

## Reconsolidation and memory infidelity in *Lymnaea*

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### Abstract

*Lymnaea stagnalis* were operantly conditioned to not perform aerial respiratory behaviour in a specific context (i.e. *context-1*). The memory for this learned response was reactivated 3 days later in *context-1*. During the 1 h *reconsolidation* period following memory reactivation, randomly picked snails were either maintained in *context-1* or exposed to a new context (i.e. *context-2*). One hour later in the *post-reconsolidation* period, snails in *context-1* were placed for 1 h in *context-2* and vice-versa. In neither the hypoxic *reconsolidation* nor the *post-reconsolidation* periods did snails receive a reinforcing stimulus when they opened their pneumostome. All snails were blindly tested for memory 24 h later period in *context-2*. Only those snails that had been exposed to *context-2* during the *reconsolidation* period exhibited ‘memory’ for *context-2*. That is, memory *infidelity* was observed. Snails exposed to *context-2* in only the *post-reconsolidation* period did not show memory for *context-2*. The immediate cooling of snails after their exposure to the new context in the *reconsolidation* period blocked the formation of the implanted memory. Snails trained in *context-1* and exposed to *context-2* in the *consolidation* period only, also did not have memory for *context-2*. However, the memory for *context-1* could still be recalled following successful implantation of the ‘new’ memory. All data presented here are consistent with the notion that during the reconsolidation process memory can be updated.

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### 1. Introduction

Hebb (1949) and later Lewis (1979) noted that memory (i.e. the ability to encode and retrieve information) was a dynamic brain process. As Lewis (1979) pointed out memory existed in one of two states: (1) A *labile* (i.e. active), and (2) A *stable* (i.e. inactive) one. In the *active* state memory could be modified or even lost, while in the *stable-inactive* state memory was held relatively inviolate. It is widely held that the *active*, more easily modifiable memory is made into a *stable* permanent memory via a process requiring altered gene activity and new protein synthesis. This is referred to as the consolidation process (Dudai, 2004, 2006; McGaugh, 2000; Squire, 1987). Recently there has been renewed

interest in what happens after a formed stable memory has been retrieved (i.e. made active, Dudai, 2006; Nader, 2003). In many instances an activated memory, re-enters a *labile* state and must go through a ‘reconsolidation’ process to again *stabilize* and make it again permanent. The initial observation that following recall a memory was again subject to disruption by electro-convulsive shock (ECS) was made by the Lewis lab in 1968 (Misanin, Miller, & Lewis, 1968). Similar results regarding the disruptability of a memory made active by a number of different amnesiac agents, including protein synthesis blockers, has been demonstrated in rodents (Nader, Schafe, & LeDoux, 2000; Przybylski & Sara, 1997; Taubenfeld, Milekic, Monti, & Alberini, 2001), chicks (Anokhin, Tiunova, & Rose, 2002), crab (Pedreira, Perez-Cuesta, & Maldonado, 2002), *Hermisenda* (Child, Epstein, Kuzirian, & Alkon, 2003), and our model system, *Lymnaea stagnalis* (Sangha, Scheibestock, & Lukowiak, 2003a). In addition to preserving memory, the so-called reconsolidation process has been

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hypothesized to allow this now *active, labile* memory to be modified or updated (Dudai, 2006; Nader, 2003; Tronel, Milekic, & Alberini, 2005). We explore here one of the potential consequences of the ability to update or alter the original memory. We hypothesize that as a result of memory-updating the ability to implant a new memory (e.g. *memory infidelity*) without further operant conditioning in *Lymnaea* is possible.

In the pond snail, *L. stagnalis*, a 3-neuron central pattern generator (CPG), whose sufficiency and necessity have been demonstrated (Syed, Bulloch, & Lukowiak, 1990, 1992), drives aerial respiratory behaviour. This behaviour, which predominates over cutaneous respiration in a hypoxic environment (Lukowiak, Ringseis, Spencer, Wildering, & Syed, 1996), can be operantly conditioned by applying a tactile stimulus to the respiratory orifice area (the pneumostome) as the snail attempts to open it to breathe. However, because respiration still occurs cutaneously snails trained not to perform this aerial respiration are not harmed. Following associative learning long-lasting memory forms and the duration of this associative memory is dependent on the specific training procedure used (Lukowiak et al., 1996, 1998, Lukowiak, Adatia, Krygier, & Syed, 2000). Both the consolidation and reconsolidation processes of long-term memory (LTM) in our model system have been shown to be dependent on altered gene activity (i.e. transcription) and *de novo* protein synthesis (i.e. translation) using both specific blockers (e.g. actinomycin D and anisomycin, respectively) as well as by cooling to 4°C for 1 h (Sangha, Scheibenstock, McComb, & Lukowiak, 2003c, 2002). Moreover, transcriptional and translational processes within one of the three CPG neurons, RPeD1, have been shown to be a necessary for associative LTM formation, (Scheibenstock, Krygier, Haque, Syed, & Lukowiak, 2002) its reconsolidation (Sangha et al., 2003a), memory extinction (Sangha, Scheibenstock, Morrow, & Lukowiak, 2003b), and forgetting (Sangha et al., 2005). In addition, *Lymnaea* also exhibits context-specific learning and memory (Haney & Lukowiak, 2001). That is, snails trained in the one context perform as naïve snails do when tested in a different context. However, snails trained on consecutive days in two different contexts have the ability to remember in both contexts (Haney & Lukowiak, 2001).

Since we can easily change the context snails experience during the *reconsolidation* period following activation of the ‘original’ memory (i.e. for *context-1*); we may be able to determine if as a result of experiencing a new context (i.e. *context-2*) during the *reconsolidation* period snails’ perform as though they were trained in the new context. We hypothesize that as a consequence of memory reactivation in *Lymnaea* it is possible to cause *memory infidelity*. Infidelity in the sense that the snail has memory for something it did not undergo training for. That is, do they have memory for the *context-2* even though they did not receive operant conditioning in *context-2*. We also will have the ability of determining whether the ‘old’ memory (i.e. LTM for *context-1*) has been obliterated by the new memory.

## 2. Experimental procedures

### 2.1. Snails

*Lymnaea stagnalis* were bred and raised in the snail facility at the University of Calgary from a strain of *Lymnaea* originally obtained from *Vrije Universiteit* in Amsterdam. The ancestors of these snails were obtained from canals in a polder located near Utrecht in the early 1950’s. All snails used (2.5–3.0 cm) were maintained at room temperature (20–22 °C) and had continuous access to lettuce in their home eumoxic (i.e. normal levels of O<sub>2</sub>; 6 ml O<sub>2</sub>/l) aquaria. Individually labeled snails were placed in a 1-liter beaker containing 500 ml of room temperature hypoxic (<0.1 ml O<sub>2</sub>/l) pond-water (made hypoxic by bubbling N<sub>2</sub> through it 20 min prior to and during training and testing). Hypoxic pond-water significantly increases aerial respiratory drive (Lukowiak et al., 1996).

### 2.2. Training procedures and specific contexts

All snails were first given a 10-minute acclimatization period in the hypoxic pond-water. They could perform aerial respiration without receiving the negative reinforcement stimulus. The onset of operant conditioning training was initiated by gently pushing the snails beneath the water surface. In all of the operant conditioning training sessions and tests for savings (i.e. the Memory test [MT]) a gentle tactile stimulus (using a sharpened wooden applicator) was applied to the pneumostome area (the respiratory orifice) every time the snail began to open its pneumostome to perform aerial respiration; hence the term ‘attempted pneumostome opening’. This tactile stimulus only evoked pneumostome closure; it did not cause the animal to withdraw its foot and mantle area (i.e. the whole-animal withdrawal response). Pneumostome stimulation also did not cause the snails to sink to the bottom of the beaker. The time of each attempted opening was recorded and tabulated.

We did not monitor the snails’ breathing behaviour during the periods they were in their eumoxic home aquaria. Unless otherwise noted snails were administered three 45-minute operant conditioning sessions. The first two training sessions were given on the same day 1 h apart. The following day, the third training session was given. This training regime produces a memory that lasts up to 5 days (McComb et al., 2002). In between the operant conditioning training sessions snails were placed in eumoxic water where they were allowed to freely perform aerial respiration.

We also utilized a ‘carrot context’ procedure. In the ‘standard’ hypoxic training procedure N<sub>2</sub> was directly bubbled into the training beaker. In the ‘carrot context’, N<sub>2</sub> was first bubbled through a 750 ml Erlenmeyer flask with blended carrots and water before being bubbled into the training beaker (see Haney & Lukowiak, 2001 for complete details). The term ‘change of context test’ (CC) means that snails were tested in the context that they were *not* operantly conditioned in. Snails perceive this as a different context and respond as if they have not received training (Haney & Lukowiak, 2001; Sangha et al., 2003a, 2003b, 2005).

The terms *context-1* and *context-2* are used throughout the manuscript. *Context-1* refers to the context that the snails received operant conditioning training in; whilst *context-2* refers to the new context snails experienced in the *consolidation*, *reconsolidation*, or *post-reconsolidation* periods (see below for definitions). The ‘standard’ or ‘carrot’ context could be used as either *context-1* or *context-2*.

### 2.3. Procedure for testing memory infidelity

Snails were trained (three 45 min sessions over a 1.5 day period) in *context-1*, which was either the ‘standard context’ or the ‘carrot context’. Three days later snails received a 45-minute savings test (i.e. memory was tested; MT) in *context-1*. In this session snails continued to receive the tactile stimulus to their pneumostome area as they attempted to open the pneumostome. This treatment is sufficient to reactivate memory and the reconsolidation process is necessary in order for the memory to be retained (Sangha et al., 2003a). Immediately after MT the snails were randomly divided into a control and an experimental group.

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