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Conditioning of Rotational Behavior After the Administration of a Single Dose of Apomorphine in Rats With Unilateral Denervation of the Dopaminergic Nigrostriatal Pathway: Relevance to Drug Addiction

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CASAS, M., T. GUIX, G. PRAT, S. FERRE, J. CADAFALCH AND F. JANE. *Conditioning of rotational behavior after the administration of a single dose of apomorphine in rats with unilateral denervation of the dopaminergic nigrostriatal pathway: Relevance to drug addiction.* PHARMACOL BIOCHEM BEHAV **31**(3) 605–609, 1988.—Our aim is to study the relationship of drug activation of the dopamine neurotransmission system and the conditioning of environmental stimuli present at the time of drug administration. We injected a singe dose of apomorphine (0.05 mg/kg SC) in rats with the nigrostriatal dopamine pathways unilaterally denervated with 6-hydroxydopamine, which generates rotational behavior contralateral to the lesioned hemisphere. We observed rotational behavior without apomorphine administration when animals were reexposed at different time intervals to the same environment in which they performed turning behavior. The present findings show that this rotational behavior can be conditioned to environmental stimuli in a strong and long-lasting way. In light of the relationship between opioids and the dopaminergic system, similar conditioning could take place in the learning processes implicated in drug addiction.

Apomorphine Rotational behavior Animal model Drug addiction Dopamine Classical conditioning

ONE of the greatest problems regarding treatment of patients addicted to any substance of abuse, especially alcohol and opiates, is the frequent relapse of even detoxified patients. This may, in part, be due to premorbid psychopathological disorders (28), protracted syndrome (19,20), or social, family and occupational problems (17). In addition, it has long been accepted that objects, places and events associated with the experience of neural states induced by addictive drugs, when presented to detoxified patients without drug administration, can reproduce the effects induced by the actions of the drugs themselves and that they can become involved in compulsive drug-seeking behavior relapse (1, 24, 31, 43). This justifies a therapeutic approach which includes isolating recently detoxified patients from the familiar drug-associated environment since a large percentage of individuals relapse when they return to such environments, even after prolonged absence.

At present, in the field of drug addiction, many authors have centered their attention on the ability of addictive drugs to directly reinforce behavior through their pharmacological effect on brain reinforcement systems, particularly those mediated by dopaminergic pathways. Available data argue for at least three rewarding brain systems which involve dopaminergic neurotransmission: 1) The ventral tegmental system is possibly the substrate which mediates both positive reinforcement and approach behavior. This area is involved in the rewarding effects of some drugs such as psychomotor stimulants and opiates (13,27) although it does not seem to be implicated in physical dependence (6,26). 2) The nigro-pallido-striatal system is also involved in positive reinforcement, motor behavior (13,27) and in the regulation of food and water intake, unconditioned reinforcers in behavioral psychology (5, 27, 38). 3) The periventricular grey system is involved in negative reinforcement and

avoidance behavior and has been implicated in physical dependence (4,5).

Based on these implications, it has been suggested that the dopamine neurotransmission system is involved in the maintenance of the abuse habits of opiate addicts (2, 18, 33, 41), through an opiate-mediated increase in dopaminergic neurotransmission (14, 22, 36) or an opiate-induced supersensitivity of central dopamine receptors (2, 9, 12).

To study the relationship between activation of the dopamine system, which may be involved in drug addiction, and environmental conditioning, we have used the "turning behavior" animal model (38,40). We administered a single dose of apomorphine (0.05 mg/kg SC) to rats with the nigrostriatal DA pathway unilaterally denervated with 6-OHDA. This dose induces sedation and drowsiness in a naive rat (30) but, because of the unilateral supersensitivity of striatal dopamine receptors in the denervated rat, it induces intense rotation contralateral to the lesioned hemisphere. We attempted to evoke rotational behavior without apomorphine administration when animals were reexposed at different time intervals to the same environment in which they had previously received apomorphine.

METHOD

Animals

Male Sprague-Dawley rats were used in all experiments. The animals were housed, four per cage, with free access to rat chow and water. They were maintained in a temperature and humidity controlled environment on a 12 hr light/dark cycle when not in experimental sessions.

Surgical Procedure, 6-OHDA Denervation

Rats weighing 145–160 g were anesthetized with pentobarbital IP and placed in a David Kopf stereotaxic frame with the skull oriented according to the König and Klippel atlas (16). They were given lesions in the left nigrostriatal pathway (coordinates relative to bregma: A −4.4, L −1.2, V −7.8) by means of stereotaxic injection of 8 μg 6-OHDA-HCl (calculated as free base) dissolved in 4 μl of saline (with 0.2% of ascorbic acid) into the area ventralis tegmenti, which contains the bundle of axons leaving the mesencephalic dopamine cell bodies. The lesion extensively denervates the forebrain dopamine-innervated areas unilaterally (40).

Rotational Behavior

Rotational behavior for the selection of animals was measured in a smooth-semispheric rotometer and a rough-flat rotometer, both of which are modified versions (described below) of the original rotometer of Ungerstedt and Arbuthnott (37). Rotational behavior was measured by observation. Results are expressed as mean number of completed 360 degree turns.

Animal Selection

Our aim was to select from an original group of approximately 300 animals those with a high degree of both denervation of the dopaminergic nigrostriatal pathway and supersensitivity of striatal dopamine receptors. We therefore included those animals which, 4 weeks after surgery, exhibited rotational behavior of more than 200 complete turns (360 degree continuous rotation) following administration of a single dose of apomorphine (0.05 mg/kg SC) either while in a smooth-semispheric rotometer or a rough-flat rotometer, and which 7 months after surgery exhibited more than 400 complete turns in a "two-peak" pattern of rotational behavior following apomorphine (0.05 mg/kg SC) administration. [Criteria for the denervation of greater than 95% of the nigrostriatal dopaminergic axons is elaborated by Ungerstedt and Herrera-Marschitz (40).] Sixty-seven animals were selected in this manner.

TABLE 1

Days	7	14	28	90	180
No. animals Group A	8	5	8	9	6
No. animals Group B	7	9	6	7	2

General Procedure

Four weeks after receiving a lesion, animals were injected between 9:00 and 10:00 a.m. (as with all other reexposures to a rotometer) with a single dose of apomorphine (0.05 mg/kg SC), either while in the smooth-semispheric rotometer (Group A) or the rough-flat rotometer (Group B). Rotation was measured for 90 min. Each 180 degree right or left turn was recorded with a detector using infrared photocell barriers. The pulses from the detector electronics were fed into a microcomputer for immediate storage and display. After completion of the experiments, the data were transferred to the main computer for permanent storage on diskettes. The rotational behavior of each individual animal was plotted as the number of 360 degree turns/min for the entire period of observation. Examination of the rotational behavior formed the basis for selecting successfully denervated rats.

Rats exhibiting more than 200 complete turns were divided at random into 10 subgroups of 14 animals each. Five subgroups received apomorphine in the smooth-semispheric rotometer and five subgroups were injected in the rough-flat rotometer. Either 7, 14, 28, 90 or 180 days later, each subgroup from Group A was transferred to rough-flat rotometers while each subgroup from Group B was transferred to smooth-semispheric rotometers for a period of 30 minutes. After this, all rats were placed for another 30 minute period in the rotometers where apomorphine was administered. No drugs were administered for these rats. Turning behavior during both periods was constantly observed and recorded. After both rotometer exposures, animals were housed until 7 months after 6-OHDA administration. Then they were administered apomorphine (0.05 mg/kg SC) once a week for 4 weeks.

Data from animals that exhibited more than 400 rotations in a "two-peak" pattern following the last apomorphine administration were included in the study. The presence of a "two-peak" contralateral rotational pattern induced by apomorphine indicates that there is at least 95% depletion of nigrostriatal dopamine (40). Consequently, we have chosen this criterion to demonstrate good nigrostriatal unilateral denervation.

For statistical analysis we used the software MICROSTAT and results were calculated with a two-way ANOVA procedure.

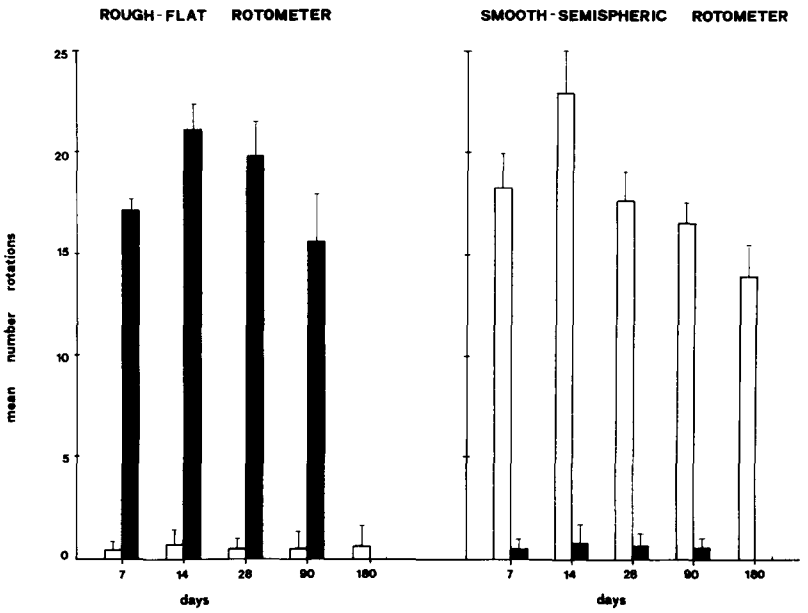

FIG. 1. The mean (±SEM) number of 360 degree spontaneous rotations made by each group in two different test environments. Group A: open columns; Group B: shaded columns.

RESULTS

The number of rats which fulfilled the animal selection requirements are shown in Table 1.

For statistical analysis, five rats from each group were selected at random (Fig. 1). The 180-day subgroup of Group B was excluded because it only consisted of two animals. In Group A we found greater rotational behavior in the smooth-semispheric rotometer than in the rough-flat rotometer ($p<0.00001$). In Group B we found a greater number of contralateral turns in the rough-flat rotometer than in the smooth-semispheric rotometer ($p<0.00001$). Moreover, the rotational response in the smooth-semispheric rotometer by Group A and also that in the rough-flat rotometer by Group B was greatest on the 14th day following apomorphine administration ($p<0.05$, in both cases).

DISCUSSION

Our results suggest that the rotational behavior, induced by a single dose of apomorphine in unilaterally 6-OHDA denervated rats, can be conditioned to an environmental situation through a classical conditioning paradigm. We observed rotational behavior without drug administration in animals reexposed at different time intervals to the same environment where they received apomorphine. This long-lasting conditioning which reaches its maximum effect 14 days after drug administration, is in agreement with the results of Silverman and Ho (32).

The finding that a unique pairing between the environmental situation and the effect of the administration of a single dose of apomorphine is enough to generate a strong, long-lasting conditioned rotational behavior emphasizes the possibility that the behavioral effects induced by pharmacological activation of the dopaminergic CNS can be conditioned to environmental stimuli present at the time of drug administration. Our results also show that this learned process is long-lasting. Furthermore, as some authors have reported that any conditioned stimuli (3), including those associated with the ingestion of drugs, may affect the activity of the dopamine neurons (29), our study suggests that environmental stimuli, conditioned to a behavioral response elicited by an increased dopamine function, could likewise reproduce this response when presented in isolation. In this regard, it has already been reported that the study of conditioned pharmacological responses using the "turning behavior" animal model could have therapeutical applications in Parkinson's disease (7,8).

Available data suggest that administration of opiates causes an enhancement of dopamine cerebral function mainly through two mechanisms: an increase of the dopaminergic neurotransmission and a development of dopamine receptor supersensitivity. Microinjections of morphine into the ventral tegmental area produce a contralateral rotation (15,36), indicating increased dopaminergic transmission. Furthermore, the cell firing rates of dopamine-containing cells of the ventral tegmental area increase either with systemic or intracranial administration of morphine (14,21) and systemic administration of opiates increases striatal and limbic dopaminergic neurotransmission (2,22).

At present, the exact mechanisms producing opiate-mediated supersensitivity of the dopamine receptors are unknown. Some authors have reported an increased striatal binding of labelled neuroleptics (11) while others have found no changes in rats chronically treated with opiates (10). It has also been reported that opiates produce an enhancement of striatal dopamine-sensitive adenylcyclase responsiveness to dopamine agonists (25), probably due to a prolonged inhibition of the adenylate cyclase activity (42), and apparently involving the nucleotide regulatory protein (25). Chronic treatment with opiates can induce supersensitivity of the striatal dopamine receptors that can be measured through the enhancement of stereotypes produced by the adminis-

tration of dopamine agonists (10,12), and rats withdrawn from chronic treatment of morphine develop supersensitivity of dopamine receptors, with the degree of the supersensitivity depending on the length of the abstinence period (2). Recently a behavioral supersensitivity to a systemic injection of morphine after chronic treatment with different neuroleptics has been described (35), suggesting that the reinforcing effect of opiates may actually be enhanced by chronic dopamine blockade that usually implies the development of dopamine receptor supersensitivity.

From the present experiment, one can infer that the effects of the activation of supersensitized dopaminergic receptors are easily conditionable to environmental stimuli. As opiates produce a supersensitivity of dopaminergic receptors, those processes associated to the activation of these opiate-induced supersensitized dopaminergic receptors are probably more easily conditioned to environmental stimuli. This fact can be implicated in the learning processes underlying relapse in toxic habits of addicts.

We suggest the use of the "turning behavior" as an animal model for the study of the learning processes in the conditioning of behavioral responses to environmental events. This animal model could later be useful in the study of the factors involved in the relapse by abstinent addicts when reexposed to the setting where addiction developed.

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