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# Roles of Catecholamine Terminals and Intrinsic Neurons of the Ventral Tegmentum in Self-Stimulation Investigated in Neonatally Dopamine-Depleted Rats

T. TAKEICHI, S. KURUMIYA, M. UMEMOTO[1] AND M. E. OLDS

*Psychology Division, Faculty of Letters, Osaka City University, Sugimoto Sumiyoshi-ku, Osaka 558, Japan*
*and Division of Biology, California Institute of Technology, Pasadena, CA 91125*

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TAKEICHI, T , S KURUMIYA, M UMEMOTO AND M E OLDS *Roles of catecholamine terminals and intrinsic neurons of the ventral tegmentum in self-stimulation investigated in neonatally dopamine-depleted rats* PHARMACOL BIOCHEM BEHAV 24(4) 1101–1109, 1986 —Three series of experiments were undertaken to determine whether the residual catecholamine (CA) terminals or intrinsic neurons of ventral tegmentum (VT) in rats given 6-hydroxydopamine (6-OHDA) after desmethylimipramine (DMI) in the lateral ventricles at birth, mediated VT self-stimulation (SS) In Experiment I, male pups were injected bilaterally on days 3 and 5 with 6-OHDA (total dose 200 μg) or with the vehicle after pretreatment with DMI (50 mg/kg, IP) 30 min earlier Each subject, 150 days old, was implanted bilaterally in the VT with electrode-cannula units Both the dopamine (DA)-depleted and control groups yielded similar percentages of self-stimulators The rate of responding was, however, slightly but significantly lower in the DA-depleted group than in the controls In Experiment II, 8 DA-depleted and 7 control rats were pretreated with pargyline (50 mg/kg, IP) and then given unilateral injections of 6-OHDA in the VT, in the tissue below the SS electrode These intracerebral injections of 6-OHDA had no effect on VT SS in both groups Seventeen controls and 12 DA-depleted rats, in Experiment III, were given injections of kainic acid (KA, 5 nM) either ipsilaterally or contralaterally The ipsilateral injection abolished SS (14 days of testing), whereas the contralateral injection had no effects on ipsilateral SS in both groups. Histochemical fluorescence study in Experiment I and II showed that the neonatal treatment with DMI + 6-OHDA had reduced the number of DA-containing perikarya in the VT and that reinjection of 6-OHDA into the VT caused the disappearance of the residual CA terminals in tissue surrounding the electrode tip Conventional histology for rats in Experiment III showed the destruction of cell bodies in tissue below the tip of the SS electrode after KA These findings suggest that the intrinsic neurons in the VT play a critical role in SS of the VT

Self-stimulation Ventral tegmentum 6-Hydroxydopamine Desmethylimipramine Kainic acid

SEVERAL lines of the evidence have implicated central dopamine (DA) systems in the control of self-stimulation (SS) behavior. For example, mapping studies [9, 14, 33, 37–39] have shown that SS can be obtained from the nigrostriatal and mesocorticolimbic DA systems which arise from the A9 and A10 regions [25,49]. Lesion studies with 6-hydroxydopamine (6-OHDA) after pretreatment with desmethylimipramine (DMI) also have shown that damage specific to the DA systems depressed nigrostriatal SS behavior [5,30]. Another verification of the significant role for DA systems in the control of SS behavior derives from pharmacological studies [51]. Especially local injections of DA receptor blocker such as haloperidol or spirperidol into the nucleus accumbens or the nucleus caudatus attenuate or suppress SS in DA regions [26, 27, 35, 46]. However, these pharmacological data must be interpreted with caution, because DA receptor blockade has been reported to interfere with voluntary movements [10, 17, 36]. It is reported that the disruptive effects of these drugs on SS may to a large degree reflect a disruption of lever responding [17, 29, 36] The involvement of the DA neurons in SS has also been questioned by the fact that responding can be reinstated transiently soon after the treatment with DMI + 6-OHDA [8,31] Furthermore, some recent electrophysiological and lesion studies

[1]Requests for reprints should be addressed to Dr M Umemoto, Psychology Division, Faculty of Letters, Osaka City University, Sugimoto, Sumiyoshi-ku, Osaka 558, Japan

TABLE 1

SELF-STIMULATION IN THE VT IN ADULT RATS NEONATALLY TREATED WITH DMI+6-OHDA ON THE LAST DAY OF ADDITIONAL 5 SS SESSIONS

| Group | SS Subject N | Current ($\mu$A) Means ± S D | Response Rate/Min Means ± S D |
|---|---|---|---|
| Control | 28 | 15 3 ± 6 8 | 75 6 ± 7 7 |
| DA-depleted | 24 | 15 6 ± 7 3 | 61 9 ± 6 3 |

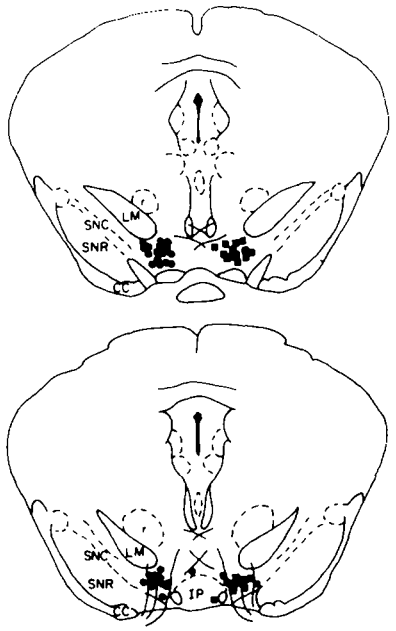



FIG 1 Localization of SS site in the ventral tegmentum of control (●) and DA-depleted (■) rats Abbreviations CC crus cerebri, IP, interpeduncular nucleus, LM, lemniscus medialis, SNC, substantia nigra, zona compacta, SNR, substantia nigra, zona reticulata

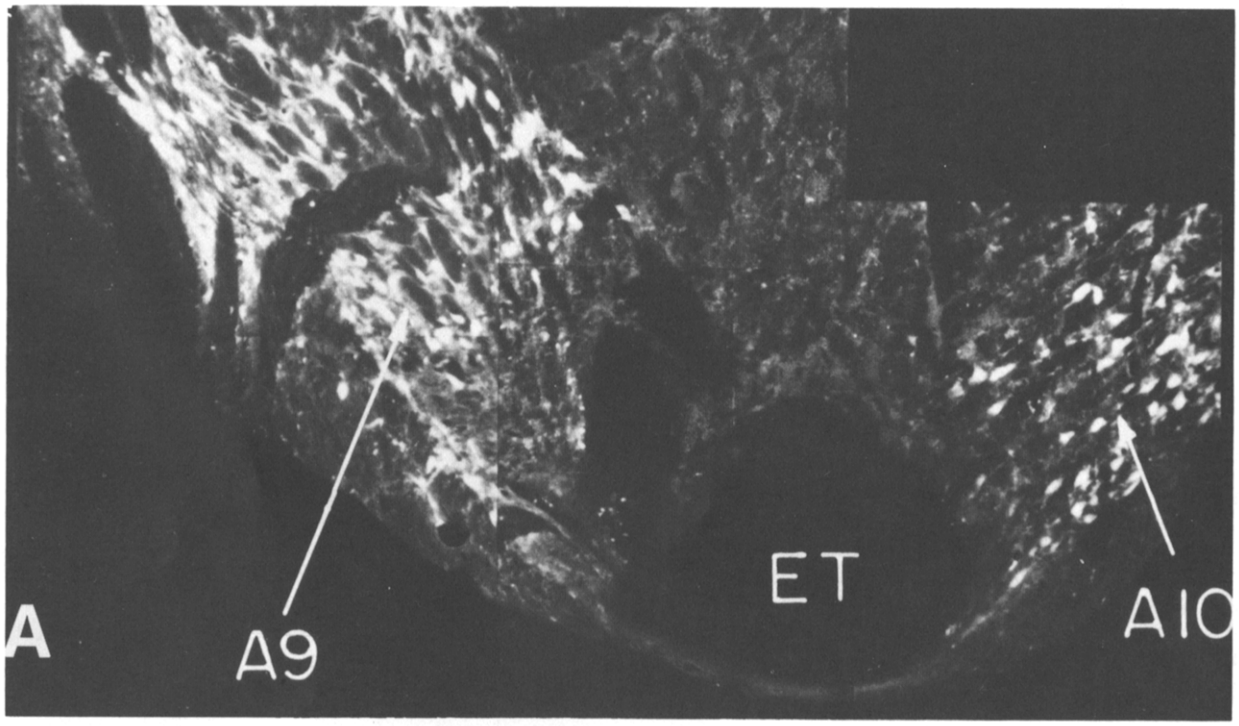
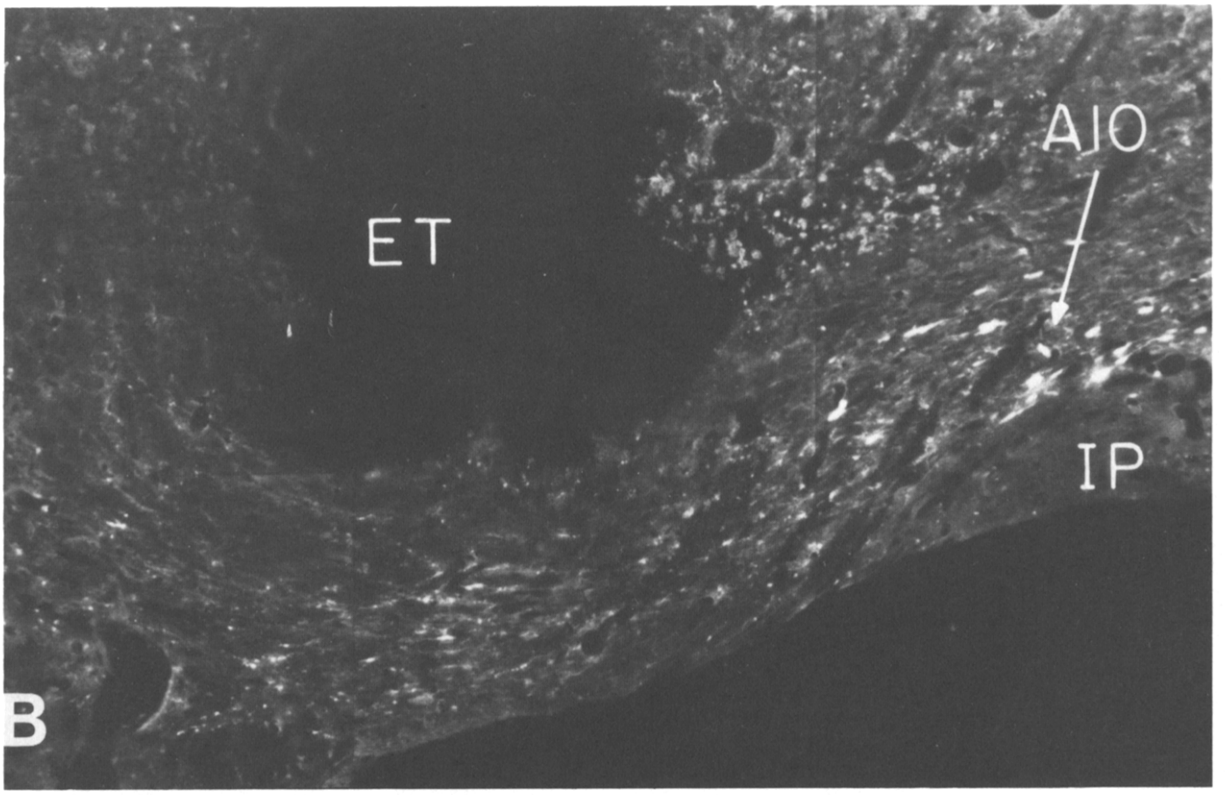



FIG 2 Photomicrographs illustrating the CA-containing cell bodies around the tip of the electrode in a control (A) and a neonatally DA-depleted (B) rat Fine CA fluorescence varicosities (C) believed to contain NE can be seen in the vicinity of the electrode track of the same animal as in B ET, electrode track, IP, interpeduncular nucleus (Figure 2 B, C on facing page )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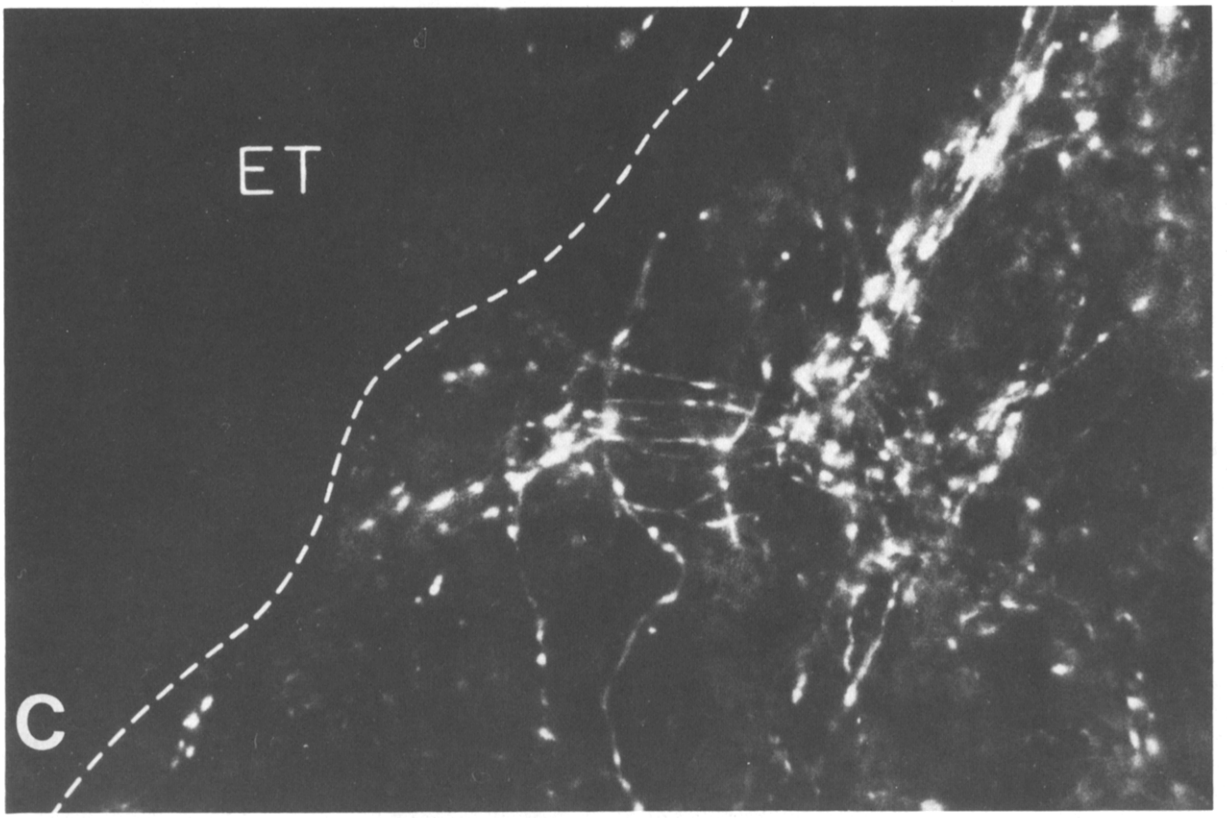
ET
C

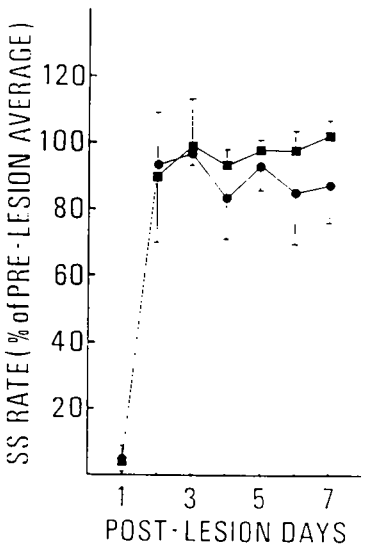


FIG 3 Rates of self-stimulation in the ventral tegmentum after 6-hydroxydopamine, ●——● controls (n=7), ■——■ DA-depleted (n=5) group Data expressed as the daily group mean % of prelesion (5 days) average score ±S E M

suggest that SS is supported by non-catecholaminergic (CA) fibers [3, 7, 19, 20, 32, 43] Taking these investigations into account, it can be pointed out that the evidence for the DA hypothesis, that DA plays crucial role for motivational aspects in SS, is far from conclusive

In a previous study [48] designed to evaluate the role of the DA projection to the medial prefrontal cortex (MFC) in SS of this same region, rats were injected neonatally with 6-OHDA in both lateral ventricles after pretreatment with DMI, given to induce selective damage to the DA terminal fields in the forebrain In adulthood, these animals gave no evidence of deficits in SS in the MFC The pattern of SS in these rats was similar to the pattern in the controls These findings suggested a critical role for the intrinsic neurons rather than the DA terminals of the MFC in SS If this hypothesis was valid, then SS in the ventral tegmentum (VT), the origin of the DA projection to the MFC, should likewise not depend on the integrity of the DA neurons in that region, but on the non-CA neurons, and in rats treated neonatally with DMI + 6-OHDA, SS should be comparable in the VT to that in the control rats

The purpose of the present study was to evaluate this hypothesis In Experiment I, 6-OHDA was injected intraventricularly (IVT) into the rat during the neonatal stage after DMI pretreatment The neonatal approach was selected because it results in more severe damage to the DA systems than injections in adulthood [12] In Experiment II, rats from Experiment I whose DA terminal fields had been neonatally damaged, were injected in adulthood—after pargyline treatment—with 6-OHDA It was injected into the VT, ipsilateral to the SS site The local VT injections in adulthood, given after the neonatal damage inflicted by the IVT injections, were designed to destroy the ascending axons of the ventral noradrenergic (NE) bundle *en passage* in the VT and the DA cell bodies of the VT which had survived the neonatal damage In Experiment III, kainic acid (KA) was injected into the VT on the side either ipsilateral or contralateral to the VT SS sites in order to investigate the role of the intrinsic neurons in the VT in SS

## METHOD

### *Experiment I*

*Subjects* The subjects were sixty-six male rats of the Wistar strain The behavioral tests were carried out on the adult animal in which the neurotoxin had been injected shortly after birth Pregnant females, and subsequently mothers and their pups, were housed in rodent breeding cages with sawdust bedding After weaning, the animals were housed in groups of 5–6 in the same cages until electrode implantation in the fifth or sixth months Thereafter, the animals were housed individually in stainless steel cages with food and water ad lib

*Neonatal injections of 6-OHDA after pretreatment with DMI* Each litter was divided into two groups a DA-depleted (N=37) and a control (N=29) The procedures used to deplete DA in the brain were the same as those used in our previous studies [47,48] Briefly, the rat pups were lightly anesthetized with metofane, and were given bilateral injections on days 3 and 5 after birth After the skin over the skull was retracted and bregma was identified, the needle of a Hamilton syringe (10 μl) was lowered first into one lateral ventricle to give an injection of 6-OHDA, and following completion of that injection, it was lowered into the other lateral ventricle When the bilateral IVT injections were completed, the skin was sutured and the animal was placed in a recovery box for about 0 5–1 hr and then returned to the care of its mother Each animal of the DA-depleted group had been pretreated with DMI (25 mg/kg, IP) 30 min before the injection of 6-OHDA (total dose, 200 μg, 50 μg/2 5 μl of artificial cerebrospinal fluid [CSF 26] containing 0 1% of ascorbic acid ×4) Each animal in the control group was pretreated with DMI and then given 4 injections of CSF (2 5 μl/injection) with the ascorbate The stereotaxic coordinates for the IVT were 0 2 mm posterior to bregma, 0 5 mm lateral to the midline, and 1 0 mm down from the top of the skull on day 3, and 1 5 mm on day 5

*Implantation of the electrode-cannula unit* DA-depleted and control animals at least 150 days old were implanted bilaterally with electrode-cannula unit aimed at the VT The stereotaxic coordinates for the VT [24] were 6 0 mm posterior to bregma, 1 0 mm lateral to the midline, and 7 8 mm down from the top of the skull The bipolar twisted electrode used in the SS test was made from 2 strands of 100 μm insulated stainless steel wire glued to a 23-gage stainless steel guide cannula The cannula was 2 mm shorter than the electrode The injection needle (a 30-gage cannula), when inserted, came to rest 0 5 mm above the electrode tip

*SS procedures* The procedures were the same as those described in detail in a previous study of this series [48]

The chamber used for the SS test measured 19×32×60 cm and was made of Plexiglas, with a metal floor It was fitted with a lever, 10×5 cm, about 3 cm above the floor on the front wall

Each depression of the lever was followed by the delivery of a 0 2 sec train of 60 Hz sine waves A high resistor across the output of the stimulator insured current stability After recovery from the surgery (at least 10 days), the rats were given SS training sessions for 2 hr for five consecutive days During these training sessions, the threshold current yielding SS was determined by varying stimulus intensities up and down by steps of 5 μA, and the rats which showed rates of

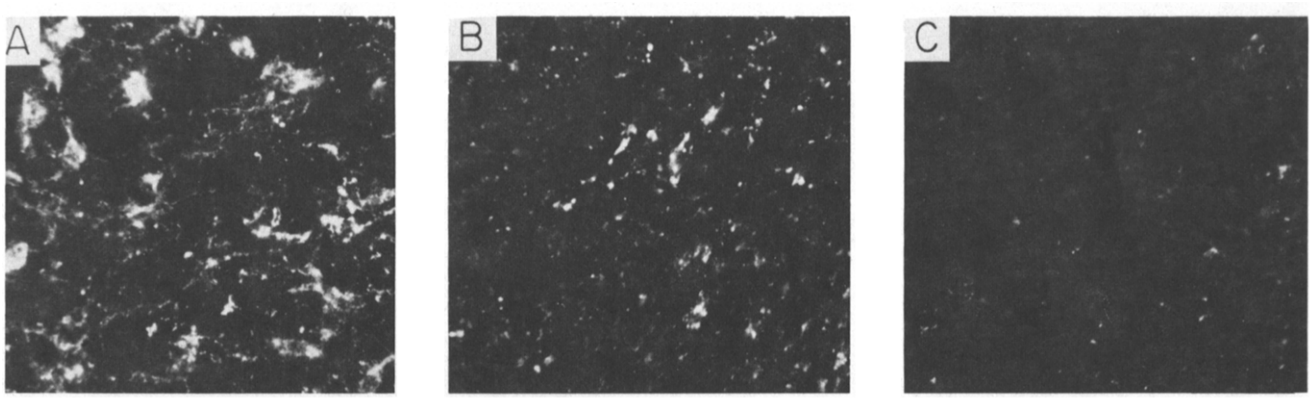


FIG 4 Histochemical CA fluorescence in the ventral tegmental tissue (A10) just below the electrode tip A Neonatally DA-depleted control rat B Contralateral VT to 6-OHDA injection in a neontally DA-depleted rat C Ipsilateral VT in the same rat as in B

responding of more than 10 responses/min for one hour in each session were classified as self-stimulator The animals which showed SS for two electrodes implanted in both sides of the brain were given five additional SS sessions lasting one hour During this and succeeding SS sessions in Experiment II and III the current intensities available for SS were set at 5 $\mu$A above the threshold intensities

*Histochemical fluorescence verification* The brains of eight self-stimulators, that is, four controls and four DA-depleted, were processed by a modified formaldehyde-glutaraldehyde perfusion cryostat method [18,22] to visualize the CA fluorescence cell bodies in the immediate vicinity of the electrode tip

The remaining subjects of Experiment I were randomly divided into two groups, and used as the subjects of Experiment II and III.

*Experiment II*

*Injection of 6-OHDA in the VT after pretreatment with pargyline* Eight neonatally DA-depleted and seven control self-stimulators which had completed Experiment I were given ipsilateral injections of 6-OHDA into the VT using one of the two electrode-cannula units. Each rat was pretreated with pargyline, 50 mg/kg IP and then, 30 min later, the stylet in the ipsilateral cannula was removed and replaced with the injection needle Each animal received an injection of 6-OHDA, 4 $\mu$g/2 $\mu$l of CSF which contained 0 1% ascorbic acid The duration of the injection was 6 min but the needle was left in place for an additional 4 min to optimize conditions for diffusion of the solution in tissue below the cannula. The animal was then returned to its home cage

*SS testing* Daily testing for SS was resumed on the day after the VT injections, and continued for seven days.

*Histochemical fluorescence verification* When SS testing was completed, brains of all subjects tested were processed by the glyoxylic acid-perfusion cryostat method [4] for visualization of CA fluorescence varicosities in the area surrounding the electrode tip

*Experiment III*

*The injection of KA after pretreatment with diazepam* Twelve DA-depleted and seventeen control self-stimulators which had completed Experiment I were given ipsilateral or contralateral injection of KA (5 nM/$\mu$l/injection) 30 min after

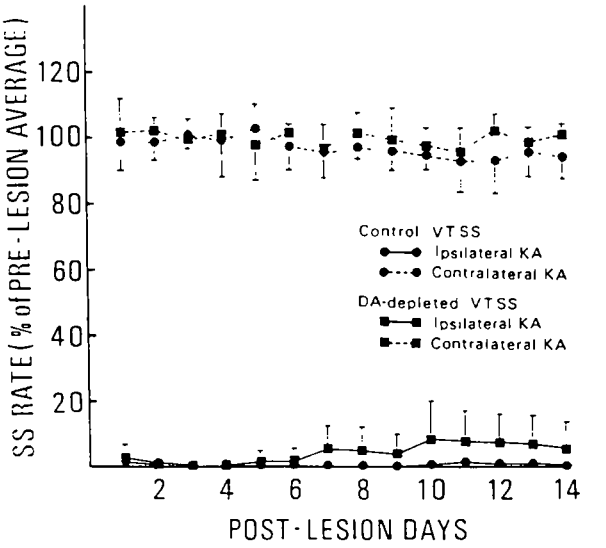


FIG 5 Suppression of self-stimulation in the ventral tegmentum after injections of kainic acid ●——● Ipsilateral SS to the injection in control rats (N=5) ●— —● Contralateral SS to the injection in control rats (n=6) ■——■ Ipsilateral SS to the injection in neonatally DA-depleted rats (n=6) ■— —■ Contralateral SS to the injection in neonatally DA-depleted rats (n=5) Data expressed as the daily group mean % of prelesion (5 days) average scores ±S E M

pretreatment with diazepam (20 mg/kg, per os), given to minimize the induction of remote effects of KA [2,41]. The injection lasted for 6 min, but the needle was left in place for another 4 min before being withdrawn. The animals were not anesthetized during these injections as diazepam, at the dose given, induced sedation Contralateral injections were given to the control group to offset the possibility that the effects of KA on SS were due to behavioral toxicity

*SS testing* The rats were given 14 consecutive daily sessions of SS on the ipsilateral or the contralateral probe

*Histological verification* At the end of these tests, the animals were sacrificed by giving a lethal dose of pentobarbital followed by cardiovascular perfusion first with physiological saline and then with 10% formalin The brains were sec-

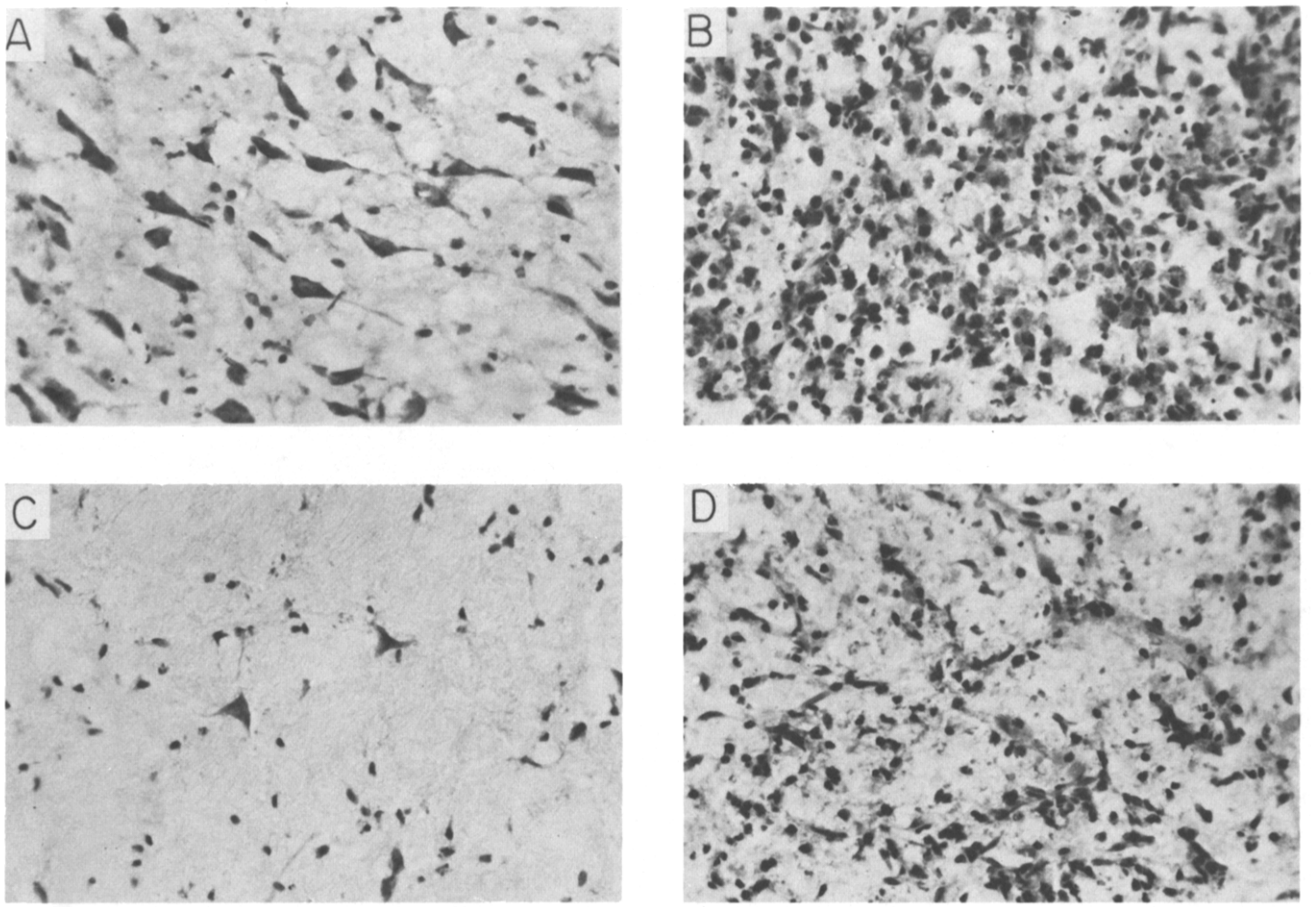


FIG 6 Photomicrographs of Nissl-stained section in the ventral tegmentum (VT) after kainic acid injection A Contralateral to the injection in a control rat B Ipsilateral to the injection in a control rat C Contralateral to the injection in a neonatally DA-depleted rat D Ipsilateral to the injection in a neonatally DA-depleted rat

tioned on a freezing microtome to obtain 30 $\mu$m sections which were subsequently mounted and then stained with cresyl violet.

*Statistical procedures* When statistical analysis was needed, Student's $t$-test, Chi-square test and an analysis of variance were used to evaluate the difference between the groups [52]

## RESULTS

### *Experiment I*

Of the sixty-six rats prepared, 52 were selected for bilateral SS with probes rightly placed in the VT. These 52 contained 28 controls and 24 neonatally DA-depleted subjects The results show that there was no difference between the percentages of animals that met the SS criterion in the DA-depleted and the control groups Although responding was stable from day to day in the subjects from both groups that met the criterion, the rate of responding on the last day of the SS session was slightly but significantly lower in the DA-depleted animals, $t(50)=5.50$, $p<0.001$ Neither was there a difference in the average current intensities needed to maintain SS in the two groups (Table 1) Thus, the neonatally induced DA-depletion did not block SS in the VT, but it reduced the rate of responding

The probes of all subjects used in Experiment I were entered on drawings of the rat brain [24] following verifications of electrode placement For Experiment I and II the verification was on sections processed by the histochemical fluorescence method; for Experiment III, on sections processed by standard histological procedures The results show that there was no systematic difference between the placement of the electrodes in the controls or the neonatally DA-depleted subjects (Fig 1) The sites yielding SS were localized lateral to the interpeduncular nucleus, medial to the substantia nigra and ventral to the lemniscus medialis

The histological results also show that the neonatal treatment with DMI+6-OHDA induced severe damage to the A9 and A10 cell groups Figure 2 shows the distribution of DA cell bodies in histochemical fluorescent-processed tissue taken from the VT near the electrode tip of control and DA-depleted animals In the control subject (A), fluorescence cell bodies are present in an area that is part of the A10 region, and which is on the right side of the electrode track Fluorescence cell bodies are also present in the A9 region which is on the left side of the electrode track In the DA-depleted subject (B), fluorescence cell bodies cannot be seen either in the A9 or A10 regions. Some are however visible in the area just dorsal to the interpeduncular nucleus Figure 2 C shows an example of the network of fine fluorescence

varicosities that was observed in the vicinity of the electrode track in the same material as in B under high magnification. This fine network most likely represented the NE fibers that traverse the VT on their way to the telencephalon.

*Experiment II*

Three of the eight neonatally DA-depleted animals died after receiving the ipsilateral injection of 6-OHDA into the VT. The DA depletion caused reduction of the body weights. Before the intracerebral injection, the average weight of the controls was 452.1±39.5 g and DA-depleted, 360 0±40.5 g, $t(10)=3\ 59$, $p<0.01$ Twenty-four hours after the injection of 6-OHDA into the VT, the weight of the controls was 427.1±38.7 g and of the DA-depleted rats, 327.0±46 9 g, indicating that the rate of body weight reduction after the intracerebral injection of 6-OHDA (% reduction values) did not differ between the control and the neonatally DA-depleted groups, $t(10)=1.79$, n s

Neither was there a difference between the two groups in the pattern of SS after the injection of 6-OHDA into the VT (Fig. 3) Both groups gave evidence of marked suppression of responding on the first day after the injection, with dramatic recovery to prelesion response rates on the second day. The stability of these rates was evident in the tests on subsequent days (for 2nd–7th post lesion days, DA-depleted; $F(5/20)=0.345$, n s , Controls, $F(5/30)=1\ 076$, n s )

The histochemical verification of the action of 6-OHDA injected into the VT in the adult animal indicated that the injection in the neonatally DA-depleted rats caused the disappearance of the residual CA varicosities in tissue surrounding the site of injection. Figure 4 shows representative examples of the CA innervation in the area just below the SS probe in a control (A) and a neonatally DA-depleted subject (B, C) In the control subject, a dense network of fluorescence CA varicosities and numerous cell bodies are present (A) In the neonatally DA-depleted subject, on the side contralateral to the intracerebral 6-OHDA injection (B), small fine varicosities are visible which are likely to be part of the CA innervation that survived the neonatal damage The effects of the neurotoxin on the CA innervation in the vicinity (ipsilateral VT) of the injected site (C) shows a striking loss of CA fluorescence varicosities The residual CA innervation seen on the contralateral side (B) is absent on the ipsilateral side (C).

These results, taken together, show that damage to the residual CA innervation present in tissue surrounding the tip of the electrode, had no effects on SS.

*Experiment III*

Eleven of the 17 controls, and eleven of the 12 neonatally DA-depleted rats survived after the injection of KA. The difference in mortality between the two groups was not statistically significant, $\chi^2=2\ 79$, n s

The weight of all the animals decreased after the KA injection. Before the injection of KA, the average weight of the controls was 430.5±33 7 g, while that of the neonatally DA-depleted animals was 369.0±48.6 g, $t(19)=3.94$, $p<0\ 01$; 24 hr after the KA injection, it was 391 8±34.8 g for the control group and 331.5±48.9 g for the DA-depleted group. The difference for the rate of body weight reduction between the two groups was not statistically significant, $t(19)=1\ 43$, n s

In the subjects which received ipsilateral injection of KA in the VT, SS was blocked in tests conducted over a 14-day period. But SS in the animals which had received the contralateral injection of KA showed no deficits (Fig. 5). The same differences in the effects of ipsi- and contralateral injections of KA were observed in both the control and the neonatally DA-depleted rats.

The tissue injected with KA showed necrosis in a circumscribed area around the tip of the injection needle, which never extended contralaterally. Figure 6, B and D, shows typical examples of the absence of cell bodies in tissue below the electrode tip of a control and a neonatally DA-depleted rat produced by an ipsilateral KA injection Figure 6 A shows that a contralateral injection of KA caused no damage to ipsilateral tissue at the electrode tip in a control rat, as evidenced by the presence of numerous cell bodies and relatively few astrocytes. Figure 6 C shows also the lack of effects of contralateral KA injection on ipsilateral tissue, in a neonatally DA-depleted rat. In Fig. 6 C, cell bodies are present, though reduced in number by the neonatal DA-depletion, and astrocytes are not as numerous as with ipsilateral KA injections.

## DISCUSSION

In Experiment I, 6-OHDA was injected bilaterally in two stages in the lateral ventricles at a dose of 200 $\mu$g. In an earlier study using identical procedures [48], the level of DA in the frontal cortex in adulthood was approximately 8% of the control level. Therefore, it is reasonable to assume that the DA level in the VT was similarly low in the neonatally DA-depleted animals of Experiment I Furthermore, our histochemical fluorescence findings pertaining to the tissue surrounding the electrode tip in the VT showed a loss of CA flourescence cell bodies, except in an area just dorsal to the interpeduncular nucleus. These results of Experiment I may suggest that the DA systems originating with either the A9 or A10 cell bodies are not necessary for SS in the VT region.

Experiment I does not exclude the possibility that CA-containing fibers in the VT are responsible for SS, as fine network of CA fluorescence varicosities was observed in the VT of the neonatally DA-depleted animals (Fig. 2 C). However, in Experiment II, we destroyed CA terminals in the VT by injecting 6-OHDA locally. Yet, VT SS was not abolished. It is evident that the CA innervation of the VT does not play a critical role in VT SS. It is noteworthy that the local infusion of 6-OHDA in the VT was given in animals pretreated with pargyline and not with DMI This treatment does not restrict the action of the neurotoxin to the DA element; instead, the substrate for this treatment is the CA innervation of the VT which includes NE and DA. with preference being given to terminals rather than perikarya, and thus to the NE terminals. The results of Experiment II thus conform to the view that the NE systems do not mediate the reinforcing effects of brain stimulation in the VT [16,53]. For the VT, the NE input derives in large part from the ventral NE bundle [15,49], and therefore, the present findings show that this bundle serves no function in VT SS.

In Experiment III, SS was abolished following ipsilateral injection of KA in tissue below the electrode. The effect was not caused by motor dysfunction or the induction of a general pathological state since the contralateral injection of KA was ineffective. The available neurochemical and histological results indicate that KA causes selective damage to local neurons, sparing the terminals and axons present in tissue at the site of the injection and nearby [21,44]. Such selective action of KA probably produced, in Experiment III, destruction of the perikarya in the tissue surrounding the elec-

trode tip, but not of the terminals and fibers in that area Since KA injected ipsilaterally led to the complete suppression of SS, this implies that the activation of the cell bodies, but not the terminals or fibers *en passage*, in the VT, was responsible for the brain reward

Intrinsic neurons of the VT, here assumed to be critically responsible for SS, are most likely to be non-dopaminergic cells with glutamate receptors on them However, involvement of DA neurons in SS cannot be excluded completely After DA-depletion in Experiment I, some compensatory process might occur to supplement DA neurons Indeed, it has been reported that DA-containing neurons which have survived the neonatal damage retain the capacity to sprout axon terminals, and that their metabolism is increased concurrently with adjustments in the number of and affinity of receptors for these neurons [1, 6, 11, 13, 34, 54] However, this possibility is unlikely as the few DA neurons which survived the neonatal DMI + 6-OHDA treatment in Experiment I were dorsal to the interpeduncular nucleus, while the SS sites were lateral to it Furthermore the KA-lesioned area in Experiment III is not likely to have extended far enough to include these surviving DA containing cell bodies (see Fig 6) The present finding that the SS rates from the VT were slightly but significantly reduced in the DA-depleted animals in comparison to those of control animals may be interpreted as suggesting that, even though DA neurons are not essential in effecting SS, they play a role in maintenance of the VT SS

ACKNOWLEDGEMENTS

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