ELECTRICAL STIMULATION OF REWARD SITES IN THE VENTRAL TEGMENTAL AREA OF THE RAT INCREASES DOPAMINE TRANSMISSION IN THE NUCLEUS ACCUMBENS AS MEASURED BY *IN VIVO* MICRODIALYSIS

By

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Abstract

In vivo microdialysis with HPLC-ED was used to measure dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the nucleus accumbens of the rat, prior to, during, and after 15-min periods of electrical brain-stimulation at sites in the ventral tegmental area (VTA) that supported intracranial self-stimulation (ICSS). In the first experiment, both ICSS and yoked-stimulation of the VTA evoked significant increases in extracellular concentrations of DA, its metabolites, and 5-HIAA. Comparable results from ICSS and yoked groups were interpreted as evidence that the rewarding properties of VTA stimulation were a causal factor in the elevated DA transmission in the nucleus accumbens, rather than intense operant behavior. Further evidence for this hypothesis came from a second set of data in which changes in extracellular DA levels during the measurement of rate/intensity functions for ICSS were positively correlated. 5-HIAA concentrations also increased during ICSS but these changes were not correlated with either ICSS rate or current intensity, suggesting that changes in serotonin metabolism were unlikely to subserve brain-stimulation reward in the VTA. These results provide further evidence that stimulated mesolimbic DA release is a sufficient condition for reward.

TABLE OF CONTENTS

List of Tables

List of Figures

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INTRODUCTION

The discovery that rats would work to stimulate electrically discrete brain areas led to the idea that intracranial self-stimulation (ICSS) could be used to uncover the neural mechanisms of reward (Olds & Milner, 1954). Rewarding stimuli have two main organizing effects on behavior (Carr, Fibiger, and Phillips, 1989). First, rewarding stimuli can elicit approach responses. Second, rewards can produce reinforcement. Based upon empirical evidence, Schneirla (1959) proposed two basic motivated behaviors in all animals: approach and withdrawal. Similarly, Young (1959) polarized behaviors into either approach-maintaining or avoidance-terminating patterns. Rewards elicit these approach patterns of behavior. An initial observation that led to the discovery of self-stimluation was that rats would return to the area of the testing box where they received brain stimulation. By this definition, the brain stimulation the rats received is rewarding. In operant terms, reinforcement describes the ability of rewards to increase the probability that the responses that preceed it will be repeated (Skinner, 1938). In the context of this thesis, reinforcement describes how animals perform a well-learned self-stimulation response. According to this perspective, reinforcement can also be used as a measure of reward. Increased rates of operant responding reflect the the rewarding quality of the stimulation. These two organizing effects of reward on behavior are interdependent and appear to share a common underlying physiological mechanism. The perception of a rewarding stimulus results in both the appreciation that the stimulus is a reward (eliciting approach behaviors and reinforcement) and a modulation of response depending upon the attractiveness of the reward.

A number of scientific findings led to the catecholamine (CA) theory of reward first proposed by Stein (1964, 1968). Early mapping studies showed that ICSS could be obtained from a wide variety of structures (Crow, 1972a). The

ICSS could be obtained from a wide variety of structures (Crow, 1972a). The medial forebrain bundle (MFB) supported notably high rates of ICSS (Fibiger, 1978; Crow, 1972a). Newly developed histochemical techniques allowed the topographical mapping of monoaminergic neurons in the nervous system and ascending catecholaminergic fibers were found to travel within the MFB to many forebrain structures (Fuxe, 1965). It became apparent that there was a good correlation between the location of ascending CA-containing neurons and areas that supported ICSS (Dresse, 1966). Given that CA neurons, especially those containing noradrenaline (NA), project widely throughout the brain, it is not surprising that a correlation with ICSS was found (Fibiger, 1978). Although correlational findings cannot imply causality, it was an important starting point for subsequent research.

More convincing evidence came from pharmacological experiments on ICSS. Drugs that enhance central CA transmission, such as amphetamine (Stein, 1964), cocaine (Crow, 1970), and monoamine oxidase inhibitors (Poschel, 1969), were found to facilitate ICSS rates of responding. Drugs that impair central CA transmission, such as reserpine (Olds, Killam, & Bach-y-Rita, 1956), tetrabenazine (Crow, 1972a), alpha-methyl-p-tyrosine (Cooper, Black, & Paolino, 1971), and chlorpromazine (Crow, 1972a) attenuated ICSS rates.

Stein (1964) offered arguments for both NA and for dopamine (DA) as the primary CA mediating ICSS but settled on a noradrenergic hypothesis of brain-stimulation reward based primarily on pharmacological and anatomical evidence. First, drugs which increased and decreased NA transmission facilitated and attenuated ICSS, respectively. Second, the site of origin of ascending noradrenergic fibers corresponded to the main ascending limb of a limbic midbrain circuit which Nauta had identified as an important part of a reward-punishment system in the central nervous system (Stein, 1968). Moreover, the ascending

"noradrenergic" ventral tegmental area (VTA) cells in this circuit were identified by Stein as playing an important role in mediating the effects of drugs on ICSS. Dresse (1966) proposed that it was primarily ascending NA, not DA, neurons that mediated ICSS. This conclusion was due, in part, to an early and erroneous report that most neurons of the VTA, an area Dresse found to support high rates of ICSS, were noradrenergic (Fuxe, 1965). Third, ICSS could be obtained from the locus coeruleus (LC, a noradrenergic nucleus in the brainstem) at points along the ascending course of its axons in the dorsal noradrenergic bundle (DNB) prior to it merging with the MFB, and in a number of terminal regions of this pathway (e.g. dorsal hippocampus) (Phillips & Fibiger, 1989). A neurochemical study using the push-pull cannula technique provided further support for the NA hypothesis. Stein & Wise (1969) found that in unanesthetized rats given an intraventricular injection of tritiated-NA, MFB stimulation caused an increase in radioactivity in perfusates from the lateral hypothalamus or amygdala. Non-rewarding brain stimulation did not.

Subsequently, an overwhelming amount of evidence was collected that refuted the noradrenergic hypothesis (see Fibiger, 1978). First, a histofluorescence study determined that the cell bodies of the VTA contain DA (Andén, Dahlström, Fuxe, Larsson, Olson, Ungerstedt, 1966), and not NA terminals as previously claimed (Fuxe, 1965). Second, because early pharmacological studies used drugs that act on both noradrenergic and dopaminergic systems, it was impossible to discern whether one or both neurotransmitters mediated ICSS reward (Fibiger, 1978). When specific noradrenergic and dopaminergic drugs were used, initial studies failed to use methods that made a clear distinction between reward and motor/performance factors (Stellar & Rice, 1989). One study that used the specific NA receptor antagonist phenoxybenzamine and extinction as a rate-free measure of reward showed that DA, but not NA, blockade, impaired MFB self-stimulation

(Fouriezos, Hanson, & Wise, 1978). The extinction method assumes that reducing reward with drugs will produce an extinction curve similar to one produced when stimulation is turned off in a self-stimulating animal. Further, it was found that the relative potencies of neuroleptics to decrease ICSS reward was better correlated to DA, rather than NA, receptor binding properties (Gallistel & Davis, 1983). Third, a series of lesion experiments provided compelling evidence that NA was not necessary for brain-stimulation reward. Bilateral lesions of the DNB using 6-hydroxydopamine (6-OHDA), a selective catecholaminergic neurotoxin, failed to disrupt LC self-stimulation despite reducing hippocampal and cortical NA by 96.7% (Clavier, Fibiger, & Phillips, 1976). Ipsilateral or bilateral electrolytic lesions of the LC did not effect self-stimulation of the DNB despite reducing cortical NA by over 80% (Clavier & Routtenberg, 1976). Finally, 6-OHDA lesions of the DNB, which reduced hippocampal NA by 97%, had no effect on hippocampus self-stimulation or its facilitation by amphetamine (Phillips, van der Kooy, & Fibiger, 1977; van der Kooy, Fibiger, & Phillips, 1977). Given these data, it was clear that the NA hypothesis of brain-stimulation reward was not tenable.

Just as anatomical experiments correlating self-stimulation sites with noradrenergic neurons provided support for the NA theory of reward, the same strategy provided the first evidence for a dopaminergic substrate in brain-stimulation reward (Phillips & Fibiger, 1989). Although the hypothesis that DA neurons exclusively mediate ICSS reward can be rejected on purely anatomical grounds, the possibility remains that stimulation of these neurons is sufficient for reward (Fibiger, 1978). Early studies had shown that ICSS could be obtained in ventral mesencephalic areas corresponding to the VTA and SN (Routtenberg & Malsbury, 1969; Olds & Olds, 1963). Crow (1972b) conducted a detailed mapping study of the ventral mesencephalon confirming positive ICSS sites in the VTA and SN and, noting that these were DA rather than NA-containing cell bodies,

concluded that DA neurons support ICSS. Further evidence came from a moveable stimulating electrode study which found that positive ICSS sites in the mesencephalon were confined to an area of DA-containing cell bodies and that ICSS thresholds and response rates were proportional to the density of dopaminergic neurons at the electrode tip (Corbett & Wise, 1980). The major terminal fields of the mesotelencephalic DA systems have also been found to support ICSS, including the nucleus accumbens (Phillips, Brooke, & Fibiger, 1975), striatum (Phillips, Carter, & Fibiger, 1976), prefrontal cortex (Rolls & Cooper, 1973), central amygdala (Wurtz & Olds, 1963), and septum (Olds & Milner, 1954).

Pharmacological experiments have shown that systemically-administered neuroleptics attenuate MFB-stimulation reward and, in some cases, produce operant motor/performance deficits (Stellar & Rice, 1989; Fibiger, 1978). This illustrates a pervasive problem in research investigating the pharmacological basis of ICSS. Since DA systems are known to serve important motor functions (Fibiger, 1978) and also appear to play a role in brain-stimulation reward, care must be taken to distinguish between the attenuating actions of neuroleptics on ICSS reward and their impairment of general operant motor performance. It was not surprising that early studies investigating neuroleptic-induced attenuation of ICSS provided equivical results (Fibiger, 1978).

The introduction of novel rate-free and reward-specific behavioral measures led to more convincing evidence for the involvement of DA in ICSS reward. Fouriezos et al. (1978) had shown that certain doses of pimozide attenuated reward independent from its impairing effects on motor performance. Franklin (1978) used a rate-frequency curve-shift method to further confirm that pimozide could selectively reduce reward. In this method, lateral and vertical curve shifts occured independently and reflected changes in reward and performance, respectively. The reward-attenuating effect of pimozide was also shown to be

counteracted by amphetamine treatment (Gallistel & Karras, 1984). Similarly, direct injections of the neuroleptic *cis*-flupenthixol into the nucleus accumbens were found to reduce MFB-stimulation reward while producing only mild motor impairments, as assessed by a rate-frequency curve shift (Stellar & Rice, 1989).

Another paradigm where animals could regulate electrical stimulation using on and off responding, provided further evidence that neuroleptic administration produced both reward and motor effects (Liebman, Hall, & Prowse, 1982). Neuroleptics also were found to raise ICSS stimulation thresholds using an autotitration-of-threshold method (Neill, Garr, Clark, & Britt, 1982), a self-adjusting procedure where the stimulation current is decreased after a fixed number of responses. In this case, animals could adjust the level of stimulating current to a value that was perceivably rewarding by pressing an adjacent lever. It is worth noting, however, that in contrast to systemic injections, direct injections of neuroleptics into the nucleus accumbens lowered ICSS stimulation thresholds (Neill et al., 1982).

The conditioned place preference technique is another rate-free behavioral paradigm which can test undrugged animals after a training period where they have learned to either avoid or approach a distinctive environment that had been repeatedly paired with aversive or rewarding stimuli, respectively. Using this paradigm, Ettenberg & Duvauchelle (1988) found that rats developed a preference to places paired with ICSS, an effect which could be blocked by systemically-administered haloperidol in a dose-dependent manner.

Studies which have examined the effects of direct and indirect DA agonists on ICSS behavior have also provided convincing evidence of DA involvement. Amphetamine and cocaine have been shown to increase reward by lowering stimulation thresholds without altering stimulation detection thresholds (Kornetsky & Esposito, 1981). Using the self-regulation of duration paradigm, amphetamine

(Liebman, Gerhardt, & Prowse, 1982b) and nomifensine (Gerhardt & Liebman, 1985) were found to increase ICSS reward. Amphetamine injection into the nucleus accumbens also enhanced reward (Stellar & Rice, 1989). ICSS was also facilitated by the specific DA uptake blocker GBR-12909 (Phillips, Blaha, & Fibiger, 1989). Apomorphine, a direct DA receptor agonist, can produce increases in self-stimulation behavior, if administered in small doses, but generally has been found to disrupt ICSS (Wauquier & Niemegeers, 1973). The latter effect may have resulted from a non-contingent reward signal competing with ICSS (Stellar & Rice, 1989). Collectively, the application of rate-free behavioral paradigms and pharmacological data have supported a role for DA in brain-stimulation reward.

Ipsilateral versus contralateral DA pathway lesion experiments have also contributed to understanding neurochemical substrates of brain-stimulation reward independent of performance effects. Phillips & Fibiger (1978) found that ICSS of the VTA was attenuated severely by selective ipsilateral destruction of DA pathways at the level of the hypothalamus using 6-OHDA. Further, self-stimulation at terminal sites of this pathway (i.e. nucleus accumbens and medial prefrontal cortex) in these lesioned animals was only marginally affected which suggested that DA does not exclusively mediate ICSS in these two regions (Phillips & Fibiger, 1989). A subsequent study using ipsilateral and contralateral 6-OHDA lesions, in combination with rate-intensity measures, found that only lesions ipsilateral to the VTA electrode reduced reward (Fibiger, LePiane Jakubovic, & Phillips, 1987). A drastic attenuation of asymptotic ICSS response rates on only the side of the lesion led to an important observation. Specifically, that this aspect of the curve-shift method, traditionally used as an index of performance, does not work with manipulations which block all reward (Phillips & Fibiger, 1989).

Various lesion experiments also suggested a dissociation between the two major ascending dopaminergic pathways in the brain: the mesocorticolimbic and

nigrostriatal systems. Unilateral kainic acid lesions of the dorsal striatum preferentially reduced brain-stimulation reward in the SN but not the VTA (Phillips, LePiane, & Fibiger, 1982). Ipsilateral 6-OHDA lesions of the mesencephalic DA pathways were found to produce only a transient attenuation in ICSS rates from the SN (Clavier & Fibiger, 1977). Since contralateral lesions produced similar impairments, ICSS attenuation was thought to be due to motor deficits. Also, amphetamine facilitated ICSS in the SN contralateral and not ipsilateral to the lesion. Collectively, these results suggested that although dopaminergic neurons may not exclusively mediate SN-stimulation reward, they are involved (Clavier & Fibiger, 1977).

There is still much debate over whether stimulated release of DA is sufficient for reward (Phillips et al., 1989; Fibiger & Phillips, 1987; Gallistel, 1986). *Ex vivo* and *in vivo* biochemical analyses provide a more direct approach in addressing this issue.

Ex Vivo Experiments

Quantitative 2-deoxyglucose (2-DG) autoradiography has been used to map functional neural pathways based on altered rates of glucose utilization, a marker of metabolic activity (Kennedy, Des Rosiers, Jehle, Reivich, Sharpe, & Sokoloff, 1975). A selective pattern of metabolic activation in dopaminergic terminal fields following self-stimulation of the VTA and SN has been shown using the 2-DG technique (Porrino, 1987; Porrino, Esposito, Seeger, & Crane, 1985; Porrino, Esposito, Seeger, Crane, Pert, & Sokoloff, 1984). In contrast, other 2-DG studies (Gallistel, Gomita, Yadin, & Campbell, 1985; Gallistel, Karreman, & Reivich, 1977; Yadin, Guarini, & Gallistel, 1983) found little evidence for activation of dopaminergic projections by self-stimulation. Methodological differences between these studies may account for these seemingly equivocal results. First, whereas

Porrino et al. directly stimulated dopaminergic neurons in the ventral mesencephalon, Gallistel and Yadin stimulated portions of the MFB. The MFB is a heterogenous bundle with widespread ascending and descending components (see Nauta & Haymaker, 1969) whereas the mesencephalic dopamine system is well-defined in terms of its projections and topography (Fallon, 1988; Domesick, 1988; Swanson, 1976). Second, Gallistel and Yadin used brief pulses (0.1 msec) of square wave current. Porrino et al. used longer pulses corresponding to a 60 Hz sine wave current (about 0.8 msec, peak to peak). It has been suggested that current parameters commonly used in self-stimulation experiments (i.e. square wave, brief pulses), are inadequate to excite the thin, unmyelinated DA fibers (Yeomans, Maidment, & Bunney, 1988). Further, square wave stimulation of the MFB is only effective in releasing striatal DA when pulses longer than 0.5 msec are used (Millar, Stamford, Kruk, & Wightman, 1985).

Another technique that has been used to map active brain areas in response to chronic MFB self-stimulation is cytochrome oxidase (CO) histochemistry (Bielajew, 1991). Active areas are denoted by an increase in CO, a key enzyme in cellular metabolism. Bielajew found that only self-stimulation with longer pulse durations (2.0 msec) increased metabolic activity in dopaminergic projection areas such as the frontal cortex and the olfactory tubercle. It is interesting that increased CO activity was observed in the nucleus accumbens with only brief pulse durations (0.1 msec).

Ex vivo measurements of DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the nucleus accumbens immediately following self-stimulation have provided some more direct evidence for increased DA transmission during ICSS (Phillips et al., 1989). Increased DOPAC/DA ratios in the nucleus accumbens and prefrontal cortex after self-stimulation of the VTA have also been reported (Simon, Stinus, Tassin, Lavielle, Blanc, Thierry, Glowindki, & Le

Moal, 1979). In contrast to the above studies, increases in DOPAC were observed in the olfactory tubercle, but not in the nucleus accumbens or striatum following VTA self-stimulation (Mitchell, Nicoleau, Arbuthnott, & Yates, 1982). This study also failed to see changes in DOPAC in the nucleus accumbens or striatum after posterior hypothalamic self-stimulation. In another study, however, increased DA turnover was observed in the nucleus accumbens and striatum after self-stimulation of the hypothalamus in mice (Garrigues & Cazala, 1983).

The finding that some types of operant behavior may increase the rate of transport of DOPAC in some brain regions (Heffner, Vosmer, & Seiden, 1984) raised an important cautionary point. That is, ICSS-related increases in DA metabolism may be due to the motoric act of lever pressing. These problems have been partially addressed in two *ex vivo* studies. In the first, increases in tyrosine hydroxylase (TH) activity, as measured by accumulated 3,4-dihydroxyphenylalanine levels, were observed in dopaminergic terminal fields such as the nucleus accumbens, striatum, and olfactory tubercle after lever pressing for stimulation of the VTA (Phillips, Jakubovic, & Fibiger, 1987). No changes in TH activity were seen after lever pressing for food on a fixed-ratio schedule that produced operant rates comparable to ICSS of the VTA. Results indicated a role for DA in VTA-ICSS independent of operant behavior. A second study incorporated a group of "yoked" animals which received non-contingent electrical stimulation to the VTA to control for increases in dopaminergic acitivty due to operant responding. Fibiger, Jakubovic, LePiane, & Phillips (1987) found comparable DOPAC and HVA to DA ratios in the ipsilateral nucleus accumbens, striatum, and olfactory tubercle in both self-stimulating and yoked animals.

It has been suggested that DOPAC may not be related to neuronal activity and is an unreliable index of DA release (Westerink, Hofsteede, Damsma, and de Vries, 1988; Zetterström, Sharp, Collin, & Ungerstedt, 1988). DOPAC, lacking an

active uptake mechanism, is able to diffuse more readily through extracellular space and may be partly derived from distal tissue. Microdialysis (MD) experiments investigating voltage-dependent release of neurochemicals suggest that only 50% of basal DOPAC formation is related to neuronal activity (Westerink, Tuntler, Damsma, Rollema, and de Vries, 1987). Further, a major source of DOPAC comes from intracellular catabolism of newly synthesized or cytoplasmic DA and, therefore, may reflect metabolism of DA prior to release (Butcher, Fairbrother, Kelly, & Arbuthnott, 1988).

An obvious problem with *ex vivo* analyses is relating behavior with neurochemical levels in post-mortem tissue. Also, tissue assays may not be an accurate reflection of what is happening in the extracellular space, the critical site of action of transmitters. The recent development of techniques such as electrochemistry and MD provide a way in which the neurochemistry in the extracellular space can be monitored *in vivo*.

In Vivo Experiments

Many studies have examined the efflux of DA after MFB stimulation using *in vivo* electrochemistry. This technique measures changes in oxidation current proportional to the concentration of electroactive species, such as DA, in the vicinity of an electrochemical probe (Blaha & Phillips, 1990). Millar et al. (1985) reported that both sine and square wave stimuli were equally effective in releasing DA in the striatum but only when pulse widths greater than 0.5 msec were used. Many electrochemistry studies have used 50 to 75 Hz sine wave stimuli successfully to induce DA efflux in the dorsal and ventral striatum (Williams & Millar, 1990; Stamford, Kruk, & Millar, 1988, 1986a, b; May & Wightman, 1989; Kuhr, Wightman, & Rebec, 1987; Kuhr, Bigelow, & Wightman, 1986; Stamford, Kruk, Millar, & Wightman, 1984).

Gratton, Hoffer, & Gerhardt (1988) found that stimulation (200-800 μA, 20-100 Hz, 0.1 msec cathodal square wave, 500 msec trains) of the VTA or MFB in anesthetized rats with a previous history of ICSS resulted in increases in the electrochemical measurement of DA. Further, the ICSS-induced increase in the electrochemical signal was potentiated by nomifensine. A series of electrochemical experiments examining the effect of experimenter-administered stimulation and self-stimulation of the VTA on DA efflux in the nucleus accumbens in unanesthetized rats provide more convincing support for the role of DA in VTA-stimulation reward (Blaha & Phillips, 1990). Stimulation (10-30 μA, 60 Hz sine wave, 0.2 sec trains) of the VTA caused increases in the DA oxidation current. Blaha & Phillips (1990) emphasized some important correlations. First, there was a close relationship between the threshold current for ICSS and the threshold for producing a change in the electrochemical signal in the nucleus accumbens. Second, there was a positve correlation between the current intensity, the ICSS rate, and the DA oxidation signal. Finally, the DA uptake blockers, nomifensine, cocaine, and GBR-12909, facilitated both the ICSS rate and the DA oxidation signal in a correlative manner.

In vivo MD is a widely-used technique that permits the monitoring of neurotransmitters and their metabolites in the extracellular space of discrete brain regions of freely-moving animals (Ungerstedt, 1991; Benveniste & Hüttemeier, 1990; DiChiara, 1990; Westerink, Damsma, Rollema, De Vries, & Horn, 1987). In this method, a hollow dialysis fiber tube is implanted stereotaxically in a specific brain area. Low molecular weight compounds diffuse down their concentration gradients from the extracellular fluid across the dialysis membrane and into a physiological salt solution that perfuses through the dialysis fiber at a constant rate. The fluid (dialysate) is then collected and assayed using standard analytical procedures. The most popular methods of analysis combine compound separation

via high performance liquid chromatography (HPLC) and compound quantification via electrochemical (ED) or fluorometric detection.

MD has been applied to the study of DA efflux and metabolism after electrical brain stimulation (Cenci, Kalén, Mandel, & Björklund, 1992; Manley, Kuczenski, Segal, Young, & Groves, 1992; Tepper, Creese, & Schwartz, 1991). Tepper et al. (1991) reported that MFB stimulation (3-100 Hz) activating antidromically-identified SN neurons could produce changes in striatal extracellular DA as measured by MD. Similarly, Manley et al. (1992) found increases in extracellular DA levels in response to stimulation of the MFB using frequencies in the range of normal dopaminergic cell firing (4-10 Hz). Both these studies observed DA augmentation using MD perfusates containing high calcium concentrations (2.3-2.4 mM). However, when a lower calcium concentration was used (1.2 mM), no increases in extracellular DA were observed (Tepper et al., 1991).

Using MD, Nakahara, Ozaki, Miura, Mieura, & Nagatsu (1989a) found that ICSS (100 Hz; biphasic square pulses, 0.3 msec duration) of the MFB resulted in increases in nucleus accumbens levels of the DA metabolites, DOPAC and HVA, and the serotonin metabolite, 5-HIAA, but no significant increases in DA. This group suggested that the small increases in DA due to ICSS were masked by an ICSS-induced facilitation of reuptake of released DA (Nakahara, 1991). In a subsequent experiment, ICSS caused a markedly increased efflux of DA in the nucleus accumbens in rats pretreated with the DA uptake blocker, nomifensine (Nakahara, Ozaki, Vapoor, & Nagatsu, 1989b). These rats were re-implanted with a MD probe three days later and, after nomifensine pretreatment, experimenter-administered stimulation caused a similar increase in extracellular DA (Nakahara et al., 1989b). These results suggested that elevations in nucleus accumbens DA were due to the rewarding effects of brain stimulation and not to the motoric act of

lever pressing. Additionally, a recent experiment has shown a preferential activation of the mesolimbic DA system by MFB ICSS that resulted in increased DA levels in the medial frontal cortex and nucleus accumbens, but not the striatum, in nomifensine-pretreated rats (Nakahara, Fuchikami, Ozaki, Iwasaki, & Nagatsu, 1992). Moreover, Miliaressis, Emond, & Merali (1991) found increases in extracellular DA in the nucleus accumbens as a result of MFB or VTA ICSS when longer pulse durations were used. When shorter pulse durations were employed, ICSS failed to produce detectable increases in dialysate DA. To date, MD studies have yielded equivocal results concerning the role of DA in ICSS.

The present series of experiments were designed to investigate whether rewarding electrical stimulation of the VTA is accompanied by increases in extracellular DA in the nucleus accumbens as measured by MD. Important methodological differences from previous ICSS-MD studies included: 1) stimulating electrodes in close proximity to the DA perikarya in the VTA rather than the MFB, 2) allowing a sufficient amount of time to elapse after implantation of the MD probe (18-24 hrs) to maximize release-dependent DA, 3) the use of physiological levels of calcium (1.3 mM) in the MD perfusate, and 4) the testing of drug-free animals (i.e. no DA uptake blockers).

Experiment 1 incorporated two important control groups. The inclusion of a "yoked" group, which received stimulation non-contingently, controlled for the possibility that increases in DA transmission in the nucleus accumbens could be due to intense operant activity accompanying VTA-ICSS. Both self-stimulating and yoked animals were compared to unstimulated control animals which had a similar history of self-stimulation.

Experiment 2 further tested the hypothesis of a dopaminergic substrate for brain-stimulation reward by examining the effect of varying current intensities on VTA-ICSS and DA efflux in the ipsilateral nucleus accumbens as measured by MD.

A positive correlation would be expected if extracellular DA in the nucleus accumbens mirrors the rewarding value of current intensity and self-stimulation response.

GENERAL METHODS

Subjects

Adult male Long Evans rats (300-400 g, Charles River Canada, Inc., St. Constance, Québec) were housed individually in Plexiglas boxes (dried corn cob bedding) on a 12:12 h light-dark cycle with free access to food and water.

Surgery

Animals were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) prior to stereotaxic surgery. A bipolar stimulating electrode (Plastic Products Co.) was inserted into the VTA (coordinates from interaural zero, flat skull: anterior 3.5 mm, dorsal 1.8 mm, lateral 0.5 mm from midline) and a MD probe guide cannula (15 mm, 19 g) was implanted above the ipsilateral nucleus accumbens (coordinates from Bregma, flat skull: anterior 1.8 mm, lateral 1.0 mm, ventral 1.0 mm from dura). Both implants were secured chronically to the skull with six set screws and dental acrylic.

Apparatus

ICSS testing was conducted in a Plexiglas box (24 cm x 25 cm x 30 cm) with a wire mesh floor. A single removeable lever (4 cm x 8 cm) was mounted on one wall 3 cm from the floor. The Plexiglass box was housed in a modified ventilated

and insulated plastic chamber (Colbourn Instruments) which served to shield the animal from distracting stimuli. A small hole on top of the chamber allowed the passage of MD tubing and electrical cables from the animal to a dual electrical/double channel liquid swivel (modified Instech 375D) located outside the box.

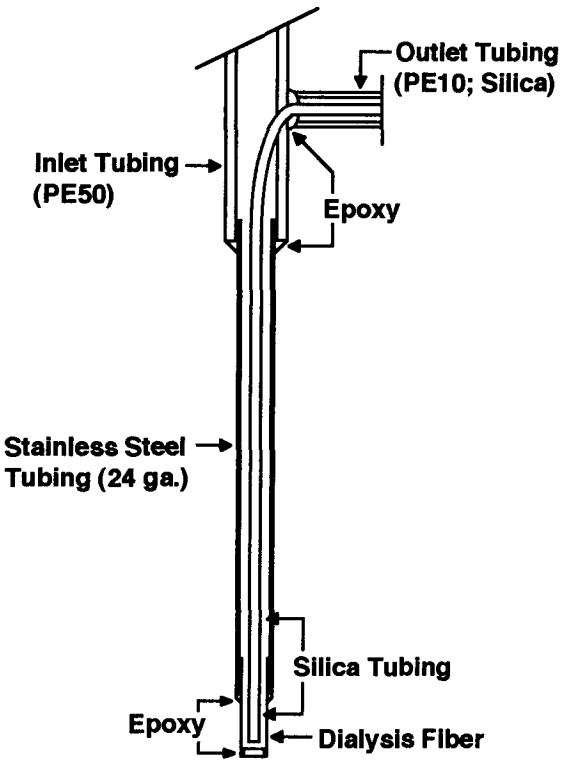
Microdialysis Probe Characteristics

MD probes (Fig. 1A) used in Experiment 1 were of a concentric design consisting of a semi-permeable hollow fiber (340 μm o.d., 65 000 M.W. cut-off, Filtral 12, Hospal), a PE50 inlet tubing, a fused silica outlet tubing (75 μm i.d x 150 μm o.d.) and a 24 g stainless steel cannula (34 mm). Epoxy (Devcon 2-Ton) was used to seal joints and plug the dialysis fiber tip. The inlet tubing was connected to a syringe pump (Harvard, model 22). MD probes used in Experiment 2 were identical to those used in Experiment 1 except that a long PE10 outlet tubing was glued over a shortened fused silica tubing (10 cm). The outlet tubing was then connected to the second liquid channel of the swivel which led to an automatic injector (Valco Instruments). Typical *in vitro* recovery at 21°C and a 1.5 μL/min flow rate was 15% for DA and 11% for the metabolites.

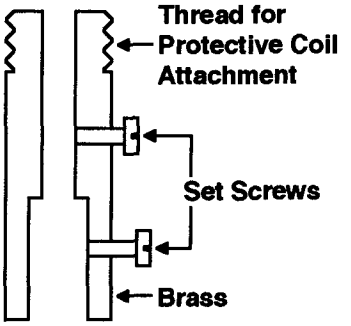
A MD probe guide collar (Fig. 1B) secured the MD probe inside the guide cannula (Fig. 1C).

Figure 1. Diagram of the microdialysis probe assembly. The microdialysis probe (A) is secured in the microdialysis probe collar (B) by a top-end set screw. The probe is then inserted into the guide cannula (C) which has been implanted over the area of interest. The probe-collar assembly is then secured over the guide cannula by a bottom-end set screw.

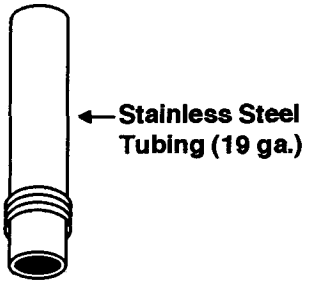
A. Microdialysis Probe



B. Microdialysis Probe Collar



C. Guide Cannula



High Performance Liquid Chromatography and Electrochemical Detection

Microdialysate analytes were separated by reverse phase chromatography (Beckman ultrasphere column, ODS 3 μm, 7.5 cm, 4.6 mm i.d.) using a 0.083 M sodium acetate buffer (pH 3.5, 3% methanol). The glassy carbon working electrode was set at +0.650 V. The apparatus consisted of a Spectra Physics 8810 HPLC pump, a Rheodyne 7125 injector (Experiment 1) or a Valco Instruments 2-Position autoinjector (EC10W) and Digital Valve Sequence Programmer (Experiment 2), an EGG 400 electrochemical detector, and a Shimadzu CR3A integrator.

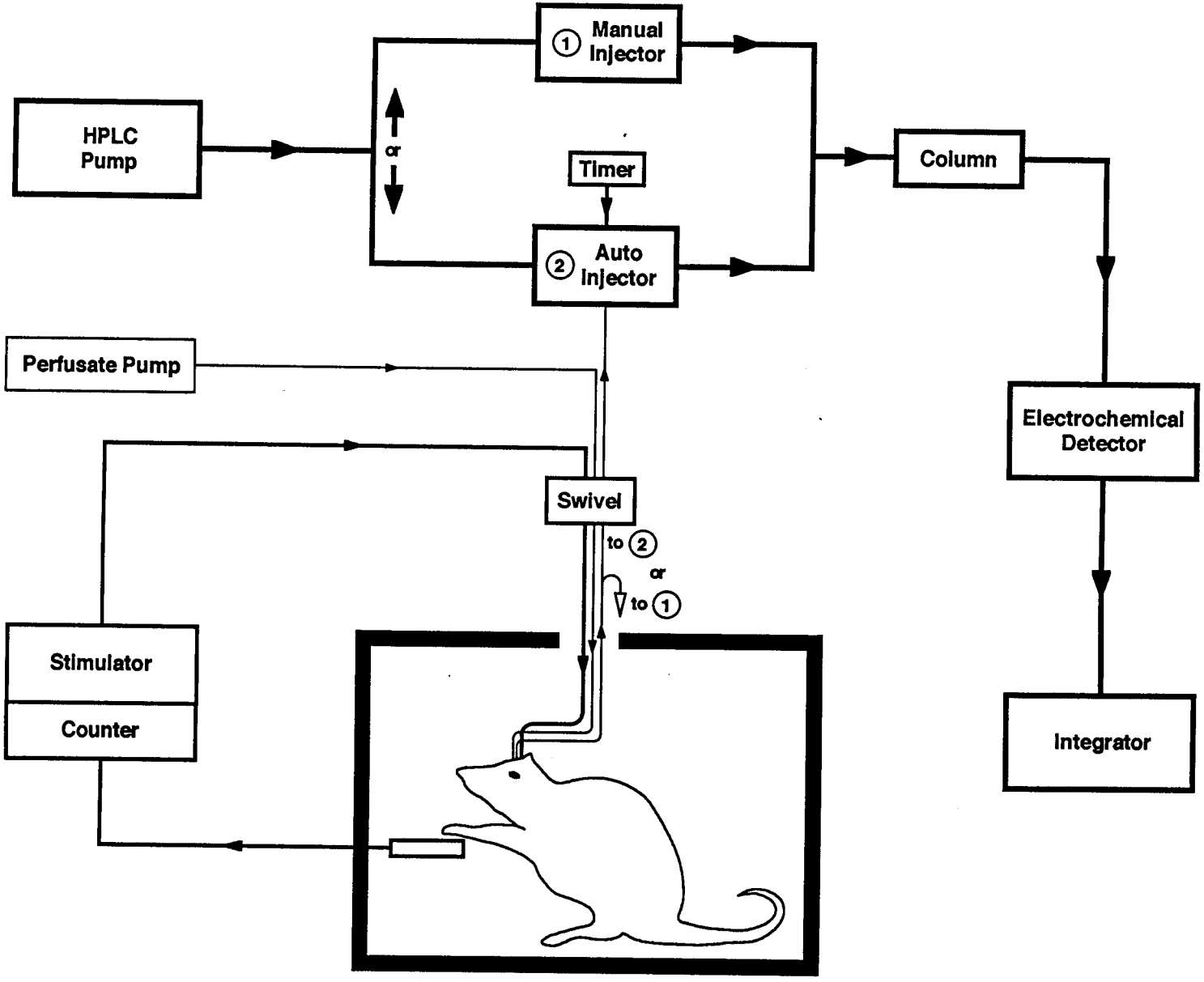
Intracranial Self-Stimulation Training

One week after surgery, animals were screened for ICSS behavior in the operant box connected via leads to the swivel. The stimulation current ranged from 6-26 μA (60 Hz sine wave; 200 ms trains). Responsive animals were then tested on an ascending current intensity program (5 min stimulation periods; 2 μA steps) for 3-5 days. Rats (n=23) that maintained stable rates of ICSS (>750 presses/15 min) were selected for the MD experiments.

Instrumentation Overview

Fig. 2 is a schematic illustration of instruments used in Experiments 1 and 2.

Figure 2. A schematic illustration of the experimental set-up. A perfusion solution was pumped through a swivel to an implanted microdialysis probe. In Experiment 1, the microdialysis samples were collected in tubes and manually injected into an HPLC-ED system (1). In Experiment 2, samples continued through the swivel into an autoinjector (2) and were injected into an HPLC-ED system every 15 min. The HPLC-ED system consisted of an HPLC pump, an injector, a column, an electrochemical detector, and an integrator. Animals received electrical stimulation either non-contingently or by pressing a lever mounted in a Plexiglas box. Current passed from the stimulator through a swivel to an implanted stimulating electrode.


HPLC Pump
or
① Manual Injector
Timer
② Auto Injector
Column
Perfusate Pump
Electrochemical Detector
Swivel
to ②
or
to ①
Stimulator
Counter
Integrator

Statistical Analysis

Neurochemical data, expressed as a percentage of the average of the first three baseline samples, were analyzed using analyses of variance (ANOVA) with repeated measures. A significant interaction in the ANOVA's was followed by post-hoc comparisons using Student *t*-tests. All tests were performed using a statistical software package (BMDP Inc.).

Histology

After the MD experiment, animals were sacrificed with an overdose of chloral hydrate and transcardially perfused with physiological saline (0.9% NaCl in distilled water) and phosphate buffered formalin (4% paraformaldehyde). Brains were quick-frozen with CO_2 and sliced (40 μm sections) using a cryostat microtome. Sections were mounted on glass slides (coated with 2% gelatin) and stained with cresyl violet for subsequent examination under a light microscope.

EXPERIMENT 1

Effects of ICSS or yoked-stimulation of the VTA on DA, DOPAC, HVA, and 5-HIAA in the ipsilateral nucleus accumbens

Experiment 1 examines whether rewarding electrical stimulation of the VTA produces any changes in DA transmission in the ipsilateral nucleus accumbens as measured by MD. This experiment includes a "yoked" stimulation group as a control for the possibility that lever-pressing, rather than brain-stimulation reward, increases DA efflux during ICSS. An unstimulated control group with comparable surgical and behavioral experience is also included. Important methodological considerations include 1) stimulating electrodes in close proximity to the DA perikarya in the VTA, 2) MD probes implanted 18-24 hrs prior to behavioral testing to maximize release-dependent DA, 3) the use physiological levels of calcium (1.3 mM) in the MD perfusate, and 4) testing of drug-free animals.

Methods

Animals were assigned randomly to three groups: 1) ICSS (n=5), 2) yoked (n=5), and 3) unstimulated controls (n=5). ICSS animals performed a bar press response to receive VTA stimulation. Each yoked animal was paired with a self-stimulating animal exhibiting a similar rate-intensity profile and the two animals were placed in separate test boxes at the same time. Only the yoked animal of the pair was implanted with a MD probe. No lever was available to yoked animals during testing. Although current intensity was the same for both animals, yoked animals received stimulation at a rate determined by their ICSS partner. Rats in

the unstimulated control group were connected to a stimulation lead but did not receive stimulation during testing. Control subjects had a comparable experience of ICSS prior to the MD experiment.

MD probes were inserted 18-24 h prior to the experiment (ventral 7.8 mm from dura). Each probe was then connected to the swivel and the animal remained in the test box overnight with free access to food and water. No lever was present in the test box overnight. The probe was flushed overnight at 0.15 μL/min with perfusate (10 mM sodium phosphate buffer, pH 7.4, 1.3 mM $CaCl_2$, 3.0 mM KCl, 1.0 mM $MgCl_2$, and 147 mM NaCl). On the day of the experiment, the flow rate of the perfusate was increased to 1.5 μL/min and dialysate samples were collected at least one hour later. If an ICSS animal was being tested, the lever was remounted in the test box. Dialysate samples were collected every 15 min in Eppendorf microtubes positioned just below the swivel. The samples were injected manually onto an HPLC-ED system.

Combined neurochemical and stimulation protocols for the ICSS and yoked groups consisted of: 1) baseline measures of neurotransmitter and metabolites (minimum of 60 min); 2) the first stimulation period (15 min) during which each animal either engaged in bar pressing for ICSS or received yoked brain-stimulation (current = 22 μA); 3) a post-stimulation recovery period with no brain-stimulation available for 90 min; and 4) a second stimulation period conducted with identical stimulation parameters to the first test phase. Dialysate samples were collected from unstimulated control animals for at least 4.5 h.

Results

VTA-Stimulation

The ICSS group averaged 1281 trains of brain-stimulation (range = 1058 to 1560) in the first stimulation period and 1568 (range = 999 to 2153) in the second stimulation period 90 min later. The yoked group received an average of 1821 stimulations (range = 1720 to 1977) in the first period and 1987 stimulations (range = 1685 to 2240) in the second. There was no significant difference in the average number of stimulations received between the ICSS and the yoked groups or between stimulation periods for each group.

Both ICSS and yoked animals exhibited approach and exploratory behaviors such as forward locomotion, rearing, and sniffing during stimulation periods.

Neurochemical Analyses

Mean baseline concentrations of DA, DOPAC, HVA, and 5-HIAA are given in Table 1A.

Dopamine

There were no significant differences in basal values between the three groups. The ANOVA revealed that during and following each of the stimulation periods, DA in both the ICSS and yoked-stimulation groups differed significantly from the unstimulated control group ($F_{1,8} = 10.44$; $p < 0.01$ and $F_{1,8} = 26.43$; $p < 0.01$). There were no significant differences in dialysate DA concentrations between the ICSS and yoked control groups over each phase of the experiment. Post-hoc comparisons revealed significant increases in DA relative to unstimulated

Table 1. Mean basal values for DA, DOPAC, HVA, and 5-HIAA in (A) Experiment 1 and (B) Experiment 2. Values are represented as the mean of the averages of three baseline samples from each animal. Range values are means of each baseline sample across animals. Values are uncorrected for probe recovery.

A. EXPERIMENT 1.

Analyte	*Group*	*Mean (nM)*	*Range (nM)*
Dopamine	Control	0.50 ± 0.03	0.49 ± 0.03 to 0.52 ± 0.04
	ICSS	0.85 ± 0.19	0.81 ± 0.17 to 0.87 ± 0.20
	Yoked	0.84 ± 0.19	0.83 ± 0.05 to 0.87 ± 0.09
DOPAC	Control	302.7 ± 74.6	292.1 ± 71.2 to 313.4 ± 77.0
	ICSS	346.5 ± 66.7	343.8 ± 63.6 to 350.4 ± 68.7
	Yoked	499.0 ± 126.4	486.4 ± 126.8 to 506.0 ± 127.8
HVA	Control	147.9 ± 25.2	145.9 ± 23.2 to 149.0 ± 25.3
	ICSS	107.9 ± 5.5	105.2 ± 5.8 to 110.3 ± 7.2
	Yoked	134.2 ± 28.4	132.0 ± 25.9 to 136.3 ± 29.7
5-HIAA	Control	110.5 ± 18.0	110.0 ± 18.4 to 111.1 ± 18.7
	ICSS	100.1 ± 15.6	98.2 ± 13.9 to 103.0 ± 18.5
	Yoked	104.9 ± 11.2	104.2 ± 11.8 to 106.3 ± 11.1

B. EXPERIMENT 2.

Analyte	*Mean (nM)*	*Range (nM)*
Dopamine	0.72 ± 0.12	0.71 ± 0.11 to 0.73 ± 0.13
DOPAC	675.1 ± 117.2	659.4 ± 108.2 to 686.8 ± 120.6
HVA	212.2 ± 23.1	209.5 ± 21.1 to 214.3 ± 23.1
5-HIAA	143.9 ± 10.2	142.9 ± 10.0 to 144.8 ± 10.2

controls that peaked during each ICSS period. Fig. 3A shows that in the first ICSS period, stimulation of the VTA was accompanied by a 96.5 ± 28.2 % increase in extracellular DA above baseline control values. DA remained elevated above pre-stimulation values for 30 min post-stimulation. The second ICSS period again produced an increase in extracellular DA of 178.3 ± 48.1 %. In this instance, DA remained significantly elevated for 60 min post-stimulation.

Stimulation of the VTA in yoked animals (Fig. 3B) was also associated with increases in extracellular DA of 93.6 ± 16.7 % and 114.8 ± 14.1 % in the first and second stimulation periods, respectively. DA remained significantly elevated for up to 75 min after each stimulation period.

Dopamine Metabolites

DOPAC

Self-stimulation produced maximal increases in extracellular DOPAC of 63.3 ± 5.0 % and 54.3 ± 14.4% after the first and second stimulation periods, respectively (Fig. 4A). Maximal increases were obtained in the first sample after the stimulation period and these values differed significantly from those of the unstimulated control group ($F_{1,8} = 130.78$; $p < 0.0005$). Significant increases were observed during the stimulation periods and DOPAC remained elevated for 60 min (first stimulation period) and 30 min (second stimulation period) post-stimulation.

Stimulation in the yoked group also resulted in extracellular increases in DOPAC that also reached maximal values (144.5 ± 8.5%) in the first post-stimulation samples (Fig. 4B). The first stimulation period produced a significant increase in DOPAC lasting 60 min post-stimulation. The second stimulation period also produced a significant increase in DOPAC which remained significantly elevated for 15 min post-stimulation. The maximum

Figure 3. Effect of (A) self-stimulation (ICSS) or (B) yoked-stimulation (Yoked) of the ventral tegmental area on extracellular dopamine (DA) in the nucleus accumbens. Stimulation-induced effects are expressed as percent change relative to the average of four pre-stimulation baseline measures (100%). Bar graphs (bottom panels) show the number of stimulations received in each 15-min stimulation period. $+p<0.10$, $*p<0.05$, $**p<0.01$, $***p<0.001$.

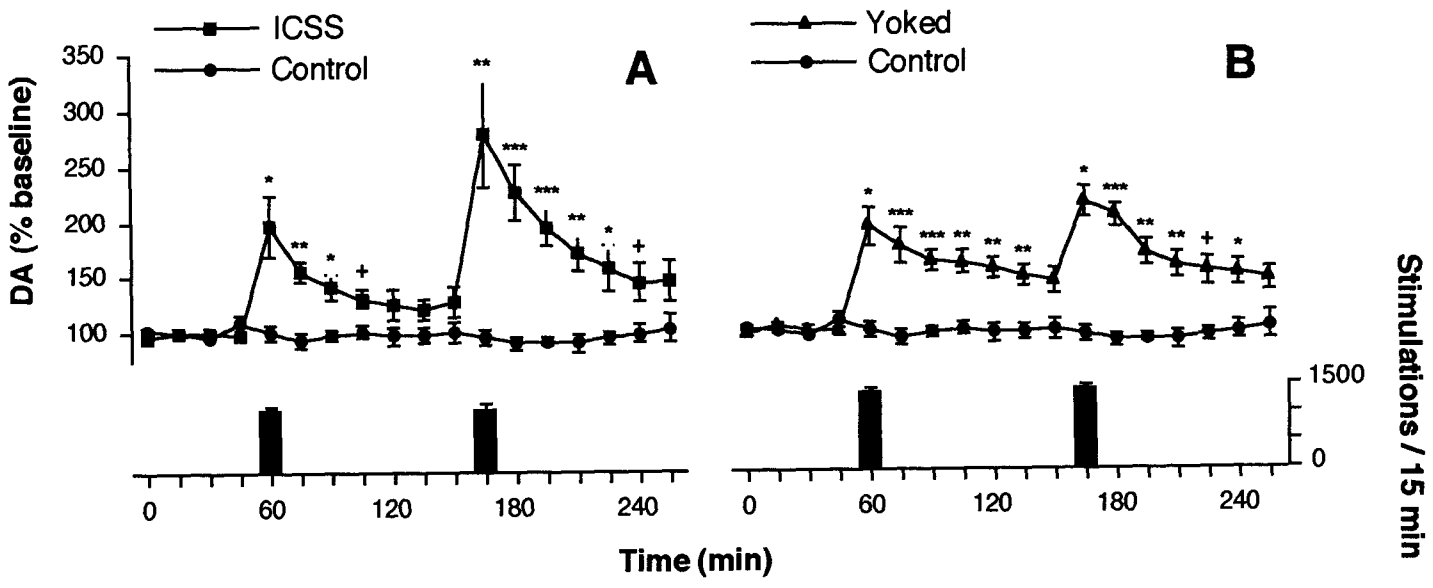
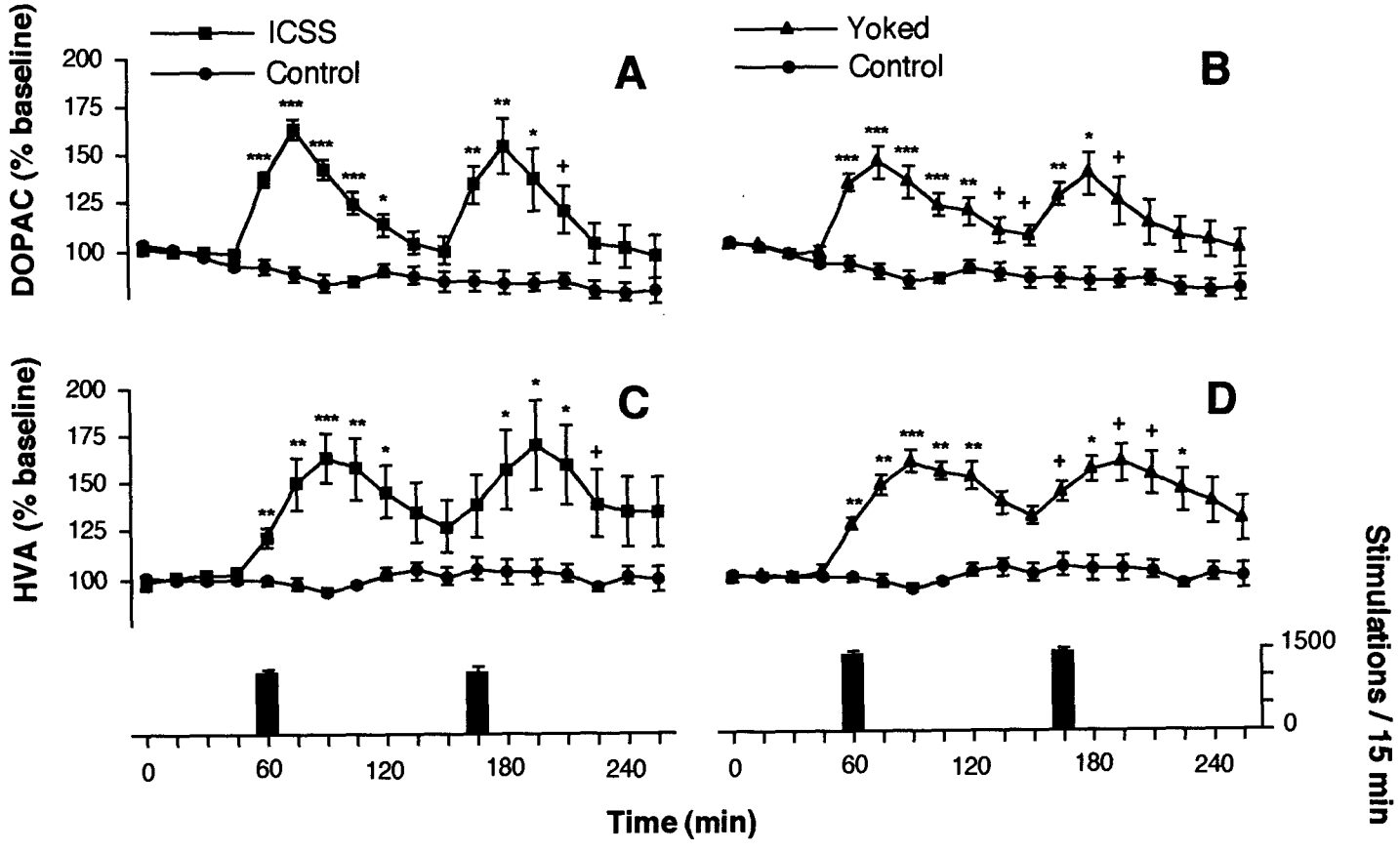

ICSS
Control
A
Yoked
Control
B
DA (% baseline)
350
300
250
200
150
100
1500
0
Stimulations / 15 min
0
60
120
180
240
Time (min)

Figure 4. Effect of self-stimulation or yoked-stimulation of the ventral tegmental area on extracellular DOPAC and HVA in the nucleus accumbens. Stimulation-induced effects are expressed as percent change relative to the average of four pre-stimulation baseline measures (100%). The top panels show DOPAC changes in the (A) ICSS and (B) Yoked groups as compared to the unstimulated Control group. The middle panels show HVA changes in the (C) ICSS and (D) Yoked groups as compared to the unstimulated Control group. Bar graphs (bottom panels) show the number of stimulations received in each 15-min stimulation period. $+p<0.10$, $*p<0.05$, $**p<0.01$, $***p<0.001$.


ICSS
Control
Yoked
Control
A
B
C
D
DOPAC (% baseline)
HVA (% baseline)
200
175
150
125
100
1500
0
0
60
120
180
240
Time (min)
Stimulations / 15 min

increase was 38.1 ± 11.4%. All of these values differed significantly from those of the unstimulated control group ($F_{1,8} = 35.25$; $p < 0.0005$).

HVA

Self-stimulation produced significant increases above baseline (Fig. 4C) in extracellular HVA (63.3 ± 13.5%, first stimulation period and 69.7 ± 23.2%, second stimulation period). The maximum increases occured 30 min post-stimulation. Significant increases were seen during ICSS in the first period and levels remained significantly elevated for 60 min post-stimulation. Following the second stimulation period, a significant increase in HVA was seen only after the stimulation session and levels remained significantly elevated for 30 min.

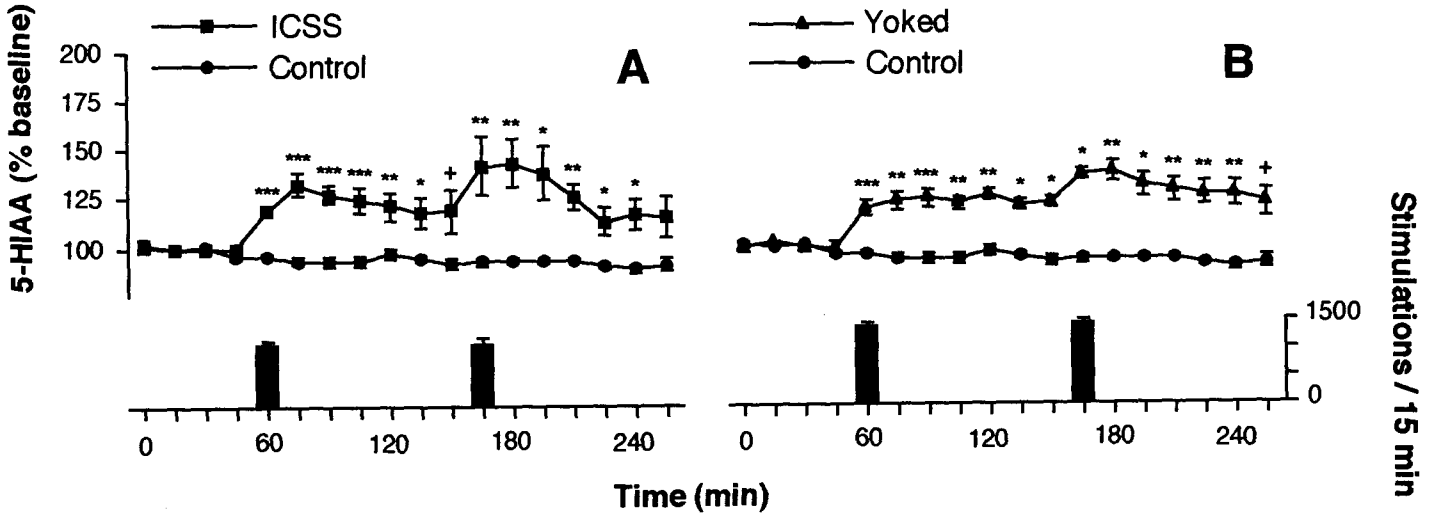
Yoked-stimulation also resulted in significant increases ($F_{1,8} = 126.89$; $p < 0.0005$) in extracellular HVA (Fig. 4D). The first stimulation produced an immediate and significant increase in HVA that persisted for 60 min. The maximal increase was 60.1 ± 5.6%. The second stimulation period produced an increase in HVA but only reached significance ($p < 0.05$) in two samples: 15 and 60 min post-stimulation.

Serotonin Metabolite

5-HIAA

ICSS was accompanied by long-lasting increases in extracellular 5-HIAA concentrations ($F_{1,8} = 38.00$; $p < 0.001$). Significant increases in 5-HIAA were seen during ICSS periods and these had not returned to pre-stimulation baseline values after 75 min (Fig.5A). Maximal increases were obtained in the samples obtained immediately following the stimulation periods. Increases of 32.3 ± 6.0% (first stimulation period) and 42.6 ± 12.5% (second stimulation period) were observed.

Figure 5. Effect of (A) self-stimulation (ICSS) or (B) yoked-stimulation (Yoked) of the ventral tegmental area on extracellular 5-HIAA in the nucleus accumbens. Stimulation-induced effects are expressed as percent change relative to the average of four pre-stimulation baseline measures (100%). Bar graphs (bottom panels) show the number of stimulations received in each 15-min stimulation period. $+p<0.10$, $*p<0.05$, $**p<0.01$, $***p<0.001$.


5-HIAA (% baseline)
ICSS
Control
A
Yoked
Control
B
200
175
150
125
100
1500
0
0
60
120
180
240
Time (min)
Stimulations / 15 min

As with ICSS, yoked-stimulation (Fig. 5B) resulted in increases in 5-HIAA that were persistent and relatively stable ($F_{1,8} = 32.20$; $p < 0.001$). The first stimulation caused an immediate increase in 5-HIAA which lasted for 90 min post-stimulation. This elevated level of 5-HIAA was stable and increases ranged from 18.6 ± 8.6% (first stimulation period) to 25.3 ± 4.8% (fourth post-stimulation sample). The second stimulation period caused a further increase in 5-HIAA to a maximum of 37.2 ± 5.2% (first sample post-stimulation). Extracellular 5-HIAA remained significantly different from baseline control values for 90 min following the second period of VTA-stimulation, at which point the experiment was terminated.

Histology

Histological examination of brain sections confirmed that MD probes were in the nucleus accumbens. Placements extended from about 1.2 to 2.2 mm anterior to Bregma (mode = 1.7 mm) and, based on the deepest point of insertion, the exposed membrane of the MD probe was fully in the nucleus accumbens. Fig. 6 shows a representative placement of a MD probe and extent of damage in the nucleus accumbens.

The tips of the bipolar electrodes were in the VTA in all animals used in the microdialysis experiments. Fig. 7 shows electrode tip placements for ICSS and yoked animals in Experiment 1. Control animal electrode tips (Fig. 8) were in the same areas.

Figure 6. Representative placement of a microdialysis probe (2 mm exposed dialysis membrane) and extent of damage in the right nucleus accumbens. Approximately 1.7 mm anterior to Bregma (flat skull). Coronal section redrawn from Paxinos & Watson (1982).

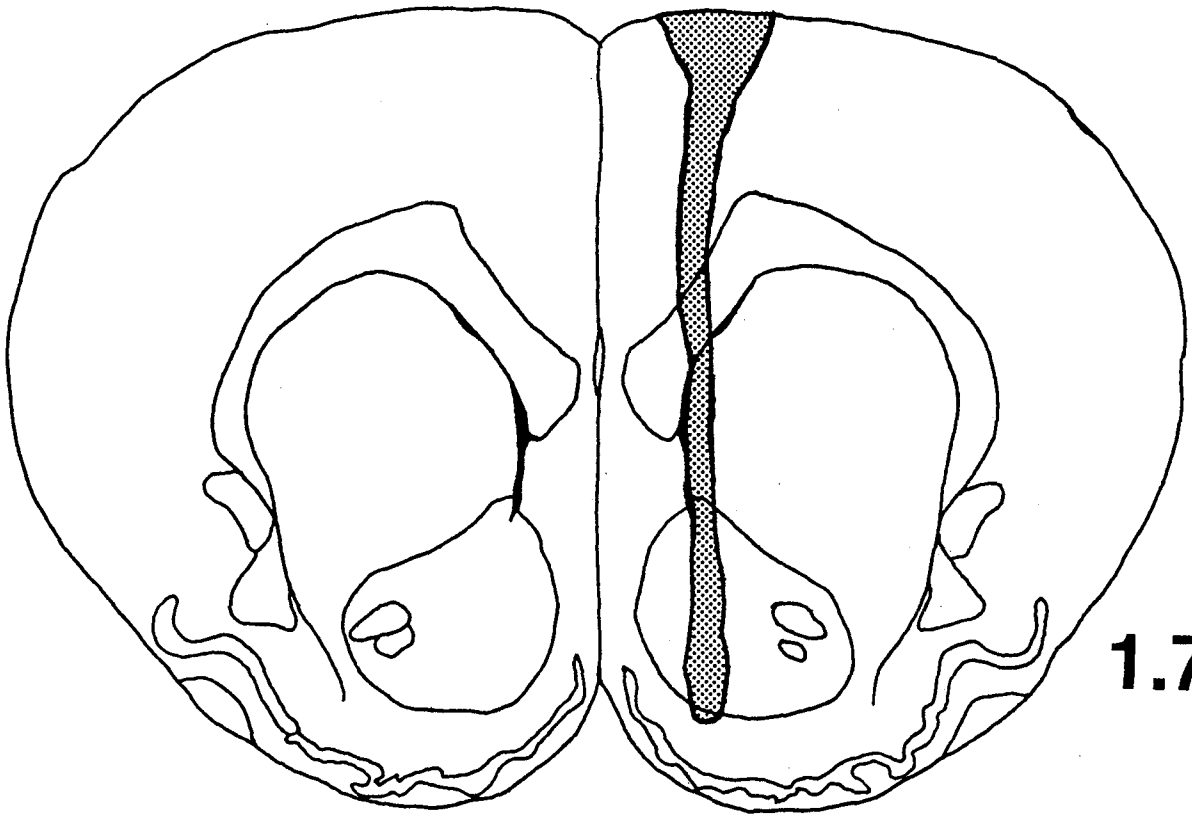

1.7 mm

Figure 7. Location of the tips of bipolar stimulating electrodes for the ICSS (squares) and Yoked (triangles) groups in Experiment 1. All electrodes were implanted in the right hemisphere. Yoked placements are drawn on the left side for clarity. Serial coronal sections redrawn from Paxinos and Watson (1982). The numbers on the right of each section refer to their position relative to Bregma (mm).

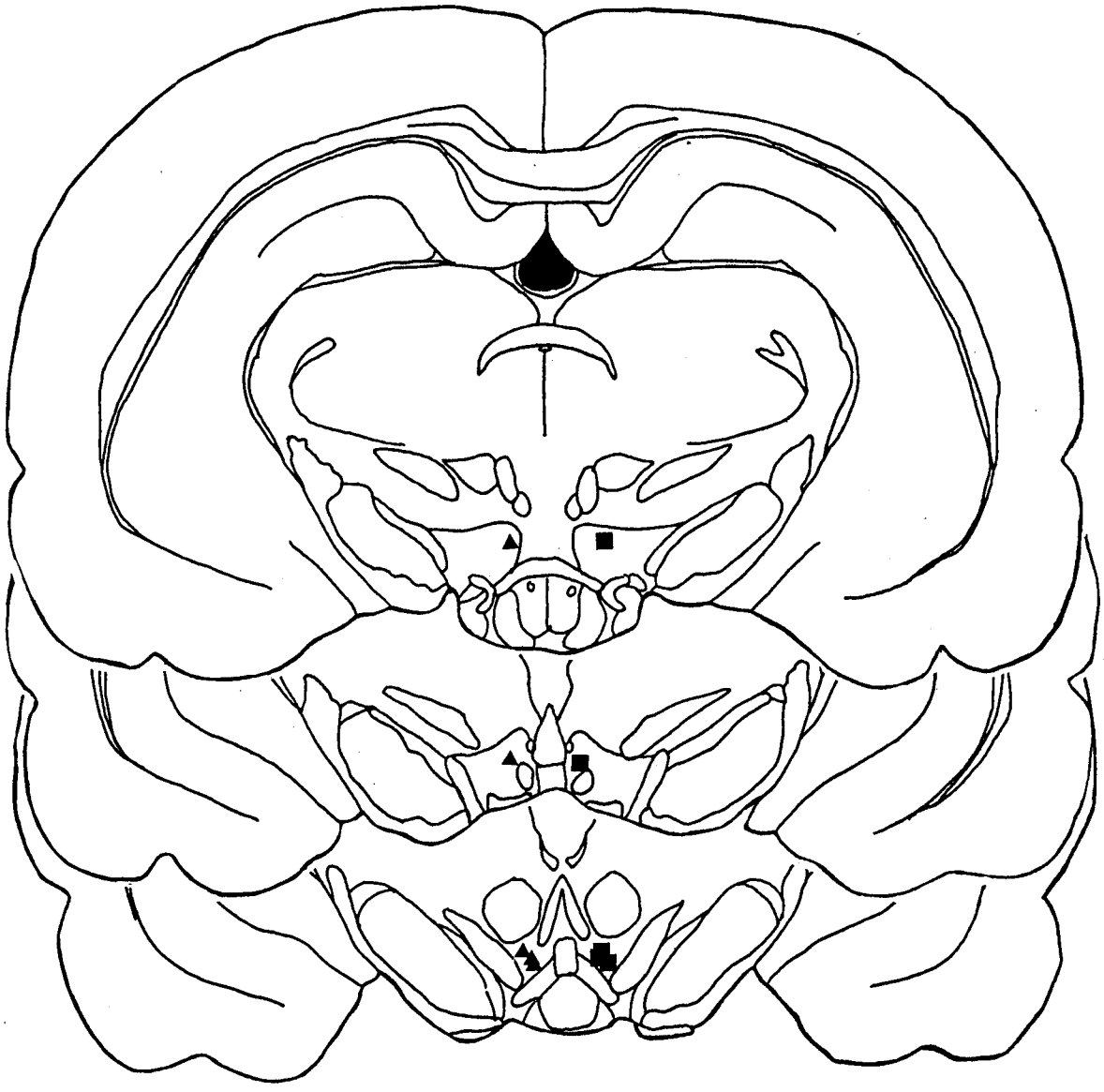
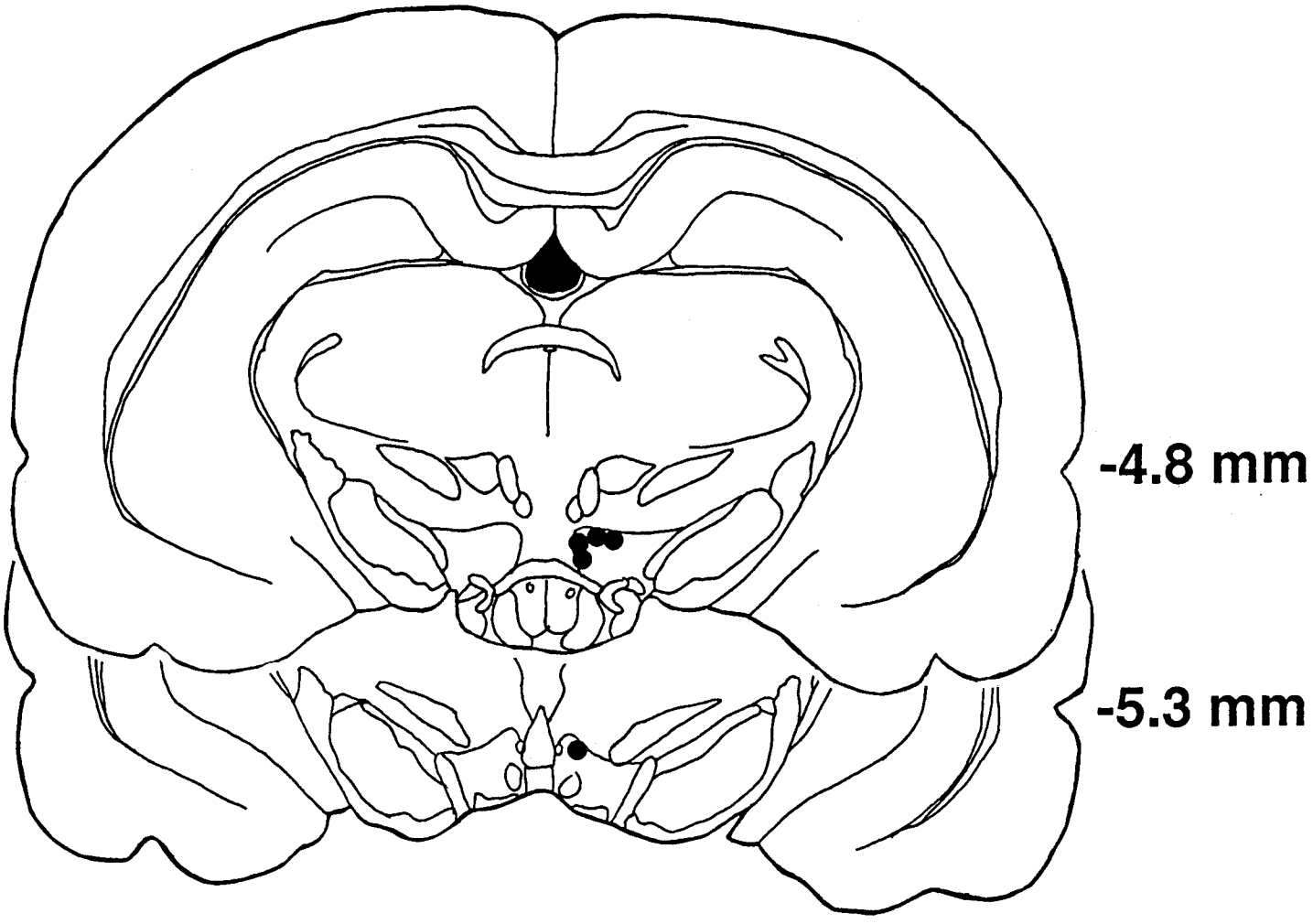

-4.8 mm
-5.3 mm
-5.8 mm

Figure 8. Location of the tips of bipolar stimulating electrodes for the unstimulated Control animals in Experiment 1. All electrodes were implanted in the right hemisphere. Coronal sections redrawn from Paxinos and Watson (1982). The numbers on the right of each section refer to their position relative to Bregma (mm).


-4.8 mm
-5.3 mm

Discussion

Self- or yoked-stimulation of the VTA in drug-free rats was accompanied by significant increases in extracellular DA concentrations in the ipsilateral nucleus accumbens as assessed by MD. Increased concentrations of the DA metabolites, DOPAC and HVA, also were associated with VTA-stimulation. The pattern of change of DOPAC and HVA relative to the 15-min stimulation session differs from DA in that the maximum increases were not observed during this period, but in the first and second post-stimulation periods, respectively. Significant increases in the serotonin metabolite, 5-HIAA also were observed following VTA-stimulation. Although the increases in 5-HIAA concentrations coincided with stimulation periods, the sustained elevation of the metabolite, unlike DA or DOPAC, suggests that serotonin activity in the nucleus accumbens may not be associated directly with brain-stimulation reward.

The inclusion of two important control groups in this experiment aided in discerning neurochemical changes due to rewarding brain-stimulation from those that might be attributed to non-specific environmental stimuli and handling (i.e. the unstimulated control group) or from operant responding (i.e. the yoked control group). Aside from an early influential report that non-contingent electrical stimulation of ICSS sites was aversive (Steiner, Beer, & Shaffer, 1969), many studies have demonstrated that brain-stimulation can be rewarding if administered non-contingently to animals with a previous history of ICSS (Ettenberg & Duvauchelle, 1988; Tsang & Stutz, 1984; Ettenberg, Laferrière, Milner, & White, 1981). In an elegant and convincing experiment, Ettenberg & Duvauchelle (1988) showed that rats developed a conditioned place preference to a location associated with non-contingent MFB stimulation at self-produced rates. Since the place preference technique has been shown to be sensitive to both rewarding and

aversive treatments, it would be difficult to defend the suggestion that non-contingent brain-stimulation is aversive.

Therefore, it is reasonable to attribute increased DA efflux in yoked animals during VTA-stimulation to the rewarding effects of brain-stimulation. It follows, then, that increased DA efflux in ICSS animals during self-stimulation is due to brain-stimulation reward rather than operant behavior. Both ICSS and yoked animals engaged in locomotion, rearing, and sniffing during VTA-stimulation. The possibility that these behaviors may increase DA release in the nucleus accumbens still exists.

EXPERIMENT 2

Effect of varying current intensity during VTA-ICSS on DA, DOPAC, HVA, AND 5-HIAA in the ipsilateral nucleus accumbens

Experiment 2 examined the effects of varying current intensities on DA efflux in the ipsilateral nucleus accumbens as measured by microdialysis. The current intensities ranged from low intensities, just above ICSS threshold, to high intensities producing optimal ICSS rates. The order of intensities used during training and on a given test day was randomized to minimize any contrast effects.

A positive correlation between ICSS responses and the magnitude of the increase in extracellular DA concentrations in the nucleus accumbens would provide further support for a dopaminergic substrate for VTA-ICSS.

Methods

Once stable rate-intensity profiles were obtained, animals (n=7) were tested for 6 days on a three-intensity program which would be used in the microdialysis phase of the experiment. The program consisted of three, 15-min stimulation periods, each at a different intensity (i.e. high, H, medium, M, or low, L, current), spaced 90 min apart. The order of intensities used in the program was randomized and a specific combination was not repeated over the 6 days. The L current was the intensity at which an animal responded at approximately 100-150 presses/ 5 min. The H current was the intensity at which the animal's pressing rate reached an asymptote. The M current was the midpoint value between the L and H current.

As in Experiment 1, each animal was implanted with a microdialysis probe and connected to the swivel and left in the test box overnight with free access to food and water. No lever was present in the test box overnight. The probe was flushed overnight at 1.5 μL/min with perfusate.

The day of the experiment, the lever was remounted in the test box. Dialysate samples were injected automatically into the HPLC-ED system every 15 min. Testing consisted of: 1) baseline measures of neurotransmitter and metabolites (minimum of 60 min); 2) an ICSS period (15 min) where each animal engaged in bar pressing for VTA stimulation; 3) a post-ICSS recovery period with no brain-stimulation available for 90 min; and 4) a second and third 15 min ICSS period, each followed by a 90 min recovery period. A different intensity was used for each ICSS period. Again, the order of intensities used on a given test day was randomized across animals with at least one subject in each of the following six sequences: LMH, LHM, MLH, MHL, HLM, HML.

Results

VTA-Stimulation

The mean current intensities for the three stimulation conditions were: L=18.0 ±1.7 μA; M=22.0 ± 1.7 μA; and H=26.7 ± 2.0 μA. During ICSS, the mean number of lever presses per 15 min at each intensity were: L intensity, 502 ± 61; M intensity, 1133 ± 109; and H intensity, 1629 ± 80. There was a positive correlation between the number of lever presses and the current intensity (Pearson's P=0.0048; r=0.5781).

Neurochemical Analyses

Mean baseline levels of DA, DOPAC, HVA, and 5-HIAA are presented in Table 1B.

Dopamine

Fig. 9. shows baseline DA levels and increases associated with ICSS at the three current intensities. The DA level at time = 0 min for each current intensity corresponded to the fourth baseline sample. Statistical comparisons were made between this baseline sample and changes in DA following ICSS. Further comparisons made between samples following ICSS and the sample preceding stimulation, although not shown, yielded almost identical results. Self-stimulation at the L intensity resulted in an increase in extracellular DA of 22.0 ± 3.6% ($p<0.05$). After the stimulation period, there was an immediate return to values that were not statistically different from baseline. A greater increase of 48.0 ± 6.3% was observed following ICSS at the M intensity and these levels remained significantly elevated for 15 min following the stimulation period. Self-stimulation at the H intensity was accompanied by an increase to 185.2 ± 13.7% of pre-stimulation baseline. Again, the augmentation outlasted the ICSS bout by 15 min ($p<0.10$). By the second post-stimulation sample, DA concentrations were still elevated but were approaching baseline values.

Dopamine Metabolites

DOPAC

The top panel of Fig.10 shows DOPAC concentrations before, during and after ICSS at each of the three stimulation intensities. Although small increases were seen after ICSS at both the L and M intensities, the only statistically significant changes were obtained after ICSS at the H intensity. In this case,

Figure 9. Effect of self-stimulation at low (18μA), medium (22μA), and high (27μA) current intensities on extracellular dopamine (DA). Stimulation-induced effects are expressed as percent change relative to the average of four pre-stimulation baseline measures (100%). Bar graphs (bottom panels) show the number of stimulations received in each 15-min stimulation period. +$p<0.10$, *$p<0.05$, **$p<0.01$.

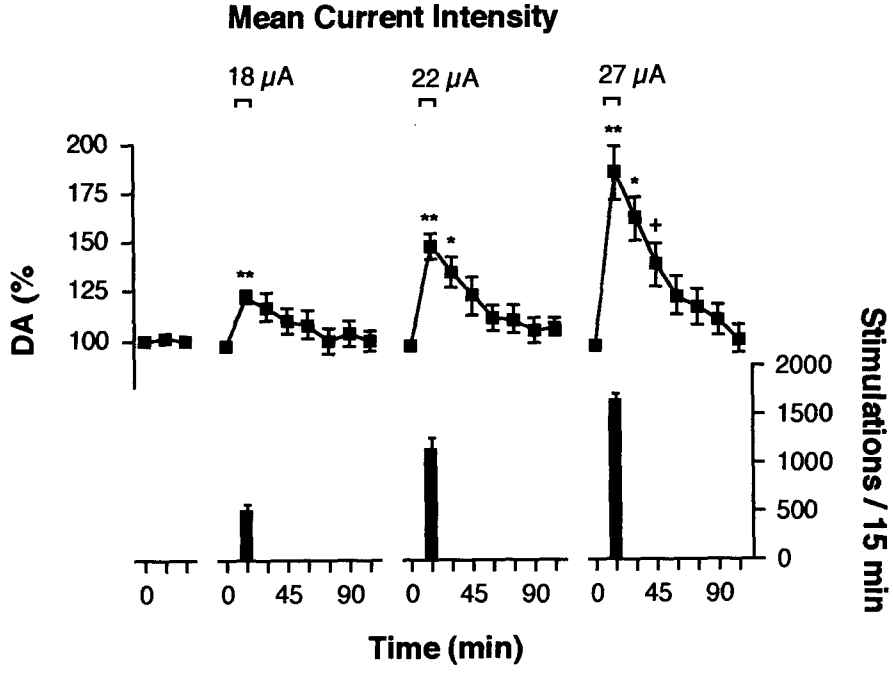
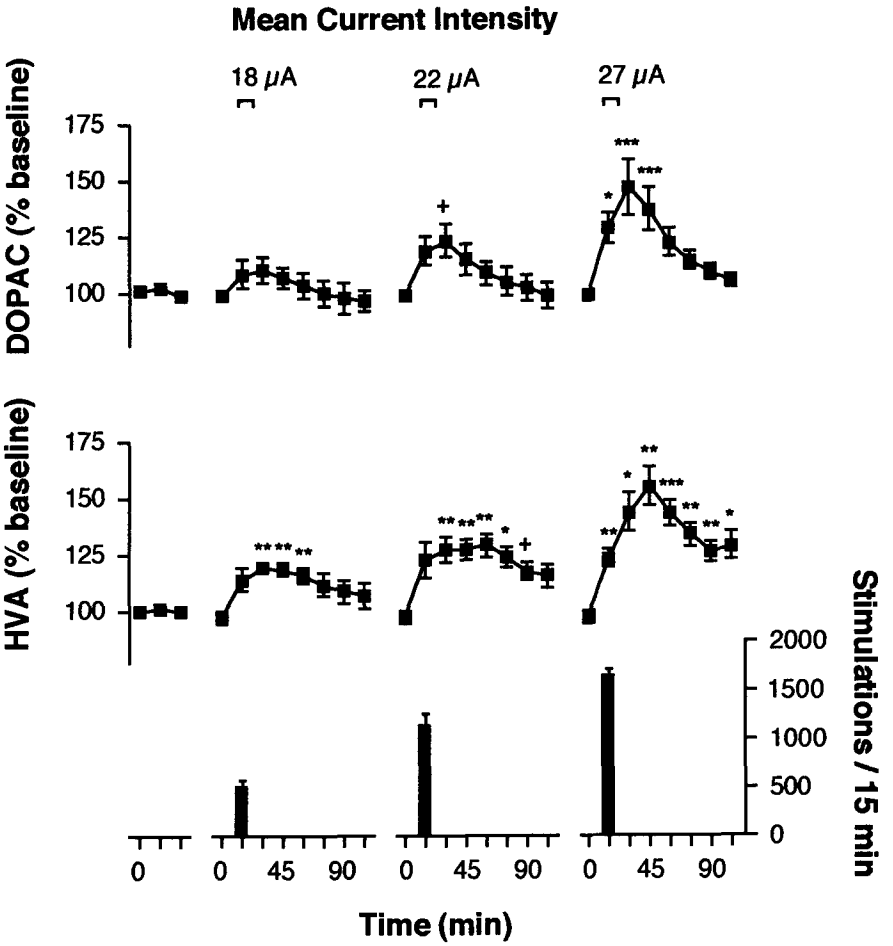

Mean Current Intensity
18 μA
22 μA
27 μA
DA (%
200
175
150
125
100
**
**
*
**
*
+
2000
1500
1000
500
0
Stimulations / 15 min
0
0
45
90
0
45
90
0
45
90
Time (min)

Figure 10. Effect of self-stimulation at low (18μA), medium (22μA), and high (27μA) current intensities on extracellular DOPAC (top panels) and HVA (middle panels). Stimulation-induced effects are expressed as percent change relative to the average of four pre-stimulation baseline measures (100%). Bar graphs (bottom panels) show the number of stimulations received in each 15-min stimulation period. +$p<0.10$, *$p<0.05$, **$p<0.01$.


Mean Current Intensity
18 μA
22 μA
27 μA
DOPAC (% baseline)
HVA (% baseline)
175
150
125
100
Stimulations / 15 min
2000
1500
1000
500
0
0
45
90
Time (min)

significant increases in DOPAC were seen during the ICSS period and maximum increases (146.9 ± 12.7%) were obtained in the first post-stimulation sample. Levels remained elevated for 30 min post-stimulation.

HVA

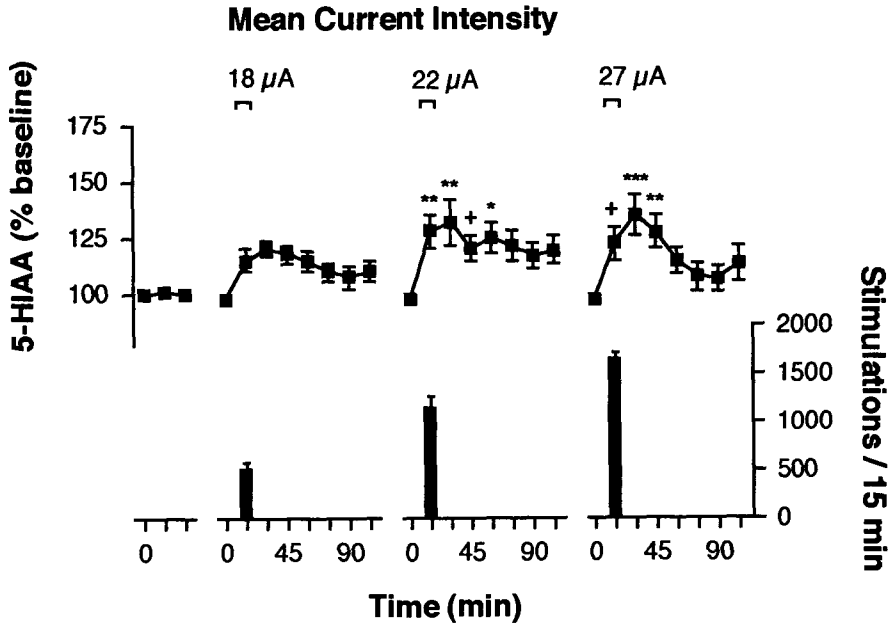
Self-stimulation resulted in significant increases in HVA at each of the three current intensities (lower panel, Fig. 10). After an ICSS period at the L current, HVA increased by 19.0 ± 2.6% (first post-stimulation sample) and remained elevated for 45 min post-stimulation. Self-stimulation at the M current elevated HVA which persisted for 60 min post-stimulation. The maximal increase at this intensity was 29.1 ± 5.0%. At both L and M intensities, changes in HVA did not reach statistical significance until the sample following the ICSS period. ICSS at the high current intensity resulted in an immediate increase in HVA which reached a maximum (154.5 ± 8.6%) in the second sample post-stimulation. After H intensity stimulation, HVA remained significantly elevated for 90 min following the ICSS period.

Serotonin Metabolite

5-HIAA

Although increased 5-HIAA concentrations were observed following L intensity ICSS, they were not statistically significant. Significant elevations in extracellular 5-HIAA were observed with both M and H intensities (Fig. 11). Self-stimulation at M intensity was accompanied by a significant increase during the ICSS period and in the first and third post-stimulation samples. The maximum increase in 5-HIAA at M intensity was 31.2 ± 10.2%. Self-stimulation at the H intensity was followed by a significant increase in 5-HIAA in the first sample post-stimulation (34.9 ± 8.9%) and they remained elevated for 30 min post-stimulation.

Figure 11. Effect of self-stimulation at low (18μA), medium (22μA), and high (27μA) current intensities on extracellular 5-HIAA. Stimulation-induced effects are expressed as percent change relative to the average of four pre-stimulation baseline measures (100%). Bar graphs (lower panels) show the number of stimulations received in each 15-min stimulation period. +$p<0.10$, *$p<0.05$, **$p<0.01$, ***$p<0.001$.

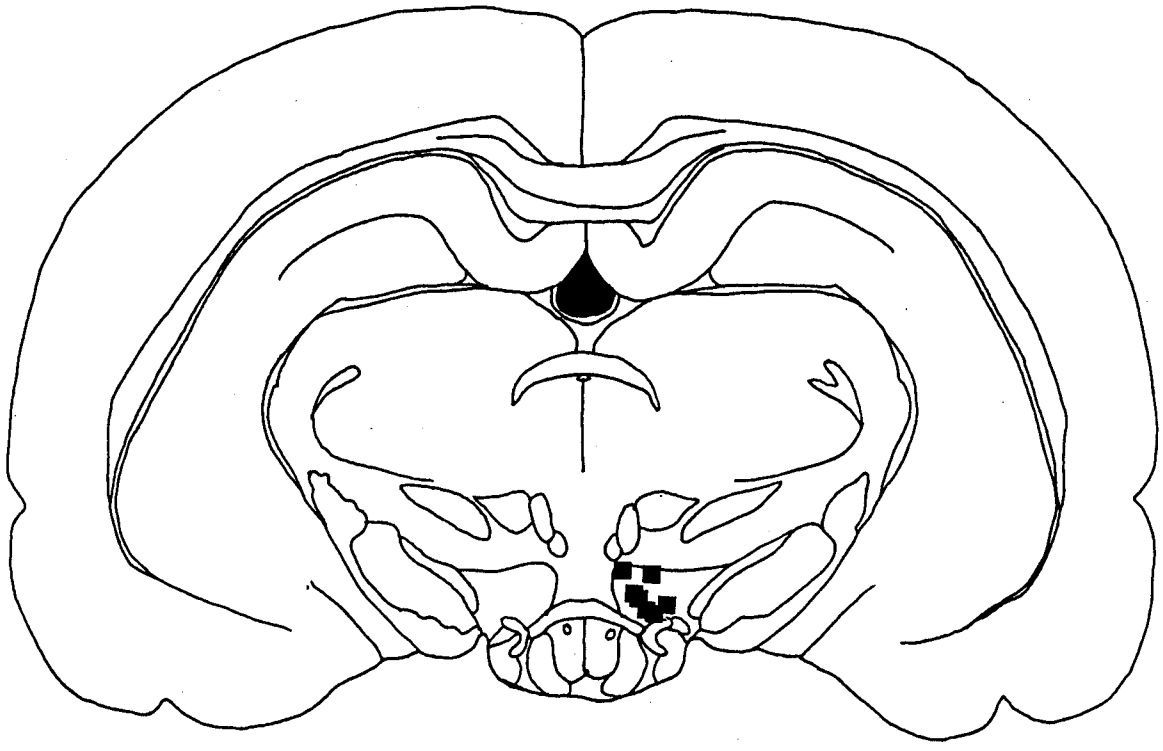

Mean Current Intensity
18 μA
22 μA
27 μA
5-HIAA (% baseline)
175
150
125
100
2000
1500
1000
500
0
Stimulations / 15 min
0
0
45
90
0
45
90
0
45
90
Time (min)

Histology

Histological examination of brain sections confirmed that microdialysis probes were in the nucleus accumbens. Placements were in the same range of sections as those in Experiment 1 and extended from about 1.2 to 2.0 mm anterior to Bregma (mode = 1.7 mm). Based on the deepest point of insertion, the exposed membrane of the microdialysis probe was fully in the nucleus accumbens.

The location of the electrode tips from animals in Experiment 2 are shown in Fig.12.

Figure 12. Location of the tips of bipolar stimulating electrodes for ICSS animals in Experiment 2. All electrodes were implanted in the right hemisphere and placements were distributed within 0.3 mm of this section. Coronal section redrawn from Paxinos and Watson (1982). The number on the right of the section refers to its position relative to Bregma (mm).



-4.8 mm

Discussion

As in Experiment 1, self-stimulation of the VTA was accompanied by significant increases in extracellular DA concentrations in the ipsilateral nucleus accumbens. There was a positive correlation between the magnitude of extracellular DA and the number of lever presses. Augmented elevations of extracellular DA at higher intensities is likely due to an increased recruitment of dopaminergic fibers carrying the reward signal.

DA concentrations associated with ICSS remained elevated above pre-stimulation baseline values for extended periods. A comparison of the temporal patterns of extracellular DA concentrations at the three current intensities suggests that the more intense the brain-stimulation, the longer DA values remain elevated. Such sustained increases in DA have also been observed in dialysates from the nucleus accumbens of male rats following a 30-min session of copulation (Damsma, Pfaus, Wenkstern, Phillips, and Fibiger, 1992; Pfaus, Damsma, Nomikos, Wenkstern, Blaha, Phillips, and Fibiger, 1990). DA returned to pre-copulation values within 20-30 min after receptive females were removed from the test chamber. The hysteresis of nucleus accumbens DA following strong stimulation by either natural stimuli or electrical brain-stimulation may have important implications for the neurochemical functioning of this system and for theories of the functional correlates of dopaminergic transmission. First, the prolonged increases in extracellular DA may indicate temporary saturation of the DA uptake processes. Second, since this condition occurs during copulation in the male rat, it may have functional significance. For example, it may be a prerequisite for the activation of immediate-early genes, such as c-fos, whose expression is induced by both copulation in the male rat (Robertson, Pfaus, Atkinson, Matsumara, Phillips, and Fibiger, 1991) and VTA-ICSS (Fiorino, Robertson, Phillips, Fibiger,

and Swindale, 1992). The large and sustained increase in DA following strong stimuli is also consistent with a parasynaptic (Herkenham, 1987) or neuromodulatory role for extracellular DA.

GENERAL DISCUSSION

Self-stimulation of VTA was accompanied by significant increases in extracellular DA concentrations in the ipsilateral nucleus accumbens. This result is in general agreement with MD (Nakahara et al., 1992, 1991, 1989a, 1989b; Miliaressis et al., 1991), electrochemistry (Blaha & Phillips, 1990; Phillips et al., 1989), *ex vivo* analysis of DA/DOPAC ratios (Fibiger et al., 1987), and *in vivo* tyrosine hydroxylase activity experiments (Phillips et al., 1987). Important controls for operant responding (i.e. yoked animals) and non-specific environmental stimuli (i.e. unstimulated control animals) strengthen the argument that the observed increases in extracellular DA after VTA-ICSS were due to rewarding properties of brain-stimulation.

There was a prolonged elevation of extracellular DA above pre-stimulation baseline values after VTA-stimulation, sometimes lasting up to 60 min post-stimulation at high current intensities. A sustained elevation in extracellular DA after VTA-ICSS has also been observed in electrochemistry experiments (Blaha & Phillips, 1990). Whereas the decline in lever press rate occured quickly (i.e. within 3 min) after a 5 μA decrement in stimulation current intensity, oxidation current corresponding to extracellular DA concentration declined more slowly (i.e. 10 min). As mentioned previously, this phenomenon may have functional significance including a role in parasynaptic transmission which may be facilitated by large and prolonged elevations of DA. Another function may be the regulation of gene expression via the induction of immediate-early genes such as c-fos. There is evidence that Fos (the protein product of c-fos) and Jun proteins form heterodimers which, upon binding, may regulate the expression of selected target genes in the cell (Morgan & Curran, 1990). Cell culture studies suggest that the proenkephalin gene may be regulated by Fos and Jun (Sonnenberg, Rauscher, Morgan, and

Curran, 1990). Given that opiate peptides, such as enkephalin, activate DA cells to increase accumbens-dependent behaviors (Stellar & Rice, 1989), it is possible that, following large increases in extracellular DA, a cascade of gene expression occurs culminating in the production of substances that can potentiate dopaminergic activity at a different level.

The temporal pattern of changes in extracellular DOPAC concentration after ICSS is consistent with the assumption that DOPAC is mainly a product of intracellular metabolism of recently released DA, recaptured by the presynaptic terminal via an uptake mechanism. Regardless, many findings suggest that DOPAC is a poor index of DA release (Westerink et al., 1988; Zetterström et al., 1988).

Evidence for serotonergic involvement in ICSS is equivocal (Stellar & Rice, 1989). Experiments 1 and 2 demonstrated that VTA-stimulation was associated with increased 5-HIAA concentrations. Unlike DA or DOPAC, however, 5-HIAA concentrations never returned to pre-stimulation baseline values following the stimulation period. Although neurotransmitter release may not be inferred directly from metabolite concentrations, this finding suggests that if serotonin is involved in VTA ICSS, its role is limited and/or indirect.

Results from Experiment 2 concerning the positive correlation between extracellular DA concentrations and ICSS responding should be interpreted with caution. It is assumed that increasing current intensity results in increased recruitment of neurons that are activated by stimulation near the tip of the electrode. Increasing extracellular DA concentrations in the nucleus accumbens, such as after administration of cocaine or amphetamine, results in increased VTA-ICSS rates. The larger increases in DA concentrations observed after VTA-ICSS at higher intensities may likely reflect a combination of an increased number of dopaminergic neurons being activated and increased rates of ICSS responding

due to the reinforcing properties of augmented DA concentrations in the nucleus accumbens. The confound is difficult to avoid. One alternative is to keep the number of stimulations constant while varying the current intensity, possibly by employing a higher fixed-ratio of responding at higher intensities. Another approach is to use varying stimulation frequency while holding current intensity constant. In this way, the number of neurons activated by stimulation would be held relatively constant. This approach, however, may yield confounded results for other reasons. The terminals of many VTA neurons contain both DA and the neuropeptides, cholecystokinin (CCK) and neurotensin (Lundberg & Hökfelt, 1983). CCK, for example, has excitatory and inhibitory effects on DA release in the medial posterior and anterior nucleus accumbens, respectively (Crawley, 1991). It has been suggested that the differential release of classical neurotransmitters, such as DA, and colocalized neuropeptides, such as CCK, may be frequency-coded. That is, low frequency firing may favor the release of classical neurotransmitters, while high frequency firing may favor peptide release (Hökfelt, Everitt, Holets, Meister, Melander, Schalling, Staines, and Lundberg, 1986). If this is true, release of peptides during high frequency stimulation may attenuate or facilitate dopaminergic modulation of reward, depending on the locus of action in the nucleus accumbens.

While other MD studies have provided evidence for and against a dopaminergic substrate of brain-stimulation reward, methodological problems in these experiments raise questions about their results. Although Nakahara et al. (1992, 1991, 1989a, 1989b) observed increased concentrations of DOPAC and HVA in the nucleus accumbens after self-stimulation of the VTA, they found significant increases in DA only in rats pre-treated with the uptake blocker, nomifensine. Some aspects of their methodology may account for this finding. First, their perfusate calcium concentration was 2.3 mM. The physiological

concentration of extracellular calcium of rat striatum has been measured at 1.2 mM (Moghaddam & Bunney, 1989). Perfusate solutions containing high calcium concentrations have been shown to enhance basal release of DA (Cenci et al., 1992; Moghaddam & Bunney, 1989; Westerink, Tuntler, Damsma, Rollema, & De Vries, 1988) and alter the pharmacological response of DA systems (Timmerman & Westerink, 1991; Moghaddam & Bunney, 1989). One would predict, however, that the use of a perfusate high in calcium would facilitate larger increases in neurotransmitter after electrical stimulation. This was not the case. A better explanation involves the issue of the latency to begin MD testing after implantation of the probe. Nakahara began dialysing three hours after probe implantation. It has been suggested, based upon histological, functional, metabolic, and blood flow changes after probe insertion, that the optimum time to begin MD is 8 to 48 hours post-implantation (Benveniste & Hüttemeier, 1990). At this time, brain tissue is tetrodotoxin- and calcium-sensitive, both indices of voltage-dependent neurotransmitter release (Benveniste & Hüttemeier, 1990). A large, release-independent pool of DA may have masked any increase in extracellular DA due to VTA-ICSS. Further, a recent experiment questioning the validity of using repeated MD probe insertions (Camp & Robinson, 1992) casts doubt on the integrity of the comparison made between ICSS rats and rats which received experimenter-administered stimulation.

The MD study by Miliaressis et al. (1991) found increases in nucleus accumbens DA as a result of MFB- or VTA-ICSS only when longer pulse durations of stimulation were used. First, this group did not mention the calcium concentration of their MD perfusate. The importance of this element has been illustrated above. Second, much of their experiment was performed on a small number of animals (four) over two days after the probe was implanted. Dramatic reactions such as edema, hemorrhaging, and gliosis in the tissue adjacent to the

probe have been reported within two days of implantation and the reliability of microdialysis at this point becomes questionable (Benveniste & Hüttemeier, 1990). Finally, this group did not include histological verification of electrode placements. Their stereotaxic coordinates from the Paxinos and Watson stereotaxic atlas (1982) appear to place their electrodes somewhere in the cerebellum. Even if their electrodes were located in the MFB and VTA, differences between the characteristics of brain-stimulation reward at these two sites have been noted and are not trivial (Phillips & Fibiger, 1989). Although Miliaressis et al. (1991) conclude that "dopaminergic cells do not constitute a substantial component of the pathway that relays the reward signal", their findings may be interpreted, putting methodological problems aside, as evidence for multiple systems of reward (see Phillips, 1984).

In conclusion, the present experiments employed an improved methodological protocol and have shown that extracellular DA increases in the nucleus accumbens during rewarding stimulation of the VTA. These results provide further evidence that stimulated mesolimbic DA release is a sufficient condition for reward.

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