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Persisting Behavioral and Neurochemical Deficits in Rats Following Lesions of the Basal Forebrain[1]

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BERMAN, R F , R D CROSLAND, D J JENDEN AND H J ALTMAN *Persisting behavioral and neurochemical deficits in rats following lesions of the basal forebrain* PHARMACOL BIOCHEM BEHAV **29**(3) 581–586, 1988 —The effects of excitotoxic lesions of the nucleus basalis magnocellularis on cortical cholinergic activity and passive avoidance performance were examined in rats at 6, 14, 84 and 180 days after lesioning Lesioned rats showed significant impairment of passive avoidance retention at every time point tested, with no evidence of behavioral recovery compared to unoperated and sham-lesioned (i e , vehicle-injected) control rats Cortical choline acetyltransferase (CAT) activity was reduced relative to controls at all time points examined, with the greatest reduction (i e , 28%) occurring at approximately 14 days after lesioning The levels of CAT activity at 180 postlesioning remained reduced compared to control animal levels, but less so than at 14 days after lesioning, indicating partial recovery No changes in cholinergic muscarinic binding were observed at any time following lesioning The results indicate that the behavioral and neurochemical effects of NbM lesions persist for at least 6 months following lesioning, but that partial, gradual recovery of cholinergic activity occurs

Basal forebrain Excitotoxin lesions Cholinergic system Passive avoidance Choline acetyltransferase Muscarinic cholinergic receptors

RECENT studies have described a basal forebrain cholinergic system (BFCS) comprised of large multipolar neurons which stain intensely for acetylcholinesterase [6, 12, 42] and which react with specific antibodies for choline acetyltransferase [20, 36, 38] In rats, this BFCS extends along the base of the forebrain from the lateral preoptic area rostrally, to the ansa lenticularis caudally, and includes the region known as the nucleus basalis magnocellularis (NbM) These forebrain neurons provide the major source of cholinergic innervation for the olfactory bulbs and neocortex. A continuous grouping of magnocellular cholinergic neurons also exists in the vertical limb of the diagonal band of Broca and the medial septum which similarly provide the major cholinergic innervation to the hippocampus [2, 12, 21]

The importance of the cholinergic system for learning and memory in laboratory animals and in man has been recognized for some time [3, 7, 8, 18] Lesions of the BFCS in the region of the NbM have been reported by several groups to reduce cholinergic innervation of the forebrain [22,23], and to impair memory in rats across a variety of tasks [1, 13, 24, 25, 28, 29, 31, 32, 35, 45] These findings have taken on greater importance following the report that Alzheimer's patients show an early, relatively selective degeneration of basal forebrain cholinergic neurons [46], suggesting a link between the cognitive impairments observed with the disease and the loss of cholinergic activity

While there has been general agreement that lesions of the BFCS produce memory impairments and reduce the levels of accepted biochemical markers for cholinergic activity, such as choline acetyltransferase (CAT) and acetylcholinesterase (AChE), there is controversy concerning the permanence of the behavioral and neurochemical deficits Wenk and Olton [44] have reported relatively rapid recovery of CAT and AChE activities in the cortex following unilateral NbM lesions, while other laboratories have failed to find evidence for such recovery [5, 11, 13] Flicker *et al* [13] found behavioral recovery without neurochemical recovery several months after NbM lesions, and Hepler, Olton and Coyle [19] have interpreted their data as support for some behavioral recovery following NbM lesions. In the present experiments the question of behavioral and neurochemical recovery following NbM lesions in rats was systematically examined over approximately a 6 month period. We find persisting impairments for memory of passive avoidance training with

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evidence for partial neurochemical recovery when animals are examined 180 days after lesioning

METHOD

Subjects

A total of 226 adult, male Sprague-Dawley rats (300–350 g) obtained from Zivic Miller breeding labs (Allison Park, PA) were used for the experiments described below Animals were individually housed in stainless steel cages under 12 12 hr light dark (7 00 a m –7·00 p m) low-level illumination with food and water available ad lib throughout the experiment

Surgery

Seventy-eight rats were surgically anesthetized with sodium pentobarbital (45 mg/kg, IP) and stereotaxically injected bilaterally into the region of the nucleus basalis magnocellularis (NbM) with a total of 14 μg (7 0 μg/side) of ibotenic acid dissolved in 1 0 μl of pH 7.4 phosphate buffered saline [43] Sixty-nine rats were similarly injected, bilaterally with the phosphate buffered saline vehicle only, and the remaining seventy-nine rats served as non-injected controls All injections were made via a microliter syringe fitted with a 26 gauge needle, and at a rate of 0 1 μl/min The stereotaxic coordinates were 6 8 mm anterior to the interaural line, 3 0 mm lateral to midline, and 6 8 mm ventral to the dural surface Ibotenic acid was prepared immediately before use each day Animals were trained in the shock avoidance task after one of several delays following lesioning (i e , 6, 14, 84 or 180 days) Non-injected and vehicle-injected control animals were matched with lesioned animals and similarly housed over the delay prior to behavioral testing and subsequent neurochemical measurements of cholinergic activity

Training

Lesioned animals, along with the matched non-injected and vehicle-injected controls, were trained to avoid the darker of a two-compartment shuttle box either 6, 14, 84 or 180 days after injection of ibotenic acid The shuttle box (76 0×34.0×19 5 cm) was constructed of black Plexiglas The floor of the box was constructed of stainless steel bars through which a scrambled 1 mA footshock could be delivered (Grayson-Stadler Series 700) A guillotine door separated the two chambers The smaller of the chambers was illuminated by 5 bulbs (1 5 Watt) positioned along the top of the rear wall The other chamber was dark Animals were placed in the rear of the lighted chamber and 10 sec later the guillotine door was opened. Animals were allowed to cross over into the darker chamber (i e , all 4 paws entered), after which the guillotine door was closed and a 3 sec 1.0 mA inescapable footshock was delivered Immediately following footshock animals were returned to their individual cages to await retention testing Any animal failing to cross over to the darker chamber within 5 min was removed from the study

Retention Testing

Twenty-four hr after training, animals were tested for retention of the footshock experience under extinction conditions (i e , no footshock). Briefly, each animal was placed into the lighted chamber of the shuttle box, and after 10 sec the guillotine door was again opened Each animal's latency to cross over into the darker compartment was recorded as the step-through latency (STL). Long STL were interpreted as evidence of good retention of the footshock experience Animals remaining on the lighted side of the shuttle box for 600 sec were assigned a maximum STL score of 600 sec and then were removed from the apparatus

Neurochemistry

Within 3 days of retention testing, all animals were sacrificed by decapitation and their brains were removed An additional group of lesioned animals, along with their respective controls, were sacrificed 3 days after lesioning for neurochemical evaluation only Slices of frontal cortex (both hemispheres) were rapidly dissected on a cooled plate (4 0°C) and frozen at −70°C for later determination of choline acetyltransferase (CAT) activity and measurement of muscarinic cholinergic binding The remainder of the brain was stored in 10% phosphate-buffered formalin for histological verification of the lesion and extent of damage produced by ibotenic acid injection

Frozen cortices were homogenized in cold 50 mM sodium phosphate pH 7 5 at approximately 20 mg tissue/ml. The homogenates were assayed immediately following homogenization for CAT activity according to the methods of Fonnum [14] Tritiated quinuclidinyl benzilate, (^{3}H)QNB, binding was assayed 3, 6, 14, and 84 days after lesioning as described by Yamamura and Snyder [48] with minor modifications Briefly, homogenized cortical tissue (1 mg) from rats in the various treatment conditions was incubated with 0 8 nM (^{3}H)QNB (40 2 Ci/mmol, New England Nuclear, Boston, MA) for 1 0–1 5 hr and processed as described Nonspecific binding was measured by including 1 μM atropine sulfate in the incubation mixture Protein concentration was determined by the method of Lowry, Rosebrough, Farr and Randall [30]

Histological Examination

Brains were frozen, sectioned at 40 microns, mounted on slides and stained with cresyl violet The extent of the lesion was determined from microscopic examination of serial sections through the forebrain of animals in both the ibotenic and the vehicle-injected groups

Data Analysis

Statistical analysis of overall treatment effects was conducted using a two factor (group × day) analysis of variance Planned post hoc comparisons of individual group effects were made with the conservative Scheffe's test [47] The minimum level of statistical significance was $p<0$ 05 (two-tailed criterion)

RESULTS

A brief, 2–3 day period of aphagia and ataxia followed the injection of ibotenic acid into the region of the NbM In no case did these effects persist beyond 72 hr after lesioning

Behavior

The effects of ibotenic acid injections on retention of passive avoidance training are shown in Table 1 A statistically significant difference was found between groups,

TABLE 1

RETENTION LATENCIES (SEC) OF SHOCK AVOIDANCE TRAINING*

Group	Days After Lesioning 6	14	84	180
Control	352 1 ± 74 4	445 2 ± 47 7	575 3 ± 14 1	511 4 ± 53 2
N=	(14)	(28)	(15)	(7)
Vehicle	247 3 ± 67 4	281 5 ± 62 3	483 6 ± 60 7	496 0 ± 54 9
N=	(15)	(21)	(10)	(7)
Lesioned	131 5 ± 52 3	158 3 ± 54 7†	147 2 ± 31 9†‡	132 3 ± 49 5†‡
N=	(16)	(20)	(14)	(12)

*Mean ± SEM step-through latencies (sec) to enter shock compartment 24 hr after foot-shock
†$p<0$ 01 compared to control group
‡$p<0$ 01 compared to vehicle injected group

TABLE 2

EFFECT OF NBM LESIONS ON CORTICAL CAT ACTIVITY*

Groups	Days After Lesioning 3	6	14	84	180
Control	100 0 ± 2 9	100 0 ± 3 0	100 0 ± 2 3	100 0 ± 4 1	100 0 ± 3 4
N=	(15)	(14)	(28)	(15)	(7)
Vehicle	98.6 ± 2 2	97 2 ± 3 9	92 0 ± 4 3	96 4 ± 2 9	102 8 ± 4 9
N=	(16)	(15)	(17)	(10)	(7)
Lesioned	86 7 ± 3 7†	79 8 ± 4 4†‡	72 3 ± 3 1†‡	76 0 ± 4 7†‡	90 1 ± 3 1§
N=	(16)	(16)	(20)	(14)	(12)

*CAT activity is expressed as a percent of mean CAT activity in the control group for each time point Data are mean ± SEM Average control cortical CAT activity across all time points was 54 nmol acetylcholine synthesized per hr per mg protein
†$p<0$ 05 compared to control group
‡$p<0$ 05 compared to vehicle injected group
§$p<0$ 05 compared to CAT levels at 14 days after lesioning

$F(2,163)=32$ 12, $p<0$ 001 As is evident in the table, lesioned animals showed impaired retention of passive avoidance training at each test day after lesioning compared to controls, and this impairment reached statistical significance on days 14, 84 and 180 ($p<0.01$) Retention latencies for lesioned animals were also significantly shorter at the 84 and 180 day tests compared to vehicle-injected controls ($p<0$ 01), but not at 6 and 14 days. In an earlier report we found that vehicle injections into the NbM alone can produce a small, but measurable impairment of passive avoidance retention in rats tested two weeks after lesioning [1]. Since the performance of vehicle-injected animals was intermediate between control and lesioned animals at 6 and 14 days after lesioning, the data suggest that a small lesion effect in the vehicle-injected animals had recovered by 84 and 180 days after lesioning

The overall differences in latencies across days apparent in Table 1 did not reach statistical significance, $F(3,175)=2.23$, $p<0.09$

Neurochemistry

The effect of bilateral injections of ibotenic acid into the NbM on cortical CAT activity across time is shown in Table 2 Data are expressed as a percentage of control values at each time point As shown in the table, overall cortical CAT activity was significantly reduced in lesioned animals, $F(2,167)=31$ 93, $p<0.001$ Individual comparisons indicated that CAT activity in lesioned animals was significantly lower than that of controls at 3 ($p<0$ 05), 6, 14 and 84 days ($p<0.01$) after lesioning, but not at 180 days. The level of CAT activity in lesioned animals was also significantly lower than that of vehicle-injected rats at 6 ($p<0$ 05), 14 and 84 ($p<0.01$) days after lesioning, but not at 3 and 180 days The maximum reduction (i.e , 28%) in cortical CAT activity occurred at 14 days after lesioning, with relatively less reduction observed before (i e , 3 or 6 days), or after (i.e., 84 and 180 days) this time point Thus, cortical CAT activity remains reduced for at least 180 days after injection of ibotenic acid into the NbM. However, the level of CAT activity in lesioned animals at 180 days was not significantly different from control values, $F(2,23)=3$ 53, $p>0$ 10, and was significantly greater than that at 14 days after lesioning, $F(4,43)=10.23$, $p<0$ 05, suggesting some recovery Vehicle injections alone produced a small (i.e , 8%), but nonsignificant, $F(2,62)=3.30$, $p>0.10$, reduction in cortical CAT activ-

TABLE 3

EFFECT OF NBM LESIONS ON CORTICAL MUSCARINIC BINDING*†

Group	Days After Lesioning 3	6	14	84
Control	100 0 ± 1 9	100 0 ± 3 4	100 0 ± 3 9	100 0 ± 2 2
N=	(15)	(14)	(20)	(15)
Vehicle	94 7 ± 3 3	94 7 ± 4 1	103 1 ± 4 7	99 0 ± 2 0
N=	(16)	(15)	(13)	(10)
Lesioned	100 0 ± 3 1	99 8 ± 3 1	95 4 ± 2 7	100 7 ± 2 6
N=	(16)	(16)	(14)	(14)

*Specific muscarinic binding is expressed as percent of mean specific muscarinic binding in the control group for each time point Data are mean ± SEM Average control specific muscarinic binding over all time points was 752 fmol [^{3}H]QNB bound per mg protein

†No statistically significant differences were found for any group on any day

ity 14 days after injection This small reduction in vehicle-injected animals replicates our findings in an early report which also demonstrated small reductions in cortical CAT activity following vehicle injections into the NbM [1]

Table 3 shows levels of cortical muscarinic binding measured 3, 6, 14 and 84 days after lesioning the NbM Binding was not measured at the 180 day time point No statistically significant differences in (^{3}H)QNB binding were observed for any group, $F(2,166)=0\ 39$, $p>0\ 60$, at any time point examined, $F(3,166)=0\ 87$, $p>0\ 80$

The correlation between cortical CAT activity and step-through latencies across NbM-lesioned animals was determined ($r=-0\ 19$), but was not found to be statistically significant, $t(60)=1\ 48$, $p>0\ 10$

Histology

Histological examination of the lesioned brains demonstrated marked gliosis in the vicinity of the injection site, with an absence of neurons with clearly discernible nucleoli These lesions were similar to those previously described from this laboratory [1] The center of the lesion corresponded to approximately A6060 of the Konig and Klippel [26] rat brain atlas The lesion produced neuronal degeneration in the ventral globus pallidus, but did not appear to extend into the striatum to any extent A cylinder of neuronal degeneration 0 2 to 0 3 mm in diameter was also observed extending up the axis of the injection track to the cortex No additional damage to other brain regions (i e , hippocampus) was observed under microscopic examination

DISCUSSION

The results demonstrate that basal forebrain lesions in rats significantly reduce the levels of CAT activity in frontal cortex, leave cholinergic muscarinic receptors in cortex unaltered, and markedly impair retention of passive avoidance training These findings are similar to those previously published by this laboratory [1] and by others [13, 17, 29]

The reductions in cortical CAT and the impaired passive avoidance performance persisted for at least 6 months, indicating that the damage to the NbM is relatively permanent Thus, the impairment of memory which follows such lesions can not be attributed to a transient neurochemical or electrophysiological disturbance, but probably represents the direct effect of loss of an important forebrain cholinergic system for normal memory

The present data also demonstrate a definite time course for the loss of cortical CAT activity following NbM lesions and for a gradual, but partial recovery in cortical CAT activity A significant decrease in cortical CAT activity was already evident by 3 days after lesioning (i e , approximately 13%), which further declined to a maximum of 28% by 14 days Cortical CAT levels also showed partial recovery, possibly beginning as early as 84 days post-lesioning and reaching statistical significance by 180 days Similarly, the small decrease in cortical CAT activity in the vehicle-only injected animals was maximal 14 days after lesioning and had recovered to the levels of unoperated control animals by 180 days

There have been previous reports of both partial and complete recovery of cortical CAT activity following NbM lesions Wenk and Olton [44] reported complete recovery of cortical CAT activity and high affinity choline uptake (HACU) in rats 12 weeks following unilateral NbM lesions Recovery occurred although extensive neuronal cell loss in the NbM was still evident They suggested that surviving NbM neurons may arborize at cortical terminals, or that CAT levels may increase in surviving cholinergic neurons as a compensatory response Pedata *et al* [33] also examined cortical CAT activity and HACU after NbM lesioning They found little evidence for recovery of CAT activity in the ipsilateral cortex at 20 days, but CAT activity had significantly increased in the contralateral cortex In addition, HACU showed complete recovery in the parietal cortex, and partial recovery in the frontal cortex 20 days after lesioning They suggested that such recovery may reflect collateral cholinergic sprouting from ipsilateral cholinergic neurons, but provided no direct evidence for such a possibility Ridley *et al* [34] examined the effects of ibotenate lesions of the basal forebrain on serial reversal learning in the Marmoset They reported significant reduction of CAT activity in frontal (60%) and temporal (40%) cortex By 6–8 weeks the reduction in CAT activity in bilaterally lesioned animals was no longer statistically significant, although still evident (i e , 27% of control levels) Animals were also significantly impaired in serial reversal learning during the first post-operative week of training and testing, but not when tested 2 5 weeks after lesioning Considered together, the present

data and those described above suggest that some recovery of CAT activity and HACU may occur following NbM lesions, but that recovery is typically not complete and in the rat may require an extended post-operative period However, even at 180 days post-operatively we did not find evidence for behavioral recovery although levels of CAT were significantly increased relative to levels measured at 14 days.

Previous studies have provided evidence for additional compensatory changes in the basal forebrain cholinergic system following NbM lesions [15, 27, 29] Specifically, Gardiner *et al* [15] reported that the decrease in K^+-evoked release of acetylcholine from frontal and parietal cortex observed following basal forebrain excitotoxin lesions showed complete recovery in parietal cortex at 102 days, and partial recovery in frontal cortex at 128 days after lesioning Stephens *et al* [39] reported a return to near normal CAT levels in the NbM 120 days after unilateral decortication although cell bodies and dendrites remained shrunken This recovery was attributed to adaptive changes in undamaged NbM neurons Finally, Lamour *et al* [27] found that the percentage of cortical neurons excited by acetylcholine was increased 2–3 weeks after bilateral NbM lesions in rats The effect appeared to be due to a supersensitivity of muscarinic cholinergic cortical neurons These reports indicate that the basal forebrain cholinergic system can undergo several types of changes in response to damage

Recovery of hippocampal cholinergic enzyme activity following lesions of septal nuclei is well documented and has been suggested to involve sprouting of spared cholinergic neurons [10] The critical factors involved in recovery within this neural system are the extent of the lesion and the duration of the recovery period [9,10] Similarly, recovery of activity in the cholinergic system following NbM lesions may well depend upon the relative completeness of the lesion, with larger lesions resulting in substantially less recovery than smaller lesions Bilateral lesions of the NbM would be expected to result in less recovery than unilateral lesions Even in Alzheimer's disease cholinergic neurons innervating the hippocampus are apparently capable of sprouting, resulting in an intensification of acetylcholinesterase activity in the dentate molecular layer [16]

These arguments take on added importance in view of recent reports which fail to find evidence for neurochemical recovery following NbM lesions El-Defrawy *et al* [11] failed to find evidence for recovery of cortical CAT activity or HACU by 12 weeks after NbM lesioning. Bartus *et al* [4,5] also report a lack of recovery of cortical CAT activity or HACU measured up to 7 months after lesioning These findings appear to contrast with those of the present study However, El-Defrawy *et al* [11] only examined post-lesion effects out to 12 weeks Our results indicate only slight recovery of CAT activity at this time point The NbM lesions described by Bartus *et al* [4,5] produced greater depletion of CAT activity than that reported in the present study, and may have been larger and more complete As discussed above, larger lesions would be expected to result in somewhat less recovery and this may explain the contradictory findings among the various studies

No significant correlations between cortical CAT activity and passive avoidance performance were found This failure is similar to our previous findings [1] and to those recently reported by Thompson, Biggs, Ristis, Cotman and Yu [40] A lack of correlation clearly weakens arguments that passive avoidance impairments found in NbM lesioned rats may be due to specific damage to cortical cholinergic projections from the NbM Several possibilities could explain the absence of significant correlations First, the areas of cortex typically sampled for CAT activity (i e , frontal region) may be too large, or may not include the critical cortical region Second, the behavioral effects of NbM lesions may show better correlation with loss of cholinergic activity in some other brain region also innervated by basal forebrain cholinergic neurons (e g , amygdala) Finally, the behavioral deficits may result only indirectly from damage to the cholinergic system, and may actually be due to undetected damage to another system It will clearly be important to find answers to these questions

In conclusion, lesions of the NbM result in passive avoidance deficits in rats which persist, apparently without evidence of significant recovery, for up to 6 months after lesioning In contrast, the loss and recovery of CAT activity following lesioning of the NbM appear to follow a definite time course This recovery of CAT activity indicates that the cholinergic system is dynamic and can undergo what may be compensatory changes following damage However, the degree of compensation is likely to be a function of the degree of damage initially produced These conclusions are consistent with a large body of evidence in the septal-hippocampal cholinergic projection indicating recovery following lesioning

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