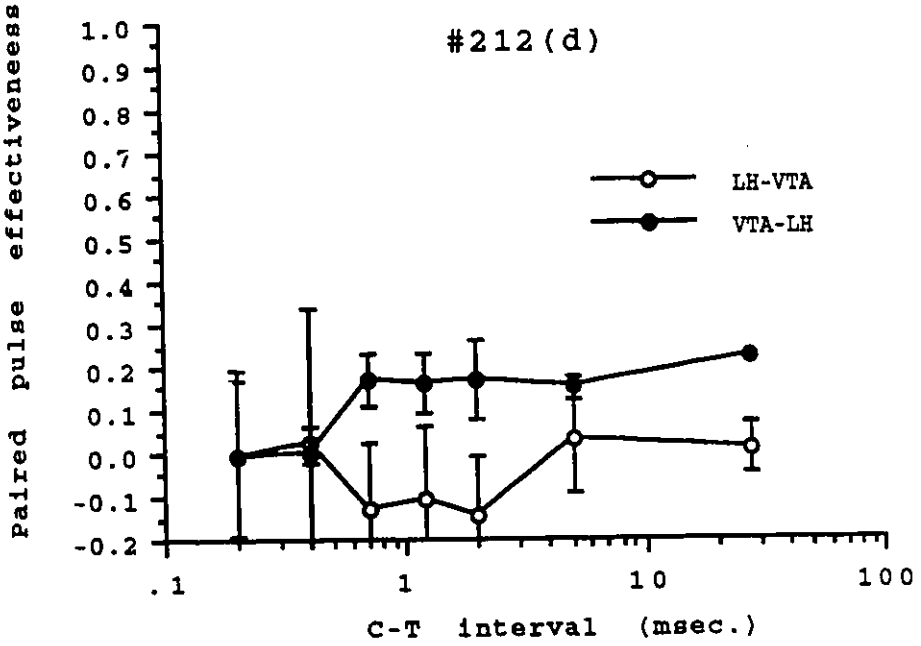

#212(d)
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
LH-VTA
VTA-LH
.1
1
10
100
C-T interval (msec.)

Fig. 11

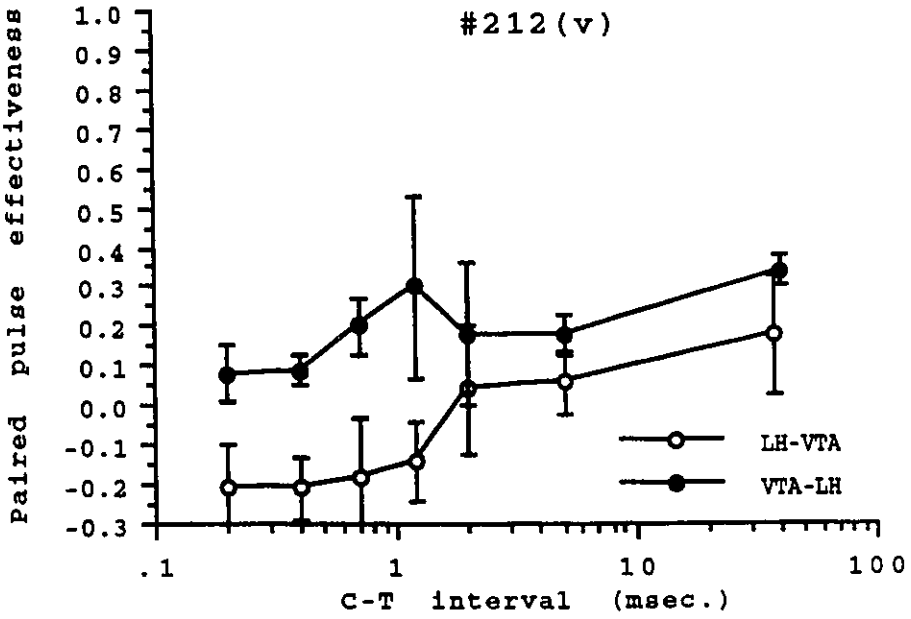

#212(v)
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 12

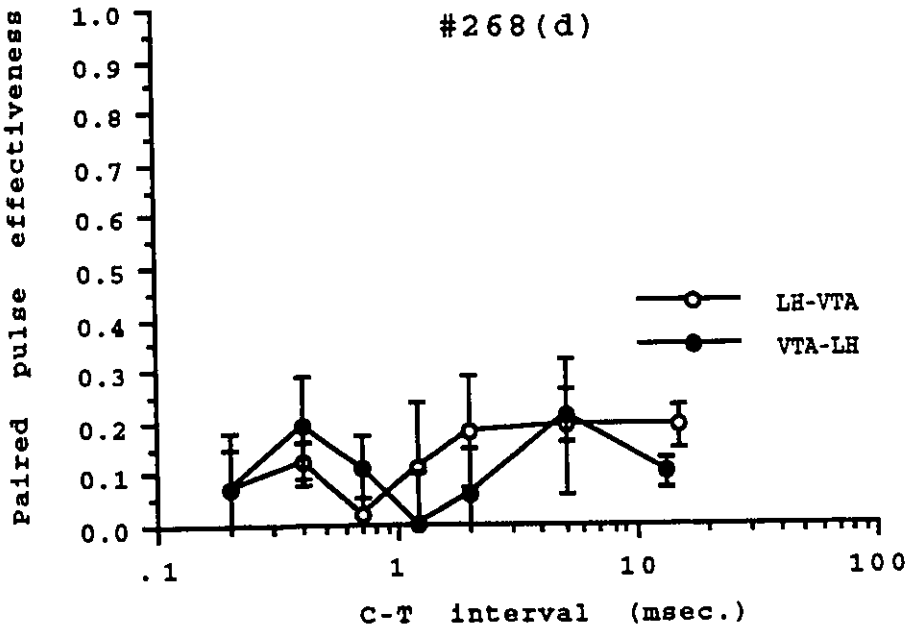

#268(d)
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 13

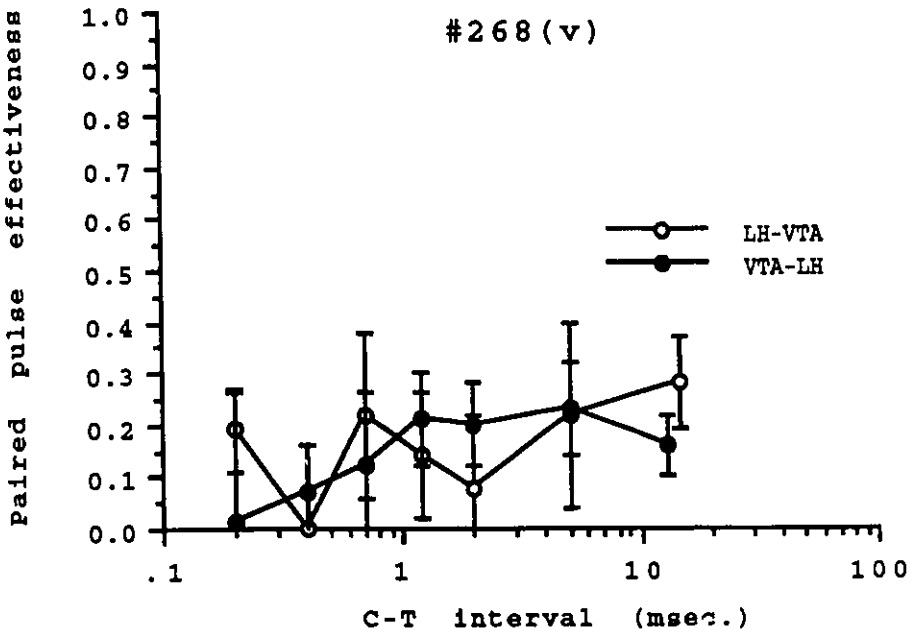

#268(v)
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
LH-VTA
VTA-LH
.1
1
10
100
C-T interval (msec.)

Fig. 14

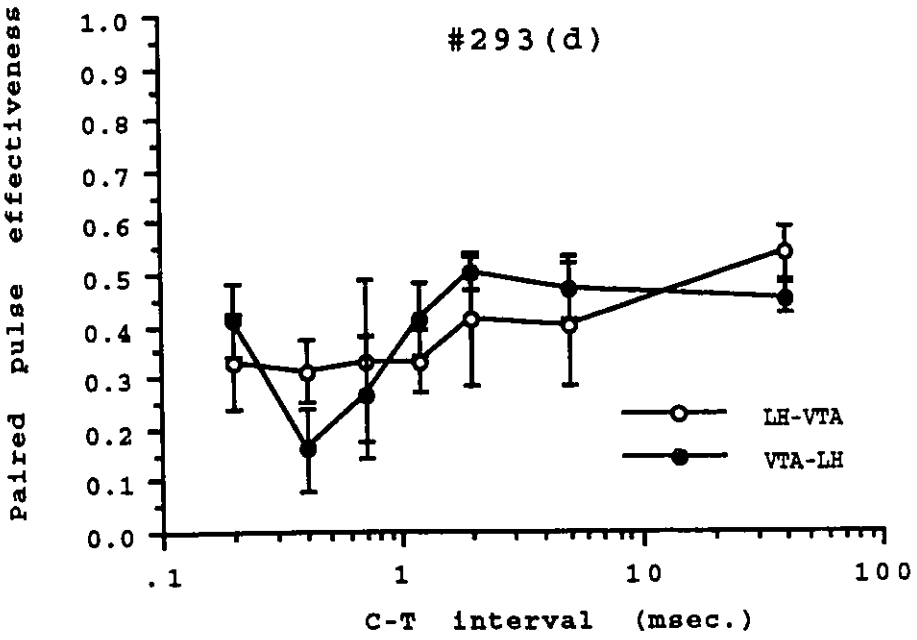

#293(d)
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 15

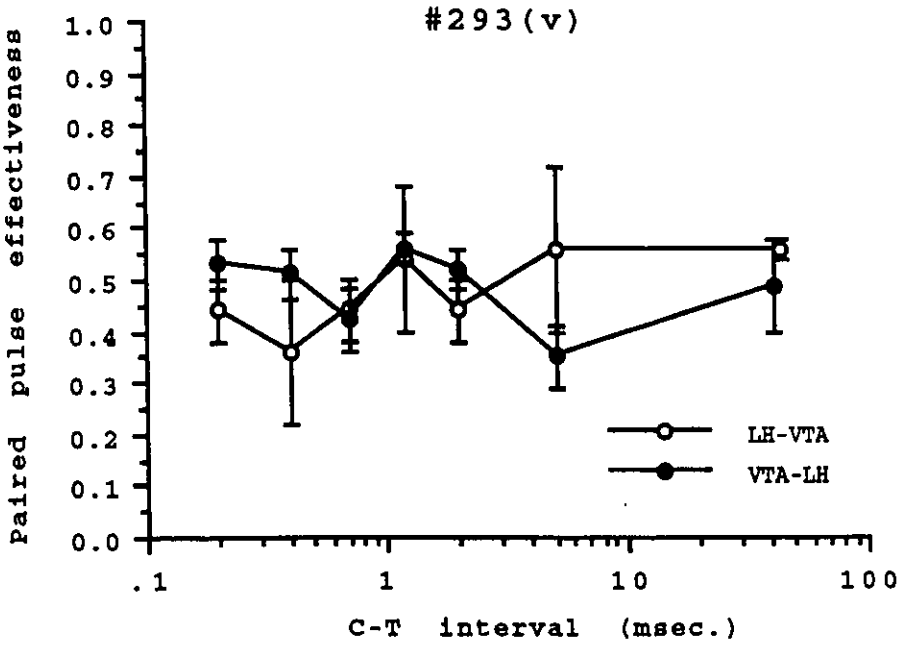

#293(v)
Paired pulse effectiveness
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
.1
1
10
100
C-T interval (msec.)
LH-VTA
VTA-LH

Fig. 16

A collision effect, indicating that the two electrodes excited common axons, is typically characterized by a lower plateau of paired pulse effectiveness at short C-T intervals, a dynamic segment lasting the duration of the refractory periods at intermediate C-T intervals, and an upper plateau at longer C-T intervals. Such a clear profile was not obtained between any of the pairs of sites tested. Due to the variability of the data, collision curves were not fitted.

To determine if there was a significant rise in paired pulse effectiveness, the difference between the mean of the shorter (less than 0.7 msec) and the higher (greater than 1.5 msec) C-T intervals was calculated using the correlated groups t-test. In the cases where both the shorter and longer means did not differ significantly between the LH-VTA and VTA-LH condition [#90, 91(d), 91(v), 125, 159, 212(d), 268(d), 268(v), 293(d), 293(v)], the data were pooled for the t-test.

A significant increase in paired pulse effectiveness was obtained for subjects #90, 91(d), 125, 151, 159, 212(v) and 293(d; $p < .05$). In subjects #177 and 193, a significant increase in paired pulse effectiveness was obtained only in the LH-VTA and VTA-LH condition, respectively.

In the remaining subjects, no significant rise in paired pulse effectiveness was obtained. The degree of summation in these subjects was obtained by calculating the mean paired pulse effectiveness across all C-T intervals. Moderate summation (ranging from 0.25 to 0.48 paired pulse effectiveness) was obtained in subjects #91(v), 148 and 293(v). These results can be contrasted with the very small amount of summation (ranging from -0.04 to 0.16) observed in subjects #212(d) and 268(d) and 268(v).

Discussion

A robust collision effect, characterized by a lower plateau at short C-T intervals, a dynamic segment lasting the duration of the refractory periods at intermediate C-T intervals and an upper plateau at longer C-T intervals, was not obtained between any of the pairs of sites tested. Thus, the collision test failed to reveal the presence of substantial anatomical linkage between any of the

anterior and posterior medial forebrain sites. Thus whether equal preference is observed when both electrodes are stimulating identical reward axons remains undetermined.

When perfect collision is not obtained, no convention exits on how to analyze collision data. We chose to use a correlated groups t-test to determine if there was a significant difference between the paired pulse effectiveness means at shorter and longer C-T intervals. With this method, a significant rise in paired pulse effectiveness was obtained in seven subjects. This small (albeit significant) collision effect indicated that the anterior and posterior electrodes were likely stimulating a small portion of common fibres.

In two subjects, a significant increase in paired pulse effectiveness was obtained in either the LH-VTA or the VTA-LH condition. Such an asymmetry may indicate that a synapse exists between both electrodes. Unfortunately, given the variability of the data, we cannot ascertain if a genuine asymmetry exists. The paired pulses of three subjects summated moderately across all C-T intervals. This effect suggests that a moderate proportion of the rewarding signals have converged at some common point. On the other hand, minimal paired pulse effectiveness was obtained for the remaining three subjects, indicating that the rewarding signals from the two electrodes failed to summate.

Shizgal and colleagues (1980), Bielajew and Shizgal (1982), Durivage and Miliaressis (1987) and Murray and Shizgal (1994) were more successful in obtaining strong collision between the LH and VTA. The discrepancy with our results might be due to the use of smaller electrode assemblies (rather than larger moveable electrodes), permitting closer proximity on the anterior-posterior plane in the other studies. Moreover, in the earlier studies, researchers prescreened for a collision effect. For example, if a 20% difference in paired pulse effectiveness was not obtained between very short and very long C-T intervals in Shizgal and colleagues' (1980) study, the current intensity was increased in order to maximize the chances of finding a collision effect. In our study, we chose to keep the intensities the same as in Experiment Two to avoid confound.

In sum, a strong and clear collision effect was not evidenced in any of the subjects. The small number of replications and resulting variable data are unlikely to account for the absence of robust collision. Therefore, whether LH and VTA threshold frequencies activating a large proportion of common rewarding fibres are equally preferred remains undetermined. Given the slight collision effects observed between some pairs of sites tested, no prediction may be made about collision magnitude and preference data, as the electrodes may simply have been misaligned within a single, functionally homogenous reward system. A better approach would have been to perform the collision test first and test for preference in two groups of subjects differing greatly in collision magnitude. Nevertheless, no correlation existed between small collision effects or moderate summation and preference behavior. In other words, rats showing a significant rise in paired pulse effectiveness were no more likely to equally prefer LH and VTA threshold frequencies than the rest.

In conclusion, a significant rise in paired pulse effectiveness from shorter to longer C-T intervals does not appear to predict preference behavior. Alternatively, factors other than anatomical linkage may play a role in rats' preference. The influence of aversive effects is explored in Chapter Five.

Chapter Five

THE BI-DIRECTIONAL INTERACTION BETWEEN REWARDING AND AVERSIVE STIMULATION: INFLUENCE ON PREFERENCE BEHAVIOUR

Rich the treasure,
Sweet the pleasure,
Sweet is pleasure after pain.

John Dryden

EXPERIMENT FOUR

Introduction

Chapter Four revealed no correlation between small collision effects and preference data. In the present chapter, we investigated the possibility that a reward-irrelevant factor at one site contributed to the observed lack of equal preference in an otherwise single system of reward. One variable known to affect self-stimulation is motoric reactions. Such reactions were occasionally noted in some of the animals tested in Experiment Two, especially at higher frequencies. However, in 1987, Miliaressis and Rompré found that the presence of motor reactions (elicited by concomitant stimulation of the reticular formation, a region dense in motor fibres) only affected performance, and not raphé stimulation reward efficacy. In other words, raphé self-stimulation rates decreased but thresholds remained unchanged. Furthermore, Malette and Miliaressis (1990) showed that two LH threshold frequencies, obtained by decreasing current from a high value to a low one, were equally preferred, even though the lower current (but higher frequency) elicited more pronounced motoric reactions. In brief, motoric reactions do not appear to affect brain stimulation reward efficacy or preference.

A second variable known sometimes to co-exist with reward is aversion. For example, Cazala, Bendani and Zielinski (1985) and Cazala (1986) showed that mice will seek dorsal central grey, medial hypothalamic, medial lemniscal, lateral tegmental and reticular formation stimulation, despite their aversive component. Interestingly, in a Y-maze choice experiment, central grey-implanted mice preferred a higher intensity, despite its stronger aversive component. However, when animals were given a choice between central grey and low intensity LH stimulation, they always preferred the latter, regardless of central grey current intensity (Cazala et al., 1985). In fact, the greater the increase in central grey stimulation, the less often it was chosen. Unfortunately, this experiment does not permit us to determine if aversion per se was prompting the rats to choose the more "pure" LH stimulation, or if the qualitative attributes of LH reward were simply favored over those of the central grey, regardless of aversion.

The objective of this chapter was to investigate the role of aversion in preference behavior. Specifically, the interactions between rewarding VTA stimulation1 and aversive hindbrain stimulation using the curve-shift paradigm were investigated. The hindbrain electrode was implanted in the nucleus reticularis gigantocellularis (Gi), an aversion-implicated structure whose neurons purportedly subserve responses to noxious natural somatic stimuli (Casey, 1969, 1971; Guilbaud, Besson, Oliveras & Wyon-Maillard, 1973; Cross, 1994). In order to verify the presence of aversion, rats' latency to escape from continual Gi stimulation was first measured as a function of current intensity. Rate-frequency curves for VTA (single pulse condition) and combined VTA+Gi (paired pulse condition) were gathered separately in a second phase. Putative interactions included: 1) Gi aversion altering VTA self-stimulation rates, but not the threshold frequency, 2) Gi aversion altering the VTA threshold frequency and possibly rates, 3) Gi aversion having no effect on the VTA rate-frequency curve or 4) Gi aversion abolishing altogether VTA self-stimulation. Finally, a preference test between VTA and VTA+Gi stimulation thresholds was conducted in some subjects. It was hypothesized that if aversion alters preference, then rats will prefer VTA over VTA+Gi threshold frequencies.

Materials and Method

Subjects and surgery

Seven naive male Sprague-Dawley rats weighing approximately 300 g at the time of surgery were stereotaxically implanted with two monopolar moveable electrodes, as in Experiment One. The stereotaxic coordinates for the VTA electrode were the same as in the first two experiments. For the Gi electrode, the coordinates were 11.6 mm posterior to bregma, 0.7 mm lateral to the sagittal suture, and 9.1 mm below the cranial surface.

The LH would have been an equally appropriate candidate for this experiment. However, in 1982, Carr and Coons conducted a similar experiment to ours using the LH, whereas the VTA has never been investigated in this context.

Apparatus, stimulation parameters and shaping

Subjects were shaped for self-stimulation in the same apparatus and according to similar stimulation parameters as in Experiment One.

The single pulse rate-frequency function

The VTA rate-frequency function was collected as in Experiment One. The mean of three consecutive stable curves was used to infer a threshold, defined as the pulse frequency required to elicit 48-75% (depending on the subject) of the maximum self-stimulation rate.

The latency to escape-intensity function

Functions relating latency to escape from continual Gi stimulation to the pulse intensity were collected. The frequency of Gi pulses was fixed at the lowest value supporting maximum VTA self-stimulation (determined from the mean rate-frequency function). This frequency was chosen to ensure that subjects would receive pairs of VTA+Gi pulses that carried both rewarding and aversive effects in a later phase of the experiment. Subjects were placed in one compartment of a two-compartment operant chamber. They were then administered continual Gi stimulation consisting of the fixed number of pulses and a starting intensity of 100 uA, until they changed compartments. Immediately following escape, the stimulation was discontinued for 60 sec. If the rats failed to escape, stimulation was terminated after 60 sec., in which instance, the latency to escape was recorded as 60 sec. A new trial was then performed with a higher current. Current intensity was increased in increments of 100 uA, until the latency to escape was lower than 7 sec. The first latency-intensity curve was intended to be a training session and was discarded for all subjects. An escape threshold, defined as the pulse intensity corresponding to latency of 7 sec. was therefore inferred from the second escape-intensity curve.

Latency to escape from continual Gi stimulation was also measured across several days in a group of three rats never having received any VTA stimulation. This phase was conducted to ensure the stability of escape thresholds across time in the absence of VTA stimulation. Note that following this phase, one animal (#295) was subjected to the single and paired pulse rate-frequency function phases.

The paired-pulse rate-frequency function

Prior to conducting the preference test, the rate-frequency self-stimulation function was again collected as explained earlier, with however, one exception: This time, each train of VTA pulses was followed 0.2 msec. or 2.0 msec. later (depending on the subject) by a train of Gi pulses. The intensity for the VTA pulses was identical to that used in the single pulse condition, whereas that for the Gi corresponded to the escape threshold. The paired-pulse rate-frequency function was obtained intermittently over several days. The rate-frequency function using VTA pulses only was also gathered periodically to assess the stability of this control curve. Threshold frequencies for single VTA and combined VTA+Gi pulses (corresponding to 48-75% of the maximum self-stimulation rate, depending on the subject) were established from the mean rate-frequency curves for the upcoming preference test. At the termination of all testing, escape thresholds, in the absence of VTA stimulation, were measured once again.

The preference test

A second lever was added to the operant chamber, and a psychophysical preference test was conducted as in Experiment One. Rats were primed and trained as in the first experiment. The Gi electrode was disconnected for the training session only. When the rats were sufficiently trained, the VTA threshold frequency was held fixed (the STD stimulus) at the new lever. The second lever delivered a variable COMP frequency of paired (VTA+Gi) pulses that was either greater, lesser or equal to the STD frequency. Each trial lasted 5 minutes, separated by 60 seconds during which no stimulation was available. Self-stimulation rates and time spent barpressing for the STD and COMP frequencies were recorded as a function of the number of COMP pulses/train. However, in order to remain consistent with Chapters Two and Three, only the time spent barpressing was plotted. The preference test was conducted in two subjects only (see Results and Discussion sections for further explanation).

Data analysis

For the preference test, data were analyzed as in Experiment One.

Histology

After completion of testing, the animals were perfused, and brain slices were subsequently stained as in Experiment Three.

Results

Histology

Figure 1 depicts the location of electrode tips (closed circles) on plates reproduced from Paxinos and Watson's (1986) stereotaxic atlas. Subjects #231, 302, 303 and 312 lost their electrode assembly. Consequently, only the VTA electrode in subject #312 and the Gi electrode in #303 could be correctly localized. The left and right plates show the anterior (VTA) and posterior (Gi) location for each electrode pair. The number on each individual plate refers to the millimetric

distance behind bregma. All anterior electrode tips landed in the VTA, except for #284, whose electrode landed in the posterior hypothalamus. The posterior electrodes landed in the vicinity of the Gi, or in the intermediate reticular nucleus.

Figure 1: The location of each stimulation site is depicted by a closed circle on the corresponding plate of Paxinos and Watson's (1986) stereotaxic atlas. The number on each individual plate indicates the millimetric distance behind bregma. The left and right plates show the anterior and posterior locations for each electrode pair, respectively. The number beside each stimulation site identifies the animal. 7g: genu facial nerve; 7n: facial nerve; cp: cerebral peduncle, basal; Gi: nucleus reticularis gigantocellularis; GiA: alpha nucleus reticularis gigantocellularis; irt: intermediate reticular nucleus; ml: medial lemniscus; ph: posterior hypothalamic area; vta: ventral tegmental area.

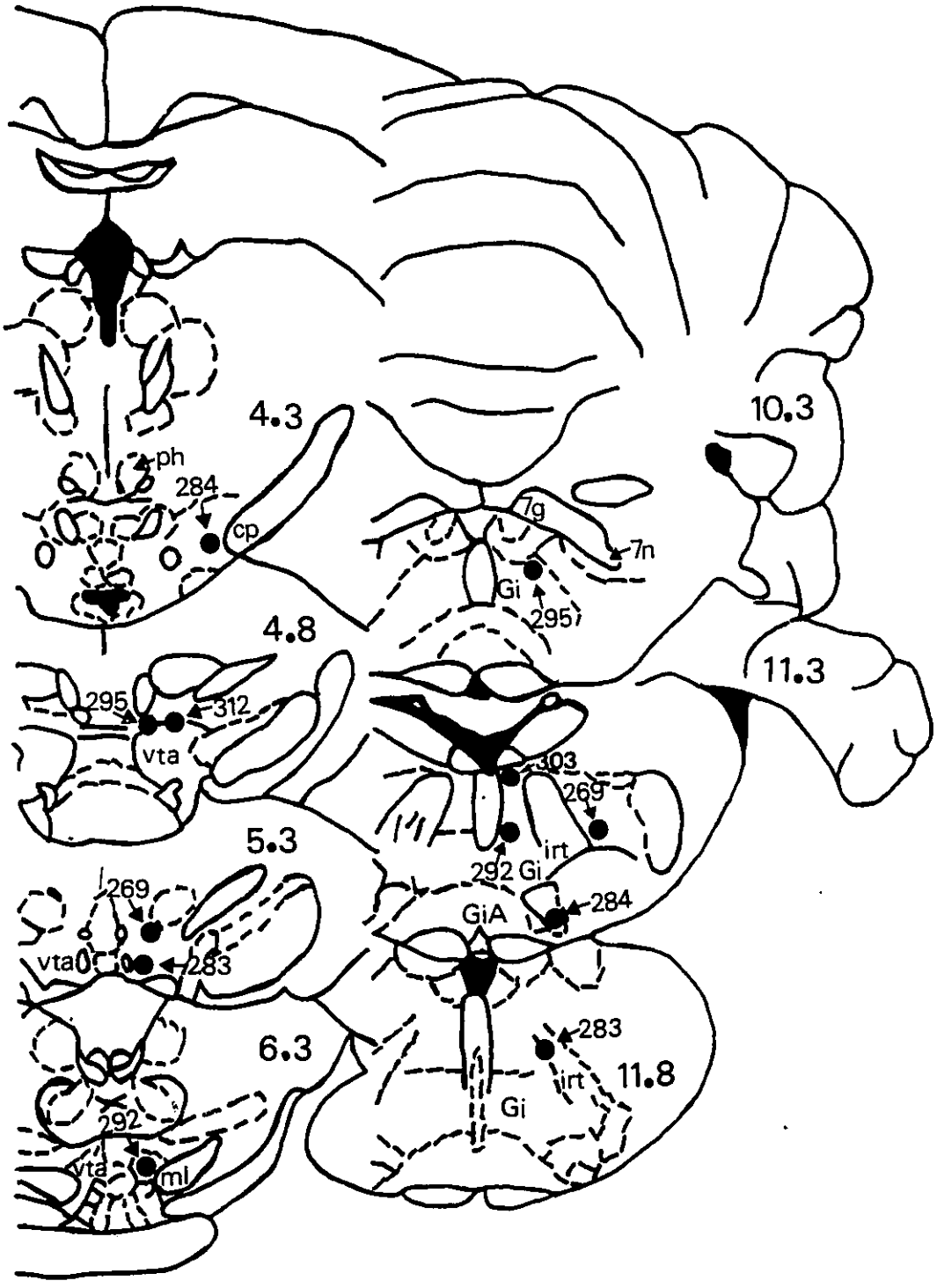

4.3
ph
284
cp
4.8
295
312
vta
5.3
269
vta
283
6.3
292
vta
ml
7g
7n
Gi
295
10.3
11.3
303
269
irt
292 Gi
284
GiA
283
irt
11.8
Gi

Fig.1

Escape thresholds

Higher intensities of Gi electrical stimulation were confirmed to be aversive and to elicit natural-like pain behaviors (defined by Casey, 1971) such as defecation, urination, scratching at the chamber walls, and jumping upon initial stimulation during the early trials. Rats also withdrew into the far corner of the opposite compartment and tried to escape. A hyperbolic function was obtained when latency to escape was plotted as a function of intensity (data not shown) from which the escape threshold (defined as the current intensity required to elicit escape within 7 sec.) was inferred.

The paired-pulse rate-frequency function

When the VTA and Gi were stimulated concomitantly, escape was abolished. In fact, rats self-stimulated for trains of Gi pulses at the same current intensity and higher pulse frequency than that originally eliciting fast escape, as long as the VTA was stimulated concomitantly. Figures 2-8 shows the rate-frequency functions obtained with single (VTA) and paired (VTA+Gi) pulses, according to the number of days (D) elapsed after the last VTA (single pulse) curve was gathered. The number at the top of each graph identifies the subject. The delay between VTA and Gi pulses is provided in brackets. Standard errors of the mean were omitted to enhance visual inspection.

Note that initially, the presence of Gi pulses shifted the self-stimulation curve to the right. This shift occurred in all rats, regardless of the size of the delay between VTA and Gi pulses. Note also that the capacity of Gi pulses to shift the function decreased progressively with time and/or repetitive testing. Interestingly, the last combined rate-frequency function culminated slightly to the left of the control function in those rats that were tested for the longest periods of time. Relatively little effect was observed on the maximum barpressing rate in five out of seven rats.

Figures 2-8: Barpressing rate as a function of pulse frequency and time. The label at the top of each graph identifies the subject. The letter D represents the number of days elapsed following the gathering of the last ventral tegmental (VTA) curve. The delay between VTA and nucleus reticularis gigantocellularis (Gi) pulses is provided in brackets.

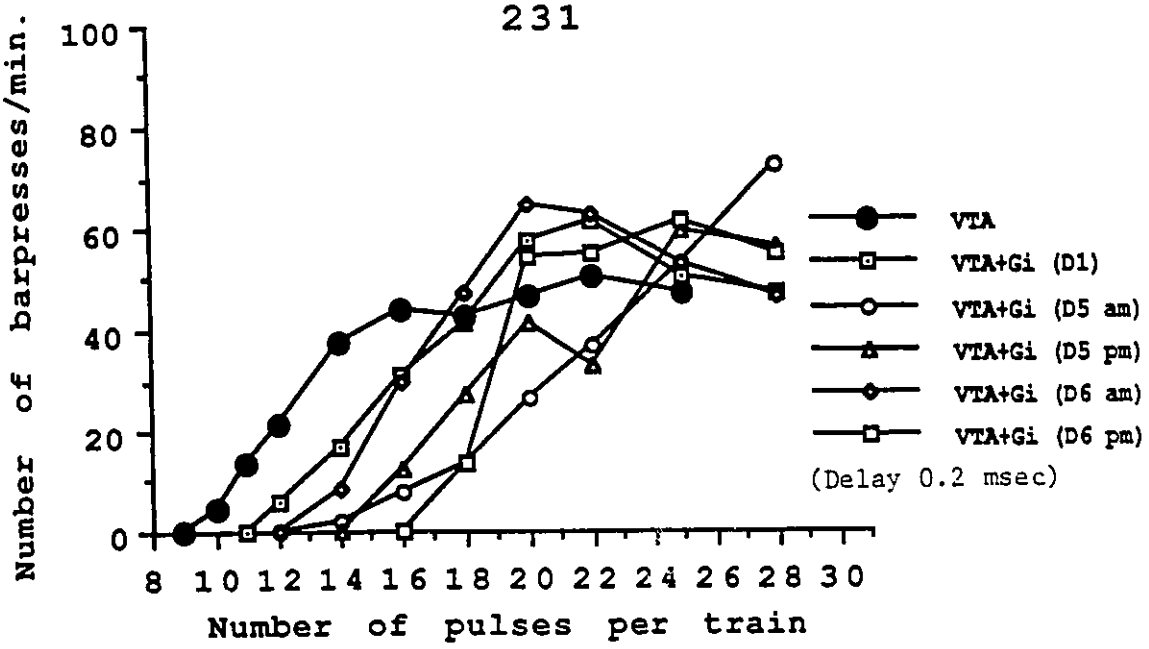

Number of barpresses/min.
100
80
60
40
20
0
8 10 12 14 16 18 20 22 24 26 28 30
Number of pulses per train
VTA
VTA+Gi (D1)
VTA+Gi (D5 am)
VTA+Gi (D5 pm)
VTA+Gi (D6 am)
VTA+Gi (D6 pm)
(Delay 0.2 msec)

Fig. 2

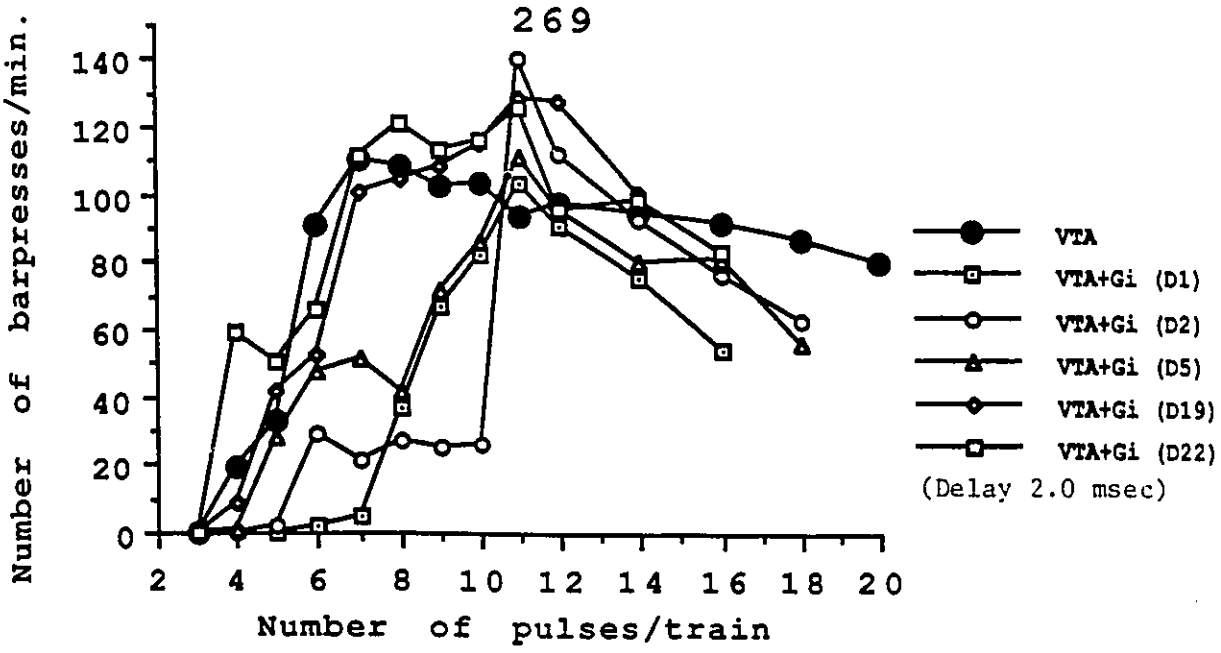

269
Number of barpresses/min.
140
120
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
Number of pulses/train
VTA
VTA+Gi (D1)
VTA+Gi (D2)
VTA+Gi (D5)
VTA+Gi (D19)
VTA+Gi (D22)
(Delay 2.0 msec)

Fig. 3

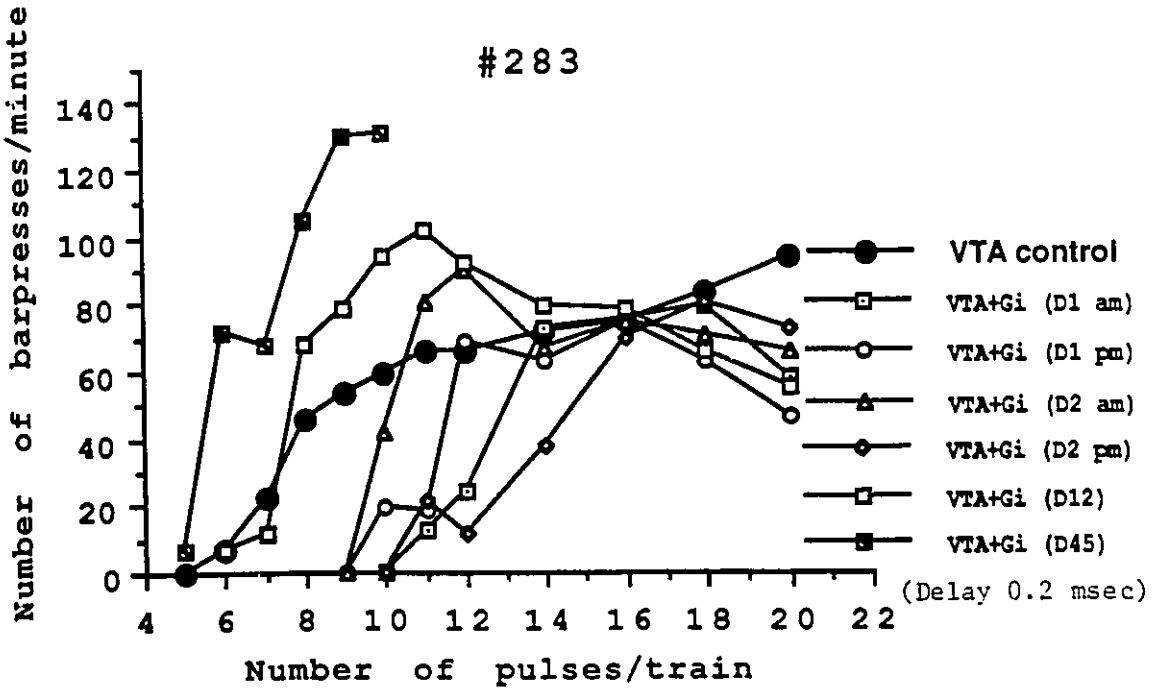

#283
Number of barpresses/minute
140
120
100
80
60
40
20
0
4
6
8
10
12
14
16
18
20
22
Number of pulses/train
VTA control
VTA+Gi (D1 am)
VTA+Gi (D1 pm)
VTA+Gi (D2 am)
VTA+Gi (D2 pm)
VTA+Gi (D12)
VTA+Gi (D45)
(Delay 0.2 msec)

Fig. 4

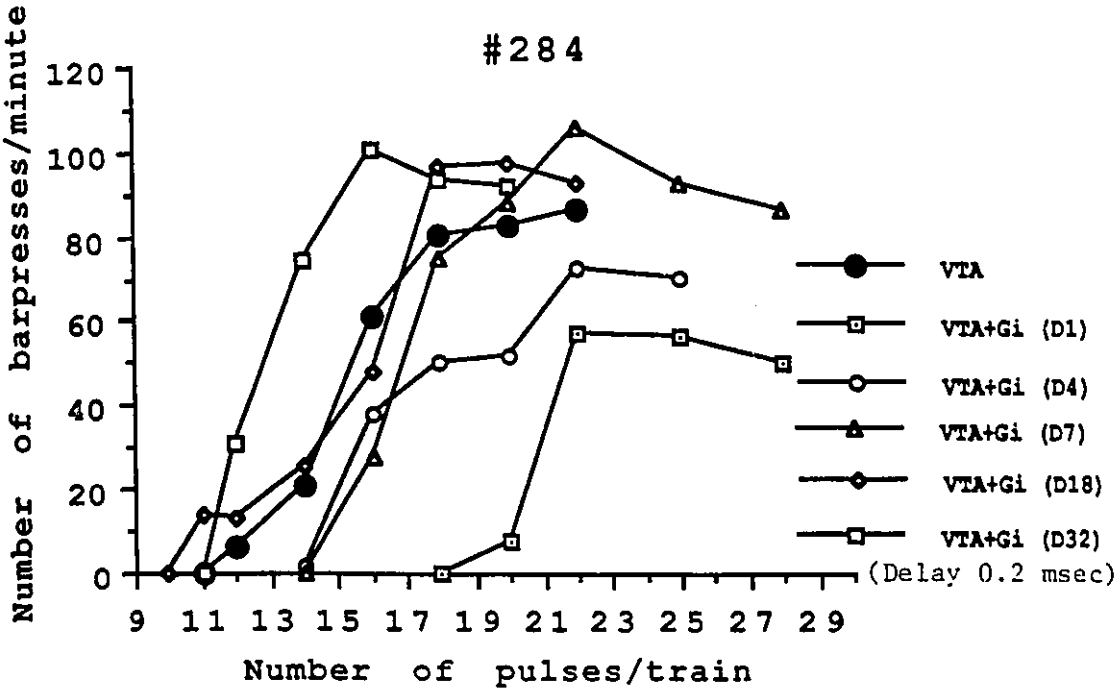

#284
Number of barpresses/minute
120
100
80
60
40
20
0
9 11 13 15 17 19 21 23 25 27 29
Number of pulses/train
VTA
VTA+Gi (D1)
VTA+Gi (D4)
VTA+Gi (D7)
VTA+Gi (D18)
VTA+Gi (D32)
(Delay 0.2 msec)

Fig. 5

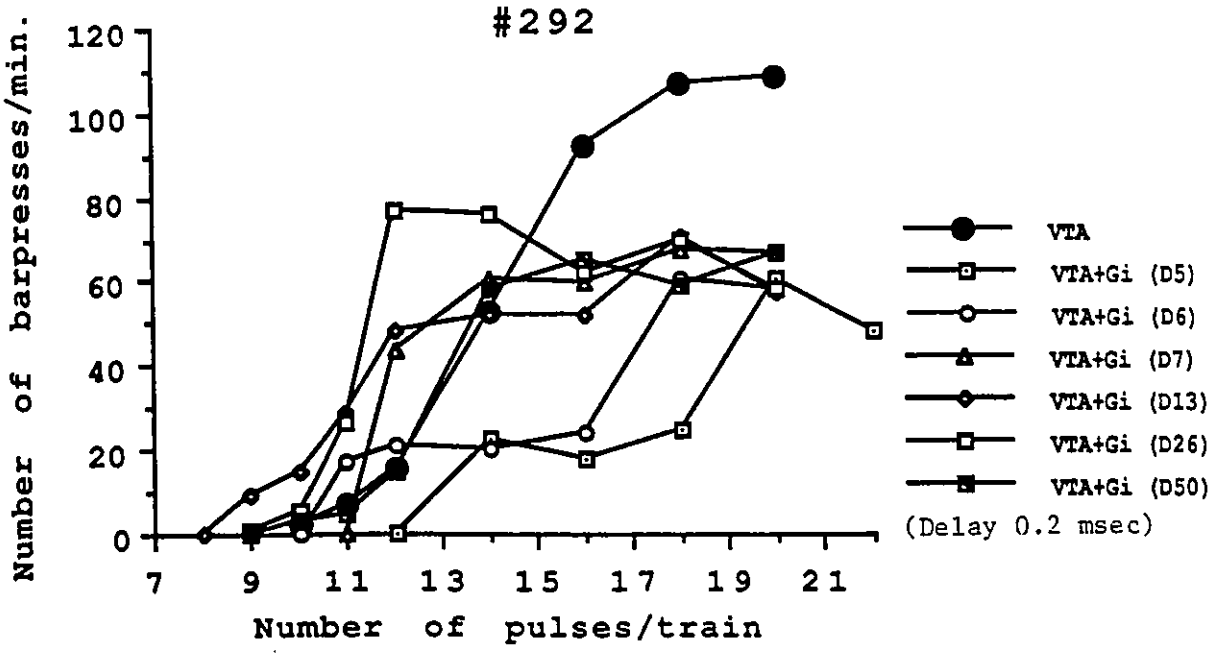

#292
Number of barpresses/min.
120
100
80
60
40
20
0
7
9
11
13
15
17
19
21
Number of pulses/train
VTA
VTA+Gi (D5)
VTA+Gi (D6)
VTA+Gi (D7)
VTA+Gi (D13)
VTA+Gi (D26)
VTA+Gi (D50)
(Delay 0.2 msec)

Fig. 6

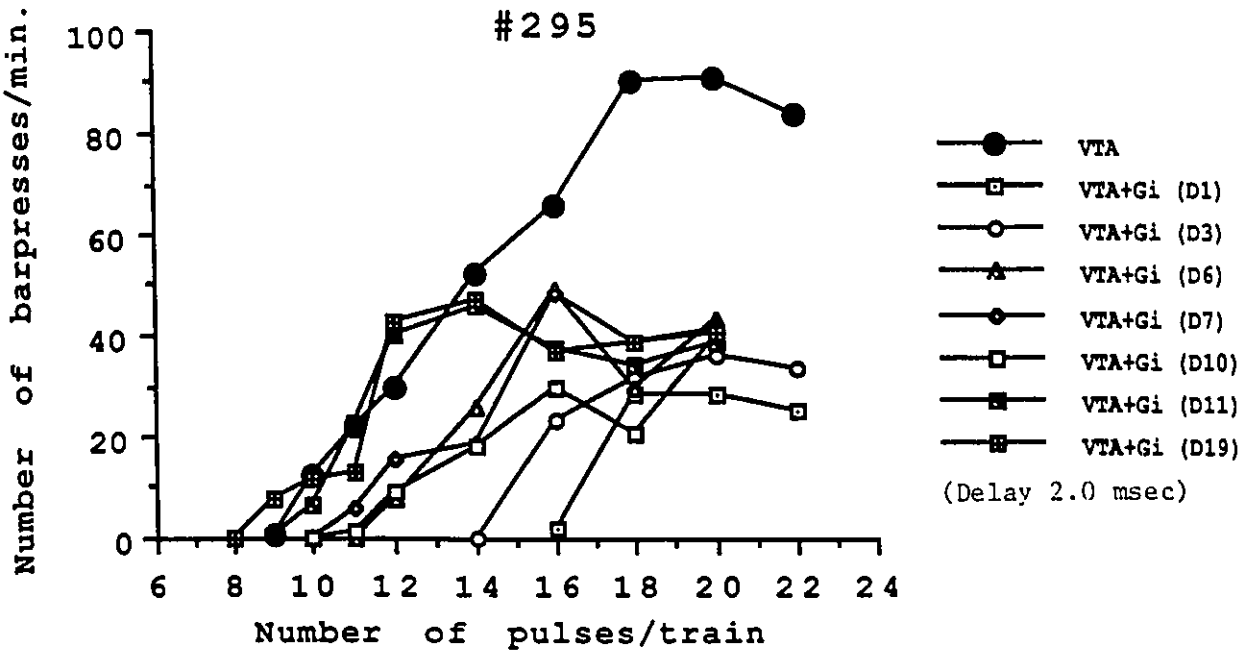

#295
Number of barpresses/min.
100
80
60
40
20
0
6
8
10
12
14
16
18
20
22
24
Number of pulses/train
VTA
VTA+Gi (D1)
VTA+Gi (D3)
VTA+Gi (D6)
VTA+Gi (D7)
VTA+Gi (D10)
VTA+Gi (D11)
VTA+Gi (D19)
(Delay 2.0 msec)

Fig. 7

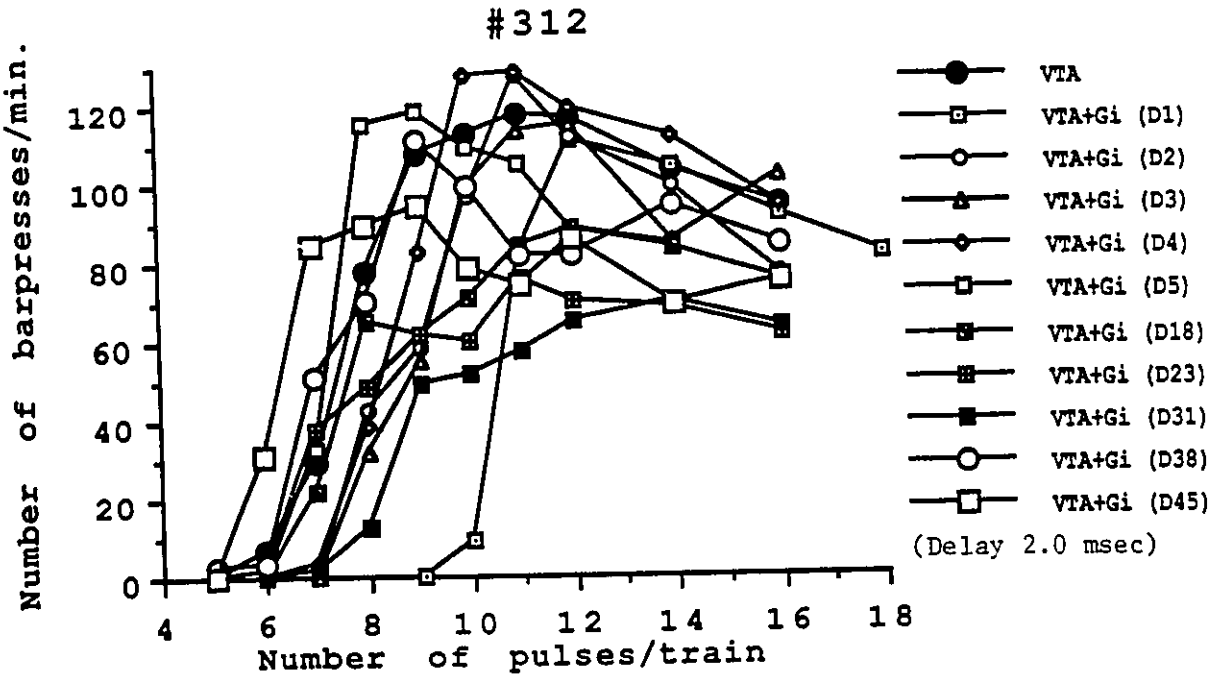

#312
Number of barpresses/min.
120
100
80
60
40
20
0
4
6
8
10
12
14
16
18
Number of pulses/train
VTA
VTA+Gi (D1)
VTA+Gi (D2)
VTA+Gi (D3)
VTA+Gi (D4)
VTA+Gi (D5)
VTA+Gi (D18)
VTA+Gi (D23)
VTA+Gi (D31)
VTA+Gi (D38)
VTA+Gi (D45)
(Delay 2.0 msec)

Fig. 8

Figures 9-15 plot the logarithmic change in frequency threshold for the seven animals, as a function of testing day. Positive and negative values indicate a shift of the rate-frequency function to the right and left of the VTA baseline curve (zero line), respectively. The number at the top of each graph identifies the subject.

Figures 9-15: Logarithmic change in self-stimulation threshold frequency, as a function of day following the ventral tegmental (VTA) baseline condition (zero line). Positive values indicate that the nucleus reticularis gigantocellularis (Gi) pulses increased the threshold, whereas negative values indicate that the threshold was decreased. The number at the top of each graph identifies the subject.

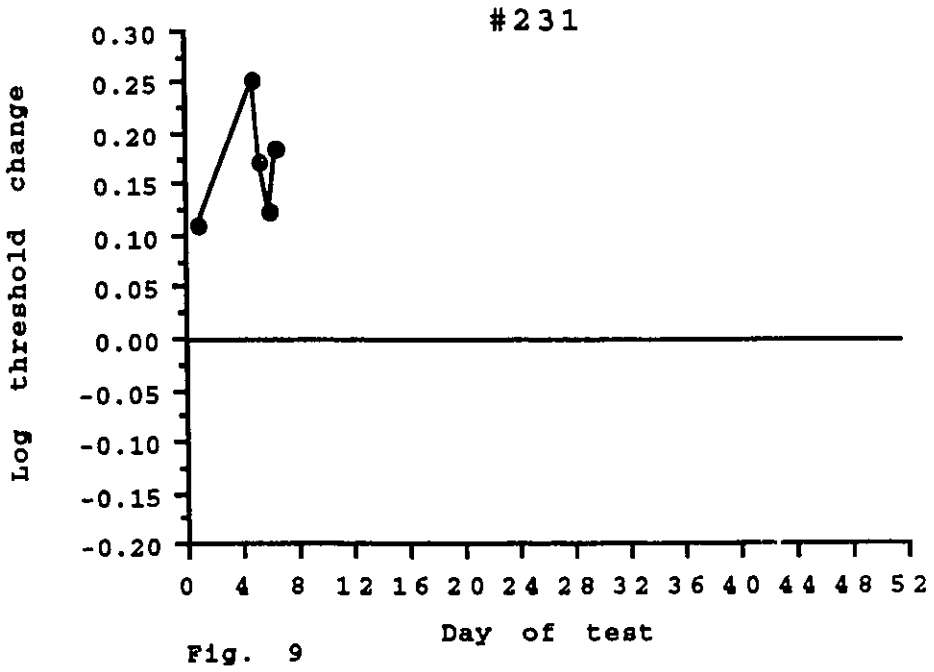

#231
Log threshold change
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0 4 8 12 16 20 24 28 32 36 40 44 48 52
Day of test

Fig. 9

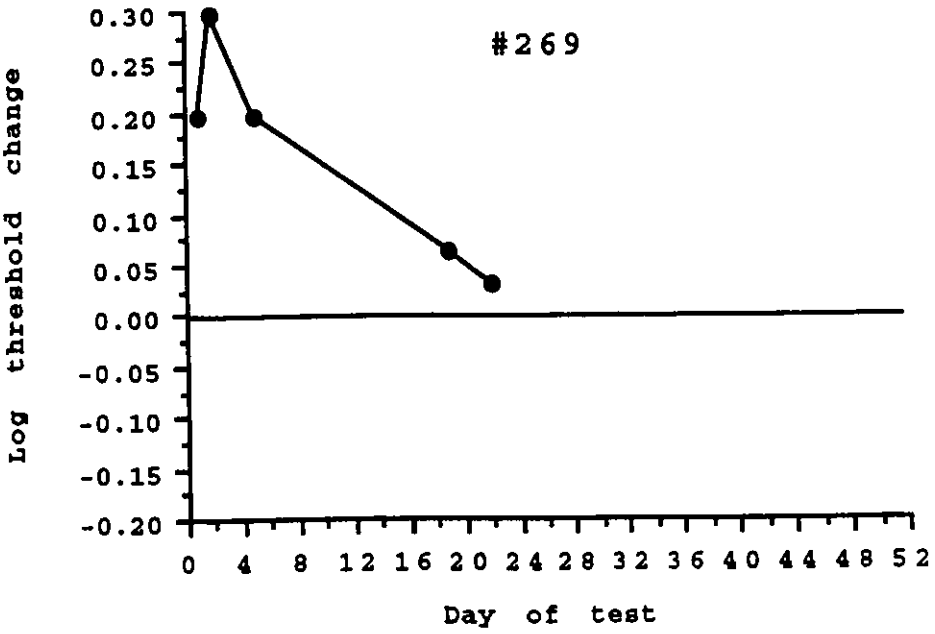

#269
Log threshold change
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0 4 8 12 16 20 24 28 32 36 40 44 48 52
Day of test

Fig. 10

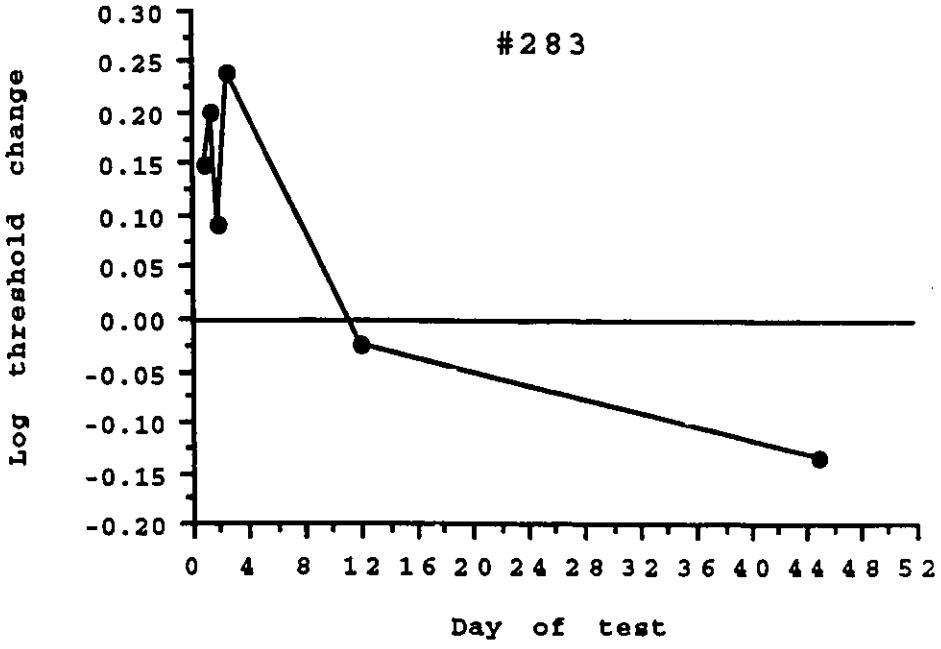

#283
Log threshold change
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0 4 8 12 16 20 24 28 32 36 40 44 48 52
Day of test

Fig. 11

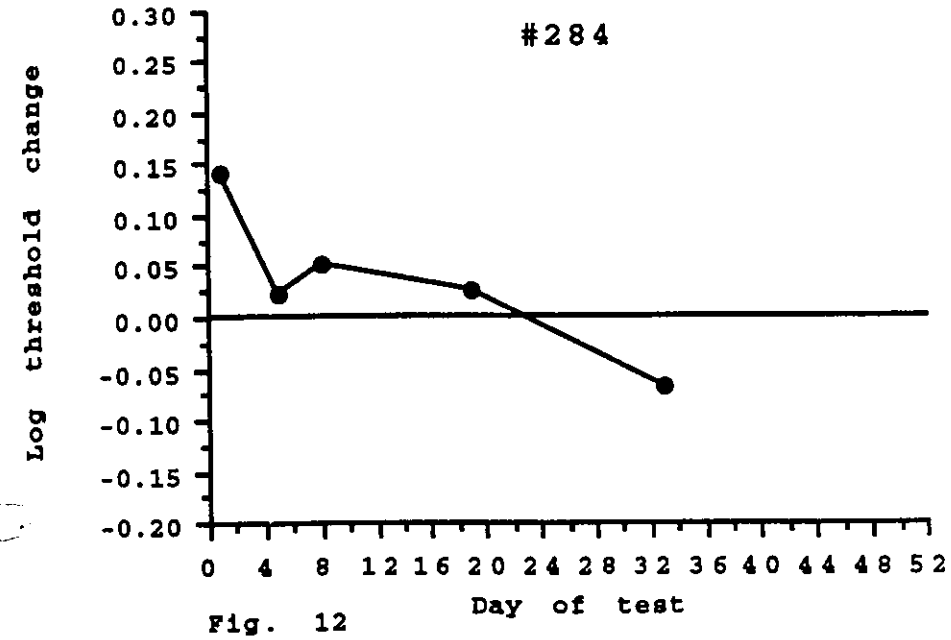

#284
Log threshold change
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0 4 8 12 16 20 24 28 32 36 40 44 48 52
Day of test

Fig. 12

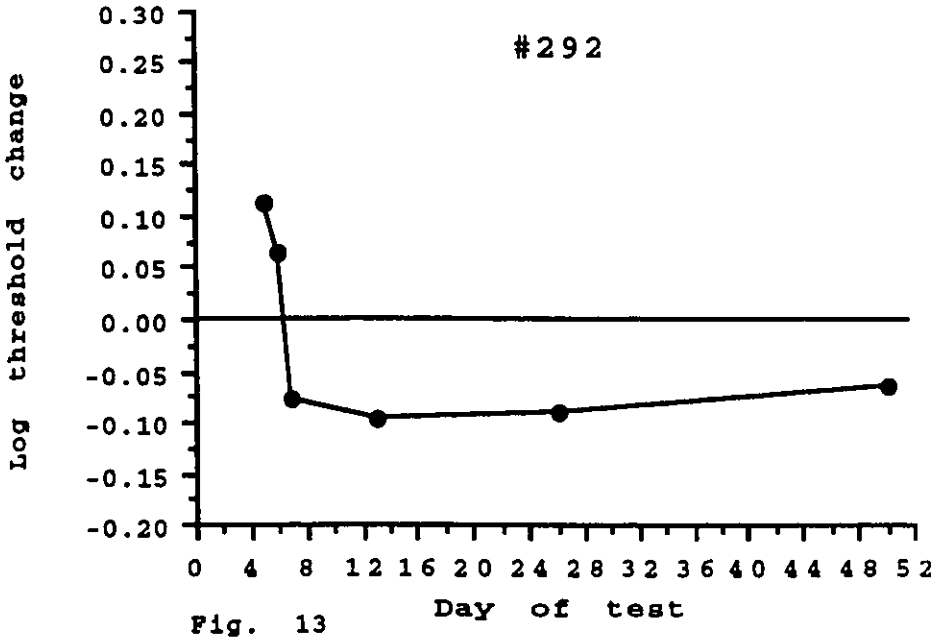

#292
Log threshold change
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0 4 8 12 16 20 24 28 32 36 40 44 48 52
Day of test

Fig. 13

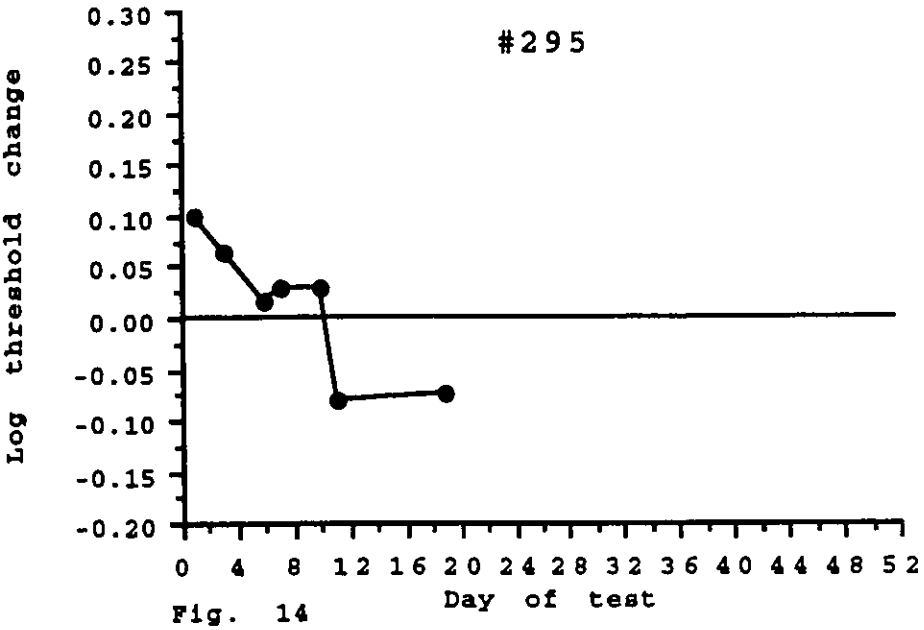

#295
Log threshold change
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0 4 8 12 16 20 24 28 32 36 40 44 48 52
Day of test

Fig. 14

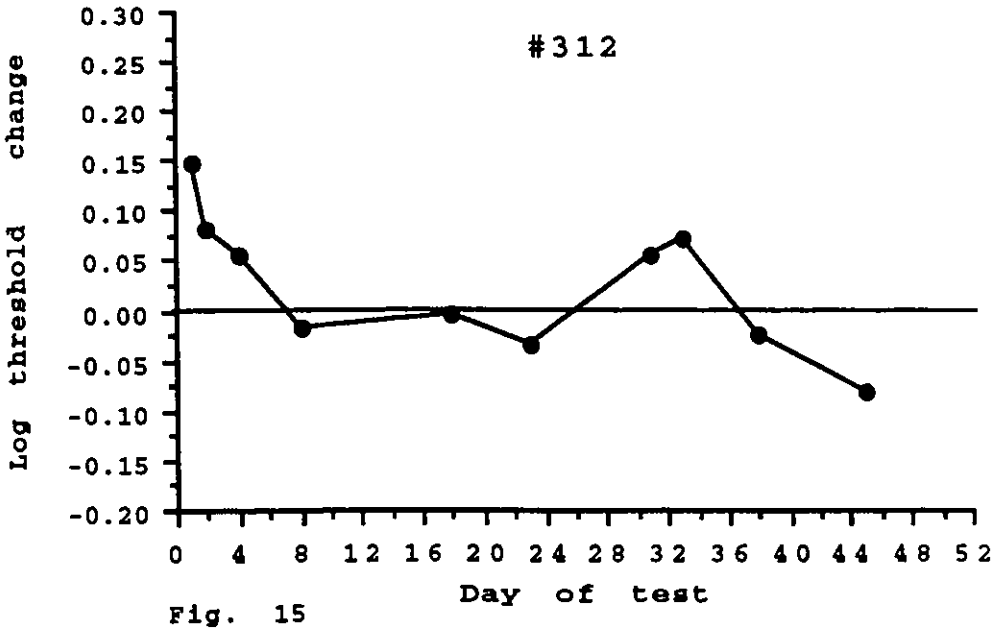

#312
Log threshold change
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0 4 8 12 16 20 24 28 32 36 40 44 48 52
Day of test

Fig. 15

The following three phenomena are of interest: First, as noted above, the frequency threshold was initially increased under the effect of Gi pulses. The largest change (+0.3 logfrequency units) was noted in subject #269, indicating a twofold increase in threshold frequency. Second, the threshold elevation dissipated with repeated testing. Depending on the subject, the data returned near the baseline or intercepted it after 2 to 5 repetitions, regardless of the day of testing. This observation suggests that the number of stimulation sessions, not the time elapsed, is the critical variable.

Third, in the five subjects that were tested for longer periods, the threshold decreased below the baseline value. In other words, the combined VTA+Gi rate-frequency function was shifted to the left of the control VTA function. The largest change, noted in subject #283 (-0.14 logfrequency units) indicates a 28% decrease in threshold.

The preference test

In order to conduct the preference test, stable thresholds across three consecutive rate-frequency tests were required. This criterion was met in the VTA (single pulse) condition, but not for the combined VTA+Gi condition (as shown in Figures 2-8). Nevertheless, a preference test was conducted between the mean VTA and VTA+Gi threshold frequencies for subject #231 (on day 12) and #283 (on day 5) in violation of the stable threshold requirement. Refer to Figures 16 and 17 for results. For the upper graphs, the number at the top of the graph identifies the subject. The solid and stippled lines correspond to the time spent barpressing for the STD (VTA) and COMP (VTA+Gi) stimulus, respectively. The frequency of the STD is provided in brackets. The number preceded by "m" represents the proportion of the maximum self-stimulation rate elicited by that frequency. The predicted point of equality, or mean threshold frequency for the COMP is also provided in brackets. The closed arrow points to the point of subjective equality. The bottom graphs show the previously shown corresponding rate-frequency functions with single (VTA) and paired (VTA + Gi)pulses, according to the number of days (D) elapsed after the last single pulse.

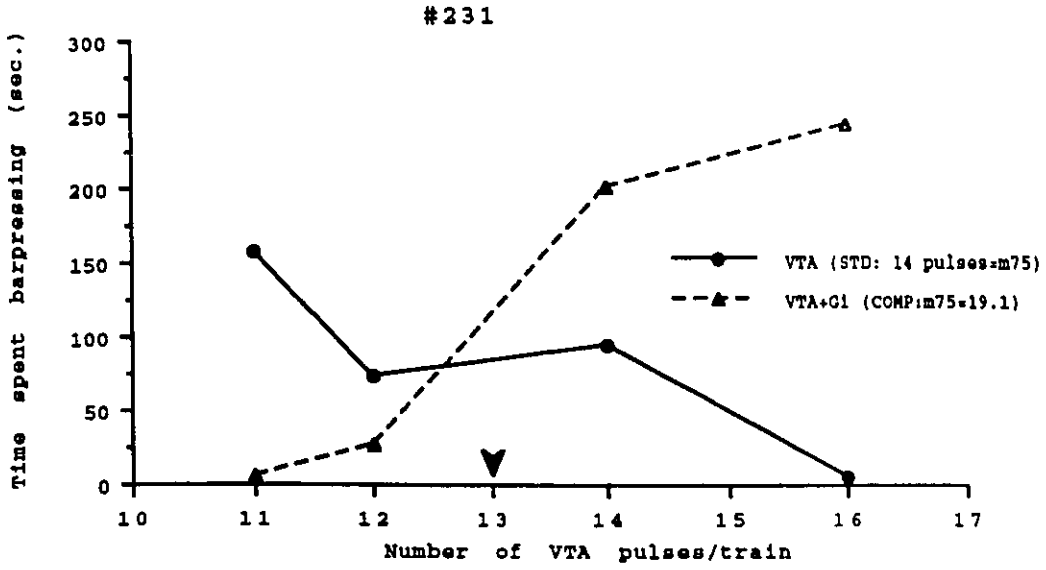
The delay between VTA and Gi pulses is provided in brackets. The asterisks denotes the approximate day on which the preference test was conducted.

As seen in Figure 16, #231 equally preferred 12 VTA and 13 VTA+Gi pulses. However, using the rationale followed in Experiment One, #231 should have equally preferred 12 VTA and 19.1 (mean VTA+Gi threshold frequency) VTA+Gi pulses. The log constant error between the predicted and subjective points of equality was 0.15 logfrequency units. Thus, despite shifting the VTA curve in the single-lever box, the presence of aversive Gi pulses did not appear to significantly affect VTA reward in the two-lever box. This paradox is difficult to explain, and is not consistent with the curve-shift paradigm. A more likely explanation for these results is that the VTA+Gi threshold frequency returned to baseline just before or during the preference test. In other words, perhaps the predicted point of equality of 19.1 VTA+Gi pulses was no longer accurate. Unfortunately, #231 lost his electrode assembly before this hypothesis could be tested by conducting another VTA+Gi rate-frequency curve. The fact that the VTA+Gi thresholds returned to baseline after 2-5 stimulation sessions in the other six rats is strong evidence in favor of this speculation. The results of this subject also prompted us to follow VTA+Gi threshold frequencies across time in all remaining subjects.

A preference test was also conducted after the gathering of the fourth combined rate-frequency curve in subject #283. Refer to Figure 17 for results. Again, aversive Gi pulses had no significant effect on preference, despite shifting the VTA curve. Note in Figure 11, however, that the VTA+Gi threshold frequency determined immediately after the preference test was below baseline. Thus the VTA+Gi threshold frequency seemed to decrease during the preference test. Due to the unstable VTA+Gi threshold frequencies, the preference test was not conducted in the remaining subjects.

Figures 16-17: The upper graph plots the time spent barpressing as a function of the standard (STD) ventral tegmental (VTA) and comparison (COMP) VTA+nucleus reticularis gigantocellularis (Gi) stimuli. The number at the top of the graph identifies the subject. The solid and stippled lines correspond to the time spent barpressing for the STD and COMP stimulus, respectively. The frequency of the STD is provided in brackets, along with the percent of maximum barpressing rate it elicits (preceded by "m"). The predicted point of equality, or mean threshold frequency for the COMP is also provided in brackets. The closed arrow points to the point of subjective equality.

The bottom graphs depict the previously shown corresponding rate-frequency functions with single (VTA) and paired (VTA + Gi)pulses, according to the number of days (D) elapsed after the last single pulse. The delay between VTA and Gi pulses is provided in brackets. The asterisks denotes the approximate day on which the preference test was conducted.


#231
Time spent barpressing (sec.)
300
250
200
150
100
50
0
10
11
12
13
14
15
16
17
Number of VTA pulses/train
VTA (STD: 14 pulses=m75)
VTA+Gl (COMP:m75=19.1)

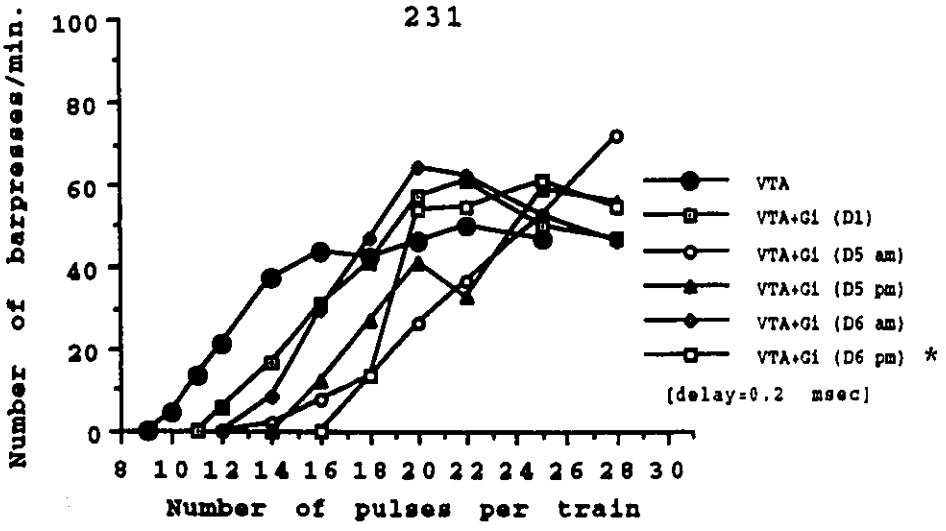
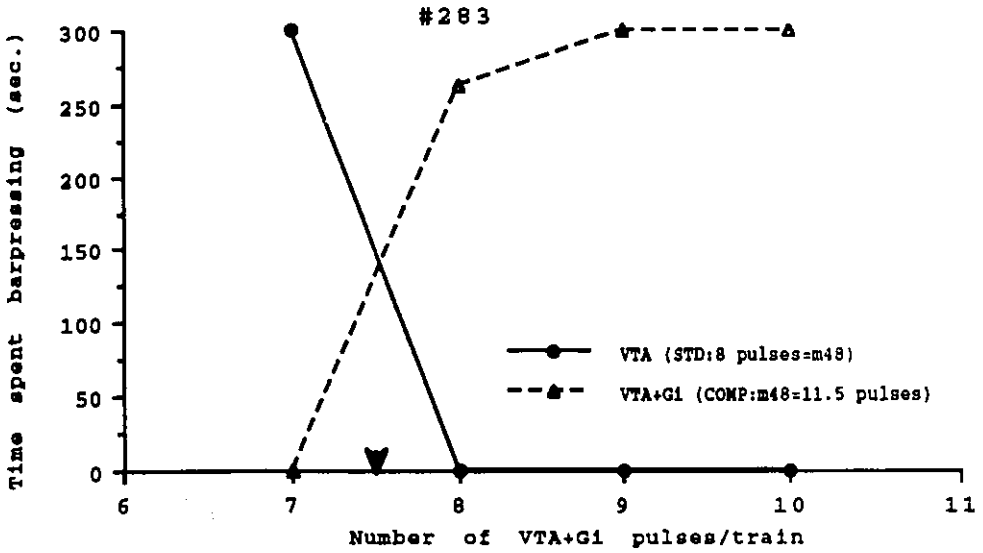

231
Number of barpresses/min.
100
80
60
40
20
0
8 10 12 14 16 18 20 22 24 26 28 30
Number of pulses per train
VTA
VTA+Gl (D1)
VTA+Gl (D5 am)
VTA+Gl (D5 pm)
VTA+Gl (D6 am)
VTA+Gl (D6 pm) *
[delay=0.2 msec]

Fig.16


#283
Time spent barpressing (sec.)
300
250
200
150
100
50
0
6
7
8
9
10
11
Number of VTA+G1 pulses/train
VTA (STD:8 pulses=m48)
VTA+G1 (COMP:m48=11.5 pulses)

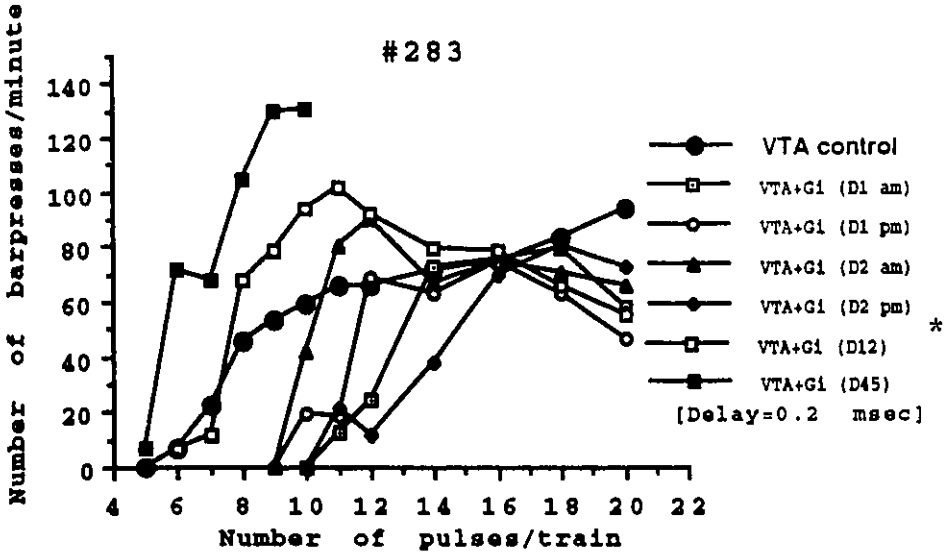

#283
Number of barpresses/minute
140
120
100
80
60
40
20
0
4
6
8
10
12
14
16
18
20
22
Number of pulses/train
VTA control
VTA+G1 (D1 am)
VTA+G1 (D1 pm)
VTA+G1 (D2 am)
VTA+G1 (D2 pm)
VTA+G1 (D12)
VTA+G1 (D45)
[Delay=0.2 msec]
*

Fig.17

Escape measures following the combined rate-frequency function

Escape from Gi stimulation, was measured a second time after completion of the paired-pulse condition. It was noted that all subjects except for #295 failed to escape from continual Gi stimulation across all intensities, even though the VTA was no longer being stimulated. In fact, if the rats' Gi was stimulated in the operant box, they approached the lever and barpressed a few times.

Escape thresholds in the control group

The contention that aversion dissipated due to repeated Gi stimulation rather than to the contribution of VTA pulses was investigated in a group of three subjects that had never been stimulated in the VTA. Figures 18-20 depict latency to escape from continual Gi stimulation as a function of pulse intensity and testing day in these control rats. The number at the top of each graph identifies the subject. Ten to 12 latency-intensity functions were obtained per animal for a maximum period of 76 days. In order to enhance visual inspection, only four (first, last and two middle) of the functions were plotted for each rat. Note that repeated testing shifted the latency-intensity function downward rather than upward, indicating that the animals escaped more rapidly. We attribute this change to practice.

Figures 18-20: Latency to escape from continual nucleus reticularis gigantocellularis (Gi) stimulation in 3 rats never having received any VTA stimulation, as a function of pulse intensity and day of testing. The number at the top of each graph identifies the subject.

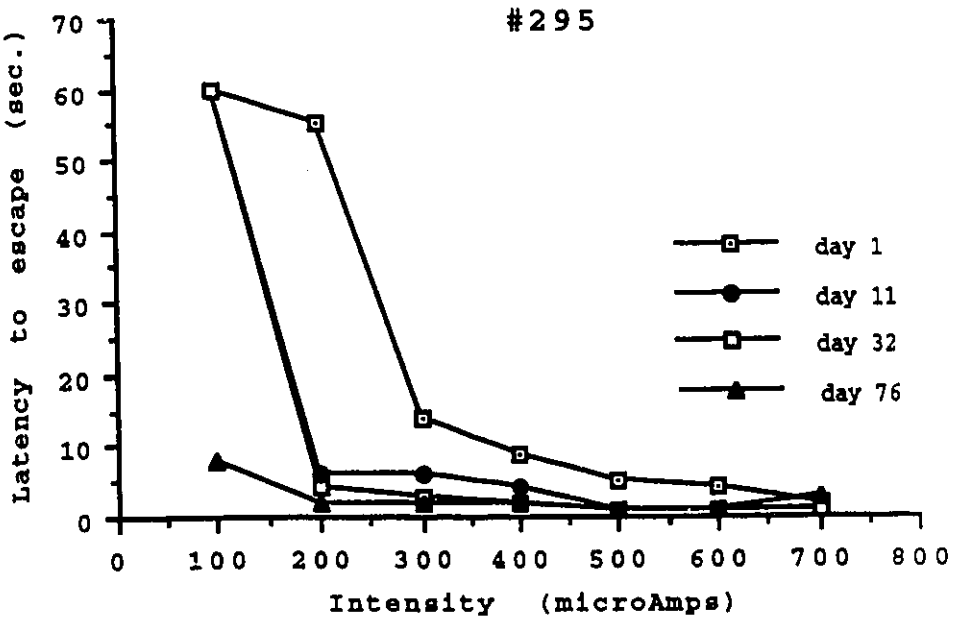

#295
Latency to escape (sec.)
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
Intensity (microAmps)
day 1
day 11
day 32
day 76

Fig.18

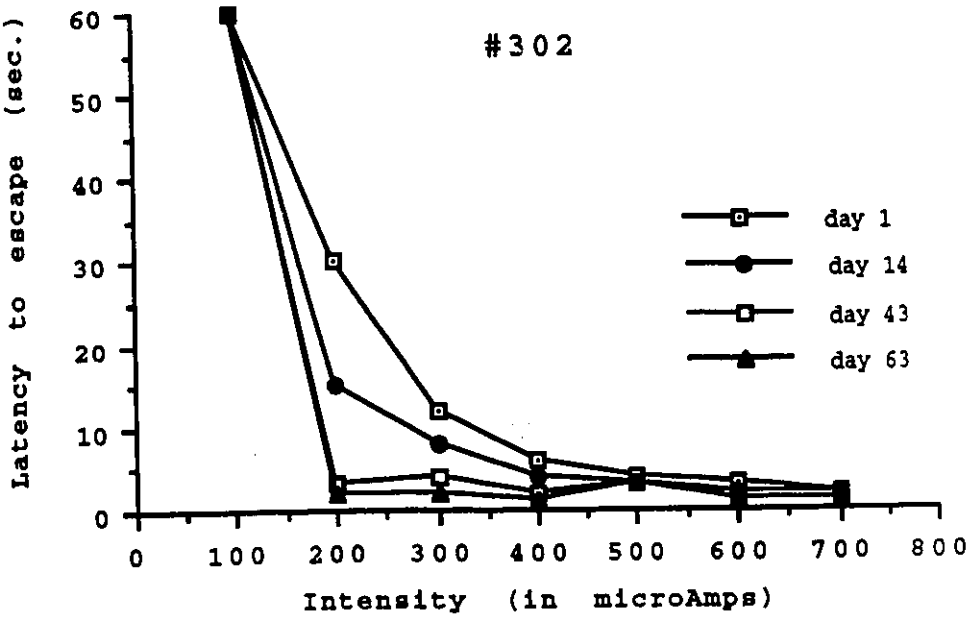

#302
Latency to escape (sec.)
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
Intensity (in microAmps)
day 1
day 14
day 43
day 63

Fig.19

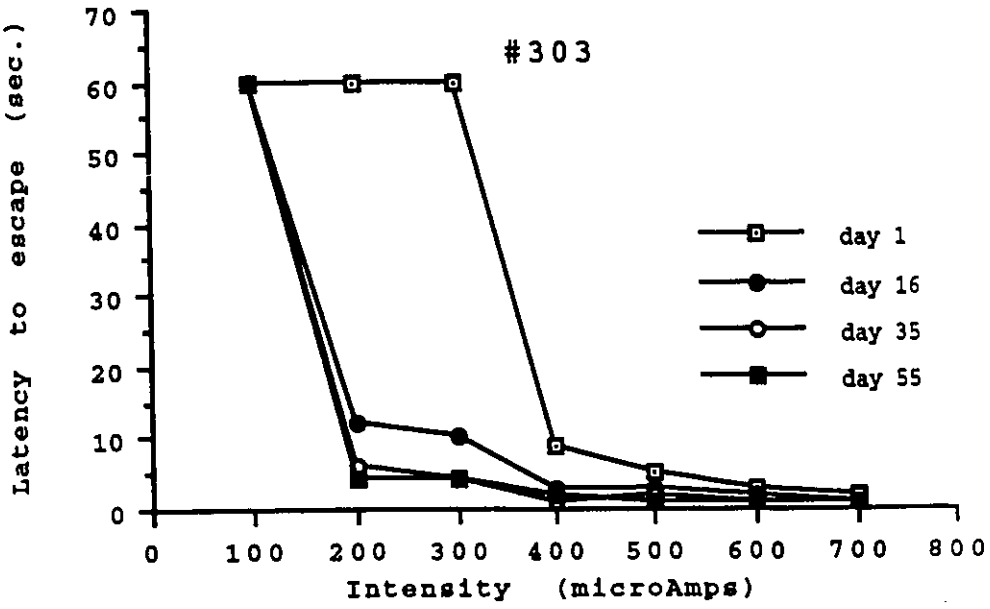

#303
Latency to escape (sec.)
70
60
50
40
30
20
10
0
0
100
200
300
400
500
600
700
800
Intensity (microAmps)
day 1
day 16
day 35
day 55

Fig.20

Discussion

The data support the view of a bi-directional interaction between rewarding and aversive impulses: Activation of the Gi initially increased the VTA self-stimulation threshold, whereas stimulation of the VTA abolished Gi aversion. Depending on the subject, the Gi pulses initially increased self-stimulation threshold by 0.12 to 0.3 logfrequency units. The largest figure indicates a cutting half the VTA rewarding stimulation efficacy. This initial interaction is interpreted as algebraic summation, namely that two opposing signals mutually modified each other, resulting in a single signal with the sign of the strongest component. A lack of summation would have manifested itself by a decrease in the maximum self-stimulation rate without a change in threshold frequency, a profile already shown following combination of rewarding VTA pulses and reticular pulses sustaining motoric reactions (Miliaressis and Rompré, 1987). Alternatively, the Gi pulses did not alter VTA reward per se, but made VTA stimulation less palatable, thus increasing the self-stimulation threshold.

Interestingly, after several sessions of paired stimulation, the Gi pulses progressively lost their ability to shift the VTA rate frequency function to the right. Furthermore, the combined curve culminated to the left of the control VTA curve. This finding is suggestive of a reversal from Gi aversion to mild reward. A reversal effect was also noted by Bishop, Elder and Heath (1964), who found that the aversive amygdaloid electrodes became rewarding upon repeated stimulation, as reported by patients. It is worth noting that, at least in rats, most of the amygdaloid complex also contains brain stimulation reward elements (Kane, Coulombe & Miliaressis, 1991). Consistent with this interpretation is the finding that when rats were stimulated in the escape chamber with Gi pulses only after the last paired pulse condition, they actually pressed the lever a few times. One parsimonious explanation of this set of observations is that the Gi electrode also activated a small population of reward neurons, whose contribution only became measurable once the aversive component of Gi stimulation had dissipated. Furthermore, this decrease in threshold remained permanent in all rats tested for longer periods of time. Finally, reward and aversion may be related, as Blake and Stein (1987) found that the nucleus accumbens, claustrum, claustrocortex,

perirhinal cortex and VTA showed increased [3H]Dpr binding (indicating decreased opioid release) due to both VTA self-stimulation and foot shock.

One may attribute the progressive loss of Gi aversiveness to the simple passage of time or to prolonged Gi stimulation only, rather than to rewarding VTA self-stimulation. However, direct evidence suggests otherwise. In a group of three control rats never having received any VTA stimulation, escape thresholds were measured approximately every two days for a maximum of 76 days, a protocol that matched the paired pulse rate-frequency phase. No increase in escape threshold intensity was noted in any of these rats. Rather, a slight decrease was noted, an effect attributed to practice. In brief, stimulation of the Gi and the passage of time were not sufficient to induce loss of aversion.

In summary, aversive Gi stimulation transiently increased the VTA self-stimulation threshold whereas, the activation of VTA permanently reduced Gi aversion.

The influence of aversion on preference

As stated above, one explanation of the curve-shift data is that rewarding mesencephalic and aversive hindbrain impulses summate into a single reward message. The fact that escape from Gi stimulation was abolished indicates that at least the motivational (aversive) component of this stimulation was suppressed. However, we cannot exclude the possibility that the subject still recognized the Gi signal as negative based on past experience, but not aversive. Such a phenomenon is reminiscent of dissociative analgesia exemplified by patients reporting "It hurts, but I'm not in pain" (Franklin, Abbott, English, Young & Jeans, 1988 in Franklin, 1989). Thus, "pure" rewarding signals cannot be distinguished from "mixed" rewarding signals based on a single rate-frequency function. In sum, the presence of a mixed effect at either structure tested in the LH-VTA preference test (Experiment Two) could feasibly have prompted the rat to choose the alternative more "pure" frequency threshold. A psychophysical preference test between VTA and VTA+Gi thresholds in all rats would have provided definitive evidence for or against this hypothesis. Unfortunately, VTA+Gi thresholds never remained stable long enough to conduct

such an experiment. Nevertheless, the preference data obtained with two subjects showed approximately equal preference for the same number of VTA and VTA+Gi pulses at a time when the VTA+Gi rate-frequency curve had returned to baseline (for at least one of these subjects). In other words, the presence of a residual mixed effect, if any, was not able to alter the animal's preference. Whether this conclusion generalizes to types of aversion other than Gi stimulation remains to be seen.

The cumulative analgesic properties of VTA stimulation reward

The bi-directional interaction between rewarding and aversive impulses was an unexpected finding that merits further discussion. Aversive Gi stimulation has often been attributed to the elicitation of pain. In particular, the reticular small-diameter, myelinated A+ and A-delta neurons of the Gi are thought to be involved in the motivational aspects of noxious, natural somatic stimuli (as opposed to the discrimination of pain; Casey, 1969, 1971; Guilbaud, Besson, Oliveras & Wyon-Maillard, 1973; Cross, 1994). Based on this assumption, electrical stimulation of the VTA may have abolished painful Gi stimuli because it is analgesic.

The VTA's role in analgesia is controversial. Using the tail pressure test, Szreniawski, Meszaros, Gajewska, Tarchalska-Krynska (1977) found no direct involvement of the VTA in the primary mechanism of morphine analgesia. They based this conclusion on the fact that morphine increased pain thresholds in both VTA- and sham-lesioned rats. Similarly, Morgan and Franklin (1989) found that morphine produced potent analgesia in both sham- and VTA-6-hydroxydopamine-lesioned rats, while using the tail flick test. Finally, Altier and Stewart (1993) showed that intra-VTA infusions of DiME-C7, a substance P analog, failed to attenuate pain responses using the tail flick test. However, electrical stimulation of the VTA was found to be partially analgesic to tail pinching (Mayer & Liebeskind, 1974) and foot shock (Moreau, Cohen & Lieblich, 1983; Blake & Stein, 1987).

The tail pressure, tail flick, tail pinch and foot shock tests are all measures of phasic pain, or reflex withdrawal from noxious stimuli. According to Franklin (1989), the VTA may play a

greater role in tonic pain. Using the formalin test, a measure of tonic pain characterized by formalin injections into to the paw, Morgan and Franklin (1989) found that morphine did not produce analgesia in VTA-lesioned rats, only in sham-lesioned rats. Similarly, Altier and Stewart (1993) found that intra-VTA infusions of DiME-C7 also attenuated pain responses using the formalin test. The last two experiments suggest that activation of mesolimbic dopamine neurons may play a role in the suppression of tonic, but not phasic pain. In addition, the Gi is known to contain neurons that respond to noxious stimuli and is purported to be involved in the affective-motivational aspects of pain (Casey, 1969; Casey, 1971; Guilbaud, Besson, Oliveras & Wyon-Maillard, 1973; Cross, 1994). If the escape suppressive effect of our VTA electrode was due to the induction of analgesia, then this study represents the first account of cumulative and long-lasting analgesia following brain stimulation.

Unfortunately, this experiment does not reveal if rewarding or some other component of VTA stimulation is responsible for the suppression of aversion. Nevertheless, these results may have an impact for chronic pain therapy. Traditional medical and surgical interventions for chronic pain are often inadequate (Bonica, 1990a & b). Due to risks such as polysurgical addiction and iatrogenic dependency, many individuals, regardless of etiology, must simply learn to live with chronic pain (Bonica 1990a). Furthermore, prolonged experiences with pain have been shown to have quite a debilitating effect on personality functioning and adaptation. For example, depression and/or anxiety ensue in a majority of patients suffering from chronic pain syndrome (Sternbach, Murphy & Akeson, 1973; Turner & Romano, 1984; Romano & Turner, 1985). These inadequacies leave room for a more effective approach to chronic pain therapy.

The application of focal electrical brain stimulation in humans has provided many patients with excellent, long-term relief from certain kinds of chronic, disabling pain related to lower-back injury (Hosobuchi, 1980), cancer (Myerson, Boethius & Carlsson, 1979; Heath, 1964), lumbar-disc surgery (Plotkin, 1982), and painful dyskinesia (Andy, 1980).

Deep brain stimulation has been shown to be a viable chronic pain therapy, especially for whom conventional treatment has been unsuccessful, or undefined (Turnbull, 1984). The

advantages of deep brain stimulation in humans over more traditional forms of chronic pain therapy include the ability to activate second, or even third-order neurons which remain viable when primary sensory neurons have been destroyed by the lesion causing the pain (Turnbull, 1984). Secondly, this technique has proven helpful for irregular types of pain, such as phantom pain. For example, the sensory thalamus is organized in a way that allows the part of it representing the painful part of the body to be stimulated even if the painful region no longer exists (Turnbull, 1984). In addition, patients can use their stimulator daily for years without the threshold changing (Turnbull, 1984), and the platinum-iridium electrodes cause little tissue damage, even when implanted chronically (Gybels, Dom & Cosyns, 1980).

To date, the most common sites of deep brain stimulation therapy in humans include the periventricular grey matter and the thalamus. However, most stimulation-induced analgesia lasts for a maximum of eight hours before additional stimulation is required (Turnbull, 1984). Considering the high correlation between analgesic sites in animals and humans (Turnbull, 1984), the complete and permanent inhibition of aversion in six out of seven rats in this experiment suggests that electrical stimulation of the VTA may have therapeutic effects for those suffering chronic (motivational) pain.

Chapter Six

SUMMARY AND GENERAL DISCUSSION

General Discussion

Since the discovery of brain stimulation reward in 1954, researchers have attempted to determine if reward circuitry is composed of multiple, functionally different systems that may relate to separate natural reward functions. Investigations linking self-stimulation to natural rewarding events, as well as lesion, pharmacological and electrophysiological studies seem to favor the notion of multiple brain stimulation reward systems. Moreover, compelling evidence from a recent preference test conducted by Leon and Gallistel (1992) suggests that the medial forebrain bundle itself terminates in multiple reward-integrator systems. This conclusion was based on the observation that the function relating the subjective magnitude of brain stimulation reward to the current differed across electrode sites in the medial forebrain bundle within the same rat. Unfortunately, these authors could not discern if these multiple integrators ultimately converge on a common group of cell bodies or if a distinct group of cell bodies exists at various anatomical sites within the medial forebrain bundle.

The main objective of this series of experiments was to determine if multiple reward systems exist within the medial forebrain bundle. In order to accomplish this goal, rats were allowed to barpress for either LH or VTA threshold frequencies in a two-lever chamber. In a first experiment, the capacity of rats to discriminate between rewarding pulse frequencies delivered to the same reward site was determined using the psychophysical preference test. Results showed that identical rewarding signals are equally preferred by rats within 0.009 logfrequency units. Secondly, rats can discriminate between stimuli that differ by only 0.016 logfrequency units when delivered to a single reward substrate. Based on these criteria, it was hypothesized that if rats fail to equally prefer LH and VTA threshold frequencies, then each site may not belong to the same reward system. Such a situation can be expected when the two brain structures are involved in different reward functions and when these functions are differentially ranked in the animal's decisional scale. For example, as mentioned earlier, an animal may seek a sexual and a food reward with the same vigor if presented individually, but may show a clear preference if for one of them if presented with a choice.

In Experiment Two, we found that LH and VTA equipotent (threshold) frequencies were equally preferred at one third of the pairs of sites tested. At another third of the pairs of sites tested, rats failed to equally prefer the anterior and posterior medial forebrain bundle threshold frequencies. In three cases, the posterior site was preferred, whereas in the remaining cases, the anterior site was preferred. In these rats, the two electrodes may have activated systems related to different reward functions.

A collision test was conducted in Experiment Three between all pairs of sites tested previously in order to determine if the number of common reward fibres between electrodes was correlated with preference behavior. Unfortunately, a substantial collision effect was not noted in any of the subjects. Nevertheless, no correlation existed between modest collision effects or summation and preference behavior.

Lack of equal preference for equipotent anterior and posterior medial forebrain bundle stimuli may alternatively have been caused by reward-irrelevant variables such as the activation of aversive fibres by one electrode. Experiment Four showed that reward can indeed be decreased by aversion, at least temporarily. Curve-shift data showed that aversive and rewarding impulses combined together to produce a net rewarding effect. However, we concluded that aversive impulses were unlikely to affect preference behavior, due to their bi-directional interaction with rewarding impulses. This bi-directional interaction was characterized as follows: (a) initially, concomitant aversive Gi pulses increased the VTA self-stimulation threshold, (b) however, the capacity of Gi pulses to increase VTA threshold decreased progressively with repeated testing. Furthermore, in those rats tested for longer periods of time, the Gi pulses were shown to actually decrease the VTA self-stimulation threshold. Consistent with this interpretation is the finding that when subjects were stimulated in the escape chamber with only Gi pulses upon completion of all paired-pulse testing, they actually pressed the lever a few times. These last two observations indicate a reversal from Gi aversion to mild reward.

The preference test between VTA and VTA+Gi thresholds in all rats would have determined if the presence of a mixed effect in Experiment Two could prompt rats to choose the

more "pure" threshold frequency. Unfortunately, the VTA+Gi thresholds were not stable, thus preventing the preference test from being conducted. Nevertheless, preference data obtained in violation of the stability requirement with two subjects showed approximately equal preference for the same number of VTA and VTA+Gi pulses. We therefore concluded that the presence of a residual mixed effect did not alter the animal's choice for equally rewarding stimuli. Nevertheless, we cannot exclude the possibility that medial forebrain bundle-induced aversion, different from that of the Gi, could affect animals' choice.

In summary, rats failed to equally prefer anterior and posterior medial forebrain bundle threshold frequencies at at least one third of the pairs of sites tested. Secondly, the presence of Gi aversion could not account for these results. Therefore, the medial forebrain bundle may convey distinct rewarding signals that are ranked unequally on the subjects' decisional scale. In conclusion, this series of experiments supports the notion that multiple reward systems exist within the medial forebrain bundle.

Significance of Findings

These results suggest that the organization of brain reward circuitry may be much less redundant than previously thought. We have shown that a single pathway, namely the medial forebrain bundle, may house at least two distinct systems of reward. Uncovering the precise geography of these systems may help to explain why lesion experiments have been plagued by such contradictory findings. Specifically, in Experiment Two, we have seen that a slight lowering of an electrode can penetrate a new reward system, as evidenced by a shift in preference behavior. This finding suggests that unless the size and site of a lesion are very carefully controlled across studies, conclusions may differ dramatically. In closing, the role of reward in behavior is paramount. The ability to seek a variety of rewards, from food to sexual contact, is crucial to a species' survival. Having multiple, distinct reward systems in the brain is advantageous considering that a circumscribed lesion of one system is unlikely to affect motivation at all levels.

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