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Resistance of Schedule-Induced Behaviours to Hippocampal Lesions

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TANG, C., M. WALLACE, G. SINGER AND L. MACKENZIE. *Resistance of schedule-induced behaviours to hippocampal lesions.* PHARMACOL BIOCHEM BEHAV **20**(4)537–541, 1984.—It has been reported that electrolytic lesions of the hippocampus accelerate the onset of schedule-induced drinking (SID) and also lead to significant increases in adrenal weights [2]. In the present experiment three groups of Long Evans rats received electrolytic or 6-Hydroxydopamine or sham lesions of the hippocampus and one group received electrolytic cortical lesions. Half of each group were tested 1 hr/day for 10 days under scheduled food delivery and the other half received food in a single presentation. It was found that both electrolytic hippocampal and cortical lesions reduced the level of SID compared with sham and 6-Hydroxydopamine lesions which did not differ from each other. However, there is support for the suggestion that hippocampal catecholamine neurones are involved in corticosterone regulation as shown by a significant increase in plasma corticosterone levels in non-scheduled, 6-Hydroxydopamine lesioned rats.

Schedule-induced drinking Hippocampus 6-OHDA lesions Plasma corticosterone Electrolytic lesions Catecholamine neurones

DATA from recent experiments [6, 7, 8] show that the acquisition of a range of schedule-induced behaviours is dependent on an intact catecholaminergic pathway in the nucleus accumbens septum (NAS). It has also been shown that following schedule-induced drinking (SID) sessions, plasma corticosterone levels are elevated and that this increase in corticosterone is abolished by 6-Hydroxydopamine (6-OHDA) lesions of the NAS [8]. Devenport [2] suggests that the hippocampus may be involved in the regulation of corticosterone since electrolytic lesions of the hippocampus which accelerate the onset of SID also lead to significant increases in adrenal weights.

We have shown a relationship between SID and plasma corticosterone levels which is dependent on an intact dopaminergic pathway in the NAS [8]. Devenport has investigated the same question with regard to hippocampal neurones, SID and corticosterone levels but his findings provide no direct and only ambiguous evidence on the relationship. He reported increases in adrenal weight following hippocampal lesions, but also reported that adrenalectomy and steroid replacement produced no clear cut effects on SID.

In the present experiment we have investigated the effects of electrolytic and catecholaminergic lesions of the hippocampus on SID and plasma corticosterone levels in rats under conditions of scheduled and non-scheduled (i.e., massed) food presentation.

METHOD

Subjects

Seventy-two male hooded Long-Evans rats with average body weight 355.5 g±33 were used. Twenty-four rats received bilateral 6-OHDA hippocampus lesions: eight of them were used as the non-scheduled food group (NS.6-OHDA), 16 of them served as the schedule group (S.6-OHDA). Of the 16 schedule group rats, 8 were randomly selected and perfused for the fluorescence histochemical localization of catecholamines; the other 8 rats were killed by decapitation, and their trunk blood collected for corticosterone assay. Sixteen rats received bilateral sham hippocampal lesions. Of these, 7 served as the schedule group (S.Sham), 9 were the non-schedule (NS. Sham). Fifteen rats underwent bilateral electrolytic lesions of the cortex overlying the hippocampus, 8 as schedule (S.Elec.C) and 7 as the non-schedule group (NS.Elec.C). Seventeen rats underwent electrolytic hippocampal lesions, 11 as schedule (S.Elec.HC) and 6 as non-schedule (NS. Elec. HC). Rats were housed individually with water available at all times. A 12-12 light-dark cycle was initiated at 0700 hours and the laboratories were maintained at 22°C±1. After at least three days acclimatisation to the laboratory, the rats were reduced over a fourteen-day period of restricted food intake to 80% of their initial body weight. This body weight was maintained throughout the experiment.

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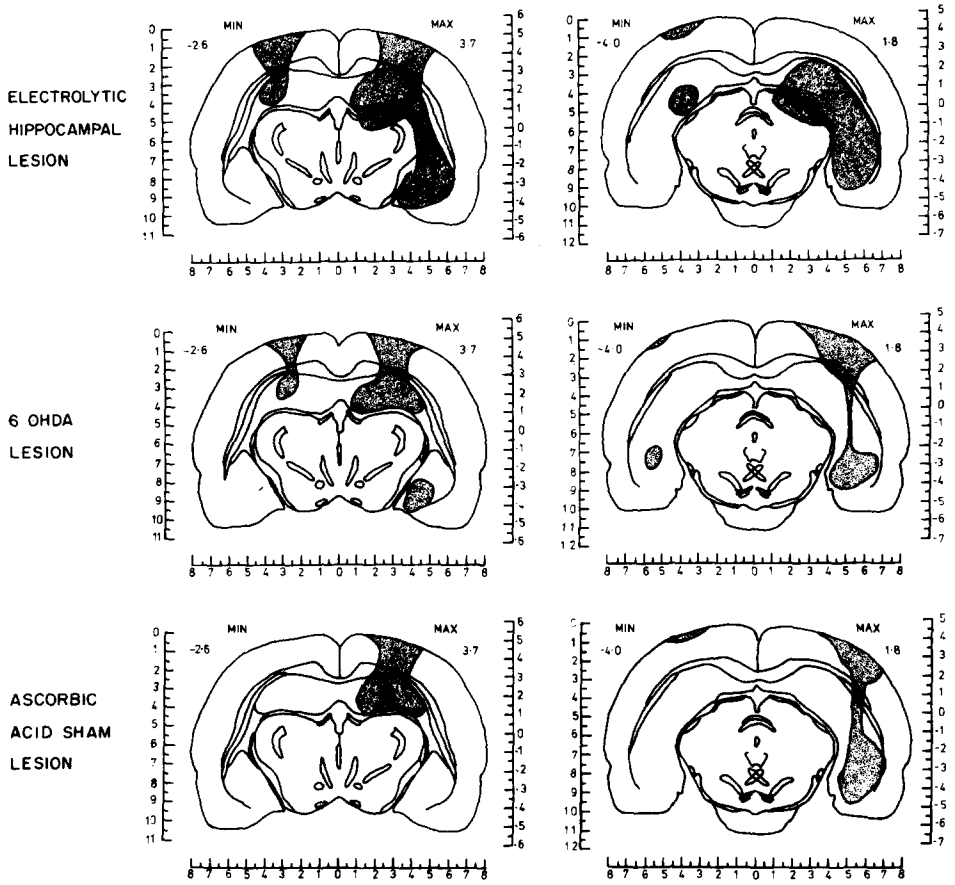

FIG. 1. Minimal and maximal extent of the anterior and posterior hippocampal lesions. A. Electrolytic B. 6-OHDA C. Ascorbic Acid Sham.

Apparatus

Experimental sessions were conducted in eight 35×23×34 cm chambers housed in sound-attenuating boxes. A food cup was located on one end wall of the chamber approximately 3.5 cm above the floor. A 2.0 cm diameter hole was located at the same height and 6 cm to the left of the food cup through which a water spout protruded 1 cm into the chamber. Each chamber was illuminated by a 40 watt globe and ventilation fans within each provided masking noise against external sounds. Noyes standard formula 45 mg food pellets were used. Pellet delivery was non-contingent and was automatically controlled by standard relay circuitry.

Surgery

Rats were anaesthetized with 60 mg/kg of Sodium Pentobarbital. Twenty-four animals received bilateral 6-OHDA injections in both the dorsal and the ventral hippocampus. The co-ordinates of the hippocampal lesion sites were as follows: dorsal, 2.5 mm posterior to bregma, 2.0 mm lateral to the midline and 3.3 mm below skull; ventral, 4.00 mm posterior to bregma, 5.00 mm lateral and 6.5 mm below skull. Stereotactic injections were made from a 10 μl Hamilton syringe through a 30-gauge stainless steel cannula. Each injection of 6-OHDA (2,4,5-Trihydroxyphenylamine 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 an ascorbic acid/NaCl mixture. The rate of injection was 1 μl/min. Hippocampus sham animals underwent the same surgical procedure but were given an ascorbic acid injection into the hippocampus.

Following surgery, the rats were returned to their home cages and allowed a post-operative recovery period of 5 to 10 days. Water consumption was recorded for each 24 hr period for 3 days preceding surgery and throughout the post-operative period. Throughout testing supplementary chow was provided to maintain the animals at 80% of their pre-deprivation body weight.

Seventeen rats received bilateral anodal lesions (1.5 mA) through a No. 2 stainless steel insect pin insulated except for 0.5 mm at the tip, at four anterior and four posterior sites. Co-ordinates for anterior placement were 2.6 mm posterior to bregma, ±1.6 mm and ±2.6 mm lateral to midline, 4.0 mm and 3.6 mm below skull respectively. Current was passed for 35 sec at each site. Posterior lesions were 4.7 mm posterior to bregma ±4.6 mm lateral, 4.0 mm and 7.0 mm below skull. Current was passed for 45 sec. Following surgery, the rats were returned to their home cages and allowed a recovery period of 12–14 days. In the last 4–6 days their body weight was reduced to 80% of their initial body weight. Fifteen rats received bilateral anodal lesions of the cortex overlying the hippocampus by the same method using the same coordinates except for the horizontal which was 1 mm below skull.

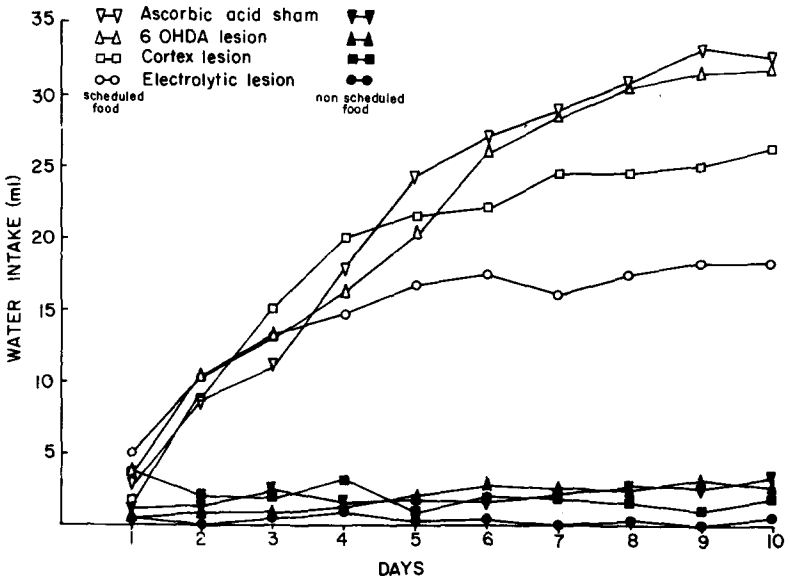

FIG. 2. Mean water intake during one hour of exposure to scheduled or non-scheduled food for 8 groups of lesioned or sham lesioned rats.

Behavioural Procedure

The rats were randomly assigned to either a schedule group or a non-schedule group. Animals were exposed to the food delivery session one hour per day for 10 consecutive days between 1030 and 1230 hours. During the one hour session, rats in the four schedule groups received one 45 mg pellet each minute (fixed time-60 seconds), whereas the four non-schedule groups were given 60 pellets in a single amount at the beginning of the one hour session. Water consumption was recorded at the conclusion of the session.

At the completion of the tenth session, all rats, except the perfused rats, were killed by decapitation and trunk blood was collected for corticosterone assay. Their brains were removed and placed in a 10% formalin solution.

Histology

An anatomical evaluation of the lesion site was undertaken for each animal. Serial frozen sections of 50 microns were cut horizontally through the hippocampus, each third section being retained and mounted on slides, and stained with cresyl violet. The extent and location of tissue damage was determined by projecting the stained slides onto copies of plates taken from the Pellegrino and Cushman Atlas [4] of the rat brain (see Fig. 1).

Eight 6-OHDA lesion rats were anaesthetized with Sodium Pentobarbital and perfused with flushing and Faglu solution. Frozen sections (25 μm) were cut through the hippocampus, each third section being retained and mounted on slides and allowed to dry, before being inspected for fluorescence.

Biochemical Assay

Rats were killed by decapitation. Trunk blood was collected in heparinized tubes and centrifuged immediately at 3200 rpm for 20 min. The plasma was stored at −80°C until assayed. Pearson-Murphy's method [3] was used to determine plasma corticosterone levels.

RESULTS

The minimum and maximum extent of the anterior and posterior hippocampal lesions for the 6-OHDA lesion group, ascorbic acid sham lesion group and the electrolytic hippocampal lesion group are shown in Fig. 1. Damage was mainly confined to the hippocampal region although in the electrolytic lesion group damage tended to be more extensive and in a few animals there was partial damage to the thalamus and one animal sustained damage to the geniculate bodies. In the ascorbic acid sham lesion group all animals except three sustained some hippocampal damage. Fluorescent histochemistry showed that all damage in the 6-OHDA lesion group was specific to the catecholaminergic terminals.

Test hour water consumption data for 10 days of testing for all eight groups (sham, 6-OHDA, Elec. HC. and Elec.C with and without scheduled food) are shown in Fig. 2.

An ANOVA (schedule condition × treatments) for water intake on day 1 showed that there was a significant difference between scheduled and non-scheduled conditions $F(1,64)=8.5$, $p<0.01$, but there was no significant difference between treatments, nor was there a significant treatment and schedule interaction. A similar analysis on water intake for day 2 also showed a significant schedule effect, $F(1,64)=33.6$, $p<0.01$, but no treatment or interaction effects.

An ANOVA of the means for the last 3 days of the 10 day test period showed a significant effect for schedule vs. non-schedule, $F(1,64)=405.5$, $p<0.01$, for lesions, $F(3,64)=8.9$, $p<0.01$, and for interaction, $F(3,64)=5.5$, $p<0.01$. A Newman Keuls post hoc analysis showed that the mean for the electrolytic lesioned scheduled hippocampal group was significantly smaller than the means for the other 3 scheduled groups ($p<0.05$) and the mean for the scheduled electrolytic cortex group was significantly smaller than the means of the 6-OHDA and ascorbic sham schedule groups ($p<0.05$) which were not significantly different from each other ($p>0.05$).

Mean home cage water intake was significantly higher for non-scheduled rats (28.6 ml) compared with scheduled rats

TABLE 1

MEANS AND STANDARD DEVIATIONS OF PLASMA CORTICOSTERONE LEVELS (μl/100 ml) FOR LESIONED AND SHAM LESIONED RATS GIVEN EITHER SCHEDULED OR NON-SCHEDULED FOOD

Condition	6-OHDA Hippocampal Lesion	Ascorbic Acid Sham Hippocampal Lesion	Electrolytic Cortex Lesion	Electrolytic Hippocampal Lesion
Schedule	24 (±6) N=8	27 (±10) N=7	30 (±14) N=8	21 (±10) N=12
Non-schedule	32 (±12) N=8	8 (±5) N=7	16 (±12) N=8	9 (±9) N= 6

(23.9 ml), $F(1,64)=6.7, p<0.05$, and also for the four lesioned conditions, $F(3,64)=5.9$, $p<0.05$. Newman Keuls tests showed no significant difference between any two means and no significant interaction ($F<1$).

Means and standard deviations of plasma corticosterone levels (μg/100 ml) are given in Table 1. A two way ANOVA showed that the level of plasma corticosterone was significantly higher in schedule groups, $F(1,56)=11.09$, $p<0.01$. There was a significant effect for lesions, $F(3,56)=4.6$, $p<0.01$, and a significant interaction, $F(3,56)=4.8$, $p<0.01$. A post hoc Newman Keuls analysis showed that the 6-OHDA lesion non-schedule group had significantly higher plasma corticosterone levels than the other non-schedule groups. The scheduled groups did not differ significantly from each other in corticosterone levels.

DISCUSSION

Onset of schedule-induced drinking, as defined by Devenport [2] in terms of a pellet delivery criterion, occurred on the first day. After 60 pellets had been delivered all scheduled groups had a significantly higher water intake than non-scheduled groups, but there were no significant lesions or interaction effects. This clearly shows that none of the lesions accelerated the onset of schedule induced drinking. The data for the second experimental day confirm this. These findings are contrary to the reported accelerated acquisition of polydipsia in rats with electrolytic hippocampal lesions [2]. However, data for the last three days of the 10 day test period show that the electrolytic hippocampal lesion leads to a significantly lower water intake by days 8, 9 and 10 of scheduled food delivery in comparison with water intake by rats which received a cortical electrolytic lesion.

The data on 6-OHDA lesions are more difficult to interpret since the water intake for this group is not different from that of the ascorbic acid sham group, but both groups are significantly higher than the cortical lesion group. Although it has been reported that frontal and occipital lesions reduce schedule induced water intake [1], a later study found no attenuation of polydipsia for any group with neo cortical damage [5]. Data from a previous study in our laboratory [8], (unoperated rats exposed to the same schedule had a mean water intake for days 8, 9 and 10 of 27.4, 25.5 and 30.1 ml respectively) seem to justify using cortical lesion intake levels as a baseline. This suggests that destruction of hippocampal neurones can lead to either an increase or decrease in schedule-induced drinking. Neither change can be specific to catecholaminergic neurones. Taking into account the earlier findings [2] the observed effects must relate to the extent of lesions. It appears that large, non-specific electrolytic lesions of the hippocampus lead to lower levels of polydipsia than the smaller, more specific 6-OHDA lesions which themselves do not differ in effect from sham ascorbic lesions.

In the control (non-schedule) rats, 6-OHDA lesions significantly increase plasma corticosterone levels above those found in sham lesioned or electrolytic lesioned non-scheduled rats. When food is presented on a schedule, plasma corticosterone levels are increased significantly, regardless of lesion or sham lesion. The levels found in these rats are similar to those found in other experiments in unoperated animals exposed to scheduled food delivery [8]. Therefore hippocampal neurones (catecholaminergic or others) do not seem to be involved directly in SID, contrary to Devenport's claim. On the other hand, there is support for the suggestion that hippocampal catecholamine neurones appear to be involved in corticosterone regulation as shown by the plasma corticosterone level increase in non-scheduled 6-OHDA lesioned rats.

Home cage water intake showed a small but significant difference between intake by scheduled and non-scheduled animals which is not unusual and no significant differences between any of the lesioned groups, which indicates that normal water intake was not affected by lesions.

From the data reported here we conclude that catecholaminergic neurones in the hippocampus have an inhibitory function on corticosterone release. Dopamine depletion releases this inhibition in non-scheduled rats. It also appears that the inhibition is released to the same extent by scheduled food presentation and that this release is not additive with that brought about by dopamine depletion.

Early work on the identification of anatomical pathways involved in schedule-induced drinking was focused on the hypothalamus [9]. Data from those studies showed that hypothalamic neurones are involved in the regulation of all drinking behaviours. More recent studies of dopaminergic neurones in the NAS show a specific involvement of NAS pathways in a broad spectrum of schedule-induced behaviours and not in deprivation induced drinking. The findings from the present experiments do not support the earlier report [2] of a direct involvement of hippocampal neurones in SID but since only a limited number of hippocampal neurones have been investigated in this and the early study, an involvement of other areas of the hippocampus is not precluded.

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