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Cholinergic Basal Forebrain Is Critical for Social Transmission of Food Preferences

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ABSTRACT: Studies using selective lesions of basal forebrain cholinergic neurons suggest that these neurons play a role in attentional processing, but not learning and memory. However, the tests of learning and memory used thus far have been restricted largely to spatial tasks. In the present study, we examined whether the cholinergic basal forebrain plays a role in a form of nonspatial associative memory, the social transmission of food preferences. Sham-operated control rats were compared to rats with 192 IgG-saporin lesions of the medial septum/diagonal band cholinergic projections to hippocampus or nucleus basalis magnocellularis/substantia innominata cholinergic projections to neocortex. Both lesions impaired 24-h retention of a learned social food preference relative to controls, despite performance on an immediate retention trial that was indistinguishable from controls. Moreover, 24-h retention of the socially learned food preference correlated strongly with cholinergic enzymatic activity in the neocortex, but not in the hippocampus. Immunohistochemical data confirmed significant and selective lesion-induced cholinergic depletions in the intended brain regions. These data provide evidence that the cholinergic basal forebrain, particularly the cholinergic projection to neocortex, is involved in the formation and/or retrieval of social memories related to food preference, and suggest a role for cortical acetylcholine in consolidation of associative memory processes. *Hippocampus 2000;10:729–738.* © 2000 Wiley-Liss, Inc.

KEY WORDS: medial septum; nucleus basalis; olfaction; 192 IgG-saporin; nonspatial learning and memory

INTRODUCTION

The role of the cholinergic basal forebrain in the formation or retrieval of memories has been the subject of considerable debate recently (Chappell et al., 1998; Wrenn and Wiley, 1998). Studies using nonselective neurotoxins such as ibotenic acid to lesion the basal forebrain report lesion-induced impairments on a variety of tasks (Dekker et al., 1991; Gallagher, 1997). Furthermore, in some cases, the magnitude of the cholinergic decrease is associated with the magnitude of the memory impairment (Dunnett et al., 1987; Fibiger, 1991). However, because the toxins used in these earlier studies damage both cholinergic and noncholinergic neurons in the basal forebrain, it has been difficult to assess the specific contributions of cholinergic neurons to memory processes. More recent studies using the selective cholinergic toxin 192 immunoglobulin G (IgG)-saporin to lesion the basal forebrain have yielded a different pattern of results from that of earlier studies. 192 IgG-saporin lesions of the basal forebrain produce little or no impairments on spatial reference memory tasks (Berger-Sweeney et al., 1994b; Torres et al., 1994; Wenk et al., 1994; Baxter et al., 1995) or on working memory tasks (Wenk et al., 1994; Walsh et al., 1995; Dornan et al., 1997; McMahan et al., 1997; Chappell et al., 1998; Wrenn et al., 1999), despite depletions of cholinergic markers more severe than those produced by nonselective neurotoxins. The general lack of impairments after selective cholinergic lesions has led many researchers to question whether the cholinergic basal forebrain plays any role in learning and memory processes.

A series of elegant studies from several laboratories suggests that the cholinergic system may play a role in selective attention rather than memory (Chiba et al., 1995; McGaughy et al., 1996; Baxter et al., 1997). Extensive depletions of neocortical acetylcholine from lesions to the nucleus basalis magnocellularis/substantia innominata (nBM/SI) result in robust impairments in sustained attention (McGaughy et al., 1996) and in incremental attentional processing of conditioned stimuli (Chiba et al., 1995). In contrast, extensive depletion of hippocampal acetylcholine from lesions to the medial septum/vertical limb of the diagonal band of Broca (MS/VDB) impairs decremental attentional processing of conditioned stimuli (Baxter et al., 1997). These studies point toward a role of the cholinergic basal forebrain in cognitive attentional processes, but not necessarily learning and memory.

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Several studies, primarily using pharmacological manipulations, suggest that social learning and memory may be modulated by the central cholinergic system. In rodents, social recognition of a conspecific is a task dependent on short-term olfactory-related memory processes (Ravel et al., 1994). Generally, drugs that reduce central cholinergic transmission impair the ability of an animal to recognize and remember previously encountered conspecifics, whereas drugs that enhance cholinergic transmission improve short-term social recognition memory in a dose-dependent fashion (Perio et al., 1989; Gheusi et al., 1994; Ravel et al., 1994; Winslow and Camacho, 1995; Levy et al., 1997). Cholinergic stimulation of muscarinic receptors in the olfactory bulbs of rats appears to be necessary for chemosensory reception and the formation of short-term olfactory memory (Ravel et al., 1994). Interestingly, the neural processing of odor cues is synchronized to ongoing theta rhythms recorded in the hippocampus; cholinergic cells of the medial septum that project to hippocampus serve as pacemakers for theta rhythms (Bland, 1986). In total, these studies suggest that the central cholinergic system is critically involved in processing of odor cues and the formation and/or retrieval of social recognition memories; however, the specific brain structures in which cholinergic modulation is critical have not been identified.

In the current study, we sought to examine the role of the cholinergic basal forebrain system in a social learning and memory task that uses olfactory cues, but does not involve aversive stimuli or spatial learning. This task, which examines a socially transmitted food preference, exploits an ethologically meaningful behavior: an animal's ability to learn quickly and remember information pertaining to social olfactory cues (Galef and Wigmore, 1983; Strupp and Levitsky, 1984). In this task, an "observer" rat encounters another "demonstrator" rat that recently has eaten a food with a distinctive scent. Then the observer rat is placed in a separate cage and is given a chance to select food with the same scent smelled on the demonstrator's breath or food with a different and unfamiliar scent. Most observers will show a strong preference for the food smelled on the demonstrator's breath, presumably because this indicates that the food consumed by the demonstrator was not harmful. This form of social learning involves the formation of a specific stimulus-stimulus association in a single acquisition, without any primary reinforcement, and then the expression of the memory in a different context (Bunsey and Eichenbaum, 1995). This form of social learning appears to be dependent on an intact hippocampus (Winocur, 1990; Bunsey and Eichenbaum, 1995). Therefore, we hypothesized that basal forebrain lesions affecting cholinergic projections to hippocampus and neocortex would impair socially transmitted food preference. We compared the performance of control rats to rats with selective lesions to MS/VDB cholinergic projections to hippocampus, or to nBM/SI cholinergic projections to neocortex. After behavioral testing, the extent and selectivity of the lesion were examined using radioenzymatic assays of choline acetyltransferase (ChAT) activity and immunohistochemistry. Finally, correlation analyses were performed to determine whether neocortical and hippocampal cholinergic activity predicted social food preference behavior.

MATERIALS AND METHODS

Subjects

Thirty-eight male, 6-week-old Wistar rats were purchased from Charles River, (Raleigh, NC). They were housed singly (4) or in pairs (34) in a temperature- and humidity-controlled room on a 12/12-h light/dark schedule. Before pretraining for the social learning task began, the rats were allowed food (Harlan Teklad 22/5 Rodent Diet-W, Harlan Teklad, Madison, WI) and water ad libitum. Surgery and behavioral testing were performed during the light phase of the cycle. Rats were housed in an AAALAC International accredited facility, and procedures were approved by the Wellesley College Animal Care and Use Committee.

Surgery

At 9 weeks of age, animals were randomly assigned to one of four groups. The initial numbers of animals in each group were: MS/VDB lesion, n = 10; nBM/SI lesion, n = 10; sham lesion to MS/VDB, nBM/SI, or intracerebroventricular n = 10; no surgery, n = 8. Rats that received no surgery were designated as demonstrators, whereas the remaining rats were designated as observers. Animals assigned to surgical groups were anesthetized with 80 mg/kg ketamine and 5 mg/kg xylazine (i.m.), then placed in a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). Lesion and sham surgeries were conducted by injection as described below using a motorized stereotaxic injector (Stoelting, Wood Dale, IL) and 28-gauge needle Hamilton syringe filled with either 0.175 μg/μl 192 IgG-saporin in sterile phosphate-buffered saline (lesion surgeries) or sterile phosphate-buffered saline (sham surgeries).

For MS/VDB surgery, two holes were drilled in the skull at stereotaxic coordinates anterior posterior (AP) = +0.45 mm and medial lateral (ML) = ± 0.6 mm from Bregma (Paxinos and Watson, 1998). Injections were made at two depths at each site, dorsal ventral (DV) = −7.8 mm and −6.2 mm from the surface of the skull. Solutions were delivered at a rate of 0.05 μl/min, injecting a total of 0.3 μl at each of the DV = -7.8 mm sites, and a volume of 0.2 μl at each of the DV = −6.2 mm sites. The syringe was left in place for 6 min after each 0.3 μl injection and for 4 min after each 0.2 μl injection to limit diffusion of solution into the needle track.

For nBM/SI surgery, four holes were drilled in the skull at stereotaxic coordinates AP = −0.75 mm and ML = ±2.3mm (medial sites) and ML = ±3.3 mm (lateral sites) from Bregma. Injections were made at two depths from the skull surface, DV = −7.8 mm at the medial sites, and at depth DV = −8.1 mm at the lateral sites. Solutions were delivered at a rate of 0.1 μl/min, injecting a total of 0.2 μl at each site. The syringe was left in place for 3 min after each injection to limit diffusion of solution into the needle track.

For intracerebroventricular sham surgery, one hole was drilled in the skull at stereotaxic coordinates AP = −1.0 and ML = +1.5 mm from Bregma. Injections were made at a depth of DV = −4.5 mm from the skull surface. The syringe needle was filled with

saline. Saline was delivered at a rate of 1.0 μl/min, injecting a total of 8.0 μl.

Behavioral Apparatus

Testing was conducted in 46-cm-long × 24-cm-wide × 16-cm-high cages equipped with mesh tops. Straight-side wide-mouth 125-ml polycarbonate jars (Nalgene 2116-0125, Nalge Nunc International, Rochester NY) were attached with Velcro® Sticky Back® hook and loop fasteners (Velcro USA Inc., Manchester, NH) to 15.5 × 19-cm acrylic food presentation trays cut from 2-mm-thick shatter-resistant plastic (Lucite®-ES, ICI Acrylics, Cordola, TN extruded acrylic safety glazing sheet). Rat chow was ground coarsely, then ground finely in a coffee mill. Dried sweet basil leaf (Spice Islands, San Francisco, CA) was ground finely, whereas ground thyme (Durkee, San Francisco, CA) was used without further processing. Ground rat chow was flavored by addition of either ground basil leaf (0.7% by weight) or ground thyme (2% by weight).

Behavioral Testing

The rats were allowed to recover from surgery for 1 week, were habituated to the experimenter for at least 1 week, and subsequently were tested in an open field exploration task and in the Morris water maze, the results of which are reported elsewhere (J. Berger-Sweeney et al., submitted). Five weeks later, the rats were shaped to eat ground chow from plastic dishes, and social learning testing continued during the next 4 weeks. Food was withdrawn 18 h before the start of pretraining and the presentation/demonstration trial and 19 h before the start of the 24-h retention trial; food was made available as soon as the rat had finished its participation in a trial. We have found that this method of food deprivation provides a stronger motivation in this task than keeping the rat at 85% normal body weight. Water was available ad libitum at all times except during the trial sessions.

During pretraining, the rats were presented with food cups containing ground, unflavored rat chow. One food cup was fastened with Velcro to the center of a food tray (for demonstrator rats) or two food cups were fastened side by side, 1.5 cm apart in the center of the food tray (for lesion and sham observer rats). A rat was placed in a cage and allowed to eat for 30 min. Then, the rat was returned to its home cage and the amount of food eaten was determined. Only rats that ate at least 2 g of food during the 30 min of pretraining were included in the study; no rats were excluded from the current study based on this criterion.

Each rat in the lesion and sham groups was assigned randomly to be cued on either basil- or on thyme-flavored food. Before this choice, the rats were assessed on an anise vs. celery and clove vs. marjoram food preference. However, the rats showed a distinct preference for one of the pair over the other, rendering tests using these odors uninformative. In contrast, basil and thyme were eaten with about equal preference. Therefore, the lesion groups were balanced over the two flavors of food (basil and thyme). Rats that were housed together in the same cage were assigned to be cued on the same food and tested at the same time.

Training and testing proceeded in four distinct phases. In phase I, a demonstrator rat was allowed access to the cued food (basil- or thyme-flavored food) for 30 min. The food cup was weighed to ensure that the animal had eaten at least 1 g of food. In phase II, the demonstrator was placed in a second cage with one observer rat from the lesion or sham group. The two rats were allowed to interact for 20 min. In phase III, the observer rat was placed alone in a cage and given a choice between two cups of food—one containing the cued food and the other containing the noncued food. The observer was allowed to eat for 30 min, then returned to its home cage. The two food cups were removed and the amount of each food eaten was recorded. The *percent cued food selected* was determined as 100 × the weight of cued food eaten/the total weight of cued + noncued foods eaten. In phase IV, which occurred 24 h after phase III, the observer again was given a choice between the cued and noncued food, and allowed to eat for 30 min. Then the rat was returned to its home cage, the food cups were weighed, and the percent cued food selected was calculated as in phase III.

Neurochemical Analyses

Immunohistochemistry or radioenzymatic assays of ChAT activity were performed to determine the extent and specificity of the lesion.

Immunohistochemistry

A subset of rats (n = 2 each in MS/VDB and nBM/SI groups) were killed by an overdose of sodium pentobarbital (100 mg/kg) and were transcardially perfused with approximately 100 ml ice-cold 0.9% saline followed by approximately 350 ml ice-cold 4% freshly depolymerized paraformaldehyde at a flow rate of 18 ml/min. Brains were postfixed in 4% paraformaldehyde for 2 h and then were placed in a solution of 20% sucrose in phosphate-buffered saline. Brains were sectioned on a freezing-sliding microtome at a nominal thickness of 60 μm. Sections through the basal forebrain were processed for immunohistochemistry for ChAT (Chemicon polyclonal goat anti-ChAT, Chemicon International, Temecula, CA) or parvalbumin (Sigma monoclonal mouse anti-parvalbumin, Sigma, St. Louis, MO) according to standard avidin–biotin complex methods (Baxter et al., 1995). Immunohistochemistry for ChAT served to confirm the location of the lesion within the basal forebrain. Immunohistochemistry for parvalbumin, which colocalizes with GABAergic neurons in the MS/VDB (Freund, 1989), was used to examine the selectivity of the lesion for cholinergic neurons. Brain tissue from unoperated rats was processed in parallel as a positive control.

ChAT radioenzymatic assay

Rats were sedated with 70% CO_2, a procedure that does not affect ChAT activity (Berger-Sweeney et al., 1994a) and were decapitated. The brains were removed rapidly on ice. Frontoparietal cortex and hippocampus were dissected, weighed, frozen on dry ice, then stored at −70°C until the assay. Using the method of Fonnum (Fonnum, 1975), ChAT activity was determined by mea-

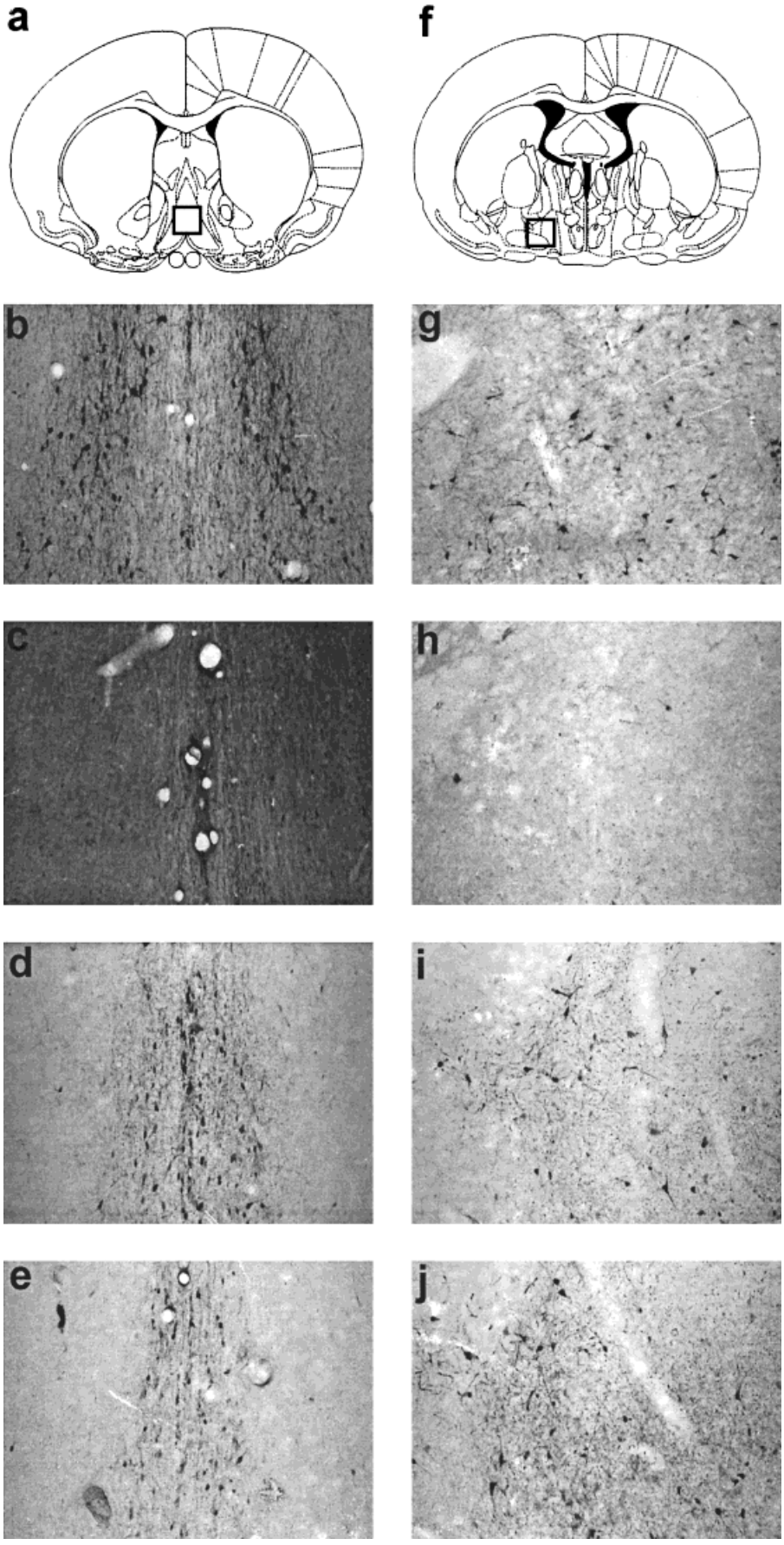


FIGURE 1. Immunohistochemistry reveals choline acetyltransferase (ChAT)-immunopositive neurons in the medial septum/vertical limb of the diagonal band of Broca (MS/VDB) of a control rat (b) that are absent in the MS/VDB of a rat that received a 192 IgG-saporin injection into this region (c). Parvalbumin-immunopositive, GABAergic (Freund, 1989) neurons are present in the MS/VDB of both control (d) and MS/VDB-lesioned (e) rats. Similarly, ChAT-immunopositive neurons are present in the nucleus basalis magnocellularis/substantia innominata (nBM/SI) of a control rat (g) and are not seen in the nBM/SI of a rat that received a 192 IgG-saporin injection into this region (h); whereas the distribution of parvalbumin-immunopositive neurons does not differ between control (i) and nBM/SI-lesioned (j) rats. The approximate locations of the photomicrographs are shown as boxes on the sections illustrated in (a) for the MS/VDB and in (f) for the nBM/SI, respectively; these diagrams are adapted from Paxinos and Watson (1998) copyright Academic Press, by permission.

TABLE 1.

Choline Acetyltransferase Activity in Hippocampus and Neocortex

Group	Hippocampus	% Depletion hippocampus[a]	Neocortex	% Depletion neocortex[a]
Control	117.3 ± 5.7	—	74.8 ± 3.9	—
MS/VDB	9.4 ± 2.4[b]	92.0%	63.1 ± 4.1[c]	15.6%
nBM/SI	124.3 ± 4.2	−6.0%	29.4 ± 2.7[d]	60.7%

[a]% Depletion versus control.
[b]$P < .01$ versus control and nucleus basalis magnocellularis/substantia innominata.
[c]$P < .05$ versus control.
[d]$P < .01$ versus control and medial septum/vertical limb of the diagonal band of Broca.

suring the radiolabeled acetylcholine produced in brain homogenates from [^{14}C]acetyl coenzyme-A and choline, as described elsewhere (Arters et al., 1998). Protein content of the brain homogenates was determined by Bradford microtiter assay (Arters et al., 1998).

Statistical Analyses

Data analyses were performed with SuperANOVA 1.11 (Abacus Concepts, Inc., Berkley, CA). Two-factor analyses of variance (ANOVAs) were performed with lesion status and cued food as independent variables and percent of cued food, grams of cued food, or total food eaten as the dependent variables. When significant differences were identified, post hoc analyses were performed using Student-Newman-Keuls tests. ChAT data were assessed using separate one-factor ANOVAs with lesion status as the independent variable. A repeated-measures ANOVA was performed with the immediate and 24-h retention trials. A one-sample *t*-test against a constant was used to determine if the percent of cued food eaten in the initial trials was greater than chance levels (50%). Univariate correlations were performed comparing the cued food preference in the immediate and 24-h trials to neocortical and hippocampal ChAT.

RESULTS

Subjects

The initial number of animals in each lesion category was MS/VDB n = 10, nBM/SI n = 10, and sham lesion (controls) n = 10. All of these animals were tested in the behavioral studies, but one animal in the MS/VDB group and one in the nBM/SI group were eliminated from both behavioral and chemical data analyses because the ChAT assay showed that these animals did not exhibit the expected depletion in ChAT activity in the appropriate brain. Therefore, the numbers of animals included in the statistical analysis of the behavioral studies are MS/VDB n = 9, nBM/SI n = 9, and sham lesion (control) n = 10. Two from each of the two saporin lesion groups (MS/VDB and nBM/SI) were killed for histological examination. One animal from the sham lesion control group died of unknown causes between the time of behavioral testing and chemical analysis. The remaining rats that were included in the ChAT neurochemical analysis and in the behavior/chemistry correlations are MS/VDB n = 7, nBM/SI n = 7, and sham lesion (control) n = 9.

Histological and Neurochemical Analyses

Immunohistochemistry confirmed that the area of intended lesion (MS/VDB or nBM/SI) was depleted of cholinergic (ChAT-

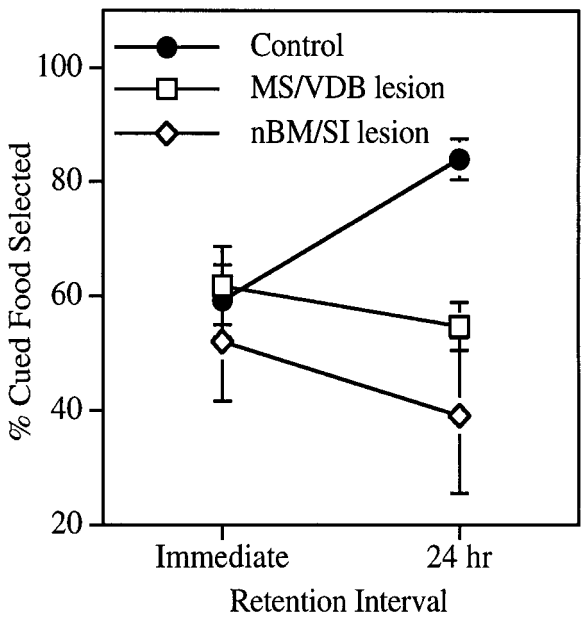


FIGURE 2. The percent cued food selected (100 × weight of cued food eaten/total weight of cued + noncued foods eaten) at two different retention intervals. Performance of control rats is compared to that of the medial septum/vertical limb of the diagonal band of Broca (MS/VDB) and nucleus basalis magnocellularis/substantia innominata (nBM/SI) lesion groups. There are no significant differences among the groups during the immediate trial. During the 24-h retention trial, controls performed significantly better than both lesion groups ($P < .05$ with respect to MS/VDB and $P < .01$ with respect to nBM/SI).

immunopositive) neurons (Fig. 1). In the MS/VDB rats, small areas of nonspecific tissue necrosis were present in the MS. Such an effect of saporin infusions has been noted in other experiments using this toxin (McGaughy et al., 1996). However, parvalbumin-immunoreactive neurons were present around the borders of the area of necrosis, and no alteration in the density or distribution of parvalbumin-immunoreactive neurons was noted outside the areas of tissue necrosis, despite an absence of ChAT-positive neurons (Fig. 1). No tissue necrosis or nonspecific damage was observed in the nBM/SI of rats with 192 IgG-saporin injections into this region. Parvalbumin-immunoreactive neurons were present throughout the entire area of the lesion, and no alteration in the density or distribution of these neurons was noted. The lesions in the two nBM/SI cases analyzed immunohistochemically also extended rostrally to the horizontal nucleus of the diagonal band (HDB), a source of basal forebrain projections to the olfactory bulbs and piriform cortex (Záborszky et al., 1986; Okoyama et al., 1987). This damage was not consistent; it was complete in one case and restricted to the posterior HDB in the other case. The HDB sustained unilateral damage in one MS/VDB case and was undamaged in the other case.

One-factor ANOVAs revealed no significant differences in either cortical or hippocampal ChAT activity among the sham lesion subgroups. Therefore, the data were collapsed into a single control group for subsequent analyses. ChAT enzymatic assays confirmed that the hippocampus and neocortex, the targets of MS/VDB and nBM/SI cholinergic projections, respectively, had significant cholinergic depletions (Table 1). ChAT activity was significantly different among the different groups in the hippocampus [$F(2,20) = 179$, $P = .0001$] and in the neocortex [$F(2,20) = 40$, $P = .0001$]. In MS/VDB rats, hippocampal ChAT activity was reduced more than 90% versus controls, and in nBM/SI rats, neocortical ChAT activity was reduced more than 60%. In addition, there was a small (15%) but statistically significant depletion of neocortical ChAT in the MD/VDB rats. One animal in the MS/VDB lesion group and one in the nBM/SI group had ChAT activities that were much less depleted in the target regions than the rest of the animals in their respective groups (ChAT activities of each of these animals was <2 SD higher than the mean ChAT activity of all animals in its group). These two animals were excluded on this basis from behavioral and chemical analyses.

Behavioral Performance

On the initial (immediate) retention trial after a single social training/demonstration, all of the observer rats showed a slight preference for the cued food [$t(27) = 2.3$, $P = .03$] (Fig. 2). There were no significant differences among the three groups (control, MS/VDB, or nBM/SI) in the proportion of cued food eaten. Also, the rats showed no distinct preference for thyme or basil [$F(1,22) = .25$, $P = .62$]. Figure 3 shows that there was a significant difference in the total grams of food eaten by the different groups [$F(2,22) = 9.0$, $P = .001$]. The nBM/SI group ate significantly less total food (1.7 ± 0.2 g) than the MS/VDB (4.2 ± 0.5 g) or control (3.6 ± 0.4 g) groups (Ps < .01).

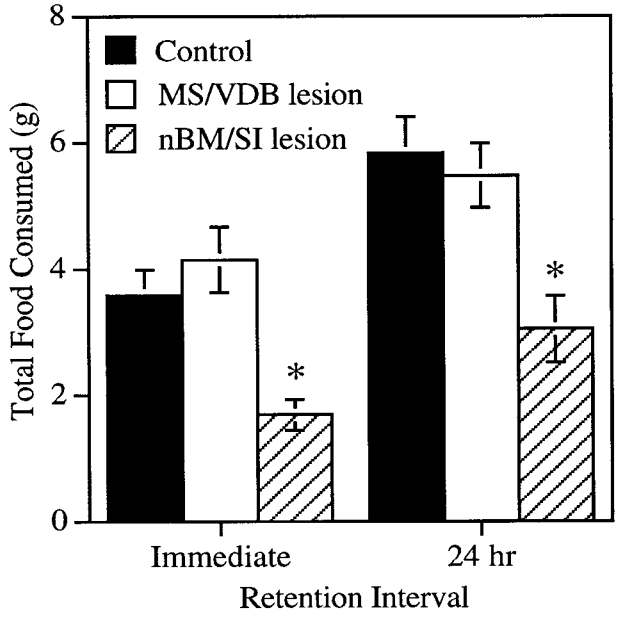


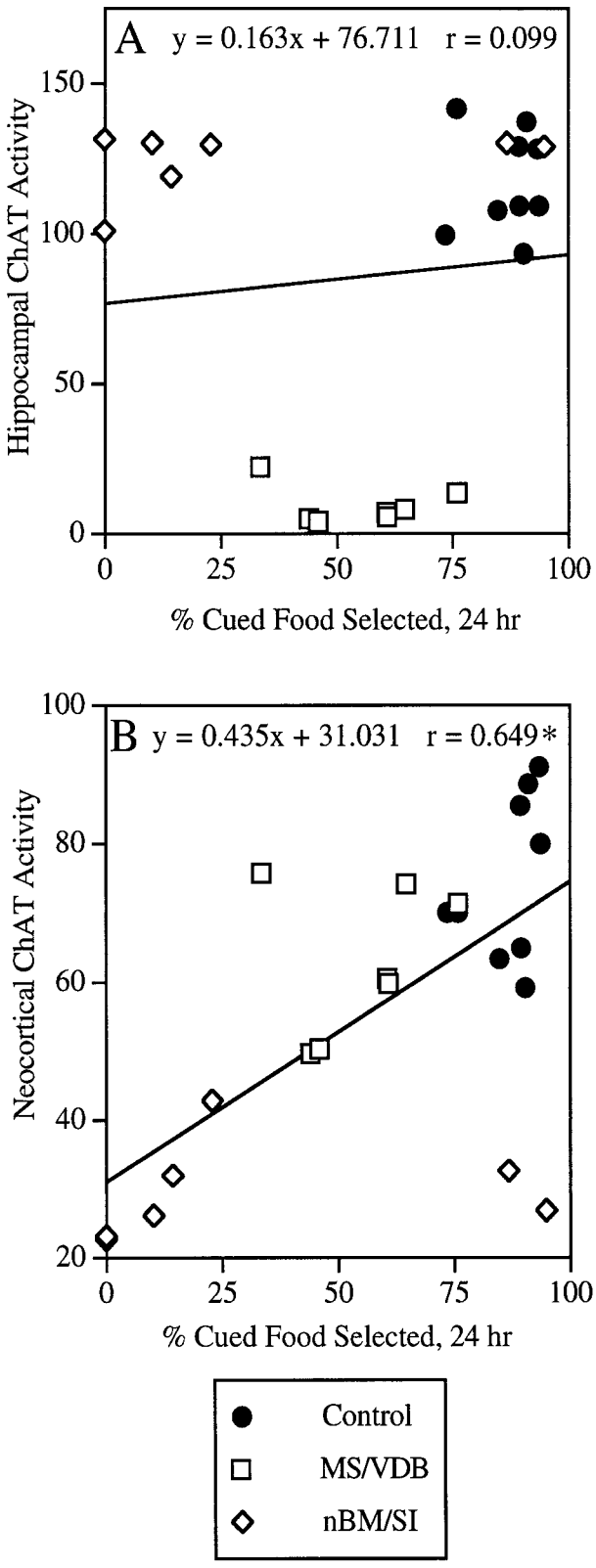
FIGURE 3. The total grams of food consumed at two different retention intervals. The nucleus basalis magnocellularis/substantia innominata (nBM/SI) group ate significantly less food (total of cued and noncued) than control or medial septum/vertical limb of the diagonal band of Broca (MS/VDB) groups (Ps < .01) at both retention intervals.

In the 24-h retention trial, only the control group showed a strong preference for the cued food (Fig. 2). During this trial, there were significant differences in the proportion of cued food eaten among the groups [$F(2,22) = 7.3$, $P = .004$]. Both the MS/VDB and nBM/SI lesioned groups showed considerably less preference for the cued food than did controls ($P < .05$ and $P < .01$, respectively). The rats cued equally well to thyme or basil when it was the cued scent. Similar to the immediate trial, there was a significant difference in the total grams of food eaten by the different groups [$F(2,22) = 8.0$, $P = .002$] (Fig. 3). The nBM/SI group ate significantly less total food (3.0 g ± 0.5) than the MS/VDB (5.6 g ± 0.5) or control (5.8 g ± 0.6) group (Ps < .01).

A repeated-measures ANOVA revealed that the groups performed significantly differently on the immediate vs. 24-h retention trials [$F(2,25) = 4.5$, $P = .02$]. The significant treatment group × retention interval interaction [$F(2,25) = 4.0$, $P = .03$] suggested that the performance of controls, but not that of either lesion group, improved from the immediate to 24-h retention trial. Subsequent post hoc analyses revealed that the nBM/SI group was significantly different from that of controls ($P < .01$) and that only in the control group did performance improve significantly between the immediate and 24-h retention trial ($P < .01$).

Behavior/Chemistry Correlations

Correlation analyses were performed to determine whether the amount of ChAT activity in the neocortex or hippocampus was associated with performance on the immediate or 24-h retention trial. On the immediate retention trial, the percentage of cued food



eaten (the cued food preference) did not correlate highly with either neocortical (r = .22) or hippocampal (r = −.20) ChAT (data not shown graphically). In contrast, during the 24-h retention trial, the percent of cued food eaten (the cued food preference) was highly correlated with neocortical (r = .65, $P = .0005$) but not with hippocampal (r = .10) ChAT (Fig. 4). In the neocortex, high ChAT activity predicted a strong cued food preference in the 24-h retention trial.

DISCUSSION

The current study reveals that rats with selective lesions of the cholinergic projections to hippocampus and neocortex exhibit impaired 24-h retention of a learned social food preference as compared to controls, despite intact performance on an immediate retention trial. Moreover, 24-h retention of this socially learned food preference correlates strongly with cholinergic activity in the neocortex, but not the hippocampus. The enzymatic and immunohistochemical data confirm that the lesions depleted the intended brain regions, and suggest that lesions were selective for cholinergic neurons.

Social Learning vs. Memory vs. Attention

Our data point toward a lesion-induced deficit in social memory, rather than social learning, because the two lesion groups showed a deficit relative to controls on the 24-h but not the immediate retention trial. In other words, all groups exhibited the ability to learn a food preference when tested immediately after acquisition, but the lesioned groups were not able to recall this knowledge with an intervening 24-h delay. Admittedly, a clear interpretation of these data regarding a learning vs. memory deficit is confounded by three factors. First, the cued preference on the immediate trial was not robust; all groups showed only a slight preference (about 60%) for the cued food. This percentage is similar to that in another study with a single acquisition/demonstration trial (Popik and Van Ree, 1993) and lower than that in one previous study with several cued acquisition/demonstration trials (Bunsey and Eichenbaum, 1995). In the 24-h retention trial, the controls displayed an 80% preference for the cued food, suggesting that the controls rats continued to learn during, or consolidated the information after, the immediate "retention" trial. The preference in controls was in sharp contrast to that in the lesion groups (55 ± 4% for the MS/VDB group and 39 ± 14 % for the nBM/SI group). It is impossible, therefore, to distinguish absolutely

FIGURE 4. Correlations between choline acetyltransferase (ChAT) activity in hippocampus (A) or neocortex (B) and the percent cued food selected in the 24-h retention trial. Hippocampal ChAT activity was not correlated with the percent of cued food selected. In contrast, neocortical ChAT activity was significantly correlated ($P < .01$) with the percent of cued food selected. MS/VDB, medial septum/vertical limb of the diagonal band of Broca; nBM/SI, nucleus basalis magnocellularis/substantia innominata.

whether the lesion-induced deficit at 24 h resulted because the lesioned rats could not form a useful social memory in the immediate trial, could not consolidate the memory after the immediate trial, or failed to retrieve the memory during the 24-h trial. Second, the nBM/SI group ate less total food than the other groups. Despite the fact that the nBM/SI group ate *proportionally* less cued food, the decreased amount of food eaten by the nBM/SI group could reflect a reduction in arousal or motivation in food consumption. Therefore, it is impossible to eliminate nBM/SI-induced alterations in food motivation or the processing of olfactory- and/or gustatory-related information in the nBM/SI group as a possible confound. However, nBM/SI lesions do not disrupt acquisition of a spatial navigation task (Baxter et al., 1995) nor the acquisition of Pavlovian conditioned responses to a food-conditioned stimulus (Chiba et al., 1995), suggesting that nBM/SI rats do not display nonspecific motivational deficits. Interestingly, the MS/VDB group ate an amount of food similar to that of controls, yet ate *proportionally* less cued food. The latter finding suggests a dissociation between impaired food preference and decreased food intake. Third, it appears that five nBM/SI rats avoided the cued food (<25% of cued food selected), whereas two nBM/SI rats preferred the cued food (>75% of cued food selected). If we assume that both avoidance and preference of the cued food requires memory of the cued food, then the nBM/SI group may be demonstrating retention. However, it is important to point out that generally each rat has a preference for one food in the food pair. The thyme/basil food pair was selected because *overall*, the rats had no strong preference for one over the other in the pair. As such, the nBM/SI rats that appear to either prefer or avoid a food may be expressing their individual relative preferences for the two foods. The demonstration of the cued food in their case was not strong enough to override this preference, perhaps because the cued scent was not learned robustly, or the memory was not consolidated properly.

Can these data also be interpreted as a lesion-induced attentional deficit? The lack of a food preference at 24 h in the lesioned groups could be caused by an attentional deficit in initial encoding of relevant olfactory information necessary to exhibit a later food preference. Such an interpretation is consistent with several other studies in which attentional capabilities are impaired after 192 IgG-saporin lesions to MS and/or nBM (McGaughy et al., 1996; Baxter et al., 1997). However, in the current study the lesioned rats did display a slight preference for the cued food in the immediate trial. This preference suggests that the lesioned rats were capable initially of attending to odor cues and forming an association between the odor and safe food, but then had difficulty retrieving this association 24 h later. As such, we interpret this performance deficit as an impairment in memory rather than attention.

It is important to note that neocortical ChAT activity predicted performance of the social food preference task quite strongly at 24 h, but not immediately after training. This strong correlation ($r = .65$) provides evidence of an association between the neocortical cholinergic system and delay-dependent food preference. The delay-dependent nature of the deficit points toward a consolidation or retrieval deficit. The evidence provided here, along with considerable pharmacological evidence (Soffié and Lamberty, 1988; Ravel et al., 1992; Gheusi et al., 1994; Ravel et al., 1994; Winslow and Camacho, 1995), provide convincing evidence of the involvement of the central cholinergic system in the consolidation or retrieval of social memories.

Neocortex vs. Hippocampus

Two previous reports suggest that damage to the hippocampus impairs long-term retention of the social transmission of food preference (Winocur, 1990; Bunsey and Eichenbaum, 1995). In these studies, severe deficits in social transmission of food preference were noted after electrolytic lesions to dorsal hippocampus (Winocur, 1990) or after ibotenic acid lesions that included the hippocampus proper, dentate gyrus, and subiculum (Bunsey and Eichenbaum, 1995). Furthermore, in the latter study, the extent of damage to the hippocampal region correlated highly ($r = .55$) with food preference on the 24-h retention test. As such, we predicted that MS/VDB cholinergic lesions would impair 24-h retention of food preference. Our results support this prediction; food preference for the cued food in the 24-h retention trial in MS/VDB-lesioned rats was less than that of controls. Interpretation of our results is obscured by the fact that the MS/VDB-lesioned rats had small, but significant, cholinergic depletion in neocortex as well as hippocampus. In addition, hippocampal ChAT activity, a marker of the extent of the cholinergic lesion, was not correlated with performance of the food preference task. Although socially mediated food preference may involve the hippocampus, we do not provide strong support here that the cholinergic septohippocampal system is critically involved.

On the other hand, neocortical ChAT activity was highly correlated ($r = .65$) with food preference on the 24-h retention trial. The current study represents the first report, to our knowledge, that the neocortical cholinergic system is critical for social memory. Furthermore, our evidence suggests that the neocortical cholinergic system may be more important than the hippocampal cholinergic system in consolidating or retrieving social memories related to food preference. In support of our data, Hunter and Murray (Hunter and Murray, 1989) showed that simple olfactory learning and memory involve cholinergic mechanisms, but these processes are unlikely to be mediated via the septohippocampal system. Additionally, increasing evidence supports a role for cortical acetylcholine in consolidation of memory traces (Woolf, 1998; Hasselmo, 1999). Furthermore, consolidation-induced synaptic modifications are assumed to be slower in neocortex than hippocampus (Hasselmo, 1999). The latter fact may account for the more dramatic deficits in the nBM/SI vs. MS/VDB group in the 24-h retention trial in the current study. The potential involvement of the cholinergic system in social memory is supported by anatomical data demonstrating that the olfactory system (critical for social memory) receives cholinergic projections from the nBM/SI (Mesulam and Mufson, 1984; Záborszky et al., 1986; Luiten et al., 1987) in addition to projections from the more rostrally located HDB (Záborszky et al., 1986; Okoyama et al., 1987). In total, the current data support that the basocortical cholinergic system is associated more strongly with socially mediated food preference than the septohippocampal cholinergic system, al-

though a contribution of HDB projections to olfactory structures (olfactory bulb and piriform cortex) cannot be excluded.

Olfactory Social Learning and Memory

Rats living in natural settings use olfactory cues constantly to communicate with each other. These olfactory cues are used to provide critical information about the environment, individual recognition, and an individual's health (Morrison and Ludvigson, 1970). Because rats reportedly solve olfactory problems more rapidly than visual ones and use olfaction as a primary mode of communication (Slotnick and Katz, 1974), olfaction is a valuable tool to study learning and memory in rodents (Eichenbaum, 1998; Taylor et al., 1999). In the social food preference task, a rat alters its choice of food after an encounter with a conspecific that has recently eaten a particular food. The odor of the recently eaten food on the demonstrator's breath combined with carbon disulfide, a natural odorant in rat's breath, provides the stimulus for the observer rat (Galef et al., 1988). The observer rat then demonstrates learning of the odorant/safe food association in a different context, and without the use of reinforcement or aversive stimuli. Memory appears to be formed as a consequence of internally motivated, incidental learning (Popik and van Ree, 1998).

A plethora of behavioral studies using the specific cholinergic toxin 192 IgG-saporin have reported little or no disruption of reference or working memory, leading to suggestions that the cholinergic basal forebrain system does not play a major role in learning or memory, particularly of spatial information (Baxter and Chiba, 1999). Possible reasons for the abundance of negative results include the following.

1. The strong aversive stimuli involved in water maze tasks, commonly used to assess cholinergic-induced memory deficits, induce stress and trigger compensations that could mask subtle memory deficits (Everitt and Robbins, 1997; McMahan et al., 1997).
2. Visuospatially oriented tasks such as water and radial arm mazes do not provide the optimal behavioral setting for assessing cholinergic lesion-induced deficits.

Our data suggest that the social transmission of food preference task may provide a more appropriate setting in which to examine cholinergic-induced memory deficits. This task can be used to examine memory in a nonaversive, nonspatial, and ethologically significant fashion.

In conclusion, we provide evidence that the cholinergic basal forebrain system is critical for the retrieval of social memories used to alter food choice patterns. These data add support to the idea that acetylcholine plays a critical role in consolidation of memories, including nonspatial associative memories.

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