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The roles of RNA synthesis and protein translation during reconsolidation of passive-avoidance learning in the day-old chick

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ABSTRACT

This series of experiments investigated the role of protein translation and RNA synthesis on consolidation and reconsolidation of passive avoidance learning (PAL) in day-old chicks. Although it is well established that protein translation is required after a reminder, there are conflicting reports in the literature concerning the requirement for RNA synthesis at this time. Day-old male New Hampshire × White Leghorn chicks were trained on a single trial passive avoidance task. The results confirmed the requirement for protein translation during reconsolidation with memory deficits induced by anisomycin (ANI) (10 mg/kg) detected at 60 min post-reminder. It was also established that RNA synthesis was required for consolidation of PAL through inhibition by 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (0.075 μg/kg), administered at or after training. The same dose of DRB was also found to inhibit memory post-reactivation. However injections were required before the reminder trial and memory deficits were evident by 60 min, consistent with that found for ANI post-reminder. As with ANI, the DRB-induced memory deficit post-reminder was also transient, and resolved by 24 h post-reminder. For both reconsolidation drug studies, the memory deficit was wholly dependent on the memory being reactivated by a reminder-trial. The study highlights an important role for RNA synthesis following memory reactivation.

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1. Introduction

It is well established that RNA synthesis and protein translation is required for the consolidation of a new memory trace following learning (Bailey et al., 1999; Davis and Squire, 1984; Sangha et al., 2003b). Recent research also suggests that protein translation is again required after retrieving a memory, a process termed "reconsolidation". Protein translation inhibitors have been shown to impair memory following reactivation trials in rats (Bernardi et al., 2007; Debiec et al., 2002; Milekic and Alberini, 2002; Nader et al., 2000), mice (Judge and Quartermain, 1982), chicken (Anokhin et al., 2002), snails (Child et al., 2003), crab (Pedreira et al., 2002) and medaka fish (Eisenberg et al., 2003).

Although, the nature of the observed memory deficit can vary according to the age of the memory, the length of the reactivation trial and the strength of the original trace (Suzuki et al., 2004), it is generally agreed that protein translation is required to restabilise the memory trace after activation (however see Cammarota et al., 2004; Lattal and Abel, 2001; Rodriguez-Ortiz et al., 2005 for exceptions). However, there is debate within the literature about the requirement for RNA synthesis activity during reconsolidation. Studies have reported that the transcription factor, c-AMP response element-binding protein (CREB), is required for reconsolidation post-reminder in mice (Kida et al., 2002). A more recent study has shown that mRNA synthesis inhibition impairs fear conditioning when injected into the lateral amygdala of rats. The effect was present when injections were administered in association with training and with reminder trials, indicating effects on both consolidation and reconsolidation processes (Duvarci et al., 2008). Another transcription factor, *Zif268*, has also been shown to be involved in reconsolidation, but not consolidation (Lee et al., 2004). Additionally, RNA synthesis and protein translation inhibitors induced reconsolidation deficits post-reminder in the sea slug *Hermissenda* (Child et al., 2003). Finally, the RNA synthesis inhibitor, actinomycin-D (ACT-D), impaired reconsolidation in the snail *Lymnaea stagnalis* (Sangha et al., 2003a). The authors of this study extended their findings by showing that ablation of the neuron's soma also impaired reconsolidation. This suggests that, for *Lymnaea*, RNA synthesis and altered gene activity in the soma is a necessary component of reconsolidation.

In contrast to these studies, many others have reported no effect from RNA synthesis inhibitors post-reminder, prompting the argument that proteins translated in the dendrites may be sufficient to support reconsolidation. Parsons et al. (2006) administered anisomycin (ANI) (protein translation inhibitor), ACT-D or 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (RNA synthesis inhibitors) after initial learning and after memory reactivation. All three compounds induced deficits in memory consolidation. However ANI was the only compound that disrupted memory recall after a reminder. Another study examined the effect of 1-β-D-arabinofuranosylcytosine triphosphate (ara-CTP), a compound that blocks DNA recombination and

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replication, on reconsolidation. The authors confirmed the impairment induced by ANI reported in other studies, but failed to show a memory impairment post-reactivation with application of ara-CTP (Colon-Cesario et al., 2006). Finally, it has been reported that the transcription factor, CCAAT enhancer binding protein β (C/EBPβ), is required for consolidation but not reconsolidation in the hippocampus (Taubenfeld et al., 2001).

As with the studies reported in other species, protein translation is a requirement for reconsolidation of passive avoidance learning (PAL) in the day-old chick (Anokhin et al., 2002; Litvin and Anokhin, 2000). Both ANI and cycloheximide (CXM), when injected just prior to a reminder trial, induce memory reconsolidation deficits by 60 min following the reminder. To date, RNA synthesis inhibitors have not been examined in this species or using this paradigm. However, one study has suggested indirectly that RNA synthesis is not required for reconsolidation of PAL. These authors examined the effect of Colchicine on PAL after a reminder trial. Colchicine acts to block the axonal transport of proteins from the soma to the dendrite, and transiently disrupts consolidation of PAL, which requires RNA synthesis and protein translation in the cell soma. Colchicine, administered 15 min after a reminder, showed no subsequent effect on memory reconsolidation (Mileusnic et al., 2005). This suggests that only local synthesis at the dendrite is required to stabilise a memory trace post-reactivation under these experimental procedures.

The current study examined the effect of DRB and ANI on reconsolidation following a reminder trial in the day-old chick. DRB has been shown to potently prevent the increase in both messenger RNA (mRNA) and heterogenous nuclear RNA in chick embryo (Granick, 1975). DRB has also been used recently to examine the effect of inhibiting RNA synthesis in the auditory thalamus during memory consolidation of fear learning in rats (Apergis-Schoute et al., 2005) and appears to have less neurotoxic effects compared to other inhibitors such as ACT-D (Wetzel et al., 1976). ANI was also examined post-reminder to confirm the findings of other laboratories, which utilise slightly different experimental protocols (Gibbs et al., 2008).

2. Method

2.1. Animals and experimental housing

Day-old New Hampshire × White Leghorn chickens (*Gallus Domesticus*) were obtained from a local hatchery on the morning of each experiment. Cockerels were always employed as they are excess to food production of this egg laying strain. The chicks were housed in pairs to eliminate the confound of stress caused by social isolation (Andrew, 1991). One chick from the pair was marked with a black marker to assist with identification and recording. 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. Chick mash was made available *ad libitum*, and water was provided when the chicks were kept for more than 24 h.

2.2. Drug preparation and administration

ANI and DRB were 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 intermediate medial mesopallium (IMM; Reiner et al., 2004), and the location of the injection site was determined using bony landmarks on the skull (Gibbs et al., 2003). Doses of ANI and DRB were prepared in saline or dimethyl sulfoxide (DMSO) respectively to a total injection volume of 10 µl per hemisphere. Control animals received saline or DMSO. The experimenter was blind to the pharmacological treatment of each group 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

All procedures were approved by the La Trobe University Animal Ethics committee (AEC07/39(P)) and all efforts were made to minimise suffering in accordance with ethical guidelines. Chicks were trained on a modified version of the single-trial passive avoidance task (Crowe and Hale, 2002). The task involved four components: pretraining, training, reminder and retention.

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 birds to peck at bright, rapidly moving 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 10 s, with the number of pecks to this bead recorded using 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 pre-training phase, the experimental chicks were trained to avoid a red bead visually identical to the one used in the pre-training trial, but which was coated in concentrated (i.e. 100%) methyl anthranilate (MeA). Chicks that pecked at the aversive bead showed a disgust reaction that included behaviours such as beak wiping, head shaking and distress calls, clearly indicating exposure to an aversive experience.

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, to control for any effects of the drug not related to memory processes. This is particularly important when avoidance ratios are used as the dependent variable because if the drug affects pecking rates, for example through sedation, the chicks will appear to be avoiding the bead but the 'avoidance' would be independent of memory processes.

Pecks to both the MeA and the water-coated training beads were counted on a behavioural event recorder connected to an on-line computer, consistent with other laboratories using this task (Gibbs et al., 2008). Chicks that failed to peck at either of the training beads within a 10-second period in either the MeA or the water training conditions were excluded from later analysis as they were deemed not to have learned the task. Although each group initially contained 20 chicks, approximately 10% of the sample was excluded on this basis in a non-dose dependent manner, consistent with previous research (see Gibbs et al., 2008 for review). Specific group sizes for each data point are indicated within the figures.

2.3.4. Reminder (reactivation) trial

Where appropriate, memory reconsolidation for the learned stimulus was activated by reminder trials that involved the presentation of a visually identical dry red bead to that used in training which was exposed for approximately 10 s (see Summers et al., 2003 for a full description of this method). Possible lateralisation effects were avoided by ensuring that the bead was seen with both eyes. Chicks were not permitted to peck at this 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 at a behavioural observation level that the presentation of the dry bead was a sufficient stimulus to reactivate the memory for the original learned experience.

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