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6-Hydroxydopamine treatment does not affect the young rat's ability to modify its response when changing from food to water deprivation

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In this study we examined whether depletion of forebrain noradrenaline would affect latent learning as predicted on the basis of the role attributed to the dorsal noradrenergic bundle in attention. Either 6-hydroxydopamine (6-OHDA) or saline was injected into the lateral ventricles of male Wistar pups on postnatal day 12. Immediately after weaning the rats were food deprived and trained to choose the left arm of a Y-maze which contained food while the right arm provided access to water. Once they had learnt to enter the left arm they were allowed access to food but not to water for one day and then tested again. All the rats quickly adjusted their behavioural response to the fact that they were deprived of water. The 6-OHDA-treated rats' behaviour did not differ significantly from that of the controls.

The noradrenergic innervation of the forebrain via the dorsal noradrenergic bundle has been attributed a role in attention. Mason (ref. 3, p. 277) has suggested that '... neural activity in the dorsal noradrenergic bundle serves to screen out irrelevant sensory stimuli impinging on the organism'. This view is supported by the fact that 6-hydroxydopamine (6-OHDA)-treated rats only perform worse than controls in behavioural tasks in which the rat must attend exclusively to a certain stimulus dimension, and even perform better than controls when the predictor of reward is subsequently changed to one of a different stimulus dimension[2,3]. A question that arises from this description of the function of the dorsal noradrenergic bundle is whether a stimulus is designated as generally 'irrelevant', in which case the animal will not respond to that stimulus under any circumstance, or whether the 'relevance' of sensory stimuli depends on other factors affecting the rat, such as other factors in the environment or 'internal' variables such as hunger, thirst, etc.

We undertook to study this problem by training food-deprived rats to respond to an environmental situation in a certain way to obtain food. We then tested the rats when they were deprived of water in the same environmental situation (latent learning). If a rat has designated all cues in this environment that do not lead to the food as 'irrelevant' one cannot expect it to adjust its response to the fact that it has been deprived of water (in which case the 6-OHDA-treated rats should modify their response considerably better than the controls). However, a rat that only attaches a 'relevance' to a stimulus under certain conditions, in this case the condition of food deprivation, may quite readily modify its response to the familiar environment when changing from

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food to water deprivation (in which case the controls should perform at least as well as the 6-OHDA-treated rats).

Two groups of 8 male rats were used in this study. They were cross-fostered on the day of birth to obtain two litters with 8 male pups each. One group (6-OHDA) received bilateral intraventricular[5] injections of 50 μg 6-OHDA in 2 μl of saline when 12 days old. A second group (SAL) received the same injections without the 6-OHDA. The rats were weaned when they were 25 days old and weighed on day 26. From then on they were deprived of food for all but 1 h each day (immediately after behavioural testing).

The rats were tested in a plastic Y-maze consisting of a starting alley (10 cm wide, 67 cm long), a 'triangular' choice compartment (sides of 37, 37 and 60 cm) and two goal boxes (25 × 25 cm). The goal boxes, which the rats could enter through doors in the 60 cm side of the triangular compartment, were opposite the starting alley. There were food pellets (Noyes 45 mg) in the box on the left and there was a water bottle in the box on the right throughout the experiment. The walls and floor of the right side of the choice compartment and of the goal box on the right were covered by a metal grid. The rats were put into the maze with both doors open on postnatal day 32. From then on they were put in the starting alley 6 times daily with an intertrial interval of several seconds.

Once the rat entered a goal box the doors were closed and the rat was left in that box for 1 min. If the rat did not enter a goal box within 1 min, it was taken out of the maze and testing was resumed several minutes later. When the rat entered the box on the right, we also noted whether it drank any water. The rats were not allowed to enter the same box on all trials: if the rat chose the left (food) side 5 times, the left door was closed on the sixth trial, to make sure that each rat visited each goal box at least once per session. Once a rat chose the left side on the first 5 trials on two consecutive days (criterion), it was deprived of water for one day (with free access to food) and then tested in one session of 6 trials.

After the behavioural tests (on day 69) the rats were killed and their brains were dissected and frozen. The noradrenaline and dopamine contents of the rats' cerebral cortex and brainstem were determined using a radioenzymatic method[6]. The body weights and the behavioural results were evaluated using two-tailed Mann–Whitney U-tests without correcting for ties. The catecholamine contents were compared using *t*-tests.

One 6-OHDA-treated rat died during training (data not included). We found a significant reduction in body weight after 6-OHDA treatment ($U_{8,7} = 4.5$), but no significant effect (at the 5% level) on any of the behavioural measures (Table I). 6-OHDA-treated rats learnt to enter the food side of the maze for a food reward in as many sessions, and they entered the food

TABLE I

Body weight when 26 days old, performance while food deprived and latency to enter a goal box on the first trial of the test session (i.e. when deprived of water)

Number of sessions to criterion, number of times the rat entered the right side and of times the rat drank during the initial training (i.e. while deprived of food), as well as the latency to enter the left side on the last of these sessions (median with range within brackets; body weight in grams, latencies in seconds).

Group	*Number of rats*	*Body weight*	*Sessions to criterion*	*Times right side entered*	*Times rat drank*	*Latency on last training session*	*Latency on test session*
SAL	8	73 (67–81)	10 (6–15)	13 (7–19)	3 (0–8)	3.4 (3–6)	6.6 (2–11)
6-OHDA	7	66* (60–68)	10 (5–11)	11 (5–16)	4 (1–7)	3.0 (2–10)	7.2 (3–10)

Mann–Whitney U test: * $P < 0.01$.

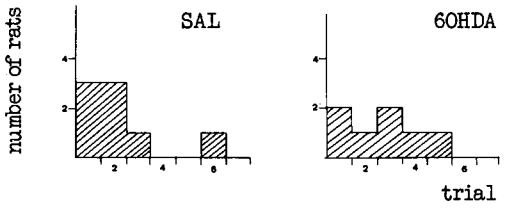

Fig. 1. Trial on which the rat first entered the 'water side' when deprived of water.

side as fast as the controls did on the last training session, and also entered a box as fast as the controls did during the first trial of the testing session. Furthermore, the 6-OHDA-treated rats entered the water side as often and also drank as often as the controls did. Fig. 1 shows the trial on which each rat chose the right (water) side for the first time in the test session. Five of the 15 rats (3 controls, 2 6-OHDA-treated) immediately adjusted their response to the fact that they were deprived of water. The 6-OHDA-treated rats did not take fewer trials to enter the water side than the controls. Table II shows that the 6-OHDA treatment reduced the NA content of the cortex to 16% (confirming considerable destruction of the dorsal bundle) and increased that of the brainstem to 132% of that of the controls. Furthermore, this treatment reduced the cortical dopamine content to 37% of that of the controls. The procedure for dissecting the cortex included the amygdala and part of the olfactory tubercle.

The rats that were treated with 6-OHDA on day 12 did not show any learning deficit several weeks later (on days 33–49). This is in agreement with many studies using adult rats, in which 6-OHDA treatment is shown not to affect learning (see ref. 3, p. 192–231). Furthermore all the rats quickly adjusted their behavioural response to the fact that they were deprived of water: most of the rats entered the water side within 3 trials while they had been trained to enter the food side during the previous sessions. Moreover, although the rats were slower at entering a goal box on the first trial of the testing session than they were at entering the food side during the last training session, this was equally the case for 6-OHDA treated rats and controls. Three control rats entered the water side on the first test trial with latencies of 6,7 and 8 s, and two 6-OHDA treated rats did so with latencies of 7 and 9 s. Taken together this suggests that the control rats have not neglected stimuli of the water side due to their being irrelevant under the training conditions.

This led us to consider the following problem: if the dorsal noradrenergic bundle is to screen out irrelevant stimuli, it must receive information on which stimuli are relevant at that moment, and must be able to activate or block sensory channels on the basis of this information. However, if the situation is changed the rat must learn a new set of relevances for the sensory stimuli, as well as learning to perform an appropriate response to the relevant stimulus. This would require two steps since the rat can only ignore the 'irrelevant' stimuli after having found out which stimuli are relevant. Another possibility is that on-going behaviour modifies neural activity in the dorsal noradrenergic bundle in such a way that this activity decreases the threshold for detection of stimuli in the sensory channels that have been used during that behaviour in comparison with those of other channels. There is convincing evidence that noradrenergic neurotransmission is responsible for adjusting the flow of blood within the brain to the distribution of neural activity[1,4]. If 6-OHDA-treated rats cannot redirect the blood flow following changes in neural activity, their capacity to continue sampling the same (relevant) stimuli will diminish as neural activity in the area involved declines due to lack of exchange of nutrients such as oxygen, CO_2, glucose, or what-

TABLE II

Mean noradrenaline and dopamine contents of the cerebral cortex and brainstem in ng/mg wet weight (with standard deviations)

Group	*(n)*	*Noradrenaline*		*Dopamine*	
		Cortex	*Brainstem*	*Cortex*	*Brainstem*
SAL	(8)	0.137 (0.045)	0.351 (0.063)	0.401 (0.142)	0.029 (0.007)
6-OHDA	(7)	0.022*** (0.011)	0.463* (0.097)	0.150** (0.099)	0.024 (0.005)

Two-tailed *t*-tests: $^{*}t_{13} = 2.70$, $P<0.05$; $^{**}t_{13} = 3.91$, $P<0.01$; $^{***}t_{13} = 6.71$, $P<0.001$.

ever the limiting factor may be. An interpretation of the role of the dorsal noradrenergic bundle in attention along this line is not fundamentally different from Mason's view (cited in the Introduction), as far as the interpretation of the behavioural effects of 6-OHDA treatment are concerned, but it may help us to find a physiological basis for the role of the dorsal bundle in attention.

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