

Effects of Dopaminergic Nucleus Accumbens Lesions on the Acquisition of Schedule Induced Self Injection of Nicotine in the Rat

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SINGER, G., M. WALLACE AND R. HALL. *Effects of dopaminergic nucleus accumbens lesions on the acquisition of schedule induced self injection of nicotine in the rat.* PHARMAC. BIOCHEM. BEHAV. 17(3) 579-581, 1982.—Data from recent experiments show that dopaminergic depletion of the nucleus accumbens septum (NAS) decreases the frequency of schedule induced drinking and wheel running. In the present experiment a group of 8 rats with 6-OHDA lesions in the NAS showed a significantly lower rate of schedule induced nicotine self injection than sham operated controls. These findings extend the number of schedule induced behaviors which appear to depend for their occurrence on an intact dopamine system in the NAS.

Schedule induced Nicotine self injection Nucleus accumbens septum Dopamine 6-OHDA

IN a recent paper [6] drug intake patterns for a number of psychoactive drugs were described as forming four distinct groups. The group classification was based on research into self injection in the presence and absence of intermittent food delivery. For drugs in the first group, which included opiates and alcohol, acquisition of self injection behavior was accelerated by the presence of a schedule. In the second group, which included nicotine and Δ^9 -THC, the presence of a schedule was a necessary condition for the acquisition of self injection behavior. For the remaining two groups, one of which included stimulants, the presence of the food delivery schedule did not affect the acquisition of drug self injections.

Data from a series of recent experiments show the involvement of a dopamine system in the nucleus accumbens septum (NAS) in schedule induced drinking and schedule induced wheel running ([4], also Wallace *et al.*, unpublished data). NAS lesions reduced schedule induced drinking and schedule induced wheel running but did not change deprivation induced or normal home cage water intake and wheel running frequencies. NAS lesions also led to a reduction in plasma corticosterone levels which are usually elevated during schedule induced drinking.

If the schedule is a necessary condition for the acquisition of self injection behavior for drugs in group two and if an intact dopaminergic NAS system is necessary for schedule induced behaviors to occur, schedule induced self injection (SIS) of drugs in this group should be reduced by NAS lesions.

In this paper we report an experiment showing the effects of 6-OHDA lesions of the NAS on the acquisition of nicotine self injections.

METHOD

Animals

Two groups of 8 naive male Long-Evans rats were reduced to 80% of free feeding body weight 360-400 g. Rats were housed individually in temperature controlled conditions ($22^{\circ}\text{C} \pm 1$) with a 12 hour light/12 hour dark cycle. Water was freely available at all times.

Apparatus

The test chamber was made of clear perspex with a stainless steel barred floor, and measured $35 \times 32 \times 32$ cm. A food pellet dispensing unit and a bar were attached to one side wall. The bar operated a syringe infusion pump (Sage Instruments Model 341) which delivered 0.07 ml of drug solution IV when triggered. A timing device was incorporated into the system so that any further bar presses during the five second infusions could not initiate another infusion. Bar presses and infusions were recorded on a chart recorder during the test sessions. Noyes food pellets (45 mg) were delivered to the animal on a FT-60 non-contingent schedule.

Surgery

All animals were anaesthetized with an IP injection of Sagatal (60 mg/kg) (May & Baker).

All animals were catheterised by implantation of a polyethylene (SP28) catheter in the right jugular vein. Catheters were held in place by leather jackets.

Prior to catheterisation 8 animals were lesioned by the bilateral stereotactic injection of 6-OHDA (2,4,5 trihydrox-

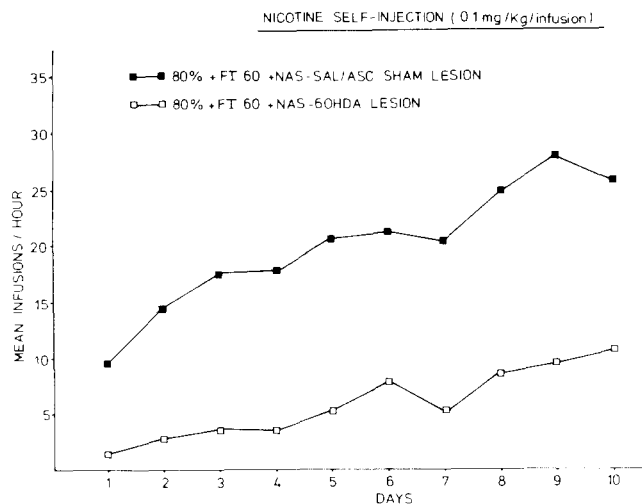


FIG. 1. Mean number of nicotine self infusions for 6-OHDA lesioned and sham lesioned groups over 10 days.

iphenalamine hydrochloride, Sigma) into the NAS. The co-ordinates of the NAS lesion site were 3.4 mm anterior to the bregma, 1.7 mm lateral to the mid line and 7.2 mm below the dura at the point of penetration. Pellegrino and Cushman [3] stereotaxic injections were made from a 10 μ l Hamilton syringe through a 30 gauge stainless steel cannula. Each injection of 6-OHDA consisted of 2 μ l of an 8 μ g/ μ l solution, thus a total dosage of 16 μ g was delivered into each site. The rate of injection was 1 μ l/min. The 6-OHDA was dissolved in a 2 μ g/ μ l solution of ascorbic acid and brought to isotonicity with sodium chloride. All solutions were prepared on the morning of surgery. Eight animals were sham lesioned using an ascorbate/NaCl mixture.

Fluorescent Histochemistry

An aqueous aldehyde method was used for the fluorescence histochemical localization of catecholamines. The method used is a modification of the method described in Furness, Heath and Costa [1].

Perfusion

The animals were intraperitoneally anaesthetized with Nembutal (200 mg/kg), the chest opened and the heart exposed. A 16 gauge stainless steel cannula was inserted into the aorta from the left ventricle and held in place by a clamp across the heart. An incision was made at the right auricle to provide an exit point for blood and perfusate. The blood was flushed out with 50–100 ml of flushing solution (1% NaNO₂ in 0.01 M phosphate buffer, pH 7.0), and then perfused with 200 ml of Faglu (4% formaldehyde, 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0). The perfusion pressure was maintained at 100–120 mmHg. After complete perfusion the brains were removed, placed in Faglu, and stored in a refrigerated compartment.

Cutting and Examination of Fluorescence

Frozen sections (50 μ m) were cut on a sledge microtome at the plane of Pellegrino and Cushman [3]. The sections

TABLE 1
HISTOCHEMICAL VERIFICATION OF NAS LESIONS

Group	CPU		NAS		OT	
	Right	Left	Right	Left	Right	Left
6-OHDA lesions*	5.86	5.3	2.57	2.86	7.0	8.0
Sham lesions	10	10	9.75	9.88	10	10

*Histochemical analysis showed that the dopaminergic depletion of the NAS was not successful in one rat (C-1, data shown below).

Rat C-1 10 10 10 8 10 10
Data from this rat was excluded.

were placed in Faglu, mounted on slides, and allowed to dry (1 hour in air, then 2 hours in a desiccator), before being examined for fluorescence. Fluorescence was examined under a Zeiss microscope (Zeiss Instruments Germany) using mercury lamp as a light source.

Fluorescence was examined in sections at 3.4 mm anterior to bregma (Pellegrino and Cushman [3]), in the nucleus accumbens septum (NAS), caudate putamen (CPU) and olfactory tubercle (OT). Fluorescence was semiquantified by assigning fluorescence values of "10" to the NAS, CPU, and OT in unlesioned brains of control animals, and a fluorescence value of "0" to the corpus callosum. A single blind experimental comparison was used to estimate the fluorescence value (relative to controls) of lesioned animals, taking both fluorescence brightness and volume into account.

Procedure

After 2 days recovery from surgery the eight 6-OHDA lesioned and eight sham lesioned rats were given a daily 1 hour session in the test chamber on a FT-60 food delivery schedule.

Nicotine hydrogen (+) tartrate (0.1 mg/kg/infusion, 0.889 mmol/L) (BHD), was available to all animals. Each animal was primed with an initial dose prior to each experimental session. After 10 days the animals were perfused for fluorescence histochemistry.

RESULTS AND DISCUSSION

The nicotine self injection rates for the 6-OHDA lesioned and sham lesioned groups over a 10 day acquisition period are shown in Fig. 1. ANOVA shows a significant reduction in nicotine self injection resulting from the lesions, $F(1,13)=88.0$, $p<0.001$, significant increase in self injection for both groups over the 10 day period, $F(9,117)=10.7$, $p<0.001$, and no significant interaction. Table 1 shows mean fluorescence ratings for the NAS, CPU and OT for 6-OHDA and sham lesioned rats. The 6-OHDA lesioned rats showed massive loss of fluorescence, whereas the sham operated ones were not affected by the treatment.

The data show a significant reduction in acquisition of nicotine self-injection behavior which is maintained over a 10 day period. Comparison of data from the 6-OHDA lesioned group with saline self injection levels obtained in other experiments in this laboratory [2,5] suggests that lesions of the NAS reduce intake to placebo levels.

In an earlier experiment [5] it was shown that there are three necessary conditions for the acquisition of nicotine self injection. These are: the availability of nicotine in the injection solution, reduced body weight and the presence of a food delivery schedule. (None of these is a sufficient condition.) Other data show that an intact dopamine system in the NAS is necessary for schedule induced drinking [4] and schedule induced wheel running (Wallace, *et al.*, unpublished) to occur. In this experiment we have shown that the acquisition of nicotine self injection which is dependent on the presence of a schedule, is prevented when a neuronal pathway in the NAS is dopamine depleted. The data also

confirm that the presence of a schedule is a necessary condition for nicotine self injection, which underlies the importance of the interaction of drug and environmental factors in acquisition of self-injection behavior. The present findings extend the range of schedule induced behaviors which appear to depend for their occurrence on intact dopaminergic neurons in the NAS. We are currently investigating the effect of NAS lesions on the schedule induced self injection of other drugs.

ACKNOWLEDGEMENTS

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