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Facilitation of the Suppressing Effect of Dopamine Upon a Motor Conditioned Response by 6-Hydroxydopamine Applied Into the Caudate Nucleus in Cats

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REYES-VÁZQUEZ, C. AND H. BRUST-CARMONA. *Facilitation of the suppressing effect of dopamine upon a motor conditioned response by 6-hydroxydopamine applied into the caudate nucleus in cats.* PHARMAC. BIOCHEM. BEHAV. 13(1) 97–101, 1980.—It has been described that the application of DA in the Caudate Nucleus improves the learning of an inhibitory motor conditioned response (SMCR). However, the application of 6-hydroxydopamine (6-OHDA) at low doses of 5, 10 and 20 μg produced a clear increment of the inhibition, while higher doses of 80 or 160 μg diminished markedly the suppression of the MCR. We postulated that although low doses of 6-OHDA produce lesions in the dopaminergic structures they cause hypersensitization by denervation. To prove this hypothesis we observed the effect of DA application into the CN before and after applying one of the lower doses of 6-OHDA. Cats were trained to press a lever (MCR) to obtain 0.5 ml of milk when a conditioned discriminative stimulus (light) was on, and to suppress the response when the light was off (SMCR). The various applications of 10 μg of DA in both CNs, always produced a decrement in the lever pressing rate only in the SMCR situation. The effects of the DA applications after the 6-OHDA application were more significant ($p<0.01$) than when applied before the 6-OHDA. These findings further support the postulation that catecholamines in the CN improve the inhibitory actions required for the suppression of motor responses.

Sensibilization by 6-OHDA Caudate nucleus Inhibitory motor conditioning
Behavioral inhibitory actions of catecholamines Catecholamines and learning

THE involvement of central catecholaminergic systems in different learning processes has been shown by different experiments. As for example, in active avoidance conditioning [7,12] in passive avoidance conditioning and in classical alimentary responses [15], as well as in self-stimulation responses [19] and in maze responses [1]. On the other hand, it has also been described that these substances participate in the control of motor and emotional activity in rats [21] and cats [4]. Even more, it has been suggested, that learned motor responses can be modified by changing the concentration of Dopamine (DA) or Norepinephrine (NE) [2]. Specifically, it has been observed that the topical application of DA into the head of the Caudate Nucleus (CN) improves the learning ability to suppress (inhibit) a motor conditioned response (MCR), such as lever pressing [24]. In a previous paper [20] we tried to demonstrate that the diminution of DA content in the Caudate Nucleus of cats would interfere with their ability to learn to suppress this type of instrumental motor conditioning. This was indeed observed, but using high doses of 6-OHDA (40, 80, 160 μg), while the lower doses (20 μg) caused an increase on the suppression, i.e. lever pressing rate diminished. We explained this effect, as a result of sensitization by denervation due to a partial lesion of the dopaminergic systems, an effect which has been widely described for peripheral synaptic structures [23] and some similar effects have been obtained in central structures [6]. In the present paper we provide data which support this interpretation.

METHOD

Fifteen cats (2.0–3.0 kg of 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.

The time allowed for rewarded lever pressing was 12 min during which a luminous signal (discriminative stimulus, CS) placed above the lever remained on (CS-on, MCR). 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 (CS-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

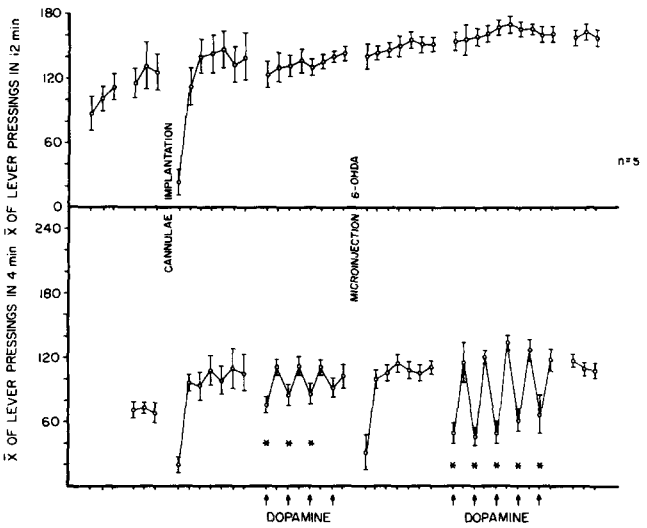

FIG. 1. Average (N=5) of the lever pressings, during the rewarding situation (upper part of this and all figures) and the non-rewarding situation (lower part of all figures) for the DA-6-OHDA-DA group. In this and subsequent figures the abscissas represent the session number and the arrows indicate the microinjections. Note the decrement in lever pressings after DA application. This effect is enhanced ($p<0.005$ for the first two and 0.01 for the next two) by the sole application of 6-OHDA into the ventral part of the head of the CN.

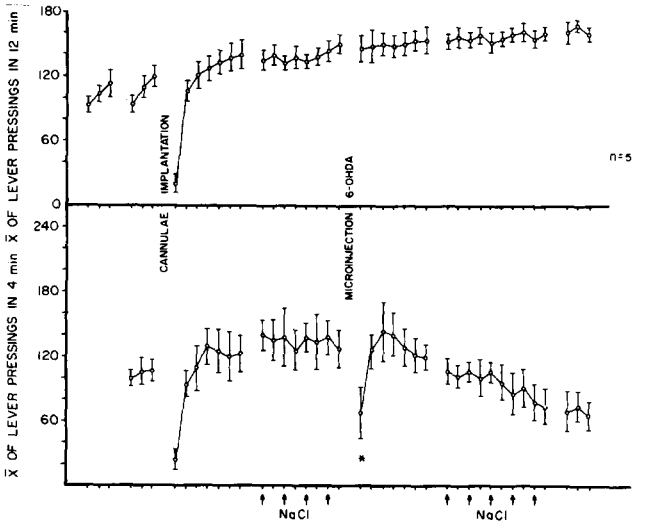

FIG. 2. Average (N=5) of the lever pressings for the NaCl-6-OHDA-NaCl group. Note the decrement in the lever pressings after 6-OHDA application in the non rewarding situation ($p<0.05$). The application of NaCl has no effect upon the conditioned responses.

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 (37 mg/kg, IP) and placed in the stereotaxic apparatus. Stainless steel cannulae (0.7 mm external diameter) were implanted into the anteroventral medial part of the head of the CN, (A=16.0, L=4.5 and H=4.5) according to the Jasper and Ajmone Marsan atlas [13]. The cannulae were fixed to the skull using dental acrylic and their stability was improved by means of 3 screws fixed to the frontal sinus. In general, the conditioning sessions were reinitiated one-two days after the implantation and continued for 7 more days.

On the eighth session, in 10 Ss a series of 4 microinjections of DA was started, using a stainless steel tube adjusted to reach the lower end of the cannula which was connected by polyethylene tubing to a microliter syringe. Five cats were injected with 5 μl of NaCl 0.9%. These microinjection sessions were alternated with sham injections which consisted of introducing the injecting tube during 20 seconds without applying any substance 10 min before the conditioning session. The DA solution was prepared dissolving 2 mg of DA-HCl (3, 4-dihydroxyphenylethylamine HCl, Nutritional Biochemicals Corporation) in 1 ml of bidistilled water immediately before application. The dosage used was 10 μg in a volume of 5 μl. The conditioning session was begun 10 min after the microinjection. The microinjections were applied over a period of 20 sec for each side, and they were always bilateral. At the end of this period of 4 DA microinjections and 4 sham injections, at the next session, the 5 cats which had been injected with DA and the 5 cats injected with NaCl received a sole microinjection of 20 μg of 6-OHDA. The 6-OHDA solution was prepared by dissolving 2 mg of 6-hydroxydopamine bromide (3, 4, 5-trihydroxyphenethylamine hydrobromide crystalized SIGMA) in 0.5 ml of bidistilled water immediately before application. The other five subjects, which had received DA, received a sole microinjection of 5 μl of NaCl 0.9% instead of the 6-OHDA microinjection, ten minutes before the conditioning session was begun. This type of conditioning session was repeated 6 more times.

At the end of this conditioning session, the animals were submitted to a second series of 5 microinjections of DA or NaCl, according to their grouping, using the same procedures as in the first microinjections period, the sessions were performed daily. This originated three groups of 5 subjects each one. (a) received NaCl, 6-OHDA, NaCl; (b) received DA, 6-OHDA, DA; and (c) received DA, NaCl, DA, microinjections.

After completing the experiment a lethal dose of sodium pentobarbital was given intraperitoneally, and the brains were perfused through the left ventricle, first with saline solution 0.9% and then with 10% Formalin. The brains were kept in Formalin 4% for 2–4 weeks, and then sectioned coronally (20 to 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 [14].

Statistics

The mean lever pressing of the different group was compared using the F test. The differences of lever pressing among each session of DA, NaCl or 6-OHDA microinjections and sham injections were compared by the correlated *t* Student test. At last the difference of the decrement of the lever pressing rate consecutively to the DA application was compared with sham injection (Lever pressing in sham injection minus lever pressing with DA) before and after the 6-OHDA or NaCl microinjections, using the *t* Student test. All the calculations were performed using a PDP 11/40 computer.

RESULTS

After the bilateral implantation of the cannulae into the

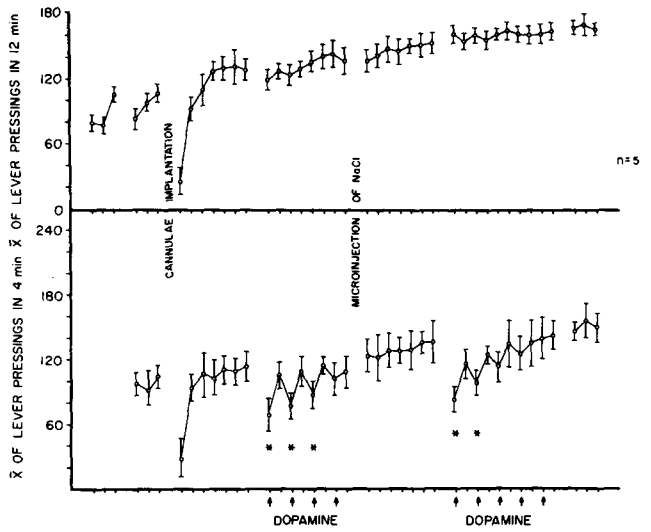

FIG. 3. Average (N=5) of the lever pressings, for the DA-NaCl-DA group. Note that the DA microinjections produce a decrement in lever pressing during the SMCR situation (no reinforcement). The DA effect is more pronounced during the first microinjections.

head of the CN (24–48 hrs) we observed a decrement in the number of lever pressing in both situations (MCR, CS-on and SMCR, CS-off). However, the subjects recovered the average lever pressing rate after 2–3 days (sessions). As we mentioned, the conditioning sessions were begun 10 min after the microinjections. During this waiting time, we observed, occasionally after the DA application, distal tremors of the tail and extremities, with light rigidity of the extensor muscles and less frequently rotation of the head to either side. These effects were observed only in 7 subjects after the first or sometimes after the second DA microinjections and disappeared rapidly. When the animals were placed in the conditioning chamber no motor disturbances could be observed. It is important to mention, that no changes in vegetative functions such as, salivation, micturition, defecation or piloerection were observed.

The DA treated animals pressed the lever in the CS-on situation similarly to the pretreatment sessions (first section of the upper part of Fig. 1). But, in the suppression situation, CS-off, they pressed the lever fewer times (first section of the lower part of Fig. 1). This effect is statistically significant at the level of $p<0.01$ for the first and second microinjections and $p<0.05$ for the third one and non significant for the fourth microinjections. The NaCl microinjections did not produce any important changes, neither in the spontaneous activity nor in the conditioned responses MCR or SMCR. This is illustrated in the first part of Fig. 2.

The application of the sole dose of 20 μg of 6-OHDA produced slight rigidity of the extensor muscles and small involuntary movements, specially in the anterior extremeties, which were more apparent when the subject was at rest. However, 10 min afterwards all the animals were able to eat by themselves. Consecutively to the 6-OHDA application we observed a decrement in the number of lever pressings during the SMCR, and comparing this number of lever pressings with the one obtained after the NaCl microinjections the difference is statistically significant at the level of $p<0.05$. The DA microinjections after the 6-OHDA application produced a more marked reduction of the lever pressing rate during the SMCR situation. Comparing the lever pressing rate after the sham injection with the one obtained after the DA application the difference is statistically significant at the level of $p<0.005$ for the first and second microinjections and of $p<0.01$ for the third and fourth microinjections. This effect is shown in the second part of Fig. 1 (i.e. after a sole application of 6-OHDA) for the DA-6-OHDA-DA group. Even more, if we compare the decrements (difference between lever pressing in sham injection and lever pressing

TABLE 1

COMPARING THE LEVER PRESSINGS BETWEEN THE 4 SHAM INJECTIONS SESSIONS AND THE NUMBER OF LEVER PRESSINGS AFTER THE DA APPLICATION

	DA-6-OHDA-DA group (N=5)					Da-NaCl-DA group (N=5)			
	Lever pressings in sham injection	Lever pressings with DA	$p<$	Differences		Lever pressings in sham injection	Lever pressings with DA	$p<$	Differences
1st.	112 ± 15.24	81.2 ± 13.55	0.01	30.8 ± 4.14		107.4 ± 15.0	73.2 ± 12.32	0.01	34.2 ± 19.8
2nd.	112.2 ± 13.3	86.0 ± 13.58	0.01	26.2 ± 2.16		110.2 ± 23.0	82.0 ± 15.0	0.05	28.2 ± 14.5
3rd.	101.0 ± 8.22	84.8 ± 10.4	0.05	16.2 ± 5.63		110.8 ± 14.4	90.6 ± 17.4	0.05	20.2 ± 19.33
4th.	103.6 ± 16.33	94.8 ± 7.43	NS	8.8 ± 7.69		108.8 ± 19.03	98.2 ± 24.18	NS	10.6 ± 13.24
	After 6-OHDA application					After NaCl application			
			$p<$		$p<$			$p<$	
1st.	116.8 ± 21.0	49.0 ± 17.03	0.005	67.8 ± 17.06	0.01	116.8 ± 27.5	81.2 ± 22.3	0.05	35.6 ± 13.6
2nd.	121.6 ± 14.4	46.2 ± 16.9	0.005	75.4 ± 10.69	0.01	124.2 ± 19.3	97.6 ± 24.3	0.05	26.6 ± 21.1
3rd.	139.8 ± 12.0	49.0 ± 18.3	0.01	90.8 ± 15.8	0.01	134.6 ± 44.1	112.4 ± 27.8	NS	22.2 ± 31.3
4th.	126.8 ± 21.1	61.8 ± 20.8	0.01	65.0 ± 30.87	0.01	134.4 ± 45.7	124.6 ± 31.9	NS	9.8 ± 13.9
5th.	118.4 ± 17.2	67.6 ± 31.2		50.8 ± 28.12		139.6 ± 27.2	136.8 ± 41.5		2.8 ± 7.9

Note that the lever pressings diminish consecutively to the DA application; this decrement is statistically significant during the first 3 sessions. After the application of 6-OHDA (lower right part) the decrement caused by DA is enhanced. If we compare the decrement between the sham injections and the DA application, with the decrement after the 6-OHDA the difference is statistically significant. In contrast this effect is not observed after the NaCl application.

in DA) produced by the DA application before and after the 6-OHDA application, the difference is statistically significant at the level of $p<0.01$. In contrast, this potentiating effect of DA is not observed after the sole application of NaCl. This is shown in the second part of Fig. 3 for the DA-NaCl-DA group. Comparing the decrement in lever pressing rate during the SMCR situation produced by the DA application, before and after the sole application of NaCl, the difference is not statistically significant. Finally, it is noteworthy to observe that the NaCl microinjection before or after the 6-OHDA application (NaCl-6-OHDA-NaCl group) did not produce changes in the conditioned responses studied (2nd part of Fig. 2). All these data are depicted in Table 1.

Histological sections showed that the microinjections were made into the ventromedial part of the head of the CN between A 15 to 16.5; L=4 to 6.5 and H=4 to 5 (according to the Jasper and Ajmone Marsan atlas, [13]).

DISCUSSION

The data described here, further support the functional participation of the CN in the motor learned responses, and suggest the existence of two regulatory systems for this type of conditioning. The drugs used, DA and 6-OHDA, catecholaminergic agents, affected exclusively the suppression of the MCR, in other words, their actions were exerted only upon the inhibitory regulatory system, leaving intact the facilitatory system upon the motor activity. Since the animals continued to press the lever for milk reinforcement, it can be stated that the actions of these drugs is not exerted through an immobilizing effect upon the animals. Another interesting observation of this paper is that the repeated application of DA diminishes its effects (tolerance?). A tentative explanation for these observations could be the formation of a state of subsensitivity caused by the prolonged use of dopaminergic agents. This effect has been described and confirmed from a neurochemical point of view [11,17], although it has not been analyzed yet, from a behavioral stand point. Apparently, this phenomenon disappears when the DA application is interrupted (but it reappears afterwards and develops faster) and does not appear after the application of 6-OHDA (although we only analyzed 5 DA microinjections after the 6-OHDA application). As has been described in a previous paper [20] the NaCl-6-OHDA-NaCl group showed a consistent decrement ($p<0.01$) of the response in the suppression situation after the 6-OHDA application. This has been interpreted as the effect of supersensitization by denervation, an effect which is being studied by several authors using synaptic blockers such as haloperidol [8,9], DA synthesis blockers [5]; or by means of chemical lesions [6]. This hypothesis seems supported by the data obtained from the DA-6-OHDA-DA group, in which the DA action was always greater when applied after 6-OHDA than when applied before ($p<0.01$).

The DA action analyzed in this experimental study is probably restricted to its intracaudal effect, since the time elapsed between its application and the behavioral observations is barely sufficient for its diffusion inside the CN [3,18].

These changes in the sensitivity of the dopaminergic receptor, probably a beta receptor, in the CN [16] evidence the close balance between the availability of the neurotransmitter and the sensitivity of the receptor, and indicate how the receptor is capable of regulating its activity depending on the amount of chemical transmitter available. It is possible that not only post-synaptic mechanisms but also presynaptic ones [8,22] play a role in this regulatory action. The behavioral model for the study of the DA action presented in this paper might be useful for the study of the dynamics of these chemical mediators and other dopaminergic precursors or antagonists.

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