Available online at www.sciencedirect.com
SCIENCE DIRECT
Behavioural Brain Research 170 (2006) 148–155
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

Post-training intra-striatal scopolamine or flupenthixol impairs radial maze learning in rats

Glenn Legault [a,d,*], Carlyle T. Smith [b], Richard J. Beninger [a,c]

[a] *Department of Psychology, Queen's University, Kingston, Ont., Canada K7L 3N6*
[b] *Department of Psychology, Trent University, Peterborough, Ont., Canada K9J 7B8*
[c] *Department of Psychiatry, Queen's University, Kingston, Ont., Canada K7L 3N6*
[d] *Department of Neurology, Sunnybrook and Women's College H.S.C., Room M1 600, 2075 Bayview Avenue, North York, Ont., Canada M4N 3M5*

Received 6 September 2005; received in revised form 1 February 2006; accepted 11 February 2006
Available online 20 March 2006

Abstract

Systemic treatments with acetylcholine (ACh) or dopamine (DA) receptor antagonists during hours 0–4 but not during hours 5–8 following training on a radial arm maze (RAM) or lesions of the dorsal striata impair learning. This suggested that intra-striatal infusions of ACh or DA receptor antagonists during hours 0–4 following training may impair learning. Rats were randomly assigned to groups (ns = 5–11) receiving dorsal striatal infusions of the ACh receptor antagonist scopolamine (0–18 μg/μL at 0 and 2 h or at 4 and 6 h after training), the DA receptor antagonist *cis*-flupenthixol (0–25 μg/μL at 0, 4 or 12 h after training) or the inactive isomer *trans*-flupenthixol (6 μg/μL at 0 h after training). Scopolamine and *cis*-flupenthixol impaired the habit-learning version of the task. Given after hours 0–4 following training, the effects of scopolamine were diminished but those of *cis*-flupenthixol were not. *Trans*-flupenthixol produced less impairment than *cis*-flupenthixol. Results suggest that ACh and DA receptors in the dorsal striatum during hours 0–4 following training play a role in habit learning.

Keywords: Acetylcholine; Dopamine; Flupenthixol; Learning; Paradoxical sleep window; Radial maze; Scopolamine; Striatum

In their 1993 paper reporting a triple dissociation, Macdonald and White showed that learning of different tasks on the radial arm maze (RAM) required different brain structures. Learning of a conditioned cue preference (CCP) task required an intact amygdala and learning a win-shift task required an intact hippocampus. Of interest to the current research was the observation that learning of a win-stay task required an intact dorsal striatum. The type of learning undertaken in this latter task requires that the animal forms associations between stimuli and responses, or habit learning. Others have identified the striatum as important to this type of learning [1,2].

Rapid eye movement (REM) sleep has been differentially implicated in learning RAM tasks. It has been shown that rats need to experience REM sleep during a discrete interval, termed the paradoxical sleep window (PSW) [3], following training for successful learning. For a CCP task, REM sleep deprivation during hours 9–13 after training but not earlier impaired memory [4] and for a habit-learning version of the RAM task, REM sleep deprivation during hours 0–4 following training, but not later, impaired learning [5,6]. In conjunction with findings implicating the dorsal striatum in learning the win-stay version of the RAM task by McDonald and White [7] these results suggested that pharmacological manipulations of the dorsal striatum might have greater effects on habit learning when they occurred during the PSW for that task.

McDonald and White [7] showed that the amygdala was required for learning of a CCP task. Kenton and Smith [8] infused the acetylcholine (ACh) receptor blocker scopolamine into the rat amygdala during the PSW (hours 9–13 after training) of a CCP task and reported impaired learning; when the same treatment was given outside of the PSW, it had no significant effect. Systemic injections of scopolamine given during, but not outside of the PSW for a habit-learning RAM task also impaired learning [6]. Animals that received systemic injections of the dopamine (DA) receptor antagonist *cis*-flupenthixol after training also were impaired but the effect extended beyond the PSW

* Corresponding author. Tel.: +1 416 480 6100x2461; fax: +1 416 480 4674.
E-mail address: Glenn.Legault@sw.ca (G. Legault).

0166-4328/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2006.02.010

(unpublished results). These findings implicated ACh during but not beyond the PSW in learning the CPP and habit version of the RAM.

The experiments reported here examined the PSW for the RAM task first described by Smith et al. [5] and later confirmed by Legault et al. [6]. The habit-learning task differed from that used by McDonald and White [7]; however, since PSWs are task and species specific [3], we used the same version of the RAM that was used to determine the PSW. Others have previously used the four-arm baited paradigm to investigate habit learning [2].

Numerous studies have shown that post-training drug injections impair learning. For example, Izquierdo and his coworkers, using the inhibitory avoidance task, implicated a wide range of neurotransmitters and signaling molecules in a number of brain regions in learning by making post-training injections (e.g., [9–11]).

McDonald and White [7] found that electrolytic lesions of the dorsal striatum, but not the hippocampus or amygdala, impaired win-stay learning in the RAM task. In electrophysiological studies in cats, dorsal striatal neurons were found to be active during REM [12]. In combination with our previous findings that ACh or DA receptor antagonists systemically injected during the PSW impaired learning [6], these observations suggested the hypothesis that striatal ACh or DA receptor blockade during the PSW will impair learning the RAM task.

To test this hypothesis, animals received bilateral dorsal striatal infusions of scopolamine or *cis*-flupenthixol following daily training on the RAM. The geometric isomer *trans*-flupenthixol that is inactive at the DA receptors also was tested [13]. The results described here are the first to implicate both cholinergic and dopaminergic neurotransmission within the dorsal striatum during a discrete temporal interval in learning new habits.

1. Methods

1.1. Subjects

Three-month-old male Sprague–Dawley rats (average mass = 303 g, standard deviation = 22 g) were obtained from Charles Rivers Laboratories and allowed to acclimatize to the Trent University Animal Facility for 7 days. Rats were individually housed on soft texture paper chip bedding in opaque plastic cages (45 cm (l) × 25 cm (w) × 20 cm (d)) located in a temperature controlled (21 ± 1.5 °C) colony room maintained on a 12-h light/12-h dark cycle with lights on at 07:00 h. Prior to any experimental manipulation or surgery, animals were handled for 3–5 min each day for 5 consecutive days. The Trent University Animal Care Committee approved all animal-related procedures a priori.

1.2. Surgery

Rats were anaesthetized with halothane and placed onto a stereotaxic frame with the skull level between lamda and bregma. A surgical incision was made and small stainless steel machine screws were placed into the skull to act as anchors for holding the skullcap in place. Holes were drilled at 1 mm anterior to bregma and bilaterally at 2.6 mm from the midline. Using a stainless steel template, 18 gauge (1.25 mm) needles cut to 11 mm in length were inserted to a ventral coordinate of 5.5 mm below the skull surface. Dental acrylic was applied to fix the guide cannulae in place. Sterile stainless steel wire was cut to length and inserted to block the cannulae between infusions. A total of 0.009 mg of buprenorphine hydrochloride for post-operative analgesia was given, as was 0.3 cc of penicillin G to combat post-operative infections. Animals were recovered in a warmed environment for 24 h prior to being returned to their cages where they were allowed to recover for 1 week prior to training.

1.3. Apparatus

1.3.1. Radial arm maze

The RAM, elevated 36 cm above the floor, consisted of a central platform (23 cm diameter) with eight arms (49 cm × 9 cm) radiating from it. Fastened to the floor at the end of each arm was a small food dish (4.4 cm diameter). The central platform and the arms were painted flat grey. The RAM was situated in a room measuring 2.9 m × 2.1 m divided by a black curtain so that the area containing the maze had the dimensions 1.7 m × 2.1 m. That area had posters on each of the remaining three walls to provide visual cues. A Hitachi™ video camera was positioned at the junction between the rod holding the curtain, the ceiling, and one of the walls to monitor the animals on the maze. On the other side of the curtain were a chair, desk and video screen. An opaque plastic cylinder (22 cm diameter × 30 cm high) was placed over the central platform and the experimenter raised this by a pulley system from the monitoring station at the beginning of each trial.

1.4. Drugs

Scopolamine hydrobromide was obtained from Sigma, Oakville, ON and *cis*- and *trans*-flupenthixol was obtained from Lundbeck A/S, Copenhagen. These compounds were diluted with 0.9% saline to concentrations as indicated below.

1.5. Procedure

1.5.1. Food restriction

Three days before training in the RAM, the animals were restricted to approximately 20 g of rat chow per day. On the 1st day of exposure to the RAM the animals were further food restricted to approximately 10 g of rat chow per day plus whatever food they obtained from the maze. The animals' masses were monitored frequently to ensure that their health was maintained. An endpoint mass of 250 g was established and no animals were removed from the study because of weight loss.

1.5.2. Groups

For the scopolamine study, animals were randomly assigned to one of the following groups. Each group had 10 or 11 rats originally assigned but the n's reported in this section indicate the numbers of animals included in the statistical analyses. All injections were intra-striatal:

(i) saline (1.0 μL/side) 0 and 2 h after training (SAL; $n = 10$);
(ii) scopolamine (18 μg/μL/side) 0 and 2 h after training (SCOP 18; $n = 8$);
(iii) scopolamine (4.5 μg/μL/side) 0 and 2 h after training (SCOP 4.5; $n = 9$);
(iv) scopolamine (18 μg/μL/side) 4 and 6 h after training, i.e., outside of the putative PSW (SCOP OW; $n = 8$).

Animals received two injections, spaced 2 h apart, as scopolamine has a half-life of 2.9 h thus ensuring muscarinic blockade during hours 0–4 following training [14].

For the flupenthixol study, animals were randomly assigned to the following groups. The SAL group from the scopolamine study served as controls here also:

(i) *cis*-flupenthixol (25 μg/μL/side) 0 h after training (FLU 25; $n = 5$);
(ii) *cis*-flupenthixol (6 μg/μL/side) 0 h after training (FLU 6; $n = 6$);
(iii) *cis*-flupenthixol (1.5 μg/μL/side) 0 h after training (FLU 1.5; $n = 10$);
(iv) *cis*-flupenthixol (6 μg/μL/side) 4 h after training (FLU 4 h OW; $n = 10$);
(v) *cis*-flupenthixol (6 μg/μL/side) 12 h after training (FLU 12 h OW; $n = 9$);
(vi) *trans*-flupenthixol (6 μg/μL/side) 0 h after training (FLU trans; $n = 11$).

1.5.3. RAM procedure

All behavioural testing took place between 10:00 and 14:00 h. All animals were exposed to the unbaited RAM for 10 min on each of 2 consecutive days. On each of the next 10 days, rats were individually placed onto the central platform

of the RAM and contained there using the plastic cylinder. A trial began when the experimenter, using the pulley system, raised the cylinder. For each rat, the same four arms of the maze for each exposure to the RAM were baited each with one-half of a piece of the breakfast cereal Froot Loops (approximately 0.05 g). The baiting pattern was different from rat to rat. The animal had a maximum of 6 min to find all of the baits within the RAM. The animal was then removed from the maze and subjected to an experimental manipulation as detailed in Section 1.5.2.

1.6. Histology

After the 10th day of training, animals were deeply anaesthetized and decapitated. The brains were removed and stored in a 10% formalin solution for a minimum of 3 days. When fixed, the brains were sliced into 60 μm sections, mounted on gelled slides and stained with thionine. Twenty-four hours after staining, the histological preparation was sealed with a coverslip. The slides were then evaluated by a researcher who was blind to the results for individual rats to determine correct cannulae placements. Animals that did not have cannulae in the dorsal striatum were excluded from further analyses.

1.7. Analyses

The data that were recorded included the latency to the consumption of the last bait or 6 min, whichever occurred first and the number of the arm into which an animal entered. The latter was used to calculate other relevant metrics. In addition to the number of the arms entered, the extent to which an animal travelled along arms in a given trial was also recorded. This information was used to calculate the ratio of food pellets consumed to the total number of completed arm traversals per trial (Eq. (1)). This was termed as the Performance Index (PI); as rats learned the task, its value approached 1. A small number (0.001) was added to the denominator of the PI to avoid the possibility of the denominator equaling zero:

$$\mathrm{PI} = \frac{\text{total no. of baits consumed in a trial}}{\text{total no. of arm entries in a trial} + 0.001} \quad (1)$$

In addition, the numbers of entries into baited and unbaited arms were evaluated so as to investigate such paradigms as working and reference memory.

All statistical analyses were conducted using Statistica '99. The dependent variables described above were subjected to mixed-design analysis of variance (ANOVA). Hartley's F_{max} was calculated and the F-ratios for each within-subjects level were computed to assess the homogeneity of variance assumption. When a significant value of F_{max} was reported for a given day, no post hoc analyses were calculated for that level. Otherwise, significant main effects were evaluated post hoc using Neuman–Keuls pairwise comparisons. Significant between- and within-measure interactions were investigated using ANOVA for simple effects with the planned contrasts interface offered by Statistica '99. For the ANOVA for simple effects calculated on a given day, contrast coefficients were applied to group means such that an omnibus F-ratio was obtained. When those omnibus F-ratios were significant, further post hoc analyses were conducted by applying different contrast coefficients to specific group means within a level to directly compare them. Another F-ratio was obtained in that fashion and when it was significant, it was interpreted that the specific group means being contrasted were different from one another.

For each of the above analyses, we were specifically interested in the performance of the groups receiving the same dose during hours 0–4 following training and beyond hours 0–4 following training. Accordingly, an ANOVA was planned for the two contrasts for each experiment that had the same dosage but on different administration schedules. Similarly, the effect of the inactive isomer *trans*-flupenthixol was directly compared to the active isomer *cis*-flupenthixol when given at the same concentration during hours 0–4 following training.

Parametric statistical tests were supplemented by survival analyses using the latency data. This allowed use of the results from all 10 trials in the analyses. The criterion for censoring an animal within any group was the first time that an animal scored less than 360 s in the latency measure. This would require that the animal consume all four food pellets.

2. Results

2.1. Histology

Animals with signs of infection at the infusion site or with inaccurately placed cannulae were excluded from statistical analyses. Placements of the animals that were included are shown in Fig. 1. See Section 1.5.2 for specific group n's used in the analyses.

2.2. Intra-striatal scopolamine study

The duration of times required each day (maximum 6 min) for a rat to collect all four pellets of food provided one index of learning (Fig. 2A). Data collected during the first 5 days of training were averaged because latencies showed little change during this period in most groups. Results showed that animals in the SAL group learned best, those in the SCOP 18 group failed to learn, with the SCOP 4.5 and SCOP OW being intermediate between the other two. A mixed-design ANOVA revealed a significant main effect of group ($F(3,31) = 28.5$, $p < 0.001$), day ($F(5,155) = 23.1$, $p < 0.001$) and a significant interaction ($F(15,155) = 3.74$, $p < 0.001$). F_{max} testing revealed significant violations of the homogeneity of variance assumption required in using ANOVA and therefore, no post hoc analyses were conducted. Because of the lack of variability in the SCOP 18 group, the planned comparison of that group with the group that received the same dose at hours 4 and 6 following training (SCOP OW) was not carried out. However, it is clear from Fig. 2A that the SCOP OW group learned whereas the SCOP 18 group did not. A one-way within-measures design ANOVA for the SCOP OW group confirmed that it improved over days ($F(5,35) = 10.2$, $p < 0.001$).

A survival analysis (Fig. 3A) was conducted using the 1st day that an animal scored less than 360 s on the latency measure as the censoring criterion. The SCOP 18 group had no animals that ever completed the RAM task in less than 360 s and was removed from this analysis. Results showed that the SAL group learned the task quickly relative to either the SCOP 4.5 or SCOP OW groups. Survival analysis revealed a significant difference among groups ($\chi^2(2, N = 27) = 17.2$, $p < 0.001$). The SAL animals had a median day of 3 and both SCOP 4.5 and SCOP OW had a median day of 9 suggesting that a low dose of scopolamine (4.5 μg) given into the striatum during hours 0–4 following training or a high dose (18.0 μg) given 4 and 6 h following training delayed acquisition of this task; fewer animals in the SCOP 4.5 group met the criteria for censoring compared to the SCOP OW group.

Another index of performance was the PI. Results indicated that all animals showed some improvement over days but that animals receiving the highest concentration of scopolamine (SCOP 18) improved least and the SAL group improved most with the other two groups being intermediate between these two (Fig. 4A). A mixed-design ANOVA showed a significant main effect of group ($F(3,31) = 26.2$, $p < 0.001$) and day ($F(5,155) = 17.1$, $p < 0.001$) but the interaction was not significant ($F(15,155) = 0.908$, $p > 0.05$). Post hoc pairwise

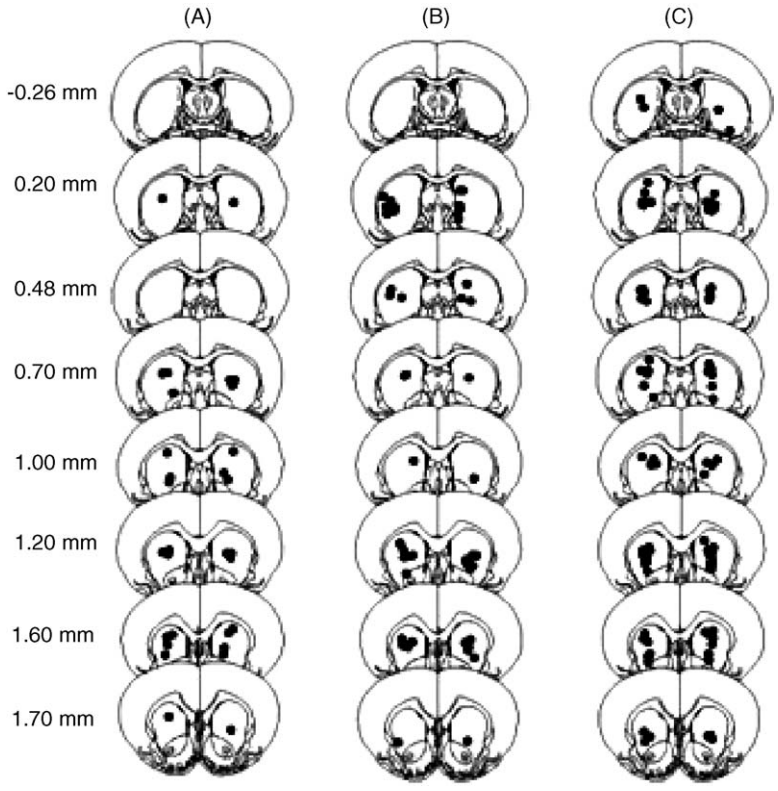

Fig. 1. Illustration of the guide cannulae tip placement. Column A shows animals in the SAL group, column B shows animals in the scopolamine groups and column C shows the flupenthixol groups.

comparisons (Neuman–Keuls) indicated that the SCOP 18 group performed worse than the other three groups and that the three drug groups performed worse than the SAL group. The comparison of the groups receiving the same dose of scopolamine (18 μg/μL/side) either 0 and 2 h or 4 and 6 h after training was not calculated as the results of the Neuman–Keuls test above showed them to differ; the SCOP OW group performed better than the SCOP 18 group. In summary, results indicate that scopolamine administration to the dorsal striatum during hours 0–4 following training impaired acquisition of the RAM task.

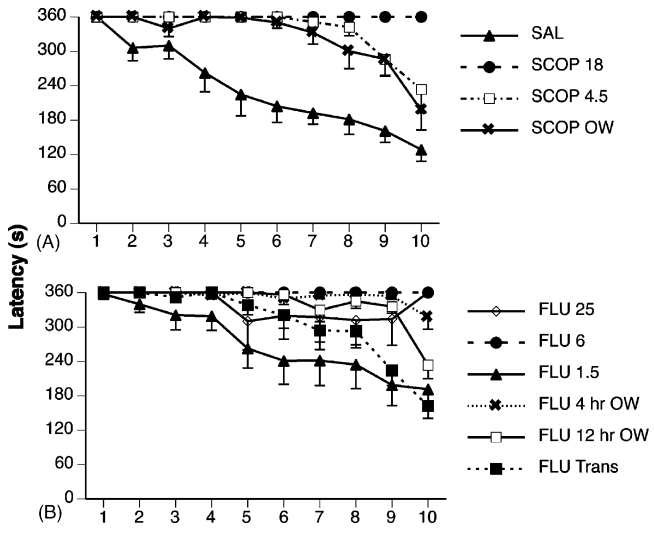

Fig. 2. Latency to completion of the RAM task: (A) scopolamine study and (B) flupenthixol study. *Note*: the same SAL group was used in statistical analyses of the scopolamine and flupenthixol studies and is omitted from Panel B for clarity.

2.3. Intra-striatal flupenthixol study

Fig. 2B shows the latency data for groups in the intra-striatal flupenthixol study. For analysis, Days 1–5 were averaged. While ANOVA showed a significant main effect of group ($F(6,54) = 11.5$, $p < 0.001$), day ($F(5,270) = 20.5$, $p < 0.001$) and a significant interaction ($F(30,270) = 3.35$, $p < 0.001$), F_{max} testing revealed that the assumption of homogeneity required for using ANOVA was violated on all days and accordingly, no post hoc analyses were conducted.

To investigate day-by-day differences, a survival analysis was calculated. As the FLU 6 group had no animals that reached the criteria for completion of the task, it was excluded from the analysis. Results showed that the SAL and FLU 1.5 groups acquired the RAM task faster than all other groups. Survival analysis (Fig. 3B) revealed significant differences among groups ($\chi^2(2,N = 55) = 27.4$, $p < 0.001$). The median day for acquisition of the task was Day 3 for the SAL group, 5.5 for FLU 1.5, Day 8 for FLU trans, Day 9 for FLU 12 h OW and Day 10 for both FLU 25 and FLU 4 h OW. The planned comparison of the *cis*- and *trans*-flupenthixol groups was not conducted, as the FLU 6 group had no animals that ever completed the task. Thus, *cis*-flupenthixol had a greater impact on learning than an equal dose of *trans*-flupenthixol within hours 0–4 following training.

The PI (Fig. 4B) provides another index of learning. Although there was variability over days, there appeared to be two clus-

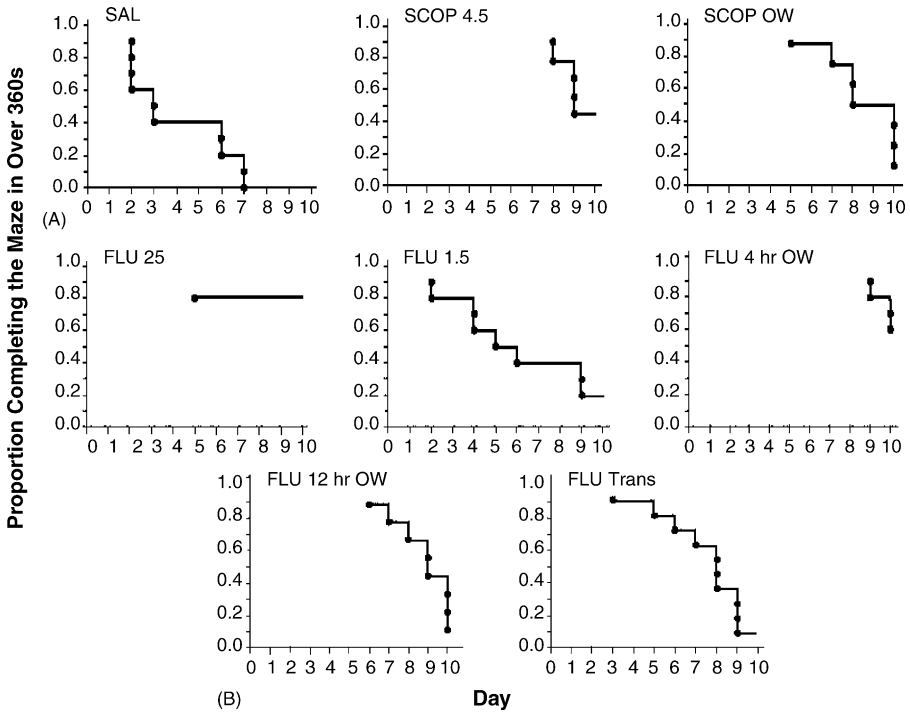

Fig. 3. Kaplan–Meier plots of latency data: (A) intra-striatal scopolamine study and (B) intra-striatal flupenthixol study. *Note*: the same SAL group was used in statistical analyses of the scopolamine and flupenthixol studies and SCOP 18 and FLU 6 groups were excluded from survival analysis.

ters and the SAL group. The SAL group performed best and the FLU 25, FLU 6 and FLU 4 h OW groups performed worst. The FLU 1.5 group, FLU trans and FLU 12 h OW groups performed intermediate between the other cluster and the SAL group. ANOVA revealed a significant main effect of group ($F(6,54) = 7.08$, $p < 0.001$), day ($F(5,270) = 15.5$, $p < 0.001$) and a significant interaction ($F(30,270) = 1.52$, $p < 0.05$). F_{max} testing revealed no significant F-ratios. Neuman–Keuls testing of the main effect of group showed that FLU 25, FLU 6, FLU 4 h OW and FLU 12 h OW groups performed poorly relative to the SAL group while the FLU 1.5 and FLU trans groups did not differ from the SAL group. ANOVA for simple effects post hoc testing showed that group differences existed for each within-measure interval (Days 1–5: $F(1,54) = 38.1$, $p < 0.001$; Day 6: $F(1,54) = 42.0$, $p < 0.001$; Day 7: $F(1,54) = 54.0$, $p < 0.001$; Day 8: $F(1,54) = 73.5$, $p < 0.001$; Day 9: $F(1,54) = 113$, $p < 0.001$; Day 10: $F(1,54) = 114$, $p < 0.001$). For Days 1–5, post hoc analyses showed that the SAL group was different from all groups but the FLU 1.5 group. On Day 6, the SAL group was different from all groups except for the FLU 1.5 and FLU trans groups. Post hoc analysis of the data for Day 7 showed that the SAL animals were different from all groups except the FLU 1.5, FLU trans and FLU 12 h OW groups. Day 8 analyses showed that the SAL group was different from FLU 6 and FLU 4 h OW groups but not significantly different from the FLU 25, FLU 1.5, FLU trans or FLU 12 h OW groups. Day 9 analyses showed that the SAL group was different from FLU 6, FLU 4 h OW and FLU 12 h OW but not different from FLU 25, FLU 1.5 and FLU trans groups. On Day 10, the SAL group was different from all other groups. To assess the performance of groups receiving the same dose of flupenthixol (6 μg/μL/side) but given at different times, groups FLU 6, FLU 4 h OW and FLU 12 h OW were compared. ANOVA showed a main effect of day ($F(5,110) = 5.45$, $p < 0.001$) but a non-significant main effect of group and interaction. The comparison of the inactive isomer versus the active isomer of flupenthixol given at the same dose during hours 0–4 following training yielded a

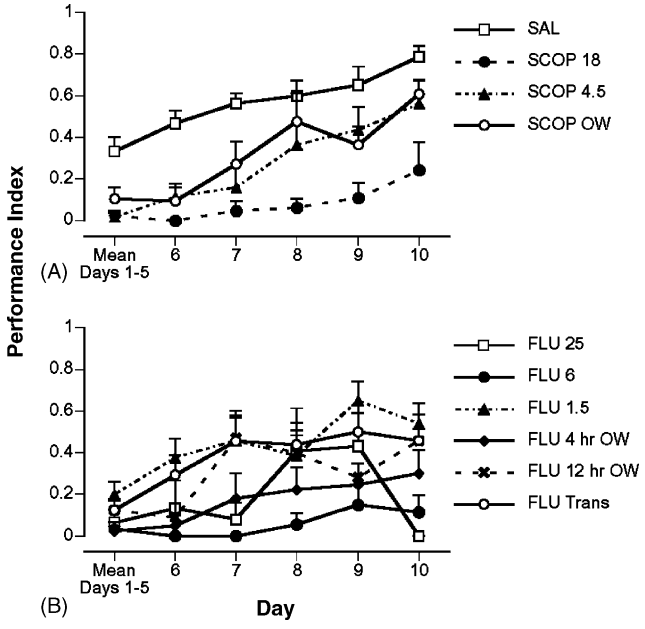

Fig. 4. Ratio of food pellets eaten to total number of completed arm traversals (PI): (A) intra-striatal scopolamine study and (B) intra-striatal flupenthixol study. *Note*: the same SAL group was used in statistical analyses of the scopolamine and flupenthixol studies and is omitted from Panel B for clarity.

Table 1
Group means over all days of training showing the mean ± S.E.M. numbers of unbaited arm entries (UAE) and always baited arm re-entries (BAR)

Group	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 8		Day 9		Day 10	
	UAE	BAR	UAE	BAR	UAE	BAR	UAE	BAR	UAE	BAR	UAE	BAR	UAE	BAR	UAE	BAR	UAE	BAR	UAE	BAR
SAL	1.6 (0.6)	1.7 (0.86)	1.7 (0.52)	1.4 (0.37)	2.0 (0.49)	1.4 (0.70)	1.9 (0.48)	1.8 (0.59)	2.1 (0.55)	2.3 (1.2)	2.1 (0.43)	1.2 (2.9)	2.2 (0.32)	1.1 (0.38)	2.0 (0.33)	1.7 (0.92)	1.8 (0.47)	1.3 (0.75	1.1 (0.31)	0.11 (0.31)
SCOP 18	0.50 (0.38)	0.25 (0.25)	0.88 (0.35)	0.00 (0.00)	0.63 (0.42)	0.13 (0.13)	0.75 (0.53)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.25 (0.25)	0.00 (0.00)	0.88 (0.44)	0.25 (0.25)	0.25 (0.16)	0.25 (0.16)	0.50 (0.38)	0.38 (0.26)	0.75 (0.49)	0.25 (0.25)
SCOP 4.5	0.22 (0.15)	0.33 (0.33)	0.22 (0.15)	0.22 (0.22)	0.44 (0.44)	0.11 (0.11)	0.44 (0.44)	0.00 (0.00)	0.44 (0.34)	0.11 (0.11)	1.0 (0.44)	0.11 (0.11)	0.44 (0.34)	0.22 (0.22)	2.9 (1.4)	1.2 (0.49)	1.2 (0.55)	1.2 (0.57)	1.8 (0.52)	0.67 (0.29)
SCOP OW	0.38 (0.18)	0.25 (0.25)	1.1 (0.58)	0.38 (0.26)	0.75 (0.49)	0.75 (0.53)	1.0 (0.57)	0.75 (0.53)	0.75 (0.53)	0.50 (0.38)	1.3 (0.62)	0.38 (0.26)	1.0 (0.57)	0.38 (0.18)	1.4 (0.63)	0.88 (0.48)	1.5 (0.50)	1.0 (0.38)	2.0 (0.38)	0.63 (0.26)
FLU 25	0.60 (0.60)	0.40 (0.40)	0.00 (0.00)	0.00 (0.00)	0.20 (0.20)	0.00 (0.00)	0.80 (0.58)	0.20 (0.20)	0.00 (0.00)	0.00 (0.00)	0.40 (0.40)	0.00 (0.00)	0.60 (0.60)	0.20 (0.20)	0.4 (0.24)	0.20 (0.20)	0.00 (0.00)	0.40 (0.24)	0.00 (0.00)	0.00 (0.00)
FLU 6	0.33 (0.21)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.17 (0.17)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.17 (0.17)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.50 (0.50)	0.17 (0.17)	0.67 (0.49)	0.00 (0.00)	0.83 (0.54)	0.50 (0.34)
FLU 1.5	1.9 (0.60)	1.4 (0.50)	1.1 (0.28)	1.0 (0.56)	1.8 (0.44)	0.70 (0.33)	2.0 (0.61)	1.9 (0.57)	2.2 (0.39)	1.1 (0.31)	1.1 (0.38)	1.1 (0.43)	1.0 (0.33)	0.60 (0.31)	2.2 (0.47)	1.0 (1.0)	1.0 (0.30)	0.30 (0.15)	1.9 (0.48)	0.9 (0.38)
FLU 4 h OW	1.0 (0.47)	1.3 (0.62)	0.70 (0.58)	0.70 (0.58)	0.40 (0.31)	0.30 (0.30)	0.60 (0.34)	0.20 (0.20)	0.40 (0.31)	0.20 (0.20)	0.60 (0.34)	0.20 (0.13)	0.40 (0.22)	0.00 (0.00)	0.40 (0.22)	0.40 (0.22)	1.1 (0.50)	1.0 (0.45)	1.3 (0.52)	0.20 (0.20)
FLU 12 h OW	1.4 (0.48)	0.56 (0.44)	0.11 (0.11)	0.00 (0.00)	0.67 (0.44)	0.11 (0.11)	0.00 (0.00)	0.22 (0.22)	0.00 (0.00)	0.11 (0.11)	0.22 (0.15)	0.22 (0.22)	0.56 (0.24)	0.22 (0.15)	1.8 (0.49)	0.67 (0.33)	2.7 (0.60)	2.6 (0.80)	2.0 (0.50)	0.56 (0.38)
FLU trans	2.1 (0.48)	1.0 (.38)	2.0 (0.49)	1.1 (0.61)	2.3 (0.53)	1.2 (0.48)	2.3 (0.49)	1.3 (0.45)	2.3 (0.47)	1.4 (0.43)	1.5 (0.47)	0.55 (0.21)	1.2 (0.40)	0.34 (0.20)	2.6 (0.47)	2.1 (0.73)	1.9 (0.44)	1.0 (0.33)	2.8 (0.23)	1.4 (0.34)

significant main effect of group ($F(1,15) = 14.3$, $p < 0.005$) and day ($F(5,75) = 3.00$, $p < 0.05$) and a non-significant interaction ($F(5,75) = 1.34$, $p > 0.05$). Fig. 4B shows that animals receiving the inactive isomer (FLU trans) consistently performed better than the FLU 0.6 group during the latter training sessions. In summary, *cis*- but not *trans*-flupenthixol impaired RAM learning and the effects of *cis*-flupenthixol were dose-dependent. However, the effects of *cis*-flupenthixol were not confined to hours 0–4 following training.

2.4. Working and reference memory

Previous studies have investigated learning of the RAM by quantifying the numbers of entries a rat might make into always baited or always unbaited arms and/or the number of "errors" the animal makes by revisiting an arm from which it previously had taken the food (e.g. [5]). Table 1 shows for each day of training the group means of the numbers of complete arm traversals into arms that were either always unbaited (UAE) or to normally baited arms from which the animal had already consumed the food (BAR).

Examination of Table 1 reveals that animals receiving high doses of the drugs made fewer complete arm traversals than did animals in the other experimental groups suggesting that animals in the high-dose groups were, in addition to not learning the task, also not exploring the maze. However, the animals' activity that included partial forays or the extension of the animals' heads and/or front paws into an arm was not included in Table 1. To explore the possibility that animals in these groups were demonstrating locomotor deficits, several derived metrics were statistically evaluated. None of the resultant analyses suggested that the animals' overall activity was reduced as a function of drug administration (calculations not shown).

It is important to note that we studied *acquisition* of the RAM task in the present experiments whereas most studies that evaluate working and reference memory use well-trained rats. Before the rats have attained knowledge of the baited and unbaited arms (reference memory) it is not meaningful to classify errors as of the working or reference memory type (see Section 3). Thus it is not surprising that Table 1 revealed no systematic group differences for either type of arm entry and that statistical analysis revealed no significant effects.

3. Discussion

Results showed that blocking cholinergic or dopaminergic receptors within the dorsal striatum after training led to deficits in learning of the RAM task. Findings suggested that cholinergic and dopaminergic neurotransmission within the striatum is required during the first 4 h following training and that dopaminergic neurotransmission is required for an interval beyond the first 4 h after training for normal learning to occur. As treatments were given after training, they were unlikely to have affected sensory-perceptual, motor or motivational functions during the next training session given on the following day.

The RAM task used here, involving consistently baiting four of the eight arms, has frequently been used to assess working

and reference memory [15]. However, assessments of different types of memories are usually made in well-trained rats (e.g., [16]). By definition, reference memory refers to information that is useful for all trials whereas working memory refers to information that is useful only for a single trial [15]. In well-trained rats, information about the subsets of four of the eight arms that are consistently baited and unbaited constitutes reference memory; information about which of the four baited arms had already been visited on any trial constitutes working memory. *Before a rat had learned that there were two subsets of arms, it would not be possible to differentiate the two types of memory*. Thus, we studied *acquisition* of the RAM task. The present results provide few relevant data for discussing working and reference memory.

It might be argued that impaired learning observed in the groups receiving drugs immediately after training resulted from impaired exploration of the maze, given the role of the basal ganglia in motor control [17]. However, the greatest impairment was seen in groups that received intra-striatal scopolamine or flupenthixol immediately after training and less impairment was seen in the groups that received the drugs later after training. As these latter groups would have received drug injections closer to the time of testing on the next day but learned better, it cannot be the case that the drugs directly produced a lack of exploration leading to impaired learning.

Intra-striatal injections of *cis*-flupenthixol dose-dependently impaired RAM learning and injection of an effective dose (6 μg/μL/side) immediately or 12 h after training impaired learning implicating striatal dopamine in learning of the RAM task. The dopaminergic nature of this effect was confirmed by the observation that the group receiving *trans*-flupenthixol during hours 0–4 following training at a dose comparable to that of *cis*-flupenthixol (6 μg/μL/side) showed significantly less impairment. The further observation that the FLU trans group performed significantly less well than the SAL group may reflect the anti-cholinergic actions of this compound. Both *cis*- and *trans*-flupenthixol have anti-cholinergic effects but the *trans* isomer is more potent in this regard [13]. The finding that the *cis* isomer produced a greater impairment of learning than the *trans* isomer when comparable doses were injected immediately following training rules out the possibility that the actions of *cis*-flupenthixol can be attributed to the blockade of muscarinic receptors. Results implicate dopaminergic neurotransmission in the dorsal striatum immediately after and for some time following daily training in the RAM.

McDonald and White [7] reported a triple dissociation among the dorsal striatum, amygdala and hippocampus in mediating habit learning, the learning of biologically significant events and relationships between stimuli and events, respectively; habit learning was studied using a modified RAM task. Others have demonstrated that the striatum is important for habit learning. For example, using a lever pressing paradigm, Yin et al. [18] showed that dorsolateral striatal lesions impaired habit learning. Faure et al. [19] extended those observations by implicating DA neurotransmission within the nigrostrial pathway in the formation of habits. Clearly, the dorsal striatum is required for tasks that require the learning of stimulus–response associations. Our results confirm the importance of the striatum and of DA in this type of learning.

The RAM task used in the present study had similarities to that used by McDonald and White [7] in their win-stay paradigm. We showed that dorsal striatal cholinergic and dopaminergic neurons contributed to the learning of reinforced stimulus–response associations in agreement with McDonald and White [7] finding for this structure. This is also consistent with Faure et al. [19] who implicated striatal DA in learning this type of task. We further showed that the learning impairment produced by post-training ACh receptor blockade was most pronounced when applied in the 4 h immediately following training. As REM sleep deprivation during the same interval (i.e., the first 4 h following training) impaired learning [6], perhaps ACh in the dorsal striatum is involved in the consolidation that putatively occurs during REM.

The PSW for the RAM task used here was discovered by Smith et al. [5] and confirmed by Legault et al. [6]. Systemic injections of scopolamine during but not after the PSW impaired acquisition implicating cholinergic neurotransmission during the PSW in learning [6]. Electrophysiological studies have shown that cholinergic neurons located in the midbrain are active during REM sleep [21]. Our present observation that scopolamine injected into the dorsal striatum during the period corresponding to the PSW produced a dose-dependent and significantly greater impairment than similar injections of the effective dose outside of the PSW suggests that cholinergic neurotransmission in the dorsal striatum may play a critical role in memory consolidation that putatively occurs during the PSW. It is noteworthy that differential contributions of striatal subregions to memory have been reported (e.g., [20]). Whether regional variation in the mnemonic effects of dorsal striatal microinjections given in the 4 h period following training also will be found will have to await further study.

It is important to note that the present findings do not directly link neuronal activity during REM sleep and consolidation processes within the dorsal striatum. Previous studies have reported that REM sleep deprivation during but not following the interval described here impair RAM learning [5,6]. Electrophysiological studies in the cat have shown that striatal neurons are active during REM [12]. It will be the task of future studies to assess the effects of ACh and DA receptor antagonists on striatal neuron activity during REM.

Others have found learning impairments as a result of post-training central infusion of various agents; however, a link to REM was not hypothesized. For example, Farr et al. [22] showed that muscarinic antagonists, given into the hippocampus immediately following training on a footshock avoidance task, impaired learning. Barros et al. [11] infused scopolamine into the hippocampus of rats 4 min after one-trial step-down inhibitory avoidance training and found that it impaired long-term memory. Perhaps these findings of impaired learning following post-training treatments will also be found to be consistent with a role for REM in memory consolidation.

In conclusion, the present results showed that for normal RAM learning to occur cholinergic and dopaminergic neurotransmission within the dorsal striatum is required immediately

following training. Also for normal RAM learning to occur, REM sleep is required during hours 0–4 (the PSW) after training [5,6]. Results suggest the possibility that activity at ACh and DA receptors may be required during REM for learning to occur.

Acknowledgement

Funded by a grant from the Natural Sciences and Engineering Research Council of Canada to R.J.B.

References

[1] Udo T, Ugalde F, DiPietro N, Eichenbaum HB, Kantak KM. Effects of persistent cocaine self-administration on amygdala-dependent and dorsal striatum-dependent learning in rats. Psychopharmacology 2004;147(2):237–45.
[2] Sakamoto T, Okaichi H. Use of win-stay and win-shift strategies in place and cue tasks by medial caudate putamen (MCPu) lesioned rats. Neurobiol Learn Mem 2001;76(2):192–208.
[3] Smith CT. Sleep states and learning: a review of the animal literature. Neurosci Biobehav Rev 1985;9(2):157–68.
[4] Vallance K, McDonald RJ, Smith C. Effects of paradoxical sleep on the memory for a conditioned cue preference task in rats. Sleep 1999;22:S243.
[5] Smith CT, Conway JM, Rose GM. Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. Neurobiol Learn Mem 1998;69:211–7.
[6] Legault G, Smith C, Beninger RJ. Scopolamine during the paradoxical sleep window impairs radial arm maze learning in rats. Pharmacol Biochem Behav 2004;79(4):715–21.
[7] McDonald RJ, White NM. A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. Behav Neurosci 1993;107(1):3–22.
[8] Kenton L, Smith C. Intra-amygdala scopolamine infusions during a paradoxical sleep window impairs conditioned cue preference acquisition. Actas Fisiol 2001;7:124.
[9] Vianna MRM, Barros DM, Silva T, Choi H, Madche C, Rodrigues C, et al. Pharmacological demonstration of the differential involvement of protein kinase C isoforms in short- and long-term memory formation and retrieval of one-trial avoidance in rats. Psychopharmacology 2000;150:77–84.
[10] Barros DM, Mello e Souza T, de Souza MM, Choi H, DeDavid e Silva T, Lenz G, et al. LY294002, an inhibitor of phosphoinositide 3-kinase given into rat hippocampus impairs acquisition, consolidation and retrieval of memory for one-trial step-down inhibitory avoidance. Behav Pharmacol 2001;12(8):629–34.
[11] Barros DM, Pereira P, Medina JH, Izquierdo I. Modulation of working memory and of long- but not short-term memory by cholinergic mechanisms in the basolateral amygdala. Behav Pharmacol 2002;13:163–7.
[12] Sarkadi A, Tram Anh DT, Nagy A, Tomka I. Activity of the corpus striatum of cats during natural sleep: a correlation analysis study. Acta Physiol Acad Sci Hung 1974;45(3–4):233–42.
[13] Moller Nielson I, Pedersen V, Nymark M, Franck KF, Boeck V, Fjalland B, et al. The comparative pharmacology of flupenthixol and some reference neuroleptics. Acta Pharmacol 1973;33:353–62.
[14] Benet LZ, Oie S, Schwartz JB. Design and optimization of dosage regimens; pharmacokinetic data. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Gilman AG, editors. Goodman & Gillman's The pharmacological basis of therapeutics. 9th ed. New York: McGraw Hill; 1996. p. 1707–92.
[15] Olton DS, Becker JT, Handelmann GE. Hippocampal function: working memory or cognitive mapping? Physiol Psychol 1980;8:230–46.
[16] Wirsching BA, Beninger RJ, Jhamandas K, Boegman RJ, El-Defrawy SR. Differential effects of scopolamine on working and reference memory of rats in the radial maze. Pharmacol Biochem Behav 1983;20:659–62.
[17] Groenewegen HJ. The basal ganglia and motor control. Neural Plast 2003;10(1–2):107–20.
[18] Yin HH, Knowlton BJ, Balleine BW. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. Eur J Neurosci 2004;19(1):181–9.
[19] Faure A, Haberland U, Conde F, El Massioui N. Lesion to the nigrostriatal dopamine system disrupts stimulus–response habit formation. J Neurosci 2005;25(11):2771–80.
[20] Reading PJ, Dunnett SB, Robbins TW. Dissociable roles of the ventral, medial and lateral striatum on the acquisition and performance of a complex visual stimulus–response habit. Behav Brain Res 1991;45(20):147–61.
[21] Steriade M, McCarley RW. Brainstem control of wakefulness and sleep. 1st ed. NY: Plenum;1990.
[22] Farr SA, Flood JF, Morley JE. The effect of cholinergic, GABAergic, serotonergic, and glutamatergic receptor modulation on posttrial memory processing in the hippocampus. Neurobiology of Learning and Memory 2000;73:150–167.