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 (Fenstra et al. 1999), all reactions that are elicited by footshock itself (Lim et al. 1982; McCarty and Baucum 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 Maak 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.
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; Martínez 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 (Martínez 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 $[F(3,21) = 6.26, P = 0.003]$; panel C: 0.4 mA shock—subgroup by training day $[F(3,21) = 25.13, P = 0.002]$ and training day $[F(3,21) = 10.42, P < 0.001]$; panel D: 0.8 mA shock—subgroup by training day $[F(3,21) = 6.79, P = 0.002]$, subgroup $[F(3,21) = 14.83, P = 0.006]$ 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 =...
The Non-Avoider and Avoider subgroups. ANOVA revealed a significant interaction between group and phase $[F_{(2,81)} = 16.81, P < 0.001]$, 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 2, 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 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 $[F_{(6,243)} = 112.97, P < 0.001]$. 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 $[F_{(6,243)} = 62.54, P < 0.001]$. 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.

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.
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 predictive 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 3](image-url)

**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.

![Figure 4](image-url)

**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.
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 (Fenestra et al. 1999; McNally et al. 1999; Dombrowski et al. 2013; Diaz-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 Leticia). The heated surface (22 x 22 cm) was maintained at 50°C ± 2.1°C (Plobe 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.

**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 a learned helplessness scenario: (If novel side is left) (left arm exploration − 1 min novel object 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 VF, Rezende FS, de Jesus Seno MD, de Carvalho MC, de Oliveira CC, dos Santos LTC, de Castro MC, Kuroki MA, Teixeira MJ, et al. 2020. Dopamine modules individual differences in avoidance behavior: a pharmacological, immunohistochemical, neurochemical and volumetric investigation. Neurobiol Stress 12:100219. doi:10.1016/j.ysts.2020.10.019
Baldin N, Walter M, Holohan 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 25:183–202. doi:10.1111/anab.12905
Balsam PD, Drew MR, Yang C. 2002. Timing at the start of associative learning. Learn Motiv 33:141–155. doi:10.1016/S0024-379X(01)00056-4
Balsam PD, Drew MR, Gallistel C. 2010. Time and associative learning. Comp Cogn Behav Rev 8:1–22. doi:10.3819/ccbr.2010.50001
Boles RC, Fancella MS. 1980. A perceptual-defensive-recoverative model of fear and pain. Behav Brain Sci 3:291–310. doi:10.1017/S0140525X0000491X
Burgess PE, Vinay 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:10.1101/2017.06.16610
Conti LH, Maciver CR, Ferkany JW, Abreu ME. 1990. Footshock-induced freezing behavior in rats as a model for assessing anxieties. Psychopharmacology (Berl) 102:492–497. doi:10.1007/BF02247130
Coofer GD, Ursin H. 1973. Plasma-cortisosterone levels during active-avoidance learning in rats. J Comp Physiol Psychol 82:170–174. doi:10.1037/h0037790
de Lima MNM, Presti-Torres J, Dornelles A, Siciliani Scalco F, Roesler R, Choi JS, Cain CK, Ledoux JE. 2010. The role of amygdala nuclei in the recognition. 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.

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.ysts.2016.02.004
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. 2003. Memory enhancement produced by post-training exposure to sucrose-conditioned cues. F1000Res 2:22. doi:10.12688/f1000research.22.v1
Holland PC. 2000. Trial and intentional 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 CY, Yu L, Chen HI, Lee HL, Kao YM, Jen CJ. 2012. Blood pressure variations real-time reflect the conditioned fear learning and memory. PLoS One 7:e2855. doi:10.1371/journal.pone.0032855
Huston JP, Mondadori C, Waser PG. 1974. Facilitation of learning by reward of post-trial memory processes. Expierientia 30:1038–1040. doi:10.1007/BF01938996
Huston JP, Mueller CC, Mondadori C. 1977. Memory facilitation by posttrial hysterical simulation and other reinforcers: a central theory of reinforcement. Biobehav Rev 1:143–150. doi:10.1016/0375-755X(77)90014-1
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/h0027966
Keller DP, Hunt RC, Ressler KJ, Marvar PJ. 2015. The physiology of fear: reconsolidation of the role of the central amygdala in fear learning. Physiology 38:389–401. 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
Krivane JK, McLaugh JG. 1969. Facilitating effects of post- and pretrial amphetamine administration on discrimination learning in mice. Agents Actions 1:36–42. doi:10.1007/BF01979764
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, Moscarrello J, Sears R, Campea V. 2017. The birth, death and resurgence of avoidance: a re-conceptualization 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 the central amygdala. Neurobiol Learn Mem 109:56–61. doi:10.1016/0149-7634(77)90079-5
Leong KC, Packard MG. 2014. Post-training re-exposure to fear conditioned stimuli enhances memory consolidation and biases rats toward the use of dorsolateral striatal-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 Pharmacol Psychopharmacol 27:292–301. doi:10.1177/1040358012475266
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 β-endorphin. Am J Physiol 243:R16–25. doi:10.1152/ajplegacy.1982.243.1.R16
Maier SF, Watkins LR. 1991. Conditioned and unconditioned stress-induced analgesia: stimulus preexposure and stimulus change. Anim Learn Behav 19:299–304. doi:10.3758/BF03197890
Manaro 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:10.1101/1117590
Martinez BCR, Gupta N, Lázaro-Muñoz G, Sears RM, Kim S, Moscarrello JM, LeDoux JE, Cain CK. 2013. Active vs. reactive threat responding is associated with differential c-fos expression in specific regions of the amygdala and prefrontal cortex. Learn Mem 20:446–452. doi:10.1101/031047.113
