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Propranolol disrupts consolidation of emotional memory in *Lymnaea*

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ABSTRACT

The therapeutic efficacy of the synthetic β-adrenergic receptor blocker, propranolol, for the treatment of post-traumatic stress disorder (PTSD) is currently being debated. Mixed results have been published regarding propranolol's ability to disrupt the consolidation and reconsolidation of memories. Here, we use the invertebrate model *Lymnaea* to study propranolol's ability to disrupt consolidation of memories formed under varying various types of stress which cause differing degrees of emotional memory. We show that when propranolol is administered immediately following operant conditioning, only the consolidation process of memories enhanced by individual stressors (i.e. a non-emotional memory) is susceptible to disruption. However, when propranolol is administered prior to training, only memories enhanced by a combination of stressors leading to an emotional memory are susceptible to disruption. These data suggest that the time of propranolol administration, as well as the type of memory formed play a key role in propranolol's ability to obstruct memory consolidation.

1. Introduction

Emotional memories created under highly stressful conditions can be invasive and result in the development of disorders such as post-traumatic stress disorder (PTSD; Breslau, 2009). The ability of stress to alter the ways in which memory is formed and maintained is well known (Hebb, 1955). Similarly, it has been repeatedly demonstrated that emotion enhances memory encoding and facilitates its later recall (Mueller & Cahill, 2010). However, it is not clear how the mechanism (s) underlying the consolidation of memories created during highly stressful, emotional situations differs from the mechanism(s) underlying the consolidation of memories created under 'typical' (i.e. normal, 'non-stressful') circumstances. During exposure to trauma, release of endogenous stress hormones results in over-consolidation of the traumatic memory (Pitman & Orr, 1990). As a result, this memory may later be reactivated much too easily by contextual cues, causing strong conditioned emotional responses (Pitman, 1989).

One method under investigation to decrease the impact of an emotional memory leading to PTSD involves the use of the synthetic β-adrenergic receptor blocker, propranolol, to disrupt the consolidation and reconsolidation of memory. Consolidation occurs when memories that initially exist in a fragile state are strengthened over time (Nader, Schafe, & Le Doux, 2000; Sangha, Scheibenstock, & Lukowiak, 2003a; Sangha, Scheibenstock, McComb, & Lukowiak, 2003b). Reconsolidation, on the other hand, occurs when recalled memories enter a transient labile phase and undergo a new stabilization process before once again returning to a stable state (Sangha et al., 2003a). Propranolol is a synthetic molecule that crosses the mammalian blood–brain barrier and exerts central inhibitory effects on protein synthesis and peripheral effects on the noradrenergic system (Przybyslawski, Roullet, & Sara, 1999). Protein synthesis is required for both the consolidation of short-term memories that are in the fragile state into long-term memories (LTM) as well as the reconsolidation of memory (Nader et al., 2000; Sangha et al., 2003a). The use of propranolol as a treatment for PTSD has been tested in human populations (Lonergan, Olivera-Figueroa, Pitman, & Brunet, 2013) as well as in animal model systems such as the rodent (Cahill, Pham, & Setlow, 2000; Debiec & Ledoux, 2004; Przybyslawski et al., 1999) and the snail (Hughes, Shymansky, Sunada, & Lukowiak, 2016). Mixed results have been reported with regards to propranolol's therapeutic efficacy in human populations (Lonergan et al., 2013).

In studies investigating PTSD, propranolol is commonly used to disrupt the reconsolidation of emotional memories (Debiec & Ledoux, 2004; Hughes et al., 2016; Kindt, Soeter, & Vervliet, 2009; Przybyslawski et al., 1999). The Lukowiak lab has previously shown that only memories created under certain stressful conditions, which create an 'emotional memory', are susceptible to disruption by propranolol following reactivation (i.e. reconsolidation, Hughes et al., 2016). These results are similar to what is seen in humans, where some memories are more susceptible to disruption by propranolol than others. Human studies report that propranolol's amnesic effect is more sizable on memories created under highly emotional conditions compared to neutral conditions (Lonergan et al., 2013; Schwabe, Nader, Wolf, Beaudry, & Pruessner, 2012).

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A second method used to lessen the effect of an emotional memory is to interfere with the initial consolidation process of is memory formation, resulting in a degraded memory trace (Lonergan et al., 2013). Studies have found that when propranolol is administered prior to viewing a series of emotionally upsetting images, the heightened recall of these images is prevented (Cahill, Prins, Weber, & McGaugh, 1994). However, much like with reconsolidation, debate still exists as to whether using propranolol to disrupt the consolidation of memory is a potential clinical treatment. For example, a recent mouse study found that propranolol affected memory consolidation in non-aversive tasks such as object recognition and object location, but had no effect on moderately aversive (Morris water maze) and highly aversive (passive avoidance, conditioned taste aversion) tasks (Villain et al., 2016). A meta-analysis in healthy human participants, on the other hand, found that when compared with placebo, propranolol given prior to memory consolidation resulted in a reduction of subsequent recall for negatively valenced stories, pictures and word lists (Lonergan et al., 2013). If propranolol can be shown to reliably disrupt the consolidation of human PTSD memory, it could prove valuable in clinical settings, such as the emergency department, to reduce the likelihood of developing PTSD after a traumatic event. Thus, further investigation is warranted.

Lymnaea is a valuable neurobiological model for investigating learning and memory because it allows one to easily study the heterogeneity of memories (Lukowiak & Dalesman, 2012). For example, at the single neuron level a memory lasting only 3 h can be seen to be different than a memory that persists for 24 h even though at the behavioural level they do not appear to be different in regards to the percentage decrease from their initial level of responsiveness on the first training session (Braun & Lukowiak, 2011). *Lymnaea* are bi-modal breathers; they are capable of respiration through both cutaneous and aerial means (Lukowiak, Ringseis, Spencer, Wildering, & Syed, 1996). Aerial respiration can be operantly conditioned (a form of associative learning), resulting in a decrease of this behavior. Our standard training procedure (two 0.5 h training sessions separated by 1 h) results in a LTM that persists for at least 24 h; while a single 0.5 h training session is only sufficient to produce an intermediate-term memory (ITM) that persists for 3 h. Whereas ITM is dependent on new protein synthesis, LTM is dependent on both altered gene activity and new protein synthesis (Sangha et al., 2003b).

Some stressors are capable of enhancing memory formation such that if presented prior to or during training, a single 0.5 h training session becomes sufficient for LTM formation (Lukowiak, Sunada, Teskey, Lukowiak, & Dalesman, 2014; Martens, De Caigny, et al., 2007; Martens, Amarell, et al., 2007). That is, some stressors can enhance memory formation such that a 0.5 h training session that does not normally cause LTM now results in LTM. These stressors include thermal stress, predator detection or application of potassium chloride (KCl) (Teskey, Lukowiak, Riaz, Dalesman, & Lukowiak, 2012; Martens, De Caigny, et al., 2007; Martens, Amarell, et al., 2007; Orr & Lukowiak, 2008). Hughes et al. (2016) hypothesized that in *Lymnaea* certain combinations of stressors caused a different form of memory to be made, which they termed an emotional memory. Importantly for our present study, reconsolidation of this emotional memory was blocked by an injection of propranolol; whereas with 'normal' memories propranolol did not block reconsolidation.

The question may arise in some as to whether an invertebrate such as *Lymnaea* can have an emotion or an emotional memory. Emotion in invertebrates remains poorly understood, even though Darwin (1872) in his book on emotion suggested that invertebrates possessed emotions. Many species of invertebrates (e.g. crayfish, ants, bees) display physiological and behavioural changes similar to what is considered to be an emotion in a vertebrate. When we use the word emotion here, we are not suggesting that snails and other invertebrates have feelings. Simple animals that have the capacity for emotion may not necessarily be capable of 'feeling' (perceiving what happens in the body and mind when emoting; Damasio, 2010). Thus, simple organisms can have emotions without experiencing feelings (Damasio, 2010; LeDoux, 2012). Several recently published studies (e.g. Fossat, Bacqué-Cazenave, De Deurwaerdère, Delbecque, & Cattaert, 2014; Perry, Baciadonna, & Chittka, 2016) suggest that invertebrates do in fact exhibit not only negative affect but also positive emotion-like states.

In this study, we explore propranolol's efficacy in disrupting the memory consolidation process following exposure to different stressors or combinations of stressors which may lead to an emotional memory. We hypothesize that propranolol will only disrupt the consolidation of memories created under conditions that lead to an emotional memory. Further, we hypothesize that propranolol injected prior to or immediately following a memory training procedure will impede recall of memories created under conditions that lead to an emotional memory.

2. Methods

2.1. Snails

The *Lymnaea* used in this study were bred from a laboratory strain maintained at the University of Calgary Biology Department. These animals were originally collected in the 1950s from a polder near Utrecht, The Netherlands. Snails were kept in home aquaria containing oxygenated artificial pond water (0.25 g/L Instant Ocean, Spectrum Brands, Madison, WI, USA; 0.34 g/L $CaSO_4$, Sigma-Aldrich, St-Louis, MO, USA) at a room temperature of 20 °C. Romaine lettuce was provided ad libitum. A total of 180 naïve snails were used in the study. It is important to note that a snail was only used in a single experiment.

2.2. Drug exposure

(±)-Propranolol hydrochloride (TLC) powder was obtained from Sigma-Aldrich (St. Louis, MO, USA). The concentration of propranolol was chosen based on pilot studies previously done in the Lukowiak lab, and is consistent with the published literature (Hughes et al., 2016). Immediately prior to injection, snails were placed in an ice bath for 5 min in order to anesthetize them. Propranolol-treated snails were injected into their foot with 0.1 mL of 50 μM propranolol dissolved in *Lymnaea* saline and saline treated snails (vehicle controls) were injected with of 0.1 mL *Lymnaea* saline. Injections were either performed prior to or following the training session (TS). If injections were done prior to TS, snails were returned to their eumoxic (6 mL O_2/L) home aquaria for 1 h after injection to recover before undergoing a 0.5 h training session. If injections were performed following TS, snails were simply placed back into their eumoxic home aquaria and remained there until the memory test (MT) 24 h later. Injection of propranolol at the concentration used here has previously been demonstrated to not affect homeostatic breathing behavior in *Lymnaea* (Hughes et al., 2016). Finally, it has previously been shown (e.g. Hughes et al., 2016; Sunada et al., 2017) that the injection of saline before or after training does not alter (i.e. neither enhancing or obstructing) memory formation.

2.3. Aerial respiratory behavior

In eumoxic conditions (6 mL O_2/L) *Lymnaea* primarily acquire oxygen by means of cutaneous respiration. In hypoxic conditions, on the other hand, with low dissolved concentration of oxygen (< 0.1 mL O_2/L), *Lymnaea* shift to aerial respiration and use their lung which is connected to the atmosphere via a structure called the pneumostome.

2.4. Standard operant conditioning procedure

Each snail was labelled 24 h prior to the training session. Snails were placed in a 1L beaker filled with 500 mL of artificial pond water made hypoxic by bubbling N_2 gas through the water for 20 min prior to a training session. Animals were allowed to acclimatize for 10 min in the beaker prior to the initiation of the training session. During the