Amphetamine acts within the lateral hypothalamic area to elicit affectively neutral arousal and reinstate drug-seeking

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Abstract
Psychostimulants, including amphetamine (AMPH), exert robust arousal-enhancing, reinforcing and locomotor-activating effects. These behavioural actions involve drug-induced elevations in extracellular norepinephrine (NE) and dopamine (DA) within a variety of cortical and subcortical regions. The lateral hypothalamic area (LHA), including the lateral hypothalamus proper, perifornical area and adjacent dorsomedial hypothalamus, is implicated in appetitive- and arousal-related processes. The LHA is innervated by both NE and DA projections and systemically administered AMPH has been demonstrated to activate LHA neurons. Combined, these and other observations suggest the LHA may be a site of action in the behavioural effects of psychostimulants. To test this hypothesis, we examined the degree to which AMPH (10 nmol, 25 nmol) acts within the LHA to exert arousing, locomotor-activating and reinforcing actions in quietly resting/sleeping rats. Although intra-LHA AMPH robustly increased time spent awake, this occurred in the absence of pronounced locomotor activation or reinforcing actions, as measured in a conditioned place preference (CPP) paradigm. Arousing and stressful conditions or drug re-exposure can elicit relapse in humans and reinstate drug-seeking in animals. Given the LHA is also implicated in the reinstatement of drug-seeking behaviour, additional studies examined whether AMPH acts within the LHA to reinstate an extinguished CPP produced with systemic AMPH administration. Our results demonstrate that AMPH action within the LHA is sufficient to reinstate drug-seeking behaviour, as measured in this paradigm. Collectively, these observations demonstrate that psychostimulants act within the LHA to elicit affectively neutral arousal and reinstate drug-seeking behaviour.

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Introduction
Psychostimulants are a widely abused class of drugs, representing a major public health concern (Gonzales et al., 2010; Marshall and Werb, 2010). These drugs exert robust arousal-enhancing and reinforcing effects that contribute to their use and abuse. The behavioural actions of psychostimulants, including amphetamine (AMPH), involve drug-induced elevations in extracellular norepinephrine (NE) and dopamine (DA) within multiple cortical and subcortical regions. For example, the locomotor-activating and reinforcing effects of psychostimulants involve actions of both DA in the nucleus accumbens and NE in the prefrontal cortex (Blanc et al., 1994; Darraçq et al., 1998; Dickinson et al., 1988; Drouin et al., 2002a,b; Pascoli et al., 2005; Snoddy and Tessel, 1985) while NE action within the medial septal and medial preoptic areas contributes to the arousal-promoting actions of these drugs (for review, Berridge, 2006).

A significant challenge in the treatment of psychostimulant abuse is the prevention of relapse. Relapse, or the reinstatement of drug-seeking behaviour, can be triggered by re-exposure to the drug, stress and/or contextually-conditioned cues in humans and animals (Brown et al., 1995; Jaffe et al., 1989; Ludwig et al., 1974; Shaham et al., 2000; Sinha, 2001). NE and DA also play a central role in the reinstatement of psychostimulant-seeking via actions within several basal forebrain regions, including areas related to...
arousal and autonomic processes, such as the medial septal area and bed nucleus of the stria terminalis (for review, Stewart, 2000; Weinschenker and Schroeder, 2007).

The lateral hypothalamic area (LHA; i.e. the lateral hypothalamus proper, adjacent perifornical area and dorsomedial hypothalamus), has long been hypothesized to participate in a variety of behavioural processes, including arousal and appetitively-motivated behaviour (for review, Boutrel et al., 2010; Marchant et al., 2012). Recent evidence further implicates the LHA in the reinstatement of drug-seeking behaviour (Boutrel et al., 2005; Harris et al., 2005). This dorsal hypothalamic region receives both NE and DA input (Baldo et al., 2003; Yoshida et al., 2006) and systemically-administered AMPH activates neurons within the LHA (Estabrooke et al., 2001; Fadel et al., 2002). Moreover, NE was recently demonstrated to act within the LHA to promote arousal (Schmeichel and Berridge, 2013). Combined, these observations suggest the LHA may be a site of action in the arousal-promoting and other behavioural effects of psycho-stimulants, including AMPH.

To test this hypothesis, we examined the degree to which microinfusion of AMPH into the LHA elicits arousal-promoting, locomotor-activating and reinforcing (as measured in a conditioned place preference paradigm; CPP) actions. Additional studies determined whether AMPH acts within the LHA to reinstate previously-extinguished drug-seeking, as measured by CPP. Our results indicate that AMPH acts within the LHA to promote affectively neutral arousal and to reinstate drug-seeking behaviour.

Methods

Animals and surgery

Sixty-eight male Sprague–Dawley rats (260–280 g, Charles River, USA) were pair-housed for at least 7 d prior to surgery with ad libitum access to food and water on an 11:13 h light:dark cycle (lights on 0700 hours). Animals were anesthetized with isoflurane and 25 gauge guide cannulae (Component Supply Company, USA; Plastics One, USA) were stereotaxically implanted, aimed bilaterally at the LHA (flathead; in mm, −2.8 A/P, range of ±1.8–2.15 M/L, −3.8 V/D; angled 6° from vertical). A subset of animals was also implanted with a bipolar electroencephalographic (EEG) electrode into the frontal cortex (+2.7 A/P, ±1.5 M/L) and a bipolar electromyographic (EMG) electrode into the dorsal neck muscle. All coordinates are relative to bregma.

The cannulae and EEG/EMG electrodes (when applicable) were cemented into position using acrylic cement (Esschem, USA). Animals were given buprenorphine (0.01 mg/kg; Reckitt Benckiser Pharmaceuticals Inc., USA) post-surgically to alleviate pain and were allowed to recover for 5–7 d before testing. All efforts were made to minimize the number of animals used. All facilities and procedures were in accordance with guidelines regarding animal use and care put forth by the National Institutes of Health and approved by the Institutional Animal Care and Use Committee of the University of Wisconsin.

Experimental procedures

Sleep/wake effects of intra-LHA AMPH

Testing procedures were similar to those described previously (Berridge and Foote, 1996). Briefly, on the day prior to testing, rats were transferred to sound-attenuated testing chambers and a stainless steel coil spring was threaded onto the cannula via plastic threaded sleeves (Plastics One, USA). The other end of the spring was attached to a counterbalance outside the outer chamber. Animals were housed individually overnight with free access to food and water.

On the morning of testing, between 0900–1100 hours, the 33 ga infusion needle was connected to PE20 tubing protected by a stainless steel coil spring (Plastics One) and loaded with vehicle (artificial extracellular fluid (AECF); in mM: NaCl 147, CaCl2 1.3, MgCl2 0.9, KCl 2.5) or drug dissolved in AECF with a 50 nl air bubble separating drug or vehicle from water. The infusion needle was inserted into the guide, extending 4.0 mm beyond the end, and secured. EEG/EMG electrodes were connected to a field effect transistor headstage. Baseline EEG/EMG recordings were initiated at a point where the animal had recovered from the arousing effects of needle insertion and had returned to quiet-rest/sleep. Infusions of 250 nl (125 nl/min) were made using a microprocessor-controlled pump (Harvard Apparatus, USA), following at least 1 h of quiet-rest/sleep. Behavioural data were collected continuously onto polygraph and video recording tape for at least 1.5 h following infusion.

Cortical electroencephalograms (EEGs) and electromyograms (EMGs) were recorded as described previously (Berridge and Foote, 1996). EEG/EMG recordings were scored for the following behavioural state categories: (i) slow-wave sleep (high-voltage EEG, low voltage EMG); (ii) rapid-eye movement (REM) sleep (low-voltage EEG combined with EMG activity that was approximately 50% lower amplitude than that observed in slow-wave sleep, with occasional
short-duration, large-amplitude deflections due to muscle twitches); (iii) waking (low-voltage EEG, sustained high-voltage EMG of an average amplitude at least twice that observed in slow-wave sleep, often with large-amplitude movement deflections; Fig. 1). To be scored as a distinct state, the EEG/EMG activity patterns needed to persist for $\geq 15$ s.

Time spent in each state was scored for each 30 min epoch, including two epochs immediately prior to infusions (PRE1=0–30 min; PRE2=30–60 min) and three epochs immediately following infusions (POST1=0–30 min; POST2=30–60 min; POST3=60–90 min). EEG/EMG recordings were scored by an observer blind to experimental conditions.

**Locomotor analyses**

Locomotor activity (i.e. quadrant entries and rears) was scored in animals that received the highest dose of AMPH infused unilaterally and bilaterally. Behaviour was recorded continuously using black and white, low-level illumination video camera, starting after insertion of the infusion needle and continuing for at least 2 h following infusion. Locomotor activity was scored from videotape by a trained observer blind to treatment conditions using a computer-based event recorder (The Observer, Noldus Information Technology, The Netherlands). The frequency of quadrant entries and rears (both wall and free) was scored for three 30 min epochs, including one epoch prior to infusion (PRE1=0–30 min) and two epochs following infusion (POST1=0–30 min; POST2=30–60 min). These measures are similar to those used by others to characterize the motor-activating effects of systemically-administered psychostimulants (Segal, 1975).

**CPP testing with systemic and intra-LHA AMPH**

The reinforcing actions of systemic and intra-LHA AMPH were assessed in two separate groups of animals using a CPP apparatus consisting of two distinct chambers (each $34 \times 24.5 \times 30$ cm; black walls/textured floor; black and white striped walls/smooth floor) and a neutral, free-choice entry zone ($24.5 \times 17 \times 10$ cm). Animals were tested over three phases: pre-conditioning (1 d; 10 min session, access to both chambers), conditioning (8 d; 20 min session, access restricted to one chamber) and post-conditioning (1 d; 10 min session, access to both chambers). For systemic administration, conditioning consisted of eight consecutive treatment days during which animals received s.c. injections of a wake-promoting dose of AMPH (0.25 mg/kg) and vehicle (saline) on alternating days. For intra-LHA AMPH, conditioning consisted of eight consecutive treatment days during which animals received bilateral intra-LHA infusions (250 nl/120 s) of a wake-promoting dose of AMPH (25 nmol) and vehicle (AECF) on alternating days. All treatments were administered in a transfer cage (similar to the home cage) 15 min prior to placement in the CPP apparatus. Testing sessions were recorded on a digital video recorder (JVC Enverio, USA). Time spent in each chamber for both pre- and post-conditioning test days was scored by a trained observer using a computer-based event recorder (The Observer,
Noldus Information Technology). Animals were tested in a biased design paradigm, with drug paired to the non-preferred chamber as determined by the pre-conditioning test.

CPP reinstatement with systemic AMPH

A separate group of animals was used to examine the effects of systemic AMPH on reinstatement of CPP. In these studies, a CPP was first established using systemically-administered AMPH (0.25 mg/kg s.c.), as described above. Following establishment of a CPP, animals underwent extinction training during which they received s.c. saline for six consecutive days in both the AMPH- and saline-paired chambers (alternating days; 20 min session, access restricted to one chamber). For the next two days, animals received a saline injection followed by a free-explore extinction test (10 min session, access to both chambers). Time spent in the drug-paired chamber was measured. If extinction criterion was not met (<50% of post-conditioning test value for two consecutive days), extinction training continued with a saline injection prior to free-explore extinction tests every 1–3 d. After meeting extinction criteria (range 12–25 d, average 16.6±1.6 d), animals received systemic AMPH (0.10 mg/kg s.c.) and were tested for reinstatement of CPP 15 min later (10 min session, access to both chambers).

CPP reinstatement with intra-LHA AMPH

An additional group of animals was used to examine the effects of intra-LHA AMPH on reinstatement of CPP. In these studies, CPP (AMPH; 0.25 mg/kg s.c.) and extinction were established as described above. After meeting extinction criteria (range 8–28 d, average 15.1±2.5 d), animals received a bilateral intra-LHA infusion (250 nl/120 s) of AMPH (25 nmol) or vehicle (AECF) and were tested for reinstatement 15-min following infusion (10-min session, access to both chambers). The order of intra-LHA infusions (AMPH or AECF) was counterbalanced across animals, with extinction training occurring prior to each treatment. Limited pilot studies indicated that initial intra-LHA needle insertions had the propensity to alter reinstatement outcomes. Thus, before receiving intra-LHA infusions, a mock infusion/needle-drop was performed on the day prior to reinstatement testing. This permitted acclimation to mild infusion restraint and minimized tissue damage associated with insertion of a needle on the day of testing.

Drugs

d-Amphetamine sulfate (AMPH; Sigma, USA) was dissolved in either AECF for intra-tissue infusions or saline for systemic administration. Doses used in the intra-tissue infusion studies were based on previous studies demonstrating this dose range elicited robust waking when infused into the medial septal and medial preoptic areas (Berridge et al., 1999) and locomotor-activating and reinforcing effects when infused into the nucleus accumbens (Carr and White, 1983; Gerdjikov and Beninger, 2005, 2006; Newman et al., 2013; Schild et al., 1998). For CPP studies, systemic doses were used that have been demonstrated to elicit pronounced arousal-promoting actions in the absence of a strong locomotor activation (Berridge et al., 1999; Berridge and Stalnaker, 2002). In the case of systemic AMPH reinstatement of CPP, a lower dose of AMPH (0.10 mg/kg for reinstatement vs. 0.25 mg/kg for conditioning) was used similar to previous studies of psychostimulant-induced reinstatement in animals (Cruz et al., 2008; Mueller and Stewart, 2000).

Histology and data selection

Following experimentation, animals were deeply anesthetized and perfused transcardially with at least 60 ml of 4% formaldehyde. The brain was removed and placed in fixative for at least 24 h, frozen and 40 μM sections were collected throughout the rostral-caudal extent of the LHA. The sections were stained with Neutral-Red dye. The location of the ventral-most extent of the needle track was identified. Data were included in the analyses for those cases in which both the EEG/EMG recordings were electrically adequate (when applicable) and the histological analyses verified accurate placements of infusion needles (Fig. 1).

Statistical analyses

AMPH-induced changes in EEG/EMG-based measures of sleep/wake state and locomotor activity were analysed statistically with a two-way mixed-design analysis of variance (ANOVA) with drug treatment as the between-subjects variable and time as the within-subjects variable. Where between-subjects omnibus tests proved significant, pairwise post-hoc group comparisons for each time epoch were made using a one-way ANOVA. CPP-based measures of reinforcement were analysed statistically with a matched-pair t test. For CPP reinstatement, a one-way within-subject ANOVA was used (4 levels: pre-conditioning, conditioning, extinction, reinstatement).
post-conditioning, extinction, reinstatement) followed by planned pairwise post-hoc group comparisons.

Results

Effects of intra-LHA AMPH on waking

To assess the arousal-promoting effects of AMPH, rats received 250 nl intra-LHA infusions of either vehicle (AECF; n=8), 10 nmol AMPH (n=7) or 25 nmol AMPH (n=8) and EEG/EMG-indices of sleep/wake state were analysed. In prior work with subcortical infusions of AMPH or NE receptor agonists, we observed that unilateral infusions were sufficient to elicit waking (Berridge et al., 1999; Schmeichel and Berridge, 2013). Thus, to minimize the number of animals needed for these studies, all infusions were unilateral, with each animal receiving a different treatment in each hemisphere on separate days, except for one group that received bilateral intra-LHA 25 nmol AMPH (n=5). As reported previously (Berridge and Foote, 1996; Berridge and O’Neill, 2001), animals spent a majority of time asleep prior to infusions under these testing conditions.

No significant differences in time spent awake or asleep were observed prior to any treatment or following vehicle infusions into the LHA (Fig. 2). In contrast, intra-LHA infusion of AMPH elicited dose-dependent increases in waking and decreases in slow-wave and REM sleep (Fig. 2; Awake: treatment, F(3,24)=19.49, p<0.001; time, F(4,96)=22.47, p<0.001; treatment×time, F(12,96)=3.92, p<0.001; Slow-Wave Sleep: treatment, F(3,24)=11.54, p<0.001; time, F(4,96)=21.13, p<0.001; treatment×time, F(12,96)=3.57, p<0.001; REM: treatment, F(3,24)=5.76, p<0.01; time, F(4,96)=4.21, p<0.01; treatment×time, F(12,96)=1.10, p=0.37). When infused unilaterally at 25 nmol, the average latency for AMPH-induced waking was 265±54 s from the start of the 120 s infusion with a range of 96–494 s. Bilateral intra-LHA infusions of 25 nmol AMPH produced larger increases in waking and decreases in slow-wave and REM sleep than that seen with unilateral infusions of this dose (Fig. 2). As shown in Fig. 3, unilateral AMPH elicited waking when infused into the majority of the medial-lateral extent of the dorsal hypothalamus also known to contain neurons implicated in the modulation of sleep/wake state (i.e. HCRT and melanin-concentrating hormone synthesizing neurons; Swanson et al., 2005), including the lateral hypothalamus proper, adjacent perifornical area, and dorsomedial hypothalamus. In contrast, infusions outside of this region, including ventrally, dorsally and laterally, were substantially less effective/consistent at eliciting waking beyond levels observed in either pre-infusion epochs or following vehicle treatment (i.e. <1200 s, assigned values of 1 and 2; see Fig. 3). Bilateral 25 nmol AMPH infusions were placed centrally within the LHA (i.e. perifornical region; not shown).

Effects of intra-LHA AMPH on locomotor activity

The above-described studies observed maximal waking with bilateral intra-LHA infusion of 25 nmol AMPH.
To assess whether AMPH-induced waking is associated with locomotor activation, we scored the number of quadrant entries and rears in these same animals prior to and following unilateral vehicle (AECF; \( n = 7 \)) or 25 nmol AMPH (\( n = 8 \)), or bilateral 25 nmol AMPH (\( n = 5 \)) infusions. One case was dropped from the vehicle group due to a video-recording malfunction. As described above, animals were tested during the resting phase and thus spent the majority of time asleep prior to the infusion, resulting in low locomotor activity.

Despite the robust arousal-promoting effects of intra-LHA AMPH (Fig. 2), infusion of AMPH into the LHA did not elicit a pronounced locomotor activation. The only increase in locomotor activity was a modest, yet statistically significant, increase in the number of quadrant entries during the first 30 min following bilateral 25 nmol AMPH infusion (Table 1; Quadrant entries: treatment, \( F_{(2,17)} = 8.42, \ p < 0.01 \); time, \( F_{(2,34)} = 8.81, \ p < 0.01 \); treatment \times \ time, \( F_{(4,34)} = 3.22, \ p < 0.05 \); Rears: treatment, \( F_{(2,17)} = 3.67, \ p < 0.05 \); time, \( F_{(2,34)} = 5.73, \ p < 0.01 \); treatment \times \ time, \( F_{(4,34)} = 1.65, \ p = 0.19 \)). There were no differences in the magnitude of locomotor activity observed following AMPH infusions in the lateral vs. perifornical vs. medial portions of the dorsal hypothalamus. Importantly, the low level and pattern of locomotor activity observed in this study is similar to that seen with spontaneous waking (Berridge and Foote, 1996) and is substantially below levels seen with behaviourally-activating doses of systemically-administered psychostimulants (Kuczenski et al., 1991, 1997; Segal, 1975; Segal and Kuczenski, 1997) or with AMPH infusion into the nucleus accumbens at doses similar to those used in the current studies (Newman et al., 2013; Schildein et al., 1998). No evidence of stereotypy was observed with either unilateral or bilateral AMPH infusions.

**Effects of intra-LHA AMPH on reinforcement (CPP)**

To determine the degree to which AMPH action within the LHA is reinforcing, we examined whether intra-LHA AMPH infusion elicits a CPP. We first confirmed that systemically-administered AMPH (0.25 mg/kg s.c.) elicits a CPP under these testing conditions. Prior studies in our laboratory demonstrated that this systemic dose of AMPH produces sustained increases in waking in the absence of a prominent locomotor activity.
activation (Berridge and Stalnaker, 2002). AMPH administration \((n=18)\) produced a significant increase in the time spent in the drug-paired chamber, demonstrating reinforcing actions of this systemic dose of AMPH (Fig. 4, Left panel; \(t_17=-9.70; p<0.001\)).

In a separate group of animals \((n=9)\), we then examined the degree to which intra-LHA AMPH elicited a CPP. For these studies, we targeted the central region of the LHA (i.e. perifornical area), a region within which AMPH acts to promote arousal (see above) and that has been implicated in reward-related processes. Animals received alternating bilateral infusions of AMPH (25 nmol) and vehicle (AECF) into the LHA during the conditioning phase (see Methods above). In contrast to that seen with systemically-administered AMPH, intra-LHA infusion of an arousing dose of AMPH did not elicit a CPP (Fig. 4, Right panel; \(t_8=0.73; p=0.49\)). This lack of reinforcement following intra-LHA AMPH infusion also contrasts with the robust reinforcing effects of similar doses of AMPH infused into the nucleus accumbens (Carr and White, 1983; Cunningham and Kelley, 1992; Gerdjikov and Beninger, 2005, 2006; Kelley and Throne, 1992; Schildein et al., 1998).

**Effects of intra-LHA AMPH on reinstatement of drug-seeking (CPP)**

The above-described studies demonstrate that AMPH acts within the LHA to exert arousal-promoting but not reinforcing actions. High arousal conditions that are not reinforcing (e.g. stress) re
behaviour in humans and animals. Therefore, additional studies examined whether AMPH acts within the LHA to reinstate a previously extinguished AMPH-induced CPP. For these studies, animals underwent conditioning with AMPH (0.25 mg/kg s.c.) followed by extinction training. We first confirmed that a low, but arousing dose of systemic AMPH (0.10 mg/kg s.c.) would reinstate a previously-extinguished CPP under the current testing conditions (n=7). In this experiment, there was an overall significant effect of testing stage (e.g. pre- and post-conditioning, extinction, reinstatement; F(3,18)=13.87, p<0.001). All animals showed an initial CPP (Fig. 5; t<sub>6</sub>=10.20; p<0.001) and a significant reduction in time spent in the drug-paired chamber following extinction (AMPH extinction: t<sub>6</sub>=14.47; p<0.001). As has been shown elsewhere (Cruz et al., 2008; Mueller and Stewart, 2000), systemic AMPH reinstated the extinguished CPP (Fig. 5; t<sub>6</sub>=2.67; p<0.05).

We then examined the degree to which bilateral intra-LHA infusion of vehicle (AECF) and AMPH (25 nmol) reinstated an extinguished CPP in a separate group of animals (n=7) that underwent conditioning with systemic AMPH (0.25 mg/kg) and extinction as described above. For intra-LHA reinstatement testing, each animal received vehicle and AMPH infusions into the central region of the LHA, an area implicated in the arousal-promoting actions of AMPH (see above) as well as reinstatement of drug-seeking behaviour (Boutrel et al., 2005; Harris et al., 2005). There was an overall significant treatment effect (Fig. 6; F(5,30)=7.88, p<0.001) and all animals showed a significant increase in time spent in the drug-paired chamber following the initial systemic AMPH conditioning (t<sub>6</sub>=12.66; p<0.001) and a significant reduction in time spent in the drug-paired chamber following extinction (extinction preceding vehicle infusion: t<sub>6</sub>=6.50; p<0.001; extinction preceding AMPH infusion: t<sub>6</sub>=6.82; p<0.001). Following extinction, intra-LHA vehicle infusion did not significantly alter time spent in the drug-paired chamber (t<sub>6</sub>=0.04; p=0.97). In contrast, intra-LHA infusion of AMPH reinstated drug-seeking, producing a significant increase in time spent in the drug-paired chamber compared to extinction (t<sub>6</sub>=2.53; p<0.05). The magnitude of intra-LHA AMPH reinstatement was similar to that seen with systemic AMPH (Figs 5 and 6).

**Discussion**

The dorsal hypothalamus, including the lateral hypothalamus proper, perifornical region and adjacent dorsomedial hypothalamus (i.e. LHA, collectively), has long been suggested to play a role in the regulation of behavioural state and state-dependent processes (Marchant et al., 2012; Olds, 1962; Stellar, 1954). More recently, the discovery of the LHA hypocretin (HCRTr) system has re-focused interest on the LHA, particularly in the context of arousal, reward and reinstatement/relapse (Boutrel et al., 2010). Thus, it is of interest that systemically-administered psychostimulants activate LHA neurons (Estabrooke et al., 2001; Fadel et al., 2002). Collectively, these and other observations suggest a potentially prominent role of the LHA in the behavioural actions of psychostimulants, including AMPH. The current studies provide the first evidence that AMPH acts directly within the LHA to elicit affectively neutral arousal in the absence of a pronounced locomotor activation. Furthermore, although AMPH infused into the LHA failed to elicit reinforcing effects as measured by CPP, intra-LHA AMPH nonetheless reinstated an extinguished CPP. Combined, these observations demonstrate that the LHA is a site involved in the arousing and reinstating effects, but not the reinforcing or locomotor-activating effects of AMPH and, presumably, other psychostimulants. These observations provide new insight into the neurobiology of psychostimulants and the LHA, and provide further support for a role of the LHA in the reinstatement of psychostimulant use. Furthermore, given narcolepsy is associated with dysregulation of the LHA (Nishino et al., 2000; Thannickal et al., 2000), the current observations also suggest this region...
may participate in the therapeutic, arousal-promoting actions of psychostimulants in the treatment of this disorder.

**AMPH acts within the LHA to elicit arousal-enhancing but not locomotor-activating or reinforcing effects**

The arousal-enhancing effects of psychostimulants contribute to their widespread use. Previous studies indicate that psychostimulant-induced arousal involves enhanced noradrenergic signaling within the medial septal and medial preoptic areas (for review, Berridge, 2008). The current studies demonstrate that the LHA is also a site of action in AMPH-induced arousal. Moreover, recent studies demonstrate additive wake-promoting actions of noradrenergic α₁- and β-receptors within the LHA, similar to that seen in the medial septal and medial preoptic areas (Schmeichel and Berridge, 2013). Collectively, these observations suggest a likely role for NE in the arousal-promoting effects of AMPH within the LHA. However, DA also likely contributes to the arousal-promoting actions of psychostimulants (Isaac and Berridge, 2003; Monti et al., 1990; Trampus et al., 1991; Wisor et al., 2001). The degree to which DA acts within the LHA to modulate arousal state remains to be determined.

Even at the highest dose, intra-LHA AMPH had no major impact on locomotor activity. In prior work, we have described that spontaneous waking in rats under testing conditions identical to those used in the current studies is associated with low levels of locomotor activity interspersed with bouts of eating, grooming and quiet-resting before an animal returns to sleep (Berridge and Foote, 1996; España et al., 2001). Following intra-LHA AMPH, we observed a comparable level and pattern of locomotor activity that both qualitatively and quantitatively differs from that seen with behaviourally-activating doses of systemically-administered psychostimulants (Kuczenski et al., 1991, 1997; Segal, 1975; Segal and Kuczenski, 1997). As such, we believe the low level of locomotor activity observed in the current studies largely reflects the wake-promoting effects of intra-LHA AMPH infusion. Despite the LHA being implicated in appetitive processes (Harris et al., 2005; Marchant et al., 2012), when infused directly into the LHA, 25 nmol AMPH lacked reinforcing properties, as measured by CPP. Although testing only a single dose of AMPH is a potential limitation of the current study, these results are in contrast to the robust reinforcing effects of a similar dose of AMPH when infused into the nucleus accumbens (Carr and White, 1983; Cunningham and Kelley, 1992; Gerdjikov and Beninger, 2005, 2006; Kelley and Throne, 1992; Schildein et al., 1998). Combined, these observations provide further evidence that the arousing, reinforcing and locomotor-activating effects of AMPH can be anatomically dissociated.

Fig. 6. Amphetamine (AMPH) directly infused into the lateral hypothalamic area (LHA) reinstates an extinguished conditioned place preference (CPP). **Left panel:** systemically administered AMPH (systemic; 0.25 mg/kg s.c.) elicited a significant CPP (PRE COND vs. POST 0.25 mg/kg). After conditioning, these same animals underwent extinction training (EXTINCTION). Following extinction, one-half of these animals then received bilateral intra-LHA vehicle infusions (REINST VEH) while the other half received bilateral intra-LHA AMPH (REINST AMPH 25 nmol). Following each treatment, animals were re-tested for extinction, and then received the other treatment (e.g. AMPH if they had first received vehicle). Middle panel: bilateral infusion of vehicle into the LHA had no effect on time spent in the drug-paired side in extinguished animals. Right panel: in contrast, bilateral intra-LHA AMPH (25 nmol) reinstated the extinguished CPP, significantly increasing time spent in the drug-paired side. Bars display mean (±S.E.M.) time (s) spent in the drug-paired side over a 10 min testing period. **p<0.01 vs. PRE COND; ##p<0.01 vs. POST COND; #p<0.05 vs. respective EXTINCTION.
Previous work suggests a topographical organization of LHA HCRT neurons, with neurons in the dorsomedial and perifornical hypothalamic areas linked to arousal and neurons in the lateral hypothalamus associated with reward processes (for review, Harris and Aston-Jones, 2006). In the current studies, we did not obtain evidence for a topographic organization for either the wake-promoting effects or the lack of reinforcing actions of intra-LHA AMPH. This is consistent with previous work that failed to observe a topographic organization in the activation of HCRT neurons under high-arousal conditions (España et al., 2003). These observations could either suggest that a topographic organization of the LHA is not a universal feature of this region or that the arousing and reinstating effects of intra-LHA AMPH do not involve HCRT.

**AMPH acts within the LHA to reinstate an extinguished CPP**

Re-exposure to a drug can elicit relapse, or the reinstatement of drug-seeking behaviour. Reinstatement can be also be triggered by non-reinforcing, arousing/stressful conditions (Shaham et al., 2000). Stress-related relapse is consistent with evidence indicating a prominent role of negative affect in addiction (Baker et al., 2004; Koob and Volkow, 2010). Alternatively, the ability of stress to reinstate drug-seeking could implicate elevated arousal per se, in relapse/reinstatement. In support of this latter hypothesis, the current studies demonstrate that although intra-LHA AMPH infusion failed to elicit either a CPP or conditioned place aversion, these infusions were potently arousing and reinstated drug-seeking. These observations are consistent with the participation of arousal/autonomic-related neurocircuitry in stress-related reinstatement of drug-seeking behaviour (Highfield et al., 2000; Leri et al., 2002). As such, these observations suggest arousal-related mechanisms, independent of affective state, may be sufficient to reinstate drug-seeking. Moreover, these observations suggest that prevention of relapse could involve pharmacological targeting of arousal-related neural systems.

**Potential LHA neuronal populations involved in the behavioural actions of AMPH**

HCRT neurons are implicated in appetitive behaviour, including drug-seeking (Aston-Jones et al., 2010; Harris et al., 2005; Zhou et al., 2008, 2012) and psychostimulant-induced synaptic plasticity (Rao et al., 2013; Yeoh et al., 2012). Furthermore, HCRT has been demonstrated to robustly promote arousal (España et al., 2001, 2002; Hagan et al., 1999; Piper et al., 2000). This raises the question of whether HCRT is involved in the arousal-promoting or reinstating actions of intra-LHA AMPH. In recent work we demonstrated that activation of noradrenergic α1-receptors within the LHA promotes waking, although this was not associated with HCRT neuronal activation (Schmeichel and Berridge, 2013). These observations could suggest the arousal-promoting actions of intra-LHA AMPH are independent of HCRT activation. However, given AMPH also elevates DA signaling, it remains possible that the behavioural effects of intra-LHA AMPH involve actions of HCRT neurons. Alternatively, the LHA contains melanocortinergic neurons implicated in the induction/maintenance of sleep (Adamantidis and de Lecea, 2008; Modirrousta et al., 2005). Thus, psychostimulant action within the LHA may involve inhibition of MCH neuronal signaling.

Finally, recent evidence indicates a role for HCRT neurotransmission in the reinstatement of drug-seeking. For example, central HCRT administration reinstates previously extinguished drug-seeking (Boutrel et al., 2005; Harris et al., 2005), while treatment with an HCRT-R1 antagonist prevents stressor-induced reinstatement of cocaine-induced drug-seeking (Boutrel et al., 2005). Interestingly, unpublished studies in our laboratory indicate that arousal-enhancing doses of HCRT fail to produce either a CPP or conditioned place aversion. Thus, the ability of intra-LHA AMPH to promote reinstatement while lacking reinforcing/rewarding actions is similar to that seen with HCRT administration and could suggest HCRT participates in the behavioural effects of intra-LHA AMPH.

**Methodological considerations for CPP reinstatement**

The current studies used CPP to assess the reinforcing and reinstating actions of intra-LHA AMPH. An alternative approach to assessing the reinforcing and reinstating actions of a drug is self-administration. As reviewed elsewhere (Aguilar et al., 2009), there are key differences in the type of learning, route of administration and degree of motivation/effort associated with the two paradigms. For example, self-administration involves instrumental learning and active drug taking and likely evaluates the primary, or innately, rewarding/reinforcing properties of a drug (Bardo and Bevins, 2000; Di Chiara, 1999). CPP, on the other hand, involves passive Pavlovian drug-conditioning and likely measures the incentive motivational properties of the drug-associated context.
Nonetheless, there is considerable concordance regarding the reinforcing effects of various drugs and drug-associated contexts/cues across these two paradigms (for review, Bardo and Bevins, 2000). However, there is some evidence for potentially divergent effects of context- and stress-related reinstatement when comparing studies across the paradigms (for review, Aguilar et al., 2009).

The fact that intra-LHA AMPH is not reinforcing but reinstates an extinguished CPP could suggest that the LHA does not play a role in the primary reinforcing effects of psychostimulants, but rather is involved in the secondary effects or the incentive value of the drug-associated context. Regardless of the conceptual framework, the current studies clearly indicate that the LHA is a region involved in the reinstatement of a psychostimulant-induced CPP. As such, these observations further support a role of the LHA in drug abuse/relapse.

Summary

The current studies demonstrate that AMPH acts within the LHA to elicit affectively neutral arousal and reinstate drug-seeking behaviour, as measured by CPP. These results identify the LHA as a site involved in psychostimulant-induced reinstatement and provide further support for a potential role of the LHA in drug abuse. Moreover, these observations suggest that arousal per se, independent of affective state, may play an important role in relapse, at least under certain conditions.

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Statement of Interest

None.

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