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# Effects of Excitotoxic Lesions of the Nucleus Basalis Magnocellularis on Conditioned Taste Aversion and Inhibitory Avoidance in the Rat

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LÓPEZ-GARCÍA, J. C., J. FERNÁNDEZ-RUIZ, M. L. ESCOBAR, F. BERMÚDEZ-RATTONI AND R. TAPIA. *Effects of excitotoxic lesions of the nucleus basalis magnocellularis on conditioned taste aversion and inhibitory avoidance in the rat.* PHARMACOL BIOCHEM BEHAV **45**(1) 147–152, 1993.—The role of the nucleus basalis magnocellularis (NBM) in a variety of learning tasks is well known. Lesions of this nucleus result in a reduction of cholinergic transmission throughout a vast portion of the cortex. Because cholinergic transmission in the insular cortex seems to be important for the acquisition of conditioned taste aversion, the aim of the present work was to study the effects of bilateral chemically induced lesions of the NBM on this conditioning, as correlated with some cholinergic markers in the insular cortex. The effect on inhibitory avoidance was also studied. Lesions prevented the acquisition of the aversion and disrupted retention of the task in previously trained animals. Learning in the inhibitory avoidance paradigm was also notably affected. Postlesion reductions of choline acetyltransferase and acetylcholinesterase activities and of $K^+$-stimulated [$^3$H]acetylcholine release were found in the insular cortex. Further, in intact rats labeling of NBM neurons was observed by retrograde tracing after injection of Fluoro-Gold into the insular cortex. These findings indicate that the NBM is involved in the neural integration of feeding behavior and that its cholinergic projection to the insular cortex is one of the implicated neurotransmitter systems.

Nucleus basalis magnocellularis  Insular cortex  Conditioned taste aversion  Choline acetyltransferase  Acetylcholine release

THE nucleus basalis magnocellularis (NBM) is the major source of cholinergic projections to the cerebral cortex (25, 26,30) and it is known to be involved in a variety of learned behaviors (5,6,17,27,31,34,36).

Conditioned taste aversion (CTA) is a learning paradigm in which animals acquire aversion to a taste cue when it is followed by digestive malaise (14). The brain structures involved in CTA learning have been well established (19). The agranular insular cortex (IC), a region of the temporal cortex in the rat, corresponding to Krieg's areas 13 and 14 and referred to as gustatory neocortex (4), has been implicated as a neural substrate of CTA (4,20). Recently, it has been demonstrated that the IC is also involved in the acquisition and consolidation of spatial and inhibitory avoidance learning tasks (2,3).

We have previously shown that the adult IC is able to release significant amounts of radioactive acetylcholine (ACh) after $K^+$-depolarization in a $Ca^{2+}$-dependent manner and possesses choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activities (21). However, it is not known whether this cholinergic input arises from the NBM and whether such projection is involved in CTA learning. Hence, the aim of the present work was to study the existence of a cholinergic pathway arising from the NBM to the IC and its possible role in the flow of taste information. For this purpose, we analyzed the effect of excitotoxin-induced lesions of the NBM on CTA and its relationship with neurochemical alterations in the cholinergic neurotransmission in the IC.

## METHOD

### *Subjects and Surgical Procedure*

Adult, male Wistar rats weighing 250 ± 10 g at the start of the experiment were used. They were housed individually,

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under an inverted 12 D : 12 L cycle, with food and water ad lib except when indicated.

Under pentobarbital anesthesia (50 mg/kg), bilateral lesions of the NBM were made in 40 animals by stereotaxic injection of 0.5 μl 0.12 M quisqualic acid (QA) dissolved in phosphate-buffered saline (PBS), using the following set of coordinates with respect to bregma: AP −1.8 mm, L ±3.2 mm, V −6.8 mm (29). Bregma and lambda were set at the same horizontal level. The use of this neurotoxin differs from most articles, in which ibotenic acid has been employed to destroy the NBM. However, it has been reported that QA shows a higher degree of specificity for NBM neurons than ibotenate and that after QA-induced lesion of the NBM cortical ACh is decreased as after ibotenate acid but without the nonspecific behavioral deficits observed with the latter (9).

The duration of the stereotaxic injection was 5 min, with a further 5-min period allowed for diffusion. In preliminary experiments, it was observed that the bilateral injection of QA during the same period of anesthesia resulted in severe loss of weight and death of the majority of animals. Therefore, a 1-week interval between the QA injection into the right and left NBM was allowed. Control rats ($n$ = 13) received injections of PBS only. Behavioral and neurochemical tests were started 2 and 4 weeks, respectively, after the second injection (see the Results section). Of the 40 rats injected, 14 were discarded because the histological examination showed that the injection site was misplaced.

### *Behavioral Procedures*

*Inhibitory avoidance.* Training for inhibitory (passive) avoidance was carried out in a two-compartment box (30 × 40 × 15 cm) divided by a sliding door. One of the chambers was illuminated by a 40-W light bulb. The other chamber was not illuminated and its floor was a metal plate through which electric shocks were delivered. During the acquisition session, animals were placed in the illuminated chamber and after 30 s the sliding door was opened, allowing the rat to enter the dark compartment. The time elapsed since the door was opened until the rat moved into the dark chamber was recorded. Then, the door was closed and a 0.8-mA DC foot-shock was delivered for 3 s. The door was opened and the animal was allowed to return to the illuminated side. Twenty-four hours later, the same procedure was followed except foot-shock was not applied (retention trial). If the rat did not enter the dark chamber within 600 s, the test was stopped and the animal was returned to its home cage.

*Conditioned taste aversion.* A previously described experimental model of CTA was used (22). Briefly, animals were deprived of water for 24 h and trained to drink water twice a day during 10-min trials for 4 days. On the fifth day, a 0.1 M LiCl solution was given instead of water to induce taste aversion. After 4 more days of baseline consumption, water was substituted by a 0.1 M NaCl solution to test the aversion. LiCl and NaCl are indistinguishable by rats (38). A decrease of NaCl intake during the test trial was considered as aversion to the salty taste.

### *Neurochemical Procedures*

*Release of labeled neurotransmitters.* The simultaneous release of [$^{14}$C]GABA and [$^{3}$H]ACh in IC slices was studied using a previously described superfusion method (21,28). Animals were decapitated and their brains quickly removed. The IC was dissected using the middle cerebral artery and the rhinal sulcus as references (4,20,37). IC slices (200 μm thickness) were obtained in a McIllwain tissue chopper and preincubated (about 15 mg tissue) for 10 min at 37°C in 1 ml of an oxygenated medium containing (in mM concentrations): NaCl 118, KCl 4.7, $Na_2HPO_4$ 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2, glucose 5.5, Tris-HCl 25, pH 7.4, aminooxyacetic acid 0.1, and eserine 0.1. After this period, the slices were incubated for another 10-min period in the presence of both [$^{14}$C]GABA (2 μCi, 0.5 μM) and [$^{3}$H]choline chloride (2 μCi, 0.5 μM) and 0.8-ml aliquots were transferred to superfusion chambers holding 0.65-μm filters. Tissue was washed by superfusion during 5 min and then the collection of 1-min fractions of the superfusate was started. At the end of the fifth fraction, the medium was substituted by one containing 47 mM KCl and 10 more fractions were collected. The radioactivity in each fraction and that remaining in the filter was counted by scintillation spectrometry, with the double-channel procedure; correction for $^{14}$C/$^{3}$H overlapping was made. Results are expressed as percent of total radioactivity released per minute. Total radioactivity is the sum of total released radioactivity plus that remaining in the filter at the end of the superfusion.

The identity of the radioactive compounds released by $K^+$-depolarization under these experimental conditions has been previously established. About 80% of the $^{14}$C label released corresponds to GABA (28), whereas more than 95% of the tritium radioactivity released represents ACh (21).

*Enzyme activities.* Water homogenates of the IC were used for measuring enzymatic activities by previously described procedures. ChAT (13,21) and glutamate decarboxylase (GAD) (1,21) activities were measured by radioisotopic techniques and AChE activity according to a spectrophotometric method (10). Protein was determined using the Folin reagent method as described (23).

### *Histology*

To assess the lesion induced by QA microinjection, some animals were anesthetized with an overdose of pentobarbital and perfused through the ascending aorta with 0.15 M NaCl followed by a 4% paraformaldehyde, 0.1% glutaraldehyde solution in PBS. The brains were subsequently immersed for 2 h in the fixation solution and then in 20% sucrose during 24 h, prior to sectioning. A series of coronal sections (40 μm thickness) were cut in a cryostat, collected in PBS, mounted on gelatin-coated slides, and stained with cresyl violet (Nissl staining) by standard procedures.

In another group of 10 nonlesioned rats, 0.5 μl of a 2% Fluoro-Gold solution (33) were unilaterally injected in the IC over a 5-min period. Four days later, animals were perfused, their brains sliced as described above, and the site of injection verified histologically by locating the needle track. The appearance of Fluoro-Gold in retrogradely labeled neurons in the NBM was studied with the aid of a fluorescence microscope.

### *Statistical Analysis*

Overall differences among the data from the behavioral experiments were analyzed using one-way analyses of variance (ANOVAs). Posthoc comparisons were made using the Fisher's least significant difference test. Biochemical data were analyzed using Student's $t$-tests. In all cases, values of $p < 0.05$ were considered significant.

### *Chemicals*

[U-$^{14}$C]Aminobutyric acid (sp. act. 192 mCi/mmol) and [acetyl-$^{3}$H]acetyl-coenzyme A (1.6 Ci/mmol) were purchased

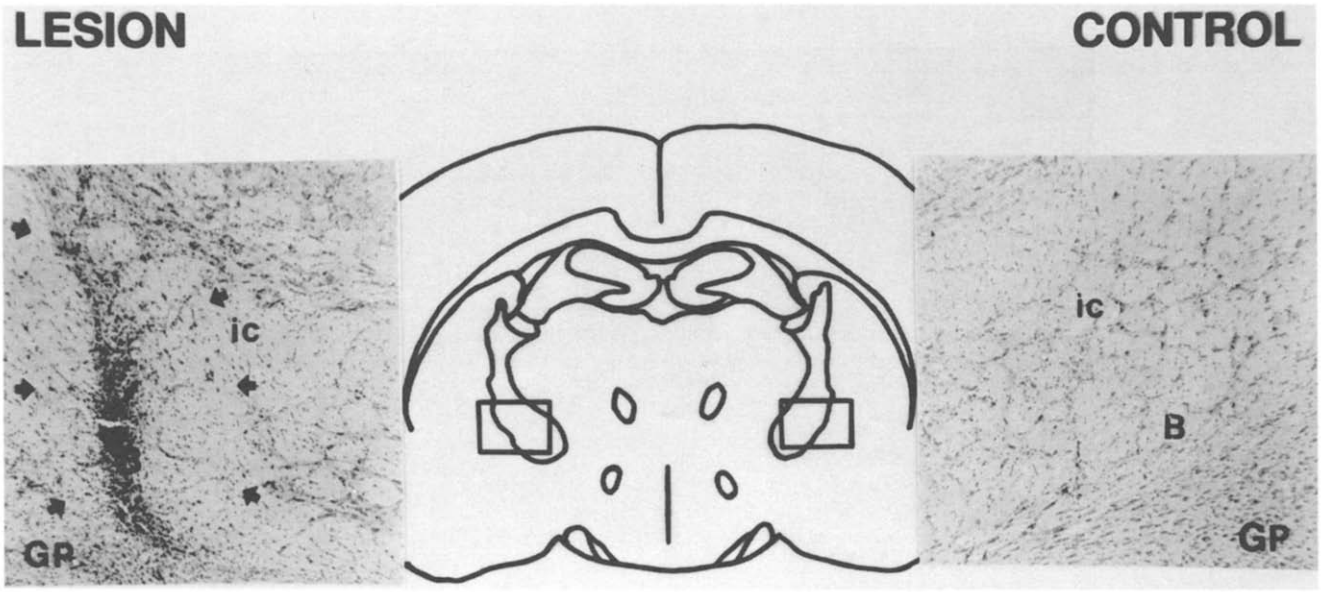


FIG. 1. Center: diagram of a section of rat brain at the NBM. Left: micrograph of a Nissl-stained section of the nucleus basalis magnocellularis (NBM) (B) 2 weeks after the lesion induced by quisqualic acid (QA) microinjection; the arrows indicate the limits of the lesion (×20). Right: micrograph of the same region in a control rat. GP, globus pallidus; ic, internal capsule. Note the important gliosis in the lesioned region.

from NEN Dupont (Boston, MA). [Methyl-$^3$H]choline chloride (80 Ci/mmol) and [1-$^{14}$C]L-glutamic acid (56 mCi/mmol) were obtained from Amersham (Buckinghamshire, UK). Quisqualic acid was purchased from Tocris Neuramin (Essex, UK). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

## RESULTS

### *Histological Observations*

*Retrograde tracing.* Injection of Fluoro-Gold into the IC of intact animals resulted in labeling of a great number of neurons in the ventroposteromedial thalamic nucleus and in the basolateral amygdala ipsilateral to the injection site, as previously described (11). In agreement with other studies (32), in the ipsilateral NBM some Fluoro-Gold containing large neurons were observed, although they were less abundant than in the former structures (results not shown).

*Assessment of the NBM lesion.* In control as well as in lesioned animals, the needle track was readily identified by the presence of gliosis. In PBS-injected animals, gliosis was also observed in the NBM area but the appearance of the neurons within the injection site was normal. The injection of QA in the NBM, however, produced loss of magnocellular neurons in the area, as revealed by Nissl staining (Fig. 1).

### *Behavioral Observations*

*Inhibitory avoidance.* The deleterious effect of NBM lesion on the inhibitory avoidance task has been previously reported (17,27). In the present study, no differences were observed in the latency to enter the dark chamber during the acquisition session between the control and the experimental group (< 30 s in most cases) and, in agreement with the previously described deficits, 70% of control rats reached the maximum latency allowed during the retention trial, with a mean value of 485 s, whereas NBM-lesioned rats entered the dark chamber within the first 200 s of the test (Table 1). All animals vocalized as a response to the electric shock during the acquisition session, indicating that pain sensitivity was not affected by the lesion.

*Conditioned taste aversion.* A small, nonsignificant reduction in the intake of the LiCl solution vs. the baseline intake (neophobia) was found in the control group (Table 2). This trend was not observed in lesioned animals. During the test, however, a highly significant reduction in NaCl intake was observed in control animals, whereas NBM-lesioned rats drank as much of the solution as during their daily intake (Table 2). As mentioned above, a decreased intake during the test trial should be considered as aversion to the salty taste.

In another series of experiments, the effect of the NBM lesion in previously trained animals was studied. In this case, the LiCl solution was given 2 days before the first QA injection into the right NBM and animals were tested with the NaCl solution 2 weeks after the injection into the left side (see the Method section). As shown in Table 2, there was a marked decrease of NaCl consumption by the control group during

TABLE 1

EFFECT OF NBM LESION ON INHIBITORY AVOIDANCE

| | Inhibitory Avoidance Latencies (seconds) | |
|---|---|---|
| | Acquisition | Test |
| Control (13) | 20.1 ± 5.0 | 485 ± 62.5 |
| Lesioned (26) | 22.6 ± 5.6 | 167 ± 44.6* |

Figures are mean values ± SEM for the number of animals shown in parentheses.

*$p < 0.001$ vs. control group.

TABLE 2

EFFECT OF NBM LESION ON CONDITIONED TASTE AVERSION

| | Acquisition | Baseline | Test | % Intake |
|---|---|---|---|---|
| Lesion before acquisition | | | | |
| Control (13) | 9.1 ± 0.9 | 10.8 ± 0.5 | 5.2 ± 0.5$^{+}$ | 49 ± 5 |
| Lesioned (26) | 10.3 ± 0.7 | 10.5 ± 0.4 | 9.4 ± 0.8* | 90 ± 7* |
| Lesion after acquisition | | | | |
| Control (10) | 9.6 ± 0.8 | 10.7 ± 0.6 | 6.3 ± 1.3$^{+}$ | 52 ± 14 |
| Lesioned (9) | 11.6 ± 0.9 | 10.4 ± 0.4 | 12.7 ± 1.1* | 122 ± 10* |

Results are expressed as absolute water intake (in ml) during each of the stages of the behavioral procedure and as percentage of water intake during the test as compared to baseline consumption (last column). Figures are mean values ± SEM for the number of animals shown in parentheses.

*$p < 0.001$ vs. control group.

$^{+}p < 0.001$ vs. baseline intake.

the test, whereas lesioned animals did not reduce the intake even though they had been previously trained.

*Biochemical Determinations*

*Release of neurotransmitters.* [$^{14}$C]GABA release from IC slices in response to high $K^+$ concentrations is shown in Fig. 2. As previously described (21), in IC slices from control rats $K^+$-depolarization produced a threefold peak increase of labeled GABA release as compared with the basal prestimulation value. The NBM lesion did not affect this release because identical results were obtained with IC slices prepared from lesioned animals.

[$^3$H]ACh release was studied in the same set of IC slices used for [$^{14}$C]GABA release experiments (see the Method section). As shown in Fig. 2, and in agreement with previous findings (21), $K^+$-depolarization resulted in a 100% peak stimulation of ACh release in control IC slices. In tissue obtained from lesioned animals, there was a 37% reduction of the $K^+$-stimulated [$^3$H]ACh release (stimulation peak related to the basal release) as compared to control tissue.

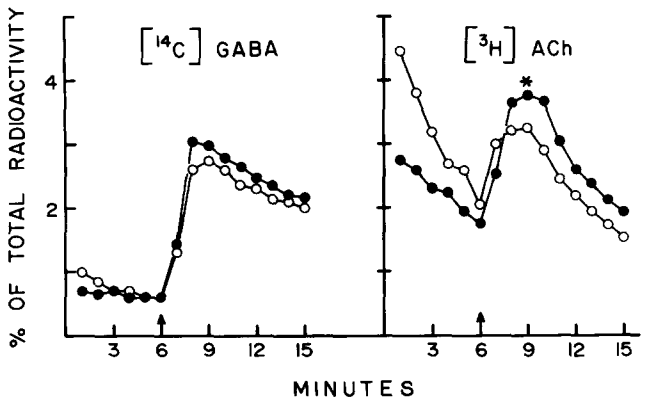


FIG. 2. $K^+$-stimulated release of labeled GABA (left) and acetylcholine (ACh) (right) from insular cortex (IC) slices obtained from nucleus basalis magnocellularis (NBM)-lesioned animals (○) and control rats (●) in the presence of $Ca^{2+}$. After loading the slices simultaneously with [$^{14}$C]GABA and [$^3$H]choline, they were superfused as described in the Method section. At 6 min (arrow), the superfusion medium was substituted by medium containing 47 mM KCl. Mean values of 26 experiments for lesioned rats and 13 for control animals. The maximum SEM was 20% of the corresponding mean but for most points it was smaller than 10%. *$p < 0.05$ vs. control group.

*Enzyme activities.* As shown in Table 3, IC homogenates from control and lesioned animals had similar GAD activity. When the incubation medium was supplemented with pyridoxal-5′-phosphate, GAD activity is enhanced by 100% and the NBM lesion had no effect on GAD activation by its coenzyme. In contrast, ChAT activity in IC homogenates was notably reduced (46% decrease as compared to controls) after the NBM lesion. AChE activity was also decreased (by 30%) in lesioned animals, although this reduction did not reach statistical levels of significance (Table 3).

## DISCUSSION

The major cholinergic input to the cerebral cortex is the NBM, and this seems to include a specific projection to the IC (32). The histological, neurochemical, and behavioral results of the present work clearly support this possibility and further indicate that this pathway may be involved in CTA. Besides the appearance of labeled neurons in the NBM when the retrograde tracer Fluoro-Gold was injected into the IC of intact rats, our neurochemical observations after the QA-induced lesion indicate the putative cholinergic nature of this pathway.

TABLE 3

EFFECT OF NBM LESION ON ENZYME ACTIVITIES IN THE IC

| | Control (13) | Lesioned (26) |
|---|---|---|
| GAD | | |
| +PLP | 45.3 ± 5.4 | 40.8 ± 6.3 |
| −PLP | 16.9 ± 2.5 | 21.0 ± 3.1 |
| ChAT | 62.0 ± 8.9 | 33.4 ± 4.2* |
| AChE | 942 ± 196 | 672 ± 88 |

Results are expressed as nmol/h/mg protein. Figures are mean values ± SEM for the number of animals shown in parentheses. GAD activity was determined in the presence and absence of pyridoxal-5′-phosphate (PLP).

*$p < 0.01$ vs. control group.

The notable reduction of ChAT activity in the IC after QA-induced lesion of the NBM is in agreement with previously reported decreases in other cortical regions using QA as the neurotoxic agent (8). In addition, we observed a 30% decrease of AChE activity, although with considerable experimental variability, and a 37% reduction in [$^3$H]ACh release. A greater release reduction was expected because ChAT activity was considerably decreased and the tissue was loaded with labeled choline. This disagreement can be explained by the remaining unlesioned cholinergic fibers, which might take up choline and synthesize and release ACh in a normal fashion. In fact, at least 20% of the ACh present in the cortex does not arise from the NBM (9,16) and it may be presumed that this value applies also to the IC.

The fact that [$^{14}$C]GABA release and GAD activity in the IC were not altered by the NBM lesion rules out the possibility of nonspecific damage of the IC and indicates that GABAergic neurotransmission, if involved, is not sufficient for CTA learning. The possibility that other neuroactive substances known to be present in the IC, such as glutamate (21), cholecystokinin, somatostatin, or enkephalin (24), may be involved in gustatory neural processing remains open.

Several avoidance and spatial learning tasks are affected by NBM destruction. The impairment of inhibitory avoidance in NBM-lesioned rats seen in the present study agrees with previous findings on the role of NBM in this model (17,27). Because lesions of the IC also disrupt passive avoidance (2,3) and because, as discussed above, there is a NBM projection to the IC, the loss of this connection may be involved in this behavioral deficit.

The main and novel behavioral finding of the present study was the marked impairment of CTA learning after NBM damage. Our results do not establish the particular stage of CTA learning in which the NBM is involved. Because central cholinergic blockade prevents the acquisition of CTA (15), the NBM projection to the IC might be the anatomic substrate of such interference. On the other hand, the NBM may also be involved in CTA information retrieval. Indeed, our present results show that animals that were trained before the surgery did not retain the CTA after NBM destruction, suggesting a role for NBM in CTA memory.

Opposite the present results, in a previous study in which the NBM was lesioned with ibotenate no effect on CTA was observed (12). This discrepancy might be due to the fact that in the latter work animals were preexposed to the conditioned taste several times prior to the induction of the digestive malaise.

Finally, it has been proposed that the learning deficits observed after NBM lesions are due to an effect on its limbic targets, mainly the amygdala, and not to the interruption of its neocortical projection (18). However, it has been shown that CTA learning is not mediated by the amygdala (3,7), and a recent report showed no participation of the NBM cholinergic projections to the amygdala in CTA (35).

In conclusion, our data indicate the existence of a cholinergic NBM projection to the IC and suggest that it may be one of the neurotransmitter systems involved in the neural integration of feeding behavior.

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