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Inhibition of cyclin-dependent kinase 5 by roscovitine impairs memory consolidation and reconsolidation in the day-old chick

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ABSTRACT

Cyclin-dependent kinase 5 (CDK-5) is reported to phosphorylate the NMDA receptor prior to the induction of long-term potentiation (LTP), among its many other effects. Application of CDK-5 inhibitors disrupts LTP and results in impaired task acquisition in behaving animals. In this study, we investigated the effect of exogenously applied roscovitine, a potent CDK-5 inhibitor, on consolidation and reconsolidation processes in day-old male chicks. New Hampshire×White leghorn cockerels were trained using a modified version of the passive avoidance learning task. Intracranial injections of roscovitine (2.5 μM) administered immediately after training induced a memory deficit that evolved from 5-minute post-training and persisted until at least 24 h following training. Injections of roscovitine (2.75 μM) administered immediately after the reminder trial induced a memory deficit observed by 30-minute post-reminder which had resolved by 24 h following the reminder. The comparison between consolidation and reconsolidation demonstrates differences both in the time of the onset of the memory deficit as well as in the permanence of this deficit. The results suggest an important, although different role for CDK-5 in consolidation and reconsolidation processes following passive avoidance learning.

1. Introduction

Consolidation and reconsolidation processes have been extensively studied in the day-old chick. Studying these two processes in tandem provides a fuller view of the dynamic nature of memory formation. The time course for the early stages of memory consolidation is well established (Ng and Gibbs, 1991; Rose, 2004). Some features of early consolidation in day-old chicks are consistent with *N*-methly-D-aspartate (NMDA) dependent long-term potentiation (LTP), a model commonly believed to reflect the process underlying physiological learning. In this model, LTP is induced by activation of NMDA receptors leading to lasting synaptic changes (Bliss and Collingridge, 1993). Research has reported an enhanced release of glutamate (Daisley and Rose, 2002) in the left intermediate medial mesopallium (IMM), an area that has been shown to be metabolically activated by passive avoidance learning (Rose and Csillag, 1985; Sedman et al., 1991), and an increase in calcium concentration in isolated synaptoneurosomes (Salinska et al., 1999). However, inhibiting passive avoidance learning with NMDA antagonists induces a memory deficit which is beyond the time expected to correlate with LTP induction (Burchuladze and Rose, 1992; Rickard et al., 1994). This suggests either that NMDA receptors are not involved in task acquisition in day-old chicks, as would be expected if the process was LTP dependent, or that LTP in the day-old chick may be NMDA receptor independent.

Many molecular events have to occur before the Ca^{2+} influx in order to "prepare" the cell for synaptic potentiation (Sweatt, 2003). One kinase proposed to participate in the events prior to actual potentiation is Cyclin-dependent kinase 5 (CDK-5) (Wang et al., 2003). Among its many hypothesised roles in learning and memory (see Angelo et al., 2006 for a review), CDK-5 phosphorylates the NMDA receptor subunit NR2 (Wang et al., 2003), which leads to an increase in NMDA receptor conductance (Li et al., 2001). Application of the potent CDK-5 inhibitor, roscovitine, has been shown to significantly inhibit LTP induction in rat hippocampal slices (Li et al., 2001). Studies examining CDK-5 activity by application of inhibitors of the enzyme in behaving animals also report that the kinase is necessary for both associative learning (Fischer et al., 2002) and contextual fear conditioning in mice (Fischer et al., 2003). Although there are eleven identified CDKs, only CDK-5 and 11 are directly involved in regulation of neuronal activity (Angelo et al., 2006).

The current study examined the effect of inhibiting CDK-5 activity on the consolidation and reconsolidation of passive avoidance learning. While there are many other memory processes involved in trace formation than these two, the passive avoidance learning task is well suited for the study of consolidation and reconsolidation in particular. This is because the event related to each (i.e. training or reminder) occurs

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within a discreet time window and memory deficits observed "upstream" can be directly attributed to the intervention applied contingent upon the training or reminder trial. Additionally, many drugs shown to inhibit memory consolidation in the chick have also been demonstrated to disrupt reconsolidation processes (Anokhin et al., 2002; Summers et al., 2003). This indicates that consolidation and reconsolidation processes may induce similar physiological processes (Nader, 2003; Sara, 2000). However, more recent studies have demonstrated that although similar, the two processes are not identical (see Crowe et al., 2008 for a recent review), perhaps reflecting the different task requirements of consolidation versus reconsolidation.

2. Method

2.1. Animals and experimental housing

Male day-old New Hampshire×White Leghorn chickens were obtained from a local hatchery on the morning of each experiment. Chicks were housed in pairs to eliminate confounds such as stress from social isolation (Andrew, 1991). Wooden boxes (20×25×20 cm) were maintained at a temperature of between 26 and 29 °C by a single 25 W white incandescent bulb suspended above each pair of chicks. Chick mash was made available *ad libitum*. Each data point was initially comprised of 20 chicks, but varied according to the number of birds in each group that successfully trained. Approximately 10% of the sample was excluded on this basis in a non-dose specific manner, consistent with previous research in our laboratory (e.g. Crowe and Hale, 2002). Cockerels are always employed in these experiments as they are excess to food production of this egg laying strain.

2.2. Drug administration and preparation

Roscovitine (2-(1-ethyl-2-hydroyethylamino)-6-benzylamino-9-isopropylpurine) was administered intracranially into the forebrain using a Hamilton repeated dispensing syringe. A plastic stopper regulated the injection depth to 3.5 mm. The target injection region was the IMM and the location of the injection site was determined using bony landmarks on the skull. Doses were prepared in DMSO to a total injection volume of 10 µl to each hemisphere. Control animals received DMSO only. The dose ranges were chosen based on previous research using rodent models of learning and memory (Fischer et al., 2002, 2003). Although there are other inhibitors of CDK-5 available, roscovitine was selected as it is the one most commonly used in behavioural models of memory. Additionally, the other available drugs are reported to have adverse effects on energy production. All drug injections were made blind, and the codes were not broken until after the behavioural data had been collected. Drugs were obtained from Sigma Chemicals (Sydney, Australia).

2.3. Procedure

The experimental protocol was approved by the La Trobe University Animal Ethics Committee (AEC04/35(P)/V6) and the procedures used were in compliance with the National Health and Medical Research Council of Australia Guidelines for Care and Use of Laboratory Animals. Every effort was made to minimise pain and stress on the animals. Chicks were trained on a modified version of the single-trial passive avoidance task (Crowe and Hale, 2002), which compares baseline levels of pecking at a pretraining bead to the pecking response at the test bead. The first series of experiments involved three components: pretraining, training and retention. The second series, examining reconsolidation, included an additional reminder trial.

2.3.1. Pretraining

Pretraining of the chicks occurred in two phases. A chrome bead (2 mm diameter) coated in water was presented to each chick for approximately 10 s to encourage the natural tendency of the chick to peck at bright, shiny objects. The procedure was repeated 20 min later to ensure optimal conditions for training. A water coated red bead (4 mm diameter) was then presented to the chicks, again for a duration of 10 s, with the number of pecks at this bead recorded on a behavioural event recorder connected to an on-line computer. The number of pecks at this bead acted as the chick's baseline level of pecking.

2.3.2. Training: experimental group

Upon completion of the pretraining phase, the experimental chicks were trained to avoid a red bead visually identical to the one used in the pretraining trial, but coated in concentrated (i.e. 100%) methylanthranilate (MeA). Chicks that peck at the aversive bead show a disgust reaction that includes behaviours such as bill wiping, head shaking and distress calls. As noted above, chicks that failed to peck at the training bead were excluded from later analysis.

2.3.3. Control group

Upon completion of the pretraining phase, the control chicks were trained on a water coated red bead visually identical to the stimulus used in the pretraining trial. Training on a water coated bead allows the determination of how the administration of the drug affects pecking rate independent of any effect that the drug may have on memory. This is particularly important when avoidance ratios are used as the dependent variable as, if the drug produces sedation, the birds will exhibit high levels of avoidance which would not be due to any effect on memory *per se*.

Chicks that failed to peck at the training bead within a 10-second period in either the MeA or water training conditions were excluded from the later analysis, as they are demonstrated not to have learned the task.

2.3.4. Reminder (reactivation) trial

Memory reconsolidation for the learned stimulus was activated by a reminder trial that involved the presentation of a visually identical dry red bead to that used in training and exposed for approximately 10 s (see Summers et al., 2003 for a full description of this method). Possible lateralisation effects (Gibbs et al., 2003) were avoided by ensuring that the bead was seen with both eyes. Chicks were not permitted to peck at the bead, thus avoiding the possibility of a new trace (i.e. the association between the bead and the absence of its reinforcement properties, or an extinction trial) being initiated. With the presentation of the reminder stimulus, chicks reacted with distress behaviour, indicating that the presentation of the dry bead was a sufficient stimulus to reactivate the memory for the original learned experience. An initial reminder time of 120-minute post-training was selected as it was deemed sufficiently temporally distant from the training trial as not to interfere with the earlier labile stages of memory (Summers et al., 2003). Additionally, research using the day-old chick has indicated that by this time, the trace has undergone sufficient stabilisation to be considered in the "long-term store" (Gibbs, 1991). In subsequent experiments the reminder time was varied according to the specific experimental protocols.

2.3.5. Retention trial

Retention for the task was measured by presenting the chicks with a dry red bead at various times following the training or the reminder trial, as stipulated by the specific experimental protocol. The dependent variable (avoidance ratio) was calculated as the number of pecks at the red pretraining bead divided by the number of pecks at the red test bead plus the number of pecks at the red pretraining bead (AR=peck pre/peck pre+peck test). Typically, a low avoidance ratio (i.e. AR=0.5) is taken to be indicative of a memory deficit. Statistical

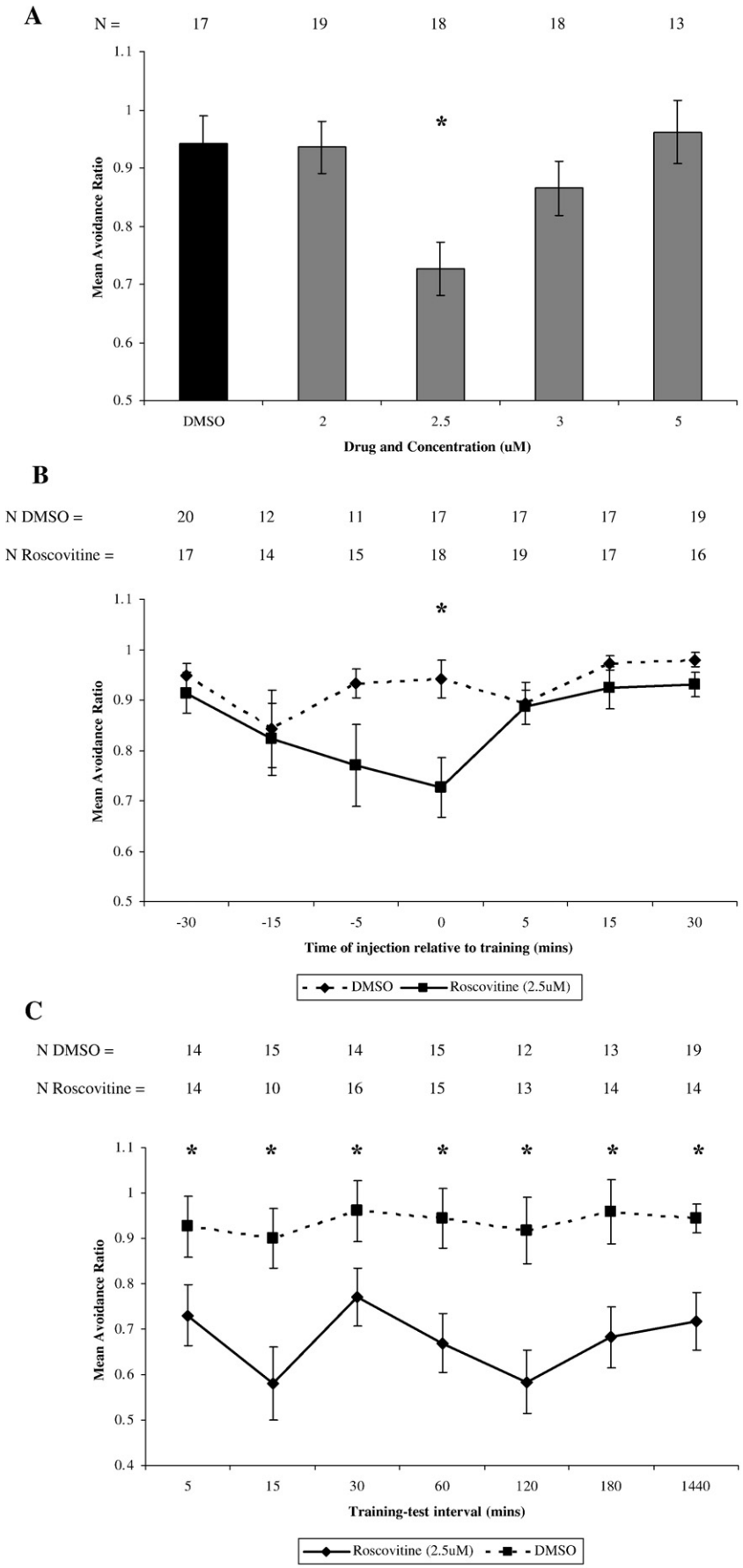

Fig. 1. Mean avoidance ratios of chicks treated with Roscovitine or DMSO. Dose response curve (A), time of injection relative to training (B) and retention at various training-test intervals (C) (100% MeA) (±SEM) (*$p<0.05$).

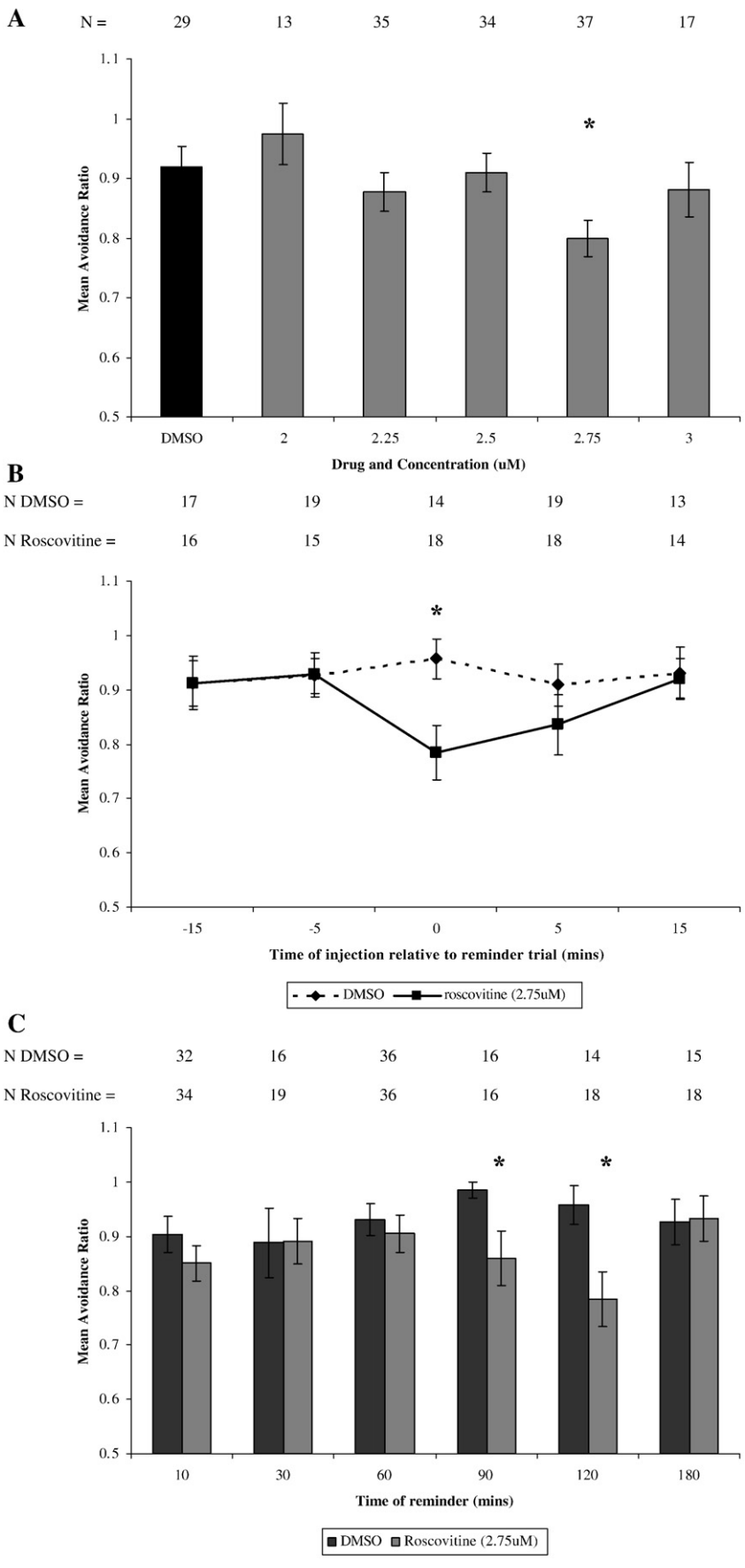

Fig. 2. Mean avoidance ratios of chicks administered Roscovitine or DMSO in association with memory reactivation. Dose response curve (A), time of injection relative to training (B), effect of varying the time of reminder presentation (C) and retention levels at various reminder-test intervals (D) (100% MeA) (±SEM) (*$p<0.05$).

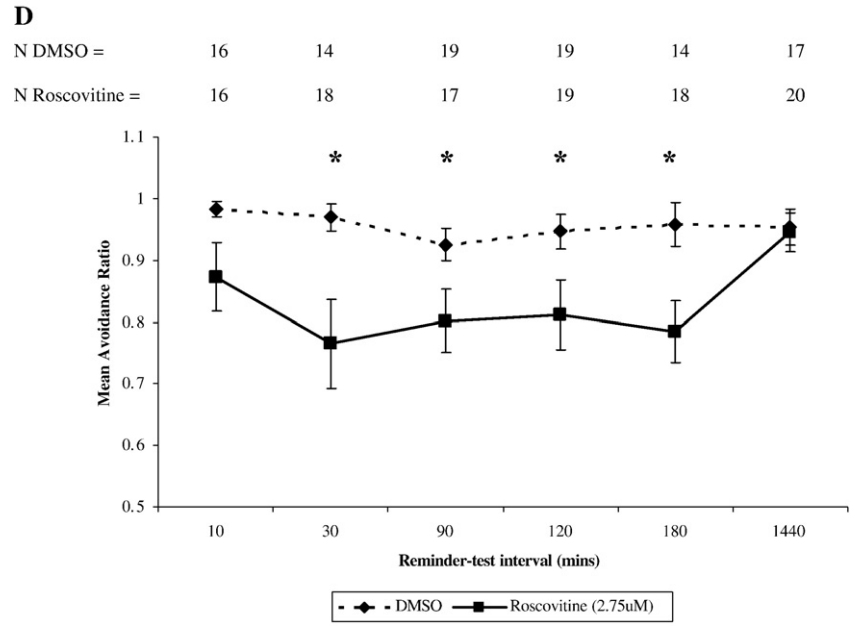

Fig. 2 (*continued*).

analysis was undertaken by univariate ANOVA with post hoc Dunnett's tests.

3. Results

3.1. Experimental series 1: inhibition of CDK-5 by roscovitine disrupts consolidation of passive avoidance learning in the day-old chick

The effect of inhibition of CDK-5 on consolidation was investigated by the application of roscovitine. Chicks were administered various doses of roscovitine (2, 2.5, 3 and 5 μM) or DMSO immediately after training to establish the most effective dose that induced memory disruption. Retention was tested at 180 min after training (see Fig. 1a).

Univariate ANOVA revealed a significant effect of dose on memory retention ($F(4,84)=4.128$, $p=0.004$, $\eta^2=0.171$). A post hoc Dunnett's test revealed a significant difference between chicks treated with DMSO and those that received a 2.5 μM dose of roscovitine ($p=0.006$). This dose was used in subsequent experiments.

A time of injection study was completed to examine if there was a window of sensitivity for drug administration. Chicks were administered roscovitine (2.5 μM) or DMSO at various times before and after training (−30, −15, −5, +0, 5, 15, 30 min) and retention was measured at 180 min following training (see Fig. 1b).

Comparisons between DMSO- and roscovitine-treated chicks at each time of injection revealed a significant difference with an injection made immediately after training ($t(28.213)=-3.043$, $p=0.005$).

To explore the parameters of roscovitine-induced memory deficit, retention levels were measured at various training-test intervals (TTIs). Chicks were administered roscovitine (2.5 μM) or DMSO immediately after training and retention was tested at various TTIs (5, 15, 30, 60, 120, 180 min and 24 h) (see Fig. 1c).

Comparisons between DMSO- and roscovitine-treated chicks at each TTI revealed a significant memory deficit at 5 ($t(17.063)=-2.203$, $p=0.042$), 15 ($t(14.124)=-3.149$, $p=0.007$), 30 ($t(17.412)=-2.381$, $p=0.029$), 60 ($t(17.887)=-3.8$, $p=0.001$), 120 ($t(22.350)=-2.505$, $p=0.02$), 180 ($t(15.259)=-2.736$, $p=0.015$) and 24 h ($t(19.132)=-3.239$, $p=0.004$) post-training.

The experimental series was repeated using water-trained controls. This measure was undertaken to control for any non-specific effects of the drug that may have impacted on the bird's ability to peck. No significant effects were detected with water-trained chicks at any of the doses tested ($F(3,60)=2.528$, $p>0.05$, $\eta^2=0.112$), at any time of injection ($F(1,60)=0.777$, $p>0.05$, $\eta^2=0.013$), or at any training-test interval ($F(3,127)=0.146$, $p>0.05$, $\eta^2=0.03$). This indicates that the administration of the roscovitine did not affect the bird's ability to peck or attend, and the memory deficits observed were exclusively associated with memory related processes.

3.2. Experimental series 2: CDK-5 inhibition via roscovitine administration disrupts "reconsolidation" of reactivated memories in the day-old chick

The second series of experiments examined the effect of roscovitine on reactivated memories. Experiment 1 examined the effect of various doses of roscovitine on memory. This experiment was completed as previous research has indicated that the concentration of drug required to inhibit consolidation may be different to that required to inhibit reconsolidation (Anokhin et al., 2002).

Chicks were administered various doses of roscovitine (2.0, 2.25, 2.5, 2.75 and 3.0 μM) or DMSO immediately after the reminder trial, which was presented at 120 min following training. Retention was tested at 180 min following the reminder trial (see Fig. 2a).

Univariate ANOVA revealed a significant effect for drug treatment ($F(5,164)=2.445$, $p=0.036$, $\eta^2=0.071$). Post hoc Dunnett's tests indicated that the memory retention of chicks that received a dose of 2.75 μM dose of roscovitine were significantly different ($p=0.046$) from those that were administered DMSO.

A time of injection study was then undertaken to determine if the period of drug sensitivity was the same in the context of a reminder trial as it was in the context of initial training. Chicks received DMSO or roscovitine (2.75 μM) at various times before and after (−15, −5, 0, +5, +15 min) the reminder trial, which was presented at 120 min following training. Retention was tested at 180-minute post-reminder (see Fig. 2b).

Comparisons between the DMSO- and roscovitine- treated chicks at each time of injection revealed a significant difference between the groups that received the injection immediately after training ($t(153)=-2.733$, $p=0.007$).

It was also of interest to determine whether the reconsolidation deficit observed was dependent on the time of the reminder trial. Chicks were given DMSO or roscovitine immediately after a reminder

trial that was presented at various times post-training (10, 30, 60, 90, 120 and 180 min). Retention was tested at 180-minute post-reminder (see Fig. 2c).

Comparisons between DMSO- and roscovitine- treated chicks at each time of reminder revealed significant differences between the groups that received a reminder trial at 90 ($t(17.697)=2.626$, $p=0.017$) and 120 min ($t(28.945)=2.807$, $p=0.009$) after training.

To explore the parameters of roscovitine-induced memory deficit following the reminder trial, retention levels were determined post-reminder. Chicks were administered roscovitine or DMSO immediately after the reminder trial at 120-minute post-training and retention was tested at various reminder-test intervals (10, 30, 90, 120, 180 min and 24 h) (see Fig. 2d) post-reminder.

Comparisons of DMSO- and roscovitine- treated chicks at each reminder-test interval revealed significant differences between the groups at 30 ($t(20.11)=2.712$, $p=0.013$), 90 ($t(24.64)=2.148$, $p=0.042$), 120 ($t(27.24)=2.141$, $p=0.041$), and 180 min ($t(28.95)=2.807$, $p=0.009$) post-reminder.

To control for the presentation of the reminder trial, an additional experiment was conducted to examine the effect of roscovitine on birds that did not receive a memory reactivation trial. These chicks received roscovitine or DMSO at 120-minute post-training in the absence of a reminder trial and retention was measured at 180-minutes post-reminder (see Fig. 3).

Univariate ANOVA revealed a significant main effect for the presentation of a reminder trial ($F(1,72)=12.386$, $p=0.001$, $\eta^2=0.152$) and a significant interaction effect between drug and reminder conditions ($F(1,72)=6.531$, $p=0.031$, $\eta^2=0.086$). Independent samples *t*-test comparing chicks that received DMSO with those that received roscovitine following the reminder trial confirmed significant differences between these groups ($t(36)=2.445$, $p=0.02$).

The experimental series was again repeated using water-trained controls. No significant effects were detected with water training across any of the doses tested ($t(33)=-0.568$, $p>0.05$), at any time of injection interval ($F(2,69)=0.685$, $p>0.05$, $\eta^2=0.021$), time of reminder ($F(2,72)=0.516$, $p>0.05$, $\eta^2=0.015$), when no reminder was presented ($t(18)=-1.352$, $p>0.05$) or at any reminder-test interval ($F(4,102)=0.836$, $p>0.05$, $\eta^2=0.035$). This indicates that roscovitine administration did not affect the bird's ability to peck or attend, and that the memory deficits observed were exclusively associated with memory processes.

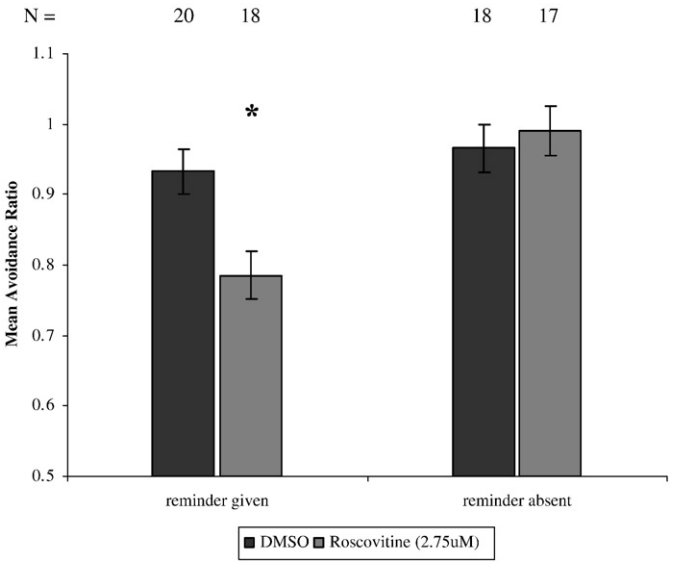

Fig. 3. Mean avoidance ratios of chicks that received either DMSO or Roscovitine at 120-minute post-training (100% MeA) in the presence or absence of a reminder trial. Retention was tested at 180-minute post-reminder trial (±SEM) (*$p<0.05$).

4. Discussion

The results of this study demonstrate that roscovitine dose dependently impairs both consolidation and reconsolidation of passive avoidance learning in the day-old chick. With injections of roscovitine immediately after training, a memory deficit was detected by 5 min, and this lasted until at least 24 h. The memory deficit induced by roscovitine when injected immediately after the reminder trial was observed slightly later, at 30-minute post-reminder, and had resolved by 24 h. The results obtained with water-trained, drug treated birds indicated that the effects observed were not due to sedation, or to other non-specific effects that impaired the chick's pecking response. The results are consistent with previous research which has examined the effect of CDK-5 inhibition on learning and memory in mammals. The results also provide further support for the proposition that consolidation and reconsolidation processes, although similar, are not identical.

Experimental series 1 demonstrated that a dose of 2.5 μM roscovitine, induced memory disruption when measured at 180-minute post-training. Although the dose response study indicated a very narrow dose range, this was replicated several times indicating that this is most likely a true effect. Some caution needs to be noted in interpreting the effect of roscovitine specifically on CDK-5, as this drug also has effects on CDKs 1, 2, 3, 7 and 9. However, none of these kinases have specific effects on neuronal functioning, therefore it seems likely that the effects on memory are due to the inhibition of CDK-5. The time of injection study indicated that active CDK-5 was necessary immediately after training. This is compatible with the notion that CDK-5 exerts its effects upstream from direct NMDA activation (Wang et al., 2003) acting to "switch on" the NMDA receptor and initiate the cascade leading to a permanent representation of the trace.

Examination of the retention levels across time indicated that roscovitine-induced memory disruption was evident by 5 min after training and lasted up until at least 24 h after training. It must be noted that a transient drop in retention levels would be expected at 15-minute post-training, as this is the hypothesised transition point between short-term (STM) and intermediate-term memory (ITM) (Ng et al., 1991). However the fact that decreased retention levels were detected at either side of this time point argues against a non-specific effect of this training-test interval. This stable and long-lasting roscovitine-induced memory deficit suggests that the early participation of CDK-5 is essential for the construction of a permanent trace.

Experimental series 2 examined the effect of roscovitine following reminder-activated retrieval. A slightly higher dose of roscovitine (2.75 μM) was required to induce memory disruption when injected immediately after a reminder trial presented at 120 min. This dose range was also more specific than expected, but once again was replicable. The time of injection study indicated that the most effective time of roscovitine administration was immediately after the reminder, consistent with experimental series 1. It was of interest to investigate whether the effect of roscovitine varied dependent upon the time of reminder presentation. Previous research has indicated that memory reactivation must occur within a specific time window in order to induce memory deficits (Sherry et al., 2005). In the current study, significant differences between DMSO- and roscovitine-treated chicks were detected only when reminder trials were presented at 90 and 120-minute post-training.

To control for the reminder presentation, the experimental protocol was repeated without a reminder trial. Under this protocol, no effect on memory retention was detected. This result suggests that: 1) no deficit was observed when no reminder was present because the memory was not reactivated; therefore no reconsolidation process could be initiated, and 2) administration of the drug at this time had

no effect on the formation of the initial trace. If it did, a deficit would have been observed regardless of whether the memory was reactivated with a reminder, because the original 'copy' of the memory would be compromised.

Examining retention levels across time indicated that roscovitine-induced memory disruption following a reminder trial was evident by 30 min, but had resolved by 24-hour post-training. The reminder-test interval of 30 min is also hypothesised to be a transition point between ITM A and ITM B (Ng et al., 1991), which may raise the question of whether the observed retention deficit was due to the drug manipulation or the time at which the test trial occurred. However no transient memory deficit has been reported or defined at this time (Ng et al., 1991). Therefore the reduced retention levels at this time point post-reminder are unlikely to be due to transient alterations in retention unrelated to the effects of roscovitine. The retention deficit observed post-reminder emerged later than that observed with a consolidation deficit and were transient, resolving by 24 h, consistent with previous research (Anokhin et al., 2002; Summers et al., 2003). In the rodent literature, a transient memory deficit following reactivation is normally interpreted to be due to impaired retrieval, rather than to impaired reconsolidation (Nader, 2003). However, intact retrieval was observed in the current study as the birds exhibited avoidance and distress behaviours with the presentation of the reminder stimulus. This was taken to indicate sufficient reactivation of the original learned experience.

It was also unlikely that the results were due to impaired extinction. In this context extinction was defined as learning (i.e. new consolidation) that the stimulus (i.e. the red bead) was no longer associated with its previously reinforcing properties (i.e. the aversive taste). If extinction was occurring, rather than reconsolidation, then the memory deficit should have appeared similar to impaired consolidation and been evident at 24 h. The fact that the memory deficit was transient argues against this explanation. Additionally, the passive avoidance learning experimental protocol protects against extinction processes as the chicks are not permitted to peck at the reminder stimulus, thus avoiding the possibility that a new trace is initiated (Crowe et al., 2008).

Compared to the memory deficit observed with injections following training, the memory deficit observed after administration of roscovitine following the reminder was delayed. Summers and colleagues have reported two distinct phases following memory reactivation: 1) an immediate process which can be inhibited by lanthanum chloride (Summers et al., 1996) and which possibly acts as a specific retrieval mechanism and, 2) a delayed onset and prolonged process possibly responsible for modification of the trace by adding new information gathered at the time of retrieval. This process was reportedly blocked by both monosodium glutamate (MSG) (Summers et al., 1995) and AP5 (Summers et al., 1997), a specific NMDA receptor antagonist. The roscovitine-induced memory deficit observed in the current study appears similar to the second phase of memory reconsolidation described by Summers et al. (2003). Considering the role of CDK-5 in activating the NMDA receptor, consistency between roscovitine and AP5 effects would be expected. This consistency suggests that CDK-5 may also participate in processes leading to the modification of the underlying trace.

It is clear from the results of the current study that CDK-5, through its inhibition by roscovitine, participates in consolidation and reconsolidation of passive avoidance learning in the day-old chick, although in slightly different ways, possibly dependent upon the specific task requirements. In consolidation, the information to be encoded is novel and so the trace requires construction from the bottom up. In contrast, when a memory is retrieved, that information is accessed from a stable store and its purpose may be very different (e.g. informing the organism, allowing trace modification). The differences between consolidation and reconsolidation memory deficits may be reflective of these alternative task requirements. Additionally, some authors have suggested that rather than the original trace being activated with retrieval, a representative trace is triggered (Sara, 2000; Summers et al., 2003). This would allow the organism to utilise the information without running the risk of permanently erasing the original trace. This proposition is consistent with the observation of a transient memory deficit, as the information contained in the original trace can once again inform the organism, after the impaired process of reconsolidation has resolved.

In experimental series 1, the observed rapid induction of memory deficit soon after training was consistent with past research examining the effect of CDK-5 on mammalian learning and memory. CDK-5 activity within the septo-hippocampal circuitry is reportedly crucial for memory consolidation of contextual fear conditioning (Fischer et al., 2003). Additionally, injections of the CDK-5 inhibitor, 4-butyrolactone, reportedly impairs the acquisition of associative learning in mice (Fischer et al., 2002). This correlates well with the LTP model of physiological learning which predicts that blockade of LTP induction would impair task acquisition. *In vitro* studies have shown that CDK-5 phosphorylates the NMDA receptor subunit, NR2A (Wang et al., 2003) and increases NMDA receptor conductance (Li et al., 2001) leading to enhanced LTP induction. Additionally, CDK-5 has been shown to phosphorylate the NR2B subunit which inhibits the activity dependent endocytosis of NMDA receptors (Zhang et al., 2008) resulting in larger populations of NMDA receptors. Comparable temporal characteristics of CDK-5-induced memory deficit between chicks and rodents, may suggest that a similar mechanism to LTP, at least in the context of consolidation, may be occurring in the avian brain.

If CDK-5 participates in the modification of the representative trace, consistent with Summers et al. (2003) hypothesised second process of reconsolidation, it is possible that this may occur through dopaminergic modulation and subsequent interaction with NMDA receptors. CDK-5 has been shown to modulate dopamine signalling and it has been suggested that the kinase may control the D1 receptor-dependent regulation of NMDA receptors (Zhang et al., 2008). D1 receptors are up-regulated in the avian brain 30 min following avoidance training (Stewart et al., 1996). Previous work in our laboratory has also demonstrated that D1 antagonists impair reconsolidation following reminder-activated retrieval (Sherry et al., 2005) in a manner similar to that observed in the current study.

The D1 receptor activates the enzyme adenylyl cyclase (Gomes et al., 2004), which subsequently increases the levels of intracellular cyclic AMP (cAMP) (Palermo-Neto, 1997). This leads to activation of cAMP-dependent protein kinase A (PKA). PKA may then act to phosphorylate both cAMP response element binding protein (CREB) (Bacskai et al., 1993) and dopamine and adenosine 3′5′ monophosphate-regulated phosphoprotein (DARPP-32) (Liu and Graybiel, 1996). When activated, DARPP-32 inhibits protein phosphatase 1 (PP-1) (Greengard et al., 1999). This compound dephosphorylates CREB (Liu and Graybiel, 1996), therefore inhibition of PP-1 by DARPP-32 acts to prolong the activity of CREB by preventing dephosphorylation. CREB is a transcription factor and may participate in the formation of structural proteins responsible for long-term memory.

CDK-5 can exert influence at many levels of this cascade. It phosphorylates DARPP-32 which converts it to an inhibitor of PKA (Bibb et al., 1999). Several studies have also identified CDK-5 as a key regulator of PP-1 (Munton et al., 2004). Additionally, stimulation of D1 receptors activates PKA and inhibits CDK-5 (Nishi et al., 2000). Modulation of this cascade at any of these points could lead to increases in CREB and modification of synapses with new information gathered at the time of retrieval. Increased levels of CREB following retrieval have been reported in mice (Kida et al., 2002) and application of protein synthesis inhibitors has been shown to impair reconsolidation in day-old chicks (Anokhin et al., 2002; Litvin and Anokhin, 2000; Salinska et al., 2004). Thus, it is feasible to suggest that the same mechanism may be responsible for encoding newly acquired

information within the underlying trace in avian reconsolidation and that CDK-5 likely participates in this process.

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