Impairment of Memory and Changes in Neurotransmitters
Induced by Basal Forebrain Lesion in Rats

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Abstract—The effect of bilateral electrolytic lesioning of the anterior and posterior parts of the basal forebrain (BF) on learning behavior and changes in neurotransmitters in the central nervous system was investigated in rats. The posterior BF lesion caused more severe impairment than the anterior BF lesion in the acquisition of conditioned avoidance response in a two-way shuttle box. A severe deficit in acquisition of passive avoidance response was produced by the posterior BF lesion. Choline acetyltransferase (CAT) activity was decreased significantly in the parietal cortex but not in the occipital cortex in anterior BF-lesioned rats. However, it was not decreased in posterior BF-lesioned rats. The contents of monoamines in the hippocampus was decreased more significantly by the posterior BF lesion than by the anterior BF lesion. These results suggest that the impairment of memory in posterior BF-lesioned rats may be related mainly to monoaminergic function rather than to cholinergic deficit.

Neurons in the basal forebrain (BF), especially those comprising the so-called nucleus basalis of Meynert, have been shown to be selectively degenerated in patients with senile dementia of the Alzheimer type (1, 2). Neurons in the BF are the major source of cholinergic innervation for the entire neocortex, hippocampus, amygdala and olfactory bulb (3-6). It has been demonstrated that rats with lesions of the nucleus basalis magnocellularis, the area which is believed to be homologous to the nucleus basalis of Meynert in humans and primates, show mild impairment in the acquisition of the passive avoidance response and show a severe deficit in its retention (7). It has also been reported that a severe deficit in the acquisition of passive avoidance response is produced by lesions of the posterior BF and that cholinomimetic drugs improve this impairment (8, 9).

On the other hand, as there are bundles of monoaminergic neurons in the BF (10, 11), not only cholinergic but also monoaminergic neurons may be degenerated by electrolytic lesioning. However, there have been no reports of changes in brain monoamine content in the BF-lesioned animals. The present study was performed in an attempt to clarify the relation between central monoaminergic and cholinergic function and memory deficit.

Materials and Methods

Animals: Male Wistar rats (250–270 g) supplied by the Shizuoka Laboratory Animal Center were housed 4 per cage in a temperature-controlled (22±1°C) environment with a regular 12-hr light-12-hr dark cycle. Food and water were given ad libitum.

Surgery: Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and placed in a stereotaxic apparatus. To produce BF lesions, insulated stainless steel bipolar electrodes (tip diameter 0.5 mm, uninsulated length 0.5 mm) were put into the BF bilaterally according to the brain atlas of König and Klippel (12), and a direct current of 3 mA was applied for 10 sec.

The stereotaxic coordinates of the BF were
anterior: 6.0 or 6.8, lateral: 3.0 and ventral 8.0 mm below the surface level of the skull. Sham operations were performed in the same way without applying electrical current.

Choline acetyltransferase (CAT) assay: Two weeks after surgery, the rats were sacrificed by decapitation, and the brains were removed and dissected. CAT activity of the occipital and parietal cortices was measured according to the method of Fonnum (13). The tissues were homogenized individually in 80 volumes of 10 mM EDTA buffer (pH 7.4), and the homogenate (10 μl) was assayed in duplicate for CAT activity. In this procedure, the homogenate was transferred to a tube containing 10 μl of choline medium (10 mM choline Cl, 300 mM NaCl, 50 mM phosphate buffer, 1 mM physostigmine sulfate and 10 mM EDTA), 5 μl of 0.5% Triton X-100 and 14C-acetyl CoA and then mixed. The tubes were then incubated at 37°C for 15 min, and 5 ml of acetylcholine solution (0.25 mg ACh) was added to stop the reaction. The mixture was then transferred to a scintillation vial, and 2 ml of acetonitrile containing 10 mg sodium tetraphenyl borate and toluene scintillation fluid were added. Radioactivity was measured with a liquid scintillation counter (Packard, Model 4430). The activity of CAT was expressed as nmol of ACh synthesized/hr/mg protein. Protein concentration was measured according to Lowry’s method (14).

Monoamine assay: Two weeks after surgery, the rat was sacrificed with a microwave applicator (TMW 6402, Toshiba), and the brain was removed and dissected. Brain monoamines were extracted according to the method of Ishikawa and McGraugh (15). Each tissue was weighed and homogenized in a three-fold volume of 0.025 N hydrochloric acid containing 0.05 M EDTA and 3.4-dihydroxy benzylamine. Next, 400 μl of homogenate was transferred to a tube containing 1 g of NaCl and 3 ml of n-butanol. After shaking and centrifugation, monoamines were extracted in 0.1 N hydrochloric acid. The eluate, 10–20 μl, was injected into a high-performance liquid chromatograph (YANACO L-4000W) with a voltammetric detector (YANACO VMD-501) and a Yanapak ODS-A column (250×4.6 mm i.d.) (Yanagimoto Manufacturing Co., Ltd). All quantitations were based on the resulting chromatogram. Ratios of the areas for the substances and the corresponding internal standard were compared for samples and standards taken through the entire extraction procedure.

Passive avoidance task: A two-chambered step-through passive avoidance apparatus was used (Muromachi Kikai Co., Ltd). The experiment was started on day 14 after the BF lesion was made. In the training trial, each rat was placed in the light chamber, and after 1 min of adaptation, the guillotine door separating the two chambers was opened. As soon as the animal placed all four paws in the dark chamber, the door was closed and a 6-mA scrambled shock was delivered to the grids for 3 sec. The rat was removed from the dark chamber and returned to its home cage. In the retention test given 24 hr later, the rat was placed in the starting chamber again, and the latency in entering the dark chamber was recorded up to a maximum cutoff time of 5 min.

Active avoidance task: Active avoidance testing was conducted in an automated two-way shuttle box. The shuttle box consisted of two compartments separated by a barrier (Muromachi Kikai Co., Ltd). Acquisition training was started on day 14 or 15 after the BF lesion had been made. Conditioned stimulus (CS) consisted of a monotone of 2000 Hz, and as an unconditioned stimulus (UCS), a 4-mA scrambled electric shock was applied to the grid floor. A presentation of CS for 5 sec was succeeded by UCS for 5 sec. The movement of the animal into the other compartment in response to CS was termed the conditioned avoidance response (CAR), and the escape after an outset of UCS was recorded as unconditioned avoidance response (UCR). A lack of response to UCS presentation during the 5-sec period was also recorded as error. After allowing 1 min for adaptation to the shuttle box, the conditioning trials were started. Each session consisted of 20 trials per day and the interval of each trial was from 10 to 20 sec.

Histology: After termination of the experiments, all animals were anesthetized with
ether and perfused with 10% formalin. The brain was removed and fixed in 10% formalin for at least 48 hr, and then it was frozen and sectioned at 60 μm. The sections were stained with cresyl violet. The location and extent of the electrolytic lesion were then verified histologically. Data from animals in which the lesion was not located at the appropriate site were excluded.

Statistical methods: The results from the CAT and monoamine assay were analyzed using the two-tailed Student's t-test. In the passive and active avoidance experiment, the results were analyzed using Mann-Whitney's U-test.

Results

Histology: The lesioned sites of the anterior BF-lesioned group were located mainly between A: 6.36 and A: 6.86 indicated in König and Klippel's brain atlas and included the substantia innominata, tuberculum olfactorium, medial forebrain bundle and ventromedial corner of the globus pallidus. In the posterior BF-lesioned group, the extent of the lesion was between A: 5.34 and A: 6.06 and located in the nucleus preopticus magnocellularis, ventromedial corner of the globus pallidus, amygdala and medial forebrain bundle (Fig. 1).

CAT activity: CAT activities in the parietal cortex of the anterior and posterior BF-lesioned groups were decreased by about 25% and 16% relative to the sham-operated rats, respectively (Fig. 2). This effect observed in the anterior BF-lesioned group was statistically significant (P<0.05). However, CAT activity in the occipital cortex was not changed in the anterior or posterior BF-lesioned group (Fig. 2).

Monoamine levels: BF lesions produced a depletion of noradrenaline (NA), dopamine

![Fig. 1. Schematic drawings of the bilateral lesions of the anterior and posterior parts of the basal forebrain (BF). cp: commissura posterior, GP: globus pallidus, ha: nucleus anterior hypothalami, FMP: fasciculus medialis prosencephali (medial forebrain bundle), am: nucleus amygdaloideus medialis, CAI: capsula interna.](image)
(DA) and serotonin (5-HT) in some regions of the brain. The reduction induced by posterior BF lesion was more remarkable than that of anterior BF lesion (Table 1). Particularly, there was a significant difference in the content of monoamine in the hippocampus between anterior and posterior BF-lesioned rats. The content of NA in the hippocampus of the posterior and anterior BF-lesioned rats was reduced to 60% and 91%, respectively. The contents of 5-HT and DA in the hippocampus of the posterior BF-

![Fig. 2. Choline acetyltransferase (CAT) in the parietal and occipital cortices in BF-lesioned rats. CAT activity was expressed as nmol ACh synthesized/hr/mg protein. The significance of the differences from the controls was assessed using Mann-Whitney's U-test. *P<0.05.](image)

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<th>Table 1. Effect of posterior and anterior BF lesions on monoamine concentrations in various brain areas of the rat</th>
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The significance of the differences from the controls was assessed using the two-tailed Student's t-test. *P<0.05, **P<0.01, ***P<0.001. mean (ng/g)±S.E.M. occ. cortex: occipital cortex.
lesioned rats decreased more significantly than those of the anterior BF-lesioned rats (Table 1).

General activity: When the BF was lesioned, rats showed a significant decrease in body weight. The decrease in the rats with posterior BF-lesions was slightly more severe than that in the rats with anterior BF lesions. The peak time of the decrease in body weight was 4–5 days after BF-lesioning, after which body weight gradually recovered. Several days after BF lesioning, rats showed slightly sedated behavior and their activity was decreased. All experiments were commenced on day 14 or 15, by which time the rats with BF lesioning had recovered their body weight almost to that of sham-operated rats. No difference in general activity between sham-operated and BF-lesioned rats was seen on day 14 or 15.

Passive avoidance: On the testing day, rats with the posterior BF lesions entered the dark chamber earlier than the sham-operated controls, whereas rats with anterior BF lesions entered the dark chamber only slightly earlier (Fig. 3).

Active avoidance: In the active avoidance test, only rats with posterior BF lesions showed delayed acquisition of CAR. The acquisition curve of the anterior BF-lesioned rats was nearly the same as that of the sham-operated rats (Fig. 4).

Discussion

The BF of primates has a direct and widespread projection towards the ipsilateral neocortex (3–6). CAT activity was decreased in the parietal cortex but not in the occipital cortex in anterior BF-lesioned rats. However, it was not decreased in posterior BF-lesioned rats. It has been reported that regional brain CAT activity following ibotenic acid lesions of the BF was significantly decreased in the temporal-parietal cortex, but not in the occipital cortex (7). Since large multipolar neurons are scattered through a number of

![Fig. 3. Effect of anterior and posterior BF lesions on the retention of passive avoidance response in rats.](image1)

![Fig. 4. Effect of anterior and posterior BF lesions on the acquisition of the shuttle box avoidance response in rats.](image2)
BF acetylcholine cell groups: the medial septal nucleus, the nucleus of the diagonal band of Broca, the magnocellular preoptic nucleus, the substantia innominata and globus pallidus (3, 5), the anterior part of the BF must contain more acetylcholine cell bodies than the posterior part of the BF.

On the other hand, the content of monoamine in the striatum, hippocampus and cortex was measured. The decrease in monoamine content that was induced by the posterior BF lesions was greater than that induced by the anterior BF lesions. Particularly, there was a significant difference in the content of monoamine in the hippocampus between anterior and posterior BF-lesioned rats. The central noradrenergic cell bodies in the locus ceruleus project to many parts of the brain, such as the neocortex, hippocampus and thalamus and so on. The pathways exist in the BF (10). In the same way, the dopaminergic cell bodies in the substantia nigra reach the striatum through the pathways included in the BF (10). Serotonergic cells mainly exist in the brain stem, especially the raphe nucleus, and they become widely spread via the BF (11). In the present experiment, the contents of NA, DA and 5-HT decreased significantly in every brain region in posterior BF-lesioned rats. It is thus conceivable that monoaminergic pathways would be destroyed by electrolytic lesioning of the posterior BF. The lesions of the BF, particularly in the posterior part, influenced the retention of passive avoidance and the acquisition of active avoidance. The common results of these experiments indicated that damage to the posterior BF was related to the impairment of memory (8, 9). The lesions of the posterior BF decreased the monoamine content in the hippocampus significantly, but not CAT activity in the cortex. Besides, lesions of the anterior BF decreased the CAT activity in the cortex but not the monoamine content in the hippocampus. It is thus assumed that the impairment of memory function is due to monoaminergic, rather than cholinergic damage.

There are many pieces of pharmacological evidence suggesting that central monoaminergic mechanisms may play an important role in memory processes (16). Anlezark et al. reported that bilateral lesions of the nucleus locus coeruleus in rats deplete the cerebral cortex of NA and significantly diminish the rate of increase of running for food reward in a sample L-shaped runway (17). Cooper et al. reported that rats treated with pargyline prior to intracisternal injection of 6-hydroxydopamine failed to display acquisition of either a shuttle box avoidance response or a one-way active avoidance response (18). Administration of α-methyltyrosine, which decreases brain DA and NA without affecting 5-HT, has also been found to decrease shuttle box avoidance responses (19). Serotonergic neurons are also related with the process of learning and memory. Zimelidine, a relatively specific 5-HT uptake blocker, attenuates the impairing effects of ethanol on learning and memory (20). Ögren reported that 5-HT terminal systems in the forebrain play an important role in avoidance learning (21).

On the other hand, it is well-known that cholinergic mechanisms also play an important role in memory processes. Anti-cholinergic drugs such as scopolamine produce memory impairment in experimental animals and humans and cholinomimetics such as physostigmine, oxotremorine and arecoline enhance memory function and antagonize the memory impairments induced by scopolamine (22–25). In the present experiment, however, a marked memory deficit occurred when monoaminergic, but not cholinergic mechanisms were damaged significantly. The impairment of memory in the posterior BF-lesioned rats may be related mainly with monoaminergic function rather than cholinergic deficit. Nagawa et al. reported that posterior BF lesions impair memory function in passive avoidance and spatial performances and that the acquisition curve was mildly enhanced by i.p. treatment with cholinergic drugs such as physostigmine, arecoline or oxotremorine (9). All these results taken together indicate that as the cholinergic function is fairly maintained even with posterior BF lesioning, improvement of memory deficit may be recognized by the administration of cholinergic-activating drugs which activate the remaining cholinergic
mechanism.

Patients with senile dementia of the Alzheimer's type have not only a deficiency of the cholinergic mechanism induced by impairment of the nucleus basalis of Meynert, but also a deficiency of the monoaminergic mechanism (26–28). Taken together, the results of the present studies suggest that lesions in the BF impair both monoaminergic and cholinergic functions, which play an important role in learning and memory.

References
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