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Investigating auditory fear memory erasure in the basolateral amygdala

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Abstract

New memories are initially fragile and need protein synthesis in order to be stabilized for long-term storage, a mechanism called cellular consolidation. When recalled, memories are re-activated and become unstable again. They therefore need to be re-stored through a process called reconsolidated. Behavioural studies in rats using auditory fear conditioning have demonstrated that propranolol, a β-adrenergic receptor antagonist, administered following memory reactivation can reduce fear expression (freezing), which has been interpreted as amnesia for the fear memory. It was recently discovered that GluR1-containing AMPA receptors are recruited into the post-synaptic membrane of the basolateral amygdala during auditory fear conditioning, suggesting that synaptic GluR1 increase may be a molecular correlate of long-term memory. The present study aims to investigate what molecular mechanisms accounts for the observed amnesia following a reconsolidation blockade by propranolol. Rats were trained in an auditory fear conditioning task, and fear memory reactivation was followed by systemic propranolol administration. Rats were then euthanized and GluR1 protein levels in baso-lateral amygdala synaptoneurosomes were quantified. We report preliminary evidence to suggest that a reconsolidation blockade by propranolol reduces fear expression with a concomitant reduction in GluR1. Such evidence suggests that a reconsolidation blockade might, at a molecular level, erase a component of the fear memory, providing support for the clinical utility of this treatment for post-traumatic stress disorder.

Memory, the ability to acquire, store and recall learned information, is a fundamental feature of human experience. Observations that new memories are initially sensitive to disruption but strengthen over time laid the foundations for the consolidation hypothesis (Muller & Pilzecker, 1900, as cited in Dudai, 2004). This theory holds that memories pass through two qualitatively different states (Ebbinghaus, 1885 as cited in Squire & Kandel, 2000). At the time of learning, a fragile memory trace is formed in short-term memory (STM) and said to be labile as it is sensitive to disruption. By way of the consolidation process, a short term memory is converted into a lasting and stable memory trace which exists in long-term memory (LTM).

The most striking evidence supporting consolidation theory comes from animal studies; amnesic agents such as protein synthesis inhibitors (Flexner, Flexner, De La Haba, & Roberts, 1965) or electro-convulsive shock (Duncan, 1949) administered to animals shortly after learning resulted in amnesia, while the same treatment after a delay caused no memory impairment. These results suggest that new memories must undergo a time-dependent process to persist in LTM stores. Consolidation is thus defined as a stabilization process that renders a newly acquired memory stable and lasting.

At the cellular level, consolidation occurs as a result of synaptic changes following acquisition of newly learned information. The synaptic plasticity hypothesis (Hebb, 1949) holds that the encoding of new memories results in structural modifications of synaptic connections, causing persistent changes in synaptic strength. Long-term potentiation (LTP) is currently the leading model for this plasticity. LTP was first demonstrated in vivo in rabbits, where it was shown that successive test pulses in the neural pathways leading to the hippocampus were shown to increase the strength of active synapses (Bliss & Lomo, 1973). Numerous subsequent studies (Rogan, Ursula, Staubli, & LeDoux, 1997) have shown that learning and LTP involve similar cellular mechanisms, suggesting that LTP may be the mechanism by which new memories stabilize over time.

LTP is a process triggered by the activation of the excitatory glutaminergic N-methyl d-aspartate receptors (NMDAr). Activation of the NMDAr results in an influx of Ca2+, which initiates a cascade of protein synthesis dependent intracellular reactions that are thought to lead to the growth of new synapses and to the insertion of receptors into the post-synaptic membrane (for review see Milner, Squire, & Kandel, 1998). Specifically, the α-amino-3-hydroxy-5-methylsoxazole-4-propionic acid receptor (AMPAr), another type of glutaminergic receptor, is inserted into the post-synaptic membrane and increases the neuron's sensitivity to glutamate, increasing the likelihood of synaptic transmission (for review see Malenka, 2003).

AMPAr are composed of four subunits, known as GluR1 to GluR4, that have different relative levels of insertion into post-synaptic membranes during LTP (Passafaro, Piech & Sheng, 2001; Rumpel, LeDoux, Zador, & Malinow, 2005; Yeh, Mao, Lin, & Gean, 2005). Both in vitro and in vivo rodent studies have demonstrated increased insertion of only the GluR1-containing AMPAr in the basolateral amygdala (BLA), the brain structure thought to be responsible for fear learning, following auditory fear conditioning (Yeh et al, 2005; Rumpel et al, 2005). Moreover, it was shown that blocking the synaptic incorporation of GluR1 (Rumpel, 2005) or knocking out the gene that codes for the GluR1 subunit (Humeau, Reisel, Johnson, Borchardt, Jensen, Gebhardt et al, 2007) impedes associative fear conditioning. These findings indicate that GluR1 may provide an essential contribution to the molecular mechanism of memory formation and maintenance, and thus may be a molecular correlate of the memory trace.

Previously, scientists believed that once the synaptic modifications necessary for consolidation were made, the memory was permanently hardwired into the brain. Misanin, Miller & Lewis (1968) challenged this hypothesis when they found that 24 hours after the acquisition of a passive-avoidance task, a cueing-procedure followed by electroconvulsive shock (ECS) resulted in memory loss. This study was the first to suggest that a consolidated memory could be susceptible to amnesic treatments. Recently, Nader, Schafe & LeDoux (2000) confirmed these results using auditory fear conditioning. This robust learning paradigm involves the pairing of a tone, the conditioned stimulus (CS), with a footshock, an aversive unconditioned stimulus (US). As such, rats learn that the CS predicts the footshock, and eventually fears the CS when it is presented alone. Nader et. al. (2000) demonstrated that post-training

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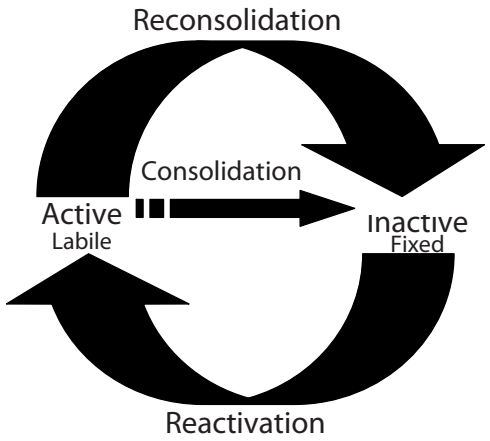

Figure 1: The dynamic nature of memory

infusion of the protein synthesis inhibitor anisomycin into the BLA immediately following memory reactivation produced amnesia. Interestingly, anisomycin infusion in the absence of reactivation did not produce amnesia, suggesting the observed amnesia hinges on memory reactivation. The authors concluded that if a protein synthesis inhibitor infused after reactivation causes amnesia, then reactivation must destabilize a well-consolidated memory and instigate a second stabilization process requiring de novo protein synthesis in order for the memory to persist in LTM. This process, termed reconsolidation, suggests that persistence of memory involves not only the storage of memories following their acquisition, but also the re-storage of these memories following recall.

Reconsolidation has been demonstrated across species ranging from C. elegans to humans, and for different types of learning, from aversive to appetitive conditioning (Nader, 2007). The memory process is therefore believed to be dynamic in nature, where a memory cycles between being in an active and an inactive state. As such, reconsolidation studies suggest that it is not the time delay following encoding that determines the durability of a memory, but its qualitative state; memories are in an active state when they are acquired and recalled, following which they require a time-dependent stabilization process to persist in LTM (see Figure 1).

The discovery of reconsolidation has important theoretical implications, as it challenges the long-standing notion that memories are fixed when consolidated, but also introduces a novel clinical treatment for disorders associated with debilitating traumatic memories, such as post-traumatic stress disorder (PTSD). Anisomycin, the amnesic agent used by Nader et. al. (2000), cannot be administered to humans, and as a result drugs with a similar amnesic effect that can be administered to humans have been investigated to determine the validity of such a treatment.

Propranolol, a β-receptor antagonist, has been examined because of the involvement of endogenous stress hormones in the memory system and because propranolol can safely be administered to humans. When a rat experiences a footshock, or when a human undergoes a traumatic experience, stress hormones are released (McGaugh, 2000). Also, β–adrenergic agonists infused into the amygdala in rats (Cahill & McGaugh, 1996) and injected in humans (Chamberlain, Muller, Blackwell, Robbins & Sahakian, 2006) enhance memory storage. Put together, these findings suggest that the release of stress hormones at the time of a traumatic event may contribute to an 'over-consolidation' process that renders fear memories highly resistant to extinction (Orr, Metzger, Lasko, Macklin, Peri, & Pitman, 2000). Following from this, being a b-receptor antagonist, propranolol was used to block the memory-enhancing effects of stress hormones during consolidation in both rats (McGaugh, 2000) and humans (Chamberlain et al., 2006). Pitman, Sanders, Zusman, Healy, Cheema, Lasko et al (2002) administered propranolol to humans immediately following an acute traumatic event, and found that this administration reduced subsequent PTSD symptoms.

Since propranolol blocks consolidation in both rats and humans it was believed that perhaps propranolol might block memory reconsolidation. Indeed, Debiec & Ledoux (2004, 2006) used auditory fear conditioning in rats and found that administration of propranolol following reactivation blocked the expression of fear memories, suggesting that propranolol blocked reconsolidation. Their study provides an animal model for the treatment of traumatic memories by way of reconsolidation blockade by propranolol, and suggests that propranolol may be an effective treatment for PTSD.

Our study aims to investigate the molecular processes that might account for the observed amnesia, as the mechanism by which reconsolidation blockade reduces fear expression in rats is currently not clear. Although it has been proposed that blocking reconsolidation actually removes a part of the memory (Nader, 2007), this has not yet been validated at a molecular level. Furthermore, since GluR1 is thought to represent a component of the memory trace,it is apt to examine whether a blockade of reconsolidation by propranolol can decrease this molecular memory tag. This would validate the hypothesis that the reduction of freezing observed by reconsolidation blockade can be directly attributed to memory erasure.

Using an auditory fear conditioning paradigm, three hypotheses were tested. Firstly, when propranolol is injected following memory reactivation, can it reduce freezing behaviour? Secondly, does fear conditioning cause a measurable increase in synaptic GluR1 three days after training? Lastly, does a blockade of reconsolidation by propranolol reverse the conditioning-induced increase in GluR1? In order to test these hypotheses, 12 rats were randomly assigned to four experimental groups (n=3) (Table 1). The rats in the propranolol group (CS+P) were habituated, trained, and were given a propranolol injection immediately following reactivation. The rats in the vehicle group (CS+V) were habituated, trained, and given a saline injection immediately following reactivation. The rats in the non-reactivated propranolol group (No CS+P) were habituated, trained, and given a propranolol injection without memory reactivation. This group is a necessary control to determine that propranolol, in the absence of reactivation, does not affect memory processing. The rats in the naïve group were habituated and administered saline. This last group provides a baseline measure of GluR1.

Methods

Subjects

Twelve adult male Sprague-Dawley rats from Charles River Laboratories, weighing 275-300g on arrival, were individually housed and maintained on a 12/12-hour light/dark cycle, with lights on at 7:00 a.m. All testing was performed during the light period. Rats were handled once a day for three consecutive days before the testing began.

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Groups	Training	Reactivation	Drug	Freezing	GluR1
Naïve	None	None	Saline	None	Baseline
CS+P	CS-US	CS	Propranolol	Low	⬇
CS+V	CS-US	CS	Saline	High	⬆
No CS+P	CS-US	No CS	Propranolol	High	⬆

Table 1: Drive efficiency of the SNOM probe as indicated by the ratio between the drive amplitude and the amplitude of the cantilever tunes

Behavioural Apparatus

Two distinct test chambers were used for this study (Med-Associates). The first chamber, (context A) was used for training. It had a metal grid floor (1.5 cm bar spacing), stainless steel sidewalls, a transparent Plexiglas front wall, and was enclosed in a custom-built sound-attenuating isolation cubicle. During training, all lights and ventilation fans in each cubicle were on.

The second chamber (context B) was used for habituation and tone testing. These chambers were located in a second room of the laboratory. Each chamber was 30 x 25 x 30 cm; two sidewalls were stainless steel and two were Plexiglas with an opaque sheet of alternating 2 cm-wide vertical black and white stripes. The floor was a Plexiglas opaque white surface, scented with peppermint before each rat was inserted. Each conditioning chamber was enclosed in a sound-attenuating isolation cubicle. The house lights were dimmed while the ventilation fans remained off. The amount of time each rat spent freezing during the 30 s interval preceding the CS presentation for each test, as well as the time spent freezing during the CS, was measured.

Molecular Apparatus

A Cryostat (Microm Instrumentation, Germany) was used to collect amygdala slices. A Teflon homogenizer was used to homogenize tissue samples in a buffer to fractionate cells. Millipore filters were used to filter the cell fractions from other cellular components. A microcentrifuge was used to separate the heavier fractions, enriched with synaptoneurosomes, from lighter ones (less than 5 µm). An electrophoresis module (Bio-rad) was used in the present study to further separate synaptic GluR1 from other cellular proteins. A transfer module (Bio-rad) allowed a current to run through the polyacrylamide gel apposed to a PVDF (Millipore) membrane, and was used to transfer proteins from the gel to the membrane. The Storm Laser scanner (Storm 860, Amersham Biosciences) was used to quantify GluR1 proteins in the PVDF membrane via Image Quant software (Blot Imaging System).

Behavioral Procedures (Figure 2)

Habituation. All rats were habituated for 15 minutes on three consecutive days in Context B. This was intended to acclimate the rats to the lab and the behavioural chambers, to minimize generalization between the two contexts, and to eliminate any contextual conditioning, ensuring that the CS is the primary predictor of US exposure.

Training. On the fourth day, only rats in the CS+P, CS+V and No CS+P were transported to a brightly lit waiting area and remained there for five minutes before training began in Context A. Rats were then individually placed in a chamber, and after a two minute acclimatizing period, they were given three forward-presented pairings of the CS-US. The CS was a 5 KHz, 65 dB, 30 s tone that co-terminated with the US, a 1.5 mA , 1 s footshock delivered through the metal grid floor. The inter-trial interval (ITI) between each tone-shock presentation was 60 s. Rats were then returned to their home cages.

Reactivation. On day five, twenty-four hours after training, when the fear memory is thought to have completed cellular consolidation (Nader et al., 2000), rats in the CS+P, CS+V and no CS+P groups were transported to a dimly lit room and remained there for five minutes. Rats in the CS+P and CS+V groups were then placed in Context B. After 120 s of acclimatization, a single tone (5 KHz, 65 dB, 30 s) was played, but no shock was given. Immediately following the memory reactivation, the animals in the CS+P group received an intraperitoneal injection of propranolol (Sigma Aldrich, Ontario), while animals in the CS+V group received a saline injection. Rats in the no CS+P group received the propranolol injection at this time, without undergoing the memory reactivation session. Propranolol was dissolved in a saline solution at a dose of 10 mg/ml and administered at a dose of 20 mg/kg, as used in Debiec & Ledoux (2004, 2006). Freezing behaviour, operationally defined as the cessation of all movement except respiratory-related movements (LeDoux, 2000), is a species-typical fear response, and is used as a measure of fear. Freezing behaviour was scored with Freeze-View software (Actimetrics) by an experimenter blind to the experimental condition.

Post-reactivation short-term memory test (PR-STM). On day five, four hours following reactivation, rats in the CS+P, CS+V and no CS+P group underwent the PR-STM test. These rats were transported to a dimly lit room and remained there for five minutes. Rats were then placed into Context A, and after a 120 s acclimatization period three tones with the same parameters as used in the reactivation trials were delivered, and the freezing behaviour scored.

Typically, PR-STM tests are performed as an internal control in order to rule out any nonspecific effects of the drug, in this case the effects of propranolol on the memory abilities of the rats (Nader et al, 2000). The rats in the CS+P, CS+V, and no CS+P groups should show no significant difference in freezing on this test, indicating that propranolol is affecting long-term memory storage without interfering with normal memory processing.

Post-reactivation long-term memory test (PR-LTM). On day six, twenty hours after the PR-STM test, rats in the propranolol, vehicle and non-reactivated propranolol group were

Figure 2: Experimental design

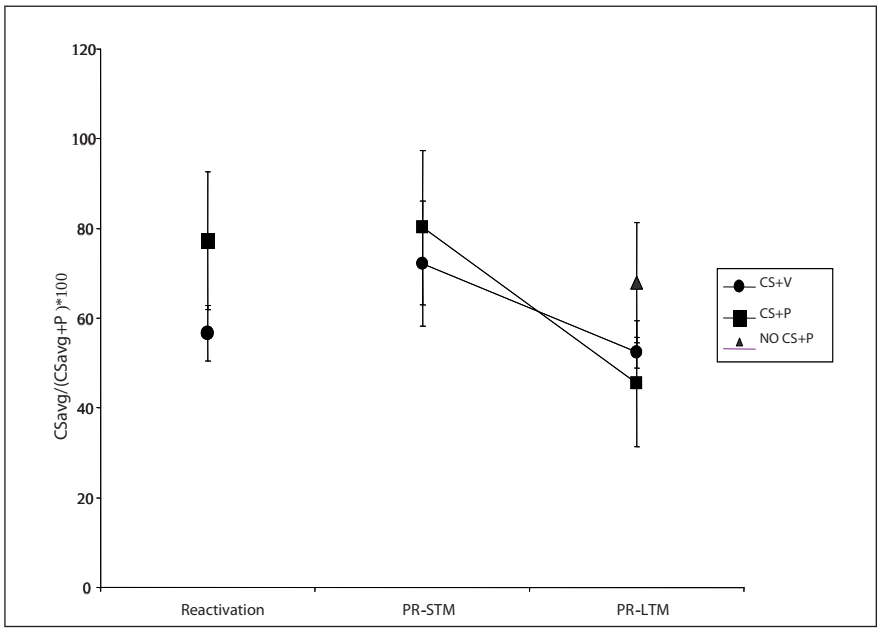

Figure 3: Averaged freezing data for the three experimental groups (CS+V, CS+P, No CS+P) in each experimental condition (Reactivation, PR-STM and PR-LTM).

transported to a dimly lit room and remained there for five minutes. Rats were placed into Context A, and after a 120 s acclimatization period, three tones with the same parameters as used in the reactivation and PR-STM trials were delivered and freezing behaviour scored.

Euthanasia. On day seven, twenty-four hours after the PR-LTM test, the animals were deeply anesthetized with urethane (1 ml/kg) and decapitated. Brains were dissected, immediately frozen on dry ice, and stored at -80 ºC for later molecular analysis.

Molecular Analysis

Brains were sliced at -20 ºC on a cryostat until the BLA was reached. BLA tissues were collected with a hollow needle and homogenized at 4 ºC using a Teflon tissue grinder in lysis buffer consisting of 10 mM Hepes/1.0 mM EDTA/2 mM EGTA/0.5 (Roche, Mississauga, ON).

Synaptoneurosome preparation. Homogenates were passed through two 100-µm-pore nylon mesh filters, then through a 5-µm-pore filter. Filtered homogenates were centrifuged at 3600 g for 10 min at 4°C. Resultant pellets were resuspended in 20 µL boiling 1% SDS for 10 min and stored at -80°C.

Western Blot Analysis. Equal amounts of proteins (30 µg) from each sample were boiled for 10 min in SDS electrophoresis sample Laemmi buffer containing beta-mercaptoethanol (Bio-Rad) and were run on a 8% SDS-polyacrylamide gel, along with a molecular weight marker (BioRad), and transferred to PVDF membranes (Bio-rad, Hercules, CA). The membranes were incubated overnight at 4°C in blocking solution (0.1 % Tween 20 and 2% bovine serum albumin in TBS), and incubated for 2 hrs at room temperature with the anti-GluR1 antibody (Chemicon). After being washed in TBS, membranes were incubated with the secondary fluorescent antibody (molecular probes).

GluR1 quantification. The membranes were scanned with a Storm Laser scanner and the signals quantified.

Results

Statistical Analysis

The behavioural data was analyzed using independent-samples t-tests or a two-way mixed analysis of variance (ANOVA), with testing interval as the within-subjects factor and group as the between-subjects factor. The molecular data was analyzed using a one-way analysis of variance (ANOVA). Significant interactions for both behavioural and molecular data were further analyzed using Tukey's post-hoc test. Type one error rate was set at 0.05. Statistica version 6.0 statistical software was used to perform all analyses.

Behavioural Results

For both the PR-STM and PR-LTM tests, the average freezing score (time spent freezing) across the three CSs was calculated (CSavg). In order to distinguish and isolate the freezing induced by the CS from the freezing induced by the context, we took the CSavg for each rat and divided it by the sum of that rat's pre-CS and CSavg freezing. This allowed us to isolate each rat's average freezing to the tone for each test, and to factor out any contextual freezing (Figure 3). All statistics were done on this modified data.

To determine that there were no significant differences between the propranolol and vehicle groups during reactivation, an independent-samples t-test was conducted. The two groups demonstrated comparable freezing during reactivation, as an independent-groups t-test, $t(4) = -1.589$, $p > 0.05$, revealed freezing of animals in the vehicle group ($M = 87.34$, $SEM = 5.67$) was not significantly different than animals in the propranolol group ($M = 73.40$, $SEM = 19.64$). A two-way mixed design ANOVA, with group (CS+P, CS+V) as between-subjects factor and test (PR-STM, PR-LTM) as within-subjects factor revealed non-significant results ($F(1, 4) = 0.15$, $p > 0.05$). However, it was observed; on the PR-LTM test, the propranolol rats froze less ($M = 45.48$, $SEM = 14.03$) than the vehicle rats ($M = 52.37$, $SEM = 5.67$).

To further quantify the amnesia observed in the propranolol group, we calculated the Amnesia Index for each group (Figure 4) (Debiec, LeDoux & Nader, 2002). This was calculated by dividing each rat's freezing score during PR-LTM by its freezing score during reactivation. A t-test, $t(4) = 0.66$, $p > 0.05$, was conducted on the averaged values and indicated no significant difference between the mean amnesia index of the vehicle group ($M = 93.13$, $SEM = 9.33$) compared to that of the propranolol group ($M = 68.98$, $SEM = 35.39$). However, the propranolol group did freeze 24.15% less than the vehicle group, indicating that propranolol treatment following reactivation produced amnesia.

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Molecular Results

We divided the BLA synaptoneurosome GluR1 levels of each rat by the average naïve value and ran the statistics on this normalized data (Figure 5). A one-way ANOVA, $F(3, 8) = 4.52$, $p < 0.05$, revealed a main effect of group. A Tukey post-hoc test revealed a significant difference between the GluR1 levels of the naïve group ($M = 100$, $SEM = 13.64$) and the vehicle group ($M = 160.72$, $SEM = 8.55$; $p < 0.05$). The Tukey post-hoc test also revealed lower levels of GluR1 in the propranolol group ($M = 125.29$, $SEM = 8.20$) as compared to the vehicle group ($M = 160.72$, $SEM = 8.55$), although these results were not statistically significant ($p > 0.05$). The non-reactivated propranolol group showed higher levels of GluR1 ($M = 136.09$, $SEM = 15.30$) than the propranolol group ($M = 125.29$, $SEM = 8.20$), and lower levels than the vehicle group ($= 160.72$, $SEM = 8.55$), but not as low as the naïve group, all of which were non-significant ($p>0.05$).

Discussion

Although not statistically significant, the results obtained demonstrate trends in the direction of our hypotheses. With regards to the first hypothesis, we demonstrated that the propranolol group demonstrated a non-significant decrease in freezing on the PR-LTM test, thereby partially replicating Debiec and LeDoux's (2004) finding. The second hypothesis was supported; trained rats that received a saline injection showed a significant increase in synaptic GluR1 levels in the BLA compared to naïve rats, which replicates the study by Yeh et al (2005). Interestingly, the results of the present study are, to our knowledge, the first demonstration that an increase in GluR1 can be detected three days after training, providing further evidence that GluR1 insertion in post-synaptic membranes can be viewed as a molecular component of the memory trace. The third hypothesis was partially supported; the propranolol group demonstrated decreased freezing with a concomitant reduction in GluR1 compared to trained rats, but these results were non-significant and thus only trends can be reported. Nonetheless, since propranolol does not affect LTM in the absence of reactivation, it appears that the effect of propranolol on the fear memory is not confounded by any effects it may have on memory systems altogether. As such. these results suggest that a blockade of reconsolidation by propranolol may reduce fear responses while reducing a molecular correlate of the memory.

This molecular information helps clarify what molecular events account for the amnesia observed when reconsolidation is blocked. Since behaviour is used as a measure of memory retention, there is some question as to the nature of the observed amnesia. Nader (2007) suggests reactivation destabilizes a memory, and blocking its reconsolidation thus interferes with its re-storage. Others suggest that the drug administration following reactivation causes amnesia by damaging the BLA. Specifically, Rudy, Biedenkapp, Moineau & Bolding (2006) cite studies showing that anisomycin can cause apoptosis, a form of programmed cell death. According to this view, the observed amnesia is not the result of deletion of a fear memory, but rather from the destruction of the tissue that would normally store it. Typically, a PR-STM test is taken four hours after reactivation and drug infusion in order to control for any nonspecific effects of the drug on the memory system altogether. This would seem to be a valid test for any lesion effects of a drug, and indeed with anisomycin, there are no such observed deficits in freezing (Nader, 2000). However, Rudy et al. (2006) argue that the lesion effect produced by anisomycin could be delayed for several hours, so that a rat would be impaired at 24 hours, but not four hours, following anisomycin infusion.

Another challenge to the observed amnesia resulting from a reconsolidation blockade was advanced by Lattal & Abel (2004), who suggest that the rats experience a transient impairment in the ability to retrieve the memory. Supporting this view, they found that rats, when tested a day after receiving anisomycin treatment following reactivation, were amnesic. However, when these animals were tested 21 days after this manipulation, the anisomycin-infused rats froze similarly to control rats, indicating that the fear memory spontaneously recovered over time. From these findings, they suggested the fear memory remains intact following reconsolidation blockade, and the amnesia observed after one day reflects a transient inability to retrieve the memory (Lattal & Abel, 2004).

The debate as to the nature of the observed amnesia results from the use of a behavioural measure of memory retention; when a rat does not exhibit fear responses following some manipulation, it is inferred that the rat is amnesic for this memory. Since there is no valid molecular measurement for the integrity of a memory, behaviour is the only way to assess memory retention. The present study, however, provides preliminary evidence to suggest that the observed amnesia is directly attributable to a reduction of GluR1, a molecular correlate of the memory. This therefore suggests that the amnesia following a reconsolidation blockade by propranolol may represent "true amnesia", in that it actually erases a component of the memory.

In light of the preliminary findings of the current study, questions still remain as to how much of the memory is erased. A recent study conducted by Rose & Rankin (2006) investigated reconsolidation in the nematode C. elegans for a nonassociative learning task called habituation. The C. elegans were repeatedly presented with a habituation stimulus (a tap). Initially, the C. elegans swam in the opposite direction of the tap, but after repeated presentations, they showed a decreased response to this stimulus. Twenty-four hours after training, the habituation memory was reactivated by the presentation of several taps followed by the delivery of a heat shock, which works like anisomycin to disrupt protein synthesis. When tested 24 hours after reactivation for the memory of the tap, these animals behaved like controls, suggesting the heat shock successfully blocked reconsolidation. Interestingly, this study also investigated GluR1 levels in the control and the heat-shock group, and found that when reconsolidation was blocked, the C. elegans not only behaved like controls, but their GluR1 levels were equivalent to that of controls. This suggested that a reconsolidation blockade actually re-set the GluR1 levels of the trained rats to that of the untrained controls.

The present study, although examining rodents using a fear conditioning paradigm, supports the results obtained in the Rose and Rankin (2006) study. Interestingly, Rose and Rankin (2006) additionally suggest that the memory trace is not only reduced, but actually erased, to the point where the C. elegans have no molecular trace for the learning of this task. This stands in contrast to the data obtained in the present study, as we demonstrate trends suggesting that propranolol reduced synaptic GluR1 to the level of the trained saline group but not to the level of the naïve rats. Importantly, Rose and Rankin (2006) provides additional evidence that the amnesia induced by a reconsolidation blockade actually decreases a molecular component of the memory, producing a "true amnesia".

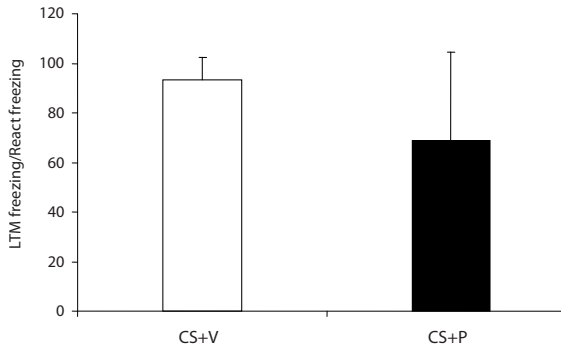

Figure 4 : Quantification of the amnesia induced by Propranolol as compared to Vehicle rats.

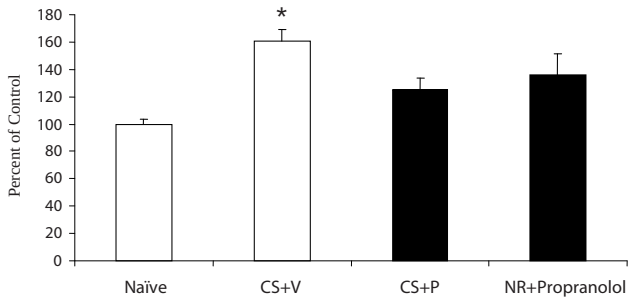

Figure 5 : GluR1 synaptoneurosome quantification in the BLA for each experimental group (Naïve, CS+P, CS+V, no CS+P) normalized to the Naïve.

Beyond these theoretical implications, our study also supports the use of propranolol in clinical treatment for PTSD. This is particularly significant because the most common treatment for PTSD is exposure-based psychotherapy, a form of extinction involving a patient's repeated exposure to the feared object or situation in the absence of any overt danger. Although believed to attenuate the associated emotional response, clinical experiments show it has a poor long-term outcome (Davis, Myers, Chhatwal, Ressler, 2006). It was initially believed that extinction represented "unlearning" at the synaptic level, in that it simply reversed the plasticity associated with acquisition. Such a theory does not reflect the literature, as extinction in both rats and humans is not long-lasting (Myers & Davis, 2002). As a result, it is currently believed that extinction is a new and distinct form of learning, resulting in the formation of an inhibitory association between the CS and US that competes with the original memory trace. This theory is more consistent with the literature, as the conditioned fear response often returns when the animals are tested in a different context, re-exposure to the US prior to testing reinstates the fear memory, and the fear responses to the CS spontaneously recover over time (Myers & Davis, 2002).

A recent study conducted by Mao, Hsaio, Ya-Hsin, Gean, & Po-Wu (2006) using a light-shock conditioning paradigm, found that extinction applied 24 hours after training reduced fear-potentiated freezing without influencing surface GluR1 levels. From this, it was proposed that although extinction reduced fear-potentiated freezing at a behavioural level, it may not affect the original memory trace at a molecular level, and this could explain why extinction training is often short-lasting. In other words, this study suggests that GluR1 might be responsible for the persistence of the memory after extinction. Interestingly, when DCS, a partial agonist for the glycine site on NMDAr was used, the rats' fear-potentiated freezing was reduced, as was the conditioning-induced increase in GluR1. From this, the authors suggested that extinction training with DCS may transform the effect of light-alone trials from inhibitory learning (extinction) to erasure (reconsolidation blockade). The preliminary results from our experiment further suggest that reconsolidation blockade may be an effective treatment for PTSD, as they indicate that a reconsolidation blockade may actually decrease a portion of the emotional component of the memory.

One limitation of the present study is that propranolol was administered systemically and not infused into brain regions of interest, as done in the Nader et. Al (2000) study with anisomycin. This raises the possibility that propranolol may exert nonspecific effects on the memory system. Although we included a control group that received propranolol without memory reactivation, propranolol may cause some long-term permanent changes in fear expression by peripheral sites of action , producing amnesia-like behaviour. Murchison, Zhang, Zhan, Lee, and Thomas (2004) addressed such a problem; prior to fear conditioning, rats were given other β-adrenergic receptor antagonists such as nadolol and sotalol, which do not readily cross the blood-brain barrier, and found no effects on freezing. This suggests that the effects of propranolol are CNS-dependent, and the observed amnesia was not due to interference with fear memory expression, but from its direct effects on the BLA. In addition, although in this study propranolol was administered systemically, we detected a specific effect on synaptic GluR1 levels in the BLA, indicating the behavioral effect of the drug was mediated at least partially via the BLA.

Another limitation of the present study concerns the increase in GluR1. Although this study is the first to our knowledge that demonstrates that levels of GluR1 remain elevated three days following the initial learning, this finding may be confounded by the test trials (reactivation, PR-STM, PT-LTM). It is possible that each test trial may reinstate GluR1, helping to maintain elevated levels of GluR1 so that it can be detected three days after the initial learning. In order to address this confound, a separate control group is required where rats are conditioned and sacrificed three days later without any test trials. This would ensure that GluR1 levels remain high even without the reminder trials, further confirming that GluR1 is a molecular correlate of the memory trace.

In conclusion, the preliminary results obtained in this experiment need to be replicated with a larger sample size in order to statistically validate the observed trends. Also, the use of central infusions of both propranolol and anisomycin into the BLA would provide further evidence that a blockade of reconsolidation actually reduces synaptic GluR1 in the BLA. This would directly correlate the observed behaviour with BLA synaptic GluR1, providing firmer conclusions. Nonetheless, the present study does demonstrate that propranolol injection following reactivation, at a behavioural level, reduces fear expression and, at a molecular level, reduces a correlate of long-term memory. These preliminary results suggest that a blockade of reconsolidation actually erases a component of the memory.

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