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ORIGINAL INVESTIGATION

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Selective immunolesioning of the basal forebrain cholinergic neurons in rats: effect on attention using the 5-choice serial reaction time task

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Abstract *Rationale:* Excitotoxic lesions of the nucleus basalis magnocellularis (nbm) in rats produce deficits in performance of the 5-choice serial reaction time (5CSRT) task, suggesting that basal forebrain cholinergic projections to the neocortex play an important role in visuospatial attention. However, non-selective damage induced by excitotoxins may have confounded the interpretation of the specific contribution of the corticopetal cholinergic neurons of the nbm to attentional processes. *Objective:* The purpose of the present study was to produce selective immunolesions of the cholinergic neurons of the nbm in order to examine more precisely the role of the cholinergic projections of the basal forebrain on attentional performance in a 5CSRT task. *Methods:* Rats received bilateral injections of the selective cholinergic immunotoxin 192 IgG-saporin (0.067 μg/μl, 1 μl) into the nbm after baseline training in the 5CSRT task. Performance of sham and nbm lesion groups was then assessed during baseline and increased task difficulty conditions. *Results:* Contrary to results previously reported, accuracy of responding and behavioral inhibition were unaffected by the immunotoxin. Rats with nbm lesions showed, however, significant increases in omissions relative to control rats, most markedly during sessions with increased difficulty of signal detection, e.g., decreased stimulus intensity or duration. Magazine and correct latencies were unaffected, suggesting that the lesion-induced omissions were not due to changes in motivation. Omissions were highly correlated with percentage of choline acetylcholine transferase depletion. Reduced premature responses were also observed when the target stimulus was made less predictable. *Conclusions:* Although the 192 IgG-saporin lesion produced a different array of behavioral deficits than previously reported, these effects nevertheless are consistent with an important role of the basal forebrain cholinergic system in attentional function, in particular with accurate timing of stimulus presentation and target detection.

Keywords Basal forebrain · 192 IgG-saporin · Attention · 5-choice serial reaction time task · Nucleus basalis magnocellularis · Signal detection

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Introduction

Increasing evidence that disorders of attention may underlie cognitive dysfunction has stimulated research into the neural bases of attention (Robbins 1997; Coull 1998; Baxter and Chiba 1999; Sarter and Bruno 2000). Information processing and attentional abnormalities are especially prominent in neuropsychiatric disorders (Filipek et al. 1997; Greenwood et al. 1997; Sarter and Bruno 1999; Berger and Posner 2000; Heimer 2000). Attention is not a unitary construct and is rather characterized by several distinct mechanisms including selective, divided and sustained attention (Koelega 1993; Bushnell 1998; Robbins 1998; Sarter et al. 2001). Several animal models have been developed to address these different attentional components including the 5-choice serial reaction time (5CSRT) task, a model designed to assess visuospatial attention by rewarding an animal for responding to a brief light signal that occurs randomly in one of five possible locations (Carli et al. 1983; Robbins 1998). The task requires the subjects to visually search for the stimulus array until stimulus presentation. The parameters of the task can be manipulated to make the stimulus presentation more brief and/or less predictable, increasing the diffi-

culty (as measured by decreased response accuracy) and presumably increasing the attentional load.

Direct administration of excitotoxins such as ibotenic and quisqualic acids or AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid) into the nucleus basalis magnocellularis (nbm) of the basal forebrain of rats trained to perform the 5CSRT task results in significant deficits in response accuracy (Robbins et al. 1989; Muir et al. 1992, 1994). The nbm constitutes one of the major sources of cholinergic efferents to neocortical areas in rodents (Mesulam et al. 1983; Everitt and Robbins 1997). Interestingly, deficits in response accuracy observed in nbm-lesioned rats performing the 5CSRT task were attenuated by cholinergic tissue grafts or administration of procholinergic drugs such as physostigmine and nicotine (Muir et al. 1992, 1994, 1995). These data support the hypothesis that cholinergic inputs originating in the basal forebrain are crucial components of the neuronal network mediating attentional performance in rats (Robbins et al. 1989; McGaughy et al. 1996). The excitotoxins used to lesion the nbm, however, are non-selective and consequently may also damage non-cholinergic cells such as GABAergic interneurons and neighboring noradrenergic neurons in the ventral pallidum (Dunnet et al. 1991; Page et al. 1995; Waite et al. 1996). Therefore, one can question the specific role of the cholinergic system in this model. The immunotoxin 192 IgG-saporin has been recently developed as a means to selectively lesion the cholinergic system, minimizing the damage to other neuronal systems (Wrenn and Wiley 1998; McGaughy et al. 2000). Saporin, a ribosome-inactivating protein, is coupled to a monoclonal antibody (192 IgG) raised against the rat nerve growth factor receptor p75, which is specifically expressed by basal forebrain cholinergic neurons and Purkinje cells in the cerebellum (Batchelor et al. 1989; Yan and Johnson 1989; Wiley et al. 1991; Waite et al. 1995). Once the immunotoxin binds to the p75 receptor, the entire immunotoxin complex is endocytosed resulting in the blockade of protein synthesis and, subsequently, apoptotic cell death (Book et al. 1994).

Following intra-cerebro-ventricular (i.c.v.) administration of 192 IgG-saporin, Waite and colleagues (1999) were unable to reproduce the degree of 5CSRT task performance deficits that were previously observed in AMPA-treated animals under basal conditions, suggesting that the basal forebrain cholinergic system may play an important but not an essential role in the modulation of attention in rats as measured by the 5CSRT task. There are three important caveats to this study however. First, i.c.v. administration, but not local administration to brain nuclei, can damage cerebellar Purkinje cells, thereby possibly confounding the outcome of the study (Torres et al. 1994; Waite et al. 1995; Wrenn and Wiley 1998; Pizzo et al. 1999). Second, the i.c.v. administration produced a lower level of depletion of cortical choline acetylcholine transferase (ChAT) activity, an indirect measure of cholinergic function, than excitotoxic lesions (Muir et al. 1994, 1995). Therefore, the cholinergic system may not have been depleted sufficiently to affect the 5CSRT task performance in rats. Third, the immunotoxin-treated rats only showed performance deficits when the difficulty of the task was increased during certain conditions.

Altogether, this implies that the basal forebrain cholinergic system may play a more subtle role in the modulation of attention in rats than was originally hypothesized. The purpose of the present study was, therefore, to further elucidate the role of the basal forebrain cholinergic system on visuospatial attention in rats by injecting the selective cholinergic immunotoxin 192 IgG-saporin directly into the nbm. This technique should yield similar levels of ChAT activity depletion as observed in previous excitotoxic lesion studies but without non-specific damage to other neuronal systems.

Materials and methods

Subjects

Fourteen male Lister hooded rats (Harlan, U.K., 300 g at the start of the study) were housed two per cage and maintained in a humidity- (50–55%) and temperature- (22–24°C) controlled facility on a 12-h/12-h light/dark cycle (lights on at 0630 hours). Water was available ad libitum, but food was restricted to that earned during the test [maximum of 100×45-mg Formula-1 pellets (Noyes, N.H.)] and 12 g standard rodent chow (Harlan-Teklad 4% rat diet 7001) at the end of the test day (approximately 1700–1800 hours). This food restriction regimen reduced and maintained body weight to 20% below normal average (i.e., relative to average body weight measured under no food restriction). All testing was conducted during the light cycle. All procedures were performed in accordance with the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee.

Surgical procedures

192 IgG-saporin was infused bilaterally into the nbm of rats previously trained to criterion performance on the 5CSRT task (see description below). A concentration of 0.067 μg/μl was selected based on pilot studies indicating that this concentration, volume of injection and batch of 192 IgG-saporin produced a depletion of ChAT activity comparable to that reported after AMPA administration into the nbm (Muir et al. 1994). Animals were anaesthetized using 2% isoflurane and placed on a Kopf stereotaxic instrument. All infusions were made via a 30-gauge cannula attached to a 25-μl Hamilton syringe (volume controlled by a Harvard Pump, 0.315 μl/min). Rats received bilateral injections into the nbm of a volume of 1 μl of either 192 IgG-saporin (Chemicon, Temecula; 0.067 μg/μl in sterile 0.9% saline, n=7) or sterile 0.9% saline (n=7). The following coordinates (Paxinos and Watson 1986) were used: AP +1.0 mm from bregma, L ±3.2 mm from the midline and DV –7.5 mm below the dura (incisor bar set at +5.0 mm above the interaural line). After each injection the cannula was held in place for an additional 2 min before retraction. Each animal was given 2 ml Lactate Ringer's solution (s.c.) immediately after surgery in order to prevent post-operative dehydration. The animals continued to receive a daily injection of Lactate Ringer's solution (2 ml, s.c.) for 3–5 days after surgery. Baked potatoes, cucumbers and oranges were supplied ad libitum for 3 days to promote feeding and minimize weight loss and dehydration.

Behavioral procedures

Apparatus

The test apparatus consisted of four 25×25-cm aluminum chambers illuminated by a 2.8-W house light located on the ceiling of each chamber (Paul Fray Ltd., Cambridge, UK). The rear wall of each chamber was curved and contained nine 2.5-cm square holes (i.e., nose-poke holes), 4-cm deep and 2.5-cm above a wire mesh floor (Carli et al. 1983). Each hole had an infrared photocell beam monitoring its entrance and was illuminated pseudorandomly by a 2.8-W lamp located at the rear of each hole. Food pellets were delivered automatically into a magazine located at the front of the chamber (25 cm from each hole). The rat collected food rewards by pushing a Perspex panel covering the magazine. The rat was placed into the chamber through a Perspex flap located above the food tray. To minimize extraneous noise, the chamber was encased in a soundproof compartment with a ventilator fan providing low-level background noise. Each chamber was automatically controlled, and data were collected via a microcomputer (Acorn System RISC PC) using the Arachnid Software (Paul Fray Ltd., Cambridge, UK).

Behavioral training

Training was conducted according to the methods described by Muir et al. (1994). Rats were introduced to a restricted diet 2 days prior to training. On the first 2 days of training, rats were placed in the chambers for 15 min with the house light on and allowed free access to 20–25 food pellets placed in the food tray. Each hole on the back of the chamber was blocked by a metal cover. On the next 2 days, the metal covers were removed from five of the holes (holes 2, 4, 6, and 8); 2–3 pellets were placed in each hole and 10–15 food pellets were placed in the food tray. The house light remained on throughout the sessions. On the fifth day, the 5CSRT task was implemented. The start of the session, which lasted for 30 min or up to 100 trials, was signaled by illumination of the house light and delivery of a single food pellet to the magazine. The act of opening the panel to collect the food pellet initiated the trial. After a fixed delay interval (inter-trial interval or ITI), the light in the back of one of the holes was illuminated for a fixed duration (stimulus duration or SD). The animal could respond during the presentation of the light stimulus or during a short period immediately after (limited hold or LH). Responses (nose pokes) made in the illuminated hole were considered as correct responses and were rewarded by the delivery of a food pellet to the magazine. A brief time out (TO), a 5-s period of darkness, followed a response in any other hole (incorrect response), and no reward was given. A failure to respond to the light stimulus (omission) and responses in the holes before stimulus presentation (premature responses) were also punished by a TO. Responses made during the TO period restarted the TO. The animals were also punished by a TO period if they continued to respond (nose poke) after the initial correct response (perseverative responses) or if they responded before the initiation of a trial. Once the TO was over or the food pellet collected, the rats had to push the magazine panel again in order to initiate the next trial. There was a 5-s delay after the food was collected during which a trial could not be started even after the push of the panel.

In the initial training session, the SD and LH were 30-s and 60-s long, respectively. The ITI and TO were fixed at 5 s throughout all training sessions. After the rats had acquired the task with these initial parameters, SD and LH were progressively reduced to 0.5 s and 5 s, respectively. The animals were considered to have reached stable performance after showing at least 80% correct responses and less than 20% errors of omission over five consecutive sessions at baseline parameters (SD=0.5 s, LH=5 s, TO=5 s). Once animals met these criteria, they were assigned to either the vehicle or immunotoxin group based on their average performance over the last 3 days of testing in order to counterbalance the groups for percentage correct, percentage omissions and correct latency (see definitions below). Two weeks post-surgery, the food restriction was resumed and the animals were re-exposed to the baseline conditions on the third week post-surgery. After post-operative baseline, one subject in the control group was dropped from subsequent experiments due to a low response rate.

Behavioral challenges

After 10 days of re-exposure to baseline conditions and after stable post-operative baseline levels were reached, the animals were exposed to various behavioral challenges. The behavioral challenges were presented in the following order: (i) variable stimulus intensity (SI, 0, 10, 33, 100% intensity); (ii) variable ITI duration (5, 6, 8, 10 s); and (iii) variable SD (0.05, 0.15, 0.25, 0.5 s).

All testing sessions were limited to 30 min or up to 100 trials. Each trial condition was presented in pseudorandom order and an equal number of times over the entire session (25 trials per condition). A baseline day of standard task parameters preceded and followed each test day. There was a minimum of two rest days between each testing period.

Performance assessment

Performance of the task was assessed using the following behavioral measures:

- Accuracy. Accuracy or percentage correct responses was defined as the number of correct responses/total number of correct and incorrect responses, expressed as a percentage).
- Omissions. Errors of omissions or "misses" were the number of trials in which the rat failed to respond within the LH period after stimulus presentation (i.e., number of missed trials/total number of trials, expressed as a percentage).
- Speed. Two measures of response speed were used. The first was correct latency measured as the time between the onset of stimulus presentation and the correct response. The second measure was the magazine latency, measured as the time between the correct response and the collection of the food pellet. Correct latency has been interpreted as a measure of motivation to perform, an indication of motor competency, and as a measure of the "processing" time for the animal to "decide" where to respond. The magazine latency is considered a measure of the animal's motivation for the food reward.
- Perseverative responses. These are any additional response(s) made in the apertures after the initial correct response. Perseverative panel pushes were defined as the number of additional magazine panel pushes after trial initiation.
- Premature responses. Any nose-poke into the appertures made after the trial was initiated (panel push), but before the light stimulus was presented, was considered as a premature response.

Both perseverative and premature responses are considered indexes of behavioral inhibition.

ChAT activity

Twenty weeks post-surgery, sham-lesioned and nbm-lesioned animals were decapitated and their brains were quickly removed and dissected free hand on an ice-cooled platform. Brain regions were dissected according to the procedure described by Muir et al. (1994) and included the parietal, medial frontal, anterior dorsolateral frontal and cingulate cortices as well as the hippocampus. A medial frontal (MF) cortex sample was taken as the area extending 3 mm posterior to the frontal pole and 1 mm laterally from the midline (corresponding to the area located +2 mm from bregma). This sample included areas Cg1, Cg3 and Fr2 according to the classification of Paxinos and Watson (1986). A sample of anterior dorsolateral frontal cortex (ADL1) was taken as the lateral area from this initial slice, extending to the rhinal fissure, and was comprised of the areas Fr2, Fr1, Fr3, and Par1. The posterior

Table 1 Effect of 192 IgG-saporin administration into the nucleus basalis magnocellularis (nbm) on cortical choline acetylcholine transferase (ChAT) activity. *MF* medial frontal cortex, *C1* anterior cingulate cortex, *C2* posterior cingulate cortex, *ADL1* anterior dorsolateral cortex, *ADL2* posterior dorsolateral cortex

Brain region	ACh formed/g tissue/h (μmol)		% ChAT reduction
	Sham (n=6)	Lesion (n=6)	
MF	1.836±0.110	0.816±0.122**	56
C1	1.064±0.065	0.616±0.105**	42
C2	0.789±0.050	0.430±0.047**	46
ADL1	1.341±0.035	0.400±0.032**	70
ADL2	1.241±0.044	0.402±0.079**	68
Parietal	1.361±0.089	0.457±0.051**	66
Hippocampus	2.661±0.192	2.856±0.139	–

**P<0.001 relative to sham, Student t-test

dorsolateral cortical area (ADL2) was taken from the beginning of the lateral area of the cingulate cortex to the rhinal fissure, and contained areas Fr1, Fr3, FL, and Par1. Two samples of postgenu cingulate cortex (anterior part C1 and posterior part C2) were collected. C1, which contained areas Cg1, Cg2, and Fr2, included the medial cortex extending 4 mm from the frontal pole and 1 mm laterally from the midline. The second sample C2, which contained areas Cg1, Cg2, Fr2, and a small anterior part of RSG, included the medial cortex from bregma and extended 2 mm posterior to bregma. Parietal cortex was taken from the lateral extent of the C2 sample to the point above the rhinal fissure and included areas Fr1, HL, FL, Par1, and Par2. The hippocampus was also dissected. Tissue weight was recorded immediately after dissection and the tissue was stored in a –80°C freezer for subsequent assay of ChAT activity. Tissue samples were homogenized in ice-cold 0.1 mM dithiothreitol in 0.2% Triton X-100 buffer solution using a Kinematic AG PT1200 Polytron homogenizer. The homogenate was then centrifuged and the supernatent was used for analysis of ChAT activity by measuring the incorporation of [^{14}C]acetyl Co-A into [^{14}C]acetylcholine according to the method reported by Fonnum (1975). All tissue samples were assayed in duplicate and data were normalized for protein content.

Histology

Following removal of brain areas for measurement of ChAT activity, the remaining intact tissue was frozen and subsequently sliced using a cryostat. Thirty-micron slices were prepared and stained with Cresyl Violet. These sections were used to verify the extent of the damaged area, necrosis, and appropriate site of injection. One immunotoxin-treated rat was removed from the study due to an incomplete lesion of basal forebrain cholinergic neurons (less than 10% cortical ChAT depletion).

Data analysis

Data were analyzed by two-way ANOVA with repeated measures following transformation of data where appropriate. Arcsine square root transformation was applied to the percentage correct and percentage omissions. Post-hoc analysis was carried out using the Newman Keuls a posteriori test. ChAT activity was analyzed using unpaired Student t-tests. All group comparisons and linear regression analyses were performed using SigmaStat (Jandel scientific software), with the criterion for significance being P<0.05.

Results

Effect of infusion of 192 IgG-saporin into the nbm on ChAT activity in cortical areas

As shown in Table 1, infusions of 192 IgG-saporin into the nbm resulted in a 40–70% reduction in ChAT activity in all cortical regions assayed, without affecting the cholinergic system within the hippocampus. These reductions were comparable to the level of ChAT activity reported by Muir et al. (1994, 1995) after injection of AMPA, a less selective toxin, into the nbm.

Effect of infusion of 192 IgG-saporin into the nbm on 5CSRT task performance

Three weeks post-surgery

Accuracy. The administration of 192 IgG-saporin did not affect the percentage of correct responses as measured for 10 days, starting 3 weeks post surgery (Fig. 1A; lesion $F_{1,10}$<1, NS; day $F_{9,89}$=1.55, NS; lesion × day $F_{9,89}$<1, NS). The lesion group did however exhibit significantly more omissions, although the number of omissions did decrease over time (Fig. 1B; lesion $F_{1,10}$=5.01, P<0.05; day $F_{9,89}$=8.79, P<0.001; lesion × day $F_{9,89}$=2.36, P<0.05). Although less pronounced, the number of omissions also decreased in the sham group over testing days, as revealed by a main effect of day. There were no significant differences in the pre-operative performance of the control and lesion groups on any of the behavioral measures (Fig. 1, % correct $F_{1,20}$<1, NS; % omissions $F_{1,20}$<1, NS).

Speed. As with accuracy, there were no effects of the lesion on correct latency (Table 2, postoperative baseline); however, there was a main effect of day (lesion $F_{1,10}$<1, NS; day $F_{9,89}$=2.88, P<0.01; lesion × day $F_{9,89}$=1.85, NS, data not shown) with both groups reducing correct latency after repeated testing. Lesions of the nbm did not affect magazine latency, although there was a significant interaction between lesion and day, which was due to the control group rats exhibiting a significantly greater latency to collect the food reward than the lesion group on day 8 (lesion $F_{1,10}$<1, NS; day $F_{9,89}$=1.92, NS; lesion × day $F_{9,89}$=4.66, P<0.001, data not shown).

Behavioral inhibition. There were no effects of lesion or interactions between lesion and day for either premature or perseverative responses (Table 2, postoperative baseline) (Premature responses: lesion $F_{1,10}$<1, NS; lesion × day $F_{9,89}$=1.79, NS. Perseverative responses: lesion

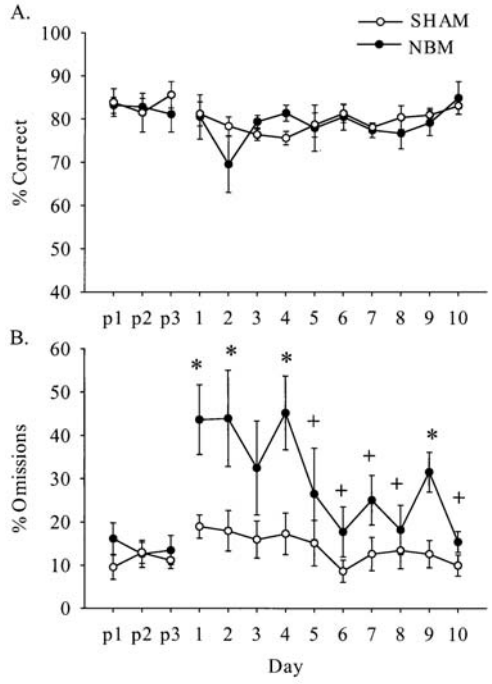

Fig. 1A, B Performance of sham controls and nucleus basalis magnocellularis (nbm)-lesioned rats on the baseline schedule of the 5-choice serial reaction time (5CSRT) task. Animals were tested 3 weeks post-surgery for 10 days using standard testing conditions (0.5-s stimulus duration, 5-s inter-trial interval). **A** Percentage of correct responses. **B** Percentage of omissions. Pre-surgical performance over the last 3 days of baseline training (p1, p2, and p3) is also indicated. Results are expressed as mean±SEM. *P<0.05 relative to sham control group; +P<0.05 relative to respective day 1, Newman Keuls post-hoc test

$F_{1,10}$<1, NS, lesion × day $F_{9,89}$=1.75, NS). Both premature and perseverative response measures were variable day to day in both groups, although the number of inappropriate responding was still low, averaging between two and ten responses per day (Table 2, postoperative baseline; day $F_{9,89}$=2.53, P<0.05; $F_{9,89}$=2.30, P<0.05, respectively).

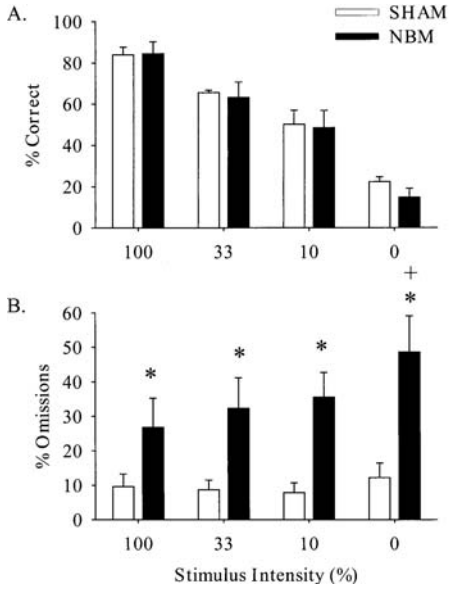

Fig. 2A, B Effects of reduced stimulus intensity (100, 30, 10 and 0% of standard intensity) on 5-choice serial reaction time (5CSRT) task performance of sham controls and nucleus basalis magnocellularis (nbm)-lesioned rats. **A** Percentage of correct responses. **B** Percentage of omissions. Results are expressed as mean±SEM. *P<0.05 relative to sham control group, +P<0.05 relative to respective 100% intensity (standard condition), Newman Keuls post-hoc test

Behavioral challenges

Variable stimulus intensity

Accuracy. As illustrated in Fig. 2A, percentage of correct responses decreased as the SI decreased. Both groups were affected equally, reaching chance level when the stimulus was not presented (0% intensity) (SI $F_{3,30}$=43.48, P<0.001; lesion $F_{1,10}$<1, NS; lesion × SI $F_{3,30}$<1, NS). Overall, the lesion group exhibited a greater level of omissions than the control group (Fig. 2B; lesion $F_{1,10}$=11.65, P<0.01; SI $F_{3,30}$=3.80, P<0.05; lesion × SI $F_{3,30}$=1.47, NS). Post-hoc analysis revealed that the number of omissions in the lesion group was significantly

Table 2 Effect of reduced stimulus intensity on measures of response speed and inhibitory responding in sham and 192 IgG-saporin-lesioned rats

		Speed (latency to respond)		Inhibitory responding	
		Correct latency	Magazine latency	Premature	Perseverative
Post-operative baseline (averaged over 10 days)	Sham	0.71±0.03	2.07±0.07	4.37±1.3	6.42±0.96
	Lesion	0.69±0.02	1.98±0.11	5.63±1.39	6.24±1.15
Stimulus intensity (%)					
100	Sham	0.57±0.03	1.82±0.07	1.50±0.56	0.83±0.31
30		0.80±0.08	1.98±1.16	1.50±0.43	1.17±0.65
10		0.99±0.14	1.96±0.11	1.67±0.33	0.67±0.42
0		2.06±0.26	2.07±0.16	1.83±0.54	0.67±0.49
100	Lesion	0.96±0.31	1.89±0.12	1.17±0.65	2.00±0.52
30		1.10±0.19	1.94±0.15	0.83±0.54	0.83±0.31
10		1.20±0.20	2.14±0.19	1.50±0.62	1.33±0.33
0		2.12±0.59	2.31±0.20	0.83±0.31	0.67±0.33

greater in the 0% than the 100% intensity conditions ($P<0.05$).

Speed. There was a main effect of SI on correct latency and magazine latency although the control and lesion groups were affected equally (Table 2, SI) (Correct latency: lesion $F_{1,10}=1.44$, NS; SI $F_{3,30}=9.17$, $P<0.001$; lesion × SI $F_{3,30}<1$, NS. Magazine latency: lesion $F_{1,10}<1$, NS; SI $F_{3,30}=4.31$, $P<0.05$; lesion × SI $F_{3,30}<1$, NS).

Premature and perseverative responses. As shown in Table 2 (SI), inappropriate responding remained unaffected by either manipulation (lesion $F_{1,10}<1.50$, NS; SI $F_{3,30}<1$, NS; lesion × SI $F_{3,30}<1$, NS; and lesion $F_{1,10}<1.20$, NS; SI $F_{3,30}<1.10$, NS; lesion × SI $F_{3,30}<1.30$, NS; for premature and perseverative responses, respectively).

Variable inter-trial interval

Accuracy. Consistent with the other behavioral challenges, the lesion group showed no differences relative to the sham group in accurate responding after increased ITIs (Table 3; lesion $F_{1,10}<1$, NS; ITI $F_{3,30}=3.67$, $P<0.05$; lesion × ITI $F_{3,30}=2.12$, NS). However, there was a main effect of lesion and an interaction with ITI on number of omissions (Fig. 3A; lesion $F_{1,10}=10.98$, $P<0.01$; ITI $F_{3,30}=2.63$, NS; lesion × ITI $F_{3,30}=7.62$, $P<0.001$). The lesion group exhibited significantly greater omissions than the control group at the 5-s and 6-s ITI trials ($P<0.05$), but not at the longer duration ITIs (8 s and 10 s). This is due to the fact that rats from the sham group significantly increased omissions at the 10-s ITI relative to the 5-s ITI trials ($P<0.05$) whereas the percentage of omissions in the lesion group was not significantly affected by ITI.

Speed. There were no effects of either manipulation on correct latency or magazine latency (Table 3; lesion $F_{1,10}<1$, NS; ITI $F_{3,30}<1$, NS; lesion × ITI $F_{3,30}<1.10$, NS; and $F_{1,10}<1$, NS; ITI $F_{3,30}<1.20$, NS; lesion × ITI $F_{3,30}<1.50$, NS; for correct and magazine latencies, respectively).

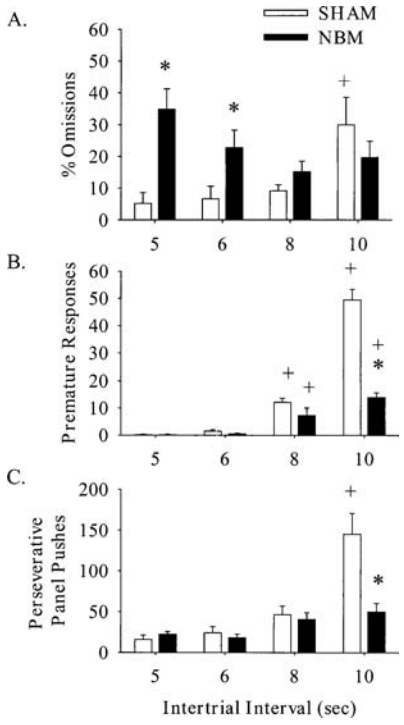

Fig. 3A–C Effects of increased inter-trial intervals [5, 6, 8 and 10 second inter-trial interval (ITI) duration] on 5-choice serial reaction time (5CSRT) performance of sham controls and nucleus basalis magnocellularis (nbm)-lesioned rats. **A** Percentage of omissions. **B** Number of premature responses. **C** Number of perseverative panel pushes. Results are expressed as mean±SEM. *$P<0.05$ relative to sham control group, +$P<0.05$ relative to 5-s ITI (standard condition), Newman Keuls post-hoc test

Premature and perseverative responding. Interestingly, the ITI manipulation was the only behavioral challenge that revealed major differences in behavioral inhibition between the lesion and control groups. There was a main effect of lesion, ITI, and a significant interaction on premature responding (Fig. 3B; lesion $F_{1,10}=44.67$, $P<0.001$; ITI $F_{3,30}=138.22$, $P<0.001$; lesion × ITI $F_{3,30}=45.65$, $P<0.001$). Post-hoc analysis revealed that both groups had significantly more premature responses than standard trials (5-s ITI) as the ITI increased to 8 s and 10 s. However, at the 10-s ITI trials, the lesion group had significantly fewer premature responses than the

Table 3 Effect of increased inter-trial interval on percentage correct, latency to respond and perseverative responding in sham and 192 IgG-saporin-lesioned rats

Intertrial interval (s)	Accuracy	Speed (latency to respond)		Inhibitory responding
	% Correct	Correct latency	Magazine latency	Perseverative
Sham				
5	69.77±2.98	0.66±0.10	1.92±0.42	0.50±0.22
6	87.96±4.55	0.55±0.04	1.95±0.16	0.50±0.50
8	82.97±3.30	0.59±0.07	1.94±0.14	1.00±0.68
10	69.52±6.90	0.92±0.36	3.15±1.09	0.33±0.21
Lesion				
5	80.66±5.54	0.82±0.12	2.13±0.17	1.17±0.48
6	82.83±1.94	0.65±0.06	2.14±0.16	1.33±0.61
8	74.29±6.51	0.75±0.06	2.05±0.15	1.17±0.40
10	70.56±1.84	0.63±0.08	2.04±0.14	1.17±0.31

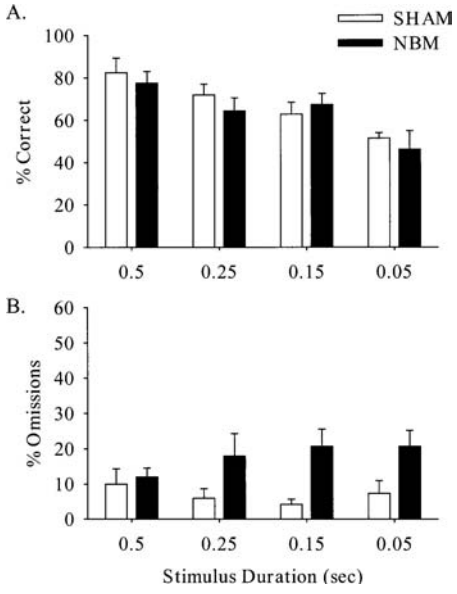

Fig. 4A, B Effects of reduced stimulus duration (0.5, 0.25, 0.15 and 0.05 s) on 5-choice serial reaction time (5CSRT) performance of sham controls and nucleus basalis magnocellularis (nbm)-lesioned rats. **A** Percentage of correct responses. **B** Percentage of omissions. Results are expressed as mean±SEM. There was a main effect of lesion found on percentage of omissions ($P<0.05$)

control group. Thus, the lesion group appeared to be less affected by increasing the interval between trial initiation and the stimulus presentation. In addition, perseverative responding was unaffected by either lesion or ITI, so the effects observed were not just due to overall decreases in ability to inhibit all inappropriate responding (Table 3; lesion $F_{1,10}=3.12$, NS; ITI $F_{3,30}<1$, NS; lesion × ITI $F_{3,30}<1$, NS). Analysis of the data also revealed that there was a main effect of ITI and an interaction between lesion and ITI on perseverative panel pushes (lesion $F_{1,10}=3.924$, NS; ITI $F_{3,30}=15.11$, $P<0.001$; lesion × ITI $F_{3,30}=6.09$, $P<0.01$), a measure that was unaffected in other behavioral challenge conditions (data not shown). As illustrated in Fig. 3C, the control group exhibited significantly more panel pushes at the 10-s ITI than the 5-s ITI (baseline), while the lesion group remained unaffected.

Variable stimulus duration

Accuracy. There was a significant main effect of SD on percentage correct, indicating that as the SD decreased, the choice accuracy decreased (Fig. 4A; $F_{3,30}=14.32$, $P<0.001$). Performance accuracy was equally affected in the lesion and control groups (lesion $F_{1,10}<1$, NS; lesion × SD $F_{3,30}<1$, NS). However, as illustrated in Fig. 4B, the lesion group had significantly greater omissions overall relative to the control group (lesion $F_{1,10}=15.54$, $P<0.01$; SD $F_{3,30}<1$, NS; lesion × SD $F_{3,30}<1$, NS).

Speed. There was a significant main effect of SD on correct latency, but no effect of lesion or an interaction (Table 4; SD $F_{3,30}=4.17$, $P<0.05$; lesion $F_{1,10}=1.45$, NS; lesion × SD $F_{3,30}<1$, NS). There were no effects on magazine latency (Table 4; SD $F_{3,30}=2.46$, NS; lesion $F_{1,10}<1$, NS; lesion × SD $F_{3,30}<1$, NS).

Premature and perseverative responding. There were no main effects of or interactions between lesion or SD on perseverative responding or premature responding (SD $F_{3,30}<2.60$, NS; lesion $F_{1,10}<1$, NS; lesion × SD $F_{3,30}<1.70$, NS; Table 4).

Regression analyses

The number of omissions during the baseline session and the most difficult behavioral challenges were compared with the ChAT activity for each animal. During the shortest SD trials of 0.05 s, the number of omissions was negatively correlated with ChAT activity in the medial frontal (R=0.68, $P<0.05$), posterior cingulate (R=0.64, $P<0.05$), and parietal (R=0.59, $P<0.05$) cortices. Correlations between cortical ChAT activity and omissions during the 10% stimulus brightness trials were significant for all cortical areas assayed (R>0.58, $P<0.05$).

Discussion

The objective of this study was to examine the effects of intra-basalis 192 IgG-saporin infusions on attentional performance as measured by the 5CSRT task in rats.

Table 4 Effect of reduced stimulus duration on measures of response speed and inhibitory responding in sham and 192 IgG-saporin-lesioned rats

Stimulus duration (s)	Speed (latency to respond)		Inhibitory responding	
	Correct latency	Magazine latency	Premature	Perseverative
Sham				
0.5	0.66±0.05	1.93±0.18	2.50±0.96	2.50±0.18
0.25	0.61±0.06	1.87±0.13	1.33±0.61	0.67±0.21
0.15	0.91±0.24	1.91±0.11	1.33±0.42	1.17±0.98
0.05	0.82±0.16	2.04±0.16	0.67±0.33	0.50±0.22
Lesion				
0.5	0.68±0.06	1.72±0.12	1.33±0.33	1.50±0.22
0.25	1.79±0.10	1.84±0.17	1.83±0.79	0.83±0.31
0.15	0.99±0.07	1.88±0.15	0.33±0.21	1.67±0.49
0.05	1.07±0.08	1.99±0.27	1.17±0.75	1.33±0.42

Nbm-lesioned rats made more omissions when re-tested under baseline training conditions after infusion of the immunotoxin, an effect which subsided with additional testing only to re-emerge under more demanding conditions when the duration and intensity of target stimuli were decreased. Nbm-lesioned rats also made fewer premature responses when challenged with a long variable ITI. Notably, these effects occurred in the absence of significant changes in discriminative accuracy and response latencies, and so contrast with the effects of intrabasalis infusions of less selective excitotoxins, such as AMPA (Muir et al. 1992, 1994, 1995) which produced a marked deficit in performance accuracy and increased correct response latencies but did not change the number of omissions. Along with the deficits in response accuracy, AMPA-treated animals also exhibited increased premature and perseverative responding, an effect which seems unrelated to attentional deficits (Muir et al. 1994, 1995). The 192 IgG-saporin-lesioned animals in the present study did not show any increases in premature or perseverative responding. This may imply that these behaviors are not, or are only partially, mediated by the cholinergic system. This hypothesis is further supported by the fact that only the 5-HT3 antagonist ondansetron was able to decrease the AMPA lesion-induced premature responses, while pro-cholinergic compounds, such as nicotine and physostigmine, had no effect on these behaviors (Muir et al. 1994, 1995).

Thus, the differences in performance deficits in the 5CSRT task after administration of excitotoxins versus immunotoxins may depend on the specificity of the lesion. These differences are unlikely to be due to differences in cholinergic denervation, as the cortical ChAT activity depletion was comparable among studies. However, it cannot be excluded that when the lesion is selective to only the cholinergic neurons, the remaining neurons may be able to compensate and therefore manage to maintain a sufficient level of accuracy across the testing session. As mentioned by McGaughy et al. (2002), there must exist some limits to this compensation mechanism. If so, one can hypothesize that a higher dose of 192 IgG-saporin, which may produce a greater level of cholinergic neuronal loss, may be required to induce deficits in response accuracy in addition to the effects observed on omissions. Indeed, a recent article published during the review process of this manuscript (McGaughy et al. 2002) reported that rats with extensive lesions of the nbm induced by a dose of 192 IgG-saporin (0.225 μg/hemisphere) 3.3 times higher than the dose used in the present study showed a severe deficit in response accuracy and correct latencies accompanied by an increase in omission, perseveration, and premature responses. Interestingly, the effect was dose dependent as a lower dose of 192 IgG-saporin produced less-prominent behavioral deficits. This suggests that the cognitive effects of a compromised basal forebrain-cortical cholinergic system are dependent on the number of neurons surviving in the nbm; although, as pointed out by McGaughy et al. (2002), the possibility of additional non-specific cell loss which may play a role in the behavioral deficits observed at the high dose of 192 IgG-saporin cannot be completely ruled out.

McGaughy et al. (2002) also reported a positive correlation between level of cortical acetylcholine efflux and accuracy, whereas no correlation was observed with cortical noradrenaline. In our study, regression analyses revealed a significant negative correlation between ChAT activity in medial frontal and parietal cortices and number of omissions. Therefore, these behavioral deficits appear to be linked to cholinergic depletion, an observation which is further supported by the existence of a significant inverse correlation between number of omissions and cortical acetylcholine efflux in animals performing the 5CSRT task (Passetti et al. 2000). Barbelivien and colleagues (2001) using brain imaging in rats performing the 5CSRT task also reported an increased (^{14}C)2-deoxyglucose uptake in the nbm and in cortical areas (i.e., frontal, cingulate and parietal cortices) innervated by the nbm-cortical pathway. Along with the present data, these studies point toward a role for the nbm-cortical cholinergic pathway in attentional performance in the 5CSRT task.

Interestingly, at a dose of 192 IgG-saporin similar to the one used in the present study (0.075 μg vs 0.067 μg, respectively), McGaughy et al. (2002) reported mild decreases in accuracy with transient increases in premature responding and no effects on omissions. The commonality with the present study is that 192 IgG-saporin induced some form of attentional deficits and that these deficits are more pronounced in testing conditions where the attentional demand of the task is increased. However, the two studies contrast in the nature of deficits reported, as the present study is reporting an increase in omissions rather than a decrease in accuracy. Unfortunately, a direct comparison of the extent of cholinergic damage between the two studies remains difficult, as the biochemical assays reporting cortical cholinergic depletion were different. Assuming that slight differences in dose and batch of 192 IgG-saporin could not account for the discrepancy in the behavioral changes reported, several interpretations can be offered to explain the present increase in omissions and the different array of behavioral deficits reported between the two studies.

In the present study, under baseline conditions, the increase in the number of omissions exhibited by nbm-lesioned animals was only apparent during initial training, a recovery being observed after 10 days of testing. This recovery phenomenon, also observed in AMPA-treated animals (Muir et al. 1994), could be attributed to a progressive functional reorganization of the neural circuitry underlying performance under repeated and identical testing conditions. The use of behavioral challenges was able to reinstate a behavioral deficit (i.e., increased number of omissions) in nbm-lesioned animals. As the animals were not exposed to behavioral challenges prior to the infusion of immunotoxin, the behavioral deficit in nbm-lesioned rats could thus be explained by an increased reactivity to novelty of the behavioral challenges. This

seems, however, unlikely for two reasons. First, deficits in omission were trial-type dependent during the ITI behavioral challenge. If novelty was an important factor, one might expect overall omissions not to depend on specific trial conditions. Second, immunotoxin-lesioned rats in the experiments of McGaughy et al. (2002) and Waite et al. (1999) only showed attentional deficits after specific behavioral challenges and not others, which again would suggest that the behavioral deficits induced by cholinergic depletion are dependent on task requirements as opposed to novel trial conditions.

Thus, the increase in omissions could imply that the cholinergic depletion selectively (1) affects the motivation for food rewards, (2) impairs the animal's ability to initiate a response following detection of the pertinent stimulus, (3) impairs target detection, or finally (4) impairs the animal's ability to accurately time stimulus presentation.

Errors of omission are most commonly associated with a lack of motivation. Pre-feeding, which presumably decreases motivation for food rewards, significantly increases omissions in the 5CSRT task (Harrison et al. 1997; Grottick and Higgins 2000). Magazine latency (latency to collect food reward) and correct latency are also considered indicators of motivation and are increased with pre-feeding (Carli and Samanin 1992; Harrison et al. 1997). In the present study, however, the lesion group did not show an increase in latency measures. Hence, if the increase in omissions was purely due to reduced motivation, one would expect effects in these measures as well. Furthermore, if motivation was decreased, one might also expect an increase in omissions toward the end of the session as the rat becomes satiated and the motivation for the food reward diminishes, a phenomenon that was not observed (data not shown). The fact that 192 IgG-saporin may impair the animal's ability to initiate a response following the detection of the pertinent stimulus is also unlikely, as the lesion had no effect on latency to respond.

However, impaired signal detection may, at least in part, explain the deficits observed in these nbm-lesioned rats. Instead of responding inaccurately, the 192 IgG-saporin-treated animals were more inclined to miss the stimulus presentation and, thus, omit responding. Although the 5CSRT task cannot easily dissociate effects on stimulus sensitivity from response bias, nbm lesions have been reported to specifically impair performance in signal detection tasks. For example, McGaughy and colleagues (1996) have demonstrated that 192 IgG-saporin lesions of the nbm disrupted performance in vigilance tasks, resulting in impairments in the ability to respond to unpredictably occurring stimuli (i.e., decrease in the number of "hits") but not in the ability to detect non-signals. This is further supported by reports of deficient signal detection in 192 IgG-saporin-lesioned animals engaged in cued signal detection tasks (Chiba et al. 1999). A signal detection deficit may also explain the differences between the excitotoxin-treated and the immunotoxin-treated rats. Because of deficits in behavioral inhibition, the AMPA-treated rats may have been more likely to respond (i.e., had a greater bias to respond) even when the signal detection was impaired, whereas the 192 IgG-saporin-treated animals in the present study had a much higher signal detection response threshold and, thus, manifested the deficit in attention through errors of omission as opposed to inaccuracy. Indeed, increased omissions in 192 IgG-saporin-treated rats and decreased accuracy in AMPA-treated rats were most consistently observed during behavioral challenges with lower SDs and consequently more difficult signal detection (Muir et al. 1994, 1995). However, the fact that the 192 IgG-saporin-lesioned rats were able, in the present study, to detect brief signals (0.05-s SD) on some trials but not on others indicates that an effect on signal detection is not sufficient to account for these inconsistent behavioral deficits.

A selective effect on timing presentation of the stimulus may be a better explanation for the present increase in omissions. The 192 IgG-saporin-lesioned rats appear to be less capable of predicting stimulus presentation and, thus, are unable to attend at the appropriate time (i.e., 5 s after trial initiation). This is supported by the fact that they do not exhibit expected behaviors relative to the control-group rats when the duration of the ITI is varied from baseline conditions (normally 5 s post-trial initiation). In fact, the control group exhibited an increased number of omissions, premature responses, and panel pushes as the ITI increased. This pattern suggests that when the stimulus does not occur at the expected time, the animals either turn around to initiate a trial (panel push) and thus miss the stimulus presentation (omissions) or "assume" it had already occurred and simply "guess" (premature responses). In contrast, the number of omissions was not significantly affected by the ITI duration in 192 IgG-saporin-treated rats, a pattern of responding that was also reported in AMPA-lesioned animals in this behavioral challenge (Muir et al. 1994), suggesting thereby that this may be due to the level of cortical cholinergic damage observed in both studies. Furthermore, premature responses and perseverative panel pushes exhibited by the lesioned group were significantly lower than in the control group (ITI of 10 s). This may be an indication that the lesioned group may not have the same expectation as the sham group for when the stimulus is normally presented (5 s). The nbm lesion group may be less able to predict or time when the stimulus will be presented and, consequently, less likely to attend to the stimulus array at the appropriate time.

Alternatively, increased conservatism may be a possible consequence of failure to detect the stimulus on some trials and may explain the different array of behavioral deficits between our study and McGaughy et al. (2002). In the procedure used by McGaughy et al. (2002), rats were tested under continuous performance conditions (no trial initiation required) without specific timeout punishment (5-s period of darkness) for inappropriate responding or omissions. This may be responsible for a shift in response bias between the two lesioned groups, with the rats in the present study more likely to be conservative in responding because of the possibility of a time out, which would

result in lost trial opportunities (all sessions were stopped after 30 min). Thus, differences in the punishment of inappropriate responding between the two studies may account for a response bias shift changing the behavioral outcome of perhaps similar underlying attentional deficits. The rats in the present study would omit responding if unsure of the target location (increased number of omissions); whereas, in the McGaughy study, the rats would be more likely to respond even with a greater degree of uncertainty (decreased accuracy). This difference in response bias may also explain why in the present study the nbm lesion did not affect inappropriate behaviors while a dose-dependent increase in premature responding was reported by McGaughy et al. (2002). Taken together, the present data indicate that lesions of the cholinergic system can produce different attentional deficits depending on the cognitive demands of the task.

In summary, although the 192 IgG-saporin lesion of the nbm produced a different array of behavioral deficits than previously reported, these effects are consistent with some impairment in attentional or target detection mechanisms and further support the hypothesis that the basal forebrain cholinergic system plays an important role in attentional function and accurate timing of stimulus presentation in visuospatial attention tasks in rodents.

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