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RESEARCH NOTE

Growth and Plasticity of Rat Cerebral Cortex after Central Noradrenaline Depletion

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Male Wistar rats (aged 31 to 33 days) received bilateral intraventricular injections of saline or 6-hydroxydopamine and were subjected to either "standard" or "enriched" rearing conditions for 42 days. The treatment reduced cerebral cortical noradrenaline by 80% and decreased the growth of the cerebral cortex. It did not, however, prevent experience in an enriched environment from enhancing the growth of the cerebral cortex.

Sensory–motor interaction with the environment is known to affect the development of the brain. One example of this effect is that the cerebral cortex of rats that have been reared in an "enriched" environment is heavier than that of rats reared in a standard (laboratory) environment (8, 14). As there seems to be a relationship between noradrenaline (NA) and environmentally induced changes in the cerebral cortex [(2, 4, 9, 12, 13) but see also (1, 5)], we examined the possibility that NA neurotransmission is involved in the enhanced growth of the cerebral cortex of rats reared in an enriched environment. In a previous study (2) we found that neonatal subcutaneous injections of 6-hydroxydopamine (6-OHDA) caused a 70% reduction in the cortical NA content and a reduction in cortical as well as in body weight. The increase in cortical weight due to the enriched environment appeared to be greater for the controls than for the drug-

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treated rats. However, as neonatal treatment is known to affect the peripheral as well as the central noradrenergic system (3), the results may have been influenced by a general growth retardation. In the present study, 6-OHDA was injected directly into the lateral ventricles of 32-day-old rats. In this way we hoped to find out whether projections of the central NA system are directly involved in cerebral cortical development and, in particular, whether or not they are directly involved in the enhanced growth of the cerebral cortex after rearing in an enriched environment.

Six litters of eight male Wistar pups, from mothers that delivered within the same 24 h, were divided at weaning (day 28) so that two pups from each litter were randomly assigned to each of four experimental groups. At 31 to 33 days of age the rats of two of the groups received bilateral intraventricular injections of 50 μg 6-OHDA in 2 μl saline while under anesthesia [fentanyl (Hypnorm) 0.1 ml/100 g body weight]. The sites of injection were 1.5 mm lateral to the sagittal sinus, 0.2 mm posterior to bregma, and 4.5 mm "downward" from the skull surface with the brain placed horizontally (6). The other two groups were injected with saline following an identical procedure but without 6-OHDA. Of the 48 pups, 7 died during anesthesia and one pup was discarded 2 days after injection of 6-OHDA as it did not have a cage-mate. All animals were housed two per cage (38 × 26 × 16 cm) at 24°C with 12 h light:12 h darkness (lights on at 1330). On days 34 to 75 one of the 6-OHDA-injected groups and one of the saline-injected groups were placed 2.5 h daily, on alternative days from 900 to 1130 and from 1130 to 1400, in large cages (75 × 75 × 85 cm; five rats per cage) in which they were exposed to a different combination of ropes, ladders, boxes, tubes, and other "toys" every day (10).

All four groups of rats were killed at 76 or 77 days of age, and their brains were immediately removed and weighed. Each brain was then dissected into eight parts in the order indicated in Table 1. The dissection and weighing were carried out without knowing the rats' group of origin. The data of one (saline-injected) pup were not included in the further analysis of the data as it had a visible cortical defect (presumably injured or infected during the injection procedure). The noradrenaline and dopamine contents of each rat's cerebral cortex were determined using a radioenzymatic method (15).

Table 1 shows the influence of the rearing environment and of the drug treatment on body weight, on regional brain weights, and on the NA and dopamine concentrations in the cerebral cortex (the "cerebral cortex" in our dissection procedure included the amygdala and large parts of the olfactory tubercle). Two-way analyses of variance showed significant drug (main) effects for the decline in total brain weight ($F[1,35] = 6.23$; $P < 0.05$), weight of the cerebral cortex ($F[1,35] = 7.68$; $P < 0.01$), and

TABLE I

Influences of an Enriched (EC) or Standard (SC) Environment in 6-Hydroxydopamine (6-OHDA) and Saline-Treated Rats[a]

Treatment	Environment:	Saline		6-OHDA	
		SC	EC	SC	EC
Number of animals		9	10	10	10
Body weight (g)					
Day 28		69 ± 2	70 ± 3	69 ± 1	70 ± 2
°Day 43		125 ± 3	116 ± 4	119 ± 3	113 ± 4
Day 75		269 ± 8	255 ± 11	264 ± 6	249 ± 9
Brain weight (mg)					
*/°°Total		1815 ± 20	1889 ± 26	1765 ± 23	1833 ± 14
Olfactory bulb		51 ± 3	54 ± 4	53 ± 2	56 ± 5
*Cerebellum		258 ± 6	266 ± 3	250 ± 3	255 ± 5
Colliculi		59 ± 3	58 ± 3	60 ± 2	61 ± 2
Hypothalamus		42 ± 3	44 ± 2	40 ± 2	43 ± 2
°°Brain stem		202 ± 3	208 ± 4	194 ± 3	210 ± 3
Hippocampus		121 ± 3	116 ± 3	116 ± 2	120 ± 3
**/° Cerebral cortex		790 ± 11	823 ± 14	760 ± 11	789 ± 10
"Rest"		232 ± 6	255 ± 8	231 ± 7	229 ± 8
Per milligram cerebral cortex					
*** Noradrenaline (ng)		0.32 ± 0.01	0.32 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
*** Dopamine (ng)		1.07 ± 0.09	1.06 ± 0.06	0.65 ± 0.04	0.72 ± 0.07

[a] Values are $\bar{x} \pm$ SE. Two-way analysis of variance showed: drug main effect—$*P < 0.05$, $**P < 0.01$, $***P < 0.001$; environment main effect—$°P < 0.05$, $°°P < 0.01$. All other main and interaction effects were not significant at the 5% level.

weight of the cerebellum ($F[1,35] = 5.24$; $P < 0.05$) as well as for the NA ($F[1,35] = 671.81$; $P < 0.001$) and dopamine ($F[1,35] = 31.11$; $P < 0.001$) content of the cerebral cortex. Significant environment (main) effects were found for the total brain weight ($F[1,35] = 10.98$; $P < 0.01$), the weight of the cerebral cortex ($F[1,35] = 6.91$; $P < 0.05$), the weight of the brain stem ($F[1,35] = 11.32$; $P < 0.01$); and the body weight on day 43 (i.e., on the 10th day of enriched rearing: $F[1,35] = 4.17$; $P < 0.05$). The environment did not significantly affect the NA content of the cerebral cortex [for a review of contradicting findings see (14)] and there was no significant interaction between the drug and the environmental treatments (interaction effect: $F[1,35] = 0.03$; $P > 0.8$).

As the drug treatment did not significantly affect the body weight in this study, we confirm that depleting the central noradrenergic projections directly impairs cerebral cortical growth [see also (7)]. The mechanism

underlying this effect is not yet known, but the reduction in cortical growth may be a result of changes in the permeability of the cerebral microvasculature as a result of the reduced NA concentration (11).

The increase in cortical weight due to experience in the enriched environment, which reflects changes in various brain parameters [see (8)] and can be considered a manifestation of cortical plasticity, was similar in drug-treated and saline-treated rats (both approximately 4%, see Table 1). Although we can not exclude the possibility that the difference in age at the time of treatment accounts for the differences between the results of this study and those of our previous study (2), an indication that the different methods of 6-OHDA application are responsible for this difference is the fact that neonatal subcutaneous 6-OHDA treatment reduced the rats' body weights significantly, whereas postweaning intraventricular 6-OHDA treatment did not. Neonatal 6-OHDA treatment may, therefore, have reduced the environmentally induced cortical growth by generally retarding the development rather than by interfering with the plasticity of the cerebral cortex.

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