Parametric and Neurological Studies of Brain Stimulation Reward

Marino Lepore

Department of Psychology

McGill University, Montreal

October, 1993

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Doctor of Philosophy

ISBN 0-315-94657-1

Canada

Statement of Originality

To my knowledge, all the experiments carried out for this thesis concerning the parametric evaluations of the self-administration of brain stimulation tehnique and the effects of lesions of the pedunculopontine tegmental nucleus and of the A8 dopamine cell group on the acquisition and maintenance of an operant task, and including the effects of these lesions on the win-shift and win-stay learning tasks, are original contributions to knowledge.

Table of Contents

Table of Contents (cont'd)

Table of Contents (cont'd)

Abstract

This thesis explored whether interpretations of the reinforcing effect of stimulation trains used in the self-administration of brain-stimulation (SABS) paradigm were artifacts of the reinforcement schedule chosen or whether it represented a genuine attempt by animals to maintain optimal levels of reward. Results demonstrate that stimulation trains used in SABS are reinforcing and that animals regulate pulse frequency to optimize the level of reward. The thesis then explored whether pedunculopontine tegmental nucleus (PPTg) lesions blocked the acquisition or maintenance of SABS, and the acquisition of eight-arm radial maze learning. Results showed that lesions confined to the PPTg block acquisition and maintenance of SABS, suggesting that the PPTg mediates the positive reinforcing effects of BSR. Further, PPTg lesions blocked win-shift and win-stay radial maze learning. However, results indicate that animals were not impaired in "shifting" or "staying" behavior. It is speculated that PPTg lesions block the reinforcing effects of food, which produce inefficient performance on both memory tasks.

Sommaire

L'objectif de cette thèse était de déterminer si les interprétations des effets renforçant de la stimulation utilisée dans le paradigme de stimulation cérébrale auto-administrer (SABS) étaient le résultat d'artifices reliés à la cédule de renforcement choisie ou si cela représentait une tentative véritable de la part des animaux de maintenir des niveaux optimals de récompense. Les résultats démontrent que les stimulations utilisées dans le paradigme SABS sont renforçants et que les animaux règlent la fréquence pulsionelle pour optimizer le niveau de récompense. Par la suite, la thèse explore si des lésions du noyau tegmental pédonculo-pontine (PPTg) empêchent l'acquisition ou le maintient de SABS, et l'acquisition dans l'apprentissage du labyrinthe radial à huit-bras. Les résultats démontrent que les lésions limitées au PPTg empêchent l'acquisition et le maintient de SABS, ce qui suggère que le PPTg est responsable des effets renforçants positifs du BSR. De plus, les lésions du PPTg empêchent l'apprentissage dans les labyrinthes radiaux "win-shift" et "win-stay". Par contre, les résultats indiquent que les comportements "shifting" et "staying" des animaux n'étaient pas affectés de façon néfastes. Il est suggéré que les lésions du PPTg bloquent les effets renforçants de la nourriture, ce qui produit une performance inéfficace sur les deux tâches de mémoire.

Acknowledgements

I wish to thank Natalie Collins, Rob McDonald, and Janet Raymond for their technical assistance during the course of this research. I would also like to thank Rhonda Amsel for her statistical advice, and Serge Arsenault for his French translation of the abstract. I am deeply indebted to the following people for their encouragement, support, and their willingness to listen to and exchange ideas which helped make this thesis possible; Natalie Collins, Rob McDonald, Lynn White, Dr. Norm White, and Dr. Keith Franklin.

I would also like to especially thank Dr. Keith Franklin for sharing with me his considerable knowledge, and for his constant support and encouragement throughout my entire graduate career.

Finally I would like to thank Fonds FCAR for their financial support through most of my Ph.D. work.

I would also like to dedicate this thesis to my family, especially my parents, and to my very significant other, Natalie Collins.

List of Figures

List of Figures (cont'd)

List of Figures (cont'd)

List of Figures (cont'd)

List of Figures (cont'd)

List of Figures (cont'd)

General Introduction

The human fascination and experience with naturally occurring or artificially produced substances that enhance or alter mood states is as old as Western civilization itself. The fascination and experience with mood-enhancing substances, however, has become one of the largest and most devastating social and medical problems of the 20th century. Conservative estimates indicate that, in the United States, there are currently 250,000 opiate abusers and over five million cocaine users (Crowley, 1987; Gawin and Ellinwood, 1988; Herridge and Gold. 1988), with a cost to society of approximately $200-$300 billion dollars per year (Kozel and Adams, 1986; Lierman, 1987). Add to this the tens of thousands of people infected with the human immunodeficiency virus through using shared needles and the cost to society becomes incalculable.

Naturally, the question that needs to be answered is why. Why, in spite of the health warnings, do people engage in such self-destructive behavior? Though part of the answer may be found in social, economic or political perspectives, it is likely that some answers lie in the study of biological processes. The fact that laboratory animals, which are not directly influenced by economic or political forces, readily initiate and maintain drug-taking behavior supports a biological contribution to drug abuse. That the microinjection of abusable substances into discrete brain loci can initiate and maintain drug-taking behavior demonstrates a **neuro**biological basis for drug abuse. Research into the neurobiological causes of drug abuse focuses on describing

the motivational forces underlying drug-taking behavior, and several theories and paradigms have been developed to elucidate the mechanisms involved in motivation.

Incentive Theory of Motivation

The approach used by most neuroscientists studying motivation and behavior can be classified as an incentive motivational approach. In this approach, incentive stimuli are viewed as being able to activate affective processes inherent in organisms (Bindra, 1968; Bindra and Stewart, 1971; Young, 1966), and animals will approach and maintain contact with stimuli that produce positive affect. With natural rewards such as food or water, reinforcing stimuli are viewed as having intrinsic incentive or "appetitive" properties that elicit an approach response. Operationally, therefore, a stimulus such as brain stimulation or a drug are rewarding if they elicit an approach response. The study of reward and reinforcement analyzes the ability of rewarding or appetitive stimuli (or stimuli that have "acquired" incentive properties) to elicit approach behavior, and how contingencies of presentation affect both approach and maintenance of contact with such stimuli.

Models of Reinforcement: Description

Several behavioral models have been developed with the purpose of measuring and analyzing motivational states produced by stimuli such as drugs or brain stimulation, and examining the underlying neural substrates involved in reward and reinforcement. Of the three models most often used, two models base their approach on the operant technique developed by Skinner. Stimuli

such as drugs or trains of brain stimulation are made contingent on the performance of some operant (e.g., lever press, nose poke or tail flick). If the stimuli are reinforcing, the probability of the operant occurring again in the future increases. When brief trains of electrical brain stimulation (Olds and Milner, 1954) or self-injections of morphine (Weeks, 1962) are made contingent on a lever press, animals readily acquire and maintain responding for BSR or morphine. Hence, brain stimulation and morphine are considered positive reinforcers in an operant paradigm and are inferred to have rewarding properties. The operant approach, along with the experimental demonstration that brain stimulation and morphine act as positive reinforcers, are the foundations for the two most widely used experimental paradigms in the study of reward and reinforcement, the ICSS and drug self-administration paradigms.

The third major approach to the study of reinforcement is the conditioned place preference (CPP) paradigm, and is based on the classical conditioning technique. An incentive stimulus such as food elicits an unconditioned approach response. By repeatedly pairing food with an environment, or more precisely, neutral stimuli that make up a particular environment, these neutral stimuli presumably acquire incentive properties. If an animal enters a compartment previously paired with food (or drug), the conditioned stimuli presumably produce conditioned affect, evidenced by the ability of these paired stimuli to elicit an unconditioned approach response. If on test day, an animal seeks out and maintains contact with these paired stimuli, it has developed a place preference, and the stimulus is inferred to have primary reinforcing

effects. For example, the discovery that brain stimulation was reinforcing was based on observations that animals approached and maintained contact with one portion of an open field (preferred that area) previously paired with brain stimulation. Since then, various stimuli such as food (Bechara and van der Kooy, 1992; McDonald and White, 1993), morphine (Beach, 1957; Bozarth, 1987b; Bozarth and Wise, 1983; van der Kooy, 1987), amphetamine (Bozarth, 1987b; Bozarth and Wise, 1983; Hiroi and White, 1991; Phillips and Fibiger, 1987), cocaine (Brown et al., 1992; Phillips et al., 1983a), plus many others, have been demonstrated to produce place preferences.

Dopamine Theory of Reward

Findings from all three models of motivation have contributed to the development of the concept of the mesolimbic/mesocortical dopamine (DA) system as a pathway which is salient to natural reward and reinforcement processes (Ettenberg, 1989; Phillips et al., 1989; Rothman, 1990; van Rossum, 1970; Wise, 1980; Wise, 1981; Wise, 1987). Although the ventral tegmental DA system and its projections are small and circumscribed (Beckstead et al., 1979; Fallon, 1988; Lindvall et al., 1978; Oades and Halliday, 1987; Phillipson and Griffiths, 1985), several lines of evidence suggest that activation of DA neurons in the ventral tegmental area and the concomitant release of DA in several limbic and forebrain nuclei is sufficient, and perhaps necessary, for the occurrence of a wide range of behaviors described as purposive, goal-directed, or motivated. Much of this evidence comes from analyzing the effects of pharmacological agents with DA-releasing or DA receptor antagonistic

properties on self-stimulation, drug self-administration, or place preference behaviors. Drugs with known direct or indirect dopaminergic agonist activity such as amphetamine, cocaine, heroin, tetrahydracannibinol, and phencyclidine (Esposito and Kornetsky, 1978; Gardner et al., 1988; Hubner et al., 1987; Kornetsky et al., 1979; Phillips et al., 1975; Wauquier and Niemegeers, 1974; Wise, 1980) have all been shown to facilitate self-stimulation behavior. That is, administration of dopamine agonists to animals responding for brain stimulation reward significantly lowers the current or pulse frequency thresholds necessary to maintain self-stimulation behavior, and increases the rate of self-stimulation (Aulakh et al., 1979; Gallistel and Freyd, 1987; Hubner et al., 1987; Neill et al., 1978; Wauquier and Niemegeers, 1973). Dopamine agonist drugs also support self-administration behavior (Dougherty and Pickens, 1976; Downs and Woods, 1974; Ettenberg et al., 1982; Roberts et al., 1977; Wilson et al., 1971) and can condition place preferences (Bozarth, 1987b; Brown et al., 1992; Phillips et al., 1983a; van der Kooy, 1987; White and Carr, 1985). For example, amphetamine and heroin are powerful dopamine-releasing agents (Arbuthnott et al., 1990; Chesselet, 1984; Groves et al., 1988; Neff et al., 1981; Rompre and Wise, 1989; van Rossum, 1970; van Rossum and Hurkmans, 1964; Wood, 1983; Wood et al., 1980) that readily initiate (Bozarth et al., 1989; Lyness et al., 1980) and maintain (Corrigall, 1987; Corrigall and Vaccarino, 1988; Dai et al., 1989; Gerber and Wise, 1989; Goeders et al., 1984; Goldberg, 1973; Spealman and Goldberg, 1978; Weeks, 1962; Weeks and Collins, 1978; Yokel and Pickens, 1974) drug self-administration behavior. Interfering with dopamine

transmission, either pharmacologically or with 6-OHDA lesions have been shown to disrupt place preference behavior (Ettenberg, 1989; Ettenberg and Duvauchelle, 1988; Phillips and Fibiger, 1990), drug self-administration (Gerber and Wise, 1989; Ljungberg, 1990; Roberts and Koob, 1982; Woolverton, 1986; Yokel and Wise, 1975) and self-stimulation (Franklin, 1978; Lynch and Wise, 1985; Phillips et al., 1975; Schaeffer and Michael, 1980). For example, neuroleptics such as pimozide or haloperidol, significantly increase the current or pulse frequency thresholds in animals responding for brain stimulation reward (Wauquier, 1979; Wauquier and Niemegeers, 1972), and decrease responding for opiate (Bozarth and Wise, 1981b) and stimulant self-administration (Amit and Smith, 1991; Roberts and Vickers, 1984).

In vivo voltammetric and *in vivo* microdialysis studies also support the correlation between reinforced responding and dopamine release (Blackburn et al., 1992; Broderick, 1991; Gratton et al., 1988; Millar et al., 1985; Nicolaysen et al., 1988; Phillips et al., 1989). In animals responding for rewarding brain stimulation, dopamine release in the nucleus accumbens has been shown to increase with increases in current intensity (Phillips et al., 1989) and pulse frequency (Nicolaysen et al., 1988) as measured by *in vivo* voltammetry. Also, animals responding for food or amphetamine (Hernandez and Hoebel, 1988a; Hernandez and Hoebel, 1988b) or cocaine reward (Pettit and Justice, 1989; Pettit and Justice, 1991) show increases in dopamine release in the nucleus accumbens as measured by *in vivo* microdialysis. Opioids and other self-administered drugs have also been shown to increase DA release in the

mesolimbic dopamine system (Carboni et al., 1989; Di Chiara and Imperato, 1986; Imperato et al., 1988).

Anatomical studies using intracerebral drug self-administration or conditioned place preference also support a role for brain dopamine in reward. Both the nucleus accumbens and prefrontal cortex receive heavy dopaminergic innervation from the ventral tegmental area (Fallon, 1988; Lindvall et al., 1978; Oades and Halliday, 1987; Phillipson and Griffiths, 1985). Opioids, known to be dopamine-releasing agents (Chesselet, 1984; Neff et al., 1981; Wood, 1983; Wood et al., 1980), maintain reliable self-administration behavior with direct VTA microinjections (Bozarth and Wise, 1981a) and morphine, enkephalin and enkephalinamide can produce place preferences when injected into the VTA (Bozarth, 1987a; Phillips and Fibiger, 1987; Phillips and LePiane, 1980; Phillips and LePiane, 1982; Phillips et al., 1983b). Self-administration behavior is also supported by direct microinjections of amphetamine (Hoebel et al., 1983), cocaine (Goeders and Smith, 1987), or opioids (Goeders et al., 1984; Olds, 1982) into the nucleus accumbens, and cocaine microinjections in the prefrontal cortex, another target of VTA dopamine cells (Lindvall et al., 1978) reliably maintains self-administration behavior (Goeders et al., 1986). Furthermore, administration of dopamine or opioid antagonist drugs in the VTA or nucleus accumbens can induce a compensatory increase in responding for heroin or cocaine self-administration (Bozarth and Wise, 1981b; Corrigall and Vaccarino, 1988; Ettenberg et al., 1982; Koob et al., 1987a; Koob et al., 1987b). This has been interpreted to suggest that receptor antagonists such as naltrexone or

pimozide partially block reward-relevant receptors in the VTA or nucleus accumbens, and that animals compensate for the reduced (rewarding) effectiveness of heroin or cocaine by increasing their drug intake. Taken together, these findings suggest that some drugs of abuse act on dopamine cells of the VTA, and that the VTA-nucleus accumbens/prefrontal cortex pathway may be a critical component underlying drug reward.

However, as more information is obtained on the functioning of DA in motivated behavior, an increasing amount of evidence reveals shortcomings of the dopamine theory of reward. While some investigators have found that dopamine antagonists disrupt opioid place preferences or opioid drug self-administration (Gerber and Wise, 1989; Kelsey et al., 1989; Rompre and Wise, 1989; Schwartz and Marchok, 1974), others have reported no effect of dopamine antagonists on either behavior (Dworkin et al., 1988a; Ettenberg et al., 1981; Ettenberg et al., 1982; Gerber and Wise, 1989; Goeders et al., 1986). Neuroleptics have been shown to disrupt cocaine self-administration (Woolverton, 1986; Woolverton and Virus, 1989) but not cocaine place preferences (Hemby et al., 1992; Mackey and van der Kooy, 1985; Morency and Beninger, 1986; Spyraki et al., 1982). Neither procaine self-administration (de la Garza and Johanson, 1982; Delfs et al., 1990; Einhorn et al., 1988; Johanson and Aigner, 1981) or place preference (Morency and Beninger, 1986) appears dependent on dopamine transmission. Furthermore, opioid place preferences can be acquired with direct injections into the nucleus accumbens (van der Kooy et al., 1982), and is believed to occur without an increase in

dopamine release, since opioids injected into dopamine terminal fields do not induce dopamine release (Ettenberg et al., 1982; Kalivas et al., 1983; Koob, 1986; Koob et al., 1987c; Vaccarino et al., 1986). 6-Hydroxydopamine lesions of the nucleus accumbens, a site believed to be involved in reward and reinforcement, have no effect on self-stimulation (Franklin and Robertson, unpubl), have variable effects on drug self-administration (Dworkin et al., 1988a; Dworkin et al., 1988b; Roberts and Koob, 1982), and have no effects on cocaine place conditioning (Hemby et al., 1992). Recent evidence from *in vivo* voltammetric or *in vivo* microdialysis studies also suggests that dopamine release occurs during the preparatory phase of behavior and not during the consummation of a reward when reward is presumably experienced (Blackburn et al., 1986; Blackburn et al., 1989; Blackburn et al., 1992). Electrochemical measurements of dopamine release during the preparatory phase of behavior have demonstrated that at least one session (i.e., experience with a reward) is required before dopamine release is evident (Blackburn et al., 1992), and that even after several sessions experience with a reward, dopamine is not released during the consumption of a reward (Blackburn et al., 1992).

These findings have two important implications; one is that dopamine does not carry the reward signal. The second implication is that dopamine release may be necessary for learning about rewards and their relationship with external stimuli. For example, in CPP, affect is repeatedly paired with a distinct environment. In order for the environment to elicit an approach response, the stimuli that make up a distinct environment must be conditior d. The

conditioning process (i.e., associating positive affect with an environment) may be dependent on dopamine transmission, whereas the positive affect produced by incentive stimuli may be generated by a different transmitter system. With operant responding, dopamine release may reinforce the stimulus (lever)-reward association, thereby increasing the probability that approach to the lever will increase, but may have nothing to do with generating or carrying the rewarding signal. In this light, the dopamine system is viewed as playing a "permissive" role in motivated behavior (Blackburn et al., 1992; Wise, 1978a). Thus, the neurochemical and anatomical evidence does not appear to support an essential role for dopamine as a reward transmitter.

Electrophysiological evidence obtained with the self-stimulation paradigm also does not support a primary role for dopamine in brain stimulation reward (BSR). Deutsch (1964), using a pulse-pair technique, demonstrated that the directly stimulated fibres in BSR had relative refractory periods between 0.5-1.2 msec. This finding has since been replicated by others (Szabo et al., 1974; Yeomans, 1975; Yeomans, 1979), and has been extended to suggest that there may be several sub-populations of neurons which underlie the rewarding effects of brain stimulation (Gallistel et al., 1981; Yeomans, 1989). Electrophysiological studies using either a variant of the pulse pair technique or the collision technique (Shizgal, 1989) have also yielded information on the characteristics of the directly stimulated fibres in BSR.

Bielajew and Shizgal (1982) demonstrated that action potentials fired by electrical stimulation are travelling at a rate of between 1 and 8 m/sec, a speed

consistent with the fibres having axonal diameters ranging between 0.5 to 2.0 μM. Moreover, impulses presumably carrying the reward signal from MFB stimulation travel in a rostral to caudal direction, to synapse at the level of the VTA or more caudally (Bielajew and Shizgal, 1986; Shizgal et al., 1980). In these experiments, the medial forebrain bundle (MFB) was lesioned at rostral and caudal positions relative to a stimulating electrode, and it was observed that only lesions caudal to the electrode site interfered with self-stimulation behavior.

Thus, electrophysiological experiments indicate that the fibres stimulated in BSR are fast-conducting, small myelinated fibres that travel in a rostral to caudal direction. This has important implications for the dopamine theory of reward outlined by pharmacological studies, since the electrophysiological characteristics of BSR neurons and DA neurons do not coincide. Dopamine fibres travel in a caudal to rostral direction, projecting from the VTA to the nucleus accumbens or prefrontal cortices (mesolimbic/mesocortical pathway) or from the substantia nigra to the striatum (nigrostriatal pathway). Furthermore, axonal diameters of dopamine neurons range from 0.1 to 0.6 μM (Gerfen et al., 1987), with refractory periods between 1.2 to 2.5 msec (Yeomans, 1989). Thus, although self-stimulation behavior can be affected by altering DA transmission, DA release or receptor activation, it is clear from electrophysiological studies that the first stage neurons involved in carrying the rewarding event produced by electrical brain stimulation are non-dopaminergic.

The Pedunculopontine Tegmental Nucleus and Reward

The increasing evidence showing the shortcomings of the dopamine theory of reward has encouraged investigators to search for other neurochemical and anatomical pathways and sites which may be involved in reward and reinforcement. The pedunculopontine tegmental nucleus (PPTg) has considerable afferent and efferent connections with the striatum, VTA, and nucleus accumbens (Parent, 1990; Semba and Fibiger, 1992; Steininger et al., 1992) - midbrain and forebrain nuclei suggested to be involved in the positive reinforcing properties of drugs (Koob et al., 1987b; Wise, 1978b; Wise, 1981; Wise, 1989). A significant projection from the lateral hypothalamus to the PPTg has also been demonstrated to exist (Semba and Fibiger, 1992), leading Bechara and van der Kooy (1989, 1992) to speculate that the PPTg may also be involved in the rewarding properties of brain stimulation. Recently, the PPTg has received considerable attention as a possible site that mediates the positive reinforcing properties of drugs of abuse (Bechara and van der Kooy, 1989; Bechara and van der Kooy, 1992). In a series of experiments, Bechara and van der Kooy (1989, 1992) tested whether lesions of the PPTg would disrupt the acquisition or retention of morphine, amphetamine, or food place preferences under motivational conditions described as deprived or non-deprived. They found that the acquisition of morphine, amphetamine, and food place preferences were blocked, but only in the non-deprived condition. Further, PPTg lesions had no effect on the retention of place preference behavior, regardless of induced-motivational conditions. These findings were

interpreted to suggest that separate neural mechanisms underlie deprived and non-deprived motivation. That PPTg lesions block food and opiate place preferences in non-deprived or drug-naive animals was interpreted to suggest that the rewarding properties of food and opiate drugs is dependent on the PPTg.

However, the CPP paradigm measures only whether stimuli can acquire incentive properties. It does not measure the primary rewarding effects of stimuli, since on the test day no primary reward is given. Although blocking the acquisition of a place preference may reflect a blockade of the incentive motivational system (Bechara and van der Kooy, 1989; Bechara and van der Kooy, 1992), it may also reflect an inability to acquire or recall a memory of the rewarding event. In other words, the unconditioned affective properties of stimuli may still be present and experienced by the animal, but the ability to form an association between the neutral stimuli and positive affect is blocked. Hence, the "conditioned" stimuli are unable to elicit an unconditioned approach response since no conditioning took place. For instance, White and Carr (1985) have demonstrated that with equally preferred (i.e., equally rewarding) solutions of saccharin or sucrose, only sucrose produced a place preference. This suggests that, as a reward, saccharin lacks some property necessary to impart incentive properties to neutral stimuli. It also suggests that reward is not a sufficient condition for place preference behavior to be established (White and Carr, 1985). In fact, primary reward may not even be a necessary condition to produce a place preference. In the report of a series of experiments performed

by Bechara and van der Kooy (1992), the authors stated that sated animals did not consume any food in the pairing chamber, yet still showed a place preference for where food was found. The authors interpreted this finding as suggesting that a survival instinct was aroused in the animal which allowed them to recall the area in which food could be found, and that this may represent a separate motivational system. Regardless of the interpretation, this finding demonstrates that animals do not need to experience the rewarding effects of a stimuli (e.g., food) in the test environment for a place preference to be produced, and calls into question what the place preference paradigm purports to measure. Bechara and van der Kooy (1992) also argue that PPTg lesions do not affect magnitude of reward but rather eliminate incentive motivation in general. That shifts in the magnitude or intensity of reward are easily detected with the CPP paradigm is a difficult claim to support because place preference behavior is more a qualitative than quantitative measure of the primary reinforcing effects of stimuli. In order to make quantitative statements using CPP, one must demonstrate that pairing a high dose of a drug (e.g., amphetamine) in one compartment will produce more of a place preference than pairing a low dose of that same drug in the other compartment. Since this type of experiment has not been performed, quantitative statements concerning place preference behavior are unsubstantiated.

The suggestion that the PPTg is involved in mediating the primary rewarding effects of stimuli could be strengthened if it could be demonstrated that PPTg lesions block the acquisition of responding in an operant paradigm.

Although operants may depend on the presence of primary and secondary reinforcement to be maintained under partial reinforcement schedules or during extinction responding (Stewart and Eikelboom, 1987), animals can acquire responding for drug self-administration or intracranial self-stimulation with only the primary rewarding effects present (discussed below). However, although in general findings from the self-stimulation and drug self-administration paradigms generally coincide, there are several differences between the operant paradigms which raise questions as to whether brain stimulation and drug rewards act on similar substrates.

Characteristics of Responding for Drugs of Abuse

One characteristic that is common to all rewarding or reinforcing stimuli is, naturally, their ability to control behavior. In drug self-administration, animals acquire and maintain self-administration of amphetamine (Lyness et al., 1980; Yokel, 1987), cocaine (Bozarth et al., 1989; Ramsey and van Ree, 1991), and heroin (Bozarth et al., 1989), but fail to acquire or respond for vehicle controls or drugs with known aversive properties (Thompson and Schuster, 1964). The acquisition of drug reward is typically slow and erratic for the first few test sessions before animals stabilize into regular drug taking patterns (Lyness et al., 1980; Stewart and Wise, 1992). When drug self-administration was discovered, it was believed that animals would ingest drugs only if they were physically dependent on them (Collier, 1968; Dole and Nyswander, 1967; Himmelsbach, 1943; Wikler, 1952). Thus, the self-injection of drugs was thought to occur because animals were attempting to either avoid or alleviate

the discomfort of a withdrawal syndrome and not because these drugs acted on some natural motivational circuit. However, it has become increasingly clear that a drug can initiate and maintain self-administration behavior in the absence of a physical dependence or withdrawal state (Bozarth and Wise, 1984; Deneau et al., 1969). For example, chronic administration of opioids in the periaqueductal gray area, which does not support opioid drug self-administration, produces an abstinence syndrome when animals are challenged with opioid antagonists (Bozarth and Wise, 1984). However, chronic administration of opioids into the VTA, which supports opioid self-administration behavior, does not produce an abstinence syndrome when animals are challenged with opioid antagonists (Bozarth and Wise, 1984). Drug-naive animals, where obviously no abstinence syndrome can or has occurred, rapidly acquire heroin or amphetamine self-administration (Lyness et al., 1980; Stewart and Wise, 1992). Furthermore, mechanisms involved in the positive reinforcing effects and the aversive or negative reinforcing effects of drugs are anatomically dissociable (Bozarth and Wise, 1984; Carr and White, 1986), and it appears that only sites mediating the positive reinforcing effects of drugs initiate and maintain self-administration (Bechara and van der Kooy, 1992; van der Kooy, 1987; Wise, 1989). Thus, it is the rewarding or positive reinforcing properties of drugs that lead to acquisition and maintenance of operant responding as measured by drug self-administration.

A second property of rewards can be identified when reinforcer magnitude is manipulated. Varying the quantity of reward changes the rate and

pattern of responding for drug reward (Pickens and Harris, 1968; Pickens and Thompson, 1968). Pickens et al. (1968) studied the effects of increasing and decreasing reward magnitude in rats self-administering amphetamine or cocaine. They found that at very low doses responding for drug increases with dose once a minimally reinforcing threshold is passed, but over a wide range of higher doses, as unit dose increases responding for drug decreases, and, if unit dose decreases responding for drug increases. This finding has since been replicated for a variety of drug reinforcers (Goldberg et al., 1971; Risner and Jones, 1976; Werner et al., 1976; Wilson et al., 1971) and has been extended to show that while increasing or decreasing the dose of a drug administered changes the number of responses performed during a test session markedly, the total amount of drug intake over the test period is relatively unaffected (Lemaire and Meisch, 1984; Van Dyke et al., 1978; Yokel and Pickens, 1974). Thus, although approach to the rewarding stimulus (measured as response rate) increases or decreases, contact with the rewarding stimulus remains quite stable. This has been interpreted to suggest that animals organize their behavior to maintain optimal levels of affect (Goldberg et al., 1971; Risner and Jones, 1976; Werner et al., 1976).

The suggestion that animals maintain optimal levels of reward is also supported when duration of effect of a rewarding stimulus is changed (Werner et al., 1976; Winger et al., 1975). Although investigators are unable to manipulate the duration of effect of a single drug, the use of drugs that fall in the same pharmacological class have been studied. In one such

demonstration, Winger et al. (1975) used a variety of barbiturates with nearly identical pharmacological activity but differing in duration of effect. They found that short-acting barbiturates produced higher rates of responding than long-acting barbiturates. In a similar vein, Werner et al. (1976) compared rates and patterns of responding for morphine and methadone, and found that morphine (half life of ≈ 90 minutes) produced higher rates of responding than methadone (half-life of ≈ 4-6 hours).

Additional support for the argument that animals maintain optimal levels of reward comes from studies investigating the neuropharmacological basis of drug reward. It has been demonstrated that a compensatory increase in the rate of self-administration occurs following pharmacological challenge with a low dose of antagonist, and ceases with higher doses of antagonist (Wise, 1987; Yokel, 1987). Animals self-administering heroin will increase their response rate in reaction to pharmacological challenge with naltrexone (Corrigall, 1987; Corrigall and Vaccarino, 1988). This increased rate of drug intake also occurs when animals are self-administering amphetamine (Yokel and Wise, 1975) or cocaine (Roberts and Vickers, 1984) and are challenged with low doses of pimozide or atypical neuroleptics. The interpretation of this effect is straightforward. If a drug produces its rewarding effects through interactions with specific receptor systems, then the partial blockade of these receptors would lead to a situation where the rewarding effect of the drug would be less than its original effect (a situation similar to reducing the unit dose of the drug). Thus, to attain an optimal level of reward animals compensate by increasing

their rate of response.

A second interpretation, though, may be that the administration of a low dose of antagonist does not reduce the rewarding effect of the drug per se, but may be antagonizing aversive properties the drug may have (Goudie, 1979; Hunt and Amit, 1987; Thompson and Schuster, 1964). Thus, animals may be increasing their rate of responding because the drug is more rewarding, owing to the elimination of aversive side effects. For example, as unit dose increases, the reinforcing effect of a drug would be expected to increase. Thus, increases in dose should lead to increases in responding for the rewarding stimulus in drug self-administration. That animals maintain relatively constant plasma drug concentrations argues against this, and supports the hypothesis that aversive side-effects limit the amount of drug an animal will ingest. Further support for this argument comes from the finding that drugs typically abused also have aversive properties. For example, morphine, amphetamine, cocaine, and nicotine have all been shown to produce conditioned taste aversions (Carr and White, 1986; Goudie, 1979; Hunt et al., 1987; Isaac et al., 1969; Stolerman, 1985; White et al., 1977). Furthermore, antagonists which induce compensatory increases in drug self-administration block the conditioned taste aversions produced by these drugs (Goudie, 1979; Hunt and Amit, 1987; Hunt et al., 1985). Thus, it is possible that over time the aversive properties of drugs become stronger, and limit the amount of drug an animal will self-administer. This implies that if one could limit the aversive or non-specific side-effects of drug intake animals would show increased responding to increases in drug

dose. Several behavioral paradigms have been used to test this hypothesis. For example, interval and ratio schedules of reinforcement are useful in minimizing non-specific side-effects of drugs (Kiser et al., 1978). With a fixed interval schedule of reinforcement, if the interval is long enough, animals are less likely to load up with drug. This results in a decrease in plasma drug concentrations maintained by the animal, and delays or minimizes the occurrence of aversive or other non-specific side effects. Using fixed interval schedules, investigators have found that the range of doses which produce increases in response rate goes up (Downs and Woods, 1974; Goldberg and Kelleher, 1976; Goldberg et al., 1971; Kelleher, 1976; Pickens and Thompson, 1968). For example, animals responding for doses of cocaine that decrease responding on a continuous reinforcement schedule (CRF) (Goldberg et al., 1971; Pickens and Thompson, 1968), show increases in response rate when these same doses are administered on a fixed interval schedule of reinforcement (Kelleher, 1976; Spealman and Goldberg, 1978). Investigators have also used progressive-ratio schedules of reinforcement, where animals are required to complete some fixed ratio before receiving a reinforcer. Once a reinforcer is received, the fixed ratio increases by some prespecified amount, and the final fixed ratio the animal completes before quitting responding is called the break point (Hodos, 1961; Hodos and Kalman, 1963). Using the progressive ratio technique, investigators have reported increases in break point with increases in dose of cocaine (Bedford et al., 1978; Griffiths et al., 1975), opioids (Hoffmeister, 1979; Spealman and Goldberg, 1978), and amphetamine

(Spealman and Goldberg, 1978; Stretch et al., 1971). Thus, a range of drug doses over which increasing dose produces decreases in responding on CRF schedules of reinforcement can produce increases in responding on partial reinforcement schedules, suggesting that as dose increases, the reinforcing effect of the drug also increases. This implies that aversive or motoric side-effects may be limiting the total amount of drug self-administered by animals.

Characteristics of Responding for Brain Stimulation Reward

With self-stimulation, approach to, and maintenance of contact with the stimulus are confounded. Trains of brain stimulation are usually so short (e.g. < 1 second) that for animals to remain in contact with rewarding stimulation, they must respond continuously. Nevertheless, several characteristics associated with responding for brain stimulation can be identified. Acquisition of self-stimulation behavior is extremely rapid, and can occur in the first few minutes of a test session (Lepore, 1990; Yeomans, 1990). Further, animals responding for BSR attain and maintain maximal rates of responding immediately upon start of a test session, and show little or no signs of response decline even after several hours of responding (Yeomans, 1990).

When duration of reinforcing trains of stimulation are increased, investigators have reported increases in responding (Deutsch and Albertson, 1974; Deutsch and Dennis, 1975; Deutsch et al., 1976), decreases in responding (Atrens and Becker, 1975; Atrens et al., 1977), or little or no effect on responding (Edmonds et al., 1974; Gallistel, 1974; Gallistel et al., 1981). Several interpretations have been put forth to explain these various findings.

First, it has been argued that train durations of more than 2 seconds are not reinforcing (Edmonds et al., 1974; Gallistel, 1974; Gallistel et al., 1981). Presumably, axons mediating BSR do not fire action potentials with trains of stimulation longer than 2-3 seconds because neural accommodation occurs (Yeomans, 1990). Hence, no changes in behavior are expected to occur. Others argue that trains of stimulation longer than 2 seconds become aversive (Atrens and Becker, 1975; Atrens et al., 1977), which explains why decreases in responding are observed with increases in train duration. However, when given the choice between short (< 1 second) and long trains (> 10 seconds) animals prefer long trains (Deutsch and Dennis, 1975; Deutsch et al., 1976). This has been interpreted to suggest that animals adapt to any aversion produced by long trains of stimulation, and when such adaptation occurs, an increase in the reinforcing effect of stimulation is detectable. In ICSS, therefore, one does not usually see an inverse relationship between magnitude or duration of reinforcing effect and responding typically observed with drug rewards. Finally, when animals responding for BSR are pharmacologically challenged with antagonists believed to block reinforcement processes, response rate invariably decreases (Franklin, 1978; Gallistel and Davis, 1983; Gallistel et al., 1982; Lynch and Wise, 1985; Schaeffer and Michael, 1980). No compensatory increases in responding are observed, again in contrast to the behavior observed with the drug self-administration paradigm.

Several explanations may be invoked to explain these apparent behavioral inconsistencies between ICSS and DSA. It is possible that drugs

produce their rewarding effects through neural mechanisms completely distinct from the mechanisms underlying the rewarding effects of stimulation. This is unlikely though, given that rewarding drugs and brain stimulation synergise to affect behavior (Colle and Wise, 1988; Esposito and Kornetsky, 1978; Gerber et al., 1981; Kornetsky et al., 1979; Wise, 1980). For example, low doses of amphetamine (Schaefer and Michael, 1988), cocaine (Aulakh et al., 1979; Frank et al., 1988; Wauquier and Niemegeers, 1974), morphine (Broekkamp et al., 1976; Esposito and Kornetsky, 1978; Levitt et al., 1977; Terando et al., 1978), heroin (Gerber et al., 1981; Hubner and Kornetsky, 1992; Koob et al., 1975), THC (Gardner, 1992; Gardner et al., 1988) and phencyclidine (Kornetsky et al., 1979) lower the intensity and frequency thresholds of rewarding brain stimulation. Furthermore, this synergism between rewarding brain stimulation and drugs occurs at sites demonstrated to be involved in the rewarding effects of drugs (Broekkamp et al., 1975; Colle and Wise, 1988; Kornetsky and Bain, 1987; Lieb:nan and Segal, 1977; Wise and Rompre, 1989), and has led to the hypothesis that drugs are rewarding because they pharmacologically activate the same substrate activated by brain stimulation reward (Koob et al., 1987b; Koob et al., 1987c; Wise, 1987; Wise and Rompre, 1989). However, if drugs and brain stimulation act on an identical reward substrate, one would expect that activation of this substrate by either brain stimulation or drug rewards would have similar characteristics. An alternate explanation is that temporal differences in the way drugs and brain stimulation activate the neural substrate of reward leads to the apparent inconsistencies in behavior observed between

brain stimulation and drug self-administration.

Self-Administration of Brain Stimulation as a Model of Reinforcement

The SABS model of drug self-administration was designed to assess the behavioral differences observed between the ICSS and drug self-administration models. It was hypothesized that differences in the time course of drugs and brain stimulation as reinforcers lead to the apparent inconsistencies in behavior. For instance, trains of brain stimulation have an immediate onset and offset, typically last from 0.250 to 1 second, and are localized to the immediate vicinity of the stimulating electrode. Drug rewards, however, take several seconds to several minutes before reaching a reinforcing threshold with the drug effect lasting anywhere from a few minutes (1-6 minutes with thiopental or fentanyl) up to several hours (12-24 hours with methadone or LAAM), and are not localized to a specific nucleus or pathway. Furthermore, animals responding for drug rewards are able to control their plasma drug concentrations, whereas animals cannot regulate ICSS. Thus, behavioral differences observed in responding for brain stimulation and drug rewards may be due to differences in the ways brain stimulation and drugs activate the reward substrate. It was reasoned that if a drug was made to act like brain stimulation or brain stimulation was made to act like a drug, the behavioral discrepancies between ICSS and drug self-administration should disappear. This hypothesis was tested using self-administration of brain stimulation (SABS), a novel brain stimulation paradigm in which brain stimulation trains were made to mimic the pharmacokinetic properties of drugs (Lepore and Franklin, 1992).

Basically, the SABS model is a hybrid of two operant paradigms - ICSS and drug self-administration (Lepore and Franklin, 1992). In SABS, animals respond for prolonged trains of brain stimulation that rise and fall in pulse frequency in a manner analogous to the rise and fall of drug concentrations in the brain. Unlike ICSS, in which the investigator controls the pulse frequency administered to the animal, in SABS it is the animal's responses that control the pulse frequency, similar to the way animals responding for drug reward control their plasma drug concentration. For example, when the animal presses the lever, the pulse frequency begins climbing from some existing level (e.g., 0 Hz) to a prespecified increment in frequency (e.g., 200 Hz) (see Figure 1). By analogy to drug self-administration, this increment in pulse frequency is referred to as the "dose" of brain stimulation. The rate of increment and decrement (or half-lives) of pulse frequency are determined by first order exponential equations for "absorption" and "elimination", respectively. The pulse frequency attained at the end of the "absorption" phase of a SABS stimulating train represents the "peak plasma drug concentration" or "peak concentration at the receptors", by analogy to drug self-administration. If, during the course of a test the animal executes another reinforced response, the "dose" of stimulation is algebraically added onto the existing portion of dose present at the time the reinforced response is executed (see Figure 1). Thus, from the beginning of the absorption phase (0 Hz) up to peak concentration (200 Hz), and from peak concentration down to 0 Hz, a train of brain stimulation models the effect of a single dose of drug, and responding for such trains of stimulation will be

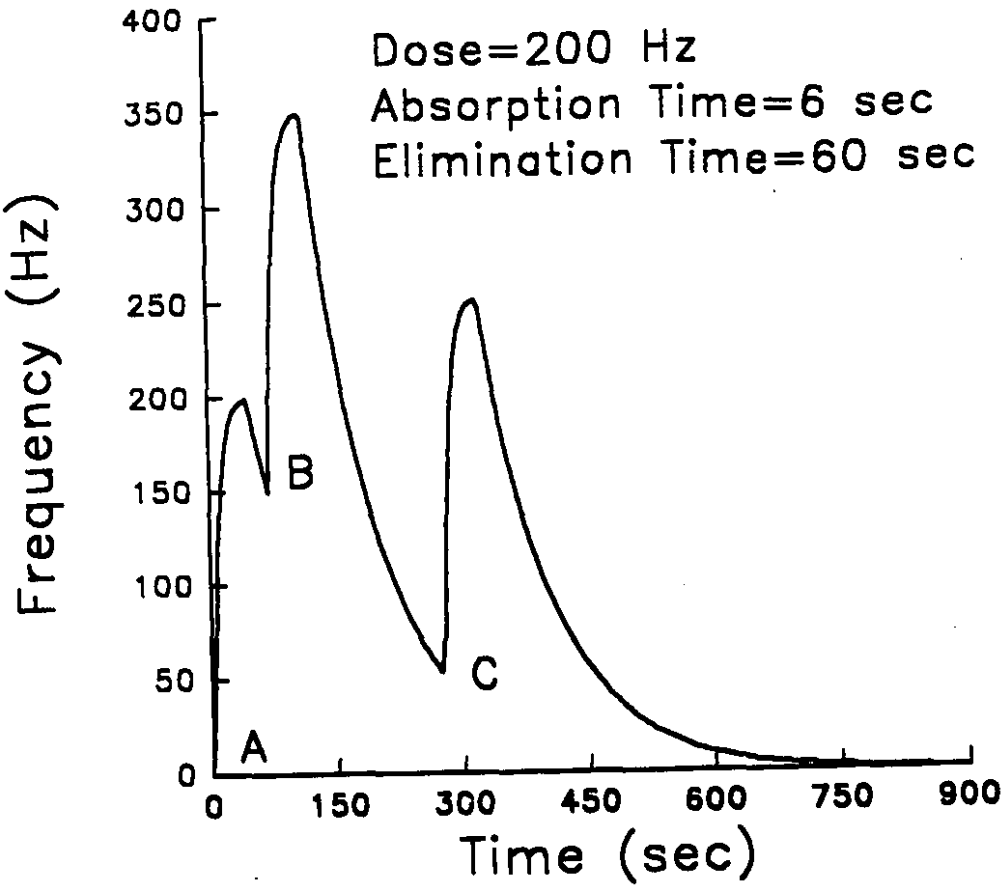


Figure 1. Time course of the changes in pulse frequency of a hypothetical train of brain stimulation generated by the SABS program. Each train is designed to mimic the effect of an hypothetical drug with an absorption half-time of 6 seconds and an elimination half-time of 60 seconds. Data points are sampled and stored by the computer once every 30 seconds. Points A, B, and C indicate the time when a reinforced response occurred.

referred to as self-administration of brain stimulation, or SABS.

Animals responding for SABS show many behaviors considered characteristic of animals responding for drug reward. For example, animals "load up" with brain stimulation at the beginning of a test session, as they do in drug self-administration, driving brain stimulation frequencies as high as 800 to 1000 Hz, before their response rate drops off and mean frequency stabilizes. Acquisition of SABS behavior also resembles acquisition of drug self-administration in that responding is erratic for several acquisition sessions before intake patterns become stable. When "doses" of brain stimulation are decreased or increased, animals will increase or decrease responding respectively, in a manner similar to animals self-administering drugs (Lepore and Franklin, 1992). A similar inverse relationship between duration of reinforcing effect and response rate also exists. When the elimination half-life of stimulation is increased from 20 to 2000 seconds, response rate decreases. Finally, administration of the neuroleptics, pimozide and cis-flupenthixol, induced an increase in both responding and mean frequency of stimulation maintained by the animal (Lepore and Franklin, 1992), similar to the way animals increase responding for opioid or stimulant drug reward when challenged with opioid or dopamine antagonist drugs (Corrigall, 1987; Corrigall and Vaccarino, 1988; Ettenberg et al., 1982; Koob et al., 1987a; Yokel and Wise, 1975). Thus, it was argued that it is the method by which brain stimulation and drugs activate the reward substrate that is producing behavioral differences among the models of motivation, rather than reflecting different processes mediating drug or brain

stimulation reward.

Results obtained with the SABS technique suggest the anatomical specificity of the ICSS technique and its interpretive power are retained in SABS. Results also suggest that SABS may be used to investigate properties of drug reward which can not be easily tested or are impossible to test using the drug self-administration paradigm. Although the initial demonstration of SABS met with success, there are several features of SABS behavior that raise questions about the interpretation of the unusual brain stimulation trains used in SABS. If the SABS model of reinforcement is to be used successfully, therefore, areas which raise concern about its interpretation need to be examined more fully.

Potential Problems of Interpretation with SABS

Though the similarities between SABS and drug self-administration are very striking, there are several features of SABS responding which raise questions with regard to the interpretation of the reinforcing effect of long frequency-modulated trains of stimulation used in SABS. One remarkable feature of responding for SABS was the tendency to drive pulse frequencies to high levels, sometimes averaging as high as 700-900 Hz over a session lasting several hours. This is puzzling because studies using BSR suggest that the optimal reinforcing frequency of a stimulating train lies within the 25 and 200 Hz range (Mason and Milner, 1985; Milner, 1978; Wood et al., 1987). Thus, the rate-frequency curve in BSR is roughly linear over approximately 0.8-1.0 log units, with the reinforcing efficacy of a stimulating train dropping off sharply at

higher frequencies (Yeomans, 1990). Indeed, it can be argued that the reinforcing effect of brain stimulation would not go on increasing beyond 700-800 Hz, because these high frequencies would be at or above the upper physiological limit of neuronal firing (Yeomans, 1990). Though recruitment of neurons in the subliminal fringe (Creed et al., 1932) might explain some of the reinforcing effects of long stimulating trains, these considerations raise the question as to whether the high average pulse frequencies maintained by animals responding for SABS reflects the animal's attempt to maintain an optimal level of reward as is generally assumed with animals responding for drug reward, (Goldberg et al., 1971; Werner et al., 1976), or whether it represents an artifact of training history or of the reinforcement schedule that was used (CRF). To examine this question, the relationship of "dose" and "elimination 1/2 time" on SABS performance was re-examined, varying the response requirement for each reinforcement on fixed ratio schedules. In addition, the rate-frequency curve was examined using FI and progressive ratio schedules reinforced with high frequency trains.

General Method for SABS

Subjects

All experiments were carried out in accordance with the guidelines for the ethical use of animals in biomedical research as outlined by the Canadian Council on Animal Care and McGill University.

Adult, male Long-Evans rats (Charles River, Que.) were anesthetized with pentobarbital (55 mg/kg) and a bipolar, stainless steel electrode (Plastics

One, VA) was implanted in the brain of each rat. Electrode coordinates for lateral hypothalamus-MFB placements taken from bregma were: -0.5 mm posterior, ± 1.5 mm lateral to the midline, and -8.3 mm ventral from the skull surface (Paxinos and Watson, 1986).

Rats were allowed at least one week recovery from surgery before behavioral testing.

Apparatus

Animals were tested in one of four conventional Skinner boxes (Coulbourn Instruments, PA) modified for delivery of brain stimulation (Vaccarino and Franklin, 1982). Each box was equipped with a single response lever (Coulbourn Instruments, PA) on which animals pressed to obtain brain stimulation. Brain stimulation was delivered by an electrically isolated constant current stimulator. Trains of 0.15 msec biphasic, square-wave pulses were generated by a variable-frequency oscillator. Oscillation frequency was controlled through a digital-to-analogue converter, and the frequency of brain stimulation could be changed in 1 Hz steps. Current intensities ranged between 75 and 150 μAmps. A microcomputer (80386) monitored the animals responses, recorded data, and controlled the parameters of brain stimulation. The computer polled the lever press detectors and set the appropriate brain stimulation frequency once every 250 msec.

Computer Control of Brain Stimulation

The SABS program was designed to deliver trains of brain stimulation, where the frequency of each train was modulated to mimic the rise and fall of

drug concentrations in the brain. Upon performance of a lever press, the computer would increase the frequency of brain stimulation from the existing level (e.g., 0 Hz) to some prespecified increment in frequency (e.g., 200 Hz). By analogy to drug self-administration, this peak increase in frequency was termed the "dose" of brain stimulation. The rate of change of pulse frequency was controlled by varying the interval of the application of successive steps of 1 Hz. These intervals were determined by a first-order kinetic equation (exponential) for "absorption", and the outputs of the kinetic equation were rounded off to whole integers before being fed to the digitally controlled oscillator. The frequency attained at the end of the absorption phase represents the peak plasma drug concentration or peak concentration at the receptors, by analogy to drug self-administration. Once absorption was complete, the computer began to decrease the frequency of brain stimulation according to kinetic equations (exponential) for elimination, such that pulse frequency was gradually decreased in steps of 1 Hz. Thus, from the beginning of the absorption phase through to peak concentration, and back down to 0 Hz modelled the effect of a single dose of drug. If the rat then executed another reinforced response, the "dose" was algebraically added to whatever portion of dose was still present the time the response was executed (see Figure 1, page 26). Thus, the rat's response rate determined the average frequency of stimulation during the test session. To prevent animals from receiving several doses in rapid succession before the effects of a single dose are experienced, an unsignalled time out period was programmed into SABS such that another

response would not be reinforced until the absorption phase was complete.

Data Sampling

The SABS program samples and collects data on 3 dependent variables: reinforced responses, total responses, and frequency of stimulation. The computer sampled each dependent variable once every 30 seconds, which was then stored in the computer RAM. At the end of the test session, all dependent measures stored in RAM were written to disk for permanent storage.

Pre-Training Procedure

Animals were allowed a minimum of 7 days recovery from surgery, whereupon daily, half-hour training sessions were carried out to screen animals for self-stimulation. Animals were initially shaped to lever press for one second trains of 0.15 msec pulse, 200 Hz stimulation, with current intensities initially set at 50 μAmps. Once self-stimulation behavior was established, animals were given one additional 1 hour session of self-stimulation, during which the current intensity (range = 75 to 150 μAmps) was adjusted to produce a moderate rate of responding (500 - 750 responses/hour). Animals were then transferred over to the SABS program, where they were allowed several sessions to familiarize themselves with the stimulation parameters appropriate for each experiment.

Histology

At the completion of each experiment, animals were sacrificed with 30% chloral hydrate, and were perfused transcardially with a 10% formol saline solution. Brains were quickly removed and placed in an additional solution of 10% formol saline for at least 24 hours before slicing. Cresyl violet staining

was then carried out and slides were examined to verify the position of the electrode tip. All animals were found to have electrode placements within 500 microns (in all directions) from the aimed coordinates.

Experiment 1

Introduction

The original parametric study of SABS was carried out using CRF schedules. Although the demonstration was instructive, evaluations of how drugs act as reinforcers often include determining the patterns of responding under various partial reinforcement (PRF) schedules. For example, it has been demonstrated that reliable drug self-administration can be elicited and maintained using a variety of PRF schedules, including fixed interval (Balster and Schuster, 1973; Dougherty and Pickens, 1976; Thompson and Schuster, 1964; Woolverton and Balster, 1983), fixed ratio (Downs and Woods, 1974; Marquis et al., 1989; Pickens and Thompson, 1968; Vanover et al., 1989) and progressive ratio schedules (Bedford et al., 1978; Griffiths et al., 1975; Hoffmeister, 1979). Further, patterns of responding are stable and comparable across both reinforcement schedules and drug classes (Morse, 1966; Morse and Kelleher, 1977; Spealman and Goldberg, 1978), although self-administration rate may change with the density of the schedule. If the SABS technique can reliably model the effects of drugs, then, both patterns of responding and relationships observed between the kinetics of stimulation and response rate should remain stable across various reinforcement schedules.

Method

Animal Subjects and Surgery

Six male Long-Evans rats were used for the following experiment. Surgery, coordinates, and post-operative care were carried out as described in the general method section.

Procedure

After screening (see General Method, page 32), animals were transferred to the SABS program, where the parameters of brain stimulation were set at 200 Hz dose, 1 second absorption half-time, and an elimination half-life of 100 seconds. Animals were run for two days at each of the following fixed ratios; 1, 3, 10 and 15. After the initial training, 3 rats were randomly assigned to an elimination half-time of 200 seconds, and 3 rats were assigned to an elimination half-life of 2000 seconds. Animals were run for two days at each of the following fixed ratios; 1, 5 or 15. Animals were then randomly assigned to one of the three test fixed-ratios and were tested for three sessions at each. The first day was used to accustom the rat to the new stimulation parameters, and the mean of the last two test sessions was used for analysis. Following completion of tests on each FR schedule, animals were switched over to the other elimination half-life, and the procedure outlined above was repeated.

Results and Discussion

Responding for SABS stimulation was maintained over a wide range of fixed ratios. Mean frequency of stimulation was inversely related to response requirement. As shown in Figure 2, increases in fixed ratio led to decreases in

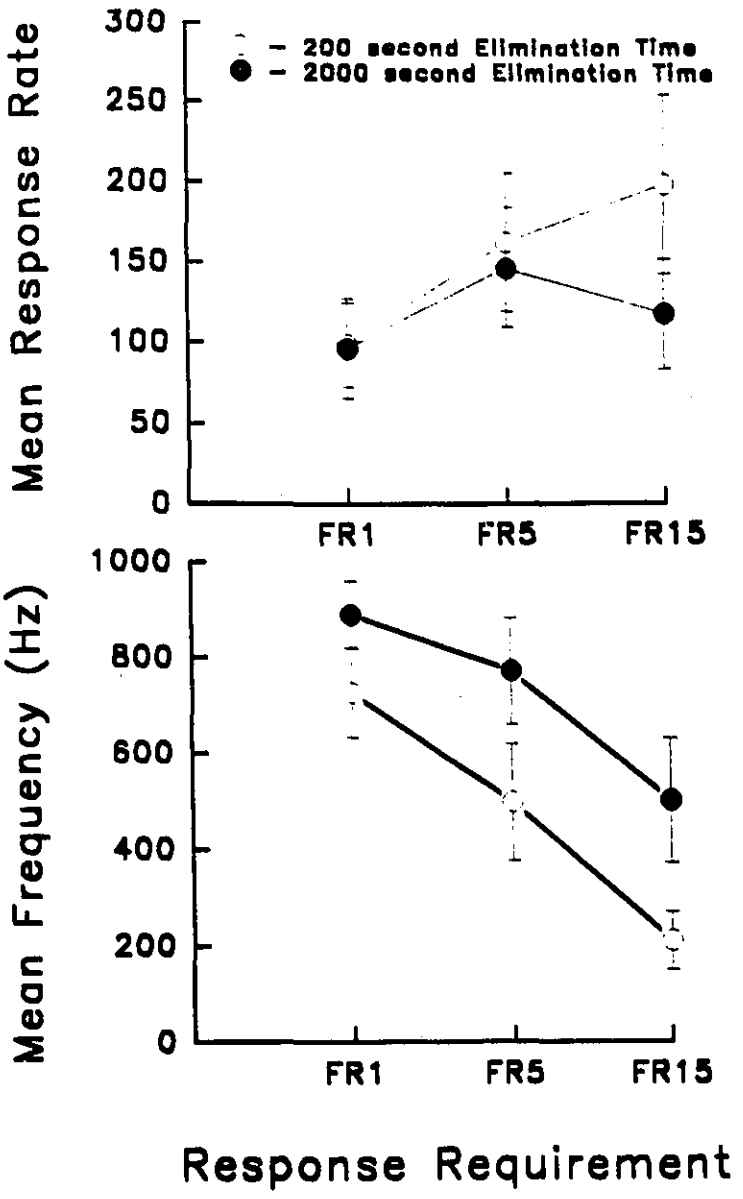


Figure 2. Relationship between response rate or pulse frequency and fixed ratio requirement during a one hr session. The top panel displays the mean number of responses at each of three fixed ratio requirements when elimination half-times are set at 200 s (open circle) and at 2000 s. The bottom panel shows the relationship between mean pulse frequency maintained by animals pressing for SABS at each of three ratio requirements when the elimination half-times are set at 200 s (open circle) and 2000 s (filled circle).

the mean pulse frequency (analogous to plasma drug concentration) maintained by animals ($F=33.5(2,10\ df)$, $p < 0.05$). Analyses with Tukeys t revealed a significant lowering of mean frequency with increases in FR size at both elimination half-times. With an elimination half-time of 200 seconds, mean frequency dropped when FR size increased from FR1 to FR5 ($t=2.82$, $p < 0.05$)and from FR5 to FR15 ($t=6.39$, $p < 0.05$). When the elimination half-time was set at 2000 seconds, mean frequency showed a reliable drop when FR size increased from FR5 to FR15($t=3.36$, $p < 0.05$), but not from FR1 to FR5. No significant interactions between FR size and elimination half-times were noted. Although mean frequency of stimulation dropped, response rates were significantly higher for FR5 and FR15 ratio requirements ($F=5.37(2,10\ df)$, $p < 0.05$) at both elimination half-times (see Figure 2).

At first glance, the relationship between total reinforcers received, FR requirement, and mean frequency does not appear consistent with our previous demonstration using a CRF schedule. We previously demonstrated that when reinforcer magnitude or duration of effect increases or decreases, animals compensate by decreasing or increasing their rate of responding (Lepore and Franklin, 1992). This suggested that animals attempt to optimize the reinforcing level of stimulation. Thus, one might expect that as FR size increases and the number of reinforcers received decreases, animals would compensate enough to maintain a constant level of stimulation. In this experiment, response rate did increase with increases in FR size, but the compensation was incomplete. Animals did not respond fast enough to maintain stimulation frequencies at high

levels though they were capable of higher response rates. This could suggest that, even though animals are capable of responding fast enough, they do not because maintaining stimulation frequencies above what is considered optimal in self-stimulation is not reinforcing. However, a more likely interpretation would be that animals do not fully compensate because a trade-off exists between the amount of work the animal has to perform and the amount of reinforcement received for that work. That is, the dose of stimulation required to maintain maximal responding under FR1 is not the same as that which maintains maximal responding under FR5 or FR15. Hence, mean frequency decreases. In order to maintain maximal responding, as FR size increases the dose of stimulation must also increase.

These results resemble those obtained in drug self-administration studies where animals responding for barbiturates (Lemaire and Meisch, 1984; Lemaire and Meisch, 1991) cocaine (Downs and Woods, 1974; Pickens and Thompson, 1968), morphine (Weeks and Collins, 1978), or phencyclidine (Marquis et al., 1989) also show a lowered plasma drug concentration (analogous with mean frequency in SABS) with increases in ratio requirement. However, this lowered plasma drug concentration is typically seen when the ratio requirement is set at relatively high ratios (Lemaire and Meisch, 1984). Furthermore, the effect of increasing FR size is less marked at high unit doses than at low unit doses, presumably because at high unit doses, more reinforcement is received with each drug injection (Moreton et al., 1977; Pickens and Thompson, 1968). The relationship between elimination half-time and mean pulse frequency was also

consistent with the previous demonstration using CRF schedules. Mean frequencies were consistently higher when animals were responding for long elimination half-times ($F=81.8(1,5\ df)$, $p < 0.05$). Analyses using Tukeys T revealed that elimination half-times of 2000 seconds produced consistently higher mean frequencies at FR5 ($t=3.397$, $p < 0.05$) and FR15 ($t=3.606$, $p < 0.05$), but not at FR1 ($t=2.05$, $p > 0.05$), when compared with elimination half-times of 200 seconds. At FR1, the mean frequencies maintained by animals in this study are nearly identical to those in the previous demonstration. Even at a ratio requirement of 15, animals responding with an elimination half-life of 2000 seconds maintained a mean frequency between 400 and 650 Hz (see Figure 2).

Figure 3 shows the pattern of responding for SABS of one animal with the elimination half-life set at 2000 seconds at the different ratio requirements. As shown, when the ratio is set at 15 (Figure 3, panel C), the pattern of intake is stable and reinforcements occur at regularly spaced intervals. It is unlikely that an animal would maintain stable and consistent responding on FR15 if high frequency stimulating trains were poor reinforcers.

It is not possible to make a quantitative comparison between responding for SABS and performance reinforced by any particular drug or dose, because the relative magnitude of the reinforcing effect of a brain stimulation train and a dose of a particular drug is not known; is a SABS dose of 200 Hz with elimination 1/2 time of 2000 seconds more or less reinforcing than 1 mg/kg of cocaine? In order to make this type of comparison, a reinforcement schedule

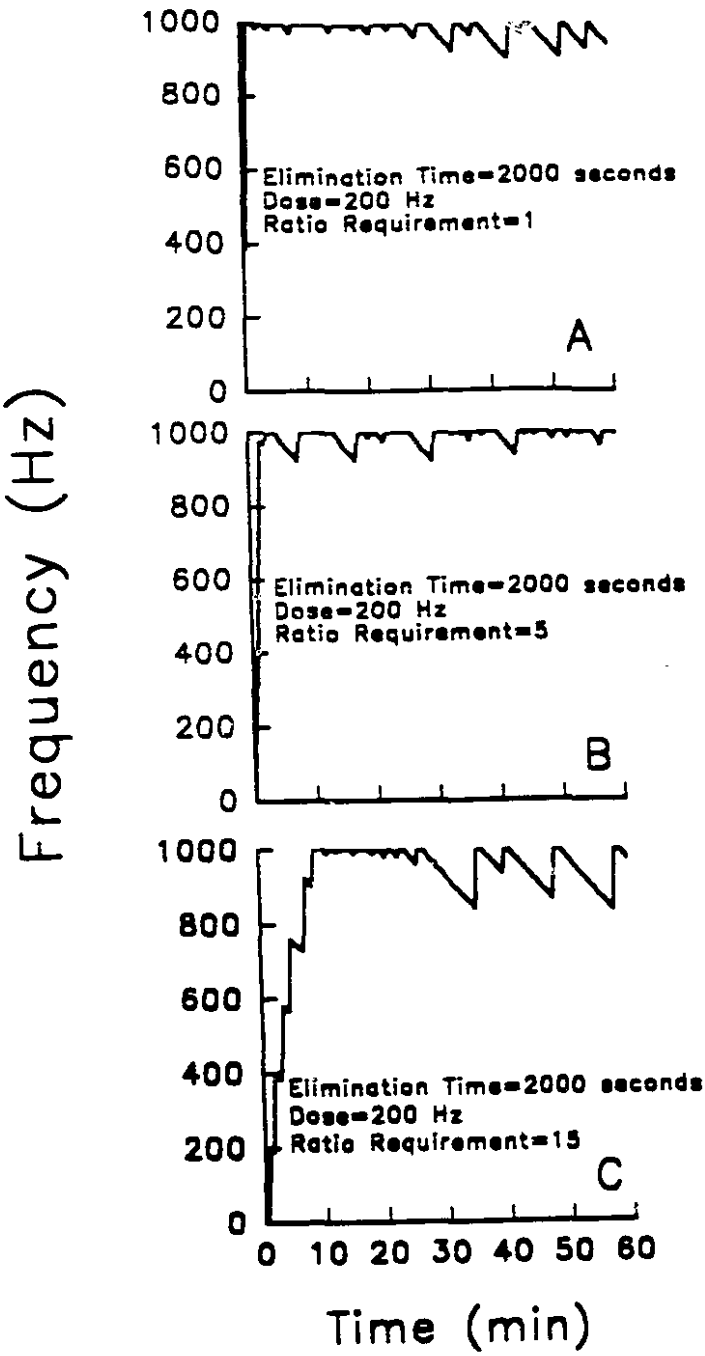


Figure 3. Pulse frequency of continuous brain stimulation maintained over a 1 h session of SABS by a single rat during sessions of responding for trains with a dose of 200 Hz, an absorption half-time of 1 s, and an elimination half-time of 2000 s. Panels A, B, and C show the records of a single rat pressing at FR1, FR5 and FR15 ratio requirements. Stimulation frequency is sampled every 30 s.

purporting to measure relative reinforcing effects of stimuli must be chosen. However, the present results show that the effect of increasing response requirement on SABS performance is similar to its effect on self-administration of a variety of drugs. Most important, the use of partial reinforcement (PRF) did not disrupt SABS performance, nor did it appear to alter the relationships between response rate and dose or elimination kinetics. The properties of SABS demonstrated on CRF are, therefore, unlikely to be artifacts of the reinforcement schedule chosen.

Experiment 2

The results of Experiment 1 demonstrated that responding for SABS remains stable across a range of ratio requirements, and that the relationship between pulse frequency and elimination half-time and response rate is consistent with our initial observations using a CRF schedule, and is consistent with the drug self-administration literature. This confirms that the frequency modulated trains of stimulation used in SABS are reinforcing. However, it has been argued that even with relatively high fixed ratios, the indirect effects of drugs can interfere with responding (Gonzalez and Goldberg, 1977; Spealman and Goldberg, 1978; Spealman et al., 1977) and, presumably, the same criticism could apply to brain stimulation trains. Furthermore, it is impossible to study the relationship between stimulation frequency and response rate in SABS because the average stimulation frequency is a dependent variable and is altered by the response rate. This is also true of plasma drug concentration in drug self-administration. To avoid this problem unit doses must be widely

separated and their rate of occurrence independent of response rate.

To minimize the problem of motoric or aversive side-effects associated with high plasma drug concentrations, investigators have been making use of so-called rate-independent schedules of reinforcement. Two widely used reinforcement schedules are fixed interval (FI) and progressive ratio (PR) schedules. Both these schedules have advantages over the use of fixed or variable ratio schedules. Fixed-interval schedules are useful in studying the motivational properties of drugs because, if the interval between drug injections is long enough and test sessions are short, animals are unable to attain a high plasma drug concentration. Consequently, undesired side effects due to accumulation of drug and/or metabolites will not interfere with responding. The same is true for progressive ratio schedules. Animals are reinforced after a fixed number of responses have been emitted and the ratio requirement increases after each reinforcement until the subject fails to complete a ratio requirement in a reasonable amount of time. The point at which animals fail to respond, the "breakpoint", is taken to reflect the reinforcing strength of the substance in question (Hodos, 1961; Hodos, 1965; Hodos and Kalman, 1963; Richardson and Roberts, 1991; Roberts, 1989; Roberts et al., 1989). Using PR schedules, investigators have shown that the break point increases with dose, and the relationship inverts (indicating the influence of side effects) only at very high unit doses (Griffiths et al., 1975; Hoffmeister, 1979). A similar relationship has been found between response rate on FI schedules and unit dose (Griffiths et al., 1975; Young and Herling, 1986). In Experiment 2, both FI and PR

schedules of reinforcement were used to examine the reinforcing efficacy of SABS trains and its relationship to peak frequency (dose).

Method

Twelve animals were prepared as described in the general methods section.

Procedure

Fixed-Interval Schedule

Rats were screened for self-stimulation as described. Following screening, six rats were trained to respond on an FI 60 second schedule for 1 second trains of 200 Hz stimulation. Once responding stabilized (within 5 sessions), rats were transferred over to the SABS program with the dose set at 200 Hz, an absorption half-time of 1 second, and an elimination half-time of 2 seconds. With these parameters, a train of stimulation lasted 16 seconds. These short trains of stimulation were used so that animals would be unable to regulate the pulse frequency within a given fixed interval period. Animals were trained on a FI 60 second reinforcement schedule until stable responding was achieved. All tests lasted 60 minutes, and thus, the total number of reinforcers an animal could obtain was 60.

Once stable responding on SABS was achieved, rats were tested at four "doses" (100, 200, 400, or 800 Hz) in random order, and were tested for three consecutive days at each dose. The absorption half-time was set at 1 second, and the elimination half-time was set at 2 seconds. The first day was used to accustom the animal to the new parameters, and the mean of the final two days

were used for analysis. Only response rate was sampled for this experiment since stimulating trains were too short to allow the animal to regulate pulse frequency.

Progressive-Ratio Schedule

Six additional animals were trained as outlined above except that animals were placed on an FR5 schedule. They were then transferred to the SABS program with the dose set at 200 Hz, an absorption half-time of 1 second, and an elimination half-time of 2 seconds.

Once stable responding was achieved, animals were tested for three days at each of four doses (i.e., peak frequencies of 100, 200, 400, or 800 Hz), in random order, on a PR schedule of reinforcement. The parameters of stimulation used for the tests were identical to those used in the FI test. For progressive ratio responding, animals started on an FR5 and after receiving a reinforcer, the ratio requirement was systematically increased by two. Thus, to obtain the reinforcer, animals had to complete an FR5, then FR7, FR9, ... and were run until break points were reached. For all animals, break points were reached by the end of three hours. Animals were run for three consecutive days at each dose, and the mean break point of the final two days was used in the analysis.

Results and Discussion

Fixed-Interval Schedule

The shape of the rate-frequency curve was found to have a shallow inverted-U shape. Responding rose from 100 to 400 Hz, with the maximum

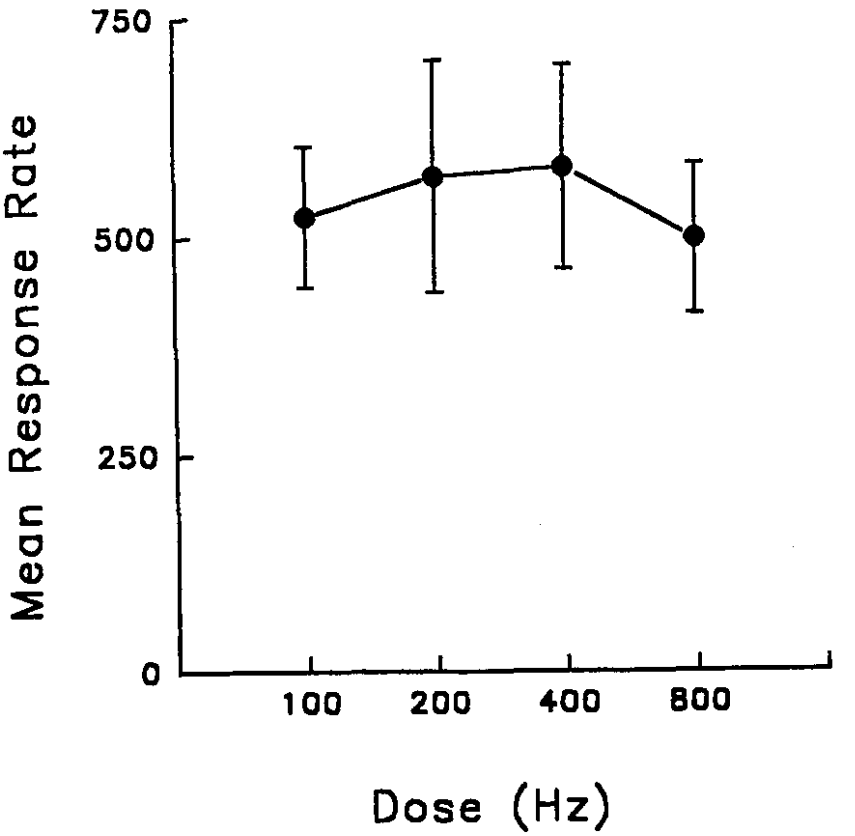


Figure 4. The relationship between mean response rate and peak pulse frequency for animals responding for 16 s trains of SABS-like stimulation (see text for further explanation) on a fixed-interval 60 s schedule of reinforcement. The parameters of each stimulating train was set at an absorption half-time of 1 s, and an elimination half-time of 2 s at each of the four peak frequencies ("dose"). Points represent the means ± S.E.M.

response rate occurring between 200 and 400 Hz (see Figure 4). At 800 Hz, response rate dropped off to a level equal to that of the lowest dose tested. These results are consistent with those found for conventional self-stimulation (Milner, 1978). Although in conventional self-stimulation, peak responding occurs between 100 and 200 Hz, the majority of rate-frequency experiments have been carried out using a CRF schedule. When a variable interval schedule of reinforcement is used (Kling et al., 1979), responding increases up to 400 Hz. In fact, pulse frequencies (Kling et al., 1979) and current intensities (Hawkins and Pliskoff, 1964) considered aversive when tested under CRF schedules (Atrens et al., 1977; Deutsch and Hawkins, 1972) maintain high rates of responding under variable interval schedules of reinforcement.

These results are also consistent with the pattern of responding observed when animals are self-administering cocaine (Balster and Schuster, 1973; Johanson, 1982), barbiturates (Johanson, 1982; Kelleher, 1976), amphetamine (Allen and MacPhail, 1991), or opioids (Thompson and Schuster, 1964) on FI schedules. Typically, response rate increases with unit dose, with only the highest unit doses producing a decrease in response rate. The decrease in responding is believed to reflect the generalized rate-decreasing effect of stimulants or opioids at high doses (Gonzalez and Goldberg, 1977; Mason and Milner, 1985), and not necessarily a decrease in the rewarding value of the drug. It is not clear whether the same interpretation could be extended to SABS.

Progressive Ratio Schedule

Break points were significantly related to stimulation frequency ($F=11.8(3,15\ df)$, $p < 0.05$). When break points were plotted against stimulation frequency, break points increase linearly from 100 to 400 Hz (see Figure 5). Maximum break points were at 400 Hz, and were significantly higher than breakpoints at 200 Hz ($t=3.45$, $p < 0.05$, Tukeys test) and 100 Hz ($t=5.21$, $p < 0.05$, Tukeys test). No reliable differences in break points were observed between 200 and 800 Hz, but break points at 200 and 800 Hz were consistently higher than at 100 Hz ($t=5.11$, $p < 0.05$, Tukeys test). It should be noted that although no differences existed between 200 and 800 Hz, a small number of animals (2/6) showed increases in break point up to 800 Hz, while others responded at the same level or slightly under that observed at 400 Hz.

These results are consistent with those obtained with the drug self-administration paradigm. Breakpoints increase with increasing unit doses of barbiturates (Griffiths et al., 1975), amphetamine (Stretch et al., 1971), and opioids (Hoffmeister, 1979), with the trend only reversing at high unit doses. The decreases in break point observed with high unit doses is also thought to represent a generalized rate-decreasing effect associated with drug side effects (Hoffmeister, 1979; Spealman and Goldberg, 1978). This pattern is also observed when animals are responding on a progressive ratio schedule for brain stimulation reward (Hodos, 1965; Keesey, 1964). Increases in train length as high as 120 seconds have been shown to produce higher break points than stimulation trains of 0.15, 1 or 10 seconds (Deutsch et al., 1976; Hodos, 1965).

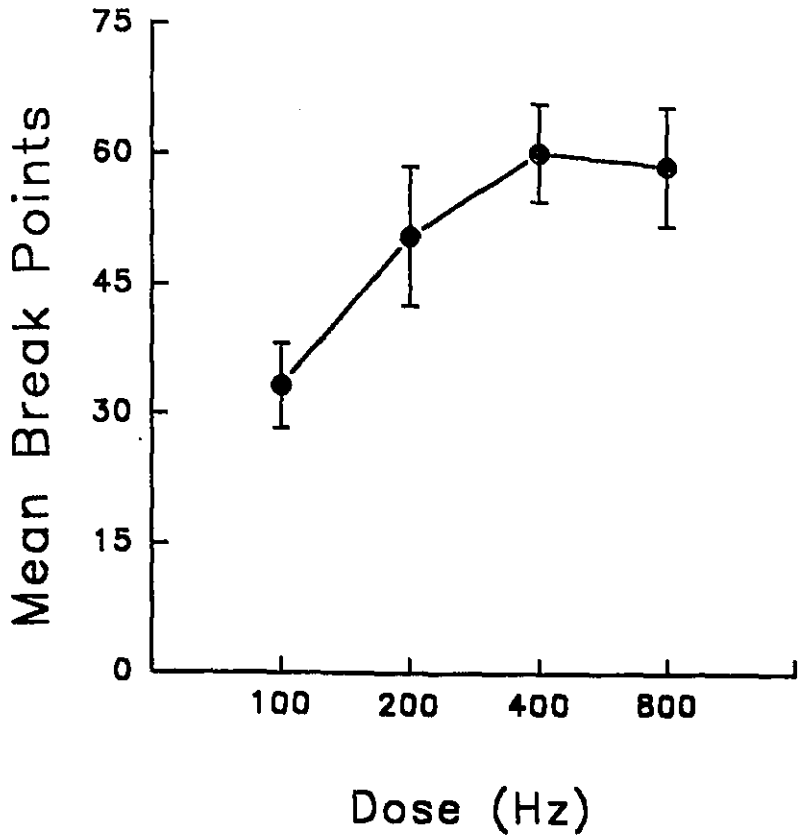


Figure 5. The relationship between mean break points and peak pulse frequency for animals responding on a progressive-ratio schedule of reinforcement for 16 s trains of SABS-like stimulation (see text for further explanation). The parameters of each stimulating train were set at an absorption half-time of 1 s and an elimination half-time of 2 s at each of four different peak frequencies ("dose"). Points represent the mean ± S.E.M.

Together with the findings from Experiment 1, these results confirm that high frequency trains used in SABS are reinforcing, and strongly suggest that high average frequencies observed with SABS represents the animals attempt at maintaining an optimal reinforcing level of stimulation. These results also demonstrate that the behavioral differences observed between drug self-administration and ICSS can be explained by procedural differences between the models. That is, when trains of stimulation are applied to the reward substrate in a fashion that mimics the time course of effect of a drug, animals responding for SABS "load up" with brain stimulation as they do in drug self-administration; animals also show an inverse relationship between stimulation "dose" or "elimination half-time" and response rate, as do animals responding for drug reward; when pimozide or cis-flupenthixol are administered to animals responding for SABS, an increase in both response rate and mean frequency is observed, similar to the increase seen in animals self-administering opioids or stimulant drugs when challenged with dopamine antagonist drugs (Corrigall, 1987; Koob et al., 1987a; Yokel and Wise, 1975); and that the acquisition of SABS behavior resembles acquisition of stimulant or opioid self-administration. Furthermore, these results demonstrate that the SABS model has characteristics of both ICSS and drug self-administration which makes SABS a potentially useful model to test hypotheses about the neural substrate of reward and reinforcement. One of the major advantages to using the SABS model is that, since the interpretation of the reinforcing effect of long frequency-modulated trains of stimulation is consistent with both the drug self-

administration and ICSS paradigms, findings from the SABS model can be extended to both brain stimulation and drug reward. That is, if a structure such as the PPTg mediates the acquisition or maintenance of SABS responding, it can be reasonably assumed that it would also mediate acquisition or maintenance of self-stimulation and drug self-administration. Further, confounds such as pharmacological dependence, aversion, or rate-interference effects of drugs is virtually non-existent at the parameters typically used with the SABS model. In the remainder of this thesis, the SABS paradigm is used to explore some neurological hypotheses concerning the substrate of drug self-administration and other reinforcers.

The Pedunculopontine Tegmental Nucleus

Introduction

The mesolimbic DA theory of reward has dominated the attention of researchers investigating the neural basis of reward and reinforcement for over a decade (Ettenberg, 1989; Koob et al., 1987c; van Rossum, 1970; Wise, 1978a; Wise, 1980; Wise, 1981; Wise, 1987; Wise and Rompre, 1989). As reviewed above, several lines of pharmacological evidence have been invoked to support the DA theory of reward. Direct and indirect DA agonist drugs increase the rate of self-stimulation behavior, interpreted to reflect a decrease in the reinforcing threshold of brain stimulation reward (Aulakh et al., 1979; Gallistel and Freyd, 1987; Hubner et al., 1987; Neill et al., 1978; Wauquier and Niemegeers, 1973); DA antagonist drugs invariably suppress responding for self-stimulation, interpreted to reflect an increase in the threshold for reinforcing brain stimulation (Corbett, 1990; Fouriezos and Wise, 1976; Franklin and McCoy, 1979; Fouriezos and Wise, 1976; Gallistel and Davis, 1983; Gallistel and Freyd, 1987; Gallistel et al., 1982; Philips et al., 1979) direct and indirect DA agonist drugs initiate (Bozarth et al., 1989; Deminiere et al., 1989; Lyness et al., 1980) and maintain (Corrigall and Coen, 1989; Gerber and Wise, 1989; Porrino et al., 1989; Roberts et al., 1977; Spear and Katz, 1991; Wise, 1981) self administration behavior, and can condition place preferences (Ettenberg, 1989; Hiroi and White, 1990; Hiroi and White, 1991; Hoffman et al., 1988; Phillips and Fibiger, 1987; Phillips et al., 1983b); and DA antagonist drugs attenuate responding for drug reward (Bozarth and Wise, 1981b; Gerber and

Wise, 1989; Roberts and Vickers, 1984; Yokel, 1987; Yokel and Wise, 1975) and can block the acquisition of place preference behavior (Acques et al., 1989; Ettenberg, 1989; Hiroi and White, 1990; Mackey and van der Kooy, 1985). Anatomical evidence using the intracerebral self-administration paradigm (Glimcher et al., 1987; Goeders and Smith, 1987; Goeders et al., 1984; Goeders et al., 1986; Hoebel et al., 1983) and intracerebral microinjection of drugs on acquisition of place preference behavior (Bozarth, 1987a; Carr and White, 1986; Phillips and LePiane, 1980; Phillips and LePiane, 1982; Phillips et al., 1983b; van der Kooy et al., 1982) also support a role for brain DA in reward. The specific pathways involved in mediating the reinforcing effects of drugs and other stimuli are thought to comprise the DA cells of the VTA and their projection to the nucleus accumbens (Bozarth, 1987a; Wise, 1980; Wise, 1987; Wise, 1989; Wise and Bozarth, 1984) and prefrontal cortex (Clavier and Gerfen, 1979; Hammer, 1989; Mora, 1978; Mora and Myers, 1977; Robertson and Mogenson, 1979; Rolls and Cooper, 1973), and the cells of the nucleus accumbens and their projection to the ventral pallidum (Ettenberg et al., 1982; Koob and Swerdlow, 1988; Strecker et al., 1982; Swerdlow and Koob, 1987).

For the first time in a decade, an extension of this reward circuitry has been proposed. The pedunculopontine tegmental nucleus has received attention as a possible output and input site of the accumbens-pallidal reward pathway that may act as a final common substrate for the reinforcing effects of drugs and other rewards (Bechara and van der Kooy, 1989; Bechara and van der Kooy, 1992; Mogenson et al., 1980). This proposal stems mainly from the

finding that bilateral ibotenate lesions of the PPTg block the conditioned place preferences produced by morphine, amphetamine, and food reward in animals who are non-deprived (Bechara and van der Kooy, 1989; Bechara and van der Kooy, 1992). The second half of this thesis describes a series of experiments exploring the role of the pedunculopontine tegmental nucleus in the positive reinforcing properties of brain stimulation by testing the effects of various lesions of the PPTg on the acquisition and maintenance of SABS responding and on other behavioral tasks.

Anatomy of the Pedunculopontine Tegmental Nucleus

The pedunculopontine tegmental nucleus was initially defined on cyto-architectural grounds as a group of large, darkly staining neurons extending as far rostrally as the pars compacta of the substantia nigra and the retrorubral field (A8 dopamine cell group) and as far caudally as the parabrachial nucleus and the red nucleus (Jackson and Crossman, 1983; Newman, 1985; Rye et al., 1987; Saper and Loewy, 1982). The PPTg is bounded along the medial plane by the periaqueductal gray area, the decussation of the brachium conjuctivum (Goldsmith and van der Kooy, 1988; Newman, 1985; Moriizumi et al., 1988) and the ascending limb of the superior cerebellar peduncle (Rye et al., 1987), and along the lateral plane by the lateral lemniscus and associated nuclei (Newman, 1985; Rye et al., 1987). The PPTg is primarily bounded dorsally by the cuneiform nucleus, and ventrally by the pontine tegmental field and the rubrospinal tract (Moriizumi et al., 1988; Newman, 1985; Rye et al., 1987). Recently, investigations of the afferent and efferent connections of the PPTg

have demonstrated direct and indirect connections with several forebrain nuclei, including the ventral tegmental area, nucleus accumbens, ventral pallidum, dorsal striatum, and the lateral hypothalamus and septal area (Beckstead et al., 1979; Garcia-Rill, 1991; Jackson and Crossman, 1983; Nauta et al., 1978; Saper and Loewy, 1982; Saper et al., 1979) - all midbrain and forebrain nuclei involved in the positive reinforcing effects of brain stimulation, food and water reward, and drugs of abuse (Gallistel et al., 1981; Hoebel, 1985; Hoebel, 1985; Koob et al., 1987b; Kornetsky and Bain, 1987; Roberts and Zito, 1987; Wise, 1981; Wise, 1989).

The pedunculopontine tegmental nucleus has also been defined[1] in neurochemical terms (Hallanger et al., 1987; Rugg et al., 1992; Semba and Fibiger, 1992; Sugimoto and Hattori, 1984), with most of the focus directed to the brainstem cholinergic system. The PPTg has approximately 1,700 acetylcholine cells per hemisphere (Rye et al., 1987), some of which project to the substantia nigra, the thalamus, and various other nuclei (Goldsmith and van der Kooy, 1988; Hallanger et al., 1987; Rye et al., 1987; Semba and Fibiger, 1992; Sugimoto and Hattori, 1984). Due primarily to the heavy cholinergic innervation of the substantia nigra and basal ganglia from the cholinergic cells of the PPTg (Beninato and Spencer, 1987; Beninato and Spencer, 1988; Clarke et al., 1987; Gould et al., 1989; Lee et al., 1988), the predominant role ascribed to this brainstem cholinergic system is the modulation or mediation of

[1] For the purposes of this thesis, the cytoarchitectural definition of the PPTg will be used.

extrapyramidal motor activity (Brudzynski et al., 1988; Garcia-Rill et al., 1987; Garcia-Rill et al., 1990; Mogenson et al., 1989; Swerdlow and Koob, 1987).

Aside from the cholinergic cell population, the PPTg also contains a relatively high number of large and small non-cholinergic cells (Spann and Grofova, 1992), some of which are believed to be glutamate cells (Clements et al., 1991; Spann and Grofova, 1992). The finding that glutamate cells make up part of the PPTg comes from experiments demonstrating that cells displaying glutamate immunoreactivity are interspersed among the cholinergic cells (Clements and Grant, 1990), and that glutamate immunoreactivity can be detected within cholinergic cells (Clements et al., 1991). These findings suggest that excitatory amino acids (EAA) may be involved in the normal functioning of the PPTg. Although the functions of the glutamate cells have yet to be identified, it is possible that glutamate efferents of the PPTg give rise to an excitatory pathway synapsing on the dopaminergic cells of the substantia nigra (Di Loreto et al., 1992). In testing this hypothesis, Di Loreto et al. (1992) found that the iontophoretic application of glutamate to the PPTg produced an excitation of nigral neurons, which was blocked by the co-administration of kynurenic acid (EAA antagonist) into the substantia nigra, but not by intra-nigral microinjections of mecamylamine or atropine (cholinergic antagonists). Though these findings have yet to be replicated, it does suggest that one of the functions of the PPTg is to modulate the activity of the nigrostriatal pathway through cholinergic or glutamatergic (or both) efferents. Of course, explorations for different types of neurotransmitter systems in this region have been limited,

and there may be as yet undefined neurochemical systems which could modulate or mediate nigrostriatal **functioning.**

Extrapyramidal Motor Activity and the Pedunculopontine Nucleus

In light of the suggestion that motivation and locomotor activity are tightly linked (Mogenson et al., 1980), and that the PPTg may be an integral brainstem substrate for both motivation and nigrostriatal extrapyramidal activity, the role of the PPTg in locomotor activity is also of interest. Based on electrophysiological and behavioral evidence (Mogenson and Wu, 1988; Mogenson et al., 1989; Swanson et al., 1987; Swerdlow and Koob, 1987), it has been suggested that dopamine-mediated locomotion in the nucleus accumbens-ventral pallidal pathway is dependent on an intact PPTg. In one such demonstration, Brudzynski and Mogenson (1988) infused amphetamine into the nucleus accumbens to stimulate locomotor activity. They found that simultaneous infusion of the local anesthetic, procaine, into the PPTg completely blocked the locomotor stimulatory effects of nucleus accumbens amphetamine administration. This suggests that one of the functions of the PPTg is to receive outflow from the nucleus accumbens-ventral pallidum pathway to mediate dopamine-induced locomotion. However, it is possible that the attenuation of amphetamine-induced locomotion by procaine results from the local anesthetic actions of procaine on the fibers of passage in that region, and not on intrinsic neurons of the PPTg (Swerdlow and Koob, 1987). Swerdlow and Koob (1987) demonstrated that electrolytic or excitotoxic lesions of the dorsomedial thalamus, but not the PPTg, partially disrupted apomorphine-

induced locomotion. Olmstead and Franklin (1992) have also demonstrated that excitotoxic lesions of the PPTg do not block amphetamine-induced locomotion. These findings suggests that the PPTg may not be receiving outflow from the accumbens-pallidal pathway to specifically mediate DA-induced locomotion.

Although there are conflicting views as to whether the PPTg mediates DA-induced locomotion by receiving output from the accumbens-pallidal pathway, there are several reports suggesting that the PPTg indirectly modulates or influences extrapyramidal motor activity in the nucleus accumbens and striatum via direct efferent projections to the ventral tegmental area and substantia nigra, respectively (Niijima and Yoshida, 1988). Niijima and Yoshida (1988) demonstrated that unilateral chemical stimulation of PPTg cells directly activated midbrain dopamine neurons to induce circling behavior, an effect blocked by i.p. administration of haloperidol and by intra-PPTg, -VTA, and -substantia nigra infusions of atropine, a cholinergic receptor antagonist. It was also reported that the activation of PPTg neurons resulted in an increase in the DOPAC/DA and HVA/DA ratios (indices of dopamine release and utilization) in both the nucleus accumbens and dorsal striatum. This direct effect of efferent cholinergic neurons of the PPTg on substantia nigra DA neurons, and consequently on DA release in the dorsal striatum, was confirmed in a study performed by Blaha and Winn (1993). In their experiment, unilateral quinolinic acid lesions of the PPTg were made and the effects of intranigral infusions of nicotine and neostigmine were determined. They demonstrated that intranigral

administration of both nicotine and neostigmine produced a reliable increase of DA efflux in the striatum. Unilateral PPTg lesions completely blocked the increase in DA release produced by neostigmine. The authors concluded that cholinergic efferents of the PPTg (and subsequent acetylcholine release) indirectly modulates the activity of the dorsal striatum via their direct modulatory activity on substantia nigra DA neurons.

Taken together, all these studies strongly suggest that the PPTg is involved in the generation of motor activity by the extrapyramidal motor system. Aside from its role in locomotion, the PPTg has also been implicated in sleep-wake mechanisms (Garcia-Rill, 1991; Hobson et al., 1986; Hoover and Jacobowitz, 1979; McGinty and Drucker-Colin, 1982; Sakai, 1980; Siegel, 1979), epilepsy (Garcia-Rill, 1991; Gloor, 1968; Harfstrandt et al., 1986; Niedermeyer, 1982), arousal and attention (Gonzales-Lima and Scheich, 1985; Steriade, 1980; Villablanca and Olmstead, 1982; Villablanca et al., 1976), sensory modulation (Hylden et al., 1985; Katayama et al., 1984; King, 1974; Lidsky et al., 1985), and learning and memory (Dellu et al., 1991; Fujimoto et al., 1989; Fujimoto et al., 1990; Fujimoto et al., 1992; Yang and Mogenson, 1987).

Learning and Memory and the Pedunculopontine Tegmental Nucleus

Although the PPTg has been proposed as a final common substrate that mediates drug- or food-induced motivation (Bechara and van der Kooy, 1989; Bechara and van der Kooy, 1992), an alternative interpretation of the PPTg results to date could be that the PPTg is involved in learning and memory

processes. As reviewed above, bilateral lesions of the PPTg block the acquisition, but not retention of, morphine-, amphetamine-, and food-induced place preference behavior (Bechara and van der Kooy, 1989; Bechara and van der Kooy, 1992). The CPP paradigm, however, may have a significant learning component associated with it (see above). If the PPTg is involved in learning and memory, it is possible that morphine- or food-induced place preference behavior is not established because the association between stimuli that make up a distinctive environment and the presentation of a reward in that environment does not or cannot occur.

Learning and memory deficits are not restricted only to classical conditioning, but also to simple instrumental conditioning (Fujimoto et al., 1989; Fujimoto et al., 1990; Fujimoto et al., 1992). Fujimoto et al. (1989, 1990, 1992) investigated whether bilateral ibotenic acid lesions of the PPTg would impair acquisition, retention, or retrieval of avoidance behavior in rats. They found that lesions of the PPTg severely impaired acquisition of both one-trial passive avoidance and two-way shuttle-box active avoidance. Pedunculopontine tegmental lesions, however, had no effect on retention or retrieval of previously learned one-trial passive or two-way active avoidance behavior. Although directly comparing avoidance behavior with place preference behavior may not be valid behaviorally, the pattern of learning deficits observed in the Fujimoto studies (1989, 1990, 1992) is basically identical to the pattern of learning deficits observed in the Bechara and van der Kooy experiments (1989, 1992). Only acquisition of avoidance or place preference behavior was blocked,

whereas retention or retrieval of either behavior remained intact.

Further evidence for learning deficits resulting from PPTg lesions comes from Dellu et al. (1991). In this experiment (Dellu et al., 1991), the effects of bilateral quisqualic acid lesions of the PPTg on Morris water maze and eight-arm radial maze learning was investigated. It was found that PPTg lesions blocked the acquisition of Morris water maze and radial maze learning, suggesting that PPTg lesions produce a hippocampal-related deficit (Dellu et al., 1991). Both the Morris water maze and eight-arm radial maze are tasks of spatial memory, which appear to be dependent on the hippocampus (Jarrard, 1993; McDonald and White, 1993; O'Keefe and Speakman, 1987; Olton and Papas, 1979; Olton and Samuelson, 1976; Olton et al., 1979; Speakman and O'Keefe, 1990) (to be discussed below). Recently, electrophysiological and behavioral evidence has been presented that shows that the nucleus accumbens-ventral pallidal area relays signals from the hippocampus to the PPTg (Yang and Mogenson, 1987). Taken together with the studies reviewed above, there is some evidence that suggests the PPTg, perhaps through the accumbens-pallidal or striatopallidal systems, mediates acquisition learning per se, and may not be specific to reward-related or motivated behaviors.

Experiment 3

Introduction

The suggestion that the PPTg mediates the rewarding effects of stimuli in non-deprived animals (Bechara and van der Kooy, 1989; Bechara and van

der Kooy, 1992) would be strengthened if it could be demonstrated that PPTg lesions also block the rewarding effects of stimuli in a different paradigm. The operant paradigm and especially the ICSS paradigm overcome some of the problems of the CPP model. For example, ICSS is sensitive to variations or shifts in the incentive value of brain stimulation, whereas the CPP model is not (Buscher et al., 1989; Koob, 1977). Furthermore, animals responding for BSR do not become physically "dependent" on brain stimulation. Thus, the potential confound of pharmacological dependence is muted using brain stimulation. Another advantage is that animals rapidly acquire responding for brain stimulation if it is above some reinforcing threshold (Yeomans, 1990). For example, implanting electrodes in the LH-MFB region almost invariably support self-stimulation behavior. As demonstrated with SABS (Lepore and Franklin, 1992), if the electrode is located in the LH-MFB and is operational, there is over a 95% chance the animals will acquire SABS responding. To date, no studies have investigated the effects of PPTg lesions on the acquisition of operant responding. The rapid spontaneous acquisition of SABS (Lepore and Franklin, 1992) provides a means to examine the role of the PPTg in the acquisition of an operant response without the problems of interpretation that might arise from experimenter bias when training or shaping procedures have to be used.

An opportunity also exists to examine the neurochemical substrate of the PPTg involved in mediating the rewarding effects of stimuli in the PPTg. A significant population of cholinergic cells can be found in the PPTg (Rugg et al., 1992; Rye et al., 1987), which to some investigators is the principal defining

feature of the PPTg (Lee et al., 1988). In the Bechara and van der Kooy (1989, 1992) experiments, ibotenate was the excitotoxic substance used to lesion the PPTg. Ibotenate, as well as NMDA, produces a roughly equivalent amount of damage to cholinergic and non-cholinergic cells (Rugg et al., 1992). However, it is possible to produce a more selective lesion of cholinergic cells than non-cholinergic cells by using quinolinic acid (Rugg et al., 1992). Experiment 3 examined acquisition of SABS behavior in groups of animals with NMDA and quinolinic acid (QA) lesions of the PPTg using SABS parameters previously found to support rapid acquisition of responding.

Method

Animal Subjects

A total of 48 male, Long evans rats were used for the following experiments. Surgical procedures, handling, and post-operative care were carried out as described in the General Method. Coordinates used for the PPTg were: -7.8 mm posterior to bregma, ± 1.5 mm from the midline, and -7.0 mm ventral from the skull surface, according to the atlas of Paxinos and Watson (1986). Stimulating electrodes were aimed and implanted in the LH-MFB region as described in the General Method.

Excitotoxic Lesions of the PPTg

Bilateral NMDA and quinolinic acid (QA) lesions of the PPTg were made in 32 animals, and bilateral vehicle sham lesions of the PPTg were made in 16 animals. Each rat was bilaterally injected over 10 minutes with 0.5 µl of either NMDA or QA (0.1 M) or vehicle solution (phosphate buffered saline, pH = 7.4),

via a 10 µl Hamilton syringe mounted on an infusion pump (Harvard Instruments). The needle was left in place for five minutes following each microinjection. The bipolar stimulating electrode (Plastics One, VA) was then implanted. When there were signs that the general anesthetic was wearing off, animals were injected with diazepam (4 mg/kg) to prevent the occurrence of seizures. Animals were allowed five days recovery from surgery before testing began.

Histology

Following completion of behavioral testing, animals were deeply anesthetized with 30% chloral hydrate and were perfused transcardially with physiological saline followed by a 10% formol-saline solution. Brains were removed and further fixed in a 10% formol-saline solution for at least 24 hours prior to sectioning. Brains were sectioned at 25 µm, and every other section (through the PPTg) was mounted and stained using the Cresyl Violet method. A detailed discussion and presentation of anatomical data will be presented later in the thesis.

Procedure

Spontaneous Acquisition Studies

Groups of ten rats that were sham-lesioned, or lesioned with either QA or NMDA were tested to determine whether spontaneous acquisition of SABS responding would occur. The parameters of each stimulating train was held constant for each group of animals, with the elimination half-time set at 100 seconds, the rise half-time set at one second, and the "dose" (peak frequency)

set at 200 Hz. The remaining groups of six rats (sham and excitotoxic-lesioned) were tested but no brain stimulation was applied (i.e. dose=0 Hz) in order to estimate the unconditioned operant rate. Animals were not preexposed to the training apparatus or to brain stimulation. Animals were placed in the operant chambers and were left for one hour each day for five days. Stimulation current was set at 100 μA for all animals, and no priming trains of brain stimulation were administered to the animals.

Results

All ten sham-lesioned animals acquired SABS behavior when the program was instituted *de novo*. All sham-lesioned and excitotoxic-lesioned animals that had a dose set at 0 Hz (no stimulation) did not increase their response rate over the five testing days. A two-way Group X Control test day ANOVA performed on response rates revealed that no significant differences in the unconditioned response rate existed between sham-lesioned or excitotoxic-lesioned animals ($F=0.132(2,15\ df)$, $p > 0.05$). Further, for all groups of animals, the number of control test days had no effect on unreinforced responding ($F=2.002(4,60\ df)$, $p > 0.05$), nor were there any significant interactions between lesion group and control test days ($F=0.801(8,60\ df$, $p > 0.05$). The unstimulated group of animals responded several times the first day of training, and had almost ceased to respond by the end of the five training days. Since unreinforced responding was negligible, data from the unstimulated control animals was not used in subsequent statistical analyses.

Inspection of the histological material revealed that in two animals in the

QA lesion group, the lesioning site was lateral to the PPTg, and in two animals in the NMDA lesion group, only unilateral lesions of the PPTg were made. In the cases where unilateral lesions were made, animals were capable of acquiring SABS behavior. However, because of incomplete lesions, the data from these animals were not included in subsequent statistical analyses. To ensure an equal n for all statistical analyses, 2 sham lesioned animals were randomly (random number table) dropped from the analyses. Of the eight animals lesioned with QA, 7 animals acquired SABS while no animals lesioned with NMDA were able to acquire SABS responding (see Figure 6). A two way Group X Acquisition Day ANOVA performed on response rate revealed that NMDA lesions of the PPTg significantly attenuated responding for SABS ($F=9.948(2,21\ df)$, $p < 0.05$). For all three groups of animals, the number of acquisition days did not significantly effect responding for SABS ($F=2.075(4,84\ df)$, $p > 0.05$), and no interactions between lesion group and acquisition days was found ($F=1.881(8,84\ df)$, $p > 0.05$). Post hoc analysis (Newman-Keuls test) performed on lesion group revealed that NMDA-lesioned animals responded less than sham-lesioned ($q=4.528$, $p < 0.05$) and QA-lesioned animals ($q=6.067$, $p < 0.05$), while no differences in response rate between sham-lesioned and QA-lesioned animals ($q=1.539$, $p > 0.05$) was found. Thus, NMDA lesions, but not QA lesions, severely impaired the acquisition of SABS.

A second Group X Acquisition day ANOVA performed on mean frequency revealed a similar trend as the response rate data (see Figure 6). NMDA lesions significantly lowered the mean pulse frequency maintained by

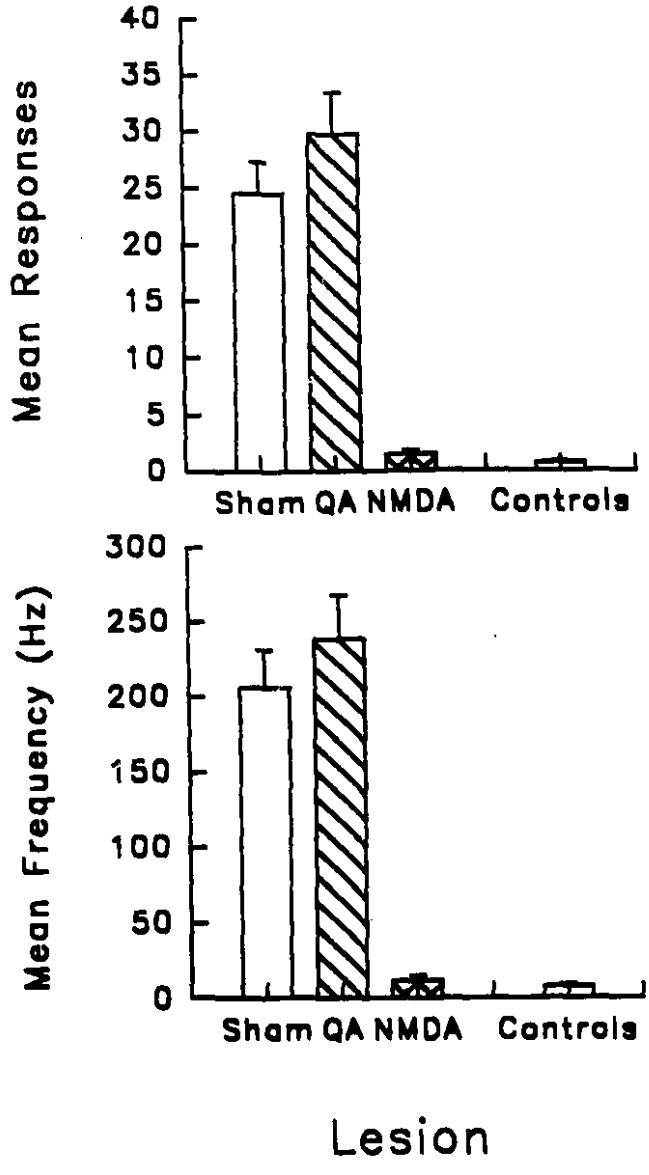


Figure 6. The effects of sham, NMDA, and quinolinic acid (QA) lesions on the acquisition of SABS. The parameters of each stimulating train was set at a dose of 200 Hz, an absorption half-time of 1 s and an elimination half-time of 100 s. The top panel displays mean number of responses (± S.E.M.) across 5 test days for each group of animals lesioned with NMDA, QA or vehicle sham solutions on SABS acquisition, and the unconditioned operant rate for NMDA-lesioned control animals. The bottom panel displays the mean pulse frequency (± S.E.M.) maintained by each group of animals across 5 test days.

animals ($F=9.177(2,21\ df)$, $p < 0.05$). Mean pulse frequency was not significantly lowered across acquisition days ($F=1.793(4,84\ df)$, $p > 0.05$), and no lesion group by acquisition day interaction was found ($F=1.530(8,84\ df)$, $p > 0.05$). Post hoc analysis using the Newman Keuls procedure showed that NMDA-lesioned animals maintained a significantly lower mean pulse frequency than sham-lesioned ($q=4.80$, $p < 0.05$) and QA-lesioned animals ($q=5.602$, $p < 0.05$), while no differences in mean pulse frequency was observed comparing sham-lesioned with QA-lesioned animals ($q=0.801$, $p > 0.05$). The results of this analysis are consistent with a previous report demonstrating that the mean pulse frequency maintained by animals responding for SABS stimulation is directly related to response rate (Lepore and Franklin, 1992).

Discussion

The results for controls and QA-lesioned animals are consistent with the previous report on the acquisition of responding for SABS stimulation (Lepore and Franklin, 1992). Performance for SABS can be acquired without pretraining, previous experience with brain stimulation, or shaping. Further, there is no increase in response rate or mean frequency across acquisition days, indicating that SABS is acquired in the first test session, similar to acquisition of self-stimulation behavior (Lepore, 1990; Yeomans, 1990).

Animals with NMDA lesions of the PPTg failed to acquire SABS behavior after 5 training days. The failure to acquire SABS behavior did not occur because NMDA lesioned animals did not sample the stimulation. NMDA lesioned animals consistently sampled SABS stimulation, responding on

average at least once every acquisition test day. Thus, it cannot be argued that NMDA-lesioned rats did not acquire SABS because they failed to create opportunities to experience the reinforcer. These results suggest the PPTg is essential for the acquisition of an operant task (SABS), and are consistent with the idea that the PPTg mediates the reinforcing effects of brain stimulation. That QA lesioned animals were no different from sham-lesioned controls suggests that the non-cholinergic neurons of the PPTg are responsible for mediating the positive reinforcing properties of brain stimulation, as suggested by Bechara and van der Kooy (1992) and Olmstead and Franklin (1992). Though cholinergic cells were not specifically counted in this experiment, quinolinic acid, at least in the PPTg, is more selective for lesioning the cholinergic cell population than NMDA. Rugg et al. (1992) demonstrated that at the concentrations of NMDA and QA used in this experiment, QA produces a relatively selective loss of cholinergic cells. Comparing the ratio of cholinergic cells to non-cholinergic cells, NMDA produces a higher non-cholinergic to cholinergic cell loss ratio (Rugg et al., 1992). Recently, barbiturate-induced anesthesia has been demonstrated to have a significant neuroprotective effect against QA in the PPTg (Inglis et al., 1993). In this experiment, animals were anesthetized with the barbiturate, sodium pentobarbital, which may have exerted a neuroprotective effect against QA, thereby producing a much smaller lesion compared to NMDA.

These results are consistent with the findings of Bechara and van der Kooy (1989, 1992) that ibotenate lesions of the PPTg blocked place

preferences produced by amphetamine, morphine, and food reward. Ibotenate and NMDA have been demonstrated to produce roughly an identical ratio of non-cholinergic:cholinergic cell loss (Rugg et al., 1992). That SABS acquisition was blocked by NMDA lesions of the PPTg is also consistent with the demonstration that NMDA lesions of the PPTg increase the rate-intensity and reinforcement thresholds of self-stimulation (Buscher et al., 1989), further supporting the role of the PPTg in mediating the reinforcing properties of brain stimulation.

It is possible that NMDA lesions spread to areas other than the PPTg and that lesioning these other brain structures may be producing the deficits in place preference or SABS acquisition. For example, Yeomans (1992) has demonstrated that administration of cholinergic antagonists can affect the reinforcing thresholds for brain stimulation, and that this effect may be mediated by the laterodorsal tegmental nucleus (LDTg). Histological results from this study show that, in animals with the largest NMDA lesions, the LDTg sustained some damage (see Figure 7). However, not all NMDA-lesioned animals impaired in SABS acquisition had damage to the LDTg. Furthermore, in animals lesioned with QA that also had damage to the LDTg, acquisition of SABS responding was no different from sham controls (see Figure 8). Thus, it is unlikely that the block of SABS acquisition occurred because the LDTg was lesioned.

The PPTg is also bordered by the periaqueductal gray (PAG) area, a site known to support self-stimulation behavior (Cazala and Zielinski, 1983; Liebman

Figure 7. NMDA lesions of the PPTg that were effective in blocking the acquisition of SABS. Open areas represent the largest observable damage and the darkened areas represent the smallest observable damage.

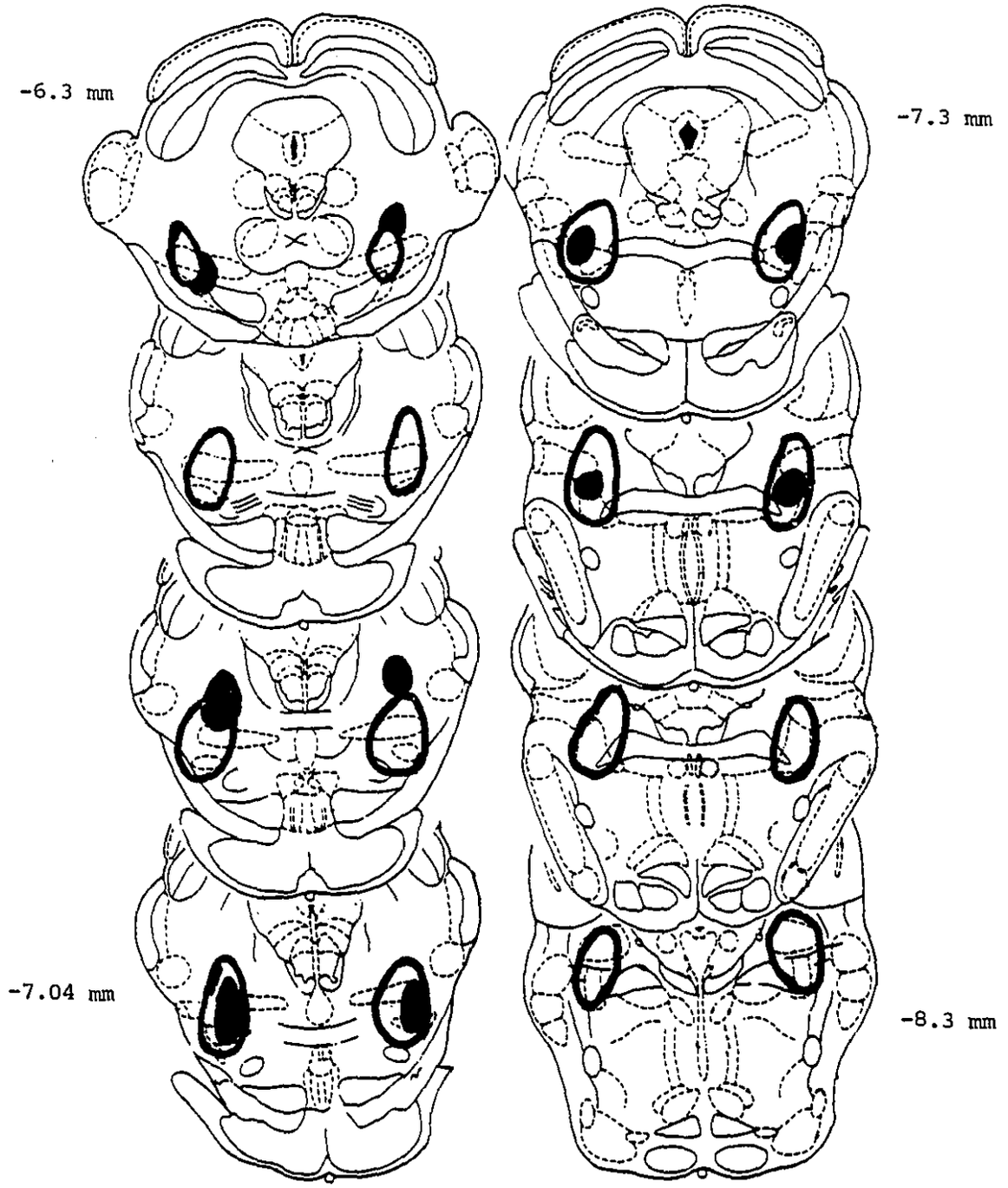

-6.3 mm
-7.3 mm
-7.04 mm
-8.3 mm

Figure 8. QA lesions of the PPTg that were ineffective in blocking the acquisition of SABS. Open areas represent the largest observable damage, and darkened areas represent the smallest observable lesions.

-7.3 mm

-7.8 mm

-8.3 mm

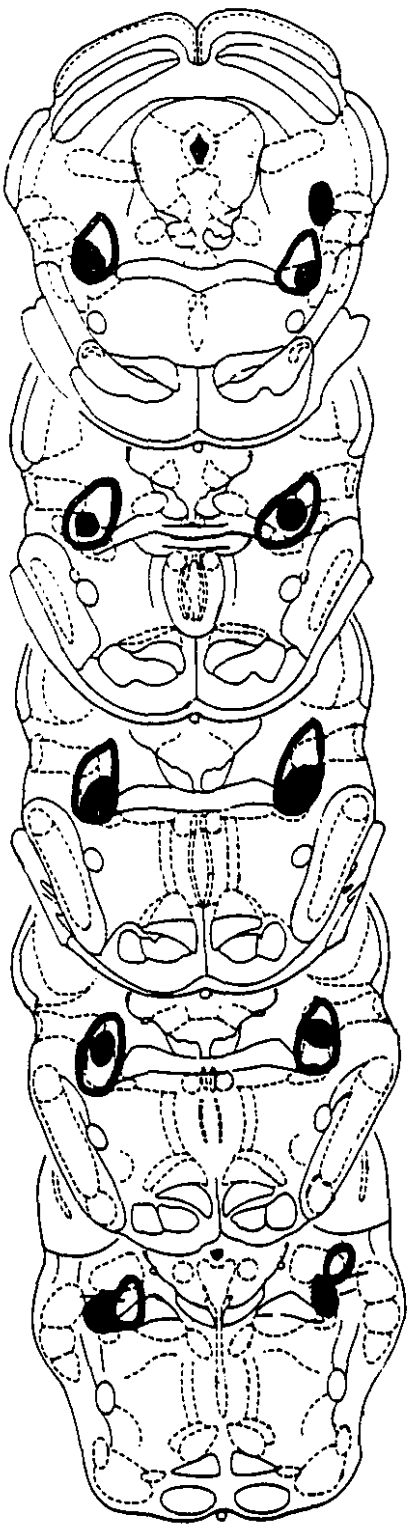
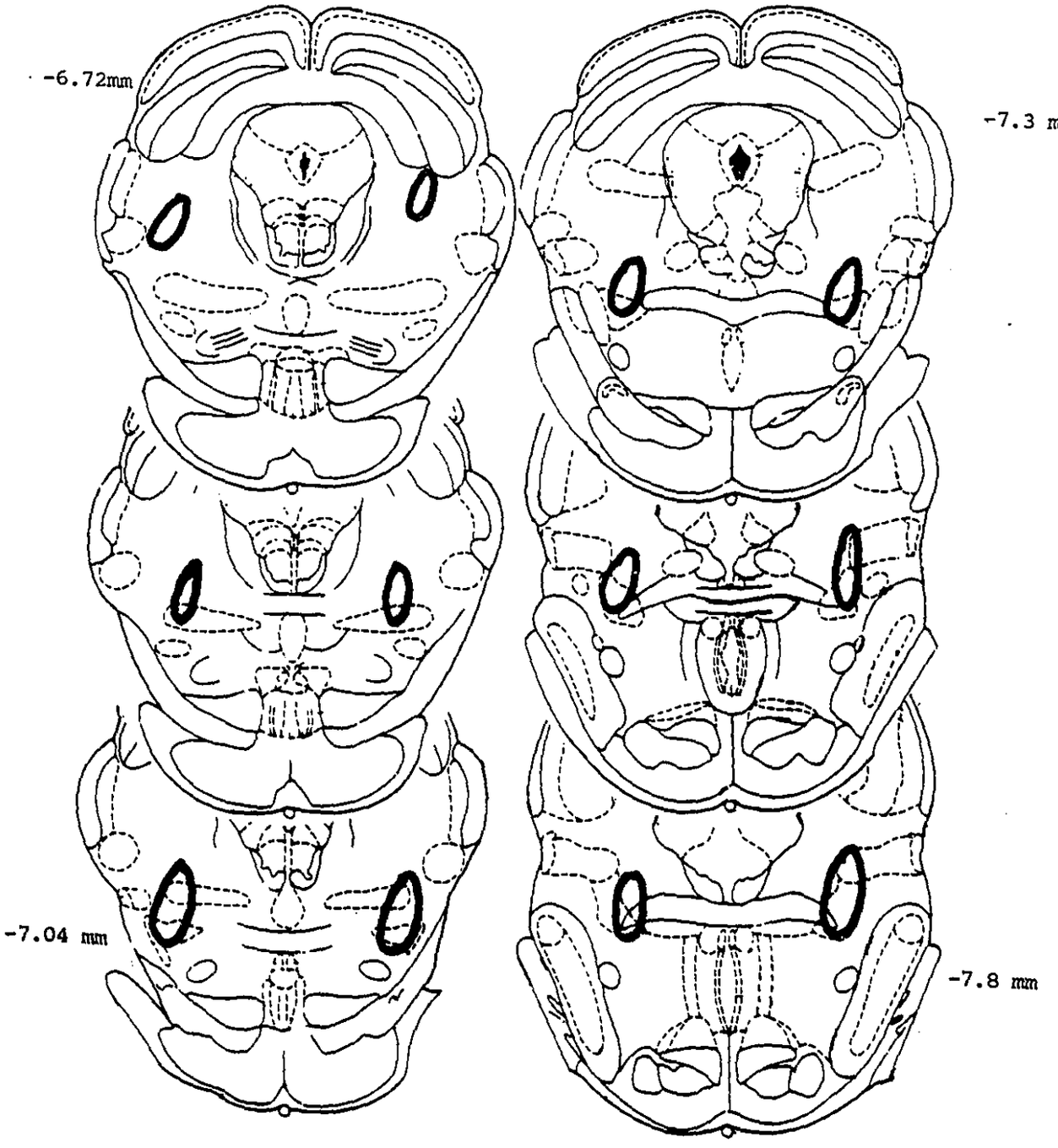


Figure 9. QA lesion of the PPTg in one animal that did not acquire responding for SABS.


-6.72mm
-7.3 mm
-7.04 mm
-7.8 mm

et al., 1973; Wolfle et al., 1971). However, it does not appear likely that damage to the PAG explains the impairment in CPP or SABS. First, in the Bechara and van der Kooy (1989) experiment, excitotoxic lesions of the PAG were also carried out and were found to have no effect on the acquisition of morphine- or amphetamine-induced place preferences. Second, in the present experiment, little or no damage of the PAG was evident, and did not correlate with the acquisition or non-acquisition of SABS responding. Thus, it appears that NMDA lesions of the PPTg block the acquisition of an operant task, supporting the notion of a role for the PPTg in mediating the rewarding effects of brain stimulation.

The results of this experiment do not favor the possibility that PPTg lesions cause a selective loss of classically-conditioned stimulus-affect learning. The results also weigh against the possibility that NMDA lesions simply disrupt long-term memory storage. Since successive lever press responses are only seconds apart, some performance should be possible on short-term memory alone. However, the results do not rule out the possibility that the blockade of place preferences or SABS acquisition reflects a generalized learning deficit and not a reward deficit. Several lines of evidence can be invoked in support of this hypothesis. Bilateral ibotenate lesions of the PPTg have been shown to impair acquisition, but not retention, of passive and active avoidance (Fujimoto et al., 1989; Fujimoto et al., 1990; Fujimoto et al., 1992). Quisqualic acid lesions of the PPTg also impair acquisition of Morris water maze and radial arm maze learning (Dellu et al., 1991).

Results from the histological analysis also showed that, in some animals, damage to the retrorubral field (A8 DA cell group) occurred (see Figures 7 and 9). As reviewed above, DA has been shown to play an important role in reward and reinforcement processes, and an extensive body of evidence supports the role of DA in learning and memory-enhancing processes (Blackburn et al., 1992; White, 1989).

While supporting a role of the PPTg in the acquisition of positively reinforced operant behavior, the results of this experiment raise two questions- (a) does the A8 dopamine cell group play a role in the acquisition of SABS and/or (b) do lesions of the PPTg produce a general learning deficit. These two issues are further explored in Experiments 4 and 5.

Experiment 4

The results of Experiment 3 are consistent with two competing hypotheses that may explain the impairment in SABS acquisition. First, it is possible that NMDA lesions of the PPTg block the incentive properties of brain stimulation reward, thereby producing an impairment in SABS acquisition. Second, it is possible that the incentive properties of brain stimulation are intact, but that NMDA lesions produce a generalized learning deficit which causes an apparent motivational deficit. It is possible to rule out one of these hypotheses by testing the effects NMDA lesions of the PPTg have on the maintenance of SABS responding. In other words, what effects would NMDA lesions have on animals that have already acquired SABS responding. The motivational deficit

hypothesis would predict that, regardless of when the lesion is made, NMDA lesions would block the incentive properties of brain stimulation and hence, attenuate responding for SABS in previously trained animals. On the other hand, if NMDA lesions result in a generalized learning impairment, lesioning the PPTg after SABS acquisition occurred should have no effect on SABS behavior. Experiment 4, therefore, examined the effects NMDA lesions of the PPTg have on animals that had previously acquired responding for SABS stimulation.

Method

Animal Subjects

A total of 24 male, Long-Eevans rats were used. Surgical procedures, handling, and post-operative care were carried out as described in the General Method. Stimulating electrodes were implanted in the LH-MFB region as described in the General Method. In addition, bilateral guide cannulae were implanted in each animal, subsequently through which NMDA or its vehicle control were infused into the PPTg following the acquisition of SABS. Coordinates used for the guide cannulae were: -7.8 mm posterior to bregma, ± 1.5 mm from the midline, and -5.0 mm ventral from the skull surface, according to the atlas of Paxinos & Watson (1986).

Excitotoxic Lesions of the PPTg

Bilateral NMDA lesions of the PPTg were made in 12 animals. Each rat was bilaterally injected over 10 minutes with 0.5 µl of NMDA (0.1 M) via a 10 µl Hamilton syringe mounted on an infusion pump (Harvard Instruments). The

needle was left in place for five minutes following each microinjection. When there were signs that the general anesthetic was wearing off, animals were injected with diazepam (4 mg/ml) to prevent the occurrence of seizures. Six sham-lesioned animals were treated in an identical fashion, but were injected with the phosphate buffered vehicle solution. Animals were allowed five days recovery from surgery before testing began.

Histology

Following completion of behavioral testing, animals were deeply anesthetized with 30% chloral hydrate and were perfused transcardially with physiological saline followed by a 10% formol-saline solution. Brains were removed and further fixed in a 10% formol-saline solution for at least 24 hours prior to sectioning. Brains were sectioned at 25 µm, and every other section (through the PPTg) was mounted and stained using the Cresyl Violet method.

Procedure

Spontaneous Acquisition

All 24 rats were tested to determine whether spontaneous acquisition of SABS responding would occur. The parameters of each stimulating train was held constant for all animals, with the elimination half-time set at 100 seconds, the rise half-time set at one second, and the "dose" (peak frequency) set at 200 Hz. Animals were not preexposed to the training apparatus or to brain stimulation. Animals were placed in the operant chambers and were left for one hour each day for five days. Stimulation current was set at 100 µA for all animals, and no priming trains of brain stimulation were administered to the

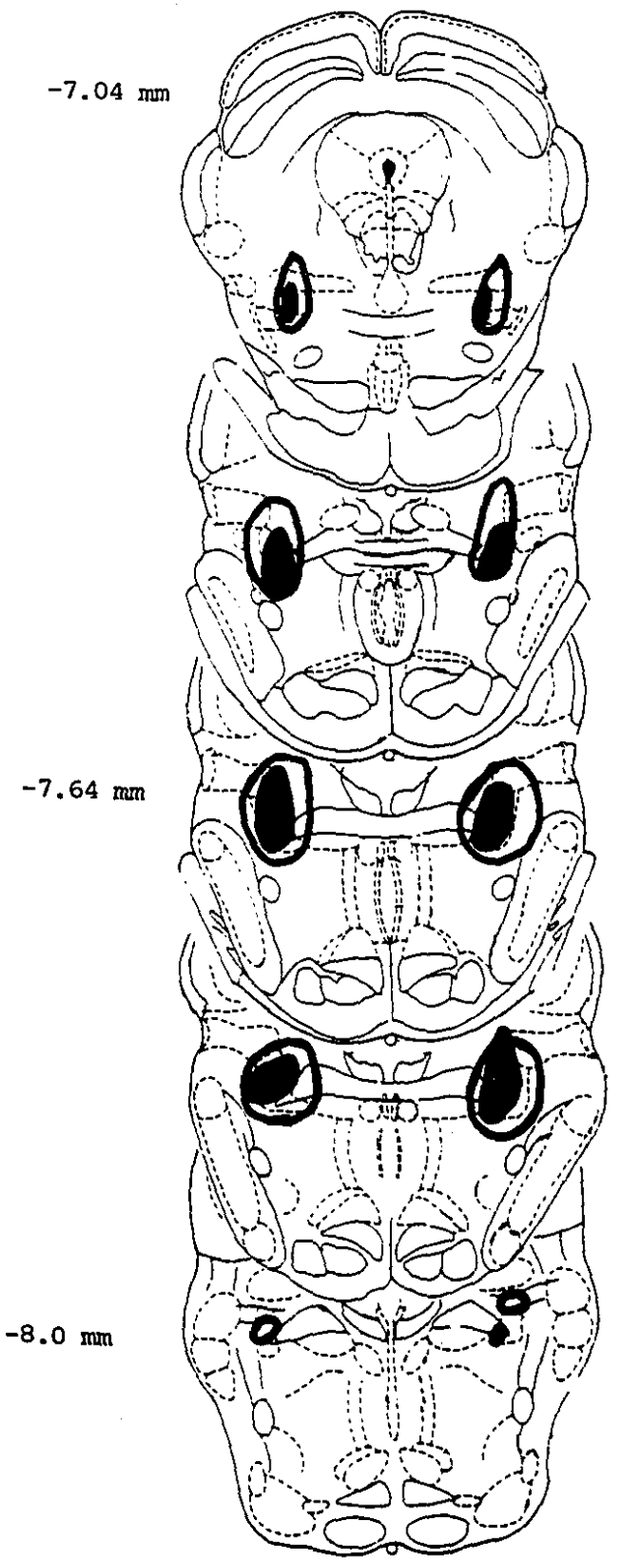
animals.

Once the five acquisition test days were completed, all animals that had acquired SABS responding underwent a second surgery to lesion the PPTg (see Method, page 74). Four out of 24 animals did not acquire SABS responding, and were dropped from the experiment. An additional two animals from the NMDA lesioned group died during the second surgery. The spontaneous acquisition data obtained on these two animals did not undergo any statistical analyses. The remaining 18 animals were allowed an additional five days recovery from surgery. Following recovery, animals were given an additional two days of testing at the same stimulation parameters used for spontaneous acquisition.

Results

Upon inspection of the histology, it was found that only 6 of 12 lesioned-animals had symmetrical, bilateral lesions of the PPTg (see Figure 10). In the remaining 6 rats, lesions were either unilateral or completely missed the PPTg. Given the histological results, the lesioned animals were subdivided into two groups; one group consisted of 6 animals with NMDA lesions of the PPTg and the second group consisted of all the other 6 animals. The third group consisted of 6 sham-lesioned animals. A Group X Acquisition day ANOVA performed on response rate for the **pre**-lesioned data revealed that, prior to lesioning, no reliable differences in response rate existed for the three groups of animals ($F=0.003(2,16\ df)$, $p > 0.05$). It was also found that no statistically significant change in response rate occurred across acquisition days

Figure 10. NMDA lesions of the PPTg that were effective in blocking the maintenance of SABS responding. The open areas represent the largest amount of observable damage, and the darkened areas represent the smallest amount of observable damage.


-7.04 mm
-7.64 mm
-8.0 mm

(F=1.452(4,76 df), $p > 0.05$), and no interaction between lesion group and acquisition days existed (F=0.602(8,76 df), $p > 0.05$). The same pattern of results was obtained when a Group X Acquisition day ANOVA was performed on mean frequency measures. The mean pulse frequency maintained by the three groups of animals prior to lesioning was not significantly different (F=0.044(2,19 df), $p > 0.05$), there was no systematic increase or decrease in mean pulse frequency across acquisition days (F=0.870(4,76 df), $p > 0.05$), and no interactions between lesion group and acquisition days was found (F=0.725(8,76 df), $p > 0.05$). Thus, prior to lesioning, the three groups of animals were not performing differently for SABS stimulation.

Only bilateral lesions of the PPTg produced a severe impairment in responding for SABS stimulation (see Figure 11). A two way Group X Days ANOVA was carried out using response rate and mean frequency data from the last two pre-lesion acquisition test days and the two post-lesion maintenance days. As shown in Figure 11, bilateral NMDA lesions of the PPTg produced a significant drop in both response rate (F=14.53(2,15 df), $p < 0.05$) and mean frequency of stimulation maintained by rats (F=15.469(2,15 df), $p < 0.05$) compared to sham-lesioned and lesioned-controls. When comparing the pre-lesion to post-lesion test sessions, NMDA lesions produced a significant attenuation of responding (F=4.028(3,45 df), $p < 0.05$) and mean frequency maintained by rats (F=5.238(3,45 df), $p < 0.05$). Further, there was a significant interaction between lesion group and time of testing on both response rate (F=2.617(6,45 df), $p < 0.05$) and mean frequency (F=2.852(6,45 df), $p < 0.05$)

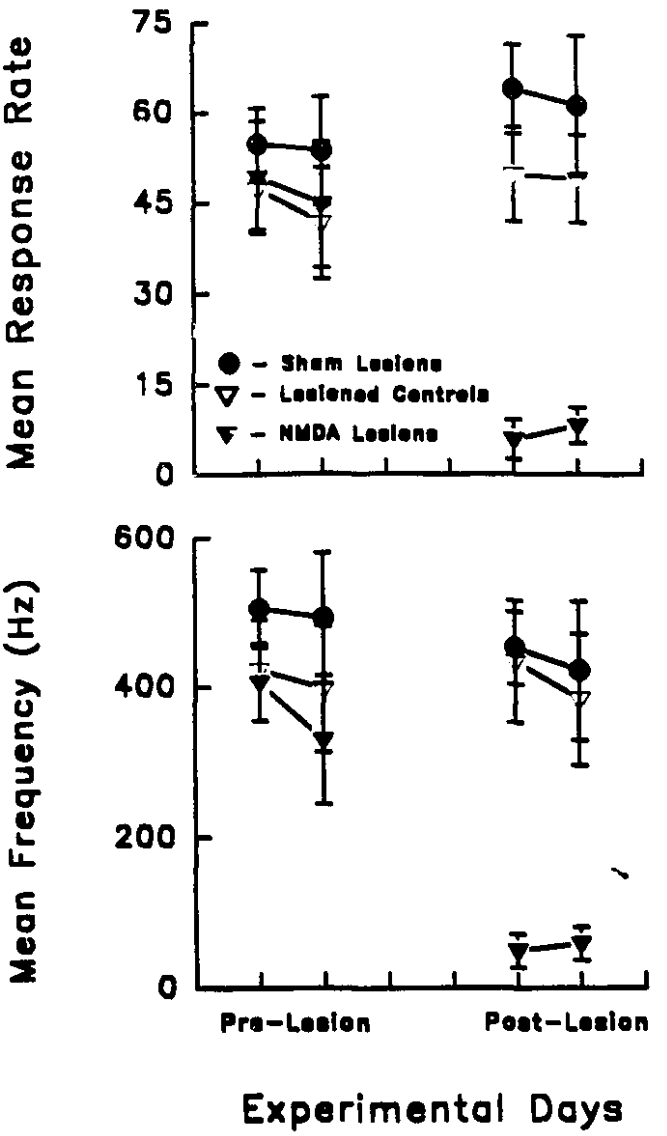


Figure 11. The effects of NMDA lesions of the PPTg in animals who previously acquired SABS responding on mean number of responses (top panel) and mean pulse frequency (bottom panel). The pre-lesion data represents the fourth and fifth days of acquisition training, and the post-lesion data represents the first and second days of SABS maintenance responding. Points represent the ± S.E.M.

measures. Post hoc analysis using the Newman-Keuls procedure showed that bilateral NMDA lesions of the PPTg severely impaired maintenance of responding for SABS from pre-lesion to post-lesion sessions ($q=3.970$, $p < 0.05$) and produced a significant drop in the mean frequency maintained by rats ($q=4.317$, $p < 0.05$), whereas sham-lesions and unilateral-lesions were without effect from pre- to post-lesion sessions (all p's > 0.05). Following the lesion, animals with bilateral lesions responded significantly less than sham-lesioned ($q=6.770$, $p < 0.05$) and unilateral-lesioned ($q=5.126$, $p < 0.05$) animals, while no differences between sham-lesioned and unilateral-lesioned animals was observed ($q=0.196$, $p > 0.05$). Bilateral-lesioned animals also maintained a much lower mean pulse frequency following the lesion when compared to sham-lesioned ($q=6.899$, $p < 0.05$) and unilateral-lesioned animals ($q=5.158$, $p < 0.05$), whereas sham-lesioned and unilateral-lesioned animals maintained relatively similar mean frequencies ($q=0.139$, $p > 0.05$).

Discussion

Only bilateral lesions of the PPTg significantly suppressed responding for brain stimulation in animals who previously acquired SABS. The finding that maintenance of SABS responding was suppressed following the lesion are not consistent with the hypothesis that the impairment seen in SABS acquisition (Experiment 3) resulted from a generalized learning deficit. A generalized learning deficit hypothesis would predict that lesions of the PPTg would have no effect on maintenance of SABS responding in animals who had previously acquired responding for brain stimulation reward. A motivational deficit

hypothesis would predict that NMDA lesions would disrupt responding for SABS regardless of whether they are carried out prior to or following acquisition. Thus, it appears likely that bilateral PPTg lesions specifically attenuate the reinforcing effects of brain stimulation.

It cannot be argued logically that the suppression of SABS maintenance was due to differences that existed in the three groups of animals prior to lesioning. Bilateral NMDA-lesioned animals were responding relatively the same as sham-lesioned and unilateral-lesioned animals, and were maintaining roughly equivalent mean pulse frequencies (see Figure 11). It is also unlikely that the impairment seen in SABS maintenance resulted from a spread of NMDA to regions dorsal and lateral to the PPTg. Animals in the unilateral-lesioned group had lesions at several different locations around the PPTg, and included such structures as the microcellular tegmental nuclei, parabigeminal nucleus, paralemniscal nucleus, and the pontine reticular nucleus, to name a few. Albeit the size of the groups which contained these lesions were comparatively small and had only unilateral damage, the finding that these lesions had no effect on the maintenance of SABS responding does not support the interpretation that spread of NMDA to regions dorsal and lateral to the PPTg produced the impairment in SABS maintenance seen in this experiment.

It also cannot be argued logically that there were differences in the size and spread of bilateral PPTg lesions in this experiment compared to Experiment 3, which may account for the deficits observed in SABS maintenance. As shown in Figure 10, the size and spread of lesions in this experiment was

roughly the same as that seen in Experiment 3 (see Figure 7, page 69). Taken together, these results support the hypothesis that the impairment seen in SABS resulted from a specific motivational deficit produced by bilateral NMDA lesions of the PPTg.

However, as in Experiment 3, inspection of the histological data reveals that NMDA not only lesioned the PPTg, but also lesioned cell nuclei rostral to the PPTg (see Figure 10). And, as in Experiment 3, damage to the A8 dopamine cell group had occurred in almost all (5/6) of the animals. Because of the importance attributed to DA in reward and reinforcement processes (see above), it is necessary to explore the possibility of whether damage to the A8 DA cell group could account for the impairment in SABS acquisition and the suppression of SABS maintenance.

Experiment 5

Introduction

Though the A8 cell group has been mapped anatomically (Beckstead et al., 1979; Deutch et al., 1988; Fallon, 1988; Swanson and Cowan, 1975), little is known about the behavioral functions of this structure. The projections of the A8 cell group are widespread. The A8 cell group projects to diverse areas such as the striatum, nucleus accumbens, the amygdala, and several frontal cortical areas (Beckstead et al., 1979; Deutch et al., 1988; Fallon, 1988; Swanson and Cowan, 1975). With this widespread distribution, it is possible that disruption of DA transmission from this cell group could have significant

effects on a variety of behaviors. Experiment 5 explored the possibility that damage to the A8 cell group was responsible for blocking the acquisition of SABS responding in animals lesioned with NMDA in the PPTg.

Method

Animal Subjects

Twenty-one male Long-Evans rats were used. Surgical procedures, handling, and post-operative care were carried out as described in the General Method. Stimulating electrodes were aimed and implanted in the LH-MFB region as described in the General Method. Lesion coordinates for the A8 cell group were -6.8 mm posterior from Bregma, ± 1.8 mm from the midline, and -7.2 mm ventral from the skull surface, according to the atlas of Paxinos and Watson (1986).

Excitotoxic Lesions of the A8 DA Cell Group

Bilateral NMDA lesions of the A8 DA cell group were made in 14 animals, and bilateral vehicle sham lesions of the PPTg were made in 7 animals. In the lesioned animals, seven rats were injected with 50 nmol NMDA, and a second group of seven animals were injected with 25 nmol NMDA. The lower dose was added to this experiment because although lesions produced by 50 nmol NMDA aimed at the PPTg did produce some damage to the A8, it was not reasonable to assume that this damage resulted from the entire 50 nmol dose. Rats were injected bilaterally over 10 minutes with 0.5 µl or 0.25 µl of NMDA (0.1 M) or vehicle solution (phosphate buffered saline, pH = 7.4), via a 10 µl Hamilton syringe mounted on an infusion pump (Harvard Instruments).

The needle was left in place for five minutes following each microinjection. A single, bipolar stainless steel electrode (Plastics One, VA) aimed at the LH-MFB was then implanted. Animals were allowed five days recovery from surgery before testing began.

Procedure

Acquisition of SABS responding was carried out as described in Experiment 3 (see Method, page 62). Animals naive to shaping, pretraining procedures and brain stimulation were placed in the operant chambers for one hour a day for five days. Stimulation parameters were set at a dose of 200 Hz, a rise half-time of one second, and an elimination half-time of 100 seconds. Stimulation current was set at 100 μA for all animals.

Histology

Following behavioral testing, brains were removed and sectioned as described in the previous experiment. In A8 preparations, every other section was mounted and stained using the Cresyl Violet method.

Results

NMDA lesions of the A8 DA cell group did not significantly prevent the acquisition of SABS responding at either the 25 or 50 nmol dose. Five of seven animals lesioned with 25 nmol NMDA acquired SABS responding, and five out of seven animals lesioned with 50 nmol NMDA acquired responding for SABS. A detailed analysis of the histology will be presented later in the discussion. The 50 nmol dose of NMDA produced a larger lesion of the A8 cell group than did the 25 nmol dose (see Figures 13 to 16). In 4/14 animals that

did not acquires SABS, a significant amount of bilateral damage to the PPTg was evident, and unilateral damage to the substantia nigra also occurred (see Figures 13 and 14). A plot of the means and variances of response rate and mean frequency for each group revealed that a correlation existed between the means and variances. A square root transformation was performed on both response rate and mean frequency measures, and the transformed data was used for all analyses.

A two way Group X Acquisition day ANOVA performed on the transformed response rate data revealed no differences between A8 lesioned-animals and sham-lesioned animals on response rate ($F=1.305(2,18\ df)$, $p > 0.05$). However, there was a significant increase in response rate across acquisition days ($F=6.494(4,72\ df)$, $p < 0.05$). Further, a significant lesion group by acquisition day interaction ($F=2.160(8,72\ df)$, $p < 0.05$) was revealed (see Figure 12).

The same pattern of results was found when a two way Group X Acquisition day ANOVA was performed on the transformed mean frequency data. A8 lesions had no effect on the mean frequency maintained by animals during acquisition ($F=1.135(2,18\ df)$, $p > 0.05$). As with response rate, though, mean frequency rose significantly across acquisition days ($F=8.832(4,72\ df)$, $p < 0.05$), and there was a significant interaction between lesion group and acquisition day ($F=2.318(8,72\ df)$, $p < 0.05$).

In analyzing the simple main effects, three one-way repeated measures ANOVAs were performed for each group of animals on the transformed

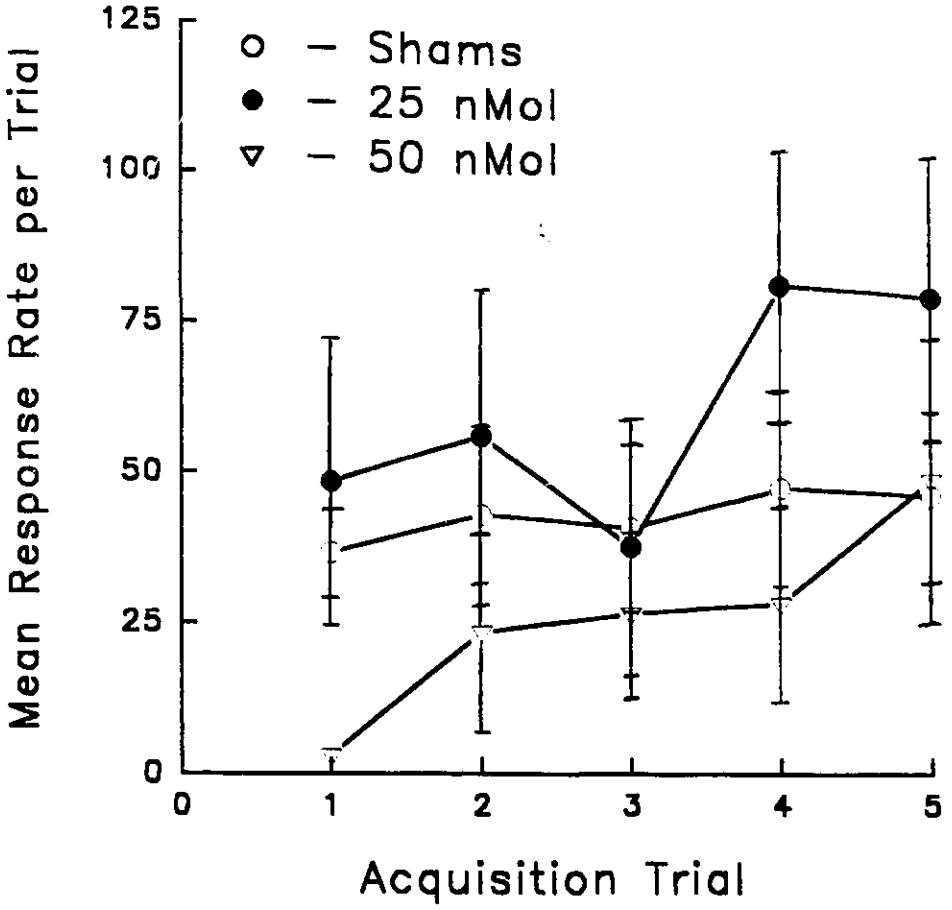


Figure 12. The effects of sham, 25 nmol, or 50 nmol NMDA lesions of the A8 dopamine cell group on the acquisition of SABS. The parameters of each stimulating train were set at a dose of 200 Hz with an absorption half-time of 1 s and an elimination half-time of 100 s. The top panel shows the effects of NMDA lesions of the A8 cell group on mean number of responses across five acquisition test days. The bottom panel shows the effects of A8 lesions on mean pulse frequency maintained by animals across five acquisition test days. Bars represent the means ± S.E.M.

response rate and mean frequency measures. In animals with 50 nmol lesions of the A8 dopamine cell group, a significant increase in both response rate ($F=4.543(4,24\ df)$, $p < 0.05$) and mean frequency ($F=7.956(4,24\ df)$, $p < 0.05$) was found across the number of acquisition days, with significant increases in response rate ($q=5.974$, $p < 0.05$) and mean frequency ($q=7.840$, $p< 0.05$) occurring from acquisition Day 1 to acquisition Day 5. Similarly, in animals with 25 nmol lesions of the A8, response rate ($F=3.834(4,24\ df)$, $p < 0.05$) and mean frequency ($F=2.839(4,24\ df)$, $p < 0.05$) rose significantly across acquisition days, with the increase in response rate occurring from acquisition Day 3 to acquisition Day 5 ($q=4.299$, $p < 0.05$). In contrast, sham-lesioned animals maintained a consistent level of responding ($F=0.162(4,24\ df)$, $p > 0.05$) and mean frequency ($F=0.186(4,24\ df)$, $p > 0.05$) across all five acquisition days (see Figure 12). Thus, though A8 lesions of the dopamine cell group did not significantly impair performance of SABS compared to controls, A8 lesions slightly impaired the rate at which SABS acquisition occurred.

Two out of seven animals in each of the 25 and 50 nmol A8 lesion groups did not acquire SABS responding. Results from the histology showed that, in these 4 animals, a significant amount of **bilateral** damage occurred in the PPTg (see Figures 13 and 14). In the remaining 5/7 rats from both the 25 nmol and 50 nmol A8 lesion groups, NMDA either did not produce bilateral damage in the PPTg or the bilateral damage was confined to mostly rostral portions of the PPTg (see Figures 15 and 16).

Figure 13. 25 nmol NMDA lesions of the A8 dopamine cell group that were effective in blocking the aquisition of SABS. Open areas represent the largest observable lesions, and the darkened areas represent the smallest observable lesions.

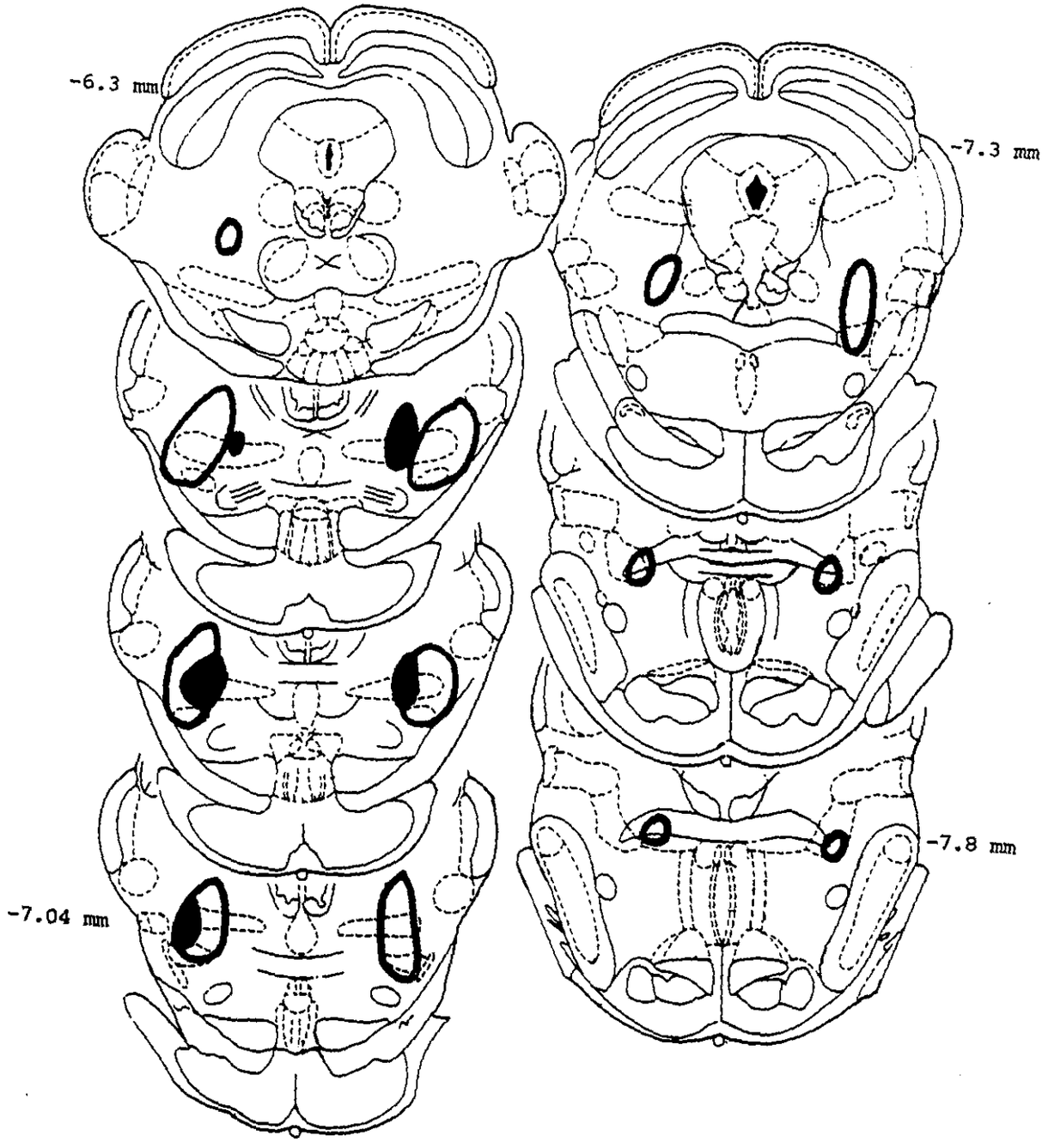

-6.3 mm
-7.3 mm
-7.8 mm
-7.04 mm

Figure 14. 50 nmol NMDA lesions of the A8 dopamine cell group that were effective in blocking the acquisition of SABS. The open areas represent the largest observable lesions, and the darkened areas represent the smallest observable lesions.

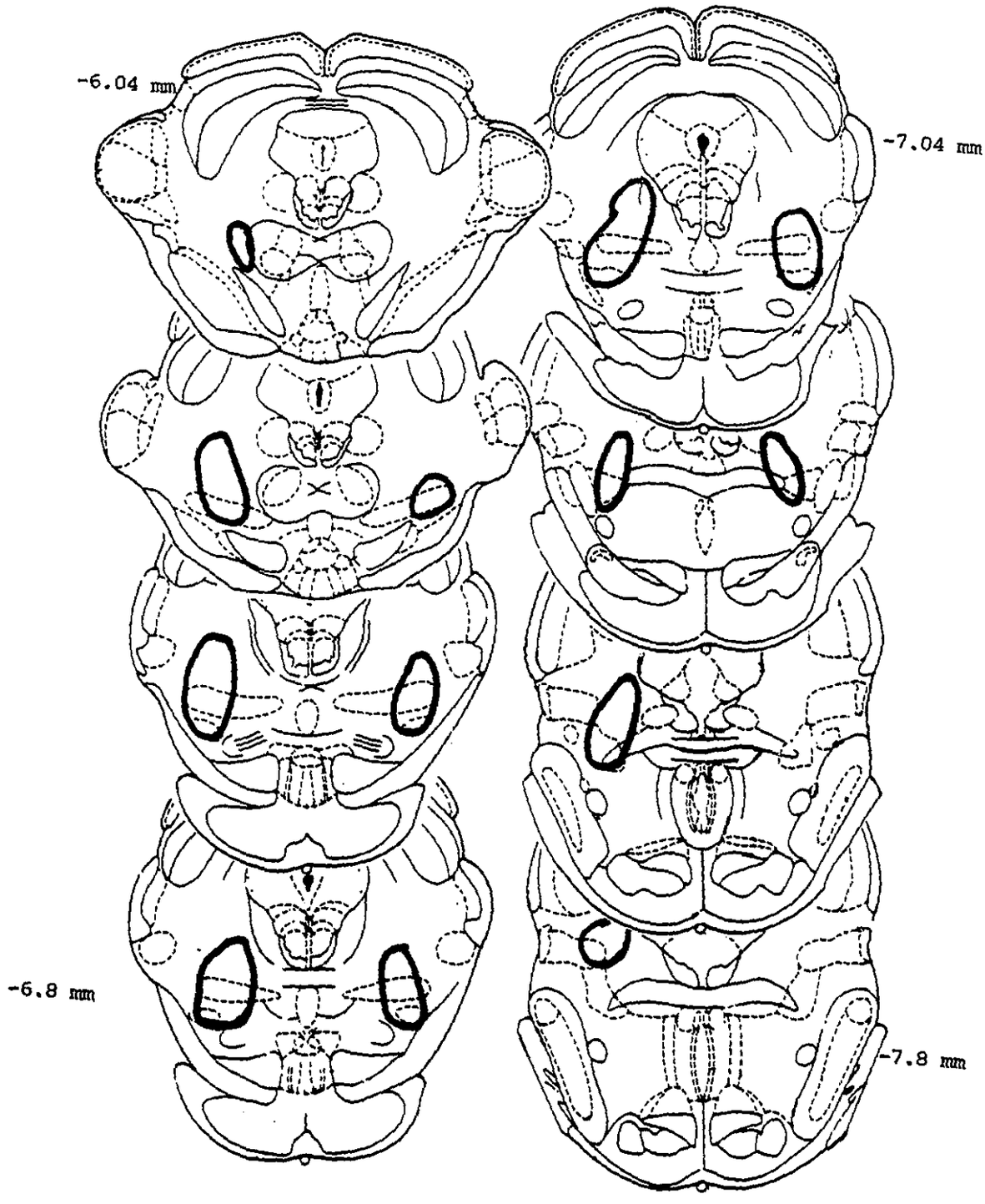

-6.04 mm
-7.04 mm
-6.8 mm
-7.8 mm

Figure 15. 25 nmol NMDA lesions of the A8 dopamine cell group that were ineffective in blocking the acquisition of SABS. The open areas represent the largest observable lesions and the darkened areas represent the smallest observable lesions.

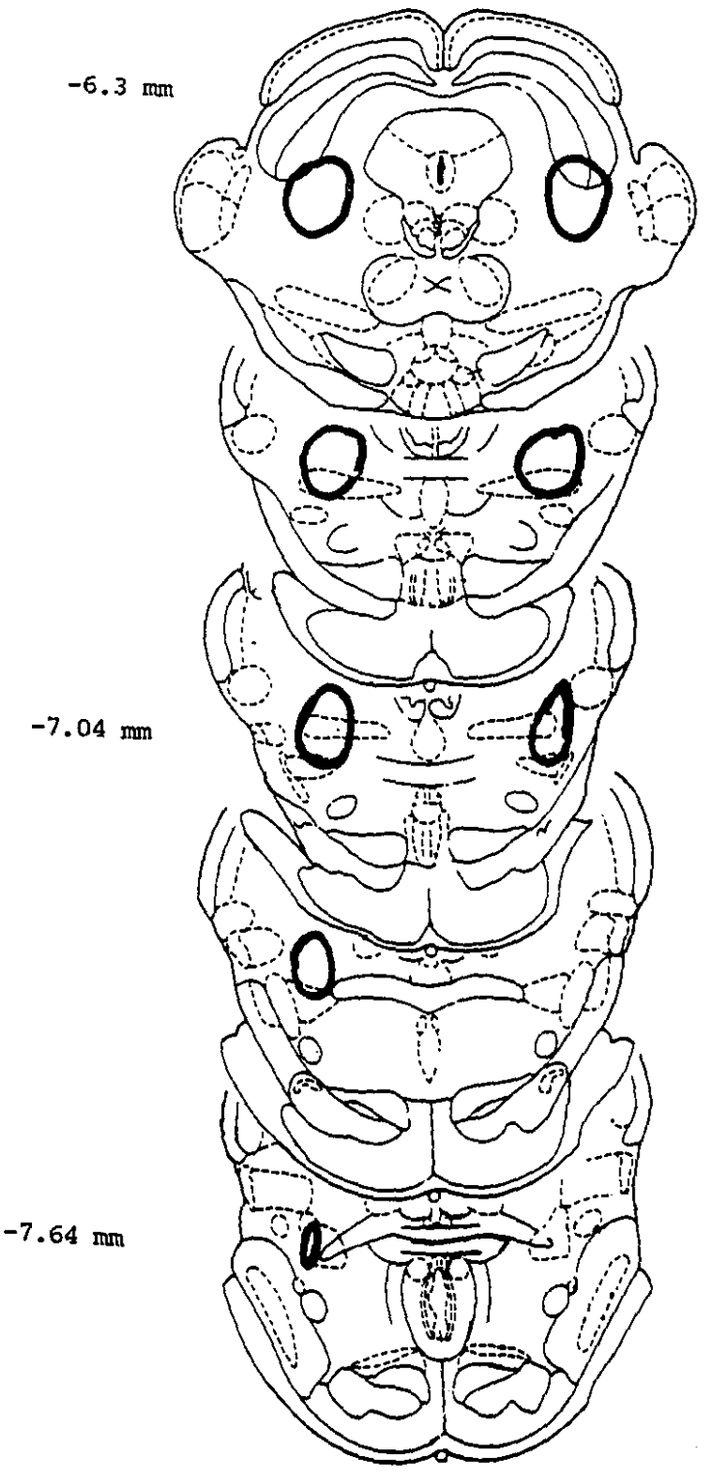
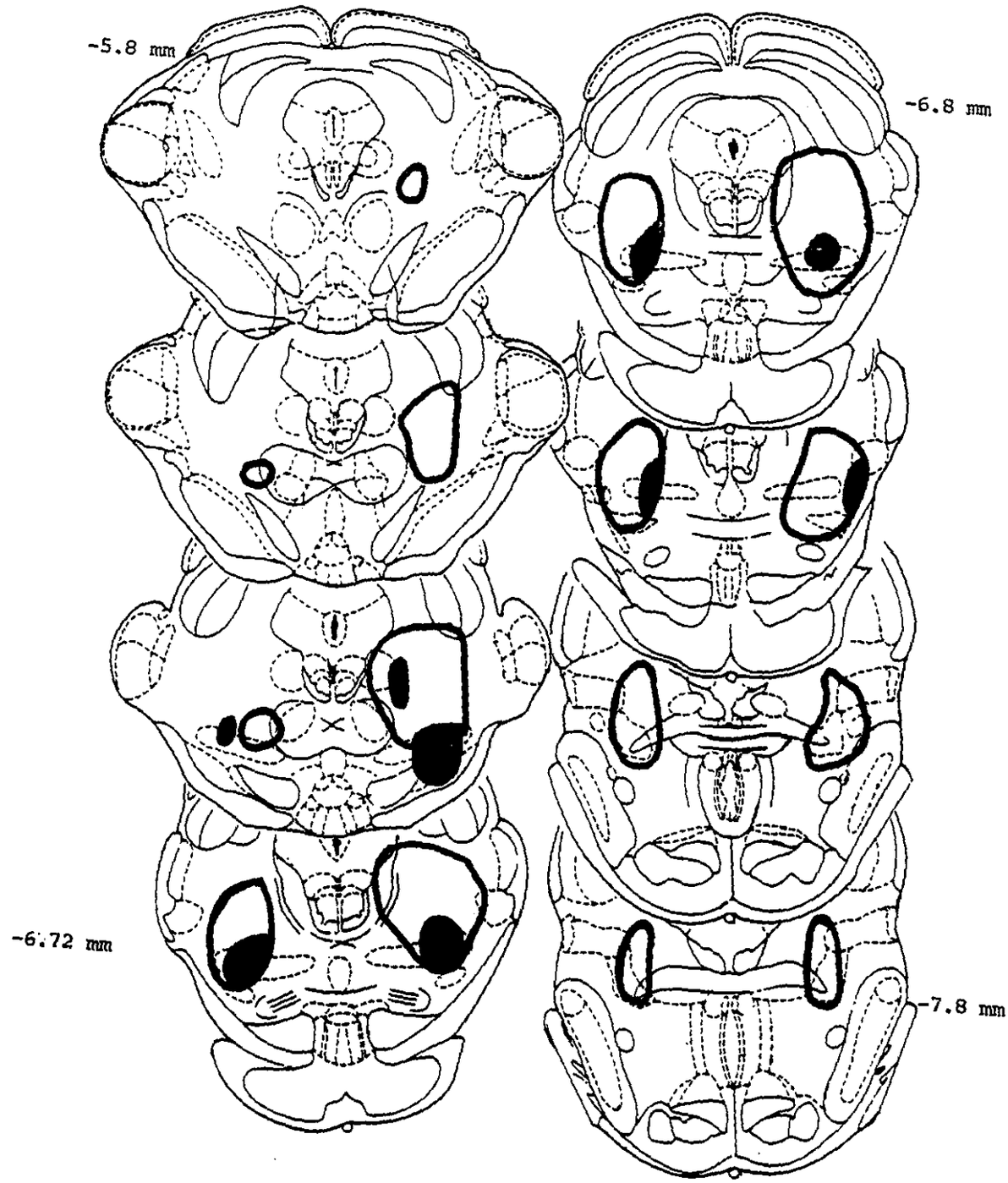

-6.3 mm
-7.04 mm
-7.64 mm

Figure 16. 50 nmol NMDA lesions of the A8 dopamine cell group that were ineffective in blocking the acquisition of SABS. Open areas represent the largest observable damage, and darkened areas represent the smallest observable damage.


-5.8 mm
-6.8 mm
-6.72 mm
-7.8 mm

Discussion

Lesions of the A8 DA cell group did not block acquisition of SABS responding unless excitotoxin-induced damage involved the PPTg bilaterally. This shows that damage confined to the A8 cell group cannot explain the block of SABS acquisition, nor the suppression of responding on SABS maintenance.

Although A8 lesions did not prevent the acquisition of SABS, they did slightly impair the rate at which SABS acquisition occurred. In this experiment, lesions of the A8 dopamine cell group were made at a caudal site within the A8. A caudal site for injecting NMDA into the A8 cell group was chosen because histological results from Experiments 3 and 4 revealed that NMDA injected into the PPTg was spreading as far rostrally as the caudal tip of the A8 (see Figures 7 and 10, pages 69 and 77, respectively). In this experiment, 25 nmol NMDA lesions damaged only approximately 25% of the A8 cell group (see Figure 15), and 50 nmol NMDA lesions damaged approximately 60-70% of the A8 cell group (see Figure 16). Moreover, damage to the A8 cell group was predominantly confined to the lateral part of the A8 cell group, sparing most of the medial portion (see Figure 15 and 16). It remains possible that a more complete lesion of the A8 cell group may have had a more severe effect on the rate of SABS acquisition or on the acquisition of SABS. Nevertheless, lesions aimed at the caudal portion of the A8 cell group do not block the acquisition of SABS. Lesions of the A8 cell group do, however, reduce the rate at which acquisition of SABS occurs.

These results support the hypothesis that it is bilateral NMDA lesions of

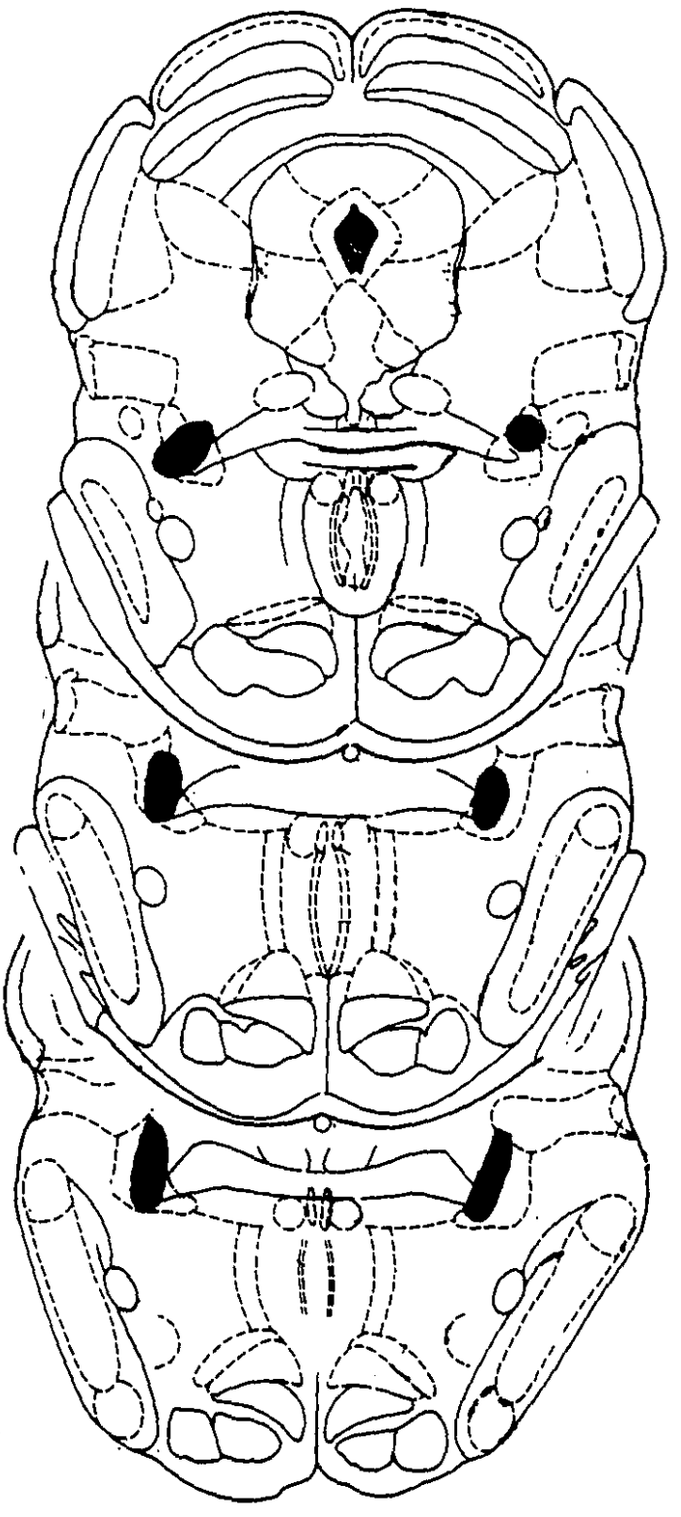
the PPTg that attenuate the positively reinforcing effects of brain stimulation reward. However, although it has been determined that spread of NMDA to the A8 cell group does not account for the impairment seen in the acquisition or maintenance of SABS, there still remains a question as to the precise anatomical location within the PPTg that mediates the reinforcing effects of brain stimulation.

As mentioned above, acquisition of SABS was impaired in 4/14 animals lesioned with NMDA in the A8 cell group. Inspection of the histology revealed that, in these 4 animals, a significant amount of bilateral damage occurred in the PPTg (see Figures 13 and 14). In an attempt to identify a common site within the PPTg that mediates the positive reinforcing effects of brain stimulation, the histological results from Experiments 3, 4, and 5 were re-analyzed. The first stage of this re-analysis consisted of plotting the common site lesioned in the PPTg in animals from Experiment 4. This was chosen as a first stage because in these animals, the lesion occurred after SABS acquisition. Thus, the possibility that the site of electrode placement was off or that electrode failure occurred is highly unlikely. As shown in Figure 17, the lesioned site that appeared to be common was located in the caudal part of the PPTg (≈ -7.64 to -8.0 mm posterior to Bregma).

The second stage of the re-analysis consisted of determining whether PPTg-lesioned, 25 nmol A8 lesioned or 50 nmol A8 lesioned animals that failed to acquire SABS had damage in the caudal PPTg. As shown in Figures 7, 13 and 14, 12 animals in these three lesion groups that failed to acquire SABS had

Figure 17. A putative common site of NMDA lesions effective in blocking the acquisition of SABS responding. This site was determined by locating an area that was common to all NMDA-lesioned animals that were suppressed in SABS maintenance (Experiment 4).

-7.64 mm



-7.8 mm

-8.0 mm

damage in this area. According to the first two stages of re-analysis, then, it appears that lesioning the caudal portion of the PPTg attenuates the positive reinforcing effects of brain stimulation. Although A8 lesioned animals with PPTg damage did not acquire SABS, it cannot be ruled out that the electrodes were simply non-functional or were misplaced. However, given that no excitotoxic-induced damage outside the PPTg (excepting A8 damage) was common to all lesioned groups, the data strongly suggest that spread of NMDA lateral, dorsal, or ventral to the PPTg was not responsible for the block of SABS acquisition, nor the attenuation in SABS maintenance.

The third and final stage of the re-analysis consisted of determining whether any of the lesioned animals with damage to this "putative" common site were able to acquire SABS responding. As shown in Figure 8 (page 70), 7/8 animals with QA-lesions of the PPTg were able to acquire SABS, and most of these animals had damage to the putative common site. However, as discussed above, QA is a relatively more selective neurotoxin for cholinergic cells than non-cholinergic cells (Rugg et al., 1992). Hence, SABS acquisition may occur simply because the brainstem cholinergic system does not mediate the positive reinforcing effects of brain stimulation. In animals with 25 nmol and 50 nmol A8 lesions that acquired SABS, animals with the largest lesions had some damage in the putative common site (see Figures 15 and 16). This finding does not necessarily pose a problem to the argument that a common site within the PPTg mediates the positive reinforcing effects of brain stimulation, since in these animals, the rate of SABS acquisition was

significantly slower than in sham-lesioned animals.

Finally, in almost all instances where impairments in SABS responding were evident, damage to both the A8 dopamine cell group and the PPTg occurred. And, although it has been determined that A8 lesions alone do not block the acquisition of SABS, it has not been determined that lesions confined to the PPTg do block SABS responding. In order to determine whether lesions confined to the PPTg block SABS acquisition, it is necessary to localize cell damage within the PPTg. One lesioning procedure capable of producing relatively confined and controllable damage is the electrolytic lesioning procedure. Experiment 6, therefore, explored whether electrolytic lesions confined to the PPTg proper, and especially, to the "putative" common site of damage, would produce impairments in SABS acquisition.

Experiment 6

Introduction

The results of the histological analysis in Experiments 3 to 5 raise questions as to the precise location of PPTg sites that presumably mediates reward and reinforcement. In almost no instances where SABS responding was impaired was the lesion confined to the PPTg. One common factor in the above three experiments is that a chemical lesioning technique was used. Although the injection volume and rate of injection was low, these procedures merely serve to minimize, and not eliminate, spread to neighbouring regions. The PPTg is bordered by a mass of fibre pathways, including the decussation

of the superior cerebellar peduncle, the fibres of the lateral lemniscus and of the brachium conjunctivum (Rugg et al., 1992; Sugimoto and Hattori, 1984). Though the chemical lesioning technique spares axons of passage, it is possible that the axons themselves act as a mechanical carrier for a chemical lesioning substance, or as a barrier to diffusion. Hence, the spread of NMDA to regions not necessarily bordering the PPTg may be considerably more or less than one would expect. Although damage to other areas may not be extensive and may be difficult to detect, it remains possible that undetected damage to a large number of nuclei or to an adjacent cell group contributes to the impairment in SABS acquisition.

In nuclei rich in cell bodies, such as the A8 cell group, there is little resistance to stop or confine the spread of NMDA. Inspection of the histological material from Experiment 5 reveals a considerable amount of spread of NMDA when injected into the A8 cell group. In many cases, NMDA spread as far rostrally as the VTA, and as far caudally as the cuneiform nucleus (see Figure 14, page 89). Given the widespread and relatively uncontrollable damage that may occur with NMDA under these circumstances, it was decided to use the electrolytic lesioning technique to lesion the PPTg in the hopes that the amount and site(s) of damage could be controlled and more precisely identified. Thus, in Experiment 6, the acquisition of SABS was examined in rats with bilateral electrolytic lesions of the PPTg using two different lesioning procedures, one procedure to produce widespread damage and a second procedure to produce more confined damage.

Method

Animal Subjects

A total of 33 male, Long-Evans rats were used for the following experiments. Surgical procedure, handling and post-operative care were carried out as described in the General Method. The lesioning electrode was aimed at the PPTg, and the coordinates were -7.8 mm posterior to Bregma, ± 1.8 mm lateral to the midline, and -7.3 mm ventral from the skull surface, according to the atlas of Paxinos and Watson (1986).

Procedure

Electrolytic Lesions of the PPTg

Seventeen animals were lesioned electrolytically, and one group of sixteen animals were sham-lesioned. Sham-lesioned animals were divided into two groups; one group of ten rats was assigned to SABS acquisition, and the remaining six rats were assigned to serve as sham-lesioned controls. Lesioned animals were assigned to one of three groups; six animals with large lesions and five animals with small lesions were assigned to SABS acquisition, and six animals with small lesions served as lesioned controls. For rats with large electrolytic lesions, the current was 0.5 mA for 20 seconds with the electrode exposed 0.03 mm at the tip. To make small electrolytic lesions, the current was 0.2 mA for 10 seconds, with only the tip of the electrode exposed. Following the bilateral lesions, a single stainless steel bipolar stimulating electrode

(Plastics One, VA) was implanted in each rat. Animals were allowed a minimum of five days recovery before testing began.

Acquisition of SABS

The procedure for SABS acquisition was identical to Experiments 3 to 5, except for rats in the control groups.

The PPTg has been demonstrated to be involved in locomotion, and is considered a part of the mesencephalic locomotor region (Coles et al., 1989; Garcia-Rill et al., 1987; Garcia-Rill et al., 1990; Vaccarino et al., 1986). To determine that disruption of normal locomotor activity does not interfere with the ability of animals to respond, sham-lesioned and lesioned-control animals were placed in the operant chamber and randomly assigned to one of two control conditions: a non-stimulated control condition and a stimulated control condition. In the non-stimulated control condition, the dose of brain stimulation was set at 0 Hz in order to estimate the unconditioned operant rate. In the stimulated control condition, sham-lesioned and lesioned-controls were primed with four trains of stimulation, one train per 15 minute period. The priming trains of stimulation were administered by the computer at 30 seconds, 15 minutes, 30 minutes and 45 minutes during the test session. The trains of stimulation were not contingent on the performance of a response, and responses emitted by the animal were counted, but not reinforced with brain stimulation. Animals were tested for one hour a day for five days in each condition, for a total of ten days. Half the animals were tested in the no-stimulation condition and were then switched to the stimulated condition, and the remaining half started in the

stimulation condition and switched to the no-stimulation condition. Following completion of both control conditions, three of the lesioned control animals were randomly selected and tested for SABS acquisition.

Results

Sham-lesioned and PPTg-lesioned control animals did not increase their operant rate over the ten control test days. No differences in operant rate in either group were found between the stimulated and non-stimulated condition.

A two way Group by Acquisition days ANOVA performed on response rate and mean frequency measures revealed that all ten animals with large or small electrolytic lesions readily acquired SABS and there were no reliable differences in response rate ($F=0.044(1,20\ df)$, $p > 0.05$) or mean frequency ($F=0.627(1,20\ df)$, $p > 0.05$) compared to sham-lesioned animals (see Figure 18). As with previous acquisition experiments (Experiment 3, Lepore & Franklin, 1992), no significant differences in response rate or mean frequency was found across training days (all p's > 0.05). Following the completion of the control conditions, the three lesioned control animals placed on SABS readily acquired SABS responding. No observable differences in response rate or mean frequency were found when comparing these lesioned control animals with all other groups (see Figure 18).

Discussion

Unlike NMDA lesions, electrolytic lesions of the PPTg do not block the acquisition of SABS responding. Gross histological comparisons of large electrolytic lesions with large NMDA lesions show considerable differences.

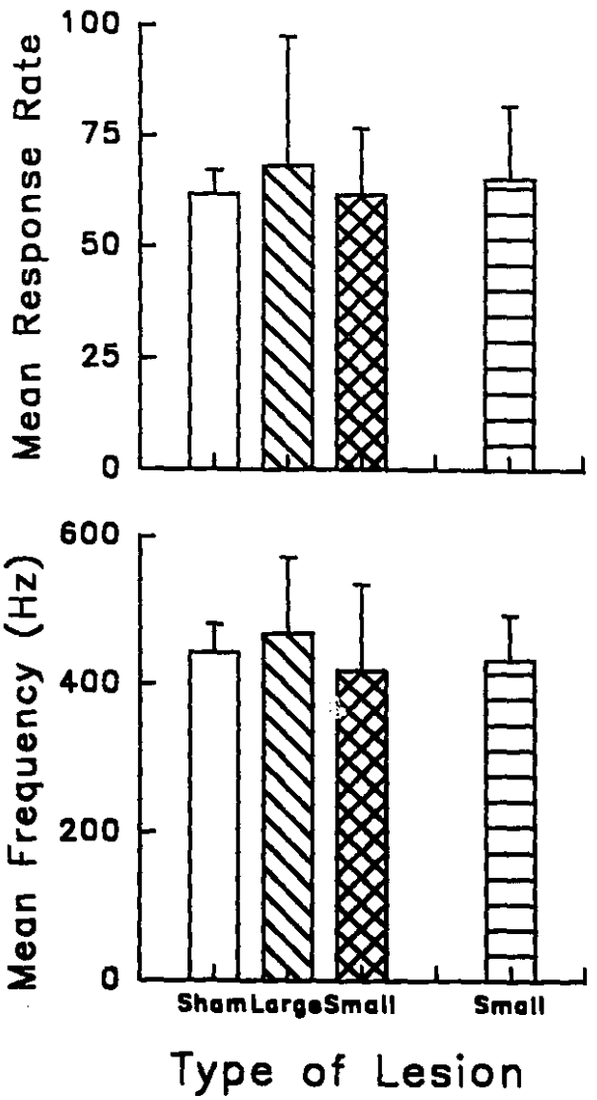
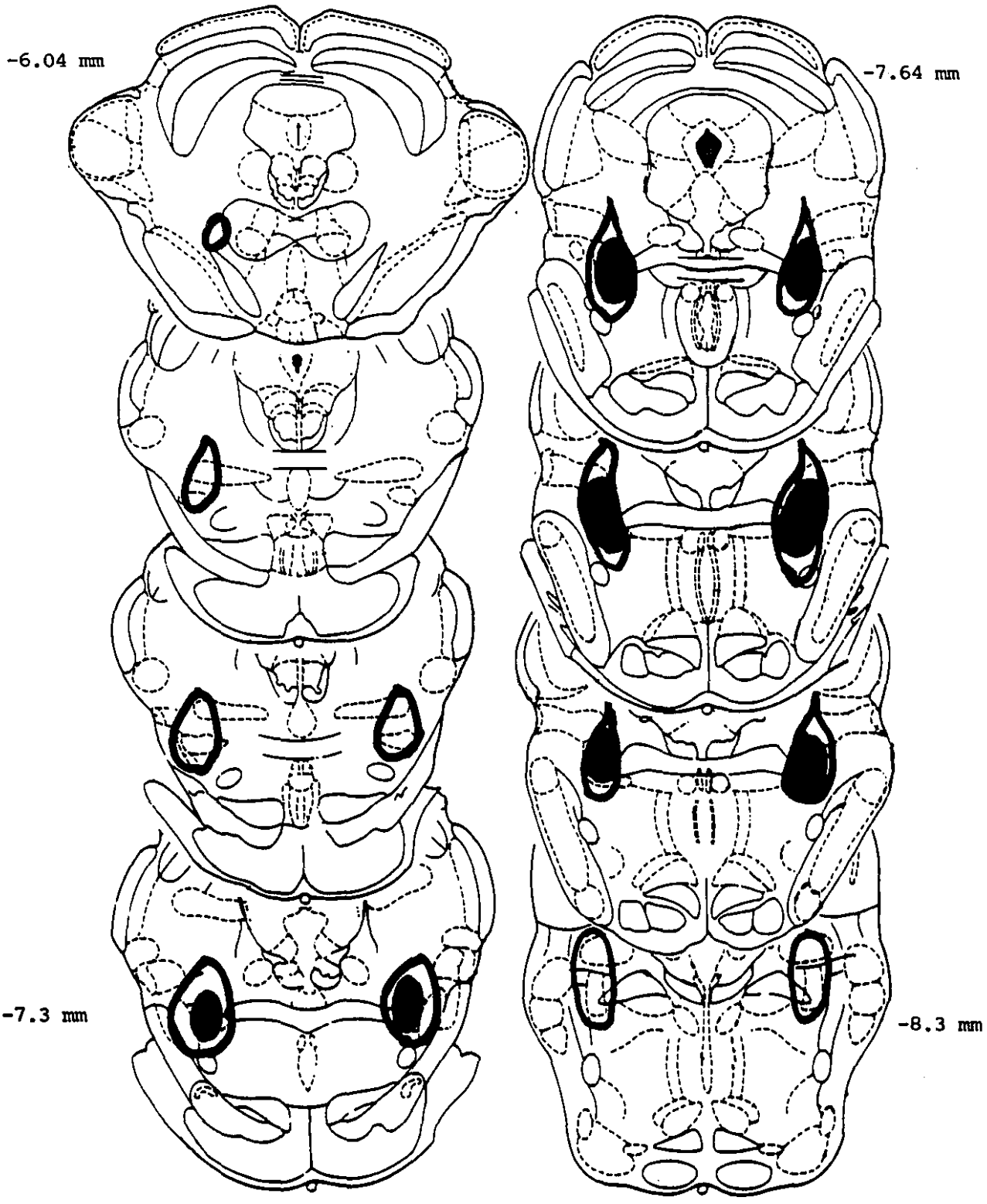


Figure 18. The effects of electrolytic lesions of the PPTg on the mean number of responses made across five acquisition sessions (top panel) and on the mean frequency maintained by animals (bottom panel). Included as a separate bar (far right, both panels) are the mean responses and mean frequency of three lesioned animals that served as controls who were later tested for SABS acquisition. Bars represent the mean ± S.E.M.

Figure 19. Electrolytic lesions of the PPTg that were ineffective in blocking the acquisition of SABS. The open areas represent the largest observable lesions, and the darkened areas represent the smallest observable lesions.


-6.04 mm
-7.64 mm
-7.3 mm
-8.3 mm

Large electrolytic lesions produce a larger amount of damage to the PPTg and to neighbouring areas than NMDA lesions. Small electrolytic lesions, however, were more confined to the PPTg proper (see Figure 19). Small electrolytic lesions did not damage the cuneiform nucleus, PAG, LDTg, or the A8 DA cell group to the same extent as NMDA or large electrolytic lesions.

These results are puzzling in view of the previous evidence obtained from Experiments 3 and 4, showing that excitotoxic lesions of the PPTg have a devastating effect on the acquisition and maintenance of SABS responding. Since the center of the electrolytic and excitotoxic lesions appeared to correspond, these results raise the question of whether the effects of NMDA lesions on acquisition of SABS are in fact due specifically to PPTg damage.

That the acquisition of SABS may be due to some abnormal locomotor effects caused by the lesions is not likely since control lesioned animals were no different from sham-lesioned controls in response rate. One slight difference to be noted, however, is that regardless of the number of days the lesioned control animals experienced either control condition, lesioned-control animals emitted at least one response in all ten control test days. In sham-lesioned animals, mean response rate per session dropped to under one by the third control day, demonstrating that at least some of the animals were not emitting any response at all. That all PPTg lesioned animals responded at least once every session for up to 10 days suggests that the lesioned animals may not be learning about the stimulus conditions surrounding the acquisition test. In other words, the observation that most of the control animals cease to respond by the

fourth control day suggests control animals have learned that nothing of any consequence will result from a lever press. Hence, they no longer approach the lever. Animals with PPTg lesions, however, do not appear to learn this since they continue to approach the lever and continue to lever press even after 10 days of unreinforced training. This suggests that animals with electrolytic lesions of the PPTg may indeed have deficits, in spite of the finding that they are capable of learning a simple operant task.

If electrolytic lesions do produce deficits in reinforcement, the deficit is not apparent using the SABS acquisition task. Although the SABS acquisition task has certain advantages, such as rapid acquisition and elimination of experimenter bias during training and shaping, the acquisition procedure itself may not be sensitive enough to pick up subtle deficits in reinforcement. For example, animals are able to acquire SABS when the absorption half-time of stimulation is extended as far as 32 seconds (Lepore and Franklin, 1992). This demonstrates that SABS can be acquired with delays of reinforcement up to 15 seconds or more. This does not, of course, demonstrate that delaying reinforcement does not effect responding for SABS. In fact, when animals are tested on a progressive ratio schedule of reinforcement, delays of reinforcement up to 16 seconds produce significantly lower break points than delays of 2 or 8 seconds (Lepore and Franklin, 1991). In order to pick up subtle deficits in reinforcement, it is necessary to use more sensitive tests of motivation. Three tasks demonstrated to be sensitive to reinforcement deficits are the extinction test, incentive contrast, and progressive ratio responding (discussed below).

Experiment 7, therefore, explored whether electrolytic-lesioned animals from experiment 6 were impaired on these three behavioral tasks.

Experiment 7

Introduction

Prior to acquiring operant responding for brain stimulation, the stimulus (e.g. lever) and the response (e.g. lever press) bear no relation to one another. During the acquisition of operant responding, however, presenting trains of rewarding brain stimulation immediately after a response has occurred (e.g. lever press) changes the relationship between the stimulus and the response. Brain stimulation reinforces the association between the stimulus and the response, and when animals have acquired operant responding, an instance of S-R learning is said to have occurred (Catania, 1968; Skinner, 1968a). Upon removal of the rewarding brain stimulation (or other reinforcer), the stimulus can still elicit a response, which is commonly referred to as extinction responding (Skinner, 1938; Skinner, 1968b). Analysis of extinction behavior can be used to demonstrate how contingencies of reinforcement affect behavior, and, under appropriately controlled circumstances, can demonstrate the reinforcing (or relative reinforcing) strength of stimuli (Skinner, 1938; Skinner, 1968b). For example, when animals trained to respond for brain stimulation are placed on extinction, responding initially increases, and is followed by a gradual cessation of responding. The pattern of responding is similar to that observed in animals undergoing extinction of food reward (Franklin, 1978; Fouriezos and Wise,

1976). Animals responding for amphetamine reward will show a "resistance" to extinction (i.e., emit more responses) when the magnitude of amphetamine reward received per operant is increased, suggesting that more amphetamine per operant has a higher reinforcing value (Yokel and Pickens, 1976). Analysis of extinction responding may, therefore, demonstrate whether the strength of an association between the lever and rewarding SABS stimulation exists and whether it incorporates information about the magnitude of reward.

Another test which has proved useful in detecting changes in reinforcement level is to test animals for contrast effects. When increases or decreases in reinforcer magnitude are presented to animals within a given session, animals show an overshoot or undershoot in response rate beyond what would be expected from the curves relating response rate to the amount of reinforcement, such as a rate-intensity or rate-frequency function (Crespi, 1952; Koob, 1977). Such overshoots or undershoots reflect positive or negative contrast effects (Crespi, 1952). These contrast effects result from changes in the reinforcement value produced by shifts in the preceding reward (Black, 1968; Koob, 1977). If shifts in the magnitude of reward do not result in a shift in incentive value, contrast effects do not occur. In ICSS, shifts in the incentive value of rewarding brain stimulation can be detected by generating rate-frequency or rate-intensity curves (Gallistel et al., 1981; Gallistel and Freyd, 1987; Hawkins and Pliskoff, 1964). Similarly, changes in the incentive value of brain stimulation can also be measured with the SABS model (Experiments 1 and 2). If lesions of the PPTg attenuate the positive reinforcing effects of brain

stimulation one would expect that animals with PPTg lesions would show a reduced contrast effect.

Another way to determine whether PPTg lesions affect the reinforcing value of stimulation is to test animals on a progressive ratio schedule of reinforcement (see above). The determination of break points can be used to demonstrate the relative reinforcing effects of various stimulation trains (Hodos, 1961; Hodos, 1965; Hodos and Kalman, 1963). In Experiment 6, lesioned animals were not significantly different from sham-lesioned animals on response rate or mean frequency measures under a CRF schedule. However, a CRF schedule is not a sensitive enough measure of the incentive value of reinforcing brain stimulation. As demonstrated in Experiment 2, the progressive ratio schedule of reinforcement is sensitive to the magnitude of reinforcement with long trains of SABS-like stimulation. Thus, if lesions of the PPTg attenuate the reinforcing effect of brain stimulation, lesioned animals might be expected to show lower break points than sham-lesioned controls.

Method

Animal Subjects

A total of twenty male, Long Evans rats from Experiment 6 were used for the following experiments.

Procedure

Extinction Test

Following five days of acquisition training in Experiment 6, all animals were given one additional day of SABS responding. On the seventh day of

acquisition, animals were allowed to press for SABS stimulation for 1/2 hour, at which point the stimulators were turned off. Response rate was sampled for the following half-hour of the extinction test.

Contrast Test

Following extinction testing four lesioned and four control animals were randomly selected to undergo contrast testing. Before contrast testing began, animals were retrained on the following SABS parameters for two additional one hour sessions; dose=200 Hz, rise half-time=1 second, and an elimination half-time of 100 seconds. Animals were given one half-hour for warm-up at a dose of 200 Hz. At the end of the half-hour (T=30 minutes), the dose of stimulation was randomly changed to 100 or 400 Hz, and animals were tested at the new dose for 30 minutes. At the end of the 30 minutes (T=60 minutes), the dose was reset to 200 Hz, and animals were allowed to respond for an additional 30 minutes. Animals completed both contrast conditions. The contrast (e.g. 200-100-200 Hz or 200-400-200 Hz) was repeated for two consecutive days. Electrolytic-lesioned and sham-lesioned animals completed both conditions.

Progressive Ratio

Of the remaining rats, four lesioned and four sham-lesioned animals were randomly selected to run on a progressive-ratio schedule of reinforcement. Animals were run for 2 days at each of the following fixed ratios: FR1, FR3, FR5, and FR10, before progressive-ratio testing began. It was planned that animals would be tested for three days at three doses of brain stimulation. However, lesioned animals did not perform well at any of the fixed ratios

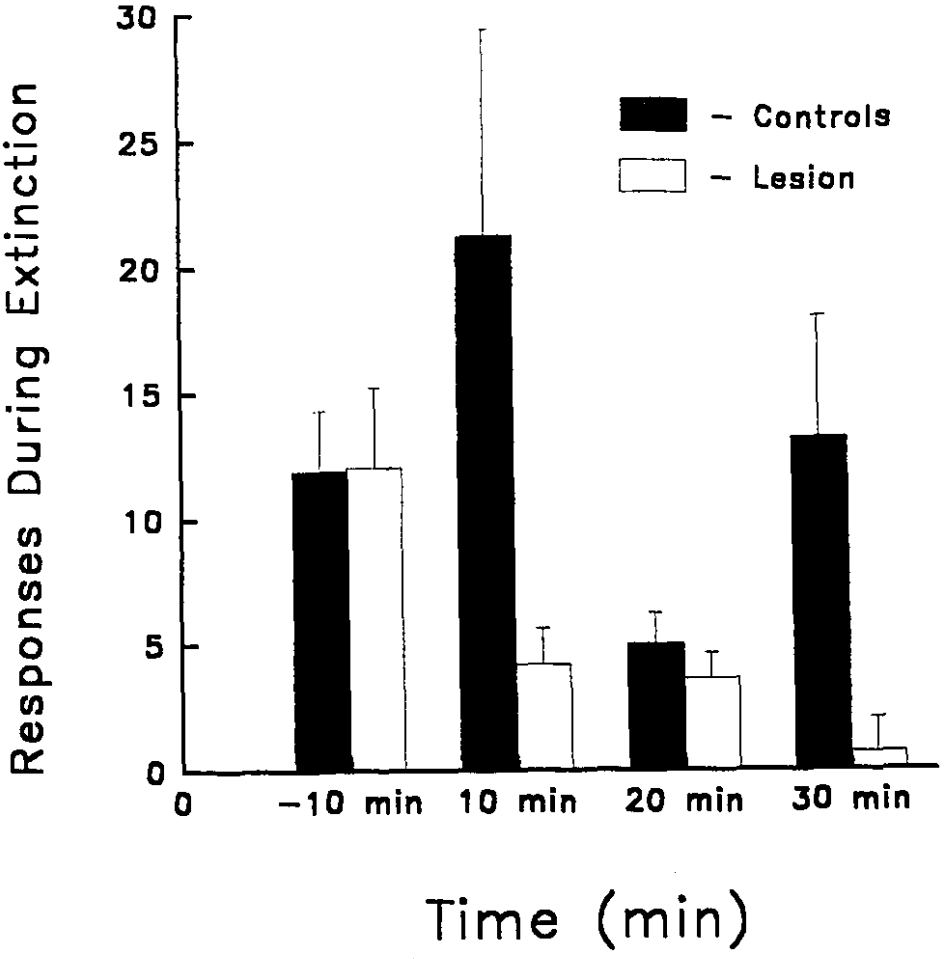


Figure 20. The effects of electrolytic lesions of the PPTg on the total number of responses made during an extinction test. The mean number of responses made by each group of animals immediately prior to (-10 min) and during extinction (10 to 30 min) were calculated for four consecutive ten minute bins. Bars represent means ± S.E.M.

(explained below), and the experiment was terminated following performance on the FR5 ratio.

Results

Extinction Test

Response rate and mean frequency were sampled in three blocks of 10 minutes. As shown in Figure 20, a significant difference in response rate was observed comparing lesioned to sham-lesioned animals. The non-parametric Wilcoxon Rank-Sum test was used to analyze the extinction data because of non-homogeneity of variance. The non-homogeneity of variance was due in large part to the fact that PPTg-lesioned animals showed very rapid extinction, with the result that there was practically no variance within that group. In the first ($p=0.046$, Wilcoxon test) and last ($p=0.0431$, Wilcoxon test) 10 minute bins, sham-lesioned animals responded more than lesioned animals during extinction. PPTg-lesioned animals responded little or not at all under extinction conditions.

Contrast Test

A two-way Lesion group X Contrast ANOVA performed on response rate revealed that animals with PPTg lesions showed no contrast effects ($F=8.285(1,6\ df)$, $p < 0.05$). Further, in each contrast condition (e.g. ascending and descending series) there was a significant interaction between lesion group and contrast condition ($F=9.246(5,30\ df)$, $p < 0.05$) was revealed.

In testing the interaction means using a regression analysis, it was found that in animals with electrolytic lesions of the PPTg, the pattern of SABS intake was not affected by changes in stimulation frequency (all p's > 0.05) (see

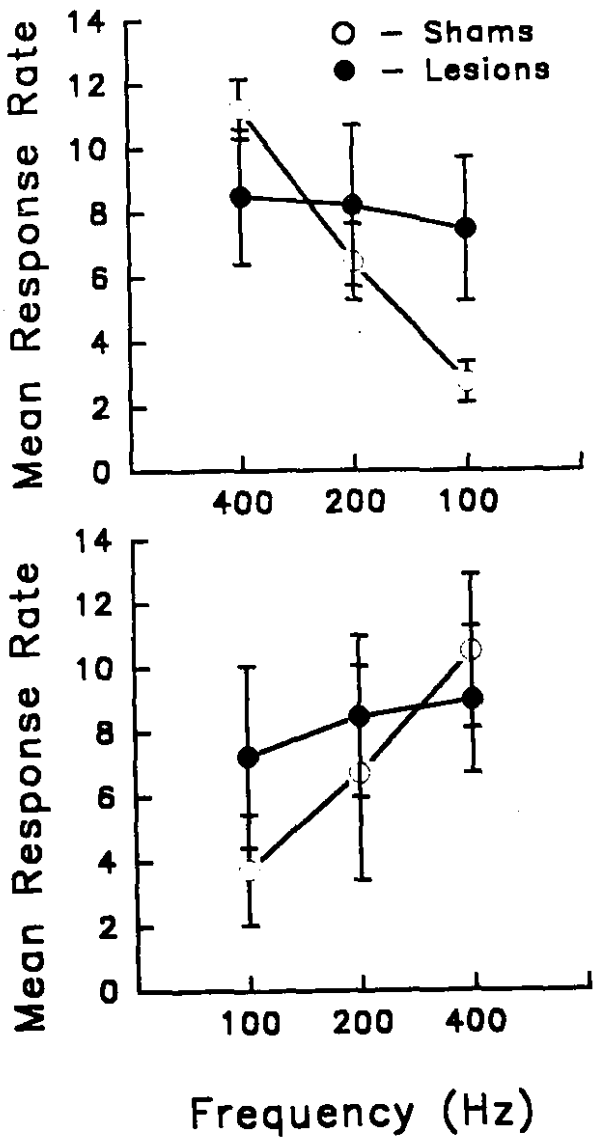


Figure 21. The effects of incentive contrasts on the mean response rate of animals with sham and electrolytic lesions of the PPTg during a one-half hour contrast test (see text for further explanation). The top panel represents the effects of the descending contrast conditions (400 Hz to 200 Hz and 200 Hz to 100 Hz). The bottom panel displays the ascending contrast condition (100 Hz to 200 Hz and 200 Hz to 400 Hz). Points represent the means ± S.E.M.

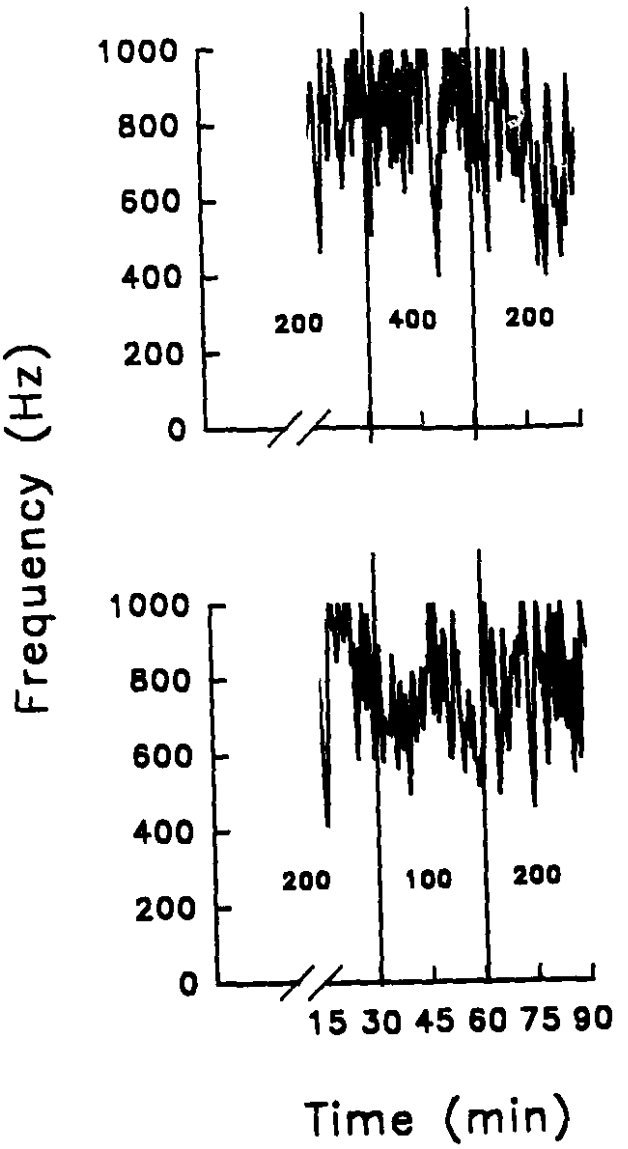


Figure 22. Pulse frequency of continuous brain stimulation maintained over a 90 min contrast test of SABS by a single rat with an electrolytic lesion of the PPTg. The parameters of each stimulating train was set at a dose of 200 Hz with an absorption half-time of 1 s and an elimination half-time of 100 s. The top panel shows the effects of changing reinforcer magnitude from 200 to 400 Hz (30 min) and the from 400 to 200 Hz (60 min). The bottom panel shows the effects of changing reinforcer magnitude from 200 to 100 Hz (30 min) and from 100 to 200 Hz (60 min). Stimulation frequency was sampled every 30 s.

Figure 21), suggesting that PPTg-lesioned animals were either unable to detect or unable to respond to changes in reward magnitude. Figure 22 show individual records of two lesioned animals responding for 400 Hz-200 Hz and 100 Hz-200 Hz brain stimulation.

In contrast, a regression analysis performed on the response rate of sham-lesioned animals for the descending contrast condition revealed a significant decrease in response rate when reinforcer magnitude was decreased ($F=11.891(2,6\ df)$, $p < 0.05$). Post hoc Newman-Keuls tests showed that animals showed a significant decrease in response rate when the frequency was changed from 400 Hz to 200 Hz ($q=4.870$, $p < 0.05$) and when frequency was dropped from 200 to 100 Hz ($q=6.64$, $p < 0.05$). In the ascending contrast condition, there was a significant increase in responding when reinforcer magnitude was increased ($F=6.941(2,6\ df)$, $p < 0.05$). Post-hoc Newman-Keuls tests revealed that response rate increased significantly when the frequency was changed from 200 to 400 Hz ($q=5.239$, $p < 0.05$), but not when the frequency was changed from 100 to 200 Hz ($q=2.135$, $p > 0.05$). Figure 23 displays the individual records of two sham-lesioned animals responding for SABS stimulation under both contrast conditions. As shown, SABS shows a significant drop in response rate when reward magnitude is changed from 400 to 200 Hz, and an increase in mean frequency when reward magnitude is changed from 200 to 400 Hz. In one case, a clear positive contrast effect can be detected immediately after the stimulation frequency is changed from 100 to 200 Hz (see Figure 23, bottom panel).

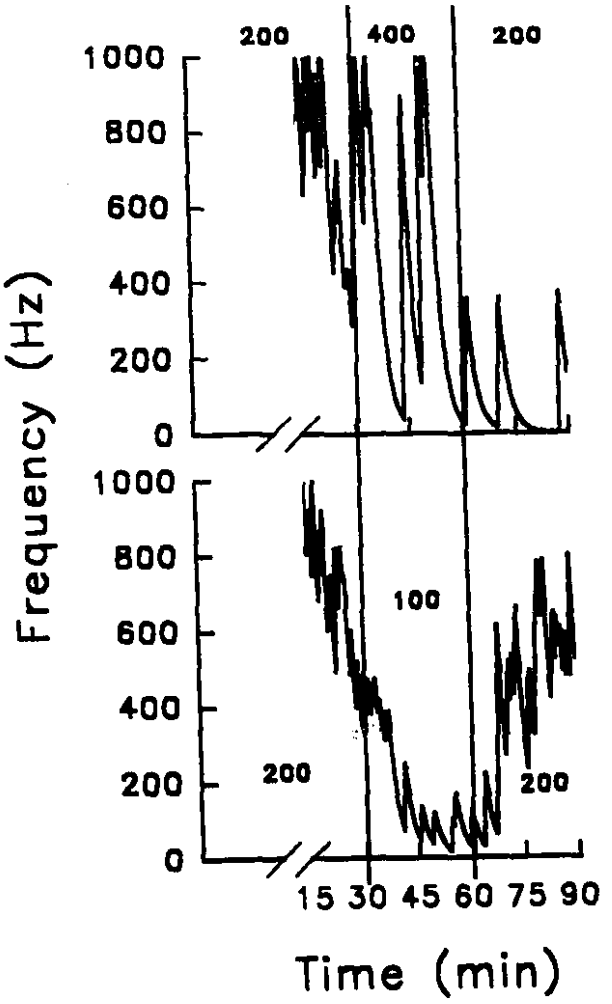


Figure 23. Pulse frequency of continuous brain stimulation maintained over a 90 min contrast test of SABS by a single rat with a sham lesion of the PPTg. The parameters of each stimulating train was set at a dose of 200 Hz with an absorption half-time of 1 s and an elimination half-time of 100 s. The top panel shows the effects of changing reinforcer magnitude from 200 to 400 Hz (30 min) and the from 400 to 200 Hz (60 min). The bottom panel shows the effects of changing reinforcer magnitude from 200 to 100 Hz (30 min) and from 100 to 200 Hz (60 min). Stimulation frequency was sampled every 30 s.

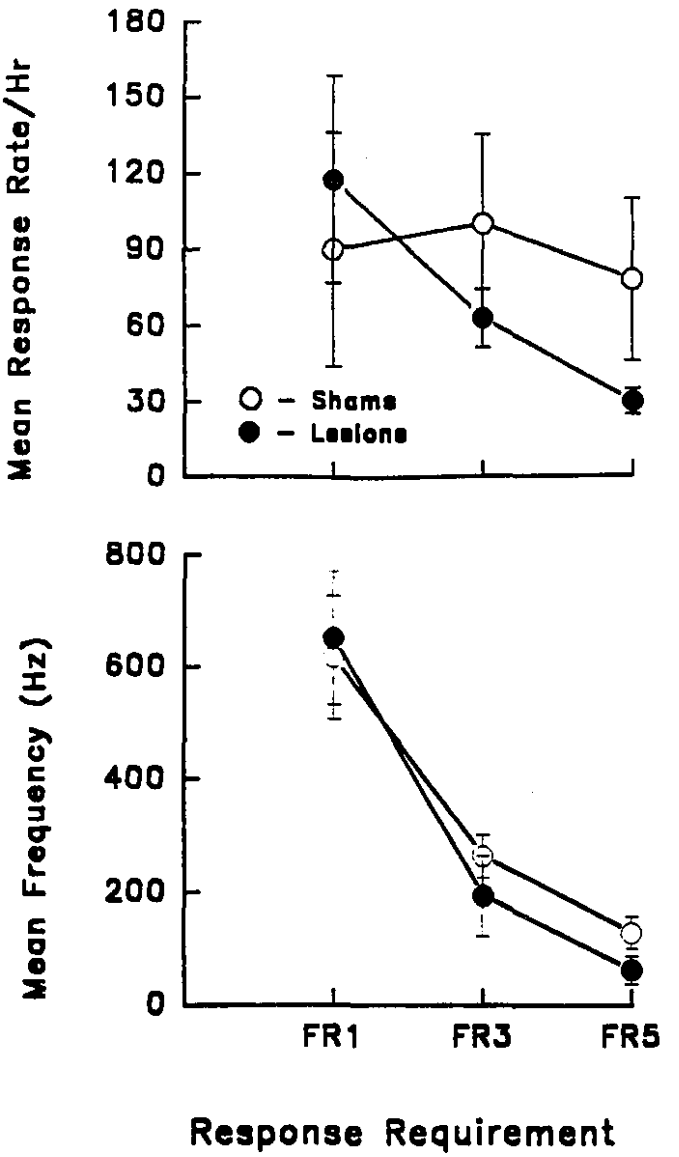


Figure 24. The relationship between response rate or mean pulse frequency at three different fixed ratio requirements for animals with sham (open circles) or electroltyic lesions (filled circles) of the PPTg. The top panel shows the effects of increasing fixed ratio requirement on mean response rate for a one hour session, and the bottom panel shows the effects of increasing ratio requirements on the mean pulse frequency maintained by sham and lesioned animals. Points represent the mean ± S.E.M.

Progressive Ratio

On the basis of previous findings (Lepore and Franklin, 1991; and see Experiments 1 and 2), animals were expected to run on a progressive-ratio schedule of reinforcement. However, it became immediately evident that as fixed ratio size increased from FR1 to FR3, and to FR5, the performance of PPTg-lesioned animals deteriorated. A two way Lesion group by Fixed-ratio size ANOVA performed on response rates revealed that PPTg-lesioned animals responded significantly less than sham-lesioned animals ($F=18.460(1,6\ df)$, $p < 0.05$), that increasing FR size led to a decrease in response rate for lesioned animals ($F=31.886(2,12\ df)$, $p < 0.05$), and that a significant interaction between lesion group and response requirement existed ($F=21.445(2,12\ df)$, $p < 0.05$) (see Figure 24). Post-hoc Newman-Keuls analysis of the interaction means revealed that, at FR1, sham-lesioned and PPTg-lesioned animals had similar response rates ($q=1.342$, $pp > 0.05$), but that at both FR3 ($q=6.167$, $p < 0.05$) and FR5 ($q=8.000$, $p < 0.05$) ratio requirements, PPTg-lesioned animals responded significantly less than sham-lesioned controls. PPTg-lesioned animals also showed a significant drop in response rate from FR1 to FR3 ($q=8.770$, $p < 0.05$) and from FR3 to FR5 ($q=5.262$, $p < 0.05$), whereas sham-lesioned animals maintained relatively stable rates of responding under all three fixed-ratio requirements (all p's > 0.05).

A similar pattern of results was obtained when a two way Lesion group X Fixed-ratio size ANOVA was performed on mean frequency measures. The results of the ANOVA revealed that electrolytic lesioned animals maintained

lower stimulation frequencies than sham-lesioned animals ($F=9.661(1,6\ df)$, $p < 0.05$), that increasing fixed-ratio size from FR1 to FR5 lowered the mean pulse frequency maintained by both groups of animals ($F=589.7(2,12\ df)$, $p < 0.05$), and that a significant interaction between lesion group and fixed ratio size existed ($F=7.01(2,12\ df)$, $p < 0.05$). Post-hoc Newman-Keuls analysis of the interaction means showed that, at FR1, sham-lesioned and PPTg-lesioned animals were maintaining similar mean frequencies ($q=2.085$, $p > 0.05$), but that at both FR3 ($q=4.487$, $p < 0.05$) and FR5 ($q=4.713$, $p < 0.05$) ratio requirements, PPTg-lesioned animals were maintaining significantly lower mean frequencies compared to sham-lesioned animals.

Discussion

Animals with bilateral electrolytic lesions of the PPTg were not impaired in the acquisition of SABS responding. They rapidly acquired and maintained responding for SABS stimulation. In fact, their performance on a CRF schedule of reinforcement was no different from sham-lesioned controls, which suggested that the rewarding or affective properties of brain stimulation had not been affected. However, when more sensitive tasks measuring the incentive value of brain stimulation were used, a specific pattern of reinforcement deficits was found in PPTg-lesioned animals.

Animals with bilateral electrolytic lesions of the PPTg showed very rapid extinction, did not show positive or negative incentive contrast effects, and performed poorly on ratio schedules of reinforcement. Sham-lesioned animals, however, responded for at least 30 minutes under extinction conditions, showed

both positive and negative contrast effects, and maintained responding for brain stimulation at different fixed-ratio requirements.

The results from these three behavioral tests can be interpreted by a motivational deficit hypothesis. In an operant task, responding under extinction conditions can reveal the strength of a reinforced S-R association (Skinner, 1938; Skinner, 1968b). The finding that electrolytic-lesioned animals showed more rapid extinction than sham-lesioned animals suggests that PPTg lesions produced a specific deficit in reinforcement. That acquisition of SABS can still occur suggests that electrolytic lesions were only partially attenuating the reinforcing effects of brain stimulation. This would also explain why PPTg-lesioned animals performed poorly compared to sham-lesioned animals on the fixed ratio schedules of reinforcement. The progressive ratio schedule of reinforcement is sensitive to shifts in the magnitude of reinforcement (Hodos, 1961; Hodos, 1965; Hodos and Kalman, 1963; Richardson and Roberts, 1991; Roberts, 1989; Roberts et al., 1989). If electrolytic lesions of the PPTg produce subtle reinforcement deficits (i.e. attenuate the reinforcing effects of brain stimulation), one would expect to see lower break points in animals with PPTg-lesions compared to sham-lesioned controls. Furthermore, the presence of primary and secondary reinforcement is required in order to maintain reliable responding under extinction-like conditions (Stewart and Eikelboom, 1987). In fact, responding can be reliably maintained solely in the presence of secondary reinforcement (Robbins and Koob, 1978; Taylor and Robbins, 1984; Taylor and Robbins, 1986). That electrolytic lesions of the PPTg disrupt responding under

partial reinforcement schedules is consistent with the interpretation that PPTg lesions attenuate the positive reinforcing effects of brain stimulation. Under conditions where an attenuation of the primary reinforcing effects of brain stimulation has occurred (e.g. PPTg lesions), it would be reasonable to assume that the secondary reinforcing effects of brain stimulation would also be attenuated.

Finally, an interpretation based on a motivational deficit hypothesis is also supported by the fact that incentive contrast effects were not detected in PPTg-lesioned animals. The contrast effect occurs when reinforcer magnitude is increased or decreased within a single test session (Black, 1968; Crespi, 1952; Trowill et al., 1969). When reinforcer magnitude is increased or decreased, animals increase or decrease responding, respectively. The increase or decrease in response rate reflects changes in the reinforcement value of the preceding reward (Black, 1968; Trowill et al., 1969). In this experiment, sham-lesioned animals show positive and negative contrasts, but PPTg-lesioned animals show neither a positive nor negative contrast (see Figure 21), suggesting that PPTg-lesioned animals cannot detect or cannot respond to changes in the reinforcement value of stimulation. Thus, the results of this experiment demonstrate that electrolytic-lesioned animals, though able to acquire SABS, show specific deficits in reinforcement. This supports the interpretation that the PPTg mediates the positive reinforcing properties of brain stimulation.

Experiment 8

Introduction

As reviewed above, the PPTg has also been suggested to be involved in learning and memory processes (Dellu et al., 1991; Fujimoto et al., 1989; Fujimoto et al., 1990; Fujimoto et al., 1992). Dellu et al. (1991) demonstrated that quisqualic acid lesions of the PPTg blocked the acquisition of Morris water maze and eight-arm radial maze learning. The Morris water maze and eight-arm radial maze are tasks of spatial memory. That PPTg lesions also block acquisition of one-way passive and two-way active avoidance (Fujimoto et al., 1989; Fujimoto et al., 1990; Fujimoto et al., 1992) raises the possibility that PPTg lesions may produce a generalized learning impairment. Although it is unlikely that a generalized learning impairment can account for the results using the SABS model, testing for acquisition of simple operant learning is not a sensitive measure of memory deficits compared to tasks designed specifically to elucidate memory processes and the neural structures which subserve those processes (McDonald and White, 1993; Packard et al., 1989). Furthermore, the finding in Experiment 7 that PPTg-lesioned animals did not respond under extinction conditions suggests the possibility that a strong S-R association was not formed, or that secondary reinforcers did not develop during acquisition.

Two memory tasks demonstrated to measure spatial versus S-R learning are the win-shift and win-stay versions of the radial maze, respectively. In both these tasks, the amount of reinforcement available is identical, but the contingencies associated with its presentation are different (Olton and

Samuelson, 1976; Packard et al., 1989). Both these tasks are run on an eight-arm version of the radial maze. The win-shift paradigm is basically a foraging task, where animals are required to approach and enter each arm in the eight-arm maze to obtain food. The contingencies associated with the presentation of food reward are such that the animal must approach and enter each arm of the maze to retrieve each food pellet after which food is no longer available from that arm (Olton and Samuelson, 1976). Once food has been retrieved from an arm, the animal must choose one of the remaining seven arms in order to retrieve another food pellet, and so on until all eight arms have been entered. Thus, the optimal strategy for an animal is to visit each arm only once, then "shift" to another arm, and so on, until all eight arms have been visited. The use of extra-maze cues (located randomly around the maze) helps the animal to navigate around the maze to achieve optimum performance. The use of extra-maze cues also ensures that no one stimulus is repeatedly or consistently paired with a correct response.

In the win-stay paradigm, an eight arm-radial maze is used but the demand characteristics of the task are changed (Packard et al., 1989). In win-stay, the animal still receives eight food pellets but in only four of the eight arms. As opposed to approaching and entering each arm at least once, animals are required to revisit an arm previously baited with food. In win-stay, extra-maze cues are no longer used. Rather, intra-maze cues (lights) are used as discriminative stimuli which signal which arms are baited. Thus, animals are required to approach the stimulus (light). After the animals have retrieved the

food in each of the four arms, they must revisit ("stay") the arms signalled by the stimulus. The use of intra-maze cues ensures that a stimulus is repeatedly and consistently paired with a correct response (Packard et al., 1989).

Both the win-shift and win-stay paradigms have been used extensively to explore the neuroanatomical and neurochemical basis of learning and memory (Jarrard, 1993; Keith and Rudy, 1990; Major and White, 1977; McDonald and White, 1993; O'Keefe and Speakman, 1987; Olton and Papas, 1979; Olton and Samuelson, 1976; Olton et al., 1979; Packard et al., 1989; Shapiro and O'Conner, 1992; Sorenson et al., 1991; Speakman and O'Keefe, 1990). There is a large body of literature which suggests that an intact hippocampus is essential for normal performance in some behavioral tasks (Bourne et al., 1989; Dunnett, 1990; Jarrard, 1993; McDonald and White, 1993; O'Keefe and Speakman, 1987; Olton and Papas, 1979; Olton et al., 1979; Packard et al., 1989; Shapiro and O'Conner, 1992; Speakman and O'Keefe, 1990). Rats with hippocampal damage are consistently impaired in the standard or place test version of the Morris water maze (Morris et al., 1982; Sutherland et al., 1982), the win-shift version of the eight-arm radial maze (Olton and Papas, 1979; Olton et al., 1979) and in contextual conditioning (Sutherland and McDonald, 1990).

Although there are different interpretations about what the hippocampus is doing (Hirsh, 1974; O'Keefe and Nadel, 1978), there is general agreement that the hippocampus is crucially involved in learning of a "cognitive" nature. By cognitive it is meant that behavior is not evoked by the stimulus directly, but

that different aspects of external stimuli, including their relation to previously experienced events, are encoded within a "representation", and that behavior is guided by the relational information contained within this representation. Recently, investigators have pointed out that behavioral tasks which are disrupted by hippocampal damage share the requirement that animals learn and use information about the relationship between external stimuli and/or events. Hence, it is argued that the hippocampus mediates the acquisition of information about the relationships among stimuli (McDonald and White, 1993; Packard et al., 1989).

Although the hippocampus is important for acquisition of a variety of behavioral tasks (e.g. win-shift), there are other tasks which can be acquired normally by rats with hippocampal damage (e.g. win-stay). In other words, acquisition and performance on a variety of other behavioral tasks seems to depend upon other memory systems. A number of these tasks can be grouped together by the criteria that an animal is consistently required to emit a response in the presence of a single stimulus (Packard et al., 1989). In other words, efficient performance in these tasks depends upon an animal being able to acquire a reinforced stimulus-response (S-R) association. There is now strong evidence that points to a role for the dorsal striatum in mediating the acquisition of S-R learning (McDonald and White, 1993; Packard et al., 1989).

Packard et al. (1989) sought to establish a double dissociation of memory functions using the win-shift and win-stay version of the radial maze. In their experiment, the performance of animals lesioned in the dorsal striatum

or fimbria-fornix was compared on both behavioral tasks. They demonstrated that, in the win-shift version of the radial maze, only animals with fimbria-fornix lesions were impaired on acquisition, whereas control animals or animals with bilateral lesions of the dorsal striatum were unimpaired. Lesions of the dorsal striatum, however, severely impaired acquisition of S-R learning (win-stay version) compared to controls, whereas fimbria-fornix lesions enhanced the learning of an S-R association (Packard et al., 1989). MacDonald and White (1992) sought to establish a triple dissociation of memory functions using the win-shift, win-stay, and conditioned cued preference (CCP) radial arm maze tasks. In this experiment, not only were the findings of Packard et al. (1989) replicated, but were extended to include the lateral nucleus of the amygdala as a third memory system which mediates stimulus-affect learning (Hiroi and White, 1991; McDonald and White, 1993). Thus, animals with fimbria-fornix lesions performed poorly on the win-shift version of the radial maze, were enhanced on the win-stay task, and fimbria-fornix lesions did not impair stimulus-affect learning. Lesions of the caudate nucleus severely impaired win-stay learning, and had no effects on either the CCP or win-shift tasks. Lesions of the lateral nucleus of the amygdala impaired CCP learning, and had no effects on either win-shift or win-stay learning (McDonald and White, 1993). These two studies support the hypothesis that there are functionally and anatomically distinct memory systems which mediate different types of learning. Further, they also demonstrate that different versions of the eight-arm radial maze are capable of dissociating distinct memory processes and the

neuroanatomical structures that underlie multiple memory systems.

Given the sensitivity of the win-shift and win-stay tasks to dissociate different types of memory impairments, the following experiment was designed to explore whether electrolytic lesions of the PPTg would produce a specific memory deficit, which would be evidenced by a selective block of either win-shift or win-stay learning, or whether PPTg-lesions would produce a generalized memory impairment, which would be evidenced by a deficit on both memory tasks.

Method

Animal Subjects

Ten animals with electrolytic lesions of the PPTg and ten sham-lesioned controls from the two previous experiments were used.

Apparatus: Win-Shift Task

The eight arm radial maze used for the win-shift task was made of wood and painted a flat grey. The height of the maze was 60 cm. The diameter of the center platform was 40 cm, and each arm was 60 cm long and 9 cm wide. A recessed food well was located at the end of each arm. A plexiglass wall (40 cm in height) enclosed the center platform. The entrance of each arm was blocked by a guillotine door, which could be lowered and raised by the experimenter. The maze was located in the center of a room which contained a variety of extramaze cues (e.g. posters, bookshelves, small writing table, etc.) randomly placed around the room.

Apparatus: Win-Stay Task

The eight arm radial maze described above (with modifications) was used for win-stay training. For the win-stay task, the radial maze was enclosed in a dark curtain. Light bulbs (7 watts) were fastened to the top of the entrance of each arm, and a system for rebaiting arms was added to the maze. One end of the tubes were connected at the end of each arm (at the site of the food wells) and the other end of the tubes were placed at a point outside the enclosed maze. When rebaiting was required, the experimenter simply dropped a piece of food into the appropriate tube.

Procedure: Win-Shift Task

Lesioned and sham-lesioned animals were randomly divided into two groups of five. One group of sham-lesioned and PPTg-lesioned animals were used for win-shift training, and the remaining two groups of animals were used for win-stay training. All animals were placed on a food-deprivation schedule, and were maintained at 35% of their free-feeding weight. During the three days of food deprivation, animals were exposed to Froot Loops (Kellogg's cereal) in their home cage. Following three days of food deprivation, each animal was placed in the radial maze apparatus for five minutes for two consecutive days, during which time the guillotine doors were manipulated randomly. No food was placed on the maze during habituation, but animals received eight Froot Loops cereal in the colony room following each habituation session. On the third day, win-shift testing began. A single piece of Froot Loops cereal was placed at the end of each arm. Each animal was placed on the center platform

with the guillotine doors closed. After ten seconds, the doors were opened and the animal was allowed to enter (choose) one of eight arms. Once the animal had made its choice, the doors to all arms were lowered except for the arm the animal entered. Once the animal returned to the center platform, the door to that arm was closed. Following a ten second waiting period, the doors to all eight arms were opened, and the animal was allowed to choose another arm. This procedure continued until all eight Froot Loops cereal were retrieved, or until ten minutes had elapsed. Animals were run for one trial per day. All arm entries made by each animal during the test were recorded. The number of revisits to arms previously entered in the first eight visits, expressed as the number of errors, was assessed for each animal. Latency to complete the task was also recorded. Daily trials continued until the number of errors made by control animals was less than one for two consecutive days.

Procedure: Win-Stay Task

All animals were placed on a food-deprivation schedule as previously described. Animals were habituated to Froot Loops cereal during food deprivation and during maze habituation. During maze habituation, animals were habituated to the maze for five minutes for two consecutive days. During this time, no food was available on the maze, the lights in the room which housed the maze were dimmed, and the lights within each arm of the maze remained unlit. Following habituation to the maze, the acquisition trials began. For each trial, only four arms of the maze were lit (randomly selected with the exception that no more than two adjacent arms could be lit) and a piece of

Froot Loop cereal was placed in the food recess at the end of each lit arm. To increase the salience of the intramaze cues, the room lights were dimmed. Each acquisition trial began by placing each animal on the center platform. Once an animal retrieved the food from the lit arm, that arm was rebaited with food. Following two arm entries into a lit arm, the light was turned off and no further food was placed there. Each trial was terminated once the animal retrieved all eight Froot Loops or once ten minutes had elapsed. All arm entries were recorded, and entries into unlit arms were scored as errors. Latency to complete the task was also recorded. Animals were tested for one trial per day, and daily testing was terminated once control animals achieved 80% or higher choice accuracy (scored as number of correct choices over total choices) for four consecutive days.

Results

Win-Shift Task

As shown in Figure 25, rats with electrolytic lesions of the PPTg were impaired on the win-shift task compared to controls. A two-way Group X Acquisition Trial ANOVA performed on the number of errors revealed that PPTg-lesioned animals made significantly more errors than sham-lesioned controls ($F=28.247(1,8\ df)$, $p < 0.05$). It was also found that, across acquisition trials, the number of errors made by both groups of animals significantly decreased ($F=11.69(9,90\ df)$, $p < 0.05$). No significant interaction between lesion group and acquisition trial was found ($F=1.834(9,90\ df)$, $p > 0.05$). Post hoc Newman Keuls tests performed on the main effect of acquisition trials

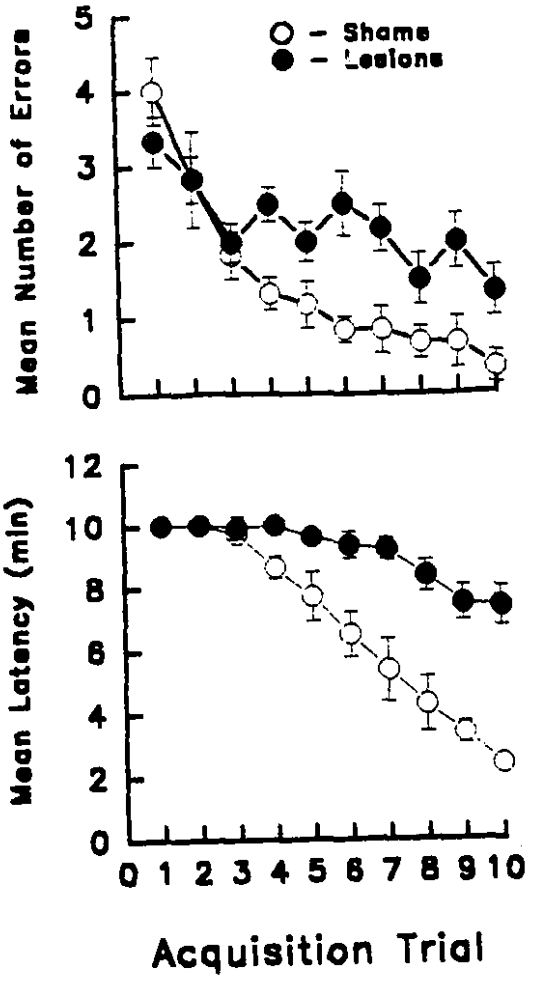


Figure 25. Mean number of errors (± S.E.M.) made by animals with sham (open circles) or electrolytic (filled circles) lesions of the PPTg on the acquisition of the win-shift task (top panel). The bottom panel displays the mean amount of time to complete the task (± S.E.m.) by sham and electrolytic lesioned animals on the acquisition of the win shift-task.

showed that by Day 3, both PPTg-lesioned and sham-lesioned animals were making significantly fewer errors when compared to acquisition Day 1 ($q=6.544$, $p < 0.05$), and fewer errors on acquisition day 10 when compared to acquisition Day 3 ($q=4.726$, $p < 0.05$). Thus, for both groups, the number of errors was significantly reduced by the end of acquisition training. However, even though both PPTg-lesioned and sham-lesioned animals were making fewer errors by the end of acquisition training, PPTg-lesioned animals were making significantly more errors than sham-lesioned animals ($q=7.526$, $p < 0.05$). These results are consistent with previous reports (McDonald and White, 1993; Packard et al., 1989) demonstrating that win-shift learning is rapidly acquired by normal animals. A two-way Group by Acquisition trial ANOVA performed on the latency to complete the task, however, revealed that PPTg-lesioned animals took longer to complete the task than sham-lesioned animals ($F=33.34(1,8\ df)$, $p < 0.05$). Latencies for both control and lesioned animals decreased across acquisition training ($F=29.92(9,90\ df)$, $p < 0.05$), and a significant interaction between lesion group and number of acquisition trials ($F=9.19(9,90\ df)$, $p < 0.05$) was found. Post-hoc tests of the simple main effects using the Newman-Keuls pairwise comparisons procedure revealed that on acquisition day 5, sham-lesioned animals were completing the task significantly faster than animals with PPTg lesions ($q=6.553$, $P < 0.05$), a trend which continued until the last acquisition trial ($q=9.496$, $p < 0.05$).

Although PPTg-lesioned animals were not performing efficiently on the win-shift task, visual examination of lesioned-animals indicated that they

maintained grossly normal body weight and were unimpaired in general performance on the maze (e.g., ate all the food, quick to initiate arm entries, etc.) compared to sham-lesioned control animals. Furthermore, although they were impaired relative to controls, lesioned-animals were making significantly fewer errors by the end of acquisition training. This demonstrates that PPTg-lesioned animals were in fact able to learn something about the win-shift task. It has been previously demonstrated, using a response distribution analysis, that animals with fimbria-fornix lesions do not distribute their responses in a manner consistent with win-shift behavior (Packard et al., 1989). To determine whether PPTg-lesioned animals were learning shift behavior but simply performing inefficiently, a response distribution analysis was carried out on the last day of acquisition testing as described by Packard et al. (1989). Animals responding efficiently (e.g., 100% accurate) would theoretically distribute their responses evenly across all eight arms (Packard et al., 1989). That is, animals would enter each arm only once and then "shift". For each animal, the percentage of visits to each arm was calculated. The group mean percentage of arm entries was then calculated and rank-ordered according to the frequency of visits. As shown in Figure 26, both sham-lesioned and PPTg-lesioned animals distributed their responses relatively evenly across all eight arms, demonstrating a high degree of shift behavior. A one way ANOVA performed on the slopes of the frequency distribution for each group revealed that no differences in slope existed between sham-lesioned and PPTg-lesioned animals ($F=0.027(1,14\ df)$, $p > 0.05$). This confirms that PPTg-lesioned animals were capable of learning

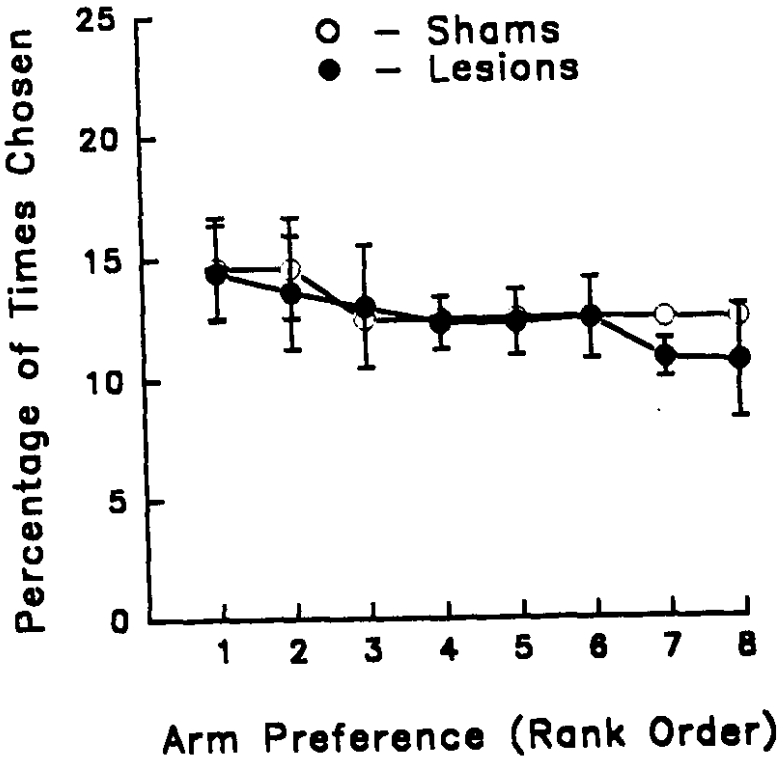


Figure 26. The effects of electrolytic (filled circles) or sham-lesions (open circles) on the response distribution analysis for the win-shift task. The analysis provides a measure of the degree of shift behavior displayed by both groups during acquisition. Points represent means ± S.E.M.

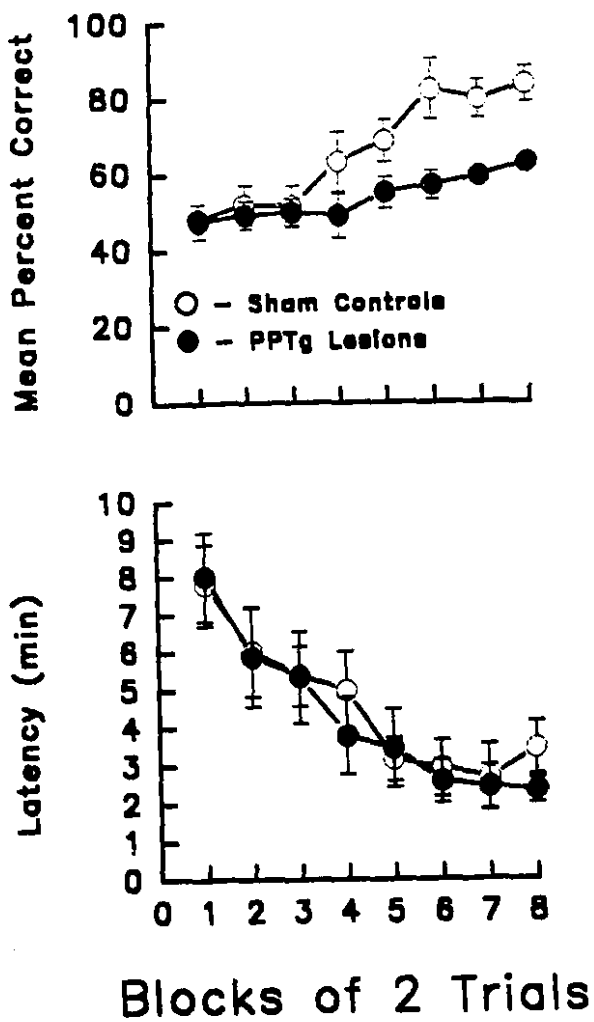


Figure 27. The top panel displays the mean percentage of correct arm entries (approach to lit arms) made by animals with sham (open circles) or electrolytic (filled circles) lesions of the PPTg on the acquisition of the win-stay task. The bottom panel shows the mean amount of time to complete the task by both groups of animals on the acquisition of the win-stay task. The data is displayed in blocks of two acquisition trials. Points represent the means ± S.E.M.

shift behavior, and points to the possibility that the impairment observed in the win-shift task was not due to a specific memory impairment.

Win-Stay

Figure 27 shows the comparison between PPTg-lesioned and sham-lesioned animals on win-stay learning. The data for the win-stay task are presented in blocks of two acquisition days. As shown, electrolytic lesions of the PPTg impair the acquisition of S-R learning. Sham-lesioned animals were able to acquire the S-R association by acquisition Day 16, consistent with previous reports (McDonald and White, 1993; Packard et al., 1989) demonstrating efficient performance on the win-stay task. A two-way Group X Trial ANOVA performed on choice accuracy scores revealed that PPTg-lesioned animals were performing poorly compared to sham-lesioned animals on choice accuracy ($F=9.595(1,8\ df)$, $p < 0.05$). The results also show that choice accuracy was significantly effected by the number of trial blocks ($F=11.37(7,56\ df)$, $p < 0.05$), and that a significant lesion group by trial block interaction existed ($F=2.625(7,56\ df)$, $p < 0.05$). Post-hoc analysis of the interaction means using the Newman-Keuls pairwise comparisons revealed that PPTg-lesioned animals were significantly less accurate than sham-lesioned animals on acquisition days 11 and 12 ($q=5.199$, $p < 0.05$), a trend which continued to days 15 and 16 ($q=4.258$, $p < 0.05$) after which acquisition trials were terminated. These results demonstrate that electrolytic lesions of the PPTg impair acquisition of win-stay learning.

Latency to complete the task was not significantly different when

comparing sham-lesioned to PPTg-lesioned animals. A two-way Group X Trial ANOVA performed on mean latency scores revealed that electrolytic lesions of the PPTg did not significantly effect latency to complete the task ($F=0.102(1,7$ df), $p > 0.05$). For both groups of animals, latencies to complete the task decreased as the number of trial blocks increased ($F=17.46(7,56$ df), $p < 0.05$). No significant lesion group by trial block interaction ($F=0.378(7,56$ df), $p > 0.05$) was observed. Post-hoc analysis using the Newman Keuls comparisons showed that, for both groups of animals, latency scores had dropped significantly by the end of acquisition training ($q=8.569$, $p < 0.05$). These results demonstrate that electrolytic lesions of the PPTg did not significantly effect motor performance on the win-stay task compared to sham-lesioned control animals. In fact, given that electrolytic-lesioned animals were making more errors but spending an equal amount of time on the maze compared to sham-lesioned animals demonstrates that electrolytic-lesioned animals were performing faster on the maze than sham-lesioned animals.

To determine whether PPTg-lesioned animals were learning "stay" behavior, a response distribution analysis was performed on the last day of acquisition training for both groups of animals. For each animal, the percentage of visits to each arm was calculated. The group mean percentage of arm entries was then calculated and rank-ordered according to the frequency of visits. For win-stay learning, animals performing at 100% choice accuracy would visit the four lit arms twice within each acquisition trial and would not approach unlit arms. Thus, animals would theoretically distribute 25% of their

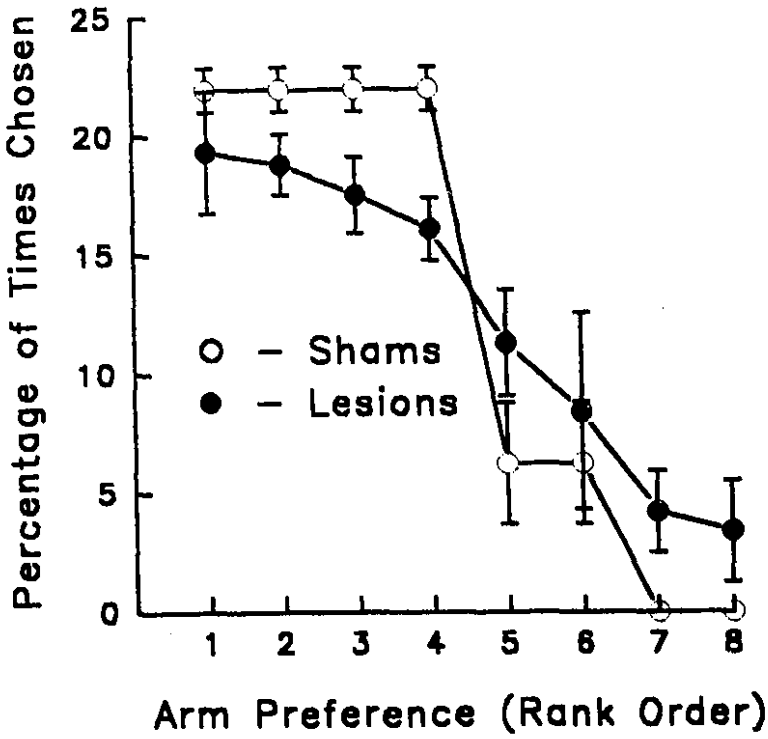


Figure 28. The effects of electrolytic (filled circles) and sham-lesions (open circles) on the response distribution analysis for the win-stay task. The analysis provides a measure of the degree towards stay behavior for both groups of animals. Points represent means ± S.E.M.

responses across the lit arms, and 0% responses for unlit arms (Packard et al., 1989). As shown in Figure 28, both PPTg-lesioned and sham-lesioned animals displayed a high degree of win-stay behavior. A one way ANOVA performed on the slopes of the frequency distribution for each group revealed that PPTg-lesioned animals did not distribute their responses differently from sham-lesioned controls ($F=0.373(1,14\ df)$, $p > 0.05$). This suggests that PPTg-lesioned animals were capable of learning stay behavior.

Discussion

The results of the win-shift and win-stay experiments demonstrate that bilateral electrolytic lesions of the PPTg impair efficient performance on both the win-shift and win-stay versions of the eight-arm radial maze. Although this could be taken as evidence that the PPTg produces specific learning and memory disturbances, it is an unlikely explanation given previous findings on win-shift and win-stay learning (Jarrard, 1993; McDonald and White, 1993; Morris et al., 1982; Olton and Samuelson, 1976; Olton and Papas, 1979; Olton et al., 1979; Packard et al., 1989; White et al., 1993). As reviewed above, the hippocampal formation has consistently been demonstrated to mediate the acquisition of spatial memory (win-shift) (Jarrard, 1993; McDonald and White, 1993; O'Keefe and Speakman, 1987; Olton and Papas, 1979; Olton et al., 1979; Packard et al., 1989), whereas the dorsal striatum has been demonstrated to be involved in the acquisition of a reinforced S-R association (Major and White, 1977; McDonald and White, 1993; Packard et al., 1989; White, 1989). In addition, the lateral nucleus of the amygdala has been

implicated in mediating the acquisition of stimulus-affect (CPP) learning (Hiroi and White, 1991; McDonald and White, 1993; White and McDonald, 1993; White and Milner, 1992). Thus, the win-shift and win-stay versions of the eight-arm radial maze, along with the conditioned place preference task, have been demonstrated to dissociate distinct memory processes and the neural structures subserving them (McDonald and White, 1993; Packard et al., 1989; White, 1989). That bilateral lesions of the PPTg impair acquisition on all three tasks (this experiment and see Bechara and van der Kooy, 1989, 1992) argues against a specific memory impairment.

There are also several reasons why a generalized learning impairment, whatever form such a deficit might take, cannot explain the present results. First, the results of Experiment 4 demonstrated that, in animals that had already acquired SABS, PPTg lesions blocked the maintenance of SABS responding, consistent with the interpretation that the PPTg is involved in mediating positive reinforcement. Second, the PPTg-lesioned animals in the present experiment were previously shown to have acquired responding for SABS (Experiment 6), demonstrating that they were capable of learning an operant task. Third, PPTg-lesioned animals that were deprived in the Bechara and van der Kooy experiments (1989, 1992) were capable of acquiring food and morphine place preference, demonstrating that there were no impairments in stimulus-affect associative learning. Fourth, the response distribution analysis carried out on the win-shift and win-stay data, combined with the finding that PPTg-lesioned animals were performing significantly better on acquisition Day 3 than

acquisition Day 1, and better on acquisition Day 10 than acquisition Day 3, demonstrate that PPTg-lesioned animals were capable of learning shift and stay behavior, but not to the same degree of efficiency as sham-lesioned animals. Although these findings are inconsistent with the Dellu et al. (1991) experiment, which reported that animals with quisqualic acid lesions of the PPTg did not improve their performance across 10 acquisition trials, one important difference between Experiment 8 and Dellu et al.'s (1991) experiment should be noted. In Experiment 8, lesions were confined to the PPTg (see Figure 19, page 102). Dellu et al."s (1991) experiment, quisqualic acid appeared to lesion a substantial portion of the caudal A8 dopamine cell group, similar to that observed in NMDA-lesioned animals from Experiments 3 and 4. It is possible that, had NMDA-lesioned animals been tested on win-shift and win-stay learning, a more severe performance deficit might have been observed. Thus, differences observed between PPTg-lesioned and control animals in this experiment are not consistent with previously reported memory impairments produced by PPTg lesions.

One possible way to interpret the results of this experiment is to examine how reinforcers function in both these memory tasks. In the win-shift task, animals are required to visit each arm only once. Hence, food is not acting in a classical fashion to increase the probability of a response (Hull, 1943; Skinner, 1938; Skinner, 1968b). Rather, animals must learn to avoid arms where reinforcement has already been received. In order to solve the task, therefore, the animal must treat the food as one of many stimulus elements in a particular

situation, and learn about the relationship food has with the many other different stimulus elements (McDonald and White, 1993; Packard et al., 1989).

In win-stay, the animals do not learn anything about the unique properties of food or its relationship with stimulus elements in the environment. Rather, food acts to reinforce approach to lit arms. In other words, food is acting in the classical fashion to increase the probability of a response to a stimulus (Hull, 1943). The consumption of food following approach to the lit arm merely serves to strengthen or "stamp in" the S-R association (McDonald and White, 1993; Packard et al., 1989). Approach to unlit arms, because they are not reinforced, should theoretically be weakened (McDonald and White, 1993; Packard et al., 1989).

As reviewed above, the hippocampus seems to be primarily involved in learning about the spatial relationship among stimuli that make up a particular environment, whereas the dorsal striatum appears to be involved in S-R learning. A response distribution analysis of FF-lesioned animals has shown that they do not distribute their responses in a manner resembling shift behavior (Packard et al., 1989), and consequently do not respond efficiently on the win-shift task. Similarly, caudate-lesioned animals do not distribute their responses in a manner resembling stay behavior (Packard et al., 1989), and are severely impaired on the win-stay task.

In Experiment 8, electrolytic lesions of the PPTg impaired efficient performance on both the win-shift and win-stay tasks, but lesioned animals appeared to be capable of learning shift and stay behavior. That is, lesioned-

animals seemed to be able to learn that shift behavior was appropriate in one task, and that stay behavior was appropriate for the other task. Perhaps if the magnitude of food reward in each arm was increased, PPTg-lesioned animals may have been able to perform more efficiently on both the win-shift and win-stay learning tasks. Thus, it is possible that the incentive properties of food, like brain stimulation (see Experiment 7), are attenuated in animals with PPTg lesions.

General Discussion

The experiments presented in the first part of this thesis explored some potential areas of concern regarding the interpretation of the reinforcing effect of the unusually long frequency-modulated trains of brain stimulation used in the SABS model. The potential problems of interpretation were directly related to the original demonstration of SABS behavior (Lepore and Franklin, 1992), and were whether the high average pulse frequency maintained by animals was an artifact of the reinforcement schedule chosen (CRF), or whether it represented an attempt by animals to maintain an optimal level of reward, as is generally assumed in drug self-administration studies (Goldberg et al., 1971; Werner et al., 1976). This problem was examined using various partial reinforcement schedules, including fixed interval, fixed ratio, and progressive ratio responding. The results of the first experiment demonstrated that, even at relatively high ratio requirements (FR15), animals consistently maintained mean stimulation frequencies above 500 Hz. Experiment 2 demonstrated that animals reliably responded for stimulation frequencies set at 400 or 800 Hz on a fixed interval reinforcement schedule, and an analysis of the breaking points demonstrated that increasing pulse frequency led to increasingly higher break points up to an asymptote at around 400-800 Hz. Taken together, the results of the first part of the thesis demonstrated that (1) the use of partial reinforcement schedules (PRF) did not disrupt SABS performance, (2) the use of PRF schedules did not alter the relationship between response rate and the kinetic parameters of "stimulation dose" or elimination half-life, (3) high frequency trains used in

SABS are reinforcing, and (4) the SABS model has characteristics of both the ICSS and drug self-administration paradigms. These results supported the interpretation that performance differences observed between the ICSS and drug self-adminstration procedures can best be put down to differences in the temporal properties of brain stimulation and drug rewards.

It seems clear from the parametric experiments that animals responding for SABS attempt to maintain optimal stimulation frequencies, even though they are higher than what is considered optimal for self-stimulation. What is not clear, however, is why animals responding for SABS maintain mean frequencies above what have been demonstrated as optimal for SABS (Experiment 2). That is, if rate-frequency curves generated in Experiment 2 show that doses of brain stimulation between 400-500 Hz produce maximal responding (FI schedule) and break points (PR schedule), why do animals consistently maintain mean pulse frequencies around 700-800 Hz (Lepore and Franklin, 1992), instead of around 400 Hz? One way to explain this finding is through a consideration of the relative reinforcing effect produced by different stimulation frequencies. To accomplish this, the rate-frequency curve generated for break points will be re-plotted using the relative reinforcing effect of stimulation (rather than rate) as a function of frequency. The relative reinforcing effect will be expressed as the ratio of the break point for that dose over the maximum break point. In this plot, the value of 0 on the ordinate represents zero reinforcing strength, and a value of 1 reflects the maximal reinforcing strength (defined by the maximum break point) (see Figure 29). For example, the relative

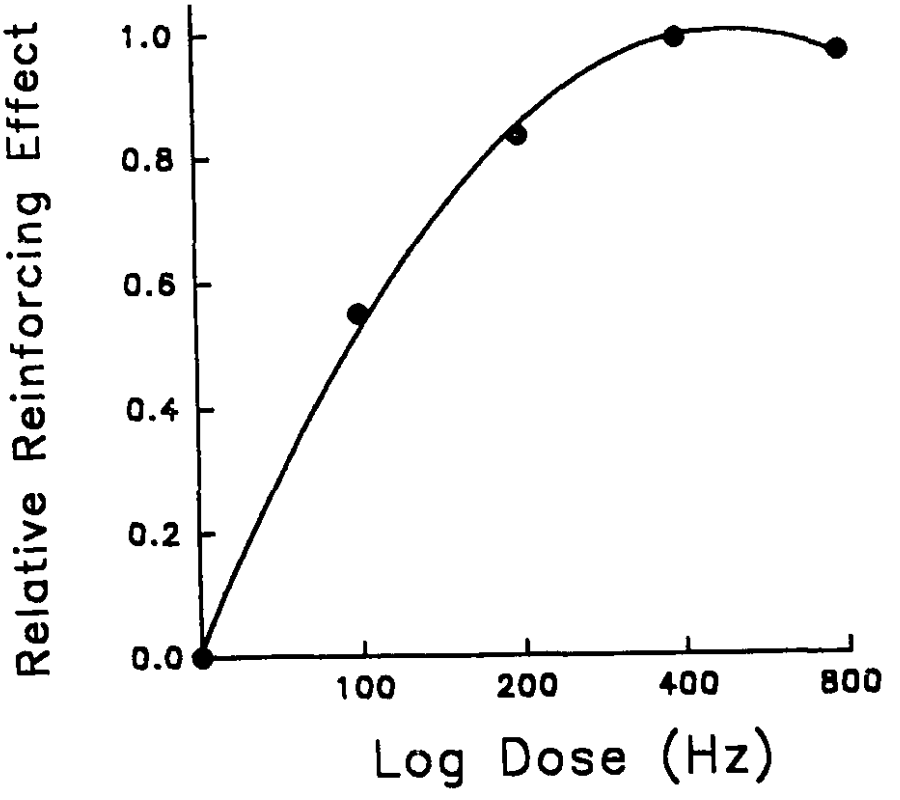


Figure 29. The relationship between relative reinforcing effect and dose of brain stimulation. Relative reinforcing effect was derived from Experiment 2 computing the ratio of break points at each of four doses to the maximally effective break point. The regression equation calculated for the above curve is $Y=0.01066 + 0.60459*X + -0.09107*X^2$, *with an* R^2 *of 0.99917.*

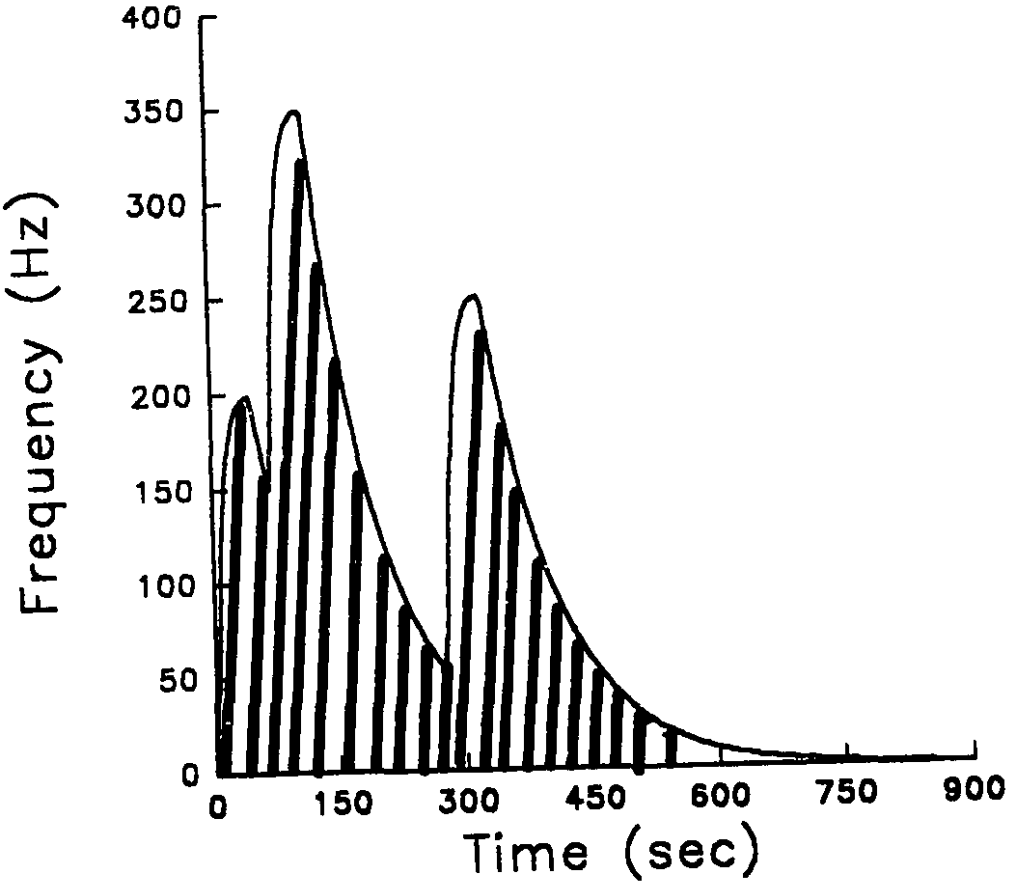


Figure 30. Time course of the changes in pulse frequency of a hypothetical train of brain stimulation generated by the SABS program. The hatched areas represent the total Area Under the Curve (AUC). The AUC was used in calculating the mean pulse frequency and the mean reinforcing effect. Points are sampled once every 30 s.

reinforcing effect of a dose of 100 Hz would be approximately 0.538, whereas a dose of 800 Hz would have a relative reinforcing strength of 0.953.

Although the following discussion of relative reinforcing effects is theoretically derived and the data are generated through an extrapolation analysis of the break point data, the assumptions made about the relative reinforcing effects of stimulation cannot be seriously in error. For example, extrapolation of the fitted curve to the ordinate in Figure 5 (Experiment 2, page 47) gave a break point of 5.675, and extrapolation to the abscissa led to a pulse frequency of 13.9 Hz. In the procedure used in Experiment 2, the first break point was set at FR5, almost identical to that calculated by extrapolation analysis (FR 5.675). Based on the fitted curve in Experiment 2, the minimum pulse frequency that would be required to reach a break point of 5 was 13 Hz. These figures match closely with what is known about minimally effective pulse frequencies (generated from self-stimulation studies) and considering the procedure used to establish the minimally reinforcing effect of stimulation using a PR schedule of reinforcement. For example, in establishing the lowest threshold for reinforcement in brain stimulation, one derives the point at which animals *begin* to respond, whereas the break point describes the point at which animals would *fail* to respond. Of the data generated in this thesis and from Lepore and Franklin (1992), the highest mean unconditioned operant rate emitted for a dose of 0 Hz was 3.8, below what is necessary to reach the first break point (FR5) given the procedure used in Experiment 2. The derived minimally effective pulse frequency (13 Hz) to reach a break point of 5 is also in

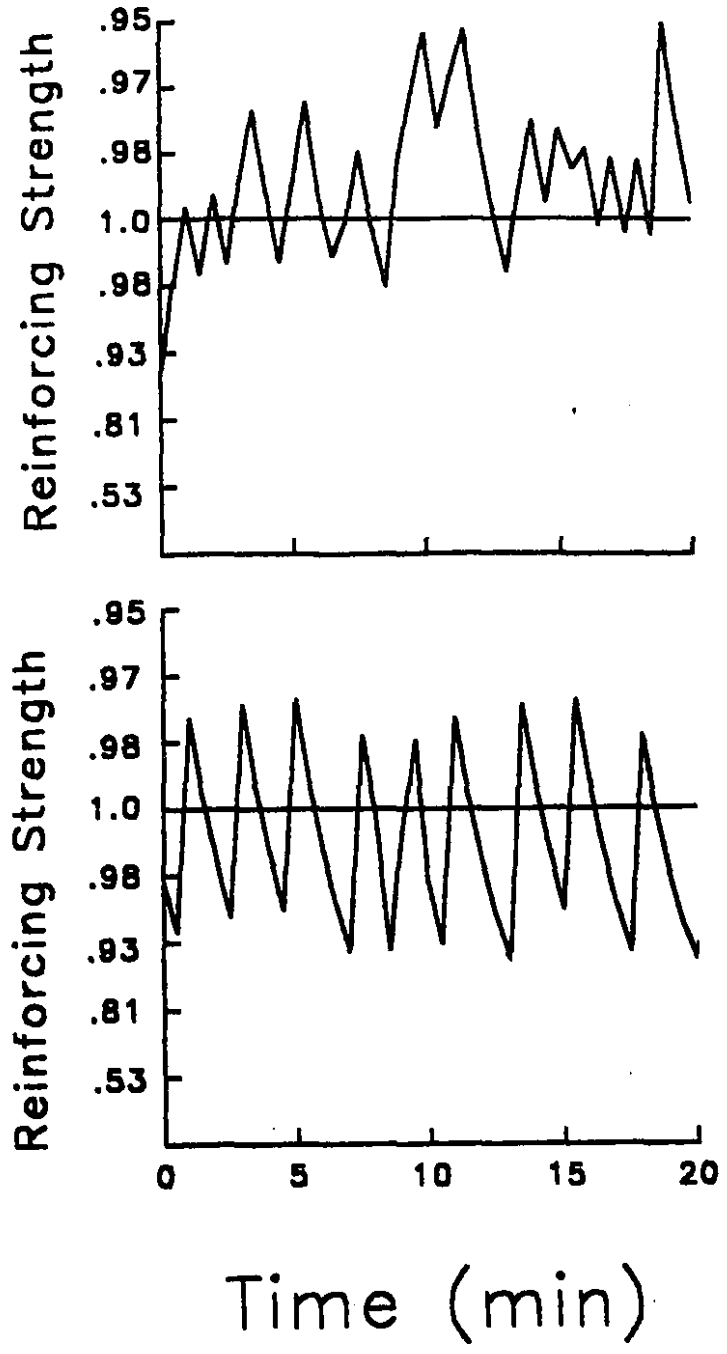


Figure 31. The top panel displays the pulse frequency of continuous brain stimulation maintained over a 20 min session of SABS by a single rat responding for trains with a dose of 200 Hz, an absorption half-time of 1 s and an elimination half-time of 100 s. The bottom panel displays the record of a hypothetical session of SABS generated by the computer at the same stimulation parameters described above. The solid horizontal line in both panels represents the optimal reinforcing effect of stimulation obtained from Figure 29 (500 Hz). Stimulation frequency is sampled once every 30 s.

line with the literature. Gallistel and colleagues (1974, 1981) have demonstrated that the minimum amount of pulses necessary for an animal to *detect* reinforcing brain stimulation is 2 pulses in one second (2 Hz), and the minimally effective frequency to initiate responding on a CRF schedule of reinforcement falls between 2-8 Hz. The minimally effective frequency to reach a break point of 5 should be somewhat higher, and the extrapolated estimate of 13 Hz would seem to be reasonable. In any case, even if the minimally effective pulse frequency is slightly off, it does not significantly affect the shape of the curve from 25 to 800 Hz.

Typically, when animals are maintaining a stable pattern of responding, the frequency fluctuates between 100-150 Hz around the mean. In other words, animals are responding on or almost on the elimination half-life (for a discussion of half-lives, see General Method, page 30). Thus, animals maintaining a mean frequency of 600 Hz will drive pulse frequencies as high as 700-750 Hz, and will let them drop as low as 450-500 Hz. In fact, the mean frequency in SABS is proportional to the total pulse frequency summed over time, or the total Area Under the Curve (AUC) (see Figure 30). By means of a simple substitution, the AUC can be converted to represent the total relative reinforcing effect summed over time. Rather than plotting pulse frequency along the ordinate, the relative reinforcing effect of a SABS train calculated from Figure 29 can be plotted. The purpose of converting frequency to relative reinforcing effect is that one can determine whether animals responding for SABS organize their behavior in such a manner as to maximize the relative

reinforcing effects of frequency during a SABS session. By doing so, it can also give an explanation of why animals maintain mean frequencies above what is considered "optimal" in SABS. Figure 31 compares the performance of one animal responding for SABS against a record of a simulated session of an animal maintaining a mean pulse frequency of 575 Hz and responding on the half-time. This frequency was chosen because extrapolation of the fitted curve from the break point data revealed that the frequency which would maintain maximal responding was 575 Hz. The top panel shows an individual record of an individual animal responding for SABS. As shown, this animals pulse frequency fluctuates above 575 Hz (horizontal line). The mean relative reinforcing effect over this 20 minute session can be calculated for this animal, and comes out to 0.989. The bottom panel shows a simulated record generated by the computer if an animal fluctuated around a mean of the optimal frequency with the typical variability (± 150 Hz). Again, the mean relative reinforcing effect over this 20 minute session can be calculated, and works out to 0.949. Comparing both sessions, it is clear that if an animal maintains the pulse frequency above what is considered "optimal" for SABS, they are generating more reinforcement over an entire session than if they fluctuated symmetrically around the optimal mean. Although not immediately obvious, the reason for this is quite simple. A look at Figure 29 clearly shows that at above 300 Hz, the curve is not that steep, and in fact, appears to asymptote. It does not appear likely that the curve "inverts" at very high frequencies for the following reason. Data generated from the progressive ratio schedule in

Experiment 2 clearly showed that even at the 800 Hz dose, animals consistently maintained higher break points than at 200 Hz. In fact, in one third of the animals tested, break points continued to rise with increasing dose, and in the remainder of the rats, break points were at, or slightly under, the break points maintained by animals responding for a 400 Hz dose. In no cases did break points fall below that obtained for the 200 Hz dose. In contrast, the ascending limb of the dose-response curve, from 25 Hz to 300 Hz, is very steep, with the majority of pulse frequencies falling well below 80% of the maximum reinforcing effectiveness. Thus, if animals maintain an average pulse frequency close to the optimal frequency, the probability that a small delay in responding will cause the relative reinforcing effect to drop off sharply is high. Whereas, if animals maintain pulse frequencies above the "optimal" level, the probability that a delay in responding will cause the relative reinforcing effect to drop off sharply is very low. Considering that the difference between the SABS sessions in Figure 31 represent only a difference of 2-3 responses emitted during the "load-up" phase of SABS, the pay off between the amount of reinforcement and the amount of work is high. That is, for the same maintenance rate of responding (17 responses in each panel of Figure 31), animals receive considerably more reinforcement by maintaining pulse frequency above 575 Hz. Thus, the results of Experiments 1 and 2, and the preceding discussion of the relative reinforcing effects of trains of brain stimulation used in SABS supports the argument that animals organize their behavior in order to maintain an optimal level of rewarding brain stimulation.

The second part of the thesis dealt primarily with the hypothesis that the PPTg mediates the reinforcing effects of brain stimulation and other rewards (Bechara and van der Kooy, 1989; Bechara and van der Kooy, 1992). Experiment 3 examined the effects of QA and NMDA lesions of the PPTg on the acquisition of SABS, Experiment 4 examined the effects of NMDA PPTg-lesions on the maintenance of SABS responding, and Experiment 5 tested the effects of NMDA lesions of the A8 dopamine cell group on the acquisition of SABS. Results from these experiments demonstrated that (1) NMDA, but not QA, lesions of the PPTg severely impaired acquisition of SABS, (2) NMDA lesions of the PPTg significantly suppressed responding in animals that had previously acquired SABS behavior, and (3) that lesions of the caudal portion of the A8 dopamine cell group did not block acquisition of SABS, but did impair the rate at which acquisition occurred. The results support the interpretation that NMDA lesions of the PPTg blocked the reinforcing effects of brain stimulation. Further, that the relatively specific cholinergic excitotoxin, QA, had no effects on SABS acquisition supports the interpretation that non-cholinergic cells of the PPTg are involved in mediating reinforcement (Bechara and van der Kooy, 1992; Olmstead and Franklin, 1992). That PPTg lesions carried out after SABS acquisition could block SABS maintenance is perhaps the strongest finding from these three experiments. In Experiment 4, misplacement of electrodes or electrode failure is a highly unlikely explanation for the impairment in SABS maintenance. Results from that experiment also argue against an interpretation based on a memory impairment produced by PPTg lesions. A

memory impairment hypothesis based on a generalized acquisition learning disorder would predict that, once acquisition had occurred, PPTg lesions would have no effect on responding. Rather, the impairment observed in SABS maintenance is more likely due to an attenuation of the positive reinforcing effects of brain stimulation produced by PPTg lesions. Although the results of Experiment 4 seem at odds with the finding from the Bechara and van der Kooy (1989) experiment that PPTg lesions carried out after food or drug conditioning have no effect on the expression of a place preference, they are in fact perfectly consistent. A place preference is normally demonstrated in a single unreinforced trial (i.e., in extinction). In the Bechara and van der Kooy (1989) experiment, the retention of a morphine place preference was unaffected by PPTg lesions, whereas once extinction of the place preference had occurred, re-acquisition of a morphine place preference was blocked. Although operant responding in the presence of secondary reinforcers can be maintained for a short while before extinguishing, steady and sustained operant responding depends on the availability and presentation of the primary reinforcer. If the primary reinforcer is withheld, or rendered ineffective, the behavior would extinguish. The results of Experiment 4 showed that lesioned animals did respond for brain stimulation when placed in the operant chamber but showed rapid extiction. This argues against an interpretation that a retention-deficit hypothesis could explain the results of Experiment 4, since animals were capable of remembering what was learned prior to the lesion, and is consistent with the findings of Bechara and van der Kooy (1989). The results of these

three experiments, therefore, are consistent with the interpretation that the PPTg mediates the primary reinforcing effects of brain stimulation.

Finally, the impairment in SABS acquisition could not be explained by the spread of excitotoxin ventral, dorsal, or lateral to the PPTg. In delineating a putative common site within the PPTg, only damage to the caudal portion of the PPTg was common in all animals that were impaired in SABS acquisition or maintenance. No other damage within the PPTg, or immediately adjacent to the PPTg was common to all animals. However, histological results from Experiments 3 to 5 indicated that, in all animals impaired in SABS acquisition or maintenance, a significant amount of damage had occurred in the caudal portion of the A8 dopamine cell group bilaterally. And, although the A8 lesions in Experiment 5 did not prevent the acquisition of SABS, they did slow the acquisition rate. Furthermore, a correlation existed between the amount of damage that occurred in the A8 and the degree of impairment in SABS acquisition. It is possible that, had a more complete lesion of the A8 cell group been made, SABS acquisition may have been blocked.

In order to resolve the issue of whether lesions confined to the PPTg, and especially to the putative common site, could block SABS acquisition, in Experiment 6, the effect of electrolytic PPTg lesions on the acquisition of SABS was explored. Results demonstrated that lesions confined to the PPTg did not impair the acquisition of SABS. This demonstrates that the rewarding effects of brain stimulation are still present in lesioned animals, and raises the possibility that the PPTg alone does not mediate the positive reinforcing effects of brain

stimulation. Histological analysis of electrolytic PPTg lesions revealed that, in all animals that had acquired SABS, damage to the putative common area had occurred bilaterally and was complete. Interestingly enough, damage to the A8 dopamine cell group did not occur in any of the lesioned animals. This does not suggest that it is the A8 cell group which mediates the acquisition of SABS, since NMDA lesions carried out in Experiment 5 did not prevent SABS acquisition. However, it does lead to two tentative conclusions. First, damage to both the A8 cell group and the common area within the PPTg must occur in order to completely prevent acquisition and maintenance of SABS. Second, given that A8 lesions alone do not block SABS acquisition (Experiment 5) unless there is concomitant damage to the PPTg, deficits in reinforcement must be occurring as a result of PPTg lesions. To demonstrate that PPTg lesions do lead to deficits in positive reinforcement, Experiment 7 made use of three behavioral tests demonstrated to be capable of detecting and measuring shifts in the reinforcing effects of brain stimulation. The results of Experiment 7 demonstrated that responding rapidly extinguished when reinforcement was withheld in animals with electrolytic PPTg lesions. They did not show positive or negative contrast effects (i.e. showed a flat rate-frequency "curve"), and were severely impaired in responding when the response requirement was increased from fixed-ratio 1 to fixed-ratio 5. These results demonstrate that lesions confined to the PPTg attenuate the positive reinforcing effects of brain stimulation, and, in conjunction with Experiments 3 to 6, confirms that the PPTg does play a role in reward and reinforcement. Moreover, that the centre of the

electrolytic lesions were within the putative common area in the PPTg adds further support to the suggestion that it is the caudal portion of the PPTg that mediates the positive reinforcing effects of brain stimulation.

Although the results from Experiments 3 to 7 support a role for the PPTg in reward and reinforcement processes, several investigators have reported memory deficits produced by PPTg lesions (Dellu et al., 1991; Fujimoto et al., 1989; Fujimoto et al., 1990; Fujimoto et al., 1992). In an attempt to rule out the hypothesis that specific memory deficits result from PPTg lesions, Experiment 8 explored the effects of electrolytic PPTg lesions on the acquisition of win-shift and win-stay learning. Results demonstrated that PPTg lesions impaired efficient performance on both the win-shift and win-stay tasks. However, a response distribution analysis performed on the win-shift and win-stay data revealed that PPTg-lesioned animals were capable of acquiring "shift" and "stay" behavior. Furthermore, the fact that the lesions indiscriminately affected performance on both tasks, and on CPP learning (Bechara and van der Kooy, 1992), argues against a specific memory deficit produced by PPTg lesions. Finally, one important difference noted between the Dellu et al. (1991) experiment and Experiment 8 was that, in Experiment 8, damage was confined to the PPTg whereas in the Dellu et al. (1991) experiment, a significant amount of damage had occurred to the caudal portion of the A8 dopamine cell group. Also, Dellu et al. (1991) did not perform a response distribution analysis, so it is unknown whether quisqualic acid lesioned animals in their experiment were able to learn the appropriate "shift" behavior. In Experiment 8, electrolytic

PPTg-lesioned animals impaired efficient performance on the win-shift and win-stay tasks, but did not impair the ability of the lesioned-animals to learn that "shift" behavior was appropriate for one task and "stay" behavior was appropriate for another. Thus, it is possible that, like brain stimulation (Experiment 7), PPTg lesions block the incentive properties of food. Though not conclusive on their own, these results also support the hypothesis that the PPTg is involved in reward and reinforcement.

One puzzling feature of the PPTg lesion experiments was the difference observed in SABS acquisition between excitotoxin and electrolytic lesions. The different effects of these two types of lesions on SABS acquisition leads to two speculations. One, already alluded to, is that in order to produce the full spectrum of deficits in SABS acquisition, both the PPTg and A8 dopamine cell group must be damaged. The second is simply that differences between the two lesioning procedures (e.g., type of damage produced) might account for the differences observed in SABS acquisition.

One of the major advantages to the electrolytic lesioning technique is that lesions can be confined within a particular cell group, whereas the relatively uncontrollable spread of excitotoxins to neighboring regions plagues the chemical lesioning technique. If the pattern of results obtained with both techniques is the same, conclusions based on the anatomical specificity of an effect are strengthened (Hiroi and White, 1991). In Experiments 6 and 7, the finding that animals were still capable of acquiring SABS demonstrates that the rewarding effects of brain stimulation were still present, but nevertheless,

electrolytic lesions of the PPTg did produce deficits in reinforcement, inconsistent with the hypothesis that lesions of the PPTg impair a specific mechanism of motivation and not the intensity or magnitude of reward (Bechara and van der Kooy, 1992). When measures that are known to be sensitive to shifts in reinforcement magnitude are used (e.g. fixed ratio and incentive contrast tests), animals with electrolytic lesions of the PPTg show severe quantitative impairments in reinforcement. Animals respond poorly on fixed ratio 3 and 5 schedules of reinforcement, and show no incentive contrast, suggesting that lesions of the PPTg may affect the intensity or magnitude of reinforcement.

One advantage to using the chemical lesioning technique is that chemical lesions spare axons of passage whereas electrolytic lesions invariably damage them. For example, experiments injecting procaine, a local anesthetic which acts to prevent axonal conduction in all neural tissue, into the PPTg have demonstrated an attenuation of amphetamine-induced locomotion (Mogenson and Wu, 1988), whereas chemical lesions of the PPTg are without effect on amphetamine-induced locomotion (Olmstead and Franklin, 1992; Swerdlow and Koob, 1987). This suggests that procaine is inhibiting axons of passage to produce an attenuation of amphetamine-induced locomotion, and, that this effect is not mediated by the cells of the PPTg. This raises the question of why lesioning both the cells and axons of passage has less effect than lesioning the cells alone on SABS acquisition. Furthermore, where are those axons originating from and projecting to which would produce this effect on SABS

acquisition, and perhaps more importantly, what kind of signals are they carrying? At the present time, the answer to these questions are unknown. However, one possibility should be considered. As reviewed above (see page 50), the PPTg is bordered by a mass of fibre pathways. One of the most predominant of the fibre pathways is the brachium conjuctivum, which conveys brainstem efferents to various parts of the mid and forebrain regions, and conveys mid and forebrain axons back down into the brainstem nuclei. The brachium conjuctivum borders the PPTg along the medio-ventral axis, and runs along almost the entire length of the PPTg. In the past few years, brainstem sites have not only received attention as possible input and output centres conveying information about rewarding events, but also, has received considerable attention as possible input sites to the forebrain relaying or conveying information about negative affective or aversive events (Bernard et al., 1991; Bernard et al., 1992; Bernard et al., 1993; Huang et al., 1993; Yamamoto, 1993). In particular, the parabrachial nucleus has received increasing attention as a possible site which generates and/or relays signals about aversive or negative affective states to forebrain nuclei (Aguero et al., 1993; Bechara et al., 1993; Huang et al., 1993; Spector et al., 1992; Yamamoto, 1993). For example, Spector et al. (1992) studied the role of the parabrachial nucleus in the formation of a conditioned taste aversion (CTA), and found that lesions of the parabrachial nucleus blocked the development of a CTA to lithium chloride. Aguero et al. (1993) induced a CTA in rats by electrically stimulating the area postrema, and found that lesions of the

parabrachial nucleus blocked the formation of a CTA. Bechara et al. (1993) tested the effects of parabrachial nucleus lesions on the development of a CTA, conditioned place aversion, and on conditioned place preference behaviors produced by morphine. They found that ibotenate lesions of the parabrachial nucleus blocked the CTA and place aversion learning produced by morphine, but had no effects on place preference behavior.

The preceding discussion on CTA is particularly relevant in light of recent publications (Bernard et al., 1991; Bernard et al., 1992; Bernard et al., 1993; Huang et al., 1993) which have demonstrated that (1) efferents from the parabrachial nucleus not only project to the central nucleus of the amygdala, but also, to the ventral pallidum, lateral hypothalamus, and lateral pre-optic area in rats -- all nuclei previously shown to be important for brain stimulation, food, and drug reward, (2) that these efferent axons projecting from the parabrachial nucleus do so via the brachium conjuctivum, and (3) these axons carry information about aversive or negative affective producing events. These three points are particularly relevant when one considers that electrolytic lesions of the PPTg (Experiment 6) damaged not only the cells that make up the PPTg, but also, the axon bundles which border the PPTg, including the brachium conjuctivum.

Previous research has shown that blocking inhibitory or aversive effects associated with brain stimulation or drug rewards can significantly increase the rate at which self-stimulation or self-administration behavior can occur, implying that aversive components of drug or brain stimulation reward suppress

responding. For instance, Hawkins and Pliskoff (1964) have demonstrated that at high current intensities the rate of self-stimulation behavior decreases. It has also been shown that, at high intensities, animals will work to turn off or escape stimulation, even though electrodes are located in "reward" areas (Atrens and Becker, 1975; Atrens et al., 1977). However, delaying and minimizing the aversive effects of high current intensities through the use of partial reinforcement schedules, they have observed increases in self-stimulation behavior at these "aversive" intensities. Lepore and Franklin (1992) have confirmed in animals responding for SABS, that addition of an aversive component of stimulation through a second electrode located in the PAG significantly suppresses responding for SABS.

There is also neurochemical data suggesting that fibres with an inhibitory/suppressive effect on reinforced responding may run through the brachium conjuctivum. Lyness et al. (1980) found that self-administration of amphetamine was significantly increased following lesions of the serotonin-containing raphe system, and Smith et al. (1986) demonstrated that amphetamine self-administration could be severely depressed in animals receiving dietary supplements of tryptophan, a treatment which significantly increases serotonin synthesis (Fernstrom and Wurtman, 1971). Furthermore, Porrino et al. (1989) have demonstrated that pre-treatment with fluoxetine (serotonin reuptake antagonist) significantly reduced amphetamine self-administration, a treatment which also has been demonstrated to significantly reduce self-stimulation behavior (Katz and Carroll, 1977). The raphe nuclei,

which lie immediately ventral and medial to the PPTg, also send heavy serotonergic projections to the forebrain via the brachium conjuctivum (Bernard et al., 1991; Bernard et al., 1993). It is possible that electrolytic lesions of the PPTg (Experiment 6), by disrupting transmission of aversive and/or suppressive signals from the parabrachial or raphe nuclei, release inhibitory control in the forebrain, and facilitate the acquisition of SABS. Thus, electrolytic lesions may simultaneously impair positive reinforcement and reduce the punishing or suppressive effects of brain stimulation so that the net effect on acquisition is weaker than neurotoxin-induced lesions that only reduce positive reinforcement.

In conclusion, the experiments described above have shown that PPTg lesions, either chemically or electrolytic-induced, produce specific reinforcement deficits in animals responding for brain stimulation reward. Even if the PPTg is not the final common substrate underlying the unconditioned rewarding effects of stimuli, it is clear that the traditional mesolimbic dopamine theory of reward will have to be expanded to include the PPTg, and perhaps, other cell nuclei such as the A8 dopamine cell group.

References

Acques, E., E. Carboni, P. Leone, and G. Di Chiara (1989) SCH 23390 blocks drug-conditioned place-preference and place-aversion: anhedonia (lack of reward) or apathy (lack of motivation) after dopamine-receptor blockade? Psychopharmacol. *99*: 151-155.

Aguero, A., M. Arnedo, M. Gallo, and A. Puerto (1993) Lesions of the lateral parabrachial nuclei disrupt aversion learning induced by electrical stimulation of the area postrema. Brain Res. Bull. *30*: 585-592.

Allen, A.R. and R.C. MacPhail (1991) Effects of triadimefon on a multiple schedule of fixed-interval performance: comparison with methylphenidate, d-amphetamine and chlorpromazine. Pharmacol. Biochem. Behav. *40*: 775-780.

Amit, Z. and B.R. Smith (1991) Remoxipride, a specific D2 dopamine antagonist: an examination of its self-administration liability and its effects on d-amphetamine self-administration. Pharmacol. Biochem. Behav. *41*: 259-261.

Arbuthnott, G.W., I.S. Fairbrother, and S.P. Butcher (1990) Brain microdialysis studies on the control of dopamine release and metabolism in vivo. J. Neurosci. Meth. *34*: 73-81.

Atrens, D.M., D.M. Ccbbin, and G. Paxinos (1977) Reward-aversion analysis of rat mesencephalon. Neurosci. Lett. *6*: 197-201.

Atrens, D.M. and F.T. Becker (1975) Assessing the aversiveness of intracranial stimulation. Psychopharmacol. *44*: 159-163.

Aulakh, C.S., B. Ghosh, and S.N. Pradhan (1979) Actions and interactions of cocaine on self-stimulation behavior in rats. Psychopharmacol. *63*: 75-79.

Balster, R.L. and C.R. Schuster (1973) Fixed-interval schedule of cocaine reinforcement: effect of dose and infusion duration. J. Exp. Anal. Beh. *20*: 119-129.

Beach, H.D. (1957) Morphine addiction in rats. Can. J. Psychol. *11*: 104-112.

Bechara, A. and D. van der Kooy (1989) The tegmental pedunculopontine nucleus: A brain-stem output of the limbic system critical for the conditioned place preferences produced by morphine and amphetamine. J. Neurosci. *9*: 3400-3409.

Bechara, A. and D. van der Kooy (1992) A single brain stem substrate mediates the motivational effects of both opiates and food in nondeprived rats but not in deprived rats. Beh. Neurosci. *106*: 351-363.

Bechara, A., G.M. Martin, A. Pridgar, and D. van der Kooy (1993) The parabrachial nucleus: a brain stem substrate critical for mediating the aversive motivational effects of morphine. Beh. Neurosci. *107*: 147-160.

Beckstead, R.M., V.B. Domesick, and W.J.H. Nauta (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Res. *175*: 191-217.

Bedford, J.A., L.P. Bailey, and M.C. Wilson (1978) Cocaine reinforced progressive ratio performance in the rhesus monkey. Pharmacol. Biochem. Behav. *9*: 631-638.

Beninato, M. and R.F. Spencer (1987) A cholinergic projection to the rat substantia nigra from the pedunculopontine tegmental nucleus. Brain Res. *412*: 169-174.

Beninato, M. and R.F. Spencer (1988) The cholinergic innervation of the rat substantia nigra: a light and electron microscopic immunohistochemical study. Exp. Brain Res. *72*: 178-184.

Bernard, J.F., M. Alden, and J.M. Besson (1993) The organization of the efferent projections from the pontine parabrachial area to the amygdaloid complex: a Phaseolus vulgaris leucoagglutinin (PHA-L) study in the rat. J. Comp. Neurol. *329*: 201-229.

Bernard, J.F., J. Carroue, and J.M. Besson (1991) Efferent projections from the external parabrachial area to the forebrain: a Phaesolus vulgaris agglutinin study in the rat. Neurosci. Lett. *122*: 157-160.

Bernard, J.F., G.F. Huang, and J.M. Besson (1992) Nucleus centralis of the amygdala and the globus pallidus ventralis: electrophysiological evidence for an involvement of pain processes. J. Neurophysiol. *68*: 551-569.

Bielajew, C. and P. Shizgal (1986) Evidence implicating descending fibers in self-stimulation of the medial forebrain bundle. J. Neurosci. *6*: 919-929.

Bindra, D. (1968) Neuropsychological interpretation of the effects of drive and incentive-motivation on general activity and instrumental behavior. Psychol. Rev. *75*: 1-22.

Bindra, D. and J. Stewart (1971) *Motivation, 2nd Edition*, Penguin, Baltimore,MD.

Black, R.W. (1968) Shifts in the magnitude of reward and contrast effects in instrumental and selective learning: a reinterpretation. Psychol. Rev. *75*: 114-126.

Blackburn, J.R., A.G. Phillips, A. Jakubovic, and H.C. Fibiger (1986) Increased dopamine metabolism in the nucleus accumbens and striatum following consumption of a nutritive meal but not a palatable non-nutritive saccharin solution. Pharmacol. Biochem. Behav. *25*: 1095-1100.

Blackburn, J.R., A.G. Phillips, A. Jakubovic, and H.C. Fibiger (1989) Dopamine and preparatory behavior: II. A neurochemical analysis. Beh. Neurosci. *103*: 15-23.

Blackburn, J.R., J.G. Pfaus, and A.G. Phillips (1992) Dopamine functions in appetitive behaviors. Prog. Neurobiol. *39*: 247-279.

Blaha, C.D. and P. Winn (1993) Modulation of dopamine efflux in the striatum following cholinergic stimulation of the substantia nigra in intact and peduncuolopontine tegmental nucleus-lesioned animals. J. Neurosci. *13*: 1035-1044.

Bourne, G.W., R. Capek, and B. Esplin (1989) Phencyclidine suppresses hippocampal long-term potentiation through stereospecific activation of phencyclidine receptors. Neuropharmacol. *28*: 49-56.

Bozarth, M.A. (1987a) Neuroanatomical boundaries of the reward-relevant opiate-receptor field in the ventral tegmental area in rats. Brain Res. *414*: 77-84.

Bozarth, M.A. (1987b) Conditioned place preference: a parametric analysis using systemic heroin injections. In *Methods of Assessing the Reinforcing Properties of Abused Drugs*, M.A. Bozarth, ed., pp. 241-273, Springer-Verlag, New York.

Bozarth, M.A., A. Murray, and R.A. Wise (1989) Influence of housing conditions on the acquisition of intravenous heroin and cocaine self-administration. Pharmacol. Biochem. Behav. *33*: 903-907.

Bozarth, M.A. and R.A. Wise (1981a) Intracranial self-administration of morphine into the ventral tegmental area in rats. Life Sci. *28*: 551-555.

Bozarth, M.A. and R.A. Wise (1981b) Heroin reward is dependent on a dopaminergic substrate. Life Sci. *29*: 1881-1886.

Bozarth, M.A. and R.A. Wise (1983) Neural substrates of opiate reinforcement. Prog. Neuro-Psychopharmacol. & Biol. Psychiat. *7*: 569-575.

Bozarth, M.A. and R.A. Wise (1984) Anatomically distinct opiate receptor fields mediate reward and physical dependence. Sci. *244*: 516-517.

Broderick, P.A. (1991) Cocaine: On-line analysis of an accumbens amine neural basis for psychomotor behavior. Pharmacol. Biochem. Behav. *40*: 959-968.

Broekkamp, C.L.E., A.J.J. Pijnenburg, A.R. Cools, and J.M. van Rossum (1975) The effect of microinjections of amphetamine into the neostriatum and the nucleus accumbens on self-stimulation behavior. Psychopharmacol. *42*: 179-183.

Broekkamp, C.L.E., J.H. van den Bogaard, H.J. Heijnen, R.H. Rops, A.R. Cools, and J.M. van Rossum (1976) Separation of inhibiting and stimulating effects of morphine on self-stimulation behaviour by intracellular microinjection. Eur. J. Pharmacol. *36*: 443-446.

Brown, E.E., G.S. Robertson, and H.C. Fibiger (1992) Evidence for conditioned neuronal activation following exposure to a cocaine-paired environment: role of forebrain limbic structures. J. Neurosci. *12*: 4112-4121.

Brudzynski, S., M. Wu, and G.J. Mogenson (1988) Modulation of locomotor activity induced by injections of carbachol into the tegmental pedunculopontine nucleus and adjacent areas in the rat. Brain Res. *451*: 119-125.

Buscher, W., M. Schugens, U. Wagner, and J.P. Huston (1989) Interhemispheric relationship between lateral hypothalamic self-stimulation and the region of the nucleus tegmenti pedunculo-pontinus. Brain Res. *487*: 321-334.

Carboni, E., A. Imperato, L. Perezzani, and G. Di Chiara (1989) Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neurosci. *28*: 653-661.

Carr, G.D. and N.M. White (1986) Anatomical dissociation of amphetamine's rewarding and aversive effects: an intracranial microinjection study. Psychopharmacol. *89*: 340-346.

Catania, A.C. (1968) *Contemporary Research in Operant Behavior*, Scott, Foresman and Company, Glenview, IL..

Cazala, P. and A. Zielinski (1983) A Y-maze test reveals the positively reinforcing properties of electrical stimulation of the mesencephalic central gray area. Brain Res. *273*: 143-146.

Chesselet, M.F. (1984) Presynaptic regulation of neurotransmitter release in the brain. Facts and hypothesis. Neurosci. *12*: 347-375.

Clarke, P.B.S., D.W. Hommer, A. Pert, and L.R. Skirboll (1987) Innervation of substantia nigra neurons by cholinergic afferents from pedunculopontine nucleus in the rat: neuroanatomical and electrophysiological evidence. Neurosci. *23*: 1011-1019.

Clavier, R.M. and C.R. Gerfen (1979) Self-stimulation of the sulcal prefrontal cortex in the rat: Direct evidence for ascending dopaminergic mediation. Neurosci. Lett. *12*: 183-187.

Clements, J.R., D.D. Toth, D.A. Highfield, and S.J. Grant (1991) Glutamate-like immunoreactivity is present within cholinergic neurons of the laterodorsal tegmental and pedunculopontine nuclei. Adv. Exp. Med. Biol. *295*: 127-142.

Clements, J.R. and S. Grant (1990) Glutamate-like immunoreactivity in neurons of the laterodorsal tegmental and pedunculopontine nuclei in the rat. Neurosci. Lett. *120*: 70-73.

Coles, S.K., J.F. Iles, and S. Nicolopoulos-Stournaras (1989) The mesencephalic centre controlling locomotion in the rat. Neurosci. *28*: 149-157.

Colle, L.M. and R.A. Wise (1988) Effects of nucleus accumbens amphetamine on lateral hypothalamic brain stimulation reward. Brain Res. *459*: 361-368.

Collier, H.O.J. (1968) Supersensitivity and dependence. Nature *220*: 228-231.

Corbett, D. (1990) Differences in sensitivity to neuroleptic blockade: medial forebrain bundle versus frontal cortex self-stimulation. Behav. Brain Res. *36*: 91-96.

Corrigall, W.A. (1987) Heroin self-administration: effects of antagonist treatment in lateral hypothalamus. Pharmacol. Biochem. Behav. *27*: 693-700.

Corrigall, W.A. and K.M. Coen (1989) Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacol. *99*: 473-478.

Corrigall, W.A. and F.J. Vaccarino (1988) Antagonist treatment in nucleus accumbens or periaqueductal grey affects heroin self-administration. Pharmacol. Biochem. Behav. *30*: 443-450.

Creed, R.S., D. Denny-Brown, J.C. Eccles, E.G.T. Liddell, and C.S. Sherrington (1932) *Reflex Activity of the Spinal Cord*, Oxford University Press, London.

Crespi, L.P. (1952) Quantitative variation of incentive and performance in the white rat. Am. J. Psychol. *55*: 467-517.

Crowley, T.J. (1987) Clinical issues in cocaine abuse. In *Cocaine: Clinical and Biobehavioral Aspects*, S. Fisher, A. Raskin and E.H. Uhlenhuth, eds., pp. 193-211, Oxford University Press, New York.

Dai, S., W.A. Corrigall, K.M. Coen, and H. Kalant (1989) Heroin self-administration by rats: influence of dose and physical dependence. Pharmacopsychiat. *32*: 1009-1015.

de la Garza, R. and C.E. Johanson (1982) Effects of haloperidol and physostigmine on self-administration of local anesthetics. Pharmacol. Biochem. Behav. *17*: 1295-1299.

Delfs, J.M., L. Schreiber, and A.E. Kelley (1990) Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. J. Neurosci. *10*: 303-310.

Dellu, F., W. Mayo, J. Cherkaoui, M. Le Moal, and H. Simon (1991) Learning disturbances following excitotoxic lesion of cholinergic pedunculo-pontine nucleus in the rat. Brain Res. *544*: 126-132.

Deminiere, J.M., P.V. Piazza, M. Le Moal, and H. Simon (1989) Experimental approach to individual vulnerability to psychostimulant addiction. Neurosci. Biobehav. Rev. *13*: 141-147.

Deneau, G., T. Yanagita, and M.H. Seevers (1969) Self-administration of psychoactive substances by the monkey: a measure of psychological dependence. Psychopharmacol. *87*: 453-457.

Deutch, A.Y., M. Goldstein, F. Baldino,Jr., and R.H. Roth (1988) Telencephalic projections of the A8 dopamine cell group. Ann. NY Acad. Sci. *537*: 27-50.

Deutsch, J.A., P.L. Roll, and F. Wetter (1976) Choice between rewarding brain stimuli of differing length. Behav. Biol. *18*: 369-377.

Deutsch, J.A. and T.E. Albertson (1974) Refractory period and adaptation in prolonged brain reward. Behav. Biol. *11*: 275-279.

Deutsch, J.A. and S.G. Dennis (1975) Adaptation of aversive brain stimulation: effects of pulse frequency. Behav. Biol. *13*: 245-250.

Deutsch, J.A. and R.D. Hawkins (1972) Adaptation as a cause of apparent aversiveness of prolonged rewarding brain stimulation. Behav. Biol. *7*: 285-290.

Di Chiara, G. and A. Imperato (1986) Preferential stimulation of dopamine release in the nucleus accumbens by opiates, alcohol, and barbiturates: studies with transcerebral dialysis in freely moving rats. Ann. NY Acad. Sci. *473*: 367-381.

Di Loreto, S., T. Florio, and E. Scarnati (1992) Evidence that non-NMDA receptors are involved in the excitatory pathway from the pedunculopontine region to nigrostriatal dopaminergic neurons. Exp. Brain Res. *89*: 79-86.

Dole, V.P. and M. Nyswander (1967) Heroin addiction-a metabolic disease. Arch. Int. Med. *120*: 19-24.

Dougherty, J. and R. Pickens (1976) Pharmacokinetics of intravenous cocaine self-injection. In *Cocaine: Chemical, Biological, Clinical, Social and Treatment Aspects*, S.J. Mule, ed., pp. 105-120, CRC Press, Cleveland,OH.

Downs, D.A. and J.H. Woods (1974) Codeine- and cocaine-reinforced responding in rhesus monkeys: effects of dose on response rates under a fixed-ratio schedule. J. Pharmacol. Exp. Ther. *191*: 179-188.

Dunnett, S.B. (1990) Neural transplantation in animal models of dementia. Eur. J. Neurosci. *2*: 567-587.

Dworkin, S.I., G.F. Guerin, C. Co, N.E. Goeders, and J.E. Smith (1988a) Lack on an effect of 6-hydroxydopamine lesions of the nucleus accumbens on intravenous morphine self-administration. Pharmacol. Biochem. Behav. *30*: 1051-1057.

Dworkin, S.I., G.F. Guerin, N.E. Goeders, and J.E. Smith (1988b) Kainic acid lesions of the nucleus accumbens selectively attenuate morphine self-administration. Pharmacol. Biochem. Behav. *29*: 175-181.

Edmonds, D.E., J.R. Stellar, and C.R. Gallistel (1974) Parametric analysis of brain stimulation reward in the rat: II. Temporal summation in the reward system. J. Comp. Physiol. Psychol. *87*: 860-869.

Einhorn, L.C., P.A. Johansen, and F.J. White (1988) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Studies in the ventral tegmental area. J. Neurosci. *8*: 100-112.

Esposito, R.U. and C. Kornetsky (1978) Opioids and rewarding brain stimulation. Neurosci. Biobehav. Rev. *2*: 115-122.

Ettenberg, A., G.F. Koob, and F.E. Bloom (1981) Response artifact in the measurement of neuroleptic-induced anhedonia. Sci. *213*: 357-359.

Ettenberg, A., H.O. Pettit, F.E. Bloom, and G.F. Koob (1982) Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacol. *78*: 204-209.

Ettenberg, A. (1989) Dopamine, neuroleptics and reinforced behavior. Neurosci. Biobehav. Rev. *13*: 105-111.

Ettenberg, A. and C.L. Duvauchelle (1988) Haloperidol blocks the conditioned place preferences induced by rewarding brain stimulation. Beh. Neurosci. *102*: 687-691.

Fallon, J.H. (1988) Topographic organization of ascending dopaminergic projections. Ann. NY Acad. Sci. *537*: 1-9.

Fernstrom, J.D. and R.J. Wurtman (1971) Brain serotonin content: physiological dependence on plasma tryptophan levels. Sci. *173*: 149-151.

Fouriezos, G. and R.A. Wise (1976) Pimozide-induced extinction of intracranial self-stimulation: response patterns rule out motor or performance deficits. Brain Res. *103*: 377-380.

Frank, R.A., S. Martz, and T. Pommering (1988) The effect of chronic cocaine on self-stimulation train-duration thresholds. Pharmacol. Biochem. Behav. *29*: 755-758.

Franklin, K.B.J. (1978) Catecholamines and self-stimulation: reward and performance effects dissociated. Pharmacol. Biochem. Behav. *9*: 813-820.

Franklin, K.B.J. and S.N. McCoy (1979) Pimozide-induced extinction in rats: Stimulus control of responding rules out motor deficit. Pharmacol. Biochem. Behav. *11*: 71-75.

Fujimoto, K.I., M. Yoshida, K. Ikeguchi, and K. Niijima (1989) Impairment of active avoidance produced after destruction of pedunculopontine nucleus areas in the rat. Neurosci. Res. *6*: 321-328.

Fujimoto, K.I., M. Yoshida, K. Ikeguchi, and M. Ogawa (1990) Impairment of passive and active avoidance produced by destruction of the cholinergic projection from the pedunculopontine nucleus to the medial thalamus. Dementia *1*: 65-73.

Fujimoto, K.I., K. Ikeguchi, and M. Yoshida (1992) Impaired acquisition, preserved retention and retrieval of avoidance behavior after destruction of pedunculopontine nucleus areas in the rat. neurosci. res. *13*: 43-51.

Gallistel, C.R. (1974) Note on temporal summation in the reward system. J. Comp. Physiol. Psychol. *87*: 870-875.

Gallistel, C.R., P. Shizgal, and J.S. Yeomans (1981) A portrait of the substrate for self-stimulation. Psychol. Rev. *88*: 228-273.

Gallistel, C.R., M. Boytim, Y. Gomita, and L. Klebanoff (1982) Does pimozide block the reinforcing effect of brain stimulation? Pharmacol. Biochem. Behav. *17*: 769-781.

Gallistel, C.R. and A.J. Davis (1983) Affinity for the dopamine D2 receptor predicts neuroleptic potency in blocking the reinforcing effect of MFB stimulation. Pharmacol. Biochem. Behav. *19*: 867-872.

Gallistel, C.R. and G. Freyd (1987) Quantitative determination of the effects of catecholaminergic agonists and antagonists on the rewarding efficacy of brain stimulation. Pharmacol. Biochem. Behav. *26*: 731-741.

Garcia-Rill, E., C.R. Houser, R.D. Skinner, W. Smith, and D.J. Woodward (1987) Locomotion-inducing sites in the vicinity of the pedunculopontine nucleus. Brain Res. Bull. *18*: 731-738.

Garcia-Rill, E., N. Kinjo, Y. Atsuta, Y. Ishikawa, M. Webber, and R.D. Skinner (1990) Posterior midbrain-induced locomotion. Brain Res. Bull. *24*: 499-508.

Garcia-Rill, E. (1991) The pedunculopontine nucleus. Prog. Neurobiol. *36*: 363-389.

Gardner, E.L., W. Paredes, D. Smith, A. Donner, C. Milling, D. Cohen, and D. Morrison (1988) Facilitation of brain stimulation reward by δ9-tetrahydrocannabinol. Psychopharmacol. *96*: 142-144.

Gardner, E.L. (1992) Cannabinoid interaction with brain reward systems - The neurological basis of cannabinoid abuse. In *Marijuana/Cannabinoids: Neurobiology and Neurophysiology*, L.L. Murphy and A. Bartke, eds., pp. 275-335, CRC Press, Boca Raton, FL.

Gawin, F.H. and E.H. Ellinwood (1988) Cocaine and other stimulants: Actions, abuse and treatment. New Eng. J. Med. *318*: 1173-1182.

Gerber, G.J., M.A. Bozarth, and R.A. Wise (1981) Small-dose intravenous heroin facilitates hypothalamic self-stimulation without response suppression in rats. Life Sci. *28*: 557-562.

Gerber, G.J. and R.A. Wise (1989) Pharmacological regulation of intravenous cocaine and heroin: a variable dose paradigm. Pharmacol. Biochem. Behav. *32*: 527-531.

Gerfen, C.R., M. Herkenham, and J. Thibault (1987) The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and nondopaminergic systems. J. Neurosci. *7*: 3915-3934.

Glimcher, P.W., A.A. Giovino, and B.G. Hoebel (1987) Neurotensin self-injection in the ventral tegmental area. Brain Res. *403*: 147-150.

Gloor, P. (1968) Generalized corticoreticular epilepsies: some considerations on the pathophysiology of generalized bilaterally synchronous spike and wave discharge. Epilepsia *9*: 249-263.

Goeders, N.E., J.D. Lane, and J.E. Smith (1984) Self-administration of methionine enkephalin into the nucleus accumbens. Pharmacol. Biochem. Behav. *20*: 451-455.

Goeders, N.E., S.I. Dworkin, and J.E. Smith (1986) Neuropharmacological assessment of cocaine self-administration into the medial prefrontal cortex. Pharmacol. Biochem. Behav. *24*: 1429-1440.

Goeders, N.E. and J.E. Smith (1987) Intracranial self-administration methodologies. Neurosci. Biobehav. Rev. *11*: 319-329.

Goldberg, S.R., F. Hoffmeister, U.U. Schlichting, and W. Wuttke (1971) A comparison of pentobarbital and cocaine self-administration in rhesus monkeys: effects of dose and fixed parameter. J. Pharmacol. Exp. Ther. *179*: 277-283.

Goldberg, S.R. (1973) Comparable behavior maintained under fixed-ratio and second-order schedules of food presentation, cocaine injection or d-amphetamine injection in the squirrel monkey. J. Pharmacol. Exp. Ther. *186*: 18-30.

Goldberg, S.R. and R.T. Kelleher (1976) Behavior controlled by scheduled injections of cocaine in squirrel and rhesus monkeys. J. Exp. Anal. Beh. *25*: 93-104.

Goldsmith, M. and D. van der Kooy (1988) Separate non-cholinergic descending projections and cholinergic ascending projections from the nucleus tegmenti pedunculopontinus. Brain Res. *445*: 386-391.

Gonzales-Lima, F. and H. Scheich (1985) Ascending reticular activating system in the rat: a 2-deoxyglucose study. Brain Res. *445*: 386-391.

Gonzalez, F.A. and S.R. Goldberg (1977) Effects of cocaine and d-amphetamine on behavior maintained under various schedules of food presentation in squirrel monkeys. J. Pharmacol. Exp. Ther. *201*: 33-43.

Goudie, A.J. (1979) Aversive stimulus properties of drugs. Neuropharmacol. *18*: 971-979.

Gould, E., N.J. Woolf, and L.L. Butcher (1989) Cholinergic projections to the substantia nigra from the pedunculopontine and laterodorsal tegmental nuclei. Neurosci. *28*: 611-623.

Gratton, A., B.J. Hoffer, and G.A. Gerhardt (1988) Effects of electrical stimulation of brain reward sites on release of dopamine in rat: an in vivo electrochemical study. Brain Res. Bull. *21*: 319-324.

Griffiths, R.R., J.D. Findley, J.V. Brady, K. Dolan-Gutcher, and W.W. Robinson (1975) Comparison of progressive-ratio performance maintained by cocaine, methylphenidate and secobarbital. Psychopharmacol. *43*: 81-83.

Groves, P., L. Ryan, M. Diana, and R. Gariano (1988) Neurophysiological consequences of amphetamine administration. NIDA Res. Mono. *90*: 213-222.

Hallanger, A.E., A.I. Levey, H.J. Lee, D.B. Rye, and B.H. Wainer (1987) The origins of cholinergic and other subcortical afferents to the thalamus in the rat. J. Comp. Neurol. *262*: 105-124.

Hammer, R.P.,Jr. (1989) Cocaine alters opiate receptor binding in critical brain reward regions. Synapse *3*: 55-60.

Harfstrandt, A., K. Fuxe, A. Cintra, L.F. Agnati, I. Zini, A.-C. Wilstrom, S. Okret, Z. Yu, M. Goldstein, H. Steinbusch, A. Verhofstad, and J. Gustafsson (1986) Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proc. Natl. Acad. Sci. USA *83*: 9779-9783.

Hawkins, T.D. and S.S. Pliskoff (1964) Brain-stimulation intensity, rate of self-stimulation, and reinforcement strength: an analysis through chaining. J. Exp. Anal. Beh. *7*: 285-288.

Hemby, S.E., G.H. Jones, D.B. Neill, and J.B. Justice,Jr. (1992) 6-hydroxydopamine lesions of the medial prefrontal cortex fail to influence cocaine-induced place conditioning. Behav. Brain Res. *49*: 225-230.

Hernandez, L. and B.G. Hoebel (1988a) Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Sci. *42*: 1705-1712.

Hernandez, L. and B.G. Hoebel (1988b) Feeding and hypothalamic stimulation increase dopamine turnover in the accumbens. Physiol. Behav. *44*: 599-606.

Herridge, P. and M.S. Gold (1988) Pharmacological adjuncts in the treatment of opioid and cocaine addicts. J. Psychoact. Drugs *20*: 233-242.

Himmelsbach, C.K. (1943) Morphine, with reference to physical dependence. Fed. Proc. *2*: 201-203.

Hiroi, N. and N.M. White (1990) The reserpine-sensitive dopamine pool mediates (+)-amphetamine-conditioned reward in the place preference paradigm. Brain Res. *510*: 33-42.

Hiroi, N. and N.M. White (1991) The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. J. Neurosci. *11*: 2107-2116.

Hirsh, R. (1974) The hippocampus and contextual retrieval of information from memory: A theory. Behav. Biol. *12*: 421-444.

Hobson, J.A., R. Lydic, and H.A. Baghdoyan (1986) Evolving concepts of sleep cycle generation: from brain centers to neuronal populations. Behav. Brain Sci. *9*: 371-448.

Hodos, W. (1961) Progressive ratio as a measure of reward strength. Sci. *134*: 943-944.

Hodos, W. (1965) Motivational properties of long durations of rewarding brain stimulation. J. Comp. Physiol. Psychol. *59*: 219-224.

Hodos, W. and G. Kalman (1963) Effects of increment size and reinforcer volume on progressive ratio performance. J. Exp. Anal. Beh. *6*: 387-392.

Hoebel, B.G., A.P. Monaco, L. Hernandez, E.F. Aulisi, B.G. Stanley, and L. Lenard (1983) Self-injection of amphetamine directly into the brain. Psychopharmacol. *81*: 158-163.

Hoebel, B.G. (1985) Brain neurotransmitters in food and drug reward. Am. J. Clin. Nutr. *42*: 1133-1150.

Hoffman, D.C., P.R. Dickson, and R.J. Beninger (1988) The dopamine D2 receptor agonists, quinpirole and bromocriptine produce conditioned place preferences. Prog. Neuro-Psychopharmacol. & Biol. Psychiat. *12*: 315-322.

Hoffmeister, F. (1979) Progressive-ratio performance in the rhesus monkey maintained by opiate infusions. Psychopharmacol. *62*: 181-186.

Hoover, D.G. and D.M. Jacobowitz (1979) Neurochemical and histochemical studies on the effect of lesion of the nucleus cuneiformis on the cholinergic innervation of discrete areas of the rat brain. Brain Res. *197*: 113-122.

Huang, G.F., J.M. Besson, and J.F. Bernard (1993) Intravenous morphine depresses the transmission of noxious messages to the nucleus centralis of the amygdala. Eur. J. Pharmacol. *236*: 449-456.

Hubner, C.B., G.T. Bain, and C. Kornetsky (1987) The combined effects of morphine and d-amphetamine on the threshold for brain stimulation reward. Pharmacol. Biochem. Behav. *28*: 311-315.

Hubner, C.B. and C. Kornetsky (1992) Heroin, 6-acetylmorphine and morphine effects on threshold for rewarding and aversive brain stimulation. J. Pharmacol. Exp. Ther. *260*: 562-567.

Hull, C.L. (1943) *Essentials of Behavior*, Yale University Press, New Haven, CT.

Hunt, T., L. Switzman, and Z. Amit (1985) Involvement of dopamine in the aversive stimulus properties of cocaine in rats. Pharmacol. Biochem. Behav. *22*: 945-948.

Hunt, T., R. Segal, and Z. Amit (1987) Differential involvement of central cholinergic mechanisms in the aversive stimulus properties of morphine and amphetamine. Pharmacol. Biochem. Behav. *28*: 335-339.

Hunt, T. and Z. Amit (1987) Conditioned taste aversion induced by self-administered drugs: paradox revisited. Neurosci. Biobehav. Rev. *11*: 107-130.

Hylden, J.L., H. Hayashi, G.J. Bennett, and R. Dubner (1985) Spinal lamina I neurons projecting to the parabrachial area of the cat midbrain. Brain Res. *336*: 195-198.

Imperato, A., G. Tanda, R. Frau, and G. Di Chiara (1988) Pharmacological profile of dopamine receptor agonists as studied by brain dialysis in behaving rats. J. Pharmacol. Exp. Ther. *245*: 257-264.

Inglis, W.L., J.S. Dunbar, and P. Winn (1993) Barbiturate anaesthesia reduces the neurotoxic effects of quinolinate but not ibotenate in the rat pedunculopontine tegmental nucleus. Neurosci. Lett. *156*: 78-82.

Isaac, W.L., A.J. Nonneman, J. Neisewander, T. Landers, and M.T. Bardo (1989) Prefrontal cortex lesions differentially disrupt cocaine-reinforced conditioned place preference but not conditioned taste aversion. Beh. Neurosci. *103*: 345-355.

Jackson, A. and A.R. Crossman (1983) Nucleus tegmenti pedunculopontinus: efferent connections with special reference to the basal ganglia, studied in the rat by anterograde and retrograde transport of horseradish peroxidase. Neurosci. *10*: 725-765.

Jarrard, L.E. (1993) On the role of the hippocampus in learning and memory in the rat. Behav. Neural Biol. *60*: 9-26.

Johanson, C.E. (1982) Behavior maintained under fixed-interval and second-order schedules of cocaine or pentobarbital in rhesus monkeys. J. Pharmacol. Exp. Ther. *221*: 384-393.

Johanson, C.E. and T. Aigner (1981) Comparison of the reinforcing properties of cocaine and procaine in rhesus monkeys. Pharmacol. Biochem. Behav. *15*: 49-53.

Kalivas, P.W., E. Widerlov, D. Stanley, G. Breese, and A.J. Prange,Jr. (1983) Enkephalin action on the mesolimbic system: a dopamine-dependent and dopamine-independent increase in locomotor activity. J. Pharmacol. Exp. Ther. *227*: 1-9.

Katayama, Y., D.S. DeWitt, D.P. Becker, and R.L. Hayes (1984) Behavioral evidence for a cholinoceptive pontine inhibitory area: descending control of spinal motor output and sensory input. Brain Res. *269*: 241-262.

Katz, R.J. and B.J. Carroll (1977) Intracranial reward after Lilly 110140 (Fluoxetine HCl): evidence for an inhibitory role for serotonin. *51*: 189-193.

Keesey, R.E. (1964) Duration of stimulation and the reward properties of hypothalamic stimulation. J. Comp. Physiol. Psychol. *58*: 201-207.

Keith, J.R. and J.W. Rudy (1990) Why NMDA-receptor-dependent long-term potentiation may not be a mechanism of learning and memory: Reappraisal of the NMDA-receptor blockade strategy. Psychobiol. *18*: 251-257.

Kelleher, R.T. (1976) Characteristics of behavior controlled by scheduled injections of drugs. Pharmacol. Rev. *307*: 323.

Kelsey, J.E., W.A. Carlezon,Jr., and W.A. Falls (1989) Lesions of the nucleus accumbens in rats reduce opiate reward but do not alter context-specific opiate tolerance. Beh. Neurosci. *103*: 1327-1334.

King, L.J. (1974) A sensory-integrative approach to schizophrenia. Am. J. Occup. Ther. *28*: 529-536.

Kiser, R.S., R.M. Lebovitz, and D.C. German (1978) Anatomic and pharmacologic differences between two types of aversive midbrain stimulation. Brain Res. *155*: 331-342.

Kling, J.W., D.G. Brownlow, S.R. Menich, and C.A. Velozo (1979) Pulse-frequency analysis of septal and hypothalamic reinforcement effects. Physiol. Psychol. *7*: 422-426.

Koob, G.F., N.H. Spector, and J.L. Meyerhoff (1975) Effects of heroin on lever pressing for intracranial self-stimulation, food and water in rat. Psychopharmacol. *42*: 231-234.

Koob, G.F. (1977) Incentive shifts in intracranial self-stimulation produced by different series of stimulus intensity presentations. Physiol. Behav. *18*: 131-135.

Koob, G.F. (1986) Separate neurochemical substrates for cocaine and heroin reinforcement. In *Quantitative Analyses of Behavior, Volume 7*, R.M. Church, M.L. Commons, J. Steilar and A.R. Wagner, eds., Lawrence Erlbaum, Hillsdale,NJ.

Koob, G.F., H.T. Le, and I. Creese (1987a) The D1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. Neurosci. Lett. *79*: 315-320.

Koob, G.F., F.J. Vaccarino, M. Amalric, and F.E. Bloom (1987b) Positive reinforcement properties of drugs: search for neural substrates. In *Brain Reward Systems and Abuse*, J. Engel and L. Oreland, eds., pp. 35-50, Raven Press, New York.

Koob, G.F., F.J. Vaccarino, M. Amalric, and N.R. Swerdlow (1987c) Neural substrates for cocaine and opiate reinforcement. In *Cocaine: Clinical and Biobehavioral Aspects*, S. Fisher, A. Raskin and E.H. Uhlenhuth, eds., pp. 80-108, Oxford University Press, New York.

Koob, G.F. and N.R. Swerdlow (1988) The functional output of the mesolimbic dopamine system. Ann. NY Acad. Sci. *537*: 216-227.

Kornetsky, C., R.U. Esposito, S. McLean, and J.O. Jacobson (1979) Intracranial self-stimulation thresholds. A model for the hedonic effects of drugs of abuse. Arch. Gen. Psychiat. *36*: 289-292.

Kornetsky, C. and G. Bain (1987) Neuronal basis for hedonic effects of cocaine and opiates. In *Cocaine: Clinical and Biobehavioral Aspects*, S. Fisher, A. Raskin and E.H. Uhlenhuth, eds., pp. 66-79, Oxford University Press, New York.

Kozel, N.J. and E.H. Adams (1986) Epidemiology of drug abuse: an overview. Sci. *234*: 970-974.

Lee, H.J., D.B. Rye, A.E. Hallanger, A.I. Levey, and B.H. Wainer (1988) Cholinergic vs. non-cholinergic efferents from the mesopontine tegmentum to the extrapyramidal motor system nuclei. J. Comp. Neurol. *275*: 469-492.

Lemaire, G.A. and R.A. Meisch (1984) Pentobarbital self-administration in rhesus monkeys: drug concentration and fixed ratio size interactions. J. Exp. Anal. Beh. *42*: 37-49.

Lemaire, G.A. and R.A. Meisch (1991) Relative reinforcing effects of pentobarbital solutions orally administered by rhesus monkeys under fixed-ratio and signalled DRL schedules. Psychol. Record *41*: 551-583.

Lepore, M. (1990) *Self-administration of brain-stimulation: An exploration of a model of drug self-administration*, M.Sc. Thesis submitted to McGill University, Montreal, Quebec.

Lepore, M. and K.B.J. Franklin (1991) Modelling drug self-administration with self-administration of brain stimulation (SABS): stability of SABS with partial reinforcement schedules. Soc. Neurosci. Abs. *17*: 1238.(Abstract)

Lepore, M. and K.B.J. Franklin (1992) Modelling drug kinetics with brain stimulation:dopamine antagonists increase self-stimulation. Pharmacol. Biochem. Behav. *41*: 489-496.

Lepore, M. and K.B.J. Franklin (1992) Effects of aversive PAG stimulation on self-stimulation and self-administration of brain stimulation. Soc. Neurosci. Abst. *18*: 710.

Levitt, R.A., J.H. Baltzer, T.M. Evers, D.J. Stilwell, and J.E. Furby (1977) Morphine and shuttle-box self-stimulation in the rat: A model for euphoria. Psychopharmacol. *54*: 307-311.

Lidsky, T.I., C. Manetto, and J.S. Schneider (1985) Consideration of sensory factors involved in motor functions of the basal ganglia. Brain Res. Rev. *9*: 133-146.

Liebman, J.M., D.J. Mayer, and J.C. Liebeskind (1973) Self-stimulation loci in the midbrain central gray matter of the rat. Behav. Biol. *9*: 299-306.

Liebman, J.M. and D.S. Segal (1977) Differential effects of morphine and d-amphetamine on self-stimulation from closely adjacent regions in rat midbrain. Brain Res. *136*: 103-117.

Lierman, T.L.,ed. (1987) *Building a Healthy America*, Mary Ann Liebert, New York.

Lindvall, O., A. Björklund, and I. Divac (1978) Organization of catecholamine neurons projecting to the frontal cortex in the rat. Brain Res. *142*: 1-24.

Ljungberg, T. (1990) Differential attenuation of water intake and water-rewarded operant responding by repeated administration of haloperidol and SCH 23390 in the rat. Pharmacol. Biochem. Behav. *35*: 111-115.

Lynch, M.R. and R.A. Wise (1985) Relative effectiveness of pimozide, haloperidol and trifluoperazine on self-stimulation rate-intensity functions. Pharmacol. Biochem. Behav. *23*: 777-780.

Lyness, W.H., N.M. Friedle, and K.E. Moore (1980) Increased self-administration of d-amphetamine after destruction of 5-hydroxytryptaminergic neurons. Pharmacol. Biochem. Behav. *12*: 937-941.

Mackey, W.B. and D. van der Kooy (1985) Neuroleptics block the positive reinforcing effects of amphetamine but not of morphine as measured by place conditioning. Pharmacol. Biochem. Behav. *22*: 101-105.

Major, R. and N.M. White (1977) Memory facilitation by self-stimulation reinforcement mediated by the nigro-neostriatal bundle. Physiol. Behav. *20*: 723-733.

Marquis, K.L., M.G. Webb, and J.E. Moreton (1989) Effects of fixed ratio size and dose on phencyclidine self-administration by rats. Psychopharmacol. *97*: 179-182.

Mason, P.A. and P.M. Milner (1985) Short- and long-term summation characteristics of electrical self-stimulation reward. Behav. Brain Res. *18*: 223-231.

McDonald, R.J. and N.M. White (1993) A triple dissociation of memory systems: Hippocampus, amygdala, and dorsal striatum. Beh. Neurosci. *107*: 3-22.

McGinty, D.J. and R. Drucker-Colin (1982) Sleep mechanisms: biology and control of REM sleep. Int. Rev. Neurobiol. *23*: 391-436.

Millar, J., J.A. Stamford, Z.L. Kruk, and R.M. Wightman (1985) Electrochemical,pharmacological and electrophysiological evidence of rapid dopamine release and removal in the rat caudate nucleus following electrical stimulation of the median forebrain bundle. Eur. J. Pharmacol. *109*: 341-348.

Milner, P.M. (1978) Models of motivation and reinforcement. In *Brain-Stimulation Reward*, A. Wauquier and E.T. Rolls, eds., North-Holland Publishing Company, Amsterdam.

Mogenson, G.J., D.L. Jones, and C.Y. Yim (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog. Neurobiol. *14*: 69-97.

Mogenson, G.J., M. Wu, and C.T. Tsai (1989) Subpallidal-pedunculopontine projections but not subpallidal-mediodorsal thalamus projections contribute to spontaneous exploratory locomotor activity. Brain Res. *485*: 396-398.

Mogenson, G.J. and M. Wu (1988) Differential effects on locomotor activity of injections of procaine into mediodorsal thalamus and pedunculopontine nucleus. Brain Res. Bull. *20*: 241-246.

Mora, F. (1978) The neurochemical substrates of prefrontal cortex self-stimulation: A review and an interpretation of some recent data. Life Sci. *22*: 919-930.

Mora, F. and R.D. Myers (1977) Brain self-stimulation: Direct evidence for the involvement of dopamine in the prefrontal cortex. Sci. *197*: 1387-1389.

Morency, M.A. and R.J. Beninger (1986) Dopaminergic substrates of cocaine-induced place conditioning. Brain Res. *399*: 33-41.

Moreton, J.E., R.A. Meisch, L. Stark, and T. Thompson (1977) Ketamine self-administration by the rhesus monkey. J. Pharmacol. Exp. Ther. *203*: 303-309.

Moriizumi, T., Y. Nakamura, H. Tokuno, Y. Kitao, and M. Kudo (1988) Topographic projections from the basal ganglia to the nucleus tegmenti pedunculopontinus pars compacta of the cat with special reference to pallidal projections. Exp. Brain Res. *71*: 298-306.

Morris, R.G.M., P. Garrud, J.N.P. Rawlins, and J. O'Keefe (1982) Place navigation impaired in rats with hippocampal lesions. Nature *297*: 681-683.

Morse, W.H. (1966) Intermittent reinforcement. In *Operant Behavior: Areas of Research and Application*, W.K. Honig, ed., pp. 52-108, Appleton-Century-Croft, New York.

Morse, W.H. and R.T. Kelleher (1977) Determinants of reinforcement and punishment. In *Handbook of Operant Conditioning*, W.K. Honig and J.E.R. Staddon, eds., pp. 174-200, Prentice Hall, Engelwood Cliffs, NJ.

Nauta, W.J.H., G.P. Smith, R.L.M. Faull, and V.B. Domesick (1978) Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. Neurosci. *3*: 385-401.

Neff, N.H., M. Parenti, S. Gentleman, and M.C. Olianas (1981) Modulation of dopamine receptors by opiates. In *Apomorphine and other Dopaminimimetics, Vol.1: Basic Pharmacology*, G.L. Gessa and G.U. Corsini, eds., pp. 193-200, Raven Press, New York.

Neill, D.B., L.A. Peay, and M.S. Gold (1978) Identification of a subregion within rat neostriatum for the dopaminergic modulation of lateral hypothalamic self-stimulation. Brain Res. *153*: 515-528.

Newman, D.B. (1985) Distinguishing rat brainstem reticulospinal nuclei by neuronal morphology. II. Pontine and mesencephalic nuclei. J. Hirnforsch. *26*: 385-418.

Nicolaysen, L.C., M. Ikeda, J.B. Justice,Jr., and D.B. Neill (1988) Dopamine release at behaviorally relevant parameters of nigrostriatal stimulation: effects of pulse frequency. Brain Res. *460*: 50-59.

Niedermeyer, E. (1982) Petit mal, primary glueralized epilepsy and sleep. In *Sleep and Epilepsy*, M.B. Sterman, M.N. Shouse and P. Passouant, eds., pp. 191-207, Academic Press, New York.

Niijima, K. and M. Yoshida (1988) Activation of mesencephalic dopamine neurons by chemical stimulation of the nucleus tegmenti pedunculopontinus pars compacta. Brain Res. *451*: 163-171.

O'Keefe, J. and A. Speakman (1987) Single unit activity in the rat hippocampus during a spatial memory task. Exp. Brain Res. *68*: 1-27.

O'Keefe, J.A. and L. Nadel (1978) *The Hippocampus as a Cognitive Map*, Oxford University Press, London.

Oades, R.D. and G.M. Halliday (1987) Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Res. Rev. *12*: 117-165.

Olds, J. and P. Milner (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J. Comp. Physiol. Psychol. *47*: 419-427.

Olds, M.E. (1982) Reinforcing effects of morphine in the nucleus accumbens. Brain Res. *237*: 429-440.

Olmstead, M.C. and K.B.J. Franklin (1992) Lesions of the pedunculopontine tegmental nucleus block drug-induced reinforcement but not amphetamine-induced motor activity. Soc. Neurosci. Abs. *18*: 364.(Abstract)

Olton, D.S., J.T. Becker, and G.E. Handelman (1979) Hippocampus, space, and memory. Beh. Brain Sci. *2*: 313-365.

Olton, D.S. and B.C. Papas (1979) Spatial memory and hippocampal function. Neuropsychol. *17*: 669-682.

Olton, D.S. and R.J. Samuelson (1976) Remembrance of places passed: Spatial memory in rats. J. Exp. Psychol. *2*: 97-116.

Packard, M.G., R. Hirsh, and N.M. White (1989) Differential effects of fornix and caudate nucleus lesions on two radial maze tasks: evidence for multiple memory systems. J. Neurosci. *9*: 1465-1472.

Parent, A. (1990) Extrinsic connections of the basal ganglia. TINS *13*: 254-258.

Paxinos, G. and C. Watson (1986) *The Rat Brain in Stereotaxic Coordinates, 2nd Edition*, Academic Press, Inc., New York.

Pettit, H.O. and J.B. Justice,Jr. (1989) Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis. Pharmacol. Biochem. Behav. *34*: 899-904.

Pettit, H.O. and J.B. Justice,Jr. (1991) Effect of cocaine self-administration behavior and dopamine levels in the nucleus accumbens. Brain Res. *539*: 94-102.

Phillips, A.G., S.M. Brooke, and H.C. Fibiger (1975) Effects of amphetamine isomers and neuroleptics on self-stimulation from the nucleus accumbens and dorsal noradrenergic bundle. Brain Res. *85*: 13-22.

Phillips, A.G., F. Mora, and E.T. Rolls (1979) Intracranial self-stimulation in orbitofrontal cortex and caudate nucleus of rhesus monkey: Effects of apomorphine, pimozide, and spiroperidol. Psychopharmacol. *62*: 79-82.

Phillips, A.G., C.L.E. Broekkamp, and H.C. Fibiger (1983a) Strategies for studying the neurochemical substrates of drug reinforcement in rodents. Prog. Neuro-Psychopharmacol. & Biol. Psychiat. *7*: 585-590.

Phillips, A.G., F.G. LePiane, and H.C. Fibiger (1983b) Dopaminergic mediation of reward produced by direct injection of enkephalin into the ventral tegmental area of the rat. Life Sci. *33*: 2505-2511.

Phillips, A.G., C.D. Blaha, and H.C. Fibiger (1989) Neurochemical correlates of brain-stimulation reward measured by ex vivo and in vivo analysis. Neurosci. Biobehav. Rev. *13*: 99-104.

Phillips, A.G. and H.C. Fibiger (1987) Anatomical and neurochemical substrates of drug reward determined by the conditioned place preference technique. In *Methods of Assessing the Reinforcing Properties of Abused Drugs*, M.A. Bozarth, ed., pp. 275-289, Springer-Verlag, New York.

Phillips, A.G. and H.C. Fibiger (1990) Role of reward and enhancement of conditioned reward in persistence of responding for cocaine. Behav. Pharmacol. *1*: 269-282.

Phillips, A.G. and F.G. LePiane (1980) Reinforcing effects of morphine microinjection into the ventral tegmental area. Pharmacol. Biochem. Behav. *12*: 965-968.

Phillips, A.G. and F.G. LePiane (1982) Reward produced by microinjection of (D-Ala2),Met5-enkephalinamide into the ventral tegmental area. Behav. Brain Res. *5*: 225-229.

Phillipson, O.T. and A.C. Griffiths (1985) The topographic order of inputs to nucleus accumbens in the rat. Neurosci. *16*: 275-296.

Pickens, R. and W.C. Harris (1968) Self-administration of d-amphetamine by rats. Psychopharmacol. *12*: 158-163.

Pickens, R. and T. Thompson (1968) Cocaine-reinforced behavior in rats: effects of reinforcement magnitude and fixed-ratio size. J. Pharmacol. Exp. Ther. *161*: 122-129.

Porrino, L.J., N.L. Goodman, and L.G. Sharpe (1989) Intravenous self-administration of the indirect dopaminergic agonist amfonelic acid by rats. Pharmacol. Biochem. Behav. *31*: 623-626.

Porrino, L.J., M.C. Ritz, N.L. Goodman, L.G. Sharpe, M.J. Kuhar, and S.R. Goldberg (1989) Differential effects of the pharmacological manipulation of serotonin systems on cocaine and amphetamine self-administration. Life Sci. *45*: 1529-1535.

Ramsey, N.F. and J.M. van Ree (1991) Intracerebroventricular naltrexone treatment attenuates acquisition of intravenous cocaine self-administration in rats. Pharmacol. Biochem. Behav. *40*: 807-810.

Richardson, N.R. and D.C.S. Roberts (1991) Fluoxetine pretreatment reduces breaking points on a progressive ratio schedule reinforced by intravenous cocaine self-administration in the rat. Life Sci. *49*: 833-840.

Risner, M.E. and B.E. Jones (1976) Characteristics of unlimited access to self-administered stimulant infusions in dogs. Biol. Psychiat. *11*: 625-634.

Robbins, T.W. and G.F. Koob (1978) Pipradol enhances reinforcing properties of stimuli paired with brain stimulation. Pharmacol. Biochem. Behav. *8*: 219-222.

Roberts, D.C.S., M.E. Corcoran, and H.C. Fibiger (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacol. Biochem. Behav. *6*: 615-620.

Roberts, D.C.S. (1989) Breaking points on a progressive ratio schedule reinforced by intravenous apomorphine increase daily following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol. Biochem. Behav. *32*: 43-47.

Roberts, D.C.S., E.A. Loh, and G. Vickers (1989) Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. Psychopharmacol. *97*: 535-538.

Roberts, D.C.S. and G.F. Koob (1982) Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. Pharmacol. Biochem. Behav. *17*: 901-904.

Roberts, D.C.S. and G. Vickers (1984) Atypical neuroleptics increase self-administration of cocaine: an evaluation of a behavioral screen for antipsychotic activity. Psychopharmacol. *82*: 135-139.

Roberts, D.C.S. and K.A. Zito (1987) Interpretation of lesion effects on stimulant self-administration. In *Methods of Assessing the Reinforcing Properties of Abused Drugs*, M.A. Bozarth, ed., pp. 87-103, Springer-Verlag, New York.

Robertson, A. and G.J. Mogenson (1979) Facilitation of self-stimulation of the prefrontal cortex in rats following chronic administration of spiroperidol or amphetamine. Psychopharmacol. *65*: 149-154.

Rolls, E.T. and S.J. Cooper (1973) Activation of neurones in the prefrontal cortex by brain-stimulation reward in the rat. Brain Res. *60*: 351-368.

Rompre, P.P. and R.A. Wise (1989) Behavioral evidence for midbrain dopamine depolarization inactivation. Brain Res. *477*: 152-156.

Rothman, R.B. (1990) High affinity dopamine reuptake inhibitors as potential cocaine antagonists: A strategy for drug development. Life Sci. *46*: 17-21.

Rugg, E.L., J.S. Dunbar, M. Latimer, and P. Winn (1992) Excitotoxic lesions of the pedunculopontine tegmental nucleus of the rat. I. Comparison of the effects of various excitotoxins, with particular reference to the loss of immunohistochemically identified cholinergic neurons. Brain Res. *589*: 181-193.

Rye, D.B., C.B. Saper, H.J. Lee, and B.H. Wainer (1987) Pedunculopontine tegmental nucleus of the rat: Cytoarchitecture, cytochemistry, and some extrapyramidal connections of the mesopontine tegmentum. J. Comp. Neurol. *259*: 483-528.

Sakai, K. (1980) Some anatomical and physiological properties of pontomesencephalic tegmental neurons with special reference to the PGO waves and postural atonia during paradoxical sleep in the cat. In *The Reticular Formation Revisited*, J.A. Hobson and M.A.B. Brazier, eds., pp. 419-442, Raven Press, New York.

Saper, C.B., L.W. Swanson, and W.M. Cowan (1979) An autoradiographic study of the efferent connections of the lateral hypothalamic area in the rat. J. Comp. Neurol. *183*: 689-706.

Saper, C.B. and A.D. Loewy (1982) Projections of the pedunculopontine tegmental nucleus in the rat: evidence for additional extrapyramidal circuitry. Brain Res. *252*: 367-372.

Schaeffer, G.J. and R.P. Michael (1988) An analysis of the effects of amphetamine on brain self-stimulation behavior. Behav. Brain Res. *29*: 93-101.

Schaeffer, G.J. and R.P. Michael (1980) Acute effects of neuroleptics on brain self-stimulation thresholds in rats. Psychopharmacol. *67*: 9-15.

Schwartz, A.S. and P.L. Marchok (1974) Depression of morphine-seeking behaviour by dopamine inhibition. Nature *248*: 257-258.

Semba, K. and H.C. Fibiger (1992) Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: A retro- and antero-grade transport and immunohistochemical study. J. Comp. Neurol. *323*: 387-410.

Shapiro, M.L. and C. O'Conner (1992) N-methyl-D-aspartate receptor antagonist MK-801 and spatial memory representation: Working memory is impaired in an unfamiliar environment but not in a familiar environment. Beh. Neurosci. *106*: 604-612.

Shizgal, P., C. Bielajew, and I. Kiss (1980) Anodal hyperpolarization block technique provides evidence for rostro-caudal conduction of reward related signals in the medial forebrain bundle. Soc. Neurosci. Abs. *6*: 422.

Shizgal, P. (1989) Toward a cellular analysis of intracranial self-stimulation: contributions of collision studies. Neurosci. Biobehav. Rev. *13*: 81-90.

Siegel, J. (1979) Behavioral functions of the reticular formation. Brain Res. Rev. *1*: 69-105.

Skinner, B.F. (1938) *The Behavior of Organisms: An Experimental Analysis*, Appleton-Century-Crofts, New York.

Skinner, B.F. (1968a) On the rate of formation of a conditioned reflex. In *Contemporary Research in Operant Behavior*, A.C. Catania, ed., Scott, Foresman and Company, Glenview, IL..

Skinner, B.F. (1968b) Are theories of learning necessary? In *Contemporary Research in Operant Behavior*, A.C. Catania, ed., Scott, Foresman and Company, Glenview, IL..

Smith, F.L., D.S.L. Yu, D.G. Smith, A.P. Leccese, and W.H. Lyness (1986) Dietary tryptophan supplements attenuate amphetamine self-administration in the rat. Pharmacol. Biochem. Behav. *25*: 849-855.

Sorenson, C.A., L.A. Raskin, and Y. Suh (1991) The effects of prenatal nicotine on radial-arm maze performance in rats. Pharmacol. Biochem. Behav. *40*: 991-993.

Spann, B.M. and I. Grofova (1992) Cholinergic and non-cholinergic neurons in the rat pedunculopontine tegmental nucleus. Anat. Embryol. *186*: 215-227.

Speakman, A. and J. O'Keefe (1990) Hippocampal complex spike cells do not change their place fields if the goal is moved within a cue controlled environment. Eur. J. Neurosci. *2*: 544-555.

Spealman, R.D., S.R. Goldberg, R.T. Kelleher, D.M. Goldberg, and J.P. Charlton (1977) Some effects of cocaine and two cocaine analogs on schedule-controlled behavior of squirrel monkeys. J. Pharmacol. Exp. Ther. *202*: 500-509.

Spealman, R.D. and S.R. Goldberg (1978) Drug self-administration by laboratory animals: control by schedules of reinforcement. Ann. Rev. Pharmacol. Toxicol. *18*: 313-339.

Spear, D.J. and J.L. Katz (1991) Cocaine and food as reinforcers: effects of reinforcer magnitude and response requirement under second-order fixed-ratio and progressive-ratio schedules. J. Exp. Anal. Beh. *56*: 261-275.

Spyraki, C., H.C. Fibiger, and A.G. Phillips (1982) Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions. Brain Res. *253*: 185-193.

Steininger, T.L., D.B. Rye, and B.H. Wainer (1992) Afferent projections to the cholinergic pedunculopontine tegmental nucleus and adjacent midbrain extrapyramidal area in the albino rat. I. Retrograde tracing studies. J. Comp. Neurol. *321*: 515-543.

Steriade, M. (1980) State-dependent changes in the activity of rostral reticular and thalamocortical elements. In *The Brainstem Core: Sensorinator Integration and Behavioral State Control Neurosci. Res. Prog. Bull*, J.A. Hobson and A.B. Scheibel, eds., pp. 83-91, MIT Press, Cambridge.

Stewart, J. and R. Eikelboom (1987) Conditioned drug effects. In *New Directions in Behavioral Pharmacology*, L. Iversen, S. Iversen and S. Snyder, eds., pp. 1-57, Plenum Press, New York.

Stewart, J. and R.A. Wise (1992) Reinstatement of heroin self-administration habits: morphine prompts and naltrexone discourages renewed responding after extinction. Psychopharmacol. *108*: 79-84.

Stolerman, I.P. (1985) Motivational effects of opioids: evidence on the role of endorphins in mediating reward or aversion. Pharmacol. Biochem. Behav. *23*: 877-881.

Strecker, R.E., D.C.S. Roberts, and G.F. Koob (1982) Apomorphine-induced facilitation of intracranial self-stimulation following dopamine denervation of the nucleus accumbens. Pharmacol. Biochem. Behav. *17*: 1015-1018.

Stretch, R., G.J. Gerber, and S.M. Wood (1971) Factors affecting behavior maintained by response-contingent intravenous infusions of amphetamine in squirrel monkeys. Can. J. Physiol. Pharmacol. *49*: 581-589.

Sugimoto, T. and T. Hattori (1984) Organization and efferent projections of nucleus tegmenti pedunculopontinus pars compacta with special reference to its cholinergic aspects. Neurosci. *11*: 931-946.

Sutherland, R.J., B. Kolb, and I.Q. Wishaw (1982) Spatial mapping: definitive disruption by hippocampal or medial frontal cortical damage in the rat. Neurosci. Lett. *31*: 271-276.

Sutherland, R.J. and R.J. McDonald (1990) Hippocampus, amygdala and memory deficits. Behav. Brain Res. *34*: 57-79.

Swanson, L.W., G.J. Mogenson, R.B. Simerly, and M. Wu (1987) Anatomical and electrophysiological evidence for a projection from the medial preoptic area to the 'mesencephalic and subthalamic locomotor regions' in the rat. Brain Res. *405*: 108-122.

Swanson, L.W. and W.M. Cowan (1975) A note on the connections and development of the nucleus accumbens. Brain Res. *92*: 324-330.

Swerdlow, N.R. and G.F. Koob (1987) Lesions of the dorsomedial nucleus of the thalamus, medial prefrontal cortex and pedunculopontine nucleus: effects on locomotor activity mediated by nucleus accumbens-ventral pallidal circuitry. Brain Res. *412*: 233-243.

Szabo, I., L. Lenard, and B. Kosaras (1974) Drive decay theory of self-stimulation: Refractory periods and axon diameters in hypothalamic reward loci. Physiol. Behav. *12*: 329-343.

Taylor, J.R. and T.W. Robbins (1984) Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacol. *84*: 405-412.

Taylor, J.R. and T.W. Robbins (1986) 6-Hydroxydopamine lesions of the nucleus accumbens, but not the caudate nucleus, attenuated enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. Psychopharmacol. *90*: 390-397.

Terando, L., N. Mirza, J. Zipnick, M. Overmeir, N. Rossi, and L.D. Reid (1978) Addictive agents and intracranial stimulation (ICS): Daily morphine, self-stimulation, and parameters of ICS. Physiol. Psychol. *6*: 65-70.

Thompson, T. and C.R. Schuster (1964) Morphine self-administration, food-reinforced and avoidance behaviors in rhesus monkeys. Psychopharmacol. *5*: 87-94.

Trowill, J.A., J. Panksepp, and R. Gandelman (1969) An incentive model of rewarding brain stimulation. Psychol. Rev. *76*: 264-281.

Vaccarino, F.J., M. Amalric, N.R. Swerdlow, and G.F. Koob (1986) Blockade of amphetamine but not opiate-induced locomotion following antagonism of dopamine function in the rat. Pharmacol. Biochem. Behav. *24*: 61-65.

Vaccarino, F.J. and K.B.J. Franklin (1982) Self-stimulation and circling reveal functional differences between medial and lateral substantia nigra. Behav. Brain Res. *5*: 281-295.

van der Kooy, D., R.F. Mucha, M. O'Shaughnessy, and P. Buchenieks (1982) Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. Brain Res. *243*: 107-117.

van der Kooy, D. (1987) Place conditioning: a simple and effective method for assessing the motivational properties of drugs. In *Methods of Assessing the Reinforcing Properties of Abused Drugs*, M.A. Bozarth, ed., pp. 229-241, Springer-Verlag, New York.

Van Dyke, C., P. Jatlow, J. Ungerger, P.G. Barashi, and R. Byck (1978) Oral cocaine: Plasma concentrations and central effects. Sci. *200*: 211-213.

van Rossum, J.M. (1970) Mode of action of psychomotor stimulant drugs. Int. Rev. Neurobiol. *12*: 307-383.

van Rossum, J.M. and J.A.T. Hurkmans (1964) Mechanism of action of psychomotor stimulant drugs: Significance of dopamine in locomotor stimulant action. Int. J. Neuropharmacol. *3*: 227-239.

Vanover, K.E., G.R. Wenger, and W.L. Woolverton (1989) Self-administration of the isomers of pentobarbital and secobarbital by rhesus monkeys. Pharmacol. Biochem. Behav. *34*: 669-671.

Villablanca, J.R., R.J. Marcus, and E.C. Olmstead (1976) Effects of caudate nuclei or frontal cortical ablation in cats. I. Neurology and gross behavior. Exp. Neurol. *52*: 389-420.

Villablanca, J.R. and C.E. Olmstead (1982) The striatum: a fine tuner of the brain. Acta Neurobiol. Exp. *42*: 227-299.

Wauquier, A. (1979) Neuroleptics and brain self-stimulation behavior. Int. Rev. Neurobiol. *21*: 335-403.

Wauquier, A. and C.J.E. Niemegeers (1972) Intracranial self-stimulation in rats as a function of various stimulus parameters. II. Influence of haloperidol, pimozide and pipamperone on medial forebrain bundle stimulation with monopolar electrodes. Psychopharmacol. *27*: 191-202.

Wauquier, A. and C.J.E. Niemegeers (1973) Intracranial self-stimulation in rats as a function of various stimulus parameters. III. Influence of apomorphine on medial forebrain bundle stimulation with monopolar electrodes. Psychopharmacol. *30*: 163-172.

Wauquier, A. and C.J.E. Niemegeers (1974) Intracranial self-stimulation in rats as a function of various stimulus parameters. V. Influence of cocaine on medial forebrain bundle stimulation with monopolar electrodes. Psychopharmacol. *38*: 201-210.

Weeks, J.R. (1962) Experimental morphine addiction: method for automatic intravenous injections in unrestrained rats. Sci. *138*: 143-144.

Weeks, J.R. and R.J. Collins (1978) Self-administration of morphine in the rat: relative influence of fixed ratio and time-out. Pharmacol. Biochem. Behav. *9*: 703-704.

Werner, T.E., S.G. Smith, and W.M. Davis (1976) A dose-response comparison between methadone and morphine self-administration. Psychopharmacol. *47*: 209-211.

White, N.M., L. Sklar, and Z. Amit (1977) The reinforcing action of morphine and its paradoxical side effect. Psychopharmacol. *52*: 63-66.

White, N.M. (1989) Reward or reinforcement: What's the difference? Neurosci. Biobehav. Rev. *13*: 181-186.

White, N.M., M.G. Packard, and J. Seamans (1993) Memory enhancement by post-training peripheral administration of low doses of dopamine agonists: possible autoreceptor effect. Behav. Neural Biol. *59*: 230-241.

White, N.M. and G.D. Carr (1985) The conditioned place preference is affected by two independent reinforcement processes. Pharmacol. Biochem. Behav. *23*: 37-42.

White, N.M. and R.J. McDonald (1993) Acquisition of a spatial conditioned place preference is impaired by amygdala lesions and improved by fornix lesions. Behav. Brain Res. *55*: 269-281.

White, N.M. and P.M. Milner (1992) The psychobiology of reinforcers. Ann. Rev. Psychol. *43*: 443-471.

Wikler, A.A. (1952) A psychodynamic study of a patient during self-regulated readdicition to morphine. Psychiat. Q. *26*: 270-293.

Wilson, M.C., M. Hitomi, and C.R. Schuster (1971) Psychomotor stimulant self-administration as a function of dosage per injection in the rhesus monkey. Psychopharmacol. *22*: 271-281.

Winger, G., M.L. Stitzer, and J.H. Woods (1975) Barbiturate-reinforced responding in rhesus monkeys: comparisons of drugs with different durations of action. J. Pharmacol. Exp. Ther. *195*: 505-514.

Wise, R.A. (1978a) Catecholamine theories of reward: a critical review. Brain Res. *26*: 49-55.

Wise, R.A. (1978b) Neuroleptic attenuation of intracranial self-stimulation: reward or performance deficits. Life Sci. *22*: 535-542.

Wise, R.A. (1980) Action of drugs of abuse on brain reward systems. Pharmacol. Biochem. Behav. *13*: 213-223.

Wise, R.A. (1981) Brain dopamine and reward. In *Theory in Psychopharmacology, Volume 1*, S.J. Cooper, ed., pp. 103-122, Academic Press, New York.

Wise, R.A. (1987) The role of reward pathways in the development of drug dependence. Pharmac. Ther. *35*: 227-263.

Wise, R.A. (1989) Opiate reward: Sites and substrates. Neurosci. Biobehav. Rev. *13*: 129-133.

Wise, R.A. and M.A. Bozarth (1984) Brain reward circuitry: four circuit elements "wired" in apparent series. Brain Res. Bull. *12*: 203-208.

Wise, R.A. and P.P. Rompre (1989) Brain dopamine and reward. Ann. Rev. Psychol. *40*: 191-225.

Wolfle, T.L., D.J. Mayer, B. Carder, and J.C. Liebeskind (1971) Motivational effects of electrical stimulation in dorsal tegmentum of the rat. Physiol. Behav. *7*: 569-574.

Wood, D.W., K.C. Retz, and M.W. Emmett-Oglesby (1987) Evidence of a central mechanism mediating tolerance to the discriminative stimulus properties of cocaine. Pharmacol. Biochem. Behav. *28*: 401-406.

Wood, P.L., M. Stotland, J.W. Richard, and A. Rackham (1980) Actions of mu, kappa, sigma, delta and agonist/antagonist opiates on striatal dopaminergic function. J. Pharmacol. Exp. Ther. *215*: 697-703.

Wood, P.L. (1983) Opioid regulation of CNS dopaminergic pathways: A review of methodology, receptor types, regional variations and species differences. Peptides *4*: 595-601.

Woolverton, W.L. (1986) Effects of a D1 and a D2 dopamine antagonist on the self-administration of cocaine and piribedil by rhesus monkeys. Pharmacol. Biochem. Behav. *24*: 531-535.

Woolverton, W.L. and R.L. Balster (1983) Effects of local anesthetics on fixed-interval responding in rhesus monkeys. Pharmacol. Biochem. Behav. *18*: 383-387.

Woolverton, W.L. and R.M. Virus (1989) The effects of a D1 and a D2 dopamine antagonist on behavior maintained by cocaine or food. Pharmacol. Biochem. Behav. *32*: 691-697.

Yamamoto, T. (1993) Neural mechanisms of taste aversion learning. Neurosci. Res. *16*: 181-185.

Yang, C.R. and G.J. Mogenson (1987) Hippocampal signal transmission to the pedunculopontine nucleus and its regulation by dopamine D2 receptors in the nucleus accumbens: an electrophysiological and behavioural study. Neurosci. *23*: 1041-1055.

Yeomans, J.S. (1975) Quantitative measurement of neural post-stimulation excitability with behavioral methods. Physiol. Behav. *15*: 593-602.

Yeomans, J.S. (1979) The absolute refractory periods of self-stimulation neurons. Physiol. Behav. *22*: 911-919.

Yeomans, J.S. (1989) Two substrates for medial forebrain bundle self-stimulation: myelinated axons and dopamine axons. Neurosci. Biobehav. Rev. *13*: 91-98.

Yeomans, J.S. (1990) *Principles of Brain Stimulation*, Oxford University Press, New York.

Yeomans, J.S., A. Mathur, and M. Tampakeras (1993) Rewarding brain stimulation: role of tegmental cholinergic neurons that activate dopamine neurons. Psychopharmacol. (In Press)

Yeomans, J.S. and A. Mathur (1993) Midbrain cholinergic neurons mediate rewarding brain stimulation via connections with dopamine cells. CSBBCS Abst. *3*: 51.

Yokel, R.A. (1987) Intravenous self-administration: response rates, the effects of pharmacological challenges, and drug preference. In *Methods of Assessing the Reinforcing Properties of Abused Drugs*, M.A. Bozarth, ed., pp. 1-33, Springer-Verlag, New York.

Yokel, R.A. and R. Pickens (1974) Drug level of d- and l-amphetamine during intravenous self-administration. Psychopharmacol. *34*: 255-264.

Yokel, R.A. and R. Pickens (1976) Extinction responding following amphetamine self-administration: determination of reinforcement magnitude. Physiol. Psychol. *4*: 39-42.

Yokel, R.A. and R.A. Wise (1975) Increased lever pressing for amphetamine after pimozide in rats: implications for a dopamine theory of reward. Sci. *187*: 547-549.

Young, A.M. and S. Herling (1986) Drugs as reinforcers: studies in laboratory animals. In *Behavioral Analysis of Drug Dependence*, pp. 9-67, Academic Press,

Young, P.T. (1966) Hedonic organization and regulation of behavior. Psychol. Rev. *73*: 59-86.