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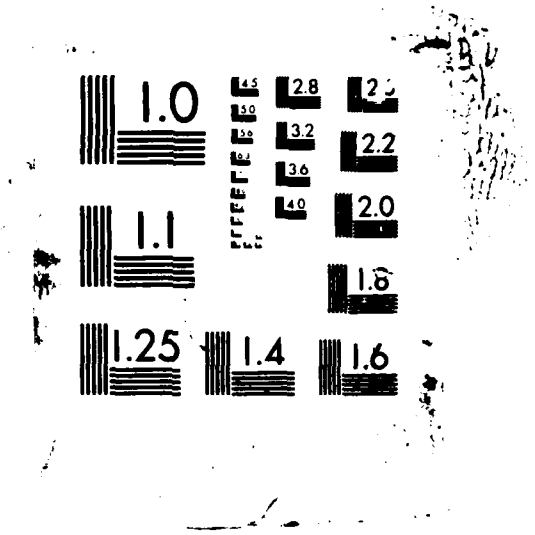
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Abstract:

Glutamic acid decarboxylase (GAD) activity was measured in the cerebral cortex of animals after acute and chronic lesions to basal forebrain cholinergic nuclei. Such lesions were shown to result in an extensive depletion of cholinergic markers in parietal cerebral cortex. A statistically significant 30% decrease in GAD activity was first detected at 6 weeks postlesion and was still measurable 8 months after the lesion. These results suggest that cholinergic inputs to cortex indirectly or directly influence GABAergic transmission in cortex.

Glutamic acid decarboxylase activity decreases in mouse neocortex after lesions of the basal forebrain

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The cholinergic projection neurons in the basal forebrain of mammals have recently received much attention due to their apparent involvement in certain degenerative diseases of the human nervous system[1,2,16,26,29,30]. The neocortical terminal fields for the different groups of cholinergic basal forebrain neurons are now rather well established and several laboratories have used lesions of these neuronal groups to study cerebral neocortex deprived of its cholinergic inputs[4,6,10-15,20,21,28].

Several different assays have been used in the past to characterize the cortical effects of basal nucleus (BN) lesions and to establish the postlesion integrity of non-cholinergic systems[12-15,28]. Glutamic acid decarboxylase (GAD) activity levels have been used as a marker for direct lesion effects on neocortex. Since this enzyme is contained in GABAergic cortical interneurons[23], activity levels should not be changed by a subcortical lesion. GAD assays have generally been performed 1 week after BN lesion, at a time when the depletion of acetylcholine (ACh) has reached its maximum. At this time, no changes in GAD activity are expected and data from several studies have failed to show any statistically significant changes in enzyme activity[12-14].

Lesions of the ventromedial globus pallidus area in the adult mouse have been performed in our laboratory to provide an ACh depleted host cortex for transplantation of embryonic cortical tissue. The present study of GAD activity in cortex was undertaken to compare the effects of our electrothermic lesions of mouse basal forebrain with those already published for rat. Since most of our

transplant experiments are analyzed 2-3 months after basal forebrain lesions, we assayed animals several months postoperatively, in addition to acutely lesioned mice. In the following paper we describe a significant decrease of GAD activity which first becomes measurable 6 weeks after the ventromedial globus pallidus lesion and which appears to persist for the lifetime of the lesioned animal. Because of the late onset of this decline in cortical GAD activity we propose that it may reflect an adjustment of cortical circuitry in response to basal forebrain damage.

All animals used in this study were male BALB/c mice derived from our own breeding colony. Mice were 2-3 months of age when the lesion was performed. Animals assayed at each time point came from several different litters.

For lesioning of the ventromedial globus pallidus area, mice were mounted in a stereotaxic apparatus with earbars at an angle of 5 degrees below the incisor bar. A hole was drilled through the skull overlying the right olfactory bulb. An electrode was mounted in the stereotaxic apparatus at an angle of 53 degrees above the horizontal and 4 degrees to the right of vertical. The electrode was lowered into the brain in a position 1 mm to the right of the midline and 2 mm anterior to the frontonasal skull suture, advanced in an anterior to posterior trajectory along the base of the brain. Lesions were made, starting 4 mm after entry into the brain by passing 1.7 mA of constant current once every mm for 4 mm at a duration of 30s each. After the last lesion, the electrode was withdrawn from the brain, the skin sutured and the animal allowed to recover from the anesthesia before returning it to its cage.

Cortical GAD activity was measured using the radioenzymatic assay of MacDonnell and Greengard[18]. In most cases, three 1.7 mg aliquots of tissue were incubated for 30 min at 38 degrees C in the presence of 1 mM (^{14}C)glutamate. The $^{14}CO_2$ that was liberated by the decarboxylation of glutamate was trapped

and counted.

For the determination of cholineacetyltransferase (ChAT) activity the assay procedure according to the protocaol of McCaman and Hunt[17] , as modified by Fonnum[8] , was used. Acetylcholinesterase (AChE) staining was performed according to Hardy et al.[9].

The lesions of the ventromedial globus pallidus result in a reproducible cholinergic depletion of parietal cortical areas. Determination of ChAT enzyme activity shows a 30-70% decrease in the hemisphere ipsilateral to the lesion when compared to the contralateral hemisphere. AChE histochemistry shows a near total depletion of the ipsilateral cortex (Fig. 1). The BN lesion induced depletion of cholinergic markers reaches its maximum by 7-9 days after the lesion and the AChE depletion remains constant for several weeks.

GAD activity levels were initially measured in animals several months after the basal forebrain lesion. To our surprise, the cortex ipsilateral to the lesion showed a significant ($P<0.05$) decrease of GAD activity compared with both the opposite hemisphere and cortex of unoperated controls. In contrast, a subsequent assay of animals lesioned 7-9 days prior to sacrifice showed no statistically significant change in GAD activity (FIg. 2). We then studied the time course of the decrease in GAD activity. Animals still did not show significant changes in cortical GAD activity by 3 weeks after the lesion. By 6 weeks after the lesion, however, significant changes in GAD activity were apparent in 2 of the 4 animals assayed. Thus, the earliest detectable decrease in GAD activity was seen at 6 weeks after BN lesions and significant decreases in enzyme activity were found in all animals with a survival period longer than 8 weeks.

The changes in GAD enzyme activity have also been measured at a substrate

concentration near saturation for the enzyme (24 mM glutamate). The statistically significant decrease of GAD activity of 30% was measurable under those conditions as well. Thus, the decrease most likely reflects a change in the amount of enzyme present in the assayed neocortex.

The present results provide evidence that lesions of cholinergic basal forebrain nuclei lead to significant biochemical changes in cortical neurons not directly affected by the lesion. The slow onset and long duration of the decrease in GAD activity makes it rather unlikely that this change is a consequence of a suppression of synthesis activity due to lowered ACh levels. A putative GABAergic pathway from the hypothalamus to neocortex has been reported[27], yet if direct destruction of this pathway would be the basis for the GAD deficit, it should have been detectable much earlier. The depletion of cortical ChAT activity for example, is maximal by 1 week postoperatively (refs. 12,13 and personal observation).

We think that the most likely explanation for the observed decrease in GAD activity is some type of secondary atrophy of GABAergic neurons in cortex. While this effect may be a consequence of depleting cortical ACh, we cannot rule out the possiblity that it results from the destruction of non-cholinergic, ascending pathways that pass through the basal forebrain. However a similar effect on cortical GABAergic cells after basal forebrain lesions has recently been documented by McGeer et al.[19]. They compared the ability of folic acid, kainic acid and electrical current injections into rat basal forebrain areas, to produce ChAT and GAD activity changes in different brain regions. Whereas folic acid injections apparently led to direct toxic effects on cortical cells, kainic acid injections into the substantia innominata, which are supposed to spare axons of passage[3], brought about GAD losses in the ChAT depleted cortical areas which are comparable to the

decreases observed in our study. However McGeer et al., found no GAD changes in animals depleted of their cortical cholinergic input by electrothermic lesions. They interpreted these results to mean that the GAD decrease was not specifically related to the depletion of cortical ACh but rather was due to the seizure activity induced by the kainate. These negative findings after electrothermic lesions may be the consequence of the postlesion survival times allowed before testing. According to our results, their maximum postlesion time points of 21 days would not be expected to yield detectable changes in cortical GAD activity. Kainic acid lesions to basal forebrain cholinergic nuclei did however result in cortical GAD losses which were detectable at an earlier time point than electrothermic lesions to the same area. This may be a consequence of the much more extensive cholinergic depletion in cortex achieved with kainic acid injections or a change in time base produced by the 'excitotoxic'[3] activity of kainic acid. We are not yet able to offer any explanations for why cortical GABAergic cells or processes are affected by the basal forebrain lesions or what the significance of this change may be for cortical function.

The occurrence of secondary changes in cortex after cholinergic degeneration is not a new phenomenon. Crutcher noted sympathetic fiber sprouting after lesions of cholinergic inputs to neocortex[4]. This sprouting of postganglionic sympathetic fibers into cerebral cortical territory, however, seems to follow a faster time course than the decrease of GAD activity. A link between sympathetic sprouting and GAD activity changes remains to be tested.

Postmortem studies on brains of Alzheimer's patients have shown decreases in GAD activity and GABA levels in several cortical areas[5,22,24,25]. These findings have been corroborated by reports of lowered GABA levels in the cerebrospinal fluid of Alzheimer's patients[7,31]. Brains from Alzheimer's patients show a loss of

cholinergic basal forebrain cells and a decrease of cortical ChAT activity[1,2,29]. Our results suggest that the decrease of GABAergic markers in brains of Alzheimer's patients may be a consequence of a chronic deterioration of cholinergic inputs to cerebral cortex.

In conclusion, one indirect effect of a basal forebrain lesion is a chronic decrease in cortical GAD activity which most likely reflects a change in GABAergic circuitry. If this decrease is due specifically to the reduction in cortical ACh it provides a striking example of a regulatory interaction between neuronal systems that produce and release different neurotransmitters.

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