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Visuospatial attentional functioning in mice: interactions between cholinergic manipulations and genotype

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Abstract

Attentional functioning in mice was assessed in an analogue of the five-choice serial reaction time task in which the requirement was to detect brief visual stimuli presented across five spatial locations. Two hybrid strains of mice were assessed; F1 C57Bl/6xDBA/2 and C57Bl/6x129sv. Both strains acquired the task to high levels of performance with, in particular, no problems due to premature responding. At performance, systematic manipulation of the task parameters indicated a pattern of effects consistent with the task, taxing aspects of visuospatial attention. There were some differential effects of task manipulations at baseline across strain. However, the pattern of effects suggested these were likely to be the result of effects on factors other than attentional functioning *per se*, such as behavioural reactivity and inhibition. There was evidence in both strains of specific, centrally mediated effects of scopolamine on attentional functioning, with the C57Bl/6xDBA/2 hybrid showing greater sensitivity to the drug manipulation. Specific effects on discriminative accuracy were observed at doses of 0.02 and 0.2 mg/kg scopolamine. At the 2 mg/kg dose, large reductions in accuracy were associated with large effects on other measures, including omissions and response latencies, suggestive of nonspecific effects on task performance. These data indicate, for the first time, the utility of operant methods in assessing visuospatial attentional functioning in mice. They confirm the importance of cholinergic mechanisms in attentional processes across species, and suggest interactions between cholinergic mechanisms and genotype in the expression of attentional phenotypes.

Introduction

Work in humans using neuropsychological and brain imaging techniques has suggested the existence of psychologically and neurally dissociable visuospatial attentional systems, serving to integrate what have been operationally defined as 'selective attention', 'vigilance' and 'divided attention' functions (reviewed by Posner & Dehaene, 1994). These studies have highlighted the role of frontal regions of brain in the ability to detect and discriminate visual stimuli accurately (Posner & Petersen, 1990; Jackson *et al*., 1994).

Work in animals has also indicated the importance of the frontal regions of the brain in processes underlying visuospatial attentional functioning, and with the advantages stemming from the experimental approach it has provided finely resolved information about key circuitry at the neural and neurochemical levels (e.g. Muir *et al*., 1992a, 1994, 1996a; McGaughy *et al*., 1994; Robbins *et al*., 1998). A significant proportion of the animal work has been conducted using rats and an analogue of the five-choice serial reaction time task (Leonard, 1959; Wilkinson, 1959) which, in requiring a discriminative response subsequent to the detection of brief visual stimuli presented across five spatial locations, has been suggested to represent a valid behavioural index of, in particular, aspects of sustained and divided visuospatial attentional functioning (Muir, 1996b; Robbins, 1998; but see also, Bushnell, 1998).

The work on rats using the five-choice task and other attentional paradigms such as the cross-modal task (Sarter & McGaughy, 1998) has revealed an important role for central cholinergic mechanisms in the processes underlying discriminative accuracy performance (Muir *et al*., 1992b, 1993, 1995; McGaughy *et al*., 1994; Jones & Higgins, 1995; Everitt & Robbins, 1997; Robbins *et al*., 1998) to the extent that the principles of function resulting from the animal work now usefully inform the human literature. Thus, a prominent role for cholinergic mechanisms has been established in humans across both normal functioning and pathological conditions where attentional functioning goes awry, such as Alzheimer's disease (Sahakian *et al*., 1993; Robbins *et al*., 1997).

The question of the extent to which attentional phenotypes are subject to genetic influences has been relatively unexplored. In humans, there are some examples of major genetic contributions to attentional deficits associated with pathological conditions, for example, the subtle 'quantitative' gene effects contributing to the constellation of symptoms present in attention deficit/hyperactivity disorder, where amongst several monoaminergic substrates, dopamine receptors of the D4 subtype appear to be of particular importance in terms of susceptibility (Smalley *et al*., 1998). However, the genetic contribution to the normal variation in attentional phenotypes within the human population is unknown. Similarly, little or no systematic work has been done in animal models.

In the present work, we made a comparison of attentional functioning in two strains of mice using an analogue of the five-choice task. The mice were kept under identical conditions and were of known genetic constitution, in that F1 crosses resulting from breeding C57Bl/6 mice with either the 129sv or DBA/2 strain of mice were used. These are inbred strains of mice for which there exists comparative databases across a range of phenotypes, including behavioural functions (Crawley *et al*., 1997). Hence, we anticipated it would be possible to go some way towards mapping observed

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behavioural phenotype(s) to particular genotypes. The hybrids also represent commonly used 'genetic backgrounds' against which targeted manipulations of the genome are made. In view of previous findings in other species, the work focused on possible interactions between genotype, attentional functioning and manipulations of central cholinergic mechanisms. The studies, which required the development of a novel five-choice task that could be used in mice, confirmed the importance of cholinergic mechanisms in attentional processes across species, and they also have important implications in suggesting the existence of genetic influences on cholinergic mechanisms underlying attentional phenotypes.

Material and methods

Subjects

Two strains of mice were used; male F1, C57Bl/6xDBA/2 ($n = 7$) and C57Bl/6x129sv ($n = 6$) generated at the Babraham Institute (Babraham, Cambridge, UK). The mice were 8 weeks old (≈ 30 g) at the outset of the experiment, and were housed in groups of three or four under temperature-controlled conditions and a 12 h light : 12 h dark cycle (lights on at 07.30 h). Standard laboratory chow was available *ad libitum*, but for most of the experiment water was restricted to 2-h access per day. This regime maintained the subjects at $\approx 90\%$ of free-feeding body weight. All procedures were conducted in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986.

Behavioural apparatus

Testing was carried out in 'nine-hole' operant chambers based on a design developed for use with rats (Carli *et al*., 1983) and modified for use with mice in collaboration with CeNeS Ltd (Cambridge, UK). The chambers were made of aluminium plate, with a clear perspex roof. The rear wall was curved and had nine 1-cm holes located at the base. For the five-choice task configuration, holes 2, 4, 6 and 8 (counted from the left) were blocked by metal caps (see Fig. 1A). The mice responded to visual stimuli, recessed into the holes, with a nose-poke, which was detected by an infrared beam crossing the entrance vertically. In the lower half of the near wall was the food-delivery panel, a 2-cm opening with a clear perspex door hinged at the top; the opening of the panel was detected by a microswitch. Food was delivered via a tube into a well set into the floor of the panel. A peristaltic pump connected to the tube delivered liquid reinforcement down to 5-μL volumes. Set into the roof was a loudspeaker and the house light. The whole chamber was housed in a sound-attenuating outer box fitted with a fan, which provided a constant low background noise. Mounted in the roof of the sound-attenuating boxes were infrared cameras (Watac WM6, Tracksys Ltd, Nottingham, UK). Illumination of the operant chambers, when dark, was provided by four roof-mounted infrared LEDS. The control of stimuli and recording of responses were managed by an Acorn Archimedes computer (CeNeS) running custom-written BASIC programmes with additional interfacing by ARACHNID (CeNeS).

Behavioural procedures

Habituation to reinforcer

Prior to any experimental work, subjects were handled for 2 weeks and their body weight monitored. After this time, subjects were put on a 22-h water deprivation schedule (2 h access in early evening) for a further 8 days until body weight had stabilized. Then the animals were habituated to the liquid reinforcer used in the operant procedures (10% solution of condensed milk, Nestle Ltd, UK). Habituation took place outside the operant chambers, over 5 days. In this way, preferences could emerge as neophobic reactivities to the novel foodstuff habituated. The subjects were given 10-min sessions with an excess of either water or 10% condensed milk presented in two small bowls the location of which (left or right) was moved from session to session. On the first day, subjects only had the option of water to drink, but on subsequent sessions they were presented with a choice between water and the 10% condensed milk solution. This design allowed both habituation to the reinforcer and any pre-existing strain differences in preference for the reinforcer to be determined prior to commencing training in the operant chambers.

Training to baseline performance

Initial shaping was carried out over five sessions. The first three sessions involved general habituation and learning that food was available in the magazine. On the final 2 days of shaping, the subject was required to make a panel push in order to initiate food delivery. On day 6, animals were moved on to the five-choice task, whereby they had to make a nose poke in the hole in which a stimuli light had been presented in order to receive a 50-μL liquid reward. The basic task sequence is shown in Fig. 1A. Briefly, a trial was initiated by a panel press at the food magazine which started a 5-s intertrial interval (ITI). At the end of the ITI, a light stimulus was presented, pseudo-randomly, at one of the five possible locations in the hole array at the rear of the chamber. A correct nose poke in the hole in which the stimulus occurred led to a 5-s delivery of reward. Incorrect responses or failure to respond (omissions) in the 5-s limited hold period (timed from the offset of the stimulus) resulted in a time-out period signified by the illumination of the house light for 5 s. Likewise, a premature nose poke, made during the ITI, also resulted in a time-out. At the beginning of training, the stimulus duration was set to 32 s; the ITI, limited hold period and time-out were all set to 5 s. A session was set to finish should the subject complete 80 trials or the run time exceed 40 min. When a subject produced three consecutive sessions of performance at criteria levels (> 50 trials, $> 80\%$ accuracy and $< 20\%$ omissions) the stimulus duration was reduced in the following pattern: 32, 16, 8, 4, 2, 1.8, 1.6 s, 1.4, 1.2, 1.0 and 0.8 s (baseline). Throughout training, video camera footage was scrutinized to ensure the subjects adopted appropriate scanning strategies in response to the increasing attentional demands occasioned by the progressively reducing stimulus durations.

Manipulations of basic task parameters

Once stable baseline performance at 0.8 s stimulus duration was achieved, a series of manipulations of the basic task were made which were designed to increase attentional load (manipulations 1–4) or alter motivational aspects of task performance (manipulation 5). Each manipulation session was presented after three consecutive days of baseline performance. The order of manipulations was as follows. (i) Altering the duration of the ITI, which increases attentional load by disrupting the temporal predictability of the stimulus onset: short ITI – 2, 3, 4 or 5 s; long ITI – 5, 6, 7 or 8 s. (ii) Reducing the stimulus duration – 0.8, 0.6, 0.4 or 0.2 s. (iii) Reducing the stimulus brightness (52, 30, 21 and 12% of full). (iv) Imposition of a 100-dB, 0.5-s burst of distracting white noise at 0, 2.5 or 5 s after the beginning of the ITI (hence, the 5-s presentation was concurrent with the onset of the stimulus). (v) Free-water – 24 h *ad-libitum* access to water prior to testing.

Pharmacological challenges

The sensitivity of baseline responding to cholinergic manipulations was assessed using the muscarinic antagonist scopolamine. Two

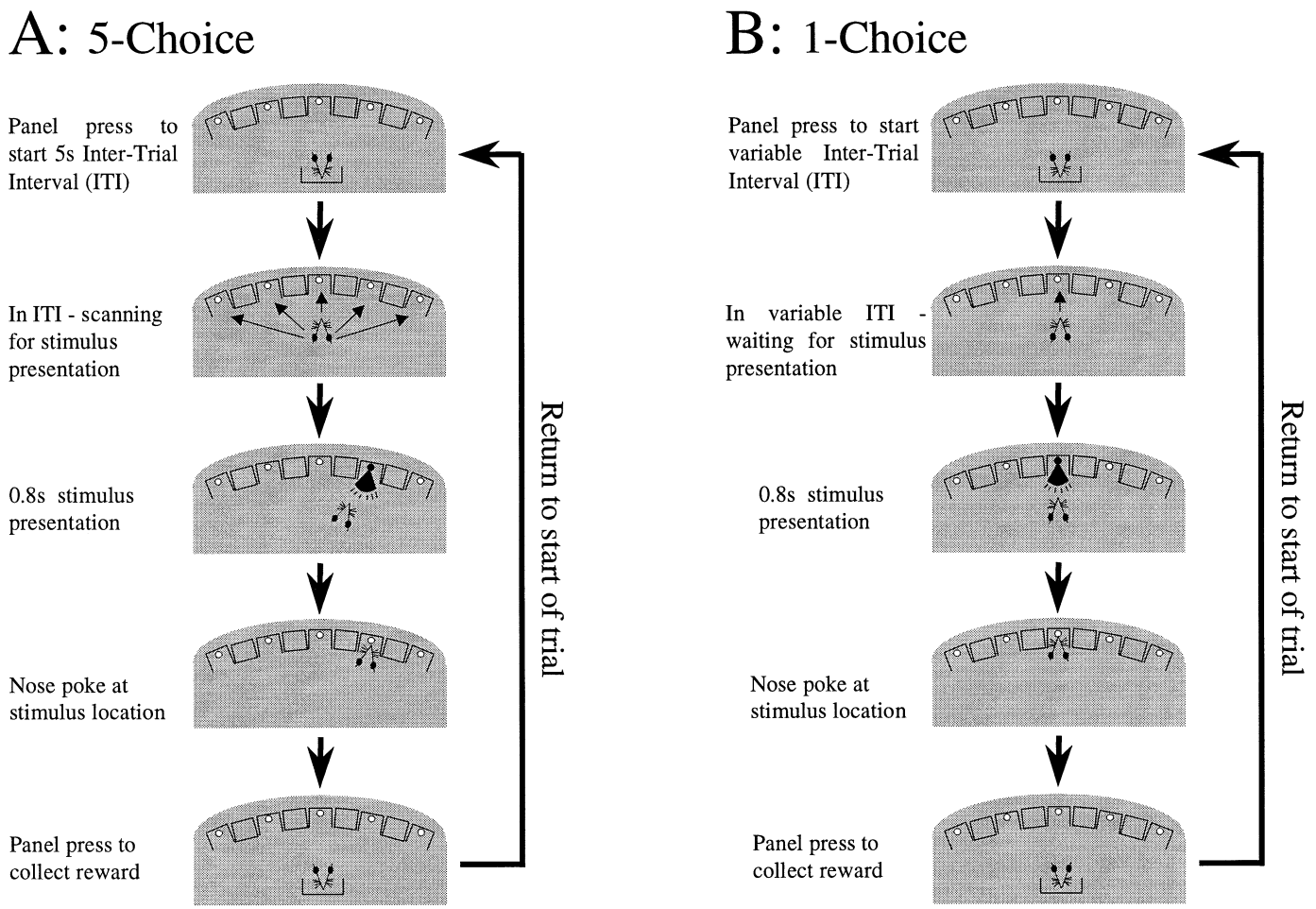

FIG. 1. Schematic diagram showing the response-hole configuration and basic sequence of actions for the mouse five-choice (A) and one-choice (B) tasks at baseline performance. In both tasks, five holes were available for responses, configured from the left of the nine holes available, as follows: 1, 3, 5, 7 and 9. The remaining four holes were blocked by metal caps. The tasks differed in that, whilst the stimulus appeared pseudo-randomly across the five-hole array in the five-choice task, in the one-choice task the stimulus always appeared in the centre hole. Also, a variable ITI (4–5.5 s.) was used in the one-choice in order to discourage anticipatory responding.

forms of the drug were used: scopolamine and its quaternary analogue, methylscopolamine (both from Sigma, UK). The latter cannot cross the blood–brain barrier and was used to dissociate central from peripherally mediated effects of scopolamine. Both forms of scopolamine were administered i.p., 20 min before testing. The drugs were given at three doses (0.02, 0.2 and 2 mg/kg). Each dose of drug was compared with saline vehicle utilizing a Latin-square design that allowed at least 3 days washout between treatments. Scopolamine is associated with a range of effects in rodents, especially motor disturbances (Mathur *et al*., 1997). Hence, in order to distinguish specific drug effects on attentional processes from those due to general disruption of the task, the effects of scopolamine were also assessed in a 'one-choice' task which was identical in terms of basic task requirements to the five-choice task, but differed in having a lower attentional load (see Fig. 1B). A direct assessment of the effects of scopolamine (again given i.p. 20 min before testing) on locomotor activity was also carried out using a battery of locomotor activity cages fitted with infra-red beams.

Data analysis and statistics

Preference for the condensed milk reinforcer on day 5 (fourth choice day) was measured as the volume of milk consumed relative to water. Discriminative accuracy in the five- and one-choice tasks was measured as the percentage of correct nose-poke responses (correct/total responses). Errors of omission were defined as number of omissions/total trials. Three latency measures were taken: correct-response latencies (time elapsing between stimulus onset and correct response); incorrect-response latencies (time elapsing between stimulus onset and incorrect response) and 'magazine latency' (time elapsing between correct response and the mouse collecting the food as registered by a panel push). Additional measures included: premature responses (responses within the ITI); perseverative responses (additional responses following a correct response) and the number of nose pokes and panel pushes per trial.

All data were analysed using the SAS statistical package (SAS Institute Inc., USA). The condensed milk preference data were subject to ANOVA with one between-group factor (Strain: C57Bl/6xDBA/2 or C57Bl/6x129sv) and one within-group factor (Solution: 10% condensed milk or water). The five- and one-choice task data were subjected to mixed design ANOVA's with between-group factor Strain and within-group factors Training Level (criteria performance at progressively reducing stimulus durations: 32, 16, 8, 4, 2, 1.8, 1.6, 1.4, 1.2, 1.0 and 0.8 s), Stimulus Position (position of light stimulus in hole: far left, near left, middle, near right and far right), ITI [intertrial interval: 2, 3, 4, 5 (baseline), 6, 7 and 8 s], Duration [stimulus duration: 0.8 (baseline), 0.6, 0.4 and 0.2 s], Brightness [intensity of

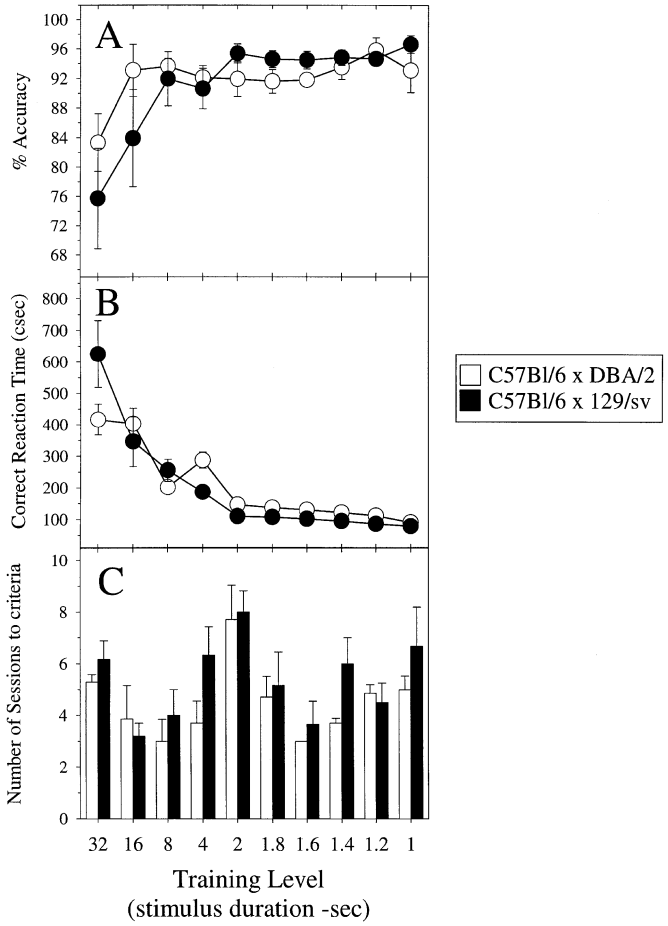

FIG. 2. The acquisition of the five-choice task by the C57Bl/6xDBA/2 and C57Bl/6x129sv hybrids as indexed by measures of discriminative accuracy (A), correct-reaction latencies (B) and the number of sessions required to reach performance criteria (C) at each of the training levels defined by the progressively reducing stimulus durations. The criteria for progressing to the next training level were: > 50 trials, > 80% accuracy and < 20% omissions. The values for discriminative accuracy and correct-reaction latency are the means ± SEM of the data taken from the last three sessions at any given training level.

visual stimulus: full (baseline), 52, 30, 21 and 12%], Noise Onset [onset of white noise relative to initiation of ITI: 0 (concurrent), 5 and 2.5 s], Water (prerestricted, 24 h *ad lib*. and postrestricted) and Dose (0.9% saline, 0.02, 0.2 and 2 mg/kg i.p. scopolamine or methylscopolamine dissolved in 0.9% saline). Locomotor activity data, as indexed by beam breaks, were analysed by ANOVA with factors Strain and Dose. Where the ANOVA revealed significant interactions the data were further analysed with *post hoc* tests.

Results

Habituation/preference for condensed milk reinforcer

The pattern of drinking of water and 10% condensed milk on day 5 (fourth choice day) indicated a large preference (≈ 90%) for condensed milk in both strains (C57Bl/6xDBA/2, 2.1 ± 0.3 mL condensed milk and 0.33 ± 0.2 mL water; C57Bl/6x129sv, 2.2 ± 0.2 mL condensed milk and 0.2 ± 0.09 mL water, significant main effect of Solution, $F_{1,25} = 88.95$, $P < 0.0001$). There were no effects of strain on this preference, as confirmed by the lack of any significant main effects or interactions involving factor Strain. Hence, prior to training, there were no pre-existing strain differences in terms of basic fluid consumption nor in the reactivity to the condensed milk reinforcer used to motivate performance in the operant tasks.

Acquisition of the five-choice task

Both strains progressed through the acquisition phase of the five-choice task, exhibiting a high degree of stimulus control in relation to the increasing attentional demands of the task. Thus, as illustrated in Fig. 2A, the mice maintained high levels of discriminative accuracy as the stimulus durations were reduced, and exhibited concomitant reductions in correct-reaction latencies down to a value of 0.8 s (Fig. 2B). The 0.8 s was chosen as the baseline condition, as the average minimum reaction time for both strains of mice was ≈ 0.7 s.

The lack of any main effects involving Strain and of interactions in the ANOVA with factors Strain and Training Level confirmed the absence of strain effects in the accuracy and correct-response latency data. However, consistent with the general tight relationship between stimulus duration and reaction time, there was a significant main effect of Training Level in the correct-reaction latency data ($F_{10,142} = 36$, $P < 0.0001$). There were also no strain differences in the overall rate at which the task was acquired to baseline (Fig. 2C). Hence, analysis of the number of sessions required to reach criteria at each of the training stages (defined by the progressively reducing stimulus durations) revealed no main effects or interactions involving the factor Strain.

The high degree of stimulus control indicated that, at baseline performance, the mice were adopting appropriate scanning strategies (i.e. scanning from a relatively fixed central point from the stimulus array) to maintain discriminative accuracy, a surmise which was confirmed by the direct video assessments of behaviour. The efficacy of the scanning strategy is shown by the sustained high levels of discriminative accuracy across the five stimulus positions (Fig. 3A) which, as confirmed by the lack of any significant interactions in the ANOVA with factors Strain and Stimulus Position, was common to both strains. There were also no between-strain differences in the correct-response latencies across stimulus location (Fig. 3B). However, consistent with the central scanning position adopted by both hybrids, there was a general increase in the correct-response latencies with increasing laterality of response location (main effect of Stimulus Position, $F_{4,64} = 3.6$, $P < 0.01$).

There was a similar lack of between-strain differences in those aspects of the task relating to response control and motivation. Hence, as detailed in Table 1, at baseline there were no differences in the premature responding exhibited by the mice, the perseverative responses, the percentage of omitted responses, the number of panel pushes per trial or the latency to collect the reward following a correct response. Taken together, these data indicate that, in terms of discriminative accuracy and concomitant response control and motivational aspects of behaviour, both strains of mice acquired the task to equivalent baseline levels of performance in essentially the same way.

Manipulations of task parameters

Variable ITI

Increasing the ITI had no systematic effects on discriminative accuracy in either hybrid (Fig. 4A), but this manipulation increased errors of omission (Fig. 4B) in both strains (main effect of ITI, $F_{3,51} = 3$, $P < 0.05$). Lengthening the ITI did, however, result in differential effects on correct-reaction latencies (Fig. 4C) in that the C57Bl/6xDBA/2 mice showed a slowing of reactions with increasing

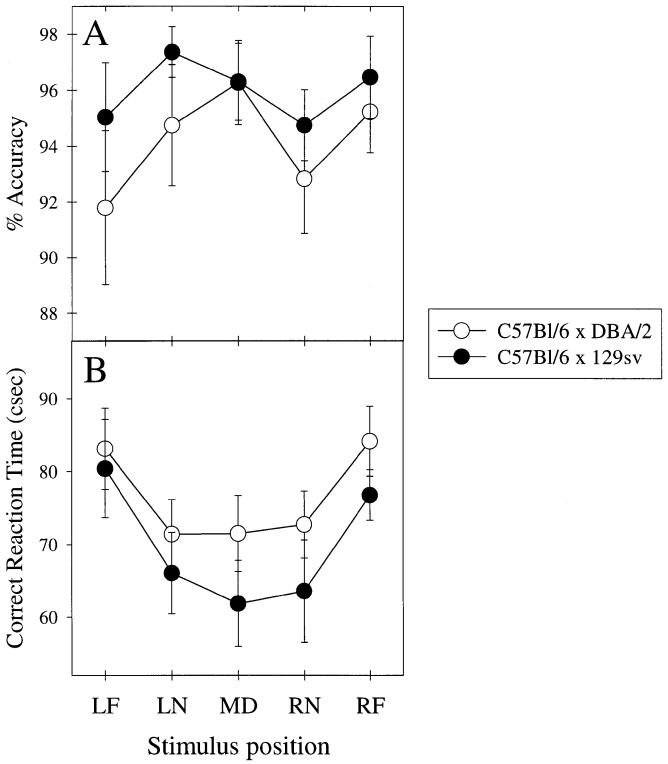

FIG. 3. The pattern of responding in the C57Bl/6xDBA/2 and C57Bl/6x129sv hybrids at baseline performance across the five stimulus positions in terms of discriminative accuracy (A) and correct-reaction latencies (B). The abbreviations define (as seen facing the stimulus array): LF, far left; LN, near left; MD, middle; RN, near right; RF, far right. Data are the mean ± SEM.

ITI (main effect of Strain, $F_{1,51} = 11.8$, $P < 0.002$) that was absent in the C57Bl/6x129sv mice. There were, in particular, no effects of lengthening the ITI on premature or perseverative responses (data not shown), these measures remaining at the low levels exhibited by both strains at the baseline ITI of 5 s. Reducing the ITI had no systematic effects on accuracy but did tend to increase errors of omission. There were no significant effects of shortening the ITI on correct-reaction latencies and premature or perseverative responding (data not shown).

Variable stimulus duration

As indicated in Fig. 5A, reducing the stimulus duration from the baseline value of 0.8 s. systematically reduced discriminative accuracy in both hybrids (main effect of Duration, $F_{3,51} = 5.26$, $P < 0.004$). In the case of the C57Bl/6xDBA/2 hybrid, these reductions in accuracy occurred in the absence of effects on omissions or correct-reaction latencies (Fig. 5B and C) except at the shortest stimulus duration of 0.2 s, where there was an abrupt increase in omitted responses. The C57Bl/6x129sv mice showed this abrupt increase at the 0.4 s. stimulus duration, resulting overall in independent main effects of Duration ($F_{3,51} = 10.39$, $P < 0.001$) and Strain ($F_{1,51} = 7.85$, $P < 0.01$). There were indications of selective effects of reducing stimulus duration on correct-response latencies, whereby the C57Bl/6xDBA/2 mice showed an increase in latency at the 0.2 s duration not paralleled by the C57Bl/6x129sv mice. However, the interaction between Duration and Strain in the correct-reaction latency data failed to reach significance. There were no effects of reducing the stimulus duration on the number of trials completed, premature or perseverative responding, the number of panel pushes made per trial or the latency to collect the reward following a correct response (though there was a nonsignificant trend common to both hybrids for this last measure to decrease with decreasing stimulus duration, data not shown). Hence, the reductions in discriminative accuracy occasioned by the manipulation did not appear to be associated with any general breakdown in basic task performance.

TABLE 1. The five-choice task. Comparison of the C57Bl/6xDBA/2 and C57Bl/6x129sv hybrids in baseline performance measures relating to response control and motivation

	C57Bl/6xDBA/2 Strain	C57Bl/6x129sv Strain
Premature responses (*n*)	0.7 ± 0.2	1.8 ± 0.8
Perseverative responses (*n*)	2.7 ± 0.4	1.9 ± 0.5
Omissions (%)	14.5 ± 1.3	12.3 ± 3.8
Panel-pushes per trial (*n*)	3.1 ± 0.1	3.4 ± 0.6
Latency to collect reward (s)	1.4 ± 0.9	1.3 ± 1.2

Data are the mean ± SEM taken from three consecutive sessions at baseline performance. See text for details of baseline conditions.

Variable stimulus brightness

Dimming the stimulus lights had differential effects on discriminative accuracy (Fig. 6A) with small effects on the C57Bl/6xDBA/2 mice but much larger effects on the C57Bl/6x129sv mice (significant main effect of Strain, $F_{1,64} = 8.7$, $P < 0.005$). The C57Bl/6x129sv mice also made more omissions with decreasing stimulus brightness (Fig. 6B, main effects of Strain, $F_{1,64} = 9.6$, $P < 0.005$, and of Brightness, $F_{3,64} = 4.8$, $P < 0.005$). The differential effects of dimming the stimuli on accuracy and omissions were accompanied by consistent effects on correct-reaction latencies (Fig. 6C), which increased in both hybrids with increasing stimulus dimness (main effect of Brightness, $F_{3,64} = 12$, $P < 0.001$). Thus, it would seem that the C57Bl/6x DBA/2 mice were able to maintain accuracy under these conditions by increasing their reaction times, whereas, despite a similar adaptation in reaction time, the C57Bl/6x 129sv mice did not. These complex effects of stimulus dimness, which were not associated with changes in premature or perseverative responding, nor in any other general aspect of task performance (data not shown), may indicate additional, strain-dependent effects on functions other than attentional processes, e.g. simple visual acuity and/or behavioural reactivity/inhibition.

White noise distractor

The effects of imposing a short burst of distracting white noise at varying times within the 5-s intertrial interval prior to the stimulus onset are shown in Fig. 7. Discriminative accuracy (Fig. 7A) was relatively unaffected by this manipulation. There were, however, large strain differences in errors of omission and correct-reaction latencies (Fig. 7B and C). As confirmed by the significant main effect of Strain ($F_{1,51} = 12.88$, $P < 0.001$), in general the C57Bl/6x129sv mice made more omissions under these conditions, with there being a tendency for larger disruptive effects to occur the closer the distractor onset was to the stimulus onset. The C57Bl/6xDBA/2 mice were, in terms of omissions, relatively indifferent to the white noise manipulation. In contrast, it was the C57Bl/6xDBA/2 mice which showed selective effects on correct-reaction latency measures, manifest as an abrupt increase in latency when the distracting burst of white noise was concurrent with the stimulus onset (significant interaction in the ANOVA with factors Strain and Noise Onset, $F_{3,51} = 5.03$, $P < 0.005$). No such increase in correct-reaction latency

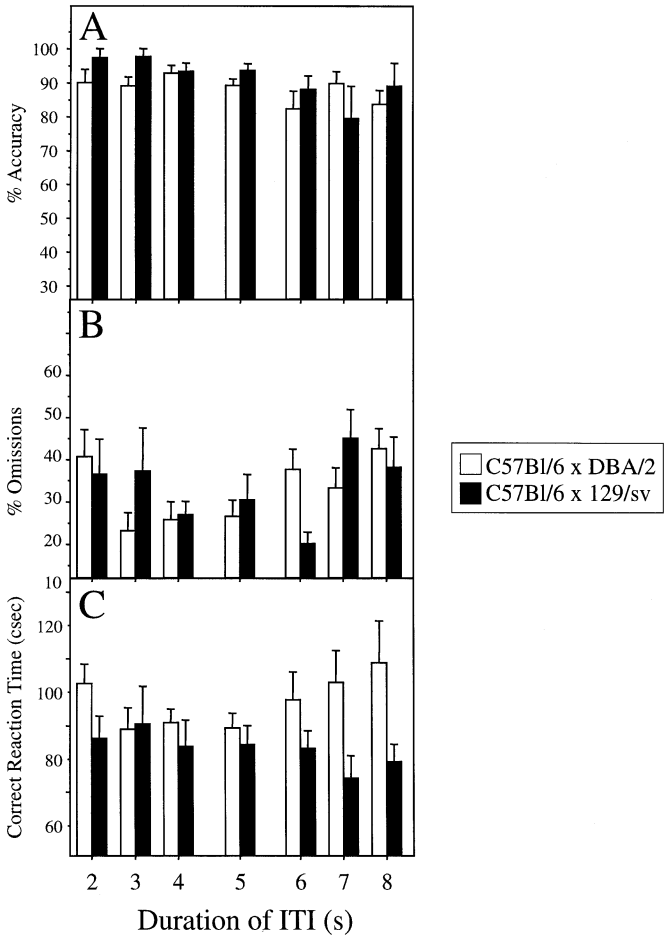

FIG. 4. The pattern of responding by the C57Bl/6xDBA/2 and C57Bl/6x129sv hybrids, in terms of discriminative accuracy (A), percentage omissions (B) and correct-reaction latencies (C), seen on manipulating the predictability of the stimulus onset by varying the ITI. The ITI under baseline conditions was fixed at 5 s. Data are the mean ± SEM

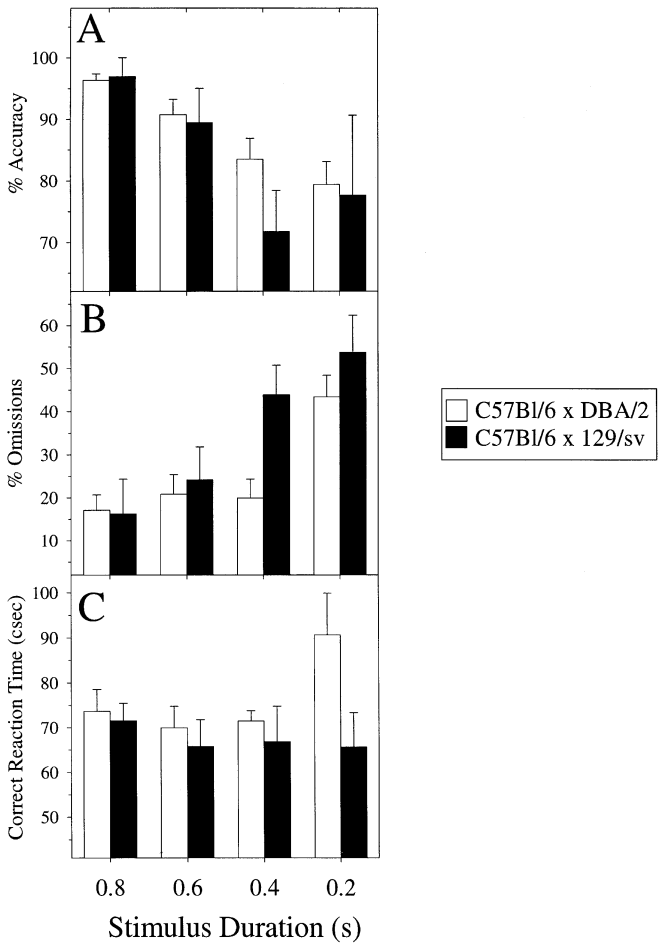

FIG. 5. The effects of decreasing stimulus duration from the baseline value of 0.8 s, in the C57Bl/6xDBA/2 and C57Bl/6x129sv hybrids, on measures of discriminative accuracy (A), percentage omissions (B) and correct-reaction latencies (C). Data are the mean ± SEM.

was observed for the C57Bl/6x129sv mice. The white noise manipulation was not associated with changes in premature or perseverative responding, nor in any other general aspect of task performance (data not shown).

Ad-libitum water for 24 h

Figure 8 shows the effects of allowing the mice free access to water for 24 h prior to testing. This manipulation had large, though reversible, effects across a wide range of measures. In both strains there was a precipitous drop in the total number of trials completed (Fig. 8A) and of those, a high proportion were omitted (Fig. 8C), resulting in main effects of Water for both measures (for trials completed, $F_{2,36} = 17.2$, $P < 0.001$; for omissions, $F_{2,36} = 38.3$, $P < 0.001$). Similarly, there were main effects of Water on correct-reaction latencies (Fig. 8D) which were longer ($F_{2,36} = 7.73$, $P < 0.01$). There were no effects on accuracy following *ad-libitum* water (Fig. 8B) and in the few trials that were completed correctly, latencies to collect the food reward were unaffected (data not shown). In no case, were there any interactions between Water and Strain. Hence, across multiple indices of discriminative accuracy, response control and motivational aspects of behaviour, the C57Bl/6xDBA/2 and C57Bl/6x129sv mice appeared equally sensitive to a general reduction in motivational drive.

Pharmacological manipulations of cholinergic functioning

Scopolamine

As shown on the left hand side of Fig. 9A, the administration of the cholinergic muscarinic antagonist scopolamine, given i.p. under baseline conditions, caused dose-dependent reductions in discriminative accuracy in both strains of mice (main effect of Dose, $F_{3,43} = 12.8$, $P < 0.001$). Moreover, the detrimental effects of scopolamine on accuracy were, at all doses tested, greater in the C57Bl/6xDBA/2 mice (main effect of Strain, $F_{1,43} = 4.35$, $P < 0.04$). These differential effects were independent of performance in the pre- and postdrug session days, and they did not interact with the order in which the doses of scopolamine were given (data not shown); in addition, there were no effects of administering saline vehicle alone to the mice. Errors of omission (Fig. 9B) were also differentially affected by scopolamine with significant main effects of Dose ($F_{3,43} = 32.46$, $P < 0.0001$) and Strain ($F_{1,43} = 4.6$, $P < 0.04$); the former due, mainly, to a common, abrupt increase in omissions at the high 2 mg/kg dosage, the latter consistent with enhanced effects of the drug in the C57Bl/6xDBA/2 mice. Both strains of mice showed a gradual lengthening of correct-reaction latencies (Fig. 9C) with ascending dosages of scopolamine (main effect of Dose, $F_{3,43} = 3.76$, $P < 0.02$). The disruptive effects of the high dosage of scopolamine did not extend to all aspects of the task, there being no

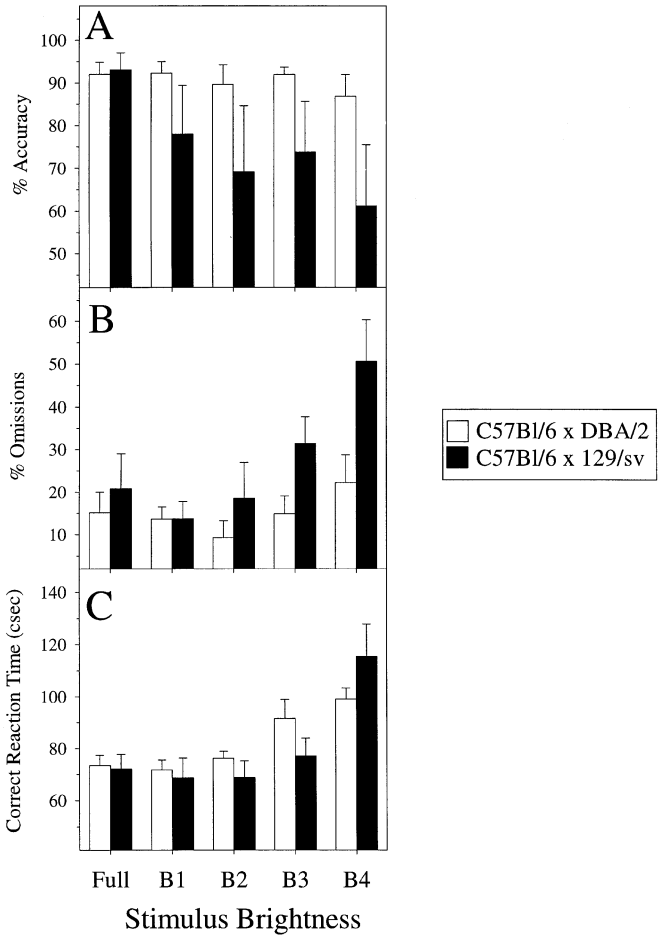

FIG. 6. The effects of dimming the stimulus lights on responding by the C57Bl/6xDBA/2 and C57Bl/6x129sv hybrids, in terms of discriminative accuracy (A), percentage omissions (B) and correct-reaction latencies (C). Data are the mean ± SEM. The abbreviations denote, as a percentages of full (baseline) brightness: B1, 52%; B2, 30%; B3, 21%; B4, 12%.

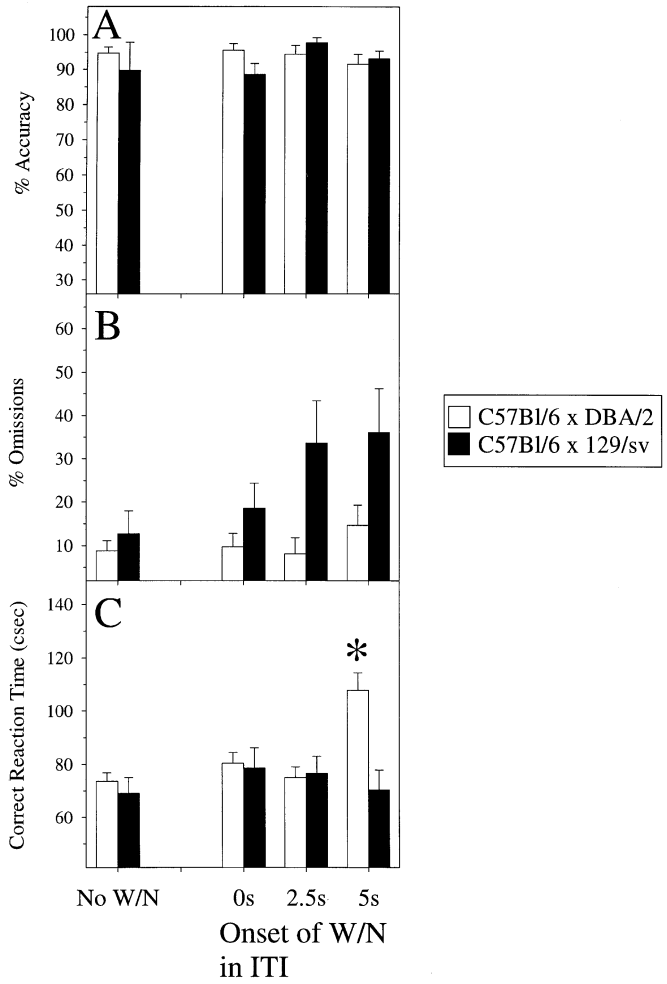

FIG. 7. The effects of introducing a 0.5-s burst of white noise (100 dB) at various intervals within the ITI on discriminative accuracy (A), percentage omissions (B) and correct-reaction latencies (C). The 0, 2.5 and 5 s indicate the white noise onset times within the ITI. Hence, the bursts of noise at 0 and 5 s occurred at the beginning and end of the ITI. Data are the mean ± SEM. *$P < 0.005$, significant difference between correct-reaction latencies in the C57Bl/6xDBA/2 mice under the 5 s condition (i.e. when the white noise was coincident with the end of the ITI, immediately prior to the stimulus onset) and all other conditions (simple main effects).

effects of scopolamine, at any dose, on the number of trials completed, premature or perseverative responding or the number of panel pushes made per trial. Therefore, it would seem that, at the low and medium doses, the effects of scopolamine on discriminative accuracy were specific to the extent that they occurred in the absence of effects on behaviours related to aspects of response control and motivation.

Methylscopolamine

As shown on the right hand side of Fig. 9, administration of equivalent doses of methylscopolamine had minimal effects on discriminative accuracy in either strain, apart from a common tendency towards reduced accuracy (Fig. 9A) at the highest, 2 mg/kg, dose of drug. Errors of omission (Fig. 9B) were not affected at the two lower doses of methylscopolamine, 0.02 and 0.2 mg/kg, but they did show an abrupt increase, that was common to both hybrids, at the highest dose of 2 mg/kg (main effect of Dose, $F_{3,39} = 5.46$, $P < 0.005$). Methylscopolamine had no effects on correct-reaction latencies (Fig. 9C). Together, these data are consistent with the view that: (i) the specific, disruptive effects of low and medium doses of scopolamine on discriminative accuracy were likely to be centrally mediated and (ii) that at least some components of the nonspecific effects of scopolamine, especially at the high 2 mg/kg dose, were due to effects on peripheral cholinergic functioning.

Effects of scopolamine on locomotor activity

Scopolamine given systemically (i.p. 20 min prior to testing) dose-dependently increased locomotor activity, as indexed by total beam breaks monitored over 2 h. At the low, 0.02 mg/kg dose, there was no difference between drug and saline vehicle (C57Bl/6xDBA/2, saline 1465 ± 52 breaks, 0.02 mg/kg scopolamine 1642 ± 61 breaks; C57Bl/6x129sv, saline 1566 ± 341 breaks, 0.02 mg/kg scopolamine 1523 ± 428 breaks). The higher doses of drug increased locomotor activity relative to the saline baseline (C57Bl/6xDBA/2, 0.2 mg/kg scopolamine 2403 ± 119 breaks; C57Bl/6x129sv, 0.2 mg/kg scopolamine 2572 ± 459 breaks; C57Bl/6xDBA/2, 2 mg/kg scopolamine 2798 ± 107 breaks; C57Bl/6x129sv, 2 mg/kg scopolamine 3006 ± 302 breaks). ANOVA of the locomotor activity data revealed main effects of Dose ($F_{3,39} = 11.8$, $P < 0.001$) but no main effects or interactions involving Strain. Thus, over the same dose range of scopolamine, the strain-dependent differences observed in the five-choice task were not apparent in the test of locomotor activity.

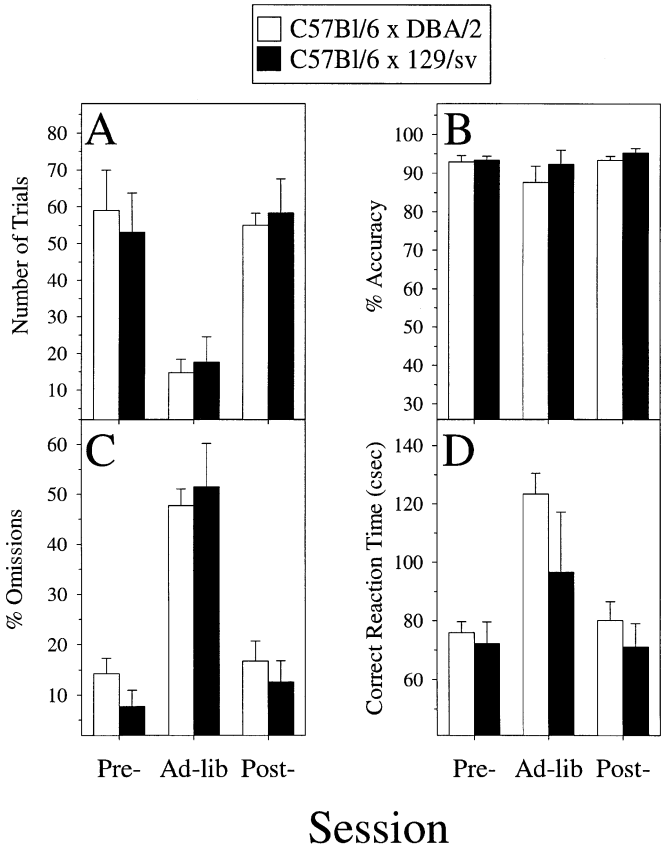

FIG. 8. The effects of allowing 24 h *ad-libitum* access to water on the overall number of trials completed in the session (A), discriminative accuracy (B), percentage omissions (C) and correct-reaction latencies (D). The data are the mean ± SEM and are presented as a comparison between performance on the day before (Pre), the day of *ad-libitum* access to water (Ad-lib) and the day following *ad-libitum* access to water (Post).

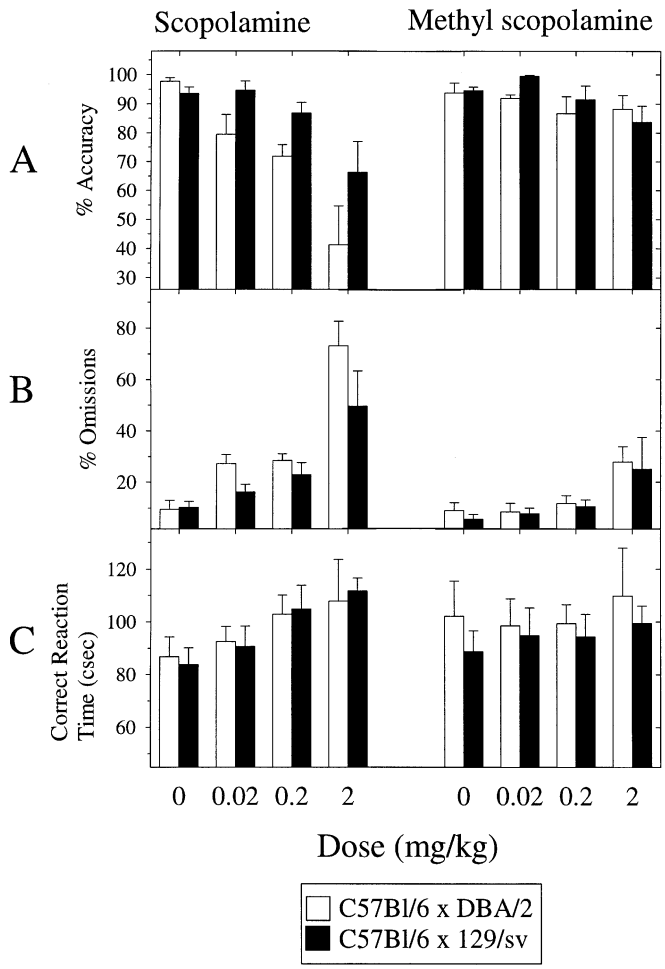

FIG. 9. The effects of scopolamine and methylscopolamine, given i.p. 20 min prior to testing, on measures of discriminative accuracy (A), percentage omissions (B) and correct-reaction latencies (C). The '0' dosage corresponds to the 0.9% saline vehicle. The drug dosages were administered utilizing a Latin-square design that allowed at least 3 days washout between treatments.

Effects of scopolamine on performance in the one-choice task

In order to assess the specificity of the effects of scopolamine on central cholinergic processes underlying discriminative performance further, the mice were re-trained on a modified version of the five-choice task. In the modified task, stimuli only appeared in the centre hole and, hence, whilst attentional demands were reduced, in terms of basic requirements, the task was identical to the five-choice one. Both strains of mice acquired the modified task rapidly (mean of about seven sessions to reach performance criteria). At baseline, there were no strain differences in measures of accuracy, omissions or correct-reaction latencies, nor in the other previously described measures of behaviour related to aspects of response control or motivation (see Table 2). In particular, there was no general increase in premature responding when compared with the five-choice task, again indicating the ability of mice to inhibit responding appropriately. Consistent with the surmise that the one-choice task had relatively low attentional demands, both strains of mice were, in terms of accuracy, indifferent to reducing the stimulus duration to values of 0.6 and 0.4 s (Fig. 10A), values that occasioned deficits in the five-choice task. At the briefest stimulus duration of 0.2 s, both strains showed an abrupt decrease in accuracy. Under the conditions prevailing in the one-choice task, scopolamine was completely without effect at doses which led to specific deficits in accuracy measures in the five-choice task (Fig. 10B). Hence, it would seem reasonable to conclude that the effects of scopolamine on measures of discriminative accuracy in the five-choice task were specific to effects on central cholinergic substrates underlying the particular form of attentional functioning assayed by the task.

Discussion

In these experiments, operant behavioural methods have been applied to the study of visuospatial attentional functioning in male mice using two F1 hybrids: C57Bl/6x129sv and C57Bl/6xDBA/2. The main findings were that, in terms of acquisition, both strains showed equal degrees of competence in learning the task to baseline levels of performance. At performance, systematic manipulation of the task parameters, designed to increase attentional load or distract the animals, indicated a pattern of effects consistent with the task, taxing aspects of visuospatial attentional functioning. The basic task manipulations did reveal strain differences, though not consistently across manipulations, and not always in terms of discriminative accuracy. There was evidence in both strains of specific, centrally mediated effects of scopolamine on attentional functioning, with the C57Bl/6xDBA/2 hybrid showing greater sensitivity to the drug manipulation. Specific effects on discriminative accuracy were observed at doses of 0.02 and 0.2 mg/kg scopolamine. At the 2 mg/kg dose, large reductions in accuracy were associated with large

TABLE 2. The one-choice task. Comparison of the C57Bl/6xDBA/2 and C57Bl/6x129sv hybrids in baseline performance measures relating to discriminative accuracy, response control and motivation

	C57Bl/6xDBA/2 Strain	C57Bl/6x129sv Strain
Accuracy (%)	96.5 ± 1.0	98.0 ± 1.1
Correct-reaction times (cs)	71.9 ± 3.8	61.2 ± 7.0
Premature responses (*n*)	0.8 ± 0.4	1.6 ± 0.4
Perseverative responses (*n*)	1.6 ± 0.6	1.5 ± 0.5
Omissions (%)	10.8 ± 1.2	7.4 ± 1.7
Panel-pushes per trial (*n*)	3.0 ± 0.1	4.7 ± 0.9
Latency to collect reward (s)	1.2 ± 0.6	1.3 ± 1.7

The baseline conditions were identical to those pertaining in the five-choice task, save for a variable ITI (4–5.5 s.) used to discourage premature responding and the fact that the stimulus always appeared in the centre hole. Data are the mean ± SEM taken from three consecutive sessions at baseline performance.

effects on other measures, including omissions and response latencies, suggestive of nonspecific effects on task performance.

Nature of the psychological processes taxed by the mouse five-choice task

Several pieces of evidence indicate that the mouse five-choice task does in fact tax aspects of visuospatial attentional functioning in this species. First, as noted above, there was the systematic and predictable pattern of effects of manipulations designed to alter attentional load, where effects on discriminative accuracy were observed in the absence of effects on other measures indicative of nonspecific effects on performance. Secondly, there was the dissociation between the effects of reducing stimulus duration in the five-choice where attentional demands were relatively high, and the one-choice task where attentional demands were relatively low. In addition, interfering with the functioning of central cholinergic systems led, at appropriates doses of scopolamine, to specific effects on discriminative accuracy, a finding consistent with previous work showing selective effects of cholinergic manipulations on processes of stimulus detection in a variety of other species, including rats (Muir *et al*., 1994; Bushnell *et al*., 1997; Robbins *et al*., 1998), monkeys (Voytko *et al*., 1994) and humans (Wesnes & Warburton, 1983; Wesnes & Revell, 1984).

The mouse five-choice task was based on methods developed to a high degree of behavioural, neural and neurochemical specification by other workers using rats (Robbins, 1998). At the outset, it was anticipated that there may be fundamental difficulties in transferring these methodologies to mice, particularly with regard to whether mice could inhibit responding appropriately in situations where motivational drive was high. Clearly, the five-choice task, in requiring the animal to scan the stimulus array and not respond until the stimulus onset, requires significant levels of response inhibition. However, in these studies, and in other unpublished work, we have encountered no difficulties due to any general inability of mice to inhibit their responding appropriately nor, for that matter, in any other aspect of response control. Indeed, if anything, under baseline conditions, the level of premature responding in mice is less than that seen typically in rats and is, moreover, resistant to task manipulations that would tend to increase premature responding in rats, such as lengthening the ITI.

Where mice seem to differ fundamentally from rats is in the response to alterations of motivational drive, to which mice seem to be much more sensitive; e.g. in the present studies allowing the mice 24 h *ad-libitum* access to water led to an almost complete breakdown

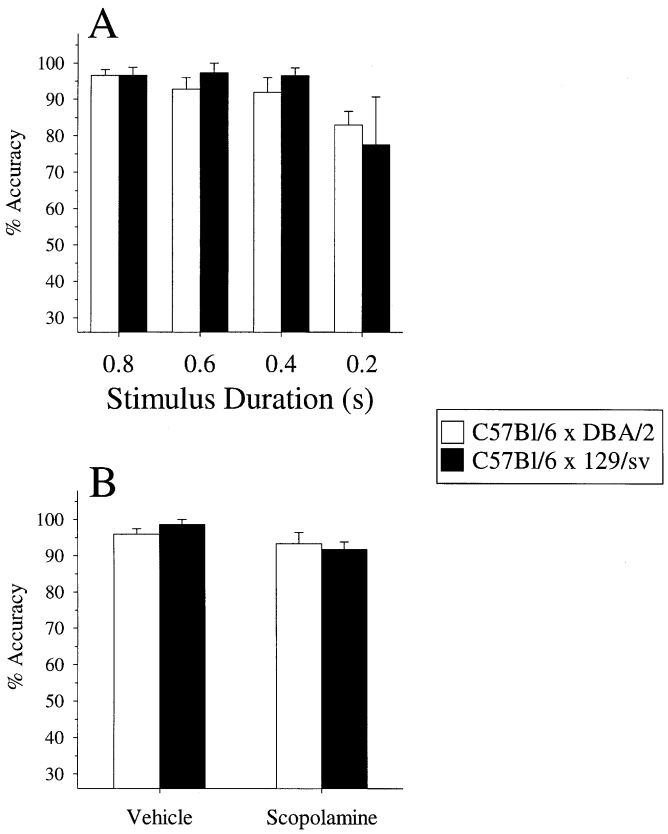

FIG. 10. The effects of reductions in stimulus duration (A) and the administration of scopolamine (B) on discriminative accuracy performance in the one-choice task at baseline. Scopolamine was given i.p. 20 min prior to testing at dosages of 0.02 and 0.2 mg/kg for the C57Bl/6xDBA/2 and C57Bl/6x129sv mice, respectively. These dosages corresponded to doses of scopolamine that were associated with specific deficits in discriminative accuracy in the five-choice task.

of task performance. For this reason, when comparing discriminative accuracy across treatments in mice it is important to be aware of possible underlying effects on motivational variables.

Strain differences in relation to manipulations of the task parameters

Though there were no strain differences in acquiring the five-choice task, at performance, manipulations of the task parameters did reveal effects related to strain. In general, the effects of these manipulations were greater in the C57Bl/6x129sv cross. However, the extent to which these differences reflect effects on attentional functioning is unclear, as there was no consistent pattern of deficits across manipulations, and in many cases the deficit was not manifest as alterations in discriminative accuracy but in terms of omissions. Therefore, it could be that, rather than reflecting effects of genotype on attentional functioning *per se*, the task manipulations revealed changes more directly related to other aspects of behaviour and/or physiology, e.g. differential general reactivities/behavioural inhibition or, in the case of the effects of dimming the stimulus lights, basic visual acuity. The idea that the C57Bl/6x129sv mice may have been less flexible in the face of increasing attentional load gains some support from the correct-response latency data, where it was noticeable that in many cases high levels of accuracy in the C57Bl/6xDBA/2 mice were accompanied by increases in correct-response latencies, an adaptation often not seen in the C57Bl/6x129sv mice. The implication would be that, whilst the C57Bl/6xDBA/2 hybrid

were able to utilize a 'speed-error' trade-off strategy under conditions of increased attentional load, the C57Bl/6x129sv mice could not.

Strain differences in relation to manipulations of cholinergic functioning

In contrast to the effects of manipulating the basic task parameters, the strain differences apparent on blocking cholinergic functioning with the muscarinic antagonist scopolamine appeared to be specific to the attentional processes taxed by the five-choice task. Hence, at doses of scopolamine shown to have specific effects on attentional functioning on the basis of differential effects in the five-choice and one-choice tasks, C57Bl/6xDBA/2 mice were more sensitive to scopolamine than the C57Bl/6x129sv hybrid. Moreover, in that both strains of mice were, at the same dose range, unaffected with respect to discriminative accuracy in the five-choice following administration of methylscopolamine, these strain-dependent effects on attentional functioning were likely to be of central origin. At the highest 2 mg/kg dose of scopolamine there was no evidence of strain differences, with large deficits in discriminative accuracy being accompanied by deficits in other measures indicative of nonspecific disruptions of performance, probably due, in turn, to the hyperactivity produced by these doses of scopolamine (present data and Mathur *et al*., 1997).

The evidence of specific interactions between genotype, central cholinergic mechanisms and attentional functioning raises several issues, not least the genetic origin of the complex phenotype. In that both the F1 crosses involved C57Bl/6, it is likely that the phenotypic differences stem from interactions between C57Bl/6 and the accompanying 129sv and DBA/2 genotypes. However, whilst the 129sv and DBA/2 strains do exhibit behavioural differences (Crawley *et al*., 1997) there are no data currently available, that would provide obvious clues as to specific gene candidates that differ between the 129sv and DBA/2 strains of mice, and which, potentially at least, could explain the cholinergic attentional phenotype in the F1 hybrids. There are data showing differential sensitivities between C57Bl/6 and DBA/2 strains to intrahippocampal injections of methylscopolamine on exploratory behaviours (Van Abeelen *et al*., 1972) and other work has shown that, in general, these two strains differ when compared across behaviours in which the hippocampus plays an important role (Gerlai, 1998). However, it is difficult to see how these previous data could be related to the differential sensitivities to scopolamine seen here in the present studies, which occur between F1 crosses containing an equal genetic contribution from C57Bl/6 and in a behavioural task which, at performance, has minimal hippocampal involvement. Overall then, there is no systematic evidence available of any general differential sensitivity to scopolamine across strains in other aspects of behaviour and physiology which would lead to the most immediate gene candidate, i.e. cholinergic receptors of the muscarinic subtype. The possibility arises therefore, that other genes may contribute to the cholinergic attentional phenotype in the F1 crosses, the identity of which could, theoretically, be revealed by cross-breeding studies mapping the genotype to the attentional phenotype.

Whatever the genetic basis of the strain-dependent differences in behaviour seen in the present studies, it would remain to be established whether the genotype was specific to the neural processes underlying attentional functioning, as opposed to being redundant effects of a genetic difference that impacted on cholinergic function generally. In this regard, the evidence of equivalent sensitivities to scopolamine, in terms of increased locomotor activity, is of relevance. Hence, it would seem that the differential sensitivities to scopolamine seen in the five-choice task by the two mice strains do show some degree of specificity when compared across behavioural functions. Additionally, of course, it would also be interesting in future studies, to extend any genetic analysis of the substrates underlying attention using other behavioural paradigms, such as the cross-model attentional task or tests of vigilance (Sarter & McGaughy, 1998), and into other neural systems, such as, for example, the noradrenaline system, which also impacts significantly on attentional functioning (Carli *et al*., 1983; Coull *et al*., 1997).

Concluding remarks

In summary, the present data indicate the utility of operant methods in assessing complex behavioural phenotypes in mice. In revealing interactions between strains and the effects of cholinergic manipulations on attentional functioning they not only add to the converging evidence suggesting a key role for cholinergic substrates in attention across species, but also suggest a significant genetic lability in the expression of such cholinergic mechanisms. The source and specificity of the genetic variability cannot be determined without additional studies. Here, both phenotype-led approaches of the kind utilized in the current work and genotype-led or -targeted approaches would be of use. Finally, within the context of the increasing use of genetically manipulated mice to study normal and abnormal behavioural phenotypes, the present work shows that it is possible to combine the advantages stemming from the genetic tractability of mice with behavioural methods allowing a high degree of stimulus control and accurate timing of responses. We anticipate that the combination of such operant methodologies with the techniques of molecular genetics will be of considerable use in unravelling the genetic contribution to a wide range of complex psychological and behavioural processes, in particular, attention but also memory and aspects of the control of behavioural output, including its inhibitory control.

Acknowledgements

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Abbreviation

ITI, inter trial interval.

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