2012

Task demands dissociate the effects of muscarinic M-1 receptor blockade and protein kinase C inhibition on attentional performance in rats

Andrea M. Robinson
William & Mary

David F. Mangini
William & Mary

Joshua A. Burk
William & Mary, jabur2@wm.edu

Recommended Citation

Robinson, A. M., Mangini, D. F., & Burk, J. A. (2012). Task demands dissociate the effects of muscarinic M1 receptor blockade and protein kinase C inhibition on attentional performance in rats. Journal of Psychopharmacology, 26(8), 1143-1150.

Original Paper

Task demands dissociate the effects of muscarinic M_1 receptor blockade and protein kinase C inhibition on attentional performance in rats

Andrea M Robinson, David F Mangini and Joshua A Burk

Journal of Psychopharmacology
26(8) 1143–1150

DOI: 10.1177/0269881111415732
jop.sagepub.com

Abstract

The cholinergic system is known to be necessary for normal attentional processing. However, the receptors and mechanisms mediating the effects of acetylcholine on attention remain unclear. Previous work in our laboratory suggested that cholinergic muscarinic receptors are critical for maintaining performance in an attention-demanding task in rats. We examined the role of the muscarinic M_1 receptor and protein kinase C (PKC), which is activated by the M_1 receptor, in attention task performance. Rats were trained in an attention-demanding task requiring discrimination of brief (500, 100, 25 ms) visual signals from trials with no signal presentation. The effects of muscarinic M_1 receptor blockade were assessed by administering dicyclomine (0–5.0 mg/kg). The effects of PKC inhibition were assessed by administering chelerythrine chloride (0–2.0 mg/kg). Dicyclomine decreased the accuracy of detecting longer signals in this attention task, including when attentional demands were increased by flashing a houselight throughout the session. Chelerythrine chloride decreased the accuracy of signal detection in the standard version of the task but not when the houselight was flashed throughout the session. The present findings indicate that muscarinic M_1 receptors are critical for maintaining performance when attentional demands are increased, and that PKC activity may contribute to some aspects of attentional performance.

Keywords

Acetylcholine, attention, basal forebrain, vigilance

Introduction

The cholinergic system is known to play a critical role in attentional processing (Everitt and Robbins, 1997; Sarter et al., 2005). Many experiments have examined, in tasks designed to assess attention, the effects of drugs that act at nicotinic receptors (Mirza and Stolerman, 1998; Rezvani et al., 2002; Turchi et al., 1995), and several experiments have begun to investigate the critical nicotinic receptor subtypes for maintaining normal attention (Blondel et al., 2000; Grottick et al., 2003; Hahn et al., 2003; Howe et al., 2010). Drugs that block muscarinic receptors also disrupt attentional performance (Mirza and Stolerman, 2000; Ruotsalainen et al., 2000) as well as performance in other procedures that are thought to be sensitive to changes in attentional processing, such as latent inhibition (Barak and Weiner, 2007, 2009). In a two-lever sustained attention task requiring discrimination of signals from trials with no signal presentation, blockade of muscarinic receptors with scopolamine has been shown to decrease accuracy, although whether the effects have been selective decreases in accuracy of signal detection (Johnson and Burk, 2006; McQuail and Burk, 2006), selective decreases in accuracy on trials with no signal presentation (Rezvani et al., 2009) or decreases in accuracy on both of these trial types (Bushnell et al., 1997) has varied across experiments.

The effects of blocking muscarinic receptor subtypes on measures of sustained attention have not been tested. The effects of drugs selective for specific muscarinic receptor subtypes have been tested on measures of memory, with the muscarinic M_1 and M_2 receptors being most thoroughly examined. Muscarinic M_1 receptors are predominantly located post-synaptically (Levey et al., 1991), and blockade of these receptors generally disrupts performance in measures of learning and memory (Aura et al., 1997; Bymaster et al., 1993; Ferreira et al., 2003; Hagan et al., 1987). Moreover, intrastriatal infusions of a selective muscarinic M_1 receptor toxin impair task switching (McCool et al., 2008). Finally, M_1 receptor knockout mice show impaired responding to a previously rewarded visual cue (Gulledge et al., 2009). Muscarinic M_2 receptors are primarily located presynaptically, acting as autoreceptors to negatively modulate acetylcholine release

Department of Psychology, College of William and Mary, Williamsburg, VA, USA

Corresponding author:
Joshua A Burk, Department of Psychology, College of William & Mary, Williamsburg, VA 23187, USA
Email: jabur2@wm.edu

(Levey et al., 1991). Muscarinic M_2 receptor antagonists have typically been associated with cognition enhancement (Gulledge et al., 2009; Quirion et al., 1995). Thus, previously observed attentional deficits induced by muscarinic receptor antagonists (references above) are most likely associated with muscarinic M_1 receptors.

Recent experiments have begun to examine the contributions of second messenger pathways in attention. For example, intra-prefrontal cortical infusions of a cAMP-dependent protein kinase inhibitor disrupt accuracy in a five-choice serial reaction time task (Paine et al., 2009). Muscarinic M_1 receptors act via the second messengers inositol triphosphate and diacylglycerol, leading to activation of protein kinase C (PKC) (Caulfield, 1993; Haas and Dokas, 1999). Chelerythrine chloride inhibits the translocation of cytosolic PKC to the membrane and therefore can be used to study the effects of decreasing PKC activity (Chao et al., 1998; Siomboing et al., 2001). Chelerythrine chloride has been shown to impair learning acquisition and memory formation (Sacchetti and Bielavska, 1998; Serrano et al., 1995) and to reverse memory impairments associated with PKC overactivity, either induced by a phorbol ester administration (Birnbaum et al., 2004) or in aged rats (Brennan et al., 2009). There are no available experiments in the literature that report the effects of manipulating PKC activity on attentional performance. These experiments are important for beginning to determine whether alterations in attention contribute to memory-related effects of PKC modulation.

The present experiments tested the effects of muscarinic M_1 receptor blockade, induced by dicyclomine administration, and PKC inhibition, induced by chelerythrine chloride administration, on attention task performance. Rats were trained in a two-lever attention task that required discrimination of visual signals from trials with no signal presentation. We hypothesized that dicyclomine would decrease the accuracy of signal detection without affecting accuracy on trials with no signal presentation, similar to the effects reported following muscarinic receptor blockade with scopolamine that were previously observed using similar conditions in our laboratory (Johnson and Burk, 2006; McQuail and Burk, 2006). Animals were tested in a 'standard' version of the task and with greater background noise to increase attentional demands. The experiment examining the effects of chelerythrine chloride on attention was more exploratory. Our goal was to test whether chelerythrine chloride produced a selective deficit in task performance, such as a decrease in signal detection, or whether there were less specific patterns of impairment (for example, decreased accuracy on all trial types).

Methods and materials

Subjects

Subjects included a total of 18 male Long-Evans rats (Charles River Laboratories Inc., Wilmington, MA, USA), weighing 151–175 g when arriving at the laboratory. Animals were housed individually in hanging wire cages in a vivarium with a 14/10 h light/dark cycle (lights on 0600–2000). All behavioral testing occurred between 0900 and 1200, for five or six days each week. Rats were given ad libitum access to standard rat chow, but were water restricted on testing days, receiving water during task performance and for 30 min following testing sessions. Rats were given overnight water access prior to days they were not trained, to maintain the animals' health throughout the experiment. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the College of William and Mary.

Apparatus

Rats were trained in one of 12 chambers (Med Associates, Inc., Georgia, VT). Each chamber was enclosed within a sound-attenuating box. One side of the chamber contained two retractable levers, a water port with a dipper to deliver water (0.01 mL) situated between the two levers, and a central panel light located above the water port. A houselight was located in the back of the chamber. Illumination levels for these chambers have been previously reported (Burk, 2004). The behavioral testing programs and data collection were managed by a personal computer utilizing Med-PC version IV software.

Behavioral training

Rats were trained in a sustained attention task developed by Bushnell et al. (1994) and validated by McGaughy and Sarter (1995). The houselight remained illuminated throughout all training sessions. In the first stage of training, the levers were extended throughout the session and the dipper was raised following each lever press. To attempt to prevent a lever bias, following five consecutive presses on a single lever, the other lever had to be pressed to receive water access. After reaching a criterion of 120 lever presses per session for three sessions, rats were trained to discriminate between signals (1 s illumination of the panel light) and nonsignals (no illumination of the panel light). After a signal or nonsignal, the levers were extended into the chamber. For half the animals, following a signal, a press on the left lever was considered a hit and the dipper was raised to allow water access, while a press on the right lever was considered incorrect, scored as a miss and the rat received no water. After a nonsignal, a press on the right lever was considered correct, scored as a correct rejection and water access was given, while pressing the left lever was scored as a false alarm and the dipper was not raised. After a lever press or a failure to press a lever within 3 s after the levers were extended (scored as an omission) the levers were retracted. The rules were reversed for the other half of the rats (a right lever press was considered correct after a signal and a left lever press was considered correct after a nonsignal). The inter-trial interval (ITI) varied (12 ± 3 s) during training to prevent the rats from anticipating the onset of the next trial. Incorrect responses were followed by a correction trial that was identical to the previous trial. Three consecutive incorrect responses triggered a forced trial where only the correct lever was extended until the lever was pressed or 90 s elapsed. When the three consecutive errors occurred on signal trials, the panel light remained illuminated while the lever was extended. Animals were trained in this task until they reached a criterion of 70% hits and 70% correct rejections for three consecutive sessions. In the next level of

training the signal duration was reduced and varied within each session (500, 100, or 25 ms). A session consisted of 162 trials with an ITI of 9 ± 3 s. The signal duration and ITI were decreased to place higher demands on attentional processing (McGaughy and Sarter, 1995; Parasuraman et al., 1987). Animals were trained in this version of the task to a criterion of 70% hits following the 500 ms signal and 70% correct rejections for three consecutive sessions in order to move to the drug administration phase of the experiment.

Preparation of dicyclomine and behavioral testing

Dicyclomine hydrochloride was dissolved in saline and injected into the intraperitoneal cavity in a volume of 1.0 mL/kg. The doses used were 0.0, 0.625, 1.25, 2.50 and 5.0 mg/kg of dicyclomine. Typical dose ranges for dicyclomine are at least 8.0 mg/kg in experiments examining the effects of this drug on learning and memory (Fornari et al., 2000; Soares et al., 2006). We chose a lower dose range because, in pilot studies, we observed that 10.0 mg/kg resulted in an almost complete failure to press a lever in the task. The dicyclomine solution was heated for approximately 5 min until the solution was visibly dissolved.

Rats ($n = 10$) received five sessions with two different task conditions: the same task the animals had trained prior to drug administration (the standard task) and the same task with the houselight flashed as a distracter throughout the session (1 s on/off). The order of the task manipulations and drug administration was randomized for each rat. Rats received each drug dose prior to the two task conditions, for a total of 10 injections. Animals were tested in the attention task 15 min after each injection. Between drug administration days, rats returned to the standard task and were required to meet a criterion of 70% hits at the 500 ms signal and 70% correct rejections before proceeding to the next drug administration day.

Preparation of chelerythrine chloride and behavioral testing

Chelerythrine chloride was administered (0.0, 1.0, and 2.0 mg/kg; ip) once the rats ($n = 8$) had reached criterion for training in the two-lever attention task. These doses are in the range used during chronic administration studies assessing the ability of PKC inhibition to attenuate stress-induced memory deficits (Hains et al., 2009). Moreover, a higher dose of chelerythrine chloride (4.0 mg/kg) substantially increased the number of omissions during pilot studies with this attention task. Chelerythrine chloride was suspended in a saline solution and placed on a vortex prior to injection. Each rat was given an injection 10 min prior to performing the two-lever attention task. Rats were trained in the attention task for at least 1 day between injection sessions. The order of drug administration was randomized and animals received chelerythrine chloride prior to the standard task and the same task with the houselight flashing (1.0 s on/off) throughout the session. Between these drug administration sessions, rats were required to reach criterion (70% hits at the 500 ms signal and 70% correct rejections) in the standard attention task.

Behavioral measures and statistical analyses

The number of hits (h), misses (m), correct rejections (cr), false alarms (fa), and omissions were recorded for each testing session. The measures of accuracy for signal trials were the relative hits (h/(h+m)) and for nonsignal trials were the relative correct rejections (cr/(cr+fa)). The relative hits were analyzed using a repeated-measures analysis of variance (ANOVA) with the factors of task (standard and flashing houselight), signal duration and drug dose. The relative correct rejections were analyzed using a repeated-measures ANOVA that included task and dose as factors. Omissions were analyzed separately from measures of accuracy. All p-values were corrected with the Huynh–Feldt procedure. Significant ANOVAs were further assessed by comparing vehicle administration sessions with performance following each drug dose, using t-tests that were corrected with the Bonferroni procedure. A level of $\alpha = 0.05$ was used to determine statistical significance.

Results

Effects of dicyclomine on attention task performance

One animal did not maintain stable performance and was not included in any data analyses. Overall, dicyclomine administration decreased accuracy on trials with the 500- and 100-ms signals (Figure 1). This observation was tested with a task × dose × signal duration ANOVA for the relative hits. This analysis yielded a main effect of signal duration, reflecting higher levels of accuracy following longer signal durations ($F(2,16) = 169.6$, $p < 0.05$). More importantly, there was also a significant main effect of dose ($F(4,32) = 3.095$, $p < 0.05$) and a significant dose × signal duration interaction ($F(8,64) = 2.936$, $p < 0.05$). The task × dose × signal duration interaction was not significant, thus the data were combined from the standard and distracter sessions for subsequent analyses of the significant dose × signal duration interaction. The basis for the dose × signal duration interaction was assessed by conducting one-way ANOVAs that included the factor dose for each signal duration. These ANOVAs yielded a main effect of dose for the 500-ms ($F(4,32) = 3.456$, $p < 0.05$) and 100-ms ($F(4,32) = 4.648$, $p < 0.05$) signals, but not for the 25-ms signal. For the 500-ms signal, the 0.625 mg/kg, 2.5 mg/kg and 5.0 mg/kg dicyclomine doses all differed significantly compared with the vehicle condition (all $p < 0.05$). For the 100-ms signal, the 2.5 mg/kg dicyclomine dose was significantly different compared with vehicle ($t(8) = 3.096$, $p < 0.05$), and the difference between vehicle and 5.0 mg/kg dicyclomine approached significance but was not significant after the Bonferroni correction was applied.

For correct rejections, there was a main effect of task, reflecting a decrease in accuracy on nonsignal trials when the distracter was presented (Standard task: 0.858 ± 0.015; Distracter task: 0.790 ± 0.019). However, there was no effect of dose nor was there a task × dose interaction for correct rejections. For omissions, a task × dose ANOVA did not yield any significant effects (Figure 1).

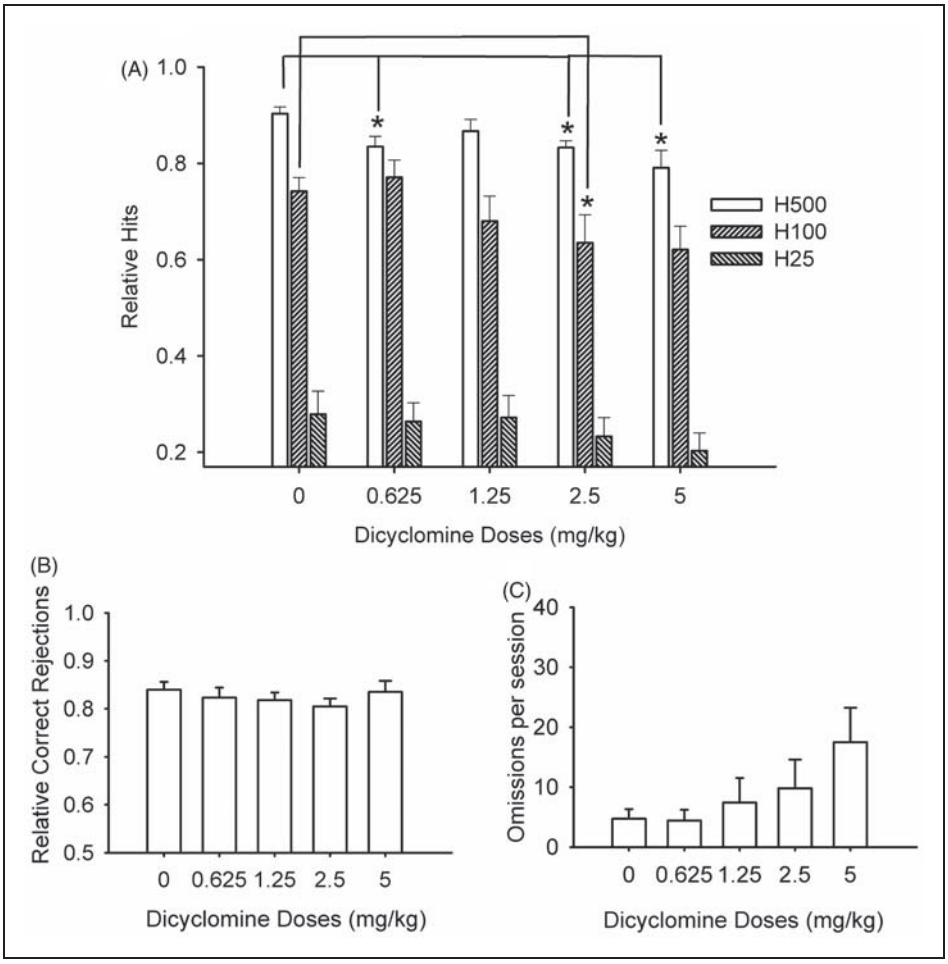

Figure 1. The figure depicts relative hits (A), relative correct rejections (B) and omissions per session (C) following each dicyclomine dose (0–5.0 mg/kg). In (A) the bars denote the different signal durations (500, 100 and 25 ms). Dicyclomine produced a significant dose × signal duration that did not further interact with task ($p < 0.05$; $n = 9$). Thus, the data shown here are combined from the standard and flashing houselight conditions. Compared with vehicle, dicyclomine (0.625, 2.5 and 5.0 mg/kg) decreased the accuracy of detecting the 500-ms signal and at one dose (2.5 mg/kg) decreased the accuracy of detecting the 100-ms signal. The asterisks denote dicyclomine doses that were significantly different compared with vehicle administration ($p < 0.05$). There were no effects of dicyclomine on correct rejections or omissions.

Effects of chelerythrine chloride on attention task performance

The data from one rat were not included in any analyses because that animal did not maintain consistent performance levels throughout the experiment. Chelerythrine chloride administration decreased signal detection in the standard task but did not affect performance when background noise was increased. A task × dose × signal duration ANOVA yielded a significant three-way task × dose × signal duration interaction ($F(4,24) = 3.90$, $p < 0.05$). To examine the basis of this interaction, we conducted separate dose × signal duration ANOVAs for the standard and distracter testing sessions. For the standard task, the dose × signal duration ANOVA yielded a main effect of signal duration ($F(2,14) = 94.6$, $p < 0.05$), reflecting decreased relative hits during shorter signal durations, and of dose ($F(2,14) = 4.39$, $p < 0.05$). Post hoc tests revealed that the relative hits following 2.0 mg/kg chelerythrine chloride were significantly lower compared with following vehicle administration ($t(7) = 2.02$, $p < 0.05$; Figure 2). For the distracter condition, the dose × signal duration ANOVA did not yield any statistically significant effects of chelerythrine chloride on the relative hits (Figure 3). Thus, chelerythrine chloride administration decreased signal detection in the standard task, but the basis for the further interaction with signal duration (as part of the significant task × dose × signal duration interaction) could not be determined. For correct rejections, there was a significant effect of task, reflecting lower accuracy on nonsignal trials during the distracter condition (Figures 2 and 3), but no main effect of dose or task × dose interaction. There were no statistically significant effects of task or dose on omissions.

Discussion

The present experiment was designed to test the effects of muscarinic M_1 receptor blockade and PKC inhibition on

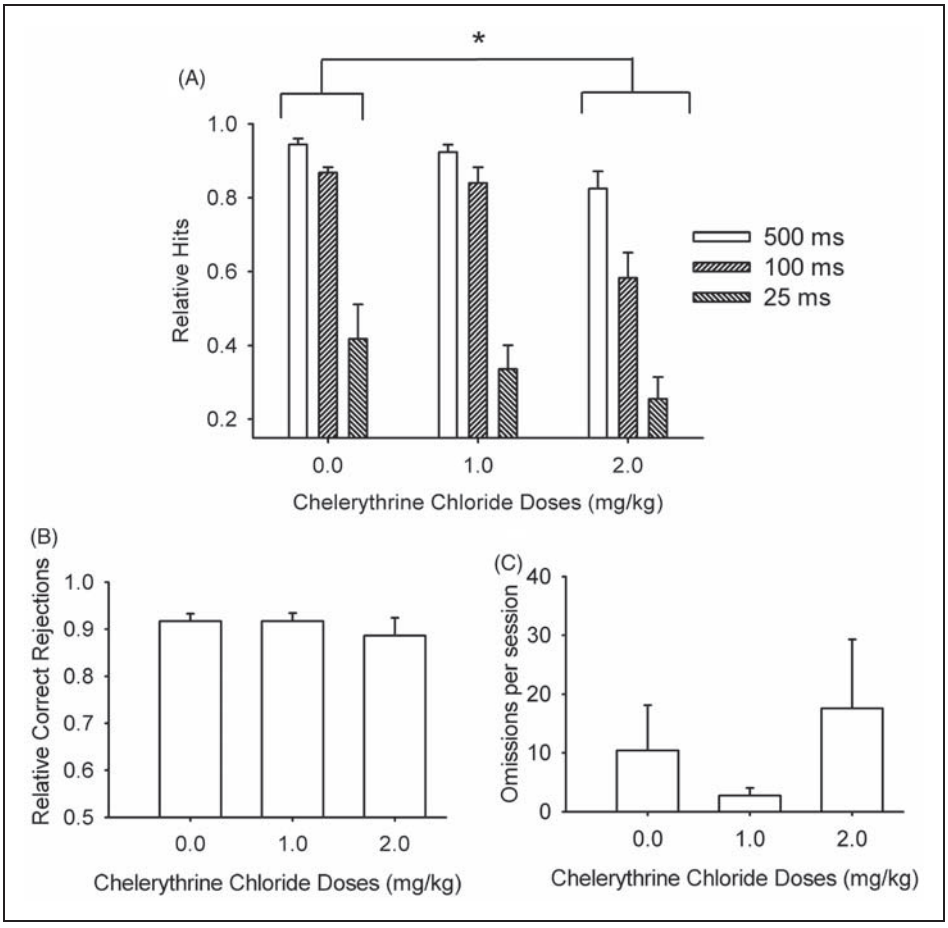

Figure 2. The figure depicts relative hits (A), relative correct rejections (B) and omissions per session (C) following each chelerythrine chloride dose (0–2.0 mg/kg) in the standard task trained prior to drug administration. In (A) the bars depict the different signal durations (500, 100 and 25 ms). The asterisk denotes a significant decrease in the relative hits following 2.0 mg/kg chelerythrine chloride compared with vehicle administration ($p < 0.05$; $n = 7$). There were no significant effects of chelerythrine chloride on correct rejections or omissions.

attention task performance. Dicyclomine-induced muscarinic M_1 receptor blockade decreased the accuracy of signal detection (relative hits) in the standard and distracter versions of this sustained attention task. The lack of drug effects on nonsignal trial accuracy indicates that dicyclomine administration did not disrupt the ability to respond based on the task rules. There were no significant effects of any drug treatments on omissions, although several of the higher drug doses produced trends for increases in omissions. The highest number of omissions remained near an average of 20 per session, which represents less than 13% of the total number of trials. Thus, it seems unlikely that the drugs had large effects on motivation or on the motoric abilities necessary for the task. Finally, dicyclomine administration decreased detection of the longer (500- and 100-ms) signals in this sustained attention task. On 25-ms signal trials, the rats typically responded (incorrectly) as if a nonsignal was presented by pressing the miss/correct rejection lever. Thus, dicyclomine administration appears to decrease signal detection, and this effect is confined to trials that are most likely to be identified by the animal as signals. Overall, this pattern of impairment is similar to the deficits observed following widespread loss of corticopetal cholinergic neurons (McGaughy and Sarter, 1998; McGaughy et al., 1996), suggesting that muscarinic M_1 receptors in multiple cortical regions may contribute to attentional performance (Sarter et al., 2005). Loss of hippocampal cholinergic projections does not affect accuracy in the standard version of this attention task (Sarter et al., 2002). Thus, the effects of dicyclomine are more likely to be mediated by the cortical muscarinic M_1 receptors compared with hippocampal muscarinic M_1 receptors.

Chelerythrine chloride selectively decreased signal detection accuracy in the standard version of the attention task. Accuracy on nonsignal trials and the number of omitted trials were unaffected by chelerythrine chloride administration, suggesting this drug produced a relatively selective effect on task performance. Interestingly, a selective decrease in signal detection in the present task is associated with pharmacological or lesion manipulations that depress the functioning of the cholinergic system (for example, McGaughy et al., 1996). Other manipulations, such as exposure to amphetamine, decrease the accuracy on nonsignal trials in this task (Kondrad and Burk, 2004). Moreover, lesions of the dorsal noradrenergic bundle do not affect performance in this two-lever sustained attention task (McGaughy et al., 1997). Thus, the selectivity of the effects of chelerythrine chloride

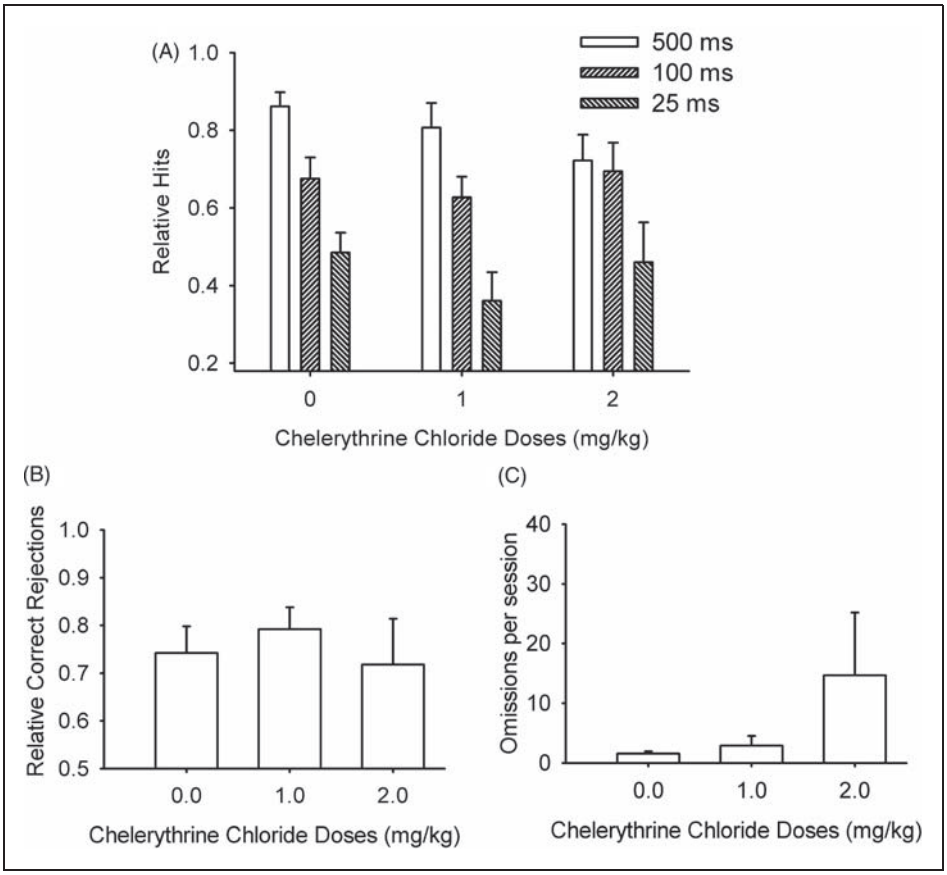

Figure 3. The figure depicts relative hits (A), relative correct rejections (B) and omissions per session (C) following each chelerythrine chloride dose (0–2.0 mg/kg) in the distracter task with the houselight flashing throughout the session. In (A) the bars denote the different signal durations (500, 100 and 25 ms). Chelerythrine chloride did not differentially affect hits, correct rejections or omissions during the distracter condition ($n = 7$).

administration suggests that it may be possible to associate the actions of PKC on attentional processing required by the standard task with specific neurotransmitter systems, and that the interactions between PKC and the cholinergic system represent a logical starting point.

Chelerythrine chloride administration did not differentially affect group performance when background noise was increased. The standard version of this task is thought to require more bottom-up, signal-driven processing, whereas increasing background noise is thought to activate top-down cognitive modulation in response to attentional challenge (Sarter et al., 2005). The present findings suggest that PKC may contribute to dissociable aspects of attentional processing, as chelerythrine chloride administration decreased the relative hits in the standard version of the task but not when background noise was increased. Collectively, our findings suggest that it is possible that PKC contributes to some aspects of processing mediated by muscarinic M_1 receptor, namely, those aspects necessary for maintaining performance in the standard task. Certainly, concurrent manipulations of the muscarinic M_1 receptor and PKC during the standard task are needed to test this hypothesis. Previous studies have shown correlations between firing rates of prefrontal and posterior parietal cortical neurons and presentation of a flashing houselight in this attention task (Broussard et al., 2009; Gill et al., 2000). Moreover, lesions of cholinergic projections to these cortical regions can alter task performance when distracters are presented, but do not affect performance in the standard task (Broussard et al., 2009; Newman and McGaughy, 2008). Infusions of dicyclomine into these discrete cortical areas would help to identify whether muscarinic M_1 receptors in these regions contribute to the present effects observed following systemic administration.

Several caveats need to be kept in mind when interpreting the present results. First, although the M_1 receptor antagonist dicyclomine shows a much greater affinity for binding to muscarinic M_1 receptors compared with muscarinic M_2 receptors (Kunysz et al., 1988), this drug has been shown to bind to M_3 receptors (Doods et al., 1987), which could contribute to the signal detection deficits observed in the present experiment following dicyclomine administration. This concern is somewhat tempered by the lower doses of dicyclomine used in the present experiment compared with in other experiments assessing learning and memory. Second, the possibility exists that if acetylcholine is released and the muscarinic M_1 receptor is occupied by an antagonist, then a greater amount of acetylcholine binds to the muscarinic M_2 receptors, leading to decreased acetylcholine release which would be expected to decrease signal detection in this attention task. Thus, the locations of muscarinic M_1 and M_2 receptors and their regulation of acetylcholine release

make it difficult to definitively associate the attentional deficits only with muscarinic M_1 receptors. Third, the effects of scopolamine on similar two-lever sustained attention tasks have not always been consistent, with some experiments demonstrating that muscarinic receptor blockade selectively decreases signal detection (Johnson and Burk, 2006; McQuail and Burk, 2006) while other experiments demonstrate that muscarinic receptor blockade decreases accuracy on trials with no signal presentation (Rezvani et al., 2009) or on both signal and nonsignal trials (Bushnell et al., 1997). The experiments presented in these papers vary in deprivation and reward procedures (food or water), in whether the signal duration or signal intensity is varied, and in apparatus (whether there is a noise generator above one of the choice levers). Examination of the effects of dicyclomine with these different methodological procedures would be useful for testing the generalizability of the effects of M_1 receptor blockade observed in the present experiment. If such studies revealed that the effects of dicyclomine, similar to those of scopolamine, vary dependent upon some experimental parameters, then it would be important to investigate which aspects of the procedures bias the sensitivity of the measures in this task that detect the effects of muscarinic receptor manipulations. Finally, a relatively narrow dose range was used with both dicyclomine and chelerythrine chloride. These dose ranges were chosen because, in pilot studies, higher doses resulted in very few lever presses during a testing session with this attention task. In this task, an increase in omissions (failure to press either lever on a trial) can be difficult to interpret, as it may reflect changes in multiple factors, including motivation for reward and motoric functioning.

Previous experiments have found that PKC overactivity can impair working memory (Birnbaum et al., 2004) and that PKC inhibition can attenuate age-related working memory deficits in rats and monkeys (Brennan et al., 2009). Thus, PKC inhibition that 'normalizes' relatively high levels of PKC may be beneficial for restoring some aspects of cognitive processing. The present results may be taken as evidence that abnormally low PKC activity may be associated with attentional deficits. Such a conclusion is consistent with observations from patients with Alzheimer's disease who exhibit a disruption of PKC activity (Battaini et al., 1999; Masliah et al., 1991; Wang et al., 1994) along with attentional deficits (Berardi et al., 2005; Parasuraman and Haxby, 1993). Interestingly, PKC activation has been proposed as a treatment to restore some cognitive deficits in patients with Alzheimer's disease (Sun and Alkon, 2010). Thus, abnormally high or low levels of PKC activity may be detrimental to cognitive processing, and restoring 'normal' levels of PKC activity may be beneficial for alleviating these cognitive deficits. In summary, the present findings support the hypothesis that muscarinic M_1 receptors contribute to normal attentional processing and also support the idea that PKC is involved in some, most likely bottom-up, aspects of performing attention-demanding tasks.

Acknowledgements

The authors wish to thank Usmaan Bashir, Sarah Baum, Will Bleser, Fuad Bohsali, Katy Easterling, Aileen Kim, Brittany Minor, Puja Parekh, Kevin Sethi, Brad Sipe, John Weeks and Bethany Wilson for assistance with behavioral testing.

Funding

This research was supported by grants from the National Institute on Aging (AG030646) and from the Jeffress Memorial Trust.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Aura J, Sirviö J and Riekkinen P (1997) Methoctramine moderately improves memory but pirenzepine disrupts performance in delayed non-matching to position test. *Eur J Pharmacol* 333: 129–134.

Barak S and Weiner I (2007) Scopolamine induces disruption of latent inhibition which is prevented by antipsychotic drugs and an acetylcholinesterase inhibitor. *Neuropsychopharmacology* 32: 989–999.

Barak S and Weiner I (2009) Towards an animal model of an antipsychotic drug-resistant cognitive impairment in schizophrenia: Scopolamine induces abnormally persistent latent inhibition, which can be reversed by cognition enhancers but not by antipsychotic drugs. *Int J Neuropsychopharmacol* 12: 227–241.

Battaini F, Pascale A, Lucchi L, Pasinetti GM and Govoni S (1999) Protein kinase C anchoring deficit in postmortem brains of Alzheimer's disease patients. *Exp Neurol* 159: 559–564.

Berardi AM, Parasuraman R and Haxby JV (2005) Sustained attention in mild Alzheimer's disease. *Dev Neuropsychol* 28: 507–537.

Birnbaum SG, Yuan PX, Wang M, Vijayraghavan S, Bloom AK, Davis DJ, et al. (2004) Protein kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science* 306: 882–884.

Blondel A, Sanger DJ and Moser PC (2000) Characterisation of the effects of nicotine in the five-choice serial reaction time task in rats: Antagonist studies. *Psychopharmacology (Berl)* 149: 293–305.

Brennan AR, Yuan P, Dickstein DL, Rocher AB, Hof PR, Manji H, et al. (2009) Protein kinase C activity is associated with prefrontal cortical decline in aging. *Neurobiol Aging* 30: 782–792.

Broussard JI, Karelina K, Sarter M and Givens B (2009) Cholinergic optimization of cue-evoked parietal activity during challenged attentional performance. *Eur J Neurosci* 29: 1711–1722.

Burk JA (2004) Introduction of a retention interval in a sustained attention task in rats: Effects of presenting a visual distracter and increasing the inter-trial interval. *Behav Processes* 67: 521–531.

Bushnell PJ, Kelly KL and Crofton KM (1994) Effects of toluene inhalation on detection of auditory signals in rats. *Neurotoxicol Teratol* 16: 149–160.

Bushnell PJ, Oshiro WM and Padnos BK (1997) Detection of visual signals by rats: Effects of chlordiazepoxide and cholinergic and adrenergic drugs on sustained attention. *Psychopharmacology* 134: 230–241.

Bymaster FP, Heath I, Hendrix JC and Shannon HE (1993) Comparative behavioral and neurochemical activities of cholinergic antagonists in rats. *J Pharmacol Exp Ther* 267: 16–24.

Caulfield MP (1993) Muscarinic receptors – characterization, coupling and function. *Pharmacol Ther* 58: 319–379.

Chao MD, Chen IS and Chang JT (1998) Inhibition of protein kinase C translocation from cytosol to membrane by chelerythrine. *Planta Med* 64: 662–663.

Doods HN, Mathy MJ, Davidesko D, van Charldorp KJ, de Jonge A and van Zwieten PA (1987) Selectivity of muscarinic antagonists in radioligand and in vivo experiments for the putative M_1, M_2 and M_3 receptors. *J Pharmacol Exp Ther* 242: 257–262.

Everitt BJ and Robbins TW (1997) Central cholinergic systems and cognition. *Annu Rev Psychol* 48: 649–684.

Ferreira AR, Fürstenau L, Blanco C, Kornisiuk E, Sánchez G, Daroit D, et al. (2003) Role of hippocampal M_1 and M_4 muscarinic receptor subtypes in memory consolidation in the rat. *Pharmacol Biochem Behav* 74: 411–415.

Fornari RV, Moreira KM and Oliveira MG (2000) Effects of the selective M_1 muscarinic receptor antagonist dicyclomine on emotional memory. *Learn Mem* 7: 287–292.

Gill TM, Sarter M and Givens B (2000) Sustained visual attention performance-associated prefrontal neuronal activity: Evidence for cholinergic modulation. *J Neurosci* 20: 4745–4757.

Grottick AJ, Haman M, Wyler R and Higgins GA (2003) Reversal of a vigilance decrement in the aged rat by subtype-selective nicotinic ligands. *Neuropsychopharmacology* 28: 880–887.

Gulledge AT, Bucci DJ, Zhang SS, Matsui M and Yeh HH (2009) M_1 receptors mediate cholinergic modulation of excitability in neocortical pyramidal neurons. *J Neurosci* 29: 9888–9902.

Haas MS and Dokas LA (1999) Muscarinic receptor- and phorbol ester-stimulated phosphorylation of protein kinase C substrates in adult and neonatal cortical slices. *Brain Res Dev Brain Res* 114: 89–98.

Hagan JJ, Jansen JH and Broekkamp CL (1987) Blockade of spatial learning by the M_1 musarinic antagonist pirenzepine. *Psychopharmacology (Berl)* 93: 470–476.

Hahn B, Sharples CG, Wonnacott S, Shoaib M and Stolerman IP (2003) Attentional effects of nicotinic agonists in rats. *Neuropharmacology* 44: 1054–1067.

Hains AB, Vu MA, Maciejewski PK, van Dyck CH, Gottron M and Arnsten AF (2009) Inhibition of protein kinase C signaling protects prefrontal cortex dendritic spines and cognition from the effects of chronic stress. *Proc Natl Acad Sci U S A* 106: 17957–17962.

Howe WM, Ji J, Parikh V, Williams S, Mocaër E, Trocmé-Thibierge C, et al. (2010) Enhancement of attentional performance by selective stimulation of alpha4beta2* nAChRs: Underlying cholinergic mechanisms. *Neuropsychopharmacology* 35: 1391–1401.

Johnson RT and Burk JA (2006) Effects of gonadectomy and androgen supplementation on attention in male rats. *Neurobiol Learn Mem* 85: 219–227.

Kondrad RL and Burk JA (2004) Transient disruption of attentional performance following escalating amphetamine administration in rats. *Psychopharmacology (Berl)* 175: 436–442.

Kunysz EL, Michel AD and Whitling RL (1988) Functional and direct binding studies using subtype selective muscarinic receptor antagonists. *Br J Pharmacol* 93: 491–500.

Levey AI, Kitt CA, Simonds WF, Price DL and Brann MR (1991) Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *J Neurosci* 11: 3218–3226.

McCool MF, Patel S, Talati R and Ragozzino ME (2008) Differential involvement of M_1-type and M_4-type muscarinic cholinergic receptors in the dorsomedial striatum in task switching. *Neurobiol Learn Mem* 89: 114–124.

McGaughy J and Sarter M (1995) Behavioral vigilance in rats: Task validation and effects of age, amphetamine, and benzodiazepine receptor ligands. *Psychopharmacology (Berl)* 117: 340–357.

McGaughy J and Sarter M (1998) Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: Effects of physostigmine and FG 7142. *Behav Neurosci* 112: 1519–1525.

McGaughy J, Kaiser T and Sarter M (1996) Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: Selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. *Behav Neurosci* 110: 247–265.

McGaughy J, Sandstrom M, Ruland S, Bruno JP and Sarter M (1997) Lack of effects of lesions of the dorsal noradrenergic bundle on behavioral vigilance. *Behav Neurosci* 111: 646–652.

McQuail JA and Burk JA (2006) Evaluation of muscarinic and nicotinic receptor antagonists on attention and working memory. *Pharmacol Biochem Behav* 85: 796–803.

Masliah E, Cole GM, Hansen LA, Mallory M, Albright T, Terry RD, et al. (1991) Protein kinase C alteration is an early biochemical marker in Alzheimer's disease. *J Neurosci* 11: 2759–2767.

Mirza NR and Stolerman IP (1998) Nicotine enhances sustained attention in the rat under specific task conditions. *Psychopharmacology (Berl)* 138: 266–274.

Mirza NR and Stolerman IP (2000) The role of nicotinic and muscarinic acetylcholine receptors in attention. *Psychopharmacology (Berl)* 148: 243–250.

Newman LA and McGaughy J (2008) Cholinergic deafferentation of prefrontal cortex increases sensitivity to cross-modal distractors during a sustained attention task. *J Neurosci* 28: 2642–2650.

Paine TA, Neve RL and Carlezon WA Jr (2009) Attention deficits and hyperactivity following inhibition of cAMP-dependent protein kinase within the medial prefrontal cortex of rats. *Neuropsychopharmacology* 34: 2143–2155.

Parasuraman R and Haxby JV (1993) Attention and brain function in Alzheimer's disease: A review. *Neuropsychologia* 7: 242–272.

Parasuraman R, Warm JS and Dember WN (1987) Vigilance: Taxonomy and utility. In: Mark LS, Warm JS and Huston RL (eds) *Ergonomics and human factors*. New York, NY: Springer-Verlag.

Quirion R, Wilson A, Rowe W, Aubert I, Richard J, Doods H, et al. (1995) Facilitation of acetylcholine release and cognitive performance by an M(2)-muscarinic receptor antagonist in aged memory-impaired. *J Neurosci* 15: 1455–1462.

Rezvani AH, Bushnell PJ and Levin ED (2002) Effects of nicotine and mecamylamine on choice accuracy in an operant visual signal detection task in female rats. *Psychopharmacology (Berl)* 164: 369–375.

Rezvani AH, Kholdebarin E, Cauley MC, Dawson E and Levin ED (2009) Attenuation of pharmacologically-induced attentional impairment by methylphenidate in rats. *Pharmacol Biochem Behav* 92: 141–146.

Ruotsalainen S, Miettinen R, MacDonald E, Koivisto E and Sirviö J (2000) Blockade of muscarinic, rather than nicotinic, receptors impairs attention, but does not interact with serotonin depletion. *Psychopharmacology (Berl)* 148: 111–123.

Sacchetti B and Bielavska E (1998) Chelerythrine, a specific PKC inhibitor, blocks acquisition but not consolidation and retrieval of conditioned taste aversion in rat. *Brain Res* 799: 84–90.

Sarter M, Draut A, Herzog CD and Bruno JP (2002) Effects of septohippocampal deafferentation on attention and learning. *Abstr Soc Neurosci* 28: 674.8.

Sarter M, Hasselmo ME, Bruno JP and Givens B (2005) Unraveling the attentional functions of cortical cholinergic inputs: Interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Brain Res Rev* 48: 98–111.

Serrano PA, Rodriguez WA, Pope B, Bennett EL and Rosenzweig MR (1995) Protein kinase C inhibitor chelerythrine disrupts memory formation in chicks. *Behav Neurosci* 109: 278–284.

Siomboing X, Gressier B, Dine T, Brunet C, Luyckx M, Cazin M, et al. (2001) Investigation of the inhibitory effects of chelerythrine chloride on the translocation of the protein kinase C betaI, betaII, zeta in human neutropils. *Farmaco* 56: 859–865.

Soares JC, Fornari RV and Oliveira MG (2006) Role of muscarinic M_1 receptors in inhibitory avoidance and contextual fear conditioning. *Neurobiol Learn Mem* 86: 188–196.

Sun MK and Alkon DL (2010) Pharmacology of protein kinase C activators: Cognition-enhancing and antidementic therapeutics. *Pharmacol Ther* 127: 66–77.

Turchi J, Holley LA and Sarter M (1995) Effects of nicotinic selective acetylcholine receptor ligands on behavioral vigilance in rats. *Psychopharmacology (Berl)* 118: 195–205.

Wang HY, Pisano MR and Friedman E (1994) Attenuated protein kinase C activity and translocation in Alzheimer's disease brain. *Neurobiol Aging* 15: 293–298.