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CORTICAL NORADRENALINE DEPLETION ELIMINATES SPARING OF SPATIAL LEARNING AFTER NEONATAL FRONTAL CORTEX DAMAGE IN THE RAT

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The possibility that cortical noradrenaline (NA) is necessary for the sparing of function that occurs after neonatal frontal cortex damage was examined. Spatial localization by rats with frontal cortex damage sustained neonatally was better than by rats with similar damage sustained as adults. The sparing was abolished in rats depleted of cortical noradrenaline by means of neonatal 6-hydroxydopamine (6-OHDA) administration. NA depletion alone did not affect spatial localization. These data are consistent with the notion that NA has some general function in maintaining some forms of plasticity in posterior cortex.

Brain damage sustained early in development often has less severe behavioral consequences than similar damage sustained in adulthood. This sparing of function has been repeatedly observed after frontal cortex damage in rats and primates [2, 4], particularly in spatial localization tasks. There is not general agreement about the neural basis of this resiliency in young animals. Recent evidence, however, suggests that sparing after frontal cortex damage in rats is dependent upon neocortical circuitry in the remaining posterior cortex [5].

Several experiments have demonstrated that noradrenaline (NA) is necessary for maintaining the neocortical plasticity that is characteristic of kitten posterior cortex during development. Specifically, depletion of NA from kitten visual cortex by intraventricular injection of 6-hydroxydopamine (6-OHDA) blocks the shift in ocular dominance which is observed in single neuron responsivity after monocular deprivation. Continuous local microperfusion of NA restores plasticity to the NA-depleted visual cortex [3].

The objective of the present experiment was to determine if cortical NA in the young rat is essential for the sparing of behavioral function associated with neonatal frontal cortex damage. A similar suggestion about the importance of NA for functional compensation after brain damage has been previously made based upon separate considerations [10].

Sixty hooded rats from seven litters bred at the University of Lethbridge served

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as subjects. Beginning within 24 h after birth, half of these rats received 3 daily injections of 6-OHDA (100 mg/kg in 0.9% saline and 0.2% ascorbic acid, s.c.) and half received only the vehicle solution. At 7–9 days of age, 30 rats were anesthetized using hypothermia and the medial frontal cortex was bilaterally aspirated according to previously described methods [5]; of these, 14 had received 6-OHDA and 16 had received vehicle injections. The remaining rats were anesthetized and their scalps incised and sutured. At approximately 100 days of age, the medial frontal cortex was aspirated in 10 rats.

Behavioral testing was conducted in the Morris water task, while the rats were between 200 and 250 days old. The details of this procedure have previously been described [12]. Briefly, rats were trained to swim to a platform that was hidden just beneath the surface of cool water in a circular pool. The inside of the pool (diam. 85 cm, height 45 cm) was painted white and the wire mesh surface of the platform was 1.5 cm below the water. There were many conspicuous cues in the room visible from the pool. Milk powder was dissolved in the water to render the platform invisible to a viewer at the water level. The rats began their search from one of 4 locations at the pool's perimeter and the order of starting locations was randomly assigned. The platform remained in the center of one quadrant of the pool, in the same place within the testing room, for the first 20 trials, thereafter (until trial 36), it was positioned in the center of the diagonally opposite quadrant. We measured the latency to find the platform (maximum 120 sec), the swim distance, the magnitude of the initial heading error after swimming 30 cm, and, on the first trial after the platform was moved, the proportion of swim path within the previously correct quadrant. An experimenter at the pool's edge recorded the rat's swim path on a map of the pool and this was analyzed on an APPLE II graphics tablet.

At the end of testing half of the rats were sacrificed, their brains processed, embedded in celloidin, and sectioned at 20 μm. Every 5th section through the lesion and every 10th section through the rest of the brain was mounted and stained with cresyl violet. The lesion extent was assessed microscopically in coronal sections. The lesions were similar in the different groups, involving bilateral damage to the likely homologue of the dorsolateral frontal cortex of primates, and damaged the prelimbic, infralimbic and anterior cingulate areas of Krettek and Price [6]. The remaining half were sacrificed for catecholamine assays. Animals were killed by decapitation, the brain was rapidly removed and chilled in 0.9% saline on ice for 30 sec. The location of a lesion was sketched during this time. Four areas of the brain were then dissected: (1) brainstem, (2) hippocampus, (3) rostral cortex, and (4) caudal cortex. Neocortex was divided into rostral-caudal pieces by a cut 7 mm from the caudal border of the neocortex.

Tissue was homogenized in 1 ml 0.05 N $HClO_4$ containing also 100 ng 3,4-dihydroxybenzylamine hydrobromide as an internal standard and 0.8 mM $NaHSO_4$ as a reducing agent. A standard curve (tissue blank plus 3 standards containing known amounts of NA and DA (free base)) was run with each assay.

Samples were centrifuged at 3500 *g* for 45 min at 4°C. After centrifugation, all of the supernatant was transferred to conical tubes containing 20 mg acid-washed alumina and 500 µl 3 M Tris-HCl (pH 8.6 at 4°C). Samples were immediately vortexed for 1 min and shaken on a reciprocal shaked for 10 min. Alumina was washed twice with 1 ml 6 mM Tris-HCl (pH 8.6 at 4°C) and 3 times with 1 ml H_2O. After completely removing the H_2O, the catecholamines were extracted with 200 µl of 0.05 N $HClO_4$ by vortexing for 1 min. An aliquot of the supernatant was stored at −20°C until assayed.

The high performance liquid chromatography system employed a Whatman ODS-3 reverse phase C-18 column and a LC-4 amperometric detector with a glassy carbon electrode (BioAnalytic Systems). The mobile phase was composed of 3 parts

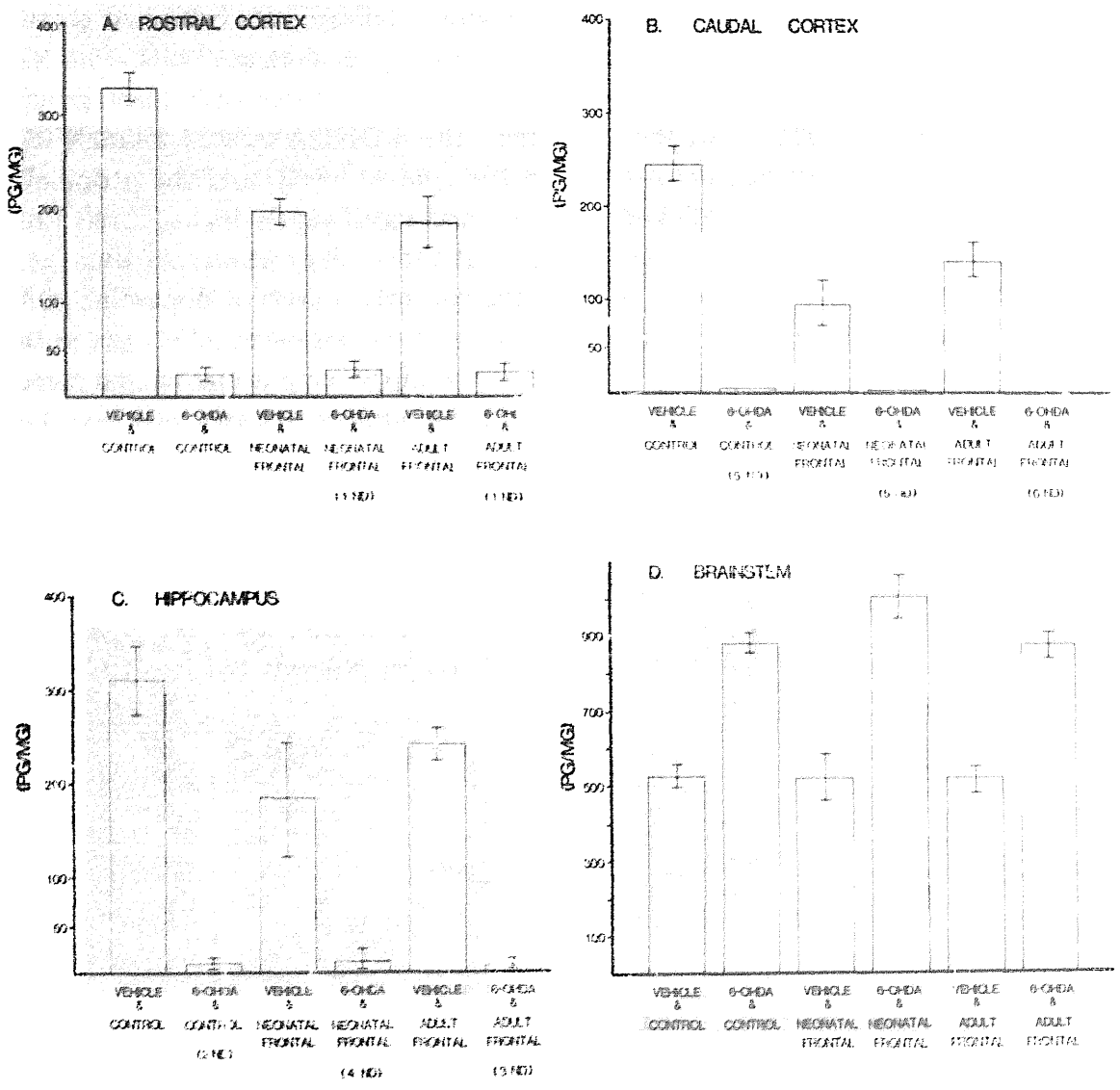

Fig. 1. The mean levels (± standard error of the mean) of noradrenaline in the rostral cortex (A), caudal cortex (B), hippocampus (C) and brainstem (D). The number of samples with no detectable (ND) noradrenaline are indicated. For statistical purposes the ND samples were assigned the value of the lowest detected level in the appropriate group.

of 0.1 M citric acid, 2 parts 0.1 M $NaHPO_4$, 0.0004% sodium octyl sulfate, and 15% methanol (pH 3.35). The mobile phase was pumped at 1 ml/min and continuously recycled. The detector potential was set at + 0.72 V vs Ag/AgCl reference electrode. Samples of 10 μl were assayed with a sensitivity of 50 pg for NA and 100 pg for DA.

Statistical evaluation of the behavioural data and the assays was conducted using an analysis of variance for repeated measures and follow-up comparisons were carried out using the LSD method with a significance level of 0.05.

The assays (Fig. 1) indicate that the 6-OHDA administration was very effective in reducing cortical NA. Significant differences in NA level were detected among the groups in caudal cortex ($F = 48.8$, $P<0.0001$), rostral cortex ($F = 89.7$, $P<0.0001$), hippocampus ($F = 25.8$, $P<0.0001$), and brain stem ($F = 30.1$, $P<0.0001$). Follow-up tests revealed that the relationship among the groups was the same for NA level in each of the forebrain regions; control rats exhibited greater concentrations than the adult and neonatal frontal cortex damaged rats, who had equivalent NA concentrations that were greater than the three equivalent groups that had received 6-OHDA. In the brainstem, the 6-OHDA-treated animals had higher concentrations of NA (170–190% of the control level), and the neonatally frontal cortex damaged, 6-OHDA-treated rats had significantly higher levels than any others. Brainstem NA levels in the groups that had adult or neonatal frontal cortex damage without 6-OHDA were equal to the controls. Levels of dopamine (DA) were not significantly different among the groups in the brainstem ($F<1$) or in the rostral cortex ($F = 2.4$, $P<0.05$). DA levels in the hippocampus and caudal cortex were similar among the groups, but because of the number of cases with very low levels, even in the control group, an ANOVA was not conducted.

The behavior of control rats in the spatial localization task (Fig. 2) was very

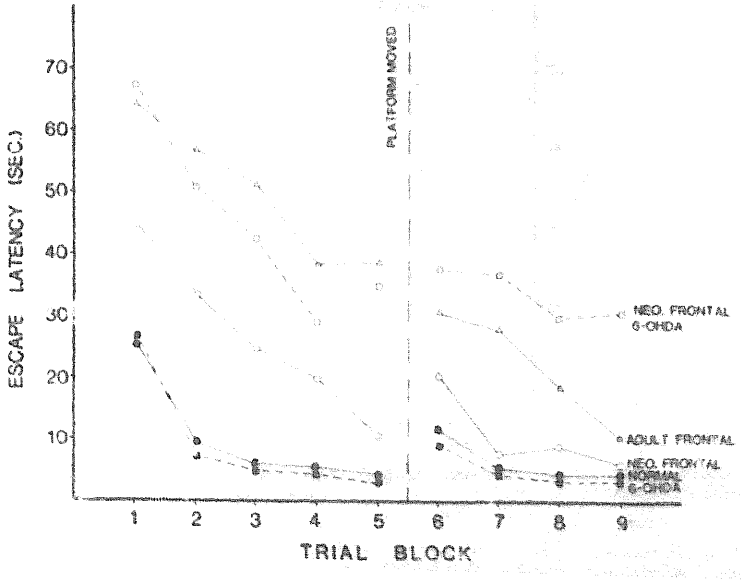

Fig. 2. Mean latency on blocks of 4 trials to find the hidden platform in the Morris water task. For clarity, the scores of the two adult operated groups have been pooled as they did not significantly differ. The vertical dotted line indicates when the platform was moved.

similar to previous reports [11, 12]. After the first block of trials they reliably swam directly to the platform, and when the platform was moved they took longer to find it, since they persisted in swimming in the previously correct quadrant. The rats who had received only 6-OHDA were not significantly different in any respect from the control group.

Neonatally operated rats showed faster acquisition in the water task than adult operates, but this behavioral sparing in acquisition of accurate localization was blocked by neonatal 6-OHDA administration, despite the fact that 6-OHDA administration alone did not affect spatial localization. The behavioral sparing associated with neonatal frontal cortex damage is not complete since the rats who did not receive 6-OHDA were significantly slower than control rats to locate the platform on trials blocks 1–4, and they were relatively less affected by moving the platform.

The overall ANOVA did reveal significant differences among the groups in the latency to find the platform ($F = 4.8$, $P<0.0022$), significant changes across blocks of trials ($F = 23.7$, $P<0.0001$), and a significant interaction between groups and trial blocks ($F = 1.6$, $P<0.03$). Follow-up comparisons revealed that the rats who had sustained frontal cortex damage as adults were significantly slower to locate the platform on every trial block except the last one. Of the two neonatally operated groups, the one sustaining only frontal cortex damage was significantly faster than the adult-operated group on trial blocks 1, 2, 3, 4, 5 and 7, indicating superior acquisition of accurate spatial localization; on the other hand, the neonatal group that had also received 6-OHDA was not significantly different from the adult-operates except on trial block 9 when they were slower.

These results clearly confirm that medial frontal cortex damage impairs the acquisition of accurate spatial localization in a novel swimming task [11], and confirm that substantial behavioral sparing occurs after neonatal damage [4]. Rats operated as neonates were significantly faster in learning to swim to a hidden platform; however, they were not as efficient as normal rats in this task.

Our novel findings were that spatial localization by rats with nearly total depletion of forebrain NA was completely normal. This result contradicts a recent suggestion that the dorsal noradrenergic system is a critical system for spatial learning [7]. This result also contradicts, at least for the case of spatial localization, a suggestion that some of the behavioral changes after frontal cortex damage are due to widespread depletion of cortical NA [8]. More importantly, however, despite the absence of a behavioral effect of 6-OHDA alone, we find that depletion of cortical NA completely blocks the sparing of behavioral function typically observed after neonatal frontal cortex damage.

These findings are consistent with those of Kasamatsu et al. [3] who found no direct effects of manipulating NA, but significant effects upon processes requiring cortical plasticity in early development. It is important to note that in rats neonatal 6-OHDA administration alone does not lead to any significant abnormalities in neocortical morphology, but does significantly increase the rate of synaptogenesis

in posterior neocortex during the first weeks of life [9]. Enhanced synaptogenesis in the dentate gyrus in response to entorhinal cortex lesions has also been reported [1] in neonatally NA-depleted rats. Thus, it is possible that NA may actually have an inhibitory effect upon synapse formation, a notion that appears to conflict with the suggestion of an important role for NA in maintaining functional plasticity. However, it should be borne in mind that a prolonged period of synaptogenesis in the young cortex may be critical for adaptive alterations in neural organization associated with early experience. Alternatively, the enhancement of synapse formation may interfere with the process of synapse elimination in posterior cortex, perhaps by reducing competition for terminal space among axons innervating the same cortical neurons.

In conclusion, neonatal frontal cortex damage, as compared with adult damage, is associated with significant sparing of the ability to learn accurate spatial localization. Neonatal depletion of cortical NA prevents this sparing of behavioral function. These results extend NA's role in cortical plasticity to the recovery of function observed after cortex damage in young rats, and may have important implications for neuropharmacological interventions in humans after brain damage.

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