LETTERS

# Morphine reward in dopamine-deficient mice

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**Dopamine has been widely implicated as a mediator of many of the behavioural responses to drugs of abuse[1]. To test the hypothesis that dopamine is an essential mediator of various opiate-induced responses, we administered morphine to mice unable to synthesize dopamine. We found that dopamine-deficient mice are unable to mount a normal locomotor response to morphine, but a small dopamine-independent increase in locomotion remains. Dopamine-deficient mice have a rightward shift in the dose–response curve to morphine on the tail-flick test (a pain sensitivity assay), suggesting either a decreased sensitivity to the analgesic effects of morphine and/or basal hyperalgesia. In contrast, dopamine-deficient mice display a robust conditioned place preference for morphine when given either caffeine or L-dihydroxyphenylalanine (a dopamine precursor that restores dopamine throughout the brain) during the testing phases. Together, these data demonstrate that dopamine is a crucial component of morphine-induced locomotion, dopamine may contribute to morphine analgesia, but that dopamine is not required for morphine-induced reward as measured by conditioned place preference.**

Three decades of research have put the dopamine neurotransmitter system squarely at the centre of our understanding of the neural mechanisms through which drugs of abuse produce their effects[1–3]. Indeed, it has been established that most drugs abused by humans increase midbrain dopamine neuron firing[4] and/or dopamine release preferentially in the nucleus accumbens (NAc)[5]. Similarly, disruptions of the dopaminergic system, either pharmacologically or through brain lesions, can inhibit drug reward[1,6–9]. Furthermore, animals will self-administer and acquire a conditioned place preference (CPP) for agents that increase dopamine receptor signalling (for example, agonists, dopamine transporter blockers) when delivered directly into the NAc[1]. It has been proposed that the rewarding properties of opiates, such as heroin and morphine, are produced via activation of μ-opiate receptors located on GABAergic midbrain interneurons that negatively regulate dopamine cell firing[10]. Activation of these inhibitory $G_{\alpha i}$-coupled μ-opiate receptors reduces the GABAergic tone onto midbrain dopamine neurons, thereby increasing their firing rate and the amount of dopamine released in the NAc. Indeed, animals will self-administer and display a place preference for opiates delivered directly into the ventral tegmental area[11,12]. Moreover, mice lacking dopamine $D_2$ receptors fail to self-administer, or demonstrate a CPP for, morphine[13,14] (but see also ref. 15). Although much evidence points to a key role for dopamine in mediating the effects of opiates, dopamine-independent mechanisms of opiate reward have been proposed[9].

The dopamine-deficient ($Th^{-/-}; Dbh^{Th/+}$, see Methods) mice generated in our laboratory[16] provide an ideal model to test whether dopamine is essential to the production of morphine-induced behavioural responses. These animals are severely hypoactive, hypophagic and require daily administration of L-dihydroxyphenylalanine (L-dopa) to prevent starvation. Therefore, to determine whether dopamine is necessary for the acute effects of morphine, we administered morphine 18–24 h after L-dopa injection, when brain dopamine levels had fallen to <1% of control mice[17].

Morphine, like most drugs of abuse, induces a locomotor response in rodents that is thought to reflect dopamine release in the striatum[18]. Administration of various doses of morphine to dopamine-deficient and control mice revealed that dopamine is a critical component of morphine-induced locomotion (Fig. 1a). At the highest dose, dopamine-deficient mice significantly increased locomotor activity but only to ~5% of control mice, indicating that morphine can stimulate a small amount of locomotion through a dopamine-independent pathway. In support of this conclusion, pre-treatment of dopamine-deficient mice with L-dopa ($30\,mg\,kg^{-1}$) restored a robust locomotor response to morphine (Fig. 1b). Amphetamine pre-treatment, which purges any residual dopamine[19], did not block the small morphine locomotor response in dopamine-deficient mice (Fig. 1c), providing further evidence that it is a dopamine-independent effect. Caffeine and other adenosine receptor antagonists stimulate locomotion when given to dopamine-deficient or control mice[20]. The effects of caffeine pre-treatment on morphine-induced locomotion by dopamine-deficient mice were approximately additive (Supplementary Fig. 1a, b), indicating that generalized locomotor activation by caffeine does not replace the requirement for dopamine, and that a robust morphine locomotor response is dependent on, and specific to, dopamine release.

Because dopamine has been implicated in the modulation of pain sensitivity[21] and certain types of morphine analgesia[22,23], we tested dopamine-deficient mice in the tail-flick task. Unlike many pain assays that require complex motor coordination, tail-flick is mediated by a spinal reflex arc, which dopamine-deficient mice are capable of performing. Dopamine-deficient mice displayed morphine analgesia, but the dose–response curve is shifted rightwards (Fig. 2a), suggesting that dopamine may mediate some of the analgesic effects of morphine. However, dopamine-deficient mice also displayed a faster tail-flick after saline injection, indicating that they may be more sensitive to pain under basal conditions. To confirm this hypothesis, we performed a tail-flick test at different temperatures. At every temperature tested, dopamine-deficient mice withdrew their tails more quickly (Fig. 2b). These data support the hypothesis that dopamine, perhaps originating from the $A_{11}$ dopamine neurons, which project directly to the spinal cord or through other indirect routes[21], provides a tonic pain suppression signal that may be mediated through $D_2$ receptors[24].

Dopamine-deficient mice are bradykinetic and severely hypoactive and thus do not perform behavioural tasks that require voluntary movement. Therefore, we used viral gene transfer to restore dopamine synthesis to the caudate putamen. These virally rescued dopamine-deficient (vrDD) mice feed and locomote in the absence of L-dopa. We used vrDD mice in the CPP assay to test whether the hedonic experience of morphine was dependent on dopamine release outside of the caudate putamen (for example, the NAc). CPP is an

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assay whereby mice are repeatedly exposed to a treatment (for example, morphine) or vehicle in distinct chambers of a conditioning box and subsequently tested for their preference to occupy either chamber. During the drug-pairing sessions, the hedonic value (reward) can become associated with the environment (dependent on associative learning), which is revealed during the testing phase. The testing phase also includes an incentive component—in the absence of motivation (drug seeking) the mouse may not manifest a preference. These vrDD mice readily explored the CPP box (Supplementary Fig. 2a, b) and displayed a strong preference for the morphine-paired chamber (Supplementary Fig. 2c). These results are consistent with previous lesioning experiments[8] and suggest that dopamine is not required in brain regions outside the caudate putamen for the formation of morphine CPP.

To test whether morphine reward requires dopamine release anywhere in the brain, we developed a paradigm (Fig. 3a) that allows the dopamine-deficient mice to perform the exploratory aspects of the task yet receive the conditioning treatments during the dopamine-depleted state. Specifically, we gave a moderate dose of caffeine to dopamine-deficient or control mice during the baseline and testing phases of the CPP paradigm, but all pairing sessions (that is saline or morphine injections) were performed 18–24 h after L-dopa and in the absence of caffeine. This dose of caffeine induces locomotion and feeding in the absence of dopamine[20]. Caffeine induced a sufficient locomotor response in dopamine-deficient mice that was ~45% of control (Fig. 3b, c). Despite the absence of dopamine during morphine administration, dopamine-deficient mice developed a CPP comparable to that of control mice at the two higher doses of morphine but not at the lowest dose (Fig. 3d). These data indicate that dopamine-deficient mice can develop a preference for the place where they receive morphine, demonstrating that dopamine release is not essential for this behaviour.

Because dopamine-deficient mice did not show a preference at the 2.5 mg $kg^{-1}$ dose of morphine, dopamine could be involved in some aspect(s) of morphine CPP. To ascertain whether dopamine is involved in the incentive (motivational or wanting) phase of

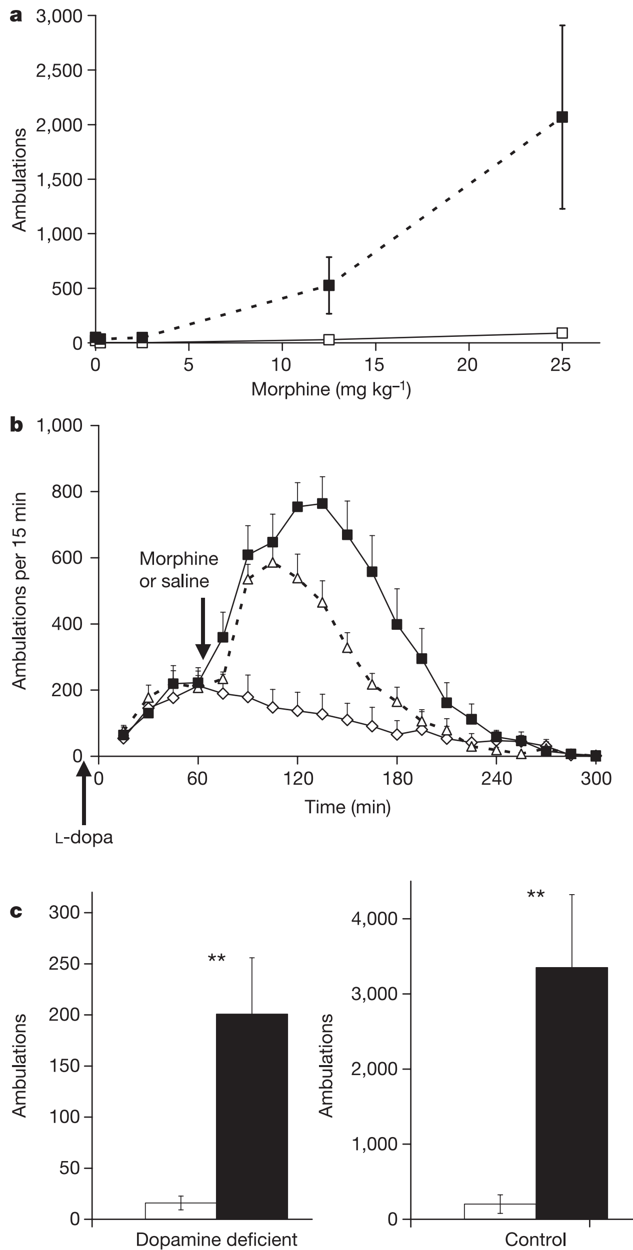


**Figure 1 | Locomotor responses to morphine in dopamine-deficient and control mice.** **a**, Cumulative 3-h morphine-induced locomotion by control ($n = 8$; filled squares) and dopamine-deficient mice ($n = 8$; open squares); dopamine-deficient mice have a severely blunted response. Repeated-measures analysis of variance (ANOVA); treatment effect $P < 0.001$; genotype effect $P < 0.05$; treatment–genotype interaction $P < 0.01$. **b**, Locomotor time course shows that 1 h L-dopa (30 mg $kg^{-1}$) pre-treatment of dopamine-deficient mice restores a robust locomotor response to morphine at 12.5 mg $kg^{-1}$ ($n = 8$; triangles) and 25 mg $kg^{-1}$ ($n = 8$; squares). Saline control shows the L-dopa response ($n = 8$; diamonds). Repeated-measures ANOVA on 3-h cumulative locomotion after morphine treatment; treatment effect $P < 0.001$. **c**, Amphetamine (3 mg $kg^{-1}$) pre-treatment does not eliminate subsequent morphine (25 mg $kg^{-1}$) response. Saline control, white bars; morphine, black bars. Repeated-measures ANOVA within genotype on 2-h cumulative locomotion after morphine treatment; treatment effect $^{**}P < 0.01$. All data are presented as means ± s.e.m.

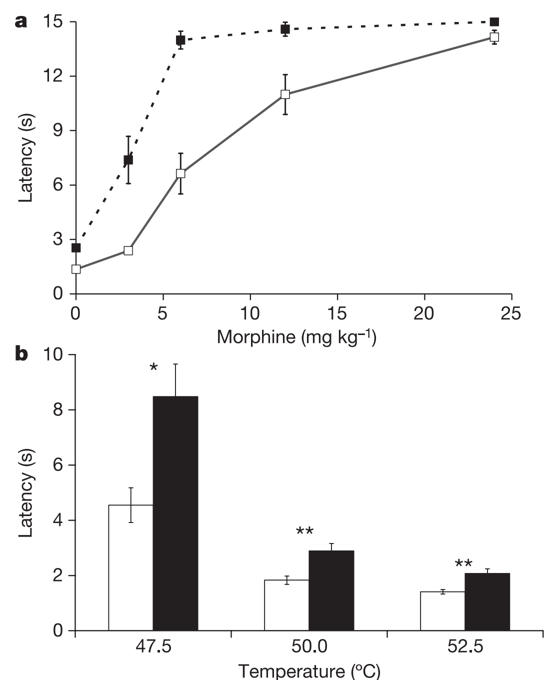


**Figure 2 | Latencies to tail-flick by dopamine-deficient and control mice.** **a**, Dose–response curve to morphine in control ($n = 8$; filled squares) and dopamine-deficient mice ($n = 9$; open squares). Repeated-measures ANOVA; treatment effect $P < 0.001$; genotype effect $P < 0.001$; treatment–genotype interaction $P < 0.001$. **b**, Heat response in dopamine-deficient ($n = 8$; white bars) or control mice ($n = 10$; black bars); dopamine-deficient mice are more sensitive to thermal stimulus. $^{*}P < 0.05$, $^{**}P < 0.01$; one-way ANOVA comparing genotypes within treatment. All data are presented as means ± s.e.m.



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morphine CPP, we restored dopamine during the baseline and testing phases (by pre-treating the animals with a moderate dose of L-dopa instead of caffeine) but continued to conduct the pairing sessions in the absence of dopamine. This treatment rescued a CPP for morphine at 2.5 mg kg$^{-1}$ (Fig. 3e), suggesting that dopamine facilitates the manifestation of place preference (the incentive phase of CPP).

Our finding that dopamine-deficient mice are capable of learning to associate the hedonic effects of morphine with a particular environment is strong evidence that dopamine is not essential for mice to experience the rewarding effects of morphine or to learn to associate hedonic experiences with a specific environment. These conclusions are consistent with our previous work showing that dopamine-deficient mice like sweets (sucrose or saccharin)[25] and can learn the location of food rewards without dopamine[26]. This result suggests a number of possibilities regarding dopamine's role in mediating the acute rewarding properties of opiates. First, dopamine may not be involved in the acute reward produced by opiates. Indeed, several lines of evidence suggest that opiates can produce reward in the absence of increased dopamine release[8,9]. Given the widespread expression of μ-opiate receptors, their activation in alternative brain regions such as the pedunculopontine tegmental nucleus[27] or NAc[28] may mediate the rewarding aspects of morphine independent of its effects on midbrain dopamine neuron firing. A second possibility is that dopamine contributes to morphine CPP but alternative neural pathways are of equal or greater importance. The fact that dopamine-deficient mice failed to develop a significant CPP at the lowest dose of morphine would be consistent with this possibility. However, because we were able to rescue CPP at the low dose of morphine by restoring dopamine only during the baseline and testing phases of the experiment, we conclude that dopamine facilitates the incentive (motivation) to seek the morphine-paired side of the conditioning chamber. A third possibility is that dopamine-independent compensatory mechanisms develop to mediate opiate reward in dopamine-deficient mice. We consider this unlikely because they are treated daily with L-dopa, which restores dopamine signalling for ~8 h each day; thus they are not chronically dopamine depleted. Furthermore, the dopamine-deficient mice are unable to compensate for other goal-directed behaviours (such as feeding) nor for the locomotor effects of morphine—although these behaviours can be rescued by acute restoration of dopamine via L-dopa.

The dopamine-deficient mice used in this study are uniquely suited to address questions about the importance of dopamine signalling throughout the brain for various biological processes, because we can maintain them with dopamine signalling (by providing L-dopa) and then study them in a dopamine-depleted state. This study demonstrates that although dopamine is a critical component of morphine-induced locomotion and modulates pain sensitivity, it is not required for mice to learn a conditioned association for morphine. We favour the hypothesis that dopamine is important for reward-seeking behaviour but is not essential for the hedonic experience of reward or for reward learning[3,25,26]. We recognize, however, that on its own, this explanation fails to account for results such as the abolition of morphine CPP in rodents when co-administered with dopamine receptor antagonists[7]. However, these pharmacological studies are often difficult to interpret because many dopamine receptor antagonists can produce place-aversion[7] and/or interfere with associative learning[29]. In contrast, our explanation could account for the results of other experimental models (for example, lesions or genetic manipulations) in which dopamine signalling was disrupted and the animals failed to manifest reward—those manipulations may have blocked the ability of the animals to demonstrate a preference rather than experience the pleasure associated with morphine treatment.

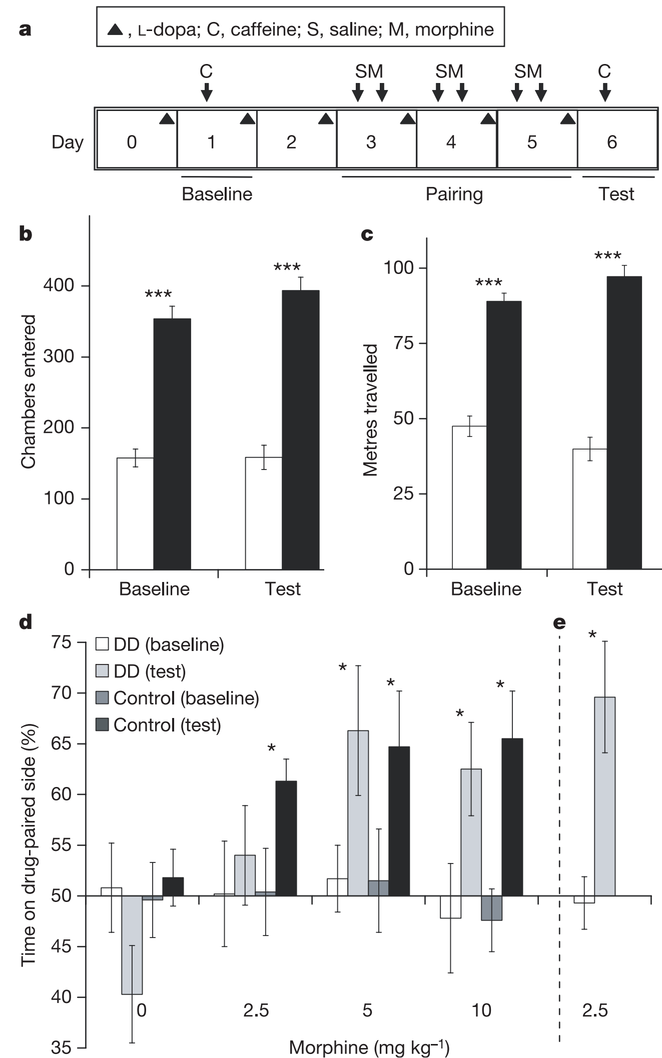


**Figure 3 | Conditioned place preference in dopamine-deficient and control mice.** **a**, Schematic illustrating the design of the CPP paradigm. **b**, **c**, Exploratory activity as measured by the number of chamber entries (**b**) or total distance travelled by dopamine-deficient ($n = 39$; white bars) or control mice ($n = 39$; black bars) during the baseline and testing phases of the caffeine CPP experiments (**c**). **d**, Dose–response to morphine (subcutaneously administered) in dopamine-deficient and control mice using caffeine as a locomotor stimulant ($n = 13$, 10, 8 and 8 for 0, 2.5, 5 and 10 mg kg$^{-1}$ doses of morphine, respectively). DD, dopamine deficient. **e**, CPP for 2.5 mg kg$^{-1}$ morphine when using L-dopa (25 mg kg$^{-1}$) instead of caffeine during the baseline and testing phases ($n = 8$). Scores are presented as the percentage of time spent in the drug-paired side compared to the saline-paired side during the pre- and post-treatments; two-tailed paired *t*-test comparing time spent on drug-paired side before and after conditioning within genotypes and doses, $^*P < 0.05$. All data are presented as means ± s.e.m.

## METHODS

**Subjects and treatments.** Dopamine-deficient ($Th^{-/-}$; $Dbh^{Th/+}$) mice carrying two inactive tyrosine hydroxylase (*Th*) alleles, one intact dopamine β-hydroxylase allele ($Dbh^{+}$), and one *Dbh* allele with a targeted insertion of the *Th* gene ($Dbh^{Th}$) were created as described[16]. Controls included animals that carry at least one intact *Th* allele and one intact *Dbh* allele. Mice were maintained on a mixed C57BL/6 × 129/SvEv genetic background. All mice were housed under a 12/12-h light/dark cycle and temperature controlled environment with food (Purina, 5LJ5) and water available *ad libitum*. Except for L-dopa (see ref. 20), all drugs were dissolved in vehicle (PBS) and administered at a volume of 10 μl g$^{-1}$. Mice were treated with PBS, morphine (RBI), amphetamine (Sigma), and/or caffeine (Sigma). All mice were treated in accordance with guidelines established by the National Institutes of Health and the University of Washington Animal Care Committee.

**Behavioural assays.** Locomotor studies were conducted in photo-beam activity cages as described[20]. Dose–response was carried out in naive mice that received



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escalating doses of morphine (0, 0.25, 2.5, 12.5, 25.0 mg kg$^{-1}$ body weight, intraperitoneally administered). All other locomotor assays were done using a Latin-square design such that each group of animals received each treatment. Tail-flick assays were performed using temperature-controlled water baths. Tails were dipped 0.5–1.0 cm beneath the water surface and the latency to withdrawal was measured with a 15-s cutoff. Each animal was tested in triplicate for each treatment and the average score was used. Escalating doses of morphine (0, 3, 6, 12, 24 mg kg$^{-1}$, intraperitoneally administered) were administered to naive mice 30 min before testing. A separate group of naive mice was used for the heat-response experiment. For CPP, we used two identical Plexiglas three-chamber boxes with two equal-sized compartments (20 × 20 cm) separated by a neutral grey chamber (20 × 7.5 cm). The boxes were separated by two sliding doors. The two large compartments had different coloured walls (black or white), different flooring (hard punched metal or stiff wire mesh), and were cued with different scents beneath the flooring (clove or ginger). These boxes were balanced such that, on average, mice tended to spend equal amounts of time in either chamber. All experiments were performed using the same schedule. On day 1 (18–24 h after L-dopa), mice were pre-treated with caffeine (15 mg kg$^{-1}$) 5 min before being placed in the centre chamber and allowed to explore the entire apparatus for 25 min. These sessions were recorded using a digital video camera (Sony). The next day, videos were analysed using Ethovision software (Noldus) to determine the time spent in each compartment, the total distance travelled, and the number of times an animal crossed from one chamber to another. Each animal was assigned one compartment for saline pairing and the other compartment for morphine pairing, such that there was minimal net difference in baseline times between compartments, within each genotype and dose (that is, unbiased paradigm). Any animal (and their littermate counterpart of the opposite genotype) that spent >65% of their time in a large compartment or >45% of their time in the neutral middle compartment were discarded from the study before assigning sides, due to the confounding factor of an endogenous preference or aversion. Conditioning was performed during days 3–5; each day each animal was treated with saline (subcutaneously administered) and confined to one side for 25 min in the morning, and treated with morphine (subcutaneously) and confined to the opposite side for 25 min in the afternoon. On day 6, animals were tested as on day 1. Data represent the per cent of time spent on the drug-paired side compared to the saline-paired side before and after conditioning (that is, the ratio does not include time spent in the centre chamber). For L-dopa rescue of CPP, the experiment was performed identically except that the mice were pre-treated with L-dopa (25 mg kg$^{-1}$) 30 min before baseline or testing instead of caffeine.

**Received 1 July; accepted 1 September 2005.**

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

**Acknowledgements** We thank our colleagues F. Perez for help generating mice, and E. Kremer for providing us with virus used for Supplementary Fig. 2. We thank the University of Washington NIDA center program for use of Noldus software. We thank our colleagues C. Chavkin, W. Watt and S. Luquet for comments on the manuscript. T.S.H. was supported in part by a grant from NIGMS.

**Author Contributions** The mouse model was developed in the R.D.P. laboratory. These experiments were designed and executed by T.S.H. with input from R.D.P. and assistance from B.N.S. for experiments shown in Fig. 1b and Supplementary Fig. 1.

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