*Pharmacology Biochemistry & Behavior*, Vol. 25, pp. 63–69, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

# Effects of Chronic Electrode Implantation on Dopaminergic Neurons *In Vivo*

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Received 19 September 1984

McCOWN, T. J., T. C. NAPIER AND G. R. BREESE. *Effects of chronic electrode implantation on dopaminergic neurons* in vivo. PHARMACOL BIOCHEM BEHAV 25(1) 63–69, 1986.—The present study evaluated the effects of chronic electrode implantation on stimulus-dependent increases of the dopamine (DA) metabolite dihydroxyphenylacetic acid (DOPAC) in relationship to a well characterized *in vivo* model which used electrical stimulation from acute electrode placements in the nigro-striatal pathway. Five days after bipolar electrodes were implanted into the nigro-striatal pathway, non-contingent electrical stimulation (100 μA, 25 Hz, 1.5 msec duration, 20 min session) did not change DA or DOPAC concentrations in the caudate nucleus, nucleus accumbens, or olfactory tubercles, whereas the same stimulation from acute electrode placements causes significant ipsilateral increases in caudate DOPAC. Although DOPAC concentrations did not change when these chronically implanted electrodes were stimulated, similar chronic electrode placement supported intracranial self-stimulation (ICSS). In order to examine the effects of self-stimulation on DOPAC concentrations, five ICSS test groups were established for comparison: implanted only, trained only, minimum response rate, 50% maximum response rate and maximum response rate. Following a 50 min test session, a comparison of either DA, or DOPAC concentrations across the different ICSS conditions revealed no change for the caudate nucleus, nucleus accumbens or olfactory tubercles. Likewise, there was no change between the stimulated and unstimulated sides within each ICSS group. When a comparison was made between implanted only and maximal ICSS response rate groups for changes in DA or DOPAC concentrations in the frontal cortex, no differences were found. Apparently, chronic electrode implantation abolished the ability to electrically stimulate nigro-striatal dopaminergic neurons under non-contingent conditions, and the relationship between dopaminergic neurons and ICSS appears to be indirect in nature.

DOPAC Stimulus-dependent Electrical stimulation Intracranial self-stimulation

USING anatomical, pharmacological and biochemical techniques, many investigations indicate a direct link between dopaminergic function and intracranial self-stimulation (ICSS) behavior. For example, electrodes placed in dopaminergic fibers or cell bodies will support ICSS [7, 9, 12, 14], but when the dopaminergic terminals or fibers are destroyed with 6-hydroxydopamine, ICSS is attenuated [5,6]. If caudate dopamine (DA) is depleted with 6-hydroxydopamine, the resulting loss of ICSS can be reversed by implantation of fetal substantia nigra tissue into the caudate [10]. Pharmacologically, studies demonstrate that blockade of DA receptors with neuroleptics attenuates ICSS [2,28]. Finally, biochemical measures, such as DA turnover or DA metabolite concentrations, have been reported to change in specific brain areas after ICSS [1, 17, 21, 22, 30, 31].

In contrast, evidence also exists which supports an indirect role for DA in ICSS. When DA levels are reduced, ICSS is attenuated from electrodes placed in noradrenergic nuclei [3, 6, 24], while 6-hydroxydopamine lesions may cause only transient decrements in ICSS [4]. In addition, ICSS from several dopaminergic terminal regions does not correlate with the DA density [25], and the optimal frequencies for ICSS far exceed the frequency capability of dopaminergic fibers [8,29]. Although these examples are only a small fraction of the vast literature on ICSS, discrepancies outlined by these examples provide a basis for the controversy surrounding the hypothesis of DA involvement in ICSS.

Certainly, one aspect of this controversy arises from the interpretational difficulties which accompany performance deficits [18], but another factor is the lack of a well characterized biochemical model of stimulus-dependent DA release *in vivo*. For example, none of the previously mentioned studies that measured DA turnover or DA metabolites after ICSS [1, 17, 21, 22, 30, 31] demonstrated a direct relationship between controlled increases in dopaminergic activity and the biochemical measures. In contrast, McCown *et al.* [20] recently showed that acute electrical stimulation of

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dopaminergic fibers in either gamma-butyrolactone anesthetized, or paralyzed, artificially respired rats, causes an increase in caudate DOPAC concentration which correlates with the artificially increased neuronal activity. If one assumes that dopaminergic neurons are essential for ICSS reinforcement, then a positive correlation should exist between dopaminergic transmission and ICSS. Therefore, we first evaluated the effects of the same electrical stimulation used by McCown *et al.* [20] from a chronically implanted electrode pair in comparison to acute electrode placement. Since the electrode placement used in these studies would support ICSS, we subsequently studied the relationship of dopaminergic transmission to ICSS.

## METHOD

### *Animals*

All animals were naive, male rats (Crl:CD(SD)Br, Charles Rivers Assoc.) between 300 and 400 g at the time of implantation. They were housed individually with ad lib access to food and water. A 12 hr light-dark cycle (0700–1900) was maintained, and all manipulations were performed during the light phase of the cycle.

### *Surgery*

For the acute study the rats were anesthetized with gamma-butyrolactone (GBL) (500 mg/kg, IP), placed into a stereotaxic frame (David Kopf Instruments), and twenty-five minutes later a bipolar electrode (Plastic Products Inc., 0.010″ diameter stainless steel wire, insulated except for blunt tips) was implanted into the right nigro-striatal pathway (4.7 interaural, 1.5 lateral, 1.2 vertical [23]). For the chronic studies, all rats were initially anesthetized with 40 mg/kg pentobarbital (IP) and then placed into a stereotaxic frame. A bipolar electrode (same as above) was implanted into the right nigro-striatal pathway (4.7 interaural, 1.5 lateral, 1.2 vertical [23]), where it was previously demonstrated that nigro-striatal neurons could be electrically stimulated [20]. The electrode was permanently anchored with cranioplastic cement to four screws placed in the skull. At least 5 days were allowed for recovery.

### *Procedure*

For the acute stimulation study, 25 minutes after the GBL administration, rats (n=4) were electrically stimulated (100 $\mu$A, 25 Hz, 1.5 msec duration, monophasic square waves) through the bipolar electrode for 20 minutes. For the chronic electrode studies, six test groups were created in order to assess the effects of chronic electrode implantation on non-contingent electrical stimulation of nigro-striatal dopaminergic neurons, the role of dopaminergic neuronal activity in ICSS reinforcement, and a variety of possible interactions between the electrode implantation and nigro-striatal dopaminergic neurons. The first group was implanted and five days later received non-contingent electrical stimulation of the nigro-striatal pathway (100 $\mu$A, 25 Hz, 1.5 msec duration, monophasic square waves) for 20 minutes. These parameters replicate those used by McCown *et al.* [20], who showed that this electrical stimulation causes an increase in caudate DOPAC concentration which is 50% of the attainable maximum. Since it was found that similar electrode placements supported ICSS, the next groups were designed to test for dopaminergic involvement in ICSS. The second group of rats was implanted, but never trained or tested (implanted only). This group controlled for any trauma effects produced by the electrode implantation procedure alone. The third group of rats was implanted and 5 days later, was determined to be trainable for ICSS responding. These animals were never tested beyond this initial assessment (trained only). The criteria for determination that an animal was trainable consisted of the animal independently responding for intracranial stimulation (60 Hz, 1.5 msec train duration, monophasic square waves) for at least one minute following the initial shaping. The difference in the stimulus frequency (60 Hz) from the frequency used for the non-contingent electrical stimulation (25 Hz) arises from the fact that lower currents will maintain ICSS responding better at 60 Hz. This different stimulation parameter should not invalidate the comparison of the non-contingent model with the ICSS model, because McCown *et al.* [20] showed that 60 Hz attains the same maximal DOPAC increase that is found for 15 Hz. The next three groups were first trained to press a lever for intracranial stimulation, using the same stimulus parameters as in the previous group. Once a stable maximum response rate (no more than 10% daily variability) was established using 50 minute test sessions, the current was adjusted daily in order to establish stable low (approximately 10% of the maximum response rate) or medium (approximately 50% of the maximum response rate) response rates. On the final test day, the animals were divided into a low (395±45 responses/50 minutes), medium (2605±350 responses/50 minutes) or maximum (5115±1115 responses/50 minutes) response rate group. The currents range from 40 $\mu$A to 80 $\mu$A across the three groups. Several animals that did not maintain stable currents for the low response rate category, but did maintain stable maximum response rate currents, were included in the maximal response rate group. Stabilization for each response rate category took from 3 to 5 weeks to complete.

Immediately after the electrical stimulation, whether non-contingent or ICSS, all rats were sacrificed by decapitation. The brains were rapidly removed and placed on ice. The left and right nucleus accumbens, olfactory tubercles and caudate nuclei were dissected rapidly, subsequently weighed and frozen on dry ice. Only the caudate was dissected for the animals in the acute electrode placement study. The rats in the implanted only and trained only groups were sacrificed one month after surgery, and the same brain areas dissected. All samples were stored at −70°C until biochemical determinations were made.

An additional experimental group was added after the above experiments were completed. These animals were implanted, shaped and trained as the above groups. When the responding stabilized, the animals were tested at their maximal response rate (7190±1210 responses/50 minutes) for 50 minutes and then sacrified by decapitation. The left and right frontal cortex, rostral to the olfactory tubercle, was removed and stored as previously described. This additional manipulation was instituted to evaluate the possible effects of the ICSS on mesocortical dopaminergic fibers.

### *Biochemical Determinations*

The DA and DOPAC concentrations in the four brain areas were determined by the methods of Kilts *et al.* [16]. Briefly, the tissue was sonically disrupted (Branson Ultrasonic probe) in 400 $\mu$l of mobile phase (0.1 M $Na_2HPO_4$, 0.075 M citrate, 0.15 mM heptane sulfonate, 17% methanol, apparent pH 4.15) and centrifuged for 5 minutes at 12,000 g.

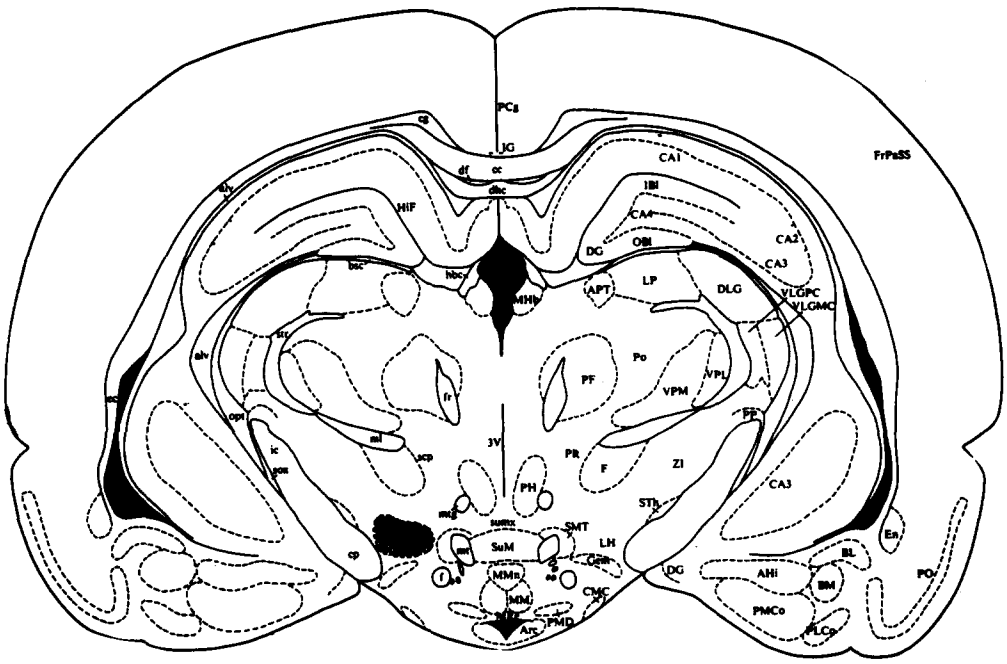


FIG. 1. The shaded area on this atlas drawing (after Paxinos and Watson [23]) shows the extent of the electrode placements used in all of the studies. This area also corresponds to the area where McCown *et al.* [20] reported acute stimulation of nigro-striatal dopaminergic fibers.

TABLE 1

THE EFFECTS OF ACUTE ELECTRICAL STIMULATION IN GAMMA-BUTYROLACTONE ANESTHETIZED RATS ON CAUDATE DA METABOLITES*

| Side† | DA (ng/mg tissue ± SEM) | DOPAC |
|---|---|---|
| Left | 18.1 ± 3.0 | 2.51 ± 0.41 |
| Right | 17.8 ± 1.5 | 7.15 ± 1.53‡ |

*Twenty-five minutes after 500 mg/kg gamma-butyrolactone, a bipolar electrode was lowered into the right nigro-striatal tract, which was then electrically stimulated (100 $\mu$A, 25 Hz, 1.5 msec duration) for twenty minutes.

†The right nigro-striatal pathway was stimulated.

‡$p<0.001$, two-tailed $t$-test.

TABLE 2

THE EFFECTS OF NON-CONTINGENT ELECTRICAL STIMULATION IN THE NIGRO-STRIATAL PATHWAY THROUGH CHRONICALLY IMPLANTED ELECTRODES ON DA AND DOPAC CONCENTRATIONS*

| Brain Area† | Side‡ | DA (ng/mg tissue ±SEM) | DOPAC |
|---|---|---|---|
| Olfactory Tubercles | Left | 9.0 ± 0.9 | 1.16 ± 0.15 |
| | Right | 7.4 ± 0.8 | 1.08 ± 0.17 |
| Nucleus Accumbens | Left | 8.7 ± 0.9 | 2.42 ± 0.16 |
| | Right | 7.0 ± 0.7 | 2.37 ± 0.27 |
| Caudate | Left | 16.2 ± 0.9 | 2.4 ± 0.16 |
| | Right | 14.8 ± 1.6 | 2.31 ± 0.12 |

*All rats had bipolar electrodes implanted into the nigro-striatal fibers five days prior to the electrical stimulation (100 $\mu$A, 1.5 msec duration, 25 Hz) during which the rats were unrestrained and unanesthetized.

†All rats were implanted on the right side. The values on the left side were not significantly different from unimplanted control rats for all brain areas (Student's $t$-test, $p>0.1$).

‡No significant differences were found between the left (unimplanted) and right (implanted) sides for all brain areas (Student's $t$-test, $p>0.1$).

Then 150 μl were injected onto a 5 micron, reverse-phase C-18 column (Applied Sciences) at a flow rate of 1.5 ml/min. The compounds were quantified by electrochemical detection at a cell potential of +0.75 V.

*Statistics*

The acute stimulation means and the non-contingent stimulation means from chronically implanted electrodes were compared by a two-tailed Student's *t*-test. For the ICSS groups, a one-way analysis of variance was used to compare the stimulated and unstimulated sides across the different ICSS groups, as well as to each other. Individual differences for between each group were compared to the appropriate control by a *post hoc* Newman Keul's comparison [32]. A probability of $p<0.05$ was required for significance.

## RESULTS

*The Comparison of Stimulation From Acute or Chronic Electrode Placements on DOPAC Concentrations*

When nigro-striatal dopaminergic neurons were stimulated from acute electrode placements shown in Fig. 1, the DOPAC concentration in the ipsilateral caudate increased by nearly three-fold in comparison to the contralateral caudate, while the DA concentration remained unchanged (see Table 1). These data replicate earlier findings of McCown *et al.* [20]. However, when unanesthetized animals received the identical electrical stimulation in the same area through chronically implanted electrodes (see Fig. 1), caudate DOPAC or DA concentrations did not change (see Table 2). Table 2 also shows that DA or DOPAC concentrations did not change in the nucleus accumbens or olfactory tubercle, suggesting that mesolimbic dopaminergic fibers were not being electrically stimulated. In addition to the lack of change attributed to the non-contingent electrical stimulation, the values for both DA and DOPAC were not significantly different in any of the three brain areas from unimplanted controls (data not shown), although the DA concentration was somewhat higher in the caudate. Thus, the electrode implantation did not cause substantial damage to the nigro-striatal dopaminergic fibers. Likewise, if animals that had chronically implanted electrodes were first anesthetized with GBL (500 mg/kg, IP) prior to the electrical stimulation, no changes were observed (data not shown).

*The Effects of ICSS on DA and DOPAC*

Although non-contingent electrical stimulation of the nigro-striatal neurons did not alter DA metabolites in the caudate, ICSS would be expected to alter DA metabolism if the dopaminergic neurons are directly involved in ICSS reinforcement. When caudate DA or DOPAC concentration was compared across the five experimental groups (implanted only, trained only, minimum response, 50% maximum response, or maximum response) no significant trends for either measure were found, either between the groups or between the stimulated and unstimulated sides (see Table 3). For example, the caudate DA or DOPAC concentration in animals that received no stimulation (implanted only) equalled the concentration in animals following maximal responding for ICSS (5115±1115 responses/50 minutes). This lack of change once again sharply contrasts the stimulus-dependent changes observed from the same electrode placement in acute preparations (Table 1, [20]).

Potential involvement of mesolimbic or A10 dopaminergic neurons in ICSS reinforcement also was evaluated. In the nucleus accumbens no significant trends of change in DOPAC or DA concentration was observed across the five groups (see Table 3). Also, no significant differences between the stimulated and unstimulated sides were found. In the olfactory tubercles, the DA concentration appears to decrease in the maximum response rate group, while the DOPAC concentration increases when compared from the minimum to maximum ICSS groups. However, this apparent trend was not significant because as seen in Table 3, the variability also increased with increasing ICSS, especially in the maximum response rate group. Also, no significant differences were found between the stimulated and unstimulated sides within any of the groups.

It has been suggested that the frontal cortex might mediate the reinforcing properties of ICSS [28], so an additional group was tested at maximal ICSS rates (7910±1210 responses/50 minutes) and compared to implanted, untested controls. As seen in Table 4, there was no change in cortical DA or DOPAC content between the animals responding maximally for ICSS and unstimulated controls. There was a tendency for the DOPAC concentration to increase on the unstimulated side of the ICSS animals, but this change was not significant, owing to the large variability contributed by one animal.

## DISCUSSION

The observation that caudate DOPAC can reflect acute, electrical stimulation of the nigro-striatal dopaminergic pathway [20] was verified in the present studies. Therefore, the same electrical stimulation through chronically implanted electrodes was expected to produced similar changes in caudate DOPAC concentration. Surprisingly, no change in DA or DOPAC content was found. The only other difference between these non-contingent stimulation groups, the presence of an anesthetic, cannot explain the lack of stimulation induced changes for two reasons. First, when animals with chronically implanted electrodes were anesthetized with GBL prior to the non-contingent electrical stimulation, no changes in DA or DOPAC were found. Likewise, the same electrical stimulation from an acutely placed electrode in paralyzed, artificially respired rats causes significant, ipsilateral increases in caudate DOPAC concentrations [20]. Since no changes were found in the olfactory tubercles or nucleus accumbens, it appears that the stimulation did not spread to A10 projections. This lack of effect was not unexpected because no changes are observed in these mesolimbic structures after stimulation from an acute electrode placement (unpublished observations). One possible explanation for these findings centers upon potential mechanical trauma which could be caused by the electrode. It is well known that electrode implantation can cause mechanical trauma to neurons [26], and acute, sham implantation of the same electrodes used in this study can cause an increase in caudate DOPAC when certain anesthetics are used [20]. Since the stimulus-dependent elevation of caudate DOPAC can only be elicited from a highly localized area [20], the chronically implanted electrode may cause damage just sufficient to impede electrical stimulation of dopaminergic fibers adjacent to the electrode, but not enough damage to cause an increase in caudate DA concentrations. Another possible explanation

TABLE 3

THE EFFECTS OF ICSS RESPONDING ON DA AND DOPAC CONCENTRATIONS IN THE CAUDATE, NUCLEUS ACCUMBENS AND OLFACTORY TUBERCLES*

| Brain Area | Side | Implanted Only N=5 | Trained Only N=5 | Minimum Response Rate N=4 | 50% Maximum Response Rate N=5 | Maximum Response Rate N=6 |
|---|---|---|---|---|---|---|
| Caudate | | | | | | |
| Dopamine | L | 9.14 ± 0.70 | 9.8 ± 1.22 | 10.6 ± 0.50 | 10.4 ± 0.81 | 9.58 ± 0.91 |
| (ng/mg | R | 7.2 ± 1.20 | 9.29 ± 0.49 | 7.5 ± 1.12 | 8.5 ± 1.92 | 8.74 ± 1.5 |
| tissue) | | | | | | |
| DOPAC | L | 1.61 ± 0.09 | 1.49 ± 0.09 | 1.69 ± 0.13 | 1.82 ± 0.10 | 1.88 ± 0.13 |
| (ng/mg | R | 1.51 ± 0.19 | 1.90 ± 0.08 | 1.17 ± 0.22 | 2.03 ± 0.53 | 1.97 ± 0.32 |
| tissue) | | | | | | |
| Nucleus Accumbens | | | | | | |
| Dopamine | L | 6.87 ± 0.38 | 6.31 ± 0.44 | 6.98 ± 0.80 | 6.97 ± 0.90 | 6.21 ± 0.31 |
| (ng/mg | R | 6.76 ± 0.40 | 5.54 ± 0.40 | 5.84 ± 0.65 | 7.08 ± 0.74 | 5.01 ± 1.21 |
| tissue) | | | | | | |
| DOPAC | L | 3.34 ± 0.34 | 2.83 ± 0.33 | 3.08 ± 0.39 | 2.86 ± 0.45 | 2.74 ± 0.33 |
| (ng/mg | R | 2.97 ± 0.11 | 3.24 ± 0.58 | 2.43 ± 0.29 | 3.49 ± 0.81 | 3.25 ± 0.33 |
| tissue) | | | | | | |
| Olfactory Tubercles | | | | | | |
| Dopamine | L | 6.25 ± 0.31 | 6.21 ± 0.27 | 6.48 ± 0.30 | 7.05 ± 0.32 | 4.69 ± 1.18 |
| (ng/mg | R | 5.56 ± 0.39 | 5.86 ± 0.33 | 5.16 ± 0.61 | 5.83 ± 1.25 | 4.57 ± 0.94 |
| tissue) | | | | | | |
| DOPAC | L | 1.09 ± 0.09 | 1.13 ± 0.12 | 1.14 ± 0.09 | 1.45 ± 0.18 | 2.03 ± 0.48 |
| (ng/mg | R | 1.12 ± 0.17 | 1.14 ± 0.12 | 0.85 ± 0.07 | 1.50 ± 0.19 | 1.91 ± 0.64 |
| tissue) | | | | | | |

*Animals were implanted, trained and tested as described in the Method section. All ICSS response groups were tested for 50 min prior to sacrifice. No significant changes were found across the ICSS stimulus groups for the stimulated or unstimulated sides (ANOVA) or between the stimulated and unstimulated sides within each group (Newman Keuls'). In the olfactory tubercles, the DA appeared to decrease in the maximum response rate group while the DOPAC tended to increase across the minimum to maximum response rate groups. However, these changes were not significant owing to the accompanying increase in variability within each group.

†All electrodes were implanted on the right side.

TABLE 4

THE EFFECTS OF MAXIMAL ICSS RESPONDING FROM ELECTRODES IMPLANTED IN THE NIGRO-STRIATAL TRACT ON FRONTAL CORTEX DA AND DOPAC CONCENTRATIONS

| | | DA (ng/mg tissue ± SEM) | DOPAC (ng/mg tissue ± SEM) |
|---|---|---|---|
| Maximal | L | 0.47 ± 0.08 | 0.23 ± 0.05 |
| ICSS | R* | 0.35 ± 0.04 | 0.12 ± 0.01 |
| Implanted | L | 0.40 ± 0.08 | 0.11 ± 0.02 |
| only | R | 0.35 ± 0.04 | 0.09 ± 0.01 |

*The right nigro-striatal tract was stimulated.

would be a change in DOPAC transport from the CNS, analogous to behaviorally-induced acceleration of DOPAC transport reported by Heffner *et al.* [15]. However, this explanation appears unlikely for several reasons. Chronic electrode implantation, alone or with stimulation, did not alter caudate DOPAC concentrations, while stimulations in an acutely implanted electrode caused ipsilateral DOPAC increases. Furthermore, animals that received non-contingent stimulation through a chronically implanted electrode exhibited no behavioral activation. These findings are not compatible with an implantation- or stimulus-induced change in DOPAC transport from the CNS.

Investigators have convincingly argued that dopaminergic transmission directly affects ICSS behavior, but the reinforcement versus performance nature of dopaminergic involvement remains difficult to differentiate. Since similar electrode placements to those used by McCown *et al.* [20] to characterize *in vivo* stimulus-dependent increases in caudate DOPAC also supported ICSS behavior, the resulting ICSS behavior should cause increases in caudate DOPAC concen-

tration if nigro-striatal dopaminergic neurons are being activated. The conjecture of such a biochemical change, in light of the previous results, was possible owing to the different stimulation parameters. However, the results showed that across a range of reinforcement conditions, the DA and DOPAC concentrations in the caudate were not changed. This finding further reinforced the previous findings of no stimulation-induced changes in the caudate from a chronically implanted electrode. The same lack of effect was found in terminal projection areas of the mesolimbic dopaminergic fibers, the nucleus accumbens and the olfactory tubercles, as well as the frontal cortex, where mesocortical dopaminergic fibers project. Thus, the DOPAC concentrations were no different in animals that had been implanted only, when compared to animals immediately following 50 minutes of maximal responding for ICSS, even though the electrode placements shown in Fig. 1 are close to placements reported by Gratton and Wise [13] to support high rates of ICSS responding.

Although both non-contingent stimulation, as well as ICSS, failed to effect significant changes in DA or DOPAC concentrations, a close examination of the ICSS findings for the olfactory tubercles (Table 3) supports recent reports from Gallistel *et al.* [11] and Mitchell *et al.* [22]. It appears that across the ICSS groups in the olfactory tubercles the DA concentration decreases in the maximum response rate group while the DOPAC concentration increases when coursing from the minimum to maximum ICSS response rate groups. These apparent changes are not significant because the variability also increases with increasing ICSS. Recently, Gallistel *et al.* [11] reported that submaximal ICSS did not alter 2-deoxyglucose utilization in forebrain structures that contain dopaminergic projections. However, if the duration and stimulus intensity of the intracranial stimulation was increased (1.5 msec pulse duration, 400–600 $\mu$A) then increased 2-deoxyglucose utilization was found in the olfactory structures. Likewise, Mitchell *et al.* [22] reported that ICSS from some electrode placements would increase DOPAC concentrations in the olfactory tubercles, while qualitatively similar ICSS from different electrode placements did not alter olfactory tubercle DOPAC. Thus, all of the data indicates that dopaminergic measures can be altered by ICSS, but the alteration appears to be indirect in nature.

Finally, it must be emphasized that the present studies do not indicate that dopaminergic neurons cannot mediate reinforcement. Amphetamine self-administration appears to depend directly upon dopaminergic transmission [27,33], and dopaminergic transmission in the nucleus accumbens appears necessary for the expression of amphetamine self-administration by rats [19]. We conclude that from the particular electrode placement used in the present studies, chronic electrode implantation abolishes the ability to electrically stimulate nigro-striatal dopaminergic neurons, and that ICSS from similar electrode placements likewise does not alter nigro-striatal, mesolimbic, or mesocortical dopamine metabolism. Therefore, deliniation of the neuronal substrate which directly mediates ICSS reinforcement from this electrode placement remains a future goal.

ACKNOWLEDGEMENTS

This work was supported by grant HD-03110 and NINCDS NSO 7166. We would like to thank Carolyn Reams for secretarial assistance in the preparation of this manuscript.

## REFERENCES

1. Arbuthnott, G. W., T. J. Crow, K. Fuxe, L. Olson and U. Ungerstedt. Depletion of catecholamines *in vivo* induced by electrical stimulation of monoamine pathways. *Brain Res* **24:** 471–485, 1970.
2. Broekkamp, C. L. E. and J. M. Van Rossum. The effect of microinjections of morphine and haloperidol into the neostriatum and nucleus accumbens on self-stimulation behavior. *Arch Int Pharmacodyn Ther* **217:** 110–117, 1975.
3. Clavier, R. M., H. C. Fibiger and A. G. Phillips. Evidence that self-stimulation of the region of the locus coeruleus in rats does not depend upon noradrenergic projections to telencephalon. *Brain Res* **113:** 71–81, 1976.
4. Clavier, R. M. and H. C. Fibiger. On the role of ascending catecholamine projections in intracranial self-stimulation of the substantia nigra. *Brain Res* **131:** 271–286, 1977.
5. Cooper, B. R., J. M. Cott and G. R. Breese. Effects of catecholamine-depleting drugs and amphetamine on self-stimulation of brain following various 6-hydroxydopamine treatments. *Psychopharmacologia* **37:** 235–248, 1974.
6. Cooper, B. R., R. J. Konkol and G. R. Breese. Effects of catecholamine depleting drugs and *d*-amphetamine on self-stimulation of the substantia nigra and locus coeruleus. *J Pharmacol Exp Ther* **204:** 592–605, 1978.
7. Corbett, D. and R. A. Wise. Intracranial self-stimulation in relation to the ascending dopaminergic systems of the midbrain: a moveable electrode mapping study. *Brain Res* **185:** 1–15, 1980.
8. Deutch, J. A. Behavioral measurement of the neural refractory period and its application to intracranial self-stimulation. *J Comp Physiol Psychol* **58:** 1–9, 1964.
9. Fibiger, H. C. Drugs and reinforcement mechanisms: a critical review of catecholamine theory. *Annu Rev Pharmacol Toxicol* **18:** 37–56, 1978.
10. Fray, P. J., S. B. Dunnett, S. D. Iversen, A. Bjorklund and U. Stenevi. Nigral transplants reinervating the dopamine-deplete neonstriatum can sustain intracranial self-stimulation. *Science* **219:** 416–418, 1983.
11. Gallistel, C. R., Y. Gomita, E. Yandin and K. A. Campbell. Forebrain origins and terminations of the medial forebrain binding metabolically activated by rewarding stimulation or by reward blocking doses of pimozide. *J Neurosci* **5:** 1246–1261, 1985.
12. German, D. C. and D. M. Bowden. Catecholamine systems as the neural substrate for intracranial self-stimulation: a hypothesis. *Brain Res* **73:** 381–419, 1974.
13. Gratton, A. and R. A. Wise. Brain stimulation reward in the lateral hypothalamic medial forebrain bundle: mapping of boundaries and homogeniety. *Brain Res* **274:** 25–30, 1983.
14. Hall, R. D., F. E. Bloom and J. Olds. Neuronal and neurochemical substrates of reinforcement. *Neurosci Res Prog Bull* **15:** 140–314, 1976.
15. Heffner, T. G., G. Vosmer and L. S. Seidner. Increased transport of 3,4-dihydroxyphenylacetic acid from brain during performance of operant behavior in the rat. *Brain Res* **292:** 85–91, 1984.
16. Kilts, C. D., G. R. Breese and R. B. Mailman. Simultaneous quantification of dopamine, 5-hydroxytryptamine, and four metabolically related compounds by means of reverse-phase high performance liquid chromatography with electrochemical detection. *J Chromatogr* **225:** 347–357, 1981.
17. Laurent, J., M. F. Roizen, E. Miliaresis and D. Jacobowitz. The effects of self-stimulation of the catecholamine concentration of discrete areas of the rat brain. *Brain Res* **99:** 194–200, 1975.
18. Liebman, J. M. Discriminating between reward and performance: A critical review of intracranial self-stimulation methodology. *Neurosci Biobehav Rev* **7:** 45–72, 1983.

19. Lyness, W. H., N. M. Friedle and K. E. Moore. Destruction of dopaminergic nerve terminals in nucleus accumbens: Effect on d-amphetamine self-administration. *Pharmacol Biochem Behav* **11:** 553–556, 1979.
20. McCown, T. J., R. B. Mueller and G. R. Breese. The effects of anesthetics and electrical stimulation on nigro-striatal dopaminergic neurons. *J Pharmacol Exp Ther* **224:** 489–493, 1983.
21. Miliaresis, E., N. B. Thoa, Y. Tizabi and D. M. Jacobowitz. Catecholamine concentration of discrete brain regions following stimulation in the ventromedial tegmentum of the rat. *Brain Res* **100:** 192–197, 1975.
22. Mitchell, J. M., N. M. Nicolaou, G. W. Arbuthnott and C. M. Yates. Increases in dopamine metabolism are not a general feature of intracranial self-stimulation. *Life Sci* **30:** 1081–1085, 1982.
23. Paxinos, C. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. New York: Raven Press, 1982.
24. Phillips, A. G., D. Van der Kooy and H. C. Fibiger. Maintenance of intracranial self-stimulation in hippocampus and olfactory bulb following regional depletion of noradrenaline. *Neurosci Lett* **4:** 77–84, 1977.
25. Prado-Alcala, R. and R. A. Wise. Brain stimulation reward and dopamine terminal fields. I. Caudate-putamen, nucleus accumbens and amygdala. *Brain Res* **297:** 265–273, 1984.
26. Ranck, J. B. Elements necessary for the electrical stimulation of nerves, a review. *Brain Res* **98:** 417–440, 1984.
27. Risner, M. and B. E. Jones. Role of noradrenergic and dopaminergic processes in amphetamine self-administration. *Pharmacol Biochem Behav* **5:** 477–482, 1976.
28. Robertson, A. and G. J. Mogensen. Evidence for a role for dopamine in self-stimulation of the nucleus accumbens of the rat. *Can J Psychol* **32:** 76–86, 1978.
29. Siggins, G. R., B. J. Hoffer, F. E. Bloom and U. Ungerstedt. Cytochemical and electrophysiological studies of dopamine in the caudate nucleus. In: *The Basal Ganglia*, edited by M. D. Yahr. New York: Raven Press, 1975, pp. 227–248.
30. Stinus, L., A. Thierry, G. Blanc, J. Glowinski and B. Cardo. Self-stimulation and catecholamines. III. Effect of imposed or self-stimulation in the area ventralis tegmenti on catecholamine utilization in the rat brain. *Brain Res* **64:** 199–200, 1973.
31. Van Hueven-Nolsen, D., L. Van Wolfswinkel, J. M. Van Ree and D. H. G. Versteeg. Electrical stimulation of the ventral tegmental area and catecholamine metabolism in discrete region of the rat brain. *Brain Res* **268:** 362–366, 1983.
32. Winer, N. *Statistical Principles in Experimental Design*. New York: McGraw-Hill, 1971.
33. Yokel, R. A. and R. A. Wise. Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. *Science* **187:** 547–549, 1976.