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BRIEF COMMUNICATION

Infusion of NMDA Into the Nucleus Basalis of Meynert, Frontal Cortex or Lateral Ventricle in Rats: Effect on Memory and Cholinergic Brain Neurochemistry

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SANTUCCI, A. C., P. D. KANOF AND V. HAROUTUNIAN. *Infusion of NMDA into the nucleus basalis of Meynert, frontal cortex or lateral ventricle in rats: Effect on memory and cholinergic brain neurochemistry.* PHARMACOL BIOCHEM BEHAV 33(2) 485–488, 1989.—The present study's aim was to examine the behavioral and neurochemical effects of damage limited to intrinsic neurons of the frontal cortex in rats. Specifically, it was of interest to evaluate the effects of N-methyl-D-aspartic acid-induced lesions of discrete frontal cortical loci on passive avoidance memory and on cortical cholinergic neurochemical markers (choline acetyltransferase—CAT and acetylcholinesterase—ACHE). The present study also compared the behavioral and neurochemical effects produced by frontal cortical damage with those effects produced by lesions of the nucleus basalis of Meynert (nbM). Results indicated that nbM lesions and lesions to a rostral frontal cortical site produced severe passive avoidance memory impairments when subjects were tested 72 hours after training. Cortical, but not hippocampal, levels of CAT and ACHE were depleted in nbM animals only. These data were interpreted as providing support for the view that intrinsic frontal cortical neurons contribute to memory.

N-Methyl-D-aspartic acid | Nucleus basalis of Meynert | Frontal cortex | Passive avoidance memory
Choline acetyltransferase | Acetylcholinesterase | Rats

TRADITIONALLY, the function of the cortex has attracted a large amount of empirical attention. Investigators have typically approached the study of cortical functioning by examining the effects of aspirative lesions on a variety of biobehavioral indices [e.g., (8, 10, 14, 18)]. However, since cortical damage inflicted by aspiration compromises cerebral blood flow and destroys not only intrinsic cortical neurons but cortical and subcortical axons of passage, results derived from aspirative lesion studies are difficult to interpret.

In order to elucidate more fully the functions of the cortex independent of the effects of damage to axons whose cell bodies lie in subcortical nuclei or in remote cortical areas, some studies have employed nonaspirative lesioning techniques (13, 15, 16). In these experiments the histological, anatomical and behavioral effects of extensive cortical damage (degeneration) produced by extradural administration of the excitatory amino acid N-methy-D-aspartic-acid (NMDA) have been examined. For example, decreases in nucleus basalis of Meynert (nbM) cell size (15,16) and shrinkage of cortical thickness (13, 15, 16) has been reported to occur following cerebral damage produced by extradural application of NMDA in rats. These findings correspond with other reports indicating a decrease in the size of neurons within the nbM of Alzheimer's disease patients (11) and aged mice (5,9).

Studies based on expansive lesions of the cortex, such as those

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produced by the topical application of NMDA, have served as useful models of gross cortical/subcortical interactions, but their lack of anatomical specificity has precluded the generation of hypotheses linking specific cortical loci to specific cognitive functions. The purpose of the present investigation was to 1) examine the mnemonic effects of discrete axon-sparing cortical lesions; 2) determine the cortical area(s) most likely to contribute to cortical lesion-induced memory deficits; 3) compare the mnemonic consequences of cortical lesions to the effects of forebrain cholinergic system lesions; and 4) ascertain the degree to which lesions of intrinsic cortical neurons affect markers of cortical cholinergic function. In keeping with previous studies of cortical lesion effects (13, 15, 16) NMDA was used as the excitotoxin. Accordingly, the consequences of NMDA-induced lesions of two discrete cortical sites were compared to the effects of NMDA-induced lesions of the nbM using a 72-hr retention of one trial passive avoidance paradigm. Lesion effects on cortical cholinergic function were determined by measurement of choline acetyltransferase (CAT) and acetylcholinesterase (ACHE) activity. To control for possible nonspecific damage effects of central NMDA infusion, NMDA was administered intracerebroventricularly to an additional group of rats.

METHOD

Subjects

Sixty-one naive male Sprague-Dawley young adult rats, weighing between 250 and 350 g and purchased from Charles River Company, served as subjects. Fifteen animals served as subjects for both the passive avoidance and neurochemistry experiments, while fifteen rats served only in the passive avoidance experiment and thirty-one rats served only in the neurochemistry experiment. Animals were maintained on a 12-hr light/dark cycle (lights on 7:00 a.m.) in group cages (3–4 per cage) and had food and water available ad lib. All subjects were allowed two weeks of habituation to the vivarium before any experimental activity.

Surgery

There were five surgical conditions: 1) bilateral lesions of the nucleus basalis of Meynert (NBM), 2) bilateral lesions of the frontal cortex, anterior site (FCTX/ANT), 3) bilateral lesions of the frontal cortex, posterior site (FCTX/POST), 4) unilateral infusions into the lateral ventricle (LV), and 5) control operations (SHAM). Subjects were anesthetized with 60 mg/kg ketamine hydrochloride (IM; KETALAR, Parke-Davis, Morris Plains, NJ) and 21 mg/kg pentobarbital (IP; Sigma, St. Louis, MO) and positioned in a Baltimore Instruments stereotaxic apparatus with the upper incisor bar set at 0. After exposing the skull, two small burr holes were made (one hole was made in the skull of animals serving in the LV condition). A 33-g stainless steel cannula was lowered into the site under study and 1 μl of NMDA was infused [50 nmol/1 μl/side in 7.4 pH phosphate buffer; dose based on (17)]. For nbM-lesioned subjects, the two burr holes were drilled at −0.3 mm from bregma and ±3.0 mm from midline, and the needle was lowered 8.1 mm from the surface of the skull. After one min, 0.6 μl of NMDA was infused slowly by hand over a period of about 2 min. One min after completion of this infusion, additional 0.2 μl infusions were made in a similar manner at 7.8 and 7.5 mm below the skull. The coordinates used for cortical infusions were +3.7, ±1.7, −2.5 mm from dura and +1.7, ±2.5, −2.1 mm from dura for the FCTX/ANT and FCTX/POST groups, respectively. The unilateral LV infusion was performed more slowly (1 μl over a 5-min period). The coordinates for unilateral LV infusions were −0.3, ±1.6, and −4.5 from skull. It should be noted that pilot data indicated 50 nmol of NMDA/1 μl to be the highest dose tolerated when infused intracerebroventricularly. To control for damage produced by the introduction of the needle into the brain, the SHAM group consisted of an equal number of animals that had the needle lowered to a point 2.0 mm above the various injection sites.

TABLE 1

EFFECT OF BILATERAL INTRACEREBRAL OR UNILATERAL INTRAVENTRICULAR INFUSIONS OF NMDA (50 nmol/1 μl/side) ON 72-HR RETENTION OF PASSIVE AVOIDANCE IN RATS (MEAN TEST LATENCIES IN SEC ± SEM)

Group (Ns = 6)	Test Latency
SHAM	366 ± 125.5
NBM	76 ± 45.5*
FCTX/ANT	109 ± 23.9†
FCTX/POST	508 ± 150.6
LV	221 ± 75.1

Group descriptions: SHAM = sham operated; NBM = bilateral lesions of the nucleus basalis of Meynert; FCTX/ANT = bilateral lesions of the frontal cortex, anterior site; FCTX/POST = bilateral lesions of the frontal cortex, posterior site; LV = unilateral intraventricular infusions (50 nmol total).

*$p = 0.025$ vs. SHAM.

†$p < 0.04$ vs. SHAM.

Passive Avoidance Task

Approximately two weeks after surgery, six subjects from each surgical condition were trained on a one-trial step-through passive avoidance task. On the day of training, subjects were brought to the experimental room and placed in a sound-attenuating cabinent with a background white noise stimulus (70 dB spl) for a 15-min period of adaptation. Training consisted of placing each animal on the white side of a 35 × 28 × 16 cm high black/white, two-compartment shuttle box. After 60 sec of confinement in the white side (15 × 28 × 16 cm high), a guillotine door that separated the two compartments was raised. When the rat crossed into the black compartment the guillotine door was lowered and a 0.6-mA 2-sec long scrambled footshock was administered through the grid floor (3-mm thick stainless steel rods spaced 1.2 cm apart). The latency (sec) to cross into the black side was recorded. Animals were confined in the black side for 60 sec and were then removed and returned to the cabinent. After the last animal in a squad (squads of 7–12 animals) completed training, all animals in that squad were kept in the cabinent for an additional 15 min and were then returned to their home cages in the vivarium.

The 15-min test session, conducted 72 hr after training, was identical to training except footshock was not administered. The latency to cross (with three paws) into the black compartment at test served as the measure of retention. If an animal failed to enter the black side at test it was removed from the apparatus and given a latency score of 900 sec. Only one animal from the SHAM group and two animals from the FCTX/POST group exhibited a test cross-through latency of 900 sec.

Neurochemistry

Subjects were sacrificed by decapitation within four weeks following surgery at which time the frontoparietal cortex (FPCTX), posterior cortex (PCTX) and hippocampus (HPC) were dissected on a cold plate. The FPCTX included the area anterior to the

TABLE 2

EFFECT OF BILATERAL INTRACEREBRAL OR UNILATERAL INTRAVENTRICULAR INFUSIONS OF NMDA (50 nmol/l μl/SIDE) ON BRAIN CONCENTRATIONS OF CAT IN RATS (MEAN nmol ACETYLCHOLINE/HR/mg PROTEIN ± SEM)

Group (Ns = 9–10)	Brain Area FPCTX	PCTX	HPC
SHAM	20.03 ± 0.45	19.67 ± 0.69	26.11 ± 0.82
NBM	13.70 ± 0.75*	17.30 ± 0.78†	26.66 ± 1.09
% difference	−31.6%	−12.0%	+2.1%
FCTX/ANT	20.93 ± 0.79	21.30 ± 0.57	27.43 ± 1.20
% difference	+4.5%	+8.3%	+5.1%
FCTX/POST	20.74 ± 0.57	20.79 ± 0.87	25.62 ± 1.03
% difference	+3.5%	+5.7%	−1.9%
LV	20.50 ± 0.84	21.21 ± 1.31	28.14 ± 0.84
% difference	+2.3%	+7.8%	+7.8%

See Table 1 for description of groups.
Brain area descriptions: FPCTX = frontoparietal cortex; PCTX = posterior cortex; HPC = hippocampus.
% difference is relative to SHAM.
*$p<0.002$ vs. SHAM.
†$p<0.05$ vs. SHAM.

TABLE 3

EFFECT OF BILATERAL INTRACEREBRAL OR UNILATERAL INTRAVENTRICULAR INFUSIONS OF NMDA (50 nmol/l μl/SIDE) ON BRAIN CONCENTRATIONS OF ACHE IN RATS (MEAN nmol/hr/mg PROTEIN ± SEM)

Group (Ns = 9–10)	Brain Area FPCTX	PCTX	HPC
SHAM	1365.2 ±43.7	1252.7 ±40.2	1929.7 ±76.9
NBM	856.8* ±64.0	1032.4† ±65.4	1817.8 ±47.7
% difference	−37.2%	−17.6%	−5.8%
FCTX/ANT	1375.4 ±45.8	1310.4 ±89.1	1900.6 ±72.9
% difference	+0.7%	+4.6%	−1.5%
FCTX/POST	1421.4 ±42.3	1324.6 ±66.0	1785.8 ±49.9
% difference	+4.1%	+5.7%	−7.5%
LV	1355.6 ±29.8	1403.4 ±106.3	1956.1 ±65.1
% difference	−0.7%	+12.0%	+1.8%

See Table 1 for description of groups and Table 2 for description of brain areas.
% difference is relative to SHAM.
*$p<0.001$ vs. SHAM.
†$p<0.02$ vs. SHAM.

bregmoidal suture and tangential to the rhinal sulcus while the PCTX included the area posterior to the bregmoidal suture and tangential to the rhinal sulcus. Once dissected, all tissue samples were stored at −80°C until assayed for CAT and ACHE according to the methods of Fonnum (2) and Johnson and Russell (6), respectively.

RESULTS

Passive Avoidance

The mean test latencies associated with the five groups of subjects are presented in Table 1.

A one-way ANOVA performed on these data revealed significant differences between the groups, $F(4,25) = 3.468$, $p<0.025$. Subsequent planned pairwise comparisons with the Student's t-test (one-tailed) indicted that the NBM, $t(10) = 2.168$, $p = 0.025$, and FCTX/ANT, $t(10) = 1.985$, $p = 0.04$, groups exhibited shorter test latencies relative to SHAM animals. In contrast, the FCTX/POST and LV groups did not differ statistically from SHAM animals ($ts<1$, $ps>0.15$). The low test scores associated with the NBM and FCTX/ANT groups were not related to differences in training cross-through latencies, $F(4,25) = 1.005$, $p>0.20$; all subjects at training readily entered the black compartment (mean = 27.5 sec) and no differences existed between the groups.

Neurochemistry

Mean concentrations of CAT and ACHE in the FPCTX, PCTX and HPC for the various groups of subjects are presented in Tables 2 and 3, respectively.

Separate one-way ANOVAs performed on these neurochemical data revealed significant treatment effects when the FPCTX and PCTX regions were analyzed [CAT: $F(4,41) = 19.406$, $p<0.001$; ACHE: $F(4,41) = 25.589$, $p<0.001$; CAT: $F(4,41) = 3.681$, $p<0.025$; ACHE: $F(4,41) = 3.284$, $p<0.025$, respectively]. No significant between-group differences were observed when CAT, $F(4,41) = 0.978$, $p>0.20$, and ACHE, $F(4,41) = 1.388$, $p>0.20$, concentrations in HPC were examined. Subsequent pairwise comparisons indicated that, relative to the SHAM group, NBM animals exhibited lower enzyme levels in the FPCTX [CAT: $t(16) = 7.265$, $p<0.001$; ACHE: $t(16) = 6.563$, $p<0.001$] and PCTX [CAT: $t(16) = 2.276$, $p<0.05$; ACHE: $t(16) = 2.868$, $p<0.02$]. No other group, including FCTX/ANT and LV animals, differed significantly from SHAM operated subjects on either neurochemical index.

DISCUSSION

Several conclusions can be drawn from the present data. First, they clearly indicated that excitotoxic-induced damage to intrinsic neurons in the frontal cortex (FCTX/ANT group), but not to more posterior frontal cortical cells (FCTX/POST group), and lesions of the nbM, produced memory impairments when rats were tested for 72-hour retention of passive avoidance. The fact that cortical damage produced mnemonic consequences similar to those seen following nbM destruction suggests that a common mechanism may account for the behavioral deficits following cortical and subcortical lesions. Given that the nbM projects to the frontal cortex (7), it could be hypothesized that a loss of cortical terminal sites for nbM projections (produced by damage to intrinsic frontal cortical cells) or removal of the normal inputs to these frontal cortical terminal sites (produced by nbM lesions) may be responsible for the observed passive avoidance memory deficits. Thus, it can be argued that the anatomical site responsible for the memory impairment resulting from either frontal cortical or nbM damage is within the frontal cortex (i.e., loss of cortical terminal sites or loss of normal inputs to cortical terminal sites). Evidence in support of this "cortical" hypothesis has already been reported (4). In this study rats prepared with ibotenic acid-induced lesions of the nbM combined with frontal cortical lesions no longer responded to the therapeutic effects of physostigmine therapy when tested in a passive avoidance paradigm. This was in sharp contrast to the

passive avoidance performance of animals with only nbM lesions who showed significant memory enhancement after physostigmine administration. Although the present "cortical" hypothesis is highly speculative and in need of further empirical support, such a hypothesis implies that certain neurodegenerative diseases (e.g., Alzheimer's disease) might have their pathophysiological origins within the cerebral cortex.

With respect to the neurochemical data, they indicated that NMDA-induced lesions of the nbM, like ibotenic acid-induced lesions [e.g., (1, 3, 7, 12)], produced cortical cholinergic abnormalities (i.e., depletions in CAT and ACHE). This finding is in agreement with the data reported by Stewart, Price, Olney, Hartman and Cozzari (17) and suggests that NMDA may be a useful tool in lesion studies examining nbM function.

The neurochemical data also indicated that the NMDA excitotoxic lesion procedure produced site-specific, axon-sparing damage. This is indicated by the findings that 1) NMDA infused into the nbM did not alter hippocampal CAT and ACHE levels and 2) cortical or hippocampal CAT and ACHE levels were unaffected when NMDA was infused into the lateral ventricle or into the frontal cortex. These results suggest that, at least with respect to the cholinergic system and those parameters used, NMDA infusion produces axon-sparing lesions without causing distal neurochemical damage.

Taken together, these data expand the results of those animal studies examining intrinsic cortical functioning (4, 13, 15, 16) by showing that axon-sparing damage to the frontal cortex produces memory impairments similar to the impairments observed following nbM lesions. More generally, it is suggested that the cortical lesion procedure employed in the present communication is appropriate for studying intrinsic cortical function and may be of use in developing animal models of certain neurodegenerative diseases.

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