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The Effect of 6-OHDA Lesions of the Nucleus Accumbens Septum on Schedule-Induced Drinking, Wheelrunning and Corticosterone Levels in the Rat

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WALLACE, M., G. SINGER, J. FINLAY AND S. GIBSON. *The effect of 6-OHDA lesions of the nucleus accumbens septum on schedule-induced drinking, wheelrunning and corticosterone levels in the rat.* PHARMACOL BIOCHEM BEHAV **18**(1) 129–136, 1983.—In a series of four experiments the relationship between 6-OHDA lesions of the nucleus accumbens septum (NAS), schedule-induced behaviors and plasma corticosterone levels was explored. Data from the first experiment show a significant decrease in water intake during a scheduled food delivery test hour for 6-OHDA lesioned groups of rats compared with sham or non-lesioned groups of rats, while during the remaining 23 hours of the day water intake was the same for 6-OHDA lesioned and sham lesioned groups. In a second experiment similar decreases in schedule-induced wheelrunning were observed for 6-OHDA lesioned rats when compared with sham lesioned rats. Data from a third experiment showed significant increases in plasma corticosterone levels of rats in the presence of a scheduled food delivery compared with rats given non-scheduled food. In a fourth experiment it was shown that 6-OHDA lesions of the NAS abolish this increase of corticosterone levels in rats on a food delivery schedule. These data extend the findings of Robbins and Koob [19] and show a more general involvement of the dopaminergic pathways of the NAS in schedule-induced behaviors and in concomitant plasma corticosterone changes.

Schedule-induced drinking Schedule-induced wheelrunning 6-OHDA Nucleus accumbens septum Plasma corticosterone

THE generality of schedule-induced behaviors has been discussed in a number of reviews [9, 24, 25, 26, 27]. In addition to excessive drinking, behaviors such as schedule-induced wheelrunning, air licking, eating, movement increases, drug self injections and many others have been reported. Schedule-induced behaviors have been observed in a number of species including monkey and man. The difficulty of explaining this behavior either in learning terms or in terms of physiological homeostasis has been discussed elsewhere [9, 24, 26, 27]. The question of why schedules are such powerful determinants of non-reinforcement contingent behavior remains unanswered.

To elucidate these questions further a number of studies have been directed towards finding anatomical and biochemical substrates involved in adjunctive behavior. It has been shown that electrolytic lesions of the hypothalamus lead to the abolition of all drinking including schedule-induced drinking [28,29]. Noradrenergic stimulation of hypothalamic structures leads to a reduction in deprivation-induced drinking but not in schedule-induced drinking [22]. Thus it is possible that the lateral hypothalamus is part of a common final pathway for drinking which is biochemically differentiated for schedule-induced and deprivation-induced drinking. Robbins and Koob [19] have reported that catecholaminergic depletion of the nucleus accumbens septum (NAS) before the rats are exposed to a schedule also leads to a dissociation between schedule-induced and deprivation-induced drinking. Destruction of dopamine neurones in the NAS after schedule-induced drinking has been established caused a reduction, compared with sham lesioned rats, in licking rates early in the between pellet interval followed by a perseveration in licking in the middle portion of the interval [20].

The first experiment reported here is an extension of the study reported by Robbins and Koob [19]. These experimenters pretreated their rats with pargyline, which increases the effectiveness of the neurotoxin, 6-hydroxydopamine (6-OHDA), to produce maximal depletion of both noradrenaline and dopamine [3]. In the present study the effects of 6-OHDA lesions of the NAS (with and without pargyline pretreatment) on schedule-induced drinking were assessed. The vehicle commonly used with 6-OHDA to reduce oxidation is ascorbic acid. Recently it has been shown [10] that ascorbic acid can have an independent physiological and behavioral effect when centrally administered. For this reason the effect on schedule-induced drinking of ascorbic acid lesions was also tested.

The second experiment was designed to test the specific-

ity of the involvement of the NAS by using the schedule to induce another behavior. In this experiment, schedule-induced and spontaneous wheelrunning were compared.

Previous work [11] suggests that corticosterone increases may be a concomitant of schedule-induced drinking and this may indicate that schedules are stressful. A third experiment was designed to investigate changes in levels of plasma corticosterone following development of schedule-induced drinking.

The fourth experiment was designed to test the effect of catecholamine depletion of the NAS on the corticosterone response.

GENERAL METHOD

Subjects

Experimentally naive, male hooded Long-Evans rats with initial ages between 90 and 120 days and body weights between 370 and 400 g were used throughout. Rats were housed individually with water available at all times. A 12 hr light/12 hr dark cycle operated (lights on at 0600 hours) and the laboratories were maintained at 22°C ±1. After four days acclimatisation to the laboratory, rats were reduced over a fourteen day period of restricted food intake to 80 percent of their initial body weights. This reduced body weight was maintained throughout the experiments.

Apparatus

The test chambers were made of clear perspex with a stainless steel barred floor. They were located separately inside larger, well ventilated sound attenuating boxes. The standard relay circuitry which automatically controlled the delivery of food pellets was located on the top of these enclosures. The chambers were lit by 40 W globes and ventilation fans within each enclosure provided masking noise against external sounds. "Noyes Precision" 45 mg food pellets were used.

The test chambers used in Experiments 1, 3 and 4 measured 35×23×34 cm. A food cup connected to the pellet dispenser was located on one end wall and a 2.0 cm diameter hole (located 6 cm to the left of the food cup) permitted access to a drinking spout attached to a calibrated 100 ml drinking tube positioned outside the chamber. The stainless steel spout protruded 1 cm into the chamber; both the food bowl and water spout were approximately 3.5 cm above the floor bars. Pellets could be delivered to the food bowl by a dispenser mounted on the same end wall.

The experimental chambers used in Experiment 2 measured 33.5×28×42 cm. A partitioned section at the rear of the chamber contained a 26 cm diameter running wheel. This partitioned section could be left open or closed via a sliding Plexiglas wall. On one side wall a graduated drinking tube was situated 2 cm from the front corner of the chamber; the drinking nipple entered the chamber 4 cm above the floor. A feeder tray was located adjacent to the drinking tube, connected by a plastic tube to a pellet dispenser located near the top of the chamber. The number of wheel revolutions was recorded on a five digit electromechanical counter.

Surgery

In Experiments 1, 2 and 4, 80 percent body weight reduced animals were subjected to injections into the NAS of either 6-OHDA or a control solution. Rats were anaesthetised with 60 mg/kg of Nembutal (Abbott Laboratories) and secured in the stereotaxic instrument at the angle of Pellegrino and Cushman [18]. The coordinates of the NAS lesion site were 3.4 mm anterior to bregma, 1.7 mm lateral to the mid line and 7.2 mm below the dura at the point of penetration. Stereotaxic injections were made from a 10 μl Hamilton syringe through a 30 gauge stainless steel cannula. Each injection of 6-OHDA (2, 4, 5-Trihydroxyphenamine hydrochloride, Sigma) consisted of 2 μl of an 8 μg/μl solution, thus a total dose of 16 μg was delivered into each site. The 6-OHDA was dissolved in a 2 μg/μl solution of ascorbic acid and brought to isotonicity with sodium chloride. Control injections were of either an ascorbate/NaCl mixture or 0.9% NaCl. The rate of injection was 1 μg/minute. All solutions were prepared freshly on the morning of surgery.

In Experiments 1 and 4 some rats were pretreated with 50 mg/kg of pargyline (a monoamine oxidase inhibitor) 30 minutes prior to surgery. Pargyline was dissolved in distilled water and administered intraperitoneally.

Following surgery the rats were returned to their home cages and allowed a post-operative recovery period of 5 to 7 days. During this period 24 hour water consumption levels were recorded and 80% body weights were restabilised.

Fluorescent Histochemistry and Histology

An aqueous aldehyde method was used for the fluorescence histochemical localisation of catecholamines. The method used is a modification of that described by Furness, Heath and Costa [12].

Perfusion

Each rat was anaesthetized with Nembutal (200 mg/kg IP), the chest opened and heart exposed. An 18 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% $NaNO_2$ in 0.01 M phosphate buffer, pH 7.0), and the rats were 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 μm) were cut on a sledge microtome at the plane of Pellegrino and Cushman [18]. The sections were placed in Faglu, mounted on slides, and allowed to dry (1 hour in air, then 2 hours in a desiccator), before being inspected for fluorescence. Fluorescence was examined under a Zeiss microscope (Zeiss Instruments, Germany) using a mercury lamp as a light source.

Fluorescence was examined in sections at 3.4 mm anterior to bregma [18] in the 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 area into account.

In Experiment 4 the collection of blood for corticosterone analysis prohibited fluorescent histochemical analysis and an

anatomical evaluation of the lesion site was carried out instead. At the conclusion of testing, rats were killed as described below in the biochemical assay section, and the whole brain removed and placed in 10 percent Formalin and saline.

Histology

An anatomical evaluation of the lesion site was undertaken for each animal in Experiment 4. Serial frozen sections of 50 microns were cut horizontally through the nucleus accumbens septi and alternate sections were mounted on glass slides using gelatine as the adhesive solution. The sections were stained using a standard cresyl violet sequence. The extent of the lesion was then mapped, by an independent observer, onto photocopied sections of the Pellegrino and Cushman [18] atlas of the rat brain. To achieve correspondence between the slides and the atlas, the block was mounted so that the cerebral hemispheres were inclined 19 degrees forward of vertical.

Biochemical Assay

The method used to determine plasma corticosterone levels was based on a competitive protein binding technique [17]. Male dog plasma provided the source of binding protein, with [^{3}H] corticosterone as the competitively bound steroid and florisil as the adsorbent for unbound steroid.

Rats were consecutively killed in a room adjoining the test laboratory by decapitation in randomly selected pairs consisting of a schedule and a non-scheduled rat. The time elapsing from entry into the laboratory to decapitation was not more than 60 seconds. The collection was sufficiently rapid to prevent a rise in plasma corticosterone as a result of the sampling procedure [7]. Trunk blood was collected in heparinized tubes and centrifuged immediately at 3200 rpm for 20 minutes. The plasma was stored at −80°C until assayed.

EXPERIMENT 1

In this experiment the effects of 6-OHDA lesions of the NAS on schedule-induced polydipsia were investigated. Robbins and Koob [19] have demonstrated that catecholamine depletion of the NAS attenuates the typical development of schedule-induced polydipsia. Depletion is produced by bilateral infusions of 6-OHDA solution in a vehicle of saline-ascorbic acid solution. However, it has been shown [10] that ascorbic acid is not necessarily a neutral vehicle. Accordingly, an ascorbic sham lesion group and a saline sham lesion group were both included in this study. Robbins and Koob [19] also used a pargyline pretreatment to potentiate the effect of the 6-OHDA. The necessity for this pretreatment was checked by the inclusion of two lesioned groups, one with pargyline pretreatment. Two unlesioned groups provided baseline data for schedule and non-schedule conditions.

Procedure

Forty-eight rats were assigned to one of 6 experimental groups (n=8). Rats in five of the groups were exposed for 1 hour per day for 13 days to a FT 60 sec non-reinforcement contingent schedule under which they received one 45 mg pellet each min. With the exception of an untreated control group (NL-Sch) each group received in addition one of the following treatments: 6-OHDA lesions (6-OHDA-Sch), saline sham lesions (SAL-Sch), 6-OHDA lesions plus pretreatment with pargyline (6-OHDA/PAR-Sch), or saline-ascorbic acid sham lesions (SAL/ASC-Sch). The remaining group was not exposed to the reinforcement schedule and received no other treatment (NL-NSch); this group was given 60 pellets in a single food presentation at the start of each one hour session.

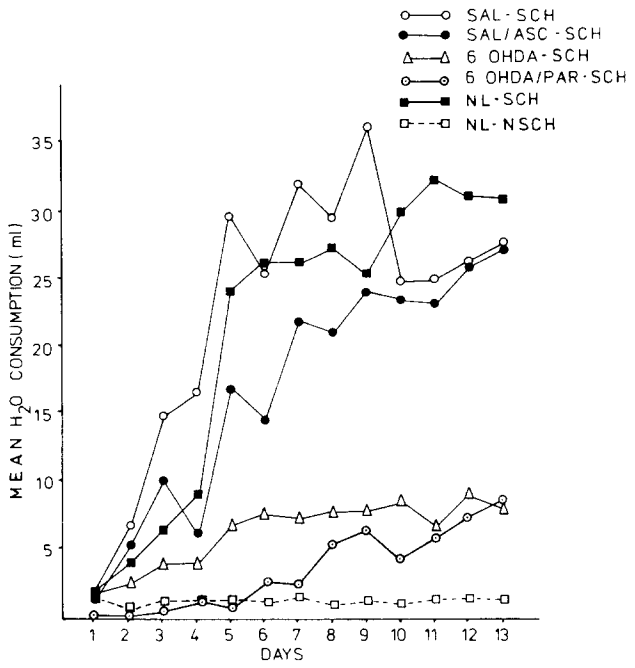

FIG. 1. Mean water consumption for the five scheduled and one non-scheduled groups during each 1 hour test period.

The handling, surgical procedures, apparatus, and drug dose levels are described in the General Method section. Water was freely available in the home cage. Both test session and 23 hour home cage water consumption levels were recorded.

RESULTS AND DISCUSSION

The mean water intake during the test hour over the 13 day period for each of the 6 conditions is presented in Fig. 1. A two way ANOVA with one repeated measure (days) indicated that there was a significant main effect, $F(5,41)=31.96$, $p<0.001$, on water intake during the test hour, and across the 13 tests sessions, $F(12,492)=39.27$, $p<0.001$. A significant interaction effect, $F(60,492)=5.21$, $p<0.001$, suggested that water consumption for some groups increased over the 13 days (Fig. 1). A simple main effects test [14] indicated that the NL-Sch, SAL/ASC-Sch and SAL-Sch groups significantly increased water intake over the 13 day period (see Fig. 1).

Although home cage 23 hour water consumption was recorded for all groups Fig. 2 shows data for 6-OHDA-Sch, 6-OHDA/PAR-Sch and NL-Sch groups only, in order to illustrate that reduced water intake by NAS lesioned rats during schedule food delivery was not due to a general drinking disability. There were no significant differences between the three groups shown in this figure.

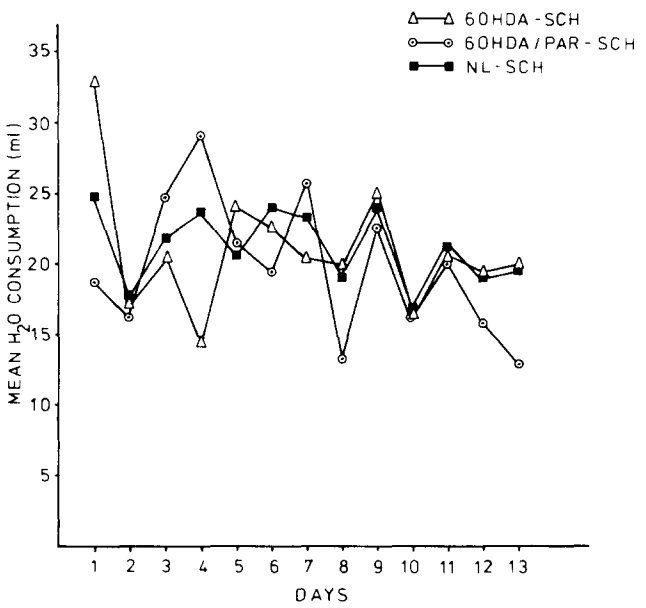

FIG. 2. Mean 23 hour home cage water consumption for three groups over 13 days.

TABLE 1
HISTOCHEMICAL VERIFICATION OF NAS LESIONS

	CPU		NAS		OT	
Group	Right	Left	Right	Left	Right	Left
NL-Sch	10	10	10	10	10	10*
SAL-Sch	10	10	9.7	9.9	10	10
SAL/ASC-Sch	10	10	9.8	9.9	10	10
6-OHDA-Sch	4.75	4.75	2	2.1	9.25	3
6-OHDA/PAR-Sch	6.1	5.9	2.7	1.5	6.4	6.8†
NL-NSch	10	10	10	10	10	10*

*NL-Sch and NL-NSch groups assigned "10" fluorescence value by definition.

†In one animal (A72) the histochemical verification revealed no depletion. Data from this animal have been omitted.

There was no difference in test hour water consumption between the two groups with 6-OHDA lesions, which indicates that the pargyline pretreatment is not a significant factor. The addition of ascorbic acid to one of the saline injected groups did not affect water intake. Therefore in the remaining experiments sham lesioned controls were given the customary ascorbic acid treatment.

The data from this experiment show a significant decrease in water intake during the test hour for the 6-OHDA lesioned groups compared with sham lesioned and non-lesioned groups in the presence of a scheduled food delivery. The intake levels for the 6-OHDA lesioned groups did not increase above the levels of non-lesioned, non-scheduled rats.

Histochemical verification of the NAS lesions revealed no depletion in one of the 6-OHDA/PAR-Sch rats. Fluorescence values, excluding this animal, are shown in Table 1. The data from this rat have not been included in the above statistical analyses.

EXPERIMENT 2

Results obtained in Experiment 1 indicate that lesions of the NAS dopaminergic projection attenuate the development of SID. It is quite possible that the NAS is involved in the mediation not only of schedule-induced drinking, but also of other schedule-induced behaviors. In this experiment, the effects of NAS lesions on a commonly studied schedule-induced behavior, schedule-induced wheelrunning, were determined. Considering that schedule-induced drinking and schedule-induced wheelrunning are quite different types of responses it is suggested that if the NAS lesion affects schedule-induced wheelrunning, then this lesion may also affect other schedule-induced behaviors. A 6-OHDA lesioned, non-schedule group of rats (6-OHDA-NSch) was included to control for the possible effects of 6-OHDA on normal locomotor activity, and an unlesioned non-schedule control group (NL-NSch) was included to obtain a baseline level of spontaneous wheelrunning activity.

Procedure

Five groups of 8 rats were tested. There were three scheduled groups, one with 6-OHDA lesions (6-OHDA-Sch), one with sham lesions (SAL/ASC-Sch) and one with no surgery (NL-Sch) and two non-scheduled groups, one with 6-OHDA lesions (6-OHDA-NSch) and one with no surgery (NL-NSch).

The treatment of these rats, surgical procedures, apparatus and drug dose levels were described in the General Method section. They were tested for one hour at the same time of day for 14 consecutive days. In this experiment, the drinking tube was not present during testing but the running wheel was accessible to the rats.

Rats in the first three conditions were exposed to a FT-120 sec food reinforcement schedule; those in the two non-scheduled conditions received an equivalent number of pellets (thirty) as a single food presentation at the start of each session. The number of wheel revolutions was recorded at the completion of each session.

RESULTS AND DISCUSSION

The mean numbers of wheel revolutions over the 14 day period for each of the 5 experimental conditions are shown in Fig. 3. A two-way ANOVA with one repeated measure (days) applied to the wheelrunning data indicated significant main effects between groups, $F(4,35)=8.74$, $p<0.001$, across days, $F(13,455)=6.83$, $p<0.001$, and a significant interaction, $F(52,455)=2.16$, $p<0.001$.

A simple main effects test indicated a significant difference between the 5 groups, $F(4,490)=2.97$, $p<0.025$, but only the NL-Sch group, $F(13,455)=7.64$, $p<0.001$, and the SAL/ASC-Sch group, $F(13,455)=6.00$, $p<0.001$, significantly increased the mean number of wheel revolutions over the 14 day test period. The number of wheel revolutions made by these two groups became significantly different from the 6-OHDA-Sch and the two non-schedule conditions by day 7 (Fig. 3).

Histochemical verification of the NAS lesions in the 6-OHDA-Sch and 6-OHDA-NSch groups was considered satisfactory. The 6-OHDA-NSch groups showed more de-

TABLE 2
HISTOCHEMICAL VERIFICATION OF NAS LESIONS

	CPU		NAS		OT	
Group	Left	Right	Left	Right	Left	Right
NL-Sch	10	10	10	10	10	10*
SAL/ASC-Sch	10	10	9.9	10	10	10
6-OHDA-Sch	7.6	8.1	3.6	3.4	9	8.4
6-OHDA/No Schedule	6.3	7.3	2.1	2.3	5.6	4.0
NL-NSch	10	10	10	10	10	10*

*NL-Sch and NL-NSch groups assigned a fluorescence value of "10" by definition.

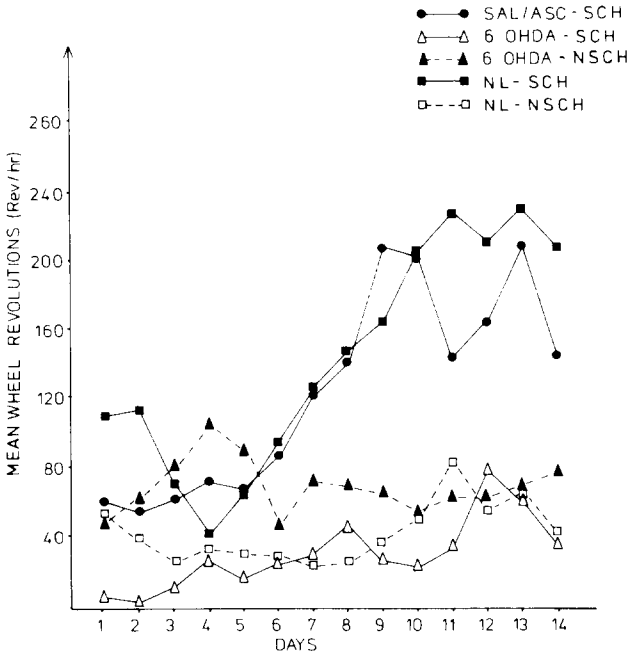

FIG. 3. Mean number of wheel revolutions for the three schedule and two non-scheduled groups during each 1 hour test period.

pletion than the 6-OHDA-Sch group in the olfactory tubercle (see Table 2).

In summary, the sham lesioned and non-lesioned groups showed a significant increase in wheelrunning in the presence of a food schedule, eventually performing in excess of 200 wheel revolutions per hour, whereas the lesioned group failed to develop a similar increase in wheelrunning activity. These results indicate that 6-OHDA lesions of the NAS attenuate the typical development of schedule-induced wheelrunning.

EXPERIMENT 3

Wayner [26,27] has suggested that schedule-induced behavior is the result of a general state of arousal which is associated with the lateral hypothalamus. One index of mild stress or arousal is an increase in plasma corticosterone levels [1]. In Experiment 3 the effects of exposure to a non-contingent food delivery schedule on corticosterone levels were investigated. The rats, apparatus, laboratory conditions and the procedure for biochemical assay are described in the General Method section.

Procedure

During the 14 day weight reduction period and for a further four days 16 naive male hooded rats were given a small quantity of food (10 Noyes 45 mg pellets) at 1100 hours. A further meal of normal laboratory rat pellets was given at 1600 hours to maintain 80% body weights.

The rats were randomly assigned to either a scheduled food delivery group or a control (single food presentation) group. Schedule and control procedures are described in Experiment 1. Water consumption was recorded at the conclusion of the session.

At the completion of the tenth test session rats were killed by decapitation as previously described and trunk blood was collected for corticosterone assay. Plasma samples for two rats were degraded and were omitted from the corticosterone analysis.

RESULTS AND DISCUSSION

Mean plasma corticosterone level in the schedule group (19.8 μg/100 ml $\pm$1.56 SEM) was significantly higher ($t=4.56$, $p<0.01$) than the mean for the control group (10.26 μg/100 ml, $\pm$1.41 SEM).

Mean water consumption during the hour of scheduled food delivery was recorded over days for both groups (Table 3). A two-way analysis of variance with one repeated measure showed a significant difference between scheduled and control animals, $F(1,14)=47.05$, $p<0.001$, and a significant difference between both groups over days, $F(9,126)=17.7$, $p<0.001$. A significant groups $\times$ days interaction, $F(9,126)=13.75$, $p<0.001$, indicates that over days the schedule group drank significantly more than the control groups.

In summary, the data show a significant increase in corticosterone levels after 10 days of 1 hr/day exposure to the schedule. This is in accordance with previous data published [4, 5, 6] and suggests that schedules are stressful. This increase is much smaller than that which follows presentation of an acute noxious stimulus, however the increase has consistently been found in this strain of rat, in our laboratories, under the same conditions [11]. The data on test hour water consumption confirm that schedule-induced drinking occurred in these rats.

TABLE 3

MEAN DAILY WATER CONSUMPTION (ml) (±SEM) DURING TEST HOUR FOR RATS IN SCHEDULED OR NON-SCHEDULED FOOD CONDITIONS

		Test Days									
		1	2	3	4	5	6	7	8	9	10
Schedule	Mean	1.25	3.25	5.13	7.13	13.25	14.75	12.88	18.63	17.38	23.88
	SEM	(0.45)	(1.02)	(1.81)	(1.69)	(3.52)	(2.27)	(2.49)	(1.89)	(2.76)	(2.15)
No Schedule	Mean	2.25	2.63	2.0	2.63	1.75	1.5	1.94	2.88	2.38	2.19
	SEM	(0.78)	(1.02)	(0.67)	(0.75)	(0.38)	(0.34)	(0.40)	(0.51)	(0.60)	(0.48)

TABLE 4

MEAN PLASMA CORTICOSTERONE LEVELS (±SEM) FOR 6-OHDA LESIONED AND SHAM LESIONED RATS AFTER 10 DAILY ONE HOUR SESSIONS OF SCHEDULED FOOD OR FOOD DELIVERED IN ONE PRESENTATION

	Schedule	No Schedule
6-OHDA	12.34	19.19
	(1.99)	(3.64)
SHAM	26.55	13.59
	(2.37)	(2.25)

TABLE 5

MEAN WATER CONSUMPTION (ml) (±SEM) DURING TEST HOUR AND DURING 23 HOURS IN HOME CAGE FOR RATS WITH 6-OHDA OR SHAM LESIONS, TESTED UNDER SCHEDULED OR NON-SCHEDULED FOOD CONDITIONS

	Schedule		Non-Schedule	
	6-OHDA	Sham	6-OHDA	Sham
Test hour	5.88	15.70	1.34	1.80
	(0.77)	(1.72)	(0.11)	(0.20)
23 hour	22.94	22.15	24.68	22.88
	(1.94)	(1.66)	(2.27)	(1.61)

EXPERIMENT 4

In Experiment 3 it was shown that corticosterone levels are increased following daily exposures to 1 hr of scheduled food delivery. This is in accordance with Wayner's general arousal explanation of schedule-induced behavior [26,27]. However, in Experiments 1 and 2 it was shown that dopaminergic pathways in the NAS are involved in schedule-induced behaviors. This finding implicates an area of the brain in addition to the lateral hypothalamus, and it is possible that the general arousal, as indexed by the corticosterone response, is dependent on an intact NAS. Accordingly an experiment was designed to assess the effects of NAS lesions on corticosterone levels in rats exposed to scheduled food delivery.

Procedure

Four group of 8 rats were assigned to the following conditions: 6-OHDA lesions and scheduled food (6-OHDA-Sch); sham (saline) lesions and scheduled food (SAL-Sch), and 2 groups given similar treatment except that they received food in a single presentation (6-OHDA-NSch) and SAL-NSch). Rats in the scheduled groups received food according to the FT-60 sec schedule previously described, whereas the non-scheduled rats were given 60 pellets in a single amount at the start of each test session.

Prior to surgery rats were reduced to 80% of the free-feeding body weight. The surgical procedure is described in the General Method section; all rats receiving 6-OHDA were pretreated with 50 mg/kg of pargyline 30 minutes prior to surgery. Isotonic saline was used for sham lesions.

Following recovery from surgery the rats were familiarised with the test apparatus for one hour daily for four days, and then exposed to a one hour test session for a further 10 days. Water consumption was recorded during testing and for the 23 hours of home cage occupation.

Upon completion of the final session, the rats were killed. Collection of plasma for corticosterone analysis did not permit perfusion and therefore histochemical analysis was precluded. Brains were removed, fixed with formol saline and sectioned. The procedures for collection of plasma for corticosterone analysis and histology are described in the General Method section.

RESULTS AND DISCUSSION

The mean plasma corticosterone levels for each of the four experimental conditions are shown in Table 4.

A two-way ANOVA showed no significant differences between the lesion and sham lesion groups, nor between the schedule and no schedule conditions. There was a significant interaction, $F(1,28)=14.03$, $p<0.001$, and data were further analysed using Newman Kuels post hoc tests. The only significant difference was that the sham lesion group in the schedule condition (SAL-Sch) was higher than both the lesioned, schedule groups, (6-OHDA-Sch), and the sham non-scheduled group (SAL-NSch).

The data show that the 6-OHDA lesions reduced corticosterone levels when compared with the sham lesioned group under conditions of scheduled food delivery, and as expected from Experiment 3, the sham lesioned, schedule group had higher corticosterone levels than the non schedule group. The 6-OHDA lesions reduced the corticosterone levels for the scheduled group to the same value as the corticosterone levels of the non schedule sham group. However it

is interesting that in the 6-OHDA lesioned group with no schedule, the plasma corticosterone level was higher, but not significantly higher, than the non-scheduled sham operated group. This is in accordance with findings reported in a recent review [30].

Mean water consumption for each group over 10 days, (1) during the daily test hour and (2) during the remaining 23 hours in the home cage, is shown in Table 5.

The data for test hour water consumption were subjected to a 2 way ANOVA with repeated measures (days). It was found that sham groups drank significantly more than lesioned groups, $F(1,28)=9.22$, $p<0.001$, and schedule groups drank more than non scheduled, $F(1,28)=31.90$, $p<0.001$. There was a significant days effect, $F(9,252)=12.77$, $p<0.001$. All the two way interactions were significant. Analysis of variance carried out on 23 hour home cage water consumption indicated no significant differences between groups on this measure, which again indicates that the lesions did not interfere with non schedule drinking behavior. This drinking pattern is consistent with Experiment 1 and with the report by Robbin and Koob [19] which showed a dissociation between schedule and deprivation induced drinking.

GENERAL DISCUSSION

The results of the first two experiments reported in this paper show that dopaminergic lesions of the nucleus accumbens reduce schedule-induced drinking and schedule-induced wheelrunning. The former finding is in accordance with the results reported by Robbins and Koob [19] and the latter implicates the NAS in a broader spectrum of schedule-induced behaviors.

Schedule-induced wheelrunning involves complex motor activities which appear to differ considerably from the oral, ingestive activities associated with scheduled-induced drinking. The observed attenuation of these behaviors supports the suggestion that the NAS dopaminergic projection is specifically involved in both. Although caution should be exercised when attributing physiological determinants to such a varied class of behaviors, it seems likely that the integrity of the NAS is necessary for their development.

One explanation for the reduction in wheelrunning is that 6-OHDA lesions of the NAS directly affect general motor activity [15]. Robbins and Koob [19] investigated the possibility that 6-OHDA affected schedule-induced drinking by way of reduced licking efficiency. They found some slight impairment of lick rates for lesioned groups when compared with control groups, but not to a degree sufficient to account for the decrease in schedule-induced drinking. In our experiment it was demonstrated that NAS lesions attenuate the development of schedule-induced drinking without affecting normal home cage water consumption. The explanation based on general motor deficit is further discredited by the present finding that the lesioned, non-scheduled group produced a greater number of wheel revolutions than the non-lesioned, non-schedule group for all but two of the 14 days (days 1 and 11). It therefore seems unlikely that the attenuation in the development of schedule-induced wheelrunning is due to 6-OHDA effects on general motor efficiency.

In contrast to findings from lesion studies of the hypothalamic nuclei [8, 28, 29] which in general fail to discriminate between deprivation and schedule-induced drinking, the NAS lesions specifically discriminate between these forms of drinking and in addition, they demonstrate a dissociation between schedule-induced and spontaneous wheelrunning. The dopaminergic specificity of our lesions is confirmed by the histochemical analyses.

It is possible that the attenuation of both forms of schedule-induced behavior might be due to a reduction in the motivating aspects of the scheduled food caused by the destruction of the NAS dopamine system. However, there was no indication in these experiments of a change in food motivation (as indexed by food intake) in the lesioned animals. Lesioned rats in scheduled drinking and wheelrunning experiments ate all the pellets in the test hour. The maintenance ration of food in home cages was always consumed and rats did not lose weight. Water intake over 23 hours and during the test hour for non-scheduled lesioned rats remained at the same level as for sham lesioned. We conclude that there was no observable effect on food intake, and that the effect is specific to schedule-induced behaviors.

The results from Experiments 3 and 4 show significantly elevated plasma corticosterone levels following sessions of schedule-induced drinking. This is in accordance with data previously published [5,31]. In one of these studies, [31], a decline in schedule-induced drinking followed bilateral adrenalectomy and adrenal demedulation, demonstrating the importance of the adrenal medulla in the maintenance of schedule-induced drinking.

Our findings are also consistent with the hypothesis proposed by Wayner [26,27] that a general increase of arousal accompanies schedule-induced behaviors. The fact that in the presence of a schedule there is a concomitant suppression of both drinking and plasma corticosterone levels in lesioned animals (Experiment 4) suggests that the dopaminergic pathways are involved in both the behavior and the hormonal response. Further experiments are necessary to establish whether there is a causal relationship or a more indirect link.

There is some evidence [7,30] of a feedback relationship between the release of corticosterone, catecholamine regulation of the pituitary adrenal axis and stress. It has been shown that anatomical lesions of hypothalamic medial forebrain bundle pathways reduce or delay the onset of corticosterone responses to acute auditory and thermal stress [21], also to leg break [13] and surgical trauma [16]. Since these areas of the hypothalamus are rich in catecholaminergic terminals, an involvement by these neurotransmitters in the regulation of corticosterone vis à vis the hypothalamo-hypophyseal axis is likely. Other data reviewed by Anisman [2] show increases of endogenous levels of noradrenaline and serotonin in mild as well as intense stress, but dopamine and acetylcholine are not affected. It has been suggested [2] that the neuronal response to stress is more complex and that it may depend on a balance of catecholamines and acetylcholine which has been shown to occur in the central nervous system [23]. At this stage there is insufficient evidence to formulate the precise relationship between central and circulating levels of catecholamines, the stress induced corticosterone response and schedule-induced drinking.

Robbins and Koob [19] suggest that the hypoactivity seen in NAS lesioned animals is an indication of reduced motivational excitement. They suggest that in this state, a behavior made dominant by deprivation will be less prone to interruptions. The lowered plasma corticosterone levels found in lesioned rats (Experiment 4) support this suggestion of reduced arousal. Reports of hypoactivity in NAS lesioned rats are based on measurements in photocell activity cages

[15,19] and in an open field [19]. These procedures do not allow detailed specification of the motor activities which are reduced. The absence of change in wheelrunning in lesioned, non-scheduled rats indicates that one specific motor activity at least is not affected. This raises problems for the generality of the Robbins and Koob [19] explanation based on hypoactivity, but may still support their "disrupted time sharing" interpretation. The present studies do not provide the type of data which would clarify this point.

In summary, the data show that acquisition of schedule-induced behaviors involves a dopaminergic pathway in the NAS. The motor component of these behaviors is not affected by dopamine depletion of the pathway; the lesions appear to affect only the portion of the behavior which is due to the presence of the schedule. It suggests that the NAS modulates that aspect of the behaviors which depends on intermittent stimulation from the environment.

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