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Central Noradrenaline Depletion During Development and its Effect on Behaviour

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BRENNER, E , M MIRMIRAN, F VAN HAAREN, B C THEUNISSE, M G P FEENSTRA, A A LAMUR, C G VAN EDEN AND J VAN DER GUGTEN *Central noradrenaline depletion during development and its effect on behaviour* PHYSIOL BEHAV **41**(2) 163–170, 1987 —Although early depletion of noradrenaline is known to affect the morphological development of various structures in the brain it is not clear what implications this has for adult behaviour In the present study, 6-hydroxydopamine (6OHDA) was injected into the lateral ventricles of 12 day old rats, permanently destroying most of the noradrenergic innervation of the spinal cord and of all the brain areas examined except for the pons/medulla, and reducing the dopamine content of the cerebral cortex considerably The noradrenaline content of the heart, as well as general developmental parameters such as food intake and body weight, were unaffected Despite the extensive noradrenaline depletion during development, these rats' spatial memory—as determined in a radial maze task—was no worse than that of controls In a lever-pressing task the 6OHDA treated rats made no more errors than did controls but performed more slowly The results indicate that at least some aspects of learning, memory and sensory-motor ability can develop normally when the noradrenergic innervation of the brain is largely destroyed

Noradrenaline Development Spatial memory Operant behaviour 6-Hydroxydopamine Receptor supersensitivity Rat

NORADRENERGIC fibres project to a variety of brain structures—such as the neocortex—early during development [5,14], suggesting that noradrenaline may play an organizing role in the development of these areas This is supported by some studies on experience dependent changes in the organization of the kitten visual cortex, which show that environmentally guided development depends on an intact noradrenergic innervation (reviewed in [25]) Moreover, several morphological studies also show that both the development of the rat's cerebellum [27] and of its cerebral cortex [6,10] are retarded by destroying the local noradrenergic innervation shortly after birth It is still unclear whether noradrenergic denervation affects the development of specific aspects of behaviour Neonatal depletion of noradrenaline in rats' brains has been reported to result in a generally impaired ability to learn (e g , [35]), but has also been reported to have no effect on behaviour at all [20,36]

In many studies, the function of certain projections are deduced from the deficits that occur when specific fibres are destroyed To do this, noradrenergic projections are often destroyed with the neurotoxin 6-hydroxydopamine (6OHDA), which is taken up by both noradrenergic and dopaminergic terminals, and destroys these projections while leaving most other cells and fibres intact During the first week after birth, systemic injections of 6OHDA destroy noradrenergic projections within the rat brain [23,24] At later ages this is no longer so, presumably due to maturation of the blood-brain barrier, and 6OHDA has to be injected into the brain, which may cause non-specific damage [41] However, systemic 6OHDA has the disadvantage of decreasing the adrenaline and noradrenaline contents of various organs [13,47] Such chemical sympathectomy may also lead to various functional abnormalities

The disadvantage of local application of 6OHDA is evident from studies on experience dependent changes in the organization of the kitten visual cortex Plastic properties of the kitten visual cortex are impaired by local perfusion of 6OHDA [4,25], but not by systemic injections [4] or injections into the locus coeruleus [1] Contradictory results were obtained with intraventricular injections [1, 15, 25] All these methods of application reduced the noradrenaline content of the visual cortex substantially In a recent study in which several doses of 6OHDA were infused into the visual cortex, it was shown that the observed decrease in plasticity is probably the result of non-specific effects of large doses of 6OHDA [49] Our own studies on a possible role for norad-

renaline in the growth and plasticity of the developing rat's cerebral cortex, however, demonstrated that other problems are encountered when interpreting effects of neonatal peripheral 6OHDA treatment [10] Exposing young rats to enriched environments stimulates the development of their cerebral cortex This increased cortical growth appeared to be reduced by systemic injections of 6OHDA, but a reduction in body weight, possibly due to disruption of peripheral noradrenergic functions, interfered with the interpretation of differences between 6OHDA treated and control rats When the study was repeated using intraventricular treatment, we found no difference in susceptibility to enriched rearing conditions between lesioned and control rats [11] Similarly lesions of noradrenergic pathways caused by a second neurotoxin, DSP4, were found to have quite different effects on behaviour than do 6OHDA lesions [3] Systemic injections of DSP4 destroy central noradrenergic pathways in adult rats, but some of the behavioural effects of this treatment may be due to changes outside the central nervous system [22,39] In order to avoid such problems, we decided to inject 6OHDA into rats' lateral ventricles, thus reducing the chance of both peripheral noradrenergic denervation and local, extensive non-specific damage at specific injection sites As the neonatal subcutaneous treatment is based on the permeability of the blood-brain barrier to 6OHDA, it is unlikely that injecting the toxin into the ventricles at that age will leave peripheral noradrenergic function unaffected so that we chose to wait until the rats were 12 days old

In behavioural studies using adult rats lesions of specific noradrenergic pathways have been demonstrated to cause specific deficits in behaviour (reviewed in [21,28]), so that even having decided to inject 6OHDA directly into the brain it is important to determine precisely which projections have been destroyed We determined the extent of the lesions in several ways, choosing the noradrenaline content of the heart as a measure for peripheral noradrenaline levels [13,47] We also kept record of rats' body weights and daily food intake, as measures of general development We used an eight-arm radial maze to test the 6OHDA treated rats' spatial working memory, an aspect of behaviour that has been reported to be affected by adult 6OHDA treatment ([2,30] but see [12,19]) Furthermore, we examined whether this form of noradrenaline depletion affects rats' ability to learn a lever-pressing task

Reducing the cortical noradrenaline content with 6OHDA has been reported to increase the noradrenergic receptor density [16, 24, 40, 46] This may compensate for some functional abnormalities that would otherwise occur after such lesions, and may mask behavioural effects of the depletion without there being substantial regeneration of noradrenergic fibres If the loss of noradrenaline is indeed compensated for by changes in receptors, 6OHDA treated rats' sensitivities to the sedative effects of noradrenergic agonists should have increased Neonatal noradrenergic denervation has been shown to cause behavioural supersensitivity to both alpha and beta adrenergic agonists [26,44] In order to check whether receptor supersensitivity could be masking behavioural effects of the denervation, we determined the sensitivity of the rats' performance in the lever-pressing task to adrenergic agonists for which pilot studies had shown sedative effects

METHOD

All the rats used in this study were Wistar rats born in our animal colony, cross-fostered at birth, and raised in groups of 5 male and 3 female pups per mother Only the male rats were used in the present study On postnatal day 12 2 μl of saline containing 50 μg of 6OHDA (HBr, Sigma, freshly dissolved for each rat) was injected into each lateral ventricle [7,8] under ether anaesthesia Controls received the same treatment except for omission of 6OHDA After weaning the male rats were housed in groups of four per cage at 21°C a humidity of 50–60% and a 12 hr light/12 hr dark cycle with lights on at 1 30 p m

Behavioural testing started when the rats were three months old They were tested at the beginning of the light period, after which they were allowed access to food for one hour Sixteen rats' spatial memory was tested in an 8 arm radial maze and another group of 16 rats was tested on a fixed-consecutive-number (FCN) lever-pressing task with a discriminative stimulus [33] The latter took place in Grason-Stadler (model 1111-L) conditioning chambers equipped with a lever and a lamp on either side of a pellet retrieval unit The radial maze was in a room with ample visual cues, and sound from a radio at a fixed position The conditioning chambers were inside (model 1101) research chests with fans providing masking noise and fresh air

Eight 6OHDA treated rats and 8 controls were tested in a radial maze with 8 arms (85 cm long 7 cm wide) radiating from a central platform Access to these arms could be obstructed by a door (for more details see [51]) On the first day, groups of four rats were placed within the radial maze for 15 minutes with ample food spread all over the floor During 3 more pre-training sessions the doors remained open, and food pellets were placed (1 per arm) at an increasing distance from the central platform Thereafter a single pellet (Noyes, 45 mg) was placed in a hollow at the end of each arm, so that it was not visible from the central platform Individual rats were placed in the central platform and the doors were opened simultaneously so that the rat could enter an arm Once the rat entered an arm (all 4 legs off the central platform), all doors were closed except for that of the arm that the rat had just entered This last door was closed as soon as the rat reentered the central platform, and there was a 5 second inter-trial interval before the doors opened again Rats remained in the maze until they had either obtained all 8 pellets or had failed to do so within 10 minutes Rats were subjected to 20 training sessions during 4 weeks

Eight other 6OHDA treated rats and 8 more controls were tested on the FCN schedule with a visual discriminative stimulus [33] Rats had to press the left hand lever 10 times for the lights to go on, and then to press that on the right in order to get the food pellet reward Sessions were terminated either after 45 minutes or when the rats had obtained 60 food pellets On the first session the lights remained on, and rats were trained to press the right lever During the next ten days the number of left-lever presses that were required to turn on the light was increased by one per day We determined the number of times that the rats pressed the wrong lever and the time it took them to obtain a food pellet Once performance had stabilized, we recorded the mean time that elapsed between (1) the first and tenth press on the left lever, (2) light onset and the response on the right lever, and (3) the rewarded right-lever press and the next press on the left lever To test for impairments due to metabolic or appetitive factors, we measured the body weights and food intake of the rats that were tested on the FCN task once a week during the 11 weeks of testing

During the next three weeks, we examined how the rats

TABLE 1

MEDIAN NORADRENALINE AND DOPAMINE CONTENTS OF VARIOUS REGIONS AS DETERMINED BY DIFFERENT METHODS

	Noradrenaline		Dopamine		
Area	Control	6OHDA	Control	6OHDA	
HPLC ng/mg Tissue (8 rats per group)					
Occipital cortex	0 45	<0 05*			
Parietal cortex	0 32	<0 01*			
Frontal cortex	0 51	0 03*			
Hippocampus	0 37	0 03*			
Amygdala	0 67	<0 30*			
Thalamus	0 61	0 49			
Hypothalamus	1 60	0 77*			
Cerebellum	0 24	0 04*			
Pons/Medulla	0 79	1 01			
Radioenzymatic† ng/mg Tissue (5 control and 4 6OHDA-treated rats)					
Neocortex	0 57	0 09*	0 28	0 12*	
Cerebellum	0 57	0 17*			
Spinal cord	0 29	0 04*	0 04	0 03	
Radioenzymatic ng/mg Protein (number of rats per group in brackets)					
Parietal cortex	1 22	0 36*	0 34	0 18*	[13]
Frontal cortex	1 79	0 21*	6 56	1 08*	[5]
Hippocampus	1 95	1 00*	0 63	0 56	[4]
Amygdala	2 33	0 58*	0 31	0 24	[5]
Thalamus	2 39	1 23*	0 81	0 77	[5]
Hypothalamus	3 30	2 11*	1 16	1 16	[13]
Cerebellum	1 83	0 25*	0 02	0 03	[5]
Pons/Medulla	5 22	5 79	0 20	0 24	[13]
Spinal cord	3 73	0 39*	0 25	0 23	[5]
Heart	33 6	33 1			[5]

*Significantly different from controls (Mann Whitney U-test 5% level)
†Rats tested on the FCN task
< Median content less than given detection limit

performance on the FCN task was affected by various doses of isoprenaline, in order to determine whether receptor supersensitivity could have compensated for the chronically reduced level of noradrenaline To do so, we injected rats with 0, 5, 10, 20 or 40 μg/kg of isoprenaline (subcutaneously in 0 1 ml saline) half an hour before the session Each dosage was given once a week, doses being presented in a random order within each week After testing for supersensitivity to peripherally injected isoprenaline, stainless steel cannulae—for central injections—were implanted into the rats' right lateral ventricles (under fentanyl anaesthesia) After several days of unlimited access to food, the rats were food-deprived as before, and then tested for two more weeks All rats that had regained their pre-operative level of performance then received the same doses of isoprenaline (in 0 5 μl of saline), injected directly into their ventricles 5 minutes before behavioural testing The procedure was otherwise identical to that described above Although it is unusual to inject central doses per unit body weight, this was done in order to ease the comparison with peripheral administration Pilot studies had already suggested that intra-ventricular injections are less effective Finally, the alpha-2 agonist clonidine (40 μg/kg) was injected into the ventricle on two sessions and subcutaneously on two subsequent sessions This drug is known to affect central neurotransmission when

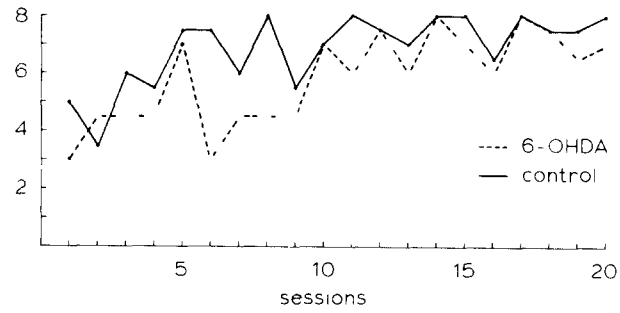

FIG 1 Number of consecutive correct responses Median number of arms entered before the first error in the radial maze

administered peripherally After the last experiment we verified that the injection procedure had released the drugs into the ventricle by injecting 0 5 μl of dye through the cannulae of four rats

After behavioural testing, catecholamine concentrations were determined in various areas of the brain The noradrenaline contents of the group that had been tested in the radial maze were measured by reversed phase high pressure

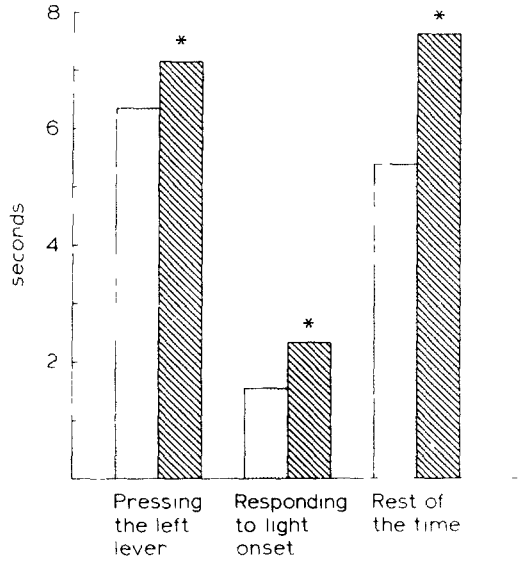

FIG 2 Time spent per reward Distribution of the time it took the rats to obtain a reward after performance in the FCN task had stabilized (9th to 11th week of testing, statistical comparison between the median values for individual rats $*p<0.05$) Dotted columns control, striped columns 6OHDA

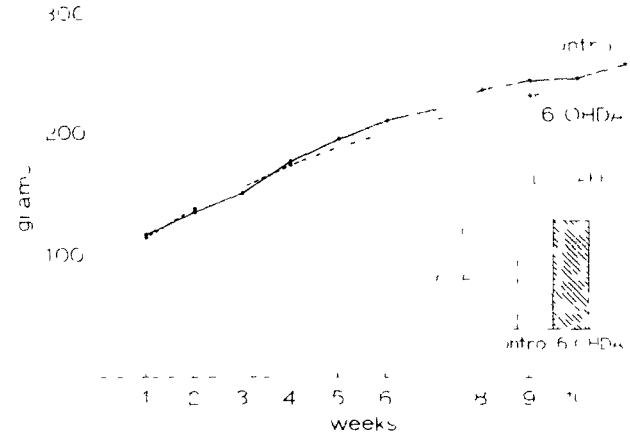

FIG 3 Median body weight and daily food intake during the period that the rats were performing the FCN task

liquid chromatography (HPLC) with electrochemical detection, using a 15 cm Nucleosil 5C18 column and a Metrohm 656 detector operated at 700 mV against a Ag/AgCl reference electrode The mobile phase (0 1 M acetate buffer pH 3 5 with 0 2 mM heptanesulphonic acid) was delivered at 0 8 ml/min by a Hewlett-Packard 1090 pump Noradrenaline was isolated from homogenates on Sephadex G10 [53] The "thalamus" in the employed dissection procedure corresponds to the midbrain in [18] (excluding colliculi)

The noradrenaline and dopamine of brains of the rats that had been tested in the operant conditioning chambers were determined radioenzymatically [50] In this case the contents were determined for the neocortex, cerebellum and spinal cord The section called "neocortex" consists of all of the dorsal part of the cerebral cortex (dissected as in [24]) Furthermore, the contents of various brain areas of a third group of rats (that had not been tested behaviourally) were also determined using this method For this third group we also determined the noradrenaline content of the heart Parietal cortex, amygdala, thalamus, and hypothalamus were dissected from a brain slice starting at the level of the optic chiasm and ending 4 mm caudally

Retrograde tracing with wheat germ agglutinin-horseradish peroxidase (WGA-HRP) was used to demonstrate that the noradrenergic fibres had actually been destroyed by the 6OHDA treatment, as this method does not depend on the noradrenaline content of the fibres Nine additional rats from the same breeding, cross-fostering and treatment series received injections of 0 2 μl of a 3% WGA-HRP solution into their occipital cortex The WGA-HRP was dissolved in Tris (pH 7 4) and injected 2 5 mm lateral to the sagittal sinus, 2 mm anterior to lambda and 1 mm downwards from the brain surface of (fentanyl) anaesthetized rats At least one 6OHDA treated and one control rat were always injected with WGA-HRP on the same day, and their brains were processed simultaneously Four days after injecting the WGA-HRP, the rats were anaesthetized with 10% urethane (ethyl carbamate, 13 ml/kg body weight) and perfused with 1 25% glutaraldehyde and 1% paraformaldehyde Seventy μm sections of the brain-stem were treated with tetramethyl-benzidine (TMB) [31], after which all stained cells in the locus coeruleus were counted

In no part of this study did the experimenter know which animals had been treated with 6OHDA and which were controls The behavioural effects of 6OHDA were evaluated with Kruskal-Wallis H-tests, except for the drug sensitivity data that were evaluated with group by dose analyses of variance on log-transformed data with rats nested within groups

RESULTS

In locus coeruleus sections of five control rats we found an average of 268 retrogradely WGA-HRP stained cells ipsilateral and 14 contralateral to the site of injection In 6OHDA treated rats we counted 16 cells ipsilateral and none contralateral to the site of WGA-HRP injection in the occipital cortex This corresponds well with the reduction in the noradrenaline content of the occipital cortex (Table 1) The dramatic reduction in the number of stained cells is unlikely to be due to methodological flaws, because the raphe nuclei always did contain stained cells in the same sections The 6OHDA treatment reduced the noradrenaline content of many brain areas and of the spinal cord considerably, and also reduced the dopamine content of the cortex (Table 1) When the HPLC method was not sensitive enough to measure the residual noradrenaline content, the detection limit in brain tissue was taken as the content value, and whenever this affected the median content, the latter was reported as being less than the detection limit (Table 1). The large depletion in spinal-cord noradrenaline is consistent with the ob-

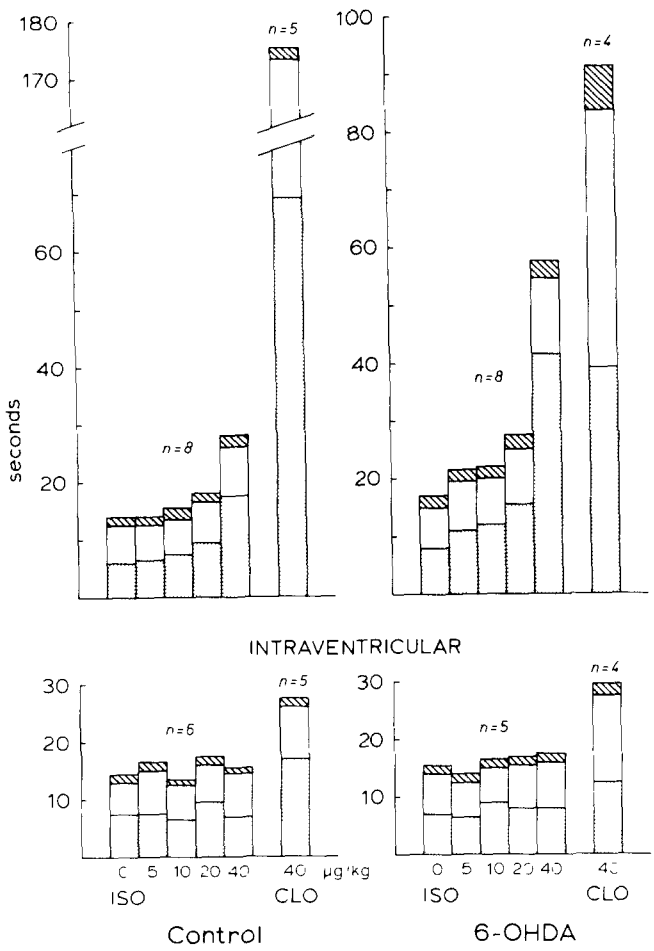

FIG 4 Time spent per reward Effects of either subcutaneous or intraventricular injections of isoprenaline (ISO) and clonidine (CLO) on the time it took the 6OHDA treated and control rats to obtain a reward Three trials per rat per dose for isoprenaline and two for clonidine n=the number of rats Striped area responding to light onset open area pressing the left lever, dotted area rest of the time

served reduction, and often even complete absence, of a post-decapitation reflex [36]

6OHDA treated rats needed as many trials to obtain all 8 food pellets in the radial maze, and performed this task as quickly as controls did In Fig 1, the median number of consecutive correct responses that the rats made on each session shows that the 6OHDA treated rats learned the task as readily as the controls did (even the decline in the 6OHDA treated rats median performance on day 6 did not lead to a significant difference between the two groups of rats on that day) Furthermore, we found no indications that lesioned rats adopt a different strategy for performing the task In the FCN task, 6OHDA treated rats learned to press the levers in the required order as quickly as the controls did, and at no stage did they make significantly more mistakes (i e , press the lever on what was the wrong side at that particular moment) than the controls did However, from the first day of testing, they took more time to obtain the food pellets On the 9th to 11th week of testing 6OHDA treated rats took significantly more time to (1) press the left lever 10 times $H(1)=3\ 19$, (2) respond to light onset, $H(1)=3\ 57$, and (3) press the left lever again, after receiving a food pellet, $H(1)=3\ 57$ (Fig 2) The 6OHDA-treated rats' body weights and food intake did not differ significantly from those of the controls (Fig 3)

The effects of central and peripheral injections of isoprenaline and clonidine on peformance in the FCN task are summarized in Fig 4 Subcutaneous injections of isoprenaline, as well as both central and peripheral injections of clonidine, increased the amount of time that it took the rats to perform the sequence of actions leading to their obtaining a food pellet, Central injection of isoprenaline did not Analysis of variance showed that the 6OHDA treated rats still took significantly more time on the 1st and 3rd measures i e , to press the left lever 10 times, $F(1,14)=8\ 74$, $p<0\ 01$, and to press the left lever after receiving a food pellet, $F(1,14)=4\ 97$, $p<0\ 05$ Furthermore, there was a significant dose main effect for all three measures, $F(4,56)=21\ 24$, 7 27 and 56 70 respectively all $p<0\ 001$ The dose-by-group interaction for the time taken to start pressing the left lever after receiving a food pellet just reached significance at the 5% level, $F(4,56)=2\ 56$, but this parameter for evaluating group differences in drug sensitivity was clearly not significant for the other two behavioural measures Both subcutaneous and intraventricular injections of clonidine increased the time that it took the rats to obtain the food pellets, but identical amounts of clonidine had much less effect when administered centrally than when administered peripherally

DISCUSSION

Injecting 6OHDA into rats' lateral ventricles on postnatal day 12 appears to be an efficient method for depleting the forebrain of noradrenaline Differences between the noradrenaline contents of some areas of the three groups of rats' brains may partly be related to differences in the dissection procedures However, we also observed some differences in local susceptibility to 6OHDA between individual rats A clear example was observed in two of the rats that were tested in the radial maze (performance similar to that of all others) that had almost normal noradrenaline contents in the cerebellum, although the contents of other areas were severely depleted The similarity between the extent of depletion found in the occipital cortex, and the decrease in the number of retrogradely stained cells after injection of the tracer into this area, confirms that the decrease in noradrenaline content can be used as a measure for the number of projections that have been destroyed Spinal cord and cerebellar noradrenaline, as well as cortical dopamine, were also drastically reduced In future studies, dopaminergic fibres may be protected by injecting a dopamine uptake blocker prior to the 6OHDA treatment [48] In contrast to what we found after neonatal subcutaneous 6OHDA treatment [10], the present treatment does not affect the rats' body weights Furthermore, the noradrenaline content of the heart remained unaffected and food intake was unimpaired It is unlikely, therefore, that behavioural effects found after this treatment are due to changes in peripheral noradrenergic functions [38]

The 6OHDA treated rats performed as well as controls in the radial maze, confirming reports that rats do not need an intact noradrenergic innervation of the forebrain to perform this task [12] In the latter study adult rats were treated with

DSP4, which caused a considerably less severe depletion of noradrenaline. Furthermore, unlike results reported for dopamine depletion [32], we found no indication of a difference in strategy between lesioned rats and controls. The strategy of choosing adjacent arms is less likely to occur in our tests, because the rats were forced to wait in the centre for 5 seconds between arm entries. During this inter-trial interval the rats usually walked around. The results support several reports of intact spatial learning on other tasks after adult 6OHDA lesions ([19,34] but see [2,29]). Rats that were treated with 6OHDA during early development have been found to be impaired at performing a delayed spatial alternation task in a Y-maze [30]. This could indicate that intact noradrenergic projections are essential for the development of mechanisms underlying spatial orientation and memory. In a previous study [9] we found that neonatal depletion of cortical noradrenaline by subcutaneous injections of 6OHDA did not prevent rats from learning a delayed spatial alternation task. The latter task was similar to that in the Y-maze, except for the fact that the response that was required of the rat was to press the two levers of an operant conditioning chamber. However, the depletion of cortical noradrenaline in that study was rather modest (50%). The present findings also do not support the developmental hypothesis: the 6OHDA treated rats of the present study showed no deficit in spatial working memory when tested in a radial maze. We conclude that noradrenergic projections are not crucially involved in spatial memory or in the development of the mechanisms underlying it.

In the FCN task, the 6OHDA treated rats learned to press the levers in the required order as readily as the controls did. However, they were slower at performing the task. Although adult dorsal noradrenergic bundle lesioned rats were not slower at this task (unpublished observations), we cannot—at present—conclude that this must be a developmental effect, as it may be the result of noradrenaline depletion in either the spinal cord or the cerebellum, and/or of dopamine depletion. Neonatal 6OHDA lesions of spinal cord noradrenergic terminals have been reported not to affect rats' complex motor behaviour either during development or as adults [36]. Depletion of cerebellar noradrenaline during development has also been reported to leave motor performance unaffected [28]. In adult rats, 6OHDA lesions of the noradrenergic innervation of the cerebellum have been reported to cause impaired acquisition of locomotor behaviour, but there was no effect on post-acquisitional performance [52]. In the present study the 6OHDA treated rats still took more time to obtain a food pellet long after performance had stabilized. The third suggested cause for the reduced rate at which the 6OHDA treated rats obtained the food pellets is the considerable reduction in cortical dopamine. Central dopaminergic systems have often been implicated in motor performance [21, 28, 37, 45]. Furthermore, dopamine depletion has been reported to result in hyperactivity during development [35]. We found no indications of hyperactivity or impaired motor performance after intraventricular 6OHDA on day 12, either in the radial maze during this study (the 6OHDA treated rats retrieved the food pellets as fast as the controls did), or in a previous study using a Y-maze [8]. In the latter study we also found no deficits in performance. We did find a similar reduction in the speed of performing a lever-pressing task after the above-mentioned neonatal subcutaneous 6OHDA treatment that reduced the noradrenaline content by about half, and which did not significantly reduce the dopamine content [9]. The fact that noradrenaline lesioned rats took more time to press the levers may therefore be a specifically developmental effect, but further research will be needed to justify this conclusion.

We failed to determine whether compensatory changes in the sensitivity of beta-adrenergic receptors were masking the behavioural effects of the lesion, because central administration of isoprenaline had no detectable effect on performance in either group. This was even so when the amount of the drug that was injected was such that it clearly reduced the rate of response when administered peripherally. These results are similar to those obtained in studies on drinking behaviour, where much larger doses of isoprenaline were needed to elicit a response when administered centrally than when administered peripherally [17,42]. We did not use higher doses, because it would have been difficult to decide whether the drug was really affecting central receptors or simply leaking out of the brain and affecting peripheral receptors.

We may not find effects after central injections of isoprenaline, because the drug is too rapidly metabolized within the brain, or is unable to reach the receptors when injected in the lateral ventricles. These suggestions could not account for the results of the above-mentioned studies on drinking behaviour. In those studies isoprenaline was injected directly into the presumed sites of action: the lateral hypothalamus [42] and the lateral septal area [17]. Although DSP4-induced noradrenergic lesions have been shown to increase the electrophysiological responsiveness to isoprenaline in hippocampal slices [54], in which case peripheral effects can obviously be excluded, our findings that (1) response rates are reduced by central injections of clonidine—which passes the blood-brain barrier more readily than does isoprenaline due to its lipid solubility, and (2) central injections of clonidine are less effective than subcutaneous injections support the idea that the agonists must enter the blood in order to affect behaviour. This may be because this is the only path by which they can reach their target areas in the brain, but may also indicate that the target areas are not within the brain. It has been shown for instance, that the increased blood glucose level after systemic injections of clonidine is not mediated by central effects [43]. We believe that the evidence is in favour of interpreting the behavioural effects of the adrenergic agonists of the present study as being mediated by peripheral adrenergic receptors. The slight change in sensitivity to subcutaneous injections of isoprenaline for one of the three measures of performance may, therefore, indicate that not all peripheral projections have been left unharmed. We conclude that at least some aspects of learning, memory and sensory-motor performance can develop normally even when noradrenergic innervation is largely destroyed.

REFERENCES

1 Adrien, J G Blanc P Buisseret, Y Fregnac, E Gary-Bobo, M Imbert J P Tassin and Y Trotter Noradrenaline and functional plasticity in kitten visual cortex a re-examination *J Physiol* **367** 73–98, 1985

2 Anderson, C D , R G Mair, P J Langlais and W J McEntee Learning impairments after 6-OHDA treatment a comparison with the effects of thiamine deficiency *Behav Brain Res* **21** 21–27 1986

3 Archer T The role of noradrenaline in learned behaviors studies using DSP4 *Scand J Psychol* Suppl **1** 61–71, 1982

4 Bear, M F , M A Paradiso, M Schwartz, S B Nelson, K M Carnes and J D Daniels Two methods of catecholamine depletion in kitten visual cortex yield different effects of plasticity *Nature* **302** 245–247 1983

5 Berger B and C Verney Development of the catecholamine innervation in rat neocortex morphological features In *Monoamine Innervation of Cerebral Cortex* New York Alan R Liss Inc 1984, pp 95–121

6 Blue, M E and J G Parnavelas The effect of neonatal 6-hydroxydopamine treatment on synaptogenesis in the visual cortex of the rat *J Comp Neurol* **205** 199–205, 1982

7 Boer, G J , H B M Uylings, A J Patel K Boer and R Kragten The regional impairment of brain development in the Brattleboro diabetes insipidus rat, some vasopressin supplementation studies In *The Brattleboro Rat,* edited by H W Sokol and H Valtin *Annals of the New York Academy of Science* Vol 394 703–717, 1982

8 Brenner, E , M Mirmiran, F Van Haaren and J Van Der Gugten 6-Hydroxydopamine treatment does not affect the young rat's ability to modify its response when changing from food to water deprivation *Behav Brain Res* **19** 89–92, 1986

9 Brenner E M Mirmiran, F Van Haaren and J Van Der Gugten Noradrenaline and delayed spatial alternation *Neurosci Lett Suppl* **22** S228 1985

10 Brenner E M Mirmiran H B M Uylings and J Van Der Gugten Impaired growth of the cerebral cortex of rats treated neonatally with 6-hydroxydopamine under different environmental conditions *Neurosci Lett* **42** 13–17, 1983

11 Brenner E M Mirmiran H B M Uylings and J Van Der Gugten Growth and plasticity of rat cerebral cortex after central noradrenaline depletion *Exp Neurol* **89** 264–268, 1985

12 Chrobak J J D L Dehaven and T J Walsh Depletion of brain norepinephrine with DSP-4 does not alter acquisition or performance of a radial-arm maze task *Behav Neural Biol* **44** 144–150 1985

13 Clark D W J R Laverty and E L Phelan Long-lasting peripheral and central effects of 6-hydroxydopamine in rats *Br J Pharmacol* **44** 233–243, 1972

14 Coyle J T and M E Molliver Major innervation of newborn rat cortex by monoaminergic neurons *Science* **196** 444–447, 1977

15 Daw N W T O Videen R K Rader T W Robertson and C J Coscia Substantial reduction of noradrenaline in kitten visual cortex by intraventricular injections of 6-hydroxydopamine does not always prevent ocular dominance shifts after monocular deprivation *Exp Brain Res* **59** 30–35, 1985

16 De Montigny, C , R Y Wang, T A Reader and G K Aghajanian Monoaminergic denervation of the rat hippocampus microiontophoretic studies on pre- and postsynaptic supersensitivity to norepinephrine and serotonin *Brain Res* **200** 363–376, 1980

17 Giardina A R and A E Fisher Effect of atropine on drinking induced by carbachol angiotensin and isoproterenol *Physiol Behav* **7** 653–655, 1971

18 Glowinsky, J and L L Iversen Regional studies of catecholamines in the rat brain *J Neurochem* **13** 655–669, 1966

19 Hagan, J J , J E Alpert, R G M Morris and S D Iversen The effects of central catecholamine depletions on spatial learning in rats *Behav Brain Res* **9** 83–104 1983

20 Isaacson R L W J Street, T L Petit and A J Dunn Neonatal treatment with 6-OH-DA affects brain NE content but not behavior *Physiol Psychol* **5** 49–52 1977

21 Iversen, S D Cortical monoamines and behavior In *Monoamine Innervation of the Cerebral Cortex* New York Alan R Liss Inc , 1984, pp 321–349

22 Jonsson, G , H Hallman, F Ponzio and S Ross DSP4 (N-(2-Chloroethyl)-N-ethyl-2-Bromobenzylamine)—a useful denervation tool for central and peripheral noradrenaline neurons *Eur J Pharmacol* **72** 173–188, 1981

23 Jonsson, G , C Pycock, K Fuxe and C Sachs Changes in the development of central noradrenaline neurones following neonatal administration of 6-hydroxydopamine *J Neurochem* **22** 419–426, 1974

24 Jonsson, G , F A Wiesel and H Hallman Developmental plasticity of central noradrenaline neurons after neonatal damage—changes in transmitter functions *J Neurobiol* **10** 337–353, 1979

25 Kasamatsu T Neuronal plasticity maintained by the central norepinephrine system in the cat visual cortex In *Progress in Psychobiology and Physiological Psychology* vol 10, edited by J M Sprague and A N Epstein New York Academic Press 1983 pp 1–112

26 Lanfumey L and J Adrien Regulation of sleep after neonatal locus coeruleus lesion functional evidence of beta-adrenergic supersensitivity *Eur J Pharmacol* **79** 257–264, 1982

27 Lovell, K L Effects of 6-hydroxydopamine-induced norepinephrine depletion on cerebellar development *Dev Neurosci* **5** 359–368, 1982

28 Mason S T *Catecholamines and Behaviour* Cambridge Cambridge University Press, 1984

29 Mason, S T and H C Fibiger Noradrenaline and spatial memory *Brain Res* **156** 382–386 1978

30 Mason, S T , C Wood and A Angel Brain noradrenaline and varieties of alternation learning *Behav Brain Res* **9** 119–127 1983

31 Mesulam, M M Principles of horseradish peroxidase neurohistochemistry and their applications for tracing neuronal pathways-axonal transport, enzyme histochemistry and light microscopic analysis In *Tracing Neuronal Connections With Horseradish Peroxidase* edited by M M Mesulam New York Wiley and Sons 1982, pp 1–151

32 Pearson D E , L A Raskin, B A Shaywitz G M Anderson and D J Cohen Radial arm maze performance in rats following neonatal dopamine depletion *Dev Psychobiol* **17** 505–517, 1984

33 Picker M L Leibold, B Endsley and A Poling Effects of clonazepam and ethosuximide on the responding of pigeons under a fixed-consecutive-number schedule with and without an external discriminative stimulus *Psychopharmacology (Berlin)* **88** 325–330 1986

34 Pisa, M and H C Fibiger Evidence against a role of the rat s dorsal noradrenergic bundle in selective attention and place memory *Brain Res* **272** 319–329 1983

35 Raskin L A Differential effects of selective dopamine, norepinephrine or catecholamine depletion on activity and learning in the developing rat *Pharmacol Biochem Behav* **19** 743–749 1983

36 Ryan C and B A Pappas Neonatal 6-hydroxydopamine lesions of spinal norepinephrine terminals fails to affect the ontogeny of swimming behavior *Dev Brain Res* **17** 258–262 1985

37 Sabol, K E , D B Neill, S A Wages, W H Church and J B Justice Dopamine depletion in a striatal subregion disrupts performance of a skilled motor task in the rat *Brain Res* **335** 33–43, 1985

38 Salmon, P C Stanford and J A Gray The effect of peripheral noradrenaline depletion on responding on a schedule of differential reinforcement of low rates of response (DRL) *Psychopharmacology (Berlin)* **87** 77–80 1985

39 Santer R M Long-lasting effects of the noradrenergic neurotoxin N-(2-Chloroethyl)-N-ethyl-2-Bromobenzylamine (DSP4) on prevertebral sympathetic neurones in the rat *Neurosci Lett* **61** 327–332, 1985
40 Schwartz, J C , J Costentin, M P Martres, P Protais and M Baudry Modulation of receptor mechanisms in the CNS hyper-and hyposensitivity to catecholamines *Neuropharmacology* **17** 665–685, 1978
41 Sievers J , H P Klemm, S Jenner, H G Baumgarten and M Berry Neuronal and extraneuronal effects of intracisternally administered 6-hydroxydopamine on the developing rat brain *J Neurochem* **34** 765–771, 1980
42 Singer, G and S Armstrong Cholinergic and beta-adrenergic compounds in the control of drinking behavior in the rat *J Comp Physiol Psychol* **85** 453–462, 1973
43 Smythe, G A , J E Bradshaw, R M Gleeson, H S Grunstein and M V Nicholson The central vs peripheral effects of clonidine on ACTH, corticosterone and glucose release *Eur J Pharmacol* **111** 401–403, 1985
44 Smythe, J W and B A Pappas Neonatal 6-hydroxydopamine potentiates clonidine's locomotor effects throughout maturation in the rat *Pharmacol Biochem Behav* **22** 1075–1078 1985
45 Spirduso, W W , P E Gilliam T Schallert M Upchurch D M Vaughn and R E Wilcox Reactive capacity a sensitive behavioral marker of movement initiation and nigrostriatal dopamine function *Brain Res* **335** 45–54 1985
46 Sporn, J R , B B Wolfe, T K Harden and P B Molinoff Supersensitivity in rat cerebral cortex pre- and postsynaptic effects of 6-hydroxydopamine at noradrenergic synapses *Mol Pharmacol* **13** 1170–1180, 1977
47 Sudo A Decrease in adrenaline content of various organs of the rat after 6-hydroxydopamine *Eur J Pharmacol* **114** 79–83 1985
48 Teicher M H N I Barber, J H Reichheld R J Baldessarini and S P Finklestein Selective depletion of cerebral norepinephrine with 6-hydroxydopamine and GBR-12909 in neonatal rat *Dev Brain Res* **30** 124–128 1986
49 Trombley P F E Allen, J Soyke C D Blaha, R F Lane and B Gordon Doses of 6-hydroxydopamine sufficient to deplete norepinephrine are not sufficient to decrease plasticity in the visual cortex *J Neurosci* **6** 266–273 1986
50 Van Der Gugten J M Palkovits H L ' M Wijnen and D H G Versteeg Regional distribution of adrenaline in rat brain *Brain Res* **107** 171–175, 1976
51 Van Gool W A M Mirmiran and F Van Haaren Spatial memory and visual evoked potentials in young and old rats after housing in an enriched environment *Behav Neural Biol* **44** 454–469 1985
52 Watson M and J G McElligott 6-OHDA induced effects upon the acquisition and performance of specific locomotor tasks in rats *Pharmacol Biochem Behav* **18** 927–934, 1983
53 Westerink B H C Analysis of trace amounts of catecholamines and related compounds in brain tissue a study near the detection limit of liquid chromatography with electrochemical detection *J Liquid Chromatogr* **6** 2337–2351, 1983
54 Zahniser, N R G R Weiner T Worth K Philpott, R P Yasuda, G Jonsson and T V Dunwiddie DSP4-induced noradrenergic lesions increase beta adrenergic receptors and hippocampal electrophysiological responsiveness *Pharmacol Biochem Behav* **24** 1397–1402, 1986