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IMPAIRED GROWTH OF THE CEREBRAL CORTEX OF RATS TREATED NEONATALLY WITH 6-HYDROXYDOPAMINE UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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Male Wistar rat pups received subcutaneous injections of either 100 mg/kg 6-hydroxydopamine (6-OHDA) or saline on days 1, 3, 5 and 7. The noradrenaline (NA) content of the cerebral cortex was reduced by 70% while sleep registration during the first two weeks of life did not show any significant differences between drug- and saline-treated animals. After weaning (day 25), both 6-OHDA- and saline-injected animals were reared under standard and enriched environmental conditions. Whereas a clear increase in cerebral cortical weight after experience with an enriched environment was found in saline-treated rats, 6-OHDA-treated animals had lower cortical weights and showed less increase due to the environment. These results demonstrate that catecholamine neurotransmission during early development influences the development and plasticity of the cerebral cortex.

Environmental experience influences the growth of the cerebral cortex in rats. Exposing rats to an 'enriched' environment after weaning increases cerebral cortical weight; morphological studies indicate that this is due to an increase in neuronal ramifications and in the number of glial cells [11]. A recent study indicates that chronic pharmacological suppression of active sleep (AS) in early postnatal life reduces the ability of the rat cerebral cortex to grow in response to sensory experience later in life [8]. AS was suppressed by injecting the rats with clonidine (an α-adrenergic agonist).

AS is a complex behavioral state in which many parts of the central nervous system are simultaneously involved. At the cortical level AS is characterized by polyneuronal bursts of action potentials [5]. The temporal relationship between AS and the activity of neurons in the locus coeruleus (the origin of noradrenergic projections to the cerebral cortex) suggests that noradrenaline (NA) neurotransmission in the cerebral cortex is correlated with the behavioral state [1]. Pharmacological attempts to suppress AS indicate that this is accomplished by interfering with α-

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noradrenergic receptor activity [3]. It is of interest, therefore, to know whether neonatal clonidine treatment reduces cerebral cortical growth [8] because it inhibits NA release in the cortex or because it interferes with the specific cortical bioelectric activity patterns which normally occur during AS [5]. NA depletion in certain brain areas (including the cerebral cortex) by means of neonatal 6-hydroxydopamine (6-OHDA) treatment has been shown not to affect the sleep pattern of the developing rat [12]. Although 6-OHDA interferes with both NA and dopamine neurotransmission, recent experiments have demonstrated that repeated subcutaneous injections of 6-OHDA during the first week of life in rats dramatically reduce cortical NA with only a slight increase in dopamine content [2]. If the cortical bioelectric activity associated with AS mediates the effect of clonidine on cortical development [8], 6-OHDA would not be expected to have the same effect on regional brain weights. If, on the other hand, early NA neurotransmission regulates cortical development, 6-OHDA should have a similar effect to that found with clonidine.

Male Wistar rat pups (from 10 mothers which delivered within approximately 24 h) were cross-fostered within one day after birth, resulting in 6 litters of 8 pups. Three of the litters received a subcutaneous injection of 6-OHDA (100 mg/kg in 0.1 ml saline) on days 1, 3, 5 and 7 [2]. The other 3 litters were injected with saline (controls). The animals were kept in standard laboratory cages at 24°C with 12 h light/12 h darkness (lights ON at 13.30 h). After weaning at 25 days of age all of the rats were housed two per cage in standard laboratory cages (38 × 26 × 16 cm). Four pups of each litter were randomly assigned to 'standard' and the other 4 to 'enriched' living conditions. As half of the litters were drug treated and the other half were controls, this resulted in 4 groups of 12 rats each. From day 30 the animals that were assigned to 'enriched' living conditions were put for 2½ h daily (09.30 to 12.00 h) in large cages (75 × 75 × 85 cm; 6 per cage) in which they were exposed to a different combination of ropes, ladders, boxes, tubes and other 'toys' every day [6]. All 4 groups of rats were sacrificed at 75 days of age, and their brains were immediately removed and weighed. Each brain was then dissected into 8 parts in the order indicated in Table I. The dissection was done without knowing the rats' group of origin. A radioenzymatic method was used to determine the NA content of the cerebral cortex [13].

Separate groups of 6-OHDA- and saline-treated pups were used for the sleep observations. In one experimental and one control litter the behavioral state was determined with the help of EMG electrodes which were implanted in the pups' neck muscles (4 pups from each litter on day 3, and another 4 on day 11). The first 8 pups were recorded daily during the light period (lights ON at 09.00 h) on days 4–8, and the other 8 pups on days 12–16. Each pup was recorded for 30 min during the light period in a recording cage situated in a sound-proof box together with two of its littermates (see ref. 7). Pups from another pair of litters were observed two at a time on days 2–12 (between 11.00 and 12.30 h; lights ON at 09.00 h) in an incubator at

30°C after at least 10 min adaptation time (52 observations). The behavioral state of the pups being observed was coded at the end of fifty 10-sec intervals per session. AS was defined by the occurrence of twitches in limbs and tail, a relaxed posture and irregular breathing.

Weekly measurements before weaning showed a retarded development of the body weight of pups treated with 6-OHDA which persisted up to the end of the experiment (Table I). In the 'enriched' environment, behavioral observations indicated that both groups actively explored their surrounding throughout the 2½ h. We did not find any significant effects on the time spent in AS for either recording method (see also ref. 12). Obviously, this does not prove that AS is completely unaffected by 6-OHDA but, in comparison to the almost total AS deprivation of clonidine treatment [7, 8], a possible effect, as far as the amount of time spent in AS is concerned, can be considered negligible.

Table I shows the influence of the rearing environment and of the drug treatment on body weight, on regional brain weights and on the NA content of the cerebral cortex. Neonatal 6-OHDA treatment reduced the cortical NA content by approximately 70%, whereas the environment appears to have had no effect on the cortical noradrenaline level. Two-way analyses of variance showed significant drug (main) effects for the decline in body weight ($F(1,44)=4.7$; $P<0.05$) and in the weight of

TABLE I

INFLUENCE OF AN ENRICHED (EC) OR STANDARD (SC) ENVIRONMENT ON MEAN REGIONAL BRAIN WEIGHTS (± S.E.M.) IN 6-OHDA- AND SALINE-TREATED RATS

Treatment	Saline		6-OHDA	
Environment	SC	EC	SC	EC
[a]Body weight (g)	248 ± 11	253 ± 5	243 ± 10	221 ± 8
[b]Brain weight (mg)	1812 ± 14	1872 ± 14	1727 ± 25	1739 ± 21
[c]Olfactory bulb	50 ± 1	54 ± 2	53 ± 2	48 ± 3
[b]Cerebellum	251 ± 3	256 ± 3	232 ± 4	229 ± 4
Colliculi	57 ± 1	58 ± 2	56 ± 2	58 ± 2
Hypothalamus	34 ± 1	38 ± 1	34 ± 2	35 ± 1
[a]Brainstem	200 ± 5	203 ± 4	196 ± 5	186 ± 5
Hippocampus	116 ± 2	119 ± 4	108 ± 3	115 ± 4
[b,d]Cerebral cortex	787 ± 7	833 ± 8	753 ± 11	772 ± 10
'Residue'	248 ± 7	243 ± 5	231 ± 12	228 ± 8
[b]Noradrenaline in the cerebral cortex (ng/mg)	0.39 ± 0.02	0.38 ± 0.01	0.12 ± 0.03	0.11 ± 0.02

Two-way analysis of variance showed the following effects:
[a]Drug main effect ($P<0.05$);
[b]Drug main effect ($P<0.001$);
[c]Drug by environment interaction ($P<0.05$);
[d]Environment main effect ($P<0.01$).
All other main and interaction effects are not significant at the 5% level.

the cerebellum ($F(1,44) = 42.5$; $P < 0.001$) as a result of 6-OHDA treatment (see also refs. 4 and 9), which had not been found after clonidine treatment [8]. This may reflect a general growth retardation due to the systemic application of the drug and/or to the early onset of the treatment.

In the cerebral cortex we found significant (main) effects for drug ($F(1,44) = 26.9$; $P < 0.001$) and for environment ($F(1,44) = 12.1$; $P < 0.01$) (Table I). The interaction effect, however, was not significant ($F(1,44) = 2.08$; $0.1 < P < 0.2$), which does not directly suggest a connection between the effects of the 6-OHDA treatment and of the environment. However, since the effect of 6-OHDA on cortical weight in this study was very similar to the effect found for clonidine, we compared the effects of the two drugs on cortical growth under the different environmental conditions. Exposure to the enriched environment led to a 6% increase in cortical weight in the saline-treated groups (controls) of both studies (see ref. 8). This effect was reduced to 3% in the 6-OHDA-treated animals and to 4% in the clonidine-treated group. Correspondingly, we found, as in the clonidine study, that *t*-tests (one-tailed) could demonstrate a significant increase in cerebral cortical weight due to environment for the control ($t(22) = 4.09$; $P < 0.001$) but not for the 6-OHDA-treated group ($t(22) = 1.27$; $0.1 < P < 0.2$). This implies, as was suggested in the clonidine study [8], that environmental experience is of less influence in the drug-treated animals.

Since early 6-OHDA and clonidine (a selective α_2-NA agonist) treatment both seem to have the same effect on cortical growth, it appears that NA neurotransmission has a regulative role in cortical development (see also refs. 2 and 10). It is interesting, therefore, to look for common mechanisms by which 6-OHDA and clonidine could act on cortical growth. Since early clonidine treatment did not change the level of cortical NA measured at 75 days of age [7], whereas 6-OHDA permanently reduced the level of cortical NA (see also ref. 2), an explanation for the reduction in the effect of the enriched environment that simply assumes that NA must be present for growth of the cerebral cortex is not sufficient, especially since the clonidine treatment was terminated before the rats were subjected to different environmental experience [8]. We conclude that normal function of NA during early development is necessary for the further development of the cerebral cortex. However, it is desirable to examine whether: (1) selective cortical NA depletion will induce similar effects to those of systemic application of the drug; (2) the effect of NA on cortical development is restricted to a particular ('sensitive') period during the early development; and (3) local perfusion of NA in previously 6-OHDA-treated animals will restore cortical plasticity. What the present study shows is that NA neurotransmission plays a crucial role in development and plasticity, and that if AS is involved, that it is probably by virtue of its effect on NA neurotransmission [1].

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