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The Roles of Endogenous Opioids in Fear Learning

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The endogenous opioid peptides and their receptors play important roles in Pavlovian fear conditioning in many species, including mice, rats, and humans. These roles are best viewed as regulating the conditions for fear learning by determining the actions of predictive error on association formation. Evidence will be reviewed showing such roles for opioid receptors in ventrolateral quadrant of the midbrain periaqueductal gray (vlPAG). These roles are shared across mammalian species because many of the effects of opioid receptor manipulations on fear learning first reported in rodents have now been documented in humans.

For the past two decades Pavlovian fear conditioning has been used extensively to study neural mechanisms of emotional learning. Exposed to pairings of a conditioned stimulus (CS) such as a tone, with an aversive unconditioned stimulus (US), such as footshock, animals learn to fear the CS as indexed by expression of co-ordinated fear responses such as species-typical defense responses, potentiated startle, and increased blood pressure upon later presentations of the CS. Fear conditioning has proven a popular model for investigations into the neural substrates of emotional learning because fear is learned rapidly, often requiring only a single trial, and because conditioned fear can persist over a long period of time. Much of this research has focussed on the role of amygdala glutamatergic neurotransmission in fear learning. This focus is unsurprising given the role of this neurotransmission in synaptic plasticity and the evidence linking amygdala synaptic plasticity to fear memory formation. However, other neurotransmitters and neuropeptides as well as other brain regions are important for fear learning. These have received significantly less empirical and theoretical attention.

This paper has two aims. The first is to review roles of the endogenous opioids in fear learning. The second is to provide a theoretical framework for understanding opioid contributions to fear learning and their potential interactions with amygdala-based glutamatergic mechanisms for fear learning. The primary focus is on rodent models of fear learning, but in recent years there has been an increase in knowledge regarding opioid contributions to fear and emotional learning in humans. These recent data strongly support the conclusions derived from non-human animal studies. This comparative research is exciting not only because it reveals common neural mechanisms for fear learning across species to underscore the important place of basic research in non-human animals, but also

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because it can help to identify novel approaches to the treatment of disorders of fear and anxiety in humans.

Learning in response to positive prediction errors: Opioids and fear learning

The opioid peptides are derived from post-translational modifications of four peptide precursors: preproopiomelanocortin, preproenkephalin, preprodynorphin, and preproorphanin. Each precursor gives rise to multiple active opioid peptides. These peptides share the common N-terminal sequence Tyr-Gly-Gly-Phe (YGGF) followed by various C-terminus extensions producing peptides ranging from 5 to 31 residues in length. The exceptions to this rule are the peptide products of proorphanin which have a Phe-Gly-Gly-Phe (FGGF) N-terminus. The major opioid peptides encoded by the precursors include β-endorphin, Met-enkephalin, Leu-enkephalin, and dynorphin. These peptides bind to at least four receptors which have been identified via molecular cloning and pharmacological studies: μ-, δ-, κ-, and ORL receptors. The peptides derived from preproopiomelanocortin, preproenkephalin, preprodynorphin bind to μ-, δ-, and κ-opioid receptors whereas the peptides derived from proorphanin bind to the ORL receptor. It is worth noting that there is a complex relationship between the opioid peptides and their receptors. The important point for present purposes is that, with the exception of the orphanin family, high affinity interactions are possible between the products of each of the peptide precursor and receptor families (for review see McNally & Akil, 2002; Williams, Christie, & Manzoni, 2001).

There is consensus that opioids are critical for regulating emotional learning and memory, with especially prominent roles in regulating fear learning. For example, in their seminal studies, Fanselow and Bolles (1979) showed that systemic administrations of the opioid receptor antagonist naloxone facilitated the acquisition of context conditioned fear learning in rodents. This finding is robust and has been replicated numerous times in many different laboratories across a variety of species. Fanselow went on to show that this facilitation of context fear conditioning was mimicked by i.c.v. infusion of a naloxone or a specific mu opioid receptor antagonist (Fanselow, Calcagnetti, & Helmstetter, 1988; Fanselow et al., 1991). The facilitation of context fear learning by opioid receptor antagonism could be subject to a number of interpretations. For example it might be suggested that opioid receptor antagonists increase fear or expression of fear conditioned responses. A closely related possibility is that the antagonists increase the aversiveness of the shock US. Alternatively, it might be suggested that the antagonists have facilitatory influences on fear memory storage and so enhance consolidation of fear memories.

Recent experiments using more complex behavioral designs have attempted to identify the specific associative process controlled by opioid receptors. This research has identified a role for opioid receptors in regulating the prediction errors which cause learning. Fear learning occurs when the actual outcome of the trial (the shock US) exceeds the expected outcome (the predicted outcome derived from the associative strengths of the CSs present). That is, when

there is a positive prediction error. When the actual outcome of the trial is not different from the expected outcome there is no prediction error and fear learning is blocked. Kamin (1968) was the first to demonstrate this effect. Kamin trained rats to fear a visual CS in Stage I. In Stage II rats received compound presentations of the visual CS + an auditory CS followed by shock. Rats in a control group received the same Stage II training but no Stage I training. Kamin showed that Stage I training blocked fear learning to the auditory CS in Stage II.

We have used the blocking design to study the effects of opioid receptor antagonism on fear learning. For example, McNally et al. (2004a) trained rats to fear a distinctive context in Stage I. Fear was measured using the species-typical defense response of freezing. In Stage II rats received auditory CS – shock pairings in that context. Prior contextual fear conditioning blocked fear conditioning to the auditory CS. Injection of naloxone prior to Stage II training prevented this blocking so that fear accrued normally to the auditory CS.

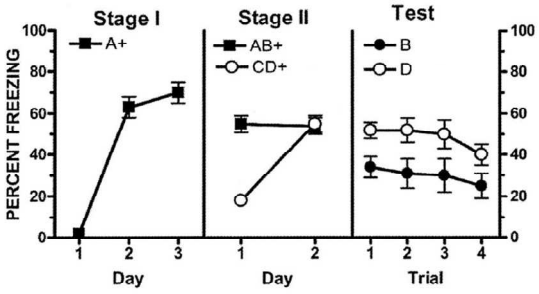
McNally and Cole (2006) used a within-subjects variant of the blocking design to study the role of opioid receptors in fear learning. This design (Figure 1) involved training rats to fear CSA in Stage I. In Stage II, for all rats, a compound of CSA+CSB was paired with shock as was a compound of CSC+CSD. Rats were tested for fear to CSB (the blocked CS) and CSD (the control CS). The logic is that Stage I training of CSA will block fear learning to CSB during Stage II. By contrast, fear learning to CSC and CSD should proceed normally because neither was paired with shock in Stage I. Blocking is shown by less fear on test to CSB as compared to CSD.

Compared with a simple experiment where an opioid receptor antagonist is administered prior to fear conditioning with a single CS, there are numerous advantages to this within-subjects design for studying fear learning. The within-subjects design isolates the contribution of predictive error to fear learning. Moreover, this design studies how the antagonist affects learning about multiple CSs in the same subjects at the same time, where those CSs differ only in their predictive error during Stage II. If opioid receptor antagonists facilitate fear learning because they increase fear, the aversiveness of the US, or facilitate consolidation of fear memories, then they will not have different effects on learning about CSB and CSD. The results of McNally and Cole (2006) showed consistently that this is not the case and instead supported a prediction error account. The results showed that: 1) blocking occurs because conditioned fear to CSB was less than conditioned fear to CSD; 2) injection of naloxone prior to Stage II prevents blocking of CSB; 3) injection of naloxone had no effect on fear learning to the control stimulus CSD.

A

Stage I	Stage II	Test
A+	AB+	B versus D
	CD+	

B



C

Group	Stage I	Stage II	Test
Saline	A+	Sal-AB+	B versus D
		Sal-CD+	
CTAP	A+	CTAP-AB+	B versus D
		CTAP-CD+	

D

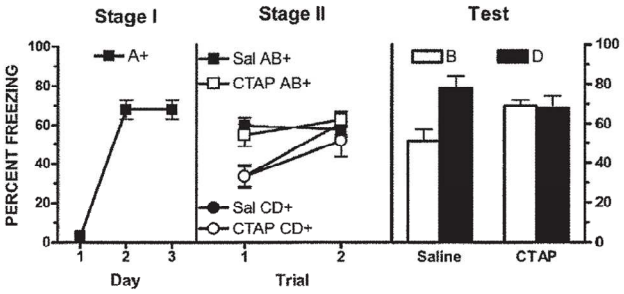


Figure 1. **A**) Behavioural design of within-subjects blocking design. **B**) Within-subjects blocking of Pavlovian fear conditioning. Stage I training of CSA blocked fear learning to CSB compared to fear learning to CSD (from McNally & Cole, 2006). **C**) Behavioural design used to investigate role of vlPAG μ opioid receptors in blocking. **D**) vlPAG infusions of a μ (CTAP) opioid receptor antagonist prevent blocking of fear conditioning (from McNally & Cole, 2006).

μ-opioid receptors in the ventrolateral quadrant of the midbrain periaqueductal gray (vlPAG) are the neuroanatomical locus for this opioid receptor regulation of fear learning. The midbrain periaqueductal gray (PAG) is an important structure for integrating defensive behavioral and autonomic responses to threats (Carrive, 1993; Fanselow, 1991; Keay & Bandler 2001, 2004). The PAG receives extensive projections from the amygdala and other forebrain structures important for learning, and it controls expression of defensive behaviors as fear CRs. The PAG is organized as a series of four longitudinal columns located dorsomedial (dm), dorsolateral (dl), lateral (l), and ventrolateral (vl) to the cerebral aqueduct that exert differential control over defensive behaviors. Both dPAG and vlPAG have been implicated in defensive responses. dPAG is important for controlling unconditioned defensive responses, whereas vlPAG is important for controlling conditioned defensive responses (Carrive, 1993). However, in addition to its well documented role in controlling fear CR expression, vlPAG plays a critical role in fear learning. We used the within-subjects blocking design to show that associative blocking of fear learning depends on endogenous opioid activation of μ-opioid receptors (Figure 1). Blocking, as measured by freezing, was prevented in a dose-dependent and neuroanatomically specific manner by vlPAG infusions of the μ-opioid receptor selective antagonist CTAP prior to Stage II training. It is worth emphasising that vlPAG infusions of CTAP did not affect the expression of fear, as measured by freezing, during Stage II. Rats infused with the μ-opioid receptor antagonist showed the same levels of fear during Stage II as control rats infused with saline. Rather, infusions of CTAP acted selectively to modulate Stage II learning by preventing the associative blocking of fear.

These results suggest that across the course of fear conditioning a fear CS acquires the ability to generate endogenous opioid signalling at μ-opioid receptors in vlPAG. This signalling acts to limit the amount of further fear learning to that CS (hence antagonists facilitate fear learning) and to block fear learning to novel stimuli conditioned in compound with that CS (hence antagonists prevent blocking). A simple, parsimonious, and theoretically coherent way to think about these results is in terms of error-correcting learning rules such as the Rescorla-Wagner model (Rescorla & Wagner, 1972). The Rescorla - Wagner model states that learning proceeds as a function of the discrepancy between the actual and expected outcomes of a conditioning trial. It provides a formal description of this discrepancy as $(\lambda - \Sigma V)$. λ is the asymptotic strength of association supported by the US, and ΣV is the summed associative strengths (V) of all conditioned stimuli present on that conditioning trial. We have suggested that opioid receptors contribute to fear learning because their activation can be specifically identified with the ΣV term in the discrepancy $(\lambda - \Sigma V)$. In other words, activation of opioid receptors contributes to encoding of the expected outcome of the conditioning trial. An opioid receptor antagonist prevents this expected outcome from regulating learning.

This approach explains why naloxone facilitates acquisition of fear learning. Across the course of conditioning the discrepancy $(\lambda - \Sigma V)$ grows smaller because the CS gains associative strength (ΣV increases). Opioid receptor

antagonists facilitate fear learning because they prevent this increase in ΣV from regulating learning on trials when the antagonist is present. In other words, opioid receptor antagonists act to maintain a large discrepancy (λ -ΣV) and so enhance learning. This same approach explains why opioid receptor antagonists prevent associative blocking of fear learning. Blocking occurs in the within-subjects design because the discrepancy (λ $-\Sigma V_A V_B$) is smaller during Stage II than the discrepancy (λ $-\Sigma V_C V_D$) due to the Stage I training of CSA. Opioid receptor antagonists increase the discrepancy (λ $-\Sigma V_A V_B$) because they prevent the V value of CSA (V_A) from regulating learning. This enables normal conditioning to CSB.

Several lines of evidence argue against the possibility that the effects of opioid receptor antagonism can be identified with an inflation of λ, or the asymptotic level of learning supported by the shock US. One such line of evidence is the fact that opioid receptor antagonists do not facilitate fear conditioning if only a single CS – US pairing (conditioning trial) is used. Rather, multiple CS – US pairings are required to detect a modulation of fear conditioning by an opioid receptor antagonist (Fanselow & Bolles, 1979). This failure of naloxone to facilitate one-trial fear conditioning is inconsistent with the possibility that opioid receptor antagonists increase λ. Instead it is consistent with the claim that these antagonist acts on a product of learning: the expected outcome, or ΣV.

Implicit in this explanation is the suggestion that fear learning which occurs following administration of an opioid receptor antagonist is different to normal fear learning. A key feature of normal fear learning is that subjects use past experience with the CS to regulate future learning about it. Opioid receptor antagonists prevent subjects from using this past experience to regulate learning. This is equivalent to suggesting that fear learning in rats treated with opioid receptor antagonists proceeds via Hebbian learning principles. For example, a key feature of Hebbian learning is that a CS will undergo increases in excitatory strength when it is paired with a US but such increases are unconstrained by predictive error, producing effectively limitless increases in fear learning. This is similar to the facilitation of fear learning by opioid receptor antagonists. Because Hebbian learning is unconstrained by predictive error, associative blocking cannot occur. Such blocking does not occur in rats treated with opioid receptor antagonists. Taken together, these data show that endogenous opioids have a critical role in regulating fear learning because they allow subjects to use past experience with the stimuli to regulate future learning about those stimuli.

It is highly unlikely that this role for opioids in predictive fear learning can be reduced simply to their role in pain modulation. Conditioned analgesia is a response to fear (Bolles & Fanselow, 1980). This analgesia can be mediated by opioid receptors (Harris, 1996). Fanselow (1998) has suggested that opioid receptor antagonists facilitate acquisition of fear because they prevent activation of descending analgesic pathways which would otherwise reduce spinal nociceptive processing of the footshock US. Several lines of evidence suggest that opioid receptors regulate predictive fear learning independently of their role in producing conditioned analgesia. For example, conditioned analgesia is frequently non-opioid (defined as insensitive to opioid receptor antagonism and lack of cross-tolerance

with morphine) (for review see Harris, 1996). Second, opioid receptor antagonists facilitate acquisition of fear to non-painful USs, such as loud noises (Cranney, 1987). Third, opioid receptor antagonists facilitate second-order fear learning which does not involve a painful US (Cicala, Azorlosa, Estall, & Grant, 1990; Cole & McNally, 2008, 2009). Fourth, opioid receptor antagonists impair fear extinction learning which involves no US (McNally & Westbrook, 2003a). Fifth, predictive fear learning is not associated with failures to detect and respond to the US, instead it is a selective alteration in learning about the affective properties of the US (Betts, Brandon, & Wagner, 1996). Thus, although opioids play an important role in conditioned analgesia, their role in regulating predictive fear learning must involve additional mechanisms to descending pain control circuits.

One such mechanism could be the large number of ascending projections from vlPAG to midline and intralaminar thalamic nuclei (Krout & Loewy, 2000). These thalamic nuclei are essential for conveying information about aversive stimuli to the amygdala, anterior cingulate cortex, prelimbic and infralimbic prefrontal cortex, as well as insula cortex. Thus, rather than viewing PAG simply as an output structure controlling fear responding via its projections to brainstem and spinal cord (e.g., LeDoux, 2000), we have suggested that PAG may be profitably viewed as part of an ascending pathway gating the transmission of aversive information to forebrain regions important for fear learning including the amygdala and prefrontal cortex (McNally, Lee, Chiem, & Choi, 2005; McNally & Cole, 2006). Recent electrophysiological data are consistent with this possibility (Johansen, Tarpley, & Blair, 2008). Lateral amygdala neurons responded strongly to a shock US but showed a diminution of this responding across the course of conditioning. This diminution was prevented by reversible inactivation of PAG, directly implicating PAG in regulating US-related activity in amygdala neurons.

The facilitation of fear learning by opioid receptor antagonism is not limited to rodents. It has also been reported in studies of fear conditioning using human subjects. Eippert , Bingel, Schoell, Yacubian, and Büchel (2008) injected human subjects with intravenous naloxone prior to fear conditioning using a discriminative (CS+/CS-) conditioning procedure with painful heat as the aversive US. Naloxone enhanced the acquisition of fear to the CS+ as measured on a reaction time task. Eippert et al. (2008) also detected diminution of US-related blood-oxygen-level-dependent (BOLD) signals in dorsal anterior cingulate across the course of conditioning which was attenuated by naloxone. Interestingly, presentations of the CS+ resulted in deactivations in rostral anterior cingulate cortex and amygdala which were prevented by naloxone. Such deactivations were observed in PAG and prevented by naloxone but these did not reach conventional levels of statistical significance. These findings are consistent not only with the behavioural data from rodents reviewed above but also with the possibility that opioid receptors in PAG exert their effects on fear learning by modulating thalamus – prefrontal cortex and thalamus – amygdala pathways.

Learning in response to negative prediction errors: Opioids and fear loss

The fear acquired through Pavlovian conditioning can be lost through learning. Fear extinction is currently the most popular animal model of this learning. In a standard extinction experiment, rats are trained to fear a CS via pairings with shock. This fear is then extinguished via repeated presentations of the CS in the absence of the US. The study of fear extinction has sparked interest among the neuroscience community due, at least in part, to its clinical importance. It is increasingly common to view anxiety-related disorders as characterised by dysregulation of fear extinction (e.g., Davis, Barad, Otto, & Southwick, 2006). Like fear acquisition, much contemporary neuroscientific research is dedicated to understanding the role of amygdala and glutamatergic neurotransmission in fear extinction learning. However, there is also evidence that the actions of opioids are central to understanding fear loss.

Systemic administrations of naloxone prior to fear extinction training prevent extinction learning. McNally and Westbrook (2003a) trained rats to fear an auditory CS via pairings with a footshock US. Fear of the CS was then extinguished via six two minute presentations of the CS. Injection of naloxone prior to extinction training impaired extinction learning as evidenced by both within-session extinction and test the following day. This impairment was dose-dependent and not due to state-dependent learning. The same injections of naloxone immediately after extinction training or prior to test for extinction had no effect on consolidation or retention of extinction. Just as the vlPAG is the key locus for opioid receptor regulation of fear acquisition, so too is it a key locus for opioid regulation of extinction. Microinjections of naloxone into vlPAG prior to extinction training produced dose-dependent and neuroanatomically specific impairments in extinction learning (McNally, Pigg, & Weidemann, 2004b). Again, μ-opioid receptors are the important subtype mediating extinction learning because infusions of μ-, but not δ-, or κ-opioid receptor antagonists into vlPAG impaired fear extinction learning (McNally et al., 2005) (Figure 2).

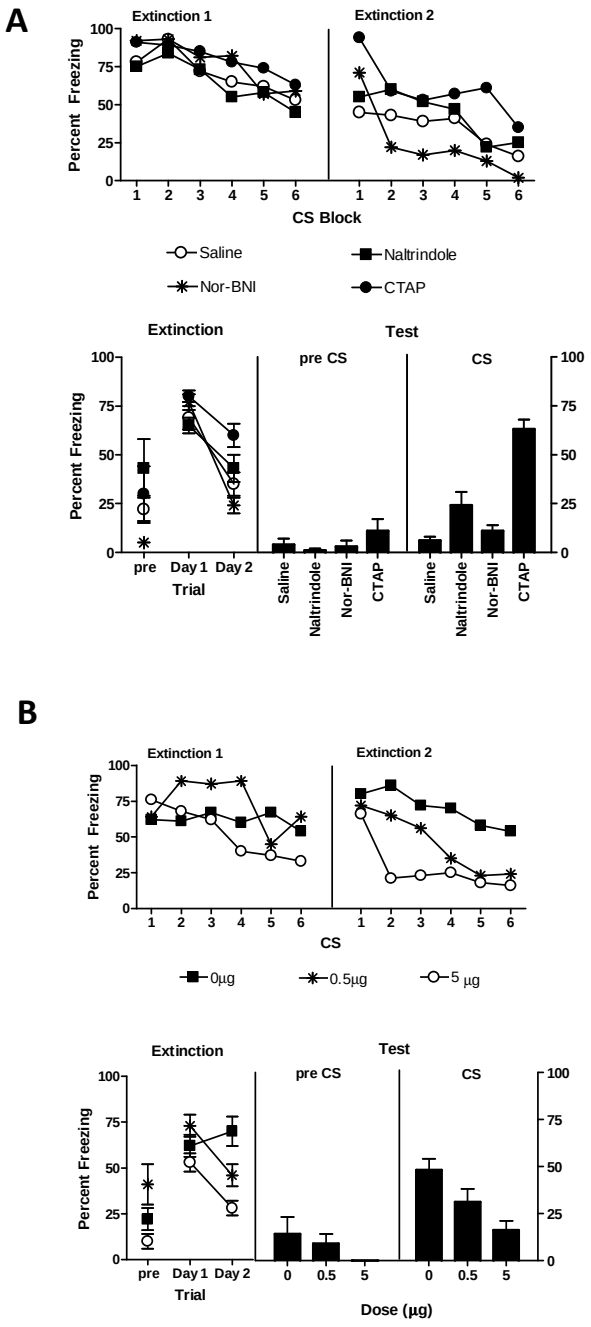


Figure 2. **A**) Effects of vlPAG infusions of opioid receptor subtype selective antagonists on extinction of Pavlovian fear conditioning. Infusions of a μ (CTAP), but not δ (naltrindole) or κ (norBNI) opioid receptor antagonist prevents extinction (from McNally et al., 2005). **B**) Effects of vlPAG infusions of RB101(S), an inhibitor of enkephalin catabolising enzymes, on extinction of Pavlovian fear conditioning. vlPAG infusions of RB101(S) dose-dependently enhance extinction (from McNally, 2005).

Further evidence for a role for vlPAG opioids in fear extinction learning comes from experiments which have studied whether fear extinction learning can be enhanced by manipulations which enhance opioid neuromodulation. One such manipulation exploits an interesting feature of opioid biology. As mentioned previously, opioid peptides have a common YGGF sequence at their N termini which is critical for determining binding to opioid receptors (Akil et al., 1984). This YGGF sequence is also a target for proteolysis by membrane bound zinc metallopeptidases (e.g., neutral endopeptidase [NEP, neprilysin, EC 3.4.24.11] and neutral aminopeptidase [APN, EC 3.4.11.2]; Roques, 2000; Turner, 2003). These enzymes are located in the PAG (Noble et al., 2001). Peptidase inhibitors can target and inhibit the enkephalin catabolising enzymes (Roques, 2000). For example, administrations of inhibitors of enkephalin catabolism such as RB101(S) and RB3001 increase extracellular levels of enkephalin in the PAG and potentiate the behavioral effects of opioids (e.g., Roques, 2000). McNally (2005) studied whether intra-vlPAG administrations of RB101(S), an inhibitor of enkephalin catabolism, would affect extinction learning. RB101(S) permits selective augmentation of the endogenous opioid peptide signal generated during fear extinction because it reduces catabolism of opioid peptides but does not have motivational effects itself (Noble, Coric, Turcaud, Fournie-Zaluski, & Roques, 1994; Noble, Fournie-Zaluski, & Roques, 1993). Infusions of RB101(S) into vlPAG significantly augmented fear extinction learning in a dose-dependent and neuroanatomically specific manner (Figure 2).

The effects of opioid receptor antagonists on fear extinction learning cannot easily be attributed to any tendency of the antagonists to increase fear. The antagonists did not inflate the fear CR nor did they reinstate extinguished fear. Moreover, manipulations which increase fear during extinction training augment prediction error and so facilitate fear extinction learning (e.g., Leung & Westbrook, 2008) whereas opioid antagonists impair this learning. Instead, these data show that the same opioid manipulations which facilitate the acquisition of fear learning impair fear extinction learning. This may seem paradoxical from the perspective that fear extinction is a learning process. However, it is entirely consistent with the predictive learning approach to understanding fear conditioning. The conditions promoting acquisition versus the extinction of fear are different. Indeed, they are opposite. Fear acquisition depends on positive predictive error whereas fear loss depends on negative predictive error. Fear extinction is the prototypical example of negative predictive error. At the start of extinction training the discrepancy between the actual outcome of the extinction trial (no shock) and the expected outcome (shock), $(\lambda - \Sigma V)$, is negative and large because $\lambda = 0$ and $\Sigma V > 0$. According to the analysis developed here, this negative prediction error should be absent in rats treated with an opioid receptor antagonist because these antagonists prevent the expected outcome (ΣV) from regulating learning on that trial. In other words, during extinction training under naloxone the discrepancy $(\lambda - \Sigma V)$ is small because ΣV has been reduced by the antagonist. The behaviour of animals subjected to fear extinction under an opioid receptor

antagonist is consistent with a third principle of Hebbian learning: fear, once acquired, cannot be extinguished.

Opioid receptor activation has diverse cellular consequences, including modulation of potassium and calcium conductances, inhibition of transmitter release, and nuclear signalling (Williams et al., 2001). Of particular relevance to understanding fear extinction learning is inhibition of adenylyl cyclase and cAMP. Inhibition of adenylyl cyclase -cAMP signalling is important for extinction learning in vlPAG because extinction learning is impaired, in a dose-dependent manner, by infusions of the membrane permeable cAMP analogue 8-Br-cAMP (McNally et al., 2005). This is interesting because activation of the adenylyl cyclase–cAMP pathway is an important mechanism for synaptic plasticity and learning in hippocampus and amygdala (Kandel, 2001; Schafe, Nader, Blair, & LeDoux, 2001). The finding that reductions of cAMP signalling in vlPAG mediate extinction learning is consistent with the fact that the circumstances promoting extinction learning (negative predictive error) are the opposite to those promoting acquisition of fear (positive predictive error).

Extinction is one example of negative prediction error. Such errors are observed under other circumstances and the actions of opioids are central to the learning that occurs under these circumstances. One example is overexpectation. In a standard overexpectation design, fear of CSA and CSB is established by pairing each with shock in Stage I. In Stage II, the experimental group receives compound presentations of CSA and CSB with shock, whereas the control groups receive either additional CSA–shock pairings or no additional training. Stage II compound training of CSA and CSB reduces the amount of fear provoked by either CS (Rescorla, 1970). This occurs because during Stage II the summed predictive strengths of CSA and CSB (ΣV) exceed the amount of learning supported by the footshock US (λ). This generates negative predictive error causing loss of fear to CSA and CSB. In many ways, overexpectation is preferable to fear extinction for study of the role of predictive error in fear loss. Simple non-reinforced presentations of a fear CS during extinction training confound the contributions of a variety of associative and non-associative processes to fear loss. For example, a key procedural difference between fear acquisition and fear extinction is the presence versus absence of the US. Absence of the US during extinction has important effects on both learning and performance during extinction training independently of its role in generating the negative prediction error which contributes to extinction learning. Overexpectation designs overcome this limitation because the shock US is present during both stages of the experiment. Thus, if a neural system, neurotransmitter, or neuropeptide is important for learning not to fear in response to negative prediction error it must serve the same role in fear overexpectation as fear extinction. We have studied the role of opioids in fear overexpectation. McNally et al. (2004a) trained rats to fear CSA and CSB via separate pairings with footshock in Stage I. In Stage II, an experimental group received compound presentations of the CSA+CSB followed by footshock. A control group did not receive Stage II training. There was evidence for

overexpectation (fear loss) on test of fear to CSA. This overexpectation was prevented by injection of naloxone prior to Stage II training.

Learning versus performance: Opioids and GABA

A consistent finding from these experiments was that an opioid receptor antagonist administered prior to test did not alter expression of fear as measured by the species-typical defense response of freezing. Neither systemic nor intra-vlPAG administrations of opioid receptor antagonists, across wide dose ranges and actual levels of fear, affected expression of fear. It is widely accepted that extinction training imposes a mask on conditioned fear. This mask reduces expression of fear after extinction training. The dissociation between the effects of opioid manipulations on acquisition versus expression of fear extinction shows that opioids are important for the learning of fear extinction but not for the expression of fear after extinction training. This can be contrasted with the role for GABAergic neurotransmission in fear loss. Harris and Westbrook (1998) reported that expression of fear extinction was prevented by pre-treatment with the benzodiazepine partial inverse agonist FG7142. This identifies GABA as a possible neurochemical substrate for masking or inhibiting fear after extinction training, a possibility supported by the demonstration that fear extinction training up regulates benzodiazepine binding and gephryin mRNA levels in the amygdala (Chhatawal, Myers, Ressler, & Davis, 2005; Heldt & Ressler, 2007).

A role for GABA in masking fear after extinction training is not specific to extinction learning. Rather, it is a product of negative predictive error. The negative prediction error during extinction training causes imposition of a GABAergic mask on fear. The evidence for this claim comes from recent studies on the mechanisms regulating expression of fear after overexpectation training. Recall that despite their procedural differences, extinction and overexpectation share a common cause (negative predictive error) and a common consequence (fear loss). Garfield and McNally (2009) trained rats to fear CSA and CSB via separate pairings with footshock in Stage I. Then, in Stage II, rats received compound pairings of CSA+CSB with footshock. This caused overexpectation of fear: there was less responding to CSA on test compared to controls which did not receive Stage II training. Garfield and McNally (2009) showed that injection of FG7142 prior to test alleviated the expression of overexpectation in a dose-dependent manner.

Taken together with the data reviewed previously there is an important difference between the effects of opioid and GABAergic manipulations on predictive fear learning. Opioids regulate *learning* in response to prediction error whereas GABA regulates *expression* of fear after negative prediction errors.

Opioids and fear loss in humans

A role for opioids in fear loss is not unique to rodents. It is also observed in humans under clinically important conditions. Exposure therapies for anxiety

disorders bear great similarity to procedures for experimental extinction of acquired fear. In both cases a feared stimulus is repeatedly presented in the absence of an aversive outcome to cause a reduction in fear. Exposure therapies have well documented efficacy for treatment of anxiety disorders (e.g., Booth & Rachman, 1992; Menzies & Clarke, 1993). Endogenous opioids mediate the therapeutic benefit of exposure therapies. Administrations of naloxone or naltrexone prior to exposure (Kozak et al., 2007; Merluzzi, Taylor, Boltwood, & Götestam, 1991) or systematic desensitization therapy (Egan, Carr, Hunt, & Adamson, 1988) for simple phobia significantly reduced treatment efficacy. Indeed, this impairment was seen regardless of whether multiple (Egan et al., 1988; Merluzzi et al., 1991) or single (Kozak et al., 2007) treatment sessions were employed indicating that it did not depend on cumulative exposure to opioid antagonists. There is important agreement between studies on fear loss via extinction training in rodents and fear loss via exposure therapy in humans: both depend critically on the actions of endogenous opioids.

The findings that opioid antagonists can impair the therapeutic efficacy of psychological treatments for anxiety disorders in humans raise the possibility that novel pharmacotherapy augmenting endogenous opioid neurotransmission may enhance the efficacy of psychological treatments for anxiety disorders. To date this possibility has not been examined. However the rodent data reviewed above using RB101(S) suggests such experiments may be worthwhile if a suitable pharmacological agent could be developed for human use. Recent data suggest that such pharmacotherapy may have additional benefit in terms of preventing the development of anxiety disorders. Bryant, Creamer, O'Donnell, Silove, and McFarlane (2008) assessed development of post-traumatic disorder (PTSD) after traumatic injury (e.g., transport accidents; assaults). Individuals had been admitted to hospital and treated with opiate analgesics. The amount of opiate exposure in the 48 hr following trauma was negatively associated with PTSD severity at 3 months. This relationship was not observed for depressive symptoms. Bryant et al. (2008) suggested that opiate exposure might have reduced later PTSD severity because it attenuated fear conditioning caused by the traumatic injury and its aftermath. This interpretation is consistent with the data reviewed here and the findings that opiates produce amnesia for fear conditioning (McNally & Westbrook, 2003b,c). This suggests that increased activity at opioid receptors in the hours following trauma may serve a protective influence against the long-term anxiogenic consequences of trauma and supports a role for opioids and their receptors in regulating human fear and anxiety.

Conclusion

The endogenous opioids acting at μ-opioid receptors in the vlPAG play important roles in regulating fear learning. μ-opioid receptor antagonists facilitate the acquisition of fear but impair the extinction, overexpectation, and blocking of fear learning. These roles are best viewed as regulating the conditions for fear learning by determining the actions of predictive error on association formation.

Manipulations which reduce μ-opioid neuromodulation in vlPAG enhance learning in response to positive predictive error and impair learning in response to negative predictive error. Conversely, manipulations which enhance μ -opioid neuromodulation in vlPAG impair learning in response to positive predictive error and facilitate learning in response to negative predictive error. Many of these effects have been observed in both non-human and human subjects. They have also been observed under clinically relevant circumstances in individuals suffering from anxiety disorders.

An important feature of the fear which is acquired in subjects treated with μ-opioid receptor antagonists is that it is divorced from the actions of predictive error. Instead this learning displays three key characteristics of Hebbian learning: apparent removal of limits on fear learning, absence of associative blocking, and absence of fear extinction. These findings show that normal opioid receptor function in vlPAG is integral to the neural mechanisms of predictive learning. During fear conditioning, vlPAG opioid receptors allow subjects to use past experience with stimuli to regulate future learning about those stimuli. We have suggested that this occurs because vlPAG opioid receptors gate transmission of information about the affective/motivational qualities of the US or its absence to amygdala, prefrontal cortex, and insular cortex via a vlPAG – midline/intralaminar thalamus pathway. In the absence of this feedback signal from vlPAG, fear learning is divorced from and proceeds independently of prediction error.

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