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Basal Forebrain Cholinergic Lesions Disrupt Increments but Not Decrements in Conditioned Stimulus Processing

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Magnocellular neurons in the basal forebrain provide the major cholinergic innervation of cortex. Recent research suggests that this cholinergic system plays an important role in the regulation of attentional processes. The present study examined the ability of rats with selective immunotoxic lesions of these neurons (made with 192 IgG-saporin) to modulate attention within an associative learning framework. Each rat was exposed to conditioned stimuli (CS) that were either consistent or inconsistent predictors of subsequent cues. Intact control rats showed increased CS associability when that cue was an inconsistent predictor of a subsequent cue, whereas lesioned rats were impaired in increasing attention to the CS when its established relation to another cue was modified. In a separate experiment designed to test latent inhibition, it was shown that removal of the corticopetal cholinergic neurons spared a decrement in associability that occurs when rats are extensively preexposed to a CS prior to conditioning. These data indicate that the cholinergic innervation of cortex is critical for incrementing, but not for decrementing attentional processing. The specific behavioral tests used to assess the role of the basal forebrain cholinergic system in the present study were previously used to identify a role for the amygdala central nucleus in attention (Holland and Gallagher, 1993b). Those studies, together with the results in this report, indicate that regulation of attentional processes during associative learning may be mediated by projections from the amygdala to the basal forebrain cholinergic system.

[Key words: substantia innominata, cholinergic basal forebrain, immunotoxin, 192 IgG-saporin, classical conditioning, attention, latent inhibition, rats]

Magnocellular neurons in the basal forebrain provide the major cholinergic innervation of cortex. The pathology associated with these neurons in Alzheimer's disease has prompted studies to shed light on the origin of deficits in dementia. Recent research suggests that this system plays an important role in the regulation of attentional processes (Pang et al., 1993; Robbins et al., 1994; Voytko et al., 1994). Concurrently, neurophysiological recording studies indicate that the activity of neurons in the basal forebrain is responsive to associations formed during learning. For example, classically conditioned neocortical activation of the electroencephalogram (that is sensitive to drugs that block cholinergic function) is tightly coupled to conditioned neural activity in the basal forebrain of rabbits (Whalen et al., 1994). Increased activity of neurons in the nucleus basalis of Meynert in monkeys also occurs to events that are associated with reward (Mora et al., 1976; Richardson and DeLong, 1986). A potentially useful framework for understanding the connection between these response characteristics of basal forebrain neurons and their role in attention is that in the process of acquiring information about relationships between events, alterations in attention to those events also occur. Contemporary theories of associative learning have identified conditions that lead to decreases in attention to cues, e.g., when those events are poor predictors of reinforcement or provide no new information, and conditions that lead to increased attentional processing, e.g., when significant relationships are first detected or when previously established expectations about future events are violated (Pearce and Hall, 1980).

The specific behavioral tests in these studies of the basal forebrain cholinergic system were previously used to identify a role for the amygdala central nucleus (CN) in the regulation of attention (Holland and Gallagher, 1993b). Those studies, in conjunction with anatomical evidence for projections from amygdala CN into the basal forebrain system (Grove, 1988), led to the proposal that amygdala regulation of attentional processes may be mediated through the magnocellular corticopetal neurons (Holland and Gallagher, 1993b). In the present experiments, rats with lesions designed to remove the cholinergic corticopetal neurons were behaviorally tested in tasks used to assess attention during conditioning. One task (Experiment 1) examined the ability to increase the associability of a cue when its established relationship to another cue was modified. The other task (Experiment 2) examined the tendency to decrease the associability of a cue when it was extensively preexposed.

Lesions of cholinergic neurons were made with 192 IgG-saporin, a monoclonal antibody to the "low-affinity" p75 nerve growth factor (NGF) receptor coupled to a ribosome-inactivating compound, saporin (Wiley et al., 1991). Microinfusion of this agent into basal forebrain nuclei removes cholinergic neurons, which bear the target NGF receptor, while sparing noncholinergic neurons at the lesion site (Torre et al., 1994; Wenk et al., 1994). Injections, in the current study, were made into the substantia innominata/nucleus basalis region (SI/nBM), where the

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Table 1. Procedures used to increment attention by altering the predictable relation between two cues

Training condition (groups)	Phase 1: consistent light (L)-tone (T) relationship	Phase 2: experimental change in light (L)-tone (T) relationship	Phase 3: Test of learning to light (L)
Consistent (CTL-C, SI/nBM-C)	L → T → food; L → T	L → T → food; L → T	L → food
Shifted (CTL-S, SI/nBM-S)	L → T → food; L → T	L → T → food; L	L → food

CTL, control. SI/nBM, lesion. L, light conditioned stimulus (CS). T, tone CS. fd, food unconditioned stimulus (US). → signifies serial relation.

majority of cholinergic neurons that innervate cortex are located (Alheid and Heimer, 1988).

Experiment 1

Experiment 1 examined whether the cholinergic neurons in the SI/nBM play a role in augmenting attentional processing. The behavioral procedure used to study attention was developed by Wilson et al. (1992) and used by Holland and Gallagher (1993a) in their investigation of the amygdala CN. In this procedure, attention is manipulated when a consistent predictive relation between two cues is shifted to a less predictive relationship. Although the conditioning procedure is somewhat complicated, its complexity permits ruling out a variety of accounts for its results that could be unrelated to increased attentional processing (see Wilson et al., 1992; Holland and Gallagher, 1993a, for discussion).

As shown in Table 1, in the first phase of training each subject was exposed to serial conditioning trials in which a light-then-tone sequence was followed by a food unconditioned stimulus (US) half of the time (light → tone → food and light → tone → nothing trials). The important feature in this phase is that the light cue is consistently followed by the tone. Because the light has a relatively poor temporal relation with food, it acquires only minimal conditioned responding, and as the light's relation to the tone is established, progressively less attention is paid to it. In contrast, the tone acquires substantial conditioned responding directed toward the food cup on the 50% reinforcement schedule.

In the second phase of Experiment 1, attention to the light was manipulated by altering its relation to the tone in one of the training conditions. The comparison groups of control and lesioned rats (groups CTL-C and SI/NBM-C) continued to receive the phase 1 procedure in which the light is invariably followed by the tone, light → tone → food, and light → tone → nothing trials. In a second pair of groups (groups CTL-S and SI/NBM-S), the light → tone → nothing trials were replaced by light-alone trials. Thus, for groups CTL-S and SI/NBM-S the light no longer reliably predicted the tone, although the light's relation to food was maintained as in phase 1. This change in the light's predictive relation to the tone, according to an associative learning theory set forth by Pearce and Hall (1980), should increase attentional processing of the light.

Attention to the light was then assessed in all four groups by pairing the light directly with food in a final test phase. To the extent that the phase 2 shift in predictive accuracy of the light in groups CTL-S and SI/NBM-S increased attention to that cue, light-food conditioning should proceed more readily in those groups in the test phase. However, to the extent that such an increment in attention is mediated by cholinergic neurons in the substantia innominata, enhanced conditioning would be observed in the control rats (group CTL-S), but not the lesioned rats (group SI/NBM-S).

Materials and Methods

Subjects. The subjects were 36 male Long–Evans rats (Charles River Laboratories, Raleigh, NC), 300–325 gm at the beginning of the experiment. After postoperative recovery, the rats were placed on a restricted feeding regimen and gradually reduced to 85% of their postoperative ad libitum weights. The rats were maintained at those weights for the remainder of the experiment.

Surgical procedure. Lesions of cholinergic neurons were made under antiseptic conditions using 192 IgG-saporin (Dr. Ronald Wiley, Nashville, TN). Each rat was anesthetized with Nembutal (50 mg/kg, i.p. sodium pentobarbital) and then placed in a Kopf sterotactic apparatus. An incision was made along the midline of the rat's head, and the underlying periosteal fascia was scraped to the side. Four holes were drilled through the skull, and a small slit was made in the underlying dura at each location to assist needle penetration. A Hamilton syringe (model 701SN, 10 μl) with a 28 gauge permanent needle was used to make four injections of 192 IgG-saporin, diluted to 0.375 μg/μl in Dulbecco's saline. Immunotoxin injections, or equal volume injections of vehicle, were made bilaterally, with the head level between lambda and bregma, at stereotactic coordinates −0.75 mm posterior to bregma, at two placements 2.3 mm and 3.3 mm lateral from the midline, and ventral from the skull surface at −7.8 mm and −8.1 mm, respectively. At each injection site, 0.2 μl was delivered over a 2 min period, followed by a 3 min period during which the needle remained at the injection site. Twenty rats received immunotoxin, and 16 rats were prepared as vehicle-injected controls. Following the completion of all injections, the skull was cleaned and the drill holes filled with gel foam, prior to suturing the wound and application of a topical antibiotic containing lidocaine. Each rat was monitored during recovery from anesthesia. Each rat was allowed 14 d of postoperative recovery, prior to the initiation of behavioral testing.

Apparatus. The behavioral apparatus consisted of eight individual chambers, each 22.9 × 20.3 × 20.3 cm, with aluminum front and back walls, clear acrylic sides and top, and a grid floor (0.48 cm stainless steel rods spaced 1.9 cm apart). A dimly illuminated food cup was recessed in the center of one end wall; a 6 W jewelled panel light, which was the source of the visual CS, was located 5 cm above the opening to that recess. Each experimental chamber was enclosed in a sound-resistant shell with acrylic windows for viewing the rats. A 6 W houselight (not used in Experiment 1) was mounted on the inside wall of the shell, 25 cm above and behind the experimental chamber, even with the end wall that contained the food cup. A speaker, used to present the auditory CS, was mounted next to the houselight. Ventilation fans provided masking noise (70 dB), and a 6 W lamp behind a red lens located opposite the house light provided continuous dim background illumination. Two low-light television cameras were mounted 2.1 m from the experimental chambers so that each could include four chambers in its field of view. Videocassette recorders were programmed to record behaviors that occurred during the 10 sec intervals before, during, and after CS presentations.

Training procedures. The rats were first trained to eat from the food cups. Twenty deliveries of two 45 mg food pellets (which served as the US) were given at random times during a single 60 min session. Then, all rats received 10 daily 64 min phase 1 conditioning sessions. In each of those sessions, the rats received four reinforced and four nonreinforced presentations of a light-tone serial compound CS, randomly intermixed, with variable intertrial intervals that averaged 8 min. The serial compound comprised a 10 sec illumination of the panel light, followed immediately by a 10 sec presentation of a 10 sec, 78 dB, 1500 Hz tone. On reinforced trials, the tone was followed immediately by two 45 mg food pellets.

In phase 2, the lesioned rats in group SI/NBM-C (n = 7) and the control rats in group CTL-C (n = 8) received 10 daily sessions identical to those given in phase 1. In each of the 10 daily 64 min phase 2 sessions, the lesioned rats in group SI/NBM-S (n = 10) and the control rats in group CTL-S (n = 8) received four light → tone → food trials like those given in phase 1, intermixed with four 10 sec presentations of the panel light alone. As in phase 1, the intertrial intervals were variable and averaged 8 min.

Each rat then received five 64 min daily test sessions that comprised phase 3. In each of those sessions, eight 10 sec illuminations of the panel light were each followed by the two-pellet food US. The intertrial intervals were variable and averaged 8 min.

Behavioral observation procedures. Observations were made from videotapes, and paced by auditory signals recorded on the tapes. Food-cup behavior, which included standing motionless in front of the recessed food cup, with the head or nose within the recessed area, and head-jerk behavior (short, rapid, horizontal and/or vertical movements of the head) oriented toward the food cup, initially occurred in response to delivery of the food US, but was rapidly acquired to CSs paired with food. For each rat, observations were made at 1.25 sec intervals during the 5 sec period immediately prior to CS presentations and during the CS presentation. Because previous data (Holland, 1977, 1984) showed that food cup behavior occurs primarily during the last 5 sec of the 10 sec CS, we report the frequency of that behavior only during that time tinerval. The index of conditioned response (CR) frequency used was percentage total behavior, obtained by dividing the frequency of the target behavior in any observation interval by the total number of observations made in that interval. Note that because the number of observations was constant within each observation interval, this measure is an absolute frequency measure, not a relative one. A single primary observer (PCH) scored all of the behavioral data reported here. To assess objectivity, a second observer also scored the data from several of the test sessions. The two observers agreed on 91% of 5184 joint observations. Neither observer was aware of the rats' lesion condition when the data were scored. Statistical analyses of all measures used two-tailed distribution-free statistics. A significance level of $p < 0.05$ was adopted.

Histological procedures. Each rat was euthanized with a lethal dose of chloral hydrate and then perfused through the ascending aorta with 300 ml of cold 0.1 M phosphate-buffered saline (PBS) solution, followed by 500 ml cold 8% buffered formalin with 2% sucrose. A final flush with 0.1 M PBS containing 10% sucrose and 1% DMSO occurred 30 min after completion of the initial perfusion. After decapitation, the brain was removed, blocked, placed in a 20% sucrose and 1% DMSO PB solution and stored at 4°C for approximately 24–48 hr. Using a sliding microtome, 50 μm sections were taken from each brain. Alternate sections were processed for either choline acetyltransferase (ChAT) or parvalbumin immunoreactivity, or for acetylcholinesterase (AChE) histochemistry.

ChAT immunostaining was used in order to confirm the presence or absence of cholinergic neurons in both vehicle-injected and immunolesioned brains. In preparation for immunostaining, sections were placed in 0.1 M phosphate buffer (PB), pH 7.4, and rinsed in three changes of buffer. Sections were first incubated in a primary antibody solution containing a monoclonal antibody to ChAT (Boehringer–Mannheim, W. Germany) at a 1:50 dilution and 2% normal rabbit serum, and gently agitated for 1 to 2 hr at room temperature. Sections were then incubated in primary antibody solution for approximately 24 hr at 4°C, followed by rinse in three changes of PB. Subsequently, sections were incubated in a 0.1 M PB solution containing 1% normal rabbit serum and 1% biotinylated secondary antibody (anti-rat made in rabbit), for 1 hr at room temperature, again followed by rinse in PB (3×). Sections were finally incubated in an avidin/biocytin complex (Rat ABC Vectastain Kit, Vector Labs, Inc., Burlingame, CA), for 45 min, followed by PB rinse (3×), prior to development in a 3,3′-diaminobendzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) solution, containing PB, 19% DAB, and 0.007% hydrogen peroxide (30%) for 2–12 min. Sections were then rinsed in three changes of 0.1 M PB, mounted on slides, dehydrated, and coverslipped using Permount.

Parvalbumin immunostaining was used to confirm the integrity of GABAergic neurons within the region of the lesion (Celio, 1986; Boegman et al., 1990). The protocol for parvalbumin immunostaining was identical to that followed for ChAT with the exception of the use of the appropriate primary and secondary antibodies. For the parvalbumin procedure, sections were incubated in a primary antibody solution containing a monoclonal antibody to parvalbumin (Sigma, St. Louis, MO) at a 1:1000 dilution and 2% normal goat serum. A PB solution containing 1% normal goat serum and 1% biotinylated antibody (anti-mouse made in goat) was used as the secondary antibody incubation solution.

The remainder of the sections from each brain were processed using an AChE staining procedure adapted from Karnovsky and Roots (1964).

Results

Histological results

Photomicrographs shown in Figure 1 depict ChAT immunostaining at the injection sites in the basal forebrain. ChAT-positive magnocellular neurons were evident throughout the sublenticular substantia innominata and the ventral pallidum in sections taken from control brains (see Fig. 1*a*). A marked absence of ChAT-immunoreactive magnocellular neurons was detected in SI-lesioned brains (see Fig. 1*b*). No difference in ChAT immunostaining throughout the remainder of the basal forebrain (medial septal area and horizontal limb of the diagonal band) was detected in comparing SI/nBM-lesioned to control brains. Also, note the intact AChE staining in the basolateral amygdala (see Fig. 2, lesioned brain in *h* compared to control brain in *g*). Recent evidence has indicated that 192 IgG-saporin spares the major cholinergic innervation of the basolateral amygdala, originating from a subpopulation of cholinergic neurons in the SI/nBM region that lack the p75-NGF receptor (Heckers and Mesulam, 1994).

Parvalbumin immunostaining revealed that the distribution of GABAergic neurons throughout the sublenticular substantia innominata and the ventral pallidum was comparable in both SI-lesioned brains and the control brains (see Fig. 1*c,d*).

Relative to control brains (see Fig. 2, left panel), acetylcholinesterase staining in SI-lesioned brains was markedly depleted throughout the entire cortical mantle, with the exception of anterior cingulate cortex (see Fig. 2, right panel). The pattern and relative density of acetylcholinesterase staining was highly similar for SI-lesioned brains and vehicle-control brains in the remainder of the brain.

Experimenter-blind histological analysis was performed on each brain. Incomplete removal of ChAT-positive neurons, or damage to tissue extending beyond the magnocellular cholinergic neurons of the SI/nBM served as a criterion for elimination from the study. Based on these criteria, three rats were eliminated from the study (2 SI/nBM lesioned and 1 control rat).

Behavioral results

The left side of Table 2 shows responding over the final two sessions of phase 1, in which all groups received partially reinforced serial conditioning trials. Note that the tone CS acquired moderate amounts of food cup CRs in all four groups, whereas the visual CS (which was more temporally remote from the US) acquired relatively low levels of this conditioned behavior. There were no differences among the groups in responding during either the light or the tone CS, Mann–Whitney $Us \geq 23$, in phase 1.

The right side of Table 2 shows conditioned responding over

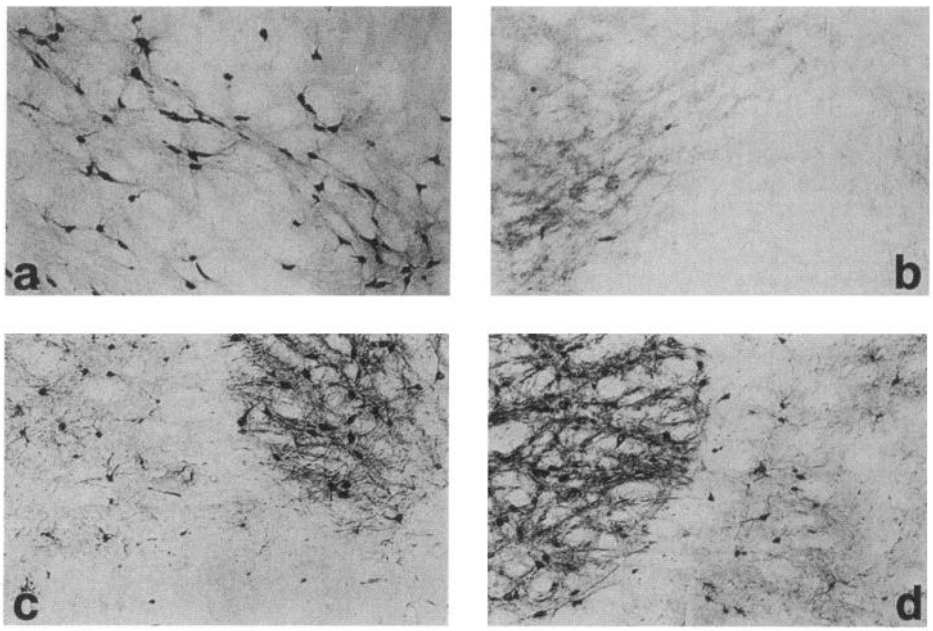

Figure 1. *a–d,* Photomicrographs taken at the level of the substantia innominata. *a,* ChAT immunostaining in a vehicle-injected control brain shows the presence of ChAT-immunopositive magnocellular cholinergic neurons. *b,* ChAT immunostaining in a SI/nBM immunolesioned brain demonstrates the absence of ChAT-immunopositive cholinergic neurons. *c* and *d,* Parvalbumin immunostaining demonstrates the presence of parvalbumin-immunopostive GABAergic neurons in a vehicle-injected control brain (*c*) as well as a SI/nBM immunolesioned brain.

the final two sessions of phase 2. As in phase 1, there were no differences among the groups in responding during either the light or the tone CS, $Us \geq 23$, and conditioned responding to the tone remained at a relatively high level compared to the light.

Figure 3 shows the primary data of Experiment 1, the acquisition of food cup behavior during the light in the light-food test sessions. Overall, conditioning was rapid; compare performance during the light CS on the first conditioning test trial (P) with performance during the first half-session block of conditioning trials during the test phase. Nevertheless, the amount of conditioned responding observed in the test phase varied as a function of both phase 2 treatment and lesion condition.

Consider first the performance of the two groups of control rats (CTL-C and CTL-S) shown in the graph on the left in Figure 3. As in previous studies with intact rats (Wilson et al., 1992; Holland and Gallagher, 1993a; Han et al., 1995), the subjects in group CTL-S, who received phase 2 training designed to enhance attention to the light, showed more food cup responding throughout the test phase than subjects in group CTL-C, $U(8,8) = 14$. However, the SI/nBM-lesioned groups (graph on the right side of Fig. 3) showed the opposite pattern of results: food cup responding was actually lower, $U(10,7) = 10.5$, in group SI/NBM-S than in group SI/NBM-C. Furthermore, although responding in lesioned (group SI/NBM-C) and control (group CTL-C) rats was equivalent with consistent training, $U(8,7) = 23$, in the shift condition, responding was reliably lower in lesioned rats (group SI/NBM-S) than in control rats (group CTL-S), $U(10,8) = 4$.

Discussion

In contrast to its effects on intact rats, a reduction in the predictive accuracy between two cues failed to enhance the associability of the CS in rats with lesions of the magnocellular cholinergic neurons in SI/nBM. The control rats in the shift condition (group CTL-S) subsequently acquired more conditioning to the light than those in the consistent condition (group CTL-C). In contrast, lesioned rats showed less conditioned responding to the light after the shift treatment (group SI/NBM-S) than after consistent training (group SI/NBM-C). This finding implicates circuitry involving the magnocellular cholinergic neurons in incrementing attentional processing of CSs when predictive accuracy is reduced.

The observation of even lower CS associability in the shift condition than in the consistent condition among lesioned rats would be anticipated if the lesions interfered with shift-induced incremental processing of the light. Several investigators have shown that when the event contingencies are not shifted, greater habituation and latent inhibition (that is, losses in attention) occur to a cue presented alone than to a cue presented in compound with another stimulus (e.g., Holland and Forbes, 1981; Lubow et al., 1982). Thus, in the absence of a shift-induced increment in attention, the light-alone presentations in group SI/nBM-S possibly reduced attention even further than consistent light-tone presentations in group SI/nBM-C.

Finally, the similar performance of lesioned and control rats in the consistent condition (groups SI/nBM-C and CTL-C, respectively) suggests that, despite abolishing the enhancement of attentional processing, the lesions had no effect on the reduction in attentional processing of a CS when its predictive value was held constant. Thus, in contrast to an essential role in incremental changes in CS processing, the magnocellular cholinergic neurons are apparently not involved in decremental attentional changes. Experiment 2 was designed to provide an independent test of this latter claim.

Experiment 2

Experiment 2 examined the effects of SI/nBM lesions on latent inhibition, the retardation of conditioning to a CS that has been extensively preexposed. Each rat received initial nonreinforced preexposure to one of two visual CSs. Then, both visual CSs were individually paired with food. Slower acquisition of conditioned responding to the preexposed CS than to the novel CS demonstrates latent inhibition.

Materials and Methods

Subjects. The subjects were 20 male Long–Evans rats (Charles River Laboratories, Raleigh, NC), 300–325 gm at the beginning of the experiment. They were maintained in the same manner as the rats in

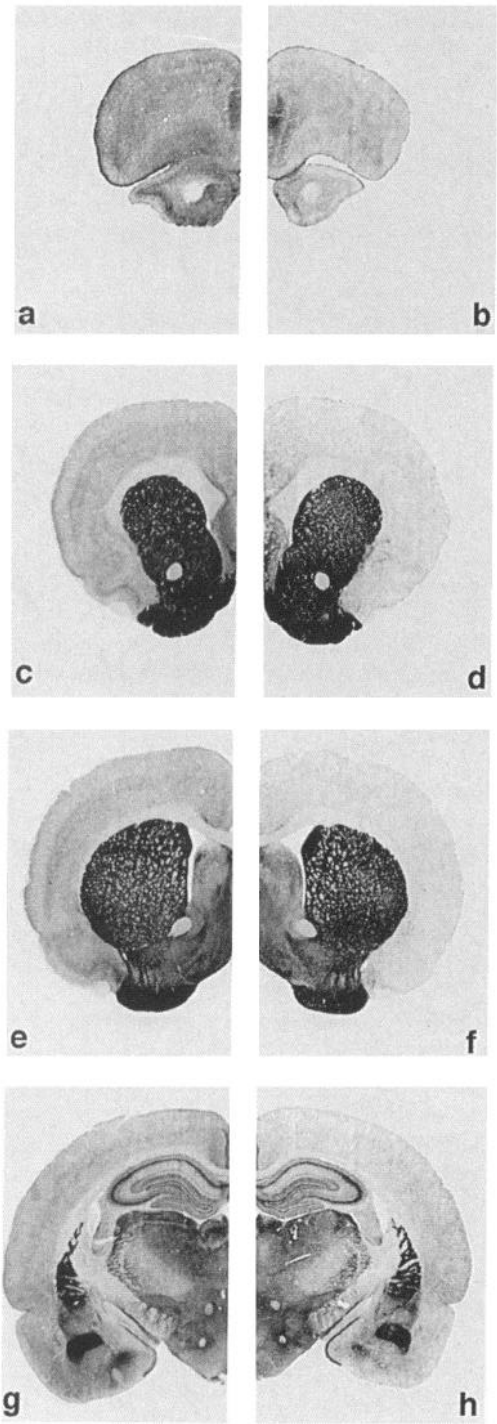

Figure 2. AChE histochemistry in photomicrographs of coronal hemisections taken from a vehicle-injected control brain (*left*) and SI/nBM immunolesioned brain (*right*). Note the extensive depletion of cortical AChE in the lesioned brain.

Experiment 1. Each rat had previous experience as a subject in an experiment using the Morris water maze (Baxter et al., 1995).

Surgical procedures and confirmation of lesions. Surgical procedures were identical to Experiment 1. A radioenzymatic assay for ChAT activity was performed on most of the brains (CTL = 8; SI/nBM = 9) in this experiment. This assay was performed on cortex, hippocampus, and striatum, dissected according to methods described elsewhere (Baxter et al., 1995). The radioenzymatic assay for ChAT was adapted from Fonnum (1960). The samples were weighed and homogenized in 20 vol of ice-cold 0.32 M sucrose. After centrifugation of the homogenate at 1000 × *g* for 10 min at 2°C, the supernatant was further homogenized in 0.25 vol of 2.0% Triton X-100 in 10 mM EDTA. An aliquot was reserved for protein determination. Samples (40 μl) were incubated at 37°C with ^{14}C acetylcoenzyme A (Dupont, NEN, Boston, MA; specific activity: 55.7Ci/mM) in 3.0 M NaCl, 0.5 M NaH_2PO_4 (pH 7.4), 100 mM EDTA (pH 7.4), 2.0 mM eserine, 10 mg/ml BSA, 160 mM choline, and 4.0 mM acetylcoenzyme A for 15 min. The reaction was stopped by adding 100 μl ice-cold H_2O. For extraction, 1.0 ml extraction solution (85:15 toluene:acetonitrile, with 5 mg/ml sodium tetraphenylboron) was added to each tube. Supernatant (650 μl) was placed into a scintillation counter. All samples were analyzed in triplicate. Histological procedures identical to those described in Experiment 1 were used for a small remaining subset of rat brains (CTL = 2; SI/nBM = 3) in Experiment 2.

Table 2. Data from phases 1 and 2 of experiment 1

	Final two sessions of phase 1		Final two sessions of phase 2	
Group	Light	Tone	Light	Tone
SI/nBM-S	13.3	53.6	14.5	58.6
SI/nBM-C	12.8	53.9	8.5	62.4
VE-S	11.5	48.1	12.5	50.4
VE-C	11.1	50.2	11.8	55.2

Data are percentages of total behavior (% CR; see text).

Apparatus. A dimly illuminated food cup was recessed in the center of one end wall; a 6 W jewelled panel light, which was the source of one visual CS, was located 5 cm above the opening to that recess. Each experimental chamber was enclosed in a sound-resistant shell with acrylic windows for viewing the rats. A 6 W house light, that ordinarily remained off, served as the other visual CS.

Behavioral observation procedures. The behavioral observation procedures for the food cup CR used in Experiment 2 were identical to those used in Experiment 1. A second category of behavior was also observed during preexposure sessions and conditioning. Rear behavior (standing on the hind legs, with both front legs off the floor, but not grooming) occurs initially as an unconditioned, orienting response to visual CSs like those used in this experiment, but also is potentiated by CS-US pairings. Given that previous data (e.g., Holland, 1977, 1984) showed that rear behavior occurs primarily during the first 5 sec period of a 10 sec CS, we report the frequencies of rear behavior during the first half of the 10 sec CS intervals. Because food-cup behavior occurs primarily during the last half of the CS interval, and we report food-

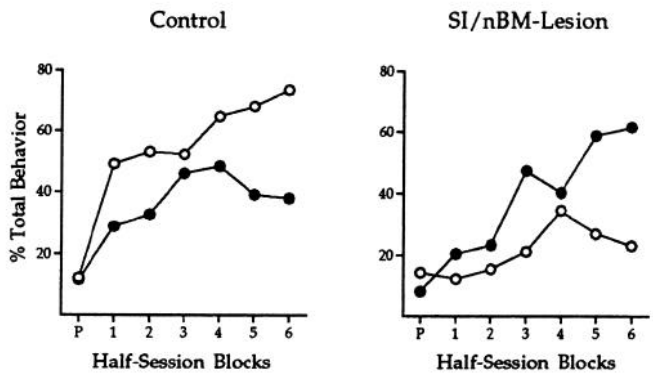

Figure 3. Acquisition of food cup behavior to the light, conditioned stimulus, during the test phase of Experiment 1. The *left* shows performance of control groups, and the *right* shows performance of SI/nBM immunolesioned groups. Groups (CTL-S and SI/nBM-S for which the lights' predictive validity was shifted in phase 2 are indicated by —○—; groups (CTL-C and SI/nBM-C) for which the lights' phase 2 treatment was consistent with that in phase 1 are indicated by —●—. The points labeled *P* on the abcissa show performance on the first test trial.

Table 3. Choline acetyltransferase activity (nmol/hr/mg protein)

	Anatomical region		
Group	Hippocampus	Cerebral cortex	Striatum
Control	74.6 ± 1.6	48.0 ± 3.9	174.6 ± 5.7
SI/nBM-Les	70.3 ± 2.8	18.0 ± 1.3*	179.6 ± 10.1

Values shown represent mean ± standard error.
* $P < 0.05$ relative to control group values.

cup behavior only in that period, there is little opportunity for competition between these two behaviors.

Training procedures. Initially, the rats were trained to eat from the food cups. Sixteen deliveries of two 45 mg food pellets (which served as the US throughout these experiments) were given at random times within a single 64 min session. Subsequently, in each of five 64 min preexposure sessions, each rat received eight 10 sec presentations of either the panel light or the house light stimulus (counterbalanced within each lesion condition). Finally, in each of ten 64 min acquisition test sessions, all rats received four 10 sec presentations of the house light, followed immediately by the food US, and four 10 sec presentations of the panel light, also followed immediately by the food US. These trials were presented in randomly intermixed orders, with variable intertrial intervals that averaged 8 min.

Experiment 2 was conducted in two identical replications, with each of the lesion and CS counterbalancing conditions represented in each replication.

Results

Confirmation of the lesions

Radioenzymatic assays for ChAT activity were performed on the most of the brains (SI/nBM = 9; CTL = 6). All lesioned rats displayed markedly reduced cortical ChAT activity relative to the control group. Data from one CTL rat were eliminated from the study based on abnormally low cortical ChAT activity. The ChAT activity data (nmol/hr/mg protein) for the SI/nBM group and the CTL group are presented in Table 3. A significant effect of lesion indicated a depletion in cortical ChAT activity [$F(1,15) = 15.2$, $p < 0.001$]. No significant effect of the lesion was evident for hippocampal or striatal ChAT activity. Based on histological analysis indicating incomplete removal of ChAT positive neurons in the substantia innominata, one SI/nBM rat was eliminated from this study. As in Experiment 1, we observed extensive loss of ChAT-positive neurons in the SI/nBM region and marked reduction of AChE staining in the two remaining SI-lesioned rats examined with these methods. In all cases parvalbumin-positive cells were visible at the lesion site and in surrounding regions of the ventral pallidum and basal forebrain.

Behavioral data

Presumably in a latent inhibition experiment, CS preexposure reduces the associability of the CS. To the extent that SI/nBM cholinergic neurons are involved in this decrement in CS processing, latent inhibition would be disrupted by SI lesions; that is, conditioning to both the preexposed and novel CSs would occur at the same rate.

Figure 4 shows conditioned responding to the two CSs in the conditioning sessions. As in previous studies that examined the effects of central nucleus lesions (e.g., Gallagher et al., 1990), the display of conditioned food-cup behavior (Fig. 4, left) was unaffected by the SI/nBM lesion: neither responding during the novel CS, $U(11,7) = 37$, nor that during the preexposed cue, $U(11,7) = 38$, differed between SI/nBM group and CTL group. Furthermore, latent inhibition was observed in both control and

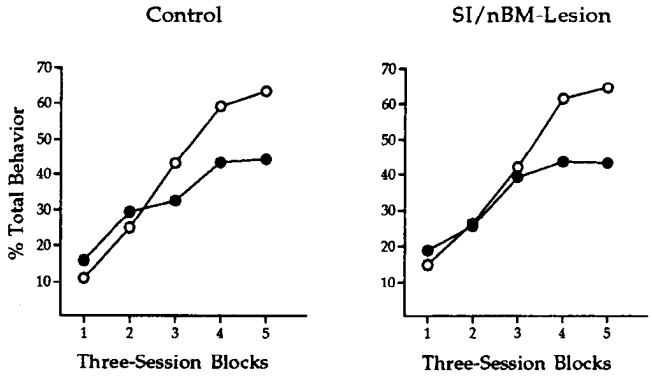

Figure 4. Acquisition of conditioned food-cup behavior in the conditioning phase of Experiment 2. Performance of control groups is displayed in the *left*, and performance of SI/nBM immunolesioned groups is displayed on the *right* (—○—, novel cue; —●—, preexposed cue).

lesioned rats: food-cup behavior was more frequent during the novel CS than during the preexposed CS in both CTL, Wilcoxon $T(7) = 1$, and SI/nBM-lesioned, $T(11) = 0$, rats. The magnitude of the latent inhibition effect (the difference between responding during the novel cue and that during the preexposed cue) did not differ between lesioned and control rats, $U(11,7) = 38$. Thus, latent inhibition, the loss of associability of a stimulus as a consequence of simple nonreinforced preexposure, was unaffected by the substantia innominata lesions.

As shown in Figure 5, during the initial preexposure sessions of Experiment 2, rearing behavior in control rats (CTL) was equivalent to that of SI/nBM immunolesioned rats (SI/nBM). The CTL rats acquired considerably lower levels of conditioned rear behavior to the two CSs during conditioning than observed in previous experiments (e.g., Gallagher et al., 1990; Holland and Gallagher, 1993a,b), making across-experiment comparisons somewhat difficult. Nevertheless, both the SI/nBM-lesioned, $T(11) = 7$, and CTL-Control, $T(7) = 3$, rats showed more rearing during the novel CS (N) than during the preexposed CS (P) over the course of conditioning, demonstrating latent inhibition of conditioned rear behavior to the preexposed cue (Fig. 6). Furthermore, there were no reliable differences between the performance of lesioned and control rats during either the preexposed, $U(11,7) = 29$, or novel, $U(11,7) = 31$, stimulus. Thus, in contrast to the

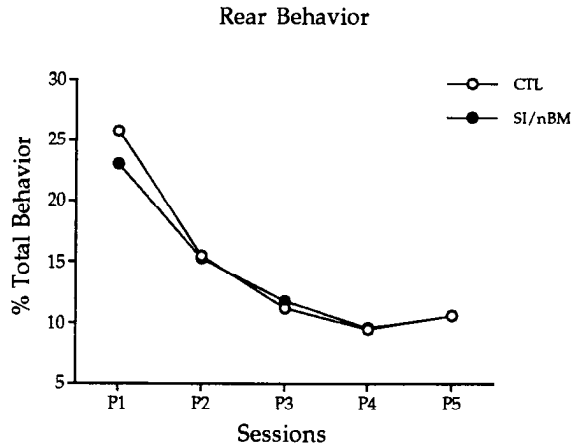

Figure 5. Rear behavior during the visual cue in the preexposure phase of Experiment 2 is displayed for both control groups (*CTL*) and SI/nBM immunolesioned groups (*SI/nBM*).

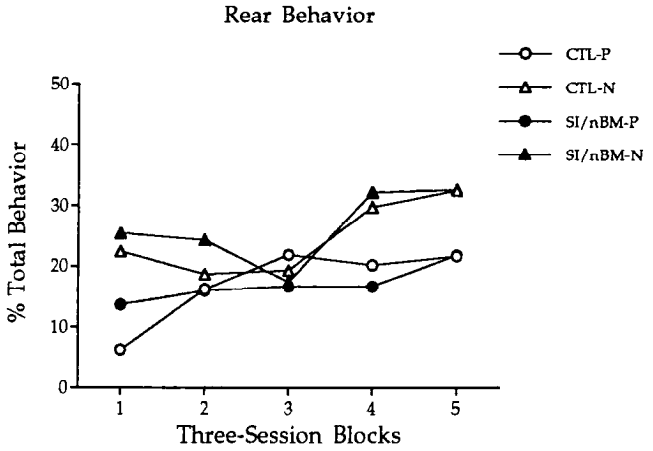

Figure 6. Acquisition of conditioned rear behavior in the conditioning phase of Experiment 2. Performance of control groups (*CTL*) and performance of SI/nBM immunolesioned groups (*SI/nBM*) is displayed (*N*, novel cue; *P*, preexposed cue).

results of previous studies that examined the effects of CN lesions (e.g., Gallagher et al., 1990), the acquisition of conditioned orienting behavior was unimpaired by SI/nBM lesions.

Discussion

The concept that basal forebrain circuitry provides a substrate for the regulation of attention is consistent with evidence from other studies using less selective lesions of the rat SI/nBM region. Neurotoxins, such as quisqualate or alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), produce substantial cortical cholinergic depletion but also damage noncholinergic neurons at the lesion site. Rats with such lesions perform less accurately, relative to intact control rats, on a multiple-choice reaction time task designed to measure attentional processes (Robbins et al., 1989; Muir et al., 1992, 1994). The effects of those lesions can be magnified by manipulations that increase attentional load (reducing the duration of signals) and, conversely, impairments can be reduced by increasing the duration of relevant signals. Inactivation of the rat nBM also produced attentional impairments on a two-choice reaction time task (Pang et al., 1993). Further evidence has implicated cholinergic mediation of attentional deficits in the multiple-choice task; intraventricular injections of hemicholinium, a high-affinity choline uptake blocker, produced behavioral impairments similar to those produced by neurotoxic lesions of the SI/nBM (Muir et al., 1992). More recently, nBM lesions in monkeys tested on a spatial attention task were found to impair the detection of targets that were predicted by cueing at inaccurate locations (Voytko et al., 1994).

The current study was designed to test whether more selective lesions of the SI/nBM cholinergic neurons alter attentional processes. The experiments were also designed to separately examine the effect of lesions on incremental and decremental processing of cues. Decreases in attentional processing were spared in rats with SI/nBM lesions. This sparing was evident in normal habituation to repeatedly presented visual cues during the preexposure sessions of Experiment 2, in the subsequent latent inhibition of conditioning to those cues, and in the loss of attention to the consistently predictive visual cue in Experiment 1, all of which were equivalent in lesioned and control rats. These results, which are consistent with the findings of Robbins and colleagues indicating that lesions of the SI/nBM produced by AMPA also spare latent inhibition (Robbins et al., 1991), support the claim that the removal of cortical cholinergic innervation does not alter decremental changes in CS processing.

In contrast, the removal of SI/nBM cholinergic neurons eliminated the ability to increase attentional processing of a cue. The failure to increase CS processing reported here is comparable to that observed in a prior study in which less selective lesions of the SI/nBM region were made with AMPA (Chiba et al., 1994). The same effect on incremental processing was previously obtained in rats with damage to the amygdala CN (Holland and Gallagher, 1993b), a nucleus that innervates the SI region in both rodent and primate brain (Russchen et al., 1985; Grove, 1988).

The concept that certain aspects of attentional processes mediated by corticopetal cholinergic neurons are regulated by input from the amygdala complex is supported by other observations in the present study that parallel findings with CN damage. In addition to the failure to increment the processing of a CS, both SI/nBM and CN lesions have no effect on decremental processing in latent inhibition (Holland and Gallagher, 1993a). Furthermore, a notable finding in Experiment I was that rats with SI/nBM cholinergic lesions showed reduced conditioning after the predictable relationship between cues was altered in group SI/nBM-S, a pattern that was opposite to the effect of that manipulation in the intact control rats. A comparable finding was also observed in rats with CN lesions (Holland and Gallagher, 1993a). In addition to these tasks, research on the effects of CN damage has used other paradigms, such as blocking and unblocking, in which the course of conditioning can be influenced by alterations in CS processing. Those experiments have provided results highly consistent with the conclusion that an incremental processing function is selectively disrupted by CN damage (Holland and Gallagher, 1993b). If attentional processes mediated by corticopetal cholinergic neurons are regulated by input from the amygdala CN, further parallels between the effects of SI/nBM and CN lesions would be predicted in those tasks.

Parallel findings between lesions of the amygdala CN and the SI corticopetal system may not exist for all behavioral components of attention. CN-lesioned rats fail to acquire conditioned orienting responses to either visual or auditory cues, whereas conditioned orienting in the latent inhibition experiment did not differ between intact rats and SI/nBM lesion rats. Because the level of conditioned orienting observed in the control group was unusually low, and because Experiment 2 did not provide a CS/US unpaired comparison to unambiguously identify that responding was associatively acquired, the apparent absence of an effect of SI/nBM lesions on conditioned rear behavior is not conclusive. As noted elsewhere, other pathways from the amygdala CN may be involved in the behavioral orienting component of attention that emerges during learning (Gallagher and Holland, 1994).

The results of these experiments also support the concept that incremental and decremental attentional processes are subserved by independent neural systems (Holland and Gallagher, 1993a,b). Interestingly, those phenomena that are considered to reflect decrements, which are unaffected by CN and/or SI/nBM lesions, have been found by others to be disrupted by hippocampal lesions (Solomon and Moore, 1975; Solomon, 1977; Rickert et al., 1978; Kaye and Pearce, 1987a,b; but see Honey and Good, 1994). However, earlier studies did not examine whether hippocampal damage, which impairs the tendency to decrease processing of cues, has any effect on the ability to increase attentional processing. As reported

elswhere (companion article) neurotoxic lesions of the hippocampus produce a double dissociation on the tasks used to assess the effects of SI/nBM lesions. In contrast to the pattern of results obtained in the present experiment, hippocampal damage was found to impair decremental attentional processes without any loss in the ability to increment attentional processing in these tasks.

The recent availability of a selective immunotoxin for cholinergic neurons bearing p75 NGF receptors should help to more clearly define the function of the basal forebrain cholinergic system. Several recent reports have indicated that lesions of the cortical cholinergic projection or lesions of the septo-hippocampal cholinergic system induced by 192 IgG-saporin largely spare performance on a number of learning and memory tasks (Berger-Sweeney et al., 1994; Torres et al., 1994; Wenk et al., 1994; Baxter et al., 1995). The research reported here supports the notion that cortical cholinergic innervation may be of considerable importance in mechanisms of attention, a concept that has been based on studies using less selective lesion methods.

References

Alheid GF, Heimer L (1988) New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal amygdaloid and corticopetal components of substantia innominata. Neuroscience 27:1–39.

Baxter MG, Bucci DJ, Gorman LK, Wiley RG, Gallagher M (1995) Selective immunotoxic lesions of basal forebrain cholinergic cells: effects on learning and memory in rats. Behav Neurosci 109:714–722.

Berger-Sweeney J, Heckers S, Mesulam MM, Wiley RG, Lappi DA, Sharma M (1994) Differential effects on spatial navigation of immunotoxin-induced cholinergic lesions of the medial septal area and nucleus basalis magnocellularis. J Neurosci 14:4507–4519.

Boegman RJ, Cockhill J, Jhamandas K, Beninger RJ (1992) Excitotoxic lesions of rat basal forebrain: differential effects on choline acetyltransferase in the cortex and amygdala. Neuroscience 51:129–135.

Celio MR (1986) Parvalbumin in most gamma-aminobutyric acid-containing neurons of the rat cerebral cortex. Science 231:995–997.

Chiba AA, Han JS, Holland PC, Gallagher M (1994) Substantia innominata lesions impair incremental attentional processing in a Pavlovian conditioning paradigm. Soc Neurosci Abstr 20:1215.

Fonnum F (1969) Radiochemical microassays for the determination of choline acetyltransferase and acetylcholinesterase activities. Biochem J 115:465–472.

Gallagher M, Holland PC (1994) The amygdala complex: multiple roles in associative learning and attention. Proc Natl Acad Sci USA 91:11771–11776.

Gallagher M, Graham PW, Holland PC (1990) The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behavior. J Neurosci 10:1906–1911.

Grove EA (1984) Neural associations of the substantia innominata in the rat: afferent connections. J Comp Neurol 277:315–346.

Han JS, Gallagher M, Holland PC (1995) Hippocampal lesions disrupt decrements but not increments in conditioned stimulus processing. J Neurosci 15:7323–7329.

Heckers S, Mesulam MM (1994) Two types of cholinergic projections to the rat amygdala. Neuroscience 60:383–397.

Holland PC (1977) Conditioned stimulus as a determinant of the form of the Pavlovian conditioned response. J Exp Psychol Anim Behav Process 3:77–104.

Holland PC (1984) The origins of Pavlovian conditioned behavior. In: The psychology of learning and motivation, Vol 18 (Bower G, ed), pp 129–173. Englewood Cliffs, NJ: Prentice-Hall.

Holland PC, Forbes DT (1980) Effects of compound or element preexposure on compound flavor aversion conditioning. Anim Learn Behav 8:199–203.

Holland PC, Gallagher M (1993a) The effects of amygdala central nucleus lesions on blocking and unblocking. Behav Neurosci 107: 235–245.

Holland PC, Gallagher M (1993b) Amygdala central nucleus lesions disrupt increments but not decrements in CS processing. Behav Neurosci 107:246–253.

Honey RC, Good M (1993) Selective hippocampal lesions abolish the context specificity of latent inhibition and conditioning. Behav Neurosci 107:22–33.

Kaye H, Pearce JM (1987) Hippocampal lesions attenuate latent inhibition of a CS and of a neutral stimulus. Psychobiology 15:294–299.

Karnovsky MJ, Roots L (1964) A "direct-coloring" thiocholine method for cholinesterases. J Histochem Cytochem 12:219–221.

Lubow RE, Wagner M, Weiner I (1982) The effects fo compound stimulus preexposure of two elements differing in salience on the acquisition of conditioned suppression. Anim Learn Behav 10:483–489.

Mora F, Rolls ET, Burton MJ (1976) Modulation during learning of the responses of neurones in the lateral hypothalamus to the sight of food. Exp Neurol 53:508–519.

Muir JL, Dunnett SB, Robbins TW, Everitt BJ (1992) Attentional functions of the forebrain cholinergic systems: effects of intraventricular hemicholinium, physostigmine, basal forebrain lesions and intracortical grafts on a multiple choice serial reaction time task. Exp Brain Res 89:611–622.

Muir JL, Everitt BJ, Robbins TW (1994) AMPA-induced excitotoxic lesions of the basal forebrain: a significant role for the cortical cholinergic system in attentional function. J Neurosci 14:2313–2326.

Pang K, Williams MJ, Egeth H, Olton DS (1993) Nucleus basalis magnocellularis and attention: effects of muscimol infusions. Behav Neurosci 107:1031–1038.

Pearce JM, Hall G (1980) A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli. Psychol Rev 106:532–552.

Richardson RT, DeLong MR (1986) Nucleus basalis of Meynert neuronal activity during a delayed response task of monkey. Brain Res 399:364–368.

Rickert EJ, Bennett TL, Lane P, French J (1978) Hippocampectomy and the attenuation of blocking. Behav Biol 22:147–160.

Robbins TW, Everitt BJ, Marston HM, Wilkinson J, Jones GH, Page KJ (1989) Comparative effects of ibotenic acid- and quisqualic acid-induced lesions of the substantia innominata on attentional function in the rat: further implications for the role of the cholinergic neurons of the nucleus basalis in cognitive processes. Behav Brain Res 35: 221–240.

Robbins TW, Roberts AC, Muir JL, Killcross AS, Everitt BJ (1991) Impaired reversal learning but preserved latent inhibition following excitotoxic lesions of the basal forebrain in the rat. Soc Neurosci Abstr 17:479.

Russchen FT, Price JL (1984) Amygdalostriatal projections in the rat. Topographical organization and fiber morphology shown using the Lectin Pha-L as an anterograde tracer. Neurosci Lett 47:15–22.

Solomon P (1977) Role of the hippocampus in blocking and conditioned inhibition of the rabbit's nictitating membrane response. J Comp Physiol Psychol 91:407–417.

Solomon P, Moore JW (1975) Latent inhibition and stimulus generalization of the classically conditioned nictitating membrane response in rabbits (*Oryctolagus cuniculus*) following dorsal hippocampal ablation. J Comp Physiol Psychol 89:1192–1203.

Torres EM, Perry TA, Blokland A, Wilkinson LS, Wiley RG, Lappi DA, Dunnett SB (1994) Behavioural histochemical and biochemical consequences of selective immunolesions in discrete regions of the basal forebrain cholinergic system. Neuroscience 63:95–122.

Voytko ML, Olton DS, Richardson RT, Gorman LK, Robin JR, Price DL (1994) Basal forebrain lesions in monkeys disrupt attention but not learning and memory. J Neurosci 14:167–186.

Wenk GL, Stoehr JD, Quintana G, Mobley S, Wiley RG (1994) Behavioral biochemical histological and electrophysiological effects of 192 IgG-Saporin injections into the basal forebrain of rats. J Neurosci 14:5986–5995.

Whalen PJ, Kapp BS, Pascoe JP (1994) Neuronal activity within the nucleus basalis and conditioned neocortical electroencephalographic activation. J Neurosci 14:1623–1633.

Wiley RG, Oeltmann TN, Lappi DA (1991) Immunolesioning: selective destruction of neurons using immunotoxin to rat NGF receptor. Brain Res 562:149–153.

Wilson PN, Boumphrey P, Pearce JM (1992) Restoration of the orienting response to a light by a change in its predictive accuracy. Q J Exp Psychol 44B:17–36.