

LETTERS

A role for lateral hypothalamic orexin neurons in reward seeking

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The lateral hypothalamus is a brain region historically implicated in reward and motivation^{1–4}, but the identity of the neurotransmitters involved are unknown. The orexins (or hypocretins) are neuropeptides recently identified as neurotransmitters in lateral hypothalamus neurons^{5,6}. Although knockout and transgenic overexpression studies have implicated orexin neurons in arousal and sleep⁷, these cells also project to reward-associated brain regions, including the nucleus accumbens and ventral tegmental area^{8,9}. This indicates a possible role for these neurons in reward function and motivation^{3,10}, consistent with previous studies implicating these neurons in feeding⁶. Here we show that activation of lateral hypothalamus orexin neurons is strongly linked to preferences for cues associated with drug and food reward. In addition, we show that chemical activation of lateral hypothalamus orexin neurons reinstates an extinguished drug-seeking behaviour. This reinstatement effect was completely blocked by prior administration of an orexin A antagonist. Moreover, administration of the orexin A peptide directly into the ventral tegmental area also reinstated drug-seeking. These data reveal a new role for lateral hypothalamus orexin neurons in reward-seeking, drug relapse and addiction.

We used a two-chamber, nonbiased, conditioned place-preference (CCP) model to measure the rewarding properties of morphine, cocaine or food^{11–13}. In this model, one chamber becomes associated with drug or food reward through repeated pairings, whereas the other chamber is associated with no reward. Preference for reward is measured by the amount of time animals spend in the reward-associated chamber minus the time it spends in the non-rewarded chamber, when given free access to both chambers after conditioning.

Orexin-expressing neurons are located in three contiguous hypothalamic regions: lateral hypothalamus, perifornical area (PFA) and dorsomedial hypothalamus (DMH)¹⁴. To determine whether orexin neurons were stimulated during the expression of preference for different rewards, we used double-label immunohistochemistry for both orexin and the immediate early gene protein, Fos (a marker of neuronal stimulation¹⁵). Conditioned animals displayed reward-seeking by spending significantly more time in the reward-paired chamber than non-conditioned animals (Fig. 1a). Only conditioned animals that exhibited a preference for the reward-paired chamber showed increased Fos activation in lateral hypothalamus orexin cells. Significant percentages (48–52%) of orexin neurons in the lateral hypothalamus were Fos-activated in these animals after preference testing for morphine, cocaine or food reward, when compared to non-conditioned animals (17%, $P < 0.01$) (Table 1; Fig. 1b, see also Supplementary Fig. 1). The percentage of Fos activation in lateral hypothalamus orexin neurons in non-conditioned animals was not statistically different from that found in naïve untreated animals (15%, $P = 0.10$). Our findings in naïve animals are similar to baseline levels of Fos activation reported previously in awake rats¹⁶.

Enhanced activation of lateral hypothalamus orexin neurons after conditioning indicates a potential involvement of these neurons in the preference for reward-related cues. In support of this idea, the amount of Fos activation in these neurons was correlated with the intensity of reward seeking. Significant positive correlations were found between the percentages of orexin neurons that were Fos-positive in the lateral hypothalamus and preferences shown by corresponding conditioned animals ($P < 0.01$; Table 1). Thus, as preference scores increased, percentages of lateral hypothalamus orexin neurons that were Fos-activated also increased proportionally, for all three rewards. In contrast, numbers of Fos-activated non-orexin neurons in the lateral hypothalamus did not correlate with preference. Moreover, despite the fact that substantial numbers of Fos-positive orexin neurons were found in PFA and DMH, no correlations were found in these areas between preference scores and the percentage of Fos-positive orexin neurons ($P > 0.20$; Table 1). This indicates that the relationship between reward-seeking and neuronal stimulation in the hypothalamus occurs specifically in

Table 1 | Orexin and Fos double labelling

Groups	Cell types	Percentage Fos-positive	Correlations <i>R</i>
Morphine-conditioned <i>n</i> = 12	Orx LH	48 ± 2*	0.72, $P < 0.01^*$
	NonOrx LH	55 ± 6	
	Orx PFA	62 ± 2	
	Orx DMH	67 ± 4	
Food-conditioned <i>n</i> = 8	Orx LH	50 ± 3*	0.87, $P < .01^*$
	NonOrx LH	47 ± 5	
	Orx PFA	42 ± 3	
	Orx DMH	47 ± 6	
Cocaine-conditioned <i>n</i> = 8	Orx LH	52 ± 5*	0.90, $P < 0.01^*$
	NonOrx LH	78 ± 7	
	Orx PFA	67 ± 3	
	Orx DMH	74 ± 3	
Non-conditioned <i>n</i> = 15	Orx LH	17 ± 2	
	NonOrx LH	43 ± 6	
	Orx PFA	52 ± 4	
	Orx DMH	59 ± 4	
Naïve <i>n</i> = 6	Orx LH	15 ± 1	
	NonOrx LH	29 ± 8	
	Orx PFA	52 ± 3	
	Orx DMH	57 ± 6	
Novelty-conditioned <i>n</i> = 6	Orx LH	18 ± 2	
	NonOrx LH	50 ± 1	
	Orx PFA	56 ± 3	
	Orx DMH	63 ± 5	

The percentages of orexin-positive cells that were also Fos-positive are indicated. Correlation coefficients for the comparisons between these percentages and the corresponding preference score in each animal. Abbreviations used: Orx, orexin-positive neurons; NonOrx, orexin-negative neurons; LH, lateral hypothalamus; PFA, perifornical area; and DMH, dorsomedial hypothalamus. The non-orexin Fos-positive neurons in the lateral hypothalamus are given as total counts not percentages. Lateral hypothalamus by group ANOVA $F_{3,39} = 33, P < 0.01$.

*Significantly different from other groups, $P < 0.05$.

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orexin neurons, but not in non-orexin neurons or in orexin neurons in adjacent hypothalamic areas.

A separate group of animals ($n = 11$) was given morphine CPP training followed by a systemic injection of the selective orexin A antagonist (SB 334867; ref. 17) on a second consecutive day of CPP testing. Administration of the orexin antagonist produced a significant reduction in preference (206 ± 23 s versus 87 ± 30 s; $t_{12} = 2.3$; $P < 0.05$) that was not found in a different group of animals given a vehicle injection instead (182 ± 21 s versus 145 ± 27 s; $t_{12} = 0.9$; $P = 0.39$). There were no differences in the original preferences for morphine between these two groups ($P = 0.46$). These data indicate that orexin has a role in the amount of preference expressed.

Not all reward-seeking behaviours were associated with enhanced stimulation of lateral hypothalamus orexin neurons. In a separate group of animals conditioned with novel object reward¹⁸, we found no enhanced Fos activation in lateral hypothalamus orexin neurons. In this model, exposure to a novel object is paired with one conditioning chamber, whereas no object is paired with the other side. This model produces a robust preference for the chamber paired with the novel object ($n = 6$ rats; preference score, 228 ± 34 s), and similar activity levels as in the drug and food conditioned animals on the test day. Despite this robust preference there was no significant Fos induction in the lateral hypothalamus orexin neurons during preference testing ($18 \pm 2\%$ of orexin neurons were Fos positive; Table 1; see also Supplementary Fig. 2). Measures of orexin versus non-orexin neurons that were Fos-activated were not statistically different from non-conditioned animals ($P > 0.20$) and there was no correlation with preference scores (Table 1). Novelty conditioning entailed more conditioning days than food or drug conditioning; however, it seems unlikely that these extra conditioning days

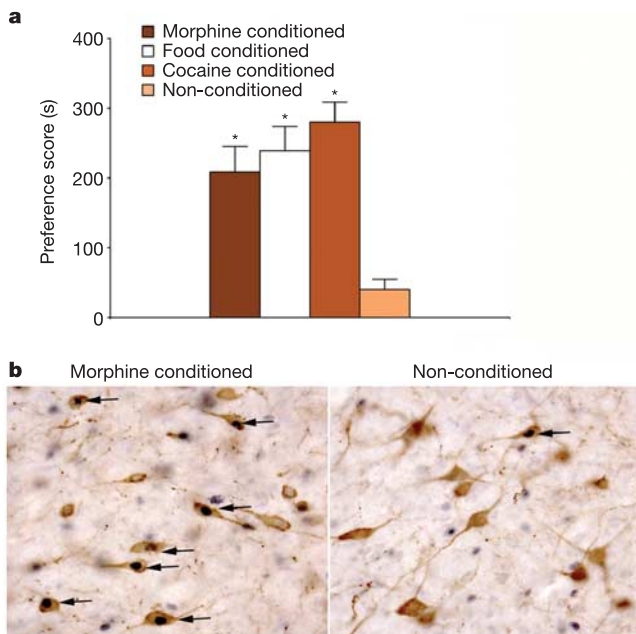


Figure 1 | Morphine conditioned animals had significantly greater place preferences and Fos activated orexin neurons in the lateral hypothalamus than non-conditioned animals. **a**, Conditioned animals showed significant preferences for the reward-paired chamber compared with non-conditioned animals ($F_{3,39} = 15$, $P < 0.01$). Preference scores for the morphine (8 mg kg^{-1}), cocaine (15 mg kg^{-1}) and food (lucky charms cereal)-paired environments expressed as the time spent in the reward-paired side minus the time spent on the non-rewarded side on the test day (mean \pm s.e.m.). **b**, High-power photomicrograph of the lateral hypothalamus showing the double labelling of orexin (brown cytoplasm) and Fos protein (black nuclei) in morphine-conditioned and non-conditioned animals. Black arrows indicate double-labelled cells.

decreased orexin neuron stimulation. These data indicate that not all rewards work through the activation of the lateral hypothalamus orexin system, and that this system may be specifically engaged with consummatory rewards (that is, food and drugs).

These results show that lateral hypothalamus orexin neurons are activated in proportion to preference for certain rewards. We reasoned that if activation of these neurons drives reward seeking, then stimulation of these cells should reinstate extinguished preference. To test this, we submitted rats to morphine place conditioning and then extinguished the morphine preference by repeatedly exposing the animals to the chambers without morphine administration¹⁹ (Fig. 2a). To activate orexin neurons, we microinfused the Y4 agonist rPP (rat pancreatic polypeptide) directly into the lateral hypothalamus orexin neuronal area. We expected rPP to stimulate orexin neurons because these cells express the Y4 receptor, and activation of this receptor by rPP potently induces Fos activation in lateral hypothalamus orexin neurons²⁰. We found that microinjecting rPP into the lateral hypothalamus robustly reinstated an extinguished morphine place preference (Fig. 2a). Similar control injections of rPP dorsal, ventral or medial to lateral hypothalamus orexin cells did not reinstate preference (Fig. 2a, b). To insure that vehicle injections alone did not produce reinstatement, five animals from the lateral hypothalamus-injected group were given vehicle injections 3 days before the rPP injection. In these cases, the vehicle

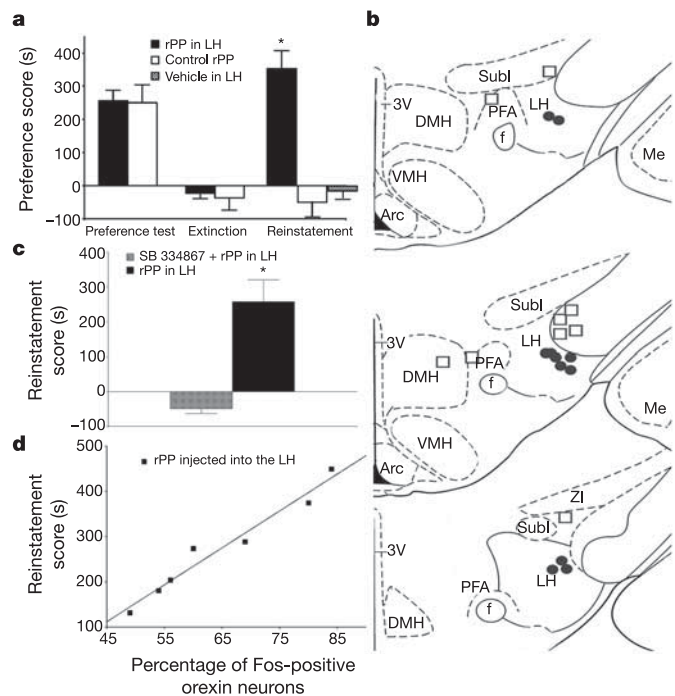


Figure 2 | Activation of lateral hypothalamus orexin neurons by rPP reinstated an extinguished preference for morphine. **a**, Preference scores are shown for both rPP- (150 nM) and vehicle-injected groups (mean \pm s.e.m. in morphine-paired side minus saline-paired side) during the initial conditioning test, after extinction and during the reinstatement test. **b**, Effective (filled circles, $n = 12$) and ineffective (open squares, $n = 9$) sites of rPP administration. **c**, The selective orexin A antagonist, SB 334867 ($20\text{--}30 \text{ mg kg}^{-1}$), blocked reinstatement by rPP ($n = 8$). Data were included only if rPP injection into lateral hypothalamus on the following day (without the antagonist pretreatment) produced reinstatement of preference. **d**, Plot of correlation between reinstatement scores and percentages of lateral hypothalamus orexin neurons that were Fos activated in rPP reinstated animals. Abbreviations used were: ZI, zona incerta; Sub I, subincertal nucleus; Me, medial amygdala nucleus; VMH, ventromedial hypothalamic nucleus; Arc, arcuate nucleus; PFA, perifornical area; DMH, dorsomedial hypothalamus; LH lateral hypothalamus; 3V, third ventricle; and f, fornix.

injections did not reinstate the CPP behaviour, whereas the later rPP injections produced robust reinstatement (Fig. 2a; reinstatement score: -47 ± 71 s for vehicle versus 465 ± 100 s for rPP). Systemic injections of morphine are a reliable method to reinstate extinguished responses to morphine cues in this model¹⁹. We found that the reinstatement by rPP in the lateral hypothalamus was similar to the reinstatement produced by systemic morphine (8 mg kg^{-1} , intraperitoneal; morphine reinstatement score was 424 ± 103 s, $n = 8$ rats and rPP reinstatement score was 353 ± 52 s, $n = 12$, $P > 0.5$).

The rPP-induced reinstatement was completely blocked by prior systemic administration of the orexin antagonist, SB 334867 (Fig. 2c), confirming a role for orexin receptors in rPP-induced reinstatement. One day later, the same animals reinstated preference when injected with rPP in the lateral hypothalamus without antagonist pretreatment. This confirmed that antagonist pretreatment blocked an otherwise effective reinstatement by rPP. In addition, we measured Fos staining in both orexin and non-orexin lateral hypothalamus cells in seven rats with significant reinstatement after rPP injections, and in six dorsal control animals that did not reinstate (reinstatement scores were 279 ± 40 s and -51 ± 46 s, respectively; $P < 0.01$). In the animals for which rPP reinstated preference, the percentage of Fos-activated orexin cells in the lateral hypothalamus was significantly greater than in control animals, for which rPP did not cause reinstatement ($P < 0.01$; lateral hypothalamus orexin neurons that were Fos-activated for reinstated rats, $66 \pm 8\%$; and for control non-reinstated animals, $11 \pm 5\%$). Moreover, a highly significant correlation was found between the preference expressed during rPP-induced reinstatement and the percentage of lateral hypothalamus orexin neurons that were Fos-activated (Fig. 2d; $R = 0.99$; $P > 0.01$). Thus, the greater the number of lateral hypothalamus orexin cells that were stimulated, the greater the intensity of reinstatement that resulted. No differences were found in the number of non-orexin Fos-positive cells between the groups ($P = 0.77$), and no correlations were found between the numbers of these non-orexin Fos-activated neurons and reinstatement scores ($R = 0.23$; $P = 0.72$).

The above evidence strongly indicates that stimulation of orexin lateral hypothalamus neurons can drive reinstatement behaviour for cues associated with drug reward. The effect of rPP on morphine

reinstatement could be due to a stress/arousal effect, or to the fact that rPP mimics the motivational effects of morphine. Our preliminary results indicate that an acute injection of morphine (but not saline) in the CPP box strongly induces Fos in lateral hypothalamus orexin neurons, whereas, exposure to footshock (using parameters that we and others find reinstates morphine CPP)¹⁹ only activated orexin neurons in the DMH and PFA but not in the lateral hypothalamus. These data support the latter interpretation (see Supplementary Table 1).

To determine whether the orexin peptide could produce reinstatement of morphine reward seeking we microinjected orexin A into the ventral tegmental area (VTA). The VTA contributes to many forms of drug reinstatement²¹ and orexin has an excitatory action on GABA- and dopamine-containing neurons in this area²². We found that orexin caused a significant reinstatement response for morphine reward only when microinjected into VTA, but not when injected into areas surrounding VTA (Fig. 3a, b). Similar vehicle injections had no effect.

Taken together these data strongly indicate that orexin neurons in the lateral hypothalamus become activated by cues associated with consummatory rewards such as food and drugs. This view is consistent with an early proposal for orexin function in feeding⁶ and recent implications in morphine dependence and withdrawal²³. Orexin receptors are expressed at high levels in reward-associated areas, including the VTA and nucleus accumbens^{24,25}. As both food and drugs of abuse work through common brain substrates^{10,26}, it is not surprising that lateral hypothalamus orexin neurons may mediate responses to cues associated with both food and drugs, but not non-consummatory rewards (for example, novelty). This is consistent with a recent report showing that treatments which increase consummatory behaviour were associated with Fos activation of lateral hypothalamus orexin cells more than exposure to novelty²⁷. Our study also indicates that different sets of hypothalamic orexin neurons may have different functions. A previous report indicated that Fos activation of orexin neurons in PFA and DMH showed diurnal changes consistent with a role in production or maintenance of arousal; lateral hypothalamus orexin neurons showed no such diurnal property²⁸. These results, together with our findings, suggest the hypothesis that orexin neurons in PFA and DMH regulate arousal whereas those in lateral hypothalamus regulate reward processing. Our data further indicate that activation of lateral hypothalamus orexin neurons may be important in the reinstatement of drug-seeking behaviours and thereby have an important role in modulating rewarding behaviour and addiction. Although orexin administration directly into VTA reinstated morphine preference, VTA may not be the only location for such orexin effects as orexin-containing fibres are found throughout areas associated with reward processing, such as the extended amygdala¹⁰ and mesocorticolimbic system⁹. We conclude that the lateral hypothalamus orexin system is an integral part of circuitry that integrates environmental cues with consummatory rewards, and that stimulation of these neurons can drive relapse of drug-seeking behaviour.

METHODS

Subjects. Male Sprague–Dawley rats (200–250 g; Harlan) were group-housed in accordance with NIH guidelines on a 12-h light/dark cycle with food and water available *ad libitum*. The Institutional Animal Care and Use Committee of the University of Pennsylvania approved all animal procedures.

Drugs. Morphine sulphate powder, provided by the National Institute on Drug Abuse, was dissolved in sterile saline and administered via intraperitoneal injection (8 mg kg^{-1}). Cocaine (Sigma-Aldrich) was dissolved in sterile saline (15 mg kg^{-1} , intraperitoneal). SB-334867 (Tocris) was dissolved in a 10% (w/v) encapsin/2% DMSO solution in water (intraperitoneal, 20 mg kg^{-1} ($n = 2$) and 30 mg kg^{-1} ($n = 18$); refs 29, 30). Orexin A (140 nM ; Tocris) and rPP (150 nM^{20} ; Anaspec) were dissolved in artificial cerebral spinal fluid (vehicle).

Surgery and histology. Rats were anaesthetized with a ketamine/xylazine cocktail and implanted with bilateral chronic indwelling guide cannulae

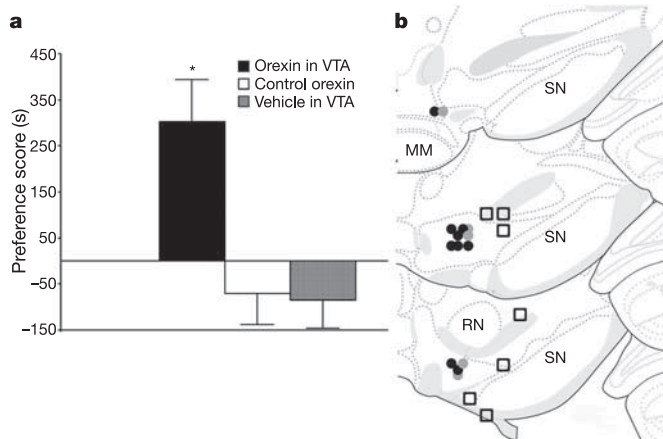


Figure 3 | Orexin administration into the VTA reinstated an extinguished preference for morphine. **a**, Reinstatement scores (time is mean \pm s.e.m. in morphine-paired side minus saline-paired side) after orexin administration (140 nM) in the VTA. Orexin in the VTA ($n = 8$) was significantly different from vehicle in the VTA ($n = 6$) or orexin outside the VTA ($n = 7$) in reinstating an extinguished morphine preference ($F_{2,18} = 11$, $P < 0.01$). **b**, Effective (filled circles) and ineffective (open squares) sites of orexin administration. Gray circles are sites of vehicle administration. Drawings were adapted from ref. 31 (plates 36–38). Abbreviations used were: SN, substantia nigra; MM, medial mammillary nucleus; and RN, red nucleus.

aimed 2 mm above the lateral hypothalamus (anterior/posterior (AP) -3.3 , medial/lateral (ML) $+2.5$ and dorsal/ventral (DV) -8.0 , 5° angle from bregma) or VTA (AP -5.3 , ML $+2.5$, DV -7.5). Surgeries were similar to previous reports¹². Control injections were made ~ 2 mm above the lateral hypothalamus or VTA. Animals were given 1 week to recover from surgery before place conditioning began. After each experiment, animals were killed with an overdose of sodium pentobarbital (100 mg kg^{-1} intraperitoneal) and cannulae locations were confirmed in histological analyses.

Conditioned place preference procedure. We used a standard two-chamber balanced design, with two conditioning sessions (for food and drugs) occurring for 3 days consecutively. Injections of drug or saline (food or no food) alternated between morning and afternoon sessions. Conditioning and testing were identical to our previously published reports for morphine¹¹, cocaine¹² and food¹³. Food conditioned animals were not food deprived and were pre-exposed to the sweet flavoured cereal one week before conditioning. Non-conditioned animals consisted of morphine-treated animals for which the environment was explicitly unpaired with morphine ($n = 7$)¹¹, and cocaine-treated animals in which conditioning failed (animals who were conditioned but whose preference scores were less than 70 s for the cocaine environment, $n = 8$). No significant differences ($P > 0.08$) were found between these two non-conditioned groups in any measures taken, and their data were pooled. Novel object conditioning¹⁸ included 10-min pairings of a novel object versus no object in distinct compartments for 8 d, with 1 h between pairings ($n = 6$). The novel objects were paper bows, a plastic ball, a piece of cotton, a newspaper, halves of socks, pieces of egg carton, a sponge and wood chips. The order of pairings (object versus no object) was alternated each day.

Extinction and reinstatement procedure. After conditioning, animals were tested daily until preference levels for the morphine-paired chamber dropped to less than 70 s difference for the two chambers for two consecutive days. rPP or vehicle was injected bilaterally into the lateral hypothalamus (or, orexin administered into the VTA) 30 min before the reinstatement test. For the orexin antagonist experiments, SB-334867 was injected 30 min before rPP. For morphine reinstatement, morphine 8 mg kg^{-1} (intraperitoneal) was injected immediately before placing animals in the conditioning boxes.

Double label immunohistochemistry. Two hours after CPP testing rats were deeply anaesthetized and perfused transcardially with paraformaldehyde as previously described¹¹. Brain sections were first processed for Fos-immunostaining with nickel ammonium sulphate intensification of 3',3'-diaminobenzidine (DAB) as we have previously described¹¹, and then processed for orexin (orexin A antibody, Santa Cruz Biotechnology; 1:1,000; biotinylated secondary, 1:500). Slices were then mounted on glass slides, dehydrated in alcohol and sealed with a cover slip. Double-labelled cells were readily identified because orexin stained the cytoplasm brown and Fos immunoreactive nuclei were black. For counting, two sections from each animal were chosen at the same levels with equivalent numbers of orexin-positive neurons. Colour photographs of the lateral hypothalamus and DMH were taken and the numbers of orexin neurons, and Fos-positive/orexin doubly labelled neurons were counted and averaged for each animal. The number of double-labelled cells were determined by both blinded and non-blinded observers; there was a 94% agreement between these two sets of observations. All orexin-labelled neurons lateral to the fornix were considered to be in the lateral hypothalamus. All orexin labelled neurons located dorsal and 0.4 mm medial to the fornix were considered to be in the PFA. All remaining orexin labelled neurons from the medial edge of the PFA region to the third ventricle, were considered to be in the DMH.

Data analyses. Place conditioning data were analysed by calculating the time spent in the reward-paired chamber minus the time spent in the other chamber. The resulting difference score was compared between groups using analysis of variance (ANOVA). The percentages of double-labelled cells were compared between groups using ANOVA. Pearson's R correlation was used to compare preference or reinstatement scores with the number of double labelled cells. Where necessary, post-hoc analysis was carried out with a Newman-Keuls test.

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- Anand, B. K. & Brobeck, J. R. Hypothalamic control of food intake in rats and cats. *Yale J. Biol. Med.* **24**, 123–140 (1951).
- Olds, J. & Milner, P. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J. Comp. Physiol. Psychol.* **47**, 419–427 (1954).
- DiLeone, R. J., Georgescu, D. & Nestler, E. J. Lateral hypothalamic neuropeptides in reward and drug addiction. *Life Sci.* **73**, 759–768 (2003).
- Petrovich, G. D. & Gallagher, M. Amygdala subsystems and control of feeding behaviour by learned cues. *Ann NY Acad. Sci.* **985**, 251–262 (2003).
- de Lecea, L. *et al.* The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl Acad. Sci. USA* **95**, 322–327 (1998).
- Sakurai, T. *et al.* Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behaviour. *Cell* **92**, 573–585 (1998).
- Chemelli, R. M. *et al.* Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* **98**, 437–451 (1999).
- Peyron, C. *et al.* Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.* **18**, 9996–10015 (1998).
- Fadel, J. & Deutch, A. Y. Anatomical substrates of orexin-dopamine interactions: lateral hypothalamic projections to the ventral tegmental area. *Neuroscience* **111**, 379–387 (2002).
- Baldo, B. A., Daniel, R. A., Berridge, C. W. & Kelley, A. E. Overlapping distributions of orexin/hypocretin- and dopamine-beta-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. *J. Comp. Neurol.* **464**, 220–237 (2003).
- Harris, G. C. & Aston-Jones, G. Enhanced morphine preference following prolonged abstinence: association with increased Fos expression in the extended amygdala. *Neuropsychopharmacology* **28**, 292–299 (2003).
- Harris, G. C. & Aston-Jones, G. Critical role for ventral tegmental glutamate in preference for a cocaine-conditioned environment. *Neuropsychopharmacology* **28**, 73–76 (2003).
- Harris, G. C. & Aston-Jones, G. Altered motivation and learning following opiate withdrawal: evidence for prolonged dysregulation of reward processing. *Neuropsychopharmacology* **28**, 865–871 (2003).
- Siegel, J. M. Hypocretin (orexin): role in normal behaviour and neuropathology. *Annu. Rev. Psychol.* **55**, 125–148 (2004).
- Herrera, D. G. & Robertson, H. A. Activation of c-fos in the brain. *Prog. Neurobiol.* **50**, 83–107 (1996).
- Espana, R. A., Valentino, R. J. & Berridge, C. W. Fos immunoreactivity in hypocretin-synthesizing and hypocretin-1 receptor-expressing neurons: effects of diurnal and nocturnal spontaneous waking, stress and hypocretin-1 administration. *Neuroscience* **121**, 201–217 (2003).
- Smart, D. *et al.* SB-334867-A: the first selective orexin-1 receptor antagonist. *Br. J. Pharmacol.* **132**, 1179–1182 (2001).
- Bevins, R. A. *et al.* Novel-object place conditioning: behavioural and dopaminergic processes in expression of novelty reward. *Behav. Brain Res.* **129**, 41–50 (2002).
- Wang, B., Luo, F., Zhang, W. T. & Han, J. S. Stress or drug priming induces reinstatement of extinguished conditioned place preference. *Neuroreport* **11**, 2781–2784 (2000).
- Campbell, R. E. *et al.* Orexin neurons express a functional pancreatic polypeptide Y4 receptor. *J. Neurosci.* **23**, 1487–1497 (2003).
- Shalev, U., Grimm, J. W. & Shaham, Y. Neurobiology of relapse to heroin and cocaine seeking: a review. *Pharmacol. Rev.* **54**, 1–42 (2002).
- Korotkova, T. M., Sergeeva, O. A., Eriksson, K. S., Haas, H. L. & Brown, R. E. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexin/hypocretins. *J. Neurosci.* **23**, 7–11 (2003).
- Georgescu, D. *et al.* Involvement of the lateral hypothalamic peptide orexin in morphine dependence and withdrawal. *J. Neurosci.* **23**, 3106–3111 (2003).
- Trivedi, P., Yu, H., MacNeil, D. J., Van der Ploeg, L. H. & Guan, X. M. Distribution of orexin receptor mRNA in the rat brain. *FEBS Lett.* **438**, 71–75 (1998).
- Marcus, J. N. *et al.* Differential expression of orexin receptors 1 and 2 in the rat brain. *J. Comp. Neurol.* **435**, 6–25 (2001).
- Carr, K. D. Augmentation of drug reward by chronic food restriction: behavioural evidence and underlying mechanisms. *Physiol. Behav.* **76**, 353–364 (2002).
- Baldo, B. A. *et al.* Activation of a subpopulation of orexin/hypocretin-containing hypothalamic neurons by GABAA receptor-mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment. *Eur. J. Neurosci.* **19**, 376–386 (2004).
- Estabrooke, I. V. *et al.* Fos expression in orexin neurons varies with behavioural state. *J. Neurosci.* **21**, 1656–1662 (2001).
- Rodgers, R. J. *et al.* SB-334867, a selective orexin-1 receptor antagonist, enhances behavioural satiety and blocks the hyperphagic effect of orexin-A in rats. *Eur. J. Neurosci.* **13**, 1444–1452 (2001).
- Haynes, A. C. *et al.* A selective orexin-1 receptor antagonist reduces food consumption in male and female rats. *Regul. Pept.* **96**, 45–51 (2000).
- Swanson, L. W. *Brain Maps: Structure Of The Rat Brain* (Elsevier, Amsterdam, 1992).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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