

Temporal-Difference Prediction Errors and Pavlovian Fear Conditioning: Role of NMDA and Opioid Receptors

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Three experiments studied temporal-difference (TD) prediction errors during Pavlovian fear conditioning. In Stage I, rats received conditioned stimulus A (CSA) paired with shock. In Stage II, they received pairings of CSA and CSB with shock that blocked learning to CSB. In Stage III, a serial overlapping compound, CSB $\rightarrow$ CSA, was followed by shock. The change in intratrial durations supported fear learning to CSB but reduced fear of CSA, revealing the operation of TD prediction errors. *N*-methyl-D-aspartate (NMDA) receptor antagonism prior to Stage III prevented learning, whereas opioid receptor antagonism selectively affected predictive learning. These findings support a role for TD prediction errors in fear conditioning. They suggest that NMDA receptors contribute to fear learning by acting on the product of predictive error, whereas opioid receptors contribute to predictive error.

Keywords: fear, temporal difference, prediction, opioid, NMDA

Learning about the predictive relations existing between events in the world is central to adaptive behavior. Predictive learning is often formally expressed by error-correction learning rules (Dickinson, 1980; Rescorla, 1988). In Pavlovian fear conditioning, these rules state that fear accrues to a conditioned stimulus (CS), which signals an aversive unconditioned stimulus (US) when the actual outcome of the conditioning trial exceeds the predicted outcome (i.e., when there is a positive prediction error). Likewise, fear of a CS is typically reduced when the actual outcome is less than the predicted outcome (i.e., when there is a negative prediction error). The blocking paradigm discovered by Kamin (1968, 1969) was central to the development of error-correction rules. Kamin (1968, 1969) subjected rats to conditioned stimulus A (CSA) paired with shock. In Stage II, rats received a compound stimulus of CSA and conditioned stimulus B (CSB) paired with shock. Fear learning to CSB during Stage II failed despite numerous AB–shock pairings. Blocking of CSB occurred because predictive error during Stage II was low: Subjects could predict the occurrence of shock from CSA, so learning about CSB was impaired. Kamin (1968, 1969) also demonstrated that blocking could be abolished by an increase in reinforcer magnitude from Stage I to Stage II (unblocking).

The Rescorla–Wagner model (Rescorla & Wagner, 1972) is among the most influential of the error-correction learning rules. According to this model, associative strength accrues to a CS on a conditioning trial (V) as a function of the discrepancy between the asymptotic associative strength supported by the US (λ) and the

summed associative strengths of all CSs present on that trial (ΣV). Learning is driven by this discrepancy between actual and predicted outcomes ($\lambda - \Sigma V$), or in other words, the prediction error. Positive prediction errors (i.e., outcome $>$ prediction) increase associative strength, whereas negative prediction errors (outcome $<$ prediction) decrease associative strength. The application of this model to blocking is straightforward. During Stage I, the pretrained CSA captures the available associative strength. During Stage II, training predictive error is low (outcome = prediction) and learning about the added CSB is blocked. Increasing the number, magnitude, or duration of the US during Stage II causes unblocking because these manipulations increase the discrepancy between the actual and expected outcomes of the trial.

Predictive error is computed in the Rescorla–Wagner model on a trial-by-trial basis. The precise temporal relations among stimuli during a trial are largely unimportant. The need to account for such relations can be illustrated by the temporal primacy effect. Egger and Miller (1962) trained rats in appetitive conditioning so that they received an overlapping serial compound training of CSB $\rightarrow$ CSA $\rightarrow$ food. Subsequent tests of the conditioned reinforcing properties of CSB and CSA indicated more learning about CSB than CSA. In other words, the temporal primacy of CSB had reduced learning to CSA. The temporal-difference (TD) model (Sutton, 1988; see also Sutton & Barto, 1981; 1990) is the most influential model of the role of temporal relations in associative learning. The TD model is a real-time instantiation of the Rescorla–Wagner or delta learning rule. The key psychological assumption is that earlier predictors (i.e., further away from the US; CSB in Egger & Miller’s experiment) are more informative and so are learned at the expense of later predictors (i.e., closer to the US; CSA in Egger & Miller’s experiment). The key computational assumption is that associative change occurs continuously during a trial. This change is the product of a momentary predictive error computed as the discrepancy between the current outcome (the sum of currently eligible associative strengths of all present CSs and/or USs; equivalent to λ in the Rescorla–Wagner

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model) and the outcome predicted to occur from the preceding periods of time (a weighted average of recent associative strengths; equivalent to ΣV in the Rescorla–Wagner model). In this way, the TD learning rule effectively identifies and supports learning about the earliest, reliable, nonredundant predictor of a US. It is precisely this identification of earlier and reliable predictors of danger that is essential to guiding defensive behavior. As Sutton and Barto (1981) noted, a predictor cotemporaneous with the dangerous event has little value in guiding defensive behavior.

An important and unique prediction of the TD model concerns an experimental design that combines the phenomena of blocking and temporal primacy (see Figure 1; three-stage blocking–unblocking design). In a standard blocking design, CSA is paired with shock in Stage I. In Stage II, a compound of CSA and CSB is followed by shock. The Rescorla–Wagner and TD models are identical at this point: CSA blocks fear learning to CSB. However, if in Stage III, CSB is arranged to precede and overlap with CSA and the compound coterminates in shock (the temporal primacy effect), the TD model predicts that CSA will lose and CSB will gain associative strength. In other words, the change in temporal relations results in increased fear (unblocking) of CSB and decreased fear of CSA. There are two TD prediction errors during each trial of Stage III. The first is a positive prediction error (outcome > prediction), generated by the change from CSB (initially low associative strength) to CSA (positive associative strength), that conditions fear to CSB. The second is a negative prediction error (outcome < prediction) produced by the US. The summed associative strengths of CSA and CSB initially exceed the

expected outcome because CSB has gained associative strength. This negative prediction error reduces fear of both CSA and CSB. Such bi-directional associative change continues until predictions from CSA and CSB are equal and their sum equals the actual outcome.

The first aim of the experiments reported here was to study whether Pavlovian fear conditioning could be used to reveal the operation of TD prediction errors. These have previously been studied for conditioning of the nictitating membrane in rabbits (Kehoe, Schreurs, & Graham, 1987) and Pavlovian appetitive conditioning (Jennings & Kirkpatrick, 2006) but to the best of our knowledge have not been explicitly studied in Pavlovian fear conditioning. It is important to study such processes in Pavlovian fear conditioning because fear conditioning remains a leading model preparation for study of the neural mechanisms of learning.

The second aim of these experiments was to characterize the roles of *N*-methyl-D-aspartate (NMDA) and opioid receptors in TD prediction errors. The neural mechanisms underlying predictive fear learning remain poorly understood (see McNally & Westbrook, 2006, for a review). The NMDA receptor is central to fear conditioning. A wealth of data from a variety of species has identified NMDA receptors, especially those in the basolateral amygdala, as critical for fear learning and for the synaptic plasticity associated with this learning (see Maren & Quirk, 2004, for a review). However, the specific role of NMDA receptors in predictive fear learning is unknown because there have been few investigations of the effects of NMDA receptor antagonists on fear learning in experimental preparations that selectively reveal the operation of predictive error. Our recent research has implicated the endogenous opioids, acting at the μ -opioid receptor in the midbrain periaqueductal gray (PAG), as important components of the neural mechanisms for predictive error during fear learning. For example, systemic administrations of an opioid receptor antagonist prevent the blocking as well as overexpectation of Pavlovian fear learning (McNally, Pigg, & Weidemann, 2004), and similar effects are observed after discrete microinjections of μ -opioid receptor selective antagonists into the ventrolateral quadrant of the midbrain PAG (Cole & McNally, 2007; McNally & Cole, 2006). However, the specific role of opioid receptors in regulating TD prediction errors remains unknown.

Experiment 1

The aim of Experiment 1 was to use the three-stage blocking–unblocking design to study the role of TD prediction errors in Pavlovian fear conditioning. The experimental design is shown in Table 1. Previous research investigating TD prediction errors has used a two-stage design, where Stage I directly precedes the Stage III shown in Figure 1 (e.g., Jennings & Kirkpatrick, 2006; Kehoe et al., 1987). Such a design is useful because even in the absence of prior compound training (i.e., Stage II), TD theory predicts that Stage III training will result in simultaneous gains to B and losses to A. However, from the perspective of blocking and unblocking, such a design is less interesting because it studies *de novo* acquisition of fear of a novel CSB, rather than the unblocking of fear of a blocked CSB. We adopted the three-stage design in the present experiments because it enabled us to study learning about CSs that had been blocked (CSB) or that had not been blocked (CSA) during Stage II. It therefore provides important insights into the

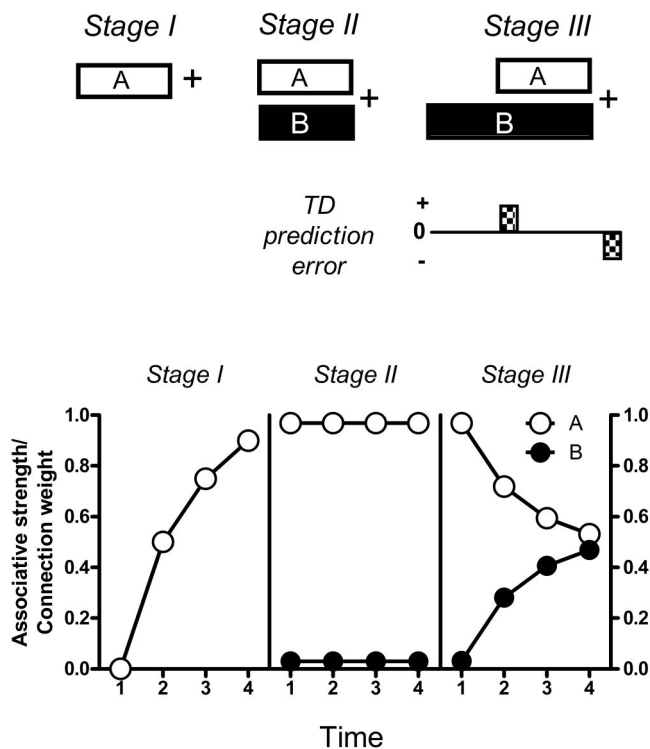


Figure 1. Representation of the three-stage blocking–unblocking design, temporal-difference (TD) prediction errors, and possible changes in associative strength/connection weights.

Table 1
Experimental Design

Experiment and group	Stage and stimulus		
	Stage I	Stage II	Stage III
Experiment 1			
TD	A+	A, B, AB+	A, B, B → AB+
Long block	A+		A, B, AB+ (60 s)
Control		A, B, AB+	A, B, AB+
Experiment 2			
MK-801	A+	A, B, AB+	A, B, MK-801: B → AB+
Saline	A+	A, B, AB+	A, B, saline: B → AB+
Experiment 3			
Naloxone	A+	A, B, AB+	A, B, naloxone: B → AB+
Saline	A+	A, B, AB+	A, B, saline: B → AB+

Note. A was a 4-Hz flashing light conditioned stimulus (CS). B was an 82-dB 10-Hz clicker CS. All presentations were 30 s unless otherwise noted. + denotes a 1-s 0.5-mA foot shock unconditioned stimulus. The *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 was injected intraperitoneally (0.1 mg/kg). The opioid receptor antagonist naloxone was injected subcutaneously (2.5 mg/kg). TD = temporal difference.

nature and consequences of the blocking (Stage II) and unblocking (Stage III) of fear learning.

The TD group were trained to fear a 30-s CSA via pairing with foot shock in Stage I. CSA was then presented in compound with a 30-s CSB and coterminated in foot shock in Stage II. In Stage III, group TD received an overlapping serial compound: CSB was presented for 60 s, with CSA being presented in compound with CSB during the last 30 s. This compound CS then coterminated in shock. The control group received no Stage I training and simultaneous reinforced 30-s compound presentations of CSA and CSB during both Stage II and Stage III. For group TD, during Stage III CSA generated a positive prediction error conditioning fear of CSB whereas the US generated a negative prediction, causing loss of fear of CSA and CSB. Unblocking is caused by establishing CSB as a predictor of CSA and enabling it to profit from the prediction error generated by CSA. However, this confounds changes in predictive relations with changes in stimulus duration that may affect blocking in other ways (e.g., Barnet, Grahame & Miller, 1993; Schreurs & Westbrook, 1982; but see Gaioni, 1982). Therefore, we included an additional control group, the long block group. Group long block received initial training with a 30-s CSA and was rested during Stage II. In Stage III, group long block received simultaneous reinforced compound presentations of CSA and CSB, with a duration of 60 s for both stimuli. The TD model predicts no significant influence of these changes in temporal duration on either CSA or CSB because neither CS is eligible for associative change until sometime after its onset. Comparisons between group TD and group long block therefore confirm that any changes to CSB and CSA during Stage III are the product of the predictive relation between CSB and CSA, not just the temporal duration of CSB. Group long block could be limited in the sense that omitting Stage II training may cause it to overestimate the unblocking observed to CSB in group TD during Stage III. However, the key prediction of the model in group TD concerns both CSA (which should show a decrease in fear during Stage III) and CSB (which should show an increase in fear). Group long block is a useful control group for the present purposes, provided

that groups TD and long block do not differ from each other in fear of CSA or CSB at the start but do differ at the end of Stage III training.

Fear of CSA and CSB was assessed during Stage III using single nonreinforced presentations of each stimulus. These presentations were intended to provide a measure of performance to each CS unconfounded by the presence of the shock US and, in later experiments, the presence of the receptor antagonists. They provide the primary data of interest during test. These presentations were introduced at the beginning of Stage II. This was done to reduce any generalization decrement that might have otherwise occurred between Stage II and Stage III (Neely & Wagner, 1974) and thereby confounded interpretation of the results. Superficially at least, inclusion of these CS alone trials causes the procedure during Stages II and III to resemble a positive patterning task (e.g., A–, B–, AB+). The relatively small amount of training makes the possibility of the subjects learning a patterning discrimination unlikely. Regardless, if subjects do acquire such discrimination, then test data should show reduced fear of CSA and CSB during Stage III. By contrast, TD theory predicts reductions in fear of CSA and increases in fear of CSB during Stage III.

Method

Subjects

The subjects were 24 experimentally naive, adult, male Wistar rats (220–280g) obtained from a commercial supplier (Gore Hill Research Laboratories, Sydney, Australia). Upon arrival, rats were housed in groups of 8 in plastic cages (67 cm [length] × 30 cm [width] × 22 cm [height]) in a colony room maintained at 20–24°C on a 12-hr light–dark cycle (lights on at 7 a.m.). Food and water were freely available. The rats were handled (1–2 min per rat per day) for 3 days prior to conditioning to habituate them to the experimenter. The procedures used in this and following experiments were conducted in accordance with the New South Wales Government Animal Research Regulation of 1995 and were approved by the Animal Care and Ethics Committee of the University of New South Wales.

Behavioral Apparatus

Conditioning and testing were conducted in a set of four identical chambers (24 cm [length] × 30 cm [width] × 21 cm [height]). The top and rear walls of these chambers as well as the front hinged door were constructed of clear Perspex, and the end walls were made of stainless steel. The floor in each chamber consisted of stainless steel rods 4 mm in diameter spaced 15 mm apart (center to center). Each chamber stood 2 cm above a tray of paper pellet bedding (Fibre-cycle, Mudgeeraba, Australia). The chambers were cleaned with water and the bedding underneath the chambers was changed between rats. These four chambers were located individually within sound attenuating boxes that were painted white. The boxes were constantly illuminated by a single red LED such that levels of illumination within the conditioning chambers were 15 cd/m². Ventilation fans provided a constant background noise (67 dB).

CSA was a flashing (4-Hz) presentation of a white fluorescent light, producing an illumination level of 75 cd/m² within the

chamber. The light was mounted on the ceiling of each box, immediately above the conditioning chamber. CSB was an 82-dB (A-scale), 10-Hz clicker delivered through speakers mounted in the ceiling of each box. The foot shock US was a 1-s, 0.5 mA unscrambled AC 50-Hz shock from a constant current generator that was delivered to the floor of each chamber. The current available to each floor could be adjusted with an in-line milliamperemeter. Digital video cameras were mounted on the rear wall of each box and connected to a digital multiplexer in an adjacent room that, in turn, was connected to a DVD recorder. The stimuli used for conditioning were controlled by computer (LabView, National Instruments, Austin, TX).

Procedure

The experiment had four stages: pre-exposure, Stage I, Stage II, and Stage III.

Pre-exposure. Prior to conditioning, rats received 2 days of pre-exposure. Each day, rats were transported to the laboratory and placed into the conditioning chambers for 15 min. During this period, there were four presentations of each CS in a counterbalanced order, with 30-s intertrial intervals (ITIs) between CS presentations. Rats were pre-exposed to the stimuli to encourage discrimination between them (Mackintosh & Bennett, 1998).

Stage I. Groups TD and long block received Stage I training on Days 1 and 2. Each day, rats were transported to the laboratory and placed into the conditioning chambers for 21 min 10 s, where they received four presentations of CSA coterminating with foot shock. The ITIs for these sessions were random, ranging from 60 to 360 s, with a mean of 211 s. Four to six hours after each conditioning session, all rats received 10-min nonreinforced exposures to the conditioning chambers to reduce levels of contextual fear. Rats in group control remained in their home cages for the duration of Stage I.

Stage II. Stage II training took place across Days 3 and 4. Each day, rats in groups TD and control were transported to the laboratory and placed into the conditioning chambers. After an adaptation period of 180 s, rats received a single 30-s nonreinforced presentation of CSA and CSB with a 30-s ITI. The order of presentations was counterbalanced across days. After another 180-s period, rats received a single 30-s reinforced AB compound presentation. Four to six hours after each conditioning session, all rats received 10-min nonreinforced exposures to the conditioning chambers to reduce levels of contextual fear. Rats in group long block remained in their home cages for the duration of Stage II.

Stage III. Stage III training took place on Days 5–8. On Days 5–7, all rats were transported to the laboratory and placed into the conditioning chambers. After an adaptation period of 180 s, rats received a single 30-s nonreinforced test presentation of CSA and CSB with a 30-s ITI. The order of test presentations (CSA or CSB) was counterbalanced across days. For groups control and long block these test presentations were followed by a single reinforced AB compound presentation of 30 s and 60 s respectively, whereas for group TD they were followed by an overlapping serial compound of 30-s CSB and 30-s CSB + CSA coterminating in shock. Four to six hours after each session, all rats received 10-min nonreinforced exposures to the conditioning chambers to reduce levels of contextual fear. On Day 8, all rats received the 30-s test presentations of A and B.

Statistics

Performance during conditioning and test was recorded. The rats were subsequently scored every 2 s as either freezing (defined as the absence of all movement other than that required for breathing) or not freezing. The number of observations scored as freezing were summed and converted to a percentage. A random sample of the data was scored by a second observer, who was unaware of group allocation. The interrater reliability, that is, the correlation between the percentages of observations each rat was scored as freezing by each observer, exceeded 0.85. The data were analyzed by means of a planned orthogonal contrast testing procedure, and the Type I error rate was controlled at 0.05 for each contrast tested.

Results and Discussion

The top panel of Figure 2 shows mean ($\pm$ SEM) levels of freezing during the first presentation of CSA on each day of Stage I training and the presentation of the AB compound on each day of Stage II training. There was no overall difference between groups TD and long block in fear of CSA during Stage I, $F(1, 14) < 1$, $p > .05$. There was a significant increase in fear of CSA across the

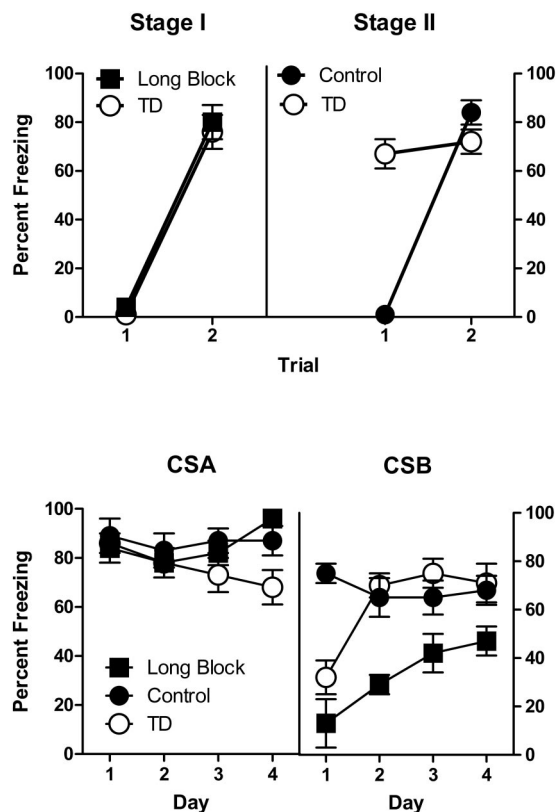


Figure 2. Mean levels of freezing in Stage I and Stage II of Experiment 1 (top panel). Mean levels of freezing during the test presentations of conditioned stimulus A (CSA) and conditioned stimulus B (CSB) across days of Stage III (bottom panel). During Stage III, freezing to CSA remained high for groups control and long block but decreased for group temporal difference (TD). Fear of CSB remained high for group control and low for group long block, but increased across Stage III for group TD. Error bars represent standard error of the mean.

course of Stage I training, $F(1, 14) = 171.3, p < .05$, but this increase was not significantly different for groups TD and long block, $F(1, 14) < 1, p > .05$. During Stage II, there was overall significantly more fear of the AB compound among group TD than group control, $F(1, 14) = 28.2, p < .05$, because group TD had received Stage I training. It is important that there was a significant increase in fear of the AB compound across Stage II, $F(1, 14) = 93.2, p < .05$, which was significantly greater for group control than group TD, $F(1, 14) = 73.3, p < .05$.

The data of primary interest are those from test presentations of CSA and CSB during Stage III. These are shown in the bottom panels of Figure 2. Inspection of these panels indicates that the key predictions of TD theory were supported. Overall, fear of CSB increased across Stage III trials, and simultaneously, fear of CSA decreased in group TD. Test data were analyzed by means of planned analyses of variance preserving the 3 (groups TD, control, long block) $\times$ 2 (CSA, CSB) $\times$ 4 (Days 1–4) factorial design. Overall, there was no significant difference in levels of freezing between group TD and group control, $F(1, 21) = 1.8, p > .05$. There was also no overall significant difference in levels of freezing between group TD and group long block, $F(1, 21) = 2.9, p > .05$. There was overall significantly more freezing to CSA than to CSB, $F(1, 21) = 155.7, p < .05$, which did not interact with the difference between group TD and group control, $F(1, 21) < 1, p > .05$, but did interact significantly with the difference between group TD and group long block, $F(1, 21) = 45.5, p < .05$. This indicates that differences in levels of fear of CSA versus CSB were significantly greater in group long block than in group TD. Across days of Stage III training, there was an overall significant linear change in freezing, $F(1, 21) = 8.4, p < .05$, which did not interact with any differences between groups. There was a significant two-way interaction between CSA versus CSB and the linear trend across days, $F(1, 21) = 18.0, p < .05$. More important, there were three-way interactions between this differential change to CSA and CSB across trials and the differences between group TD and group control, $F(1, 21) = 19.4, p < .05$, as well as the differences between group TD and group long block, $F(1, 21) = 5.5, p < .05$. This confirms that the change in fear of CSA and CSB across Stage III training was significantly greater for group TD than for both group control and group long block.

Analysis of simple effects confirmed these interpretations. For CSA, on the first Stage III test trial there was no difference between group TD versus groups control and long block, $F(1, 21) < 1, p > .05$, nor was there a difference between these latter two groups, $F(1, 21) < 1, p > .05$. This confirms that all groups showed equivalent levels of fear of CSA at the start of Stage III training. By contrast, on the last Stage III test trial, there was significantly less fear of CSA among group TD versus groups control and long block, $F(1, 21) = 10.9, p < .05$, but there was no difference between these latter two groups, $F(1, 21) < 1, p > .05$.

For CSB, on the first Stage III test trial there was a significant difference between group control versus groups TD and long block, $F(1, 21) = 30.6, p < .05$, but there was no significant difference between these latter two groups, $F(1, 21) = 2.8, p > .05$. This confirms that Stage II training produced blocking of fear of CSB in group TD: Levels of fear were significantly lower than group control, which did not receive training in Stage I. Moreover, levels of fear of CSB in group TD were not significantly different from a group that had not yet received B–shock pairings (group

long block). By contrast, at the end of Stage II training this pattern of differences had reversed. There was significantly greater fear of CSB among groups TD and control as compared with group long block, $F(1, 21) = 7.3, p < .05$, whereas group TD and group control did not significantly differ from each other, $F(1, 21) < 1, p > .05$.

The levels of freezing observed in the first 180 s of context exposure in each session across the three stages of the experiment are shown in Figure 3. Inspection of the figure indicates that pre-CS freezing was low. The analysis showed no significant difference between group TD and group long block in pre-CS freezing during Stage I, $F(1, 14) < 1, p > .05$. There was a significant difference between group TD and group control in pre-CS freezing during Stage II, $F(1, 14) = 8.5, p < .05$, reflecting the influence of Stage I training in group TD. Finally, there were no significant differences between groups in pre-CS freezing during Stage III, $F_s(1, 21) < 1, p > .05$.

The results of this experiment have confirmed the key predictions of the TD model. Pretraining of CSA in Stage I conditioned fear of CSA, which blocked fear learning of the simultaneously presented CSB in Stage II. However, arranging an overlapping serial compound of CSB $\rightarrow$ CSA during Stage III produced rapid unblocking of fear of CSB and simultaneously reduced fear of CSA. Unblocking of fear of CSB was not simply caused by the increase in Stage III CSB duration because it was observed relative to group long block, which was subject to an increase in the durations of both CSA and CSB. This within-subjects bi-directional associative change is a unique prediction of the TD model. It is worth noting that the decrease in fear of CSA in group TD was modest (approximately 20%). This is consistent with that observed in both Pavlovian appetitive conditioning (Jennings & Kirkpatrick, 2006) and rabbit nictitating membrane conditioning (Kehoe et al., 1987). This partial fear loss is relevant because the TD model (Sutton, 1988) predicts partial loss of fear of CSA during Stage III, whereas Sutton and Barto's (1981) original model predicted complete loss of fear of CSA (see Sutton & Barto, 1990, for a review).

Experiment 2

The results of Experiment 1 confirm the utility of the three-stage blocking–unblocking design to investigate TD prediction errors

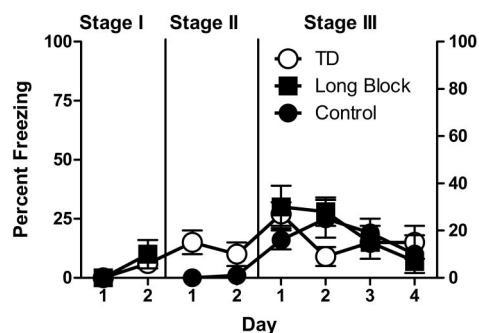


Figure 3. Mean levels of pre-conditioned stimulus across each stage of Experiment 1. TD = temporal difference. Error bars represent standard error of the mean.

during fear conditioning. In Experiment 2, we investigated the effects of an NMDA receptor antagonist on this predictive learning. NMDA receptor antagonists impair both the acquisition (e.g., Kim, DeCola, Landeira-Fernandez, & Fanselow, 1991; Miserendino, Sananes, Melia, & Davis, 1990) and extinction (e.g., Baker & Azorlosa, 1996; Falls, Miserendino, & Davis, 1992; Sotres-Bayon, Bush, & Ledoux, in press) of fear. However, the role of NMDA receptors in the predictive error that cause such learning remains unknown. For this experiment, the procedure for Group TD from Experiment 1 was used, and groups control and long block were omitted. These control groups confirmed the specificity of learning in group TD in Experiment 1. They were omitted here because the bi-directional change in learning that occurred to CSA and CSB in group TD cannot readily be explained by other associative or nonassociative mechanisms. The design is shown in Table 1. In Stage I of this experiment, rats were trained to fear CSA via pairings with foot shock. In Stage II, CSA was presented in compound with CSB and coterminated in foot shock. During Stage III, all rats received serial compound training. Stage III trials were preceded by systemic injections of the NMDA receptor antagonist MK-801 (group MK-801) or by injection of saline (group saline).

Method

Subjects were 24 experimentally naive, adult, male Wistar rats (220–280 g) obtained from the same source and maintained under the same conditions as in Experiment 1. The apparatus was identical to that described for Experiment 1.

The procedures for pre-exposure, Stage I, and Stage II were identical to those of group TD in Experiment 1. Stage III occurred on Days 5–8. On Days 5–7, all rats were transported to the laboratory and placed into the conditioning chambers. After receiving the test presentations of CSA and CSB, rats were removed from the conditioning chambers and injected intraperitoneally with 0.1 mg/kg MK-801 (Tocris-Cookson, Bristol, England) dissolved in 0.9% (wt/vol) pyrogen-free saline (MK-801; $n = 12$) or with 1 ml/kg 0.9% (wt/vol) pyrogen-free saline (saline; $n = 12$) and then returned to their home cage for 30 min. All rats were then returned to the conditioning chambers where, after another 180-s adaptation period, they received serial compound training in the manner described for group TD in Experiment 1. Four to six hours after each conditioning session, all rats received 10-min nonreinforced exposures to the conditioning chambers to reduce levels of contextual fear. On Day 8, all rats received the initial session of test presentations of CSA and CSB.

Results and Discussion

The top panel of Figure 4 shows mean ($\pm$ SEM) levels of freezing during the first presentation of CSA on each day of Stage I training and during the first presentation of the AB compound during Stage II training. There was no overall difference between groups saline and MK-801 during Stage I, $F(1, 22) < 1$, $p > .05$. There was a significant increase in fear of CSA across the course of Stage I training, $F(1, 22) = 2317.8$, $p < .05$, but this increase was not significantly different for group saline versus group MK-801, $F(1, 22) < 1$, $p > .05$. During Stage II, there was no overall significant difference between groups saline and MK-801 in freez-

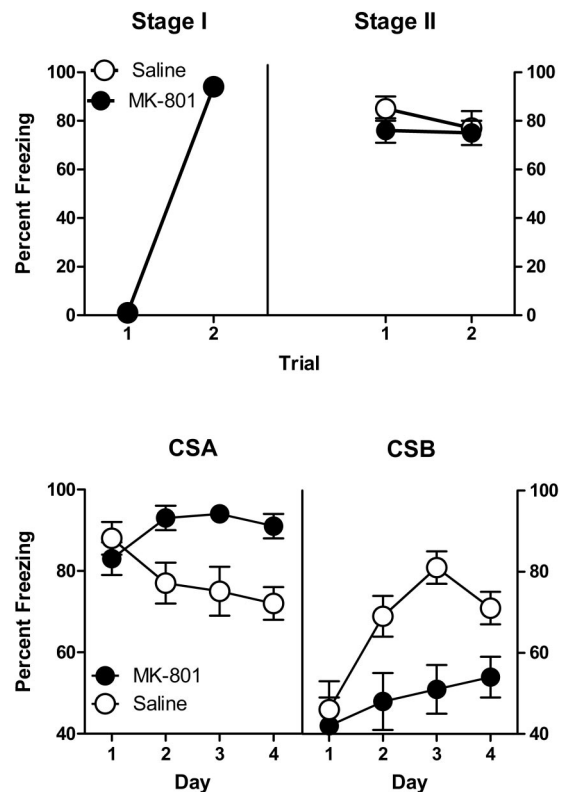


Figure 4. Mean levels of freezing in Stage I and Stage II of Experiment 2 (top panel). Mean levels of freezing during the test presentations of conditioned stimulus A (CSA) and conditioned stimulus B (CSB) across days of Stage III (bottom panel). During Stage III, freezing to CSA remained high for group MK-801 but decreased for group saline. Fear of CSB increased for group saline but remained constant for group MK-801. Error bars represent standard error of the mean.

ing to the AB compound, $F(1, 22) < 1$, $p > .05$, no significant change in fear of the AB compound across Stage II, $F(1, 22) = 1.6$, $p > .05$, and no differential change in freezing to the AB compound between groups, $F(1, 22) < 1$, $p > .05$.

The bottom panel of Figure 4 shows mean ($\pm$ SEM) levels of freezing during test presentations of CSA and CSB across days of Stage III. Inspection of these panels suggests fear of CSA decreased for group saline but not for group MK-801, whereas fear of CSB increased to a greater extent in group saline than in group MK-801. The analysis confirmed these observations. There was no overall significant main effect of group (MK-801 vs. saline), $F(1, 22) < 1$, $p > .05$. There was a significant main effect for stimulus type (CSA vs. CSB), $F(1, 22) = 81.8$, $p < .05$, so overall there was more fear of A than B. There was also a significant main effect for day, $F(1, 22) = 6.3$, $p < .05$, so overall levels of fear increased across days of Stage III. The interaction of group and stimulus was significant, $F(1, 22) = 27.3$, $p < .05$, so the difference in freezing between CSA and CSB was significantly greater for group MK-801 than for group saline. There was no interaction of group and day, $F(1, 22) < 1$, $p > .05$. The interaction of stimulus (CSA vs. CSB) and day was significant, $F(1, 22) = 22.7$, $p < .05$, so there was a greater change in fear of CSB than CSA across days. It is important that the three-way interaction of group, stimulus, and

day was significant, $F(1, 22) = 14.6, p < .05$. This interaction shows that MK-801 prevented the increase in fear of CSB and the decrease in fear of CSA.

The levels of freezing observed in the first 180 s of context exposure in each session across the three stages of the experiment are shown in Figure 5. Inspection of the figure indicates that pre-CS freezing was low. There were no significant differences between groups in levels of pre-CS freezing across the course of the experiment, $F(1, 14) < 1, p > .05$.

Experiment 3

This experiment studied the role of the endogenous opioids and their receptors in TD predictive learning. We have previously shown that systemic administrations of an opioid receptor antagonist prevent blocking as well as overexpectation of fear learning (McNally et al., 2004), and similar effects have been observed after discrete microinjections of μ -opioid receptor selective antagonists into the ventrolateral quadrant of the midbrain PAG (Cole & McNally, 2007; McNally & Cole, 2006). However, the specific role of opioid receptors in regulating TD prediction errors remains unknown. In Stage I, rats were trained to fear CSA via pairings with foot shock. In Stage II, CSA was then presented in compound with CSB and coterminated in foot shock. During Stage III, all rats received serial compound training. Stage III trials were preceded by systemic injections of the opioid receptor antagonist naloxone (group naloxone) or saline (group saline).

Method

Subjects were 32 experimentally naive, adult, male Wistar rats (220–280g) obtained from the same source and maintained under the same conditions as in Experiment 1. The apparatus was identical to that described for Experiment 1.

The procedures for pre-exposure, Stage I, and Stage II were identical to those of group TD in Experiment 1. The procedure for Stage III was similar to Experiment 2 with two exceptions. First, after removal from the conditioning chambers, rats were injected subcutaneously in the dorsal neck region with 2.5 mg/kg naloxone hydrochloride (Tocris-Cookson, Bristol, England) dissolved in 0.9% (wt/vol) pyrogen-free saline (naloxone; $n = 16$) or with 1 ml/kg 0.9% (wt/vol) pyrogen-free saline (saline; $n = 16$) and then returned to their home cage. Second, rats remained in their home

cages for 10 min prior to being placed back in the conditioning chamber for Stage III training.

Results and Discussion

The top panel of Figure 6 shows mean ($\pm$ SEM) levels of freezing during the first presentation of CSA on each day of Stage I training and during the first presentation of the AB compound during Stage II training. There was no overall difference between groups saline and naloxone during Stage I, $F(1, 30) < 1.0, p > .05$. There was a significant increase in fear of CSA across the course of Stage I training, $F(1, 30) = 1849.8, p < .05$, and this increase was not significantly different for group saline versus group naloxone, $F(1, 30) = 1.0, p > .05$. During Stage II, there was no overall significant difference between groups saline and naloxone in freezing to the AB compound, $F(1, 30) < 1, p > .05$, and no significant change in fear of the AB compound across Stage II, $F(1, 30) = 2.0, p > .05$. However, the interaction between groups and days reached significance, $F(1, 30) = 4.6, p < .05$. This was unexpected but shows simply that fear of the AB compound decreased slightly across Stage II in group naloxone and increased slightly in group saline. However, analysis of simple effects showed no significant difference in levels of freezing between groups during the first AB presentation on either Day 1, $F(1, 30) = 3.4, p > .05$, or Day 2, $F(1, 30) < 1, p > .05$.

The bottom panels of Figure 6 show mean ($\pm$ SEM) levels of freezing during test presentations of CSA and CSB across days of Stage III. Inspection of these panels suggests fear of CSA decreased for group saline but not for group naloxone, whereas fear of CSB increased for group saline, and this increase was even greater in group naloxone. The analysis confirmed these observations. There was an overall significant main effect of group (naloxone vs. saline), $F(1, 30) = 11.4, p < .05$. There was a significant main effect for stimulus type (CSA vs. CSB), $F(1, 30) = 53.2, p < .05$, so overall there was more fear of CSA than CSB. There was also a significant main effect for day, $F(1, 30) = 4.2, p < .05$, so overall levels of fear increased across days of Stage III. There was no interaction between the effects of naloxone versus saline with either stimulus or day, $F_s(1, 30) < 3.5, p < .05$. The interaction of stimulus (CSA vs. CSB) and day was significant, $F(1, 30) = 46.8, p < .05$. It is important that the three-way interaction of group, stimulus, and day was significant, $F(1, 30) = 7.1, p < .05$. This interaction shows that naloxone prevented the decrease in fear of CSA but facilitated the acquisition of fear of CSB.

The levels of freezing observed in the first 180 s of context exposure in each session across the three stages of the experiment are shown in Figure 7. Inspection of the figure indicates that pre-CS freezing was low. There was a significant difference between groups in levels of pre-CS freezing across the course of the experiment, $F(1, 14) = 8.6, p > .05$. Inspection of the figure suggests that this difference appeared early within the experiment (during Stages I and II, during which time the groups had not received differential treatment), was present on some days but not on others, and was unchanged across the course of the experiment. This was confirmed by the absence of a Group $\times$ Day interaction in pre-CS freezing, $F(1, 30) = 1.8, p < .05$. The cause of these differences in low levels of pre-CS freezing are unclear, but regardless, they are not plausible explanations for the pattern of

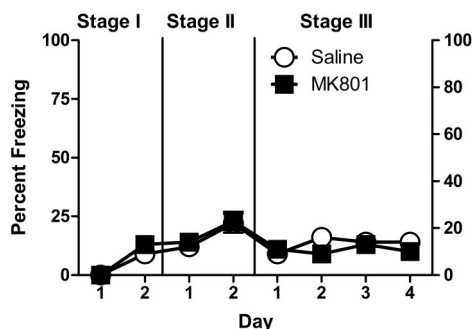


Figure 5. Mean ($\pm$ SEM) levels of pre-conditioned stimulus across each stage of Experiment 2. Error bars are obscured by the plotting symbols.

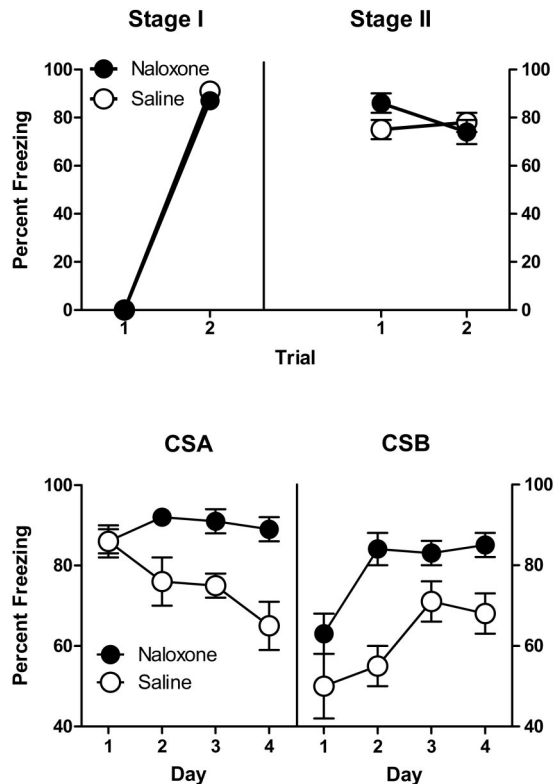


Figure 6. Mean levels of freezing in Stage I and Stage II of Experiment 3 (top panel). Mean levels of freezing during the test presentations of conditioned stimulus A (CSA) and conditioned stimulus B (CSB) across days of Stage III (bottom panel). During Stage III, freezing to CSA remained high for group naloxone but decreased for group saline. Fear of CSB increased for both groups but was greatest for group naloxone. Error bars represent standard error of the mean.

differences in freezing to CSA and CSB observed across the course of the experiment.

General Discussion

These experiments studied predictive fear learning. In Stage I, rats received CSA-shock pairings that conditioned fear of CSA. In Stage II, CSA was presented in simultaneous compound with CSB, and the AB compound was followed by shock. This Stage II training produced blocking of fear learning to CSB. In Stage III, CSB was arranged as a predictor of CSA by increasing its duration, and the compound CS was followed by shock. In each of the experiments reported here, fear of CSB increased across Stage III training, whereas fear of CSA decreased. This pattern of results can only be explained by the operation of TD prediction errors. In Stage III, CSA produces a positive prediction error (outcome > prediction) that conditions fear of CSB. This is the same mechanism that mediates second-order fear conditioning (for a review, see Rescorla, 1973). Thus, the unblocking of fear of CSB during Stage III is formally equivalent to the second-order conditioning of fear. By contrast, in Stage III the US produces a negative prediction error (outcome < prediction) that reduces fear of CSA and CSB. This is the same mechanism that mediates the overexpecta-

tion of fear learning (Rescorla, 1970). Thus, the reduction of fear of CSA during Stage III is formally equivalent to the overexpectation of fear learning. These two prediction errors cause bi-directional associative change during Stage III until the predictions from CSA and CSB equal each other and their sum equals the actual outcome.

This is the first time that temporal primacy has been found to reverse blocked fear learning. For example, Kehoe et al. (1987) as well as Jennings and Kirkpatrick (2006) showed that temporal primacy prevented the development of blocking (i.e., Stage I then Stage III), and Seymour et al. (2004), using human subjects, showed a role for temporal primacy in aversive conditioning using a single stage serial compound design, but none of these experiments used the intervening Stage II blocking training used here. The ability of a change in temporal relations to rapidly and completely reverse blocked fear learning to CSB is striking and underscores the importance of temporal factors in regulating Pavlovian fear learning.

Experiment 2 showed that antagonism of NMDA receptors had a pronounced effect during Stage III training. Administration of MK-801 prior to each Stage III training session reduced any learning during Stage III. This effect of MK-801 was nonselective in the sense that it prevented learning in response to the positive (i.e., learning to CSB) and negative (i.e., learning to CSA and CSB) prediction errors. This cannot be attributed to any effects of the drug on performance of the fear response because animals were tested during Stage III drug free. Generally, this pattern of results is consistent with the effects of systemic or basolateral amygdala NMDA receptor antagonism reported previously in the literature. Other things being equal, NMDA receptor antagonists impair first-order (e.g., Kim et al., 1991; Miserendino et al., 1990) and second-order (Gewirtz & Davis, 1997) fear conditioning, but they also impair the extinction (e.g., Baker & Azorlosa, 1996; Falls et al., 1992; Sotres-Bayon et al., in press) of Pavlovian fear conditioning. The present results show for the first time within a single experiment that NMDA receptor antagonists have the same effects on fear learning regardless of the direction (positive or negative) of predictive error causing that learning. This strongly suggests that NMDA receptors themselves do not encode predictive error during Pavlovian fear conditioning. This pattern of results is consistent, however, with other possibilities. For example, a simple and parsimonious interpretation is that NMDA receptors control the rate of fear learning independently of predictive error. Most error-

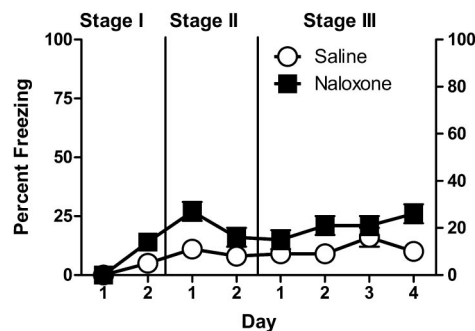


Figure 7. Mean ($\pm$ SEM) levels of pre-conditioned stimulus across each stage of Experiment 3. Error bars are obscured by the plotting symbols.

correction models of associative learning assume that the rate of associative change is at least partly independent of direction and magnitude of predictive error. For example, such models invoke rate parameters (e.g., α and β in the Rescorla–Wagner model), which influence associative change. NMDA receptor antagonists, by retarding the rate of associative change, would retard learning in response to both positive and negative prediction errors. By contrast, NMDA receptor partial agonists, such as d-cycloserine, by increasing the rate of associative change, would increase learning in response to such prediction errors. Further experiments using methods specifically designed to assess associative change (e.g., Rescorla, 2002) are needed to test this possibility.

Experiment 3 showed that antagonism of opioid receptors also had a pronounced effect during Stage III training. Administration of naloxone prior to each Stage III training session facilitated the unblocking of fear of CSB and attenuated the loss of fear of CSA. This cannot be attributed to any effects of naloxone on performance of the fear response because animals were tested during Stage III drug free. This result is consistent with the effects of naloxone on fear learning reported previously in the literature. Naloxone and μ -opioid receptor selective antagonists enhance the acquisition of first-order (McNally et al., 2004; Young & Fanselow, 1992) and second-order (Cicala, Azorlosa, Estall, & Grant, 1990) fear conditioning, and they impair the associative blocking of fear learning (Cole & McNally, 2007; McNally et al., 2004). In contrast, these same manipulations impair the extinction (McNally & Westbrook, 2003) and overexpectation (McNally et al., 2004) of Pavlovian fear learning. The facilitation of fear learning to CSB and maintenance of fear of CSA during Stage III are consistent with these opposing influences of opioid receptor antagonism on positive and negative prediction errors. Opioid receptor antagonism augments learning in response to positive prediction errors (outcome > prediction) and diminishes learning in response to negative prediction errors (outcome < prediction). Within the TD model this pattern of results could occur if opioid receptors mediated encoding of the predicted outcome (i.e., the weighted average of recent eligible associative strengths). Opioid receptor antagonism would therefore enhance the positive prediction error generated by CSA but also diminish the negative prediction error generated by the US. This would facilitate fear learning to CSB and maintain fear of CSA. The opposing influence of opioid receptor antagonism on learning in response to positive and negative prediction errors stands in marked contrast to the effects of NMDA receptor antagonism but is precisely the pattern of results expected if opioid receptors regulated predictive error. Therefore, we suggest that NMDA and opioid receptors have complementary roles in Pavlovian fear learning. Opioid receptors mediate predictive learning by regulating the predicted outcome at a given point of time in a conditioning trial. NMDA receptors, by contrast, act on the product of this predictive error to control the rate at which fear learning occurs.

In conclusion, these experiments have shown that Pavlovian fear conditioning in rats can be used to study the operation of TD prediction errors. The three-stage blocking–unblocking design used here is especially useful in this regard because it involves both positive and negative prediction errors, causing increases and decreases in fear learning to different stimuli within the same subjects. It may provide a powerful tool to probe the neural and cellular mechanisms of predictive learning. These experiments

have shown that NMDA and opioid receptors play complementary roles in fear learning. The effects of NMDA receptor antagonism on fear learning are consistent with a role for NMDA receptors in acting on the product of predictive error to control the rate at which fear learning occurs. In contrast, the effects of opioid receptor antagonism on fear learning are dependent on the direction of predictive error and are consistent with a role for opioid receptors in regulating the predicted outcome.

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