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Neural substrates of conditioned-cued relapse to drug-seeking behavior

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Abstract

Relapse to drug use following abstinence is a significant impediment in the long-term treatment of drug abuse and dependence. Conditioned stimuli are believed to be critically involved in activating drug craving and relapse to compulsive drug-taking behavior. Studies in humans and animal models have recently begun to identify the fundamental neural circuitry that mediates relapse following withdrawal from chronic drug self-administration. The current review summarizes key findings in this area that have converged on the amygdalar complex and regions of the frontal lobe as critical structures in conditioned-cued relapse. It is proposed that the amygdala is a key regulator of discrete stimulus–reinforcer associations, while the anterior cingulate and orbitofrontal cortex are critical regulators of relapse evoked by conditioned stimuli that predict drug availability. This corticolimbic circuitry may form the neural basis of multiple long-term conditioned associations produced by a variety of drugs of abuse ranging from psychostimulants to opiates. Future studies aimed at discerning the functional roles of these pathways will provide critical direction for the development of treatments for the prevention of relapse.

Keywords: Relapse; Reinstatement; Amygdala; Anterior cingulate; Orbitofrontal cortex; Addiction; Cue

1. Craving and relapse to drugs of abuse

A basic definition of relapse in the context of drug abuse and dependence is the return to drug-seeking and drug-taking behavior following a prolonged period of abstinence. High rates of relapse to drug taking are routinely reported following treatment for drug dependency. Often, the role of craving (defined as an intense desire for a specific object or experience) is invoked as a primary motivating force behind relapse. Indeed, the quantified measurement of craving states is an integral part of most studies in humans that have assessed neural substrates of relapse to drugs of abuse (Childress et al., 1999; Grant et al., 1996; Kilts et al., 2001). While craving is a widely utilized construct, the role of craving in relapse, and even the basic definition of craving, has been extensively debated in addiction research (Miller and Gold, 1994; Rohsenow and Monti, 1999). Several recent papers have addressed this issue (Anton, 1999; Drummond, 2001; Sinha et al., 2000; Tiffany and Carter, 1998). Although craving as a leading causal factor in relapse remains questionable, evidence has clearly established that various external and internal stimuli can trigger an increased motivation to drug-seeking and drug-taking behavior (Carter and Tiffany, 1999; Childress et al., 1993). As for animal models, the subjective state of craving is obviously difficult to clearly define and measure (Koob et al., 1999; Littleton, 2000; Markou et al., 1993). However, the operant task of returning to a previously established behavioral response (e.g. reinstating lever pressing previously associated with drug delivery) by means of an instigating stimulus is a well-accepted experimental method that can be readily applied to study the neural substrates of relapse. In the current paper, two general paradigms will be referred to in examining the neural substrates of relapse: (a) animal models of the reinstatement of extinguished operant responding in the presence of previously drug-paired stimuli and (b) in vivo brain imaging of drug-dependent humans exposed to various forms of drug-paired stimuli designed to evoke craving for drugs of abuse.

2. Drug-associated environmental stimuli and relapse

A number of factors contribute to the incidence of relapse, and recent theoretical models have addressed

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several issues of etiology, such as environmental stressors (Stewart, 2000) and adaptive changes in neural regulatory systems (Koob and Le Moal, 2001). One particularly salient feature that occurs during abstinence from repeated drug use is the ability of drug-associated environmental cues (e.g. locations where a drug was consumed or associated drug paraphernalia) to elicit drug craving and consequently reinstate drug seeking and drug taking. Such conditioned responses have been demonstrated for a variety of abused drugs, including cocaine (Childress et al., 1988; O'Brien et al., 1992), opiates (Childress et al., 1986) and alcohol (Drummond et al., 1990). For example, abstinent cocaine abusers report intense drug craving and physiological arousal when exposed to stimuli previously associated with cocaine use (Childress et al., 1993). Furthermore, experienced users can be experimentally trained to discriminate stimuli that are discretely paired (S+) or not paired (S−) with cocaine, as determined by both self-reports of craving and measurements of autonomic arousal (Foltin and Haney, 2000). These findings suggest that, through a process of associative learning, previously neutral stimuli acquire incentive-motivational properties following repeated pairing with drug taking. These drug-associated stimuli can elicit both subjective reports of craving and increased negative affect (Foltin and Haney, 2000; Sinha et al., 2000). Conditioned stimuli thus play a critical role in both ongoing drug-seeking behavior and during abstinence, when craving for the drug may perpetuate further drug use and relapse (Gawin, 1991). Drug addicts exposed to extinction procedures (O'Brien et al., 1990) or pharmacotherapy (Satel et al., 1995) exhibited less conditioned craving for cocaine, suggesting that a greater understanding of the role of conditioned stimuli in drug-seeking behavior will ultimately facilitate behavioral and somatic treatment approaches.

3. Animal models of relapse

Nonhuman primates and rodents have been shown to reliably self-administer most drugs abused by humans (Balster and Lukas, 1985; Spealman and Goldberg, 1978), and intravenous (iv) drug self-administration is well established as a model with good predictive and construct validity. The majority of studies utilizing animal models of drug self-administration have focused on the time when the drug is being actively administered (acquisition and maintenance phases). However, growing interest has focused on the utilization of self-administration models to study factors controlling relapse to drug-seeking behavior following prolonged drug discontinuation. The self-administration paradigm is ideally suited as an animal model for the study of relapse (Koob et al., 1999; Markou et al., 1993). During extinction and reinstatement procedures, the persistence of drug-seeking behavior in the absence of response-contingent drug infusions as measured by lever responding or nose poking on an operandum where drug was previously available represents an intuitive measure of relapse.

Three general experimental paradigms have been used for reinstatement of operant responding for drugs of abuse. One established approach is the use of noncontingent (''priming'') injections of drugs to reinstate self-administration (de Wit and Stewart, 1983; Markou et al., 1999; Worley et al., 1994). This paradigm has been found to produce a robust degree of reinstatement and is arguably a good model for pharmacologically induced relapse in addiction (Spealman et al., 1999). The drug priming model has recently been extended to explore the discrete neural substrates of cocaine-induced relapse (Cornish and Kalivas, 2000). A second paradigm employs the use of various external stressors to induce reinstatement of drug-seeking behavior (Erb et al., 1996; Mantsch and Goeders, 1999; Stewart, 2000). This paradigm provides a model for the study of stress activation of craving states as evidenced from clinical studies (Sinha et al., 2000). Recent theoretical models of drug dependence as a state of chronic dysregulation of brain reward systems have emphasized the crucial role of negative stimuli (i.e. ''stressors'') in the development and perpetuation of drug addiction (Koob and Le Moal, 2001), supporting the usefulness of the stress-induced reinstatement model in understanding causal factors of relapse. The third general model (and the focus of the current review) is the conditioned-cued model of reinstatement. This paradigm possesses good predictive and face validity for modeling the activation of craving states by conditioned environmental stimuli in drug-dependent individuals. It is likely that all three of these behavioral reinstatement paradigms engage overlapping yet distinctly different patterns of neural activity.

A few studies emerged during the 1970s and 1980s that directly addressed the issue of associative learning processes that may contribute to conditioned-cued reinstatement of drug-seeking behavior following withdrawal and extinction of active drug-taking. While not a direct model of relapse, studies using second-order schedules of reinforcement indicated the importance of drug-paired stimuli in maintaining drug-seeking behavior. Monkeys exposed to a visual stimulus previously paired with drug administration performed long behavioral sequences on second-order schedules maintained over time with only occasional drug infusions (Goldberg and Gardner, 1981). When cues were removed, responding was substantially decreased, thus demonstrating the ability of conditioned stimuli to facilitate drug-seeking behavior. Further extension of second-order schedules to rodent self-administration has proven extremely useful in determining the role of drug-paired stimuli in maintaining drug-seeking behavior (Arroyo et al., 1998; Markou et al., 1999). Davis and Smith (1976) were among the first to demonstrate the role of conditioned reinforcers in a rodent reinstatement model of self-administration by pairing a neutral stimulus with intravenous morphine or amphetamine.

They showed that conditioned stimuli could readily reinstate responding following extinction periods of 3 days. Furthermore, drug infusions previously ineffective in reestablishing responding became effective when responding resulted in the presentation of stimuli associated with previous drug injections (Davis and Smith, 1976). Subsequent studies by de Wit and Stewart (1981, 1983) demonstrated that a tone previously paired with drug infusions facilitated responding in animals who had undergone within session extinction trials. In more recent years, a number of groups have developed variants of an "extinction/reinstatement" paradigm for the study of relapse-like behaviors in the presence of conditioned stimuli (Bespalov et al., 2000; Fuchs et al., 1998; Katner et al., 1999; Meil and See, 1996; See et al., 1999; Weiss et al., 2000). In these models, animals are maintained on chronic drug self-administration followed by prolonged extinction of responding. Various stimuli (usually lights or tones) that were previously paired with drug taking are then presented in the absence of the drug as a measure of conditioned-cued reinstatement. As described below, these animal models provide a means to study the neural substrates of the associative learning that occurs with drug-paired stimuli.

4. Neural substrates of appetitive conditioning

Current concepts of learning and memory postulate multiple, overlapping pathways in appetitive conditioning, including conditioning with drugs of abuse (Everitt et al., 1999; Gallagher, 2000; McGaugh et al., 1996). For the purposes of the current review, a brief summary of some of the key structures implicated in appetitive conditioning is included here. Fig. 1 provides a schematic diagram of this circuitry. While they possess distinctive anatomical and functional characteristics, the nuclei within these circuits have extensive reciprocal interconnections. For example, the amygdala has major connections with the nucleus accumbens (NAcc; Kelley et al., 1982), and it has been postulated that environmental stimuli are translated into adaptive motor responses in part through connections of the amygdala to the NAcc (Mogenson et al., 1980). Projections from the anterior cingulate and orbitofrontal cortices also directly influence the NAcc and the amygdala (Brinley-Reed et al., 1995; Porrino et al., 1981; Sesack et al., 1989), and the amygdala has widespread inputs back to frontal cortical structures (McDonald, 1991; Price and Amaral, 1981). Key aspects of this circuitry have come under extensive study for their role in drug addiction. For example, the components identified as the extended amygdala (de Olmos and Heimer, 1999) have been implicated as undergoing long-lasting dysfunctional alterations following chronic drug exposure (Koob, 2000).

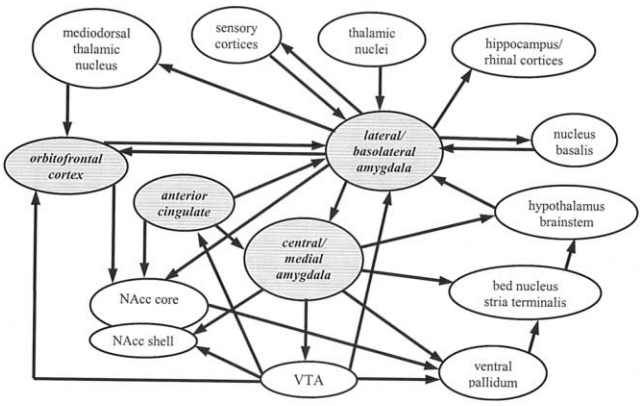

Fig. 1. Schematic of the critical brain circuitry involved in appetitive conditioning. A number of connections have been omitted for clarity. Brain regions strongly implicated in conditioned-cued relapse are indicated with grey shading.

4.1. The NAcc and appetitive conditioning

Perhaps more than any other brain structure, the NAcc has been the focus of study for the neural circuitry underlying appetitive conditioning across a wide range of natural reinforcers (Parkinson et al., 2000a; Salamone et al., 1997; Woodward et al., 2000). The NAcc is also well established as a primary site for the reinforcing properties of drugs of abuse and the associative processing of drug-paired conditioned stimuli. A number of recent detailed reviews of NAcc function in relation to drug abuse are available (Berridge and Robinson, 1998; Di Chiara et al., 1999; Kalivas and Nakamura, 1999; Wise, 1998). Two points of interest are worth mentioning in the current discussion. First, the core and the shell subregions of the NAcc appear to have clearly dissociable roles in conditioning. In conditioned reinforcement responding, selective NAcc shell lesions left Pavlovian and instrumental conditioning intact but attenuated amphetamine-potentiated conditioned responding (Parkinson et al., 1999). In contrast, NAcc core lesions impaired Pavlovian conditioning but did not alter acquisition of responding with a conditioned reinforcer. A similar dissociation between core and shell has also been recently demonstrated using a different appetitive task (Corbit and Balleine, 2000). Second, the NAcc may be preferentially involved in contextual aspects of appetitive stimuli, as witnessed by the deleterious effects of NAcc core lesions on Pavlovian approach behavior (Parkinson et al., 2000c) and NAcc pharmacological blockade of contextually conditioned locomotor activity (Franklin and Druhan, 2000), both of which are unaffected by manipulations of the amygdala.

4.2. The amygdala and appetitive conditioning

Several lines of research have extensively implicated the amygdala in the acquisition and expression of a variety of motivational tasks, both aversive (Cahill and McGaugh, 1990; LeDoux, 2000) and appetitive (Everitt et al., 2000; Gallagher, 2000). While the role of the amygdala in appetitive conditioning has long been recognized (Weiskrantz,

1956), research over the last 10 years has begun to clarify the unique roles of different amygdalar nuclei. For the sake of simplicity, the amygdala can be roughly divided into (1) the basolateral amygdala (BLA), a ''quasicortical'' area comprised of the lateral and basal nuclei, and (2) a central/medial collection of nuclei (CeA; Pitkanen, 2000). In general, the lateral/basal nuclei of the amygdala have a greater degree of direct connectivity with neocortical regions, while the CeA have greater subcortical connections (Alheid and Heimer, 1988). In addition, the CeA forms a major target of direct efferent projections from the BLA.

At a functional level, both of these regions are now recognized as having closely related yet separable roles in appetitive learning. The BLA is strongly involved in conditioned reinforcement and reward evaluation/devaluation during instrumental responding. Rats with BLA lesions showed a decrease in responding for presentations of conditioned stimuli associated with food (Cador et al., 1989) or sexual reinforcement (Everitt et al., 1989). However, responding for primary reinforcement was left intact after BLA lesions, thus indicating a selective lesion effect on conditioning and not on the ability to respond for primary reinforcers. While the BLA is critical for conditioned reinforcement, other tasks of appetitive conditioning are unaffected by BLA lesions. One example of this is Pavlovian approach behavior, whereby animals show approach behavior towards discriminative stimuli that have acquired incentive properties through repeated association with reinforcer availability. Even though these conditioned stimuli are predictive of reinforcement, they are spatially removed from the reinforcer and are presented independent of the animal's behavior. In contrast to a lack of effect of BLA lesions on this form of learning, CeA lesions attenuate conditioned approach behavior (Parkinson et al., 2000b) and conditioned orienting behaviors (Holland and Gallagher, 1999). Conversely, BLA lesions will attenuate conditioned stimulus-induced potentiation of feeding (Gallagher, 2000) and second-order Pavlovian conditioning (Everitt et al., 1989; Hatfield et al., 1996), while CeA lesions leave these tasks unaffected, further supporting a dissociation of function in these two amygdalar structures. In summary, the BLA appears to be critically important for the discrete associative pairing of neutral stimuli with primary reinforcement, while the CeA may be more critical for controlled attention, including orienting to distal conditioned stimuli.

4.3. The prefrontal cortex (PFC) and appetitive conditioning

The PFC is a key integrator of external and internal sensory information and coordinates output to various structures involved in motivational/emotional processes that guide complex behaviors (Fuster, 2000). There is a good deal of similarity between the rat and primate brains for both afferent and efferent PFC connectivity (Groenewegen et al., 1997), suggesting that rodent models can provide useful insight into frontal cortex regulation of appetitive learning. The main anatomical divisions of the rat PFC are the medial (anterior cingulate, prelimbic and infralimbic), lateral (dorsal and ventral agranular insular areas) and ventral (orbital). For the current discussion, it is worth noting several lines of evidence implicating the anterior cingulate and the orbitofrontal cortex in appetitive conditioning. The anterior cingulate cortex has extensive connections with the amygdala (Pitkanen, 2000) and the NAcc (Groenewegen et al., 1997). In appetitive conditioning tasks, it has been reported that anterior cingulate lesions will attenuate Pavlovian approach behaviors (Parkinson et al., 2000c) and discrimination task performance (Bussey et al., 1997). However, anterior cingulate lesions, in contrast to BLA lesions, do not abolish responding for a conditioned reinforcer (Burns et al., 1993). Like the anterior cingulate cortex, the orbitofrontal cortex has extensive anatomical connections with subcortical limbic structures (Haber et al., 1995). The orbitofrontal cortex has been implicated in response integration for rewarding stimuli (Tremblay and Schultz, 1999), response selection (Gallagher et al., 1999) and the formation of stimulus–reinforcer associations (Rolls, 2000). Recent evidence from clinical (Volkow and Fowler, 2000) and animal (Porrino and Lyons, 2000) studies strongly implicate both the anterior cingulate and the orbitofrontal cortex as critical components in the mediation of craving and relapse to drug-seeking behavior.

5. Neural substrates for conditioned-cued relapse

The immediate reinforcing effects of drugs of abuse have been clearly linked to mesolimbic–mesocortical dopamine (DA) function, particularly in the mesoaccumbens DA system consisting of cell bodies in the ventral tegmental area (VTA) with projecting axons to the NAcc (Fibiger et al., 1992; Koob, 1992). Evidence implicating the mesolimbic DA system in the reinforcing effects of drugs includes findings that lesions of the NAcc, the VTA and the ventral pallidum severely attenuate cocaine self-administration (Hubner and Koob, 1990; Roberts and Koob, 1982; Roberts et al., 1980). Furthermore, extracellular DA levels are reliably increased in the NAcc during self-administration of drugs such as cocaine (Meil et al., 1995; Weiss et al., 1992; Wise et al., 1995b), alcohol (Weiss et al., 1993) and heroin (Wise et al., 1995a). While assessment of the neural basis of drug-seeking behavior has centered primarily on the initiation, acquisition and maintenance of drug taking, the neural processes related to prolonged drug withdrawal and environmental conditioning have only recently come under greater scrutiny. Since the mesolimbic DA pathway is a primary site for the unconditioned effects of cocaine and other drugs of abuse, many models postulate that sensitization of mesoaccumbens DA release increases the motivational value of drug-associated stimuli (Robinson and

Berridge, 1993; Schultz et al., 1997). This possibility is supported by findings such as (a) NAcc lesions disrupt responding to motivationally relevant stimuli (Le Moal and Simon, 1991), (b) NAcc DA release can be augmented by rewarding stimuli (Mas et al., 1990) and (c) some NAcc cells show distinctive changes in firing patterns in the presence of conditioned stimuli (Carelli, 2000). However, given that prolonged drug exposure results in time-related alterations in a variety of neurotransmitter systems, it is likely that the neural processes underlying relapse are, in many respects, different from the processes that mediate immediate drug reinforcement (Koob and Le Moal, 2001; Robinson and Berridge, 2000). Several lines of evidence support such a possibility. For example, cocaine injections enhanced c-*fos* expression in the NAcc, but exposure to a cocaine-conditioned environment failed to stimulate c-*fos* expression in the NAcc (Brown et al., 1992). Furthermore, DA release in the NAcc was not elevated in rats responding for presentation of drug-paired stimuli after prolonged withdrawal from cocaine (Meil et al., 1995; Neisewander et al., 1996) or amphetamine (Di Ciano et al., 2001) self-administration or in monkeys presented with a cocaine-associated visual stimulus (Bradberry et al., 2000). Finally, it has been shown that lesions (Taylor and Robbins, 1986) or pharmacological blockade (Grimm and See, 2000) of the NAcc do not abolish responding for drug-paired conditioned reinforcers. Such evidence indicates that appetitive conditioning with drugs of abuse extends beyond the mesoaccumbens DA pathway. Accumulating evidence from clinical and preclinical studies suggests that the same structures that regulate appetitive conditioning for natural reinforcers also form the essential circuitry for mediating the maladaptive associative learning that occurs during addiction.

5.1. Human imaging data

Recent brain imaging studies in cocaine abusers have contributed evidence that relatively discrete pathways are activated in the presence of drug-associated cues that produce self-reported craving. These studies have employed different assessment techniques, including positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Furthermore, the parameters for cue presentation and self-reports have varied between studies. Table 1 summarizes some of the key findings from these imaging studies. While there are regions of nonoverlap between reported outcomes, there are clearly brain regions that show commonality across laboratories for metabolic activation by cocaine-paired cues. The salient point to note is the consistency of activation of two brain structures in response to cocaine-related cues: the amygdala and subregions of the frontal lobe. The amygdala shows greater activation in the presence of cocaine-related cues when compared with neutral cues (Childress et al., 1999; Grant et al., 1996; Kilts et al., 2001), although craving induced by cocaine itself has actually been correlated with negative signal changes as measured by fMRI (Breiter et al., 1997). Even more consistent across studies is the increased activation of specific cortical structures in the presence of cocaine-paired cues, particularly the anterior cingulate (Childress et al., 1999; Garavan et al., 2000; Kilts et al., 2001; Maas et al., 1998; Wexler et al., 2001) and the orbitofrontal cortex (Grant et al., 1996; Wang et al.,

Table 1
Summary of imaging studies of brain activation in the presence of cocaine-paired cues

Reference	Imaging method	Stimulus presentation	Areas showing increased activation during cocaine-paired stimuli presentation in cocaine users
Grant et al. (1996)	PET (glucose metabolism)	Drug paraphernalia and videotape of cocaine self-administration	amygdala[a] dorsolateral prefrontal cortex[a] orbitofrontal cortex
Maas et al. (1998)	fMRI	Audiovisual (video) of cocaine-related scenes	anterior cingulate[a] dorsolateral prefrontal cortex[a]
Childress et al. (1999)	PET (cerebral blood flow)	Audiovisual (video) of cocaine-related scenes	amygdala anterior cingulate
Wang et al. (1999)	PET (glucose metabolism)	Interactive interview about cocaine themes	insula[a] orbitofrontal cortex
Garavan et al. (2000)	fMRI	Audiovisual (video) of cocaine-related scenes	anterior cingulate inferior parietal cortex caudate/lateral dorsal nucleus several other regions[b]
Wexler et al. (2001)	fMRI	Audiovisual (video) of cocaine-related scenes	anterior cingulate frontal lobe regions[c]
Kilts et al. (2001)	PET (cerebral blood flow)	Personalized drug use scripts	amygdala anterior cingulate subcallosal gyrus NAcc

[a] Indicates areas found to correlate with self-reported craving.
[b] These areas were not content specific, in that other stimuli (sex film) produced similar activation.
[c] Indicates decreased activation in cocaine addicts.

1999). While almost all of the imaging studies of conditioned-cued brain activation have focused on changes produced during cocaine cue-induced craving, a recent study in heroin addicts examined changes in regional cerebral blood flow as measured by PET (Sell et al., 2000). Correlations with self-reported "urge to use" during viewing of heroin related cues were seen with increased regional blood flow in the orbitofrontal cortex, inferior frontal cortex, right precuneus and left insula.

Results from these imaging studies generally complement the known functional roles of these brain regions in cognitive processing, and the data lend support to current models that postulate corticolimbic dysfunction as the basis for repetitive, pathological drug-taking behavior (London et al., 1999; Volkow and Fowler, 2000). Although the anatomical resolution obtained with in vivo imaging methods remains somewhat limited, these clinical results also parallel very well with recent data derived from studies that have examined the role of the amygdala and anterior cingulate in animal models of conditioned-cued relapse.

5.2. Animal model data

Preclinical studies have only recently begun to explore the question of conditioned stimulus-induced reinstatement of responding after chronic self-administration of drugs of abuse. As with the human imaging data, evidence from animal models implicates the amygdala (Everitt et al., 1999) and areas within the frontal cortex (Porrino and Lyons, 2000).

In a series of studies, we have focused on the role of the BLA in mediating conditioned-cued reinstatement following prolonged cocaine self-administration in rats. Excitotoxic lesions of the BLA following 7 days of intravenous cocaine self-administration did not affect subsequent self-administration but significantly attenuated responding during extinction sessions and abolished the ability of drug-paired stimuli to reinstate lever responding (Meil and See, 1997). This selectivity of BLA lesions on conditioned reinstatement stands in contrast to the effects of NAcc, VTA or pallidal lesions, all of which decrease cocaine self-administration (Hubner and Koob, 1990; Roberts and Koob, 1982; Roberts et al., 1980). We have also utilized reversible inactivation with the Na^+ channel blocker, tetrodotoxin (TTX), an approach that has been successfully applied in a number of learning paradigms (Ambrogi Lorenzini et al., 1999). Reversible inactivation allows for disruption of associative processing in a discrete brain structure, without affecting these processes at later time points. In contrast, permanent lesions cannot as easily dissociate different stages of learning, such as acquisition vs. expression. We first utilized TTX inactivation in order to examine reinstatement following cocaine self-administration (Grimm and See, 2000). Following extinction, bilateral inactivation of the BLA resulted in significant attenuation of lever pressing for a cocaine-paired light + tone stimulus, but it had no effect on the reinstatement of cocaine self-administration. The exact opposite pattern was found with TTX inactivation of the NAcc, whereby TTX attenuated cocaine self-administration but left intact conditioned lever responding for the drug-paired stimuli. Finally, in order to test the role of amygdalar DA and glutamate in reinstatement, we recently examined the effects of selective DA and glutamate receptor antagonists directly infused in the BLA (See et al., 2001). A selective DA D1 antagonist (SCH23390) or a combination of SCH23390 + raclopride (DA D2/D3 antagonist) profoundly attenuated conditioned reinstatement, without affecting responding for cocaine by itself. Raclopride alone was without effect, suggesting a critical role of dopaminergic inputs to DA D1 receptors in the BLA, whereby DA may modulate the stimulus associations linked to drug reinforcement. In contrast to DA D1 antagonism, direct infusion of the glutamate receptor antagonists, AP5 (NMDA antagonist) and/or CNQX (kainate/AMPA antagonist), into the BLA did not have a significant effect on conditioned reinstatement (See et al., 2001). These glutamatergic receptors may be critical during the acquisition of drug-paired stimulus learning, but do not appear necessary for reinstatement with previously associated drug-paired stimuli.

In studies of aversive conditioning, both the acquisition and expression of conditioned associations are often examined (Amorapanth et al., 2000; Maren et al., 1996; Miserendino et al., 1990). Since most experimental paradigms in aversion learning (e.g. passive avoidance) can utilize one-trial acquisition sessions, it is relatively easy to directly test the neural substrates of acquisition by pharmacological manipulation at the time of learning as well as during later time points. Models of appetitive learning with drugs of abuse have not readily approached the issue of acquisition, since multiple conditioning trials are invariably utilized during chronic drug self-administration (Fuchs et al., 1998; Meil and See, 1996; Weiss et al., 2000; Weissenborn et al., 1995). In order to examine the neural substrates of associative learning with drugs of abuse at different stages, we recently developed a modification of our reinstatement paradigm that allows for assessment of both *acquisition* and *expression* of stimulus–drug associations. Rats are first trained to lever press for cocaine over several days in the absence of programmed stimuli. The animals then experience a separate Pavlovian conditioning session for acquisition, during which they receive noncontingent pairings (no levers available) of a light + tone stimulus discretely paired with cocaine infusions. Afterwards, animals continue for several days on cocaine self-administration in the absence of the stimuli, followed by extinction trials and reinstatement testing. In our first study, we examined whether conditioned reinstatement would occur using this approach (Kruzich et al., 2001). We found that discrete stimulus–drug pairings during prior Pavlovian conditioning sessions would later reinstate extinguished lever responding at levels seen in animals that received the paired stimulus throughout the daily self-administration sessions. Importantly, animals that

experienced presentation of the light + tone in a completely random fashion at the time of acquisition (Rescorla, 1967) or as a novel stimulus (i.e. first experience of the stimulus at the time of reinstatement testing) did not significantly increase their lever responding over extinction levels.

Using this approach of a discrete acquisition session, we applied TTX inactivation in order to assess amygdalar regulation of acquisition and expression of cocaine-paired associative learning (Kruzich and See, 2001). Separate groups of rats received bilateral TTX or vehicle infusions in the BLA or CeA just prior to the acquisition (Pavlovian conditioning trial) or expression (conditioned reinstatement) sessions. Our results show that the BLA is critical in the initial formation of stimulus–drug associations, as well as the expression of cocaine-seeking behavior, since TTX blocked reinstatement under both conditions. In contrast, TTX infused in the CeA just prior to acquisition failed to block subsequent reinstatement, although TTX just prior to expression effectively blocked reinstatement. This is the first study, to our knowledge, that has directly assessed neural circuitry in both the acquisition and the expression of drug-associated conditioned stimuli in a self-administration relapse model. We interpret this finding as a reflection of the primary role of the BLA in the initial associative formation of stimulus–reinforcer pairings. Since much of the efferent outflow of the BLA occurs via the CeA, the fact that expression is blocked by inactivation of either amygdalar nucleus suggests that expression of reinstatement follows a lateral to medial flow of stimulus processing. This interpretation fits what is known about intraamygdalar connections (Pitkanen, 2000) and supports current thinking regarding amygdalar processing of other affective learning, particularly fear conditioning (LeDoux, 2000).

Recent self-administration studies have further supported the role of the amygdala in mediating drug-seeking behavior that is initiated and maintained by conditioned stimuli. For example, excitotoxic BLA lesions had no effect on the maintenance of cocaine self-administration but attenuated second-order responding for a cocaine-paired conditioned stimulus (Whitelaw et al., 1996). In studies utilizing extinction/reinstatement procedures, increases in extracellular DA have been found in the BLA during presentation of a discriminative stimulus (Weiss et al., 2000). While Weiss et al. (2000) also found an increase in extracellular DA in the NAcc in the presence of stimuli predictive of cocaine availability, other studies have reported no effect of a drug-paired conditioned stimulus on NAcc DA in monkeys (Bradberry et al., 2000) or rats (Di Ciano et al., 2001; Neisewander et al., 1996). Furthermore, increased c-*fos* expression in the BLA, but not the NAcc, was recently reported in rats exposed to cue-induced reinstatement using cocaine-predictive discriminative stimuli (Ciccocioppo et al., 2001). Such studies further support a pivotal role for the amygdala in drug-paired cue reinstatement.

Only a few studies have begun to explore the role of cortical structures in conditioned reinstatement models of relapse. Two recent experiments have measured c-*fos* expression immediately following drug-paired stimulus presentation. In the study by Ciccocioppo et al. (2001), increased c-*fos* expression following conditioned stimulus presentation was not only seen in the BLA but also in the anterior cingulate. It has also been reported that the anterior cingulate was the one region best correlated with a cocaine-induced c-*fos* response after contextual association with a cocaine-paired environment (Neisewander et al., 2000). Excitotoxic lesions of the anterior cingulate cortex do not affect responding for cocaine per se but do disrupt normal patterns of responding in the presence of a cocaine-associated stimulus under a second-order schedule of reinforcement (Weissenborn et al., 1997). We have recently found that TTX inactivation of the anterior cingulate (but not the parietal cortex) attenuates conditioned reinstatement for cocaine-paired stimuli (unpublished observations). Thus, while the data are still very limited, it would appear that the anterior cingulate is a critical component of the circuitry that is activated during conditioned-cued reinstatement.

6. Neural circuitry of discretely paired stimuli vs. predictive, contextual stimuli

Extensive evidence indicates that appetitive conditioning involving natural reinforcers (e.g. food) occurs through differential forms of learning, mediated via interconnected yet distinctly different neural pathways. In similar fashion, associative learning involving drugs of abuse involves these different modes of learning, depending upon the relationship between unconditioned and conditioned stimuli. Discrete stimulus–response learning is considered to be one form of associative learning that occurs during the process of drug taking (Stewart et al., 1984). An example of this is a proximal cue that becomes a conditioned reinforcer (e.g. a crack pipe for cocaine or a needle for heroin). Another form of associative learning involves the predictive stimuli (which are generally more distal to the subject) that comprise the context in which drug taking occurs (e.g. a house in which the drug is consumed). These two processes of associative learning simultaneously occur during drug taking, yet they may involve different patterns of neural activation.

We have recently extended our paradigm to examine stimuli that are predictive of drug availability. Such an approach has been previously used with cocaine self-administration, whereby discriminative stimuli are established during chronic self-administration and then used to elicit reinstatement of responding after short-term (Panlilio et al., 1996) or long-term extinction (Weiss et al., 2000). We tested the same light + tone stimulus used in our original conditioned reinstatement paradigm but presented as a predictive stimulus, whereby the light + tone is constantly present until the right lever is pressed, whereupon cocaine is delivered. As in our discretely paired, contingent stimulus paradigm, subjects showed a pattern of stable responding during

chronic maintenance, decreased responding during extinction and robust conditioned reinstatement of responding when the stimulus is presented again in the absence of cocaine. However, in contrast to our results with a discrete, contingent cocaine-paired stimulus, TTX inactivation of the BLA failed to significantly block reinstatement when the same stimulus was presented in a predictive manner (Fig. 2). These results suggest that these two forms of associative learning in a relapse model can be functionally dissociated. Specifically, it appears that the BLA is most critical for discretely paired conditioned stimuli. It is predicted that the anterior cingulate (as well as other possible structures such as the CeA and NAcc) preferentially mediates predictive stimulus features of drug-paired stimuli. Furthermore, based on current understanding of cortical function and appetitive conditioning (Rolls, 2000; Tremblay and Schultz, 1999), the orbitofrontal cortex may also be important in regulating both forms of associative processing.

Referring back to the cocaine-cued imaging studies, it would be of interest to vary the presentation of drug-paired stimuli as a means of differentiating conditioned stimulus processing. A recent study reported that the largest changes in physiological measures of arousal in cocaine-dependent subjects occurred in those individuals allowed to physically interact with cocaine-related paraphernalia, as compared to audio or visual presentations alone (Johnson et al., 1998). If the amygdala plays a greater role in discrete stimulus–reinforcer associations, direct engagement of drug-paired stimuli during scanning procedures may preferentially activate the amygdala. In fact, in one of the three studies reporting amygdalar activation in cocaine addicts, subjects were exposed to drug paraphernalia as well as videos (Grant et al., 1996). Passive presentation of a broader set of contextual cues, such as those observed during drug-related videos, may be more likely to preferentially engage the anterior cingulate and orbitofrontal cortex.

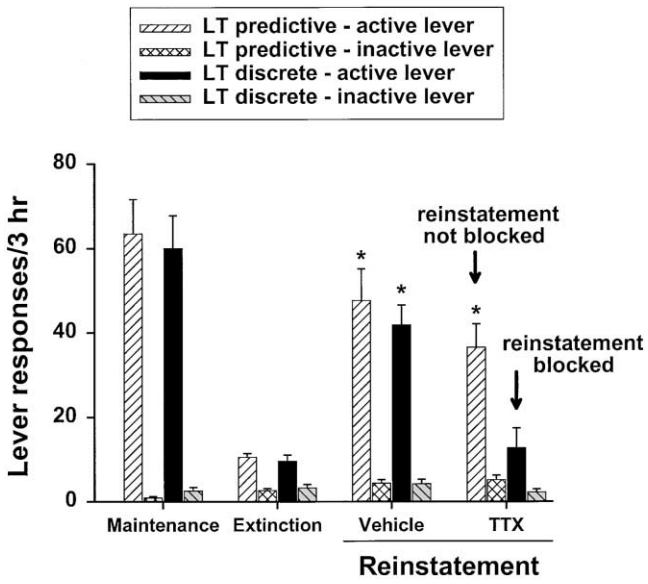

Fig. 2. TTX infused into the BLA blocks reinstatement of active lever responding to a contingent, discretely paired compound stimulus light + tone (LT) but fails to disrupt conditioned reinstatement when the LT is presented in a predictive manner (i.e. signalling drug availability). Active (drug paired) and inactive lever responses (mean ± S.E.M.) are shown for the last day of self-administration (maintenance), last day of extinction and the two test sessions. Subjects received bilateral infusions of vehicle or TTX just prior to beginning each test session. Significant increases over extinction level responding are noted (* $P < .05$; Student–Newman–Keuls' test).

7. Differences in conditioned-cued relapse across drugs of abuse

While the neural substrates of reinforcement for various drugs of abuse involve common structures such as the NAcc and VTA (Koob, 1992), functional differences in this circuitry may exist across different drug classes (Ettenberg et al., 1982; Pettit et al., 1984). If the circuitry that mediates responding for cocaine-paired conditioned stimuli constitutes one common pattern of associative learning, the circuitry should be equally critical in mediating conditioned responding for stimuli associated with other drugs, such as opiates or alcohol. However, recent findings with heroin reinforcement in rats support the possibility that appetitive conditioning with opiates may differ in some regards from psychostimulants (Everitt et al., 2000; Geist and Ettenberg, 1997; McFarland and Ettenberg, 1997). While the available data from imaging studies are quite limited (see above), the study by Sell et al. (2000) showed that heroin-related cues produced a pattern of brain metabolic activation similar to that seen with cocaine-related cues (e.g. increased in the orbitofrontal cortex) but also included areas not noted with cocaine (e.g. precuneus). A recent report found a lesser impact of discrete heroin-associated cues on heroin-seeking in rats under a second-order schedule, relative to responding seen for cocaine-associated cues (Alderson et al., 2000b). The authors suggest that predictive (i.e. contextual) cues may play a greater role in conditioning with opiates than with psychostimulants. Furthermore, in contrast to cocaine, excitotoxic lesions of the BLA failed to block heroin-seeking behavior maintained under a second-order schedule (Alderson et al., 2000a).

Our own initial results (unpublished) with heroin self-administration in an extinction/reinstatement procedure similar to that used for cocaine show robust reinstatement in the presence of a discretely paired compound stimulus. In addition, blockade of conditioned reinstatement for the heroin-paired stimulus was seen after TTX, but not vehicle, infusion into the BLA. These results do suggest a role for the BLA in conditioned reinstatement for opiate-paired stimuli, similar to that seen for psychostimulants. Although prefrontal structures have not yet been examined in a relapse model with opiates, it has been reported that excitotoxic lesions of prefrontal subregions had uniquely different effects on conditioned place preference depending upon the type of drug (Tzschentke and Schmidt, 1999). Thus, it

is possible that the cortical circuitry engaged by heroin-paired cues is different from that activated by cocaine-paired cues and may indeed involve greater associative processing of contextual cues.

Conditioned-cued craving and relapse has also been demonstrated with other drugs of abuse, including alcohol (Drummond, 2000) and nicotine (Drobes and Tiffany, 1997). However, data on the neural substrates of conditioned-cued relapse with other drugs of abuse are only beginning to emerge. One recent study using fMRI reported that alcoholics presented with alcohol-related cues showed increased activity in the dorsolateral PFC and the anterior thalamus (George et al., 2001). As for nicotine, there are as yet no studies that have explored the neural substrates of conditioned-cued relapse. Based on evidence that nicotine activates similar neural pathways as cocaine and other addictive drugs (Pagliusi et al., 1996; Pich et al., 1997; Stein et al., 1998), it is likely that the substrates for conditioned-cued relapse would also show considerable overlap. On the other hand, recent evidence from an animal model of cue-induced reinstatement in rats previously trained to self-administer nicotine has shown that nicotine-paired stimuli may play an even more critical role in the learned associations that precipitate relapse than those seen with cocaine (Caggiula et al., 2000). If such differences are of significance, then the neural substrates of relapse in the presence of nicotine-paired stimuli may show some degree of differentiation from the pattern found for cocaine or opiates.

8. Conclusions

Although much has been learned about the neural basis of reinforcement during drug self-administration, the neural circuitry that underlies conditioned-cued relapse is only now being clarified. The information gained from previous studies of associative learning with nondrug appetitive reinforcers provides a clear foundation for the hypothesis that different components of corticolimbic circuitry contribute to different aspects of conditioning in the presence of drug-paired stimuli. As the assessment of brain function in human addicts and animal models of relapse becomes more sophisticated, the exact roles of each structure in the various components of drug-paired learning across all classes of abused drugs will continue to be elucidated.

Among the many issues that remain to be examined is the role of various neurotransmitters that mediate amygdalar–cortical function during relapse. For example, acetylcholine is one of the best-characterized neurotransmitters in mediating associative learning and memory. The most densely labeled area for acetylcholinesterase staining in the rat brain is the BLA (Paxinos and Watson, 1986), with the major cholinergic projections arising from the nucleus basalis (Carlsen and Heimer, 1986; van der Zee and Luiten, 1999). While a number of studies have examined cholinergic modulation of amygdala-mediated learning (Blozovski and Dumery, 1987; Dumery and Blozovski, 1987; Ingles et al., 1993; Vazdarjanova and McGaugh, 1999), cholinergic regulation in a relapse model has never been examined. Other examples of critical neurotransmitters that remain to be explored in the context of conditioned-cued relapse include norepinephrine, serotonin and various neuropeptides.

Finally, given that conditioned-cued craving plays a significant role in relapse, a better understanding of the relevant brain nuclei that subserve these processes should yield new behavioral and pharmacological treatment approaches that can help break patterns of repetitive, compulsive drug use. The further development and application of animal models of relapse will provide a testable means for assessing treatment intervention following prolonged withdrawal from chronic drug self-administration.

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