Research

Analysis of memory modulation by conditioned stimuli

Thomas Lapointe, Michael Wolter, and Francesco Leri

Department of Psychology, Collaborative Program in Neuroscience, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Conditioned stimuli (CS) have multiple psychological functions that can potentially contribute to their effect on memory formation. It is generally believed that CS-induced memory modulation is primarily due to conditioned emotional responses, however, well-learned CSs not only generate the appropriate behavioral and physiological reactions required to best respond to an upcoming unconditioned stimulus (US), but they also serve as signals that the US is about to occur. Therefore, it is possible that CSs can impact memory consolidation even when their ability to elicit conditioned emotional arousal is significantly reduced. To test this, male Sprague–Dawley rats trained on a signaled active avoidance task were divided into "Avoider" and "Non-Avoider" subgroups on the basis of percentage avoidance after 6 d of training. Subgroup differences in responding to the CS complex were maintained during a test carried out in the absence of the US. Moreover, the subgroups displayed significant differences in stress-induced analgesia (hot-plate test) immediately after this test, suggesting significant subgroup differences in conditioned emotionality. Importantly, using the spontaneous object recognition task, it was found that immediate post-sample exposure to the avoidance CS complex had a similar enhancing effect on object memory in the two subgroups. Therefore, to our knowledge, this is the first study to demonstrate that a significant conditioned emotional response is not necessary for the action of a predictive CS on modulation of memory consolidation.

Biologically significant stimuli (unconditioned stimuli [US]) support learning and promote changes in behavior by enhancing the consolidation of memory (White and Milner 1992). Thus, stimuli such as food (Huston et al. 1974, 1977), pain (Galvez et al. 1996; Quirarte et al. 1998), and various drugs of abuse (Krivanek and McGaugh 1969; White 1996; Leri et al. 2013; Rkieh et al. 2014; Wolter et al. 2019, 2020) increase memory storage and facilitate performance on a variety of learning tasks when delivered during a window of memory consolidation that occurs following a learning experience (McGaugh and Roozendaal 2009; Roozendaal and Mcgaugh 2012; McGaugh 2015).

Interestingly, exposure to stimuli paired with both incentive (Holahan and White 2013; Wolter et al. 2019, 2020; Baidoo et al. 2020) and aversive (Holahan and White 2002, 2004; Leong et al. 2015; Goode et al. 2016) USs also enhances memory consolidation, presumably because of the conditioned emotional responses that they generate. For example, CSs that precede exposure to footshock elicit freezing (Díaz-Mataix et al. 2017), avoidance (Dombrowski et al. 2013), analgesia (McNally et al. 1999), as well as sympathetic stimulation such as increases in heart rate (Zhang et al. 2019), blood pressure (Hsu et al. 2012), and release of stress hormones (Feenstra et al. 1999), all reactions that are elicited by footshock itself (Lim et al. 1982; McCarty and Baucom 1982; Conti et al. 1990; Galvez et al. 1996; O'Doherty 2004; Lázaro-Muñoz et al. 2010). Holahan and White (2002, 2004) reported that the memory enhancing action of a shock-paired CS could be blocked by lesions of the central amygdala nucleus (CeA), a region involved in generating the behavioral and neurohormonal responses to emotionally arousing stimuli (LeDoux 2003). As well, similarly to a range of aversive USs (anxiogenic drugs, predator odor, tail shock, restraint stress; Kim et al. 2001; Elliott and Packard 2008; Leong and Packard 2014), the effect of a CS paired with footshock on consolidation was found dependent on noradrenergic activation of the amygdala (Goode et al. 2016).

However, well-learned CSs not only generate the appropriate behavioral and physiological reactions required to best respond to an upcoming US, but they also serve as signals that the US is about to occur. Temporal relationships between CSs and USs are learned rapidly during conditioning (Ohyama and Mauk 2001; Balsam et al. 2002), and these expectations modulate the expression of learned responses (Holland 2000; Balsam et al. 2010). Moreover, the ability of CSs to predict USs is heavily dependent on mesolimbic dopamine (DA) activity (Schultz et al. 1997; Flagel et al. 2011), and there is substantial evidence that mid-brain DA plays an important role in memory consolidation (White 1989; Managò et al. 2009; Redondo and Morris 2011; Yamasaki and Takeuchi 2017).

This analysis suggests that CSs can impact memory consolidation because of their predictive function, even when their ability to elicit preparatory conditioned emotionality is significantly reduced. To test this idea, the current study used a signaled active avoidance task whereby rats learn to avoid an aversive US (footshock) by crossing from one compartment of a shuttle box to another during the presentation of a warning signal. Miller (1948) posited that animals perform the shuttle response during the signal because it prevents the occurrence of the US, and this reduces the experience of conditioned fear caused by the signal. However, it has been found that avoidance persists even when the warning signal no longer elicits a measurable fear state (Kamin et al. 1963; Linden 1969; Coover and Ursin 1973; Starr and Mineka 1977; Mineka and Gino 1980), suggesting that CSs can promote robust avoidance even though conditioned emotional responses are greatly reduced. The current study also used active avoidance because it consistently reveals robust individual differences in learning (Choi et al. 2010; Lázaro-Muñoz et al. 2010; Martinez et al. 2013; Antunes et al. 2020), such that animals can be distinguished into subgroups of Avoiders and Non-avoiders by simple median split (Storace et al. 2019) on percentage avoided

Corresponding author: fleri@uoguelph.ca
Article is online at http://www.learnmem.org/cgi/doi/10.1101/lm.052407.120.

USs. Although the source of the individual differences is unknown, it has been postulated the subgroups learn different behavioral responses to the CS (Lázaro-Muñoz et al. 2010; Martinez et al. 2013; Antunes et al. 2020): Avoiders display a loss of fear responses to the CS as the avoidance response is acquired (LeDoux et al. 2017; Cain 2018), while those who fail to acquire the avoidance response continue to display conditioned fear characterized by freezing (Martinez et al. 2013).

By capitalizing on these individual differences, the current study explored whether both the predictive and preparatory functions of aversive CSs play a role in modulating consolidation of object memory using the spontaneous object recognition (OR) task. OR relies on the natural tendency of rats to explore novel objects (Winters et al. 2008) and this task was selected because it has been found sensitive to enhancement by exposure to contextual CSs paired with both incentive and aversive stimuli (Wolter et al. 2019, 2020; Baidoo et al. 2020). Given the evidence reviewed above, it was predicted that exposure to the avoidance CS complex (the training chamber, the retractable gate, the warning tone, and the cue light) would impact consolidation of object memory equally in Avoider and Non-Avoider subgroups. Avoider and Non-Avoider subgroups were tested for reactivity to thermal pain throughout avoidance training and testing using the hot-plate to provide an indirect measure of emotional reactivity to the footshock and/or to the aversive CS complex (Fig. 1). This approach was selected because fear/stress-inducing stimuli such as footshock (Maier and Watkins 1991; Rosellini et al. 1994), predator odor (Williams et al. 2005), and their CSs (Hotsenpiller and Williams 1997; McNally and Akil 2001; Ford et al. 2011), elicit stress-induced analgesia; a well-known defensive response in various species (Bolles and Fanselow 1980; Fendt and Fanselow 1999).

Results

Experiment 1

This experiment was designed to establish whether different shock intensities would impact the acquisition of signaled avoidance, whether sensitivity to thermal pain would change during acquisition, whether significant individual differences would emerge over the course of training, and whether these differences would still be observed in response to the CS complex in the absence of shock. Figure 2, A–D, represents mean (±SEM) percentage shocks avoided during training with different shock intensities (0, 0.2, 0.4, and 0.8 mA) in different groups of rats divided into Avoider and Non-Avoider subgroups based on percentage avoidance on training day 6. For 0 mA (panel A), the ANOVA revealed a significant subgroup by training day interaction [$F_{(3,21)} = 3.88$, $P = 0.024$], as well as significant main effects of subgroup [$F_{(1,21)} = 14.16$, $P = 0.007$] and training day [$F_{(3,21)} = 13.12$, $P < 0.001$]. Multiple comparisons indicated that the Avoider subgroup displayed significantly more avoidance on training day 6. The pattern of avoidance in animals tested with the other shock intensities was very similar, although significant differences between Avoider and Non-Avoider subgroups emerged earlier in training (day 3) and continued thereafter {panel B: 0.2 mA shock—subgroup by training day [$F_{(3,21)} = 6.26$, $P = 0.003$], subgroup [$F_{(1,21)} = 25.13$, $P = 0.002$] and training day [$F_{(3,21)} = 10.42$, $P < 0.001$]; panel C: 0.4 mA shock—subgroup by training day [$F_{(3,21)} = 8.30$, $P < 0.001$], subgroup [$F_{(1,21)} = 33.03$, $P < 0.001$] and training day [$F_{(3,35)} = 12.67$, $P < 0.001$]; panel D: 0.8 mA shock—subgroup by training day [$F_{(3,21)} = 6.79$, $P = 0.002$], subgroup [$F_{(1,35)} = 14.83$, $P = 0.006$] and training day [$F_{(3,21)} = 26.60$, $P < 0.001$}.

Significant subgroup differences in hot-plate latency (panels E–H) were observed only in animals trained with 0.8 mA. In fact, the ANOVA revealed a significant main effect of subgroup [$F_{(1,28)} = 19.85$, $P = 0.003$] and training day [$F_{(3,28)} = 4.86$, $P < 0.010$], and multiple comparisons on marginal means indicated that rats in the Avoider subgroup displayed significantly shorter latencies than rats in the Non-Avoider subgroup.

Figure 3A represents mean (±SEM) percentage avoidance when Avoider and Non-Avoider subgroups were tested with shockers turned off (Shock-OFF test). The ANOVA revealed a significant main effect of subgroup [$F_{(1,28)} = 24.09$, $P < 0.001$] and multiple comparisons on marginal means indicated that the Avoider subgroup displayed significantly more avoidance. Figure 3B represents mean (±SEM) hot-plate latencies assessed immediately following the Shock-OFF avoidance test. Although the 0.8-mA Avoider subgroup displayed reduced latency, the ANOVA did not reveal any significant difference.

Experiment 2

This experiment tested whether post-sample exposure to the CS complex would equally impact object memory in Avoider and Non-Avoider subgroups. In this experiment, different groups of animals were trained as in Experiment 1, except that only two shock intensities were used: 0 and 0.8 mA. The 0 mA ($n = 17$) was included because Experiment 1 indicated that even when there are no footshocks, exploration of the two chambers generates a substantial level of "percentage avoidance." This probably resulted from a possible approach component of the paradigm used that may have facilitated avoidance learning in this apparatus: when the gate connecting the chambers opens, the tone + light warning stimuli emanates from the chamber that the animals must shuttle toward. This said, the 0-mA group was also included to control for general locomotion activity, which was found to be significant in this apparatus when a group of control rats ($n = 6$) was trained with 0 mA in the absence of tone + light warning stimuli (mean ± SEM percentage avoidance: day 1 = 26.7 ± 5.2, day 3 = 27.8 ± 10.5, day 6 =

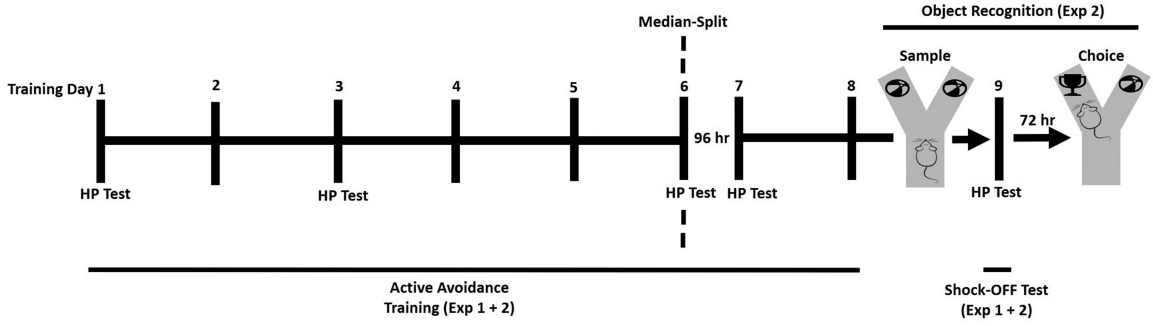

Figure 1. Experimental design used in Experiments 1 and 2.

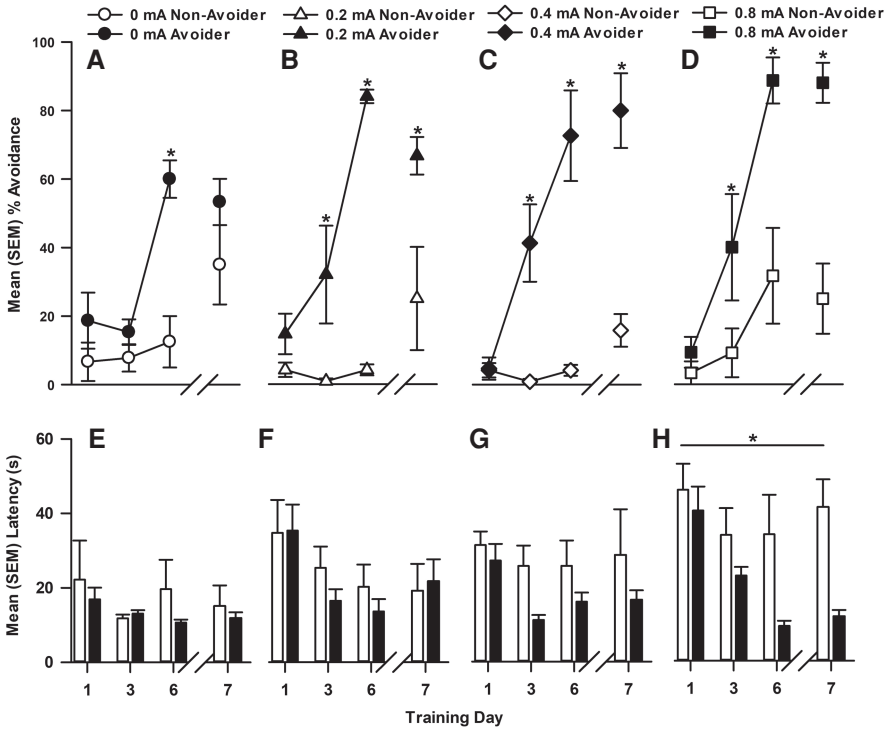

Figure 2. (*A–D*) Mean (±SEM) percentages shocks avoided across 7 d of avoidance training in Non-Avoider and Avoider subgroups trained with 0, 0.2, 0.4, and 0.8 mA shock. The asterisk denotes a significant difference compared with the Non-Avoider subgroup. (*E–H*) Mean (±SEM) hot-plate latencies assessed immediately after avoidance training. The asterisk denotes a significant difference between the Non-Avoider and Avoider subgroups.

47.2 ± 9.1, day 7 = 40.6 ± 9.0). The 0.8 mA ($n = 69$; the experiment was repeated in various cohorts) was selected because it generated the greatest individual differences in both avoidance and response to thermal pain in Experiment 1.

Figure 4A represents mean (±SEM) percentage shocks avoided during training with 0- and 0.8-mA shocks. Only sessions 1, 3, 6, and 7 are represented because rats were tested for reactivity to thermal pain only following these sessions. The median split was not performed in the 0 mA because of the lack of consistent subgroup differences observed in 0 mA group of Experiment 1. The ANOVA revealed a significant interaction between group and training day [$F_{(6,243)} = 38.20$, $P > 0.001$], as well as a significant main effect of group [$F_{(1,243)} = 101.01$, $P < 0.001$] and training day [$F_{(3,243)} = 112.97$, $P < 0.001$]. Multiple comparisons further indicated that 6 d of consecutive training significantly improved avoidance performance in the Avoider, but not in the Non-Avoider subgroups. Similarly, percentage avoidance of the Avoider subgroup was significantly higher than that of the 0-mA group from training day 3 on, while the Non-Avoider subgroup displayed significantly less avoidance than the 0 mA group from the very first day of training.

Figure 4B represents mean (±SEM) hot-plate response latencies. The ANOVA revealed a significant interaction between group and training day [$F_{(6,243)} = 3.29$, $P = 0.004$], as well as a significant main effect of group [$F_{(1,243)} = 32.91$, $P < 0.001$] and training day [$F_{(3,243)} = 62.54$, $P < 0.001$]. Multiple comparisons further indicated that latencies significantly decreased over the course of avoidance training in all groups. Moreover, latencies of the Avoider and Non-Avoider subgroups were initially identical but started to differ significantly by training day 3. Finally, while the Non-Avoider subgroup displayed higher latencies in comparison with the 0-mA group on all tests, the difference between the Avoider subgroup and the 0-mA group was no longer significant by the last training day.

Figure 5, A and B, represents avoidance performance and hot-plate latencies, respectively, when all subjects were tested for avoidance in the absence of footshock (Shock-OFF tests). For percentage avoidance, the ANOVA was significant [$F_{(2,81)} = 40.60$, $P < 0.001$] and multiple comparisons confirmed that the Avoider subgroup displayed significantly higher levels of percentage avoidance than the other groups. Moreover, both subgroups trained with 0.8 mA were significantly different from the 0 mA group, but in opposite directions. For hot-plate latencies, the ANOVA was also significant [$F_{(2,81)} = 16.81$, $P < 0.001$], and multiple comparisons indicated that the latency of the Non-Avoider subgroup was significantly different from latencies of the Avoider subgroup and the 0 mA group.

Figure 6 represents mean (±SEM) discrimination ratios on sample and choice phases of object recognition testing. All subjects received immediate postsample exposure to the CS complex in the absence of footshock (Shock-OFF tests). The ANOVA revealed a significant interaction between group and phase [$F_{(2,81)} = 3.90$, $P = 0.024$], as well as significant main effects of group [$F_{(2,81)} = 4.70$, $P = 0.012$] and phase [$F_{(1,81)} = 37.43$, $P < 0.001$]. Multiple comparisons further indicated that choice discrimination ratios of both subgroups trained with 0.8 mA were different from sample, and from choice of the 0 mA group. A separate analysis of motor activity during sample and choice phases revealed significantly lower activity in the Non-Avoider subgroup, but Non-Avoider and Avoider subgroups displayed equivalent total object exploration during sample and test (data not shown).

Discussion

Conditioned stimuli have multiple psychological functions that can potentially contribute to their effect on memory formation. It is generally believed that CS-induced memory modulation is due to conditioned emotional responses, however, well-learned CSs not only generate the appropriate behavioral and physiological reactions required to best respond to an upcoming US, but they also serve as signals that the US is about to occur. Therefore, to test the possibility that CSs can impact memory consolidation even when their ability to elicit conditioned emotionality is significantly reduced, male Sprague–Dawley rats were trained on a signaled active avoidance task and were then assigned to Avoider and Non-Avoider subgroups by median split based on percentage avoidance after 6 d of training. It was found that subgroup differences were maintained during an avoidance test carried out in the absence of the US. The subgroups also differed in hot-plate latency assessed immediately after avoidance training and testing, suggesting significant differences in conditioned emotionality. Notably, immediate postsample exposure to the CS complex had a similar enhancing effect on object memory in the two subgroups.

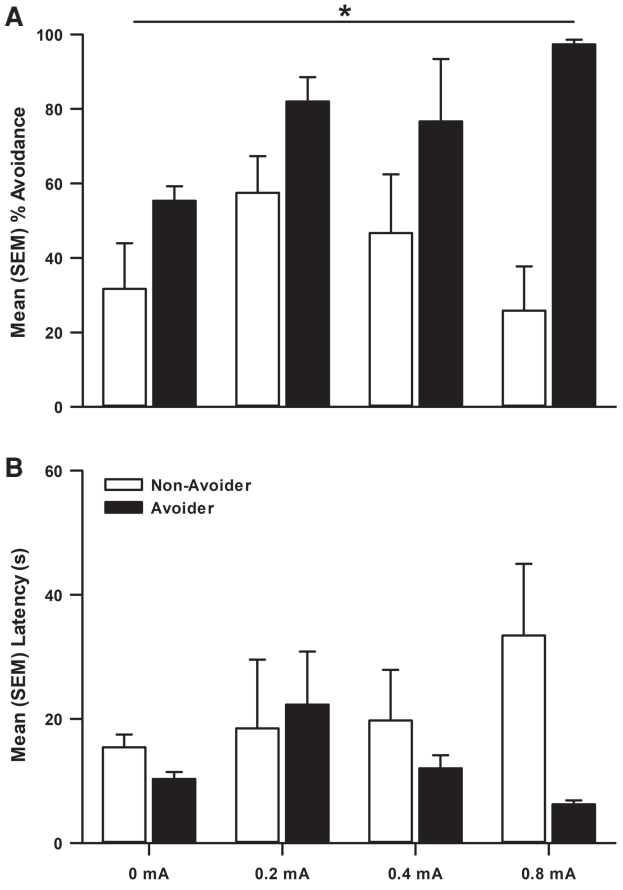

Figure 3. (*A*) Mean (±SEM) percentage avoidance during the Shock-OFF avoidance test in Non-Avoider and Avoider subgroups trained with 0-, 0.2-, 0.4-, and 0.8-mA shock. The asterisk denotes a significant difference between the Non-Avoider and Avoider subgroups. (*B*) Mean (±SEM) hot-plate latencies assessed immediately after the Shock-OFF avoidance test.

To our knowledge, this is the first study to indicate that a robust conditioned emotional response is not necessary for the action of a predictive CS on modulation of memory consolidation.

This study capitalized on the known individual differences that typically emerge when rats are trained on active avoidance tasks (Choi et al. 2010; Lázaro-Muñoz et al. 2010; Martinez et al. 2013; Antunes et al. 2020). One important conclusion is that Avoider and Non-Avoider subgroups learned different contingencies and behaviors during training: Avoiders learned to shuttle during the presentation of the warning stimulus complex, while Non-Avoiders learned to freeze in the avoidance chambers. This assertion is supported by the observations that Avoider and Non-Avoider subgroups displayed significantly higher and lower percentage avoidance in comparison with the group trained with 0 mA, respectively. This difference in response strategy had a significant impact on sensitivity to thermal pain, as longer duration of footshock exposure in the Non-Avoiders significantly enhanced their latency to respond on the hot-plate test (Fig. 4). Although interesting, this result was hardly surprising given the well-known link between pain and analgesia (Butler and Finn 2009). More interesting, however, was the observation that subgroup differences in response strategy and sensitivity to thermal pain were maintained during the test of avoidance performed in the absence of footshocks. Hence, our observations suggest that the Avoider subgroup learned a behavioral response to the warning light + tone that was performed with minimal conditioned emotionality, while the Non-Avoider subgroup learned a freezing response to the testing chambers indicative of high conditioned emotionality.

Demonstrating significant subgroup differences in conditioned emotional responses following exposure to the avoidance CS complex was essential to explore whether preparatory and predicative functions of CSs play similar roles in modulating memory consolidation. Experiment 2 found that immediate postsample exposure to the CS complex had a similar enhancing effect on discrimination ratios (DR) in both the Avoider and Non-Avoider subgroups, as both groups spent significantly more time investigating the novel objects over the familiar object on test. Importantly, animals trained with 0 mA shock did not show a change in DR indicating that previous training with 0.8 mA was necessary for the effect of the CS complex on object memory. Although the experiment did not include a group of animals that received postsample

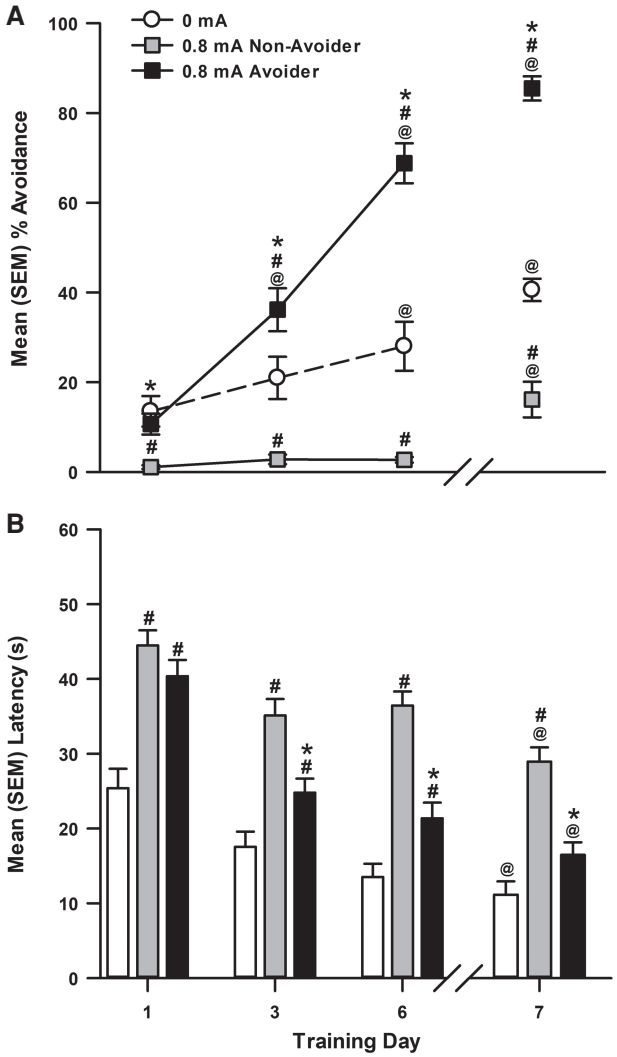

Figure 4. (*A*) Mean (±SEM) percentage shocks avoided across 7 d of avoidance training in animals trained with 0- and 0.8-mA shock. Only animals trained with 0.8-mA shock were divided into Non-Avoider and Avoider subgroups. (*B*) Mean (±SEM) hot-plate latencies assessed immediately after avoidance training. The asterisk denotes a significant difference compared with the 0.8-mA Non-Avoider subgroup. The number sign denotes a significant difference compared with the 0-mA group. The "at" sign denotes a significant difference compared with training day 1.

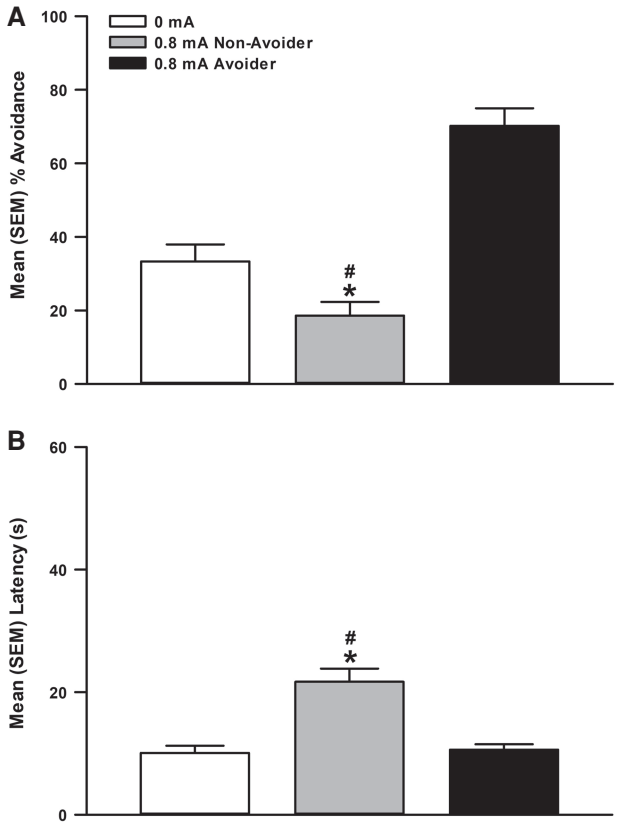

Figure 5. (*A*) Mean (±SEM) percentage avoidance during the Shock-OFF avoidance test in animals trained with 0- and 0.8-mA shock. Only animals trained with 0.8-mA shock were divided into Non-Avoider and Avoider subgroups. (*B*) Mean (±SEM) hot-plate latencies assessed immediately after the Shock-OFF avoidance test. The asterisk denotes a significant difference compared with the 0.8-mA Non-Avoider subgroup. The number sign denotes a significant difference compared with the 0-mA group.

exposure to the CS complex outside the window of consolidation (i.e., a delay group, exposed to the context/CS several hours after the sample phase), the observation that both Avoider and Non-Avoider subgroups displayed similarly elevated DRs during the choice phase, and that comparable effects have been observed in rats exposed to immediate postsample incentive (paired with nicotine, cocaine or heroin) (Wolter et al. 2019, 2020) and other aversive (paired with precipitated opiate withdrawal) (Baidoo et al. 2020) CSs, suggest that the CS complex did, in fact, modulate the consolidation of object memory.

A possible conditioned memory enhancement in the Non-Avoider subgroup was predicted by the well-known facilitatory effects of emotionally arousing stimuli on memory consolidation processes (McGaugh and Roozendaal 2002; Roozendaal and McGaugh 2012; Schwabe et al. 2012). One crucial function of a CS is to produce anticipatory responses that are relevant to the nature of the US, and it has been demonstrated that CSs paired with footshock produce conditioned states that include freezing, avoidance, analgesia, and the release of stress hormones (Feenstra et al. 1999; McNally et al. 1999; Dombrowski et al. 2013; Díaz-Mataix et al. 2017). The central amygdala nucleus (CeA) is a critical structure involved in the expression of the behavioral and autonomic reactions to emotionally arousing stimuli (LeDoux 2003) and it has an essential role in regulating the consolidation of emotional memories (Keifer et al. 2015). Therefore, it is likely that the memory enhancing effects of the CS complex in the Non-Avoider subgroup was dependent on neuromodulatory actions occurring in the CeA, as it has been shown that inactivation of the entire amygdala (Holahan and White 2004), or lesions of the CeA (Holahan and White 2002), eliminate the memory modulating effects of posttraining exposure to a footshock CS in rats.

A possible conditioned memory enhancement in the Avoider subgroup was also predicted, but from considering the role of DA in avoidance learning and memory formation. In fact, acquisition of avoidance is dependent on increased DA neurotransmission in the ventral medial striatum (VMS) during the warning signal (Oleson et al. 2012; Dombrowski et al. 2013; Oleson and Cheer 2013; Gentry et al. 2016; Wenzel et al. 2018), as well as during the safety period (Oleson et al. 2012; Stelly et al. 2019). Moreover, when the response is acquired, it likely becomes habit-like and also dependent on DA, but on nigrostriatal projections to the dorsal striatum (Wenzel et al. 2018). Importantly, mid-brain DA has a known role in memory consolidation (Redondo and Morris 2011; Yamasaki and Takeuchi 2017), contributes to spontaneous OR memory consolidation in rats (Nelson et al. 2010; de Lima et al. 2011), and has been implicated in consolidation of various other memory tasks such as one-trial inhibitory avoidance (Managò et al. 2009), spatial water maze training (Setlow and McGaugh 1998), and object-in-place associations (Nelson et al. 2010).

In summary, by capitalizing on the known individual differences in learning strategies that typically emerge when rats are trained in active avoidance tasks, this study explored whether CSs can impact memory consolidation even when their ability to elicit conditioned emotionality is significantly reduced. It was found that exposure to the avoidance CS complex similarly modulated object memory consolidation in animals that learned a behavioral response to the warning light + tone stimulus performed with minimal conditioned emotionality (Avoider subgroup), and in animals that learned a freezing response to testing chambers performed with high conditioned emotionality (Non-Avoider subgroup). An analysis of known neurobiological mechanisms involved in avoidance learning suggests the hypothesis that conditioned memory modulation may involve striatal DA in avoider animals, while in nonavoider animals it may involves stress hormones and the amygdala.

Materials and Methods

Subjects

A total of 120 male Sprague–Dawley rats (Charles River), weighing between 225 and 250 g at the beginning of the experiments were individually housed in standard rat cages (polycarbonate; 50.5 × 48.5 × 20 cm) with standard bedding and environmental enrichment, and were maintained on a reverse light–dark schedule (lights off at 07:00; on at 19:00). All testing was conducted during the dark period. Rats had access to 20 g per day of standard rat chow and water was available ad libitum in home cages. All experiments were approved by the Animal Care Committee of the University of Guelph and were performed in accordance with recommendations provided by the Canadian Council on Animal Care.

Apparatus

Avoidance Chambers

Gemini avoidance chambers (San Diego Instruments) were constructed of acrylic and aluminum walls with compartments of equal size (9.5 × 8 × 8 in) separated by a stainless-steel gate. The chambers were enclosed in acrylic and aluminum boxes (66 × 33 × 44.5 cm). Scrambled footshocks are delivered to the grid floor made of stainless-steel rods with a solid-state feedback controller, and infrared photobeams were used to detect subject location. At the beginning of every trial, the gate opens, and a

compound warning stimulus comprised of a 10-sec tone (65 db, 3000 Hz) and cue light (18 lux) is presented in the opposite compartment of the animals start position. At the end of each trial following either an escape, avoidance, or nonresponse, the gate closes and does not open until the beginning of the next trial.

Hot–plate

Response latency to thermal pain was assessed using a hot-plate apparatus (model LE7406; LSI Letica). The heated surface (22 × 22 cm) was maintained at 50°C ± 2.1°C (Plone et al. 1996). Animals were placed onto the hot-plate apparatus and removed following either a lick of the fore paw, hind paw, or if 60 sec elapsed.

Object recognition

The Y-apparatus used for OR has been described previously by Wolter et al. (2019). On each object recognition trial, the rats experienced a new set of never before seen objects comprised of plastic, ceramic, and glass ranging in height from 10 to 20 cm with varying visual and tactile qualities. Objects were fixed to the floor using odorless reusable adhesive putty and were always wiped with 50% ethanol before being placed into the apparatus to control for any olfactory cues that may influence exploration. A JVC Everio digital camera was mounted on a tripod above the apparatus to record all trials.

Procedures

Experiment 1

The avoidance protocol described below was adapted from a study that explored individual differences in learned helplessness (Storace et al. 2019), as well as from an active avoidance pilot (9 rats trained with 0.8 mA footshock) indicating that avoidance performance tends to spontaneously increase after a break (4 d) from daily training (Fig. 1). Therefore, rats were habituated to the avoidance chambers with the house light on for 15 min 1 d prior to the beginning of training. They then underwent six consecutive days of avoidance training which included 30 trials per day, with an inter-trial interval between 22–38 sec. On each trial, the compound warning stimulus was activated for 10 sec, the footshock was then activated, and both terminated 30 sec later. On each training trial, rats could: completely avoid the footshock by crossing to the adjacent compartment during the presentation of the warning stimulus, escape the footshock during the 30-sec footshock activation, or fail to cross to the other compartment altogether. Different groups of rats ($n = 9$ each) were trained with different footshock intensities: 0, 0.2, 0.4, or 0.8 mA. All animals were given a 96-h rest period, and then training resumed for 2 consecutive days. On experimental day 9, avoidance was tested with all shockers turned off (Shock-OFF test). Immediately following avoidance sessions on training days 1, 3, 6, 7, and the Shock-OFF test, hot-plate latencies were assessed.

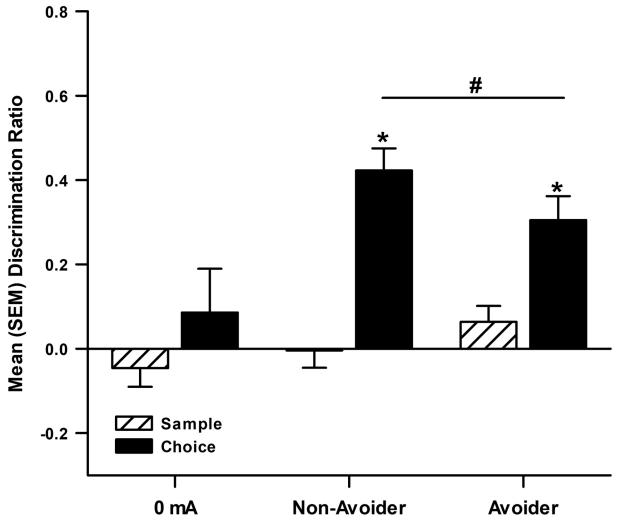

Figure 6. The mean (±SEM) discrimination ratio produced during the sample and choice phase of object recognition following exposure to the avoidance context and CS postsample. The asterisk denotes a significant difference compared with sample phase discrimination ratio. The number sign denotes a significant difference compared with the 0-mA choice phase discrimination ratio.

Experiment 2

In this experiment, different groups of animals were trained identically as Experiment 1, except that only two shock intensities were used: the 0 mA ($n = 17$) was included as a control for general motor activity in the chambers, while 0.8 mA ($n = 69$; the experiment was repeated in various cohorts over a period of time) was selected because it generated the greatest individual differences in both avoidance learning and responsivity to thermal pain in Experiment 1.

To test the effects of postsample exposure to conditioned avoidance CS on object recognition memory, all rats were habituated to the empty Y-apparatus for 5 min following avoidance training days 7 and 8 (Fig. 1). OR testing consisted of two phases: a sample phase and a choice phase, separated by a 72-h retention interval. This retention interval was chosen as a "suboptimal" condition in which treatment naïve rats do not typically express memory (Wolter et al. 2019, 2020; Baidoo et al. 2020). During the sample phase, two identical novel objects were placed into the Y-apparatus at the end of each exploration arm. Each rat was placed in the start box, and the guillotine door was opened. Rats were allotted a maximum of 180 sec to explore objects or were removed if 25 sec of total object exploration was achieved, whichever came first. Object exploration was defined as directing the nose to the object at <2 cm and/or touching the object with the nose. Ten minutes following the conclusion of the sample phase, rats were tested for avoidance with shockers turned off (Shock-OFF test) and then immediately tested for reactivity to thermal pain at the end of the avoidance session. After the 72-h retention interval in home cages, rats were returned to the Y-apparatus for a test of choice between a copy of the original sample object in one arm and a novel object in the other. This choice phase lasted 2 min, and the time spent exploring the novel and familiar objects was recorded. The positions of the objects were counterbalanced across groups.

Statistical analysis

One-factor and two-factor repeated measures analysis of variance (ANOVAs) were performed using SigmaPlot (v.12.5, Systat Software, Inc.). Significant main effects and/or interactions were further analyzed by Student Newman–Keuls post-hoc analysis. The significant alpha level for all analyses was set at 0.05. Analyses of the OR task required the calculation of a discrimination ratio (DR) to standardize for differences in individual total exploration times between the rats. A DR is a ratio of object preference, where a score of 0 means the rat shows no preference between the two objects, a positive score indicates preference of the novel object, and a negative score indicates preference for the familiar object. Exploration data were taken from the first minute of the choice phase to calculate the choice discrimination ratio [1 min novel object exploration – 1 min familiar object exploration/ 1 min novel object exploration + 1 min familiar object exploration], as previous research indicates that novelty preference is most robust during the first minute of the choice phase (Dix and Aggleton 1999). The sample DR was calculated using an if/then scenario: (If "novel side is left" (left arm exploration – right arm exploration)/(total object exploration) If "novel side is right" (right arm exploration – left arm exploration)/(total object exploration)). A minimum exploration time was not used in these calculations.

Acknowledgments

We thank Dr. Boyer Winters for consultation and technical assistance. This research was supported by the National Sciences and Engineering Research Council of Canada and the Queen Elizabeth II Graduate Scholarship in Science and Technology.

References

Antunes GF, Gouveia FV, Rezende FS, de Jesus Seno MD, de Carvalho MC, de Oliveira CC, dos Santos LCT, de Castro MC, Kuroki MA, Teixeira MJ, et al. 2020. Dopamine modulates individual differences in avoidance behavior: a pharmacological, immunohistochemical, neurochemical and volumetric investigation. *Neurobiol Stress* **12:** 100219. doi:10.1016/j.ynstr.2020.100219

Baidoo N, Wolter M, Holahan MR, Teale T, Winters B, Leri F. 2020. The effects of morphine withdrawal and conditioned withdrawal on memory consolidation and c-Fos expression in the central amygdala. *Addict Biol* e12909. doi:10.1111/adb.12909

Balsam PD, Drew MR, Yang C. 2002. Timing at the start of associative learning. *Learn Motiv* **33:** 141–155. doi:10.1006/lmot.2001.1104

Balsam P, Drew M, Gallistel C. 2010. Time and associative learning. *Comp Cogn Behav Rev* **5:** 1–22. doi:10.3819/ccbr.2010.50001

Bolles RC, Fanselow MS. 1980. A perceptual-defensive-recuperative model of fear and pain. *Behav Brain Sci* **3:** 291–301. doi:10.1017/S0140525X0000491X

Butler RK, Finn DP. 2009. Stress-induced analgesia. *Prog Neurobiol* **88:** 184–202. doi:10.1016/j.pneurobio.2009.04.003

Cain CK. 2018. Avoidance problems reconsidered. *Curr Opin Behav Sci* **26:** 9–17. doi:10.1016/j.cobeha.2018.09.002

Choi JS, Cain CK, Ledoux JE. 2010. The role of amygdala nuclei in the expression of auditory signaled two-way active avoidance in rats. *Learn Mem* **17:** 139–147. doi:10.1101/lm.1676610

Conti LH, Maciver CR, Ferkany JW, Abreu ME. 1990. Footshock-induced freezing behavior in rats as a model for assessing anxiolytics. *Psychopharmacology (Berl)* **102:** 492–497. doi:10.1007/BF02247130

Coover GD, Ursin H. 1973. Plasma-corticosterone levels during active-avoidance learning in rats. *J Comp Physiol Psychol* **82:** 170–174. doi:10.1037/h0033790

de Lima MNM, Presti-Torres J, Dornelles A, Siciliani Scalco F, Roesler R, Garcia VA, Schröder N. 2011. Modulatory influence of dopamine receptors on consolidation of object recognition memory. *Neurobiol Learn Mem* **95:** 305–310. doi:10.1016/j.nlm.2010.12.007

Díaz-Mataix L, Piper WT, Schiff HC, Roberts CH, Campese VD, Sears RM, LeDoux JE. 2017. Characterization of the amplificatory effect of norepinephrine in the acquisition of Pavlovian threat associations. *Learn Mem* **24:** 432–439. doi:10.1101/lm.044412.116

Dix SL, Aggleton JP. 1999. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav Brain Res* **99:** 191–200. doi:10.1016/S0166-4328(98)00079-5

Dombrowski PA, Maia T V, Boschen SL, Bortolanza M, Wendler E, Schwarting RKW, Brandão ML, Winn P, Blaha CD, Da Cunha C. 2013. Evidence that conditioned avoidance responses are reinforced by positive prediction errors signaled by tonic striatal dopamine. *Behav Brain Res* **241:** 112–119. doi:10.1016/j.bbr.2012.06.031

Elliott AE, Packard MG. 2008. Intra-amygdala anxiogenic drug infusion prior to retrieval biases rats towards the use of habit memory. *Neurobiol Learn Mem* **90:** 616–623. doi:10.1016/j.nlm.2008.06.012

Feenstra MGP, Teske G, Botterblom MHA, De Bruin JPC. 1999. Dopamine and noradrenaline release in the prefrontal cortex of rats during classical aversive and appetitive conditioning to a contextual stimulus: interference by novelty effects. *Neurosci Lett* **272:** 179–182. doi:10.1016/S0304-3940(99)00601-1

Fendt M, Fanselow MS. 1999. The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev* **23:** 743–760. doi:10.1016/S0149-7634(99)00016-0

Flagel SB, Clark JJ, Robinson TE, Mayo L, Czuj A, Willuhn I, Akers CA, Clinton SM, Phillips PEM, Akil H. 2011. A selective role for dopamine in stimulus-reward learning. *Nature* **469:** 53–59. doi:10.1038/nature09588

Ford GK, Kieran S, Dolan K, Harhen B, Finn DP. 2011. A role for the ventral hippocampal endocannabinoid system in fear-conditioned analgesia and fear responding in the presence of nociceptive tone in rats. *Pain* **152:** 2495–2504. doi:10.1016/j.pain.2011.07.014

Galvez R, Mesches MH, Mcgaugh JL. 1996. Norepinephrine release in the amygdala in response to footshock stimulation. *Neurobiol Learn Mem* **66:** 253–257. doi:10.1006/nlme.1996.0067

Gentry RN, Lee B, Roesch MR. 2016. Phasic dopamine release in the rat nucleus accumbens predicts approach and avoidance performance. *Nat Commun* **7:** 13154. doi:10.1038/ncomms13154

Goode TD, Leong KC, Goodman J, Maren S, Packard MG. 2016. Enhancement of striatum-dependent memory by conditioned fear is mediated by beta-adrenergic receptors in the basolateral amygdala. *Neurobiol Stress* **3:** 74–82. doi:10.1016/j.ynstr.2016.02.004

Holahan MR, White NM. 2002. Conditioned memory modulation, freezing, and avoidance as measures of amygdala-mediated conditioned fear. *Neurobiol Learn Mem* **77:** 250–275. doi:10.1006/nlme.2001.4012

Holahan MR, White NM. 2004. Amygdala inactivation blocks expression of conditioned memory modulation and the promotion of avoidance and freezing. *Behav Neurosci* **118:** 24–35. doi:10.1037/0735-7044.118.1.24

Holahan MR, White NM. 2013. Memory enhancement produced by post-training exposure to sucrose-conditioned cues. *F1000Res* **2:** 22. doi:10.12688/f1000research.2-22.v1

Holland PC. 2000. Trial and intertrial durations in appetitive conditioning in rats. *Anim Learn Behav* **28:** 121–135. doi:10.3758/BF03200248

Hotsenpiller G, Williams JL. 1997. A synthetic predator odor (TMT) enhances conditioned analgesia and fear when paired with a benzodiazepine receptor inverse agonist (FG-7142). *Psychobiology* **25:** 83–88.

Hsu YC, Yu L, Chen HI, Lee HL, Kuo YM, Jen CJ. 2012. Blood pressure variations real-time reflect the conditioned fear learning and memory. *PLoS One* **7:** e32855. doi:10.1371/journal.pone.0032855

Huston JP, Mondadori C, Waser PG. 1974. Facilitation of learning by reward of post-trial memory processes. *Experientia* **30:** 1038–1040. doi:10.1007/BF01938996

Huston JP, Mueller CC, Mondadori C. 1977. Memory facilitation by posttrial hypothalamic stimulation and other reinforcers: a central theory of reinforcement. *Biobehav Rev* **1:** 143–150. doi:10.1016/0147-7552(77)90003-1

Kamin LJ, Brimer CJ, Black AH. 1963. Conditioned suppression as a monitor of fear of the CS in the course of avoidance training. *J Comp Physiol Psychol* **56:** 497–501. doi:10.1037/h0047966

Keifer OP, Hurt RC, Ressler KJ, Marvar PJ. 2015. The physiology of fear: reconceptualizing the role of the central amygdala in fear learning. *Physiology* **30:** 389–401. doi:10.1152/physiol.00058.2014

Kim JJ, Lee HJ, Han JS, Packard MG. 2001. Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. *J Neurosci* **21:** 5222–5228. doi:10.1523/JNEUROSCI.21-14-05222.2001

Krivanek JA, McGaugh JL. 1969. Facilitating effects of pre- and posttrial amphetamine administration on discrimination learning in mice. *Agents Actions* **1:** 36–42. doi:10.1007/BF01977664

Lázaro-Muñoz G, LeDoux JE, Cain CK. 2010. Sidman instrumental avoidance initially depends on lateral and basal amygdala and is constrained by central amygdala-mediated pavlovian processes. *Biol Psychiatry* **67:** 1120–1127. doi:10.1016/j.biopsych.2009.12.002

LeDoux J. 2003. The emotional brain, fear, and the amygdala. *Cell Mol Neurobiol* **23:** 727–738. doi:10.1023/A:1025048802629

LeDoux JE, Moscarello J, Sears R, Campese V. 2017. The birth, death and resurrection of avoidance: a reconceptualization of a troubled paradigm. *Mol Psychiatry* **22:** 24–36. doi:10.1038/mp.2016.166

Leong KC, Packard MG. 2014. Exposure to predator odor influences the relative use of multiple memory systems: role of basolateral amygdala. *Neurobiol Learn Mem* **109:** 56–61. doi:10.1016/j.nlm.2013.11.015

Leong KC, Goodman J, Packard MG. 2015. Post-training re-exposure to fear conditioned stimuli enhances memory consolidation and biases rats toward the use of dorsolateral striatum-dependent response learning. *Behav Brain Res* **291:** 195–200. doi:10.1016/j.bbr.2015.05.022

Leri F, Nahas E, Henderson K, Limebeer CL, Parker LA, White NM. 2013. Effects of post-training heroin and d-amphetamine on consolidation of win-stay learning and fear conditioning. *J Psychopharmacol* **27:** 292–301. doi:10.1177/0269881112472566

Lim AT, Wallace M, Oei TP, Gibson S, Romas N, Pappas W, Clements J, Funder JW. 1982. Foot shock analgesia: lack of correlation with pituitary and plasma immunoreactive-β-endorphin. *Neuroendocrinology* **35:** 236–241. doi:10.1159/000123388

Linden DR. 1969. Attenuation and reestablishment of the CER by discriminated avoidance conditioning in rats. *J Comp Physiol Psychol* **69:** 573–578. doi:10.1037/h0028226

Maier SF, Watkins LR. 1991. Conditioned and unconditioned stress-induced analgesia: stimulus preexposure and stimulus change. *Anim Learn Behav* **19:** 295–304. doi:10.3758/BF03197890

Managò F, Castellano C, Oliverio A, Mele A, De Leonibus E. 2009. Role of dopamine receptors subtypes, D1-like and D2-like, within the nucleus accumbens subregions, core and shell, on memory consolidation in the one-trial inhibitory avoidance task. *Learn Mem* **16:** 46–52. doi:10.1101/lm.1177509

Martinez RCR, Gupta N, Lazaro-Munoz G, Sears RM, Kim S, Moscarello JM, LeDoux JE, Cain CK. 2013. Active vs. reactive threat responding is associated with differential c-Fos expression in specific regions of amygdala and prefrontal cortex. *Learn Mem* **20:** 446–452. doi:10.1101/lm.031047.113

McCarty R, Baucom K. 1982. Physiological responses of rats to footshock stress: effects of social environment. *Behav Neural Biol* **34:** 394–403. doi:10.1016/S0163-1047(82)91798-8
McGaugh JL. 2015. Consolidating memories. *Annu Rev Psychol* **66:** 1–24. doi:10.1146/annurev-psych-010814-014954
McGaugh JL, Roozendaal B. 2002. Role of adrenal stress hormones in forming lasting memories in the brain. *Curr Opin Neurobiol* **12:** 205–210. doi:10.1016/S0959-4388(02)00306-9
McGaugh JL, Roozendaal B. 2009. Drug enhancement of memory consolidation: historical perspective and neurobiological implications. *Psychopharmacology (Berl)* **202:** 3–14. doi:10.1007/s00213-008-1285-6
McNally GP, Akil H. 2001. Effects of contextual or olfactory cues previously paired with morphine withdrawal on behavior and pain sensitivity in the rat. *Psychopharmacology (Berl)* **156:** 381–387. doi:10.1007/s002130100743
McNally GP, Gorrisen MC, Low LF, Westbrook RF. 1999. Effects of contextual cues previously paired with footshock or illness on behavior and pain sensitivity in the rat. *Anim Learn Behav* **27:** 416–425. doi:10.3758/BF03209978
Miller NE. 1948. Studies of fear as an acquirable drive: I. Fear as motivation and fear-reduction as reinforcement in the learning of new responses. *J Exp Psychol* **38:** 89–101. doi:10.1037/h0058455
Mineka S, Gino A. 1980. Dissociation between conditioned emotional response and extended avoidance performance. *Learn Motiv* **11:** 476–502. doi:10.1016/0023-9690(80)90029-6
Nelson AJD, Thur KE, Marsden CA, Cassaday HJ. 2010. Dissociable roles of dopamine within the core and medial shell of the nucleus accumbens in memory for objects and place. *Behav Neurosci* **124:** 789–799. doi:10.1037/a0021114
O'Doherty JP. 2004. Reward representations and reward-related learning in the human brain: insights from neuroimaging. *Curr Opin Neurobiol* **14:** 769–776. doi:10.1016/j.conb.2004.10.016
Ohyama T, Mauk MD. 2001. Latent acquisition of timed responses in cerebellar cortex. *J Neurosci* **21:** 682–690. doi:10.1523/JNEUROSCI.21-02-00682.2001
Oleson EB, Cheer JF. 2013. On the role of subsecond dopamine release in conditioned avoidance. *Front Neurosci* **7:** 1–9. doi:10.3389/fnins.2013.00096
Oleson EB, Gentry RN, Chioma VC, Cheer JF. 2012. Subsecond dopamine release in the nucleus accumbens predicts conditioned punishment and its successful avoidance. *J Neurosci* **32:** 14804–14808. doi:10.1523/JNEUROSCI.3087-12.2012
Plone MA, Emerich DF, Lindner MD. 1996. Individual differences in the hotplate test and effects of habituation on sensitivity to morphine. *Pain* **66:** 265–270. doi:10.1016/0304-3959(96)03048-5
Quirarte GL, Galvez R, Roozendaal B, McGaugh JL. 1998. Norepinephrine release in the amygdala in response to footshock and opioid peptidergic drugs. *Brain Res* **808:** 134–140. doi:10.1016/S0006-8993(98)00795-1
Redondo RL, Morris RGM. 2011. Making memories last: the synaptic tagging and capture hypothesis. *Nat Rev Neurosci* **12:** 17–30. doi:10.1038/nrn2963
Rkieh N, Cloke JM, Gallagher N, Winters BD, Leri F. 2014. Drugs of abuse as memory modulators: a study of cocaine in rats. *Psychopharmacology (Berl)* **231:** 2339–2348. doi:10.1007/s00213-013-3390-4
Roozendaal B, Mcgaugh JL. 2012. Memory modulation. *Behav Neurosci* **125:** 797–824. doi:10.1037/a0026187
Rosellini RA, Abrahamsen GC, Stock HS, Caldarone BJ. 1994. Modulation of hypoalgesia by morphine and number of shock trials: covariation of a measure of context fear and hypoalgesia. *Physiol Behav* **56:** 183–188. doi:10.1016/0031-9384(94)90277-1
Schultz W, Dayan P, Montague PR. 1997. A neural substrate of prediction and reward. *Science* **275:** 1593–1599. doi:10.1126/science.275.5306.1593
Schwabe L, Joëls M, Roozendaal B, Wolf OT, Oitzl MS. 2012. Stress effects on memory: an update and integration. *Neurosci Biobehav Rev* **36:** 1740–1749. doi:10.1016/j.neubiorev.2011.07.002
Setlow B, McGaugh JL. 1998. Sulpiride infused into the nucleus accumbens posttraining impairs memory of spatial water maze training. *Behav Neurosci* **112:** 603–610. doi:10.1037/0735-7044.112.3.603
Starr MD, Mineka S. 1977. Determinants of fear over the course of avoidance learning. *Learn Motiv* **8:** 332–350. doi:10.1016/0023-9690(77)90056-X
Stelly CE, Haug GC, Fonzi KM, Garcia MA, Tritley SC, Magnon AP, Ramos MAP, Wanat MJ. 2019. Pattern of dopamine signaling during aversive events predicts active avoidance learning. *Proc Natl Acad Sci* **116:** 13641–13650. doi:10.1073/pnas.1904249116
Storace A, Daniels S, Zhou Y, Kalisch B, Parker L, Rock E, Limebeer C, Lapointe T, Leri F. 2019. A study of limbic brain derived neurotrophic factor gene expression in male Sprague-Dawley rats trained on a learned helplessness task. *Behav Brain Res* **376:** 112174. doi:10.1016/j.bbr.2019.112174
Wenzel JM, Oleson EB, Gove WN, Cole AB, Gyawali U, Dantrassy HM, Bluett RJ, Dryanovski DI, Stuber GD, Deisseroth K, et al. 2018. Phasic dopamine signals in the nucleus accumbens that cause active avoidance require endocannabinoid mobilization in the midbrain. *Curr Biol* **28:** 1392–1404.e5. doi:10.1016/j.cub.2018.03.037
White NM. 1989. Reward or reinforcement: what's the difference? *Neurosci Biobehav Rev* **13:** 181–186. doi:10.1016/S0149-7634(89)80028-4
White NM. 1996. Addictive drugs as reinforcers: multiple partial actions on memory systems. *Addiction* **91:** 921–950. doi:10.1046/j.1360-0443.1996.9179212.x
White NM, Milner PM. 1992. The psychobiology of reinforcers. *Annu Rev Psychol* **43:** 443–471. doi:10.1146/annurev.ps.43.020192.002303
Williams JL, Baez C, Hladky KJ, Camacho CA. 2005. Effects of a synthetic predator odor (TMT) on freezing, analgesia, stereotypy, and spatial memory. *Psychol Rec* **55:** 3–38. doi:10.1007/BF03395496
Winters BD, Saksida LM, Bussey TJ. 2008. Object recognition memory: neurobiological mechanisms of encoding, consolidation and retrieval. *Neurosci Biobehav Rev* **32:** 1055–1070. doi:10.1016/j.neubiorev.2008.04.004
Wolter M, Huff E, Speigel T, Winters BD, Leri F. 2019. Cocaine, nicotine, and their conditioned contexts enhance consolidation of object memory in rats. *Learn Mem* **26:** 46–55. doi:10.1101/lm.048579.118
Wolter M, Huff AE, Baidoo N, Jardine KH, Pulles Z, Winters BD, Leri F. 2020. Modulation of object memory consolidation by heroin and heroin-conditioned stimuli: role of opioid and noradrenergic systems. *Eur Neuropsychopharmacol* **33:** 146–157. doi:10.1016/j.euroneuro.2020.01.010
Yamasaki M, Takeuchi T. 2017. Locus coeruleus and dopamine-dependent memory consolidation. *Neural Plast* **2017:** 8602690. doi:10.1155/2017/8602690
Zhang Y, Ouyang K, Lipina T V, Wang H, Zhou Q. 2019. Conditioned stimulus presentations alter anxiety level in fear-conditioned mice. *Mol Brain* **12:** 1–12. doi:10.1186/s13041-018-0417-0

Received July 29, 2020; accepted in revised form December 2, 2020.