Cortical Cholinergic Function and Deficits in Visual Attentional Performance in Rats Following 192 IgG–Saporin-induced Lesions of the Medial Prefrontal Cortex

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Lesions of the basal forebrain (BF) cortical cholinergic system impair performance on a rodent five-choice visual attentional task. This study examines the effects on the same task of selective depletion of acetylcholine from the prefrontal cortex (PFC) using 192 IgG–saporin, the cholinergic immunotoxin. Rats were trained to detect brief visual stimuli, either presented unpredictably both temporally and spatially to increase attentional load, or under less demanding conditions where stimuli were temporally and spatially predictable. Following training, 192 IgG–saporin (50 ng or 100 ng/infusion) or its vehicle was infused bilaterally into the ventromedial PFC. The 100 ng lesion group exhibited post-operatively a transient increase in perseveration, specifically when the visual stimuli were temporally unpredictable. A vigilance decrement, as well as a reinstatement of perseverative responding occurred in both lesion groups under conditions of enhanced attentional load, specifically with high target frequency sustained over many trials. Lesioned subjects were also more impulsive with increased anticipatory errors. Systemic administration of the muscarinic receptor antagonist scopolamine further dissociated the groups with attentional accuracy in the 100 ng group decreasing relative to shams. These findings are consistent with an important modulatory influence of PFC function by BF cholinergic neurons, particularly during increased attentional demand.

Keywords: 192 IgG–saporin, acetylcholine, five-choice serial reaction time task, impulsivity, medial prefrontal cortex, muscarinic receptors, visual attention

Introduction

Magnocellular neurons in the Ch4 region of the basal forebrain (BF) provide the major source of cholinergic innervation to the rat cerebral cortex (Bigl *et al.*, 1982; Mesulam *et al.*, 1983; Eckenstein *et al.*, 1988; Lysakowski *et al.*, 1989). Understanding their discrete and separable functions in cognitive and behavioral processes may be relevant to neuropathological states involving memory dysfunction such as Alzheimer's disease (Davies and Maloney, 1976; Perry *et al.*, 1978; Coyle *et al.*, 1983; Jones *et al.*, 1992; Sahakian and Coull, 1993). Previous studies in rodents and monkeys have generally investigated such functions by administering neurotoxins directly in the vicinity of cholinergic cell bodies in the BF (e.g. Berger-Sweeney *et al.*, 1994; Muir *et al.*, 1994; Voytko *et al.*, 1994; Baxter *et al.*, 1995; Chiba *et al.*, 1995; Risbrough *et al.*, 2002; McGaughy *et al.*, 2002). Although this approach has been useful in characterizing the topography and functional organization of BF cholinergic systems, it is clearly less suitable for delineating the functional significance of cholinergic inputs in specific areas of cortex to which these groups of neurons project.

Recently, it has become possible to achieve selective lesions of cholinergic neurons by targeting them with the ribosome-inactivating toxin saporin coupled with an antibody (192 IgG) that recognizes the low-affinity p75 nerve growth factor (NGF) receptor (Wiley *et al.*, 1991; Book *et al.*, 1992; Heckers *et al.*, 1994; Bucci *et al.*, 1998). However, many of the deficits produced by intra-BF infusions of less selective neurotoxins such as NMDA and ibotenic acid, including effects on learning on memory, have not generally been replicated in BF-saporin-lesioned subjects, despite often greater reductions in functional indices of cholinergic transmission following 192 IgG–saporin infusions into the BF (Berger-Sweeney *et al.*, 1994; Torres *et al.*, 1994; Wenk *et al.*, 1994; Baxter *et al.*, 1995, 1996; Galani *et al.*, 2002; for review see Everitt and Robbins, 1997; McGaughy *et al.*, 2000). Moreover, no direct relationship has been found between cholinergic cell loss in the BF and memory impairments in rodents (Wenk *et al.*, 1992). In contrast, there is now compelling evidence for a significant role of the BF cholinergic system in attention. Thus, tasks that place explicit demands on attentional processes, rather than learning and memory *per se*, appear particularly sensitive to lesions of the BF (Pang *et al.*, 1993; Muir *et al.*, 1994; Voytko *et al.*, 1994), including lesions made using 192 IgG–saporin (Chiba *et al.*, 1995; McGaughy *et al.*, 1996, 2002; Risbrough *et al.*, 2002; Lehmann *et al.*, 2003). This claim is supported by recent *in vivo* microdialysis studies showing large and sustained elevations in cortical acetylcholine (ACh) release during established attentional performance (Himmelheber *et al.*, 2000; Dalley *et al.*, 2001; McGaughy *et al.*, 2002) and evidence for a significant positive relationship between ACh efflux in the prefrontal cortex (PFC) and attentional accuracy (McGaughy *et al.*, 2002).

The five-choice serial reaction time (5-CSRT) task, analogous to the continuous performance test in humans, has been employed in several previous investigations of BF function (Robbins *et al.*, 1989; Muir *et al.*, 1994; McGaughy *et al.*, 2002). In this procedure rats or mice are trained to discriminate a brief visual stimulus presented randomly in one of five locations, and to respond appropriately with a nose-poke to receive reinforcers. In this format, the test contains elements of a sustained attention paradigm in addition to requiring subjects to divide attention across multiple spatial domains. Based on previous investigations it is now established that different neural and neurochemical substrates contribute to different aspects of performance on this task, including sub-regions of PFC (Muir *et al.*, 1996; Chudasama and Muir, 2001; Passetti *et al.*, 2002) and striatum (Christakou *et al.*, 2001; Rogers *et al.*, 2001) and the ascending monoaminergic and BF cholinergic systems (see Robbins, 2002 for review). For example, excitotoxic lesions of the entire medial PFC in rats result in deficits in accuracy,

speed of responding and perseveration, while lesions sparing the dorsal PFC (Cg1, anterior cingulate cortex) mainly affect the number of perseverative or repeat responses following a correct trial (Chudasama and Muir, 2001; Passetti *et al.*, 2002). Moreover, it is of interest that some or all of these deficits also result from lesions of the BF (Robbins *et al.*, 1989; Muir *et al.*, 1994; McGaughy *et al.*, 2002) and that impairments in accuracy in BF-lesioned subjects are reversed by cholinergic-rich grafts implanted directly into the neocortex (Muir *et al.*, 1992).

The present study examines the consequences of a selective cholinergic lesion of the PFC produced by local infusions of 192 IgG–saporin, using immunocytochemical indices of neuronal loss and lesion specificity in conjunction with behavioral testing on the 5-CSRT task. It was hypothesized that the selective loss of prefrontal cholinergic afferents would produce deficits in performance on this task that resemble those of BF lesions. However, unlike other studies that have used this task subjects were first trained to respond to visual stimuli presented in a *single* aperture, the location of which was allocated on a random basis on each training day. Training in this way provides a sensitive baseline from which to investigate the different component processes that contribute to performance on the 5-CSRT task, including visual search, response selection and response timing. During challenge sessions the stimuli were presented either more frequently ('high event rate') or spatially as well as temporally unpredictably, manipulations that place greater demands on attentional rather than simply sensory functions (Parasuraman and Giambra, 1991; Koelega, 1989). In addition, subjects were challenged with scopolamine, a muscarinic receptor antagonist that previously has been shown to disrupt performance on the 5-CSRT task (Mirza and Stolerman, 2000), even when administered directly into the medial PFC (Robbins *et al.*, 1998). Based on previous evidence of functional compensation of cortical muscarinic receptors following immunolesioning in rats with 192 IgG–saporin (Schliebs *et al.*, 1996), which potentially could mask any behavioral deficits on the 5-CSRT task, it was predicted that scopolamine would produce differential effects in sham and 192 IgG–saporin-lesioned rats.

Materials and Methods

Subjects

The subjects were 27 male Lister Hooded rats (Charles River, Margate, Kent, UK), weighing 370–420 g at the time of surgery. The animals were housed individually in a temperature- and humidity-controlled holding room under a 12 h light/dark cycle (lights off 0730). Animals were given free access to food (laboratory chow, Purina, UK) and water for 2–3 weeks until their body weights reached 200–250 g (~2–3 months of age). Thereafter, training on the task commenced and food was restricted to 14 g/subject per day. All surgical procedures complied with the requirements of the UK Animals (Scientific Procedures) Act of 1986 (Project number PPL 80/1324).

Apparatus

The apparatus consisted of eight five-choice chambers (25 × 25 × 25 cm), each housed within a ventilated wooden sound-attenuating box (Paul Fray Ltd, UK). The rear wall of the chamber was curved with nine contiguous 2.5 × 2.5 cm apertures, 4 cm deep and set 2 cm above a wire grid floor. A metal insert blocked every alternate hole (i.e. holes 1, 3, 5, 7 and 9 were left open). A photocell beam was located at the entrance of each aperture to detect nose-poke responses. A 3 W stimulus light was located at the rear of each the five apertures. The front of the chamber contained a magazine connected to a food dispenser, entries into which were monitored by a horizontal infrared beam. The apparatus was controlled by software written in Arachnid (Paul Fray Ltd), a real-time extension to BBC Basic V running on an Acorn Archimedes computer.

Behavioral Training

Subjects were trained on a modified version of the five-choice task where responding was restricted to a single aperture, the location of which varied on a daily basis ('forced choice' paradigm). In the initial training phase, consisting of two 20 min sessions, 5–10 pellets (Noyes dustless pellets, Research Diets, UK) were placed in the magazine and in each of the open apertures to encourage the subjects to enter these locations. In subsequent sessions, subjects were trained over ~30 sessions to detect the presence of a brief light stimulus (0.5 s in duration) presented at the rear of each aperture. A Latin square design was used to determine which aperture would be used on any particular training day. This was never the same over consecutive days and once selected remained fixed for the duration of the session.

Training was facilitated by progressively shortening the stimulus duration from 30 s to 0.5 s (see Dalley *et al.*, 2002 for details of this procedure). Each session began with the illumination of the house light and the delivery of a food pellet. The collection of this pellet triggered the first trial by breaking the horizontal photobeam at the entrance of the magazine. After a fixed inter-trial interval (ITI) of 5 s, a light at the rear of the designated aperture was briefly illuminated. Responses in this aperture within a limited hold period of 5 s were recorded as 'correct' responses and were rewarded by the delivery of a single pellet in the magazine. A failure to respond within the limited hold period was deemed an 'omission' and was punished by the house light being extinguished for 5 s and no delivery of food reward. Responses in a non-illuminated hole were recorded as 'incorrect' responses; these were punished with a 5 s period of darkness and no food reward. The 'accuracy' of target detection was computed as the percentage of correct responses to the total number of correct and incorrect responses. Additional responses in any hole prior to food collection ('perseverative' responses) were recorded but not punished. Responses made in any aperture before the onset of the light stimulus (i.e. ITI responses) were deemed 'premature' and were punished by a 5 s period of darkness and no food delivery. Two measures of speed of responding were used. The first measure was latency for a correct response, defined as the time between the onset of the stimulus and the response. The second measure was latency to collect the reinforcement, defined as the time between a correct response and the first entry into the magazine. All sessions consisted of 100 trials, which took ~30 min to complete. Whilst premature responses were monitored they were not registered as complete trials.

Subjects were considered to have acquired the task when the accuracy of signal detection was greater than 85% and omissions were less than 15%. Subsequent sessions consisted of four behavioral manipulations (A, B, C, D), each repeated on six occasions prior to surgery according to a Latin square design. Subjects were run on the forced choice task on the day following each behavioral manipulation in order to re-establish baseline performance. The manipulations consisted of (A) a fixed location and constant ITI (5 s); (B) five locations (i.e. holes 1, 3, 5, 7 and 9) and a constant ITI (5 s); (C) a fixed location and variable ITI (2, 4, 6 and 8 s); (D) five locations (i.e. holes 1, 3, 5, 7 and 9) and a variable ITI (2, 4, 6 and 8 s). Each session consisted of 100 trials and lasted ~30 min. Fixed locations were allocated according to a Latin square procedure, such that subjects sampled all of the available response apertures at the same frequency. On sessions where a variable ITI was used, the trials were presented in a randomized order, balanced to give an equal number of trials for each ITI.

Surgery

Subjects were anesthetized with ketamine (Ketalar, 90 mg/kg i.p.; Vet Drug, Bury St Edmunds, UK) and xylazine (Rompun, 6.7 mg/kg, i.p.; Vet Drug) and secured in a small-animal stereotaxic frame (Kopf, Tijunga, CA) in a flat skull position (incisor bar set at –3.3 mm relative to the interaural line). A small quantity of ophthalmic ointment (Lacri-Lube; Allergan, UK) was wiped over each eye to prevent desiccation of the corneal surfaces. Six small holes were drilled through the skull at the level of the medial PFC to allow the

administration of either vehicle (0.01 M phosphate-buffered saline), 50 ng/0.5 µl or 100 ng/0.5 µl 192 IgG–saporin (Chemicon, Temecula, CA). Bilateral microinfusions (0.5 µl/site) were carried out using 31-gauge, non-beveled stainless steel injectors (Cooperneedle Works, UK) at a rate of 0.25 µl/min, commencing 2 min after lowering the injectors in the brain. The following stereotaxic coordinates were used (relative to bregma and the dural surface): site 1, AP 2.6 mm, L ±0.65 mm, V –3.5 mm; site 2, AP 3.2 mm, L ±0.65 mm, V –3.5 mm; site 3, AP 3.8 mm, L ±0.65 mm, V –3.0 mm. Following each infusion, the injector was left in place for 4 min before being slowly retracted. Subjects were housed in a recovery room overnight with free access to water. The next day, animals were returned to the stock holding room, housed in individual cages, and fed 32 g/subject of laboratory chow. Animals were given a full week to recover from surgery before being re-tested on the forced choice paradigm.

Post-operative Behavioral Manipulations

Subjects were tested on the forced choice task as before with each of the four behavioral manipulations presented on alternate days according to a Latin square design. Each manipulation was followed by a baseline training session consisting of 100 trials where the ITI was 5 s and only one of the five apertures was used, again chosen according to a Latin square design. Following three complete cycles of each of the four behavioral manipulations subjects were further challenged with sessions consisting of stimuli presented across all the five apertures, either less frequently ('low event rate' ITI = 7 s), more frequently ('high event rate' ITI = 2 s) or with a reduced duration (0.5, 0.25 or 0.125 s). The high and low event rate sessions each consisted of 200 trials and were run on alternate days with a baseline session (a fixed ITI of 5 s but a random location) separating them. Sessions employing a reduced stimulus duration were run in three blocks of 100 trials, with the stimulus duration descending across blocks.

Scopolamine Administration

One week after the last behavioral challenge subjects received a subcutaneous injection of either vehicle (0.9% saline, 1 ml/kg) or scopolamine hydrochloride (0.01, 0.03, 0.06, or 0.1 mg/kg; Sigma, UK) in the neck region. The doses were calculated as the free base and were administered according to a Latin square design. Animals were tested on the five-choice version of the paradigm (fixed ITI 5 s) 15 min after drug administration. Sessions ran on alternate days with a drug-free baseline session (five-choice version, fixed ITI 5 s) separating them. All sessions consisted of 100 trials and were ~30 min in duration.

Immunocytochemistry

Upon completion of testing, subjects were deeply anesthetized with sodium pentobarbitone (Euthatal, 200 mg/ml, Genus Express, UK) and perfused transcardically via the left ventricle with 60 ml phosphate-buffered saline (PBS 10 mM) followed by 300 ml 4% paraformaldehyde (containing 10% formalin) at a rate of ~30 ml/min. The brains were removed and stored in 4% paraformaldehyde for 24–48 h, and then in 30% sucrose until they sank. Coronal sections (40 µm) were cut on a cryostat (–15°C) from the rostral pole to the genu. Every third section was discarded.

For acetylcholinesterase (AChE) staining, sections were first rinsed in cold 0.9% saline (4°C) for 1–2 h and then immersed in a solution containing 100 mg acetylthiocholine iodide, 130 ml sodium acetate (pH 6.0, 0.1 M), 10 ml sodium citrate (0.1 M), 20 ml copper sulfate (30 mM), 20 ml potassium ferricyanide (5 mM) and 20 ml deionized distilled water for 2 h. After rinsing twice with 10 mM PBS, sections were mounted onto subbed glass slides and air-dried for 24 h. Sections were then serially dehydrated in alcohol, cleared with Histoclear and coverslipped. The specificity of the lesion was assessed by determining the number of parvalbumin-immunoreactive neurons in dorsal and ventral regions of the medial PFC (see Bucci *et al.*, 1998).

For parvalbumin staining, sections were quenched in a solution containing 10% methanol and 10% hydrogen peroxide for 5 min and, following rinsing (3 × 5 min) in Tris-buffered saline (TBS), incubated in normal goat serum (NGS, 30 µl/ml in 0.2% Triton-X100 and TBS) for 1 h. Without rinsing, sections were then incubated overnight at room temperature in primary antibody (1:200 dilution) in TBS with 0.2% Triton-X100 and 1% NGS (PARV antimouse; Sigma). Sections were then rinsed (3 × 10 min) in TBS and incubated for 2 h at room temperature in streptavidin ABC solution in TBS with 1% NGS. They were then rinsed (2 × 5 min) in 0.05 M Tris non-saline (TNS, 6 g/l Tris, pH 7.4) and incubated in a TNS solution containing diaminobenzidine (20 mg) and hydrogen peroxide (30%) for 2 h. Sections were then rinsed (3 × 5 min) in TNS, mounted onto subbed slides and air-dried for 24 h. Following dehydration in alcohol, the sections were coverslipped in DePeX. Since AChE-positive fibers were widely abundant in the sham animals as well as adjacent margins of the lesion site (see Fig. 2), individual fibers were not counted. However, visualization under a low power microscope revealed that AChE depletion was profound and localized to a relatively restricted region of the medial PFC.

Statistical Analyses

Data for each dependent variable were subjected to analyses of variance (ANOVA) using SPSS version 10 (SPSS, Chicago, IL). Significant deviations from the requirement for homogeneity of variance were corrected using the Huynh–Feldt epsilon to adjust degrees of freedom as recommended by Keppel (1991). Prior to ANOVA, percentage data were transformed using the formula for angular transformation [$x' = 2\arcsin \mathrm{SQRT}x$]; response and magazine latencies were subjected to logarithmic transformation. Post-operative effects on behavioral performance were analyzed using repeated measures ANOVA with one between-subjects factor 'lesion' (three levels: sham, 50 ng, 100 ng) and two within-subjects factors: 'session' (four levels; one pre-surgical session and three consecutive post-surgical sessions) and 'manipulation' (four levels: A, B, C, D). The high and low event rate manipulations were analyzed by two-way ANOVA with the between-subjects factor 'lesion' (three levels) and the within-subjects factor 'ITI' (two levels; low event rate ITI = 7 s, high event rate ITI = 2 s). In addition, the high event rate data was subjected to repeated measures ANOVA with factors 'lesion' and 'time' (seven levels: consecutive 5 min time bins) to assess a possible decrement in performance during time on task. Data from the reduced stimulus duration experiment and scopolamine challenge were analyzed by ANOVA with the between-subjects factor 'lesion' (three levels) and the within-subjects factors 'stimulus duration' (three levels: 0.5, 0.25, 0.125 s) or 'dose' (five levels: 0, 0.01, 0.03, 0.06, 0.10 mg/kg). Probability values less than 0.05 were considered significant. Significant interactions were further analyzed using ANOVA and, where appropriate, Bonferroni *t*-tests were used for multiple comparisons.

Results

Immunocytochemistry

Histological evaluation revealed a substantial reduction in AChE-positive fibers in the ventromedial region of the PFC with the greatest loss of AChE-positive fibers evident in the infralimbic cortex and ventral most aspects of the prelimbic cortex (see Figs 1 and 2). Dorsal regions of the prelimbic cortex, anterior cingulate cortex and supplementary motor regions were spared in both lesion groups. There was no appreciable difference in the spread of the lesion between the two lesion groups (see Fig. 1). PARV-IR neurons in prelimbic and infralimbic cortices were not significantly affected by the 192 IgG–saporin infusions (see Fig. 2) and there was no evidence of gross, non-selective neuronal damage such as cavitation.

Behavioral Effects of Prefrontal 192 IgG–Saporin

Pre-operatively, there were no significant differences between future sham and lesioned animals with respect to accuracy, premature responding, perseveration, omissions and response latency for any of the four behavioral manipulations.

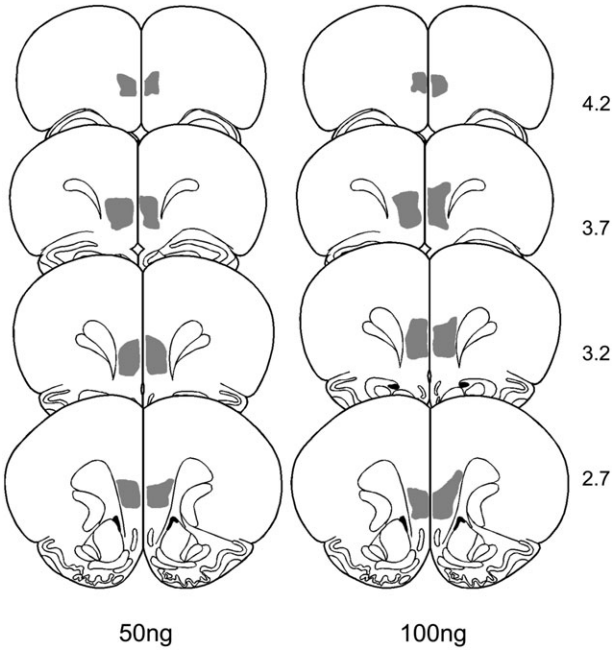

Figure 1. Schematic diagrams of the rat forebrain showing the largest extent of AChE-positive fiber loss within the medial PFC (adapted from Paxinos and Watson, 1998), following bilateral infusions of 50 ng (left hand diagrams) and 100 ng (right hand diagrams) 192 IgG–saporin. Numbers to the right of each figure refer to the anterior–posterior level forward of bregma (mm).

Target Accuracy

Figure 3 shows the effects of cholinergic deafferentation of the PFC on the accuracy of performance on the attentional task. Varying the timing and spatial location of the visual stimuli produced graded effects on the accuracy of performance [manipulation: $F_{(3,72)}$ = 126.9, $P < 0.01$]. In particular, manipulations affecting only the spatial features of the task (i.e. sessions B and D) significantly reduced accuracy relative to fixed location sessions with fixed or variable ITIs (i.e, sessions A and C). In addition, accuracy was reduced when the ITI was made variable in the case of fixed location sessions but not when the ITI was varied in the case of multiple locations. The lesion itself however, under these conditions, produced no significant effects on the accuracy of performance [lesion: $F(2,24) = 0.59$, $P = 0.56$], either across session [lesion × session: $F(6,72) = 1.55$, $P = 0.18$] or manipulation [lesion × manipulation: $F(2,24) = 0.44$, $P = 0.85$].

Perseveration

The effects of 192 IgG–saporin infusions into the medial PFC on perseverative responding are shown in Figure 4. Perseverative responding varied as a function of session, manipulation and lesion group [session × manipulation × lesion interaction: $F(18,216) = 1.78$, $P = 0.034$] with significant main effects of session [$F(3,72) = 3.06$, $P = 0.041$], manipulation [$F(3,72) = 2.90$, $P = 0.044$] and lesion [$F(2,24) = 4.73$, $P = 0.019$]. Analysis of the effects of individual manipulations by two-way ANOVA revealed that sessions with *variable* (i.e. C and D) but not *fixed* inter-trial intervals (i.e. A and B) were those associated with significant effects on perseveration. The greatest changes were evident when responding was restricted to a single aperture (i.e. manipulation C) where there was a main effect of lesion [$F(2,24) = 8.11$, $P < 0.01$] and session [$F(3,72) = 10.23$, $P < 0.01$] and a significant lesion × session interaction [$F(6,72) = 5.00$, $P < 0.01$]. *Post hoc t*-tests revealed that perseverative responding in the high lesion group (100 ng 192 IgG–saporin infusions) increased significantly relative to both sham animals and pre-operative levels of responding ($P < 0.01$); however, this effect was transient with perseverative responding falling over subsequent sessions. Although inspection of the data suggests that a similar pattern of results was evident for the 50 ng lesion group this effect failed to reach statistical significance relative to sham controls. Rats with cholinergic lesions of the medial PFC also exhibited increased perseverative responding when variable interval trials and multiple response apertures were used (i.e. manipulation D) with main effects of lesion [$F(2,24) = 3.47$, $P = 0.047$] and session [$F(3,72) = 3.31$, $P = 0.025$] and a near significant difference between the 100 ng lesion group and sham controls ($P = 0.051$).

No other significant behavioral effects of the prefrontal cholinergic lesions were found during the baseline behavioral challenges (manipulations A–D, data not shown), including speed of responding and magazine latency. However, regardless of lesion group, omissions and premature responses varied according to the manipulation used [omissions: $F(3,72) = 66.48$, $P < 0.01$; premature: $F(3,72) = 148.5$, $P < 0.01$], both being reduced during fixed ITI sessions (i.e. A and B) whilst correct responses were slower when the ITI was variable but the location fixed (manipulation C).

High and Low Event Rate Manipulations

Figure 5 summarizes the effects of low and high event rates on the behavioral performance of sham and 192 IgG–saporin-lesioned subjects on the five-choice version of the task. The accuracy of subjects varied as a function of ITI [$F(1,24) = 27.29$, $P < 0.01$] with more accurate performance under the low event rate condition. However, under high event rate conditions performance *deteriorated* significantly in lesioned subjects [lesion × ITI: $F(2,24) = 3.77$, $P = 0.038$] with reduced accuracy in the 100 ng group (low versus high event rate: $P = 0.0012$). Accuracy in the 50 ng group was not affected by changing the event rate (low versus high event rate: $P = 0.076$).

Perseverative responses were significantly increased in lesioned animals during the low and high event rate manipulations [lesion: $F(2,24) = 3.61$, $P = 0.043$], particularly in the 100 ng group, where overall levels of responding were significantly greater than sham controls ($P = 0.039$). Perseverative responses in the 50 ng group were not significantly different from the sham group ($P = 0.636$).

The number of premature responses increased when the event rate was decreased [ITI: $F(1,24) = 114.5$, $P < 0.01$], an effect which was greater in lesioned subjects than sham controls [lesion: $F(2,24) = 3.45$, $P = 0.048$; lesion × ITI: $F(2,24) = 3.55$, $P = 0.045$]. One-way ANOVA confirmed that lesioned subjects overall made more premature responses under the low event rate condition [lesion: $F(2,24) = 3.51$, $P = 0.046$].

Finally, the saporin lesions produced no significant effect on the number of omissions during the low and high event rate sessions [lesion: $F(2,24) = 2.49$; NS; lesion × ITI: $F(2,24) = 1.64$, NS].

Vigilance Decrement

The accuracy of subjects as a function of time on task during the high event session is shown in Figure 6. Sham-operated rats

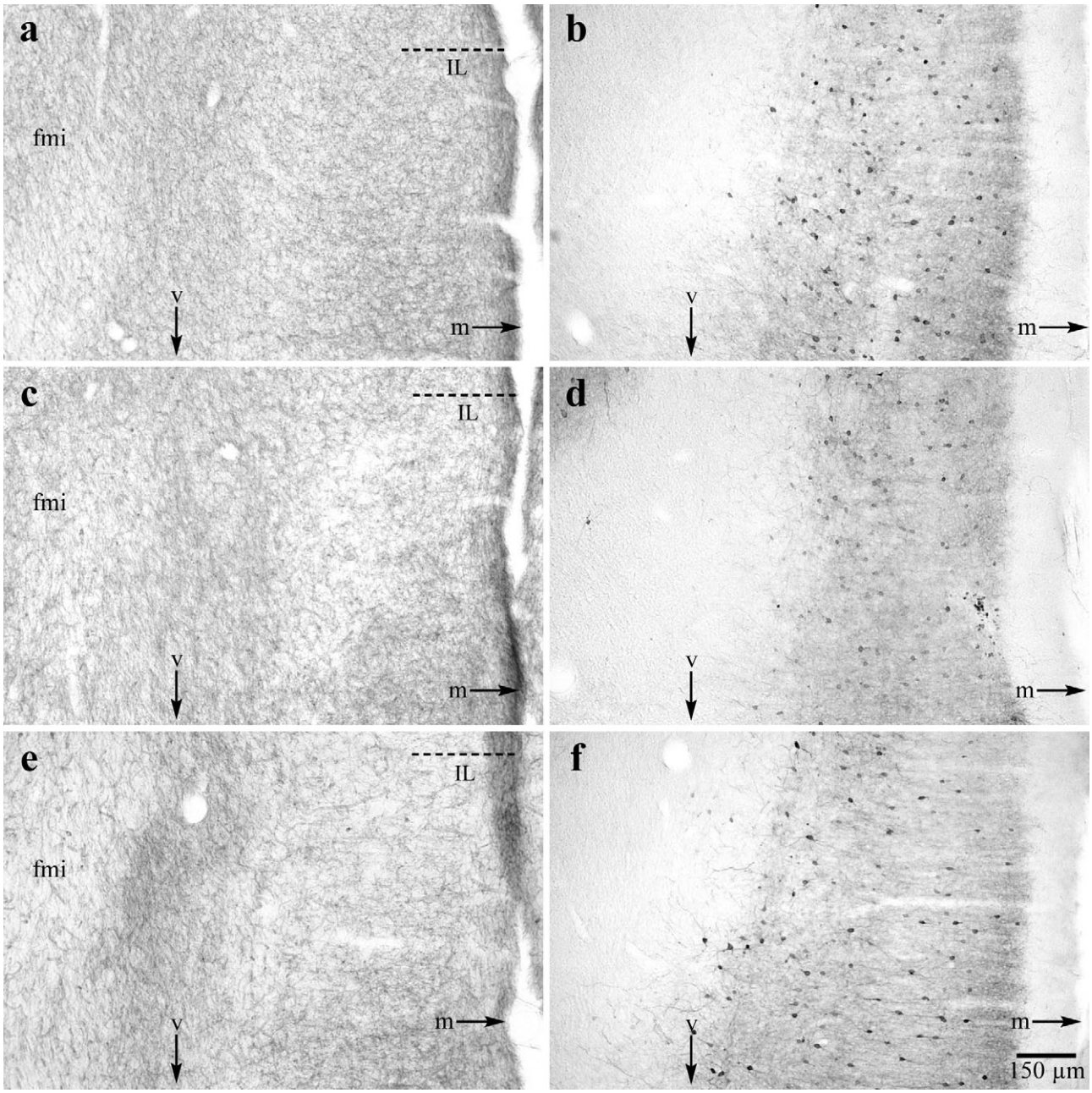

Figure 2. Representative photomicrographs showing AChE-positive fibers (*a*, *c*, *e*) and PARV-immunoreactive neurons (*b*, *d*, *f*) in the ventromedial PFC of sham (upper panels), 50 ng (middle panels) and 100 ng (lower panels) 192 IgG–saporin lesioned rats. It can be seen in 192 IgG–saporin-lesioned animals that the density of AChE-positive fibers is clearly decreased in the infralimbic cortex (IL) in both lesion groups compared to the sham group. By contrast, PARV-IR neurons are unaffected by immunotoxin infusions in this region. Abbreviations: fmi, forceps minor of the corpus callosum; m, medial; v, ventral; IL, infralimbic cortex.

showed no significant decline in performance under these conditions [time: $F(6,48) = 1.19$, $P = 0.33$] whereas the accuracy of lesioned subjects decreased significantly as a function of time on task [time: $F(6,96) = 2.70$; $P = 0.025$]. This vigilance decrement was apparent in both the 50 ng lesion group [lesion × time interaction: $F(6,96) = 2.29$, $P = 0.041$] and the 100 ng lesion group [lesion × time interaction: $F(6,96) = 2.34$, $P = 0.050$] relative to sham animals and was similar in magnitude for both lesion groups [lesion: $F(1,16) = 0.023$; $P = 0.88$)].

Reduced Stimulus Duration

The effect of stepwise reductions in the duration of the visual stimuli on accuracy is shown in Table 1. Decreasing the stimulus duration resulted in less accurate responding [stimulus duration: $F(2,48) = 79.46$, $P < 0.01$]; however, there was no significant effect of the lesion on this performance measure [lesion: $F(1,24) = 1.09$, $P = 0.35$; lesion × stimulus duration: $F(4,48) = 0.697$, $P = 0.60$].

Systemic Scopolamine Administration

The effects of systemic administration of the muscarinic receptor antagonist scopolamine on 5-CSRT task performance are shown in Figure 7. The accuracy of performance overall was diminished by scopolamine [dose: $F(4,96) = 10.30$, $P < 0.01$] with the greatest effects observed in rats with cholinergic lesions of the medial PFC [lesion: $F(2,24) = 0.304$,

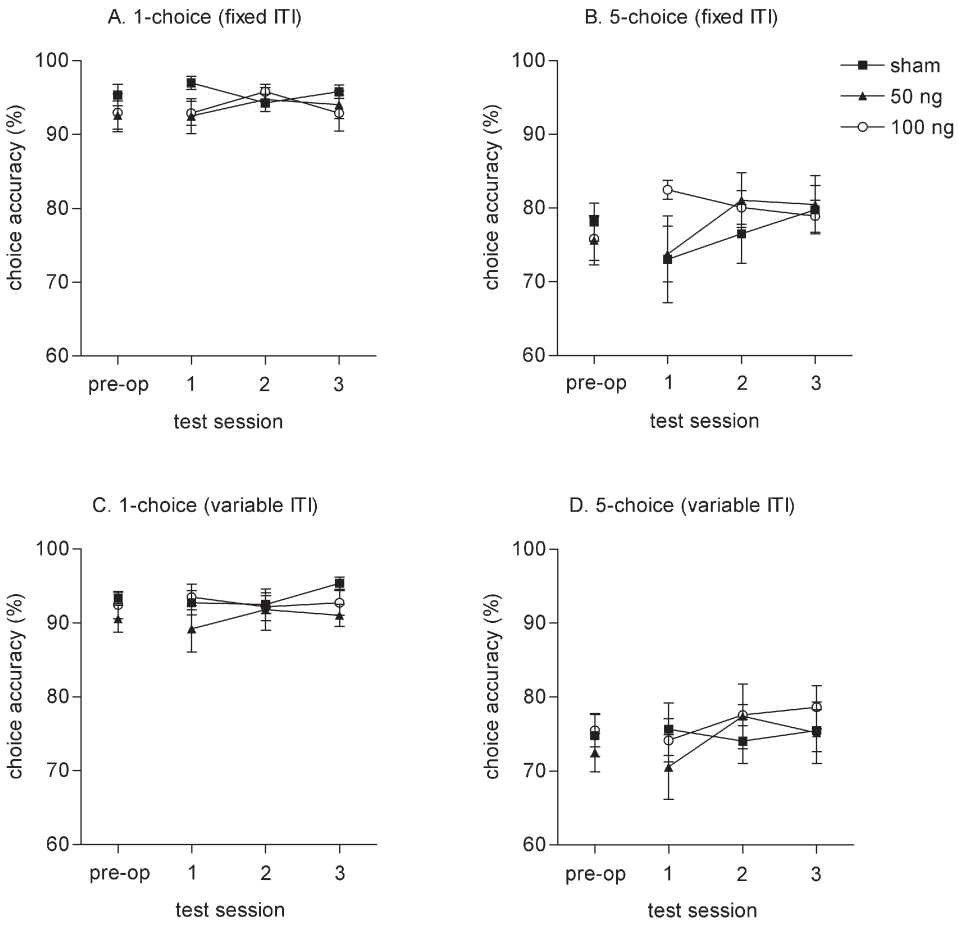

Figure 3. Effect of varying the timing and spatial location of the visual targets on the accuracy of performance of sham ($n = 9$) and 192 IgG–saporin lesioned rats (50 or 100 ng, each $n = 9$). Pre-operative data were obtained from the final session prior to surgery (mean ± SEM). Subsequent testing was conducted over three challenge sessions, each consisting of four behavioral challenges ('A' one-choice, fixed ITI; 'B' five-choice, fixed ITI; 'C' 1-choice, variable ITI; 'D' five-choice, variable ITI) separated by baseline test sessions.

$P = 0.741$; dose × lesion: $F(8,96) = 2.05$, $P = 0.049$]. Specifically, scopolamine reduced accuracy in the 100 ng group [dose: $F(4,32) = 19.01$, $P < 0.01$] with near significant deficits in the 50 ng group [dose: $F(4,32) = 2.63$, $P = 0.058$] but no effect in the sham group [dose: $F(4,32) = 1.2$, $P = 0.33$]. A *post hoc t*-test established that accuracy was significantly decreased in the 100 ng group relative to sham animals at the 0.1 mg/kg dose ($P < 0.01$). Scopolamine also increased omissions [dose: $F(4,96) = 23.61$, $P < 0.01$] to ~35% of trials after 0.1 mg/kg as well as premature [dose: $F(4,96) = 5.84$, $P < 0.01$] and perseverative [dose: $F(4,96) = 5.78$, $P < 0.01$] responses (data not shown). However, there were no significant effects of the lesion (or any interactions) on the number of omissions, premature or perseverative responses following the scopolamine challenge.

Discussion

The present study examined the effects of administering the cholinergic immunotoxin 192 IgG–saporin directly in the medial PFC on performance of rats on a visuo-spatial attentional task. The results reveal dissociable deficits of sham and 192 IgG–saporin-lesioned animals on the *accuracy* of attentional performance, particularly under conditions of increased attentional load imposed by the more frequent presentation of visual targets. Importantly, rats with cholinergic lesions of the medial PFC exhibited a significant vigilance decrement in terms of accuracy when performance was monitored over the course of a long session. Lesioned animals also exhibited differential impairments in accuracy following systemic administration of the muscarinic receptor antagonist scopolamine but not during sessions where the stimulus duration was progressively diminished. Additionally, rats with cholinergic lesions of the medial PFC showed deficits in inhibitory response control, including a transient increase in perseverative responding, specifically when the visual targets were temporally unpredictable, as well as an increase in anticipatory responses and a reinstatement of perseverative responding when the visual targets were presented either more slowly or more frequently. Notably, these effects were behaviorally specific with no significant disturbances in motivation (i.e. no change in omissions or latency to collect food), speed of responding or visual sensory function. Therefore, disruption of cholinergic transmission in the medial PFC results in a specific pattern of attentional and executive deficits on the 5-CSRT task that resemble some of the deficits produced on this task by more global disturbances of cortical cholinergic function (see Muir *et al.*, 1994; McGaughy *et al.*, 2002). Overall, these data are compatible with the hypothesis that cholinergic projections from the BF to the ventromedial sub-region of the PFC contribute to visual

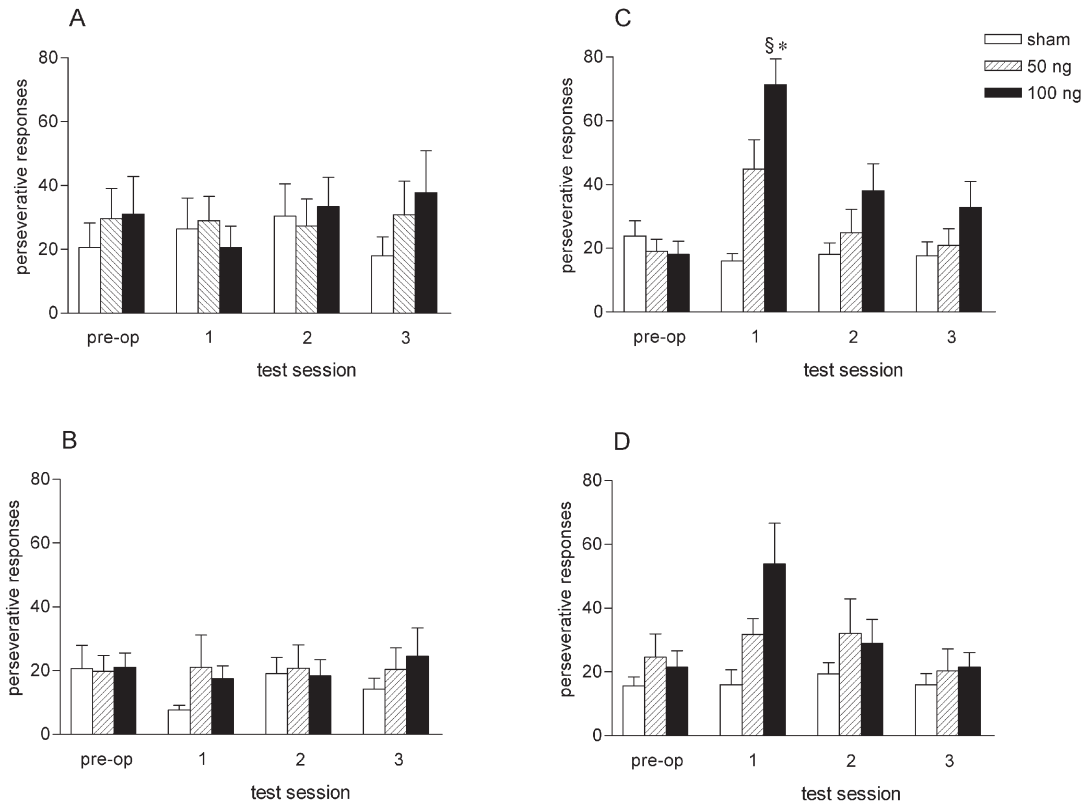

Figure 4. Perseverative responses of sham ($n = 9$) and 192 IgG–saporin lesioned rats (50 ng or 100 ng, each $n = 9$) during sessions consisting of either a fixed location and fixed ITI ('A'), five locations and a fixed ITI ('B'), a fixed location and variable ITI ('C') or five locations and a variable ITI ('D'). §$P < 0.05$ versus pre-operative lesion subjects (100 ng group). *$P < 0.05$ versus sham subjects.

attentional processes, especially under conditions of increased attentional load.

Cholinergic Specificity of the Lesion

The region of PFC most affected by the saporin infusions in the present study corresponds with areas 24a, 25 and 32 (i.e. ventral prelimbic and infralimbic cortices), as depicted by Lysakowski *et al.* (1989). Although studies have confirmed that AChE immunoreactivity parallels that of choline acetyltransferase (ChAT) in many cortical regions (Lysakowski *et al.*, 1989; Mesulam and Geula, 1992), differences have been reported in the laminar distribution of AChE and ChAT in areas 24a, 25 and 32 (Lysakowski *et al.*, 1989). In particular, ChAT staining in layers II–V is less marked than AChE staining in area 25 (equivalent to the infralimbic cortex), suggesting that AChE may be located in non-cholinergic neurons in this region, possibly in GABA-ergic cells (Hallanger *et al.*, 1986). However, our own analysis found no significant evidence for a reduction in the density of parvalbumin-stained neurons in this, or any other region of the medial PFC, indicating that GABA-ergic neurons were largely spared by the 192 IgG–saporin lesions. Nevertheless, it remains possible that other sub-populations of neurons in the medial PFC also bear the low affinity p75 NGF receptor, including intrinsic cholinergic neurons and other (non-parvalbumin) neurons, which would make them susceptible to the toxic effects of 192 IgG–saporin. Therefore, we cannot totally exclude the possibility that damage to such neurons contributed to the behavioral effects observed. It is also possible that the 192 IgG–saporin infusions affected other neurochemical projections to the medial PFC, for example, the ascending noradrenergic, dopaminergic and serotonergic systems from the midbrain. However, we consider this to be unlikely because NGF is specifically taken up and transported retrogradely to cholinergic neurons in the BF, and not the locus coeruleus, substantia nigra, raphé nuclei or thalamus, following direct administration in the frontal cortex (Seiler and Schwab, 1984). Moreover, additional evidence from a recent quantitative histochemical study lends strong support to the putative cholinergic specificity of 192 IgG–saporin when infused directly into the cerebral cortex (Holley *et al.*, 1994).

Further support for cholinergic mechanisms underlying the attentional impairments of 192 IgG–saporin-lesioned rats in the present study stems from the differential effects of the post-synaptic muscarinic receptor antagonist scopolamine in these subjects. This compound produced a number of performance deficits on the 5-CSRT task (see also Jakala *et al.*, 1992; Jones and Higgins, 1995; Mirza and Stolerman, 2000), but deleterious effects on response selection were only evident in the high-dose 192 IgG–saporin group. Clearly, the neural site mediating these effects cannot be inferred from these data but they are consistent with the effects of intra-prefrontal infusions of scopolamine on this task (Robbins *et al.*, 1998), and more general evidence that scopolamine also disrupts active forms of attentional control in humans (Dunne and Hartley, 1986). The lack of significant effects on accuracy in the low-dose 192 IgG–saporin group is possible further evidence that BF cortical cholinergic neurons have a high capacity for functional

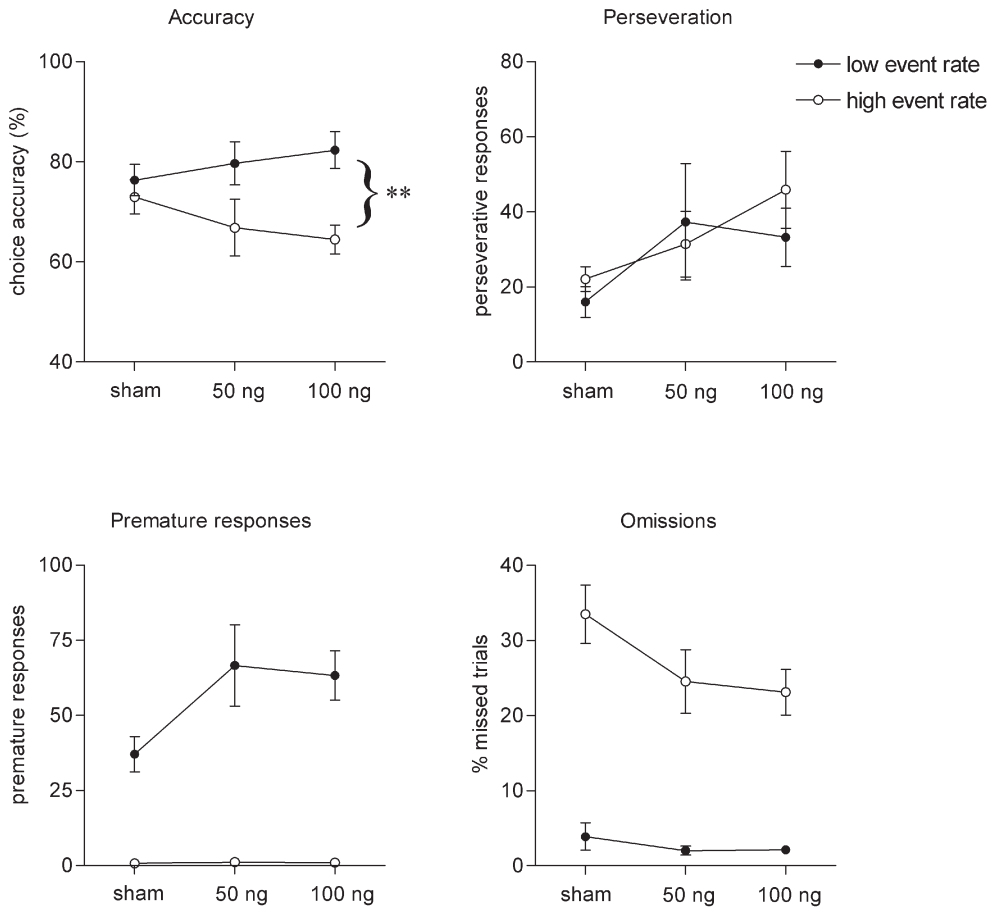

Figure 5. Performance of sham ($n = 9$) and 192 IgG–saporin-lesioned animals (50 ng or 100 ng, each $n = 9$) under conditions of high and low event rate. Stimuli were presented randomly across all five spatial locations. High and low event rate sessions consisted of 200 trials and inter-trial intervals of 2 and 7 s, respectively. $^{**}P < 0.05$ high versus low event rate (100 ng group).

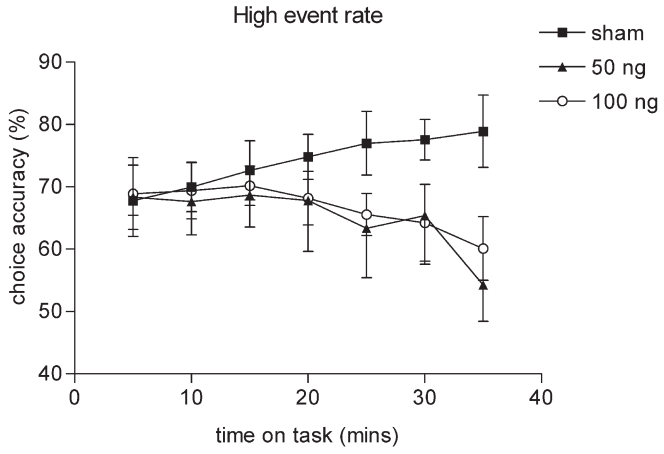

Figure 6. Vigilance decrement in rats with cholinergic lesions of the medial PFC as a function of time on task during the high event rate session. Accuracy of target detection was determined over 5 min blocks with stimuli presented randomly across all five spatial locations.

Table 1
Lack of effect of reducing the duration of the visual stimuli on choice accuracy of sham ($n = 9$) and 192 IgG–saporin PFC-lesioned animals (50 or 100 ng, each $n = 9$)

	Stimulus duration (s)		
	0.5	0.25	0.125
Sham	77.6 ± 3.2	62.8 ± 4.0	52.4 ± 3.5
50 ng	84.8 ± 4.4	69.2 ± 5.4	52.4 ± 3.8
100 ng	83.9 ± 2.5	72.6 ± 2.6	55.1 ± 4.5

The data are percentage correct responses ± SEM. Chance performance is 20%.

compensation and that this depends on task requirements. In other words scopolamine may well have disrupted attentional selectivity in the low 192 IgG-saporin group under more demanding conditions, for example under high event conditions or in the presence of an auditory distractor (Jones and Higgins, 1995).

Cortical Cholinergic Transmission and Visual Attention

Previous investigations in rats and monkeys have strongly implicated the BF cortical cholinergic system in the modulation of visual attentional performance (Olton *et al.*, 1988; Voytko *et al.*, 1994; Muir *et al.*, 1994; Robbins *et al.*, 1989). However, because of the absence of a suitably specific cholinergic neurotoxin, many early studies utilized excitotoxins such

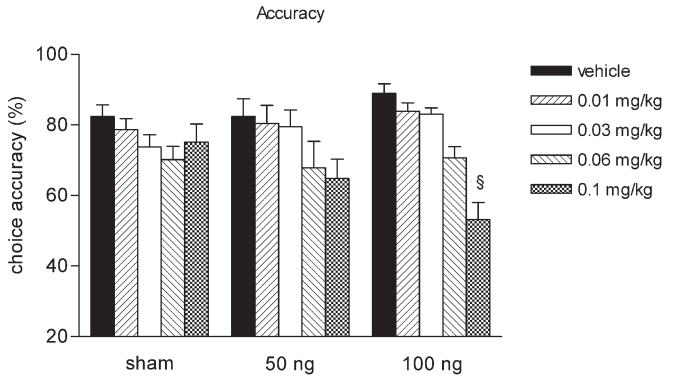

Figure 7. Accuracy of sham ($n = 9$) and 192 IgG–saporin lesioned animals (50 or 100 ng, each $n = 9$) on the five-choice task (stimulus duration 0.5 s) following systemic administration of vehicle (normal saline, 1 ml/kg s.c.) or scopolamine hydrochloride (0.01–0.1 mg/kg). ANOVA revealed a significant interaction between lesion and dose [$F_{(8,96)} = 2.05$; $P = 0.049$] and a significant main effect of dose for the 100 ng lesion group [$F_{(4,32)} = 19.01$; $P < 0.01$]. §$P < 0.05$ versus sham controls (0.1 mg/kg dose level).

as ibotenic acid, quisqualic acid and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) to lesion the principal cholinergic cell groups of the BF. Such lesions varied in their cholinergic specificity and the degree of collateral damage to neighboring brain regions such as the dorsal and ventral pallidum. More recently, the specific cholinergic immunotoxin 192 IgG–saporin has been introduced and its effects evaluated in various cognitive and behavioral settings. The results indicate that several cognitive and mnemonic functions in rodents, including spatial learning, are either insensitive to, or only modestly affected by, the substantial and selective loss of BF cholinergic neurons (Torres *et al.*, 1994; Baxter *et al.*, 1995; Galani *et al.*, 2002). By contrast, paradigms that place explicit demands on attentional processing, rather than learning *per se*, appear particularly sensitive to 192 IgG–saporin-induced lesions of the cortically projecting cholinergic neurons of the BF (Chiba *et al.*, 1995; McGaughy *et al.*, 1996, 2002; Risbrough *et al.*, 2002; Lehmann *et al.*, 2003). For example, in rats, 192 IgG–saporin lesions of the BF disrupt performance on sustained and divided attentional tasks (McGaughy *et al.*, 1996; Turchi and Sarter, 1997) and impair incremental attentional processing of discrete conditioned stimuli (Chiba *et al.*, 1995). In addition, it has recently been demonstrated that infusions of 192 IgG–saporin directly into the BF produce dose-dependent effects on attentional accuracy on the 5-CSRT task that correlate significantly with cholinergic cell loss in the BF and with reductions in ACh release in the medial PFC (McGaughy *et al.*, 2002). Indeed, there is now substantial evidence from other *in vivo* microdialysis studies that ACh release in the frontal cortex increases during established performance on sustained attentional tasks (Himmelheber *et al.*, 2000; Passetti *et al.*, 2000; Dalley *et al.*, 2001). Although increases in cortical ACh release are also observed on tasks that place less explicit demands on sustained attention, it is notable that performance on such tasks is not disrupted by selective cholinergic lesions of the BF (Himmelheber *et al.*, 2001). Therefore, the BF cortical cholinergic system is apparently required to support instrumental processes associated with explicit attentional demands.

An important aim of the present investigation was to examine whether the apparent attentional impairments produced by 192 IgG–saporin-induced cholinergic lesions of the BF could be mimicked by more restricted cholinergic lesions of the medial PFC. The results indicate that although rats with cholinergic lesions of the medial PFC were generally unimpaired on the 5-CSRT task when the visual targets were temporally, as well as spatially unpredictable, they were impaired by further increases in attentional load, specifically with high target frequency sustained over a large number of trials (i.e. a high event rate). This manipulation is known to place greater demands on attentional resources because it requires them to be maintained on a continuous basis (Parasuraman and Giambra, 1991). Lesioned rats completed as many trials as their sham counterparts and, if anything, made *fewer* omissions during the high event rate manipulation. However, they exhibited impaired response selection (significantly reduced accuracy). They also made more perseverative responses and, under the low event rate condition, were more likely to respond prematurely in anticipation of the target stimulus. These deficits suggest additional impairments in the inhibitory control of performance, normally observed with damage to more ventral regions of the rat medial PFC (Passetti *et al.*, 2002). The lesioned animals showed no deficits in attentional accuracy when stimuli were made temporally, as well as spatially, unpredictable, a schedule that prevents animals from relying on automatic processing to guide responding to the visual discriminanda (Koelega, 1989; Robbins, 2002). Therefore, cholinergic deafferentation of the medial PFC results in a rather specific set of attentional impairments that are separate from any disturbance in response speed, visual sensory function or motivation. Operational measures of motivation such as magazine latencies and omissions were generally unaffected by the 192 IgG–saporin lesions and there were no differential impairments in attentional accuracy when the duration of the stimuli was reduced.

This study is the first to demonstrate that selective cholinergic lesions of the medial PFC produce impairments in visual attentional function that resemble those produced by more global reductions in cortical cholinergic function. Rats infused intraventricularly with 192 IgG–saporin show impaired accuracy on an equivalent reaction time task, especially under conditions of high event rate or when stimuli are made temporally unpredictable (Waite *et al.*, 1999). Although interpreting the findings of this study is constrained somewhat by associated collateral damage to Purkinje cells in the cerebellum it is noteworthy that attentional deficits are also reported following selective cholinergic lesions of the BF, not only on this task (McGaughy *et al.*, 2002; Risbrough *et al.*, 2002; Lehmann *et al.*, 2003), but also on other sustained and divided attentional paradigms (McGaughy *et al.*, 1996; Turchi and Sarter, 1997). However, in contrast to previous studies where 192 IgG–saporin was administered directly into the BF (McGaughy *et al.*, 2002; Lehmann *et al.*, 2003; but see Risbrough *et al.*, 2002), rats with cholinergic lesions of the medial PFC showed no impairment in *baseline* attentional accuracy. There are several reasons for this apparent discrepancy, including differences in training and testing procedures, but the most obvious relates to the removal of cholinergic projections to widespread cortical regions, including posterior parietal cortex and other brain areas implicated in attentional processes, following selective BF lesions. In addition, the deleterious effects of BF infu-

sions of 192 IgG–saporin on 5-CSRT task performance are evidently dose-related (McGaughy *et al.*, 2002), implying that higher doses of 192 IgG–saporin in the medial PFC may have resulted in more severe impairments in attentional accuracy.

These findings reveal a remarkable degree of overlap in the pattern of behavioral deficits on a five-choice visual attentional task following selective cholinergic lesions of the BF and ventromedial PFC induced by the cholinergic immunotoxin 192 IgG–saporin. Such lesions affect attentional selectivity, especially during increased attentional load, in addition to impairing inhibitory response control. Cholinergic projections from the BF to the ventromedial PFC therefore support a continuum of cognitive functions that are sensitive to task demands and the extent of cholinergic dysfunction within different sectors of the medial PFC.

Notes

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