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The Role of Mitogen-Activated Protein Kinase in Treatment Strategies for Fear and Drug Addiction

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1. Introduction

Fear-avoidance and reward-seeking are necessary motivations to guide survival; however, both can lead to maladaptive behavior when expressed inappropriately, manifesting as anxiety disorders and drug addiction. Both disorders are a major worldwide public health concern with a high co-morbidity (M. S. O'Brien et al., 2005; Wasserman et al., 1997). Specifically, the World Health Organization (WHO) pin-pointed generalized anxiety disorder and substance abuse as the most common mental disorders across the world, ranking them highly as a cause of disease burden (WHO, 2001). Furthermore, in the recent United States National Comorbidity Survey Replication study, it was reported that 18.1% and 8.9% of adults met the 12-month Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; American Psychiatric Association, 2000) criteria for an anxiety or substance abuse disorder, respectively (Kessler et al., 2005). These staggering statistics also highlight the economic costs of anxiety disorders and substance abuse. For example, one estimate puts the yearly economic cost of alcohol abuse to be \$148 billion, and the economic cost of harmful drug use and dependence has been estimated to be \$98 billion in the United States alone (Harwood et al., 1998). Another study reported the economic cost of anxiety disorders to be \$42 billion in the United States (Greenberg et al., 1999).

Given the prevalence and the financial burden of anxiety disorders and substance abuse, a significant amount of research has been dedicated to finding effective treatments for those disorders. At present, the use of pharmacological agents has become a standard approach to attempt to ameliorate aspects of drug addiction and anxiety (Jupp & Lawrence, 2010; Reinblatt & Riddle, 2007). Unfortunately, the prevalence of these disorders still remain extremely high, although pharmacotherapy may be an effective approach to the treatment of drug addiction and anxiety disorders for some individuals. Furthermore, relapse is high in both substance abuse and anxiety disorders, even after pharmacotherapy (C. P. O'Brien, 1997b; Rachman, 1989). Thus, it has been suggested that cognitive-behavioral therapies that rely on learning mechanisms may potentially lead to more effective treatments for both

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types of disorders (Myers et al., 2011; Sutton et al., 2003). Therefore, much effort has been placed in finding an effective pharmacotherapy to combine with cognitive-behavioral therapies to effectively treat these mental disorders and prevent their relapse (M. Davis et al., 2006; Hofmann, 2007).

The importance of cognitive-behavioral therapies in treating anxiety disorders and substance abuse has been highlighted following observations that learning and memory processes play a significant role in acquisition and relapse in these disorders both in clinical settings and in the laboratory (Bouton et al., 2001; Mineka & Zinbarg, 1996; Rasmusson & Charney, 1997). For example, a common form of anxiety, posttraumatic stress disorder (PTSD), develops in vulnerable people following exposure to a traumatic event, and it is believed that these symptoms arise in part through a learning process in which cues present at the time of trauma acquire the ability to elicit fear (M. Davis & Whalen, 2001). Those cues can generalize over time to other settings and trigger pervasive fear and anxiety in inappropriate situations (Rothbaum & M. Davis, 2003). Drug addiction also involves learning - cues that are associated with the rewarding properties of a drug can trigger drug use in an individual (Rescorla, 1988; Robinson & Berridge, 2001). For example, drug paraphernalia such as a syringe, can trigger powerful drug craving that contributes to the maintenance of, and relapse to, drug use (A. R. Childress et al., 1986). Discovering that memory processes underlie anxiety disorders and drug addiction has not only led to a more successful modeling of anxiety and drug use using rodents in the laboratory, but also has guided pharmacological research. That is, considerable progress has already been made in delineating the neural bases of memory storage (Kandel, 2001, 2009), and research on anxiety disorders and drug addiction is now focused on characterizing the specific effects of proteins that have already been identified to be involved in general learning and memory. Mitogen-activated protein kinase (MAPK), in particular, refers to the entire superfamily of signaling cascades including the extracellular signal-regulated kinase (ERK) (Sweatt, 2001). The MAPK cascade is distinguished by a unique core series of three kinases: the first is MAP kinase kinase kinase (MAPKKK) that phosphorylates (i.e., activates) the second, a MAP kinase kinase (MEK). MEKs in turn activate a MAP kinase. The two main isoforms of MAPK are ERK1 and ERK2, which are 44 kDa and 42 kDa respectively (L. Lu et al., 2006). MAPK is well-known for its critical role in learning and memory as well as cellular growth, differentiation and survivability (Ahn & Krebs, 1990; Hoshi et al., 1988; Impey et al., 1999; Kornhauser & Greenberg, 1997; Sweatt, 2001). The present chapter will provide an in-depth review of the existing findings on the relationship between MAPK and fear and drug-seeking behavior using animal models. The aim of our chapter is for the audience to gain a better understanding of the significance of MAPK in anxiety disorders and drug addiction in order to facilitate the development of pharmacological adjuncts to assist in treatment of these disorders. The next section will first briefly review the research that revealed the molecular cascade underlying memory storage and formation, and the importance of MAPK signaling in that cascade.

2. The molecular biology of memory formation and storage

Memory formation has long been thought to be sub-served by a mechanism called 'synaptic plasticity', which refers to molecular and morphological changes that occur in dendrites and axons that either enhances or reduces the effectiveness of electrical and/or chemical

communication between neurons across synapses (Konorski, 1948). Due to the seminal work on Aplysia by the Nobel laureate Kandel and his colleagues, we now know that synaptic plasticity requires various molecular cascades that ultimately result in modifications of synaptic structure and efficacy (Kandel 2001, 2009). The best-studied mechanism that may potentiate these changes is N-methyl-D-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) (Sweatt, 1999). LTP refers to a lasting enhancement of the strength of synaptic connection as a result of brief repetitive activation of that synaptic pathway (Bliss & Collingridge, 1993). For example, experimentally-induced LTP involves a few trains of high-frequency stimuli to the connection between two neurons (Huang & Kandel, 1994). We know that LTP is important for memory storage because LTP disruption has been shown to impair memory consolidation in a region-specific manner. In the hippocampus, for example, antagonism of NMDARs impairs both LTP induction as well as hippocampal-dependent spatial learning in rats (Morris et al., 1986).

NMDA-dependent LTP is generally divided into three phases: short-term potentiation (or initial LTP), early LTP (E-LTP), and late LTP (L-LTP) (Sweatt, 1999). Short-term potentiation refers to the first stage of LTP that is independent of protein kinase activity for its induction or expression (Roberson et al., 1996). E-LTP starts at around 30 mins upon induction and typically lasts 60-90 mins. E-LTP requires protein kinase activation but is independent of gene transcription and protein synthesis (Frey et al., 1993). The last phase of LTP, L-LTP, lasts many hours and requires gene transcription and protein synthesis for its maintenance, and such processes are critical in the modification of synapses (Frey, et al., 1993). Although it appears that short-term potentiation is necessary for the subsequent phases of LTP, not much is known about the mechanisms underlying it (Roberson et al., 1996), therefore, the present review will focus on the molecular cascade related to E-LTP and L-LTP.

Typically, excitatory synaptic transmission occurs chemically via presynaptic release of glutamate into the synapse. Glutamate then binds to 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid receptors (AMPA) on the postsynaptic membrane, triggering the influx of positively-charged sodium ions into the postsynaptic neuron (Agranoff & Siegel, 1999). Such influx of ions causes depolarization of the cell, called the excitatory postsynaptic potential (EPSP). Likewise, the induction of E-LTP begins with NMDA receptor activation that leads to an influx of positively-charged calcium ions through ligand- and voltage-gated calcium channels into the postsynaptic neuron, causing depolarization (Ascher & Nowak, 1986). If calcium influx does not reach a threshold, E-LTP fails to occur (Lynch, 2004). The rapid rise in intracellular calcium concentration initiates the short- or long-lasting activation of several proteins that appear to be necessary for E-LTP. For example, blocking phosphorylation (i.e., activation) of calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) impairs the induction and maintenance of E-LTP (Sweatt, 1999). Protein kinase A (PKA) and mitogen-activated protein kinase (MAPK) phosphorylation also contribute to the maintenance of E-LTP (Lynch, 2004; Sweatt, 1999). Additionally, protein kinase Mζ (PKMζ), which is calcium independent, becomes activated during the maintenance of E-LTP. Those protein kinases carry out phosphorylation of existing AMPARs to increase their activity, as well as mediating insertion of AMPARs into the postsynaptic membrane (Malenka & Bear, 2004).

As mentioned previously, L-LTP is a natural extension of E-LTP. That is, long-lasting activation of some of the protein kinases mentioned above can induce L-LTP. L-LTP can last many hours, even days, and requires gene transcription and *de novo* protein synthesis in the

postsynaptic neuron (Frey et al., 1988; Lynch, 2004; Stanton & Sarvey, 1984). Those characteristics distinguish L-LTP from E-LTP because E-LTP does not require either. For example, administration of the protein synthesis inhibitor anisomycin blocks L-LTP, but not E-LTP, in the hippocampus (Frey et al., 1988). Gene transcription necessary for L-LTP is triggered by the persistent activation of MAPK. MAPK can translocate to the nucleus and activate nuclear molecules such as cyclic adenosine monophosphate (cAMP)-response element binding protein (CREB), which is a gene transcription factor (Lynch, 2004; Sweatt, 1999). ERK-mediated changes in transcription factor activity can ultimately result in the protein synthesis and morphological changes in neurites observed in L-LTP (Figure 1). For example, activity-regulated cytoskeletal element (Arc; also termed Arg3.1), is an immediate early gene that requires MAPK for its transcription (Chotiner et al., 2010). Arc has been widely implicated in experience-dependent synaptic plasticity because Arc is tightly coupled to the activation of signaling pathways implicated in learning, rather than being involved in normal cellular activity (Fletcher et al., 2006). Indeed, Arc expression in the hippocampus is critical for both LTP maintenance and the formation of hippocampus-dependent memory (Guzowski et al., 2000) and MEK inhibition suppresses experience-induced Arc mRNA levels (Chortiner et al., 2010). Further, Arc transcription can lead to protein synthesis via activation of receptors such as Notch (Alberi et al., 2011). Notch receptors and ligands are transmembrane proteins present at synapses that play a major role in changing neurite structure (Sestan et al., 1999). Blocking any one of the steps in the molecular cascade mentioned above impairs memory consolidation and or/learning and LTP (Kandel, 2001). Taken together, it appears that formation of long-term memory is linked to a molecular cascade involving pre-synaptic glutamate release, NMDA-mediated calcium entry, activation of protein kinases, gene expression and transcription, and protein synthesis (Figure 1).

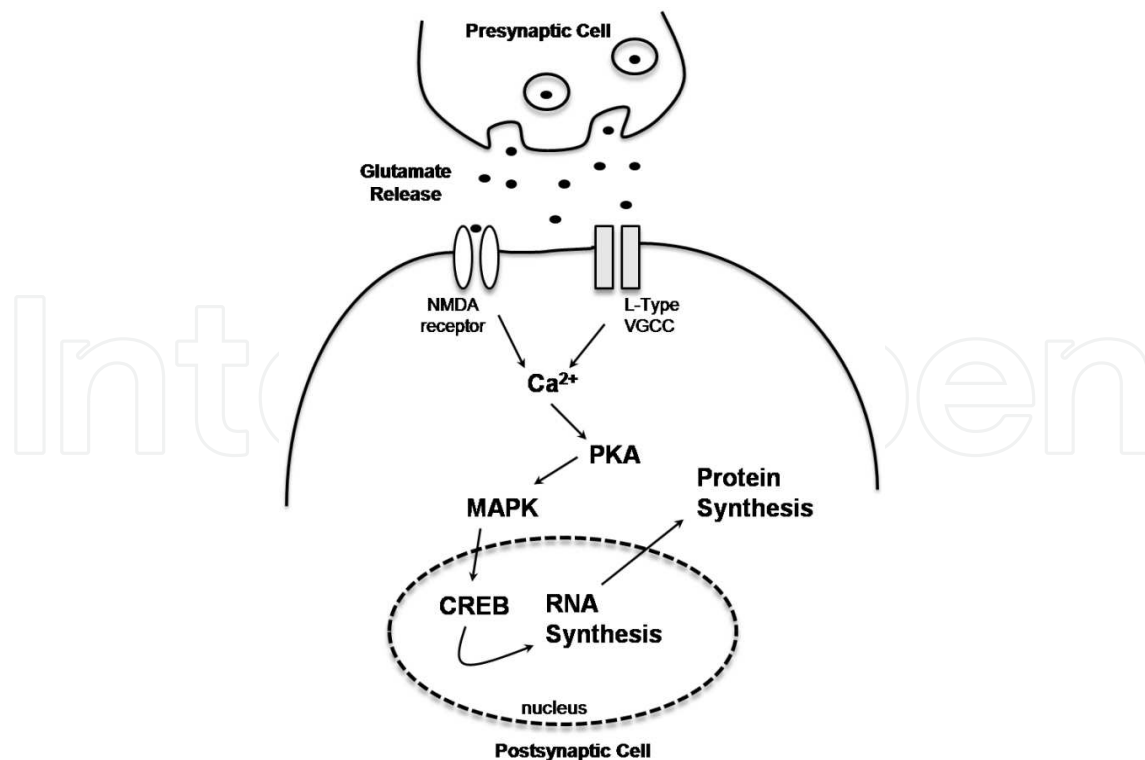


Fig. 1. Schematic diagram illustrating intracellular signalling pathway involved in the neuronal plasticity implicated in the formation of memory.

It is important to note that MAPK is critical for both L-LTP and E-LTP; MEK inhibitors significantly attenuate E-LTP and block L-LTP (S. Davis, Salin et al., 2000; English & Sweatt, 1997; Schafe et al., 2008; Sweatt, 1999; Wu et al., 1999). That is, MAPK signaling appears to be a molecular link between E-LTP and L-LTP. Indeed, many signaling cascades involved in E-LTP, including CaMKII and PKC, can converge on ERK (Kelleher et al., 2004). The strong possibility of MAPK being a link between E-LTP and L-LTP has significant implications for its role in memory. Historically, E-LTP and L-LTP are believed to be mechanisms subserving short-term memory (STM) and long-term memory (LTM), respectively, because both E-LTP and STM endure for a few hours, whereas L-LTP persists for many hours and even days, similar to LTM (Kandel, 2009). Thus, blockade of MAPK may sever any possible link between STM and LTM, and critically affect memory consolidation and storage. Therefore, it is not surprising that much attention has been given to the role of MAPK in animal models of anxiety disorders and drug addiction in the past couple of decades, as it became increasingly apparent that memory processes underlie these disorders.

3. MAPK in conditioned fear

Among the early advocates of the idea that anxiety disorders emerge from traumatic memories were Watson and Rayner (1920) who proposed that humans are born with very few 'emotional reaction patterns', and the way in which stimuli come to evoke emotion throughout one's life is through learning, or conditioning, processes. That is, animals learn to respond in distinct ways to different stimuli according to previous experiences with those stimuli. According to this notion, simple aversive learning and memory processes are critical for triggering anxiety disorders. To support this idea, in the famous 'little Albert' experiment, a baby boy was conditioned to fear a rat by sounding an aversive loud noise each time the baby touched the rat (Watson & Rayner, 1920). Before this experience, the baby was keen to touch and play with the rat. After the experience, however, the baby displayed signs of fear and anxiety (e.g., crying and reaching for mum) when in the presence of the rat. The simple pairing of the rat and the aversive noise induced fear learning in little Albert. This associative learning is referred to as classical or Pavlovian conditioning because Pavlov was the first to demonstrate such associative learning in animals (Konorski, 1948; I. P. Pavlov, 1927). Typically, Pavlovian *fear* conditioning involves pairings of a conditioned stimulus (CS), such as a tone, with an aversive unconditioned stimulus (US), such as a footshock. The CS can be discrete or diffuse, such as a localized light or a context, respectively. Initially, the CS is a neutral stimulus that has little effect on animals. On the other hand, the US is a stimulus that already elicits fear responses via physiological, autonomic and behavioral changes. These fear responses triggered by the US are referred to as unconditioned responses. When the CS and the US are repeatedly paired, the CS starts to elicit such autonomic and behavioral fear responses on its own without the US, and these responses are referred to as conditioned responses (CRs).

Such fear conditioning is readily acquired in animals, and is not easily forgotten (Gale et al., 2004). Because this form of learning can be acquired even in very immature animals (e.g., 12-day-old rats; Sullivan et al., 2000), Pavlovian conditioning provides a model for investigating the neurobiological bases of anxiety disorders, which often emerge during childhood/early adolescence in humans (Kessler, et al., 2005; Newman et al., 1996). Apart from Pavlovian conditioned fear, however, there are other laboratory models of anxiety

disorders. For example, Grillon and colleagues proposed that anxiety disorders are caused by unpredictable aversive events that lead to hyperactive fear responses that are generalized across different situations and environments (Grillon et al., 2004). Specifically, if an aversive US is not signalled reliably by a discrete CS, subjects show more fear to the training context compared to subjects that received the US signalled reliably by a CS. The elevated fear to the context exhibited by the unsignalled group reflects generalized anxiety according to this theory. Another laboratory model for anxiety disorders relies on the concept of 'unlearned' fear, which refers to the innate fear that animals have to various stimuli without any previous learning experiences with these stimuli (Boulis & M. Davis, 1989). Unlearned fear is triggered by a diffuse cue rather than a specific cue; therefore, unlearned fear can serve as a good model for anxiety disorders that may not be triggered by a specific object or a situation. Additionally, unlearned fear may explain some specific phobias that may be an exaggerated, debilitating form of natural fear (e.g. acrophobia).

Nevertheless, Pavlovian fear conditioning is the most utilized neurobiological model for anxiety, as it reflects the most common form of anxiety disorder, namely post-traumatic stress disorder (PTSD). This is partly because this preparation allows a variety of discrete stimuli to be used as the CS or the US, so that the role of each sensory modality and different neural structures in learning can be examined. Also, Pavlovian conditioning is easily acquired in many different animals across a range of different ages, unlike contextual conditioning in Grillon's model for anxiety (Grillon et al., 2004). Further, considering that anxiety disorders may be normal fear gone astray (Rosen & Schulkin, 1998), using Pavlovian conditioning as a model for fear learning provides a tool for understanding how the fear system works. It is no surprise then since Pavlov (1927) researchers have investigated the neurobiology underlying conditioned fear. Although much work has been done with the rat, it should be noted that similar neural processes are involved in learned fear across a range of species (Davis, 2000; LeDoux, 2000; Price, 2003). These findings have pin-pointed the amygdala as a central structure that is essential for the acquisition, storage and expression of conditioned fear.

The amygdala is made up of many sub-nuclei including the central (CeA) and basolateral (BLA) nuclei. The BLA is further divided into basal (BA) and lateral (LA) nuclei. During fear conditioning, perceptual (e.g., olfactory, visual) and somatosensory (e.g., noxious) information regarding the CS (discrete as well as contextual information) and US from hippocampal, cortical and thalamic structures is transmitted to the LA (LeDoux, 1993; LeDoux et al., 1990; McDonald et al., 1996; Price, 2003; C. Shi & Davis, 2001). As the result of CS-US convergence, neuronal activity in the LA is modified (Quirk et al., 1997; Quirk et al., 1995; Repa et al., 2001). Interestingly, once the CS and the US have been associated, the fear memory is stored in the BLA via similar intracellular molecular changes involved in LTP discussed earlier (Bailey et al., 1999; Campeau et al., 1991; Farb et al., 1992; Lee & Kim, 1998; C. H. Lin et al., 2001; Maren et al., 2003). Further, *in vitro* electrophysiological recordings show that LTP in the BLA is blocked by inhibition of MAPK activity without affecting basal transmission (Huang et al., 2000; Schafe et al., 2000). Hence, fear conditioning results in an enhanced functional and structural connectivity between sensory pathways and the BLA, such that future presentation of the CS alone is sufficient to activate the BLA. Such enhanced functional and structural connectivity requires MAPK signaling, as evidenced by many empirical demonstrations of the relationship between MAPK and conditioned fear.

3.1 The role of MAPK in the acquisition of conditioned fear

The involvement of MAPK in conditioned fear was initially illustrated by correlational studies that showed increases in phosphorylated MAPK (pMAPK) following fear conditioning via Western blotting and immunohistochemistry (Atkins et al., 1998; Gresack et al., 2009; Schafe & LeDoux, 2000; Sindreu et al., 2007). In one of the first studies examining the role of MAPK and conditioned fear, Sweatt and his colleagues first gave context-shock or cue-shock training to rats (Atkins et al., 1998). The memory of conditioned fear was assessed by CS-elicited freezing fear 24 hours later. Freezing is a species-specific defense response in rodents, and is characterized by an immobile, crouching position accompanied by hypervigilance, and is operationally defined as the absence of movement other than that required for respiration (Blanchard & Blanchard, 1972). At test, both context- and cue-shock trained rats exhibited robust freezing to the context or the cue, respectively. In a follow-up experiment, Western blotting revealed significant increases in pMAPK in the hippocampus (a known neural structure important for contextual and spatial learning) of rats that received either context-shock or cue-shock training compared to sham-trained rats that were merely placed in the context. This increase was significant 60 mins following conditioning, but not 1 min or 24 hours following conditioning. Interestingly, pre-conditioning systemic injection of the NMDA antagonist MK801 blocked this conditioning-related increase in pMAPK, as well as impairing both cue- and context-elicited freezing in a separate group of rats tested 24 hrs later. This finding suggests that the increase in MAPK phosphorylation in the hippocampus following fear conditioning is part of the NMDA-dependent molecular cascade that may be responsible for the formation of long-term memory. Additionally, Sindreu et al., (2007) also reported increased pERK in the CA1 area of the hippocampus following contextual fear conditioning in mice. Peak pERK was detected 30 mins following conditioning, which is different from Atkins et al. (1998) but can possibly be attributed to inter-species differences. Importantly, double immuno-labeling using the neuronal marker NeuN, interneuron marker GAD69, or astrocyte marker GFAP revealed that learning-induced ERK activation was exclusively neuronal (CA1 pyramidal cells) and not glial or interneuronal (Sindreu, et al., 2007). Further, activation of MAPK, PKA and CREB kinase was observed in the same neurons, further supporting that the molecular cascade underlying memory is likely to be involved in contextual fear memory consolidation.

The first study to examine phosphorylation of MAPK in the amygdala, a structure central to both contextual and cued fear conditioning, reported that tone-shock training significantly increased pMAPK-labelled neurons in the LA, compared to tone-only and shock-only control groups (Schafe & LeDoux, 2000). Additionally, Western blot revealed increases in phosphorylated ERK (pERK) in the conditioned group. Consistent with Atkins et al. (1998), both immunohistochemical and western blot data showed that pMAPK levels were the strongest 60 mins following conditioning. However, no rats were tested for their long-term memory following conditioning or tone-/shock-only training, making it difficult to attribute the increases in pMAPK/pERK in the LA to associative fear memory formation. Another study has provided such evidence for increases in pMAPK/pERK levels in the amygdala following Pavlovian fear conditioning as well as another type of fear learning. Specifically, Radwanska et al. (2002) trained rats on a two-way active avoidance procedure, in which footshocks were given in a compartment of a two-compartment shuttle box. During each training trial the auditory CS was presented and the rat could 'avoid' the shock by moving

into the other compartment within 5 seconds of CS onset. Failure to change the compartment within this time led to the onset of footshock US. Rats were then either sacrificed for immunohistochemistry, or were tested for avoidance memory 24 hours later. At test, rats showed good retention of two-way avoidance learning by showing increased avoidance behavior upon CS-onset at test compared to training. Increased pERK was also observed in the ventral portion of the dorsal LA compared to naive rats. In a separate, but identical experiment, increased activation of the immediate early-gene, Fos, was also observed in the same area. These results were replicated using Pavlovian fear conditioning (Di Benedetto et al., 2009; Radwanska et al., 2002). The authors suggested that the increases in pERK and c-Fos in the ventral-dorsal LA may be indicative of fear memory storage as a prior electrophysiology study observed that this specific area of the amygdala contained neurons that displayed extinction-resistant firing patterns (Repa et al., 2001).

MAPK phosphorylation in the LA as a potential marker for storage of fear memory is supported by a very recent study that examined fear memory retrieval and forgetting in developing rats (Kim et al., 2012). In that study, a memory phenomenon called 'infantile amnesia' was examined, which refers to the more rapid forgetting displayed by young animals compared to older animals (Campbell & Spear, 1972). Typically, no age differences in retention are observed when testing occurs soon after training, indicating that animals of all ages are equally able to acquire and express memory. However, when testing occurs after a delay, retention increases dramatically with maturation. This general finding has since been replicated numerous times, and is of substantial biological significance because it is an ubiquitous phenomenon that occurs in all altricial species, including humans (for a review, see Campbell & Spear, 1972). Interestingly, infantile amnesia reflects a failure to retrieve the fear memory at test because various pretest "reminder" treatments are able to reinstate the memory. For example, a single pre-test shock unconditioned stimulus (US) can reverse infantile forgetting (Campbell & Jaynes, 1966; Kim & Richardson, 2007a, 2007b). Kim et al. (2012) first showed that when postnatal day (P) 16 and 23 rats receive tone CS-shock pairings, both ages exhibited similar levels of conditioned fear when tested immediately after conditioning; when tested after 2 days, however, P16 rats showed poor CS-elicited freezing relative to P23 rats. When rats were assessed for pMAPK after test, both P23 and P16 rats given paired presentations exhibited significant elevation of pMAPK-immunoreactive (ir) neurons in the BLA compared to rats given unpaired presentations, despite P16 rats showing poor CS-elicited freezing (i.e., forgetting). In contrast, only the P23-paired group showed an elevated number of pMAPK-ir neurons in the medial prefrontal cortex (mPFC), indicating that MAPK phosphorylation in the mPFC tracks memory expression. These findings provided initial evidence suggesting that while the mPFC is involved in memory retrieval, MAPK phosphorylation in the BLA may be a persisting neural signature of fear memory storage that is forgotten but can be retrieved with reminder treatments.

The studies described above show whether learning or memory retrieval is followed by MAPK activation, providing correlational evidence for the involvement of MAPK in fear memory consolidation. In a different type of correlational study, Gresack et al. (2009) used sex differences to examine whether stronger memory is associated with greater MAPK activation following learning because it is well-established that there are sex differences in contextual fear conditioning due to differences in hippocampal dendritic and synaptic

morphology and cell excitability (Juraska, 1998; Madeira et al., 1991; M. D. Smith et al., 2002). It was first shown that male rats exhibit more long-term retention of contextual fear conditioning than female rats, as tested by context-elicited freezing 24 hours following context-shock training (Gresack, et al., 2009). Western blot then showed that pERK levels in ventral, but not dorsal, hippocampus were higher in males than females, relative to same-sex controls, 60 minutes following fear conditioning. Further, there were no sex differences in context conditioning-induced pERK increases in the amygdala, suggesting that the sex difference in pERK may be restricted to the ventral hippocampus. These findings imply different strengths of memory may be subserved by quantitative differences in MAPK activation.

Taken together, the correlational studies show phosphorylation of MAPK following fear conditioning and expression. However, they do not indicate whether MAPK is necessary for long-term fear memory. The direct upstream activator of MAPK is MEK, and various fear studies using MEK inhibitors support the hypothesis that MAPK regulation is necessary for memory consolidation (Atkins et al., 1998; Izquierdo et al., 2000; Schafe et al., 1999; Selcher et al., 1999). First, it was shown that systemic injection of SL327, a MEK inhibitor that crosses the blood-brain barrier (Favata et al., 1998), dose-dependently decreased pMAPK in the hippocampus in rats (Atkins et al., 1998). More importantly, systemic injection of SL327 one-hour before fear conditioning significantly impaired both cue- and context-elicited freezing when tested 24 hours later, compared vehicle injection. Open-field and shock sensitivity tests indicated that the effects of SL327 were not due to changes in anxiety levels or sensory perception. Importantly, memory impairment was not due to state-dependent mechanisms because injection of SL327 immediately after conditioning also blocked cue- and context-freezing. These findings were replicated in mice (Selcher et al., 1999). Finally, an intracerebroventricular (ICV) infusion study using the selective MEK inhibitor PD098059 (Seger & Krebs, 1995), definitively showed that memory impairments due to inhibition of MAPK activity was central rather than peripheral. Specifically, ICV infusion of PD098059 prior to tone-conditioning significantly reduced both tone- and context-elicited freezing when tested 24 hours, but not 1 hr, after conditioning (Schafe et al., 1999). The failure to see any memory deficit at the 1 hr test strongly suggests that MAPK activation during conditioning is necessary for LTM but not for STM. This is consistent with the idea that MAPK is particularly important for L-LTP, a putative neural mechanism underlying long-term memory. It should also be noted that in all three studies mentioned in this paragraph, there were no discernible effects of the drug during the conditioning session.

Since Schafe et al. (1999), quite a few studies have examined the necessity of MAPK activation in various neural structures that form the neural circuitry underlying conditioned fear, including the BLA, hippocampus, cortical and thalamic structures. Schafe et al., (2000) showed that bilateral intra-BLA infusion of the selective MEK inhibitor U0126 (Seger, et al., 1995) 30 mins before tone-shock conditioning dose-dependently attenuated tone-elicited fear when rats were tested 24 hours later. There was no effect of drug during conditioning and STM was unaffected. Di Benedetto et al. (2009) replicated this finding in mice using fear-potentiated startle (FPS) as the measure of conditioned fear. The startle response is evoked by an intense, unexpected stimulus (startle pulse; e.g., a brief but loud, sharp noise), and in studies with rodents, a whole body jump is usually taken as the magnitude of startle (M. Davis et al., 1993). FPS is the increase in startle response when the fear-associated stimulus

(e.g., CS previously associated with shock) is present compared to when it is absent. When U0126 was infused bilaterally into the LA 30 mins before conditioning, FPS was significantly impaired when tested 24 hrs later compared to vehicle infusion (Di Benedetto, et al., 2009). Immunohistochemical double-labeling showed that conditioning enhanced pMAPK was exclusively found in neuronal cells and not glial cells. In both of these studies, reacquisition of conditioned fear after test was not affected, indicating that the infusion of MEK inhibitors did not permanently damage the amygdala, and any drug effects were specific to the initial fear memory consolidation. Lastly, a study showed that for cued-fear conditioning, MAPK phosphorylation is critical not only in the BLA, but also in thalamic inputs into the BLA (Apergis-Schoute et al., 2005). In that study, rats were given intra-thalamic infusions of U0126 either 30 mins before or immediately after cued-fear conditioning. At both infusion intervals, findings revealed that long-term memory to the CS (assessed at 24 h) was impaired, whereas short-term memory (assessed at 1–3 h) was intact. In additional experiments, it was shown that intra-thalamic infusion of U0126 before LTP-inducing stimulation of thalamic inputs to the LA significantly disrupted LTP in the LA. Taken together, it appears that MAPK activation in the BLA and its thalamic inputs is critical for cued-fear memory consolidation.

In contrast to the BLA, intra-hippocampal infusion studies provide mixed results. For example, bilateral infusion of the MEK inhibitors PD098059 and U0126 into the dorsal hippocampus 30 mins before contextual fear conditioning was shown to have no effects on STM (tested at 1 hr) and LTM (tested at 24 hrs) (Ahi et al., 2004). On the other hand, disruption of the biphasic increase in pERK following context conditioning (at 0–1 h and 9–12 h post-conditioning) via bilateral infusion of U0126 into the dorsal hippocampus led to an impairment in freezing to the context when tested 24 hours following conditioning. STM, assessed 1 h after conditioning, was unaffected (Trifilieff et al., 2006). Both of these studies were performed in mice. It may be the case that the critical phase of MAPK activation in the hippocampus following contextual fear conditioning is rather late (i.e., hours after conditioning) compared to MAPK activation in the BLA.

Despite the inconclusive literature on the necessity of hippocampal MAPK activation on contextual fear memory consolidation, findings using the inhibitory step-down avoidance task are much clearer. Inhibitory step-down avoidance is a hippocampus-dependent task (I. Izquierdo & Medina, 1993), and its training typically involves placing the rat onto a platform, and presenting a footshock when the rat steps down. The rat learns to avoid stepping down and exploring the box. The latency to step down is used as a measure for fear memory in subsequent tests. Izquierdo and his colleagues have shown that bilateral infusion of PD098059 into the dorsal hippocampus 180 mins following training significantly impaired avoidance performance when tested 24 hrs later (Walz, Roesler, Barros et al., 1999). This effect was replicated again in the hippocampus (Walz et al., 2000), as well as in other brain structures such as the amygdala, entorhinal and parietal cortices at different time intervals following conditioning (Walz, Roesler, Barros, et al., 1999; Walz, Roesler, Quevedo et al., 1999; Walz, et al., 2000), suggesting differences in time-specificity of critical MAPK activation in different cortical structures. The same researchers also examined hippocampal MAPK involvement in the expression of inhibitory learning shortly after training compared to long-term memory expression. Bilateral infusion of PD098059 into the dorsal hippocampus 10 mins prior to test significantly attenuated retrieval of avoidance memory

when the test occurred 31 days after training, whereas STM test 3 hrs following test was unaffected (L. A. Izquierdo et al., 2000). This finding complements Kim et al. (2012) that showed retrieval of conditioned fear involves phosphorylation of MAPK. It appears that this phosphorylation of MAPK is not an indication of memory reconsolidation; rather, it is implicated in retrieval processes. Further studies are necessary to disambiguate this issue.

One study examined whether *increasing* ERK phosphorylation via an experimental manipulation affects fear memory consolidation. Giovannini et al. (2003) first showed that bath application of H2 and H3 histamine receptor agonists increased pERK in hippocampal brain slices. In a follow-up experiment, rats received bilateral hippocampal infusion of H2 or H3 receptor agonists amthamine/RAMH immediately after context-shock training. When the rats were tested 72 hours later, both H2 and H3 agonists improved rats' freezing to the trained context. Infusions of the MEK inhibitor U0126 blocked any effects of H2 and H3 agonists, further supporting the idea that any memory enhancement caused by H2/H3 agonists is due to ERK phosphorylation (Giovannini et al., 2003). Although these results do not show that MAPK phosphorylation in the hippocampus is necessary for hippocampal-dependent learning, they provide some evidence that MAPK phosphorylation in the hippocampus plays a critical role in fear memory consolidation.

Overall, the localized infusion studies described above provide a convincing case for the necessity of MAPK in fear conditioning, measured by various types of experimental paradigms. Those studies typically used MEK inhibitors that specifically inhibit MAP kinases p44MAPK (or ERK1) and p42MAPK (or ERK2) without distinguishing between the two. Therefore, research using genetic knockout (KO) or knockdown mouse models provided further clues as to which MAPK subtype is particularly important for fear learning. In the very first study using the genetic KO model, Sweatt and colleagues examined the p44 MAPK/ERK1 KO mice and observed no deficits in their hippocampal LTP or contextual- or cued-fear conditioning (Selcher et al., 2001). The authors concluded that the effects of MEK inhibition on fear learning must be due to the inhibition of ERK2 isoform of MAPK rather than ERK1. The same group of researchers then used mutant mice that displayed decreased ERK 1/2 phosphorylation in the hippocampus (Shalin et al., 2004). The mutant mice showed the same open field, rotarod, shock sensitivity behavior as the wildtypes, indicating that their motor coordination, general anxiety, and sensory perception were not different from controls. Further, within-session conditioning freezing levels did not differ between the mutant and the wildtype. However, the mutant mice displayed significant impairment in context-, but not cue-, elicited freezing when tested 24 hours later. Recently, a separate group reported that full ERK2 KO mice show almost a 100% prenatal mortality rate, thus used a specific ERK2 mutant mouse with partially reduced (20-40%) ERK2 (Sato et al., 2007). Those ERK2 mutant mice showed the same ERK1 level, anatomy and synaptic density as wildtypes. There were also no differences in open-field, elevated plus maze, Y-maze, wheel-running or circadian activity. Further, no differences were observed in within-session freezing during fear conditioning between the mutant and wildtype, indicating no differences in shock sensitivity. At test (48 h) however, the mutant mice were impaired in both cued- and context-elicited freezing compared to wildtype, although there were no differences when measured 2 h after training (STM test). These results suggest that ERK2, but not ERK1, plays an essential role in long-term memory formation.

Taken together, MAPK cascade, especially the one involving the ERK2 isoform, appears essential in fear conditioning. There is also overwhelming evidence that MAPK activation following learning is critical for memory consolidation and LTM, as STM is not affected by MEK inhibition. The next section will review in detail the role of MAPK in an animal model of treating anxiety disorders, namely fear extinction.

3.2 The role of MAPK in extinction of conditioned fear

At present, the most effective treatment for anxiety disorders is exposure therapies, which are based on the process of extinction (Hofmann, 2007). Extinction refers to the decrease or suppression in responses expressed to a stimulus due to repeated exposure to that stimulus without any reinforcement. As with conditioning, Pavlov was the first person to document extinction in the laboratory (see Pavlov, 1927, for review). He observed that animals show a robust CR after CS-US pairings; however, the CR dramatically decreased if the CS was presented repeatedly without the US. *Fear* extinction hence refers to the decrease in fear conditioned responding due to repeated presentation of the CS without any aversive outcome.

In a typical extinction session, a CS is first paired with a shock, and repeated presentation of the CS in the absence of the shock leads to a reduction of conditioned fear both in magnitude and frequency. Early theoretical models of Pavlovian conditioning suggested that this decreased response to the CS after extinction was due to the 'unlearning' or 'erasure' of the original CS-US association (Rescorla & Wagner, 1972). That is, the association between the CS and US is severed so that the CS becomes neutral again. However, it is now widely believed that the CS-US association remains intact after extinction, and that extinction reflects a new learning (CS-no US) process that inhibits the original CS-elicited behavior. The primary evidence for this view comes from behavioral studies that show performance to an extinguished CS recovers without subsequent re-training of the CS-US association, namely in the cases of *reinstatement*, *renewal* and *spontaneous recovery* (Bouton, 2002). Reinstatement refers to the recovery of extinguished fear responses via a pre-test reminder US, usually a footshock. Renewal refers to the recovery of extinguished fear responses when the subject is tested outside the extinction context. Spontaneous recovery refers to the recovery of extinguished fear responses due to the passage of time following extinction training. These findings suggest that extinction is a new learning that is mediated by internal, physical and temporal contexts. Strong evidence for new learning also comes from pharmacological and molecular studies demonstrating that extinction shares similar neural mechanisms with acquisition of learning. Specifically, formation of a new long-term memory and extinction are both linked to a molecular cascade involving pre-synaptic glutamate release, NMDA-mediated calcium entry, activation of protein kinases, gene expression and transcription, and protein synthesis (Baker & Azorlosa, 1996; Lattal et al., 2006; C. H. Lin, et al., 2001). This molecular cascade has been found to be necessary for the consolidation of fear extinction memory in neural structures important for extinction. In recent years, local infusion studies, lesion studies, *in vivo* electrophysiology studies and immunohistochemical studies in specific neural structures strongly suggest that extinction of conditioned fear involves a circuit including the amygdala and mPFC (Herry & Garcia, 2002; Milad & Quirk, 2002; Morgan et al., 1993; Sotres-Bayon et al., 2009). The hippocampus has been also incorporated into this neural circuitry, to account for the context

effects on extinction (Bouton et al., 2006; Corcoran & Maren, 2001; Myers & Davis, 2007; Sotres-Bayon et al., 2006).

Because extinction is an active learning process that involves many of the neurobiological substrates that subserve normal learning and memory, there is now accumulating evidence for the involvement of MAPK in extinction of conditioned fear. Indeed, mice exhibit significant increases in pMAPK-labelled neurons in the BLA 15, 60 and 360 mins following extinction of conditioned freezing responses compared to control groups that were not trained or did not receive any extinction (Herry et al., 2006). Additionally, increases in phosphorylation of ERK1 and ERK2 are also observed in the hippocampus 15 - 60 mins following extinction training compared to untrained mice (Fischer et al., 2007). These findings show that extinction triggers MAPK phosphorylation not unlike fear conditioning.

Interestingly, detecting the activation of MAPK has recently been used to dissociate the neural structures involved in extinction of conditioned fear in developing rats. Kim and colleagues have provided substantial evidence that fear extinction processes are dissociated across development, and have suggested that this is because some neural structures mature later than others (Kim & Richardson, 2010). To summarize, P24 rats display adult-like extinction in that they show renewal, reinstatement and spontaneous recovery of extinguished stimuli. However, pre-weanling aged rats (e.g., P17) do not show any of those behavioral phenomena. Pharmacological studies also show that reducing NMDA, γ -aminobutyric acid (GABA), and opioid neurotransmission impairs extinction in P24 rats, but extinction in P17 rats is only affected by the blocking of opioid neurotransmission. When phosphorylation of MAPK was detected using immunohistochemistry, P24 rats showed significantly increased pMAPK levels in the amygdala and mPFC compared to untrained, reactivated and context-exposure controls 1 hr following extinction (Kim et al., 2009). In contrast, extinction in P17 rats led to increases in pMAPK only in the amygdala, but not in the mPFC. The authors concluded that the differential involvement of the mPFC as shown by MAPK phosphorylation was at least partly responsible for the developmental dissociation of extinction-related phenomena. Kim and colleagues also showed that adolescent (P35) rats show impairments in long-term extinction of conditioned fear compared to pre-adolescent (P24) and adult (P70) rats, a finding that was accompanied by significantly lower levels of pMAPK in the infralimbic cortex (IL) of the mPFC in P35 rats compared to the other ages 1 hr following extinction training (Kim et al., 2011). Importantly, the long-term extinction deficit displayed by adolescent rats was overcome if adolescent rats were given double the amount of extinction training, and as a consequence pMAPK levels were also increased, supporting the notion that MAPK is directly involved in the consolidation of extinction memory.

The first study that provided evidence for the necessity of MAPK in fear extinction used the MEK inhibitor PD98059 for localized infusions (K. T. Lu et al., 2001). In that study, PD98059 was infused bilaterally into the BLA or the hippocampus 10 mins before extinction training. When rats were tested 24 hours later, results showed that BLA, but not hippocampal, infusion significantly impaired maintaining the extinction memory. This finding was replicated using the MEK inhibitor U0126 (C. H. Lin, Yeh, Lu et al., 2003). In those studies FPS was used as a measure for conditioned fear response, which typically does not allow the assessment of within-session extinction fear levels due to the obnoxious level of the startle pulse. Therefore, it is hard to conclude whether the infusion of MEK inhibitors disrupted

fear retrieval and/or extinction acquisition. Using freezing as an index for fear, Herry et al. (2006) demonstrated in mice that pre-extinction bilateral infusions of U0126 into the BLA completely blocked within-session extinction acquisition compared to vehicle infusion. Further, a high level of CS-elicited freezing was maintained at test the next day in the drug infusion group. This finding suggests that MAPK is important for the acquisition of extinction, rather than its consolidation, which is contrary to the role of MAPK in conditioned fear. It may be the case that the role of MAPK phosphorylation in the BLA is dissociated across conditioning and extinction.

In fact, studies indicate that the mPFC, rather than the BLA, is critical for the consolidation of fear extinction. For example, immediate, but not late (2 or 4 h), post-extinction infusion of PD098059 bilaterally in the mPFC resulted in a full return of conditioned freezing compared to vehicle infusions when rats were tested for their long-term extinction (Hugues et al., 2004). In a follow-up study, Hugues et al. (2006) first showed that hippocampal inputs to the mPFC display LTP-like changes following fear extinction compared to before extinction training. Further, LTP-like changes were blocked by PD098059 infusion into the mPFC. Additionally, Western blot revealed decreases in pERK in the mPFC following PD098059 infusions. These findings imply that MAPK activation in the mPFC is important for LTP-like changes in the mPFC following extinction, which may be the underlying mechanism for extinction memory consolidation (Hugues et al., 2006).

Hippocampal MAPK involvement in fear extinction is still equivocal. For example, Fischer et al. (2007) reported that immediate, post-extinction infusion of PD098059 or U0126 both impaired long-term extinction of contextual conditioned fear. On the other hand, Izquierdo and colleagues found that bilateral infusions of PD098959 prior to extinction only affected inhibitory avoidance expression during extinction, without impairing any long-term extinction (Szapiro et al., 2003). However, when they infused the p38MAPK inhibitor, SB203580, immediately after extinction training of inhibitory avoidance behavior, rats failed to show any extinction memory the next day (Rossato et al., 2006). This effect was temporally-specific, so that infusion 3 hours following extinction had no effects. The authors concluded that unlike inhibitory avoidance conditioning, extinction of inhibitory avoidance behavior is not dependent on p44MAPK and p42MAPK activation in the hippocampus.

Overall, the studies described above provide further support for the idea that fear extinction involves a new learning that relies on similar molecular cascades as fear learning. This premise led to the discovery that increasing the neurotransmission involved in memory consolidation can enhance and facilitate the consolidation of extinguished fear memory. Specifically, systemic, as well as intra-BLA, injection of the NMDA partial agonist D-cycloserine (DCS) facilitates extinction (Walker et al., 2002). It appears that increasing NMDA neurotransmission during extinction leads to a stronger extinction memory when tested the next day. Follow-up studies clarified that drugs acting on NMDA receptors are most likely to be affecting the consolidation of extinction (Ledgerwood et al., 2003; Santini et al., 2001). Using freezing as an index of fear, Ledgerwood et al. (2003) demonstrated that intra-BLA infusions of DCS had no effect on within-session extinction of conditioned freezing compared to vehicle, whilst facilitating extinction at test. Interestingly, this facilitation effect of DCS on fear extinction appears to be due, at least partly, to increases in MAPK phosphorylation (Matsuda et al., 2010; Yang & Lu, 2005). In Yang and Lu (2005), systemic DCS injection 15 mins prior to extinction training significantly facilitated extinction

the next day compared to rats injected with saline. Intra-BLA infusion of MEK inhibitors, PD098059 or U0126, however, abolished any DCS effects, when infused 5 mins following the DCS injection. Western blot revealed that compared to the context-exposure control group, extinction training significantly elevated pMAPK in the BLA. Further, administration of DCS enhanced the effect of extinction training on MAPK phosphorylation, which in turn was blocked by MEK inhibitors. These results strongly suggest that the DCS effect on extinction of conditioned fear is mediated by the MAPK-dependent signalling cascades in the amygdala. This general finding has been replicated in mice (Matsuda, et al., 2010).

Taken together, these studies provide compelling evidence for the critical role of MAPK in conditioned fear, especially cued-conditioning. There is accumulating evidence indicating that fear extinction involves activation and / or plasticity of inhibitory circuits in the amygdala (Chhatwal et al., 2005; Marsicano et al., 2002; Rosenkranz et al., 2003; Royer & Pare, 2002). It may be the case that MAPK activation acts directly on the molecular processes underlying synaptic plasticity and dendritic integration of neurons in the inhibitory circuit. For example, acquisition of extinction and its consolidation are protein synthesis-dependent (Berman & Dudai, 2001; C. H. Lin, Lee et al., 2003; Suzuki et al., 2004). Further, recent studies implicate the MAPK pathway in controlling rapid local translation during the stabilization of newly formed hippocampal memories, which is consistent with a role for MAPK / ERK-mediated control of local protein synthesis during the acquisition of fear extinction (Kelleher et al., 2004; Thomas & Huganir, 2004). Importantly, extinction induces up-regulation of the protein phosphatase calcineurin in the BLA, which depends on *de novo* protein synthesis (C. H. Lin, Yeh, Lu, et al., 2003). In fact, inhibition of calcineurin interferes with extinction (C. H. Lin, Yeh, Leu et al., 2003). Those observations indicate that acquisition of extinction may involve, at least in part, a MAPK-dependent control of mRNA translation in the BLA. Additionally, MAPK has been implicated in other forms of synaptic plasticity including long-term depression (LTD) (G. M. Thomas & Huganir, 2004). Therefore, it may be the case that MAPK is necessary not only for the maintenance of CS-no US memory in the extinction neural circuitry, but also for the reversal (that is, depotentiation) of fear conditioning-induced LTP (H. C. Lin et al., 2010; S. C. Mao et al., 2006; Rosenkranz & Grace, 2002; Rumpel et al., 2005). In either case, pharmacological modulation of MAPK activation could potentially provide novel therapeutic strategies for the treatment for anxiety disorders, assuming a lack of off-target effects following long-term systemic treatment.

4. MAPK and drug addiction

Drug addiction is more than mere drug use, rather it is a chronic, relapsing disorder that consists of a compulsive pattern of drug-seeking and drug-taking behavior that takes place at the expense of other activities. A key question in addiction research is identifying what changes in the brain underpin the transition from casual to compulsive drug use. Over the last 20 years much research has accumulated describing the various long-lasting cellular, molecular and neurochemical adaptations which occur with repeated drug administration (Kalivas, 2009; Kalivas & O'Brien, 2008; Robinson & Berridge, 1993; M. J. Thomas et al., 2008). Furthermore, there is a compelling body of evidence linking various models of addiction with synaptic plasticity in brain areas involved in reward and reinforcement (Kauer & Malenka, 2007; Kelley, 2004). Drug-induced neuroadaptations are thought to be critical in the transition to addiction, though it remains to be fully elucidated how, and to

what extent, these changes contribute to prolonged relapse vulnerability after cessation of drug use (Shaham & Hope, 2005).

A central problem facing the treatment of drug addiction is the enduring vulnerability to relapse displayed by users despite months or even years of abstinence (Dackis & O'Brien, 2001; Wagner & Anthony, 2002). Even with successful detoxification, abstinence remains an unobtainable goal for many addicted individuals with up to 90% relapsing within 12 months (DeJong, 1994; Deroche-Gamonet et al., 2004; Gossop et al., 1989). Drug craving, a subjective affective state experienced by humans, which motivates them to seek out drugs, is associated with relapse propensity (Markou et al., 1993; C. P. O'Brien, 1997a). Craving can be induced in addicted individuals by exposure to drug-related paraphernalia, images or environmental contexts (A. Childress et al., 1988; Grant et al., 1996). Repeated exposure to such cues and contexts during the initiation and maintenance of drug use is therefore thought to result in these cues acquiring incentive motivational and conditioned reinforcing value (C. O'Brien et al., 1992). Once formed, these pathological associations may ultimately contribute to the precipitation of craving and relapse upon re-exposure to drug-associated stimuli. In addition to drug-associated cues, relapse can be triggered by other factors such as stress and exposure to the drug itself (S. A. Brown et al., 1995; C. O'Brien, et al., 1992).

Research into potential treatment strategies for addiction, therefore, primarily focuses on relapse prevention. Animal models of relapse have yielded invaluable information regarding the neurobiology of drug-seeking and the persistent neuroadaptations which likely underpin this behavior, revealing a number of key molecules that appear to play a critical role. ERK is one of these molecules and the following sections in this chapter will cover the evidence which supports a role for ERK in mediating relapse to drug-seeking and the drug-induced changes which potentially underlie this behavior.

4.1 Addiction neurocircuitry

Despite their varying pharmacological profiles and properties, drugs of abuse all share the common feature of acutely enhancing neurotransmission in the mesocorticolimbic dopamine system. This interaction can be direct, as in the case of psychostimulants, or indirect, as in the case of opioids, but each ultimately result in increased levels of extracellular dopamine in the terminal fields of these neurons and this increase in synaptic dopamine is thought to be responsible for their reinforcing properties (Di Chiara & Imperato, 1988; Wise, 1996). The mesocorticolimbic system is comprised of dopaminergic neurons, the cell bodies of which originate in the ventral tegmental area (VTA) and project to the nucleus accumbens (NAc) and other limbic regions such as the hippocampus and amygdaloid nuclei. A further subdivision of these dopaminergic neurons innervates cortical areas including the mPFC, anterior cingulate and orbitofrontal cortices (OFC) (Le Moal, 1991). Different terminal regions are thought to be involved in the different aspects of addictive behavior. The NAc is thought to be involved in the mediation of the acute reinforcing effects of drugs (Di Chiara, 2002; Volkow et al., 2003), whereas changes in the PFC, OFC and anterior cingulate are related to the decreases in inhibitory control and hyper-responsiveness to drug-related cues (Kalivas & Volkow, 2005). The amygdaloid nuclei and hippocampus play an important role in conditioning and learning associations between drugs and drug-related stimuli (Fuchs et al., 2007; Fuchs et al., 2005; Rogers & See, 2007; See, 2005).

In addition to dopaminergic input, the striatum receives excitatory glutamatergic input from the BLA, the hippocampus, the mPFC, the OFC, cingulate cortex and midline thalamic nuclei (Alexander et al., 1986). Dopaminergic and glutamatergic inputs converge on the dendritic spines of GABAergic striatal efferent neurons (Sesack & Pickel, 1992; Totterdell & Smith, 1989). This allows dopamine to play a modulatory role in regulating the excitatory input from glutamate to the dendritic spines (A. D. Smith & Bolam, 1990). While dopamine is thought to be responsible for the acute effects of addictive drugs, persistent vulnerability to relapse is believed to be a result of enduring neuroadaptations in corticostriatal circuitry (Kalivas, 2009). The VTA also receives glutamatergic input from structures such as the PFC (Omelchenko & Sesack, 2007). Excitatory inputs from the PFC play an important role in regulating the activity of VTA neurons and therefore the extracellular levels of dopamine within forebrain regions (Carr & Sesack, 2000).

4.2 The role of MAPK in responses to drug-associated cues and contexts

A process of classical conditioning occurs with repeated drug use: the multiple cues and contexts associated with drug use (previously neutral stimuli) become conditioned stimuli (CS) through repeated pairings with the drug (the unconditioned stimulus, US). As this learning about drug-associated stimuli develops, the cues gain increasingly greater control over behavior and drug-associated cues can acquire the capacity to evoke salient and powerful memories of the drug-taking experience that induce craving and ultimately relapse (Jentsch & Taylor, 1999). In animals these cues have been shown to increase operant drug self-administration and precipitate relapse-like behavior (Aguilar et al., 2009; Crombag et al., 2008; Shaham et al., 2003). In the conditioned place preference (CPP) paradigm, a variety of species (even crayfish) will preferentially choose to spend more time in an environment where they have received a non-contingent injection of drug over an environment where they have received vehicle (Nathaniel et al., 2009; Tzschentke, 1998). Animal models of relapse have also demonstrated that the motivational properties of drug-associated stimuli cues can augment or “incubate” over time (Grimm et al., 2001), thereby highlighting the need to acknowledge the role of drug-associated cues when developing therapeutic interventions.

CPP is based on classical conditioning principles whereby animals are conditioned to associate a distinct environment (i.e., CS) with a drug experience (i.e., US) (Tzschentke, 1998). A place preference describes a situation where the subject spends more time in a drug-paired environment when given the choice after a conditioning period. As researchers, we make an inference regarding the rewarding or hedonic properties of a drug based on its ability to induce approach (Bardo & Bevins, 2000). Though conditioned approach towards specific stimuli, especially drugs of abuse, obviously occurs in humans, this phenomenon has never been clinically validated (Bardo, et al., 2000). As with operant self-administration, in the reinstatement version of this paradigm the association between the CS and US can be extinguished and then reinstated either by non-contingent ‘priming’ injections of the drug (Parker & McDonald, 2000) or stress (L. Lu et al., 2002). In the case of CPP however, there is no reinstatement of an instrumental task, rather the reinstatement of conditioned approach to the CS (the environment in which the appetitive stimulus was experienced).

Activation of ERK has been shown to play a role in the associative learning processes that occur upon exposure to an environment previously paired with a drug experience. Thus, ERK

activation has been shown to be required for the acquisition of CPP induced by cocaine (Valjent et al., 2000), MDMA (Salzmann et al., 2003), THC (Valjent et al., 2001), amphetamines (Gerdjikov et al., 2004; Mizoguchi et al., 2004) and morphine (Ozaki et al., 2004), but not ethanol (Groblewski, Ryabinin et al., 2011). It is most likely that the NAc is a site of action, at least for amphetamines, as intra-NAc injections of the MEK inhibitor PD98059 impair both methamphetamine and amphetamine-induced CPP (Gerdjikov, et al., 2004; Mizoguchi, et al., 2004). This is supported by the observation of increased activation of ERK in the NAc and dorsal striatum of mice that have been conditioned to methamphetamine (Mizoguchi, et al., 2004) and the NAc, dorsal striatum and prefrontal cortex of mice that have been conditioned to cocaine (Valjent, Corbille et al., 2006). Another study found that exposing mice to a previously cocaine-paired context increased phosphorylation of ERK in the dorsal hippocampus, but not the NAc. Enhanced CREB and DARPP-32 phosphorylation in the NAc, however, was observed (Tropea et al., 2008). Pre-treatment with the MEK inhibitor SL327 has also been shown to abolish the conditioned locomotor response of mice placed in a context previously paired with cocaine or D-amphetamine (Valjent, Corvol et al., 2006), indicating the role of ERK can be generalised to other contextually-driven behaviors.

It also appears that the NAc mediates consolidation of learned associations between a drug's rewarding effects (US) and the drug-paired context (CS). If rats are injected with a MEK inhibitor either systemically or into the NAc core after the test for the expression of cocaine or morphine CPP, subsequent CPP is abolished (Miller & Marshall, 2005; Valjent, Corbille, et al., 2006). Erasure of CPP expression is accompanied with blockade of ERK phosphorylation in the NAc and dorsal striatum (Valjent, Corbille, et al., 2006). This effect can be mirrored with a protein synthesis inhibitor thus providing evidence that ERK exerts this effect on memory reconsolidation through regulation of protein synthesis, presumably by controlling transcription.

4.3 The involvement of MAPK in the actions of drugs of abuse & drug-induced plasticity

The MAPK signalling cascade has been implicated in responses to most drugs of abuse, supporting the notion that drug-induced plasticity might share common mechanisms with learning and memory processes (Berke & Hyman, 2000; Girault et al., 2007; Hyman, 2005). ERK is expressed abundantly throughout the brain, including mesocorticolimbic regions (Ortiz et al., 1995). Acute and chronic administration of most drugs of abuse such as cocaine, morphine, nicotine and Δ^9 -tetrahydrocannabinol (THC) has been shown to increase activation of ERK in reward-related areas such as the VTA, NAc, PFC, central and extended amygdala and hippocampus (reviewed in Girault et al., 2007; Zhai et al., 2008). The exception is alcohol, where data are equivocal. Acute administration has actually been shown to decrease phosphorylation of ERK in the cortex and hippocampus (Chandler & Sutton, 2005) but increase phosphorylation in other areas (Bachtell et al., 2002). Chronic ethanol intake has been shown to attenuate ERK phosphorylation in the striatum, whereas withdrawal for one day potentiates ERK phosphorylation in the same region (Cui et al., 2011).

The activation of ERK in response to repeated administration of drugs of abuse suggests that ERK plays a role in drug-induced plasticity. One measure of drug-induced plasticity is the development and expression of behavioral sensitization (an enhancement of the

psychomotor-activating properties of a drug). Indeed, activation of ERK in the brain mesocorticolimbic area has been shown to be involved in sensitization and hence drug-induced plasticity (Pierce et al., 1999; X. Shi & McGinty, 2006; Valjent, et al., 2000; Valjent, Corvol, et al., 2006). Thus, pre-treatment with the MEK inhibitor SL327 prior to the repeated administration of cocaine or amphetamine prevents the subsequent expression of sensitization to their locomotor-activating effects upon receiving a challenge dose of the drug (Valjent, Corvol, et al., 2006). In contrast, the expression of sensitization was not affected when SL327 was administered before the challenge (Valjent, Corvol, et al., 2006). Therefore it appears ERK is more critical for the development of sensitization to psychostimulants. ERK activation in the VTA is implicated in this process as intra-VTA infusions of PD98059 attenuate the development of sensitization to cocaine (Pierce, et al., 1999).

ERK in the NAc also appears to be involved in drug-induced plasticity as cocaine sensitized rats display increased phospho-ERK2 during withdrawal from cocaine which is normalized upon cocaine challenge (Boudreau et al., 2007). ERK2 phosphorylation corresponded with accumbens AMPA receptor surface expression (Boudreau, et al., 2007). The authors suggest the possibility that ERK contributes to both rapid (LTP) and slowly induced (cocaine withdrawal) increases in AMPA receptor surface expression, thus contributing to addiction-related plasticity (Boudreau, et al., 2007). Similarly, withdrawal following development of sensitization to cocaine has been shown to be associated with time-dependent increases in ERK activity which correlate with the increased expression of NMDAR subunits (Schumann & Yaka, 2009). Furthermore, the increase in both GluR1 expression and ERK activity is blocked with intra-NAc infusions of a MEK inhibitor 21 d after withdrawal from cocaine. These data suggest that the development of cocaine psychomotor sensitization triggers a delayed increase in the expression of NMDAR subunits in the NAc, which in turn enhances the activity of ERK (Schumann, et al., 2009).

4.4 Drugs of abuse and mechanisms of ERK signalling

The activation of ERK by drugs of abuse in mesocorticolimbic regions has been shown to require both glutamate and dopamine signalling as it is prevented by both dopamine D1 and NMDA receptor antagonists (Berhow et al., 1996; Jenab et al., 2005; Valjent, et al., 2000; Valjent, et al., 2001; Valjent et al., 2005), and in the case of amphetamine, by group I metabotropic glutamate (mGlu) receptor antagonists (Choe et al., 2002). This is not surprising as ERK is found downstream of all these receptors. Thus, the activation of dopamine D1 receptors by dopamine increases cyclic adenosine monophosphate (cAMP) and thus cAMP-dependent protein kinase A (Bilbao et al. 2008). Activated cAMP-dependent protein kinase (Bilbao, et al. 2008) then activates ERK through the the dopamine- and cAMP-regulated phosphoprotein 32 kDa (DARPP-32) signaling cascade (Greengard et al., 1999; Svenningsson et al., 2004). In striatal neurons, the mGlu5 receptor activates ERK through Ca²⁺-dependent and -independent mechanisms, the latter involving the scaffolding protein Homer (L. Mao et al., 2005). Activation of NMDA receptors and the resulting Ca²⁺ influx activate Ca²⁺/calmodulin-dependent protein kinase II (CaMKII). CaMKII then activates Ras-GTP, which initiates a number of kinase cascades, including Raf-1, MAPK/ERK kinases and ERK (G. M. Thomas, et al., 2004; Xia et al., 1996). ERK is also activated by opioid receptors most likely via a mechanism involving Ras and beta-gamma subunits of Gi/o proteins (Belcheva et al., 1998). Once activated, ERK translocates to the cell nucleus where it regulates

the activity of the transcription factors CREB and Elk-1 which control the transcription of many genes implicated in addiction, such as *c-fos*, Δ FosB, *zif268*, *Homer* and *preprodynorphin* (Benavides & Bibb, 2004; Choe & McGinty, 2001; S. Davis, Vanhoutte et al., 2000; Girault, et al., 2007; Hurd & Herkenham, 1993; L. Lu, et al., 2006; McClung & Nestler, 2003; Nestler, 2001; Radwanska et al., 2005). Downstream effects of many drugs of abuse on these genes have been shown to be mediated via ERK (Bachtell, et al., 2002; Salzman, et al., 2003; Valjent, et al., 2000).

Evidence has accumulated which supports a coincident interaction between D1 and NMDA receptors with respect to cocaine-driven ERK signaling in the striatum. Thus, cocaine-induced ERK activation has been shown to require the coincident stimulation of D1 and NMDA receptors (Valjent, et al., 2000; Valjent, et al., 2005) and this appears to be controlled by Ras-guanine nucleotide-releasing factor 1 (Ras-GRF1), a neuronal specific activator of Ras-ERK signaling (Fasano et al., 2009). The relevance of this Ras-GRF1-mediated ERK signaling to reward and drug-induced plasticity is demonstrated by the observation that CPP and locomotor sensitization are significantly attenuated in Ras-GRF1-deficient mice, and conversely heightened in over-expressing transgenic animals (Fasano, et al., 2009).

More recently, the D1 or cocaine-mediated activation of ERK in striatal neurons was shown to depend on a potentiation of NMDA receptor function independent of cAMP (Pascoli et al., 2011). Furthermore, NMDA receptor potentiation involves tyrosine phosphorylation of the NR2B subunit via Src-family kinase (SFKs). This mechanism, demonstrated in cultured striatal neurons, was also observed *in vivo*, as ERK phosphorylation in response to D1 receptor agonism in mice was blocked by pretreatment with an NMDA receptor antagonist (Pascoli, et al., 2011). The observed potentiation by the D1 receptor potentially explains why NMDA receptor stimulation is required for ERK activation by cocaine and other drugs of abuse. As the authors suggest this mechanism has potentially significant functional consequences, providing a possible mechanism for facilitation of long-term potentiation of glutamatergic synapses in D1 receptor-expressing striatal neurons (Andre et al., 2010; Shen et al., 2008). This was evidenced by the attenuation of cocaine-induced sensitization and CPP upon inhibition of this D1R/Src family kinases/NR2B pathway (Pascoli, et al., 2011).

4.5 MAPK in reward learning and synaptic plasticity

As discussed earlier in this chapter, MAPK has been shown to be intimately involved in synaptic plasticity and hence learning and memory processes (for detailed review see G. M. Thomas, et al., 2004). As with fear-related disorders, drug addiction has been proposed by some to be a form of pathological learning (Berke, et al., 2000). Reinforcement as a concept is ultimately defined by learning processes – it is the strengthening of associations through experience. Reinforcement can also be viewed as a process that enhances memory consolidation for the association (Landauer, 1969). Either way, it is the “stamping in” (and hence learning) of associations that ultimately leads to reinforcement. The associations may manifest in a variety of ways: associations between a stimulus and a response (Thorndike, 1898), associations between a US and CS (I.P. Pavlov, 1927), or associations between a response and an outcome (Skinner, 1938). With repeated exposure drugs cause aberrant “stamping in” of associations between drug-taking, the resultant drug high and associated cues and contexts.

The striatum is a key structure which mediates instrumental (action-outcome) learning and it is glutamate-dopamine signaling which appears particularly important (Horvitz, 2002). Dopamine release in the dorsal striatum is thought to enable coding of action-outcome associations facilitating potentiation of corticostriatal synapses during learning (Di Filippo et al., 2009; Reynolds et al., 2001). The induction of corticostriatal LTP is dependent on the coincident activation of D1 and NMDA receptors (Calabresi et al., 1992; Kerr & Wickens, 2001; Reynolds, et al., 2001). Thus, corticostriatal LTP has been shown to require activation of ERK (Ferguson et al., 2006; Mazzucchelli et al., 2002; Sgambato et al., 1998) and indeed, the requirement for coincident activation of NMDA and D1 receptors for ERK activation has led some to propose that ERK functions to selectively induce plasticity at corticostriatal synapses during highly salient learning situations like reward learning, when coordinated dopamine and NMDA receptor activation is likely to occur (Girault, et al., 2007; Shiflett & Balleine, 2011).

The involvement of ERK in instrumental learning and corticostriatal LTP (Shiflett et al., 2010; G. M. Thomas, et al., 2004) suggests that ERK may play a role in the action-outcome learning which occurs with initial drug use. Indeed, a recent study showed that clinically relevant concentrations of ethanol dose-dependently attenuated the high frequency stimulation-induced LTP and ERK activation in the dorsomedial striatum (Xie et al., 2009). Relatively little is understood regarding synaptic plasticity in habit formation, but it may involve corticostriatal LTD (Shiflett, et al., 2011). The induction of LTD in the striatum was recently shown to require ERK activation (Cui, et al., 2011) which matches with findings regarding LTD in other brain regions such as hippocampus (Thiels et al., 2002). This recent study also showed that chronic ethanol intake by rats attenuated both ERK phosphorylation and LTD induction, whereas withdrawal for one day potentiated ERK phosphorylation and LTD induction (Cui, et al., 2011). By showing that chronic ethanol intake and withdrawal differentially altered synaptic plasticity in the dorsolateral striatum through the ERK signaling pathway, these findings suggest that chronic alcohol use may impact on the habit-formation process, and this neural maladaptation may consequently lead to habitual drug-seeking behavior (Cui, et al., 2011).

Persistent vulnerability to relapse in addiction is thought to be a result of a specific impairment in LTD at cortico-accumbal synapses (Kasanetz et al. 2010; Martin et al., 2006; Moussawi et al., 2009). In addition to LTD, cortico-accumbal LTP is abolished *in vivo* after withdrawal from cocaine self-administration (Knackstedt et al., 2010; Moussawi, et al., 2009). The loss of both LTP and LTD *in vivo* after cocaine self-administration has led to the hypothesis that the inability of the medium spiny neurons in the NAc core to undergo bidirectional prefrontal-induced neuroplasticity may reduce the capacity of drug addicts to adaptively organize a behavioral response that incorporates environmental contingencies that mediate against relapse (Kalivas, 2009; M. J. Thomas, et al., 2008). The following section will describe the literature supporting a role for ERK in relapse to drug-seeking behavior.

4.6 The involvement of MAPK in drug-seeking behavior

The operant self-administration procedure is a widely accepted model to study drug reinforcement and drug-seeking because it has both face and construct validity (Feltenstein & See, 2008). In the operant paradigm the animal is trained to perform an instrumental task to self-administer a drug (generally by pressing a lever or via a nose-poke) for a period of

time, thereby developing an action-outcome association between the operant task and the ensuing reward (conditioned response). Rodents will readily self-administer most drugs of abuse, including cocaine (R. M. Brown & Lawrence, 2009; McPherson et al., 2010). Accordingly, the abuse potential of a substance in humans can be predicted from self-administration in rodents (Collins et al., 1984). Moreover, similar to the laboratory model of anxiety disorders, the self-administration paradigm can incorporate a Pavlovian conditioning component by introducing CSs that signal drug delivery to represent cues/contexts that are critically related to relapse of drug-seeking (Adams et al., 2010; Jupp et al., 2011).

4.6.1 The role of MAPK in the reinstatement model of drug-seeking

As with extinction of fear, the reinforced response (e.g. lever press) can be subsequently extinguished whereby no drug is administered despite the animal responding on the drug-paired manipulandum. Responding will typically spike in what has been termed an 'extinction burst' and then gradually decline to a level whereby the behavior is deemed extinguished. Extinction learning is an active process that reduces the value or salience of these conditioned cues and contexts. Longer and repeated cue/contextual re-exposure without the associated fear or drug experience reduces conditioned responding. Extinction learning can be effective for reducing cue- and context-induced symptoms in addiction and anxiety and can improve outcomes in these disorders. Reinstatement of drug-seeking behavior as measured by responding on the drug-paired manipulandum can be precipitated in a number of ways: with non-contingent 'priming' injections of the drug (Stretch et al., 1971), stressors (Carroll, 1985; Shaham & Stewart, 1996), and drug-associated cues (W. M. Davis & Smith, 1976). In humans, drug craving and relapse have shown to be triggered by similar factors (S. A. Brown, et al., 1995; C. O'Brien, et al., 1992), giving this model an element of construct validity. This model also exhibits predictive validity, as drugs such as naltrexone that decrease relapse in abstinent drug users also decrease reinstatement of drug seeking behavior in rats (Comer et al., 2006)

As mentioned previously, drug-associated cues induce ERK phosphorylation in components of the mesocorticolimbic system (L. Lu et al., 2005; Miller, et al., 2005) and ERK has been shown to play a role in drug-induced and synaptic plasticity (G. M. Thomas, et al., 2004; Valjent, Corvol, et al., 2006) thus it is not surprising that evidence exist that support a role for ERK in cue-elicited reinstatement of drug-seeking. Cue-induced reinstatement of alcohol-seeking is associated with enhanced activation of ERK in the BLA and NAc shell (Schroeder et al., 2008). The mGlu5 receptor antagonist 2-Methyl-6-(phenylethynyl)pyridine (MPEP) was shown to block both the cue-induced drug-seeking and the associated increase in phospho-ERK (Schroeder, et al., 2008), indicating that ERK activation is dependent on mGlu5 receptor signalling and plays a key role in the effect of MPEP on drug-seeking.

A recent study demonstrated a critical role for ERK in the ability of BDNF infusions in the dmPFC to attenuate reinstatement of both cue-induced and primed cocaine-seeking, as well as cocaine-seeking following abstinence (Whitfield et al., 2011). Vehicle-infused rats showed significant decreases in phospho-ERK and CREB at the end of the cocaine self-administration period that were reversed by intra-dmPFC BDNF. This ability of BDNF to reverse cocaine-mediated decreases in ERK and CREB phosphorylation was blocked by both the MEK inhibitor U0126 and the tyrosine kinase inhibitor K252a, thus demonstrating the

behavioral effects of BDNF were most likely mediated via a TrkB receptor-ERK pathway. This study elucidates a mechanism whereby BDNF/TrkB activates ERK-regulated CREB phosphorylation in the dmPFC to counteract the neuroadaptations induced by cocaine self-administration and subsequent relapse to cocaine-seeking (Whitfield, et al., 2011).

There are no studies as yet which have specifically examined the role of ERK in extinction learning related to operant responding. There is one CPP study that showed heightened ERK activation in the amygdala following extinction of cocaine-induced CPP (Chen & Xu, 2010), which is consistent with fear extinction literature showing a key role for the amygdala (Myers, et al., 2007). In addition, dopamine D3 receptor mutant mice showed delayed CPP extinction compared with wild-type mice and this was associated with sustained ERK activation in the NAc and PFC (Chen, et al., 2010). This suggests that extinction of cocaine conditioning involves dopamine D3 receptor/ERK signaling though this finding is not necessarily readily generalized to other drugs of abuse as the MEK inhibitor SL327 has been shown to have no effect on extinction of an ethanol-induced CPP in mice (Grobowski, Franken et al., 2011).

4.6.2 The role of MAPK in the abstinence model of relapse and incubation of craving

Of the millions of people suffering from addiction, only a small proportion actually seek treatment in a formal setting. For example, in 2009 out of 23.5 million Americans who were considered to have an illicit drug or alcohol use problem, only 2.6 million (11.2%) received treatment at a specialty facility (SAMHSA, 2010). In this sense then, the reinstatement model of relapse is only relevant to those addicts who engage in new learning to overcome their addiction. The bulk of drug dependent individuals will attempt to overcome their addiction by simply attempting to abstain from drug use (also known as going 'cold turkey'). Therefore another relevant model of relapse is one that measures drug-seeking following a period of abstinence.

In 1986 it was suggested that in humans, craving induced by drug cues actually increases over the first several weeks of the withdrawal period and remains elevated over extended periods of abstinence (Gawin & Kleber, 1986). Scientists have since discovered an analogous phenomenon in laboratory animals which has been termed 'incubation of craving'. It has been demonstrated with opiates (Shalev et al., 2001), psychostimulants (Grimm, et al., 2001; Shepard et al., 2004) and ethanol (Bienkowski et al., 2004), that cue-induced drug-seeking increases over time over the first months of withdrawal. This phenomenon has also been observed with the natural reinforcer sucrose (Grimm et al., 2002), though in this case the effect peaks after 1–4 weeks, but decays after 2 months, while for cocaine, the responding peaks after 1 month and persists for up to 3 months in rats (L. Lu et al., 2004). In humans, this incubation of drug craving ultimately results in an increased propensity to relapse as a function of time spent in withdrawal. A more appropriate paradigm for this sort of relapse behavior is a modification of the reinstatement model known as the abstinence model (e.g. Brown et al., 2009; Lu et al., 2009; reviewed in Reichel and Bevins, 2009). After a period of self-administration the animal undergoes withdrawal for a period of time (typically a number of weeks) in the home cage. At the end of this period drug-seeking behavior is measured by re-introducing the subject to the operant chamber and measuring responding under extinction conditions (i.e. in the absence of drug).

Numerous cellular and molecular changes are produced by chronic drug use, which are thought to regulate drug-seeking behavior, yet only a small number thus far have been shown to be associated with time-dependent increases in drug-seeking behavior. One of these neuroadaptations is activation of ERK. Studies by Lu and colleagues have revealed that it is ERK phosphorylation in the CeA, but not BLA, that underpins incubation of cocaine and opiate craving as inhibition of CeA, but not BLA, ERK phosphorylation decreases drug-seeking after 30 days of drug withdrawal but not after 1 day of withdrawal (Li et al., 2008; L. Lu, et al., 2005). After 30 withdrawal days, inhibition of CeA ERK phosphorylation by U0126 or the NMDA receptor antagonist AP-5 decreased cue-induced cocaine seeking.

Subsequently, other studies have shown a similar enhancement of ERK phosphorylation in the NAc core (Edwards et al., 2011) and mPFC (Koya et al., 2008) as a result of exposure to a cocaine-paired context after extended abstinence in animals that had previously self-administered cocaine. In the former study, the activation of ERK was found only in rats that had self-administered cocaine and not their yoked controls, demonstrating the effect was not merely the result of cocaine exposure (Edwards, et al., 2011). Though the precise role of increased ERK activity in the NAc and mPFC in incubation of craving remains to be investigated, a key role for ERK in the amygdala has been found in Pavlovian fear conditioning and memory reconsolidation (Nader et al., 2000). ERK in the amygdala also appears to be critically involved in the long-term neuroadaptations which result in heightened reactivity to drug-associated cues. This is supported by the observation that re-exposure to an ethanol-associated context and discrete cues following abstinence increases activation of ERK in the BLA and ventral lateral amygdala (Radwanska et al., 2008). Collectively, these findings indicate that activation of ERK, particularly in the amygdala appears to be a key substrate for incubation of drug craving.

5. Concluding remarks

The studies highlighted in the present chapter clearly demonstrate the learning and memory principles underlying fear and drug-seeking behavior. Additionally, drug addiction has been emphasized as a brain disorder that involves enduring neuroadaptations that alter signal transduction within neurons as well as electrical and chemical communications between neurons and neural structures. Considering the significance of the MAPK cascade in cellular growth, differentiation and neuronal survivability (Ahn & Krebs, 1990; Hoshi et al., 1988; Impey et al., 1999; Kornhauser & Greenberg, 1997), perhaps it is not surprising that MAPK signalling also plays a seemingly important role in both the acquisition and treatment of anxiety and drug-seeking behavior as pointed out in the present chapter. Given the prevalence and financial burden of anxiety disorders and drug addiction, we propose that further research into the MAPK signalling cascade could be a fruitful strategy in finding effective treatments for anxiety disorders and drug addiction.

6. References

- Adams, C. L., Short, J. L., & Lawrence, A. J. (2010). Cue-conditioned alcohol seeking in rats following abstinence: Involvement of metabotropic glutamate 5 receptors. *Br J Pharmacol*, 159, 534-542.

- Agranoff, B. W., & Siegel, G. J. (1999). *Basic neurochemistry: Molecular, cellular, and medical aspects*. Philadelphia: Lippincott-Raven.
- Aguilar, M. A., Rodriguez-Arias, M., & Minarro, J. (2009). Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res Rev*, 59, 253-277.
- Ahi, J., Radulovic, J., & Spiess, J. (2004). The role of hippocampal signaling cascades in consolidation of fear memory. *Behav Brain Res*, 149, 17-31.
- Ahn, N. G., & Krebs, E. G. (1990). Evidence for an epidermal growth factor-stimulated protein kinase cascade in swiss 3t3 cells. Activation of serine peptide kinase activity by myelin basic protein kinases in vitro. *J Biol Chem*, 265, 11495-11501.
- Alberi, L., Liu, S., Wang, Y., Badie, R., Smith-Hicks, C., Wu, J., et al. (2011). Activity-induced notch signaling in neurons requires arc/arg3.1 and is essential for synaptic plasticity in hippocampal networks. *Neuron*, 69, 437-444.
- Alexander, G. E., DeLong, M. R., & Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu Rev Neurosci*, 9, 357-381.
- Andre, V. M., Cepeda, C., Cummings, D. M., Jocoy, E. L., Fisher, Y. E., William Yang, X., et al. (2010). Dopamine modulation of excitatory currents in the striatum is dictated by the expression of d1 or d2 receptors and modified by endocannabinoids. *Eur J Neurosci*, 31, 14-28.
- Apergis-Schoute, A. M., Debiec, J., Doyere, V., LeDoux, J. E., & Schafe, G. E. (2005). Auditory fear conditioning and long-term potentiation in the lateral amygdala require erk/map kinase signaling in the auditory thalamus: A role for presynaptic plasticity in the fear system. *J Neurosci*, 25, 5730-5739.
- Ascher, P., & Nowak, L. (1986). A patch-clamp study of excitatory amino acid activated channels. *Adv Exp Med Biol*, 203, 507-511.
- American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed.). Washington DC: Author.
- Atkins, C. M., Selcher, J. C., Petraitis, J. J., Trzaskos, J. M., & Sweatt, J. D. (1998). The mapk cascade is required for mammalian associative learning. *Nat Neurosci*, 1, 602-609.
- Bachtell, R. K., Tsivkovskaia, N. O., & Ryabinin, A. E. (2002). Alcohol-induced c-fos expression in the edinger-westphal nucleus: Pharmacological and signal transduction mechanisms. *J Pharmacol Exp Ther*, 302, 516-524.
- Bailey, D. J., Kim, J. J., Sun, W., Thompson, R. F., & Helmstetter, F. J. (1999). Acquisition of fear conditioning in rats requires the synthesis of mrna in the amygdala. *Behav Neurosci*, 113, 276-282.
- Baker, J. D., & Azorlosa, J. L. (1996). The nmda antagonist mk-801 blocks the extinction of pavlovian fear conditioning. *Behav Neurosci*, 110, 618-620.
- Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference: What does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)*, 153, 31-43.
- Belcheva, M. M., Vogel, Z., Ignatova, E., Avidor-Reiss, T., Zippel, R., Levy, R., et al. (1998). Opioid modulation of extracellular signal-regulated protein kinase activity is ras-dependent and involves gbetagamma subunits. *J Neurochem*, 70, 635-645.
- Benavides, D. R., & Bibb, J. A. (2004). Role of cdk5 in drug abuse and plasticity. *Ann N Y Acad Sci*, 1025, 335-344.
- Berhow, M. T., Hiroi, N., & Nestler, E. J. (1996). Regulation of erk (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat

- mesolimbic dopamine system by chronic exposure to morphine or cocaine. *J Neurosci*, 16, 4707-4715.
- Berke, J. D., & Hyman, S. E. (2000). Addiction, dopamine, and the molecular mechanisms of memory. *Neuron*, 25, 515-532.
- Berman, D. E., & Dudai, Y. (2001). Memory extinction, learning anew, and learning the new: Dissociations in the molecular machinery of learning in cortex. *Science*, 291, 2417-2419.
- Bienkowski, P., Rogowski, A., Korkosz, A., Mierzejewski, P., Radwanska, K., Kaczmarek, L., et al. (2004). Time-dependent changes in alcohol-seeking behavior during abstinence. *Eur Neuropsychopharmacol*, 14, 355-360.
- Bilbao, A., Parkitna, J. R., Engblom, D., Perreau-Lenz, S., Sanchis-Segura, C., Schneider, M., et al. (2008). Loss of the ca²⁺/calmodulin-dependent protein kinase type iv in dopaminergic neurons enhances behavioral effects of cocaine. *Proc Natl Acad Sci U S A*, 105, 17549-17554.
- Blanchard, D. C., & Blanchard, R. J. (1972). Innate and conditioned reactions to threat in rats with amygdaloid lesions. *J Comp Physiol Psychol*, 81, 281-290.
- Bliss, T. V., & Collingridge, G. L. (1993). A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature*, 361, 31-39.
- Boudreau, A. C., Reimers, J. M., Milovanovic, M., & Wolf, M. E. (2007). Cell surface ampa receptors in the rat nucleus accumbens increase during cocaine withdrawal but internalize after cocaine challenge in association with altered activation of mitogen-activated protein kinases. *J Neurosci*, 27, 10621-10635.
- Boulis, N. M., & Davis, M. (1989). Footshock-induced sensitization of electrically elicited startle reflexes. *Behav Neurosci*, 103, 504-508.
- Bouton, M. E. (2002). Context, ambiguity, and unlearning: Sources of relapse after behavioral extinction. *Biol Psychiatry*, 52, 976-986.
- Bouton, M. E., Mineka, S., & Barlow, D. H. (2001). A modern learning theory perspective on the etiology of panic disorder. *Psychol Rev*, 108, 4-32.
- Bouton, M. E., Westbrook, R. F., Corcoran, K. A., & Maren, S. (2006). Contextual and temporal modulation of extinction: Behavioral and biological mechanisms. *Biol Psychiatry*, 60, 352-360.
- Brown, R. M., & Lawrence, A. J. (2009). Neurochemistry underlying relapse to opiate seeking behavior. *Neurochem Res*, 34, 1876-1887.
- Brown, R. M., Short, J. L., Cowen, M. S., Ledent, C., & Lawrence, A. J. (2009). A differential role for the adenosine a_{2a} receptor in opiate reinforcement vs opiate-seeking behavior. *Neuropsychopharmacology*, 34, 844-856.
- Brown, S. A., Vik, P. W., Patterson, T. L., Grant, I., & Schuckit, M. A. (1995). Stress, vulnerability and adult alcohol relapse. *J Stud Alcohol*, 56, 538-545.
- Calabresi, P., Pisani, A., Mercuri, N. B., & Bernardi, G. (1992). Long-term potentiation in the striatum is unmasked by removing the voltage-dependent magnesium block of nmda receptor channels. *Eur J Neurosci*, 4, 929-935.
- Campbell, B. A., & Jaynes, J. (1966). Reinstatement. *Psychological review*, 73, 478-480.
- Campbell, B. A., & Spear, N. E. (1972). Ontogeny of memory. *Psychological review*, 79, 215-236.

- Campeau, S., Hayward, M. D., Hope, B. T., Rosen, J. B., Nestler, E. J., & Davis, M. (1991). Induction of the c-fos proto-oncogene in rat amygdala during unconditioned and conditioned fear. *Brain Res*, 565, 349-352.
- Carr, D. B., & Sesack, S. R. (2000). Projections from the rat prefrontal cortex to the ventral tegmental area: Target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J Neurosci*, 20, 3864-3873.
- Carroll, M. E. (1985). The role of food deprivation in the maintenance and reinstatement of cocaine-seeking behavior in rats. *Drug Alcohol Depend*, 16, 95-109.
- Chandler, L. J., & Sutton, G. (2005). Acute ethanol inhibits extracellular signal-regulated kinase, protein kinase b, and adenosine 3':5'-cyclic monophosphate response element binding protein activity in an age- and brain region-specific manner. *Alcohol Clin Exp Res*, 29, 672-682.
- Chen, L., & Xu, M. (2010). Dopamine d1 and d3 receptors are differentially involved in cue-elicited cocaine seeking. *J Neurochem*, 114, 530-541.
- Chhatwal, J. P., Myers, K. M., Ressler, K. J., & Davis, M. (2005). Regulation of gephyrin and gabaa receptor binding within the amygdala after fear acquisition and extinction. *J Neurosci*, 25, 502-506.
- Childress, A., Ehrman, R., McLellan, A. T., & O'Brien, C. (1988). Conditioned craving and arousal in cocaine addiction: A preliminary report. *NIDA Res Monogr*, 81, 74-80.
- Childress, A. R., McLellan, A. T., & O'Brien, C. P. (1986). Role of conditioning factors in the development of drug dependence. *Psychiatr Clin North Am*, 9, 413-425.
- Choe, E. S., Chung, K. T., Mao, L., & Wang, J. Q. (2002). Amphetamine increases phosphorylation of extracellular signal-regulated kinase and transcription factors in the rat striatum via group i metabotropic glutamate receptors. *Neuropsychopharmacology*, 27, 565-575.
- Choe, E. S., & McGinty, J. F. (2001). Cyclic amp and mitogen-activated protein kinases are required for glutamate-dependent cyclic amp response element binding protein and elk-1 phosphorylation in the dorsal striatum in vivo. *J Neurochem*, 76, 401-412.
- Chotiner, J. K., Nielson, J., Farris, S., Lewandowski, G., Huang, F., Banos, K., et al. (2010). Assessment of the role of map kinase in mediating activity-dependent transcriptional activation of the immediate early gene arc/arg3.1 in the dentate gyrus in vivo. *Learn Mem*, 17, 117-129.
- Collins, R. J., Weeks, J. R., Cooper, M. M., Good, P. I., & Russell, R. R. (1984). Prediction of abuse liability of drugs using iv self-administration by rats. *Psychopharmacology (Berl)*, 82, 6-13.
- Comer, S. D., Sullivan, M. A., Yu, E., Rothenberg, J. L., Kleber, H. D., Kampman, K., et al. (2006). Injectable, sustained-release naltrexone for the treatment of opioid dependence: A randomized, placebo-controlled trial. *Arch Gen Psychiatry*, 63, 210-218.
- Corcoran, K. A., & Maren, S. (2001). Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. *J Neurosci*, 21, 1720-1726.
- Crombag, H. S., Bossert, J. M., Koya, E., & Shaham, Y. (2008). Review. Context-induced relapse to drug seeking: A review. *Philos Trans R Soc Lond B Biol Sci*, 363, 3233-3243.

- Cui, S. Z., Wang, S. J., Li, J., Xie, G. Q., Zhou, R., Chen, L., et al. (2011). Alteration of synaptic plasticity in rat dorsal striatum induced by chronic ethanol intake and withdrawal via erk pathway. *Acta Pharmacol Sin*, 32, 175-181.
- Dackis, C. A., & O'Brien, C. P. (2001). Cocaine dependence: A disease of the brain's reward centers. *J Subst Abuse Treat*, 21, 111-117.
- Davis, M. (2000). The role of the amygdala in conditioned and unconditioned fear and anxiety. In J. P. Aggleton (Ed.), *The amygdala* (Vol. 2, pp. 213-287). Oxford, UK: Oxford University Press.
- Davis, M., Falls, W. A., Campeau, S., & Kim, M. (1993). Fear-potentiated startle: A neural and pharmacological analysis. *Behav Brain Res*, 58, 175-198.
- Davis, M., Myers, K. M., Chhatwal, J., & Ressler, K. J. (2006). Pharmacological treatments that facilitate extinction of fear: Relevance to psychotherapy. *NeuroRx*, 3, 82-96.
- Davis, M., & Whalen, P. J. (2001). The amygdala: Vigilance and emotion. *Molecular psychiatry*, 6, 13-34.
- Davis, S., Salin, H., Helme-Guizon, A., Dumas, S., Stephan, A., Corbex, M., et al. (2000). Dysfunctional regulation of alphacamkii and syntaxin 1b transcription after induction of ltp in the aged rat. *Eur J Neurosci*, 12, 3276-3282.
- Davis, S., Vanhoutte, P., Pages, C., Caboche, J., & Laroche, S. (2000). The mapk/erk cascade targets both elk-1 and camp response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. *J Neurosci*, 20, 4563-4572.
- Davis, W. M., & Smith, S. G. (1976). Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. *Pavlov J Biol Sci*, 11, 222-236.
- DeJong, W. (1994). Relapse prevention: An emerging technology for promoting long-term drug abstinence. *Int J Addict*, 29, 681-705.
- Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science*, 305, 1014-1017.
- Di Benedetto, B., Kallnik, M., Weisenhorn, D. M., Falls, W. A., Wurst, W., & Holter, S. M. (2009). Activation of erk/mapk in the lateral amygdala of the mouse is required for acquisition of a fear-potentiated startle response. *Neuropsychopharmacology*, 34, 356-366.
- Di Chiara, G. (2002). Nucleus accumbens shell and core dopamine: Differential role in behavior and addiction. *Behav Brain Res*, 137, 75-114.
- Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci U S A*, 85, 5274-5278.
- Di Filippo, M., Picconi, B., Tantucci, M., Ghiglieri, V., Bagetta, V., Sgobio, C., et al. (2009). Short-term and long-term plasticity at corticostriatal synapses: Implications for learning and memory. *Behav Brain Res*, 199, 108-118.
- Edwards, S., Bachtell, R. K., Guzman, D., Whisler, K. N., & Self, D. W. (2011). Emergence of context-associated glur(1) and erk phosphorylation in the nucleus accumbens core during withdrawal from cocaine self-administration. *Addict Biol*, 16, 450-457.
- English, J. D., & Sweatt, J. D. (1997). A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. *The Journal of biological chemistry*, 272, 19103-19106.

- Farb, C., Aoki, C., Milner, T., Kaneko, T., & LeDoux, J. (1992). Glutamate immunoreactive terminals in the lateral amygdaloid nucleus: A possible substrate for emotional memory. *Brain Res*, 593, 145-158.
- Fasano, S., D'Antoni, A., Orban, P. C., Valjent, E., Putignano, E., Vara, H., et al. (2009). Ras-guanine nucleotide-releasing factor 1 (ras-grf1) controls activation of extracellular signal-regulated kinase (erk) signaling in the striatum and long-term behavioral responses to cocaine. *Biol Psychiatry*, 66, 758-768.
- Favata, M. F., Horiuchi, K. Y., Manos, E. J., Daulerio, A. J., Stradley, D. A., Feeser, W. S., et al. (1998). Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J Biol Chem*, 273, 18623-18632.
- Feltenstein, M. W., & See, R. E. (2008). The neurocircuitry of addiction: An overview. *Br J Pharmacol*, 154, 261-274.
- Ferguson, S. M., Fasano, S., Yang, P., Brambilla, R., & Robinson, T. E. (2006). Knockout of erk1 enhances cocaine-evoked immediate early gene expression and behavioral plasticity. *Neuropsychopharmacology*, 31, 2660-2668.
- Fischer, A., Radulovic, M., Schrick, C., Sananbenesi, F., Godovac-Zimmermann, J., & Radulovic, J. (2007). Hippocampal mek/erk signaling mediates extinction of contextual freezing behavior. *Neurobiol Learn Mem*, 87, 149-158.
- Fletcher, B. R., Calhoun, M. E., Rapp, P. R., & Shapiro, M. L. (2006). Fornix lesions decouple the induction of hippocampal arc transcription from behavior but not plasticity. *J Neurosci*, 26, 1507-1515.
- Frey, U., Huang, Y. Y., & Kandel, E. R. (1993). Effects of camp simulate a late stage of ltp in hippocampal ca1 neurons. *Science*, 260, 1661-1664.
- Frey, U., Krug, M., Reymann, K. G., & Matthies, H. (1988). Anisomycin, an inhibitor of protein synthesis, blocks late phases of ltp phenomena in the hippocampal ca1 region in vitro. *Brain Res*, 452, 57-65.
- Fuchs, R. A., Eaddy, J. L., Su, Z. I., & Bell, G. H. (2007). Interactions of the basolateral amygdala with the dorsal hippocampus and dorsomedial prefrontal cortex regulate drug context-induced reinstatement of cocaine-seeking in rats. *Eur J Neurosci*, 26, 487-498.
- Fuchs, R. A., Evans, K. A., Ledford, C. C., Parker, M. P., Case, J. M., Mehta, R. H., et al. (2005). The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology*, 30, 296-309.
- Gale, G. D., Anagnostaras, S. G., Godsil, B. P., Mitchell, S., Nozawa, T., Sage, J. R., et al. (2004). Role of the basolateral amygdala in the storage of fear memories across the adult lifetime of rats. *J Neurosci*, 24, 3810-3815.
- Gawin, F. H., & Kleber, H. D. (1986). Abstinence symptomatology and psychiatric diagnosis in cocaine abusers. Clinical observations. *Arch Gen Psychiatry*, 43, 107-113.
- Gerdjikov, T. V., Ross, G. M., & Beninger, R. J. (2004). Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of erk or p38 map kinases in rats. *Behav Neurosci*, 118, 740-750.
- Giovannini, M. G., Efoudebe, M., Passani, M. B., Baldi, E., Bucherelli, C., Giachi, F., et al. (2003). Improvement in fear memory by histamine-elicited erk2 activation in hippocampal ca3 cells. *J Neurosci*, 23, 9016-9023.

- Girault, J. A., Valjent, E., Caboche, J., & Herve, D. (2007). Erk2: A logical and gate critical for drug-induced plasticity? *Curr Opin Pharmacol*, 7, 77-85.
- Gossop, M., Green, L., Phillips, G., & Bradley, B. (1989). Lapse, relapse and survival among opiate addicts after treatment. A prospective follow-up study. *Br J Psychiatry*, 154, 348-353.
- Grant, S., London, E. D., Newlin, D. B., Villemagne, V. L., Liu, X., Contoreggi, C., et al. (1996). Activation of memory circuits during cue-elicited cocaine craving. *Proc Natl Acad Sci U S A*, 93, 12040-12045.
- Greenberg, P. E., Sisitsky, T., Kessler, R. C., Finkelstein, S. N., Berndt, E. R., Davidson, J. R., et al. (1999). The economic burden of anxiety disorders in the 1990s. *J Clin Psychiatry*, 60, 427-435.
- Greengard, P., Allen, P. B., & Nairn, A. C. (1999). Beyond the dopamine receptor: The darpp-32/protein phosphatase-1 cascade. *Neuron*, 23, 435-447.
- Gresack, J. E., Schafe, G. E., Orr, P. T., & Frick, K. M. (2009). Sex differences in contextual fear conditioning are associated with differential ventral hippocampal extracellular signal-regulated kinase activation. *Neuroscience*, 159, 451-467.
- Grillon, C., Baas, J. P., Lissek, S., Smith, K., & Milstein, J. (2004). Anxious responses to predictable and unpredictable aversive events. *Behav Neurosci*, 118, 916-924.
- Grimm, J. W., Hope, B. T., Wise, R. A., & Shaham, Y. (2001). Neuroadaptation. Incubation of cocaine craving after withdrawal. *Nature*, 412, 141-142.
- Grimm, J. W., Shaham, Y., & Hope, B. T. (2002). Effect of cocaine and sucrose withdrawal period on extinction behavior, cue-induced reinstatement, and protein levels of the dopamine transporter and tyrosine hydroxylase in limbic and cortical areas in rats. *Behav Pharmacol*, 13, 379-388.
- Groblewski, P. A., Franken, F. H., & Cunningham, C. L. (2011). Inhibition of extracellular signal-regulated kinase (erk) activity with sl327 does not prevent acquisition, expression, and extinction of ethanol-seeking behavior in mice. *Behav Brain Res*, 217, 399-407.
- Groblewski, P. A., Ryabinin, A. E., & Cunningham, C. L. (2011). Activation and role of the medial prefrontal cortex (mpfc) in extinction of ethanol-induced associative learning in mice. *Neurobiol Learn Mem*.
- Guzowski, J. F., Lyford, G. L., Stevenson, G. D., Houston, F. P., McGaugh, J. L., Worley, P. F., et al. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *J Neurosci*, 20, 3993-4001.
- Harwood, H. J., Fountain, D., & Livermore, G. (1998). Economic costs of alcohol abuse and alcoholism. *Recent Dev Alcohol*, 14, 307-330.
- Herry, C., & Garcia, R. (2002). Prefrontal cortex long-term potentiation, but not long-term depression, is associated with the maintenance of extinction of learned fear in mice. *J Neurosci*, 22, 577-583.
- Herry, C., Trifilieff, P., Micheau, J., Luthi, A., & Mons, N. (2006). Extinction of auditory fear conditioning requires mapk/erk activation in the basolateral amygdala. *The European journal of neuroscience*, 24, 261-269.
- Hofmann, S. G. (2007). Enhancing exposure-based therapy from a translational research perspective. *Behav Res Ther*, 45, 1987-2001.

- Horvitz, J. C. (2002). Dopamine gating of glutamatergic sensorimotor and incentive motivational input signals to the striatum. *Behav Brain Res*, 137, 65-74.
- Hoshi, M., Nishida, E., & Sakai, H. (1988). Activation of a ca^{2+} -inhibitable protein kinase that phosphorylates microtubule-associated protein 2 in vitro by growth factors, phorbol esters, and serum in quiescent cultured human fibroblasts. *J Biol Chem*, 263, 5396-5401.
- Huang, Y. Y., & Kandel, E. R. (1994). Recruitment of long-lasting and protein kinase a-dependent long-term potentiation in the ca1 region of hippocampus requires repeated tetanization. *Learn Mem*, 1, 74-82.
- Huang, Y. Y., Martin, K. C., & Kandel, E. R. (2000). Both protein kinase a and mitogen-activated protein kinase are required in the amygdala for the macromolecular synthesis-dependent late phase of long-term potentiation. *J Neurosci*, 20, 6317-6325.
- Hugues, S., Chessel, A., Lena, I., Marsault, R., & Garcia, R. (2006). Prefrontal infusion of pd098059 immediately after fear extinction training blocks extinction-associated prefrontal synaptic plasticity and decreases prefrontal erk2 phosphorylation. *Synapse*, 60, 280-287.
- Hugues, S., Deschaux, O., & Garcia, R. (2004). Postextinction infusion of a mitogen-activated protein kinase inhibitor into the medial prefrontal cortex impairs memory of the extinction of conditioned fear. *Learn Mem*, 11, 540-543.
- Hurd, Y. L., & Herkenham, M. (1993). Molecular alterations in the neostriatum of human cocaine addicts. *Synapse*, 13, 357-369.
- Hyman, S. E. (2005). Addiction: A disease of learning and memory. *Am J Psychiatry*, 162, 1414-1422.
- Impey, S., Obrietan, K., & Storm, D. R. (1999). Making new connections: Role of erk/map kinase signaling in neuronal plasticity. *Neuron*, 23, 11-14.
- Izquierdo, I., & Medina, J. H. (1993). Role of the amygdala, hippocampus and entorhinal cortex in memory consolidation and expression. *Braz J Med Biol Res*, 26, 573-589.
- Izquierdo, L. A., Barros, D. M., Ardenghi, P. G., Pereira, P., Rodrigues, C., Choi, H., et al. (2000). Different hippocampal molecular requirements for short- and long-term retrieval of one-trial avoidance learning. *Behav Brain Res*, 111, 93-98.
- Jenab, S., Festa, E. D., Nazarian, A., Wu, H. B., Sun, W. L., Hazim, R., et al. (2005). Cocaine induction of erk proteins in dorsal striatum of fischer rats. *Brain Res Mol Brain Res*, 142, 134-138.
- Jentsch, J. D., & Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: Implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl)*, 146, 373-390.
- Jupp, B., Krstew, E., Dezsi, G., & Lawrence, A. (2011). Discrete cue-conditioned alcohol-seeking after protracted abstinence: Pattern of neural activation and involvement of orexin(1) receptors. *Br J Pharmacol*, 162, 880-889.
- Jupp, B., & Lawrence, A. J. (2010). New horizons for therapeutics in drug and alcohol abuse. *Pharmacol Ther*, 125, 138-168.
- Juraska, J. M. (1998). Neural plasticity and the development of sex differences. *Annu Rev Sex Res*, 9, 20-38.
- Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci*, 10, 561-572.

- Kalivas, P. W., & O'Brien, C. (2008). Drug addiction as a pathology of staged neuroplasticity. *Neuropsychopharmacology*, *33*, 166-180.
- Kalivas, P. W., & Volkow, N. D. (2005). The neural basis of addiction: A pathology of motivation and choice. *Am J Psychiatry*, *162*, 1403-1413.
- Kandel, E. R. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. *Science*, *294*, 1030-1038.
- Kandel, E. R. (2009). The biology of memory: A forty-year perspective. *J Neurosci*, *29*, 12748-12756.
- Kasanez, F., Deroche-Gamonet, V., Berson, N., Balado, E., Lafourcade, M., Manzoni, O., et al. Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science*, *328*, 1709-1712.
- Kauer, J. A., & Malenka, R. C. (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci*, *8*, 844-858.
- Kelleher, R. J., 3rd, Govindarajan, A., Jung, H. Y., Kang, H., & Tonegawa, S. (2004). Translational control by mapk signaling in long-term synaptic plasticity and memory. *Cell*, *116*, 467-479.
- Kelley, A. E. (2004). Memory and addiction: Shared neural circuitry and molecular mechanisms. *Neuron*, *44*, 161-179.
- Kerr, J. N., & Wickens, J. R. (2001). Dopamine d-1/d-5 receptor activation is required for long-term potentiation in the rat neostriatum in vitro. *J Neurophysiol*, *85*, 117-124.
- Kessler, R. C., Chiu, W. T., Demler, O., Merikangas, K. R., & Walters, E. E. (2005). Prevalence, severity, and comorbidity of 12-month dsm-iv disorders in the national comorbidity survey replication. *Arch Gen Psychiatry*, *62*, 617-627.
- Kim, J. H., Hamlin, A. S., & Richardson, R. (2009). Fear extinction across development: The involvement of the medial prefrontal cortex as assessed by temporary inactivation and immunohistochemistry. *J Neurosci*, *29*, 10802-10808.
- Kim, J. H., Li, S., Hamlin, A. S., McNally, G. P., & Richardson, R. (2012). Phosphorylation of mitogen-activated protein kinase in the medial prefrontal cortex and the amygdala following memory retrieval or forgetting in developing rats. *Neurobiol Learn Mem*, doi:10.1016/j.nlm.2011.09.005.
- Kim, J. H., Li, S., & Richardson, R. (2011). Immunohistochemical analyses of long-term extinction of conditioned fear in adolescent rats. *Cereb Cortex*, *21*, 530-538.
- Kim, J. H., & Richardson, R. (2007a). A developmental dissociation in reinstatement of an extinguished fear response in rats. *Neurobiol Learn Mem*, *88*, 48-57.
- Kim, J. H., & Richardson, R. (2007b). Immediate post-reminder injection of gamma-aminobutyric acid (gaba) agonist midazolam attenuates reactivation of forgotten fear in the infant rat. *Behav Neurosci*, *121*, 1328-1332.
- Kim, J. H., & Richardson, R. (2010). New findings on extinction of conditioned fear early in development: Theoretical and clinical implications. *Biol Psychiatry*, *67*, 297-303.
- Knackstedt, L. A., Moussawi, K., Lalumiere, R., Schwendt, M., Klugmann, M., & Kalivas, P. W. (2010). Extinction training after cocaine self-administration induces glutamatergic plasticity to inhibit cocaine seeking. *J Neurosci*, *30*, 7984-7992.
- Konorski, J. (1948). *Conditioned reflexes and neuron organization*. Cambridge: University Press.
- Kornhauser, J. M., & Greenberg, M. E. (1997). A kinase to remember: Dual roles for map kinase in long-term memory. *Neuron*, *18*, 839-842.

- Koya, E., Uejima, J. L., Wihbey, K. A., Bossert, J. M., Hope, B. T., & Shaham, Y. (2008). Role of ventral medial prefrontal cortex in incubation of cocaine craving. *Neuropharmacology*.
- Landauer, T. K. (1969). Reinforcement as consolidation. *Psychol Rev*, 76, 82-96.
- Lattal, K. M., Radulovic, J., & Lukowiak, K. (2006). Extinction: [corrected] does it or doesn't it? The requirement of altered gene activity and new protein synthesis. *Biol Psychiatry*, 60, 344-351.
- Le Moal, M. & Simon, H. (1991). Mesocorticolimbic dopaminergic network: Functional and regulatory roles. *Physiological Review*, 71, 155-234.
- Ledgerwood, L., Richardson, R., & Cranney, J. (2003). Effects of d-cycloserine on extinction of conditioned freezing. *Behav Neurosci*, 117, 341-349.
- LeDoux, J. E. (1993). Emotional memory systems in the brain. *Behav Brain Res*, 58, 69-79.
- LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual review of neuroscience*, 23, 155-184.
- LeDoux, J. E., Farb, C., & Ruggiero, D. A. (1990). Topographic organization of neurons in the acoustic thalamus that project to the amygdala. *J Neurosci*, 10, 1043-1054.
- Lee, H., & Kim, J. J. (1998). Amygdalar nmda receptors are critical for new fear learning in previously fear-conditioned rats. *J Neurosci*, 18, 8444-8454.
- Li, Y. Q., Li, F. Q., Wang, X. Y., Wu, P., Zhao, M., Xu, C. M., et al. (2008). Central amygdala extracellular signal-regulated kinase signaling pathway is critical to incubation of opiate craving. *J Neurosci*, 28, 13248-13257.
- Lin, C. H., Lee, C. C., & Gean, P. W. (2003). Involvement of a calcineurin cascade in amygdala depotentiation and quenching of fear memory. *Mol Pharmacol*, 63, 44-52.
- Lin, C. H., Yeh, S. H., Leu, T. H., Chang, W. C., Wang, S. T., & Gean, P. W. (2003). Identification of calcineurin as a key signal in the extinction of fear memory. *J Neurosci*, 23, 1574-1579.
- Lin, C. H., Yeh, S. H., Lu, H. Y., & Gean, P. W. (2003). The similarities and diversities of signal pathways leading to consolidation of conditioning and consolidation of extinction of fear memory. *J Neurosci*, 23, 8310-8317.
- Lin, C. H., Yeh, S. H., Lu, K. T., Leu, T. H., Chang, W. C., & Gean, P. W. (2001). A role for the pi-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala. *Neuron*, 31, 841-851.
- Lin, H. C., Mao, S. C., Su, C. L., & Gean, P. W. (2010). Alterations of excitatory transmission in the lateral amygdala during expression and extinction of fear memory. *Int J Neuropsychopharmacol*, 13, 335-345.
- Lu, K. T., Walker, D. L., & Davis, M. (2001). Mitogen-activated protein kinase cascade in the basolateral nucleus of amygdala is involved in extinction of fear-potentiated startle. *J Neurosci*, 21, RC162.
- Lu, L., Grimm, J. W., Hope, B. T., & Shaham, Y. (2004). Incubation of cocaine craving after withdrawal: A review of preclinical data. *Neuropharmacology*, 47 Suppl 1, 214-226.
- Lu, L., Hope, B. T., Dempsey, J., Liu, S. Y., Bossert, J. M., & Shaham, Y. (2005). Central amygdala erk signaling pathway is critical to incubation of cocaine craving. *Nat Neurosci*, 8, 212-219.
- Lu, L., Koya, E., Zhai, H., Hope, B. T., & Shaham, Y. (2006). Role of erk in cocaine addiction. *Trends Neurosci*, 29, 695-703.

- Lu, L., Zhang, B., Liu, Z., & Zhang, Z. (2002). Reactivation of cocaine conditioned place preference induced by stress is reversed by cholecystokinin-b receptors antagonist in rats. *Brain Res*, 954, 132-140.
- Lynch, M. A. (2004). Long-term potentiation and memory. *Physiol Rev*, 84, 87-136.
- Madeira, M. D., Sousa, N., & Paula-Barbosa, M. M. (1991). Sexual dimorphism in the mossy fiber synapses of the rat hippocampus. *Exp Brain Res*, 87, 537-545.
- Malenka, R. C., & Bear, M. F. (2004). Ltp and ltd: An embarrassment of riches. *Neuron*, 44, 5-21.
- Mao, L., Yang, L., Tang, Q., Samdani, S., Zhang, G., & Wang, J. Q. (2005). The scaffold protein homer1b/c links metabotropic glutamate receptor 5 to extracellular signal-regulated protein kinase cascades in neurons. *J Neurosci*, 25, 2741-2752.
- Mao, S. C., Hsiao, Y. H., & Gean, P. W. (2006). Extinction training in conjunction with a partial agonist of the glycine site on the nmda receptor erases memory trace. *J Neurosci*, 26, 8892-8899.
- Maren, S., Ferrario, C. R., Corcoran, K. A., Desmond, T. J., & Frey, K. A. (2003). Protein synthesis in the amygdala, but not the auditory thalamus, is required for consolidation of pavlovian fear conditioning in rats. *Eur J Neurosci*, 18, 3080-3088.
- Markou, A., Weiss, F., Gold, L. H., Caine, S. B., Schulteis, G., & Koob, G. F. (1993). Animal models of drug craving. *Psychopharmacology (Berl)*, 112, 163-182.
- Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., et al. (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature*, 418, 530-534.
- Martin, M., Chen, B. T., Hopf, F. W., Bowers, M. S., & Bonci, A. (2006). Cocaine self-administration selectively abolishes ltd in the core of the nucleus accumbens. *Nat Neurosci*, 9, 868-869.
- Matsuda, S., Matsuzawa, D., Nakazawa, K., Sutoh, C., Ohtsuka, H., Ishii, D., et al. (2010). D-serine enhances extinction of auditory cued fear conditioning via erk1/2 phosphorylation in mice. *Prog Neuropsychopharmacol Biol Psychiatry*, 34, 895-902.
- Mazzucchelli, C., Vantaggiato, C., Ciamei, A., Fasano, S., Pakhotin, P., Krezel, W., et al. (2002). Knockout of erk1 map kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. *Neuron*, 34, 807-820.
- McClung, C. A., & Nestler, E. J. (2003). Regulation of gene expression and cocaine reward by creb and deltafosb. *Nat Neurosci*, 6, 1208-1215.
- McDonald, A. J., Mascagni, F., & Guo, L. (1996). Projections of the medial and lateral prefrontal cortices to the amygdala: A phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience*, 71, 55-75.
- McPherson, C. S., Mantamadiotis, T., Tan, S. S., & Lawrence, A. J. (2010). Deletion of creb1 from the dorsal telencephalon reduces motivational properties of cocaine. *Cereb Cortex*, 20, 941-952.
- Milad, M. R., & Quirk, G. J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature*, 420, 70-74.
- Miller, C. A., & Marshall, J. F. (2005). Molecular substrates for retrieval and reconsolidation of cocaine-associated contextual memory. *Neuron*, 47, 873-884.
- Mineka, S., & Zinbarg, R. (1996). Conditioning and ethological models of anxiety disorders: Stress-in-dynamic-context anxiety models. *Nebr Symp Motiv*, 43, 135-210.

- Mizoguchi, H., Yamada, K., Mizuno, M., Mizuno, T., Nitta, A., Noda, Y., et al. (2004). Regulations of methamphetamine reward by extracellular signal-regulated kinase 1/2/ets-like gene-1 signaling pathway via the activation of dopamine receptors. *Mol Pharmacol*, 65, 1293-1301.
- Morgan, M. A., Romanski, L. M., & LeDoux, J. E. (1993). Extinction of emotional learning: Contribution of medial prefrontal cortex. *Neurosci Lett*, 163, 109-113.
- Morris, R. G., Anderson, E., Lynch, G. S., & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an n-methyl-d-aspartate receptor antagonist, ap5. *Nature*, 319, 774-776.
- Moussawi, K., Pacchioni, A., Moran, M., Olive, M. F., Gass, J. T., Lavin, A., et al. (2009). N-acetylcysteine reverses cocaine-induced metaplasticity. *Nat Neurosci*, 12, 182-189.
- Myers, K. M., Carlezon, W. A., Jr., & Davis, M. (2011). Glutamate receptors in extinction and extinction-based therapies for psychiatric illness. *Neuropsychopharmacology*, 36, 274-293.
- Myers, K. M., & Davis, M. (2007). Mechanisms of fear extinction. *Mol Psychiatry*, 12, 120-150.
- Nader, K., Schafe, G. E., & Le Doux, J. E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*, 406, 722-726.
- Nathaniel, Thomas I., Panksepp, Jaak, & Huber, Robert. (2009). Drug-seeking behavior in an invertebrate system: Evidence of morphine-induced reward, extinction and reinstatement in crayfish. *Behavioral Brain Research*, 197, 331-338.
- Nestler, E. J. (2001). Molecular basis of long-term plasticity underlying addiction. *Nat Rev Neurosci*, 2, 119-128.
- Newman, D. L., Moffitt, T. E., Caspi, A., Magdol, L., Silva, P. A., & Stanton, W. R. (1996). Psychiatric disorder in a birth cohort of young adults: Prevalence, comorbidity, clinical significance, and new case incidence from ages 11 to 21. *J Consult Clin Psychol*, 64, 552-562.
- O'Brien, C., Childress, A. R., Ehrman, R., Robbins, S., & McLellan, A. T. (1992). Conditioning mechanisms in drug dependence. *Clin Neuropharmacol*, 15 Suppl 1 Pt A, 66A-67A.
- O'Brien, C. P. (1997a). Progress in the science of addiction. *Am J Psychiatry*, 154, 1195-1197.
- O'Brien, C. P. (1997b). A range of research-based pharmacotherapies for addiction. *Science*, 278, 66-70.
- O'Brien, M. S., Wu, L. T., & Anthony, J. C. (2005). Cocaine use and the occurrence of panic attacks in the community: A case-crossover approach. *Subst Use Misuse*, 40, 285-297.
- Omelchenko, N., Sesack, S. R. (2007). Glutamate synaptic inputs to ventral tegmental area neurons in the rat derive primarily from subcortical sources. *Neuroscience*, 146, 1259-1274.
- Ortiz, J., Harris, H. W., Guitart, X., Terwilliger, R. Z., Haycock, J. W., & Nestler, E. J. (1995). Extracellular signal-regulated protein kinases (erks) and erk kinase (mek) in brain: Regional distribution and regulation by chronic morphine. *J Neurosci*, 15, 1285-1297.
- Ozaki, S., Narita, M., Ozaki, M., Khotib, J., & Suzuki, T. (2004). Role of extracellular signal-regulated kinase in the ventral tegmental area in the suppression of the morphine-induced rewarding effect in mice with sciatic nerve ligation. *J Neurochem*, 88, 1389-1397.

- Parker, L. A., & McDonald, R. V. (2000). Reinstatement of both a conditioned place preference and a conditioned place aversion with drug primes. *Pharmacol Biochem Behav*, 66, 559-561.
- Pascoli, V., Besnard, A., Herve, D., Pages, C., Heck, N., Girault, J. A., et al. (2011). Cyclic adenosine monophosphate-independent tyrosine phosphorylation of nr2b mediates cocaine-induced extracellular signal-regulated kinase activation. *Biol Psychiatry*, 69, 218-227.
- Pavlov, I. P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. London: Oxford University Press.
- Pierce, R. C., Pierce-Bancroft, A. F., & Prasad, B. M. (1999). Neurotrophin-3 contributes to the initiation of behavioral sensitization to cocaine by activating the ras/mitogen-activated protein kinase signal transduction cascade. *J Neurosci*, 19, 8685-8695.
- Price, J. L. (2003). Comparative aspects of amygdala connectivity. *Ann N Y Acad Sci*, 985, 50-58.
- Quirk, G. J., Armony, J. L., & LeDoux, J. E. (1997). Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. *Neuron*, 19, 613-624.
- Quirk, G. J., Reppas, C., & LeDoux, J. E. (1995). Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: Parallel recordings in the freely behaving rat. *Neuron*, 15, 1029-1039.
- Quirk, G. J., Russo, G. K., Barron, J. L., & Lebron, K. (2000). The role of ventromedial prefrontal cortex in the recovery of extinguished fear. *J Neurosci*, 20, 6225-6231.
- Rachman, S. (1989). The return of fear: Review and prospect. *Clin Psychol Rev*, 9, 147-168.
- Radwanska, K., Caboche, J., & Kaczmarek, L. (2005). Extracellular signal-regulated kinases (erks) modulate cocaine-induced gene expression in the mouse amygdala. *Eur J Neurosci*, 22, 939-948.
- Radwanska, K., Nikolaev, E., Knapska, E., & Kaczmarek, L. (2002). Differential response of two subdivisions of lateral amygdala to aversive conditioning as revealed by c-fos and p-erk mapping. *Neuroreport*, 13, 2241-2246.
- Radwanska, K., Wrobel, E., Korkosz, A., Rogowski, A., Kostowski, W., Bienkowski, P., et al. (2008). Alcohol relapse induced by discrete cues activates components of ap-1 transcription factor and erk pathway in the rat basolateral and central amygdala. *Neuropsychopharmacology*, 33, 1835-1846.
- Rasmusson, A. M., & Charney, D. S. (1997). Animal models of relevance to ptsd. *Ann N Y Acad Sci*, 821, 332-351.
- Reichel, C. M., & Bevins, R. A. (2009). Forced abstinence model of relapse to study pharmacological treatments of substance use disorder. *Curr Drug Abuse Rev*, 2, 184-194.
- Reinblatt, S. P., & Riddle, M. A. (2007). The pharmacological management of childhood anxiety disorders: A review. *Psychopharmacology (Berl)*, 191, 67-86.
- Reppas, J. C., Muller, J., Apergis, J., Desrochers, T. M., Zhou, Y., & LeDoux, J. E. (2001). Two different lateral amygdala cell populations contribute to the initiation and storage of memory. *Nat Neurosci*, 4, 724-731.
- Rescorla, R. A. (1988). Pavlovian conditioning. It's not what you think it is. *Am Psychol*, 43, 151-160.

- Rescorla, R. A., & Wagner, A. R. (1972). A theory of pavlovian conditioning: Variations in the effectiveness of reinforcement and non-reinforcement. . In Prokasy A. H. (Ed.), *Classical conditioning ii: Current research and theory*. (pp. 64-99). New York: Appleton-Century-Croft.
- Reynolds, J. N., Hyland, B. I., & Wickens, J. R. (2001). A cellular mechanism of reward-related learning. *Nature*, *413*, 67-70.
- Roberson, E. D., English, J. D., & Sweatt, J. D. (1996). A biochemist's view of long-term potentiation. *Learn Mem*, *3*, 1-24.
- Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Res Brain Res Rev*, *18*, 247-291.
- Robinson, T. E., & Berridge, K. C. (2001). Incentive-sensitization and addiction. *Addiction*, *96*, 103-114.
- Rogers, J. L., & See, R. E. (2007). Selective inactivation of the ventral hippocampus attenuates cue-induced and cocaine-primed reinstatement of drug-seeking in rats. *Neurobiol Learn Mem*, *87*, 688-692.
- Rosen, J. B., & Schulkin, J. (1998). From normal fear to pathological anxiety. *Psychol Rev*, *105*, 325-350.
- Rosenkranz, J. A., & Grace, A. A. (2002). Cellular mechanisms of infralimbic and prelimbic prefrontal cortical inhibition and dopaminergic modulation of basolateral amygdala neurons in vivo. *J Neurosci*, *22*, 324-337.
- Rosenkranz, J. A., Moore, H., & Grace, A. A. (2003). The prefrontal cortex regulates lateral amygdala neuronal plasticity and responses to previously conditioned stimuli. *J Neurosci*, *23*, 11054-11064.
- Rossato, J. I., Bevilaqua, L. R., Lima, R. H., Medina, J. H., Izquierdo, I., & Cammarota, M. (2006). On the participation of hippocampal p38 mitogen-activated protein kinase in extinction and reacquisition of inhibitory avoidance memory. *Neuroscience*, *143*, 15-23.
- Rothbaum, B. O., & Davis, M. (2003). Applying learning principles to the treatment of post-trauma reactions. *Ann N Y Acad Sci*, *1008*, 112-121.
- Royer, S., & Pare, D. (2002). Bidirectional synaptic plasticity in intercalated amygdala neurons and the extinction of conditioned fear responses. *Neuroscience*, *115*, 455-462.
- Rumpel, S., LeDoux, J., Zador, A., & Malinow, R. (2005). Postsynaptic receptor trafficking underlying a form of associative learning. *Science*, *308*, 83-88.
- Salzmann, J., Marie-Claire, C., Le Guen, S., Roques, B. P., & Noble, F. (2003). Importance of erk activation in behavioral and biochemical effects induced by mdma in mice. *Br J Pharmacol*, *140*, 831-838.
- SAMHSA. (2010). *Results from the 2009 national survey on drug use and health: Volume i. Summary of national findings*. Rockville, MD: Office of Applied Studies.
- Santini, E., Muller, R. U., & Quirk, G. J. (2001). Consolidation of extinction learning involves transfer from nmda-independent to nmda-dependent memory. *J Neurosci*, *21*, 9009-9017.
- Satoh, Y., Endo, S., Ikeda, T., Yamada, K., Ito, M., Kuroki, M., et al. (2007). Extracellular signal-regulated kinase 2 (erk2) knockdown mice show deficits in long-term

- memory; erk2 has a specific function in learning and memory. *J Neurosci*, 27, 10765-10776.
- Schafe, G. E., Atkins, C. M., Swank, M. W., Bauer, E. P., Sweatt, J. D., & LeDoux, J. E. (2000). Activation of erk/map kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning. *J Neurosci*, 20, 8177-8187.
- Schafe, G. E., & LeDoux, J. E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase a in the amygdala. *J Neurosci*, 20, RC96.
- Schafe, G. E., Nadel, N. V., Sullivan, G. M., Harris, A., & LeDoux, J. E. (1999). Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, pka, and map kinase. *Learn Mem*, 6, 97-110.
- Schafe, G. E., Swank, M. W., Rodrigues, S. M., Debiec, J., & Doyere, V. (2008). Phosphorylation of erk/map kinase is required for long-term potentiation in anatomically restricted regions of the lateral amygdala in vivo. *Learn Mem*, 15, 55-62.
- Schroeder, J. P., Spanos, M., Stevenson, J. R., Besheer, J., Salling, M., & Hodge, C. W. (2008). Cue-induced reinstatement of alcohol-seeking behavior is associated with increased erk1/2 phosphorylation in specific limbic brain regions: Blockade by the mglur5 antagonist mpep. *Neuropharmacology*, 55, 546-554.
- Schumann, J., & Yaka, R. (2009). Prolonged withdrawal from repeated noncontingent cocaine exposure increases nmda receptor expression and erk activity in the nucleus accumbens. *J Neurosci*, 29, 6955-6963.
- See, R. E. (2005). Neural substrates of cocaine-cue associations that trigger relapse. *Eur J Pharmacol*, 526, 140-146.
- Seger, R., & Krebs, E. G. (1995). The mapk signaling cascade. *FASEB J*, 9, 726-735.
- Selcher, J. C., Atkins, C. M., Trzaskos, J. M., Paylor, R., & Sweatt, J. D. (1999). A necessity for map kinase activation in mammalian spatial learning. *Learn Mem*, 6, 478-490.
- Selcher, J. C., Nekrasova, T., Paylor, R., Landreth, G. E., & Sweatt, J. D. (2001). Mice lacking the erk1 isoform of map kinase are unimpaired in emotional learning. *Learn Mem*, 8, 11-19.
- Sesack, S. R., & Pickel, V. M. (1992). Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol*, 320, 145-160.
- Sestan, N., Artavanis-Tsakonas, S., & Rakic, P. (1999). Contact-dependent inhibition of cortical neurite growth mediated by notch signaling. *Science*, 286, 741-746.
- Sgambato, V., Pages, C., Rogard, M., Besson, M. J., & Caboche, J. (1998). Extracellular signal-regulated kinase (erk) controls immediate early gene induction on corticostriatal stimulation. *J Neurosci*, 18, 8814-8825.
- Shaham, Y., & Hope, B. T. (2005). The role of neuroadaptations in relapse to drug seeking. *Nat Neurosci*, 8, 1437-1439.
- Shaham, Y., Shalev, U., Lu, L., De Wit, H., & Stewart, J. (2003). The reinstatement model of drug relapse: History, methodology and major findings. *Psychopharmacology (Berl)*, 168, 3-20.

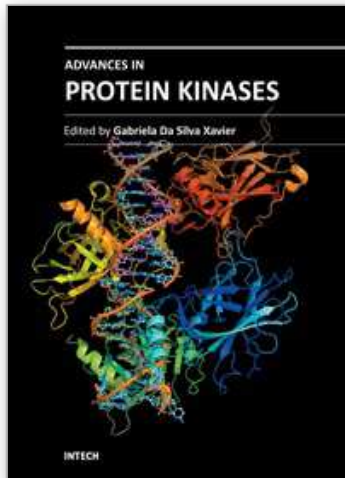
- Shaham, Y., & Stewart, J. (1996). Effects of opioid and dopamine receptor antagonists on relapse induced by stress and re-exposure to heroin in rats. *Psychopharmacology (Berl)*, 125, 385-391.
- Shalev, U., Morales, M., Hope, B., Yap, J., & Shaham, Y. (2001). Time-dependent changes in extinction behavior and stress-induced reinstatement of drug seeking following withdrawal from heroin in rats. *Psychopharmacology (Berl)*, 156, 98-107.
- Shalin, S. C., Zirrgiebel, U., Honsa, K. J., Julien, J. P., Miller, F. D., Kaplan, D. R., et al. (2004). Neuronal mek is important for normal fear conditioning in mice. *J Neurosci Res*, 75, 760-770.
- Shen, W., Flajolet, M., Greengard, P., & Surmeier, D. J. (2008). Dichotomous dopaminergic control of striatal synaptic plasticity. *Science*, 321, 848-851.
- Shepard, J. D., Bossert, J. M., Liu, S. Y., & Shaham, Y. (2004). The anxiogenic drug yohimbine reinstates methamphetamine seeking in a rat model of drug relapse. *Biol Psychiatry*, 55, 1082-1089.
- Shi, C., & Davis, M. (2001). Visual pathways involved in fear conditioning measured with fear-potentiated startle: Behavioral and anatomic studies. *J Neurosci*, 21, 9844-9855.
- Shi, X., & McGinty, J. F. (2006). Extracellular signal-regulated mitogen-activated protein kinase inhibitors decrease amphetamine-induced behavior and neuropeptide gene expression in the striatum. *Neuroscience*, 138, 1289-1298.
- Shiflett, M. W., & Balleine, B. W. (2011). Contributions of erk signaling in the striatum to instrumental learning and performance. *Behav Brain Res*, 218, 240-247.
- Shiflett, M. W., Brown, R. A., & Balleine, B. W. (2010). Acquisition and performance of goal-directed instrumental actions depends on erk signaling in distinct regions of dorsal striatum in rats. *J Neurosci*, 30, 2951-2959.
- Sindreu, C. B., Scheiner, Z. S., & Storm, D. R. (2007). Ca²⁺-stimulated adenylyl cyclases regulate erk-dependent activation of msk1 during fear conditioning. *Neuron*, 53, 79-89.
- Skinner, B.F. (1938). *The behavior of organisms : An experimental analysis* Appleton-Century, New York.
- Smith, A. D., & Bolam, J. P. (1990). The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. *Trends Neurosci*, 13, 259-265.
- Smith, M. D., Jones, L. S., & Wilson, M. A. (2002). Sex differences in hippocampal slice excitability: Role of testosterone. *Neuroscience*, 109, 517-530.
- Sotres-Bayon, F., Cain, C. K., & LeDoux, J. E. (2006). Brain mechanisms of fear extinction: Historical perspectives on the contribution of prefrontal cortex. *Biol Psychiatry*, 60, 329-336.
- Sotres-Bayon, F., Diaz-Mataix, L., Bush, D. E., & LeDoux, J. E. (2009). Dissociable roles for the ventromedial prefrontal cortex and amygdala in fear extinction: Nr2b contribution. *Cereb Cortex*, 19, 474-482.
- Stanton, P. K., & Sarvey, J. M. (1984). Blockade of long-term potentiation in rat hippocampal ca1 region by inhibitors of protein synthesis. *J Neurosci*, 4, 3080-3088.
- Stretch, R., Gerber, G. J., & Wood, S. M. (1971). Factors affecting behavior maintained by response-contingent intravenous infusions of amphetamine in squirrel monkeys. *Can J Physiol Pharmacol*, 49, 581-589.

- Sullivan, R. M., Landers, M., Yeaman, B., & Wilson, D. A. (2000). Good memories of bad events in infancy. *Nature*, *407*, 38-39.
- Sutton, M. A., Schmidt, E. F., Choi, K. H., Schad, C. A., Whisler, K., Simmons, D., et al. (2003). Extinction-induced upregulation in ampa receptors reduces cocaine-seeking behavior. *Nature*, *421*, 70-75.
- Suzuki, A., Josselyn, S. A., Frankland, P. W., Masushige, S., Silva, A. J., & Kida, S. (2004). Memory reconsolidation and extinction have distinct temporal and biochemical signatures. *J Neurosci*, *24*, 4787-4795.
- Svenningsson, P., Nishi, A., Fisone, G., Girault, J. A., Nairn, A. C., & Greengard, P. (2004). Darpp-32: An integrator of neurotransmission. *Annu Rev Pharmacol Toxicol*, *44*, 269-296.
- Sweatt, J. D. (1999). Toward a molecular explanation for long-term potentiation. *Learn Mem*, *6*, 399-416.
- Sweatt, J. D. (2001). The neuronal map kinase cascade: A biochemical signal integration system subserving synaptic plasticity and memory. *J Neurochem*, *76*, 1-10.
- Szapiro, G., Vianna, M. R., McGaugh, J. L., Medina, J. H., & Izquierdo, I. (2003). The role of nmda glutamate receptors, pka, mapk, and camkii in the hippocampus in extinction of conditioned fear. *Hippocampus*, *13*, 53-58.
- Thiels, E., Kanterewicz, B. I., Norman, E. D., Trzaskos, J. M., & Klann, E. (2002). Long-term depression in the adult hippocampus in vivo involves activation of extracellular signal-regulated kinase and phosphorylation of elk-1. *J Neurosci*, *22*, 2054-2062.
- Thomas, G. M., & Huganir, R. L. (2004). Mapk cascade signalling and synaptic plasticity. *Nat Rev Neurosci*, *5*, 173-183.
- Thomas, M. J., Kalivas, P. W., & Shaham, Y. (2008). Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. *Br J Pharmacol*, *154*, 327-342.
- Thorndike, E. L. (1898). *Animal intelligence; [electronic resource] an experimental study of the associative processes in animals*: Macmillan, New York.
- Totterdell, S., & Smith, A. D. (1989). Convergence of hippocampal and dopaminergic input onto identified neurons in the nucleus accumbens of the rat. *J Chem Neuroanat*, *2*, 285-298.
- Trifilieff, P., Herry, C., Vanhoutte, P., Caboche, J., Desmedt, A., Riedel, G., et al. (2006). Foreground contextual fear memory consolidation requires two independent phases of hippocampal erk/creb activation. *Learning & memory*, *13*, 349-358.
- Tropea, T. F., Kosofsky, B. E., & Rajadhyaksha, A. M. (2008). Enhanced creb and darpp-32 phosphorylation in the nucleus accumbens and creb, erk, and glur1 phosphorylation in the dorsal hippocampus is associated with cocaine-conditioned place preference behavior. *J Neurochem*, *106*, 1780-1790.
- Tzschentke, T. M. (1998). Measuring reward with the conditioned place preference paradigm: A comprehensive review of drug effects, recent progress and new issues. *Prog Neurobiol*, *56*, 613-672.
- Valjent, E., Corbille, A. G., Bertran-Gonzalez, J., Herve, D., & Girault, J. A. (2006). Inhibition of erk pathway or protein synthesis during reexposure to drugs of abuse erases previously learned place preference. *Proc Natl Acad Sci U S A*, *103*, 2932-2937.

- Valjent, E., Corvol, J. C., Pages, C., Besson, M. J., Maldonado, R., & Caboche, J. (2000). Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J Neurosci*, 20, 8701-8709.
- Valjent, E., Corvol, J. C., Trzaskos, J. M., Girault, J. A., & Herve, D. (2006). Role of the erk pathway in psychostimulant-induced locomotor sensitization. *BMC Neurosci*, 7, 20.
- Valjent, E., Pages, C., Rogard, M., Besson, M. J., Maldonado, R., & Caboche, J. (2001). Delta 9-tetrahydrocannabinol-induced mapk/erk and elk-1 activation in vivo depends on dopaminergic transmission. *Eur J Neurosci*, 14, 342-352.
- Valjent, E., Pascoli, V., Svenningsson, P., Paul, S., Enslen, H., Corvol, J. C., et al. (2005). Regulation of a protein phosphatase cascade allows convergent dopamine and glutamate signals to activate erk in the striatum. *Proc Natl Acad Sci U S A*, 102, 491-496.
- Volkow, N. D., Fowler, J. S., & Wang, G. J. (2003). The addicted human brain: Insights from imaging studies. *J Clin Invest*, 111, 1444-1451.
- Wagner, F. A., & Anthony, J. C. (2002). Into the world of illegal drug use: Exposure opportunity and other mechanisms linking the use of alcohol, tobacco, marijuana, and cocaine. *Am J Epidemiol*, 155, 918-925.
- Walker, D. L., Ressler, K. J., Lu, K. T., & Davis, M. (2002). Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of d-cycloserine as assessed with fear-potentiated startle in rats. *J Neurosci*, 22, 2343-2351.
- Walz, R., Roesler, R., Barros, D. M., de Souza, M. M., Rodrigues, C., Sant'Anna, M. K., et al. (1999). Effects of post-training infusions of a mitogen-activated protein kinase inhibitor into the hippocampus or entorhinal cortex on short- and long-term retention of inhibitory avoidance. *Behav Pharmacol*, 10, 723-730.
- Walz, R., Roesler, R., Quevedo, J., Rockenbach, I. C., Amaral, O. B., Vianna, M. R., et al. (1999). Dose-dependent impairment of inhibitory avoidance retention in rats by immediate post-training infusion of a mitogen-activated protein kinase inhibitor into cortical structures. *Behav Brain Res*, 105, 219-223.
- Walz, R., Roesler, R., Quevedo, J., Sant'Anna, M. K., Madruga, M., Rodrigues, C., et al. (2000). Time-dependent impairment of inhibitory avoidance retention in rats by posttraining infusion of a mitogen-activated protein kinase inhibitor into cortical and limbic structures. *Neurobiol Learn Mem*, 73, 11-20.
- Wasserman, D. A., Havassy, B. E., & Boles, S. M. (1997). Traumatic events and post-traumatic stress disorder in cocaine users entering private treatment. *Drug Alcohol Depend*, 46, 1-8.
- Watson, J.B., & Rayner, R. (1920). Conditioned emotional reactions. *Journal of Experimental Psychology*, 3, 1-14.
- Whitfield, T. W., Jr., Shi, X., Sun, W. L., & McGinty, J. F. (2011). The suppressive effect of an intra-prefrontal cortical infusion of bdnf on cocaine-seeking is trk receptor and extracellular signal-regulated protein kinase mitogen-activated protein kinase dependent. *J Neurosci*, 31, 834-842.
- WHO. (2001). Mental health: New understanding, new hope. *The world health report*: World Health Organisation.
- Wise, R. A. (1996). Neurobiology of addiction. *Curr Opin Neurobiol*, 6, 243-251.

- Wu, S. P., Lu, K. T., Chang, W. C., & Gean, P. W. (1999). Involvement of mitogen-activated protein kinase in hippocampal long-term potentiation. *J Biomed Sci*, 6, 409-417.
- Xia, Z., Dudek, H., Miranti, C. K., & Greenberg, M. E. (1996). Calcium influx via the nmda receptor induces immediate early gene transcription by a map kinase/erk-dependent mechanism. *J Neurosci*, 16, 5425-5436.
- Xie, G. Q., Wang, S. J., Li, J., Cui, S. Z., Zhou, R., Chen, L., et al. (2009). Ethanol attenuates the hfs-induced, erk-mediated ltp in a dose-dependent manner in rat striatum. *Alcohol Clin Exp Res*, 33, 121-128.
- Yang, Y. L., Lu, K. T. (2005). Facilitation of conditioned fear extinction by d-cycloserine is mediated by mitogen-activated protein kinase and phosphatidylinositol 3-kinase cascades and requires de novo protein synthesis in basolateral nucleus of amygdala. *Neuroscience*, 134, 247-260.
- Zhai, H., Li, Y., Wang, X., & Lu, L. (2008). Drug-induced alterations in the extracellular signal-regulated kinase (erk) signalling pathway: Implications for reinforcement and reinstatement. *Cell Mol Neurobiol*, 28, 157-172.

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Advances in Protein Kinases

Edited by Dr. Gabriela Da Silva Xavier

ISBN 978-953-51-0633-3

Hard cover, 374 pages

Publisher InTech

Published online 05, June, 2012

Published in print edition June, 2012

Proteins are the work horses of the cell. As regulators of protein function, protein kinases are involved in the control of cellular functions via intricate signalling pathways, allowing for fine tuning of physiological functions. This book is a collaborative effort, with contribution from experts in their respective fields, reflecting the spirit of collaboration - across disciplines and borders - that exists in modern science. Here, we review the existing literature and, on occasions, provide novel data on the function of protein kinases in various systems. We also discuss the implications of these findings in the context of disease, treatment, and drug development.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Robyn Mary Brown, Andrew J. Lawrence and Jee Hyun Kim (2012). The Role of Mitogen-Activated Protein Kinase in Treatment Strategies for Fear and Drug Addiction, *Advances in Protein Kinases*, Dr. Gabriela Da Silva Xavier (Ed.), ISBN: 978-953-51-0633-3, InTech, Available from:
<http://www.intechopen.com/books/advances-in-protein-kinases/the-role-of-mitogen-activated-protein-kinase-in-treatment-strategies-for-fear-and-drug-addiction>

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