

Brain substrates for increased drug seeking during protracted withdrawal

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Abstract

Studies are reviewed indicating that both increased anxiety and altered hedonic processing accompany protracted withdrawal from opiates. Increased anxiety may be most apparent in response to stress, whereas decreased motivation for natural rewards but increased interest in drugs reveals substantial alterations in hedonic values. Our recent work indicates that increased norepinephrine (NE) release in the bed nucleus of the stria terminalis (BNST) may underlie anxiety associated with protracted withdrawal. Altered plasticity in afferents to the ventral tegmental area (VTA; accumbens, amygdala and lateral hypothalamus), or in the VTA itself, may be involved in the altered hedonic processing that occurs during protracted withdrawal. We hypothesize that conditioned release of NE in the BNST in response to stressors (including drug-associated stimuli) may elevate anxiety which then augments the reward value of drugs by a negative reinforcement mechanism. We also propose that plasticity in VTA neurons and their afferents during chronic drug exposure and protracted withdrawal decreases the valence of natural rewards whereas sensitization occurs to the motivational effects of drugs that increases their motivational valence. The combination of anxiety, decreased valence of natural rewards, and sensitized incentive for drugs make a potent formula for relapse and drug seeking during protracted withdrawal.

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1. Introduction

Drug addiction is a chronic relapsing disorder characterized by compulsive drug seeking and use. More than 80% of addicts relapse to drug seeking and use after a period of withdrawal and abstinence during what is known as the protracted withdrawal phase. The long-lasting nature of this compulsion and the high rates of recidivism present a challenge for effective treatment. An understanding of the neurobiological basis of drug seeking and relapse is a central issue of addiction research.

We describe here an hypothesis for brain mechanisms that may underlie, at least in part, the increased drug seeking during protracted withdrawal. We propose that two important alterations occur following

dependence and withdrawal: (i) Conditioned responses of A1/A2 neurons release norepinephrine (NE) in the extended amygdala. This produces elevated anxiety which, when alleviated by drug administration, generates negative reinforcement that summates with the positive reinforcing qualities of drugs. (ii) Changes occur in the mesocorticolimbic dopamine (DA) system and its afferents that alter hedonic processing and impair either the learning of, or motivation for, natural rewards. At the same time, motivation or learning for drug reward and drug-associated cues is increased.

In support of this hypothesis, we first present our data revealing a prominent role for norepinephrine projections from the A1/A2 neurons of the caudal medulla to the bed nucleus of the stria terminalis (BNST) and central amygdala (CNA) in the affective (aversive) response to acute opiate withdrawal. Next, we review our more recent findings showing that neurons in the extended amygdala respond to drug-associated stimuli during

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protracted withdrawal in proportion to the preference expressed for these stimuli; the area of the BNST where NE is important in the aversive aspect of withdrawal is prominently implicated in this response as well. We will then briefly review evidence implicating mesocortical limbic and dopamine neurons in the processing of, and motivation for, reward. Finally, we will present results indicating that although preference for drug cues is increased the preference for natural rewards such as food is decreased during protracted withdrawal, indicating a change in overall hedonic processing. Associated changes in Fos expression in the extended amygdala and lateral hypothalamus pinpoint specific areas where altered plasticity in afferents to the VTA may underlie such alterations in hedonic function. Finally, we review our current work showing that plasticity in the VTA itself is important for learning and remembering drug-timulus associations, indicating that plasticity changes within the VTA might also be involved in hedonic changes during protracted withdrawal.

2. Role of norepinephrine in the extended amygdala in the affective response to opiate withdrawal

Previous studies have shown that NE neurons are strongly activated during opiate withdrawal. Although a great deal has been learned about the mechanisms involved in activation of these cells (Akaoka and Aston-Jones, 1991; Aston-Jones et al., 1993, 1997; Nestler and Aghajanian, 1997), until recently little was known about where elevated NE acted to produce behavioral symptoms of withdrawal. We noted that the densest NE innervation in the brain occurs in the ventral BNST (vBNST). We therefore studied the possible role of NE in the vBNST in affective and somatic responses to withdrawal from chronic morphine. As described in more detail in our publications, we found that selective beta adrenoceptor antagonists micro-injected into the vBNST eliminated the aversive response to withdrawal as measured in a place conditioning paradigm, but had little or no effect on the somatic (physical) signs of withdrawal (Aston-Jones et al., 1999; Delfs et al., 2000). Additional anatomical studies revealed that the bulk of the NE innervation to the vBNST originates in the A1 and A2 noradrenergic cell groups of the caudal medulla; relatively few inputs derive from the locus coeruleus (LC) (Delfs et al., 2000). In view of these results, we selectively neurochemically lesioned the ventral bundle of NE fiber projections from the A1/A2 neurons and confirmed that aversive, but not somatic, responses to morphine withdrawal were virtually eliminated (Delfs et al., 2000). In contrast, similar lesions of the dorsal bundle of NE fibers ascending from the LC had no significant effect on either affective or somatic withdrawal signs. Thus,

the vBNST is an important target for behavioral effects of elevated NE release during withdrawal, and the NE input from the A1/A2 neurons is critical for the aversive response to acute opiate withdrawal.

The BNST is closely related to the medial and central amygdala, so that they together with intervening areas have been proposed to be a single functional system, denoted as the extended amygdala (Alheid and Heimer, 1988; Alheid et al., 1995). This similarity between the amygdala and BNST prompted us to extend the above studies to the amygdala. We found that selective beta adrenoceptor antagonists micro-injected into the central nucleus of the amygdala also decreased the aversive response to morphine withdrawal measured with a similar place conditioning paradigm as described above (unpublished results). An important additional finding in these studies is that the elevated anxiety that accompanied abstinence withdrawal was also blocked by the intra-amygdala or vBNST beta adrenoceptor antagonist injection. These results are consistent with recent work indicating roles for the BNST and amygdala in anxiety and fear (Davis et al., 1997; Cecchi et al., 2002). Together, these considerations lead us to propose that the anxiogenic response to withdrawal is an important element in the aversive response, and that elevated NE in the extended amygdala is a key factor producing this heightened anxiety. As described below, prolonged heightened anxiety during the protracted withdrawal period may also play a role in the increased drug seeking during this time.

3. Protracted withdrawal: activation of extended amygdala and enhanced drug seeking

Vulnerability to relapse during long-term withdrawal is thought to result in part from adaptive processes within the CNS that oppose the acute actions of drugs of abuse (the homeostatic hypothesis, reviewed in Weiss et al., 2001). These changes may lead to affective changes such as anxiety during withdrawal. Unlike the acute physical withdrawal syndromes that occur during the first 24 h of drug abstinence, these affective changes can persist for considerable periods of time (weeks or longer) during the protracted withdrawal phase. The alleviation of the discomfort associated with such negative affective states can be a motivation for drug seeking (via a negative reinforcement mechanism) during protracted withdrawal (Koob and Le Moal, 1997). Although a great deal of work has defined the roles of brain areas in drug dependence and withdrawal, very few have examined how withdrawal, particularly protracted withdrawal, alters brain areas to produce the increased drug seeking that characterizes human drug addicts during abstinence. Therefore, we next asked

whether the circuits and actions identified above for acute withdrawal may also be involved in the drug seeking that occurs during protracted withdrawal.

3.1. Behavior

We first established an animal model of increased drug seeking during protracted withdrawal. As described in more detail in our recent publications, rats were made dependent with morphine pellets (denoted as M rats) or given placebo pellets (P rats) for two weeks, and then subjected to abstinence withdrawal for two (Harris and Aston-Jones, 2001) or five weeks (Harris and Aston-Jones, 2003e). After this withdrawal period, they underwent a three-day place conditioning procedure where a distinct environment was paired with an acute dose of morphine (10 mg/kg ip) on three successive days, while another environment was paired with saline injections. One week after these conditioning sessions, animals were tested in a drug-free state for their preference for morphine-paired vs. saline-paired environments. Note that during the chronic morphine exposure and subsequent abstinence withdrawal, animals remained in their home cages and there was no conditioning or exposure to the environments that were

subsequently used for place conditioning with morphine or saline.

As expected, this paradigm produced a significant preference for the morphine-paired environment in all conditioned rats. Notably, however, the preference for the morphine environment was greatly increased by chronic morphine pretreatment followed by either two or five weeks of abstinence withdrawal prior to CPP testing. All conditioned groups were significantly different from non-conditioned groups, and conditioned morphine pretreated (M) rats showed enhanced preference for the morphine-paired environment relative to conditioned placebo pretreated (P) rats (Fig. 1). The degree of preference was similar in the two- and five-week withdrawal groups such that increased preference was not decreased by the five-week withdrawal period, indicating a long-lasting effect. It is noteworthy that the stress-induced reinstatement of heroin self-administration also follows a similar time course, becoming greater at six and 12 days post-withdrawal (Shalev et al., 2001). In addition, we have found a similar increase in preference for cocaine following two weeks of withdrawal from chronic cocaine administration, indicating that increased drug seeking during protracted withdrawal may be found for several drugs of abuse (Harris et al., 2001).

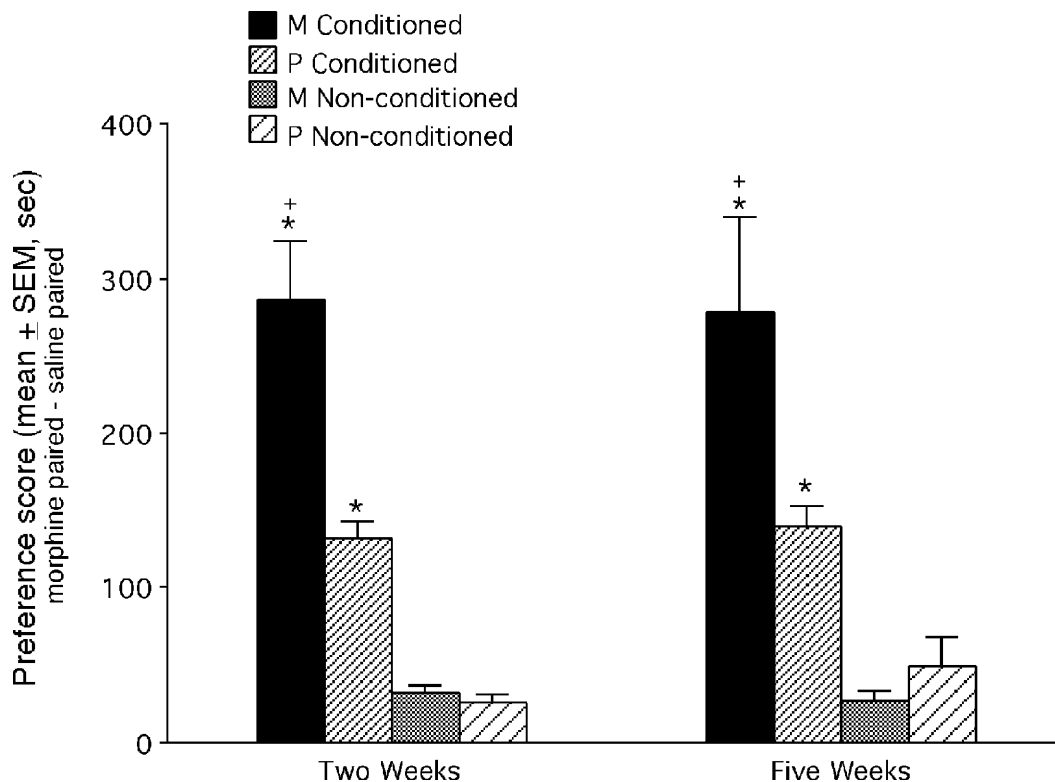


Fig. 1. Preference scores for the morphine-paired environment expressed as the mean time in seconds spent in the morphine-paired side minus the mean time in seconds spent in the saline-paired side on the test day. *Significantly different ($p < 0.01$) from non-conditioned groups; +significantly different ($p < 0.01$) from conditioned P groups. M—rats pretreated with morphine pellets; P—rats pretreated with placebo pellets. Taken from Harris and Aston-Jones (2003c).

Others have also reported that preference scores increase following prolonged exposure to morphine (Lett, 1989; Contarino et al., 1997). However, those studies did not examine preference following prolonged drug abstinence. In addition, unlike our study in which a continuous-release morphine pellet was used, the prior reports used a daily injection regimen that is known to produce substantial locomotor sensitization (Vanderschuren et al., 1997). It is interesting that both modes of morphine administration produced similar results for enhanced preference following prior morphine administration. This indicates that withdrawal from opiates may be an important factor in generating enhanced preference to opiates, whether it occurs repeatedly as in the case of daily injections or just once following a continuous exposure to opiates as in our study.

We also measured anxiety using the defensive conditioned burying paradigm (Harris and Aston-Jones, 2001). In this procedure, rats are individually placed in a chamber with an electrified prod inserted through a hole in the chamber wall. While exploring the prod, rats receive one shock, and subsequently bury the prod using bedding material from the floor of the chamber. Burying time is measured for 15 min. Enhanced anxiety is evidenced by significantly shorter latencies to begin burying, as well as by an increased in burying durations. Previous studies have validated this procedure as a measure of anxiety using conventional anxiolytic drugs (Treit, 1985). Results revealed that M animals (two weeks after withdrawal) were substantially more anxious than P animals. This is consistent with our previous finding for prolonged elevated anxiety following withdrawal from cocaine treatment (Harris et al., 2001) As described below, we hypothesize that this elevated anxiety may play an important role in the increased preference for morphine exhibited by the M animals.

3.2. Fos expression

We used immunohistochemistry for the Fos protein (produced by the immediate early gene, *cfos*) to identify neurons in the extended amygdala whose activity was associated with increased drug seeking during protracted withdrawal (Harris and Aston-Jones, 2003c). For this, M or P rats were euthanized 2 h following the CPP preference test; this is at least one week following any morphine exposure. Thus, Fos expression here (measured by counts of Fos-positive neurons) reflects response to the CPP environment previously associated with drug exposure and the animal's expression of preference. In these experiments, conditioned P and M animals showed significantly more Fos in extended amygdala regions than non-conditioned animals. Furthermore, the conditioned M group showed signifi-

cantly higher Fos levels than either the conditioned or non-conditioned P groups in the anterior cingulate cortex (Cg), nucleus accumbens core (Ac-C) and shell (Ac-S), vBNST, and central and basolateral amygdala nuclei (ACE, ABL; Fig. 2). The conditioned P group exhibited significantly greater Fos than the non-conditioned groups in the Cg, Ac-S, Ac-C vBNST, ACE, ABL and dorsal lateral BNST. In contrast, a brain area not known to be involved in drug conditioned behaviors, the paraventricular thalamic nucleus (PVT), showed no significant differences in Fos levels in any of the groups. This finding indicated that changes in Fos were specific for areas involved in drug conditioned responses and were not non-specifically distributed across brain areas.

3.2.1. Correlations Between Preference Scores and Fos Expression

To determine if the neuronal stimulation observed above (reflected in Fos activation) plays a role in the increased drug seeking during protracted withdrawal, we calculated the Pearson's product-moment correlation coefficient (r) in each group between the number of cells expressing Fos in each brain area and the degree of preference expressed for each animal (Harris and Aston-Jones, 2003c). Significant positive correlations (r values > 0.72) were found in the Cg and ABL for the conditioned P group (Table 1). In the conditioned M group, significant positive correlations between preference and Fos staining were found in the Cg, ABL and vBNST (r values > 0.81). Non-conditioned animals had no significant correlations.

It is noteworthy that the amount of preference strongly correlated with Fos levels in the ABL and Cg in both P and M conditioned groups. This may reflect activity in these regions associated with conditioning irrespective of prior morphine exposure. The vBNST was the one area where a significant positive correlation between Fos and behavior was found only in the M conditioned animals, indicating that this area may be particularly involved in the increased preference seen in subjects that were chronically pretreated with morphine and subjected to withdrawal. It is also notable that this is the same area that receives a dense noradrenergic innervation from A1/A2 neurons in the caudal medulla, a pathway that is critically involved in the aversive response to acute opiate withdrawal (Delfs et al., 1998; Aston-Jones et al., 1999) (described above) and in the action of stress to induce relapse of drug self-administration (Leri et al., 2002). This indicates a possible site where elevated NE during withdrawal may act to promote long-term changes associated with increased propensity for drug seeking.

As described above, the BNST, and in particular NE innervation of the BNST, has been recently linked to anxiety (Davis et al., 1997; Cecchi et al., 2002). We

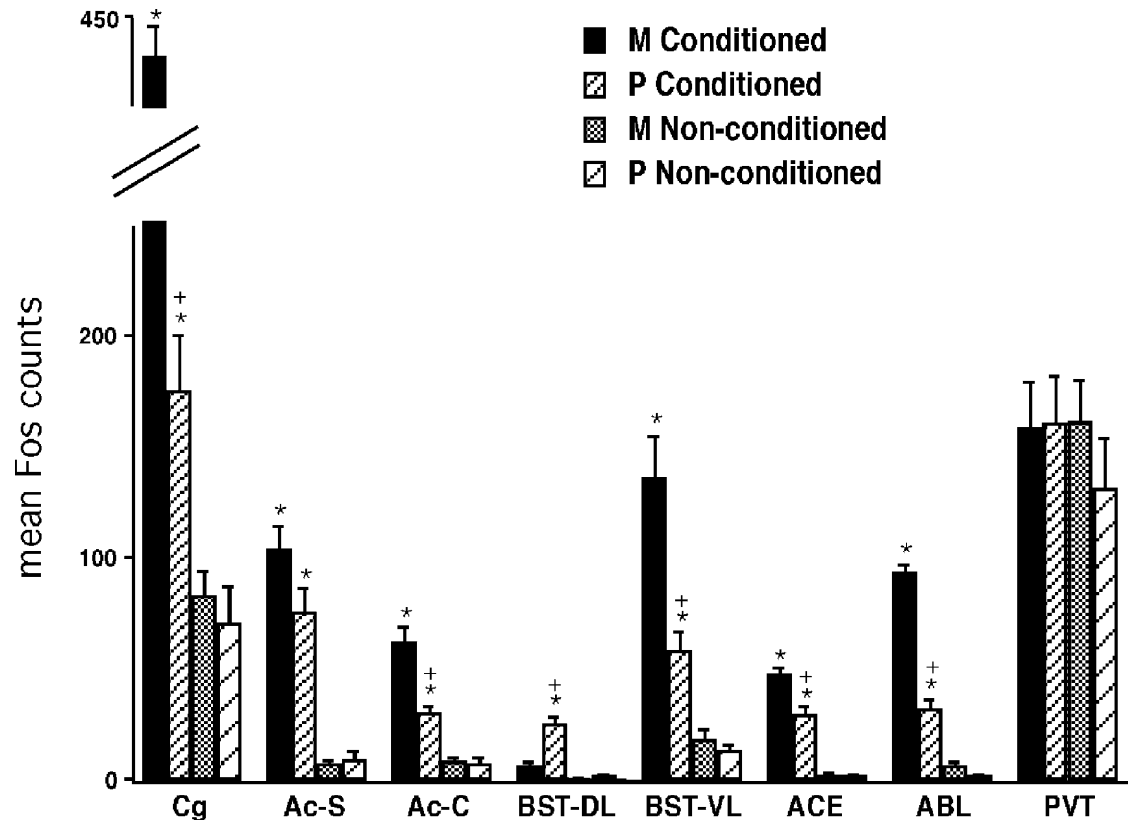


Fig. 2. Number of Fos-positive nuclei (\pm SEM) counted in the cingulate cortex (Cg), nucleus accumbens shell (Ac-S) and core (Ac-C), bed nucleus of the stria terminalis dorsal lateral (BST-DL) and ventral lateral (BST-VL), amygdala central (ACE) and basolateral (ABL) nucleus and the periventricular nucleus of the thalamus (PVT). *significantly different ($p < 0.01$) from non-conditioned groups; +significantly different ($p < 0.05$) from conditioned M groups. M—rats pretreated with morphine pellets; P—rats pretreated with placebo pellets. Taken from Harris and Aston-Jones (2003c).

propose that during protracted withdrawal stressors (including conditioned stimuli such as the drug-paired environment in the CPP test) activate A1/A2 neurons that liberate NE in the extended amygdala. In support of this idea, we found that A2 noradrenergic neurons in the nucleus tractus solitarius (the major source of

NE to the vBNST, Delfs et al., 2000) also exhibited increased Fos expression following the CPP preference test in conditioned M animals compared to either P conditioned or non-conditioned subjects (unpublished observations). This heightened anxiety may increase the reinforcing quality of drugs due to their anxiolytic

Table 1

Correlation coefficients showing the relationships between the numbers of Fos+ cells in each area and the preference for the morphine-paired side in the corresponding animal (Pearson's product-moment correlation coefficient, r). M and P refer to morphine- or placebo-pretreated animals, respectively. Statistically significant correlations are indicated in bold and with asterisks

| | Cg | Ac-S | Ac-C | BST-DL | vBNST | ABL | ACE | PVT |
|-------------------|--------------|------|-------|--------|--------------|--------------|-------|-------|
| <i>Five weeks</i> | | | | | | | | |
| M conditioned | 0.88* | 0.12 | 0.53 | -0.01 | 0.84* | 0.89* | -0.57 | -0.06 |
| P conditioned | 0.77* | 0.65 | 0.24 | -0.26 | 0.01 | 0.81* | 0.54 | 0.09 |
| M non-conditioned | 0.21 | 0.08 | 0.14 | 0.10 | 0.04 | 0.01 | 0.17 | 0.05 |
| P non-conditioned | 0.20 | 0.11 | -0.25 | 0.05 | 0.07 | 0.14 | 0.09 | 0.01 |
| <i>Two weeks</i> | | | | | | | | |
| M conditioned | 0.54 | 0.31 | 0.67 | -0.21 | 0.72* | 0.72* | 0.66 | 0.24 |
| P conditioned | 0.79* | 0.08 | -0.43 | 0.21 | 0.34 | 0.76* | -0.56 | -0.10 |
| M non-conditioned | 0.12 | 0.05 | 0.20 | 0.04 | 0.15 | 0.07 | 0.04 | 0.09 |
| P non-conditioned | 0.10 | 0.06 | 0.12 | 0.02 | 0.08 | 0.13 | 0.06 | -0.02 |

* $p < 0.05$.

actions resulting in negative reinforcement. We propose that this negative reinforcement with drugs (linked to conditioned A1/A2 neural responses) during protracted withdrawal may underlie, at least in part, the increased drug seeking that accompanies protracted withdrawal.

It is noteworthy that elevated anxiety during cocaine withdrawal is reported for the defensive burying test (as we found) but not when using the elevated plus maze (Basso et al., 1999). This may indicate that the elevated anxiety during withdrawal reflects an increased sensitivity to stress (such as the shock in the defensive burying paradigm) but is not evident in resting baseline conditions (plus maze). This is consistent with previous studies finding elevated stress sensitivity in addiction (Kreek and Koob, 1998).

4. Altered hedonic processing during protracted withdrawal

Other results indicate that altered hedonic processing may also contribute to the increased interest in drugs during prolonged abstinence. Thus, human clinical observations find that addicts have feelings of dysphoria, depression and anhedonia for prolonged periods following abstinence from drugs (Dole et al., 1966; Martin and Jasinski, 1969). Researchers have hypothesized that these symptoms of protracted opiate withdrawal involve dysregulation of hedonic processing due to altered brain chemistry (Solomon, 1977; Koob and Le Moal, 1997), and that these changes contribute to drug seeking and relapse. The following sections review evidence that corticolimbic areas linked with midbrain dopamine neurons are integrally involved in hedonic processing. We then review our data revealing altered hedonic processing during protracted morphine withdrawal in rats, and associated changes in corticolimbic brain function.

4.1. The role of mesocorticolimbic dopamine system in hedonic processing

Primary appetitive stimuli consistently increase dopamine (DA) transmission in the Ac-S as well as in the prefrontal cortex and to a lesser extent in the Ac-C (Bassareo and Di Chiara, 1997; Tanda and Di Chiara, 1998; Bassareo and Di Chiara, 1999). Considerable evidence indicates that low to moderate doses of DA antagonists, or depletions of DA in the Ac-S, can suppress instrumental responding for food while leaving appetite (primary reinforcement) intact (Salamone and Correa, 2002). It has been proposed that the Ac-S may gate corticolimbic information to the lateral hypothalamus and exert executive control over brain circuits controlling feeding behavior and related motivation

(Kelley, 1999; Petrovich, 2001). Opioid peptide neurotransmission within the accumbens modulates the hedonic impact of food reward (Glass and Billington, 1999; Pecina and Berridge, 2000; Kelley et al., 2002), supporting the notion that drugs of abuse may act on systems that evolved to mediate natural rewards. Microinjections of morphine in the Ac-S directly increases “liking”-type rat orofacial expressions elicited by sucrose (Pecina and Berridge, 2000), and alters intake consistent with enhanced food palatability (Zhang and Kelley, 2000). Therefore, perturbations in this system caused by chronic opiate exposure could alter the hedonic or incentive motivational properties of natural rewards.

4.2. Altered hedonic processing during protracted morphine withdrawal in rats

We reasoned that if opiate withdrawn animals are dysfunctional in processing hedonic events, then withdrawn animals may have problems learning about appetitive reinforcers but not about other types of reinforcement (i.e., aversive events).

In the following series of experiments, we used several paradigms to determine whether opiate abstinent animals show altered learning and hedonic processing. Rats were either made dependent on morphine with pellets as described above (M animals), or were instead implanted with placebo pellets (P animals). These results are described in more detail in our recent publication (Harris and Aston-Jones, 2003a).

In the first experiment, rats were trained to lever-press for food on an escalating fixed ratio (FR) schedule of reward beginning one week after removing the morphine or placebo pellets. As shown in Fig. 3, M animals took significantly more time to learn to bar press for food and were significantly slower in mastering most FR requirements compared to P animals. Additional experiments and analyses indicated that the slower learning rates found in the M animals were not due to a non-specific change such as a motor impairment.

We then examined animals in a conditioned suppression paradigm to determine if the delayed FR conditioning we observed in M animals reflected a general learning deficit, or a change in behavior specifically related to appetitive tasks. For this, a tone previously paired with foot shock was presented during the performance of the instrumental food task to assess the effect on responding. As can be seen in Fig. 4, M animals took significantly longer to resume responding on an FR 16 schedule for food after the presentation of the shock-associated CS. On the first test day (immediately after CS-shock pairing) only two out of seven M animals resumed responding in the 30 min time period, and all seven of the P animals resumed responding. On

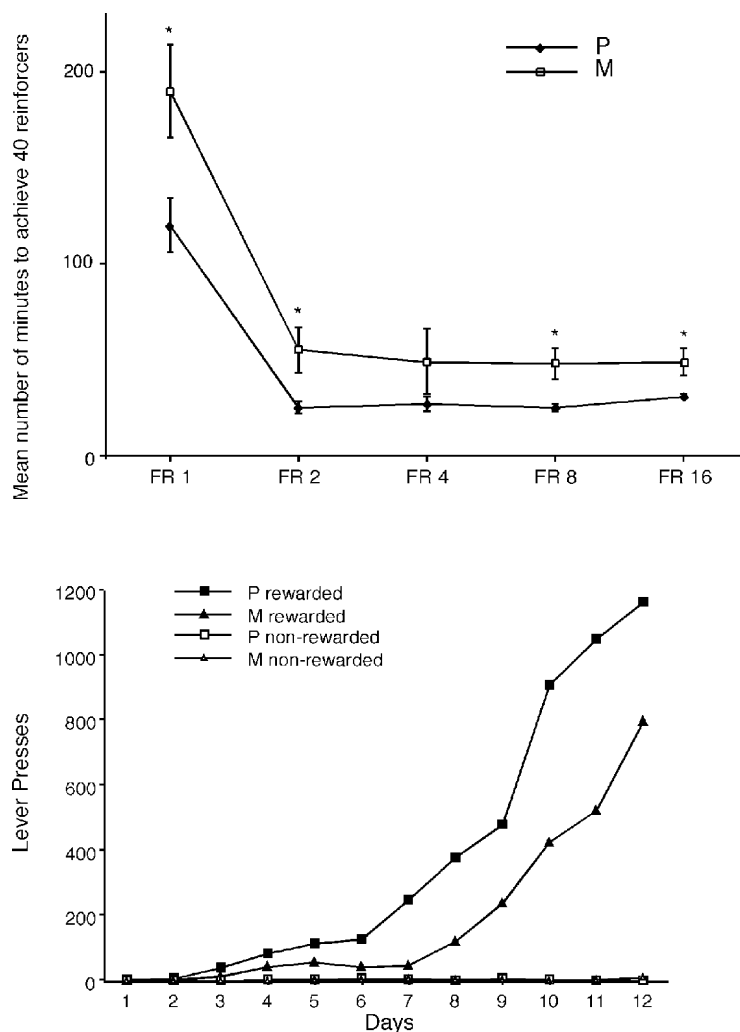


Fig. 3. Acquisition of responding for food reward at each FR schedule. *Upper panel*: the mean amount of time for each group to achieve 40 reinforcers averaged over different 30-min sessions is depicted. *significantly different ($p < 0.05$). *Lower panel*: Mean number of lever presses made by each group over the different days of training. P animals completed the criterion for the FR 16 by day 10. M—rats pretreated with morphine pellets; P—rats pretreated with placebo pellets. Taken from Harris and Aston-Jones (2003a).

the following day, four of seven M animals resumed responding in the 30 min time period compared to all seven P animals. These results show that chronic morphine dependence and protracted withdrawal does not produce a general learning deficit, because the M animals learned the association between the tone and shock at least as well (perhaps better) than P rats. Instead, these findings indicate that motivation for, or learning about, appetitive events is decreased while that for aversive events is increased during protracted withdrawal. It is noteworthy that at this time point in protracted withdrawal animals also show elevated anxiety in the defensive burying task (described above).

To further examine a possible change in learning or motivation for food during protracted withdrawal, we examined food place preference at two and five weeks post-morphine withdrawal and compared this to morphine place preference. Fig. 5 (upper panel) shows the

results of the food place conditioning study. Statistical analysis revealed that P rats expressed a significantly greater preference for the food-paired chamber than M rats. Comparison analyses of each group pre- and post-conditioning indicated that all groups except the two-week M group showed a significant place preference. A comparison of body weights in the two-week groups revealed no significant difference between M and P animals, indicating that M rats were physically recovered from withdrawal and eating normally. Fig. 5 (lower panel) shows the results of morphine place conditioning in rats withdrawn five weeks. Both M and P groups showed significant preferences for the morphine-paired environment, but M animals showed significantly greater preference than P animals. Thus, M animals showed less preference than P animals for food, but more preference than P animals for morphine.

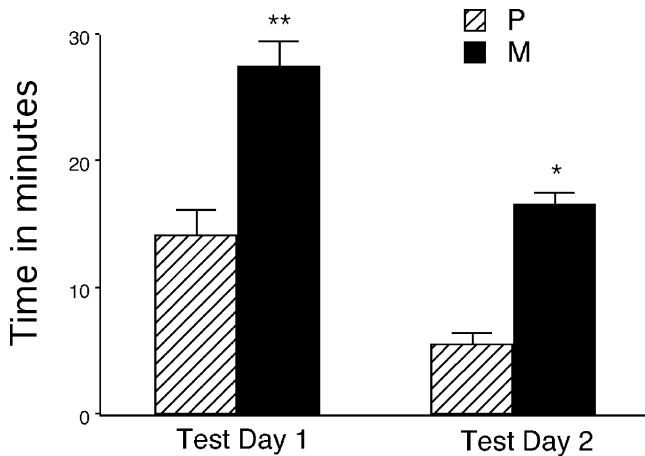


Fig. 4. Mean amount of time to resume responding for food after the presentation of a tone that had been paired with shock for M and P groups. The maximum amount of time was 30 min, *significantly different ($p < 0.05$), **significantly different ($p < 0.01$). M—rats pretreated with morphine pellets; P—rats pretreated with placebo pellets. Taken from Harris and Aston-Jones (2003a).

4.3. Opposite changes in Fos expression associated with altered food or morphine preference during protracted withdrawal

Recently, we also compared the Fos staining in the M and P groups between the food and morphine CPP paradigms. Results revealed that in morphine abstinent animals Fos induction during food CPP testing was significantly less in the Ac-S, lateral hypothalamus (LH) and ABL than that found in non-dependent animals. Conversely, Fos expression was significantly greater in these same areas in M compared to P rats that underwent morphine place conditioning. These results are illustrated in Fig. 6. Thus, significantly higher levels of Fos were found in the Ac-S, ABL and LH in the groups showing greater preferences (i.e., in M rats for the morphine-paired chamber, and in P rats for the food-paired chamber). Conversely, Fos expression was lower in these same areas in groups that exhibited decreased preference (i.e., in P rats for the morphine-paired chamber CPP, and in M rats for the food-paired chamber). Together, these results indicate that these limbic areas may play a central role in the altered reward processing that occurs during protracted withdrawal.

Notably, each of these areas send projections to the VTA, and our recent studies reveal that VTA afferents and Fos-responsive neurons are co-extensive in these brain regions. These findings raise the possibility that alterations in VTA afferents in these regions underlie the changes observed in hedonic preference during protracted withdrawal. In addition, the following studies reveal that plasticity within the VTA itself may be involved in forming such stimulus-rug associations.

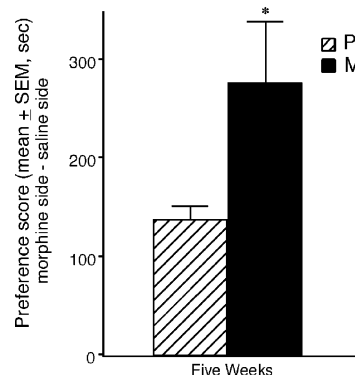
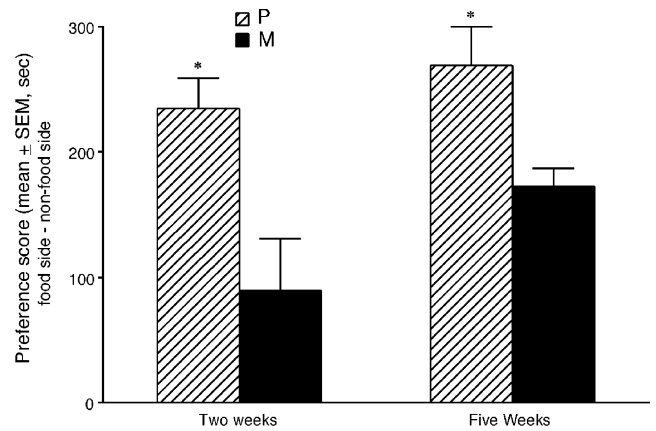


Fig. 5. Upper panel: preference scores for the food-paired environment expressed as the mean time in seconds spent in the food-paired side minus the mean time in seconds spent in the non-food paired side on the test day. Two and five weeks refer to the number of weeks of morphine abstinence at the time of testing. *significantly different ($p < 0.01$). Lower panel: preference scores for the morphine-paired environment expressed as the mean time in seconds spent in the morphine-paired side minus the mean time in seconds spent in the saline-paired side on the test day. *Significantly different ($p < 0.01$). M—rats pretreated with morphine pellets; P—rats pretreated with placebo pellets. Taken from Harris and Aston-Jones (2003a).

4.4. The role of the mesocorticolimbic dopamine system in opiate-associated learning

The mesocorticolimbic DA system has also received a great deal of attention as a possible brain substrate for the reinforcing and abuse potential of drugs. Opiates acutely increase DA release in the accumbens. A wealth of data suggests that neural elements in the region of the ventral tegmental area (VTA) and Ac-S are responsible for the reinforcing properties of opiates, and that there are DA-dependent and DA-independent mechanisms of opiate action (Glick and Cox, 1975; Bozarth and Wise, 1981; Davis and Smith, 1983; Spyraki et al., 1983; Pettit et al., 1984; Stinus et al., 1989; Shippenberg et al., 1992; Higgins et al., 1994) (reviewed in van Ree et al., 1999). A pivotal role for mesocorticolimbic dopaminergic mechanisms in opiate-induced

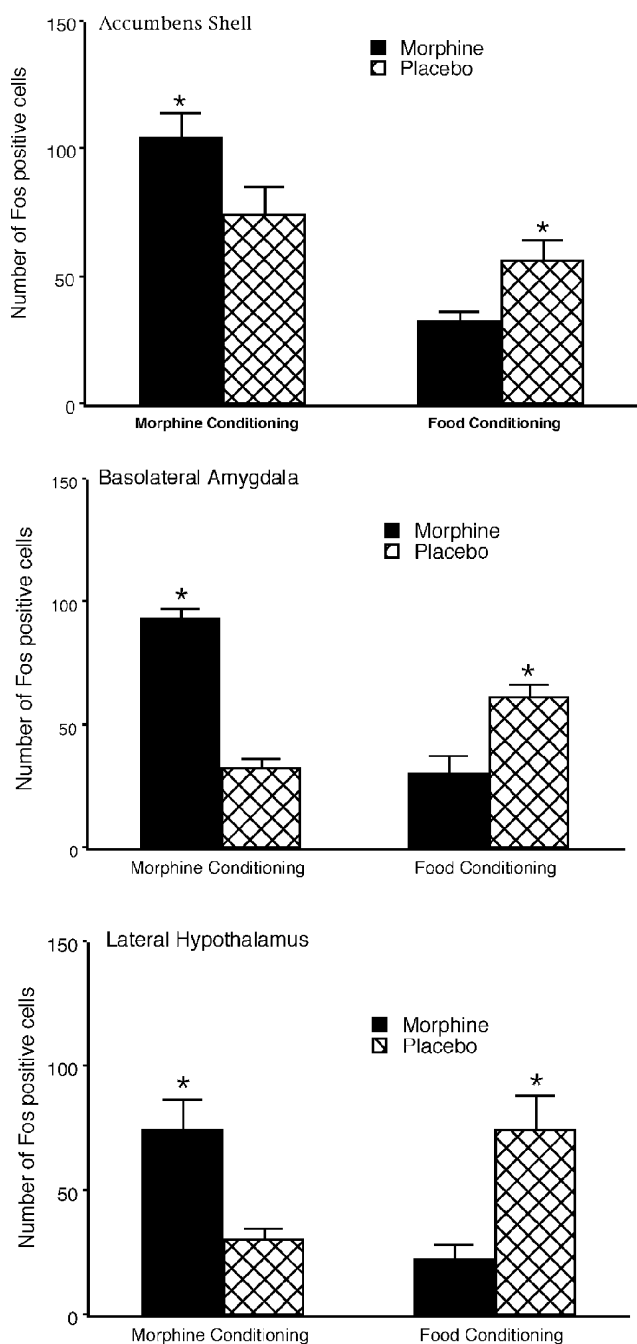


Fig. 6. Number of Fos+ nuclei (\pm SEM) counted in the Ac-S, ABL, and LH of morphine-abstinent or placebo-pretreated rats conditioned with either morphine or food in a CPP paradigm. Fos+ neurons were identified using immunohistochemistry in fixed tissue sections, as described in our recent publications (Delfs et al., 2000) (Harris and Aston-Jones, 2003c). Significantly more Fos+ neurons were found in each area in abstinent than placebo-pretreated animals that underwent morphine CPP, and conversely significantly more Fos+ neurons were found in each area for placebo than abstinent animals that underwent food conditioning ($*p < 0.001$). Morphine—rats pretreated with morphine pellets; placebo—rats pretreated with placebo pellets.

place conditioning has been shown in a number of studies (Phillips and LePiane, 1980, 1982; Bozarth and

Wise, 1981; Phillips et al., 1983; Spyraiki et al., 1983; Leone and Di Chiara, 1987; Bals-Kubik et al., 1993; Shippenberg et al., 1993; Nader et al., 1994; Maldonado et al., 1997). However, studies using the self-administration paradigm have not suggested a critical role for accumbal DA in opiate reinforcement (Ettenberg et al., 1982; Gerber and Wise, 1989; Higgins et al., 1994). Such differences may reflect the fact that the place preference paradigm involves classical conditioning of an operant approach response and requires learning, whereas in the self-administration experiments rats had already acquired the necessary operant behaviors prior to manipulations of the DA system. Thus, DA mechanisms may be primarily involved in the learning of, and motivation for, conditioned behaviors (Robinson and Berridge, 1993; Wolterink et al., 1993; Kiyatkin, 1995; Robbins and Everitt, 1996; Salamone, 1996; Nader et al., 1997; Schultz et al., 1997).

4.5. Role of plasticity in the VTA in conditioned effects of drugs

Work by Malenka and colleagues found that a single exposure of cocaine, morphine or other abused drugs to rats produced glutamate-dependent long-term potentiation (LTP) in VTA dopaminergic neurons (Ungless et al., 2001; Saal et al., 2003; Thomas and Malenka, 2003). This cellular response led us to postulate that drug-related behavioral plasticity, such as learning drug-timulus associations, may involve plasticity within the VTA. To test this idea, we administered a cocktail containing two glutamate antagonists (the AMPA antagonist CNQX, 0.12 nmol, and the NMDA antagonist AP5, 0.24 nmol, in 0.3 μ l) directly into the VTA just prior to conditioning trials of a cocaine CPP paradigm. These injections blocked the development of a cocaine CPP, indicating that acquisition of a cocaine place preference requires glutamate transmission within the VTA (Harris and Aston-Jones, 2003d) (Fig. 7). More recently, we also found that glutamate input to the VTA is crucial for both the acquisition and expression of morphine CPP (Byrne et al., 2003). These results were consistent with the idea that glutamate-dependent plasticity within the VTA is necessary for the acquisition and expression of stimulus-drug associations. In an extension of this work, we examined whether protein kinase A (PKA) activity within the VTA was involved in the importance of glutamate receptor activation in the morphine CPP task. This was based upon previous studies by others revealing that PKA is an important part of glutamate-induced synaptic plasticity in other brain regions (Kandel, 1989; Spencer and Murphy, 2002). Different groups of drug-naïve rats received microinjections bilaterally into the VTA of the PKA inhibitor Rp-cAMPS (0.6 μ g), or control injections of aCSF, immediately after each of

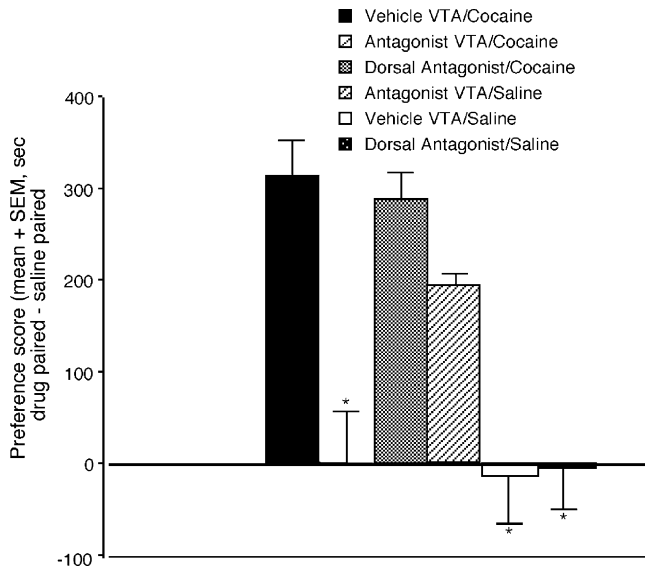


Fig. 7. Effects of intra-VTA microinjections of glutamate antagonists on cocaine preference. A cocktail of glutamate antagonists (the AMPA antagonist CNQX, 0.12 nmol, and the NMDA antagonist AP5, 0.24 nmol, in 0.3 μ l) was microinjected directly into the VTA or nearby area just prior to conditioning trials of a cocaine CPP paradigm. Preference scores for the cocaine-paired environment expressed as the mean time in seconds spent in the cocaine-paired side (or intracerebral microinjection-paired side for non-cocaine-conditioned groups) minus the mean time in seconds spent in the other side on the test day. For VTA groups, only data from animals where injection sites were confirmed to be within the VTA are included. One-way ANOVA on groups yielded $F(5,27)1/413.17$, $p < 0.001$. *Significantly different ($p < 0.01$) from vehicle VTA/cocaine, dorsal antagonist/cocaine and antagonist VTA/saline. Taken from Harris and Aston-Jones (2003b).

three morphine conditioning sessions. Microinjections were also given alone in the VTA without morphine conditioning to assess any potential rewarding or aversive effects of the PKA inhibitor itself. We found that Rp-cAMPS blocked the development of a morphine CPP when given in the VTA but not when given outside the VTA. Microinjections of this drug into the VTA without morphine conditioning were hedonically neutral and had no rewarding or aversive effects. Together, these data indicate that glutamate input to the VTA provides crucial information for the conditioning of drug effects to environmental stimuli and that this effect may be mediated through PKA activation. Note that the PKA inhibitor was effective when administered *after* the conditioning sessions. Thus, the effect of this agent presumably is to block consolidation of the memory for the drug-timulus association.

In addition, the PKA inhibitor, like glutamate antagonists, also blocked the expression of the morphine CPP when administered into the VTA just prior to the preference test session. This result indicates that glutamate input and PKA activation in the VTA are not only necessary for conditioning to occur but are

also necessary for the recall of that memory. Thus, plasticity within the VTA is involved in forming and recalling stimulus-rug associations. It is possible that this plasticity is altered during dependence and withdrawal, so that the change in hedonic processing described above in protracted withdrawal also involves alterations in plasticity within the VTA. Additional experiments are needed to test this possibility.

5. Discussion and hypothesis

5.1. Anxiety during protracted withdrawal and negative reinforcement

In the opponent process view of addiction, abstinence from chronic drug exposure uncovers neuroadaptations in brain function that contribute to an ill-defined feeling of dysphoria, anxiety or malaise that can only be ameliorated by more drug (Koob and Le Moal, 2001). Continued drug use is thought to be rewarding not only because it stimulates the natural reward circuitry but also because of the reinforcement associated with offsetting the anti-reward response (including stress hyper-responsivity and anxiety), yielding an additional reinforcing effect of abused drugs that summates with their positive rewarding effects.

Consistent with this view, we found that during the protracted withdrawal period animals exhibited elevated anxiety. This anxiety may result from an “anti-reward” response to the prior chronic presence of opiates. In addition, this heightened anxiety could involve alterations in the NE input to the BNST or amygdala. We have shown that the aversiveness and elevated anxiety associated with acute withdrawal from morphine is dependent on beta adrenoceptor stimulation in the extended amygdala. Prior studies have linked NE in the BNST to stress-induced anxiety responses (Cecchi et al., 2002). In addition, we found that A2 NE neurons in the NTS (a major source of NE to the BNST and amygdala) were stimulated by exposure to the morphine-paired environment during protracted withdrawal. Finally, we and others have demonstrated that acute morphine inhibits activity in NE neurons (North and Williams, 1983; Aghajanian and Wang, 1987; Akaoka and Aston-Jones, 1991; Shiekhatter and Aston-Jones, 1993; Ivanov and Aston-Jones, 2001). In view of these findings, we propose that drug-associated stimuli activate A1/A2 neurons during protracted withdrawal, and the ensuing release of NE in the extended amygdala elevates anxiety. This, in turn, conveys an additional reinforcing property to morphine via the alleviation of anxiety (reflecting a negative reinforcement mechanism) when these NE neurons are inhibited. In this view, part of the increased drug seeking during protracted withdrawal involves conditioned

release of NE in the extended amygdala, elevated anxiety, and the reinforcing effect of morphine achieved in reducing this anxiety.

5.2. Altered hedonic processing

Additional evidence indicates that chronic drug exposure leads to a generalized hedonic deficit for natural rewards as well as a sensitized hedonic or incentive value for drugs. The hedonic deficit is thought to generate symptoms of protracted withdrawal seen in humans including anhedonia and depression (Koob and Le Moal, 2001), whereas the sensitized hedonic drug value is proposed to increase the motivation for drug (Robinson and Berridge, 2004). We found evidence of such altered hedonic processing in rats as well. M animals withdrawn five weeks showed reduced preference for food compared to P animals. This could correspond to an “anti-reward” response to chronic drug exposure, consistent with an opponent process or allostatic hypothesis for addiction (Koob and Le Moal, 2001). However, M animals also exhibited a much larger preference for the morphine-associated environment than P animals. This cannot be explained by an “anti-reward” response but instead is compatible with theories of addiction based upon sensitization to the incentive motivational effects of drugs with chronic treatment (Robinson and Berridge, 1993, 2004). Overall, in the non-dependent P subjects, the preference for the food-associated chamber was twice as high as that found for the morphine-associated chamber. The opposite was true for the M animals, in which preference for morphine-associated cues was twice that found for food-associated cues. At five weeks post-withdrawal the lack of motivation for food-reinforced responding appeared to be diminishing, possibly indicating recovery of normal reward function. However, the motivation to seek out morphine-associated environmental cues had not decreased. This indicates that the changes that occur in hedonic processing mechanisms following opiate exposure may involve multiple systems that recover at different rates.

In a theory outlined by Di Chiara (1998, 1999), natural reinforcers release DA in the accumbens shell and this response is thought to rapidly habituate. It is further proposed that the release of DA produced by drugs such as morphine does not habituate, thereby leading to a relative strengthening with repeated drug use of the connections between environmental stimuli and drug reward. Along similar lines, we propose that morphine pretreatment may cause animals to be sensitized to learning about cues associated with drug both through an enhanced incentive motivation mechanism (Robinson and Berridge, 1993, 2004) and learning (Robbins and Everitt, 2002) as well as through the alleviation of negative affective states (as discussed

above) (Koob and Le Moal, 2001). Our results with Fos staining reveal opposite Fos responses for morphine vs. food CPP in brain areas previously associated with reward processing, including the Ac-S, ABL and LH. Such changes suggest that these areas may contribute to the altered preferences exhibited during protracted withdrawal for food and morphine reward. These areas contain numerous inputs to the VTA. Moreover, our recent studies reveal that plasticity within the VTA is critically involved in stimulus-rug associations. Thus, changes in afferents to the VTA, or in plasticity within the VTA itself, could play a critical role in altered hedonic processing during protracted withdrawal.

5.3. Conclusions

Together, these results lead us to propose that elevated drug seeking during protracted withdrawal may involve two processes: (i) prolonged elevated anxiety and other related “opponent process” responses leading to a negative reinforcement mechanism for morphine effects and decreased valence for natural rewards, plus (ii) increased incentive motivation for drug reward through a sensitization mechanism. The altered hedonic processing associated with protracted withdrawal may reflect changed plasticity within the VTA and its major inputs in the extended amygdala and hypothalamus. Future research to determine the relative importance of each of these processes, their interactions, and their sensitivity to therapeutic pharmacologic treatments, will be important in creating new and more effective clinical approaches to the treatment of addiction.

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