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Effects of Intracaudate Microinjections of 6-Hydroxydopamine Upon the Suppression of Lever Pressing and Upon Passive Avoidance Conditioning in Cats

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REYES VAZQUEZ, C., I. ZARCO-CORONADO AND H. BRUST-CARMONA. *Effects of intracaudate microinjections of 6-hydroxydopamine upon the suppression of lever pressing and upon passive avoidance conditioning in cats.* PHARMAC. BIOCHEM. BEHAV. 9(6) 747–751, 1978.—Recent studies have demonstrated that the caudate nucleus (CN) is an important element of the neuronal circuitry required for the acquisition and retention of a motor conditioned response (MCR). It has also been suggested that a normal level of dopamine in the CN is required for an animal to learn to suppress the MCR. In the experiments described here, cats were trained to press a lever (MCR) following the onset of a discriminative stimulus (light) which was reinforced with 0.5 ml of milk, and to refrain from pressing the lever in the absence of the stimulus (suppression of motor conditioned response, SMCR), since no reinforcement was given. After 3 sessions, bilateral microinjections of 5.0 μl of NaCl or different doses (5, 10, 20, 40, 80 or 160 μg) of 6-hydroxydopamine were made into the antero-ventral area of the head of CN. After 30–35 days the cats were submitted to a passive avoidance conditioning (PAC). Regarding the MCR, no differences were observed among the various groups; in relation to SMCR, the cats injected with 5, 10 and 20 μg of 6-OHDA showed a significant decrease in the number of lever pressings when compared with the NaCl group ($p<0.05$), while, in contrast, the 80 and 160 μg doses produced a significant increase ($p<0.05$). The 40 μg dose caused no effects different from the NaCl group. All the subjects acquired the PAC. It is postulated that the lower doses of 6-OHDA, although producing lesions of the dopaminergic structures, cause sensitization by denervation, thus enhancing the inhibitory effects. The higher doses probably produce a marked diminution of DA content and therefore a reduced ability to suppress a MCR. However, the subjects are capable of acquiring the PAC, thus suggesting that the lesioned structures are not involved in this type of learning.

Caudate nucleus Learning Conditioning Catecholamines 6-OHDA

THE PARTICIPATION of the caudate nucleus (CN) in the neuronal circuitry responsible for the acquisition and retention of a motor conditioned response (MCR) has been demonstrated by use of electrolytic lesions [5], topically applied KCl [19] and locally injected anesthetics [4]. It has also been shown that the CN is essential for the maintenance of an inhibitory conditioning [3]. Therefore, it has been assumed that the CN holds the necessary elements to activate the circuitry responsible for the performance of a MCR as well as those required to inhibit this circuitry [2]

Several authors have demonstrated high acetylcholinesterase as well as choline-acetylase concentrations in the striatum [7]. Furthermore, the iontophoretic application of Ach in the CN facilitates the unitary activity [1]. Prado-Alcalá *et al.* [20] found that the application of atropine in the CN has a blocking effect on a MCR. Thus, it seems that a cholinergic system in the caudate could be of a facilitatory, and an adrenergic system of an inhibitory nature. High concentrations of catecholamines, especially dopamine (DA), have been demonstrated in the CN [11, 12, 29], and the nigrostriatal pathway has been postulated as being responsible for keeping adequate levels of DA in the striatum [30]. Iontophoretic application of DA has an inhibitory effect upon the neuronal pool of CN [26,27]. Behaviorally, it has been observed that the application of DA in CN increases the inhibitory stage of the MCR (Zarco-Coronado, C. Reyes-Vázquez and H. Brust-Carmona, unpublished data) without affecting the facilitation of the response.

Recent experiments have demonstrated that 6-hydroxydopamine (6-OHDA) damages the central catecholaminergic endings [22,24]. Ungerstedt [31] has reported that the microinjection of 6-OHDA along the nigrostriatal pathways depletes the DA content of the striatum without significatively affecting the nonspecific system. However, in this type of experiments other structures of the CNS, which could also be catecholaminergic, are affected, i.e. hypothalamus and n. accumbens.

Our goal in this research was to decrease the DA content

of CN through topical microinjections of 6-OHDA and, hence, demonstrate the involvement of DA in the suppression of MCR (SMCR), and, at the same time, assess the role of DA in a passive avoidance conditioning. Since chemical lesions of the striatum [18] or of the substantia nigra [21] affect this type of conditioning, it has been postulated that the integrity of the nigro-striatal pathway is required for the acquisition and retention of an avoidance conditioning.

METHOD

Animals

Forty three cats (2.5–3.5 kg body weight) of either sex were trained to obtain 0.5 ml of milk each time they pressed a lever in a dimly illuminated Skinner type box (Lehigh Valley Electronics) enclosed in a sound-insulated chamber.

Procedure

The time allowed for pressing the lever (MCR) was 12 min during which a luminous signal (discriminative stimulus, CS) placed above the lever, remained on. The operant conditioning apparatus turned the light off for 1.0 sec at the end of each minute. This training pattern was repeated for 3 consecutive days. In the fourth session (day) after each 1 min period, the CS was turned off for 20 sec and no reinforcement was given for lever pressing during this short period (SMCR). Thus the length of the session was increased to a total of 16 min. Lever pressing in both situations was automatically recorded. After each session the cats received meat at a ratio of 35 g/kg of body weight.

After 3 consecutive combined (CS-on and CS-off) sessions, the cats were anesthetized with pentobarbital and placed in a stereotaxic instrument. Stainless steel cannulae were lowered into the caudate nucleus on both sides (A=16, L=4.5, H=+4.5) following the Jasper and Ajmone Marsan atlas [15]. Then bilateral microinjections of different doses of 6-OHDA were made over 20 sec per each injection (5, 10, 20, 40, 80 and 160 μg; n=6 for each group).

In a control group (n=6) 5 μl of saline solution were injected bilaterally. The 6-OHDA solution was prepared by dissolving 6-hydroxydopamine bromide (SIGMA) in bidistilled water immediately before its application. Twenty four hr later training was resumed and continued for at least 25 more sessions. At the end of this period (30–35 days), acquisition of a one-trial passive avoidance conditioning (PAC) was tested.

A two compartment chamber with the compartments separated by a sliding door was used for the training and testing session. One of the compartments (50×40×40 cm) had an even floor. The other compartment, of the same size, was continuously illuminated by a 50 W bulb and was provided with an electrifiable grid made of 0.5 cm dia. stainless steel bars which were separated by 2 cm intervals and connected to a high impedance stimulator (Nucleo-Electronica). Thirty seconds after placing a cat in the first compartment, the door communicating the compartments was opened and the latency to cross into the second compartment was measured. Once the animal had crossed, the door was closed and a nociceptive stimulus (60 Hz sine wave of 4–5 mA strength and 1 sec duration) was applied to the paws. Two additional shocks were delivered at 45 sec intervals. After the third shock the cat was allowed to escape into the first compartment and was then removed after 3 min. Twenty four hr later the latency to cross from the one compartment to the other was again measured over a maximum waiting time of 600 sec (learning criterion).

After completing the experiment a lethal dose of sodium pentobarbital was given intraperitoneally, and the brains perfused first with saline solution and then with 10% Formalin. The brains were kept in Formalin (4%) for one week and then sectioned coronally (20 and 60 μ) on a freezing microtome. To localize the cannulae placements, photographic prints were made of the sections by using them as negatives in an enlarger [10]. The thin sections were stained according to the Fink and Heimer technique [8] and studied for neuronal degeneration.

The averages of the total lever pressings for MCR and SMCR for each session of each group were plotted. The significance of the observed differences was evaluated by use of a U test program on a PDP 11/40 computer.

RESULTS

In some cats we observed the effect of 6-OHDA microinjections as soon as the anesthesia had worn off. A slight rigidity of the extensor muscles and some small movements mainly in the forepaws were observed while the animals were at rest. These effects were more easily seen at higher doses for which some animals remained quiet and did not eat. However, all animals recovered and were able to walk and eat unassisted after 48 hr. No further gross behavioral alterations were noticed during the month of daily observations.

Aside from an initial depression of lever pressing in all animals, neither the rate per min nor the total amount of lever pressings during the reward situation (CS-on, reinforcement on) changed with 6-OHDA or saline administration (Fig. 1). In contrast, the lever pressings during the no-reward situation (CS-off, reinforcement off) markedly changed. In the animals injected with 5, 10 and 20 μg the lever pressings clearly decreased (Fig. 2). The strongest diminution was produced with 20 μg.

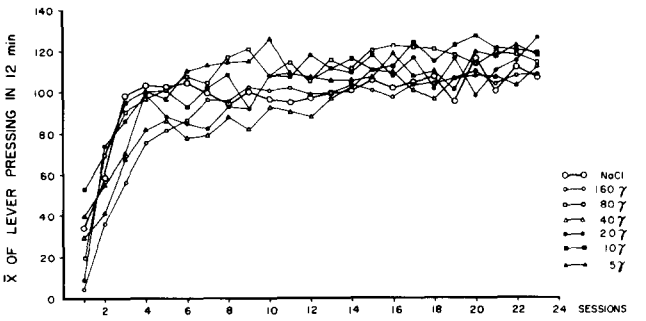

FIG. 1. The average (N=6) lever pressing per session in the reward situation (CS-on, reinforcement on) after bilateral microinjection of 5 μl of NaCl or 6-hydroxydopamine in the antero-ventral part of the head of the CN. There is no significant difference in the performance under these conditions.

Due to limitations of the computer program we pooled the lever pressings of 2 consecutive sessions of each group and then compared the differences using the U test. The difference of the lever pressing of 2 consecutive sessions for animals injected with NaCl and those injected with 20 μg of

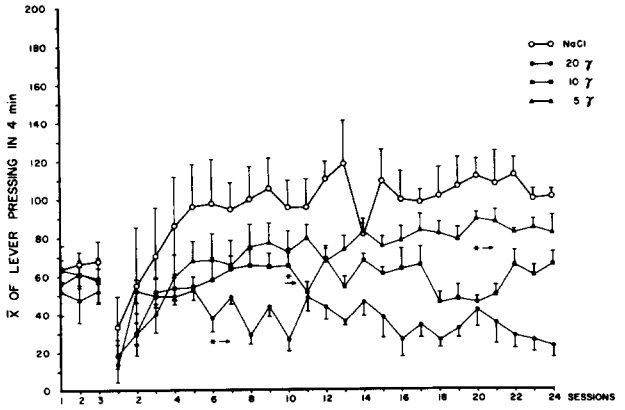

FIG. 2. The average (N=6) lever pressing in the suppression situation (CS-off, reinforcement off) for low doses of 6-OHDA. The first three sessions represent the control period which is followed by injection and the experimental sessions. The vertical lines represent one standard deviation, which for illustration purposes was drawn in only one direction. The asterisk indicates the session at which statistical significance ($p<0.05$) begins upon comparing the effects of saline with 6-OHDA (U test).

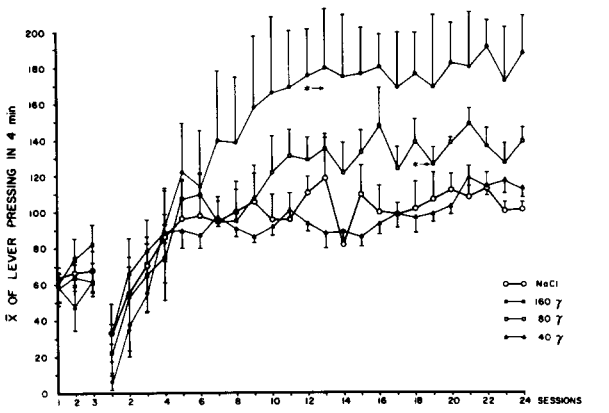

FIG. 3. The average (N=6) lever pressing in the suppression situation (CS-off, reinforcement-off) for high doses of 6-OHDA. Description of the graphs is as for Fig. 2.

6-OHDA is statistically significant ($p<0.05$) at the combined 5–6 sessions. The difference between saline and 10 μg injections is also significant at the 9–10 sessions, while that at 5 μg is not always significant; in the 11–12 sessions there is a significant difference and then again only after the 19–20 sessions.

The animals injected with 40 μg of 6-OHDA and the saline group were alike, but those injected with 80 and 160 μg of 6-OHDA increased the lever pressing rate over NaCl controls (Fig. 3). The difference is significant after the 17–18 sessions for 80 μg and after the 11–12 sessions for 160 μg. For further analysis, the total amount of lever pressing for all sessions was compared among all groups. The histogram of Fig. 4 shows the average and standard deviation obtained in each group. The groups are statistically different ($p<0.05$)

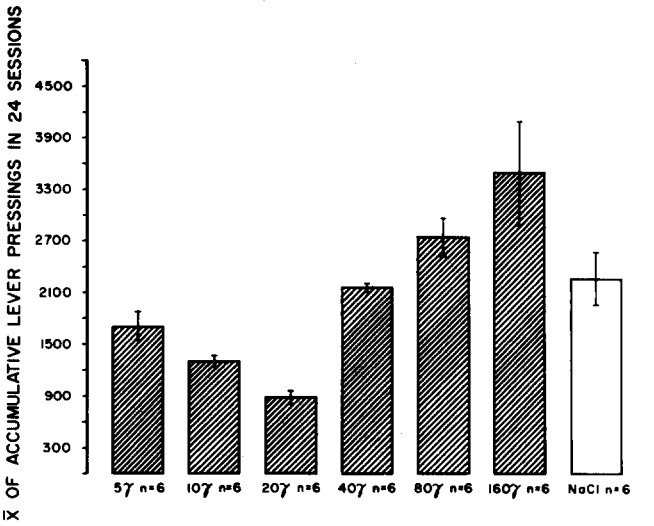

FIG. 4. Averages (N=6) of the total amount of lever pressing in the 24 sessions for all groups. The only group in which the difference from the saline treated animals is not statistically significant is the 40 μg group.

for saline compared to the 160, 80, 20, 10 and 5 μg groups, but not the 40 μg group.

In the acquisition session of the PAC all the cats crossed from the first compartment to the second in 47.2 sec on the average (range=43.0–58.1). There were no significant differences between the animals of the various groups. In the retention test none of the animals crossed during the 600 sec maximum waiting time (learning criterion).

Histological sections showed that the microinjections were made into the ventromedial anterior part of the head of CN (Fig. 5). The Fink and Heimer staining technique revealed that the lower doses (5, 10 and 20 μg) produced only intracaudal degeneration and that the number of degenerated fibers was higher in the animals injected with 20 μg. The higher doses produced a more pronounced intracaudal degeneration and some extracaudate structures were also affected; e.g.: putamen, n. accumbens and substantia nigra. These effects were more clearly seen with the 160 μg dose.

DISCUSSION

Our results show that the SMCR (CS-off, reinforcement off) is altered by intracaudate injections of 6-hydroxydopamine. Small doses (5, 10 and 20 μg) which decrease the number of lever pressings during the suppression condition seem to enhance the intracaudate inhibitory mechanisms, probably due to a partial denervation accompanied by supersensitivity. Catecholamine depletion by 6-OHDA, as well as the supersensitivity by denervation have been demonstrated by different authors [9, 27, 28, 29, 30]. In contrast, high doses produce a clear diminution of the inhibitory processes, since the animals press the lever more often. This supports the hypothesis that the inhibitory action in the CN responsible for the suppression depends on the presence of catecholamines. However, other structures, such as the putamen, n. accumbens and the substantia nigra should also be taken into account, as higher doses produce some degeneration in these structures. However, a word of

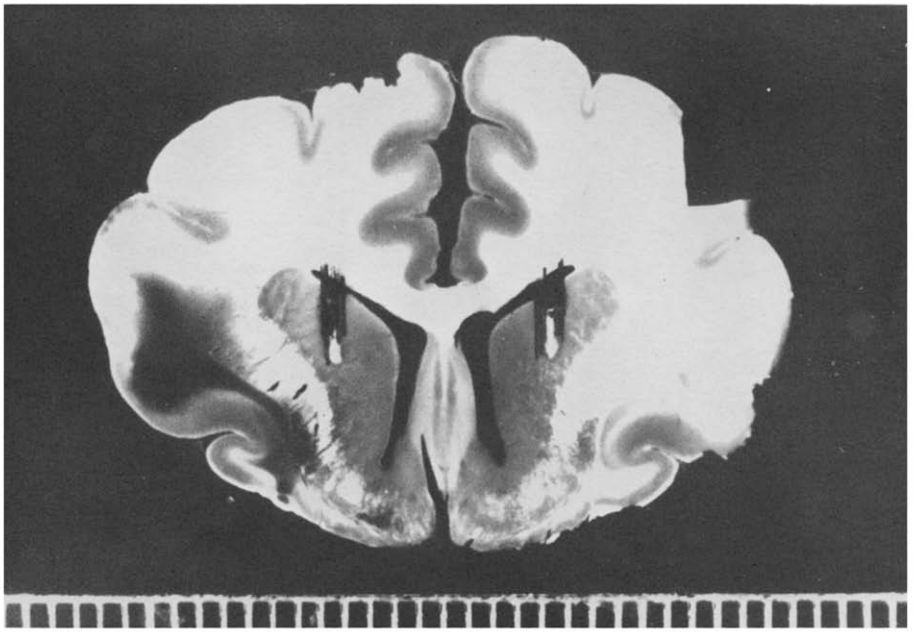

FIG. 5. The antero-medial-ventral area of the head of the CN in which injections were made. Scale: 1 mm/div. The outlined area represents the area over which the cannulae placements were found in the various cats. The anterior-posterior variation in placements was about 1.0 mm.

caution has to be said in relation to the higher doses of 6-OHDA, since the work of Willis *et al.* [32] and Evans *et al.* [6] showed that high doses of 6-OHDA, injected into various regions of the central nervous system, cause generalized damage, but damage specific to catecholamine-containing neurones, as shown by fluorescence histochemistry, was not seen. Nevertheless, the same authors showed that CN is really a structure sensitive to 6-OHDA as compared to other structures, such as the olfactory bulb. Thus, if we refer to CN only we might be on the safe side. It is noteworthy that MCR is not substantially modified by the application of 6-OHDA which indicates that the facilitatory circuits are able to maintain their functions independently from the variation of catecholamine content in the CN. Howard, Lealy and Breese [13] have shown that bar pressing on a fixed ratio for food reward in rats, is not modified by the intracisternal injection of 6-OHDA. Later, Howard, Grant and Breese [14] observed that rats injected with 200 μg of 6-OHDA persisted in pressing the bar several more times after receiving a pellet, rather than shuttling immediately to the correct goal box at the other end of the alley. This could be interpreted as a reduction of inhibition; however, in comparing their results with ours one should note that other brain structures could have been involved in their experiments, since higher doses of 6-OHDA were used and they were applied intracisternally.

It is significant that the PAC which could also be due to the inhibitory actions upon the facilitatory motor circuitry was not altered by any of the intracaudate 6-OHDA injections. This could be explained by proposing that the affected structures do not participate in this type of conditioning, although some authors [16,33] have shown such involvement. These authors have demonstrated a role of the caudate in passive avoidance conditioning, but less discriminating experimental methods were used, i.e., electrolytic lesions and electrical stimulation.

Although one can postulate recovery of neurons involved in passive avoidance conditioning or compensation by other neurons, it is more likely that the catecholamines of the antero-ventral portion of the CN are not involved either in the acquisition nor in the manifestation of a passive avoidance conditioned response. This type of conditioning may depend more on the globus pallidus, since some authors [16, 17, 18, 33] found alteration of the PAC with lesions of the corpus striatum in rats. Furthermore, Neil and Grossman [17] described certain topographical organization of the corpus striatum by use of different cholinergic blockers. Recently, in cats, similar results of topographical organization of the CN have also been described [25]. However, Runnels and Thompson [23] suggested that the effects of striatal lesions are due to the interruption of fibers crossing through the striatum which possibly were not lesioned in our experiments.

In conclusion, the results described here support the hypothesis that the suppression of a specific motor response (such as lever pressing) depends on the existence of dopamine in the antero-ventral region of the CN in cats, whereas passive avoidance is not dependent on the dopamine level. But, we recognize that direct proof of catecholamine-depletion is lacking in our experiments, thus, more investigation is needed.

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