The basolateral amygdala complex and perirhinal cortex represent focal and peripheral states of information processing in rats.

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Abstract. This study used variations of a sensory preconditioning protocol in male and female rats to test a theory that the basolateral amygdala complex (BLA) and perirhinal cortex (PRh) represent focal and peripheral states of attention, respectively. It specifically tested predictions derived from the theory regarding when learning about a stimulus that signals danger will be disrupted by BLA or PRh infusions of the *N-methyl-D-aspartate* receptor (NMDAR) antagonist, DAP5. Consistent with the theory, the effects of these infusions depended on the novelty/familiarity of the conditioned stimulus as well as the manner in which it was paired with foot shock. When a stimulus was novel, its conditioning required activation of NMDAR in the BLA and not the PRh (Experiments 2A and 2B) regardless of whether the stimulus-shock pairings were contiguous or separated in time. When a pre-exposed and, thereby, familiar stimulus was presented contiguously with shock, its conditioning again required activation of NMDAR in the BLA and not the PRh (Experiments 1A, 1B, 3A and 3B). However, when a pre-exposed stimulus was indirectly paired with shock - because it was associatively activated at the time of shock or separated from the shock by another stimulus - its conditioning required activation of NMDAR in the

PRh and not the BLA (Experiments 1A, 1B, 3A and 3B). These findings are discussed in relation to theories of information processing that distinguish between focal and peripheral states of attention/memory, and past studies that have examined the substrates of learning and memory in the PRh and BLA.

Introduction

Sensory preconditioning is a laboratory protocol that can be used to examine how the mammalian brain processes sensory and emotional information. It typically involves three stages. In stage 1, rats are exposed to pairings of two relatively harmless stimuli, S2 and S1 (e.g., a sound and light). In stage 2, rats are exposed to pairings of one of these stimuli, the S1 (e.g., the light), with foot shock. Finally, in stage 3, rats display fear or defensive responses (e.g., freezing) when tested with the S1 that had been paired with shock and the S2 that had *never* been paired with shock. Importantly, the fear of S2 is not due to generalization from S1 or any innately aversive properties of the S2, as it is absent among controls exposed to explicitly unpaired presentations of the relevant stimuli in either stage 1 or stage 2 (Holmes et al., 2013; Holmes & Westbrook, 2017; Kikas et al., 2019; Michalscheck et al., 2021; Parkes & Westbrook, 2010; Rizley & Rescorla, 1972; Wong et al., 2019, 2025). Instead, where it is observed, fear of the S2 reflects integration of the sensory and emotional information encoded in stages 1 and 2. That is, the S2-S1 (sensory) association formed in stage 1 is integrated with the S1-shock (emotional) association formed in stage 2 to drive fear responses during presentations of the S2 alone in stage 3.

Over the past 10 years, our laboratory has examined the neural substrates of the different types of information that rats encode in this sensory preconditioning protocol. Our work has focused on two regions of the medial temporal lobe - the perirhinal cortex (PRh) and basolateral amygdala complex (BLA) - and revealed that the involvement of these two regions can be quite distinct. For example, the S2-S1 association that forms in stage 1 is

encoded through activation of *N-methyl-D-aspartate* receptors (NMDARs) in the PRh, not the BLA; whereas the S1-shock association that forms in stage 2 is encoded through activation of NMDARs in the BLA, not the PRh (Campeau et al., 1992; Fanselow & Kim, 1994; Miserendino et al., 1990; Holmes et al., 2013, 2018; Rodrigues et al., 2001; see also Bang & Brown, 2008; Phillips & LeDoux, 1995; Romanski & LeDoux, 1992a, 1992b; Wilensky et al., 2006). In a recent pair of studies, we extended these findings in two ways. First, we showed that S1 does not just form an association with shock in stage 2; it also mediates an association between S2 and the shock. Thus, S2 enters testing in stage 3 already conditioned, able to elicit fear responses (Wong et al., 2019). Second, we showed that this mediated S2-shock association requires NMDAR-activation in the PRh, as well as communication between the PRh and BLA (Wong et al., 2025). These findings raise two critical questions: 1) *why* is the PRh engaged for mediated conditioning of S2 but not for direct conditioning of S1; and 2) more generally, *what* determines whether the BLA and/or PRh is engaged for conditioning of the S1 and/or S2?

One explanation for the different substrates of conditioning to S2 and S1 in our sensory preconditioning protocol is that the two stimuli access different states of attention/memory which are differentially supported by the BLA and PRh. That is, during stage 2 of our protocol, the BLA may function like a focal state of attention in processing information about the presented S1 and foot shock. By contrast, because the PRh encodes the S2-S1 pre-exposures in stage 1, this region may function like a peripheral state of attention in processing information about the S2 that is retrieved from long-term memory. Such a characterization explains why the direct S1-shock association requires activation of NMDARs in the BLA but not the PRh (S1 and shock co-occur in the focus of attention); and the mediated S2-shock association requires activation of NMDARs in the PRh as well as communication between the PRh and BLA (S2 conditions in the periphery of attention as the

shock occurs in its focus). However, it also implies that the substrates of conditioning to S2 will depend on how the S2-S1 and S1-shock pairings are presented. For example, different types of arrangements may influence the substrates of conditioning to S2 by influencing its novelty and/or its predictive value at the time of the shock, on the supposition that familiar stimuli are processed in the periphery of attention and, thereby, the PRh (Bogacz & Brown, 2003; Brown & Banks, 2015; Brown & Bashir, 2002; Martin et al., 2013; McClelland et al., 2014; Morillas et al., 2017; Murray & Wise, 2012; Robinson et al., 2010; Suzuki & Naya, 2014; Voss et al., 2009; Yang et al., 2023) whereas novel stimuli are processed in the focus of attention and, thereby, the amygdala (Holmes et al., 2018; Qureshi et al., 2023; Roozendaal et al., 2006; Rutishauser et al., 2006; Schomaker & Meeter, 2015; Wright et al., 2003). This can be achieved by presenting the S2-S1 and S1-shock pairings together as S2-S1-shock sequences. Under such circumstances, conditioning to the S2 should cease to occur in the periphery of attention as it is novel and no longer requires retrieval of information from long-term memory. Instead, this conditioning should occur in the focus of attention and, thereby require activation of NMDARs in the BLA and not the PRh (e.g., Williams-Spooner et al., 2022).

Different types of arrangements may also influence the substrates of conditioning to S2 as a function of its "distance" or temporal separation from the shock. The supposition here is that shock/danger and its immediate antecedents are processed in the focus of attention (and, thereby, the amygdala), regardless of whether the antecedents are novel or familiar; whereas stimuli that are temporally separated from the shock *can be* processed in the periphery of attention (and, thereby, the PRh) and *are more likely to be* processed there if they are familiar. This can be assessed by pre-exposing rats to S2-S1 pairings prior to conditioning with S2-S1-shock sequences. Under these circumstances, conditioning to the familiar S1 that is contiguous with shock should occur in the focus of attention and, thereby,

require activation of NMDARs in the BLA and not the PRh; whereas conditioning to the familiar S2 that is separated from the shock (by S1) should occur in the periphery of attention and, thereby, require activation of NMDARs in the PRh and not the BLA.

The present series of experiments used these variations of a sensory preconditioning protocol to test the implications of our theory that the BLA and PRh represent focal and peripheral states of attention, respectively. The first implication is that, in our standard sensory preconditioning protocol (S2-S1 pairings in stage 1, S1-shock pairings in stage 2), the substrates of conditioning to S2 and S1 will be doubly dissociable at the level of NMDARs in the PRh and BLA. That is, during the session of S1-shock pairings in stage 2, mediated conditioning of the absent S2 should occur in the periphery of attention and hence, require NMDAR-activation in the PRh but *not* the BLA, whereas direct conditioning of the presented S1 should occur in the focus of attention and hence, require NMDAR-activation in the BLA but not the PRh. This was supported by the initial experiments which found that a stage 2 infusion of the NMDAR antagonist, DAP5 into the PRh disrupts mediated conditioning of S2 but spares direct conditioning of S1 (Experiment 1A); whereas a stage 2 infusion of DAP5 into the BLA disrupts direct conditioning of S1 but spares mediated conditioning of S2 (Experiment 1B). Subsequent experiments then used variations of this protocol to examine whether the engagement of NMDAR in the PRh or BLA for Pavlovian fear conditioning is influenced by the novelty/predictive value of the stimuli at the time of the shock (second implication of theory) as well as their distance or separation from the shock (third implication of theory; Table 1). Following the arguments above, we predicted that:

1) When S2 or S1 is novel and paired with shock, its conditioning will be disrupted by a DAP5 infusion into the BLA but unaffected by a DAP5 infusion into the PRh (Experiments 2A and 2B);

2) When S2 or S1 is familiar and directly paired (i.e., co-occurs) with shock, its conditioning will again be disrupted by a DAP5 infusion into the BLA but not the PRh (Experiments 1A, 1B, 3A and 3B);
3) However, when S2 or S1 is familiar and indirectly paired with shock - because it is associatively activated at the time of shock or separated from the shock by another stimulus - its conditioning will be unaffected by a DAP5 infusion into the BLA but disrupted by a DAP5 infusion into the PRh (Experiments 1A, 1B, 3A and 3B).

Results

Experiments 1A and 1B – Encoding of the mediated S2-shock association requires NMDAR-activation in the PRh but not the BLA; Encoding of the direct S1-shock association requires NMDAR-activation in the BLA but not the PRh

We propose that the BLA and PRh represent focal and peripheral states of attention/memory, respectively. The first implication of this proposal is that, during stage 2 of our standard sensory preconditioning protocol, the substrates of conditioning to the absent S2 and presented S1 will be doubly dissociable at the level of NMDARs in the PRh and BLA: mediated conditioning of the absent S2 should occur in the periphery of attention and hence, require NMDAR-activation in the PRh but *not* the BLA, whereas direct conditioning of the presented S1 should occur in the focus of attention and hence, require NMDAR-activation in the BLA but not the PRh. Experiments 1A and 1B tested this implication. The design of these experiments is shown in Figure 1A. The two experiments differed only in that we assessed the involvement of the PRh in Experiment 1A and the BLA in Experiment 1B. In each experiment, two groups of rats were exposed to a session of S2-S1 pairings in stage 1 and, 24 h later, a session of S1-shock pairings in stage 2. Immediately prior to the conditioning

session in stage 2, rats in each experiment received an infusion of DAP5 or vehicle only into either the PRh (Experiment 1A) or BLA (Experiment 1B). Finally, all rats were tested with presentations of the S2 alone and S1 alone in stage 3. Based on our past findings and the reasoning given above, we expected that mediated conditioning of the S2 would require activation of NMDARs in the PRh (Wong et al., 2025) but not the BLA, whereas the direct conditioning of the S1 would require activation of NMDARs in the BLA but not the PRh (e.g., Rodrigues et al., 2001; Williams-Spooner et al., 2022). Hence, we anticipated that the stage 2 infusion of DAP5 into the PRh would disrupt the test level of freezing to S2 without affecting the test level of freezing to S1; and that the stage 2 infusion of DAP5 into the BLA would disrupt the test level of freezing to S1 without affecting the test level of freezing to S2.

Experiment 1A – NMDAR-activation in the PRh is required for acquisition of the mediated S2-shock association but not the direct S1-shock association

Conditioning. The baseline levels of freezing during conditioning and test sessions were low (< 10%) and did not differ between groups (largest $F_{(1,19)} = 2.876$; $p = .106$). Conditioning of the S1 was successful. Freezing to S1 increased across the four S1-shock pairings in stage 2 ($F_{(1,19)} = 74.708$; $p < .001$; $n_p^2 = .797$; 95% CI: [1.658, 2.717]). The rate of this increase did not differ between the two groups ($F_{(1,19)} = .008$; $p = .930$), and there was no significant between-group differences in overall freezing to the S1 ($F_{(1,19)} = 1.803$; $p = .195$).

Test. Figure 1B shows the mean (± SEM) levels of freezing to the S2 alone (left panel) and S1 alone (right panel) during testing in Experiment 1A, averaged across the eight presentations of each stimulus. Both groups showed equivalent levels of freezing to the directly conditioned S1 ($F_{(1,19)} = 1.423$; $p = .248$), indicating that the PRh infusion of DAP5 had no effect on this conditioning. However, rats that received a PRh infusion of DAP5 (Group DAP5) in stage 2 exhibited significantly less freezing to the preconditioned S2 than

rats that received a PRh infusion of vehicle only (Group VEH; $F_{(1,19)} = 7.136$; $p = .015$; $n_p^2 = .273$; 95% CI: [0.176, 1.447]).

Experiment 1B – NMDAR-activation in the BLA is required for acquisition of the direct S1-shock association but not the mediated S2-shock association

Conditioning. The baseline levels of freezing during conditioning and test sessions were low (< 10%) and did not differ between groups (largest $F_{(1,26)} = 1.590$; $p = .219$). Conditioning of the S1 was successful. Freezing to S1 increased across the four S1-shock pairings in stage 2 ($F_{(1,26)} = 104.494$; $p < .001$; $n_p^2 = .801$; 95% CI: [1.633, 2.456]). The rate of this increase did not differ between groups ($F_{(1,26)} = .259$; $p = .615$), however there was a significant between-group difference in overall freezing to the S1 ($F_{(1,26)} = 6.068$; $p = .021$; $n_p^2 = .189$; 95% CI: [0.094, 1.046]), indicating that the BLA infusion of DAP5 disrupted conditioning to the S1.

Test. Figure 1D shows the mean (± SEM) levels of freezing to the S2 alone (left panel) and S1 alone (right panel) during testing in Experiment 1B, averaged across the eight presentations of each stimulus. Both groups showed equivalent levels of freezing to the preconditioned S2 ($F_{(1,26)} = .013$; $p = .910$). However, rats that received a BLA infusion of DAP5 (Group DAP5) in stage 2 exhibited significantly less freezing to the directly conditioned S1 than rats that received a BLA infusion of vehicle only (Group VEH; $F_{(1,26)} = 4.775$; $p = .038$; $n_p^2 = .155$; 95% CI: [0.040, 1.308]).

Experiment 1A replicated previous findings that a stage 2 infusion of DAP5 into the PRh disrupts the acquisition of sensory preconditioned freezing to S2 without affecting the acquisition of freezing to S1 (Wong et al., 2025). By contrast, Experiment 1B showed that a BLA infusion of DAP5 disrupts the acquisition of freezing to S1 (replication) without affecting the acquisition of sensory preconditioned freezing to S2 (new result). Taken

together, these findings indicate that the involvement of NMDARs in direct and mediated conditioning is doubly dissociable at the levels of the BLA and PRh. The direct conditioning of a stimulus that co-terminates with shock requires activation of NMDARs in the BLA but does not require activation of NMDARs in the PRh. Conversely, the mediated conditioning of a stimulus representation that is retrieved from long-term memory (and, hence, indirectly paired with shock) requires activation of NMDARs in the PRh but occurs independently of NMDAR-activation in the BLA. These findings are exactly consistent with the predictions derived from our proposal that the BLA and PRh represent focal and peripheral states of attention/memory, respectively. The BLA represents a focal state and, thereby, encodes the directly conditioned S1-shock association, whereas the PRh represents a peripheral state and, thereby, encodes the indirect or mediated S2-shock association.

Experiments 2A and 2B – The roles of the PRh and BLA in Pavlovian fear conditioning when the S2-S1 and S1-shock pairings are combined into an S2-S1-shock sequence.

A second implication of our proposal is that the engagement of NMDAR in the PRh for conditioning to S2 will be influenced by its novelty/familiarity at the time of the shock. We addressed this implication by combining the S2-S1 and S1-shock pairings into S2-S1-shock sequences that were administered in a single training session (Experiments 2A and 2B). The consequence of doing so is that, in contrast to the previous experiments in which the S2 and S1 were both familiar at the time of their conditioning with foot shock, here the S2 and S1 were both novel at the time of their conditioning with foot shock. Immediately prior to the session containing the S2-S1-shock sequences, rats received an infusion of DAP5 or vehicle only into either the PRh (Experiment 2A) or BLA (Experiment 2B). Finally, all rats were tested with presentations of the S2 alone and S1 alone in stage 3. We hypothesized that, because S2 was novel and presented in sequence with S1 and shock in stage 2, it would be processed in a focal state of attention rather than the periphery. Hence, whereas conditioning

221 to a representation of the pre-exposed S2 in the previous experiment required NMDAR-
222 activation in the PRh but not the BLA, we predicted that conditioning to the novel S2 in this
223 experiment would require NMDAR-activation in the BLA but not the PRh. Specifically, we
224 expected that the stage 2 infusion of DAP5 into the PRh would have no effect on the test
225 level of freezing to either S2 or S1; and that the stage 2 infusion of DAP5 into the BLA
226 would disrupt the test level of freezing to both S2 and S1.

227 ***Experiment 2A – When the S2-S1 and S1-shock pairings are combined into S2-S1-shock***
228 ***sequences, the PRh is no longer engaged for conditioning of the S2***

229 *Conditioning.* The baseline levels of freezing during conditioning and test sessions
230 were low (< 10%) and did not differ between groups (largest $F_{(1,15)} = 3.026$; $p = .102$).
231 Conditioning was successful. Freezing to the S2 and S1 increased linearly across the four S2-
232 S1-shock sequences in stage 2 (smaller $F_{(1,15)} = 47.193$; $p < .001$; $n_p^2 = .759$; 95% CI: [1.453,
233 2.760]). The linear x Group interaction was significant for freezing to the S2 ($F_{(1,15)} = 5.117$;
234 $p < .039$; $n_p^2 = .254$; 95% CI: [0.056, 1.868]), but not for freezing to the S1 ($F_{(1,15)} = .009$; $p =$
235 $.926$). This suggests that the PRh infusion of DAP5 slowed acquisition of freezing to the S2
236 without affecting freezing to the S1. However, there were no significant between-group
237 differences in overall freezing to the S2 ($F_{(1,15)} = 4.125$; $p = .06$) or S1 ($F_{(1,15)} = 2.726$; $p =$
238 $.120$).

239 *Test.* Figure 2B shows the mean (± SEM) levels of freezing to the S2 alone (left
240 panel) and S1 alone (right panel) during drug-free testing in Experiment 2A, averaged across
241 the eight presentations of each stimulus. Both groups showed equivalent levels of freezing to
242 the S2 ($F_{(1,15)} = 1.292$; $p = .274$) and S1 ($F_{(1,15)} = .838$; $p = .374$).

243 ***Experiment 2B – When the S2-S1 and S1-shock pairings are combined into S2-S1-shock***
244 ***sequences, the BLA is engaged for conditioning of the S2***

Conditioning. The baseline levels of freezing during conditioning and test sessions were low (< 10%) and did not differ between groups (largest $F_{(1,15)} = .576$; $p = .460$). Conditioning was again successful. Freezing to the S2 and S1 (smaller $F_{(1,15)} = 62.211$; $p < .001$; $n_p^2 = .806$; 95% CI: [1.093, 1.902]) increased linearly across the four S2-S1-shock sequences in stage 2. The linear x Group interaction was significant for freezing to the S2 ($F_{(1,15)} = 9.477$; $p = .008$; $n_p^2 = .387$; 95% CI: [0.337, 1.852]) and S1 ($F_{(1,15)} = 12.771$; $p = .003$; $n_p^2 = .460$; 95% CI: [0.548, 2.167]), suggesting that the BLA infusion of DAP5 slowed acquisition of freezing to both stimuli. However, there were no significant between-group differences in overall freezing to the S2 ($F_{(1,15)} = 2.999$; $p = .104$) or S1 ($F_{(1,15)} = 3.747$; $p = .072$).

Test. Figure 2D shows the mean (± SEM) levels of freezing to the S2 alone (left panel) and S1 alone (right panel) during drug-free testing in Experiment 2B, averaged across the eight presentations of each stimulus. Rats that received a BLA infusion of DAP5 (Group DAP5) in stage 2 exhibited significantly less freezing to both the S2 ($F_{(1,15)} = 8.660$; $p = .010$; $n_p^2 = .366$; 95% CI: [0.349, 2.182]) and S1 ($F_{(1,15)} = 12.089$; $p = .003$; $n_p^2 = .446$; 95% CI: [0.507, 2.114]) compared to the vehicle-infused controls.

Experiments 2A and 2B showed that combining the S2-S1 and S1-shock pairings into S2-S1-shock sequences altered the roles of the PRh and BLA in conditioning to the S2. In the previous experiments, the S2-S1 (stage 1) and S1-shock (stage 2) pairings were separated by 24 hours and acquisition of freezing to S2 required NMDAR-activation in the PRh but not the BLA. Here, the acquisition of freezing to S2 (and S1) across S2-S1-shock sequences required NMDAR-activation in the BLA but not the PRh. These findings are consistent with our proposal that the BLA and PRh function like different states of attention/memory. The BLA represents a focal state and, thus, processes information about novel stimuli that are presented in a sequence that co-terminates with shock: hence it was engaged for conditioning to both

270 the S2 and S1 in the current experiment. By contrast, the PRh represents a peripheral state
271 and maintains the trace or representation of a pre-exposed stimulus: hence, it was not
272 engaged for conditioning to either the S2 or S1 in the current experiment.

273 **Experiments 3A and 3B – The roles of the PRh and BLA in Pavlovian fear conditioning**
274 **when rats are pre-exposed to S2-S1 pairings prior to conditioning with S2-S1-shock**
275 **sequences.**

276 A third implication of our proposal is that pre-exposure can alter the way that stimuli
277 are processed in the focus/periphery of attention and, thereby, the substrates of their
278 conditioning in the PRh and BLA. The additional implication is based on decades of work
279 showing that pre-exposure to a stimulus can reduce its rate of conditioning (Holmes & Harris,
280 2009, 2010; Kennedy et al., 2021; Leung et al., 2013a, 2013b; Lubow, 1973; Lubow &
281 Moore, 1959) while increasing the context dependence of any responding that develops
282 (Holmes & Westbrook, 2014; Rosenberg et al., 2011; Westbrook et al., 2000). We addressed
283 this implication by pre-exposing rats to S2-S1 pairings in stage 1 followed by conditioning
284 with S2-S1-shock sequences in stage 2. We specifically examined whether pre-exposing rats
285 to S2-S1 pairings reduces processing of the S2 in the focus of attention when it is presented
286 as part of S2-S1-shock sequences in stage 2: i.e., whether it reduces the ability of S2 to access
287 the focus of attention (unlikely given the S1 results in Experiments 1A and 1B) and/or
288 accelerates the rate at which S2 transitions to the periphery when it is replaced by
289 presentation of the S1. If so, the substrates of conditioning to S2 in the current experiments
290 should differ from the substrates of conditioning to S2 in Experiments 2A and 2B. That is,
291 whereas conditioning of the novel S2 in Experiments 2A and 2B required NMDAR-
292 activation in the BLA and not the PRh, conditioning of the pre-exposed S2 in the current
293 experiments should require NMDAR-activation in the PRh and not the BLA.

The design of Experiments 3A and 3B is shown in Figure 3. Briefly, rats were exposed to a session of S2-S1 pairings in stage 1 and, 24 h later, a session of S2-S1-shock sequences in stage 2. Immediately prior to the latter session, rats received an infusion of DAP5 or vehicle only into either the PRh (Experiment 3A) or BLA (Experiment 3B). Finally, all rats were tested with presentations of the S2 alone and S1 alone in stage 3. We hypothesized that pre-exposure to S2-S1 pairings prior to conditioning with S2-S1-shock sequences would *not* affect the substrates of directly conditioned fear to S1: that is, just as the direct conditioning of a pre-exposed S1 in Experiments 1A and 1B required NMDAR-activation in the BLA but not the PRh, here again we expected that direct conditioning of the pre-exposed S1 would require NMDAR-activation in the BLA but not the PRh. Such a result would suggest that, with the level of pre-exposure used here, the S1 accesses the focus of attention: hence its conditioning requires activation of NMDAR in the BLA and not the PRh. By contrast, we hypothesized that pre-exposure to S2-S1 pairings prior to conditioning with S2-S1-shock sequences would alter the substrates of conditioning to S2: that is, whereas conditioning to the novel S2 in the previous experiments required NMDAR-activation in the BLA but not the PRh, here we expected that conditioning of the pre-exposed S2 would require NMDAR-activation in the PRh but not the BLA. Such a result would suggest that a pre-exposed S2 more rapidly transitions from the focus of attention to its periphery when it is replaced by S1: hence its conditioning ceases to require activation of NMDAR in the BLA and now requires activation of NMDAR in the PRh.

Experiment 3A – When rats are pre-exposed to S2-S1 pairings prior to conditioning with S2-S1-shock sequences, the PRh is engaged for acquisition of freezing to S2 but not S1

Conditioning. The baseline levels of freezing during conditioning and test sessions were low (< 10%) and did not differ between groups (largest $F_{(1,22)} = 3.143$; $p = .090$). Conditioning was successful. Freezing to the S2 and S1 increased across the four S2-S1-

shock sequences in stage 2 (smaller $F_{(1,22)} = 110.127$; $p < .001$; $n_p^2 = .833$; 95% CI: [1.877, 2.802]). The rate of this increase did not differ between groups (largest $F_{(1,22)} = 1.397$; $p = .250$), and there were no significant between-group differences in overall freezing to the S2 and S1 (largest $F_{(1,22)} = .620$; $p = .439$).

Test. Figure 3B shows the mean (± SEM) levels of freezing to the S2 alone (left panel) and S1 alone (right panel) during testing in Experiment 3A, averaged across the eight presentations of each stimulus. Both groups showed equivalent levels of freezing to the directly conditioned S1 ($F_{(1,22)} = .226$; $p = .639$). However, rats that received a PRh infusion of DAP5 (Group DAP5) in stage 2 exhibited significantly less freezing to the S2 than rats that received a PRh infusion of vehicle only (Group VEH; $F_{(1,22)} = 4.644$; $p = .042$; $n_p^2 = .174$; 95% CI: [0.025, 1.330]).

Experiment 3B – When rats are pre-exposed to S2-S1 pairings prior to conditioning with S2-S1-shock sequences, the BLA is engaged for acquisition of freezing to S1 but not S2

Conditioning. The baseline levels of freezing during conditioning and test sessions were low (< 10%) and did not differ between groups (largest $F_{(1,18)} = 2.045$; $p = .170$). Conditioning was again successful. Freezing to the S2 and S1 increased across the four S2-S1-shock sequences in stage 2 (smaller $F_{(1,18)} = 36.409$; $p < .001$; $n_p^2 = .669$; 95% CI: [1.035, 2.141]). The rate of this increase did not differ between groups (largest $F_{(1,18)} = 1.474$; $p = .240$), and there were no significant between-group differences in overall freezing to the S2 ($F_{(1,18)} = 1.056$; $p = .318$) and S1 ($F_{(1,18)} = .071$; $p = .793$).

Test. Figure 3D shows the mean (± SEM) levels of freezing to the S2 alone (left panel) and S1 alone (right panel) during testing in Experiment 3B, averaged across the eight presentations of each stimulus. Both groups showed equivalent levels of freezing to the S2 ($F_{(1,18)} = 2.078$; $p = .167$). However, rats that received a BLA infusion of DAP5 (Group

343 DAP5) in stage 2 exhibited significantly less freezing to the directly conditioned S1 than rats
344 that received a BLA infusion of vehicle (Group VEH; $F_{(1,18)} = 5.899$; $p = .026$; $n_p^2 = .247$;
345 95% CI: [0.114, 1.571]).

346 Experiments 3A and 3B showed that pre-exposure can alter the way that stimuli are
347 processed in the focus/periphery of attention and, thereby, the substrates of their conditioning
348 in the PRh and BLA. In the preceding experiments (2A and 2B) where rats were conditioned
349 with S2-S1-shock sequences in the absence of any stimulus pre-exposure, the acquisition of
350 freezing to S1 and S2 required NMDAR-activation in the BLA but not the PRh. By contrast,
351 in the present experiments where rats were conditioned with S2-S1-shock sequences 24 h
352 after having been pre-exposed to S2-S1 pairings, the substrates of conditioning to S1 and S2
353 were distinct: acquisition of freezing to S1 required NMDAR-activation in the BLA but not
354 the PRh, whereas acquisition of freezing to S2 required NMDAR-activation in the PRh but
355 not the BLA. These findings are consistent with our proposal that the BLA and PRh function
356 like different states of attention. The BLA functions like a focal state and, thus, processes
357 information about stimuli that are directly paired with shock: hence it was engaged for
358 conditioning to the pre-exposed S1 which co-terminated with foot shock but was not engaged
359 for conditioning to the pre-exposed S2 which was separated from the shock by S1. By
360 contrast, the PRh represents a peripheral state and, as such, maintains a trace/representation
361 of a pre-exposed stimulus when it is replaced by another stimulus: hence, it was not engaged
362 for conditioning to the pre-exposed S1 which co-terminated with foot shock but was engaged
363 for conditioning to the pre-exposed S2 which was separated from the shock by S1.

364 **Experiments 4A and 4B – The roles of the PRh and BLA in Pavlovian fear conditioning**
365 **when rats are pre-exposed to S2-S1 pairings prior to conditioning with S2-[trace]-shock**
366 **sequences.**

367 In Experiments 3A and 3B, conditioning of the pre-exposed S1 required NMDAR-
368 activation in the BLA and not the PRh; whereas conditioning of the pre-exposed S2 required
369 NMDAR-activation in the PRh and not the BLA. We attributed these findings to the fact that
370 the pre-exposed S2 was separated from the shock by S1 during conditioning of the S2-S1-
371 shock sequences in stage 2: hence, at the time of the shock, S2 was no longer processed in the
372 focal state of attention supported by the BLA; instead, it was processed in the peripheral state
373 of attention supported by the PRh.

374 Experiments 4A and 4B employed a modification of the protocol used in Experiments
375 3A and 3B to examine whether a pre-exposed S1 influences the processing of a pre-exposed
376 S2 across conditioning with S2-S1-shock sequences. The design of these experiments is
377 shown in Figure 4A. Briefly, in each experiment, two groups of rats were exposed to a
378 session of S2-S1 pairings in stage 1 and, 24 hours later, a session of S2-[trace]-shock pairings
379 in stage 2, where the duration of the trace interval was equivalent to that of S1 in the
380 preceding experiments. Immediately prior to the trace conditioning session in stage 2, one
381 group in each experiment received an infusion of DAP5 or vehicle only into either the PRh
382 (Experiment 4A) or BLA (Experiment 4B). Finally, all rats were tested with presentations of
383 the S2 alone in stage 3. If the substrates of conditioning to S2 are determined only by the
384 amount of time between presentations of this stimulus and foot shock in stage 2, the results
385 obtained in Experiments 4A and 4B should be the same as those obtained in Experiments 3A
386 and 3B: acquisition of freezing to S2 will require activation of NMDARs in the PRh and not
387 the BLA. If, however, the presence of S1 in the preceding experiments (Experiments 3A and
388 3B) accelerated the rate at which processing of S2 transitioned from the focus of attention to
389 its periphery, the results obtained in Experiments 4A and 4B will differ from those obtained
390 in Experiments 3A and 3B. That is, in contrast to the preceding experiments where

acquisition of freezing to S2 required NMDAR-activation in the PRh and not the BLA, here acquisition of freezing to S2 should require NMDAR-activation in the BLA but not the PRh.

Experiment 4A – When rats are exposed to S2-S1 pairings in stage 1 and trace conditioning of S2 in stage 2, the PRh is NOT required for encoding of the S2-shock association

Conditioning. The baseline levels of freezing during conditioning and test sessions were low (< 10%) and did not differ between groups (largest $F_{(1,27)} = 1.095$; $p = .305$). Conditioning was successful. Freezing to the S2 increased across the four S2-[trace]-shock pairings in stage 2 ($F_{(1,27)} = 120.496$; $p < .001$; $n_p^2 = .817$; 95% CI: [1.966, 2.871]). The rate of this increase did not differ between the two groups ($F_{(1,27)} = .332$; $p = .569$), and there was no significant difference between them in overall freezing to the S2 ($F_{(1,27)} = .323$; $p = .575$).

Test. Figure 4B shows the mean (± SEM) levels of freezing to the S2 alone (left panel) during testing in Experiment 4A, averaged across the eight presentations of this stimulus. Both groups showed equivalent levels of freezing to the trace conditioned S2 ($F_{(1,27)} = 1.505$; $p = .230$).

Experiment 4B – When rats are exposed to S2-S1 pairings in stage 1 and trace conditioning of S2 in stage 2, the BLA is required for encoding of the S2-shock association

Conditioning. The baseline levels of freezing during conditioning and test sessions were low (< 10%) and did not differ between groups (largest $F_{(1,24)} = 3.823$; $p = .062$). Conditioning was successful. Freezing to the S2 increased across the four S2-[trace]-shock pairings in stage 2 ($F_{(1,24)} = 47.799$; $p < .001$; $n_p^2 = .666$; 95% CI: [1.069, 1.979]). The rate of this increase did not differ between the two groups ($F_{(1,24)} = 3.421$; $p = .077$), however there was a significant between-group difference in overall freezing to the S2 ($F_{(1,24)} = 7.040$; $p = .014$; $n_p^2 = .227$; 95% CI: [0.168, 1.348]).

415 *Test.* Figure 4D shows the mean (± SEM) levels of freezing to the S2 alone (left
416 panel) during testing in Experiment 4B, averaged across the eight presentations of this
417 stimulus. Rats that received a BLA infusion of DAP5 (Group DAP5) in stage 2 exhibited
418 significantly less freezing to the trace conditioned S2 than rats that received a BLA infusion
419 of vehicle only ($F_{(1,24)} = 7.390$; $p = .012$; $n_p^2 = .235$; 95% CI: [0.213, 1.554]).

420 Together with the results of the preceding experiments (3A and 3B), the present
421 findings show that when a pre-exposed S2 is paired with shock, the substrates of its
422 conditioning are determined by more than just the time between its offset and the shock: they
423 are additionally determined by what happens in the interval between the two events. When
424 the interval is blank, as it was in the present experiments (4A and 4B), the acquisition of
425 freezing to S2 requires NMDAR-activation in the BLA, not the PRh. When the interval is
426 occupied by a presentation of the pre-exposed S1, as it was in the preceding experiments (3A
427 and 3B), the acquisition of freezing to S2 requires NMDAR-activation in the PRh, not the
428 BLA. These findings can be understood in terms of our proposal that the PRh and BLA
429 function like different states of attention: the presence of S1 in the preceding experiments
430 (Experiments 3A and 3B) accelerated the rate at which processing of S2 transitioned to a
431 peripheral state of attention, hence its conditioning in those experiments required NMDAR-
432 activation in the PRh and not the BLA. This and other implications of our proposal will be
433 considered further in the General Discussion.

434

435 **General Discussion**

436 This series of experiments used variations of a sensory preconditioning protocol in
437 rats to test the implications of our proposal that the BLA and PRh represent different states of
438 attention: the BLA functions like a focal state in processing stimuli that are physically

439 present, regardless of their associative history; whereas the PRh functions like a peripheral
440 state in supporting the traces or representations of pre-exposed stimuli that are not physically
441 present. The first implication was that, in our standard sensory preconditioning protocol, the
442 substrates of conditioning to S2 and S1 would be doubly dissociable at the level of NMDARs
443 in the PRh and BLA: during the session of S1-shock pairings in stage 2, mediated
444 conditioning of the absent S2 would occur in the periphery of attention and hence, require
445 NMDAR-activation in the PRh but *not* the BLA; whereas direct conditioning of the presented
446 S1 would occur in the focus of attention and hence, require NMDAR-activation in the BLA
447 but not the PRh. In these experiments, rats were exposed to a session of S2-S1 pairings in
448 stage 1 and, 24 hours later, to a session of S1-shock pairings in stage 2. We have previously
449 shown that, across the session of S1-shock pairings, the S1 is directly associated with foot
450 shock and mediates an association between the *absent-but-expected* S2 and foot shock. Here,
451 we replicated our previous findings that formation of the direct S1-shock association requires
452 activation of NMDARs in the BLA but not the PRh (Williams-Spooner et al., 2022; Wong et
453 al., 2025); and extended these findings to show that formation of the mediated S2-shock
454 association requires activation of NMDARs in the PRh but not the BLA. Specifically, we
455 found that a BLA infusion of the NMDAR antagonist, DAP5, immediately prior to the
456 training session in stage 2 disrupted freezing to the S1 at test without affecting the level of
457 freezing to S2; and, conversely, a PRh infusion of DAP5 immediately prior to stage 2
458 disrupted freezing to the S2 at test without affecting the level of freezing to S1. These
459 findings were taken to mean that the BLA regulates conditioning to a stimulus that is directly
460 paired with danger and, hence, processed in the focus of attention; whereas the PRh regulates
461 conditioning to a stimulus that is indirectly paired with danger and, hence, processed in a
462 peripheral state of attention/memory. Consequently, blocking NMDAR in the BLA impaired
463 direct conditioning of the S1 but spared mediated conditioning of the S2; and blocking

464 NMDAR in the PRh impaired mediated conditioning of the S2 but spared direct conditioning
465 of the S1.

466 The second implication of our proposal was that combining the S2-S1 and S1-shock
467 pairings into S2-S1-shock sequences should selectively alter the substrates of conditioning to
468 S2. Under these circumstances, the novel S2 would be more effectively maintained in the
469 focus of attention along with the S1: hence, conditioning to both stimuli would occur in the
470 BLA and not the PRh. This prediction was supported in Experiments 2A and 2B. Here, rats
471 were exposed to S2-S1-shock sequences and the acquisition of freezing to both S1 and S2
472 was disrupted by a DAP5 infusion into the BLA but unaffected by a DAP5 infusion into the
473 PRh. That is, combining the S2-S1 pairings and S1-shock pairings into S2-S1-shock
474 sequences had no impact on the substrates of conditioning to S1: as in Experiments 1A and
475 1B where the S2-S1 and S1-shock pairings were separated by 24 hours, acquisition of
476 freezing to S1 required NMDAR-activation in the BLA but not the PRh. It did, however, alter
477 the substrates of conditioning to S2: in contrast to Experiments 1A and 1B where acquisition
478 of freezing to S2 required NMDAR-activation in the PRh but not the BLA, here acquisition
479 of freezing to S2 required NMDAR-activation in the BLA but not the PRh.

480 The remaining implications of our proposal concerned the effects of pre-exposing rats
481 to S2-S1 pairings 24 hours prior to conditioning with S2-S1-shock sequences. We predicted
482 that, relative to the results of Experiments 2A and 2B, pre-exposing rats to S2-S1 pairings
483 would again alter the substrates of conditioning to S2 without affecting the substrates of
484 conditioning to S1. Specifically, we predicted that at the end of each S2-S1-shock sequence
485 in conditioning (i.e., the time of the shock), the pre-exposed (and thereby familiar) S2 would
486 be processed in the periphery of attention while the S1 is processed in its focus: hence,
487 conditioning to S2 would occur in the PRh and not the BLA, whereas conditioning to S1
488 would occur in the BLA and not the PRh. These predictions were supported in Experiments

489 3A and 3B. Rats were pre-exposed to S2-S1 pairings and, 24 hours later, exposed to S2-S1-
490 shock sequences under the influence of a DAP5 infusion into the PRh or BLA. The test
491 results revealed that the acquisition of freezing to S2 was disrupted by a DAP5 infusion into
492 the PRh but unaffected by a DAP5 infusion into the BLA; whereas the acquisition of freezing
493 to S1 was disrupted by a DAP5 infusion into the BLA but unaffected by a DAP5 infusion
494 into the PRh. That is, pre-exposing rats to S2-S1 pairings prior to conditioning with S2-S1-
495 shock sequences again had no effect on the substrates of conditioning to S1: as in
496 Experiments 2A and 2B where rats received no pre-exposure, acquisition of freezing to S1
497 required NMDAR-activation in the BLA but not the PRh. It did, however, alter the substrates
498 of conditioning to S2: in contrast to Experiments 2A and 2B where acquisition of freezing to
499 the novel S2 required NMDAR-activation in the BLA but not the PRh, here acquisition of
500 freezing to the pre-exposed S2 required NMDAR-activation in the PRh but not the BLA.

501 The results of Experiments 2A, 2B, 3A and 3B provide support for our idea that the
502 BLA and PRh function like focal and peripheral states of attention (respectively) by showing
503 that the locus of conditioning in one region or the other is determined by novelty/familiarity
504 of the stimulus (or whether it has been pre-exposed or not) and the nature of stimulus-shock
505 pairings (whether they are direct or indirect). The results of Experiments 4A and 4B extended
506 these findings by showing that the substrates of conditioning to a pre-exposed stimulus are
507 also influenced by what happens in the period between presentations of the stimulus and
508 shock. Here, rats were pre-exposed to S2-S1 pairings and, 24 hours later, exposed to S2-
509 [trace]-shock pairings under the influence of a DAP5 infusion into the PRh or BLA. Thus,
510 these experiments were like the preceding ones (3A and 3B) in that S2 was separated from
511 the shock by 10 s during its conditioning; but critically differed from the preceding
512 experiments in the fact that the 10 s interval between the S2 and shock was *not* filled by a
513 presentation of the S1. The consequences of leaving this interval blank were evident at test,

514 where the pattern of results produced by infusions of DAP5 into the PRh and BLA was
515 exactly opposite to that obtained in Experiments 3A and 3B. That is, whereas Experiments
516 3A and 3B showed that freezing to S2 was disrupted by a DAP5 infusion into the PRh but
517 unaffected by a DAP5 infusion into the BLA, here the test level of freezing to S2 was
518 disrupted by a DAP5 infusion into the BLA but unaffected by a DAP5 infusion into the PRh.
519 These results were taken to mean that the substrates of conditioning to a pre-exposed S2 are
520 influenced by more than just the interval between presentations of this stimulus and foot
521 shock: it is also influenced by events that occur in that interval. If the interval is blank, the
522 pre-exposed S2 may remain in a focal state of attention: hence its conditioning requires
523 NMDAR-activation in the BLA and not the PRh (4A and 4B). If the interval is occupied by
524 S1, the pre-exposed S2 more rapidly transitions to a peripheral state of attention: hence its
525 conditioning requires NMDAR-activation in the PRh and not the BLA (3A and 3B).

526 Taken together, the results of the present study are generally consistent with theories
527 of information processing that distinguish between focal and peripheral states of attention
528 (e.g., Atkinson & Shiffrin, 1968; Posner & Snyder, 1975; Shiffrin & Schneider, 1977).
529 Importantly, these theories were developed to explain the different characteristics of
530 information processing; they were not developed to explain changes in the substrates of
531 learning and memory with variations in the circumstances of training. They do, however,
532 provide an excellent framework for thinking about the present findings. For example,
533 according to one type of theory (Wagner, 1981), stimuli are represented as a series of
534 elements that transition between states of activity and inactivity; and these transitions are
535 governed by formal rules. Briefly, when a stimulus is presented, its elements are activated to
536 the focal state of attention (A1) from which they decay to a peripheral state (A2) before
537 returning to a state of inactivity. Importantly, the capacity of A1 is limited and much less than
538 that of A2; stimuli processed in A1 can prime their associates to A2; and the rate at which

539 stimulus elements decay from A1 to A2 is influenced by their familiarity (a result of context-
540 induced priming) as well as other stimuli that are present (i.e., the presence of other stimuli
541 increases the processing demand on A1 and, thereby, the rate of information decay to A2).
542 Given these features and our proposal that the BLA and PRh represent the focus and
543 periphery of attention, a version of this theory can accommodate the full pattern of results in
544 this study (Holland, 1983). When rats are exposed to S2-S1-shock sequences in the absence
545 of any pre-exposure (Experiments 2A and 2B), the elements of both S2 and S1 overlap with
546 those of the foot shock in the focal state of attention. Hence, conditioning to each stimulus
547 requires activation of NMDAR in the BLA and not the PRh. When rats are conditioned with
548 S1-shock pairings in stage 2 after pre-exposure to S2-S1 pairings in stage 1 (Experiments 1A
549 and 1B), S1 elements are activated to the focus of attention and prime S2 elements to the
550 peripheral state. Hence, conditioning to S1 requires NMDAR-activation in the BLA and not
551 the PRh, whereas conditioning to S2 requires NMDAR-activation in the PRh and not the
552 BLA. When rats are conditioned with S2-S1-shock sequences in stage 2 after pre-exposure to
553 S2-S1 pairings in stage 1 (Experiments 3A and 3B), the S2 elements are initially processed in
554 the focus of attention but are rapidly shunted to the periphery during presentations of the S1.
555 Hence, conditioning to S1 requires NMDAR-activation in the BLA and not the PRh, whereas
556 conditioning to S2 requires NMDAR-activation in the PRh and not the BLA. Finally, when
557 rats are conditioned with S2-[trace]-shock pairings after pre-exposure to S2-S1 pairings in
558 stage 1 (Experiments 4A and 4B), the S2 elements are initially processed in the focus of
559 attention and naturally decay to the periphery during the trace interval. However, as the trace
560 interval is relatively short, most S2 elements remain in the focal state at the time of foot
561 shock: hence, conditioning to S2 requires NMDAR-activation in the BLA and not the PRh.
562 With a longer trace interval, the theory predicts that there will be a greater accumulation of
563 S2 elements in the peripheral state: hence, conditioning to S2 would cease to require

564 NMDAR-activation in the BLA and now require NMDAR-activation in the PRh. This will be
565 tested in future research.

566 An additional point to consider in relation to Experiments 3A, 3B, 4A and 4B is the
567 level of surprise that rats experienced following presentations of the familiar S2 in stage 2.
568 Specifically, in Experiments 3A and 3B, S2 was followed by the expected S1 (low surprise)
569 and its conditioning required activation of NMDA receptors in the PRh and not the BLA. By
570 contrast, in Experiments 4A and 4B, S2 was followed by omission of the expected S1 (high
571 surprise) and its conditioning required activation of NMDA receptors in the BLA and not the
572 PRh. This raises the possibility that surprise, or prediction error, also influences the way that
573 S2 is processed in focal and peripheral states of attention. When prediction error is low, S2 is
574 processed in the peripheral state of attention: hence, learning under these circumstances
575 requires NMDA receptor activation in the PRh and not the BLA. By contrast, when
576 prediction error is high, S2 is preserved in the focal state of attention: hence, learning under
577 these circumstances requires NMDA receptor activation in the BLA and not the PRh. The
578 impact of prediction error on the processing of S2 could be assessed using two types of
579 designs. In the first design, rats are pre-exposed to S2-S1 pairings in stage 1 and this is
580 followed by S2-S3-shock pairings in stage 2. The important feature of this design is that, in
581 stage 2, the S2 is followed by surprise in omission of S1 and presentation of S3. Thus, if a
582 large prediction error maintains processing of the familiar S2 in the BLA, we might expect
583 that its conditioning in this design would require NMDA receptor activation in the BLA (in
584 contrast to the results of Experiment 3B) and no longer require NMDA receptor activation in
585 the PRh (in contrast to the results of Experiment 3A). In the second design, rats are pre-
586 exposed to S2 alone in stage 1 and this is followed by S2-[trace]-shock pairings in stage 2.
587 The important feature of this design is that, in stage 2, the S2 is not followed by the surprising
588 omission of any stimulus. Thus, if a small prediction error shifts processing of the familiar S2

589 to the PRh, we might expect that its conditioning in this design would no longer require
590 NMDA receptor activation in the BLA (in contrast to the results of Experiment 4B) but,
591 instead, require NMDA receptor activation in the PRh (in contrast to the results of
592 Experiment 4A). Future studies will use both designs to determine whether prediction error
593 influences the processing of S2 in the focus versus periphery of attention and, thereby,
594 whether learning about this stimulus requires NMDA receptor activation in the BLA or PRh.

595 Finally, the present findings add to a body of work which shows that the neural
596 substrates of Pavlovian conditioned fear critically depend on the type of conditioning (Keidar
597 et al., 2023; Lay et al., 2018; Leake et al., 2024; Leidl et al., 2018; Williams-Spooner et al.,
598 2019, 2022); and extend our previous work in sensory preconditioning by identifying how the
599 mediated S2-shock association is encoded at the level of the PRh and BLA. Specifically, our
600 previous work showed that formation of this association requires neuronal activity in the PRh
601 (Wong et al., 2019), as well as communication between this region and the BLA (Wong et
602 al., 2025). Here, we have extended these findings by showing that NMDAR-activation is a
603 critical component of the processing requirement in the PRh; but is not a component of the
604 processing requirement in the BLA. Future work will identify the nature of the processing
605 requirement in the BLA and exactly how the distinct signaling requirements in the PRh and
606 BLA combine to generate the mediated S2-shock association.

607 In summary, the present study has shown that conditioning to a novel stimulus
608 requires activation of NMDAR in the BLA but does not require activation of NMDAR in the
609 PRh. By contrast, the substrates of conditioning to a pre-exposed stimulus are influenced by
610 several factors, including its contiguity with shock and events interpolated between its
611 presentations and shock. The overall pattern of results is consistent with our proposal that the
612 BLA and PRh function like focal and peripheral states of attention/memory, respectively; and
613 generally consistent with information processing theories which specify how events come to

614 be represented in the focal and peripheral states (Holland, 1983; Wagner, 1981). Future
615 research will test the additional implications of these theories and continue to examine
616 commonalities and differences in the processes through which mediated and direct
617 associations are encoded and stored in the medial temporal lobe.

618 **Materials and methods**

619 **Subjects**

620 Subjects were experimentally naïve male and female Long-Evans rats, obtained from
621 a colony maintained by the Biological Resources Centre at the University of New South
622 Wales. The rats were housed by sex in plastic cages (40 cm wide x 22 cm high x 67 cm long),
623 with four rats per cage and food and water continuously available. The cages were located in
624 an air-conditioned and humidity-controlled colony room maintained on a 12 h light-dark
625 cycle (lights on at 7 a.m. and off at 7 p.m.) at a temperature of approximately 21°C. All
626 procedures were approved by the Animal Care and Ethics Committee at the University of
627 New South Wales and conducted in accordance with the Australian code for the care and use
628 of animals for scientific purposes (published by the National Health and Medical Research
629 Council of Australia). Please note that these subject details (source, housing conditions, and
630 animal maintenance) are exactly the same as those reported in past studies by our laboratory
631 (e.g., Kennedy et al., 2024; Wong et al., 2025).

632 **Surgery**

633 Prior to behavioral training and testing in each experiment, rats were surgically
634 implanted with cannulas targeting either the perirhinal cortex (PRh; Experiments 1A, 2A, 3A
635 and 4A) or basolateral amygdala complex (BLA; Experiments 1B, 2B, 3B and 4B). Rats were
636 anesthetized using isoflurane, which was delivered in a steady stream of oxygen. The rat was
637 then mounted onto a stereotaxic apparatus (David Kopf Instruments) and incisions made over

638 the skull. Two holes were drilled through the skull and 26-gauge guide cannulas (Plastic
639 Ones) were implanted into the brain, one in each hemisphere. For Experiments 1A, 2A, 3A
640 and 4A, the tips of the cannulas targeted the PRh at coordinates 4.30 mm posterior to
641 Bregma, 5.00 mm lateral to the midline, 8.4 mm ventral to Bregma, and angled at
642 approximately 9° (Paxinos & Watson, 2007). For Experiments 1B, 2B, 3B and 4B, the tips of
643 the cannulas targeted the BLA at coordinates 2.40 mm posterior to Bregma, 4.90 mm lateral
644 to the midline, 8.40 mm ventral to Bregma (Paxinos & Watson, 2007). Guide cannulas were
645 secured in place with four jeweller's screws and dental cement. A dummy cannula was kept
646 in each guide cannula at all times except during drug infusions. Immediately after surgery,
647 rats received a subcutaneous (SC) injection of a prophylactic dose (0.1 ml/kg) of Duplocillin
648 (Merck & Co, NJ, USA). Rats were allowed seven days to recover from surgery, during
649 which they were monitored and weighed daily. Please note that these surgery details are
650 exactly the same as those reported in our earlier studies (e.g., Wong et al., 2019; Wong et al.,
651 2025).

652 **Drug infusions**

653 In each experiment, the NMDA receptor antagonist, DAP5, or vehicle was infused
654 bilaterally into the PRh or BLA. For these infusions, infusion cannulas were connected to 25
655 µl Hamilton syringes via polyethylene tubing. These syringes were fixed to an infusion pump
656 (Harvard Apparatus). The infusion procedure began by removing the dummy caps from the
657 guide cannulas on each rat and inserting 33-gauge infusion cannulas in its place. The pump
658 was programmed to infuse a total of 0.5 µl of DAP5 at a rate of 0.25 µl/min, which resulted
659 in a total infusion time of two min. The infusion cannulas remained in place for an additional
660 two min after the infusion was complete to allow for diffusion of the drug into the PRh or
661 BLA tissue and, thereby, avoid reuptake when the infusion cannulas were withdrawn. This
662 resulted in a total infusion time of four min. After the additional two min, the infusion

663 cannulas were removed and replaced with dummy cannulas. The day prior to infusions, the
664 dummy cannulas were removed and the infusion pump was activated to familiarize the rats
665 with the procedure and thereby minimize any non-specific effect of the procedure on the day
666 of infusions. Please note that these drug and infusion details are exactly the same as those
667 reported in the earlier study by Wong et al. (2025).

668 **Drugs**

669 The NMDA receptor antagonist, DAP5 (Sigma, Australia) was prepared in the
670 manner described by Williams-Spooner et al. (2022). Briefly, it was dissolved in ACSF to a
671 concentration of 10 μg/μl and injected into the PRh or BLA as described above.

672 **Histology**

673 After behavioral training and testing, rats were euthanized with a lethal dose of
674 sodium pentobarbital. The brains were extracted and sectioned into 40 μm coronal slices.
675 Every second slice was mounted onto a glass microscope slide and stained with cresyl violet.
676 The placement of the cannula tip was determined under a microscope using the boundaries
677 defined by Paxinos and Watson (2006). Please note that the details for histology are exactly
678 the same as those reported in the earlier studies by Wong et al. (2019, 2025).

679 Rats with misplaced cannulas were excluded from statistical analysis. Figure 5 shows
680 placement of the most ventral portion of these cannulas in the BLA for all rats that were
681 included in the statistical analyses of data from Experiments 1B, 2B, 3B and 4B. Figure 6
682 shows placement of the most ventral portion of these cannulas in the PRh for all rats that
683 were included in the statistical analyses of data from Experiments 1A, 2A, 3A and 4A. The
684 numbers of rats excluded from each experiment based on misplaced cannulas were 3 rats in
685 Experiment 1A, 12 rats in Experiment 1B, 9 rats in Experiment 2A, 11 rats in Experiment 2B,
686 6 rats in Experiment 3A, 6 rats in Experiment 3B, 3 rats in Experiment 4A, and 5 rats in

Experiment 4B. A further nine rats were excluded from all experiments due to issues with their head caps and/or health issues prior to the infusion day. The final *n*s for each group are shown in the figure legend for each experiment.

Behavioral apparatus

All experiments were conducted in four identical chambers (30 cm wide x 26 cm long x 30 cm high). The side walls and ceiling were made of aluminum, and the front and back walls of clear plastic. The floor consisted of stainless steel rods, each two mm in diameter and spaced 13 mm apart (center-to-center). A waste tray containing bedding material was located under the floor. At the end of each session, the chambers were cleaned with water and any soiled bedding was removed from the waste trays and replaced with fresh bedding. Each chamber was enclosed in a sound- and light-attenuating wooden cabinet. The cabinet walls were painted black. A speaker and LED lights within a fluorescent tube were mounted onto the back wall of each cabinet. The speaker was used to deliver a 1000 Hz square-wave tone stimulus, presented at 75 dB when measured at the center of the chamber (digital sound meter: Dick Smith Electronics, Australia). The LED lights were used to deliver a flashing light stimulus, presented at 3.5 Hz. A custom-built, constant current generator was used to deliver a 0.5 s duration, 0.8 mA intensity shock to the floor of each chamber. Each chamber was illuminated with an infrared light source, and a camera mounted on the back wall of each cabinet was used to record the behavior of each rat. The cameras were connected to a monitor and a high-definition digital image recorder located in an adjacent room. This room also contained the computer that controlled stimulus and shock presentations through an interface and appropriate software (MATLAB, MathWorks, USA). Please note that the behavioral apparatus used for this study was exactly the same as that used in the earlier studies by Wong et al. (2019, 2025) and a previous study by Kennedy et al. (2024).

Behavioral procedure

712 *Experiments 1A and 1B*

713 *Context exposure.* On Days 1 and 2, rats received two 20 min exposures to the chambers, one
714 in the morning and the other approximately three hours later in the afternoon.

715 *Stage 1: Sensory preconditioning.* On Day 3, rats were exposed to eight paired presentations
716 of the stimuli designated S2 and S1. For half the rats, the S2 was a sound and the S1 was a
717 light. For the remaining rats, the S2 was a light and the S1 was a sound. Each presentation of
718 the S2 was 30 s in duration, each presentation of the S1 was 10 s in duration, and the stimuli
719 were paired in such a way that the offset of S2 co-occurred with the onset of S1. The first S2
720 presentation occurred five min after rats were placed into the chambers, and the interval
721 between each S2-S1 pairing was five min (measured from the offset of the S1 to onset of the
722 next S2). After the last S1 presentation, rats remained in the chambers for an additional one
723 min. They were then returned to their home cages.

724 *Stage 2: First-order conditioning of the S1.* On Day 4, rats were exposed to four paired
725 presentations of the S1 and foot shock. The first S1 presentation occurred five min after rats
726 were placed in chambers, each 10 s S1 co-terminated with the 0.5 s foot shock, and the
727 interval between the S1-shock pairings was five min. Rats remained in the chambers for an
728 additional one min after the final S1-shock pairing and were then returned to their home
729 cages.

730 *Context extinction.* On Day 5, rats received two 20 min exposures to the chambers, one in the
731 morning and the other three hours later in the afternoon. These exposures were intended to
732 extinguish any freezing elicited by the context alone so that the level of freezing to the S2 and
733 S1 could be assessed unconfounded by context elicited freezing. On Day 6, rats received a
734 further 10 min extinction exposure to the context.

735 *Testing.* On Day 6, approximately two hours after the context extinction session, rats were
736 tested for their levels of freezing to the preconditioned S2. On Day 7, rats were tested for
737 their levels of freezing to the conditioned S1. For each test, the first stimulus was presented
738 two min after rats were placed in the chambers. Each test consisted of eight stimulus alone
739 presentations, with a three min interval between each presentation. Each S2 presentation was
740 30 s in duration and each S1 presentation was 10 s. Rats remained in the chamber for an
741 additional min after the final stimulus presentation.

742 *Experiments 2A and 2B*

743 *Context exposure.* On Days 1 and 2, rats received two 20 min exposures to the chambers in
744 the manner described for the previous experiments.

745 *Serial-order conditioning of S2 and S1.* On Day 3, rats were exposed to four S2-S1-shock
746 sequences. For half the rats, the S2 was a sound and the S1 was a light. For the remaining
747 rats, the S2 was a light and the S1 was a sound. Each presentation of the S2 lasted for 30 s,
748 each presentation of the S1 lasted for 10 s, and each presentation of foot shock lasted for 0.5
749 s. The stimuli were presented in such a way that offset of the 30 s S2 co-occurred with onset
750 of the 10 s S1, which then co-terminated with the 0.5 s foot shock. The first S2 presentation
751 occurred five min after rats were placed into the chambers, the interval between each S2-S1-
752 shock sequence was five min (measured from the offset of the S1 to onset of the next S2),
753 and rats remained in the chambers for an additional one min after exposure to the final S2-S1-
754 shock sequence. They were then returned to their home cages.

755 *Context extinction.* On Day 4, rats received two 20 min exposures to the chambers, one in the
756 morning and the other three hours later in the afternoon. These exposures were intended to
757 extinguish any freezing elicited by the context alone so that the level of freezing to the S2 and

758 S1 could be assessed unconfounded by context elicited freezing. On Day 5, rats received a
759 further 10 min extinction exposure to the context.

760 *Testing.* On Day 5, approximately two hours after the context extinction session, rats were
761 tested for their levels of freezing to the S2. On Day 6, rats were tested for their levels of
762 freezing to the S1. The details of these test sessions were identical to those described for the
763 previous experiments.

764 *Experiments 3A and 3B*

765 *Context exposure.* On Days 1 and 2, rats received two 20 min exposures to the chambers in
766 the manner described for Experiments 1A and 1B.

767 *Stage 1: Sensory preconditioning.* On Day 3, rats received a single session of sensory
768 preconditioning in which they were exposed to eight paired presentations of the stimuli
769 designated S2 and S1. For half the rats, the S2 was a sound and the S1 was a light. For the
770 remaining rats, the S2 was a light and the S1 was a sound. The details for this session were
771 identical to those described for the sensory preconditioning session in Experiments 1A and
772 1B.

773 *Stage 2: Serial-order conditioning of the S2 and S1.* On Day 4, rats received a single session
774 in which they were exposed to four S2-S1-shock sequences. The details for this session were
775 identical to those described for the serial-order conditioning session in Experiments 2A and
776 2B.

777 *Context extinction.* On Day 5, rats received two 20 min sessions of context extinction; and
778 this was followed by an additional 10 min context extinction session on Day 6. The details
779 and purpose of these sessions was exactly as described for each of the previous experiments.

780 *Testing.* On Day 6, approximately two hours after the context extinction session, rats were
781 tested for their levels of freezing to the S2. On Day 7, rats were tested for their levels of

782 freezing to the S1. The details of these test sessions were exactly as described for each of the
783 previous experiments.

784 *Experiments 4A and 4B*

785 *Context exposure.* On Days 1 and 2, rats received two 20 min exposures to the chambers in
786 the manner described for Experiments 1A and 1B.

787 *Stage 1: Sensory preconditioning.* On Day 3, rats received a single session of sensory
788 preconditioning in which they were exposed to eight paired presentations of the stimuli
789 designated S2 and S1. For half the rats, the S2 was a sound and the S1 was a light. For the
790 remaining rats, the S2 was a light and the S1 was a sound. The details for this session were
791 identical to those described for the sensory preconditioning session in Experiments 1A and
792 1B.

793 *Stage 2: Trace conditioning of the S2.* On Day 4, rats received a single session in which they
794 were exposed to four S2-[10 s]-shock pairings. The stimuli were presented in such a way that
795 offset of the 30 s S2 was followed by a 10 s trace interval, which co-terminated with a 0.5 s
796 foot shock. The first S2 presentation occurred five min after rats were placed into the
797 chambers, the interval between each S2-[10 s]-shock pairing was five min, and rats remained
798 in the chambers for an additional one min after exposure to the final S2-[10 s]-shock pairing.
799 They were then returned to their home cages.

800 *Context extinction.* On Day 5, rats received two 20 min sessions of context extinction; and
801 this was followed by an additional 10 min context extinction session on Day 6. The details
802 and purpose of these sessions was exactly as described for each of the previous experiments.

803 *Testing.* On Day 6, approximately two hours after the context extinction session, rats were
804 tested for their levels of freezing to the S2. On Day 7, rats were tested for their levels of

freezing to the S1. The details of these test sessions were exactly as described for each of the previous experiments.

Scoring and statistics

Conditioning and test sessions were recorded. Freezing, defined as the absence of all movements except those required for breathing (Fanselow, 1980), was used as a measure of conditioned fear. Freezing data were collected using a time-sampling procedure in which each rat was scored as either 'freezing' or 'not freezing' every two seconds by an observer that was unaware of the rat's group allocation. A second naïve observer also scored all of the test data. The correlation between the two observers' scores was high (Pearson > 0.9). The data were analyzed using a set of planned orthogonal contrasts (Hays, 1963), with the Type 1 error rate controlled at $\alpha = 0.05$. Standardized 95% confidence intervals (CIs) are reported for all significant results, and Cohen's *d* is reported as a measure of effect size (where 0.2, 0.5, and 0.8 is a small, medium and large effect size, respectively). The required number of rats per group was determined during the design stage of the study. It was based on our prior studies of sensory preconditioning which indicated that eight subjects per group (or per comparison in the contrast testing procedure) provides sufficient statistical power to detect effect sizes > 0.5 with the recommended probability of 0.8-0.9. Please note that the details for scoring and data analysis are exactly the same as those reported in the earlier study by Wong et al (2025).

References

Atkinson RC, Shiffrin RM. (1968). Human memory: a proposed system and its control processes. *Psychology of Learning and Motivation*, *2*, 89-195.

Bauer EP, Schafe GE, LeDoux JE. (2002). NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. *Journal of Neuroscience*, *22*, 5239-49.

Bogacz R, Brown MW. (2003). Comparison of computational models of familiarity discrimination in the perirhinal cortex. *Hippocampus*, *13*(4), 494-524. doi: 10.1002/hipo.10093.

Brown MW, Banks PJ. (2015). In search of a recognition memory engram. *Neuroscience and Biobehavioral Reviews*, *50*, 12-28. doi: 10.1016/j.neubiorev.2014.09.016.

Brown MW, Bashir ZI. (2002). Evidence concerning how neurons of the perirhinal cortex may effect familiarity discrimination. *Philos Trans R Soc Lond B Biol Sci*, 357(1424), 1083-95. doi: 10.1098/rstb.2002.1097.

Campeau S, Miserendino MJ, Davis M. (1995). Intra-amygdala infusion of the N-methyl-D-aspartate receptor antagonist AP5 blocks acquisition but not expression of fear-potentiated startle to an auditory conditioned stimulus. *Behavioral Neuroscience*, *106*, 569-74. doi: 10.1037/0735-7044.106.3.569.

Fanselow MS, Kim JJ. (1994). Acquisition of contextual Pavlovian fear conditioning is blocked by application of an NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid to the basolateral amygdala. *Behavioral Neuroscience*, *108*, 210-12. doi: 10.1037/0735-7044.108.1.210.

Gewirtz JC, Davis M. (1997). Second-order fear conditioning prevented by blocking NMDA receptors in amygdala. *Nature*, *388*, 471-74.

Holland PC. (1983). Representation-mediated overshadowing and potentiation of conditioned aversions. *Journal of Experimental Psychology: Animal Behavior Processes*, *9*, 1-13.

Holmes NM, Harris JA. (2009). The loss of latent inhibition across compound conditioning. *Journal of Experimental Psychology: Animal Behavior Processes*, *35*(3), 328-39. doi: 10.1037/a0014537.

Holmes NM, Parkes SL, Killcross AS, Westbrook RF. (2013). The basolateral amygdala is critical for learning about neutral stimuli in the presence of danger, and the perirhinal cortex is critical in the absence of danger. *Journal of Neuroscience*, 33, 13112-25. doi: 10.1523/JNEUROSCI.1998-13.2013.

Holmes NM, Raipuria M, Qureshi OA, Killcross S, Westbrook F. (2018). Danger changes the way the mammalian brain stores information about innocuous events: a study of sensory preconditioning in rats. *eNeuro*, *5*(1), ENEURO.0381-17.2017. doi: 10.1523/ENEURO.0381-17.2017.

Holmes NM, Westbrook RF. (2017). A dangerous context changes the way that rats learn about and discriminate between innocuous events in sensory preconditioning. *Learning and Memory*, *24*, 440-48. doi: 10.1101/lm.044297.116.

Holmes NM, Westbrook RF. (2013). ABA renewal is greater when extinction occurs in the same context as cue pre-exposure. *Journal of Experimental Psychology: Animal Learning and Cognition*, *40*(3), 369-79. doi: 10.1037/xan0000024.

Keidar T, Williams-Spooner MJ, Wong FS, Westbrook RF, Holmes NM. (2023). NMDA receptors in the basolateral amygdala complex are engaged for Pavlovian fear conditioning when an animal's predictions about danger are in error. *Journal of Neuroscience*, *43*(39), 6679-96. doi: 10.1523/JNEUROSCI.0067-23.2023.

Kennedy NGW, Holmes NM, Westbrook RF. (2021). Latent inhibition is facilitated when a target stimulus is preexposed in compound with a nontarget stimulus, but only when the

two stimuli coterminate: a test of the Hall-Rodriguez theory. *Journal of Experimental Psychology: Animal Learning and Cognition*, *47*(2), 91-103. doi: 10.1037/xan0000289.

Lay BPP, Westbrook RF, Glanzman DL, Holmes NM. (2018). Commonalities and differences in the substrates underlying consolidation of first- and second-order conditioned fear. *Journal of Neuroscience*, *38*(8), 1926-41. doi: 10.1523/JNEUROSCI.2966-17.2018.

Leake J, Leidl DM, Lay BPP, Fam JP, Giles MC, Qureshi OA, Westbrook RF, Holmes NM. (2024). What is learned determines how Pavlovian conditioned fear is consolidated in the brain. *Journal of Neuroscience*, *44*(2), e0513232023. doi: 10.1523/JNEUROSCI.0513-23.2023.

Leidl DM, Lay BPP, Chakouch C, Westbrook RF, Holmes NM. (2018). Protein synthesis in the basolateral amygdala complex is required for consolidation of a first-order fear memory, but not for consolidation of a higher-order fear memory. *Neurobiology of Learning and Memory*, *153*(B), 153-65. doi: 10.1016/j.nlm.2018.04.001.

Leung HT, Killcross AS, Westbrook RF. (2013a). A further assessment of the Hall-Rodriguez theory of latent inhibition. *Journal of Experimental Psychology: Animal Behavior Processes*, *39*(2), 117-25. doi: 10.1037/a0031724.

Leung HT, Killcross AS, Westbrook RF (2013b). Error correction in latent inhibition and its disruption by opioid receptor blockade with naloxone. *Neuropsychopharmacology*, *38*(12), 2439-45. doi: 10.1038/npp.2013.144.

Lubow RE. (1973). Latent inhibition. *Psychological Bulletin*, *79*(6), 398-407. doi: 10.1037/h0034425.

Lubow RE, Moore AU. (1959). Latent inhibition: the effect of nonreinforced pre-exposure to the conditional stimulus. *Journal of Comparative and Physiological Psychology*, *52*, 415-9. doi: 10.1037/h0046700.

Martin CB, McLean DA, O'Neil EB, Köhler S. (2013). Distinct familiarity-based response patterns for faces and buildings in perirhinal and parahippocampal cortex. *Journal of Neuroscience*, *33*(26), 10915-23. doi: 10.1523/JNEUROSCI.0126-13.2013.

McLelland VC, Chan D, Ferber S, Barense MD. (2014). Stimulus familiarity modulates functional connectivity of the perirhinal cortex and anterior hippocampus during visual discrimination of faces and objects. *Frontiers in Human Neuroscience*, *8*:117. doi: 10.3389/fnhum.2014.00117.

Miserendino MJ, Sananes CB, Melia KR, Davis M. (1990). Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature*, 345, 716-18. doi: 10.1038/345716a0.

Morillas E, Gómez-Chacón B, Gallo M. (2017). Flavor and object recognition memory impairment induced by excitotoxic lesions of the perirhinal cortex. *Neurobiology of Learning and Memory*, *144*, 230-34. doi: 10.1016/j.nlm.2017.08.002.

Murray EA, Wise SP. (2012). Why is there a special issue on perirhinal cortex in a journal called hippocampus? The perirhinal cortex in historical perspective. *Hippocampus*, *22*(10), 1941-51. doi: 10.1002/hipo.22055.

Parkes SL, Westbrook RF. (2010). The basolateral amygdala is critical for the acquisition and extinction of associations between a neutral stimulus and a learned danger signal but not between two neutral stimuli. *Journal of Neuroscience*, *30*, 12608-18. doi: 10.1523/JNEUROSCI.2949-10.2010.

Phillips RG, LeDoux JE. (1995). Lesions of the fornix but not the entorhinal or perirhinal cortex interfere with contextual fear conditioning. *Journal of Neuroscience*, *15*, 5308-15. doi: 10.1523/JNEUROSCI.15-07-05308.1995.

Posner MI, Snyder CRR. (1975). Attention and Cognitive Control. In *Information Processing and Cognition: The Loyola Symposium*, (pp.55-85), 1975, Hillsdale, NJ: Lawrence Erlbaum Associates.

Qureshi OA, Leake J, Delaney AJ, Killcross S, Westbrook RF, Holmes NM. (2023). Danger changes the way the brain consolidates neutral information; and does so by interacting with processes involved in the encoding of that information. *Journal of Neuroscience*, *43*(16), 2934-49. doi: 10.1523/JNEUROSCI.1796-22.2023.

Robinson J, Whitt EJ, Horsley RR, Jones PM. (2010). Familiarity-based stimulus generalization of conditioned suppression in rats is dependent on the perirhinal cortex. *Behahvioral Neuroscience*, *124*(5), 587-99. doi: 10.1037/a0020900.

Rodrigues SM, Schafe GE, LeDoux JE. (2001). Intra-amygdala blockade of the NR2B subunit of the NMDA receptor disrupts the acquisition but not the expression of fear conditioning. *Journal of Neuroscience*, *21*, 6889-96.

Romanski LM, LeDoux JE. (1992a). Bilateral destruction of neocortical and perirhinal projection targets of the acoustic thalamus does not disrupt auditory fear conditioning. *Neuroscience Letters*, *142*, 228-32. doi: 10.1016/0304-3940(92)90379-L.

Romanski LM, LeDoux JE. (1992b). Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning. *Journal of Neuroscience*, *12*, 4501-09. doi: 10.1523/JNEUROSCI.12-11-04501.1992.

Roozendaal B, Okuda S, Van der Zee EA, McGaugh JL. (2006). Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral

amygdala. *Proceedings of the National Academry of Sciences USA*, *103*(17), 6741-46. doi: 10.1073/pnas.0601874103.

Rosenberg H, Holmes NM, Harris JA, Westbrook RF. Pre-exposure enhances recovery of conditioned responding after extinction. *Learning and Behavior*, *39*(3), 212-23. doi: 10.3758/s13420-011-0019-5.

Rutishauser U, Mamelak AN, Schuman EM. (2006). Single-trial learning of novel stimuli by individual neurons of the human hippocampus-amygdala complex. *Neuron*, *49*(6), 805-13. doi: 10.1016/j.neuron.2006.02.015.

Schomaker J, Meeter M. (2015). Short- and long-lasting consequences of novelty, deviance and surprise on brain and cognition. *Neuroscience and Biobehavioral Reviews*, *55*, 268-79. doi: 10.1016/j.neubiorev.2015.05.002.

Shiffrin RM, Schneider W. (1977). Controlled and automatic human information processing: II. Perceptual learning, automatic attending and a general theory. *Psychological Review, 84*(2), 127–190. doi: 10.1037/0033-295X.84.2.127.

Suzuki WA, Naya Y. (2014). The perirhinal cortex. *Annual Review of Neuroscience*, 37, 39-53. doi: 10.1146/annurev-neuro-071013-014207.

Voss JL, Hauner KK, Paller KA. (2009). Establishing a relationship between activity reduction in human perirhinal cortex and priming. *Hippocampus*, *19*(9), 773-8. doi: 10.1002/hipo.20608.

Wagner AR. (1981). SOP: A model of automatic memory processing in animal behavior. In In: Spear, N.E., Miller, R.R. (Eds.), Information Processing in Animals: Memory Mechanisms. (pp. 5-47).

Westbrook RF, Jones ML, Bailey GK, Harris JA. (2000). Contextual control over conditioned responding in a latent inhibition paradigm. *Journal of Experimental*

Psychology: Animal Behavior Processes, *26*(2), 157-73. doi: 10.1037//0097-7403.26.2.157.

Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE. (2006). Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. *Journal of Neuroscience*, *26*, 12387-96. doi: 10.1523/JNEUROSCI.4316-06.2006.

Williams-Spooner MJ, Delaney AJ, Westbrook RF, Holmes NM. (2022). Prediction error determines whether NMDA receptors in the basolateral amygdala complex are involved in Pavlovian fear conditioning. *Journal of Neuroscience*, *42*(21), 4360-4379. doi: 10.1523/JNEUROSCI.2156-21.2022.

Wong FS, Westbrook RF, Holmes NM. (2019). 'Online' integration of sensory and fear memories in the rat medial temporal lobe. *eLife*, 8:e47085. doi: 10.7554/eLife.47085.

Wong FS, Thomas AB, Killcross S, Laurent V, Westbrook RF, Holmes NM. (2025). Integration of sensory and fear memories in the rat medial temporal lobe. *eLife*, 13:RP101965. doi: 10.7554/eLife.101965.

Wright CI, Martis B, Schwartz CE, Shin LM, Fischer H H, McMullin K, Rauch SL. (2003). Novelty responses and differential effects of order in the amygdala, substantia innominata, and inferior temporal cortex. *Neuroimage*, *18*(3), 660-9. doi: 10.1016/s1053-8119(02)00037-x.

Yang H, McRae K, Köhler S. (2023). Perirhinal cortex automatically tracks multiple types of familiarity regardless of task-relevance. *Neuropsychologia*, *187*, 108600. doi: 10.1016/j.neuropsychologia.2023.108600. Epub 2023 May 29.

Tables and Figures

Table 1. Designs and predictions for each experiment in the series.

EXPERIMENT	TRAINING		PREDICTIONS	
	STAGE 1	STAGE 2	FOR S2	FOR S1
1A	S2-S1	● S1-shock	Disrupted	intact
1B	S2-S1	● S1-shock	Intact	disrupted
2A	…	● S2-S1-shock	Intact	intact
2B	…	● S2-S1-shock	Disrupted	disrupted
3A	S2-S1	● S2-S1-shock	Disrupted	intact
3B	S2-S1	● S2-S1-shock	Intact	disrupted
4A	S2-S1	● S2-[trace]-shock	Intact	
4B	S2-S1	● S2-[trace]-shock	Disrupted	

Notes. ● *= PRh infusion of DAP5;* ● *= BLA infusion of DAP5. In Experiments 1A, 1B, 3A and 3B, S2 is familiar and indirectly paired with shock, whereas S1 is familiar and directly paired with shock. In Experiments 2A and 2B, S2 is novel and indirectly paired with shock, whereas S1 is novel and directly paired with shock. In Experiments 4A and 4B, S2 is familiar and more-directly paired with shock (compared to Experiments 3A and 3B).*

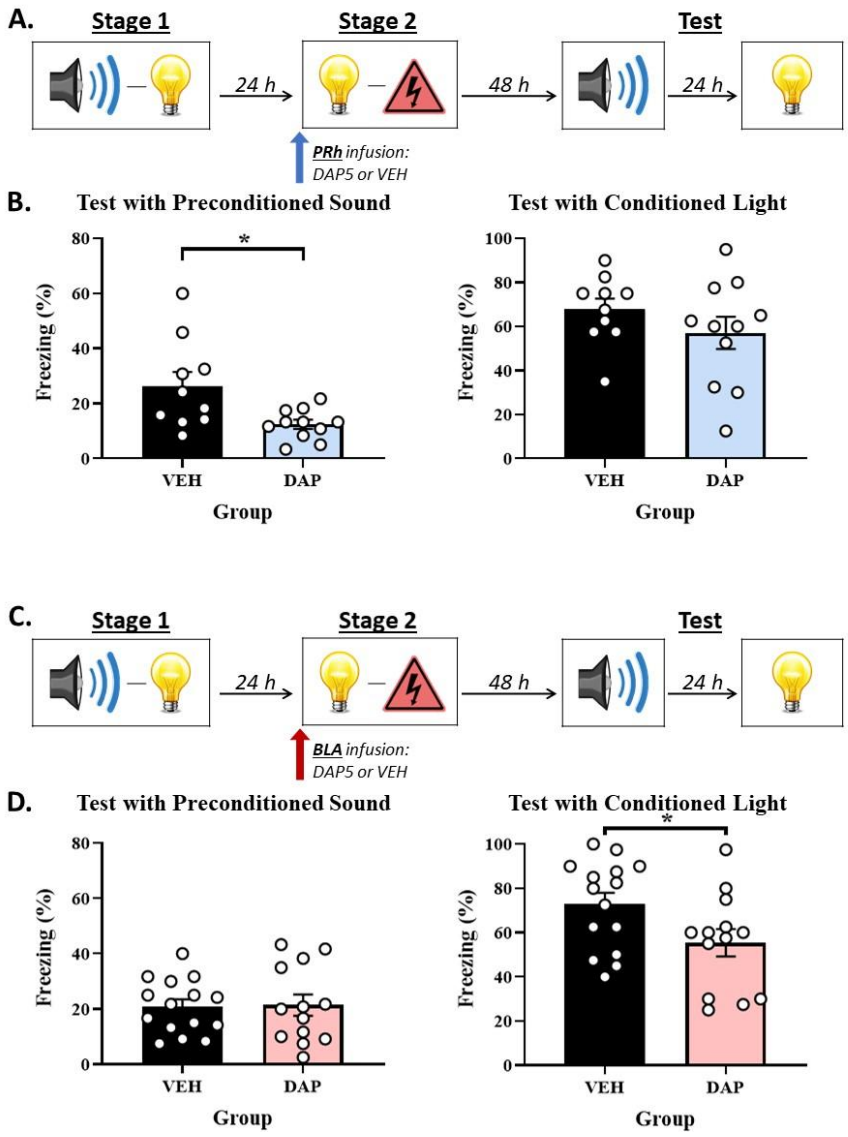

Figure 1. The neural substrates of the mediated S2-shock and direct S1-shock associations that form in stage 2 of sensory preconditioning are doubly dissociable at the level of NMDAR-activation in the PRh and BLA. **(A, C)** Schematics of the protocols used to assess the involvement of NMDAR in acquisition of freezing to S2 and S1 in the PRh (Experiment 1A) and BLA (Experiment 1B). The protocols differed only in whether rats received an infusion of the NMDAR antagonist, DAP5, into the PRh (in A) or BLA (in C). **(B)** Test results showing that a stage 2 infusion of DAP5 into the PRh disrupts freezing to S2 without affecting freezing to S1. **(D)** Test results showing that a stage 2 infusion of DAP5 into the BLA disrupts freezing to S1 without affecting freezing to S2. The bars in each histogram show the mean level of freezing in each group across repeated presentations of S2 or S1 under conditions of extinction. The error bars show the standard error of the mean, and the overlaid data points represent the mean level of freezing for individual rats. The final group

1013 sizes were n = 10 for Group VEH and n = 11 for Group DAP5 in Experiment 1A; and n = 15
1014 for Group VEH and n = 13 for Group DAP5 in Experiment 1B.

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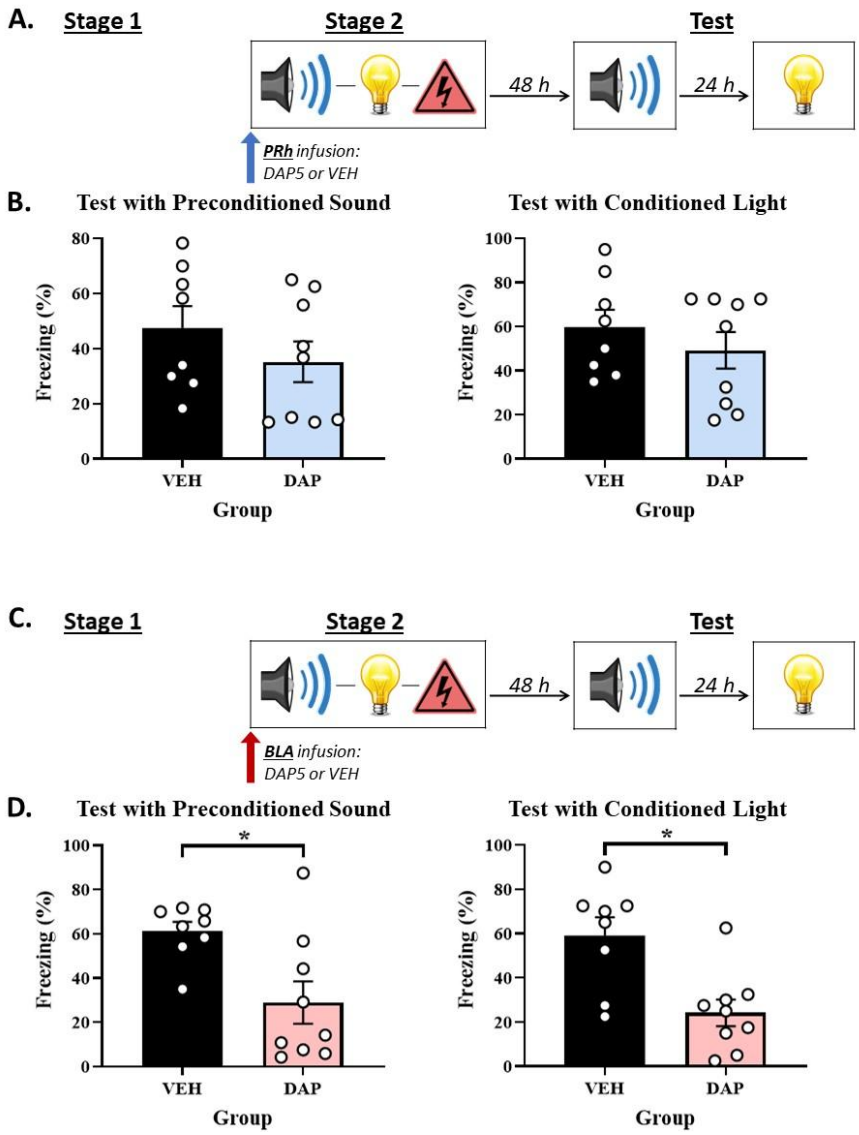


Figure 2. Combining the S2-S1 and S1-shock pairings into S2-S1-shock sequences alters the substrates of conditioning to the S2 in the PRh and BLA. **(A, C)** Schematics of the protocols used to assess when NMDAR are involved in acquisition of freezing to S2 and S1 in the PRh (Experiment 2A) and BLA (Experiment 2B). The protocols differed only in whether rats received an infusion of the NMDAR antagonist, DAP5, into the PRh (in A) or BLA (in C). **(B)** Test results showing that a stage 2 infusion of DAP5 into the PRh has no effect on levels of freezing to either S2 or S1. **(D)** Test results showing that a stage 2 infusion of DAP5 into the BLA disrupts levels of freezing to both the S2 and S1. The final group sizes were $n = 8$ for Group VEH and $n = 9$ for Group DAP5 in Experiment 2A; and $n = 8$ for Group VEH and $n = 9$ for Group DAP5 in Experiment 2B.

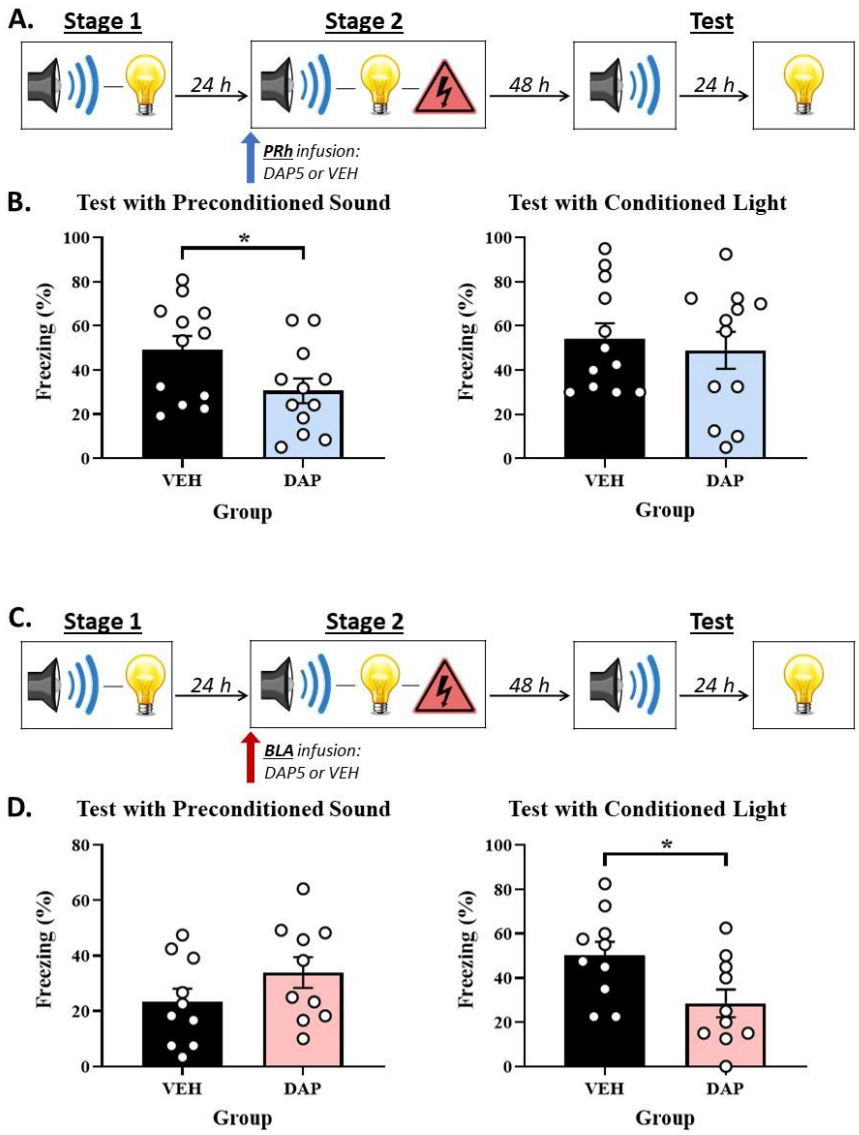


Figure 3. Pre-exposing rats to S2-S1 pairings prior to a session of S2-S1-shock sequences re-engages NMDAR in the PRh for conditioning of the S2. **(A, C)** Schematics of the protocols used to assess when NMDAR are involved in acquisition of freezing to S2 and S1 in the PRh (Experiment 3A) and BLA (Experiment 3B). The protocols differed only in whether rats received an infusion of the NMDAR antagonist, DAP5, into the PRh (in A) or BLA (in C). **(B)** Test results showing that a stage 2 infusion of DAP5 into the PRh disrupts freezing to S2 without affecting freezing to S1. **(D)** Test results showing that a stage 2 infusion of DAP5 into the BLA disrupts freezing to S1 without affecting freezing to S2. The final group sizes were n = 12 for Group VEH and n = 12 for Group DAP5 in Experiment 3A; and n = 10 for Group VEH and n = 10 for Group DAP5 in Experiment 3B.

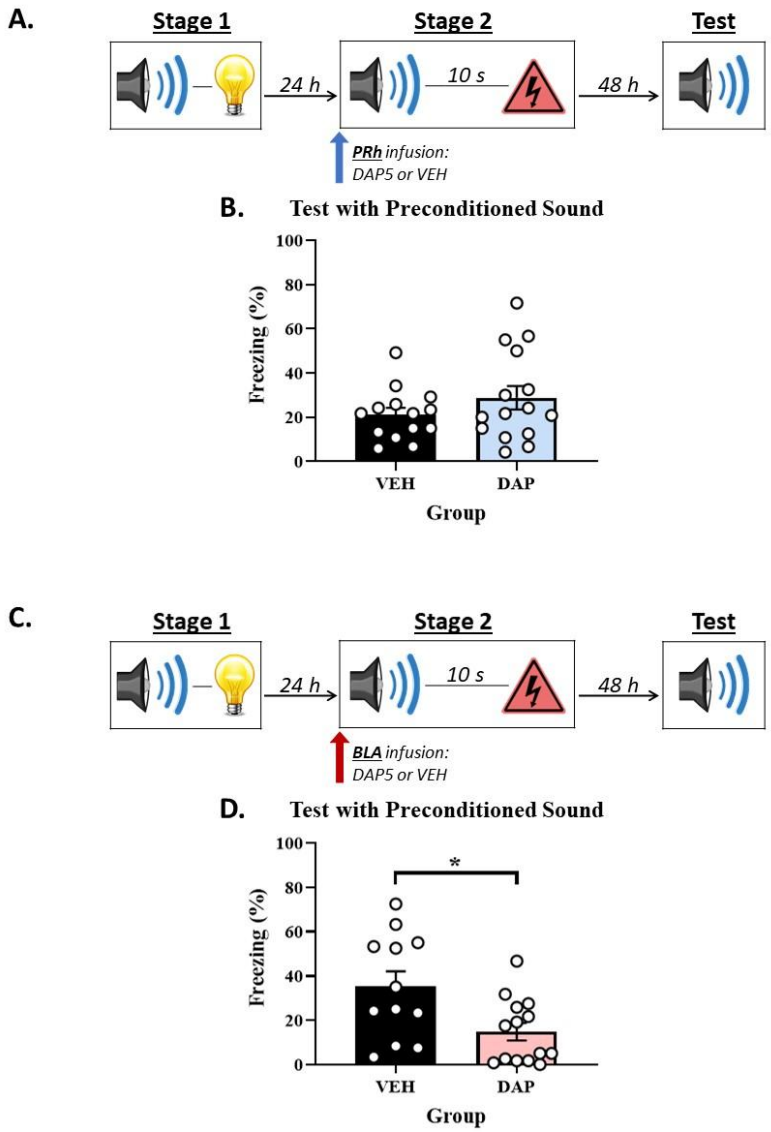


Figure 4. Pre-exposing rats to S2-S1 pairings prior to a session of S2-[trace]-shock pairings re-engages NMDAR in the BLA for conditioning of the S2. **(A, C)** Schematics of the protocols used to assess when NMDAR are involved in acquisition of freezing to S2 and S1 in the PRh (Experiment 4A) and BLA (Experiment 4B). **(B, D)** Test results showing that a stage 2 infusion of DAP5 has no effect on the level of freezing to S2 when injected into the PRh (in B) but disrupts the level of freezing to S2 when injected into the BLA (in D). The final group sizes were n = 14 for Group VEH and n = 15 for Group DAP5 in Experiment 3A; and n = 12 for Group VEH and n = 14 for Group DAP5 in Experiment 3B.

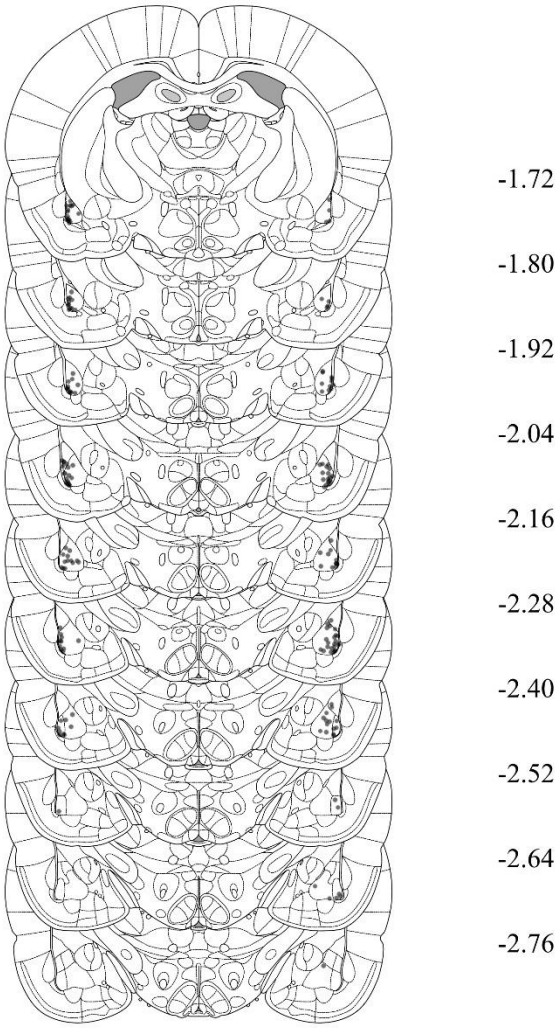


Figure 5. Cannula placements in the PRh for rats in Experiments 1B, 2B, 3B and 4B. The most ventral portion of the cannulas are marked on coronal sections based on the atlas of Paxinos and Watson (2006).

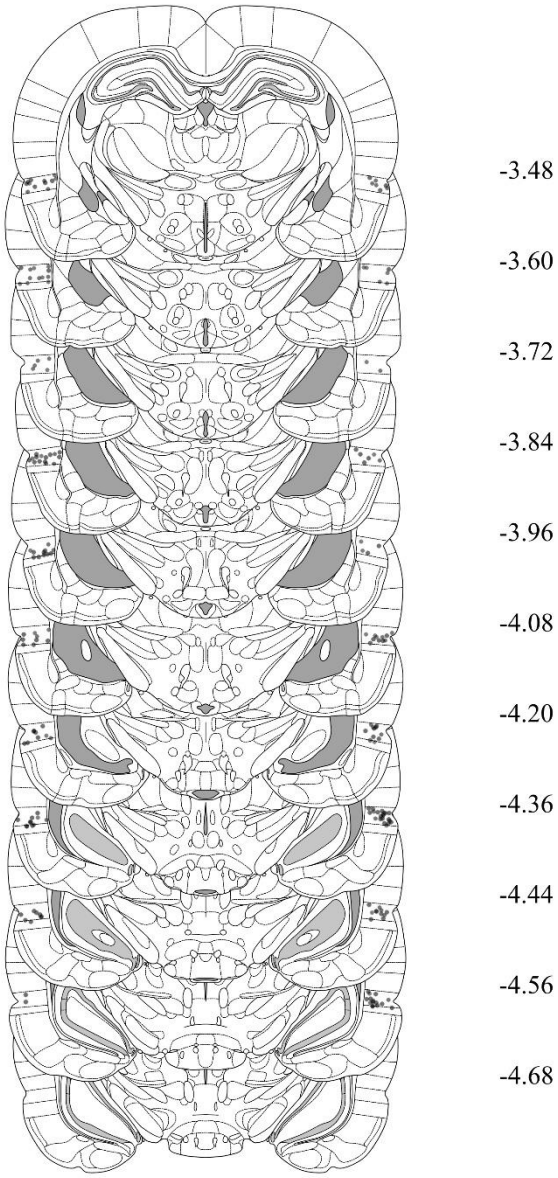
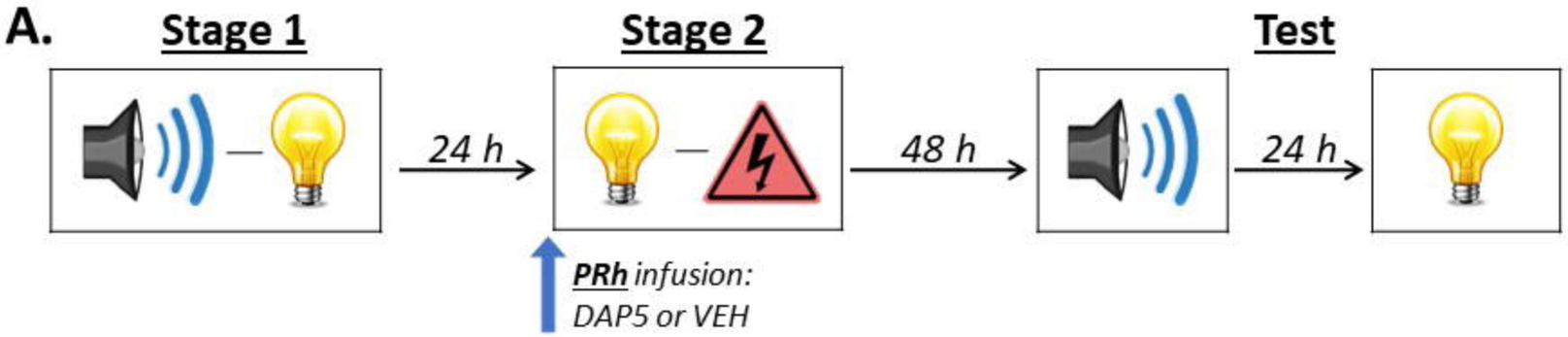
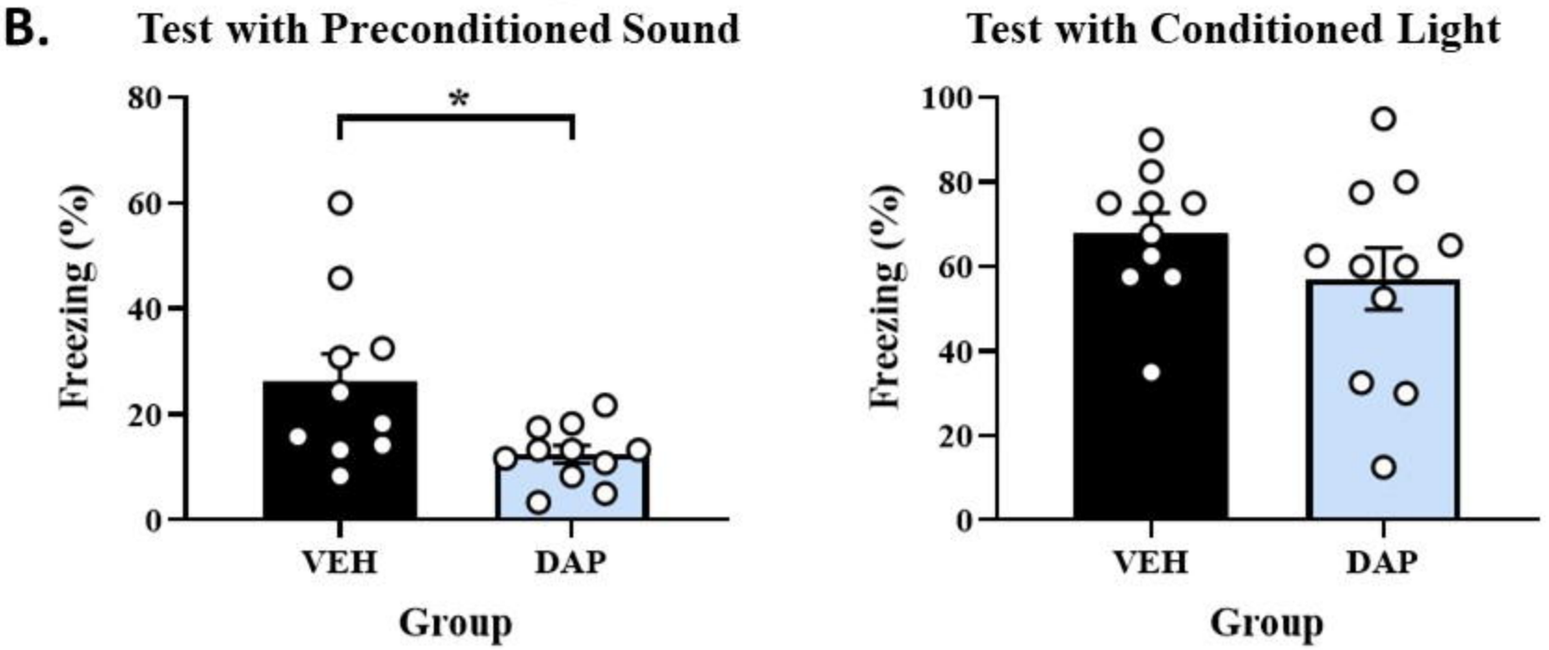
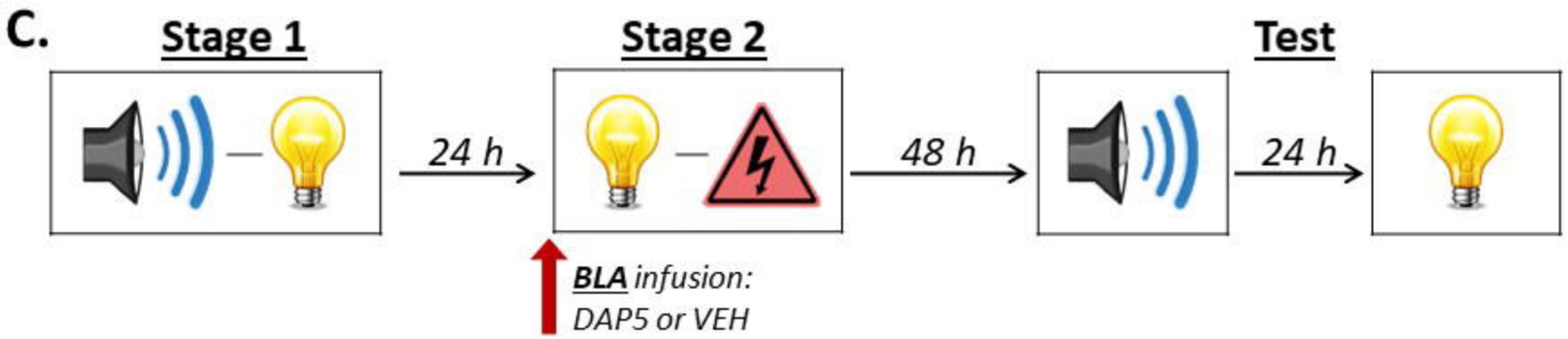
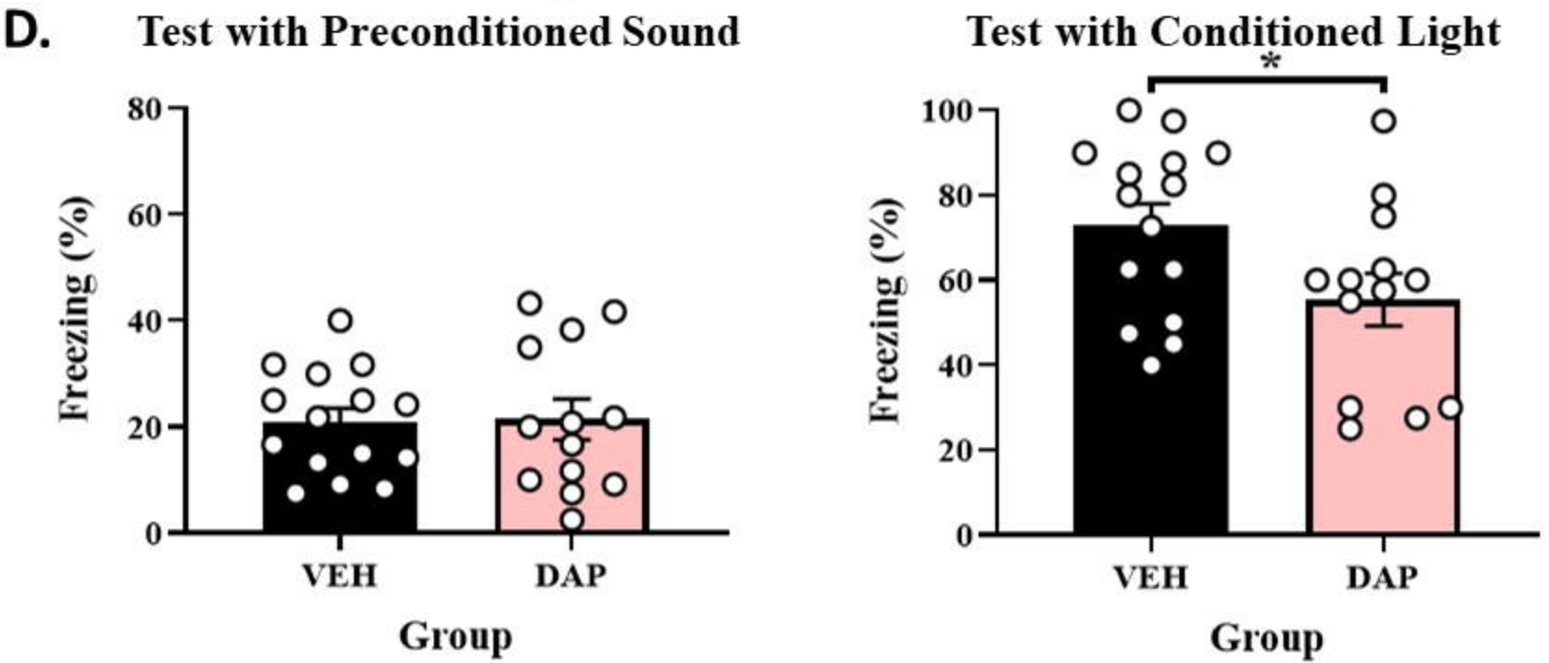


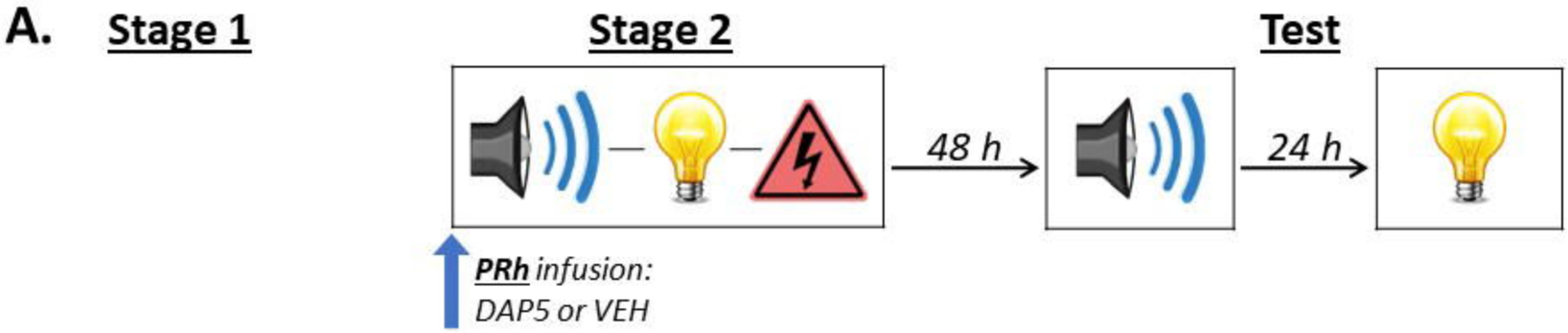
Figure 6. Cannula placements in the PRh for rats in Experiments 1A, 2A, 3A and 4A. The most ventral portion of the cannulas are marked on coronal sections based on the atlas of Paxinos and Watson (2006).

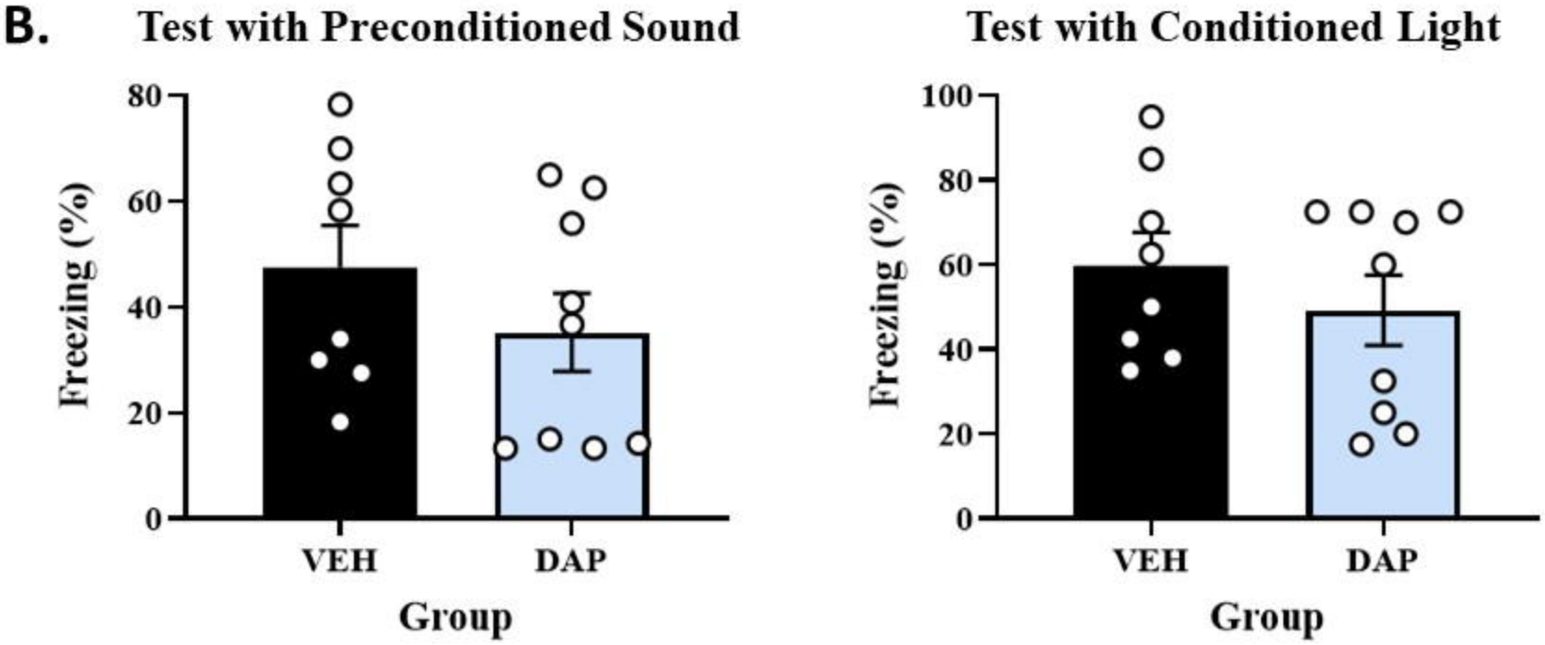

A.
Stage 1
Stage 2
Test
24 h
48 h
24 h
PRh infusion:
DAP5 or VEH

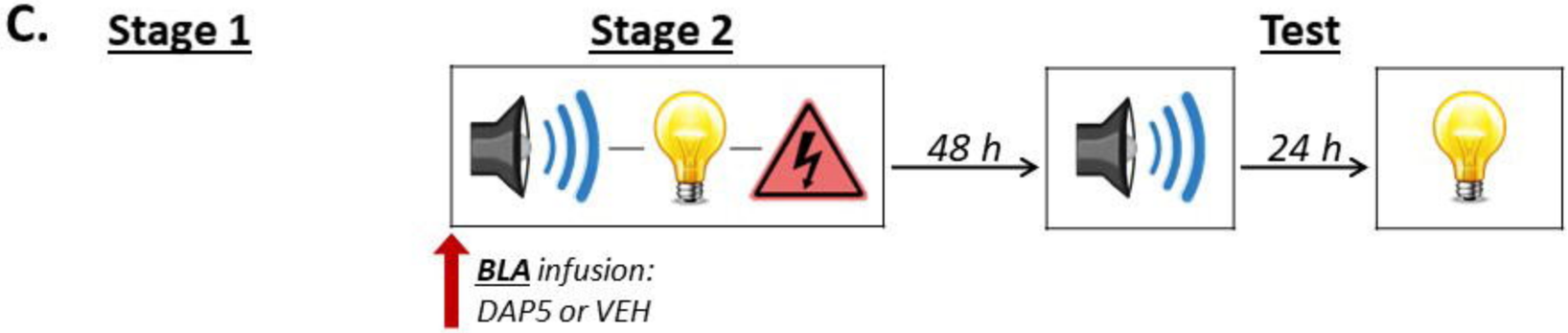

B.
Test with Preconditioned Sound
Test with Conditioned Light
Freezing (%)
80
60
40
20
0
100
*
VEH
DAP
Group

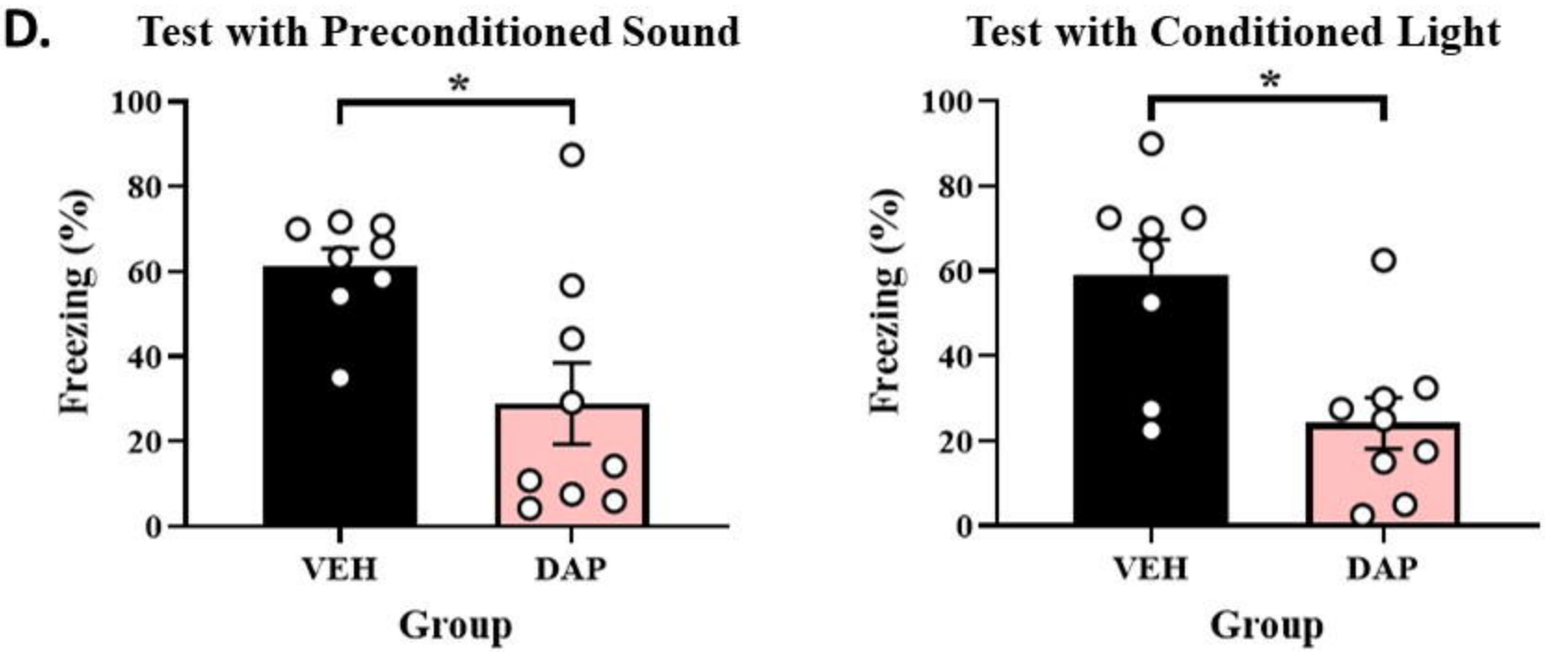

C.
Stage 1
Stage 2
Test
24 h
48 h
24 h
BLA infusion:
DAP5 or VEH

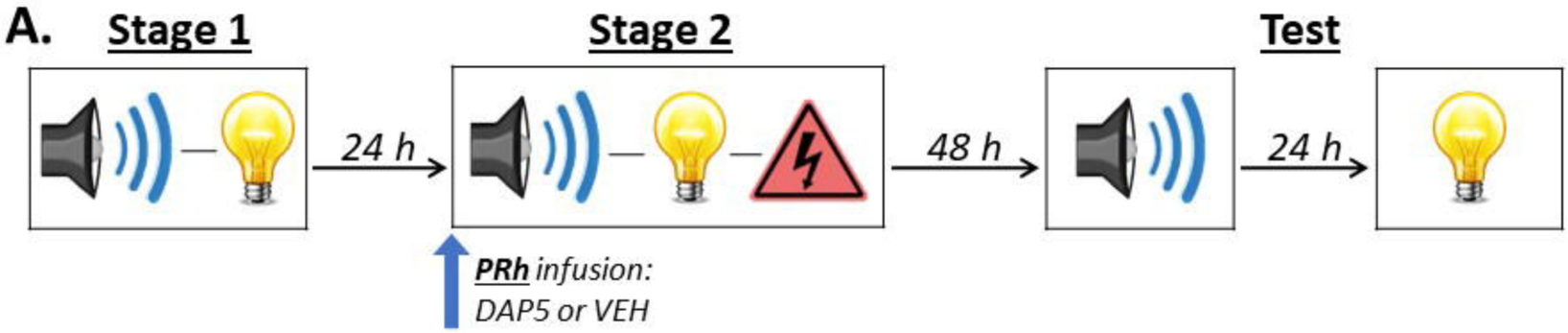

D.
Test with Preconditioned Sound
Test with Conditioned Light
Freezing (%)
80
60
40
20
0
100
*
VEH
DAP
Group

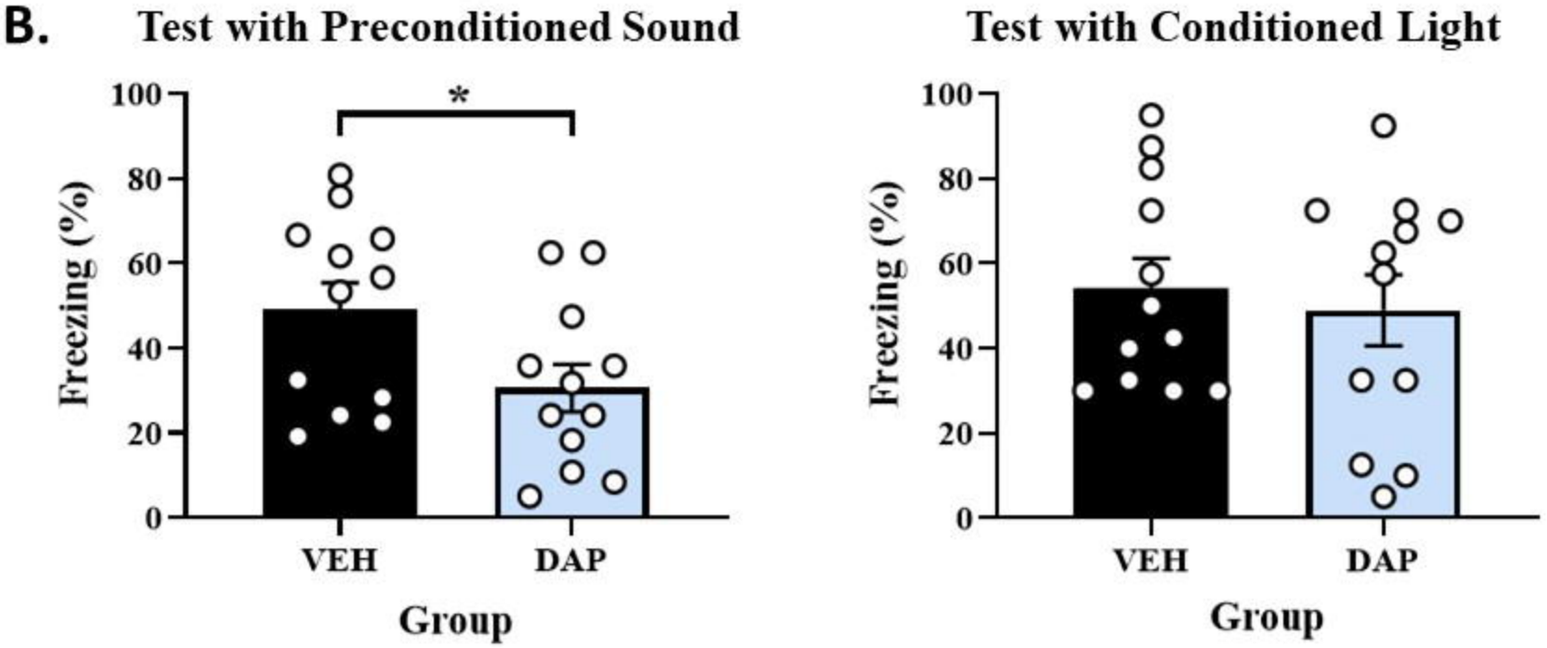

A.
Stage 1
Stage 2
Test
48 h
24 h
PRh infusion:
DAP5 or VEH

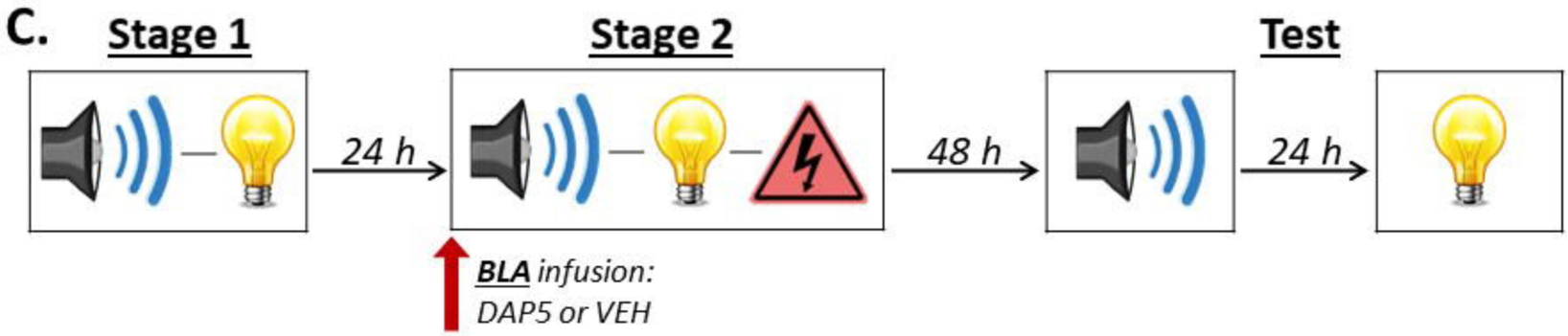

B.
Test with Preconditioned Sound
Test with Conditioned Light
Freezing (%)
0
20
40
60
80
100
VEH
DAP
Group

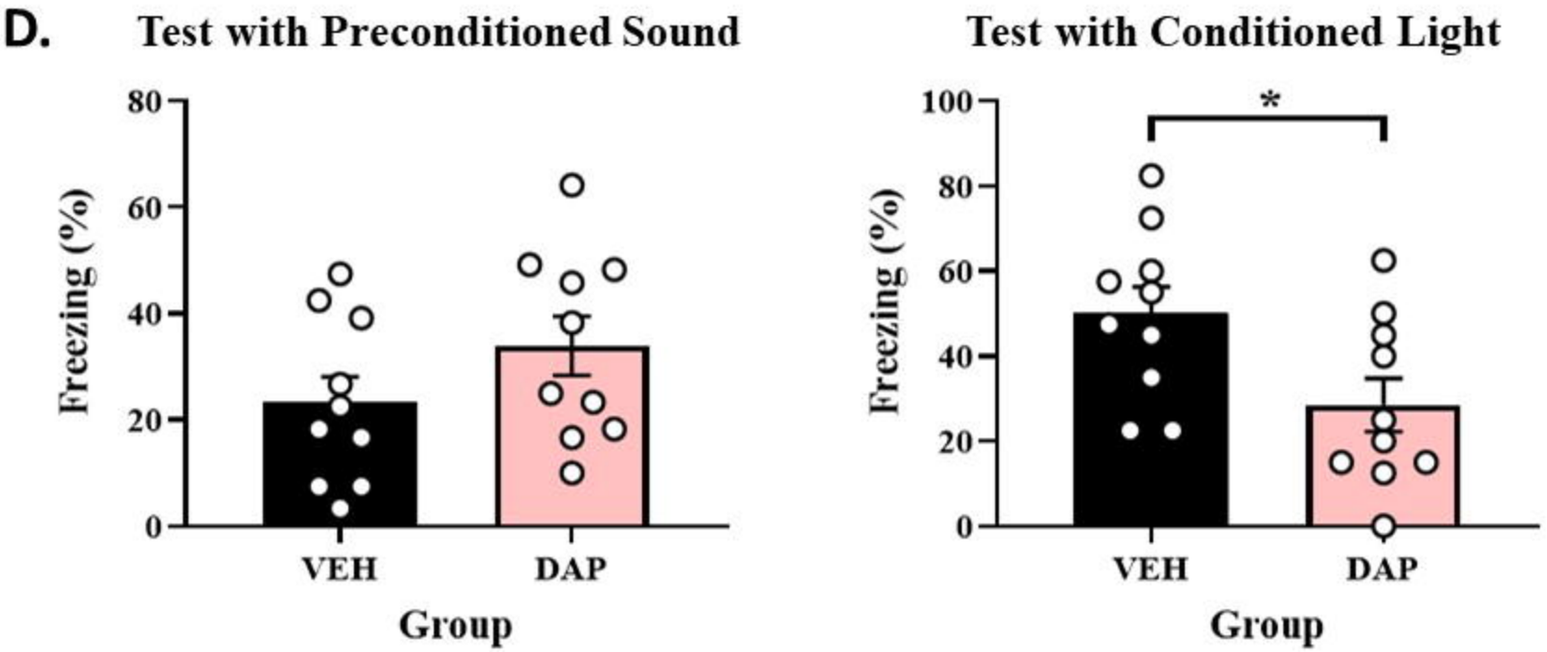

C.
Stage 1
Stage 2
Test
48 h
24 h
BLA infusion:
DAP5 or VEH

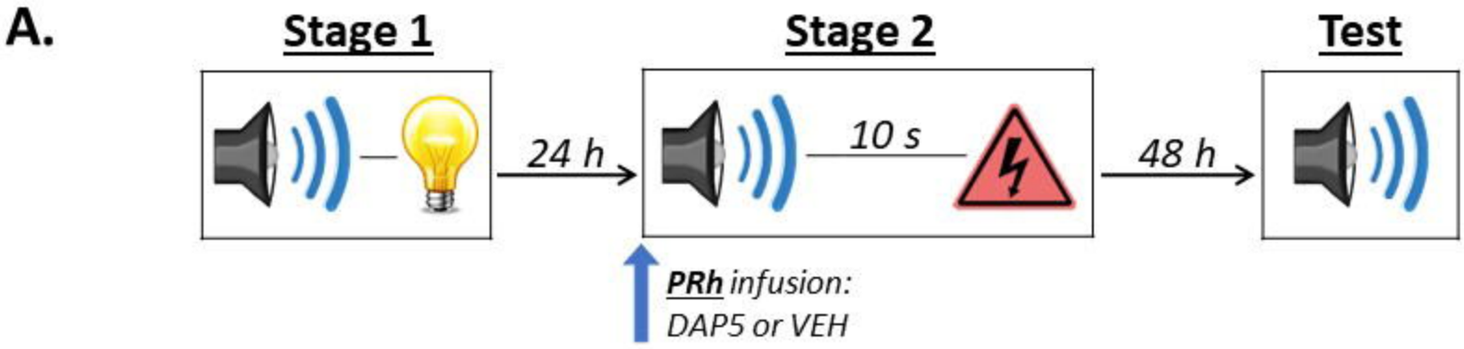

D.
Test with Preconditioned Sound
Test with Conditioned Light
*
*
Freezing (%)
0
20
40
60
80
100
VEH
DAP
Group

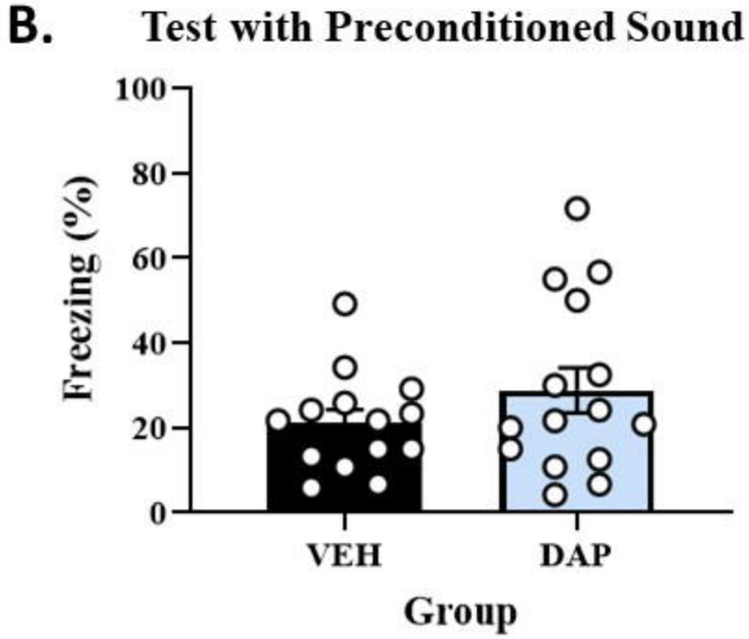

A.
Stage 1
Stage 2
Test
24 h
48 h
24 h
PRh infusion:
DAP5 or VEH

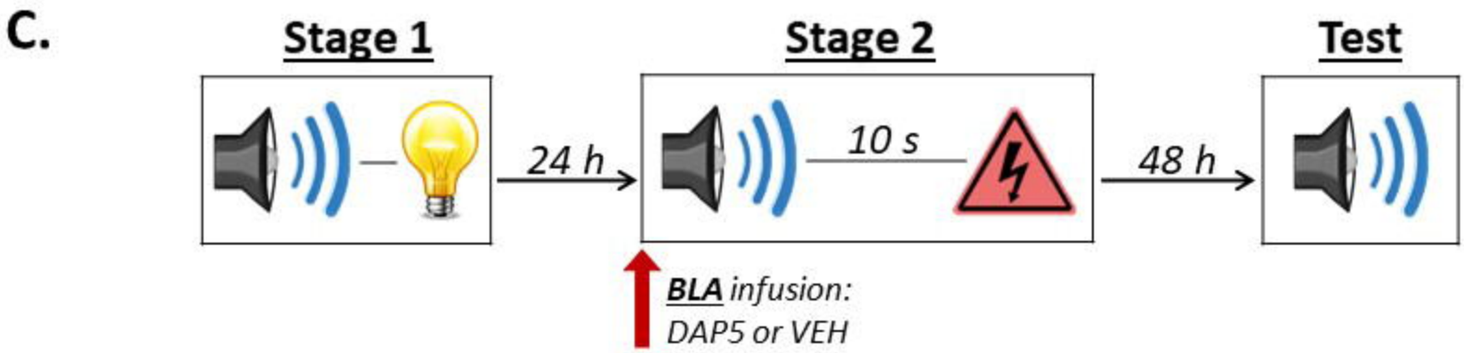

B.
Test with Preconditioned Sound
Test with Conditioned Light
Freezing (%)
100
80
60
40
20
0
*
VEH
DAP
Group

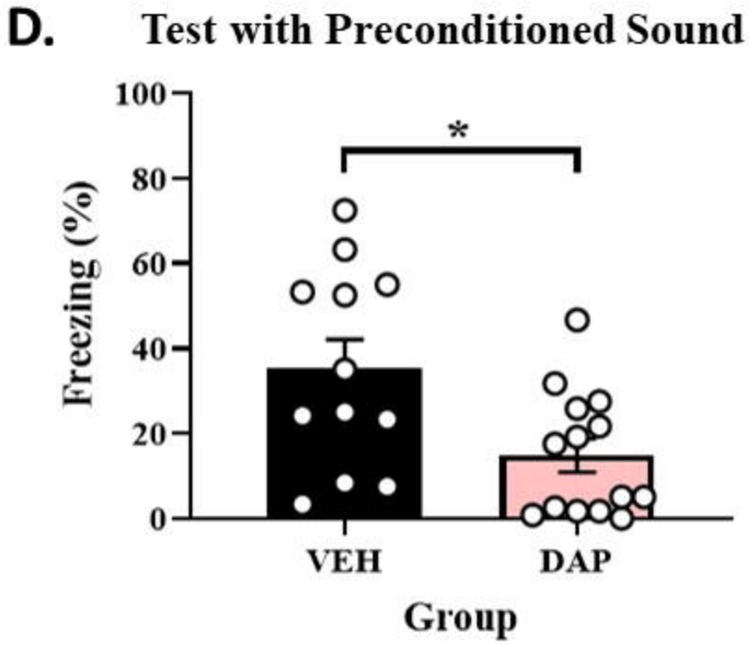

C.
Stage 1
Stage 2
Test
24 h
48 h
24 h
BLA infusion:
DAP5 or VEH

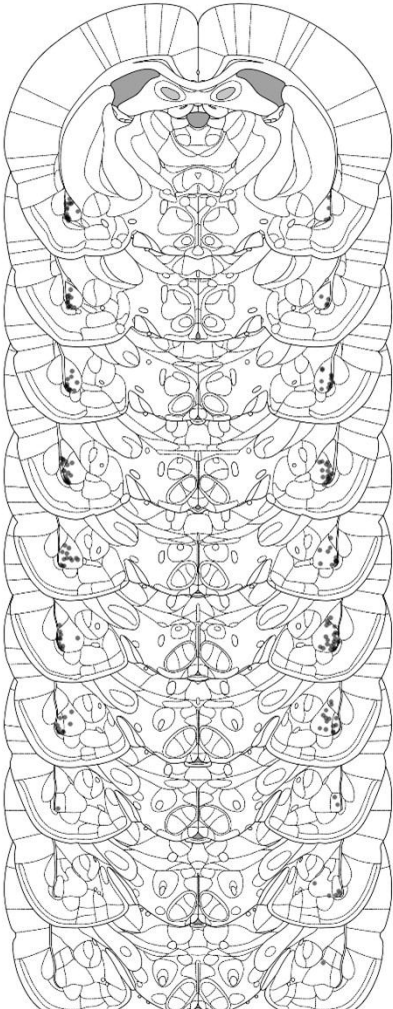

D.
Test with Preconditioned Sound
Test with Conditioned Light
Freezing (%)
100
80
60
40
20
0
*
VEH
DAP
Group

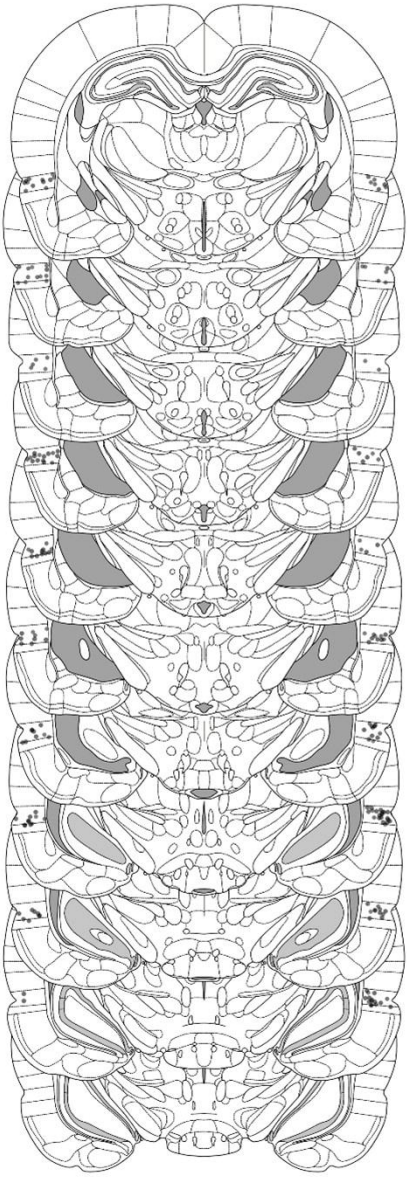

A.
Stage 1
Stage 2
Test
24 h
10 s
48 h
PRh infusion:
DAP5 or VEH


B.
Test with Preconditioned Sound
Freezing (%)
100
80
60
40
20
0
VEH
DAP
Group


C.
Stage 1
Stage 2
Test
24 h
10 s
48 h
BLA infusion:
DAP5 or VEH


D.
Test with Preconditioned Sound
Freezing (%)
100
80
60
40
20
0
*
VEH
DAP
Group


-1.72
-1.80
-1.92
-2.04
-2.16
-2.28
-2.40
-2.52
-2.64
-2.76


-3.48
-3.60
-3.72
-3.84
-3.96
-4.08
-4.20
-4.36
-4.44
-4.56
-4.68