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The Amygdalo-Hypothalamic Interaction
in Brain-Stimulation Reward

Frank M. KANE

A thesis submitted to the School of Graduate Studies
of the University of Ottawa as partial fulfilment of
the requirements for the degree of Doctor of Philosophy



Frank M. Kane, Ottawa, Canada, 1991



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**This thesis is dedicated to my dad
Frank B. Kane (1922-1970)**

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Amygdalo-Hypothalamic Interaction in Brain-Stimulation Reward

Summary

In the first experiment, the extent and distribution of self-stimulation foci within the amygdala were mapped using moveable electrodes. Amygdaloid rate-frequency functions were obtained for all positive sites tested. The pulse period required to maintain criterial responding was then calculated using a curve fitting procedure. Self-stimulation was found throughout the amygdaloid complex, with the exception of the lateral amygdaloid nucleus. Depending on brain site, maximum self-stimulation rates varied from 3 to 37 presses/min whereas threshold frequencies varied from 9.2 to 40 pulses per train. No correlation was found between these two aspects of self-stimulation. The majority of threshold estimates lay within the range of 10 to 20 pulses per train, suggesting a relatively homogenous distribution of rewarding stimulation efficacy within the positive areas. The lowest threshold estimates are comparable to those usually obtained for the lateral hypothalamus, suggesting that the amygdala is an important focus for self-stimulation.

In a second experiment, the effect of concurrent stimulation of the amygdala and lateral hypothalamus was assessed using a curve-shift method. In general, combining equi-rewarding stimuli had no effect on self-stimulation rate and threshold. However, when current to the lateral hypothalamus was adjusted such that the hypothalamic pulses were subthreshold for self-stimulation, under concurrent amygdalo-hypothalamic stimulation, a dramatic increase in self-stimulation rate was obtained. The magnitude of this rate augmentation was positively correlated with hypothalamic pulse intensity, however, independent of the order of stimulus presentation

and intrapulse pair interval.

The rate enhancing effect of combining low intensity hypothalamic stimulation with threshold amygdaloid self-stimulation was subsequently investigated in a series of experiments. In the first experiment, subjects were given a choice between a pulse frequency yielding maximal amygdaloid rate and a series of higher pulse frequencies. Subjects consistently preferred the higher frequency values attesting that the maximal rate in the amygdaloid rate-frequency function was not constrained by a saturating reinforcing effect. In a second experiment, subjects were given a choice between amygdaloid stimulation and concurrent amygdalo-hypothalamic stimulation, using low intensity hypothalamic pulses. Subjects showed no preference for either stimulation condition, although rates were higher for the latter condition. These findings suggest that the maximal rate for amygdaloid stimulation was constrained by factors interfering with bar-pressing and that the effect of these factors was attenuated by co-activation of the lateral hypothalamus. The nature of this constraint was then investigated. Administration of Phenobarbital (10, 15 & 20 mg/kg; i.p.) mimicked the rate-enhancing effect of concurrent amygdalo-hypothalamic stimulation for 2 of the 4 subjects tested. This finding suggests that the hypothalamic pulses contributed to attenuate seizure activity accompanying amygdaloid self-stimulation. In a final experiment, amygdaloid self-stimulation rates were also increased by co-activation of rewarding sites in the rostral medial forebrain bundle but not the dorsal raphe suggesting an anatomical specificity of this effect.

It is concluded that within the circumscribed area of brain stimulation reward the amygdala is a separate reward system, the behavioral expression of which can be modulated by concurrent activation of rewarding foci in the lateral hypothalamus or medial forebrain bundle.

Table of Contents

Acknowledgements	iii
Summary	iv
List of illustrations	viii
List of tables	xi
Chapter 1:	
General Introduction	
Primary links between the amygdala and hypothalamus	2
Electrophysiological data: confirmation of a functional relationship between the amygdala and hypothalamus	3
Behavioral significance of the anatomic and electrophysiological data	6
Thesis research objectives	12
Chapter 2:	
Amygdaloid self-stimulation: a moveable electrode mapping study	
Introduction	22
Method	23
Results	27
Discussion	37
Chapter 3:	
The effects of concurrent amygdalo-hypothalamic stimulation: a moveable electrode mapping study	
Introduction	43
Method	44
Results & Discussion	47
Chapter 4:	
The rate-enhancing effect of amygdalo-hypothalamic self-stimulation: further investigation	

<i>Experiment 1: Rate limiting factors in amygdaloid self-stimulation</i>	
Introduction	75
Method	75
Results & discussion	77
<i>Experiment 2: Competition for amygdaloid versus amygdalo-hypothalamic stimulation</i>	
Introduction	83
Method	83
Results & discussion	84
<i>Experiment 3: The effects of Phenobarbital on amygdaloid self-stimulation</i>	
Introduction	90
Method	92
Results & discussion	93
<i>Experiment 4: Regional anatomic specificity of the rate enhancing effect of amygdalo-hypothalamic stimulation</i>	
Introduction	99
Method	99
Results & discussion	102
Chapter 5:	
Anomalies: implications for self-stimulation research	
Introduction	110
Results & discussion	110
Chapter 6:	
General Discussion	126
References	141

List of Illustrations

Chapter 2

- Figure 1:* Number of brain sites and asymptotic self-stimulation rate as a function of threshold frequency 30
- Figure 2:* Stimulation efficacy as a function of electrode position for subject's H72 and H68 32
- Figure 3:* Stimulation efficacy as a function of electrode position for subject's H69, H65 and H67 36
- Figure 4:* Location of dorso-ventral electrode positions for subject's H85 and H82 39

Chapter 3

- Figure 5a:* Location of amygdaloid and hypothalamic test sites for subject's A37, A26, A13, A31, A57 and A58 49
- Figure 5b:* Location of amygdaloid and hypothalamic test sites for subject's I89, I88, I81 and I65 51
- Figure 6:* Typical families of rate-frequency functions for the amygdala and hypothalamus 53
- Figure 7:* Rate-frequency functions under amygdaloid, lateral hypothalamic and concurrent amygdalo-hypothalamic stimulation conditions 56
- Figure 8:* Location of amygdaloid and hypothalamic test sites where concurrent "equi-rewarding" stimulation had no effect on self-stimulation threshold 58
- Figure 9:* Rate-frequency functions under amygdaloid, lateral hypothalamic and concurrent amygdalo-hypothalamic stimulation conditions 62
- Figure 10:* Location of amygdaloid and hypothalamic test sites where concurrent stimulation dramatically increased self-stimulation rates 64

<i>Figure 11:</i> Rate-frequency functions obtained under amygdaloid, lateral hypothalamic and concurrent amygdalo-hypothalamic stimulation with varying hypothalamic intensity	68
<i>Figure 12:</i> Rate-frequency functions obtained under amygdaloid and concurrent amygdaloid + lateral hypothalamic stimulation, with varying intervals and pulse order reversed	72
Chapter 4	
<i>Figure 13:</i> Histological data for subject's P83, P80 and P74	79
<i>Figure 14:</i> Bar-pressing rates for a fixed pulse frequency (the standard: corresponding with the asymptote of the amygdaloid rate-frequency function) and for a variable pulse frequency (the alternative) as a function of the frequency in the alternative stimulus	82
<i>Figure 15:</i> Histological data for subjects A57 & A58	86
<i>Figure 16:</i> Bar-pressing rates for a fixed frequency of amygdaloid pulses (the standard) and a variable frequency of amygdaloid and hypothalamic pulse-pairs, as a function of the frequency of the latter	88
<i>Figure 17:</i> Histological data for subject's P83, D75, D81 and D84	95
<i>Figure 18:</i> Effects of Phenobarbital on the amygdaloid rate-frequency function	97
<i>Figure 19:</i> Histological data for subject's B48, B50, B73 and B74	104
<i>Figure 20:</i> Rate-frequency functions for amygdaloid (AMY), medial forebrain bundle (MFB), dorsal raphe (DR), and concurrent AMY+MFB, AMY+DR and AMY+ hippocampal stimulation	106
Chapter 5	
<i>Figure 21:</i> Location of amygdaloid and lateral hypothalamic stimulation sites the concurrent stimulation of which resulted in spurious lateral shifts of the amygdalo-hypothalamic self-stimulation function	112

<i>Figure 22:</i> Amygdaloid (AMY), lateral hypothalamic (LH), medial forebrain bundle (MFB) and concurrent AMY+LH and AMY+MFB rate-frequency functions under low and high intensity amygdaloid stimulation conditions	114
<i>Figure 23:</i> Hypothalamic and concurrent amygdalo-hypothalamic self-stimulation functions at two different hypothalamic pulse intensities	116
<i>Figure 24:</i> Families of amygdaloid rate-frequency functions obtained at different pulse intensities	120
<i>Figure 25:</i> Effect of stimulation of the lateral amygdaloid nucleus on the hypothalamic self-stimulation function	123

List of Tables**Chapter 3**

Table 1: Threshold frequencies (X_i) and asymptotic rates (A_s) obtained from rate-frequency data fitted with Gompertz's model 59

Table 2: Threshold frequencies (X_i) and asymptotic rates (A_s) obtained from rate-frequency data fitted with Gompertz's model 65

Table 3: Threshold frequencies (X_i) and asymptotic rates (A_s) obtained from rate-frequency data fitted with Gompertz's model 69

Chapter 4

Table 4: Threshold frequencies (X_i) and asymptotic rates (A_s) obtained from rate-frequency data fitted with Gompertz's model 107

CHAPTER 1:
General Introduction

General Introduction

Primary links between the amygdala and hypothalamus

The amygdala and hypothalamus are two distinct groupings of nuclei located in the rostral temporal lobes and ventral diencephalon, respectively. Two main fiber systems link the amygdala to the hypothalamus: the stria terminalis and the ventral amygdalofugal pathway.

The stria terminalis arises predominately from the cortico-medial portion of the amygdala and, in part, from the basal amygdaloid nuclei, with a small contribution from the lateral amygdaloid nucleus. Sites of termination include the ventromedian nucleus of the hypothalamus, the pre-commissural area of the septum and, in part, the ventral pre-mamillary area (Lammers, 1972). Heimer and Nauta (1969) have identified a further subdivision based on a pre- and post-commissural component with the pre-commissural component terminating in the medial preoptic area and anterior hypothalamic region as well as in the ventromedial hypothalamic nuclei and ventral pre-mamillary region. The caudal component of the stria terminalis terminates in the bed nucleus of the stria terminalis. It also sends collaterals to the anterior hypothalamus (Heimer & Nauta, 1969). The sites of origin of the post-commissural component are in the medial and basolateral amygdala (Leonard & Scott, 1971). The stria terminalis also contains afferents originating in the hypothalamic region and in the bed nucleus of the stria terminalis (Nauta, 1961; Cowen, et al., 1965; Van Alpen, 1969); these fibers terminate in the corticomедial as well as in the basolateral nuclei of the amygdala.

The ventral amygdalofugal pathway projects to the medio-frontal cortex, the nucleus

accumbens, the olfactory tubercle, the diagonal band of Broca, as well as the preoptic and anterior hypothalamic area (Cowen et al., 1965). In the lateral preoptic and hypothalamic regions, these efferents merge with the medial forebrain bundle (MFB). In addition, a portion of these fibers (ventral amygdalofugal) turn dorsally to end in the dorsomedial thalamic nuclei (Lammers, 1972).

The amygdaloid nuclei also have connections with MFB fibers that descend through the lateral hypothalamus to reach the ventral tegmental area and more caudal regions including the substantia nigra and periaqueductal grey (Hopkins & Holstedge, 1978; Krettek & Price, 1978).

Electrophysiological data: confirmation of a functional relationship between the amygdala and hypothalamus

Links between the hypothalamus and amygdala have been confirmed by electrophysiological studies. Researchers employing extracellular microelectrode techniques have observed neuronal responses in various parts of the hypothalamus following amygdaloid stimulation (Dreifuss & Murphy, 1968; Murphy, 1968; Dreifuss, 1972; Egger, 1967 & 1972; Gloor, 1975; Renaud, 1976 a & b; Dauth et al., 1976; Oomura et al., 1970).

Early studies focused on the relationship between the ventromedial hypothalamus (VMH) and the amygdala. For example, Dreifuss, Murphy & Gloor (1968) found evidence of an antagonistic control of the amygdala on VMH activity in the cat. With stimulating electrodes located in the amygdala and recording electrodes in the VMH two patterns of activity were obtained from VMH neurons following stimulation of different areas of the amygdala.

Stimulation of the basolateral amygdala resulted in an increase in VMH firing rates (a short latency activation followed by a period of inhibition) whereas stimulation of the corticomедial amygdala inhibited spontaneous VMH activity. Moreover, knife cuts revealed that these antagonistic influences were mediated via two distinct pathways: the ventral amygdalofugal and stria terminalis, respectively.

This antagonistic control, however, has not been replicated in the rat (Renaud, 1976a). Stimulation of the amygdala and stria terminalis (in the rat) evoked similar VMH response profiles regardless of the location of the stimulation focus within the amygdala; most neurons studied exhibited either excitation or excitation-inhibition sequences; other VMH cells were inhibited by the stimulation. In addition, lesions of the stria terminalis eliminated these modulatory effects suggesting that the source of any modulatory control of VMH activity originates in amygdaloid nuclei that contribute fibers (either directly or indirectly via internuclear connections) to the stria terminalis. Anatomical or functional divisions within this pathway may account for differential effects on VMH activity (Renaud, 1976a).

Amygdaloid stimulation also elicits single-cell discharges of hypothalamic neurons in areas outside the ventromedial nucleus (Egger, 1967; Murphy, Dreifuss and Gloor, 1968; Van Atta and Sutin, 1971, 1972). For example, Van Atta and Sutin recorded from single units in the lateral hypothalamus following amygdaloid stimulation and found that patterns of hypothalamic responses varied depending on the location of the recording electrode and the frequency of the amygdaloid stimulation. Repetitive stimulation, in particular, greatly modified the response patterns of hypothalamic neurons: depending on the units studied and the frequency of the stimulation, either an increase or decrease in lateral hypothalamic excitability profiles was

observed. This has led Gloor (1975) to suggest that "the influence of the amygdala upon hypothalamic neurons is thus highly complex, excitatory and inhibitory influences being exerted upon a large population of hypothalamic neurons, the type of response being partially dependent upon the part of the amygdala stimulated and partially upon the rate of repetitive stimulation. To what extent these complexities reflect the normal conditions ... remains unknown" (Gloor, 1975, p.30).

More recent research has focused on the interaction between the amygdala, VMH and lateral hypothalamic nuclei. Spontaneous firing rates of lateral hypothalamic units are typically reduced by stimulation of the basolateral amygdaloid nucleus (Egger, 1967; Oomura et al., 1967; Van Atta & Sutin, 1972). Increased firing rates or a sequence of excitation/inhibition (similar to that recorded in the VMH) have also been observed (Egger, 1967; Murphy et al., 1968; Van Atta & Sutin, 1971). It is unclear whether lateral hypothalamic activity is directly influenced by amygdaloid activity or whether modulatory influences are mediated by connections between the VMH and lateral hypothalamus. In an attempt to answer this question Oomura and Ono (1982) recorded evoked potentials in the lateral hypothalamus following stimulation of the basolateral nucleus of the amygdala. They obtained a negative-positive wave which increased as the recording electrode was lowered into the lateral hypothalamus. Differences in spatial distribution of the negative and positive aspects of the evoked potential as well as differences in thresholds for these two forms suggested that these effects were mediated by different pathways. Furthermore, lesions of the VMH left the pattern relatively intact suggesting that the effects are mediated by a direct amygdalo-lateral hypothalamic pathway. Subsequent lesions of the stria terminalis eliminated the positive aspect of the wave but not the negative one, indicating that

these two effects are mediated by separate pathways. These data suggest that the inhibition typically observed following stimulation of the basolateral nucleus is mediated via a direct connection between the basolateral nucleus and the lateral hypothalamus. The effect of stimulation of other regions of the amygdala on lateral hypothalamic neurons was not, however, considered by these investigators.

Behavioral significance of the anatomical and electrophysiological data

Comparatively few studies have been done on the behavioral significance of an amygdalo-hypothalamic interaction. Theoretical accounts have attempted to differentiate the respective roles of these structures. Some authors have proposed that the amygdala might modulate hypothalamic activity (Mogenson, 1973) while others have postulated that the amygdala might be a link or an interface between cortical centers and diencephalic control systems (Powell & Hines, 1974).

Various authors have found evidence for an amygdalo-hypothalamic interaction in the control of eating (Fonberg, 1974; Fonberg, 1975; Kling & Hutt, 1958; Morgane & Kosman, 1960; Sclafani, 1970; White & Fisher, 1969; Montgomery & Singer, 1975) and drinking behaviour (Grossman, 1964; Singer & Montgomery, 1969).

Early work identified the amygdala as a satiety center (eg. Kling & Schwartz, 1961). Electrical stimulation of this structure sometimes resulted in lowered food intake (Fonberg & Delgado, 1961; Grossman, 1964; Robinson & Mishkin, 1962). This suggested to some authors that the amygdaloid influence on food intake acted via the hypothalamic "feeding centers". In

an attempt to clarify the relationship between the amygdala and hypothalamus in the control of eating, White & Fisher (1969) evaluated the effects of stimulation of the amygdala and lesions of the VMH. Continuous bilateral stimulation of the cortico-medial-pyriform transition zone was first shown to suppress food intake in deprived rats. Interestingly, destruction of the VMH or severance of the stria terminalis prevented the suppression of eating by amygdaloid stimulation. These authors proposed that the amygdaloid stimulation acts via the stria terminalis to increase the level of activity in the VMH. This in turn results in a suppression of lateral hypothalamic activity which results in lowered food intake. Two references in support of this hypothesis are cited (Anand, 1963; Oomura et al., 1964).

Sclafani et al. (1970) have shown that rats with VMH lesions, in addition to becoming hyperphagic, showed a decreased latency to eat in a novel environment. Lesions of the corticomедial amygdala also inhibited feeding in a novel environment, however, had no effect on ad lib food intake in the home cage. Rats with combined lesions of the amygdala and VMH responded to food like VMH lesioned animals suggesting that the influence of the amygdaloid mechanisms on feeding may be mediated by the VMH.

More extensive lesioning of the amygdaloid complex has suggested that the amygdala both facilitates and inhibits alimentary function in dogs (Fonberg, 1974). Lesions restricted to the dorsomedial amygdala produced aphagia with adipsia, decreases in body weight and impairment of both classical and instrumental retention. Similar results were obtained following lesioning of the lateral hypothalamus. Lesions involving the lateral portion of the amygdala produced hyperphagia and an increase in body weight: a syndrome typically associated with the VMH. Moreover, combined lesions of the amygdaloid and hypothalamic nuclei greatly

exaggerated these effects. Finally, lesions of the lateral amygdaloid area following lesions of either the dorsomedial amygdala or lateral hypothalamus restored food intake and instrumental reactions. In discussing these data in a subsequent paper, Fonberg (1975) has suggested several hypotheses that may explain these results including interaction effects between the amygdala and hypothalamic "feeding and satiety centers". More recently, Fonberg (1981) has suggested that alterations in alimentary function following dorsomedial ablation may be due to diminished motivation or a decrease in the reinforcing value of the food. This latter hypothesis is consistent with the more extensive literature on the role of the amygdala in learning and complex behaviours (eg. Cormier, 1981; Isaacson, 1982).

Data on the effects of noradrenergic stimulation of the amygdala and lateral hypothalamus provide further support for a close amygdalo-hypothalamic involvement in the control of eating (Montgomery and Singer, 1975). Injections of noradrenaline increased food intake in the satiated rat. Concurrent noradrenergic stimulation of the amygdala cortical nucleus augmented this increase. However, simultaneous anti-adrenergic blockade in the amygdaloid cortical nucleus reduced eating to control levels. The lack of response of the amygdaloid cortical nucleus to adrenergic stimulation in the satiated rat, under simultaneous stimulation of the lateral hypothalamus with either a placebo or an adrenergic blocker, suggest that the cortical nucleus of the amygdala exercises a modulatory influence on eating behaviour which is dependent on the level of activity in the lateral hypothalamus. This modulatory control of the amygdala over cholinergic mechanisms in the lateral hypothalamus had been shown previously for drinking, initially by Grossman (1964), and later by Singer and Montgomery (1969).

The amygdalo-hypothalamic interaction has also been investigated in self-stimulation.

In one study (Szabo et al., 1972), the effect of concurrent amygdaloid stimulation on lateral hypothalamic self-stimulation rates and thresholds was examined. Ipsilateral amygdaloid stimulation increased bar-pressing rates and decreased thresholds for lateral hypothalamic self-stimulation. This was interpreted as evidence that the amygdala facilitated hypothalamic reward. A complementary experiment utilizing contrast elation measures (negative & positive) supported this interpretation. Furthermore, it was suggested (by these authors) that the amygdala represents a secondary rewarding focus; amygdaloid self-stimulation results from a direct or indirect activation of the lateral hypothalamic-medial forebrain bundle reward circuitry. Some of the electrophysiological data reported above are consistent with this hypothesis.

However, in the monkey (Jackson & Gardner, 1974) concurrent amygdaloid stimulation had no effect on lateral hypothalamic self-stimulation when the stimulating electrode was located in the basolateral amygdaloid nucleus. An inhibitory effect on lateral hypothalamic self-stimulation, characterized by a decrease in response rate and an increase in threshold, was observed when the test site was located in the cortico-medial nucleus.

One explanation for these contradictory data (apart from species difference) is rooted in methodological differences between the two experiments. For example, Szabo et al. (1972) employed "biologically weak" stimulation of amygdaloid sites that supported self-stimulation. The amygdaloid sites tested in the Jackson & Gardner (1974) experiment apparently did not support self-stimulation. Moreover, the location of the stimulating electrodes as well as the frequency and duration of the limbic stimulation may have been critical factors in the latter experiment. These investigators used long duration high intensity stimulation of various limbic foci including the amygdala.

An implicit assumption of both studies is that the amygdala (Szabo et al., 1972) and the hippocampal-amygdaloid complex (Jackson & Gardner, 1974) modulate lateral hypothalamic reward related activity. Support for this hypothesis (within the circumscribed area of self-stimulation) was provided by Kelly (1974) who produced bilateral lesions in the basolateral region of the amygdala in the rat. This resulted in an attenuation or complete abolishment of hypothalamic self-stimulation. However, this effect was transient and a return to normal threshold values was observed within a few days. To further investigate amygdaloid modulation of hypothalamic self-stimulation, Kelly (1974) injected local anaesthetic (5% procaine hydrochloride) bilaterally into the amygdala. Within 60 sec. of the injection the subjects stopped hypothalamic self-stimulation altogether, and self-stimulation could not be reinstated until higher current was delivered. In discussing these findings, Rolls (1975) suggests that the amygdala appears to modulate rather than control brain stimulation reward - a suggestion that is consistent with the finding of Ward (1961) that bilateral ablation of the amygdala does not prevent the appearance of tegmental self-stimulation from electrodes implanted later. Parallel findings have been obtained from the eating literature. Stimulus bound eating and drinking produced by lateral hypothalamic stimulation was also found to be attenuated or abolished by anaesthetization of the amygdala (Rolls, 1975), with control experiments showing no effect on locomotor activity. However, lesion studies, especially those performed by Rolls & Rolls (1973 a,b) have shown that the basic controls of eating and drinking (including homeostatic control) are not directly impaired by amygdaloid lesions. Only in particular situations in which previous experience appears to modulate or control such behaviours did abnormalities emerge. These experiments "indicate that when previous experience affects ingestive behaviour, as in neophobia and learned

aversion, the amygdala is involved " (Rolls, 1975,P.56). Rolls (1975) further proposes that "because of the close relationship of the amygdala to reward, it is likely that it controls food intake on the basis of previous experience by adjusting reward level". This suggestion is consistent with the above-cited findings that show a sudden cessation of hypothalamic self-stimulation until a new set of reward parameters are introduced. Thus the amygdala "can be considered as a system which allows a sensory stimulus to influence reward level" (Rolls, 1975, P.57).

However, the posited modulatory function of the amygdala and the contention that amygdaloid self-stimulation is a secondary consequence of an indirect activation of the lateral hypothalamic-medial forebrain bundle circuitry can be challenged by the following data: the threshold (frequency) for amygdaloid self-stimulation is typically lower than that for the hypothalamus using comparable current (Kane et al., in press). Furthermore, Rolls, Burton and Mora (1980) have shown that in addition to lateral hypothalamic neurons firing following stimulation of rewarding foci in the amygdala, amygdaloid neurons are also activated by stimulation of self-stimulation sites in the lateral hypothalamus, nucleus accumbens, mediodorsal nucleus of the thalamus and orbitofrontal cortex. This would suggest that these interconnected sites are part of a common reward circuit; the finding that lateral hypothalamic and amygdaloid reward neurons have similar refractory periods (Rolls, 1972 and personal unpublished data) further supports this conjecture.

More recent research (Waraczynski et al., 1990) has suggested that amygdaloid and caudal medial forebrain bundle self-stimulation may be subserved by different directly stimulated substrates. Extensive unilateral lesioning of the amygdala had no effect on lateral hypothalamic

or ventral tegmental area self-stimulation thresholds (for all but one subject) intimating that the directly stimulated substrate subserving amygdaloid and caudal medial forebrain bundle self-stimulation may be differentially organized or are subserved by a different reward integrator.

Finally, the possibility that the amygdala represents a separate reward system cannot be discounted since the apparent modulatory control cited above may have been attributable to processes other than reward (performance factors, for example).

Thesis research objectives, theoretical orientation and general methodology

The basic technique and methods employed in this research evolved from the early work of Deutsch (1964). Deutsch trained animals to bar-press at 'threshold' sustained rates for trains of paired pulses. The first pulse of each pair is called the C or conditioning pulse; the second is called the T or test pulse. The interval between the pairs of pulses (the C to T interval) was then varied and the resulting effect on the animal's self-stimulation performance monitored. Deutsch reasoned that if the T pulse followed the C pulse too closely then it would find the directly stimulated fibers refractory to further stimulation; at longer delays both pulses should be equally effective in eliciting neuronal responses. This latter configuration would be analogous to doubling the frequency of the stimulation resulting in an increase in the reward efficacy of the stimulation. By systematically varying the C to T interval Deutsch was able to identify the shortest interval that produced a measurable difference in the reward efficacy of the stimulation manifested as an increase in self-stimulation rate. This interval was believed to reflect the refractory period of neurons subserving the self-stimulation response. Two companion

experiments (a preference experiment and a voltage scaling procedure) failed, however, to replicate the original estimates. A more sophisticated method of assessing the effectiveness of the T pulse (and thus refractoriness, unconfounded by the use of rates as the dependent variable) was later developed (Yeomans, 1975).

The basic logic of Yeomans's approach has its origins in classical psychophysics. His method requires that T pulse effectiveness be inferred not from a consequent effect on rates but rather by the compensatory change in a second electrical stimulation parameter (pulse frequency) required to sustain a constant self-stimulation performance. Yeomans's model assumes a monotonic relationship between pulse frequency and reward efficacy. Decreases in paired pulse effectiveness at short C to T delays (because of refractoriness) is evidenced by an increase in the number of paired pulses required to maintain threshold rates. The more paired pulses required at a particular C to T interval the less effective the T pulse. Conversely, at long C to T intervals both pulses are equally effective. By carefully contrasting the required frequency for single versus paired pulses at various C to T delays Yeomans was able to obtain a more precise estimate of refractoriness.

The double pulse technique was subsequently used by Shizgal et al. (1980) to determine whether two substrates that support self-stimulation along an identified neural pathway were anatomically linked. They employed a collision paradigm based on the classic work of Lucas (1913). In their design electrodes were located at a distance from one another along the trajectory of the medial forebrain bundle. The double pulse technique described above was then performed except that in this experiment one pulse of each pair was delivered through one electrode and the second pulse through the second electrode. As before, the within pair interval

was varied and effectiveness evaluated using pulse frequency as the scaling "off-set" variable. Effectiveness varied as a function of delay suggesting that at a critical delay some of the excitation originating from one of the electrodes collided with excitation originating from the other, hence, the term collision. The collision interval was subsequently used to estimate conduction velocities, the data of which was used to infer axonal properties (Bielajew and Shizgal, 1982). In addition to cases of collision, summation effects in some studies have been documented (reviewed in Shizgal and Murray, 1989 and documented below). Summation effects imply that the reward relevant neural signals generated at the two electrodes converge on a common reward integrator.

A fundamental assumption underlying most parametric work in contemporary self-stimulation research is the counter model of reward integration (Gallistel, 1978). According to this model the integrator subserving self-stimulation sums the neural effects of pulses. With current fixed (and hence the size of the excitation field) the number of action potentials generated by the electrical stimulus is proportional to the number of pulses in a train of fixed duration. The trade-off between current and frequency (Gallistel, 1978; Gallistel, Shizgal & Yeomans, 1981) supports this model. Decreases in current are typically compensated for by increases in frequency. In fact, in most experiments (including those performed in our own lab) a linear function is obtained. The integrator thus appears to sum the neural effects of pulses independent of the spatial or temporal configuration of the pulses. This model has important heuristic value because it allows for a quantitative evaluation and neurophysiologically based interpretation of the effects of brain stimulation. If following, for example, a transection of a fiber bundle a two fold increase in the number of pulses is required to maintain a predetermined (critical) self-

stimulation performance then it can be said that the effectiveness of the stimulation has been halved. Moreover, this model can be used to estimate the density of reward relevant neurons in the vicinity of the tip of a stimulating electrode (Miliaressis et al., 1982; Rompré and Miliaressis, 1985). The more pulses required to maintain criterial rates, and assuming homogeneity with respect to membrane thresholds, the less behaviorally relevant neurons are believed to reside within the effective radius of the current.

Constraints, however, have recently been imposed on the model. Schindler (1983) has shown that the trade-off between current and frequency occasionally fails; electrode placement has been identified as one source of nonreciprocity. For example, electrodes located near the rim of the medial forebrain bundle are less effective than electrodes located centrally (Yeomans, et al., 1988). In addition, summation both within the medial forebrain bundle and bilaterally is only partial (Bielajew & Shizgal, 1982; Shizgal et al., 1980; Fouriezos & Wise, 1984). Spatial summation also varies as a function of inter-electrode distance within the medial forebrain bundle: the further apart the electrodes, and therefore the stimulation sites, the less summation (Fouriezos, 1981). Finally, temporal summation is not always perfect. Frequencies in excess of 200 Hz alter refractory period data (Yeomans & Davis, 1975). Despite these shortcomings the counter model remains the most plausible and parsimonious explanation of the "intensity-number" trade-off data.

According to Gallistel (1988), the self-stimulation preparation is an amalgam of processes that extends from the initial neural effects of the stimulation to a response generation stage. Intermediate stages include spatial-temporal integration and a conversion process that transduces some aspect of the signal into an engram - the record of reward. This quantity

combined with the initial energizing effect of the stimulation (conventionally referred to as prime) determine in the words of Gallistel "reward affinity". This latter quantity combined with performance factors (such as the health of the subject, performance obstacles etc.) determine speed of running, bar-pressing rate etc. The rate at which an animal performs for a given level of reward does not depend solely on the reward value of the stimulation but rather on an amalgam of processes that trade-off experiments such as the ones described above attempt to penetrate.

A method of evaluating the reward specific effects of a drug or other manipulation such as a lesion on self-stimulation performance has been proposed by Edmonds and Gallistel (1974). These authors plotted running speed (in a runway apparatus) as a function of number of pulses in train of fixed duration and obtained a sigmoidal shaped speed-frequency function. Systematic increases in the number of pulses in the reward train (obtained at the goal end of the run way apparatus) increased speed of running until a maximal running rate was observed. Subjects were then challenged with several performance variables (including low pre-trial priming stimulation, an increase in the grade of the runway, and partial paralysis induced by curare or methocarbamol) and the rate-frequency function determination repeated. Interestingly, none of these performance hindering variables appreciably altered the location of the sharp rise in the self-stimulation function. Rates, however, decreased and asymptotic performance varied as a function of the intensity of the performance variable. A decrement in current (the amplitude of the pulses), however, shifted the curve to the right (towards higher pulse frequency values) indicating that a decrease in the size of the stimulation field and thus the number of neurons fired by the stimulation decreased the reward value of the stimulation. The resultant curve shift

is a further example of a trade-off described above: Increases in pulse frequency were required to offset the decrease in current and thus reinstate the self-stimulation performance function. The power of the curve shift technique is thus its apparent ability to penetrate the amalgam of performance factors that intervene between the first stage neurons and performance. Subsequent validation work (Miliaressis et al., 1986; Miliaressis & Rompré, 1987; Malette & Miliaressis, 1990) has further substantiated this claim.

The explanation for why performance challenges fail to shift the locus of rise of the self-stimulation function is related to the narrow range within which the function reaches its asymptote. Initially it was thought that reward does not rise beyond the stimulus value for which the self-stimulation performance function reaches an asymptote (Gallistel, 1983 & 1988; Campbell et al., 1985). Experimental manipulations unrelated to reward cannot shift the function beyond the frequency at which the self-stimulation function reaches its maximum (typically between .2 and .3 log units). This assumption has since been challenged by two choice experiments (Miliaressis & Malette, 1987; Waraczynski et al., 1987). In these experiments, subjects were given a choice between a fixed pulse frequency corresponding with the asymptote of the rate-frequency function and higher pulse frequencies. Both studies showed that reward (as reflected in preference) continued to increase beyond the point at which the performance function reaches its maximum (in the order of $0.20 + \log$ units in the latter study) indicating that performance factors may, in fact, shift curves. However, despite this finding, these researchers agree that this failure of the performance function to faithfully follow the underlying reward summation function does not by itself present a serious challenge to the paradigm since validation work done to date has not revealed any significant shift (greater than

.1 log units) following performance challenges (Campbell et al., 1985; Edmonds & Gallistel, 1974; Miliaressis et al., 1986; Stellar & Neeley, 1982; Yanovski et al., 1987). Moreover, Miliaressis & Malette (1987) have argued that such a constraint on the curve-shift paradigm may not really be necessary. They base their argument on the observation that the rate-frequency function typically rises within a relatively narrow range (an interval of approximately 0.3 log units or less). Preference for pulse frequencies within this range (the dynamic interval of the self-stimulation function) indicate that reward summates steeply. Thus, summation properties of the substrate make lateral shifts following performance challenges unlikely because such challenges can be compensated for by small increases in pulse frequency.

Fouriezos et al. (1990) have collected data that impose statistical constraints on the interpretation of curve-shift data collected in the Skinner box. Increasing lever weight and thus the force required to activate the manipuladum in a Skinner box was shown to shift rate-frequency curves in a weight dependent manner. Maximum shifts did not, however, exceed 0.1 log frequency units. This finding has important statistical value since curve shifts within this range cannot be unambiguously interpreted.

To date the curve-shift paradigm has been used to test the specificity of drugs on self-stimulation behaviour and the results of knife cuts and lesions of pathways and nuclei suspected of either carrying or giving rise to the neurons subserving self-stimulation. In the former, neuroleptics that block dopaminergic transmission centrally (eg. Pimozide) have shifted curves in a dose dependent manner (eg. Miliaressis et al., 1986). The curve-shift method has been used to test the effect of medial forebrain bundle lesions on self-stimulation reward obtained from this bundle (eg. Stellar and Neeley, 1982; Janas and Stellar, 1984; Waraczynski, 1988) and

consequently have pointed the way towards the identification of the origin of the hypothalamic reward circuit. Glimcher (1989) used lesions in combination with psychophysical methods and neuroanatomical tracing techniques to identify the source of the self-stimulation pathway; his data suggest that cell bodies within the dorsal medial hypothalamic area give rise to the first stage fibers subserving medial forebrain bundle self-stimulation reward. The curve-shift method has also been used by Rompré & Miliareisis (1985) to map the substrate for self-stimulation in the caudal brain stem.

The present series of experiments utilizes the curve-shift measurement procedure in combination with moveable electrodes in order to further elucidate the role of the amygdala in self-stimulation reward and its relationship to the hypothalamic-medial forebrain bundle self-stimulation substrate.

The first experiment was designed to assess the relative importance of the amygdala as a focus for self-stimulation using a quantitative rather than qualitative approach. This approach is particularly significant in light of previous claims that the amygdala plays a secondary role in self-stimulation (Szabo et al., 1972; Rolls, 1975).

The second experiment was undertaken to reevaluate the effect of concurrent activation of the amygdala and lateral hypothalamus. The rationale behind this experiment is that if amygdaloid and hypothalamic self-stimulation foci are linked to a common reward integrator or if as Szabo et al. (1972) has suggested amygdaloid self-stimulation is a secondary consequence of an indirect activation of the hypothalamic reward circuitry, then combining stimulation of these two substrates should shift rate-frequency curves to the left; fewer pulses should be required to sustain threshold amygdalo-hypothalamic self-stimulation. Put differently, combining

amygdaloid and hypothalamic self-stimulation should decrease pulse frequency threshold for self-stimulation. The advantage of using the curve-shift method rather than single threshold measurements is that this procedure allows one to monitor, in addition to changes in threshold frequency (inferred from the location of the curve on the x-axis), the dynamic interval of the function and its asymptote. A simple threshold statistic obscures these latter variables.

It is noteworthy that summation effects reported previously in the literature depend on such factors as the location of the electrode tip (see above). Furthermore, electrophysiological data reviewed earlier indicate that amygdaloid regions differ with respect to their potential modulatory influence on hypothalamic activity. To test the possibility of dual effects (similar to those described in the electrophysiological and more general neurobehavioral literature) moveable electrodes were used and the experiment repeated at a number of sites throughout the amygdala, including the stria terminalis.

Finally, supplementary experiments were performed to test the validity of interpretations drawn from the curve-shift data as well as to elucidate the nature and regional anatomical specificity of any interaction.

Taken together these data are expected to provide a more accurate portrait of the role of the amygdala in brain stimulation reward and its relationship to the hypothalamic-medial forebrain bundle reward circuit.

CHAPTER 2:

**Amygdaloid self-stimulation:
A moveable electrode mapping study**

Introduction

Relatively few studies have been devoted to the involvement of the amygdaloid complex in self-stimulation. Early attempts at mapping this area for self-stimulation began with Wurtz & Olds (1963) who reported self-stimulation with electrodes in the medial, central, and cortical nuclei, and escape with electrodes in the lateral and basolateral nuclei. More recently, Prado-Alcala and Wise (1984), in an effort to further elucidate the role of dopamine in self-stimulation of various brain regions, identified several sites in the anterior amygdaloid area and basal amygdaloid nucleus that supported self-stimulation; in addition, a few sites in the lateral nucleus were also positive for self-stimulation. High thresholds and low rates characterized these latter reward sites. Interestingly, a positive correlation between catecholamine density (as revealed by fluorescence histochemistry) and self-stimulation thresholds was also found (Prado-Alcala and Wise, 1984).

Miliaressis et al. (1982) proposed the use of the curve shift paradigm in mapping neural substrates of self-stimulation. In this paradigm, the rewarding efficacy of the stimulation is inferred from the lateral position of the function relating bar-pressing rate to the frequency of stimulating pulses. The frequency of short cathodal rectangular pulses (delivered in a train of constant duration) was proposed as the stimulus variable because the number of action potentials generated in the directly excited neurons is directly proportional to the magnitude of this variable. Pulse intensity (an alternative parameter commonly used) lacks this simple but essential attribute. Furthermore, the rewarding effect of a constant-duration burst of pulses is believed to depend solely on the total number of action potentials generated in the directly excited neurons (Gallistel, Shizgal & Yeomans, 1981). Thus, the lateral position of the rate-

frequency function represents a quantitatively undistorted and physiologically interpretable index of the rewarding stimulation efficacy.

In the present study, we used moveable electrodes and a curve-shift method to determine the rewarding efficacy of amygdaloid stimulation.

METHOD

Subjects:

Seven male Long-Evans rats weighing approximately 300 to 325 g at the time of surgery were used. The subjects were housed individually in plastic cages under a 12 hr on / 12 hr off lighting schedule. Food and water were available ad lib throughout the experimental period, except during testing.

Materials:

Subjects were tested in an operant box, located within a sound attenuating chamber, constructed of clear Plexiglass with a metallic grid floor. A lever (2 x 3 cm) protruding from one wall, 6 cm. above the floor, was connected to a constant current stimulator programmed to deliver rectangular cathodal pulses of fixed duration (0.1 msec) and variable frequency; current and train duration were fixed at 200 μ A and 400 msec., respectively. Stimulation parameters were controlled by a microcomputer through a digital interface. In addition to controlling the stimulator, the computer provided all the timing functions and also recorded the bar-press responses of the subjects.

The stimulating electrodes were of two types: monopolar moveable electrodes (0.25 mm in dia.)(Model SME-01, Kinetrods, Ottawa) and fixed monopolar constructed from stainless steel

wire (0.25 mm in dia.) and capped with a miniature amphenol pin. Both electrodes were insulated with EpoxyLite except for the conically shaped tip.

Procedure

Surgery:

Subjects were stereotaxically implanted (under general anaesthesia with sodium pentobarbital; 50 mg/kg) with a moveable monopolar electrode aimed at the dorsal surface of the amygdala and a fixed monopolar electrode aimed at the contralateral lateral hypothalamus. With the skull held horizontal, hypothalamic coordinates were 1.9 mm posterior to bregma, 2 mm lateral to the midline and 7.9 mm below skull surface. Various anterior-posterior and medial-lateral coordinates for the amygdaloid implants were selected so as to provide a representative sampling of the nuclei that comprise the amygdaloid complex (see histology). The current return consisted of flexible stainless steel wire wrapped around 4 stainless steel skull screws and soldered to an Amphenol miniature plug. The electrode assembly was chronically fixed to the skull with dental cement.

Training & Testing:

Phase 1:

One week following surgery, subjects were trained to bar-press for electrical stimulation of the lateral hypothalamus. Stimulation frequency was adjusted until stable responding on a continuous reinforcement (CRF) schedule was observed. Rate-frequency functions were then obtained by systematically varying pulse frequency (in steps of approximately 0.05 log units) in two alternate ascending and descending sequences. An average of 16 pulse frequencies covering the full range of responses of the subject (ie. from no responses to maximal response rate) were

tested. Each rate-frequency function was based on the mean of 4 sweeps. Immediately preceding each trial or pulse frequency test the animals received five non-contingent priming stimulations (1 prime per sec); the pulse frequency of the priming stimulation was the same as that used for the succeeding trial. Each trial was 60 sec in duration (during which stimulation at the predetermined frequency was available) followed by a 60 sec time-out period (during which stimulation was not available). A 0.1 sec interval was imposed between the depression of the lever and delivery of the pulse train. Rate-frequency function determinations were repeated until stable threshold and asymptotic performance were achieved.

Phase 2:

During phase 2 of the experiment, subjects initially bar-pressed for lateral hypothalamic stimulation on a CRF schedule (warm-up period); the stimulation was then diverted to the contralateral amygdaloid electrode through a separate channel and lead (the lead to the lateral hypothalamus was disconnected). If the subject continued to bar-press for stimulation of the amygdala, the site was deemed positive for self-stimulation and a rate-frequency function employing 0.1 logarithmic increases and decreases in pulse frequency for that site was obtained. Twenty-four hours later, a second rate-frequency function was obtained using 0.05 unit changes in pulse frequency. The purpose of this manipulation was to ensure that the obtained threshold was independent of the logarithmic increment in pulse frequency used. If a replication was obtained the electrode was lowered 160 μm and a second site tested 24 hours later. If a discrepant threshold was obtained, the procedure (rate-frequency determination) was repeated until a stable threshold measure was demonstrated. If the subject seized, the session was aborted and re-tests were performed the following day. Finally, if after repeated testing (an average of

8 sweeps from low to high frequency; approximately 2 hours of testing) a subject failed to bar-press, the site was deemed negative for self-stimulation. This procedure was repeated until the full dorso-ventral amygdala had been tested.

Histology:

At the conclusion of the experiment, animals were injected with a lethal dose of sodium pentobarbital. The location of the terminal stimulation site was then marked as follows: a direct anodal current (0.1 mA in amplitude and 15 sec. in duration) was passed through the tip of the electrode. The animals were then perfused intracardially with physiological saline (50 cc flush) followed by a 50 cc solution of potassium ferrocyanide (3 %), potassium ferricyanide (3 %), and trichloroacetic acid (0.5 %) in 10 % formalin. The brains were then extracted and stored in 10 % formalin for a minimum of 3 days, followed by 2 days in a 30 % sucrose solution. The brains were then sliced in a cryostat microtome (at - 20 ° C) and the sections containing the electrode tract mounted on microscope slides. Drawings of fresh sections were then made on calibrated paper under 10X magnification. Selected sections were subsequently stained with thionine for further detailed histological analysis.

The successive dorso-ventral positions of the electrode were inferred from the terminal marked site and drawn on a calibrated representation of the brain. The accuracy of site representation using the above procedure has been documented in a previous report (Rompré & Miliaressis, 1985).

Statistical treatment:

Two aspects of the rate-frequency data obtained in this experiment were considered for

data analysis. The lateral position of the rate-frequency function on the frequency axis, and the maximal rate. These aspects were analyzed by fitting the rate-frequency data to the following variant of the Gompertz sigmoid model (Coulombe & Miliaressis, 1987),

$$f(x) = ae^{-e^{b(x_i-x)}}$$

When this equation is used to fit a rate-frequency function, a represents the maximum (asymptotic) rate, X_i (X at inflection) represents the pulse frequency at the point of maximum acceleration of the curve (that is at $0.3679a$), b represents an index of the dynamic interval, and e is the base of natural logarithms. The fits to the modified Gompertz model were obtained using Marquardt's algorithm for non-linear regression (Marquardt, 1963). From these analyses, estimates of parameters a and X_i were computed, together with their respective confidence intervals and the usual regression statistics. The reciprocal of threshold frequency ($1/X_i$) was used to plot the reinforcing efficacy of the stimulation (Miliaressis et al., 1982).

RESULTS

The number of brain sites tested with a given electrode varied from 11 to 23. Seventy-one out of 126 tested sites supported self-stimulation. Of the positive sites, 50 were found in various amygdaloid compartments. Self-stimulation was frequently accompanied by epileptiform manifestations such as wet dog shakes and freezing responses. However, despite the occurrence of these competing behaviours, rate-frequency functions were remarkably stable. Only exceptionally did the animals convulse. Maximal self-stimulation rates were generally very low.

The threshold frequency and maximal rate estimates for the 71 positive sites are presented in Figure 1. Threshold (top panel) varied from 9.2 to 40 pulses/train, depending on brain site. Most of these estimates, however, were confined in a range of 10 to 20 pulses/train. Asymptotic rate (lower panel) varied from 3 to 37 presses/min., depending on brain site. The mean asymptotic rate (17.96 ± 0.88) is consistent with Prado-Alcala and Wise's estimate of approximately 22 presses/min. However, contrary to these authors, no correlation was found between asymptotic rates and threshold frequencies ($r = 0.111$, $t = 0.919$, $p > 0.05$).

The reconstructed stimulation sites and associated stimulation efficacy are shown in Figures 2 to 4 according to the following format: Each subject is identified at the upper part of an anatomical plate traced from Paxinos and Watson's (1982) stereotaxic atlas. Other numbers designate the millimetric position of the plate behind bregma. The successive dorso-ventral positions of the moveable electrode are presented by circles. These positions are designated by numbers in text with the implantation site designated as 1. Positive and negative sites are identified by filled and open circles, respectively. The graph beside each plate shows the reinforcing stimulation efficacy (Xi^{-1} , x-axis) as a function of the electrode position (y-axis). By convention, a value of zero for stimulation efficacy means that self-stimulation could not be obtained. The rate-frequency functions for one of the subjects are also shown as an illustration (Fig.2).

Figure 2 presents the anatomical findings and associated efficacy graphs for the two most rostral implants. The rate-frequency data for subject H72 are also shown. Where discernable, the brain site number is given alongside its corresponding curve. Depending on the brain site, asymptotic rates and frequency thresholds (as estimated from the fits) varied from 5.4 to 37.4

Figure 1:

Top panel: Number of brain sites as a function of threshold frequency (X_i). Bottom panel: Asymptotic self-stimulation rate as a function of threshold frequency (X_i). Each point represents a brain site.

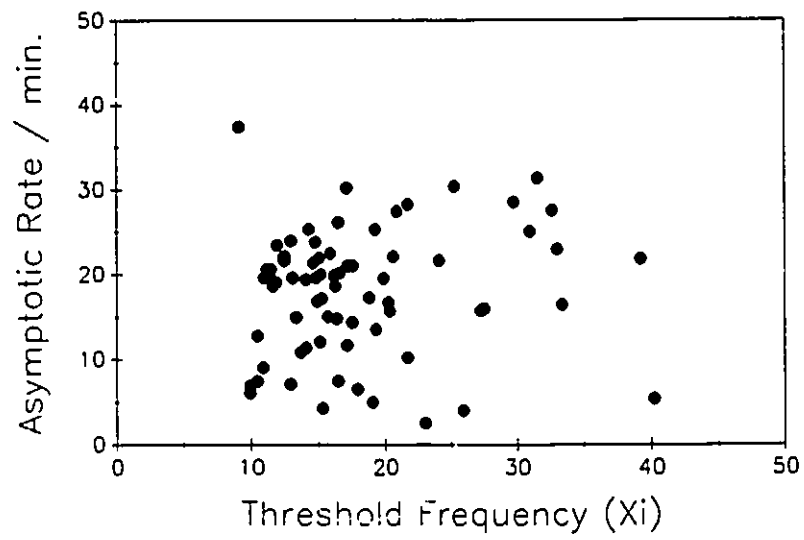
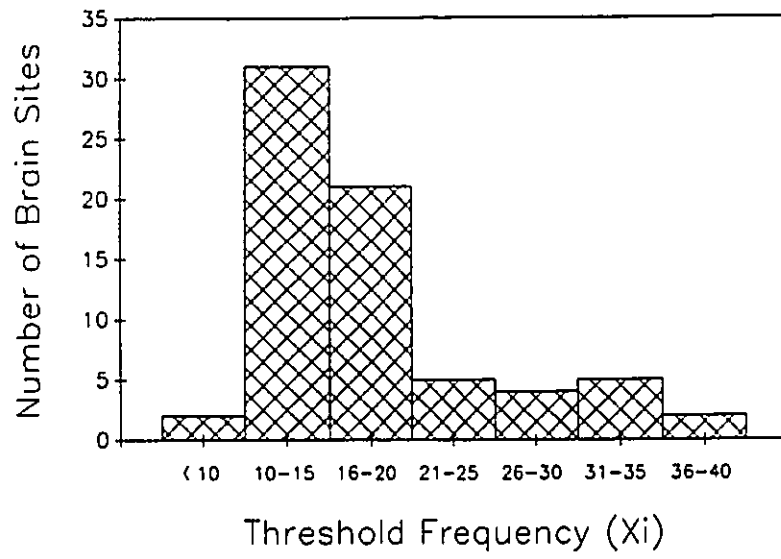
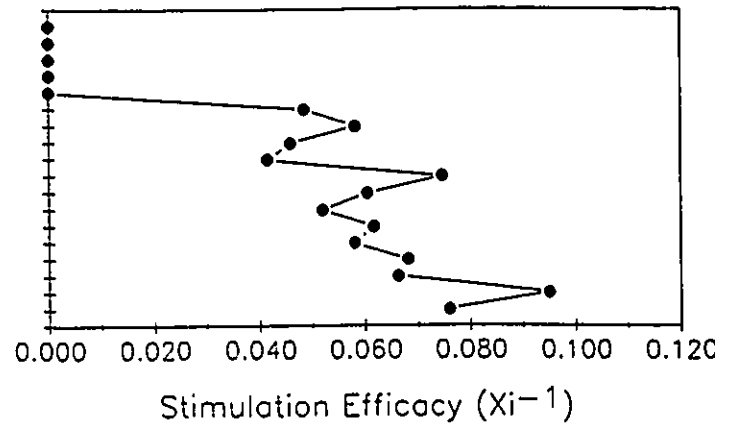
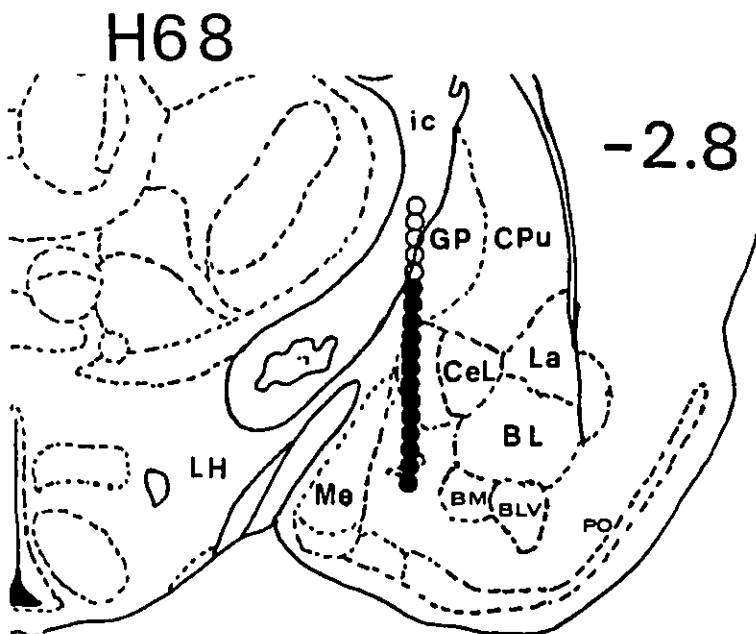
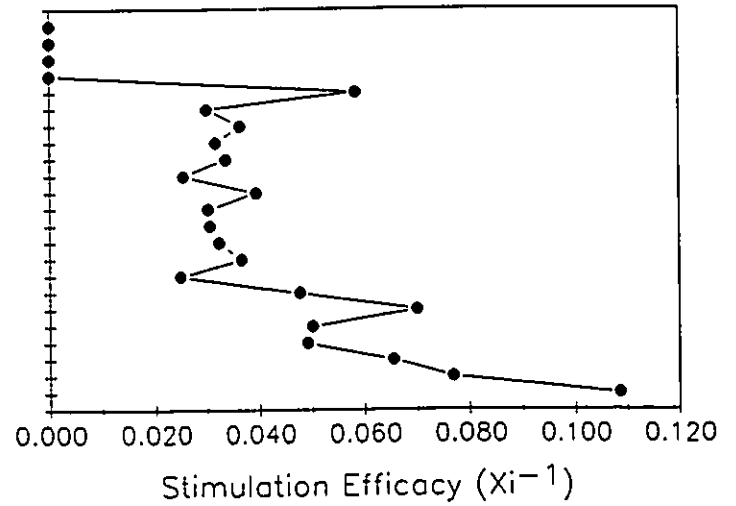
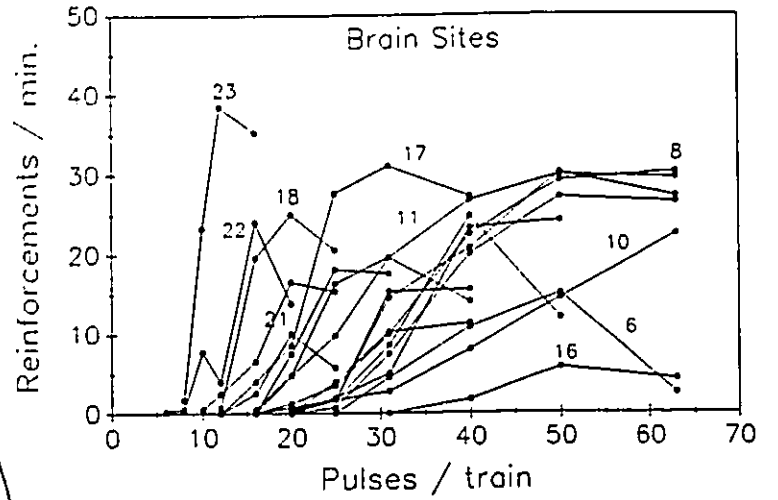
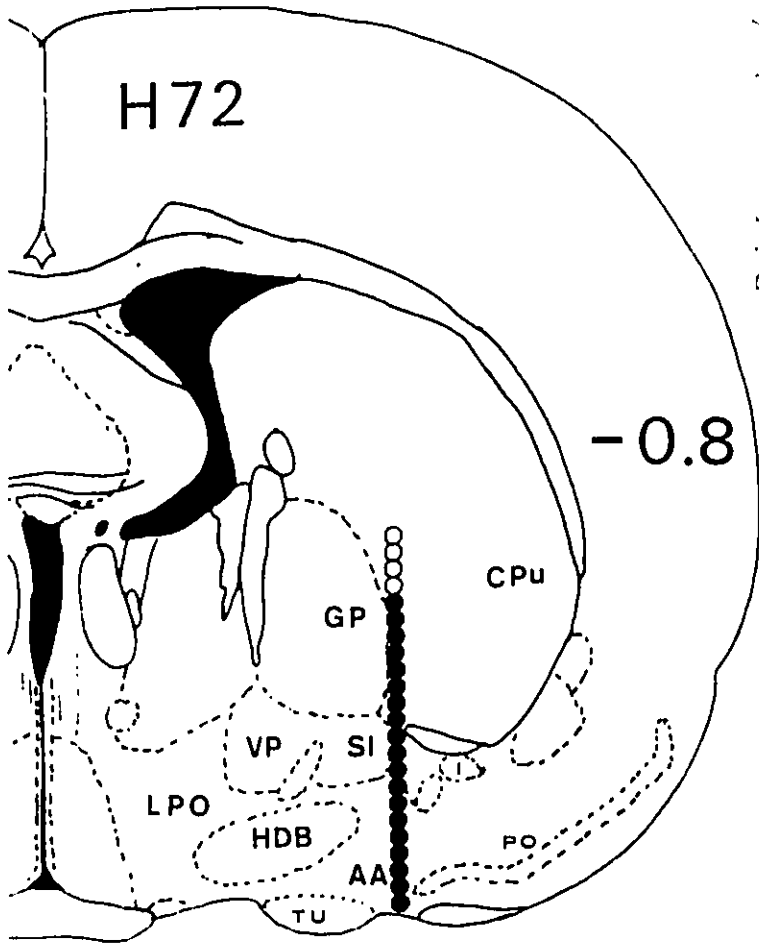


Figure 2:

The location of stimulated sites and associated rewarding stimulation efficacy for the two most rostral implants (subjects H72 and H68). The rate-frequency data for subject H72 are also shown. Positive and negative sites for self-stimulation are represented by filled and open circles, respectively. The number at the top of each anatomical plate identifies the subject. The number at the rightmost part of the plate designates the millimetric distance of the plate from Bregma, according to Paxinos and Watson's (1982) stereotaxic atlas of the rat brain. The reinforcing stimulation efficacy (the reciprocal of threshold frequency) is shown on the x-axis. By convention, a value of zero means that self-stimulation could not be obtained.



presses/min and from 9.2 to 40 pulses/train, respectively. Site 23 showed the highest asymptotic rate and the lowest threshold among all sites and subjects.

The electrode track of subject H72 was located 0.8 mm behind Bregma and approximately 3.6 mm from the midline. A total of twenty-three brain sites were tested, from the caudate-putamen down to the floor of the brain, near the anterior amygdaloid area. The upper 12 sites were on the medial wall of the caudate-putamen. The eight most ventral of these sites supported self-stimulation. Sites 13 & 14, located in the ventral pallidus-fundus striata region were also positive for self-stimulation. The remaining nine sites, covering the full dorso-ventral extent of the anterior amygdaloid area, supported self-stimulation. Efficacy estimates decreased after the first positive site and then tended to stabilize close to site 18 (dorsal amygdaloid area) where an abrupt increase was noted. Further increases were noted as the electrode moved into the most ventral amygdaloid area, between the olfactory tubercle and the primary olfactory cortex.

The electrode tract of subject H68 was located 2.8 mm behind Bregma and 3.8 mm lateral to the midline. The electrode coursed through the internal capsule, medial wall of the globus pallidus, lateral edge of the basal nucleus of Meynert, medial part of the central nucleus, and intercalated nucleus of the amygdala. Sites 1 to 5, encompassing the internal capsule and upper medial aspect of the globus pallidus, did not support self-stimulation. Sites 6 to 8, located at the junction of the basal nucleus of Meynert and globus pallidus, supported self-stimulation. The remaining 10 sites (all within the amygdala) were positive for self-stimulation. The efficacy profile obtained across this region showed a two-fold gradual staggered increase. Maximum efficacy was obtained at the second from last site, located in the amygdaloid intercalated nucleus.

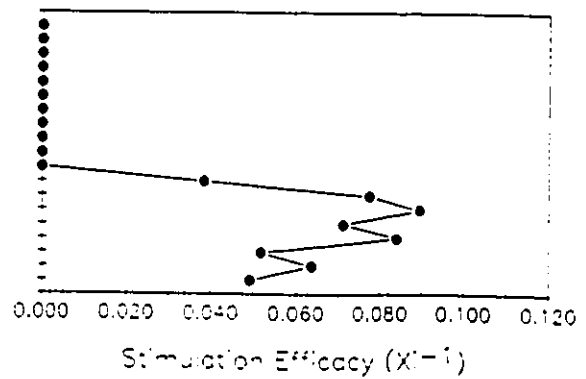
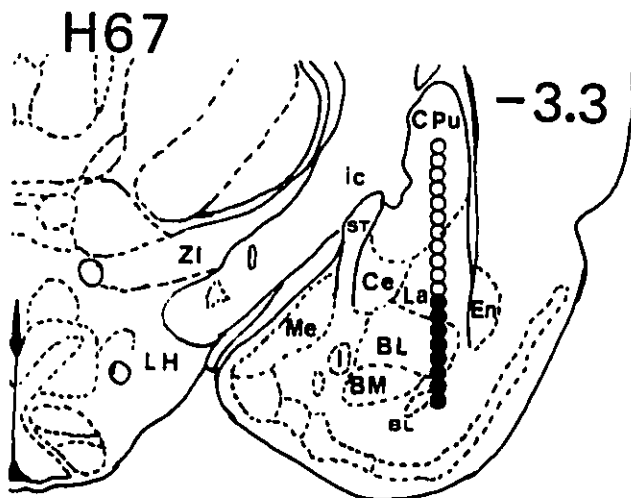
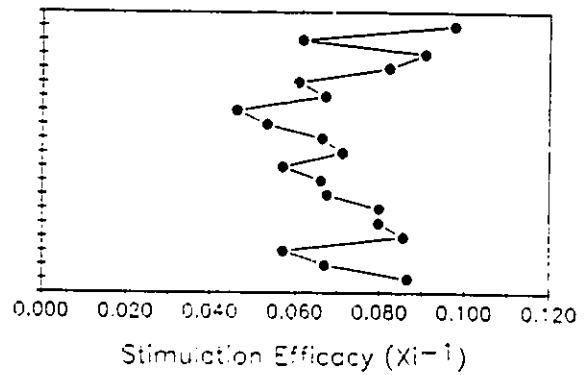
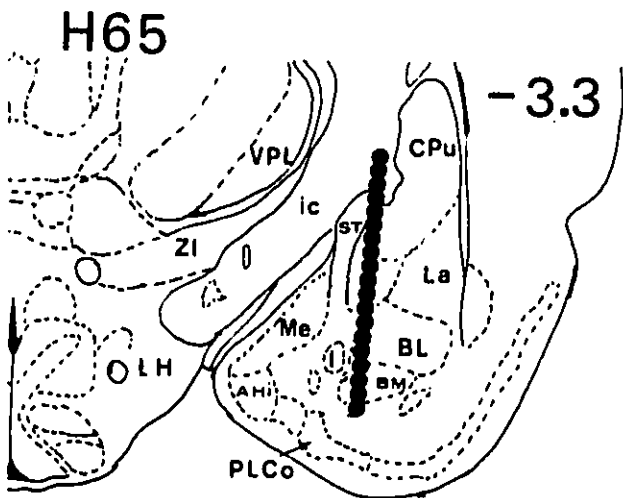
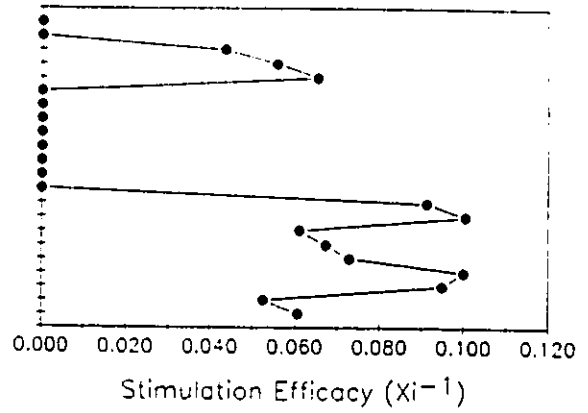
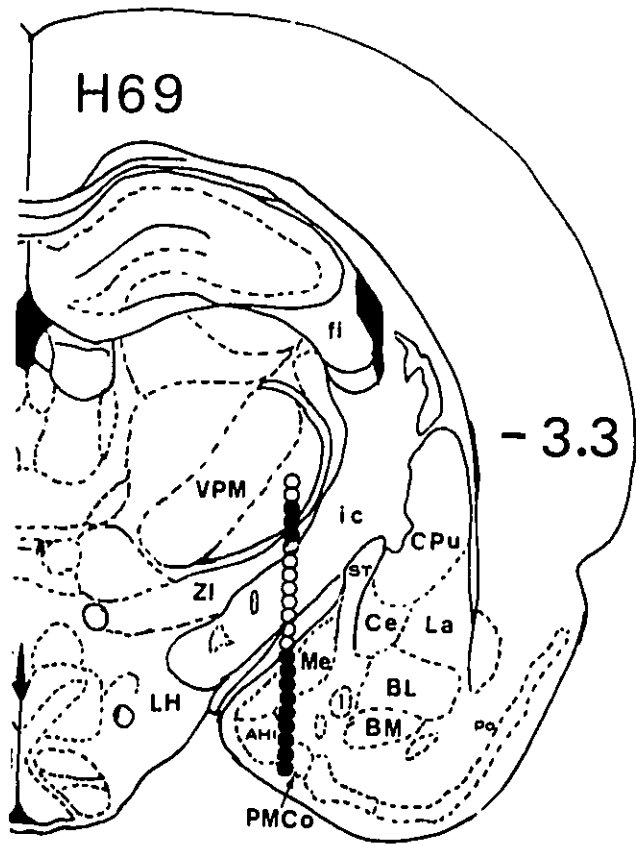
Figure 3 shows the histological plates and efficacy profiles for 3 subjects whose electrodes were found 3.3 mm behind Bregma. The electrode of H69 was found 3.2 mm lateral to the midline. Its path covered a region between the ventral posterolateral thalamic nucleus and the lower boundary of the posteromedial cortical amygdaloid nucleus. Twelve out of twenty-two tested sites supported self-stimulation. Among them, 2 sites were located in the ventral portion of the posterolateral thalamic nucleus and 1 site in the reticular thalamic nucleus. Sites 6 to 13 corresponding to the internal capsule and optic tract failed to support self-stimulation. Marked motor effects accompanied the stimulation of these sites. Sites 14 to 22 located in the middle portion of the medial amygdaloid nucleus, periamygdaloid area and posteromedial cortical amygdaloid nucleus supported self-stimulation. The stimulation efficacy showed a staggered profile within the amygdaloid complex.

The electrode track of subject H65 was found slightly slanted, 0.8 to 1 mm lateral to the previous subject. The region examined extended from the globus pallidus-internal capsule region down to the lower boundary of the basomedial amygdaloid nucleus. Self-stimulation was found throughout the entire electrode path. The first two sites were in the globus pallidus neighbouring the internal capsule. Sites 3 to 5 were located at the point where the stria terminalis emerges from the amygdala. The remaining sites (with one exception) were located in the central amygdaloid nucleus and medial aspects of the basolateral and basomedial amygdaloid nuclei. The data show a two-fold variation in stimulation efficacy, without any identifiable trend across sites.

The electrode track of subject H67 was found approximately 5 mm lateral to the midline. The region examined included sites in the caudate-putamen, the lateral and basolateral

Figure 3:

Stimulation efficacy as a function of electrode position for subjects H69, H65 and H67. Other details, as in figure 2 and text.



amygdaloid nuclei, and the piriform cortex. Eleven out of nineteen tested sites failed to support self-stimulation. These sites were located in the caudate-putamen and the lateral amygdaloid nucleus. A single site (#12) in the latter nucleus supported self-stimulation. The remaining seven sites, located in or near the basolateral amygdaloid nucleus, all supported self-stimulation. Stimulation efficacy showed a marked increase when the electrode penetrated the upper aspect of the basolateral nucleus. A staggered gradual decrease followed as the electrode coursed through the medial and ventral portions of this nucleus.

Finally, the histological data for the most posterior implants (subjects H85 and H82) are shown in Figure 4. None of the sites, most of which were located either in the anterior hippocampal region or ventral hippocampus, supported self-stimulation. Several sites in the zona incerta and internal capsule (subject H82) were also negative.

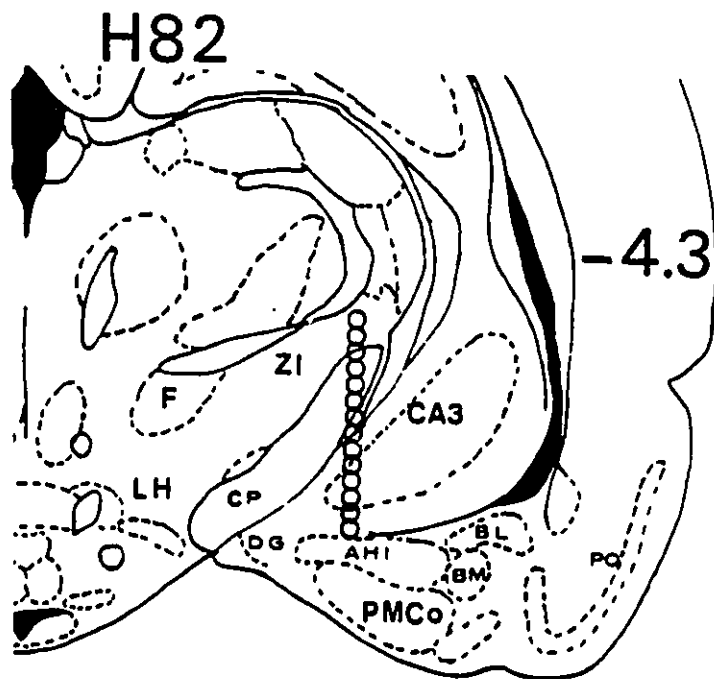
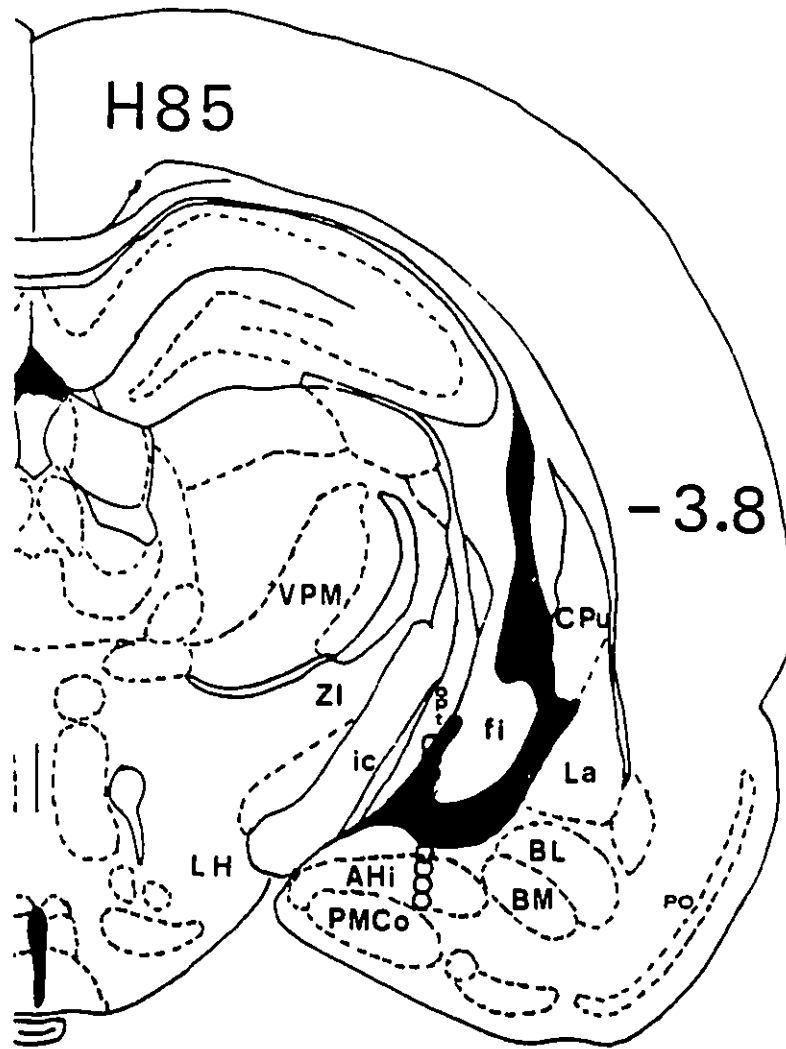
DISCUSSION

The present results are in agreement with Prado-Alcala & Wise's (1984) finding that maximal self-stimulation rates in the amygdala are generally low.

No correlation was found between maximal rates and the index of reinforcing stimulation efficacy. This finding is of primary importance because contemporary reports occasionally use rates to make decisions on the relative importance of various brain regions in reward. In addition to the present data, a series of recent reports (Malette & Miliaressis, 1990; Miliaressis et al., 1982; Miliaressis & Rompré, 1987; Rompré & Miliaressis, 1985) have extensively substantiated Hodos & Valenstein's (1962) early assertion that rates are not an accurate reflection of the reinforcing stimulation efficacy.

Figure 4:

The location of successive dorso-ventral electrode positions for subjects H85 and H82. None of the tested sites supported self-stimulation. Other details, as in figure 2 and text.



Approximately 70% of the sites in the amygdaloid complex supported self-stimulation. In the majority of the cases threshold frequency was confined in the range of 10 to 20 pulses/train. These figures are comparable to those obtained by the same method for the traditional most rewarding brain areas, namely the ventral tegmental area and dorsal raphe (Rompré & Miliaressis, 1985) and the lateral hypothalamus (Kane et al., in press). In the latter of these studies animals were implanted with a pair of amygdaloid and lateral hypothalamic electrodes. Although a systematic comparison of threshold frequencies fell outside the scope of the experiment, it was noted that in most of the cases the threshold frequency was lower in the amygdala.

According to Miliaressis et al. (1982), a difference in threshold frequency between two brain sites is mainly attributable to a difference in the density of reward neurons around the electrode tip, assuming homogeneity with respect to: 1) neuronal excitability 2) the density of arborization and 3) the total post-synaptic effect.

All amygdaloid nuclei, with the exception of the lateral nucleus supported self-stimulation. Although there was a slight trend for an increase in stimulation efficacy as the electrode penetrated deeper in the amygdaloid complex, notably in the anterior amygdaloid area and periamygdaloid regions (a rich source of ventral amygdalofugal fibers; De Olmos, 1972), there was no clear boundaries of a primary low frequency focus. When compared to the ventral tegmental and dorsal raphe pontine substrates, the stimulation efficacy distribution in the amygdaloid complex and at the source of its efferents (the stria terminalis and amygdalofugal pathways) seems relatively homogeneous.

The present failure to detect self-stimulation in the dorsal and middle compartments of

the lateral amygdaloid nucleus is inconsistent with the findings of Prado-Alcala and Wise (1984) who reported a few self-stimulation sites in this region. It may be that neural elements of this nucleus involved in epileptiform or other secondary reactions were more sensitive to the specific stimulation parameters of our study.

In summary, all amygdaloid compartments except the lateral nucleus supported self-stimulation with pulse frequencies comparable to those used in the traditional most rewarding brain areas. This finding along with other supporting elements is inconsistent with the view that the amygdala represents a secondary focus of reward. The putative dependence of the amygdaloid self-stimulation substrate on hypothalamic mechanisms of reward is examined in the following chapter.

CHAPTER 3:

**The Effects of Concurrent Amygdalo-Hypothalamic stimulation:
a moveable electrode mapping study.**

Introduction

The effect of concurrent activation of the lateral hypothalamic and amygdaloid self-stimulation substrates has been previously evaluated (Szabo et al., 1972; Jackson & Gardner, 1974). As indicated in the general introduction, discrepant findings were obtained. Szabo et al. (1972) reported rate increases and threshold decreases under concurrent amygdalo-hypothalamic activation; Jackson & Gardner (1974) reported either inhibitory or no effects. The use of different methodologies may account for these inconsistent findings. For example, Szabo et al. (1972) used low level (contingent) stimulation of the amygdala, whereas, Jackson & Gardner (1974) employed continuous high intensity stimulation of the amygdala. Alternatively, their measuring methods may have biased data interpretation. For example, the rate increase shown by Szabo et al. (1972) may well have been attributable to performance factors and the reported "intensity" threshold shifts to summation rather than facilitation as suggested by these authors. The rate effects observed by Jackson & Gardner (1974) could have been similarly misinterpreted. Moreover, the number and distribution of sites tested in these experiments are not necessarily indicative of amygdaloid functioning as a whole. It is possible that some regions of the amygdala interact differently with the hypothalamus; the work of Fonberg (1974; 1975; 1981) is consistent with this hypothesis. The amygdalo-hypothalamic interaction therefore warrants reexamination.

Stimulation with trains of pulse pairs (each pulse being delivered through a different electrode) can be used to investigate the existence of direct neural connectivity or convergence of reward signals into a common integrator (collision technique; Shizgal et al., 1980). Summation of reward signals elicited by different electrodes is assumed when the rate-frequency

function obtained under concurrent stimulation lies to the left of the function obtained with each separate electrode. Changes in the upper asymptotic rate can normally be expected when activation of one site alters the animal's ability to perform the task.

The present experiment was designed to test whether previously reported results of concurrent amygdalo-hypothalamic stimulation are attributable to genuine reward effects or performance factors.

Moveable electrodes were used and the experiment repeated at a number of sites throughout the amygdala. This allowed for a more complete anatomical assessment of the effect of the stimulations.

METHOD

Subjects:

Ten male Long Evans rats weighing between 300 and 325 g at the time of surgery were used. The subjects were housed individually in plastic cages under a 12 hr on / 12 hr off lighting schedule. Food and water were available ad libitum throughout the experimental period, except during testing.

Materials:

Testing apparatus was the same as that used in the preceding experiment, except that the double-pulse feature was used.

Procedure

Surgery:

Subjects, under sodium pentobarbital anaesthesia (50 mg/kg), were stereotaxically

implanted with a fixed monopolar electrode aimed at the lateral hypothalamus and a moveable, monopolar electrode aimed at the dorsal surface of the ipsilateral amygdala. With the skull held horizontal, the implantation coordinates (relative to bregma, the midline, and the surface of the skull at bregma) for the hypothalamic electrodes were: - 2mm, 2 mm, 7.9 mm, respectively. Variable amygdaloid coordinates were selected so as to provide a representative sampling of the nuclei of the amygdaloid complex (see histology). The indifferent electrode consisted of a stainless steel wire attached to 4 stainless steel screws (installed in the skull) and soldered to an Amphenol miniature plug. The electrode assembly was held in place with dental cement.

Training & Testing:

Phase 1:

Subjects were initially trained to bar-press for stimulation of the lateral hypothalamus. Depression of a 2 x 3 cm lever was followed 0.1 sec later by the delivery of a 0.3 sec train of rectangular cathodal pulses of fixed duration (0.1 msec) and variable frequency and intensity. Subjects were allowed to self-stimulate for periods of 60 sec separated by a time out period of the same length. Rate-frequency functions were obtained by systematically varying pulse frequency (while holding intensity constant) in two ascending and two descending sequences. Each 60 sec trial was immediately preceded by five (non-contingent) stimulation trains (1 train/sec). A family of rate-frequency functions was obtained by replicating the above procedure with different pulse intensities.

Subjects were then trained to bar-press for stimulation of the amygdala. If the implantation site failed to support self-stimulation (after two hours of repeated shaping) the site was designated a neutral placement. If the site supported self-stimulation, a series of rate-

frequency functions were obtained at different pulse intensities.

Phase 2:

Following completion of the above, the rate-frequency function was obtained under concurrent stimulation of the amygdala and lateral hypothalamus. The concurrent stimulation consisted in the delivery of trains of pulse pairs, with the first pulse of each pair being delivered in the amygdala and the second pulse, in the lateral hypothalamus. The intra-pair interval was fixed at 2.5 msec. The intensities of the amygdaloid and hypothalamic pulses were selected on the basis of the two families of rate-frequency functions in order to enable us to investigate a) the presence of summation between equi-rewarding amygdaloid and hypothalamic stimuli and b) the effect of self-stimulation subthreshold hypothalamic pulses on the amygdaloid rate-frequency function. Subjects were randomly assigned to either condition.

Phase 3:

The amygdaloid electrodes were then lowered 0.16 mm (0.32 in two subjects) and the experiment repeated. The number of sites tested in each subject varied. A total of 61 sites across subjects were tested.

Three additional parameters of the stimulation were varied at randomly selected sites in some subjects, namely, current to the hypothalamus and amygdala, inter-pulse interval and order of presentation of the stimuli (BA vs. AB).

Histology:

At the conclusion of the experiment, the terminal stimulation site was marked using the previously described ferrocyanide marking technique. The brains were then extracted from the cranium and stored in 10 % buffered formalin for at least one week. Brain sections were then

obtained (40 μ M in thickness) in a cryostat microtome and drawings of the unstained sections immediately made (under magnification). Sections were later stained with thionine for further histological analysis. The locations of the test sites were then determined using the procedure described in the previous chapter.

Statistical Analysis:

Rate-frequency curves were fitted using the previously described Gompertz's equation and estimates of threshold (X_i), asymptotic performance and associated confidence intervals computed. On some occasions, the logistic model was used instead of the Gompertz in order to improve the characteristics of the fit, as described by Coulombe and Miliaressis (1987).

RESULTS & DISCUSSION

Figures 5a and 5b show the location of the stimulation sites for all subjects that participated in this experiment. Closed circles identify sites that supported self-stimulation whereas open circles identify sites that did not support self-stimulation. All intended hypothalamic electrodes with one exception (subject I89) were located in the lateral hypothalamus. Subject I89's electrode was located in the bed nucleus of the stria terminalis at the level of the decussation of the anterior commissure. Several sites in the caudate-putamen were also tested.

Figure 6 illustrates two typical families of rate-frequency functions obtained with amygdaloid and lateral hypothalamic electrodes. The observed differences between these two sets of curves, discussed below, were found in most subjects.

Figure 5a:

Location of amygdaloid and hypothalamic test sites for subjects A37, A26, A13, A31, A57 and A58. Open circles denote test sites that did not support self-stimulation (SS); filled circles identify sites that supported SS. Letter and number in upper left corner (of each pair of plates) identifies subject; number and sign at bottom of plate denotes distance (in mm) and direction from Bregma according to the atlas of Paxinos & Watson (1982).

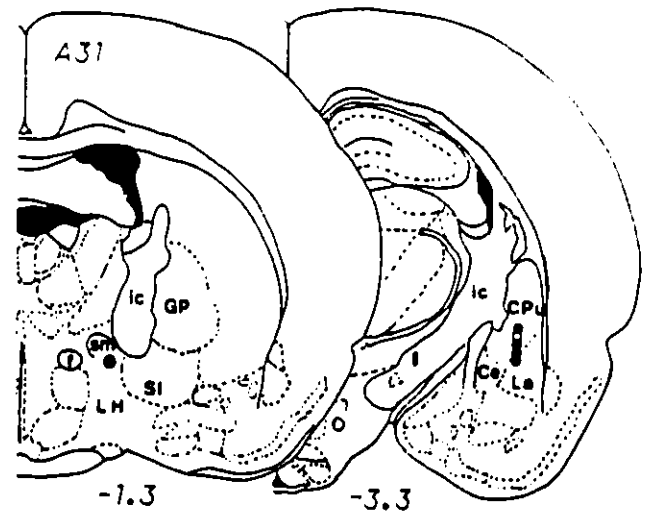
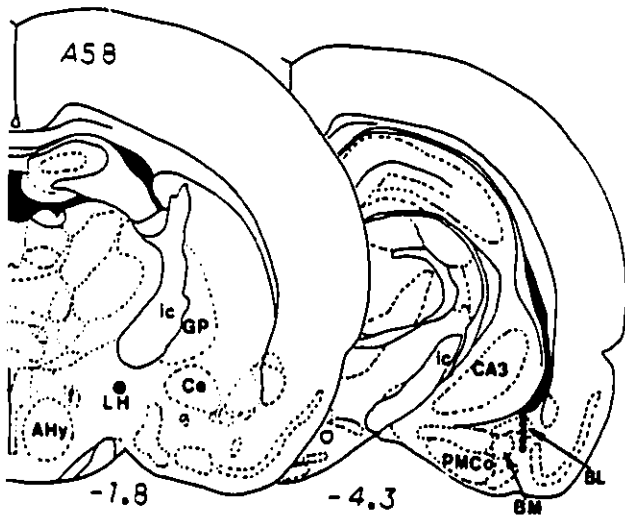
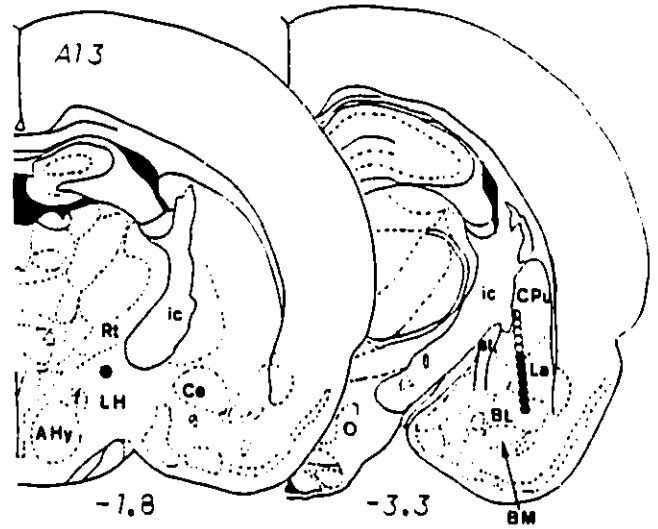
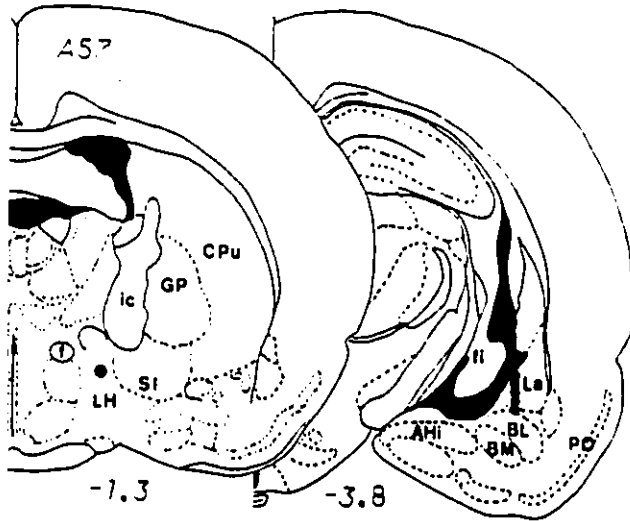
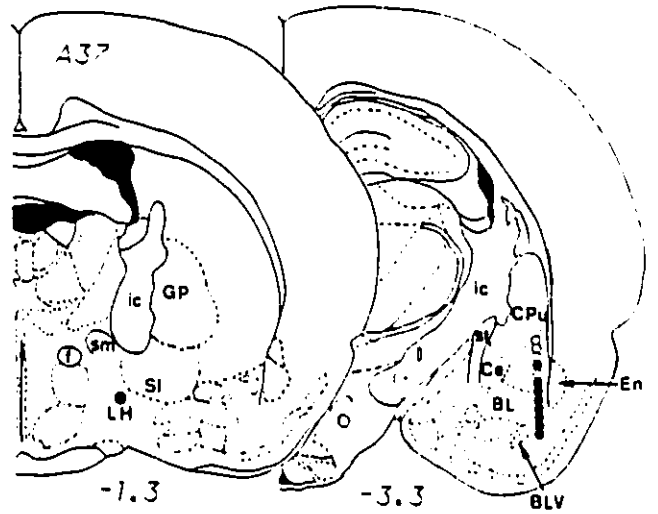
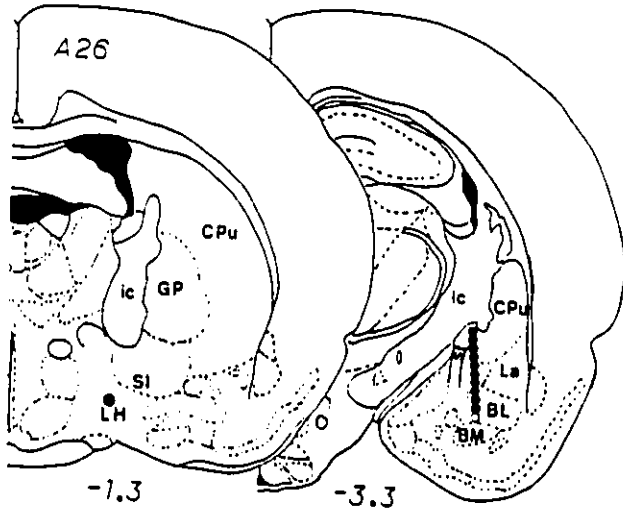


Figure 5b:

Location of stimulation sites for subjects I89, I88, I81 and I65. Open circles denote test sites that did not support self-stimulation (SS); filled circles identify sites that supported SS. Letter and number in upper left corner (of each pair of plates) identifies subject; number and sign at bottom of plate denotes distance (in mm) and direction from bregma according to the atlas of Paxinos and Watson (1982).

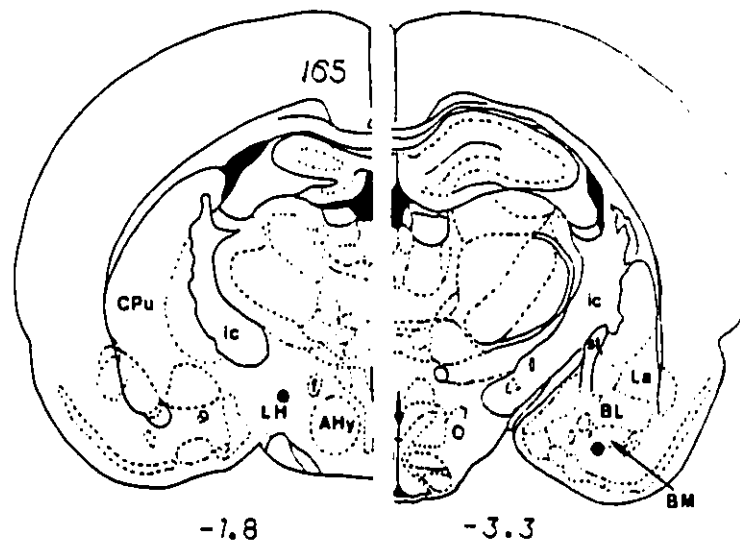
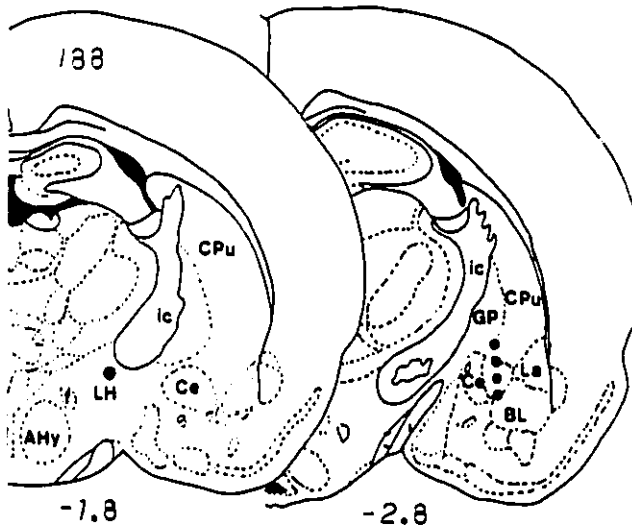
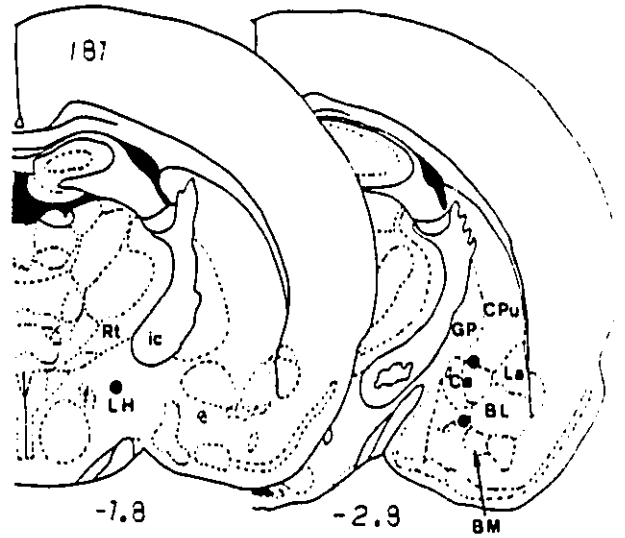
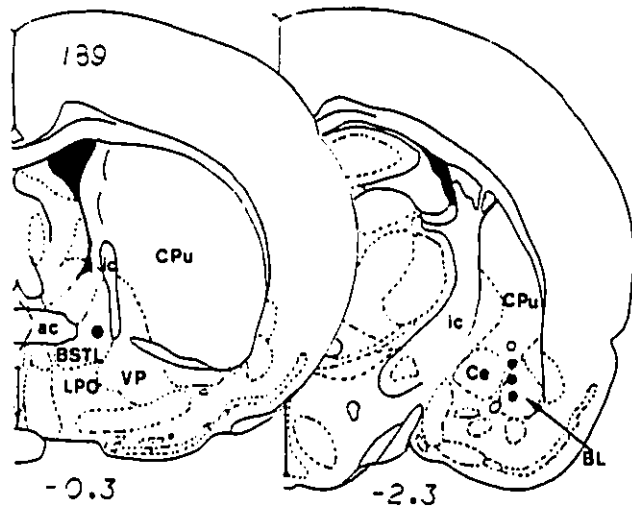
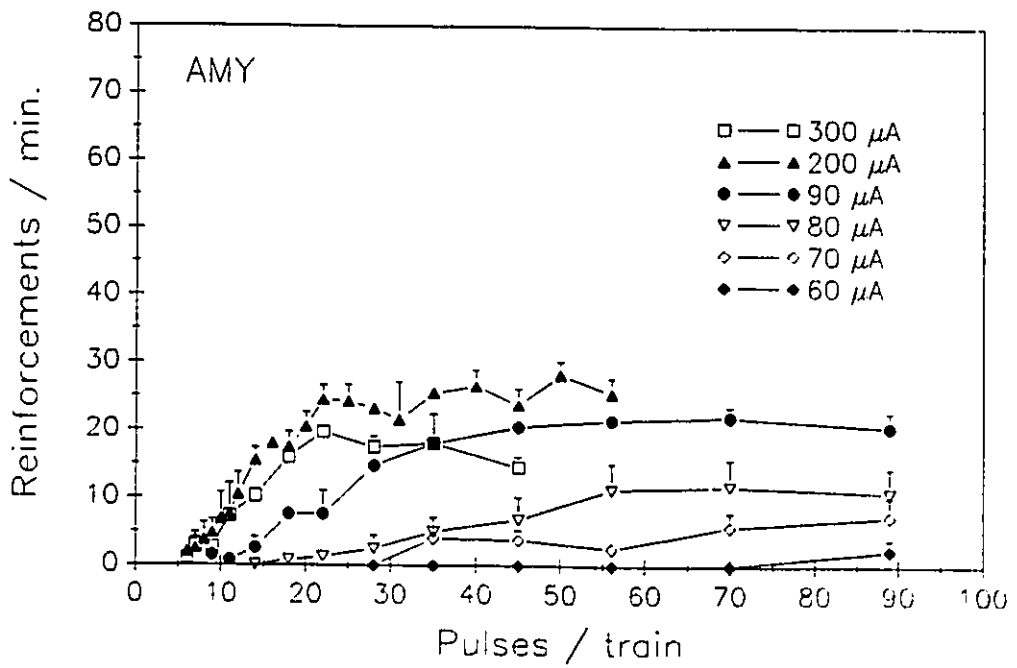
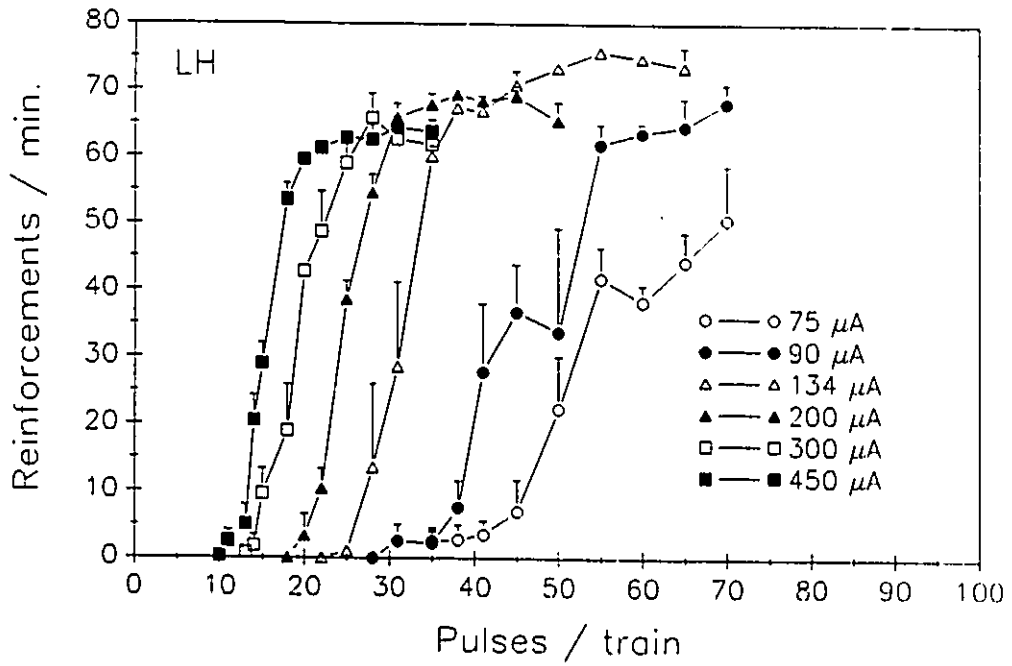


Figure 6:

Typical families of rate-frequency functions for the lateral hypothalamus (LH, upper panel) and amygdala (AMY, lower panel). Numbers refer to the pulse intensity in microamperes.



First, the bar-pressing rates for amygdaloid stimulation were considerably lower than those for hypothalamic stimulation. Second, the rate-lowering effect of decreasing current, often observed in hypothalamic sites (Miliaressis and Malette, 1987; Malette and Miliaressis, 1990), was more pronounced with amygdaloid electrodes. Third, the self-stimulation frequency threshold was generally lower in the amygdala, as shown by the lateral position of amygdaloid and hypothalamic functions obtained with comparable pulse intensities. For example, the amygdaloid function obtained with 200 μ A departs from zero at approximately 6 pulses/train as opposed to 18 pulses/train for the hypothalamic function.

Figure 7 shows representative rate-frequency functions for amygdaloid, lateral hypothalamic and concurrent amygdalo-hypothalamic stimulation, in a condition in which the pulse intensities were chosen to yield comparable threshold frequencies for the two brain structures. Figure 8 identifies the stimulation sites (arrows point to the amygdaloid test sites that match the behavioral data described below). A lower intensity of amygdaloid pulses was also used for subject I81. Table 1 presents the relevant statistics.

Due to a frequency shift that occurred between training and testing (at this site), the amygdaloid curve of subject I89 lies slightly to the left of the hypothalamic curve. Data inspection and analysis (Table 1) show that concurrent amygdalo-hypothalamic stimulation failed to produce any appreciable lateral shift of the rate-frequency function, suggesting a lack of summation between the rewarding effects of the two stimuli. In addition, the asymptotic hypothalamic rate was not decreased, suggesting that factors limiting amygdaloid self-stimulation were not active under the concurrent stimulation condition. Note also that subthreshold amygdaloid pulses (subject I81, lower panel) had no effect on any aspect of the hypothalamic

Figure 7:

Rate-frequency functions under amygdaloid (AMY), lateral hypothalamic (LH) and concurrent (AMY+LH) stimulation conditions. Numbers in parentheses refer to the pulse intensity in μA .

Numbers in upper left hand corner identify the subject.

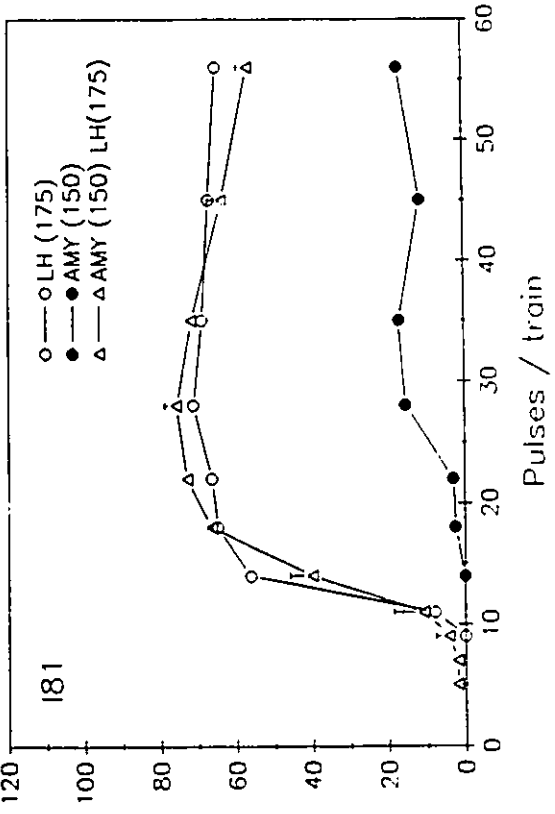
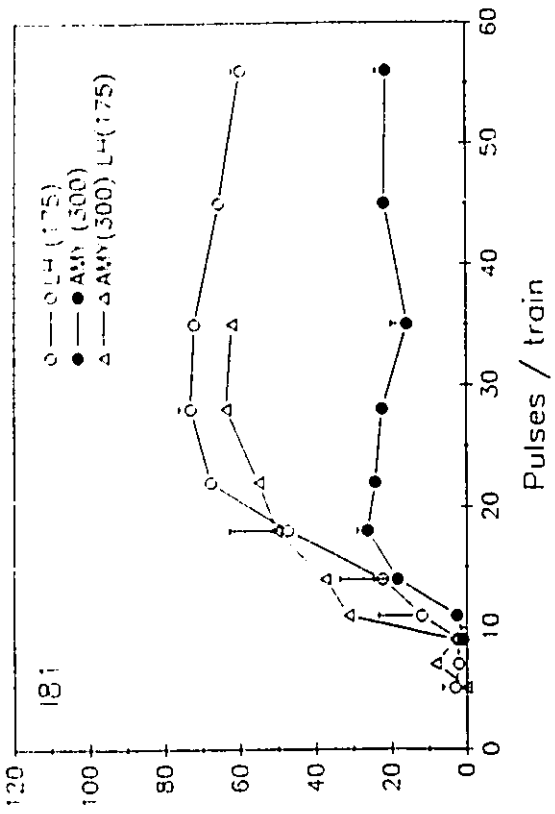
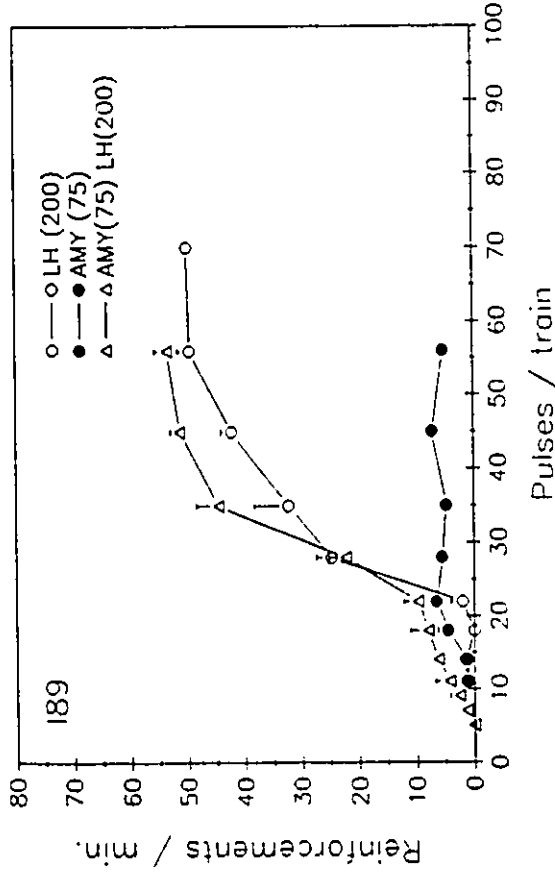
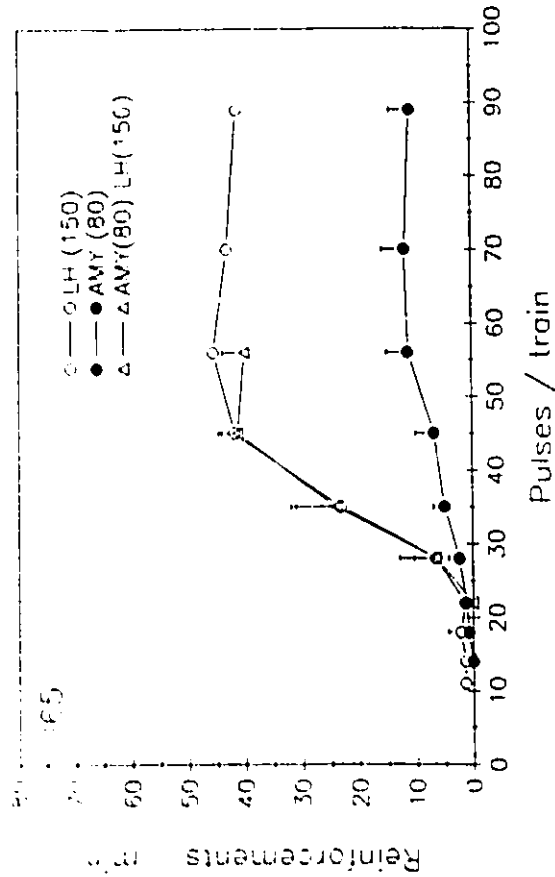


Figure 8:

Location of amygdaloid and hypothalamic test sites where combining equi-rewarding hypothalamic and amygdaloid stimulation had no effect on self-stimulation thresholds. Arrows point to the stimulation sites that match the behavioral data shown in figure 7. Letter and number in upper left corner of each pair of plates identify the subject. Number and sign at bottom of plates denotes distance (in mm) and direction from bregma according to the atlas of Paxinos and Watson (1982).

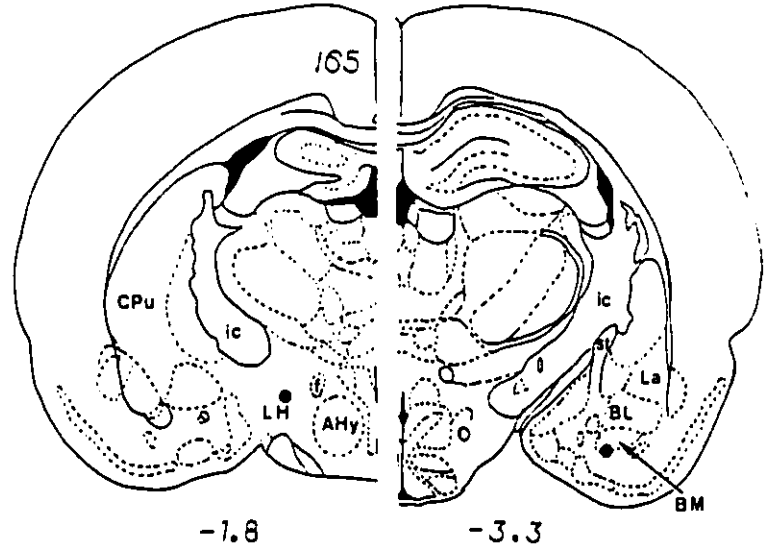
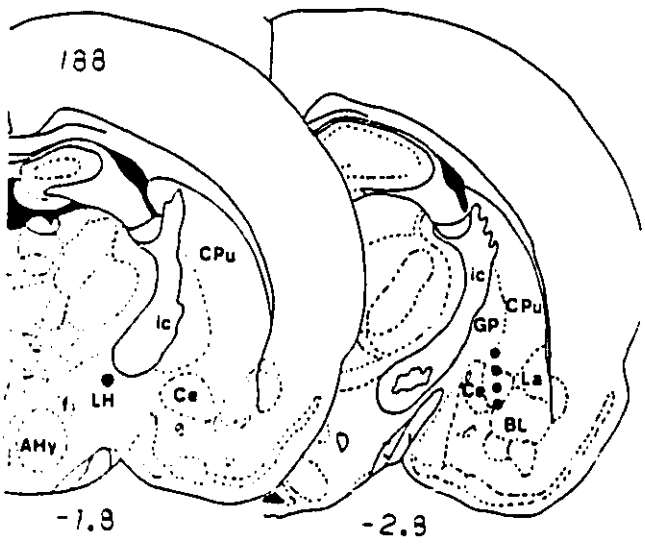
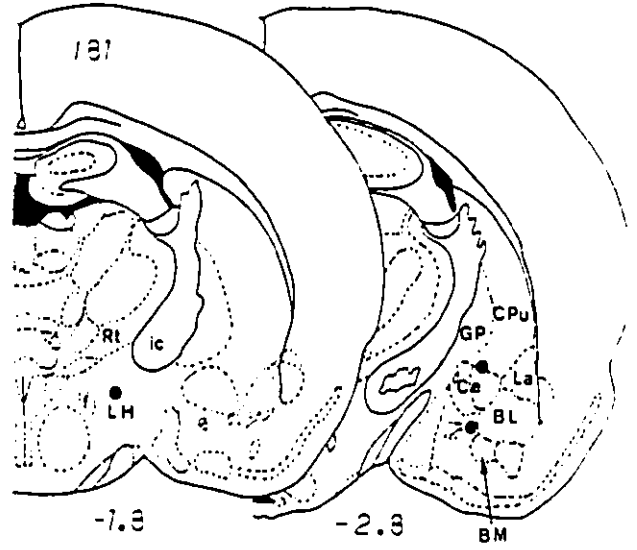
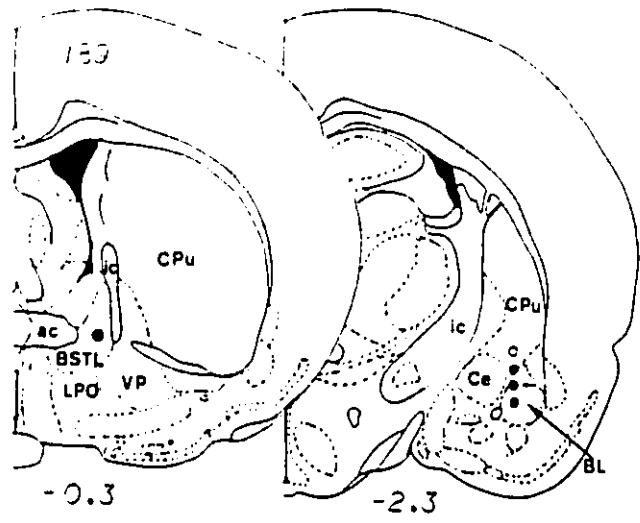


Table 1

Expected threshold frequencies (X_i) and asymptotic rates (A_s) obtained from rate-frequency data fitted with Gompertz's model.

Subject	Stim. site	Intensity (μ A)	X_i CI	A_s CI
I65	LH	150	34.39 ± 0.84	43.45 ± 1.72
	AMY	80	33.41 ± 4.10	11.81 ± 1.55
	AMY+LH		31.5 ± 1.04	41.98 ± 4.22
I89	LH	200	27.36 ± 1.17	48.92 ± 4.80
	AMY	75	15.00 ± 2.05	11.79 ± 3.87
	AMY+LH		25.04 ± 1.53	58.04 ± 9.23
I81a	LH	175	13.86 ± 0.52	68.56 ± 5.53
	AMY	300	11.83 ± 0.69	21.91 ± 2.72
	AMY+LH		10.92 ± 0.59	61.86 ± 7.78
I81b	LH	175	12.66 ± 0.20	67.39 ± 1.56
	AMY	150	23.59 ± 3.18	15.33 ± 2.94
	AMY+LH		13.44 ± 0.39	68.41 ± 4.85

Abbreviations: AMY= Amygdaloid stimulation. LH= lateral hypothalamic stimulation. AMY+LH = Concurrent stimulation of the two brain structures. CI= Confidence interval (95%).

rate-frequency function.

Figure 9 illustrates representative amygdaloid, hypothalamic, and concurrent amygdalo-hypothalamic rate-frequency functions where current was chosen as to enable the amygdaloid function to rise within a frequency range below that of the hypothalamic function. Figure 10 identifies the stimulation sites (arrows point to the amygdaloid test sites that match the behavioral data described below). Table 2 presents the relevant statistics.

The data show that concurrent amygdalo-hypothalamic stimulation with hypothalamic pulses below self-stimulation threshold failed to shift the amygdaloid function to lower frequencies for any of the subjects and sites identified in Figure 10. For subject A13, A57 & A58 (at the sites identified with arrows in figure 10), the X_i index was marginally increased. The fact that subthreshold hypothalamic pulses failed to shift the amygdaloid function to lower frequencies was predictable from the results of the previous experiment. In the latter, the rewarding effects of above-threshold hypothalamic frequencies failed to summate over the effect of amygdaloid stimulation. However, significant differences were observed in the present experiment when comparing asymptotic performance for amygdaloid stimulation and for concurrent amygdalo-hypothalamic stimulation. Statistical support was provided by non-overlapping confidence intervals (Table 2). Maximum performance rates were consistently higher under the concurrent amygdalo-hypothalamic condition than under the amygdala only condition (an increase varying from 191% to 308%, depending on the subject).

Figure 11 illustrates the effect of varying the current to the hypothalamus while holding constant the current to the amygdala. The relevant statistics are presented in table 3.

For subject A37, the concurrent amygdalo-hypothalamic function with the highest

Figure 9:

Rate-frequency functions under amygdaloid (AMY), lateral hypothalamic (LH) and concurrent (AMY+LH) stimulation conditions. Numbers in parentheses refer to the pulse intensity in μA .

Numbers in upper left hand corner identify the subject.

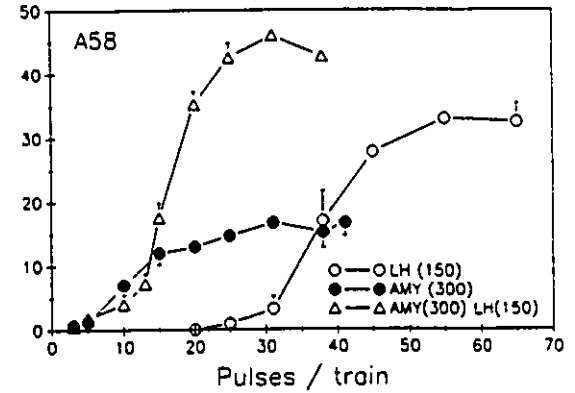
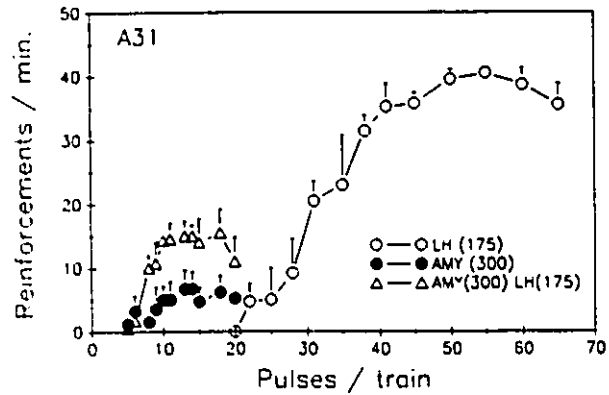
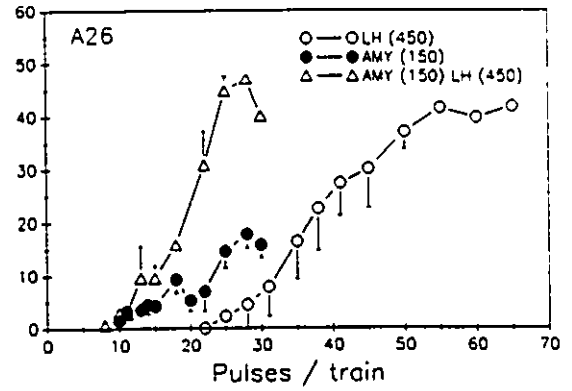
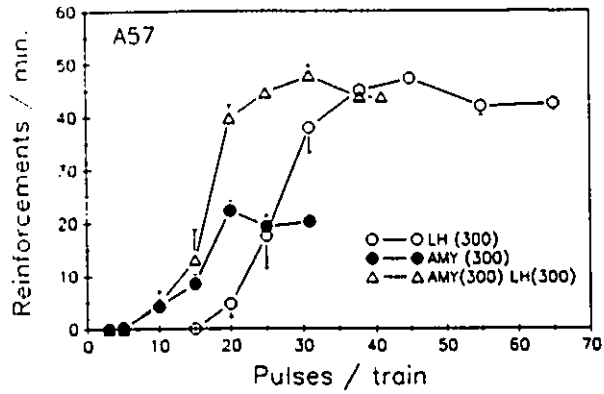
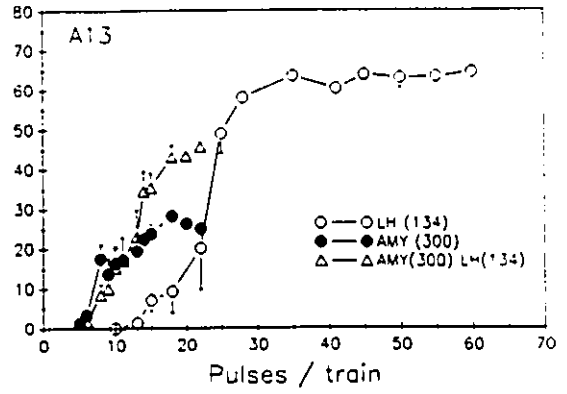
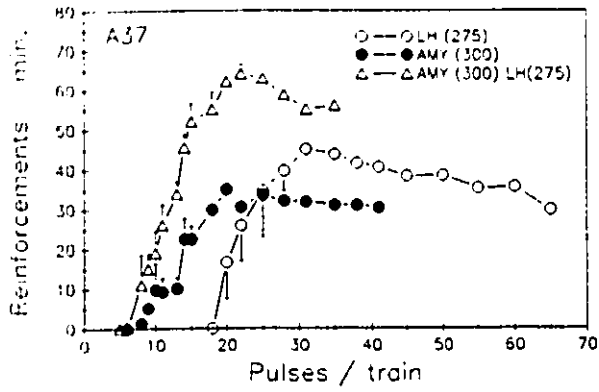


Figure 10:

Location of amygdaloid (AMY) and lateral hypothalamic (LH) test sites the concurrent stimulation of which dramatically increased self-stimulation rates without a concomitant lateral shift of the concurrent AMY-LH self-stimulation function. Arrows point to the stimulation sites that match the behavioral data shown in figure 9. Letter and number in upper left corner of each pair of plates identify subject. Number and sign at bottom of plates denotes distance (in mm) and direction from bregma according to the atlas of Paxinos and Watson (1982).

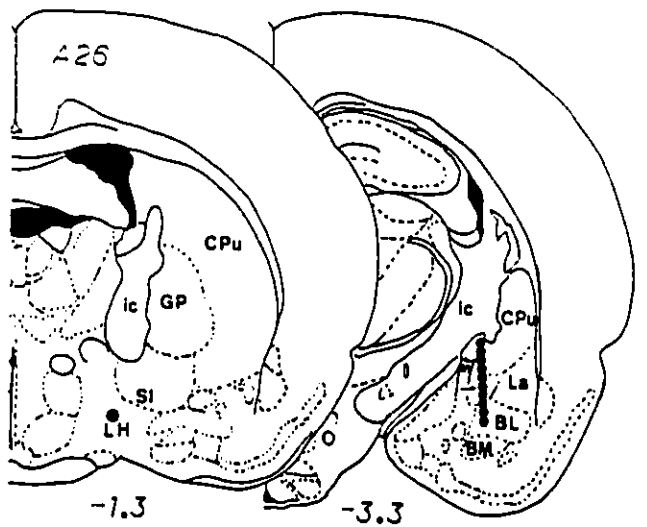
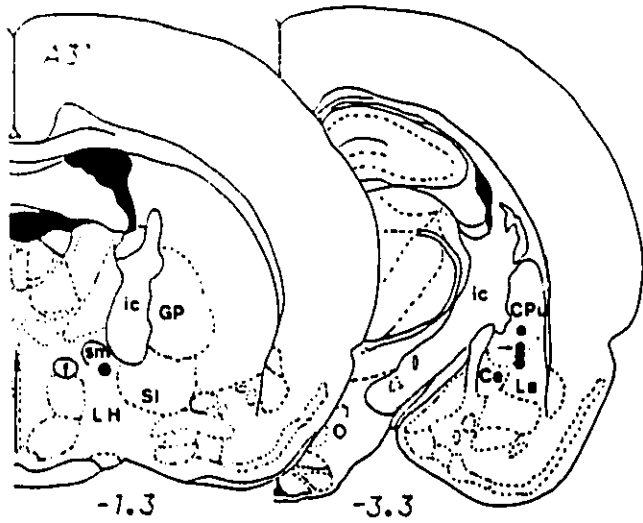
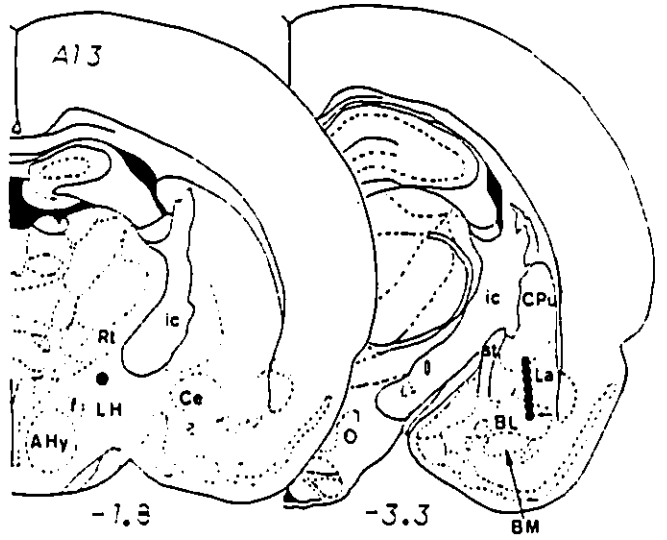
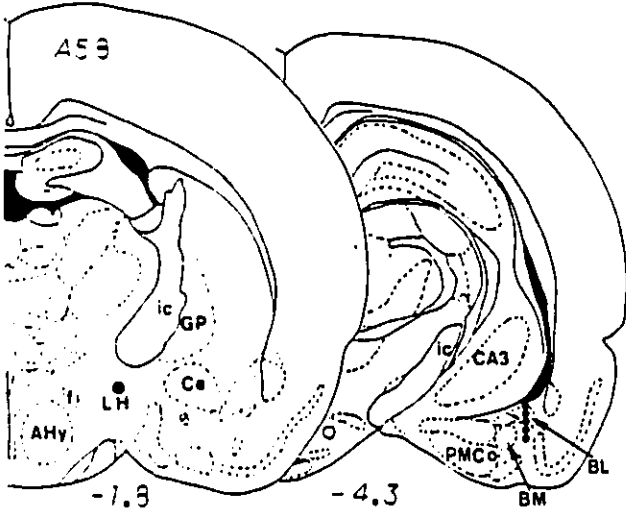
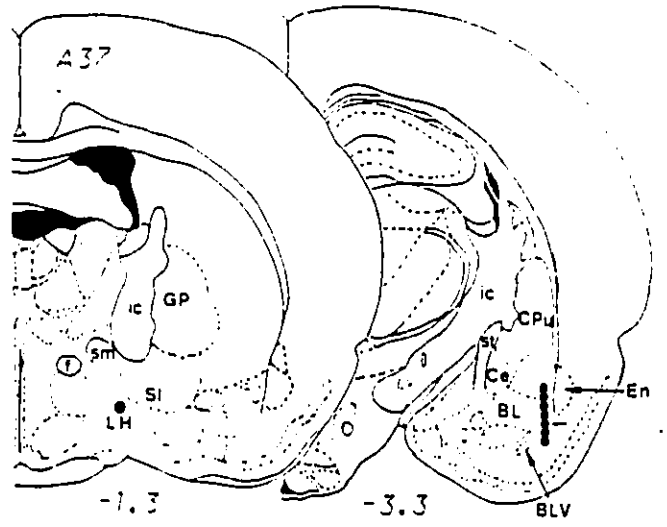
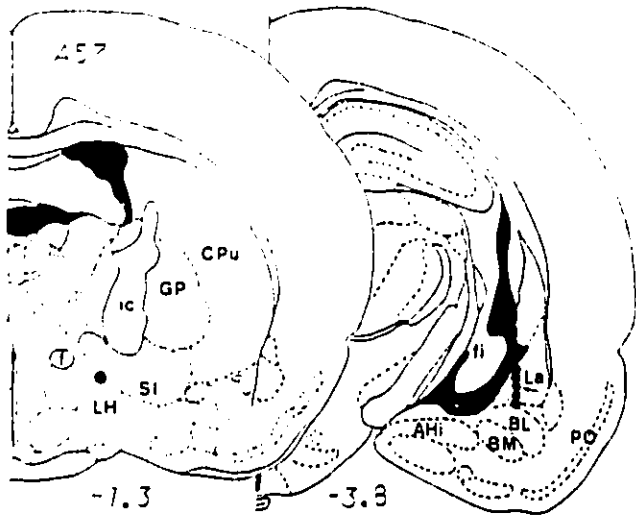


Table 2

Expected threshold frequencies (X_i) and asymptotic rates (A_s) obtained from rate-frequency data fitted with Gompertz's model.

Subject Number	Stim. Site	Intensity (μ A)	X_i CI	A_s CI
A26	LH	450	34.15 ± 0.72	41.12 ± 1.86
	AMY	150	20.00 ± 4.00	17.00 ± 2.00
	AMY+LH		19.34 * ± 1.07	46.70 * ± 7.47
A31	LH	175	29.64 ± 0.84	40.64 ± 2.89
	AMY	300	6.43 ± 1.90	6.23 ± 1.75
	AMY+LH		6.97 ± 0.41	15.01 ± 0.75
A37	LH	275	20.06 ± 0.42	39.67 ± 2.14
	AMY	300	11.56 ± 0.48	32.56 ± 2.14
	AMY+LH		10.49 ± 0.29	63.51 ± 3.72
A58	LH	150	35.72 ± 0.67	33.28 ± 0.87
	AMY	300	9.27 ± 1.28	16.09 ± 0.97
	AMY+LH		14.82 ± 0.50	44.59 ± 2.76

Table 2 (continued)

A57	LH	300	24.30 ±0.46	44.63 ±2.79
	AMY	300	13.09 ±1.69	21.42 ±5.31
	AMY+LH		16.52 ±0.53	45.20 ±2.06
<hr/>				
A13	LH	134	22.27 ±0.36	62.98 ±3.11
	AMY	300	7.39 ±0.62	25.92 ±2.90
	AMY+LH		10.87 ±0.53	49.63 ±5.86

Abbreviations as for Table 1 and text. An asterisk indicates that the estimate was obtained from the Logistic Model.

Figure 11:

Rate-frequency functions obtained under amygdaloid (AMY), lateral hypothalamic (LH), and concurrent (AMY+LH) stimulation with varying hypothalamic intensity. Numbers in parentheses refer to the pulse intensity in μA . Numbers in upper left corner identify the subject.

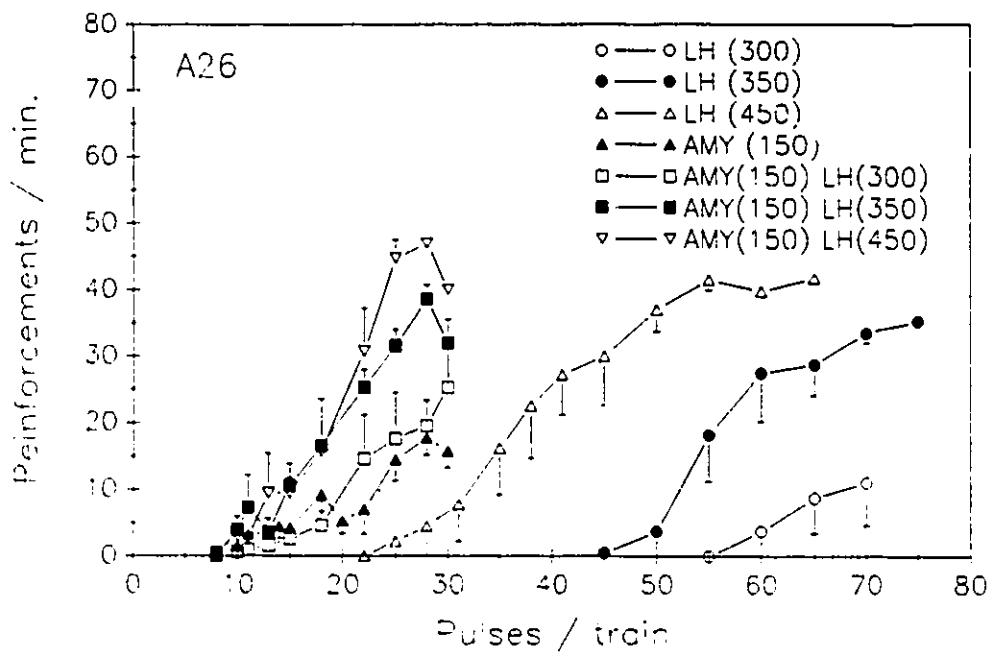
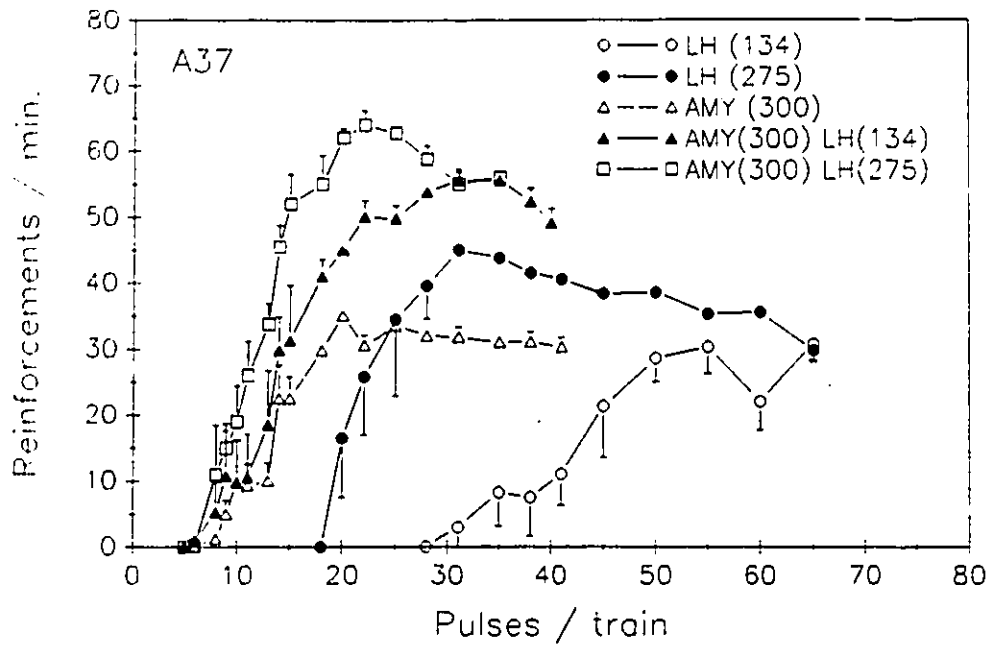


Table 3

Expected threshold frequencies (X_i) and asymptotic rates (A_s) obtained from rate-frequency data fitted with Gompertz's model.

Subject Number	Stim. Site and Intensity (μA)	X_i CI	A_s CI
A26	AMY 150	20.00 ± 4.00	17.00 ± 2.00
	AMY 150 + LH 250	22.01 * ± 1.71	25.53 * ± 4.83
	AMY 150 + LH 350	18.75 * ± 1.43	37.15 * ± 6.57
	AMY 150 + LH 450	19.34 * ± 1.07	46.70 * ± 7.47
A37	AMY 300	11.56 ± 0.48	32.33 ± 2.14
	AMY 300 + LH 134	12.36 ± 0.36	53.84 ± 2.42
	AMY 300 + LH 275	10.09 ± 0.32	57.66 ± 3.47

Abbreviations as for Table 1 and text. An asterisk indicates that the estimate was obtained from the logistic model.

hypothalamic current was, when compared to the amygdaloid function, shifted marginally to lower frequencies (see Table 3). However, the most apparent feature of the data is a positive correlation between hypothalamic current and asymptotic performance for concurrent amygdalo-hypothalamic stimulation.

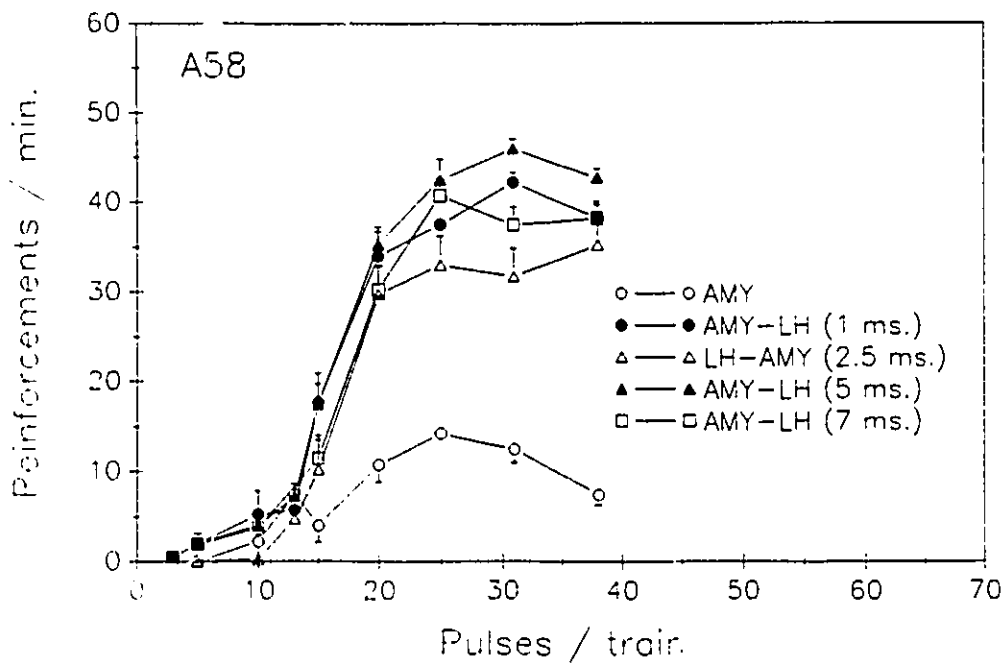
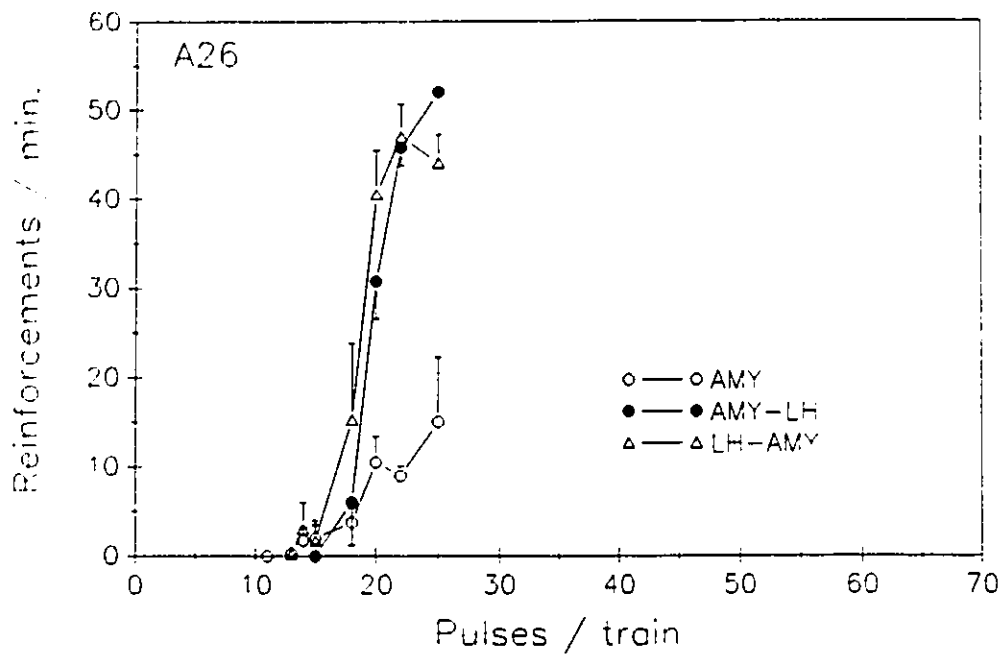
Finally, the effects of varying the presentation order in the pulse-pairs (amygdaloid pulse first vs hypothalamic pulse first) and the intra-pair interval are illustrated in Figure 12. Reversing the order of presentation or varying the intra-pair interval had no appreciable effect on any aspect of the concurrent amygdalo-hypothalamic rate-frequency function.

In summary, no evidence of summation between amygdaloid and lateral hypothalamic rewarding effects (at any of the sites identified in figures 8 and 10) was obtained. The most striking and robust effect of combining lateral hypothalamic and amygdaloid pulses (at the sites identified in figure 10) was an increase in amygdaloid self-stimulation rate. The latter were changed by approximately the same proportion for all frequencies (a scalar transformation of the rate-frequency function). The magnitude of this effect correlated positively with hypothalamic current but was insensitive to the presentation order of the two stimuli or the delay between them. The rate-enhancing effect of concurrent stimulation does not reflect a shift of the hypothalamic function to lower frequencies because the lateral position of the concurrent function was insensitive to the hypothalamic current. In addition, no shift of the concurrent function was observed in the experiment in which the hypothalamic and amygdaloid current were chosen so as to enable the two functions to rise over the same frequency range.

The simplest interpretation of these data is that hypothalamic pulses have attenuated some secondary rate-limiting effect of amygdaloid stimulation. This hypothesis is plausible,

Figure 12:

Rate-frequency functions obtained under amygdaloid (AMY) and concurrent amygdaloid + lateral hypothalamic (AMY+LH) stimulation, with varying intervals between AMY and LH pulses and the presentation pulse order reversed. Numbers in upper left corner identify the subject.



considering that amygdaloid self-stimulation was frequently accompanied by wet dog shakes and other epileptiform behaviours. The contention that the maximum rate for amygdaloid stimulation was limited by some performance debilitating factor rather than reward saturation is examined in the next chapter.

CHAPTER 4:

**The rate-enhancing effect of amygdalo-hypothalamic stimulation:
further investigation**

EXPERIMENT 1:

Rate limiting factors in amygdaloid self-stimulation

Introduction

If the maximum of the amygdaloid rate-frequency function was constrained by a saturating reinforcing effect, not performance factors, subjects should then show no preference for either of two frequencies drawn from the asymptotic portion of the rate-frequency function. This possibility was explored using Miliaressis & Malette's *isopreference test* (1987). These authors have shown that rats learn easily to discriminate between two levers delivering unequal frequencies of the same pulses. In this situation, a rat does not select the most rewarding stimulus but rather alternates bar-pressing according to a ratio that seems to reflect the difference in rewarding potency of the competing stimuli. Isopreference was found to occur for identical frequencies, as normally expected.

METHOD

Subjects:

Three male, Long Evans rats weighing between 300 and 325 g at the time of surgery were used. All other particulars have been described previously.

Materials:

The test chamber (30 X 25 X 30 cm. in dimension) was constructed of clear plexiglass and equipped with two levers (2 X 3 cm.), protruding from one wall, 3 cm above the floor. The separation between levers was 12.5 cm. Each lever triggered an independent channel of a twin

constant current stimulator. Stimulation parameters and response rates were controlled and registered by a microprocessor.

Procedure

Surgery:

Subjects, under Sodium Pentobarbital anaesthesia (50 mg/kg), were stereotaxically implanted each with a moveable monopolar stimulating electrode (described previously) aimed at the amygdala. Implantation coordinates were: - 3.3 mm posterior to bregma, 4.5 mm lateral to the midline and 7.5 mm below skull surface at bregma for all three subjects. All other aspects of the surgery including the construction of the indifferent electrode have been described previously.

Training & Testing:

Subjects were trained to bar-press for amygdaloid stimulation using standard shaping procedures. If necessary, the amygdaloid electrode was lowered until a self-stimulation site was found. Stimulation consisted of 0.3 sec trains of cathodal rectangular pulses of fixed pulse duration (0.1 msec) and variable frequency. Current intensity was fixed at 300 μ A for subjects P74 & P83 and 600 μ A for subject P80. Rate-frequency functions were then obtained (as described in previous chapters). Three consecutive replications of the rate-frequency function were required before the functions were deemed stable.

Subjects were then trained to alternate in the twin-lever choice box. During training, the pulse frequency delivered by one lever was fixed at an arbitrary value chosen from the rate-frequency function. The frequency delivered by the second lever varied from lower to higher values every 5 min. A training session usually lasted an hour. Spontaneous alternation usually

occurred within the first minutes of the session. Training was deemed complete once a consistent pattern of responding was noted for each pair of fixed and variable frequencies. During the testing phase, the subjects were given a choice between a fixed frequency, corresponding to the initial levelling of the rate-frequency function (the standard stimulus), and a variable (lower and higher) frequency (the alternative stimulus). The alternative frequency was varied in ascending and then descending order every 5 min. Testing sessions were repeated daily for a period of one week. The lever-to-stimulus connections were reversed daily to control for place preference. The bar-pressing rates per 5 min for the standard and alternative stimuli were plotted as a function of the frequency in the alternative stimulus.

Histology:

At the conclusion of the experiment, subject's P80 & P74 were sacrificed with an overdose of pentobarbital and perfused intracardially using the ferrocyanide procedure described previously. The brains were then extracted and stored in 10 % formalin for at least a week. Brain sections were then obtained (40 μ M in thickness) in a freezing microtome and drawings of the unstained sections and electrode tracts made under magnification. Sections were later stained using the Laskey procedure for further histological analysis. Subject P83 was preserved for participation in a subsequent experiment.

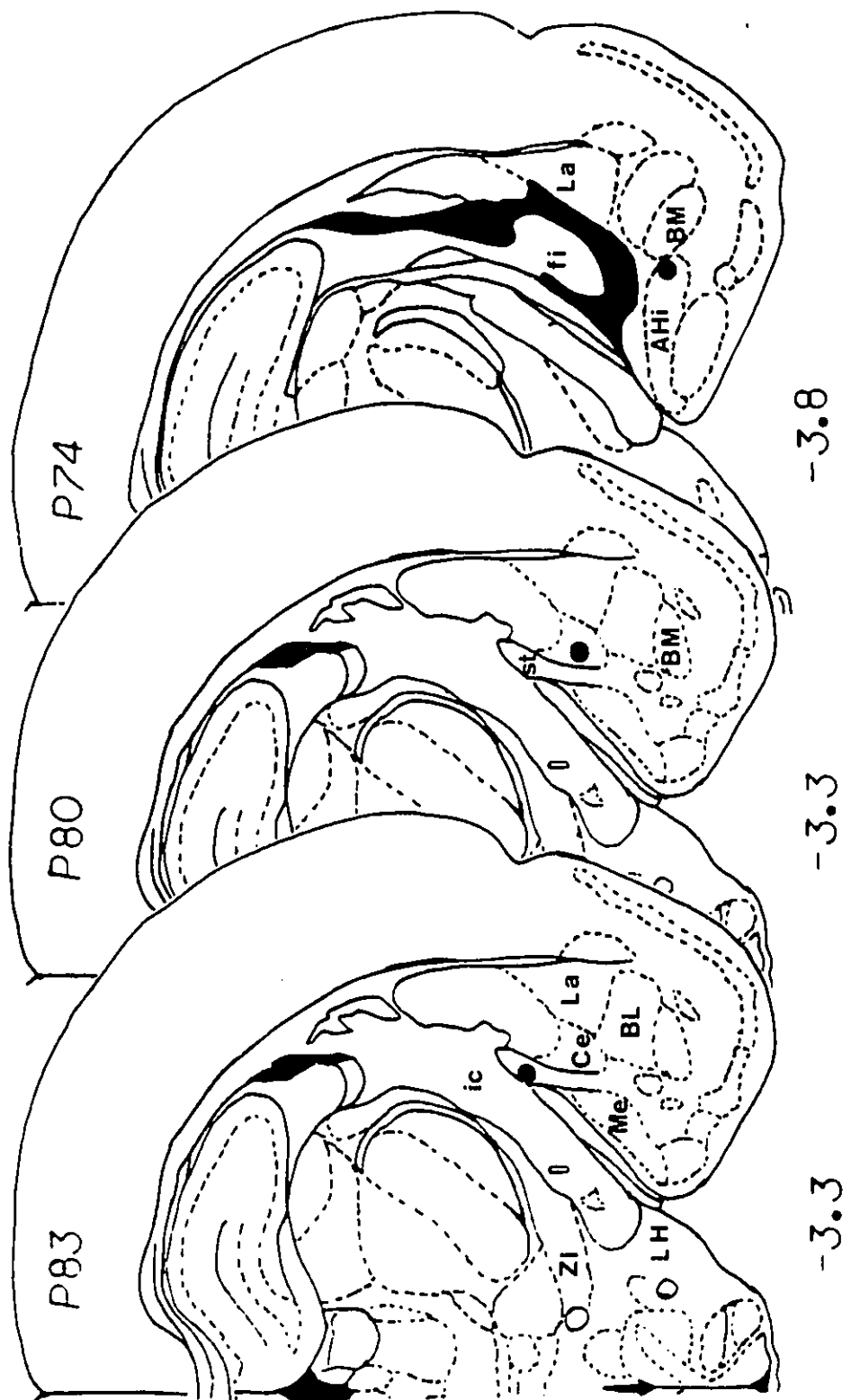
Results & Discussion

Histological Results:

The histological results (including subject P83's who was later sacrificed) are presented in Figure 13. All stimulation sites were within the amygdala.

Figure 13:

Histological data for subjects P83, P80 and P74. Closed circles identify the location of the electrode tips. Letter and number in upper cortex identifies subject; number and sign at bottom of plate denotes distance (in mm) and direction from Bregma according to the atlas of Paxinos and Watson (1982).



Behavioral Results:

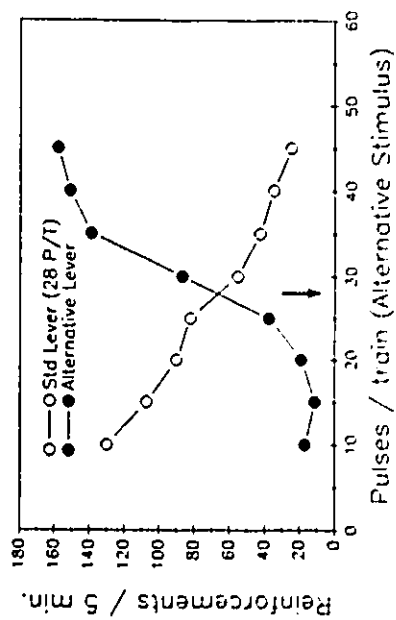
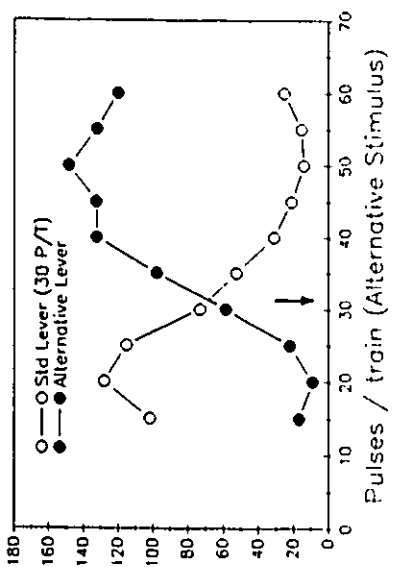
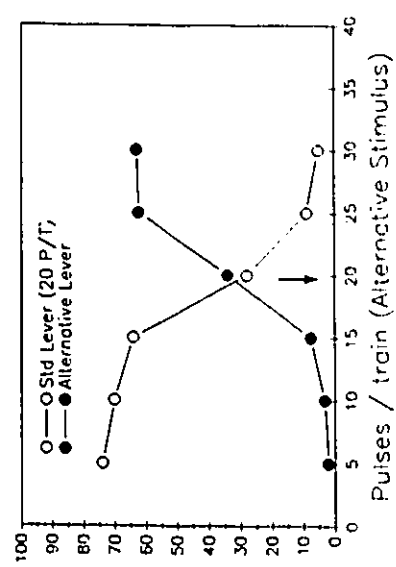
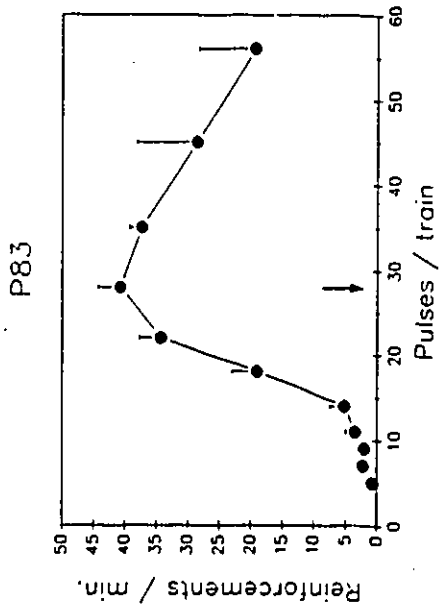
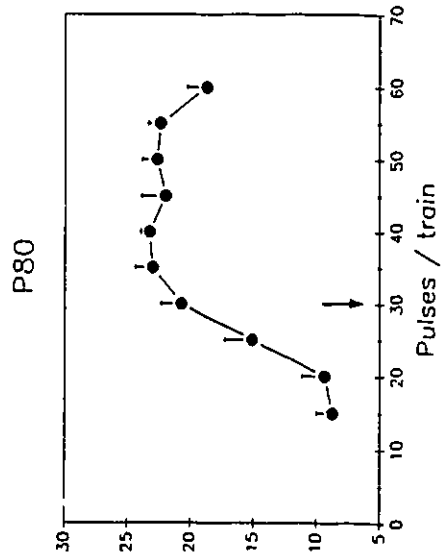
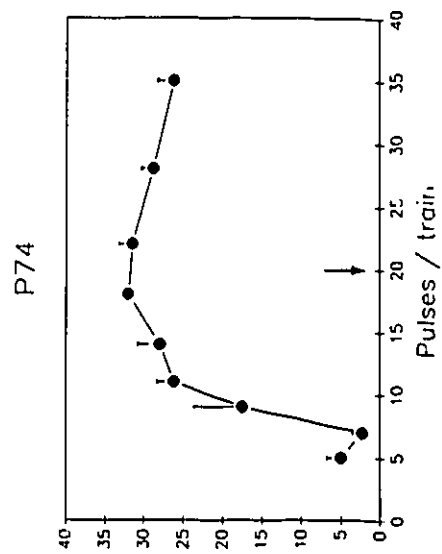
The behavioral data are presented in Figure 14. Upper panels show the rate-frequency functions. The arrows point to the frequencies used as the standard in the isopreference test. Lower panels show the bar-pressing rates for the standard and alternative stimuli as a function of the frequency in the alternative stimulus. Isopreference is shown by the intersection of the two data lines. The arrows point to the projection of this intersection point on the x-axis.

Note that the two data lines intercept each other at a frequency of the alternative stimulus virtually identical to that of the standard stimulus. In other words, isopreference was shown for stimuli of the same physical magnitude, as normally expected. Most relevant to the present work is the fact that the animals preferred the alternative stimulus when its frequency was higher than that of the standard stimulus. This phenomenon provides clear indication that the asymptote of the rate-frequency function was not due to a saturation in the reinforcing efficacy of the stimulation. If saturation had occurred, bar-pressing for the alternative stimulus should then have reached an asymptote for a frequency equal to the frequency of the standard stimulus.

This experiment indicates that the asymptotic self-stimulation rate in the amygdala was not constrained by a saturating effect in the reinforcing stimulation efficacy. The next experiment provided an alternative complementary test of this interpretation.

Figure 14:

Upper panels: Rate-frequency functions for amygdaloid stimulation. The arrows on the x-axis point to the pulse frequency used as the standard stimulus in the isopreference test shown below. Lower panels: Bar-pressing rates for a fixed pulse frequency (the standard stimulus) and for a variable pulse frequency (the alternative stimulus), as a function of the frequency in the alternative stimulus. Isopreference for the two stimuli is shown by the intersection of the two data lines. The arrow represents the projection of the intersection point on the x-axis. Numbers on top of each set of figures identify the subject. Abbreviations: Std (standard), P/T (pulses per train).



EXPERIMENT 2:

Competition for amygdaloid versus amygdalo-hypothalamic stimulation

Introduction

If the rate-enhancing effect of hypothalamic pulses was due to an increase in the reinforcing efficacy of the amygdaloid stimulation, animals then should prefer a frequency of amygdalo-hypothalamic pulse pairs over the same frequency of amygdaloid pulses. Since the isopreference test is based on rates, it might be considered inappropriate for the present purpose since the rates for the two competing stimuli could be markedly different. However, it has recently been found that isopreference for identical frequencies occurred even when bar-pressing for one of the stimuli was substantially limited by adding weight to one of the levers (Malette & Miliaressis, 1990). Isopreference occurs because the animals self-stimulate for longer periods with the heavy lever.

METHOD

Subjects:

Subjects A57 & A58 from the previous mapping experiment (chapter 3) were used.

Materials:

The testing apparatus was the same as that used in the previous experiment.

Procedure

Training & Testing:

The amygdaloid electrodes for both subjects were lowered 0.16 mm and a new set of

amygdaloid and concurrent amygdaloid-hypothalamic rate-frequency functions was obtained, as described previously. The amygdaloid current was adjusted to obtain a rate-frequency function with a frequency range lying below that for the hypothalamic function (a replication of condition b; method section; chapter 3). Animals were then tested in the twin-lever box. One lever delivered a fixed frequency of amygdaloid pulses (the standard stimulus) whereas the second lever delivered a variable frequency of amygdalo-hypothalamic pulse pairs (the alternative stimulus). The pulse frequency of the amygdaloid pulses was fixed at the value found to yield approximately 80-90 % of maximal rate. The frequency of the amygdalo-hypothalamic pulse pairs was varied every 5 min, in two ascending and two descending sequences. The number of bar-presses for the competing stimuli was recorded every 5 min. Testing sessions were repeated daily for a period of one week. Following completion of the above, the amygdaloid electrode of subject A57 was lowered 0.16 mm and the entire procedure was replicated.

Results & Discussion

Histological Results:

Histological data are shown in Figure 15. Note that for subject A57 two sites were tested in the amygdala.

Behavioral Results:

The upper and lower panels of Figure 16 show the rate-frequency functions and preference data, respectively. The arrows show that isopreference was obtained for frequencies of amygdalo-hypothalamic pulse pairs equal to (subject # A57-2) or higher (subjects A57-1 and A58) than the fixed frequency of amygdaloid pulses. If a frequency of amygdalo-hypothalamic

Figure 15:

Histological data for subjects A57 and A58. The location of the LH electrodes are shown on the left histological plates; the location of the amygdaloid test sites are shown on the right plates. Letter and number in upper cortex identify subject; number and sign at the bottom of plate denotes distance (in mm) and direction from Bregma according to the atlas of Paxinos and Watson (1982).

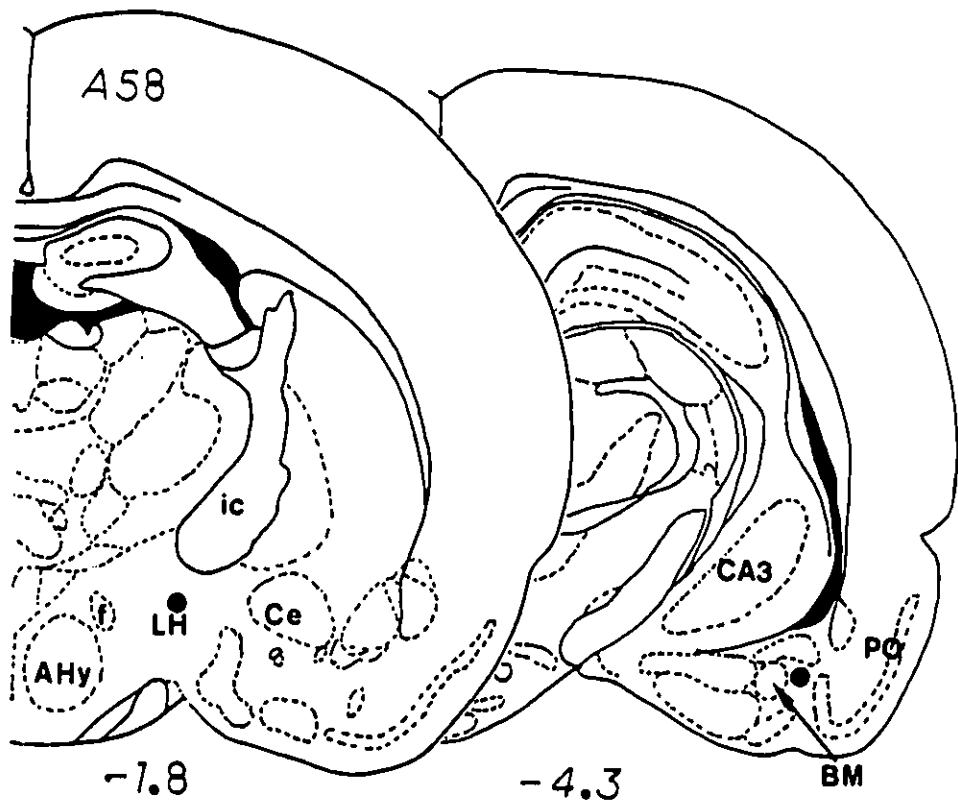
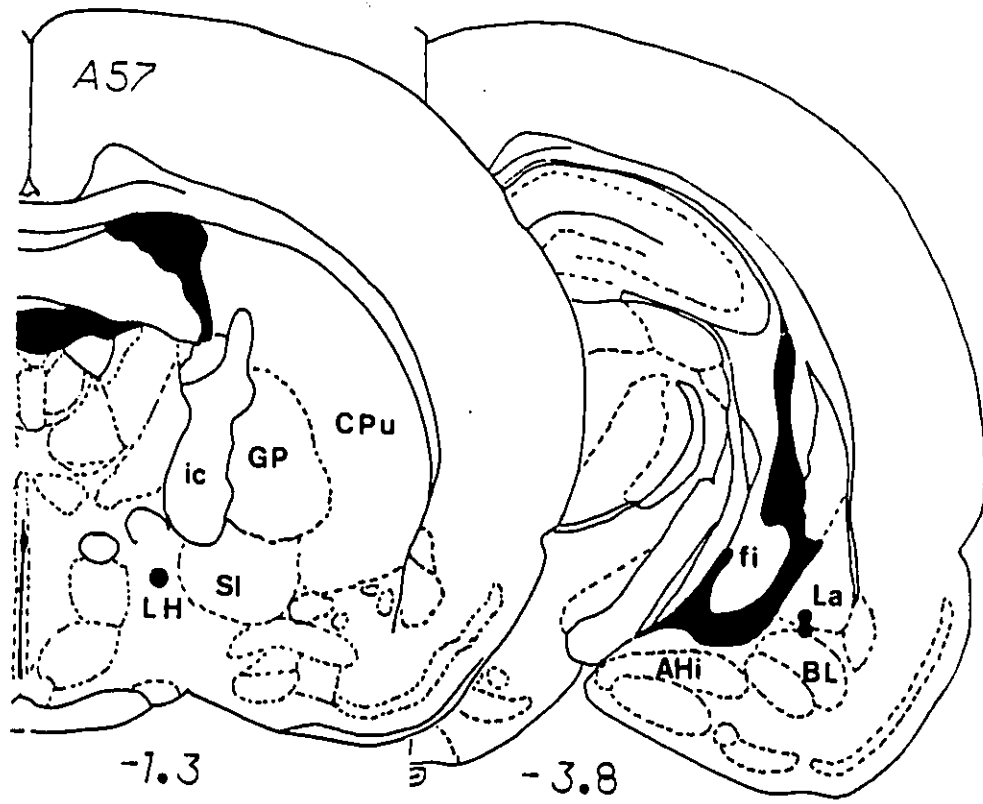
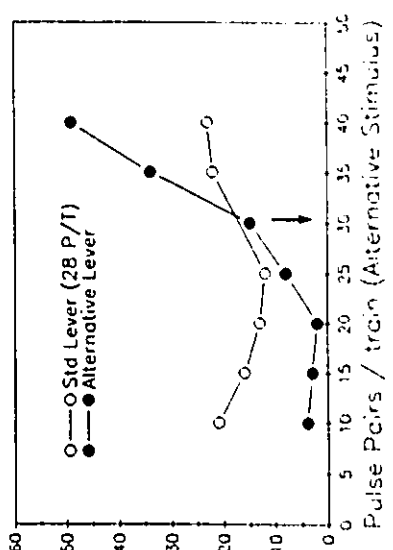
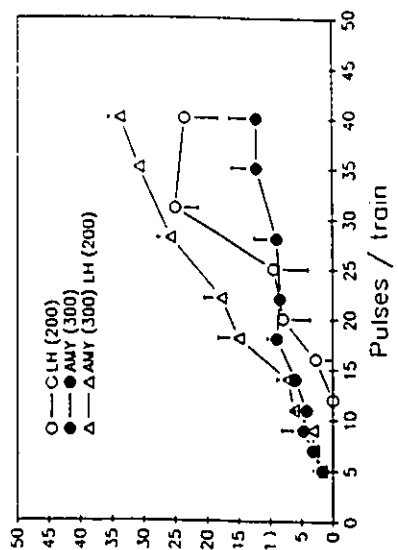


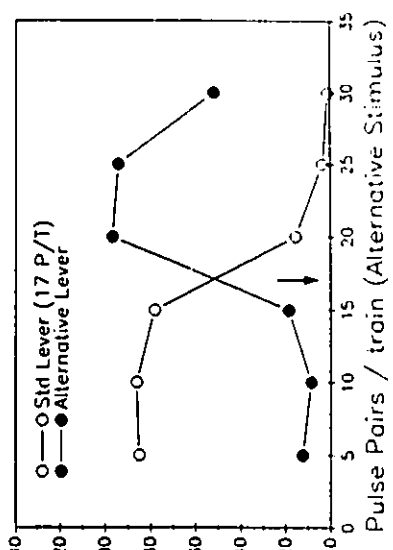
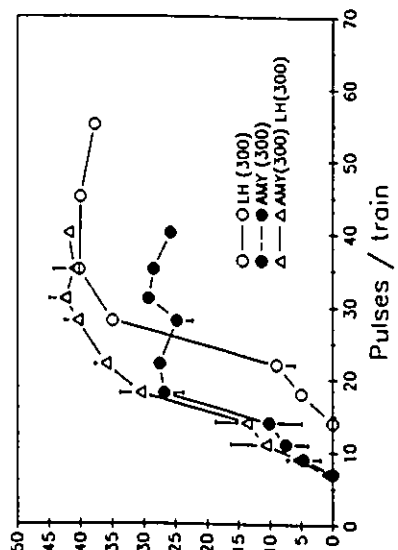
Figure 16:

Upper panels: Rate-frequency functions for amygdaloid (AMY), lateral hypothalamic (LH) and concurrent AMY+LH stimulation. Numbers in parentheses refer to the pulse intensity in μA . Lower panels: Bar-pressing rates for a fixed frequency of AMY pulses (the standard stimulus) and a variable frequency of AMY+LH pulse pairs (the alternative stimulus), as a function of the frequency in the latter. Isopreference for the two competing stimuli is shown by the intersection of the two data lines. The arrows represent the projection of this intersection point on the X-axis. Numbers on top of each set of figures identify the subject. Abbreviations: Std (standard), P/T (pulses per train).

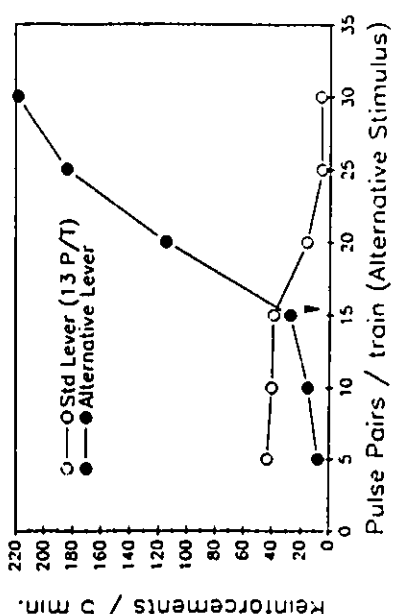
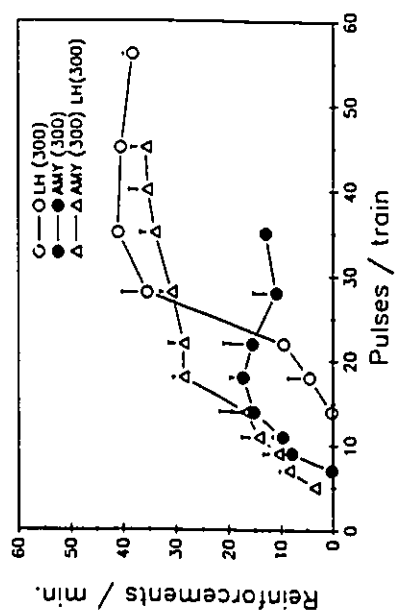
A58



A57-2



A57-1



pulse pairs was more rewarding than the same frequency of amygdaloid pulses, the equi-preferred frequency should then be expected to shift to a value lower than the fixed frequency of amygdaloid pulses. The data thus add substance to the view according to which the rate-enhancing effect of hypothalamic pulses, documented extensively above, was due to the inhibition of some performance contaminating factor, not to reward summation. The nature of this putative factor was explored in the next experiment.

EXPERIMENT 3:

The effects of Phenobarbital on amygdaloid self-stimulation

Introduction

Epileptiform activity (for example, wet dog shakes and freezing responses) was often displayed by our animals bar-pressing for amygdaloid self-stimulation. However, despite the occurrence of these contaminative behaviours, amygdaloid rate-frequency functions were remarkably stable. Animals might have limited lever-pressing to maintain excitation levels below threshold for overt seizures. This would explain the low level of responding that characterizes self-stimulation in the amygdala. A much different behavioral profile, however, emerged under concurrent amygdalo-hypothalamic stimulation: The frequency of these contaminative influences diminished and bar-pressing rates increased. Furthermore, the higher the hypothalamic current the lower the incidence of these contaminative behaviours. The behavioral facilitation observed under the conjoint amygdalo-hypothalamic pulse condition might then be attributable to a suppression of epileptiform activity, originating in the amygdala. Indirect evidence in support of this conjecture has been published by Dubicka et al., (1978). In their study, electrical stimulation of the lateral hypothalamus attenuated a carbachol induced convulsive syndrome in the amygdala. The possible involvement of mutually antagonistic noradrenergic and cholinergic systems was presented as a explanatory model.

Various drugs have been shown effective in controlling epileptiform seizures. These drugs include the hydantoins (Diphenylhydantoin, Mephentoin and Ethotin), barbiturates (Phenobarbital, Mephobarbital and Metharbital), and benzodiazepines (Diazepam and

Chlordiazepoxide). Interestingly, many of these drugs have been shown to influence various aspects of self-stimulation behaviour. The fact that hypothalamic self-stimulation can be impaired by stimulation-induced seizures was demonstrated by Reid et al. (1964), who observed facilitation of self-stimulation following injections of four anti-convulsant drugs (Phenobarbital, Tridione, Mysoline and Phenurone). Olds (1970) and Domino and Olds (1972) observed a dose-dependent effect of chlordiazepoxide and diphenylhydantoin on self-stimulation. A low dose of the former drug produced a facilitation, while a high dose produced a depression of self-stimulation. The latter drug, on the other hand, seemed to depress this behaviour. More recently, Albright and Burnham (1980) showed that diazepam and phenobarbital were more effective than phenytoin (diphenylhydantoin) in controlling either amygdala or cortical-generalized seizures. Phenytoin was more effective in controlling cortical than generalized seizures. Finally, Robertson et al (1982) showed that both diazepam and phenobarbital had no effects on responding for self-stimulation of the prefrontal cortex, but attenuated the facilitative effect of prefrontal cortex stimulation on the development of self-stimulation in that area.

In preparing the present experiment we reasoned that if the effect of lateral hypothalamic stimulation was to suppress epileptiform activity associated with amygdaloid self-stimulation, a chemical agent with proven anti-convulsant properties should then mimic the hypothalamic effect. Phenobarbital was chosen for this purpose because this compound has been found to be more effective than diphenylhydantoin in controlling either amygdaloid or cortical-generalized seizures (Albright & Burnham, 1980).

METHOD

Subjects:

Four male, Long Evans rats were used. Three rats were naive and the fourth (subject P83) had been used previously (Experiment 1 above). All other particulars have been described previously.

Materials:

Testing apparatus and peripherals were described in the previous chapter.

Procedure

Surgery:

Subjects were implanted each with a monopolar stimulating electrode (described previously) aimed at the amygdala. Implantation coordinates relative to bregma, the midline and skull surface at bregma were: - 3.8 mm, 5.3 mm & 7.9 mm for subject #D75; - 3.3 mm, 4.5 mm & 7.9 mm for subject D81; and - 3.3 mm, 4.5 mm & 7.5 mm for subject D84. Subject P83's coordinates were presented previously (method section of experiment 1).

Training & Testing:

Rats were trained to bar-press for electrical stimulation of the amygdala. Stimulation parameters consisted of .3 sec trains of cathodal rectangular pulses of fixed duration (0.1 msec) and variable frequency.

Rate-frequency functions were then obtained following intraperitoneal injection of physiological saline or Phenobarbital (Abbot Laboratories) at varying doses. Phenobarbital was administered in increasing doses (10, 15 & 20 mg/kg), one dose every other day. A drug-free saline day preceded each drug administration. Self-stimulation began fifteen min after the

injection of saline or of the drug.

Histology:

At the conclusion of the experiment, the location of the tips of the stimulating electrodes were marked using the previously described ferrocyanide marking technique. The brains were then extracted from the cranium and stored in 10 % formalin for 1 week. Brain sections, 40 μM in thickness, were obtained in a cryostat at -20°C ., and drawings of the unstained sections immediately made under magnification. Sections were later stained with thionine for further histological analysis.

Results & Discussion

Histological Results:

Histological data are presented in Figure 17. All stimulation sites were within the anatomical boundary of the amygdaloid complex.

Behavioral Results:

Figure 18 illustrates the rate-frequency functions obtained under saline and varying doses of phenobarbital. The rate-frequency function did not differ under the various saline injections. Therefore, the mean saline function is presented. The data show that phenobarbital increased rates for two of the subjects (D75 and P83). Missing doses indicate that no difference was observed between the effect of 10 and 15 mg/kg, for these subjects. A dose-dependent effect was noted for subject P83. The absence of a lateral shift and the magnitude of the rate-enhancing effect are reminiscent of the effect obtained under concurrent amygdalo-hypothalamic stimulation. However, for the remaining two subjects, a rate decrease was noted. In addition,

Figure 17:

Histological data for subjects P83, D75, D81 and D84. Black dots show the location of the electrode tips. Letter and number in upper cortex identify subject; number and sign at bottom of histological plate denotes distance (in mm) and direction from bregma according to the atlas of Paxinos and Watson (1982).

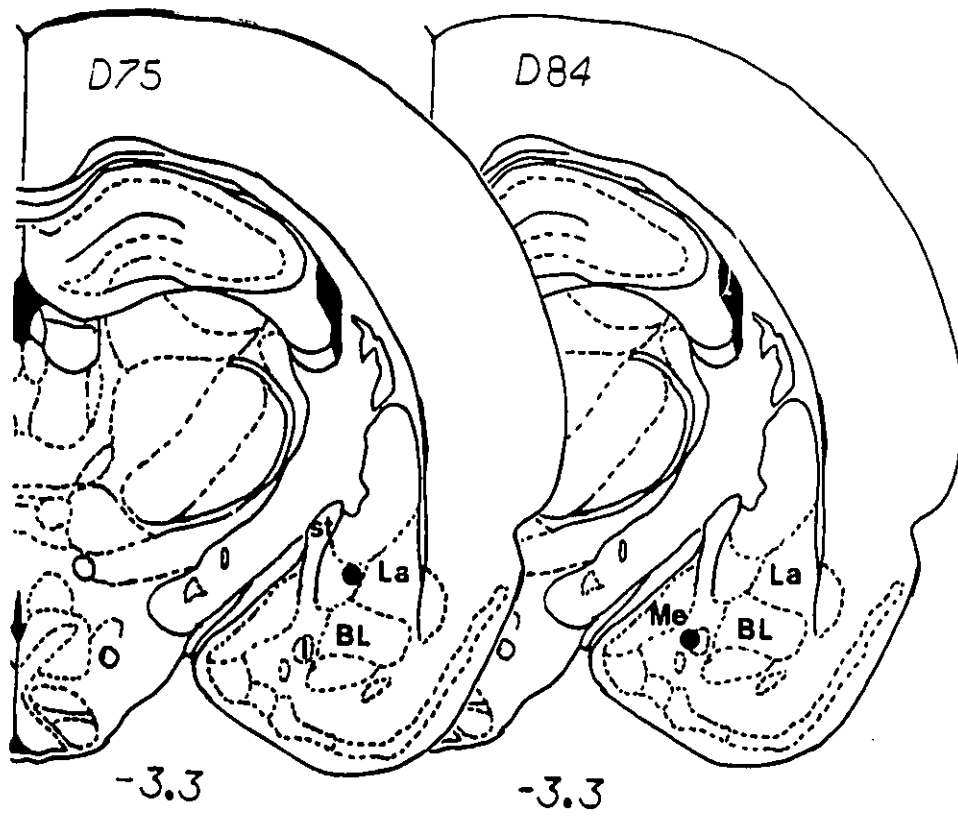
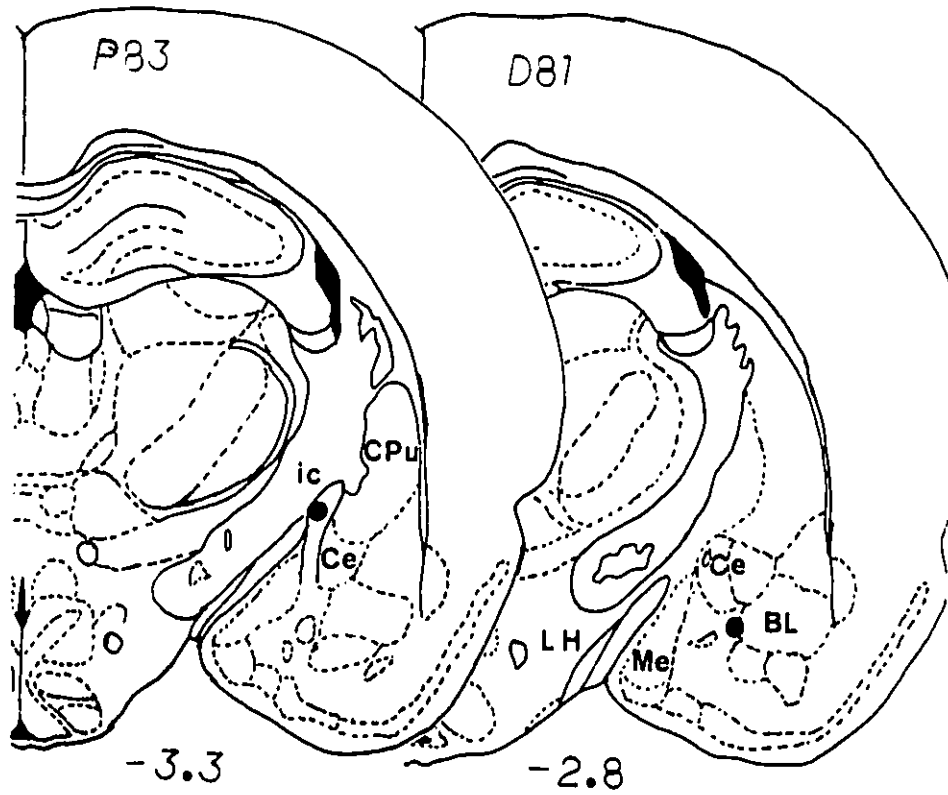
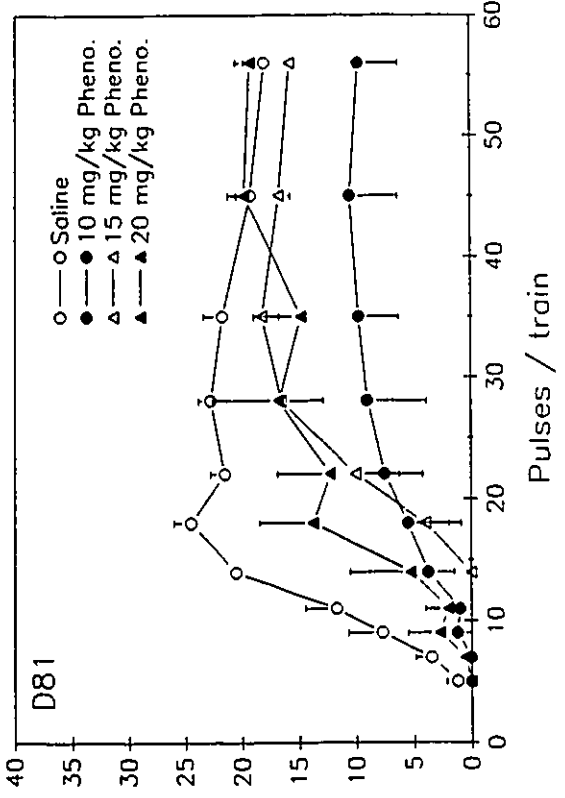
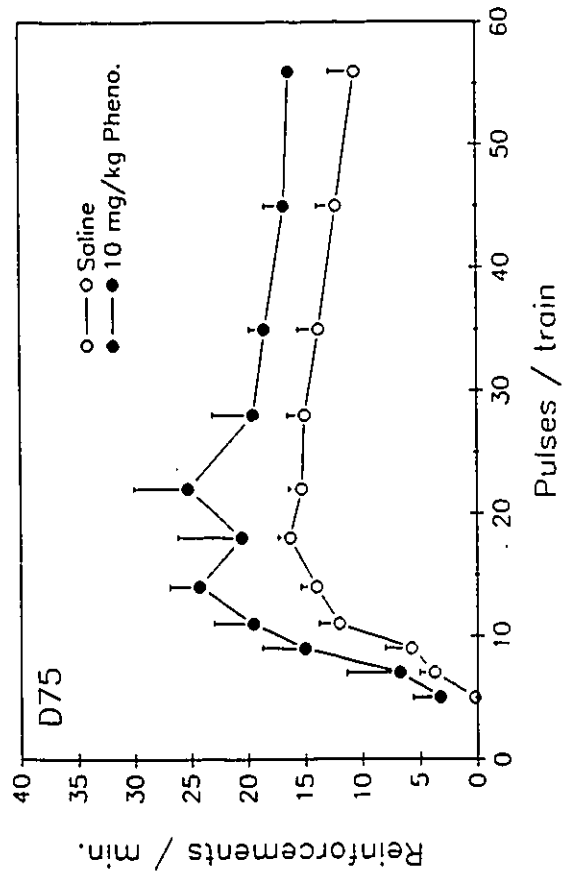
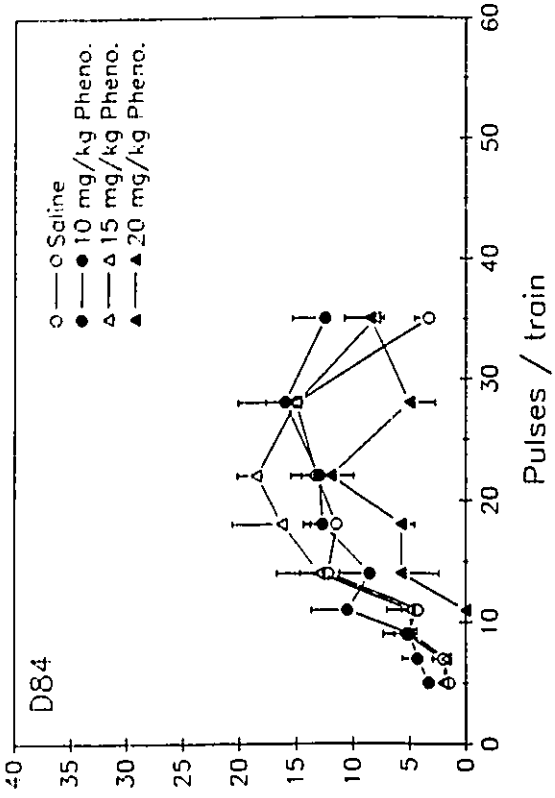
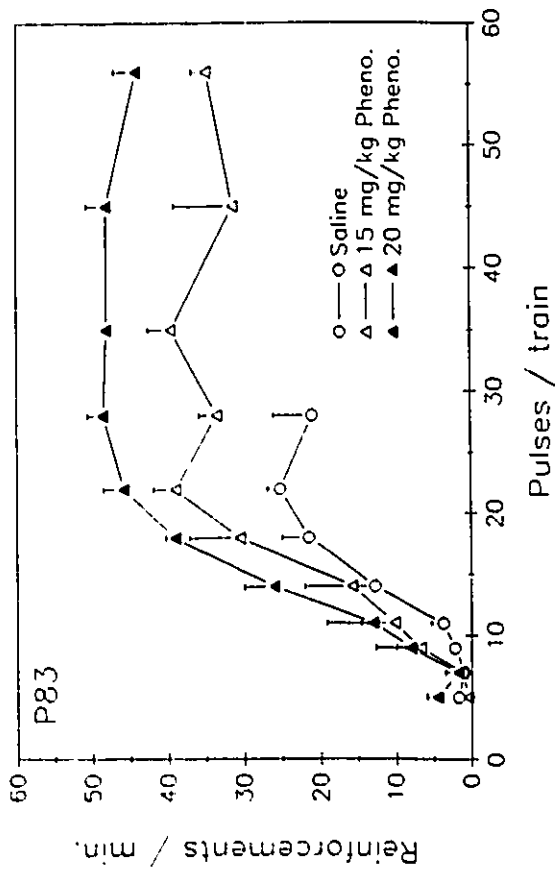


Figure 18:

Effect of Phenobarbital on the amygdaloid rate-frequency function. Numbers in upper left corner identify the subject. Abbreviation: Pheno. (Phenobarbital).



a dose of 15 or 20 mg/kg shifted the rate-frequency function toward substantially higher frequencies.

One explanation for the discrepant results obtained between subjects may be that some amygdaloid sites were more responsive to the anti-convulsant effects of phenobarbital than others, more specifically, at the doses used in this experiment. The sites that supported the facilitative effect were located dorsally (see histology). Manifest epileptiform activity was virtually absent for subject P83 at the highest dose tested, suggesting that the drug was effectively suppressing covert seizure activity. On the other hand, epileptiform activity was still visible for subjects D81 and D84 self-stimulating under phenobarbital.

The data obtained in the present experiment may suggest, although equivocally, that the rate-enhancing effect of lateral hypothalamic pulses was due to the ability of this structure to suppress epileptiform activity accompanying amygdaloid self-stimulation. Alternatively, the hypothalamic rate-enhancing effect may reflect the attenuation of fear-inducing properties of amygdaloid stimulation. The question might be better approached by monitoring the electroencephalographic recording in animals bar-pressing for amygdaloid and concurrent amygdaloid-hypothalamic stimulation.

EXPERIMENT 4:

Anatomical specificity of the rate-enhancing effect

Introduction

In the final experiment of this series, the regional anatomical specificity of the observed rate-enhancing effect was investigated in a small group of animals. We wanted to know more specifically whether this effect might be mimicked by concurrent activation of extra-hypothalamic reward areas. For this purpose, rats were prepared with two electrodes, one in the amygdala and the other in a site several mm rostral or caudal to the lateral hypothalamus. Moveable electrodes were used and a number of sites tested in each subject.

METHOD

Subjects:

Four, male, Long Evans rats were used. All other particulars have been described previously.

Materials:

Testing apparatus was the same as that used in the experiment described in chapter 3.

Procedure

Surgery:

Under anaesthesia (Sodium Pentobarbital: 50 mg/kg), subjects (300 to 325 g at the time of surgery) were implanted with miniature, moveable electrodes (Kinetrods, Ottawa). Two subjects received implants aimed at the rostral medial forebrain bundle (MFB) at the level of the

decussation of the anterior commissure and the amygdala. The implantation coordinates relative to bregma, the midline, and skull surface at bregma were : -3.8 mm, 5.3 mm & 7.5 mm for the amygdala and + .2 mm, 2.5 mm & 8.7 mm for the MFB for both subjects B50 & B48. The remaining two subjects were implanted with miniature moveable electrodes aimed at the dorsal raphe and the amygdala. The implantation coordinates were: - 3.3 mm, 4.5 mm & 7.5 mm for the amygdala and - 8.3 mm, 0 & 6.5 mm for the dorsal raphe for both subjects B73 and B74. All other particulars, including construction of the indifferent electrode, have been described previously.

Training & Testing:

Following a post-operative recovery period of one week, subjects were trained to bar-press for stimulation of the rostral MFB and dorsal raphe. If required, the electrode was lowered in increments of .16 mm until self-stimulation was observed. Subjects were then trained to bar-press for stimulation of the amygdala. Again, if required, the electrode was lowered. Stimulation parameters for both substrates consisted of .3 sec. trains of cathodal rectangular pulses of fixed duration (0.1 msec.) and variable frequency.

Rate-frequency functions were then obtained for all sites and subjects as described previously (with the exception of one intended amygdaloid target). Current was selected such that the MFB and dorsal raphé rate-frequency curves were located to the right of the amygdaloid curves on the frequency axis. This pattern, in the location of the rate-frequency curves, essentially replicates condition "b" of the previous mapping experiment (Chapter 3). The stimulation parameters used to obtain these functions were then combined to obtain the conjoint amygdalo-MFB and amygdalo-dorsal raphe self-stimulation functions. The inter-pulse interval

was set at 2.5 msec..

The amygdaloid electrode (for subject B74) was then lowered .16 mm and the conjoint experiment repeated. The dorsal raphe electrode for subject B73 was lowered .16 mm twice and the double pulse experiment repeated at both these sites. Then, the amygdaloid electrode was lowered .16 mm (subject B73) and a final test performed. The following combination of sites was therefore tested:

Subject B74: amygdaloid test site # 1; dorsal raphe site # 1

amygdaloid test site # 2; dorsal raphe site # 1

Subject B73: amygdaloid test site # 1; dorsal raphe site # 1

amygdaloid test site # 1; dorsal raphe site # 2

amygdaloid test site # 1; dorsal raphe site # 3

amygdaloid test site # 2; dorsal raphe site # 3

Histology:

At the conclusion of the experiment, the terminal stimulation site was marked using the ferrocyanide marking procedure described previously. All other procedural particulars have been described previously.

Statistical Treatment:

Rate-frequency curves were fitted using the regression procedure described previously and estimates of threshold (X_i) and asymptotic performance computed.

Results & Discussion

Histological Results:

Histological data are presented in Figure 19. Stimulation sites for three of the 4 intended amygdaloid targets were located in the amygdala. The fourth electrode was misplaced in the ventral hippocampus (B50). The two MFB electrodes (subject B48 & B50) were located in the course of this bundle at the level of the decussation of the anterior commissure. The intended raphe electrodes (subject's B73 & B74) were located in the dorsal raphe at the level of the decussation of the superior cerebellar peduncle. Arrows identify sites that match behavioral data described below.

Behavioral Results:

Representative rate-frequency functions and relevant statistics are presented in Figure 20 and table 4, respectively.

The pattern of responding under concurrent amygdalo-MFB stimulation for subject B48 is reminiscent of that obtained previously under amygdalo-lateral hypothalamic stimulation: Bar-pressing rates increased scalarly as a function of the MFB intensity. Concurrent stimulation of the MFB and the ventral hippocampus (a brain region that failed to support self-stimulation) produced a converse scalar effect, suggesting that hippocampal stimulation impeded the animal's ability to perform the task, or that the animal adjusted bar-pressing rate so as to minimize some secondary effect of the stimulation.

The response profiles for the combined amygdala-dorsal raphe stimulation (subjects B73 & B74) were markedly different. Concurrent stimulation of the dorsal raphe had no effect on amygdaloid self-stimulation rates and thresholds, as indicated in Figure 20 and statistically

Figure 19:

Histological data for subjects B48, B50, B73 and B74. Location of MFB electrodes are shown on the left of each pair of plates; amygdaloid and hippocampal test sites are shown on the right. Letter and number in upper cortex identify subject; number and sign on bottom of plate denotes distance (in mm) and direction from bregma according to the atlas of Paxinos and Watson (1982).

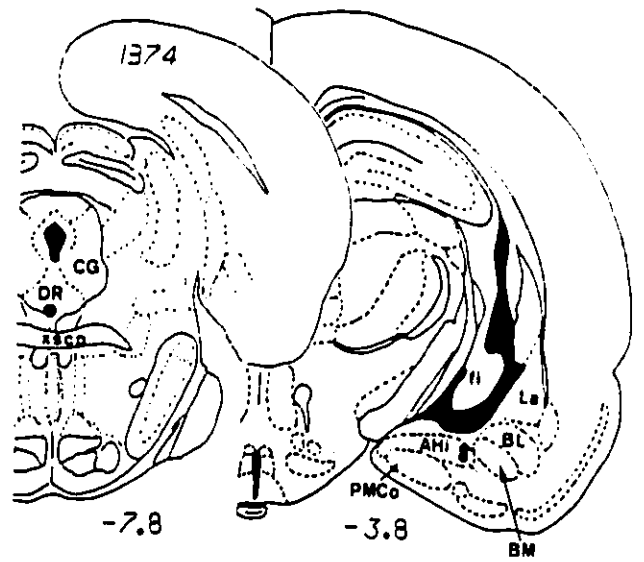
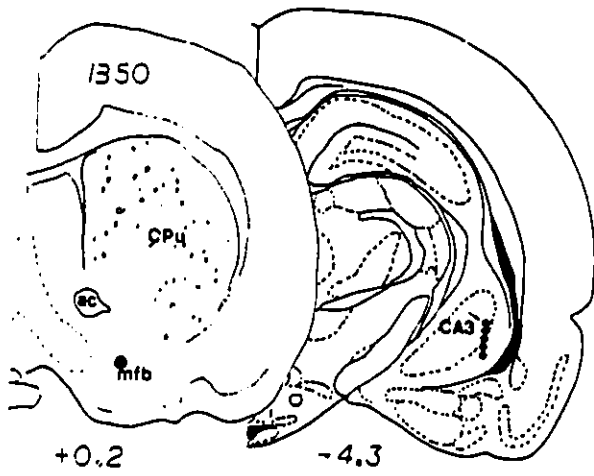
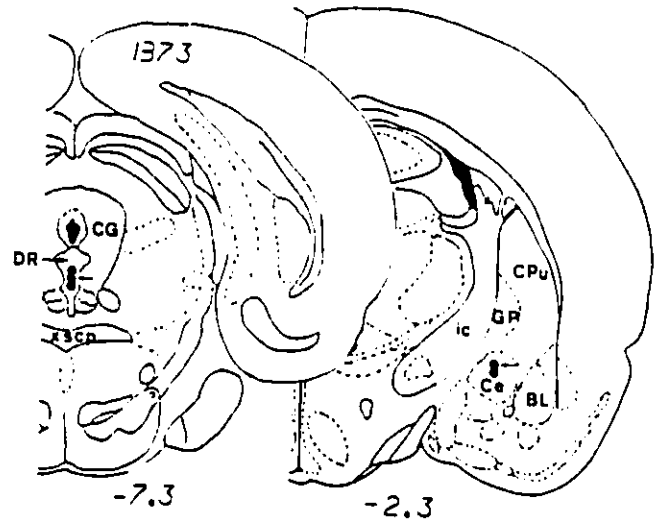
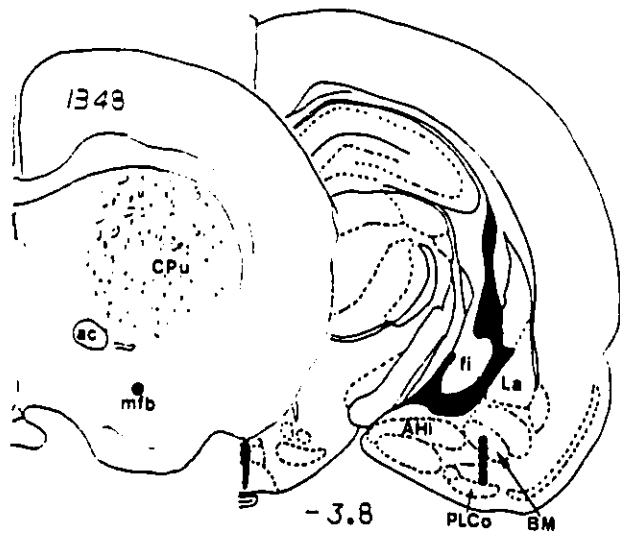


Figure 20:

Rate-frequency functions for amygdaloid (AMY), medial forebrain bundle (MFB), dorsal raphe (DR), and concurrent AMY+MFB, AMY+DR and AMY+Hippocampal stimulation. For subject B50, the intended amygdaloid implant was misplaced in a hippocampal (HIP) area that failed to sustain self-stimulation. Numbers in parentheses indicate pulse intensity in μA . Numbers in upper left or right corner of each box identify the subject.

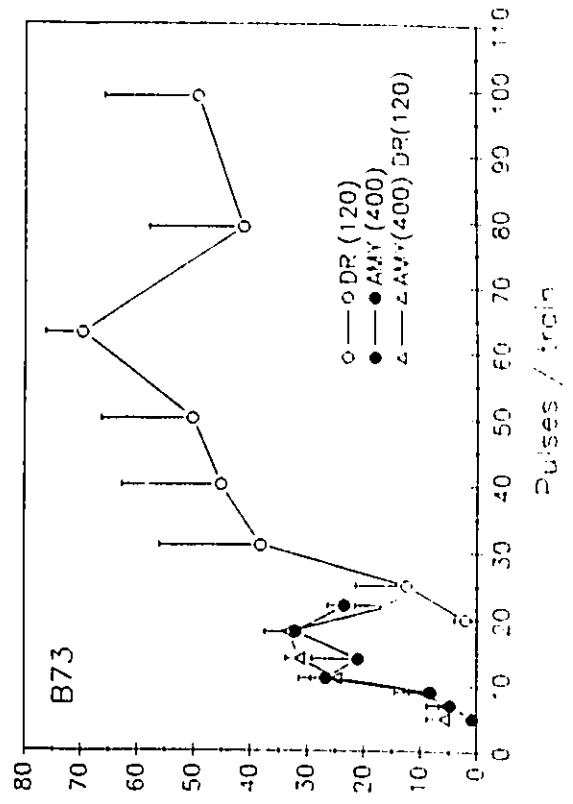
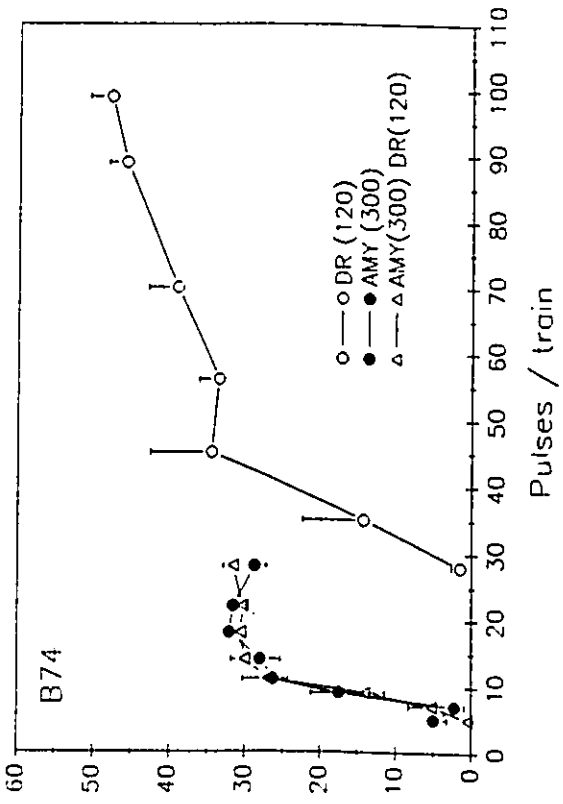
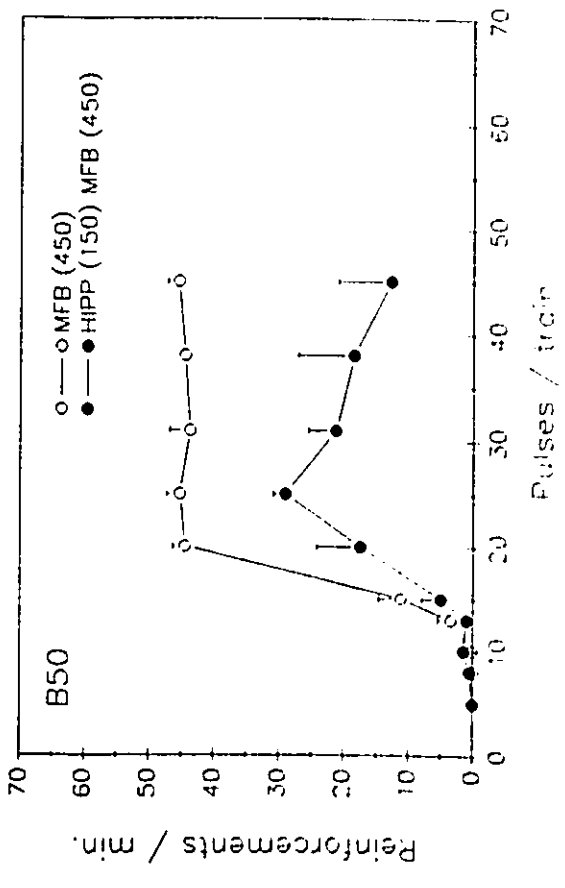
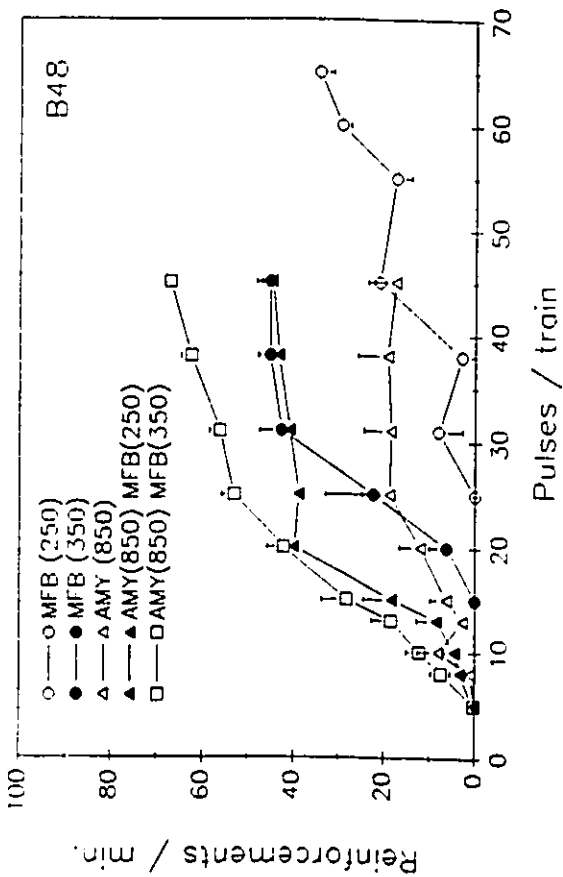


Table 4

Expected threshold frequencies (X_i) and asymptotic rates (A_s) obtained from rate-frequency data fitted with Gompertz's model.

Subject Number	Stim Site	Intensity (μ A)	X_i CI	A_s CI
B73	Raphe	120	26.35 ± 1.35	52.03 ± 9.42
	AMY	400	9.06 ± 0.52	34.51 ± 5.85
	CONC		8.99 ± 0.80	35.59 ± 11.10
B74	Raphe	120	36.14 ± 2.01	43.77 ± 5.89
	AMY	300	8.23 ± 0.42	30.19 ± 2.74
	CONC		8.27 ± 0.33	31.07 ± 1.59
B48	AMY	850	14.37 ± 2.07	19.56 ± 4.30
	CONC MFB	250	14.33 ± 0.49	42.66 ± 2.92
	CONC MFB	350	14.17 ± 0.58	65.89 ± 3.36
B50	MFB	450	16.13 ± 0.50	44.93 ± 0.81
	CONC HIP	150	16.75 ± 1.87	20.24 ± 4.90

Abbreviations: CONC = concurrent stimulation of the amygdala and dorsal raphe. CONC MFB = concurrent stimulation of the amygdala and MFB with the intensity of the MFB pulse identified. CONC HIP = concurrent hippocampal-MFB stimulation with the intensity of the hippocampal (HIP) pulse shown. All other abbreviations as in text.

confirmed in table 4.

The finding of a correspondence between the effects of lateral hypothalamic and rostral MFB stimulation on the amygdaloid rate-frequency function was not surprising when considering the close anatomical and functional relationship between these two brain regions. The fact that raphe stimulation failed to alter the amygdaloid rate-frequency function indicated that the hypothalamic rate-enhancing effect is not shared by all self-stimulation sites.

CHAPTER 5:

Anomalies: implications for self-stimulation research

Introduction

During the course of the mapping of the effects of concurrent amygdalo-hypothalamic stimulation, several site specific apparent reward effects were obtained ie. shifts of the hypothalamic self-stimulation function following stimulation of non-reactive sites in the lateral amygdaloid nucleus. Note that these effects were obtained from only a few test sites. The present chapter presents a detailed description and analysis of these data. A discussion of the methodological and theoretical implications of these data is also elaborated.

METHOD

Subjects, materials and procedures have been described previously in chapter 3.

Results & Discussion

Figure 22 and the lower panel of Figure 23 show three different apparent reward specific effects obtained from concurrent stimulation of the amygdala and lateral hypothalamus-MFB. The location of the stimulation sites are identified in Figure 21. The upper panel of Figure 22 shows a case of apparent reward summation. Combining hypothalamic self-stimulation with low current amygdaloid self-stimulation (essentially a replication of Szabo et al.'s design (1972)) shifted the self-stimulation function laterally towards lower pulse frequencies. In fact, based on X_i threshold estimates, a 70.66% decrease in the required frequency was obtained. Note, however, what happened when current to the amygdala was increased by a factor of 2 and the experiment repeated. The amygdaloid rate-frequency curve shifts to the left in accordance with

Figure 21:

Location of stimulation sites the concurrent stimulation of which resulted in spurious lateral shifts of the concurrent self-stimulation function. Arrows point to the stimulation sites that match the behavioral data shown in figures 22 and 23. Letter and number in upper left cortex (of each pair of histological plates) identifies subject. Number and sign at bottom of each plate denotes distance (in mm) and direction from bregma according to the atlas of Paxinos and Watson (1982).

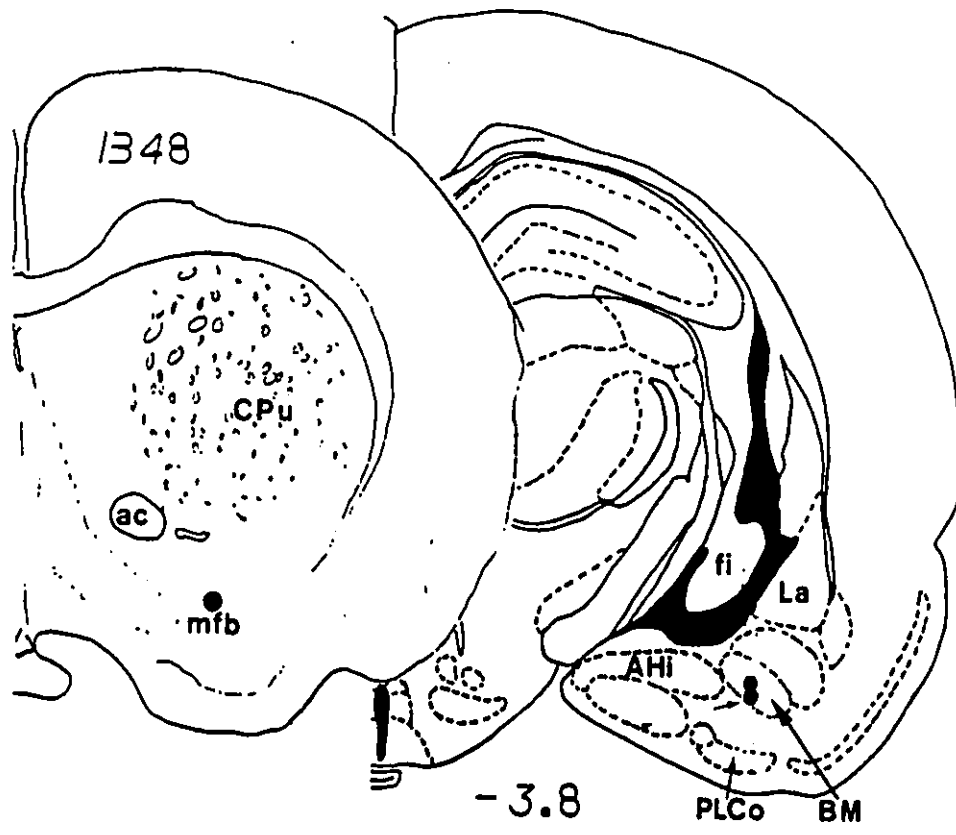
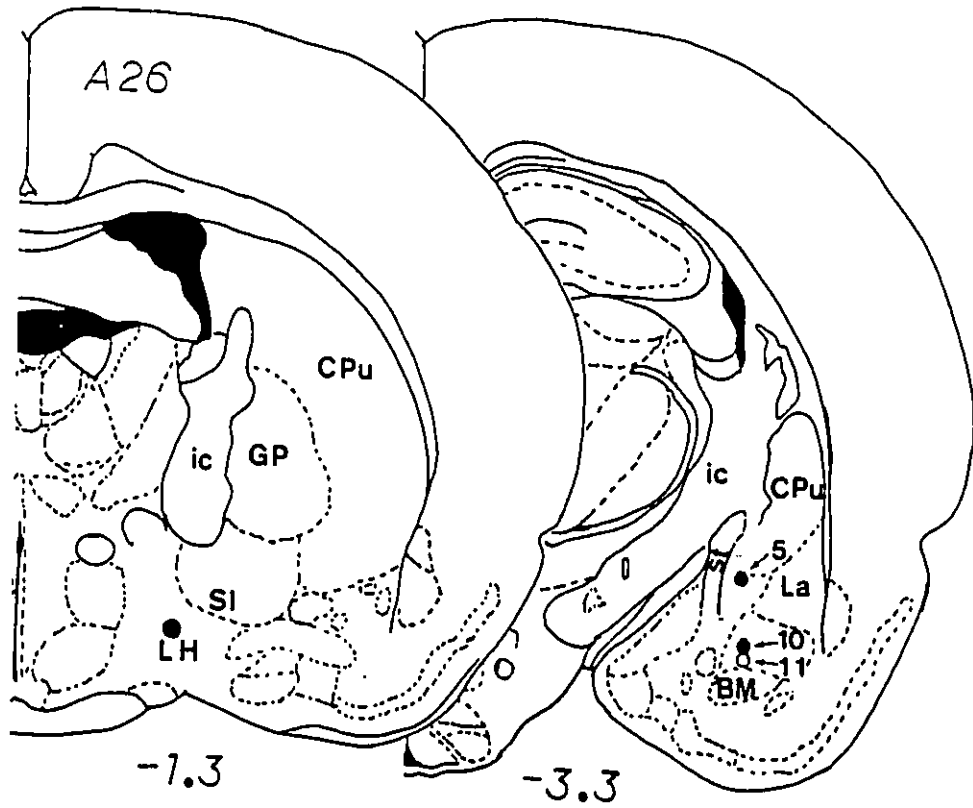


Figure 22:

Upper panel: Amygdaloid (AMY), lateral hypothalamic (LH) and concurrent AMY+LH rate-frequency functions under low and high intensity amygdaloid stimulation conditions. Lower panel: AMY, medial forebrain bundle (MFB) and concurrent AMY+MFB self-stimulation (SS) functions under subthreshold and threshold (for SS) amygdaloid stimulation. Numbers in parentheses (right side of box) refer to the intensity of the pulses in μA . Letter and number in upper left corner of each box identifies subject; number in parentheses identify amygdaloid test site according to figure 21.

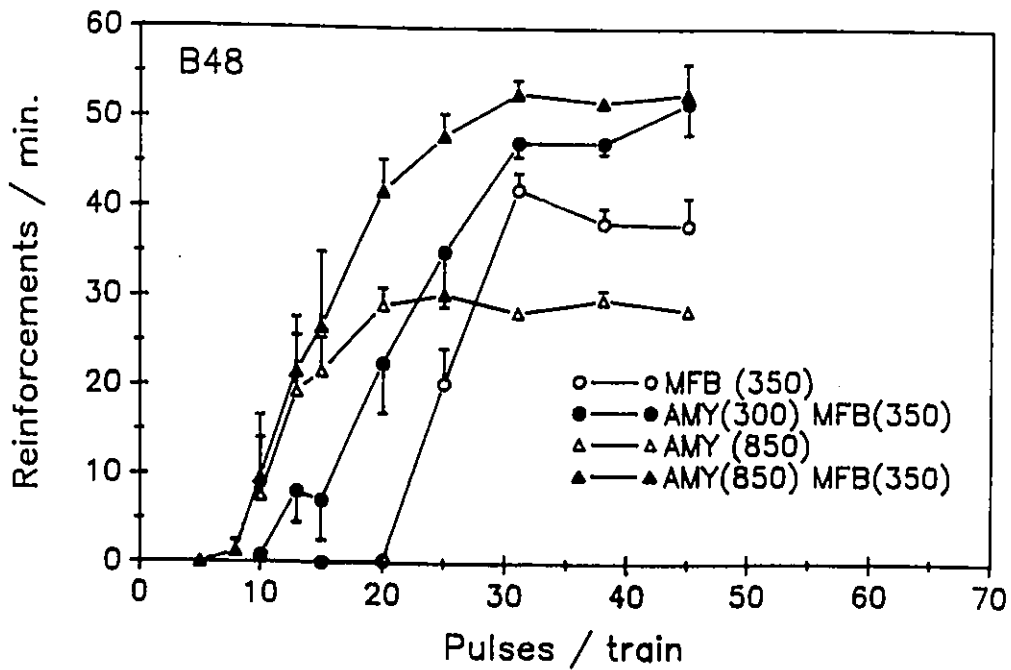
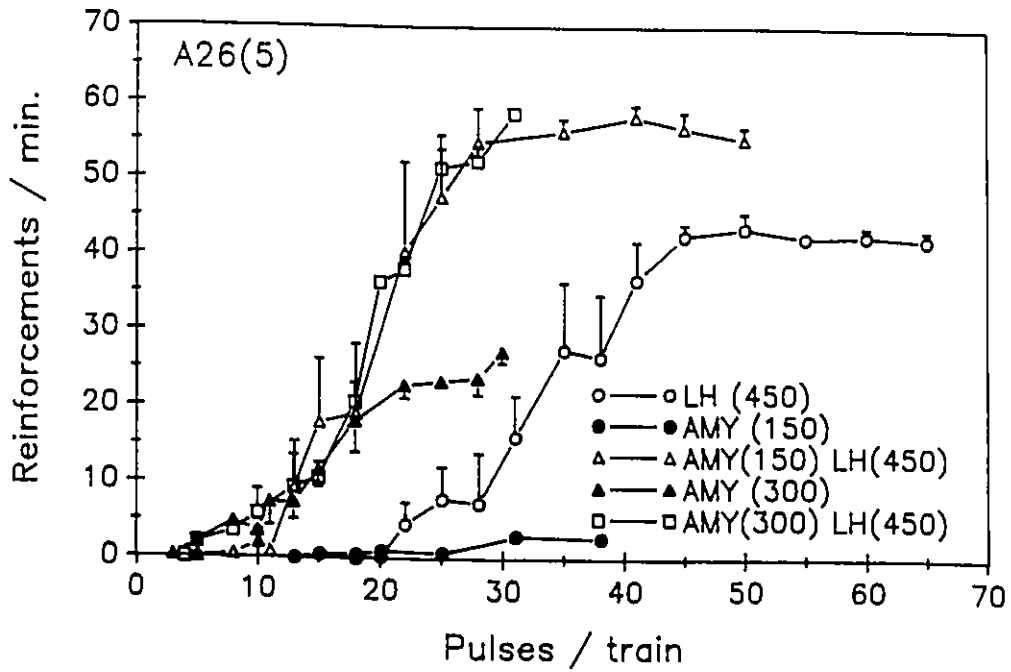
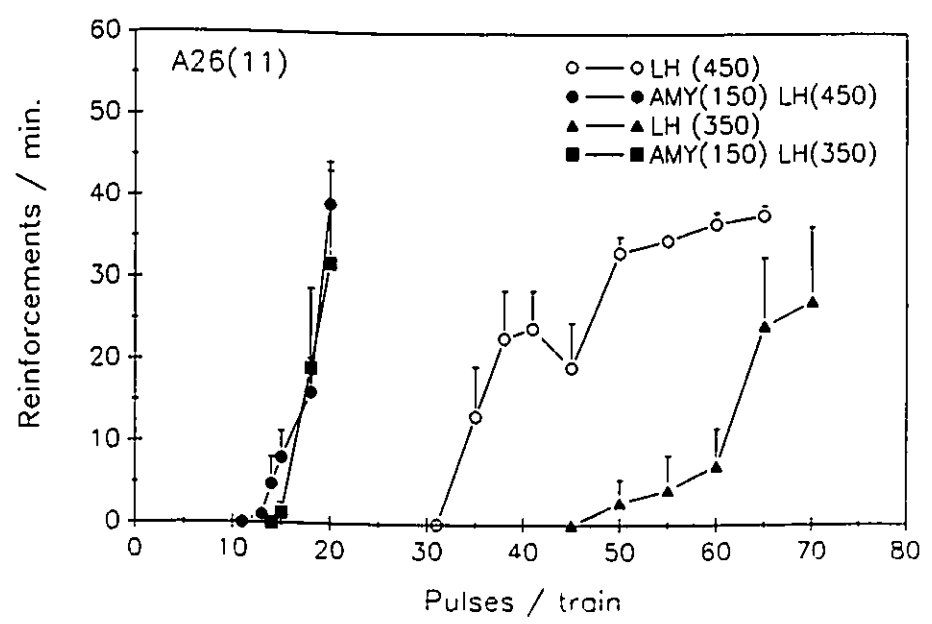
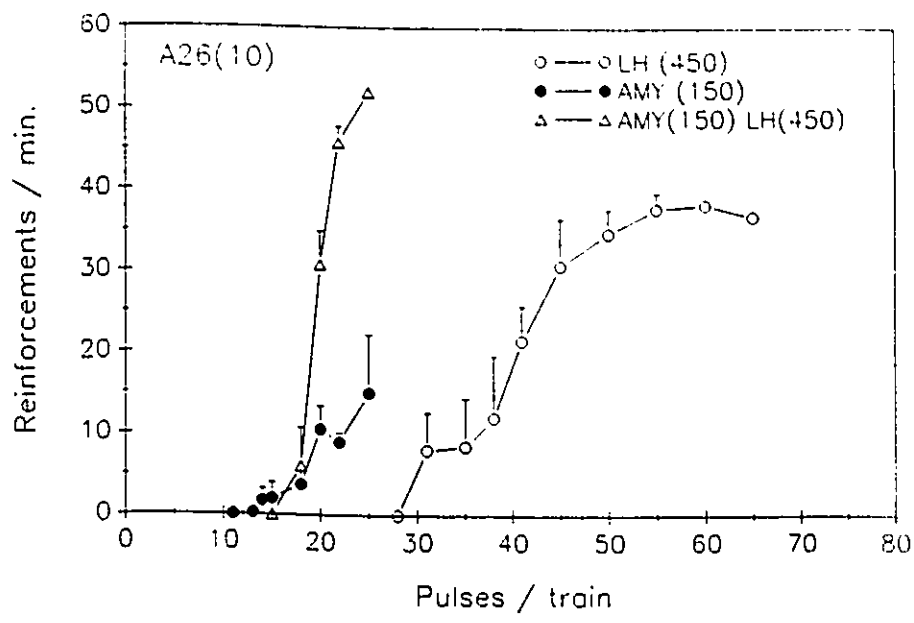


Figure 23:

Upper panel: Lateral hypothalamic (LH), amygdaloid (AMY), and concurrent AMY-LH self-stimulation (SS) functions for AMY test site # 10 (Subject A26). Lower panel: Hypothalamic and concurrent amygdalo-hypothalamic SS function at two different LH pulse intensities for AMY test site # 11 (same subject). Numbers in parentheses refer to the intensity of the pulses in μA (right side of box). Letter and number identify subject; number in parentheses (left side of box) identify amygdaloid test site (according to figure 21).



the principle of spatial-temporal integration described previously. Repeating the double pulse test under this configuration failed to shift the self-stimulation function further. Instead a transformation of the amygdaloid function was obtained. The lower panel of this figure shows a similar case obtained while mapping the effects of amygdalo-MFB stimulation. Combining subthreshold (for self-stimulation) amygdaloid stimulation with MFB self-stimulation produced an apparent shift of the MFB self-stimulation function indicating reward facilitation (similar to the rate-intensity shifts reported by Szabo et al., 1972). However, when the experiment was repeated using above threshold amygdaloid pulses no further lateral shift of the resulting self-stimulation function is observed. A scalar rate effect is rather apparent.

Figure 23 shows a more convincing case of the spurious nature of these shifts. The upper panel shows the effect of subthreshold lateral hypothalamic stimulation on amygdaloid self-stimulation. The rate effect documented in Chapter 3 was obtained. In accordance with the methodology described in Chapter 3, the amygdaloid electrode was then lowered (.16 mm) and an attempt was made to elicit self-stimulation from this new site. Current greater than 150 μ A induced seizures; no further attempt was made to elicit self-stimulation with higher current. The effect of subthreshold (for self-stimulation and seizures) amygdaloid stimulation on lateral hypothalamic self-stimulation was then assessed. Results are shown in the lower panel of Figure 23. Paradoxically, a dramatic lateral shift of the hypothalamic self-stimulation function was obtained. Interestingly, the location of the self-stimulation function at this site did not differ from that obtained at the previous site (shown in the upper panel of this figure) where amygdaloid self-stimulation could be elicited (using the same current intensity). The experiment was then repeated using lower intensity hypothalamic stimulation. Note that decreasing the

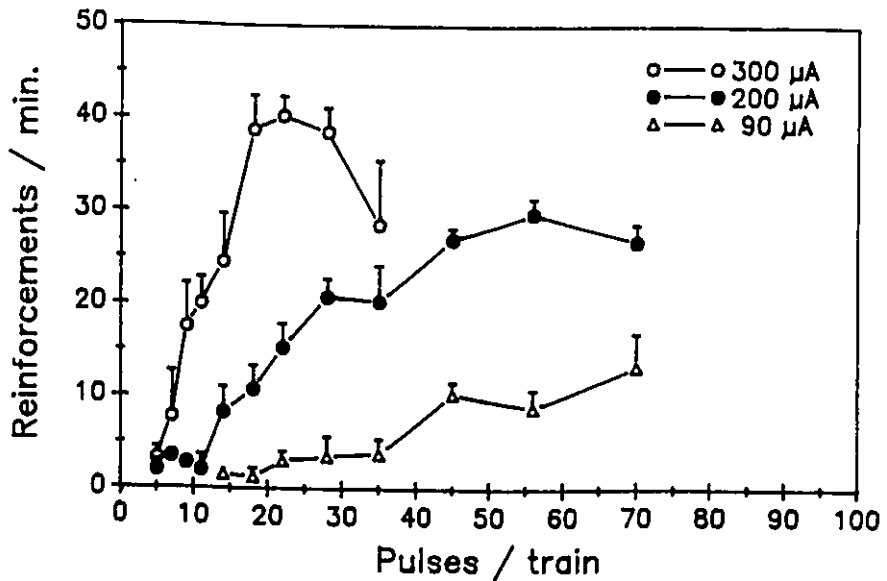
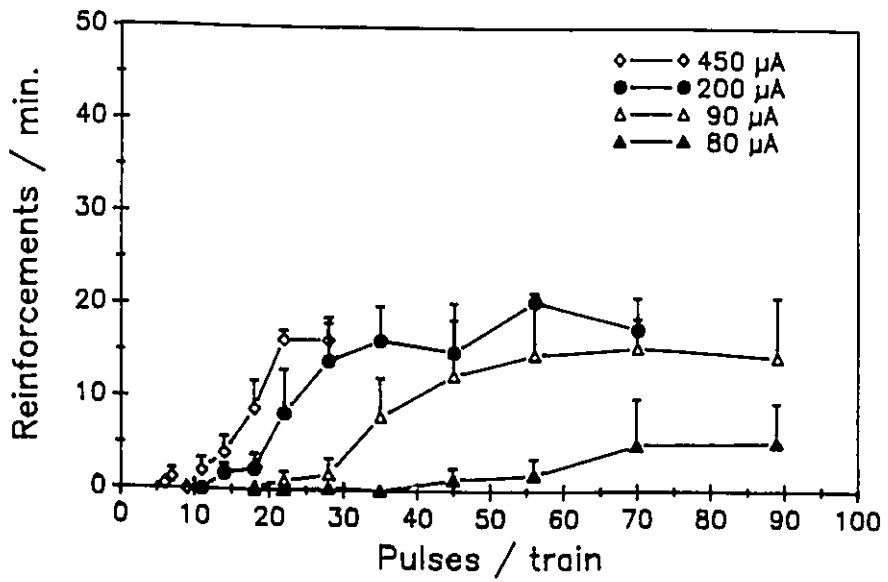
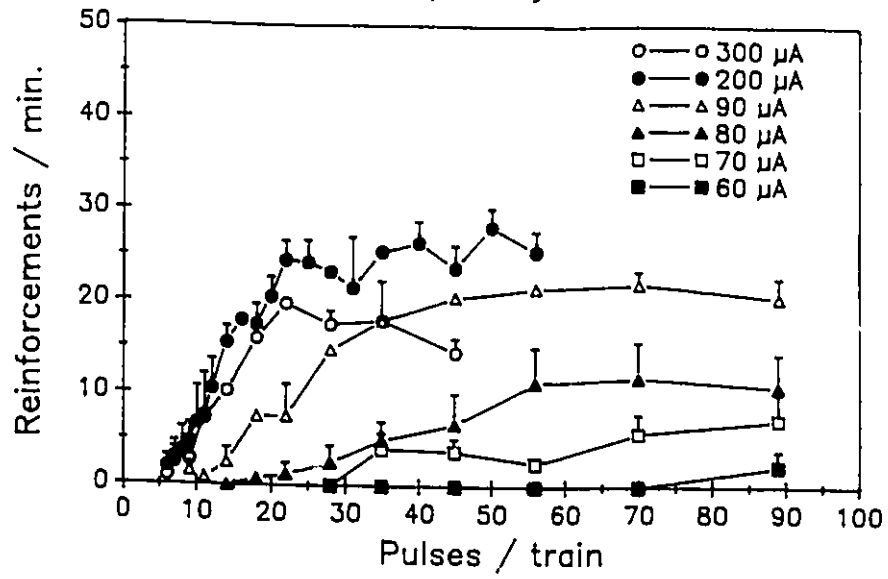
intensity of the hypothalamic pulse shifted the hypothalamic curve to the right. Combining stimulation of the amygdala (150 μA) and low intensity hypothalamic stimulation produced a shift virtually identical to that obtained with the high intensity hypothalamic stimulation. The fact that equivalent shifts were obtained with low and high hypothalamic current is counter to the well established principles of reward integration. An alternative explanation for these data is required.

Consider data obtained during the mapping experiment. Families of typical amygdaloid functions obtained from three different subjects are shown in Figure 24. Two characteristics relevant to the present analysis are evident. Decreases in pulse intensity shifted the rate-frequency curves towards higher pulse numbers; curve shift were therefore obtained consistent with the spatial-temporal integration principle discussed previously. However, unlike the typical current-frequency trade-off profile obtained from the lateral hypothalamus, a marked decrease in asymptotic performance accompanied amygdaloid intensity-frequency curve shifts. For example, for the subject shown in the middle panel of Figure 24, rates dropped dramatically during testing with 80 μA at high frequencies. Error bars indicate that none of the data points comprising the self-stimulation function are significantly different from 0. Variance is accounted for by the observation that animals periodically stopped bar-pressing at these frequencies (evidently because of manifest epileptiform activity). For the animal shown in the lower panel of this figure self-stimulation current-frequency trade-off data for intensities less than 90 μA could not be obtained. The frequencies required to offset further decreases in current induced seizures. The current-frequency trade-off profile is therefore limited by the occurrence of epileptiform manifestations at high frequencies. Furthermore, the sensitivity of

Figure 24:

Families of rate-frequency functions obtained at different pulse intensities for three different subjects. Numbers refer to the pulse intensity in microamperes.

Rate-Frequency Functions

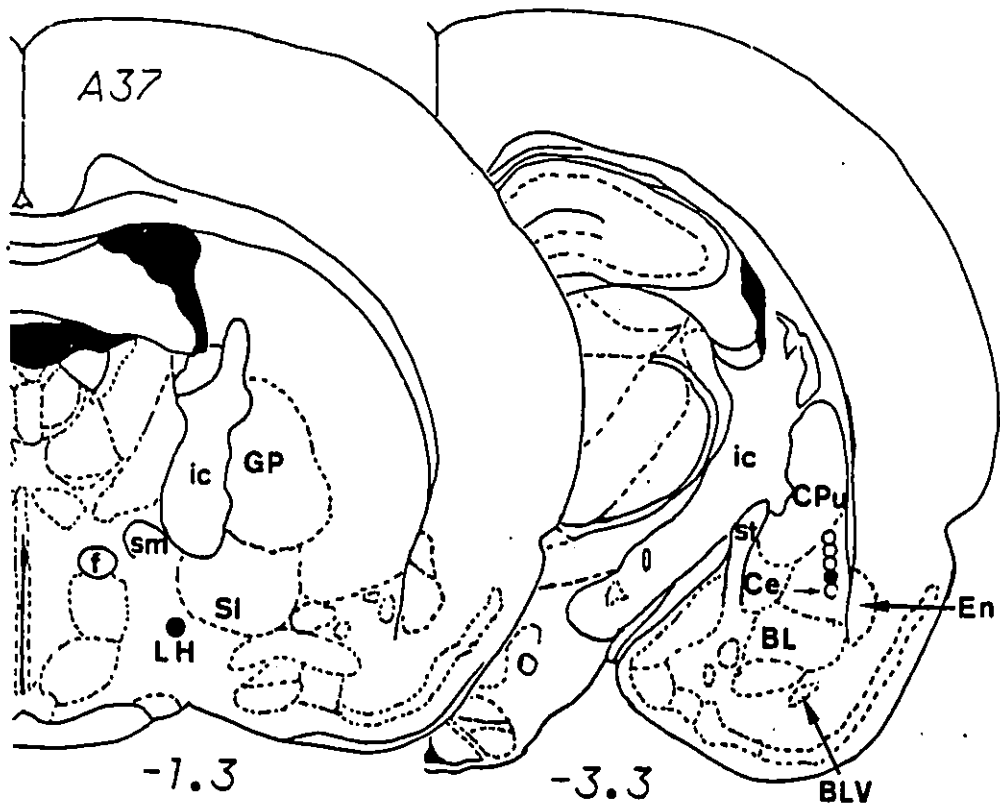
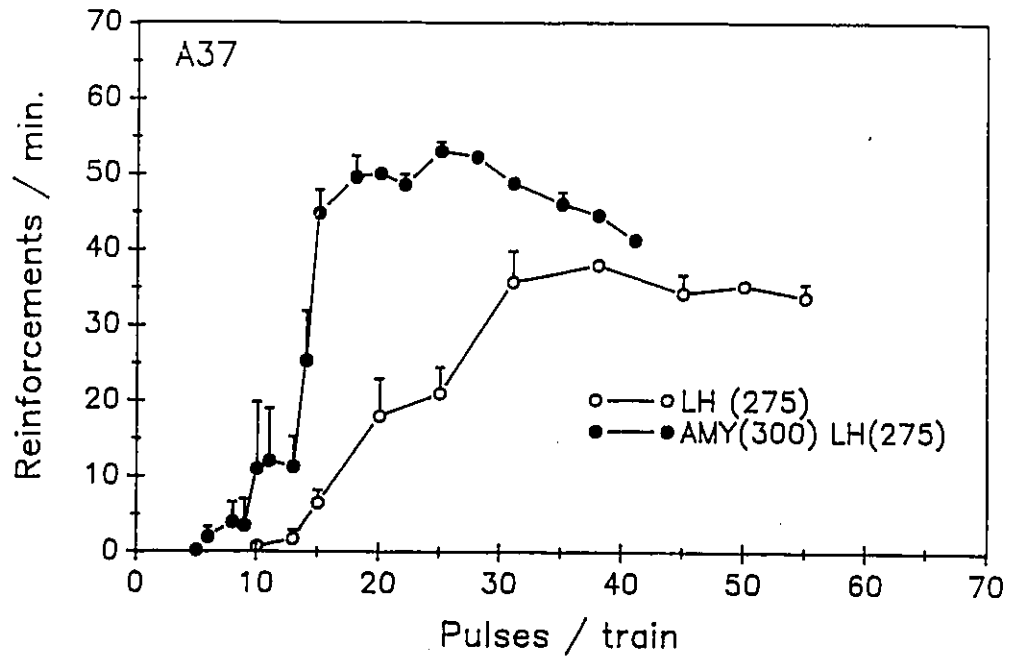


amygdaloid test sites with respect to this variable varied. It is therefore feasible that at those sites where lateral shifts were observed, amygdaloid reward signals were actually being generated but epileptiform activity prevented their expression; concurrent hypothalamic stimulation would then be expected to suppress this factor thus disinhibiting amygdaloid self-stimulation performance. The resultant curve shift thus represents a resurgence of the amygdaloid self-stimulation performance function, not a shift of the hypothalamic self-stimulation function. Such an interpretation is consistent with the data reported in the previous chapter.

Finally, the effect of concurrent stimulation of the lateral amygdaloid nucleus on hypothalamic self-stimulation is shown in upper panel of Figure 25. The location of the stimulation sites is shown in the lower panel. The most apparent feature of these data is an increase in self-stimulation rate under concurrent stimulation. Curve fitting indicated that at a low performance level (eg., 1 bar-press per min) a negligible change in the calculated value of threshold was obtained. Confidence limits around this estimate indicated a 1 pulse difference between the fitted estimates for the two functions. However, at higher performance levels, significant differences in threshold estimates were noted. For X_i (defined as 37 % of the maximal rate), a significant decrease in the required frequency was obtained (a shift from $19.67 \pm .57$ to $12.22 \pm .41$ pulses per train). Even higher discrepancies were found for the more traditionally employed half-maximum criteria: a difference of 13 pulses or .32 log units: a value well above the statistical criteria of a .1 log unit shift. In short, the magnitude of the inferred shift of the self-stimulation function increased as a function of level of performance used in the calculation of pulse frequency threshold and, thus, the reward value of the stimulation. These data cannot be unambiguously interpreted since an unbiased estimate of pulse frequency

Figure 25:

Upper panel: Effect of stimulation of the lateral amygdaloid nucleus on the hypothalamic self-stimulation function. Lower panel: location of amygdaloid and hypothalamic stimulation sites the concurrent stimulation of which altered the slope of the hypothalamic self-stimulation function. Arrow points to the stimulation site that matches the behavioral data shown in the upper panel. Letter and number in upper left cortex identify the subject; number and sign at bottom of plate denotes distance (in mm) and direction from Bregma according to the atlas of Paxinos and Watson (1982).



threshold cannot be calculated.

In a recent analysis of the curve-shift paradigm in self-stimulation, Miliaressis et al. (1986) showed how different experimental manipulations can effect the rate-frequency function. For example, it was shown that increasing the weight of the lever (and thus the force required to initiate the stimulation) or increasing the inter-trial interval depressed the asymptote of the rate-frequency function and altered the range over which the function rose from zero behaviour to asymptotic rates. The net effect of these manipulations was a confusion as to the reward specific nature of these shifts. At low performance levels little or no shift of the function was observed; however, at higher levels shifts were evident: in fact, the magnitude of the shift increased with the increase in the required number of bar-presses; in effect, the arbitrary choice of criteria for the interpolation of 'required frequency' determined the reward specificity of the manipulation.

CHAPTER 6:
General Discussion

The body of data presented in the previous chapters provide a congruent data base for a discussion of the role of the amygdala in brain stimulation reward and its relationship with the hypothalamic-MFB reward circuit.

The amygdaloid self-stimulation substrate

The results of the first experiment showed that all amygdaloid nuclei with the exception of the lateral amygdaloid nucleus supported self-stimulation behaviour. Self-stimulation can, however, be obtained from the lateral nucleus providing the electrode is located within its medial compartment (histological data reported in chapter 3) or ventral compartment (Prado-Alcala & Wise, 1984). The lateral quadrant, however, does not support self-stimulation behavior.

Amygdaloid self-stimulation rates varied from 3 to 37 presses per min depending on brain site, whereas threshold frequencies varied from 9.2 to 40 pulses per train. No correlation was found between these two aspects of self-stimulation. This finding is of primary importance because contemporary reports occasionally use rate as a measure of the relative importance of various brain regions in reward. It is noteworthy that self-stimulation rates were highest in the basolateral region of the amygdala, an area that Wurtz and Olds (1963) had previously identified as aversive. It is further noteworthy that self-stimulation was also obtained from periamygdaloid regions as well as from electrode placements proximal to the base of the temporal cortex.

In general, self-stimulation frequency thresholds were unexpectedly low throughout the amygdala. Pulse frequency thresholds for most positive sites varied between 10 and 20 pulses per train. These values are comparable to those typically obtained from more extensively

studied brain regions, including the ventral tegmental area and dorsal raphé (Rompré & Miliareisis, 1985). Moreover, in the second experiment reported in this thesis, pulse frequency thresholds were shown to be lower in the amygdala than in the lateral hypothalamus (using comparable current). These findings are inconsistent with the traditional notion that the amygdala plays a secondary role in brain stimulation reward.

The amygdalo-hypothalamic interaction in self-stimulation

A direct test of the putative dependence of the amygdaloid self-stimulation substrate on hypothalamic mechanisms of reward was made by concurrently stimulating the two substrates. In general, combining equi-rewarding hypothalamic and amygdaloid stimuli had no effect on self-stimulation rates and thresholds. However, when current was chosen as to enable the amygdaloid function to rise within a frequency range below that of the hypothalamic function amygdaloid self-stimulation rates increased dramatically. Effective sites were located in the basolateral and central nuclei and surrounding periamygdaloid area. A comparatively modest effect was obtained from the medial aspect of the lateral nucleus. It was further shown that the magnitude of the rate-effect increased as a function of hypothalamic current intensity. Despite the dramatic rate-effect, no significant lateral shift of the concurrent self-stimulation function was observed (apart from the spurious shifts documented in Chapter 5). Moreover, the rate-effect was independent of the order of presentation of the stimuli and inter-pulse interval (at least within the range of values tested). These latter data suggest that the rate-enhancing effect of co-activation of the amygdala and lateral hypothalamus-MFB was not a result of a direct

monosynaptic activation of the hypothalamic-MFB reward substrate.

The nature of the amygdalo-hypothalamic interaction in self-stimulation

The observation that self-stimulation rates increased when subthreshold hypothalamic pulses were paired with threshold amygdaloid pulses suggested that amygdaloid self-stimulation is constrained by some reward-irrelevant factor and that co-activation of the lateral hypothalamus attenuates this factor.

Within the traditional interpretation schema, a vertical alteration of the self-stimulation function reflects a change in the subject's ability to perform the self-stimulation response. Performance challenges typically reduce the rate at which subjects self-stimulate. I know of no study that has shown a rate increase following concurrent stimulation of two reward foci.

Choice data have shown that different self-stimulation substrates have different reward saturation points (Miliaressis & Malette, 1987). Reward saturates earlier in the hypothalamus than in the central grey (Miliaressis & Malette, 1987). It is therefore possible that saturation occurs even earlier in the amygdala and that the effect of the lateral hypothalamic stimulation may have been to shift the amygdaloid saturation point. Alternatively, if reward saturation occurs beyond the point at which the amygdaloid self-stimulation function reached its maximum then it can be assumed that amygdaloid self-stimulation is constrained by a "performance" factor the effects of which are attenuated by co-activation of the lateral hypothalamus. The data of experiment 1 (Chapter 4) clearly showed that the underlying amygdaloid reward signal grows beyond the pulse frequency at which self-stimulation performance reaches its' maximum,

suggesting that amygdaloid self-stimulation is constrained by a "performance" factor.

As a further test of the performance nature of these vertical shifts of the concurrent self-stimulation function, subjects were given a choice between amygdaloid self-stimulation and conjoint amygdalo-hypothalamic stimulation; subjects showed no preference for either stimulus.

The nature of the putative performance constraint was investigated in a third experiment. This experiment was inspired by the serendipitous discovery of a correlation between hypothalamic current levels and incidences of overt epileptiform activity: the higher the lateral hypothalamic current under co-activation of the amygdala and lateral hypothalamus the less epileptiform activity was apparent. It is a well known fact that threshold for stimulation induced seizure activity is lower in the amygdala than in most other regions of the brain, especially the hypothalamus. It is therefore not surprising that epileptiform activity accompanied amygdaloid self-stimulation. It is also not surprising that amygdaloid self-stimulation rates are generally low, since high rates of self-stimulation would likely precipitate seizures. These observations suggested that the nature of the behavioral facilitation obtained under the conjoint stimulation condition is somehow related to the ability of the lateral hypothalamus or MFB to suppress epileptiform activity arising from self-stimulation of the amygdala. Support for this hypothesis has been supplied by Dubicka et al. (1978). In their study, concurrent electrical stimulation of the lateral hypothalamus was found to attenuate a carbachol induced convulsive syndrome in the amygdala. The possible involvement of mutually antagonistic noradrenergic and cholinergic systems was presented as an explanatory model. Our data only in part supports this hypothesis. As shown in Figure 18, pre-treatment with phenobarbital increased asymptotic response rates in 2 of 4 subjects tested. The magnitude of the facilitation was correlated with dose for one

subject. These discrepant data were discussed in terms of variability of the sensitivity of the amygdala to seizures and the doses of drug used in this experiment. Previous research (Reid et al., 1964) has shown that doses of phenobarbital within the range used in the present experiments did facilitate responding; however, the brain regions stimulated (the hypothalamus and septum) are far less excitable than the amygdala.

Regional anatomical specificity of the rate-enhancing effect of concurrent amygdalo-hypothalamic stimulation

Evidence in support of the lateral hypothalamic-MFB specificity of the rate-effect was obtained in a final experiment. In general, the response profiles obtained under concurrent amygdalo-rostral MFB stimulation were similar to those shown for the amygdalo-hypothalamic. Self-stimulation rates increased scalarly and the correlation between MFB current levels and the magnitude of the rate effect was shown. Superficially, these data confirm the close anatomic and functional relationship between the rostral MFB and the lateral hypothalamus. On an analytic level, the results of this experiment raise the important question whether direct activation of the MFB may have been responsible for much of the data reported in this thesis or whether the MFB (through synaptic input onto lateral hypothalamic cells) activated intrinsic lateral hypothalamic circuits which in turn gave rise to these effects. This is a more complex question that can only be answered by a more sophisticated experiment designed to dissociate the relevant contribution of these two neuronal systems, for example, by selectively lesioning lateral hypothalamic cells with kainic acid and repeating the experiment.

The finding of a rate-decrement under co-activation of the hippocampus-MFB indicates that direct activation of the ventral hippocampus impedes MFB self-stimulation performance; the nature of this impediment may be related to hippocampal afterdischarge (a frequently reported consequence of electrical stimulation of the hippocampus (eg. Isaacson, 1982)). Destrade et al. (1985) have shown that severance of the temporo-ammonic perforant path (the major efferent from the entorhinal cortex to the hippocampus) facilitates entorhinal cortex self-stimulation in mice. Phenobarbital produced similar effects suggesting that intrinsic hippocampal circuits exert an inhibitory influence on entorhinal self-stimulation. Direct activation of these hippocampal circuits could similarly affect MFB self-stimulation through a non-fornix amygdalofugal hippocampal pathway. Furthermore, reward, reflected in threshold, did not change under concurrent hippocampal-MFB stimulation intimating that the response decrement obtained is unrelated to neural processing mediating operant reinforcement. It is noteworthy that Miliareisis & Rompré (1987) obtained a similar response profile under co-activation of motoric elements in the reticular formation.

Finally, dorsal raphe stimulation had no effect on amygdaloid self-stimulation rates and thresholds. This suggests that the rate-effect (documented extensively in Chapter 3) is not shared by all self-stimulation sites. It remains to be known if the rate-enhancing effect is linked to the reward nature of the stimulation.

Anomalies: amygdaloid current-frequency trade-off profiles

Spurious lateral shifts of the self-stimulation function under co-activation of the amygdala and lateral hypothalamus-MFB were explained in terms of the rate-effect reported above. Current-frequency trade-off data suggest that the amygdaloid self-stimulation substrate is constrained by epileptiform manifestations. Sites that are more sensitive to the effects of high frequency stimulation would not support self-stimulation until the lateral hypothalamic attenuating effects were introduced. All the data reported in this dissertation converge on this conclusion.

The current-frequency trade-off data may also explain the discrepancies between our findings and those of previous investigators (Szabo et al., 1972; Jackson & Gardner, 1974). As indicated previously, Jackson & Gardner tested the effects of long duration (2 min trains) high frequency (60 cycles per sec.) stimulation of limbic loci on hypothalamic self-stimulation. At some loci no effect was observed; whereas at other loci (notably the corticomедial amygdaloid nucleus) a marked decrease in hypothalamic self-stimulation rate was observed. It is therefore possible that at these latter amygdaloid sites the threshold for epileptiform activity was lower and the effect of the stimulation disruptive. Although encephalographic recordings were made from both cortical and deep electrodes, no mention is made of the location of these recording electrodes. Disruption effects are discussed solely with reference to the hippocampus and hippocampal afterdischarge suggesting that the encephalographic recording electrodes were located in this region. No mention of afterdischarge activity following stimulation of the amygdala was made suggesting that either no direct recording of the amygdala was performed

or that the posited disruptive effects of the amygdaloid stimulation did not spread beyond the amygdala to either the hippocampus or cortex. It is unlikely given the intensity of the stimulation that amygdaloid afterdischarges did not occur. The postulated modulatory control of the amygdala on hypothalamic reward may then have been artifactual and a result of an activation of epileptiform sensitive fibers in the amygdala.

The threshold and rate effects reported by Szabo et al. (1972) may be similarly explained. In Szabo et al.'s design a rate intensity paradigm was used to assess the effect of stimulation of the amygdala on lateral hypothalamic self-stimulation. Current intensity to the amygdala was fixed: values ranged between 115 to 150 μA , depending on subject. Current to the lateral hypothalamus was varied between 60 and 500 μA , depending on subject. However, most currents tested were within the range of 100 to 200 μA ; in some cases current varied between 50 and 100 μA . The frequency of the stimulation was fixed at 200 HZ for both substrates. The effect of concurrent stimulation of the amygdala and lateral hypothalamus was then assessed at different hypothalamic current levels. Given that the threshold for amygdaloid self-stimulation is lower than that for the lateral hypothalamus-MFB and that comparable current used to stimulate both substrates it is possible that the shift in threshold obtained under co-activation of the amygdala and lateral hypothalamus-MFB reflected a disinhibition of amygdaloid self-stimulation performance by co-activation of the lateral hypothalamus. The rate increases observed at the different current settings would then be comparable to the rate-effect reported in this dissertation. Contrast effects reported by these authors could be similarly interpreted. It is noteworthy that none of the rate-intensity curves reported by these authors has an identifiable intercept on the abscissa. It is therefore not possible to assess whether the shift of

the rate-intensity curves is analogous to the rate-effect reported in Chapter 3.

The relative independence of the amygdaloid and hypothalamic self-stimulation substrates

The present data converge on the notion that the substrates for amygdaloid and lateral hypothalamic-MFB self-stimulation are relatively independent. Our data also show that self-stimulation of the amygdala does not arise as a direct activation of the lateral hypothalamic-MFB reward substrate since no evidence of a reward interaction was obtained. Moreover, the minor role typically ascribed to the amygdala as a focus for self-stimulation has not been substantiated by these data; based on frequency thresholds, the amygdala represents an important self-stimulation focus.

Further evidence in support of the relative independence of the amygdala and hypothalamus within the circumscribed area of brain stimulation reward has also been obtained by Waraczynski et al. (1990). In this study the effects of unilateral amygdaloid lesions on self-stimulation of the lateral hypothalamus and ventral tegmental area was assessed using the curve-shift method. Extensive lesioning of the amygdala had no effect on self-stimulation thresholds for all but one subject. This latter subject ceased self-stimulating altogether. It is noteworthy that in previous work (Kelly, 1974) bilateral anaesthetization of the amygdala arrested lateral hypothalamic self-stimulation; however, rates and thresholds later recovered.

Interestingly, the substrates for medial-prefrontal cortex self-stimulation appears to be distinct from the hypothalamic self-stimulation substrate (Schenk & Shizgal, 1982). A further distinction between the medial prefrontal cortex and the hippocampal self-stimulation substrates

has also been identified (Robertson et al., 1986). No evidence of summation between the amygdala and dorsal raphe was observed in the present experiments. The relative independence of the amygdala from the lateral hypothalamus-MFB and dorsal raphe (as well as the data dissociating the lateral hypothalamus and medial frontal cortex self-stimulation substrates) suggests that some of the pathways subserving self-stimulation, at least in the rat, may be independently organized. 2-deoxyglucose (2-DG) data further suggest a relative independence between the amygdala and MFB (Gallistel et al., 1985).

Existence of separate reward pathways: theoretical implications

The existence of separate self-stimulation pathways implies that natural reinforcement systems may be similarly organized. There exists a growing consensus in the literature that the pathways subserving electrical brain self-stimulation also mediate the effects of naturally occurring reinforcers (Hoebel & Thompson, 1969; Hoebel 1975; Rolls et al., 1980; Shizgal and Murray, 1989). Evidence of a differential organization of pathways at least within the MFB with respect to drives and self-stimulation performance have been identified (Deutsch, & Howarth, 1962; Hoebel, 1968 ; Gallistel & Beagley, 1971). In the latter study, electrode preference varied as a function of drive state. Water and food deprived subjects preferred stimulation of different sites within the MFB.

The bulk of the neurobehavioral data converge on the notion that the amygdala and hypothalamus are closely involved in the regulation of visceral and higher order cerebral functions. Lesions to either structure typically result in a disruption of these behaviours

(reviewed in Isaacson, 1982). It is noteworthy that the effects of lesions of the amygdala particularly on ingestive behaviour are usually only transient. According to Fonberg (1974), disruptive effects (notably aphagia) usually do not endure beyond 3 weeks, suggesting that these two systems are hierarchically organized with respect to eating and drinking. This has suggested to some researchers (eg. Rolls, 1975) that the amygdala modulates hypothalamic mediated appetitive behaviours by adjusting the reward value of natural reinforcers.

The hierarchical organization of functional systems within the brain may explain the relative independence of the amygdala and lateral hypothalamus-MFB within the circumscribed area of self-stimulation and the close functional relationship between these two brain regions in the expression of other reward relevant behaviours. According to Maclean (1970), anatomical, neurochemical and evolutionary data suggest a tripartite separation of the mammalian brain into protoreptilian, paleomammalian and neomammalian quarters. The protoreptilian brain includes such structures as the upper spinal cord, parts of the midbrain, the diencephalon, and basal ganglia. The paleomammalian brain is essentially the limbic system; the neomammalian brain represents neocortical developments. It is noteworthy that self-stimulation reward has been obtained at all these levels. The protoreptilian brain (or R-complex) is thought to be responsible for stereotyped behaviours based on "ancestral learning and ancestral memories". The limbic brain represents nature's first attempt towards an awareness of the internal conditions of the organism: a visceral consciousness (Maclean, 1949). It is also thought to have functional significance for more elaborate neocortical formations. It is capable of overriding neural information arising from the R-complex. The neocortex, at least in higher primates, operates independently of the internal world and acts as a nonemotional analyzer of the environment

(Maclean, 1978).

According to Isaacson (1982), the limbic system is a strong modulator of the R-complex. Information processed on one level is further conditioned at higher levels. The limbic system is considered an ideal location for the integration of impulses arising from the R-complex and information relevant to the external environment descending from the neocortex.

The fact that learning occurs at all levels of the tripartite brain has been extensively documented. For example, in creatures with minimal neural tissue learning has been demonstrated (McConnell & Jacobson, 1973). Invertebrates with relatively simple nervous systems have been shown to learn relatively simple tasks quite efficiently (Morrow & Smithson, 1969). Conditioning has been demonstrated in rats with extensive cortical, striatal and limbic damage (Huston & Borbely, 1973). Moreover, these "thalamic" subjects have been trained to self-stimulate using tail movements as the required operant. The fact that self-stimulation can be obtained independently of higher structures further suggests that reward pathways may be hierarchically organized within the mammalian brain. The fact that a convergence of influence does not occur under co-activation of the R-complex and limbic system self-stimulation rewarding foci suggests that the rewarding nature of the two stimulations may differ. Isaacson (1982) has suggested that conditioning at the level of the R-complex involves simple stimulus-response associations. Once an association is made it perseverates even in the absence of reward (Huston & Borbely, 1973); the limbic system appears to be required to re-orient subjects towards new, more situationally relevant, rewards. The mechanism whereby the limbic system re-orient behaviour toward new rewards has been suggested by Cormier (1981). According to Cormier (1981), the limbic system evaluates reward relevant stimuli through the coordinated action of

three key limbic structures: the amygdala, hippocampus and septum. The amygdala is designated the reinforcement system and is thus critically important to stimuli acquiring conditioned reinforcing properties. "only stimuli processed through this system can become conditioned reinforcers" (Cormier, 1981; p. 4). The hippocampus processes nonsalient cues and either attributes them secondary reinforcing properties or habituates them (Cormier, 1981). The septum is believed to coordinate the interaction between these two substrates; the output of which converges on hypothalamic motivational mechanisms (Cormier, 1981).

It is therefore the postulated hierarchical organization of reward pathways that may explain the amygdala's ability to modulate reward relevant hypothalamic mediated responses. Associations learned at the protoreptilian level can be overrun by limbic processing of relevant internal and impinging external stimuli. Alterations in appetitive responses may then occur following amygdaloid manipulation because of its ability to adjust reward value to new environmental contingencies. The data of Rolls (1975) is consistent with this conjecture as well as more recent speculations by Fonberg (1981) on the nature of feeding deficits in dogs following amygdaloid ablations.

Alterations in amygdala functioning cannot by themselves be responsible for many of the effects reported in the literature; other structures are also involved. For example, in operant conditioning involving food reward, functional interactions between the inferotemporal cortex, amygdala and lateral hypothalamus have been documented (Fukuda et al., 1987). Their data suggest a dynamic interaction between these three brain regions in the visual recognition of food reinforcers.

The contention that self-stimulation reward pathways subserves naturally occurring

reinforcement processes is intuitively sound. Rolls (1980), in particular, has provided data in support of this. The present thesis provides further support for the existence of relatively independent reward pathways. The more general neurobehavioral literature supports the notion that these pathways are hierarchically organized. Although the data reported in this dissertation do not directly support the hypothesis of hierarchical organization of reward pathways they do support the notion of independent reward systems in the brain. The existence of such systems and their hierarchical nature require further investigation.

Finally, Gallistel (1975) has suggested that the self-stimulation circuit provides a direct link to the substrate(s) that condition memory for rewards. The general literature converge on the notion that the limbic system, in particular, plays a crucial role in the formation of memories (Isaacson, 1982). The enduring memory for rewards may therefore involve structures such as the amygdala (suggested on the basis of M. Mishkin and colleagues' work: Mishkin, 1978; Spiegler & Mishkin, 1981 and Mishkin & Elizabeth, 1983). This is particularly compelling in view of the fact that *bilateral* anaesthetization of the amygdala abolishes hypothalamic self-stimulation until a new set of reward parameters is introduced (Kelly, 1974). This is not to say that memory processes do not involve diencephalic and other brain stem structures but rather that the hierarchical nature of memory processes (iconic vs. short term vs. long term) may be differentially organized within the mammalian nervous system. These and related hypotheses require more systematic study.

Summary & Conclusion

In summary, the amygdala represents a potent reinforcement center. The amygdaloid and hypothalamic self-stimulation reward substrates appear to be independently organized. The hierarchical organization of reward relevant pathways has been suggested as the means whereby the amygdala modulates hypothalamic mediated behaviour despite the relative independence of these two substrates in self-stimulation reward.

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