mulated a greater amount of proline than the less drought resistant species.

Recently, however, the adaptive role of proline in drought resistance has been questioned. Tal *et al.* (1979) found that under water stress more proline accumulated in plants of cultivated tomato (*Lycopersicon esculentum* Mill.) than in plants of two of its wild relatives, which are more xerophytic. In a study of several dessication resistant species of vascular plants, Tymms and Gaff (1979) showed that some of them accumulated proline when they were air dried, and others did not.

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EFFECT OF NEOCORTICAL INSULT ON FOREBRAIN CATECHOLAMINE CONTENT IN THE RAT

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ABSTRACT

Experimental ischemic ablations in rats have been reported to produce changes in catecholaminergic innervation of the forebrain which are not predictable simply from knowledge that the brain has been damaged. The effects of surgical removal of portions of neocortex on forebrain norepinephrine (NE) and dopamine (DA) have not been examined. In the present experiment, neocortical aspiration ablations were produced in rats. Spectrofluorometric procedures were used to assess the effect of these ablations on forebrain concentration of NE and DA ninety days after the ablations had been made as compared to a group of intact rats. The forebrain concentration of both NE

and DA was found to be significantly higher in the group of rats with neocortical ablation. These results are relevant to inventigations of the effects on cognition and behavior of surgical destruction of portions of neocortex in humans and animals.

INTRODUCTION

Ischemic lesions produced experimentally in rats by unilateral ligation of the middle cerebral artery produce alterations in the pattern of neocortical catecholaminergic innervation. Histofluorescent light microscopy reveals that 40 days after the ligation the number of fluorescing varicosities increases above control values in intact neocortical regions and in the cerebellum (Robinson *et al.,* 1977). Robinson *et al.* interpret this increase in number of catecholaminergic varicosities as representing a case of "'compensatory" sprouting of uninjured axons. Devor and Schneider (1975) developed the concept of "compensatory" sprouting and use it to indicate that, if a portion of the terminal field of a neuron is destroyed, then the remaining arborizations of the axon will sprout in order to maintain the total number of axonal branches constant.

Biochemical measurements also indicate changes in catecholamine content of the forebrain after experimental unilateral neocortical infarct. These changes vary with time after the trauma. For example, Robinson *et al.* (1980) found that norepinephrine (NE) concentrations decreased between 50% and 70% in neocortex by 12 h after ligation of the middle cerebral artery, but returned to levels which did not differ from control values by 40 days after the operation. Although these experimenters did not find increased NE levels in ligated rats, which might have been expected because of their previous observations using histofluorescent methodology, their biochemical observations are still consistent with the "'compensatory" sprouting hypothesis. The area of the lesion was included in the assayed tissue. This area was essentially devoid of NE innervation yet the neocortex as a whole demonstrated virtually normal NE values. This strongly suggests that the NE content outside of the damaged area had increased above normal values.

The experiment reported in this communication assessed the biochemical effects of a different form of neocortical insult. Whereas Robinson and his colleagues produced ischemic infarcts, aspiration ablations were produced in the present experiment. This type of lesion is produced both in neurosurgical procedures for treatment of intractable focal epilepsy in humans, and is used experimentally in animals to assess brain function in behavior. Therefore, it seemed reasonable to determine the consequences for forebrain NE and dopamine (DA) concentrations of this type of lesion and to compare these effects to the consequences of ischemic infarcts produced by Robinson and his colleagues.

METHODS

Surgical Procedures.

Twenty-two male Long-Evans hooded rats weighing between 385 and 472 g at the time of surgery served as subjects for this experiment. Ten of these rats served as unoperated controls (Group N). Twelve rats were given bilateral aspiration lesions of the dorsolateral neocortex (Group C). The procedure for producing aspiration lesions of rat neocortex, as followed in our laboratory, has been described in detail in a recent publication (Isaacson and Woodruff, 1976) and will be presented only briefly here.

Each rat in Group C was anesthetized with an intraperitoneal injection of 60 mg of sodium pentobarbital (Nembutal) for each kg of rat body-weight. The scalp was shaved and the rat's head positioned in a Kopf stereotaxic instrument. The scalp was then incised along the midline and the dorsum of the skull was exposed by retraction of the muscles and fascia overlying the skull. Bilateral holes were drilled in the skull with a dental burr approximately 2 mm posterior to bregma. The holes were expanded with rongeurs until they were about 4 mm long by 3 mm wide. The dura under the opening was then removed with forceps and the exposed neocortex was gently removed by aspiration through a blunt 22 gauge hypodermic needle. The aspiration was concluded when the corpus callosum in the region beneath the skull opening had been exposed. Bleeding was arrested with Gelfoam. The muscles and fascia were replaced and the scalp was sutured. Antibiotics were given to combat possible postoperative infections.

Biochemical Procedures.

Ninety days after the lesions were produced in the rats in Group C, each rat in Groups C and N was killed by means of cervical dislocation and the brain was removed rapidly over ice. The forebrain was separated from the hindbrain at the level of the intraquadrigeminal ridge and the pes pedunculi and assayed for DA and NE content by means of a modification of the fluorometric method of Shellenberger and Gordon (1971). The forebrain was first weighed and then homogenized in 2.5 ml of 0.5 N perchloric acid. The homogenate was centrifuged and the 2.0 ml aliquots of the supernatant thereby obtained were placed in conical centrifuge tubes with 500 mg of alumina. Four ml of 0.5 M Tris buffer was added to the tubes to adjust the pH to 8.2 to 8.5 and the tubes were shaken for 10 min. Subsequently the tubes were centrifuged and the supernatant discarded. The alumina in each tube was then washed with 5 ml of glass distilled water, shaken for 5 min and centrifuged. The supernatant was again discarded. The final step in purification of NE and DA was accomplished by eluting the catecholamines from the alumina with 0.2 N acetic acid (2.5 ml/tube of washed alumina), then shaking and centrifuging the tubes.

Two 1.0 ml aliquots of the resulting supernatant were placed into separate test tubes for the oxidation procedure. One aliquot served as a test sample and the other as a tissue blank. The pH in all tubes was adjusted to approximately 7.0 by addition of 1.0 ml of 0.1 M EDTH—1.0 sodium acetate. The test samples were then oxidized to form quinone derivatives by the addition of 0.1 ml, 0.1 N ethanolic iodine. The oxidation reaction was stopped exactly 3 min after it was begun by addition of 0.2 ml alkaline bisulfite. The oxidation procedure is thought to cause formation of fluorescent derivatives of NE and DA, but these are unstable in alkaline solution. For this reason 0.2 ml of 5 N acetic acid was added exactly 2 min after the alkaline bisulfiite to adjust the pH to approximately 5.4.

Reverse oxidation was produced in each tissue blank aliquot to prevent the oxidation of catecholamines to fluorescent compounds. In addition to the samples and tissue blanks, five internal standards containing known amounts of NE and DA and a reagent blank containing 1.0 ml of 0.2 N acetic acid and 1.0 ml of 0.5 N perchloric acid were carried through the entire purification, elution and oxidation sequence. Moreover, a blank and five external standards containing known amounts of NE and DA were prepared prior to the oxidation procedure. The final volume of these standards was adjusted to 1.0 ml with 0.2 N acetic acid, and replicate samples were oxidized or reverse oxidized.

Upon completion of oxidation all of the tubes were placed into boiling water for 20 min after which they were cooled for half an hour. All tubes were then read in quartz curvettes at 30 sec intervals in a Farrand Spectrofluorometer. Activation and emission slits were set at 20 mm/40 mm and 10mm/20 mm respectively. The excitation wavelength for NE was 380 mμ and the emission wavelength 485 mμ. The tubes were then replaced in the boiling water bath for 5 min, allowed to cool for 30 min, and reread for DA (excitation wavelength 310 mμ, emission wavelength 380 mμ).

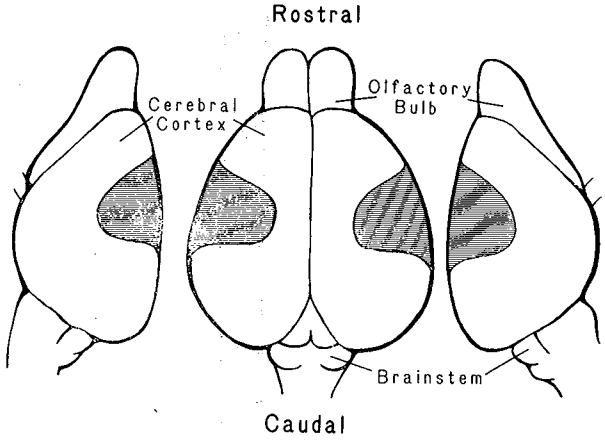

FIG. 1: *Schematic reconstruction of a typical bilateral neocortical ablation produced in the rats in Group C. Cross-sectional reconstructions of such lesions have been published by Woodruff and Isaaacson (1972).*

Results

The extent of the neocortical lesions was similar to the size of lesions previously described in reports from this laboratory (e.g. Woodruff and Isaacson, 1972). A schematic reconstruction of a typical lesion is presented in Figure 1. The ablated area is roughly comparable to the surface area damaged as a consequence of ligation of the middle cerebral artery in the experiments of Robinson and his co-workers. However, the effect of the lesions on forebrain NE and DA was somewhat different from the results reported by Robinson's group.

TABLE I: *Mean $\overline{(X)}$ and standard error (S.E.) of the mean forebrain content of norepinephrine and dopamine in ng/g of tissue weight in neocortically ablated and intact rats.*

	Norepinephrine	Dopamine
Neocortically Ablated Rats	$\overline{X}$=554.8	$\overline{X}$=1032.3
	S.E.= 21.1	S.E.= 26.8
Unoperated Rats	$\overline{X}$=405.6	$\overline{X}$= 798.8
	S.E.= 12.6	S.E.= 28.1

Extraction efficiency (internal standard slope divided by external standard slope) for NE ranged from 67% to 91% and for DA from 83% to 95%. The spectrofluorometer readings were converted to nanograms after reverse oxidation blank readings were subtracted from each sample reading. Conversion was made by the formula $x=\frac{y-b}{m}$, where b is the y intercept and m is the slope calculated from the external standards; y is the fluorometry reading corrected for recovery, and x is the amount of NE or DA for the sample. The mean and standard error of the mean ng NE and DA per g of tissue weight is presented in Table 1. T-tests (two-tailed) were conducted to compare NE and DA forebrain content of Group N to Group C and the results were very statistically significant: NE; t=5.81, df=20, $p < .001$; DA; $t = 7.97$, $df = 20$, $p < .001$. As can be seen from Table 1, the forebrain content of both catecholamines was significantly higher in the neocortically ablated rats than in the control animals.

Discussion

The principal finding of the present experiment is that bilateral ablation of a portion of the cerebral neocortex of the rat results, 90 days after the operation, in significant increases in the amount of NE and DA contained in the forebrain. Although the relatively long postoperative period indicates that the increase in transmitter content is probably permanent (Moore *et al.*, 1971), the methodology employed in this experiment does not permit exact elucidation of the cause of this increase in forebrain transmitter content and at least two explanations may be offered to account for this observation. Both of the explanations proposed below rely upon the assumption that some form of compensatory change, either biochemical or morphological, or both, occurs in the axons and terminal boutons in intact brain regions after neocortical insult.

The cell bodies of origin of the NE-and Da-containing axons and terminals destroyed by the ablation are found in the pons and midbrain (Dahlstrõm and Fuxe, 1965; Lindvall and Bjorkland, 1974) and each of these neurons has a relatively large terminal field. Therefore, destruction of the terminal field of some of these neurons could lead either to enhanced levels of transmitter in the remaining axons and terminals, or to acutal increase in the number of terminals (compensatory sprouting).

Catecholaminergic neurons have been reported to exhibit a profound ability to undergo reconstruction after damage to terminal areas (e.g. Moore *et al.*, 1971; Woodruff and Baisden, 1980) and the work of Robinson *et al.* (op. cit.) indicated that the number of axonal varicosities increases significantly as a result of ischemic neocortical damage. For these reasons, it seems more likely that the increases in NE and DA observed in the present experiment are due to actual morphological expansion of terminal zones of catacholaminergic axons remainnig after the ablation, than to simple enhanced content of catecholamines in existing morphologically static, neurons. However, histofluorescent microscopy should be applied to test this hypothesis.

The results of this experiment are not inconsistent with those obtained by Robinson *et al.* (op. cit.). However, neither are they entirely compatible with those earlier results. Although they reported an increase in the number of fluorescing catecholaminergic varicosities, Robinson *et al.* did not find differences between rats with neocortical ischemic lesions and control rats when biochemical measurement was made of NE and DA in neocortex. The tissue samples used in the present experiment included the entire forebrain and the olfactory bulbs. Therefore, it could be that increases in NE and DA were found in the present experiment, and not by Robinson *et al.* (op. cit.), because the number of brain structures included in our assay allowed incorporation of more areas where compensatory increases occurred. It may also be that the dif-