Published in final edited form as:

Biol Psychiatry. 2017 May 01; 81(9): 778–788. doi:10.1016/j.biopsych.2016.12.022.

Mu opioid receptors in GABAergic forebrain neurons moderate motivation for heroin and palatable food

Pauline Charbogne^{1,2}, Olivier Gardon¹, Elena Martín-García³, Helen L. Keyworth⁴, Aya Matsui⁵, Anna E. Mechling^{6,7}, Thomas Bienert⁶, Taufiq Nasseef², Anne Robé¹, Luc Moquin², Emmanuel Darcq², Sami Ben Hamida², Patricia Robledo³, Audrey Matifas¹, Katia Befort^{1,10,11}, Claire Gavériaux-Ruff¹, Laura-Adela Harsan^{6,8,9}, Dominik Von Everfeldt⁶, Jurgen Hennig⁶, Alain Gratton², Ian Kitchen⁴, Alexis Bailey^{4,12}, Veronica A. Alvarez⁵, Rafael Maldonado³, and Brigitte L. Kieffer^{1,2,*}

¹Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/Université de Strasbourg, 1 rue Laurent Fries, 67404 Illkirch, France

²Douglas Mental Health Institute, Department of Psychiatry, McGill University, 6875 boulevard LaSalle, H4H 1R3 Montreal, QC, Canada

³Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, PRBB, C/Dr. Aiguader 88, 08003 Barcelona, Spain

⁴Faculty of Health and Medical Sciences, AY Building, University of Surrey, Guildford, Surrey GU2 7XH, UK

⁵Section on Neuronal Structure, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD, USA

*Corresponding author. Douglas Mental Health Institute, Department of Psychiatry, McGill University, 6875 boulevard LaSalle, H4H 1R3 Montreal, QC, Canada, Phone: 514 761-6131 ext.: 3175; fax: 514 762-3033, brigitte.kieffer@douglas.mcgill.ca. Address correspondence to Brigitte L. Kieffer, Ph.D., Douglas Hospital Research Center, Perry Pavilion Room E-3317.1, 6875 Boulevard LaSalle, Montreal, Quebec H4H 1R3, Canada; brigitte.kieffer@douglas.mcgill.ca.

¹⁰Laboratoire de Neurosciences Cognitives et Adaptatives (LNCA), Université de Strasbourg Faculté de Psychologie, 12 rue Goethe, F-67000

¹¹LNCA UMR 7364, CNRS, 12 rue Goethe, F-67000 Strasbourg

¹²Institute of Medical & Biomedical Education, St. George's University of London, Cranmer Terrace, London, SW17 0RE, UK
From the Institut de Génétique et de Biologie Moléculaire et Cellulaire (PC, OG, AR, AMati, KB, CGR, BLK), Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Université de Strasbourg, Illkirch, France; Douglas Mental Health University Institute, Department of Psychiatry, McGill University, Montreal, QC, Canada (PC, LM, ED, SBH, AG, BLK); Departament de Ciències Experimentals i de la Salut (EMG, PR, RM), Universitat Pompeu Fabra, Barcelona, Spain; Faculty of Health and Medical Sciences (HLK, IK, AB), Department of Biochemistry and Physiology, University of Surrey, Guildford, Surrey, United Kingdom; Section on Neuronal Structure, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health (AMats, VAA), Bethesda, MD, USA; Department of Radiology (AEM, TB, LAH, DVE, JH), Medical Physics, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Germany; Faculty of Biology (AEM), University of Freiburg, Freiburg, Germany; Laboratory of Engineering (LAH), Informatics and Imaging (ICube), Integrative multimodal imaging in healthcare, UMR 7357, University of Strasbourg, France; University Hospital Strasbourg (LAH), Department of Biophysics and Nuclear Medicine, Strasbourg, France; Laboratoire de Neurosciences Cognitives et Adaptatives (KB), Université de Strasbourg, Faculté de Psychologie, Strasbourg, France; LNCA UMR 7364 (KB), CNRS, Strasbourg, France; and Institute of Medical & Biomedical Education (AB), St. George's University of London, London, United Kingdom.

FINANCIAL DISCLOSURES

The authors declare no biomedical financial interests or potential conflicts of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

⁶Department of Radiology, Medical Physics, Medical Center – University of Freiburg, Faculty of Medicine, University of Freiburg, Germany

⁷Faculty of Biology, University of Freiburg, Freiburg, Germany

⁸Laboratory of Engineering, Informatics and Imaging (ICube), Integrative multimodal imaging in healthcare (IMIS), UMR 7357, University of Strasbourg, France

⁹University Hospital Strasbourg, Department of Biophysics and Nuclear Medicine, Strasbourg, France

Abstract

BACKGROUND—Mu opioid receptors (MORs) are central to pain control, drug reward and addictive behaviors, but underlying circuit mechanisms have been poorly explored by genetic approaches. Here we investigate the contribution of MORs expressed in GABAergic forebrain neurons to major biological effects of opiates, and also challenge the canonical disinhibition model of opiate reward.

METHODS—We used *Dlx5/6*-mediated recombination to create conditional *Oprm1* mice in GABAergic forebrain neurons. We characterized the genetic deletion by histology, electrophysiology and microdialysis, probed neuronal activation by c-Fos immunohistochemistry and resting state-functional magnetic resonance imaging, and investigated main behavioral responses to opiates, including motivation to obtain heroin and palatable food.

RESULTS—Mutant mice showed MOR transcript deletion mainly in the striatum. In the ventral tegmental area (VTA), local MOR activity was intact, and reduced activity was only observed at the level of striatonigral afferents. Heroin-induced neuronal activation was modified at both sites, and whole-brain functional networks were altered in live animals. Morphine analgesia was not altered, neither was physical dependence to chronic morphine. In contrast, locomotor effects of heroin were abolished, and heroin-induced catalepsy was increased. Place preference to heroin was not modified, but remarkably, motivation to obtain heroin and palatable food was enhanced in operant self-administration procedures.

CONCLUSIONS—Our study reveals dissociable MOR functions across mesocorticolimbic networks. Thus beyond a well-established role in reward processing, operating at the level of local VTA neurons, MORs also moderate motivation for appetitive stimuli within forebrain circuits that drive motivated behaviors.

Keywords

mu opioid receptor; conditional gene knockout; opiate; reward; motivation; dopamine

INTRODUCTION

Mu opioid receptors (MORs) mediate biological effects of morphine and heroin, including their powerful analgesic and euphorogenic properties (1). These receptors are also major players for recreational drug use, contributing to rewarding effects of other drugs of abuse (2) and also mediate naturally pleasurable stimuli including food and social reward (3). Several brain areas responsible for MOR-mediated reward have been identified by

intracranial pharmacological manipulations (4), however underlying circuit mechanisms are unclear, and the exact nature of key MOR-expressing neurons remains elusive.

The mesolimbic dopaminergic circuitry is recognized as a main brain network in the processing of appetitive behaviors and drug reward. Central to this circuit is the well characterized ventral tegmental area-nucleus accumbens (VTA-NAc) pathway (5), involving midbrain dopamine (DA) neurons of the VTA projecting massively to GABAergic medium spiny neurons (MSNs) of the ventral striatum, which project back to the VTA either directly or indirectly (6). MORs are densely expressed at both VTA and NAc sites (4, 7), mostly in GABAergic neurons (8–11), and an abundant literature has established that MORs in the VTA are essential for opiate reward (12). In brief, the canonical disinhibition model posits that opiates produce reward by acting at MORs expressed in GABAergic VTA interneurons, an action that in turn relieves the local inhibitory tone and disinhibits dopaminergic neurons (8, 13). However MORs are expressed at multiple pre- and postsynaptic sites within the VTA (14) with distinct synaptic actions on reward, or even aversive (15, 16) processes. In addition, other mechanisms likely contribute and, in particular, MOR agonists are self-administered in VTA, NAc and also elsewhere (4, 12), and heroin is self-administered even in the absence of dopamine terminals in the NAc (17). Here we tested whether MORs expressed in GABAergic forebrain neurons contribute to central effects of opiates with particular emphasis on opiate reward.

METHODS AND MATERIALS

Drugs

See Supplemental Information.

Mutant animals

Dlx-MOR mice were created by breeding our floxed *Oprm1* (*Oprm1^{f/f}*) mice (18) with Dlx5/6-Cre mice (19), obtained from Beat Lutz (Institute of Physiological Chemistry, Johannes Gutenberg University, Germany) in our vivarium. Details in Supplemental Information.

mRNA analyses

In situ hybridization in wild type mice. See Supplemental Information. *Quantitative real-time PCR.* RNA extraction, reverse transcription and cDNA quantification were performed as previously described (20, 21), and detailed in Supplemental Information.

Autoradiographic binding

Ctl, Dlx-MOR and constitutive males and females KO mouse brains were used for determination of total binding for MORs using [³H]DAMGO (D-Ala²-MePhe⁴-Gly-o¹⁵ enkephalin). MOR binding was carried out as described previously (22) with minor modifications (details in Supplemental Information).

Electrophysiology

Electrophysiology was performed as described previously (23). See Supplemental Information for details.

C-Fos protein immunoreactivity

See Supplemental Information.

Resting-state functional Magnetic Resonance Imaging

Animal preparation, data acquisition and analysis were performed as described previously (24, 25), and directionality analysis conducted as described in Supplemental Information.

Dopamine microdialysis

DA microdialysis after cocaine and heroin administration were performed as described in Supplemental Information. Only mice with correct probe placements (see Supplemental Figure S4) were used in the study.

Behavior

Nociception (1), physical dependence and withdrawal (1, 26), locomotor activity (20), locomotor sensitization (27), bar test (28), conditioned place preference (CPP) (29) and SA procedures (20, 30, 31) are described in Supplemental Information.

Statistical analyses

Data are presented as mean $\pm$ standard error (SEM). Statistical significance was achieved by $p < 0.05$. See details in Supplemental Information.

RESULTS

Dlx-MOR mice show a major MOR deletion in the striatum

In the adult mouse striatum, the *Oprm1* mRNA, encoding MOR, is mainly co-expressed with *Drd1* (NAc, $64.71 \pm 1.68\%$; dorsal striatum or DS, $54.96 \pm 2.11\%$) and *Drd2* (NAc, $27.02 \pm 1.35\%$; DS, $37.02 \pm 2.11\%$) or both (NAc, $3.13 \pm 0.41\%$; DS, $3.33 \pm 0.70\%$) transcripts (Figure 1A and Supplemental Figure S1) considered canonical markers of GABAergic MSNs. To inactivate the *Oprm1* gene in these neurons, and preserve MOR expression in local VTA neurons, we bred floxed *Oprm1* mice with Dlx5/6-Cre driver mice that express Cre recombinase in GABAergic forebrain neurons (19, 20). *Oprm1* mRNA level was assessed across the brain of mutants (conditional Dlx-MOR and constitutive CMV-MOR) knockout mice using quantitative real-time (qRT) PCR. *Oprm1* expression was significantly decreased at the level of striatum, including the NAc ($t_2 = 44.65$, $p < 0.001$) and the DS ($t_1 = 267.5$, $p < 0.001$), and was intact in the midbrain, hindbrain and the spinal cord ($p > 0.05$; Figure 1B) that do not express Cre recombinase. The transcript was undetectable in all the regions for CMV-MOR mice ($p < 0.001$). Transcripts for other components of the opioid system also expressed in the striatum (delta and kappa opioid receptors, preproenkephalin and prodynorphin) were otherwise unchanged ($p > 0.05$; Figure 1C).

We next examined MOR binding levels throughout the brain using [^3H]DAMGO binding autoradiography, rather than MOR protein by immunohistochemistry as the latter is less reliable in brain tissues. Autoradiograms showed strong receptor deletion in the striatum, whereas binding seemed intact in more posterior sections (Figure 1D). Quantification using one-tailed Mann-Whitney analysis showed significant reduction of [^3H]DAMGO binding in the NAc core ($U=0$, $p=0.029$) and NAc shell ($U=0$, $p=0.029$), and no change was detected in mid/hindbrain areas ($p>0.05$; Figure 1E). MOR proteins expressed in MSNs are normally transported to ventral pallidum (VP) and VTA, along striatopallidal and striatonigral pathways respectively (6). [^3H]DAMGO binding was reduced in the VP of mutant mice ($U=0$, $p=0.029$), but no change could be detected in the VTA ($U=1$, $p=0.057$; Figure 1E), likely because MSN-MORs at VTA terminals only represent 10–20% total VTA receptors (14).

We then used VTA slice electrophysiology to determine whether MOR activity is modified at VTA terminals of *Dlx-MOR* mice. As expected, recordings of local VTA GABA interneurons in Ctl mice showed a significant decrease in the amplitude of evoked IPSCs in response to DAMGO. However these synaptic currents were not inhibited in *Dlx-MOR* mice (unpaired two-tailed *t* test, $t_{16}=6.645$, $p<0.001$, Figure 1F), demonstrating loss of MOR-mediated inhibition over GABA inputs on these neurons in mutant mice. In contrast, recordings of DA neurons showed similar DAMGO-mediated inhibition of eIPSCs in the two groups ($t_{16}=0.257$, $p=0.80$, Figure 1G), supporting the notion that inputs to DA neurons are regulated by MORs expressed by other neuron populations, most likely local GABA neurons (12, 14).

In sum, the data together indicate that *Dlx-MOR* mice show a major MOR deletion in the striatum. Within VTA, presynaptic MORs on MSN afferents are lacking, while MORs expressed by local VTA interneurons or more dorsal projections are intact (Figure 1H).

Neuronal activity is modified in *Dlx-MOR* mice

To probe the striatum-VTA circuitry, we tested neuronal reactivity to acute heroin administration at the level of DS, NAc and VTA by c-Fos immunohistochemistry. Two-way ANOVA revealed a significant effect of genotype, treatment, as well as interaction in both the dorsolateral striatum (DLS, genotype $F_{1,20}=8.032$, $p=0.010$; treatment $F_{1,20}=4.72$, $p=0.042$; interaction $F_{1,20}=8.71$, $p=0.008$) and VTA (genotype $F_{1,19}=8.44$, $p=0.009$; treatment $F_{1,19}=44.65$, $p<0.001$; interaction $F_{1,19}=5.70$, $p=0.028$). In the NAc shell, two-way ANOVA revealed genotype and treatment effects (genotype $F_{1,20}=12.92$, $p=0.002$; treatment $F_{1,20}=17.72$, $p<0.001$; interaction $F_{1,20}=1.77$, $p=0.20$). In Ctl animals, Student Newman Keuls (SNK) *post hoc* analysis showed a significant increase of c-Fos in response to heroin treatment in NAc ($p<0.001$), DLS ($p<0.01$) and VTA ($p<0.001$). In mutant animals, heroin-induced c-fos activation was undetectable in NAc and DLS, and reduced in the VTA (SNK $p<0.01$, Figure 2A, B), indicating perturbed responses to opiates within mesolimbic dopaminergic circuitry. NAc core, dorsomedial, ventrolateral and ventromedial striatum did not respond to heroin for both genotypes (not shown).

We further probed the status of whole-brain network activities in live animals, as previously done in (25). Resting-state functional Magnetic Resonance Imaging (Rs-fMRI) and the

analysis of whole-brain connectivity (24) showed that the recruitment of DS, NAc and VTA as functional hubs differed in the brain of Dlx-MOR compared to Ctl mice (Figure 2C). This hypothesis-free Rs-fMRI analysis also identified significant modifications of network hubs at other brain sites (Supplemental Figure S2) indicating that the conditional *Oprm1* gene deletion influences neural network activities within and also beyond the VTA-NAc circuit. Note however that forebrain nodes other than striatal hubs were minimally modified, supporting the notion of a major striatal MOR deletion in the forebrain. Directional analysis of functional connectivity (32, 33) across 6 seed regions confirmed significant modifications of information flow within addiction circuitry and revealed reversed VTA-DS directionality (Figure 2E), consistent with behavioral modifications found in mutant mice (see legend and discussion).

Dlx-MOR mice show intact morphine analgesia and dependence

We then investigated behavioral responses to opiates. Morphine analgesia was unchanged in Dlx-MOR mice using both spinal and supraspinal nociceptive testing (Figure 3A–C), consistent with intact MOR expression at major brain sites for pain control. Mutant mice were also subjected to a chronic morphine regimen. Naloxone-precipitated withdrawal did not differ from Ctl mice, suggesting that forebrain GABA MORs do not contribute significantly to this massive behavioral adaptation to opiates (Figure 3D and Supplemental Figure S3).

Dlx-MOR mice show modified motor responses to heroin

The striatum is critical for motor functions, therefore we investigated locomotor stimulant effects of heroin, a potent diacetylated morphine derivative. During 2h-recording sessions, heroin dose-dependently increased locomotor activity in Ctl mice, as expected (34), but not in Dlx-MOR mice (Figure 3E). Two-way ANOVA revealed treatment effect ($F_{7,160}=7.37$, $p<0.001$), genotype effect ($F_{1,160}=23.79$, $p<0.001$) and interaction (genotype X treatment, $F_{7,160}=7.44$, $p<0.001$). Bonferroni's multiple comparisons test showed no locomotor effect of heroin in Dlx-MOR mice ($p>0.05$). Treatment effect in Ctl mice was observed at 6, 8 and 10mg/kg heroin ($p<0.01$, $p<0.05$ and $p<0.001$ respectively) compared to saline-treated groups. Genotype effect was observed at 6 and 10 mg/kg ($p<0.05$ and $p<0.001$ respectively). Further, sensitization to heroin-induced hyperlocomotion developed in Ctl mice, as classically reported, but Dlx-MOR mice remained unresponsive to heroin in successive sessions (Figure 3F). Three-way repeated measures ANOVA revealed a genotype effect ($F_{1,78}=39.51$; $p<0.001$), a treatment effect ($F_{1,78}=52.91$; $p<0.001$) as well as session X genotype X treatment interaction ($F_{3,085,240.62}=11.01$; $p<0.001$). SNK *post hoc* analysis between the four groups showed no difference between Ctl group treated with saline and Dlx-MOR groups ($p<0.001$). Heroin-treated Ctl group showed a significant difference with the three other groups ($p<0.001$). Finally heroin-induced catalepsy, typically observed in Ctl mice (28), was potentiated in mutant mice (Figure 3G). Two-way ANOVA revealed genotype effect ($F_{1,116}=14.78$; $p=0.002$), treatment effect ($F_{3,116}=39.12$; $p<0.001$) as well as genotype X treatment interaction ($F_{3,116}=12.78$; $p<0.001$). Bonferroni's multiple comparisons test showed a significant treatment effect at 6 and 10 mg/kg ($p<0.001$) and a difference between Dlx-MOR and Ctl mice at 10 mg/kg heroin ($p<0.001$).

Because locomotor activity engages DA neurotransmission in the striatum (35, 36), and compensatory mechanisms in the DA system were described upon constitutive deletion of MOR (37), we examined *Drd1* and *Drd2* mRNA levels in DS and NAc, DA-mediated locomotor activation by amphetamine, and cocaine-induced increase of extracellular NAc dopamine levels, however none of these parameters was modified in *Dlx-MOR* mice (Supplemental Figure S4).

Striatal MORs, therefore, do not seem to modify basal dopaminergic function; however this particular receptor population is essential for the opiate-mediated regulation of motor function.

***Dlx-MOR* mice show intact place conditioning to opiates**

Next, we focused on opiate reward, defined as a subjective hedonic feeling whose experience leads to increase probability of further action to gain the same stimulus (12). We first used CPP, where drug-context association develops upon non-contingent drug delivery. *Dlx-MOR* and *Ctl* mice showed comparable preference for the heroin-paired compartment (Figure 4A): three-way ANOVA showed a conditioning effect ($F_{1,166}=10.61$; $p<0.001$) and a conditioning X treatment interaction ($F_{3,166}=3.28$; $p=0.022$), a tendency for a treatment effect ($F_{3,166}=2.62$; $p=0.052$) and no genotype effect ($F_{1,166}=0.076$; $p=0.78$). These results suggest overall that forebrain GABAergic MORs are not involved in appetitive effects of opiates, nor do they seem to contribute to context learning.

Opiates, as other drugs of abuse, are known to trigger DA release in the NAc which is traditionally associated to drug reward and CPP (38). We investigated heroin-induced (10 mg/kg) DA release in the NAc using microdialysis. Two-way repeated measures ANOVA analysis revealed a time effect ($F_{15,300}=5.820$; $p<0.001$) and neither a genotype effect ($F_{1,20}=0.13$; $p=0.72$) nor genotype X time interaction ($F_{15,300}=0.73$; $p=0.76$). Two-tailed Mann-Whitney analysis of the area under the curve (AUC, inset) confirmed that heroin significantly increased extracellular NAc DA ($U=59$, $p=0.73$) in the two groups over the 3-hour session (Figure 4B and Supplemental Figure S5) in line with the CPP data. We also confirmed the absence of hyperlocomotor activity induced by heroin in *Dlx-MOR* animals concurrently to microdialysis measures (right inset, AUC, two-tailed unpaired t test, $t_{21}=3.064$, $p=0.006$).

Altogether, neither CPP testing nor DA microdialysis allowed detecting any *Dlx-MOR* phenotype in response to heroin, suggesting that MORs in forebrain GABAergic neurons are not involved in opiate reward. This is in agreement with the broadly accepted disinhibition model of opiate reward (12) and our observation that VTA MORs are mostly intact in *Dlx-MOR* mice. Whether hedonic reactions to heroin, known to involve the NAc shell (39) are altered in *Dlx-MOR* mice deserves further investigation.

***Dlx-MOR* mice show enhanced motivation for heroin**

The CPP paradigm evaluates conditioned behaviors essentially driven by drug reward. Operant SA, however, also measures other aspects of drug reinforcement related to the acquisition, maintenance, motivation for, and reinstatement of drug seeking behaviors. Thus, operant responses are not only associated with the primary reward circuit, but also recruit

other brain networks interfacing the striatum, which contribute to decision-making and behavioral control (40). We thus tested Dlx-MOR mice for heroin SA (Figure 4C–E and Table 1 for statistics). At the starting high dose (0.1 mg/kg/infusion), Dlx-MOR mice stabilized their nose-poking into active holes to a level significantly higher than Ctl animals (fixed ratio 1, FR1 schedule of reinforcement), resulting in overall higher level of heroin intake (Figure 4C). When the heroin dose was reduced in subsequent sessions (0.05 to 0.006 mg/kg/infusion), the two groups maintained hole discrimination, and increased their nose-poking behavior as expected. Dlx-MOR mice consistently showed a higher number of active nose-pokes (three-way ANOVA, significant at 0.1 and 0.025 mg/kg/infusion) (Figure 4C). When further tested in a progressive ratio schedule of reinforcement (PR), Dlx-MOR mice again nose-poked for heroin more actively and achieved a higher breaking point (two-tailed Mann Whitney, $U=48$, $p=0.0172$) (Figure 4D), with breaking point/acquisition ratios differing significantly between genotypes (Ctl 0.49 ± 0.21 ; Dlx-MOR 3.04 ± 1.33 ; U-Mann-Whitney = 37.50, $p<0.05$). Finally, mutant mice gradually decreased their heroin SA under extinction conditions; they reinstated this behavior upon cue exposure while Ctl mice did not (two-tailed Mann Whitney, $U=25.50$, $p<0.001$) (Figure 4E). Covariance analysis of cue-induced reinstatement with heroin infusion numbers prior to reinstatement sessions further confirmed significant genotype difference ($F_{1,26}=6.01$, $p<0.05$).

These data showing a higher breaking point and stronger reinstatement to heroin SA in Dlx-MOR mice indicate that forebrain GABAergic MORs significantly regulate motivation to obtain heroin. Together with CPP data, therefore, SA data suggests that this particular MOR population is not involved in rewarding properties of heroin but rather contributes to motivational aspects of heroin consumption.

Dlx-MOR mice show enhanced motivation for palatable food

Because the increased motivation of Dlx-MOR mice to seek heroin was particularly striking and novel, we finally investigated whether forebrain GABAergic MORs are involved in motivated behaviors for a natural reward (Figure 4F, G and Table 2 for statistics). We examined acquisition and maintenance of operant responding for chocolate, a highly palatable food reward. Overall operant responding was more frequent for palatable food than for heroin in the two groups, due to low satiety induced by each food pellet compared to powerful and durable effects of a single heroin infusion. On FR1 schedule, Dlx-MOR mice and their controls acquired and maintained stable operant responding for chocolate-flavored food pellets, with higher nose-poking for Dlx-MOR mice significant at session 5 and 10 (Figure 4F). When shifted to a FR5 schedule of reinforcement, the two groups increased and stabilized their active responses to a higher level, which was significantly greater for Dlx-MOR mice for all the sessions (Figure 4F). Mutant mice also showed more responses in a subsequent PR session (two-tailed Mann Whitney, $U=58$, $p=0.0064$) (Figure 4G).

Therefore, as for heroin, Dlx-MOR mice showed a higher motivation to obtain palatable pellets. MORs in GABAergic forebrain neurons therefore also moderate motivation to obtain natural rewards, suggesting that this particular MOR population may generally moderate motivation for appetitive stimuli.

DISCUSSION

In this study, we targeted the *Oprm1* gene in forebrain GABAergic neurons, and obtained a deletion of the MOR mRNA mainly at the level of the striatum while the transcript was intact in mid/hindbrain including the VTA. Our behavioral analysis of these conditional mutant mice shows that this particular MOR population does not mediate morphine analgesia or physical morphine withdrawal, but regulates locomotor and motivational effects of heroin. These results extend our previous study showing that ectopic rescue of MOR expression in striatal pDyn neurons of MOR knockout mice is sufficient to restore locomotor morphine effects and morphine SA, but not morphine analgesia and withdrawal (41), and further substantiate the notion of an essential role for striatal MORs in these specific behavioral responses. In addition, our data further show that these receptors do not contribute to heroin positive reinforcement, but modulate motivation to obtain heroin and food reward, revealing a yet unreported role for MORs within addiction circuits.

In the brain, MOR expression was mostly described in GABAergic neurons, including in the VTA (8, 10), bed nucleus of the stria terminalis (9), VP (42), and striatum (11). *Dlx5/6-Cre* mice, previously used to delete cannabinoid CB1 (19) and delta opioid (20) receptors in forebrain GABAergic neurons, produced a massive *Oprm1* mRNA deletion in the striatum where MOR and Cre expression overlap, while mRNA levels remained intact elsewhere. In the striatum, MOR transcripts were almost totally deleted in both NAc and DS (93 and 98%), but substantial levels of MOR binding sites were still detectable (30 and 48%). Remaining *Oprm1* mRNA may originate from cholinergic interneurons (43, 44), and remaining protein from presynaptic receptors on glutamatergic projecting neurons from the cortex (45). Overall we conclude that our targeting strategy led to a major MOR deletion in striatal projection neurons, and that behavioral modifications in these mice mainly arise from the lack of striatal MOR activity. Further supporting this conclusion is our observation that network hubs are mainly modified at the level of VTA-striatal seed regions with minimal modifications at cortical level, and also the fact that behavioral responses tested in this study primarily recruit striatal-mediated responses. We cannot exclude, however, that limited MOR deletion also occurred elsewhere in the forebrain but was undetected, and may also partially contribute to behavioral responses that have not been investigated here.

Total *Oprm1* knockout leads to complete suppression of morphine analgesia (1, 46). In this study, *Dlx-MOR* mice showed intact morphine-induced antinociception for both spinal and supraspinal responses, indicating that MORs in forebrain GABAergic neurons are not implicated in acute morphine analgesia. MORs are expressed throughout nociceptive pathways in the brain, spinal cord and sensory neurons (7) and our data are in accordance with previous studies indicating that major sites for these responses to morphine include periaqueductal gray (PAG) and spinal cord (47) where receptors are spared. Recent attention has focused on the implication of brain reward circuits in chronic pain (48) and a recent study used optogenetic manipulations to show that corticostriatal circuits regulate both nociceptive and affective aspects of neuropathic pain (49). *Dlx-MOR* mice responses to persistent pain will therefore be of interest in future investigations.

Physical dependence induced by chronic morphine administration is abolished in total MOR knockout mice (1). Here, morphine-dependent Dlx-MOR mice develop the full spectrum of physical withdrawal signs upon naloxone administration. Main neuronal sites for opioid withdrawal include locus coeruleus and PAG for somatic signs (50, 51). In accordance, our data suggest that striatal MORs do not contribute to the expression of somatic withdrawal signs, or that a minor contribution of these receptors is not detected under our conditions. Whether the aversive states of withdrawal are modified in mutant mice has not been tested, as yet. A recent study showed that expression of opiate withdrawal-associated aversion involves projections from the paraventricular nucleus of the thalamus to the NAc rather than striatal cell bodies (52) and further investigation will be necessary to evaluate if striatal MORs modulate the negative affect of opiate withdrawal.

In mice, acute heroin injection increases locomotor activity (53), and this behavior is not observed in total MOR knockout mice (34). Dlx-MOR mice in this study show no locomotor response to heroin, consistent with the lack of heroin-induced c-fos activation in the NAc shell [Figure 2A, B and (54)]. Further, we observed a weak but significant heroin-induced cataleptic state in Ctl mice (bar test), as reported and well described for morphine in the literature (28), and this effect was dramatically enhanced in Dlx-MOR mutants. It is possible that heroin-induced hyperactivity normally hides catalepsy in Ctl mice, and that heroin catalepsy is unmasked in Dlx-MOR animals that do not show locomotor activation, as suggested in (55). Heroin-induced locomotor activation and catalepsy, therefore, may engage two separated neural mechanisms. Further, mechanisms underlying locomotor effects of heroin have been proposed to involve DA transmission (56, 57). In our study, the lack of heroin locomotor stimulant effects in Dlx-MOR mice, which was observed both during activity testing (Figure 3E) and along the microdialysis experiment (Figure 4B), was not accompanied by any alterations of extracellular DA levels in the NAc, suggesting that heroin effects on DA transmission in the NAc and locomotor activation are independent processes.

Rewarding and motivational properties of opiates, as measured by CPP and SA procedures, are both abolished in total MOR knockout mice (1, 58, 59). In Dlx-MOR mice, heroin place conditioning was intact, indicating that both opiate reinforcement and opiate-context association are preserved. This is further supported by intact heroin-induced increase of NAc extracellular DA observed in mutant mice. MORs expressed in GABAergic forebrain neurons, therefore, do not contribute to opiate reward. This conclusion is in accordance with broad literature pointing at VTA as a major site for opiate reinforcement [(60, 61) for review see (4)]. It is also in agreement with the well-accepted disinhibition model of opiate reward where MORs expressed in local VTA GABA interneurons (62), which are intact in Dlx-MOR mice, play a central role in opiate reward.

Importantly however, data from the heroin SA experiments reveal a strong phenotype for Dlx-MOR mice. First, operant responding for heroin was slightly enhanced; second, breaking point in the PR schedule was remarkably higher; and third, cue-induced reinstatement readily occurred in mutant but not Ctl mice, demonstrating together higher motivation to gain heroin. The MOR in GABAergic forebrain neurons, therefore, is implicated in motivation for heroin rather than heroin reward. This is in accordance with recent evidence that striatonigral fibers originating from striosomes, and receiving inputs

from cortical regions related to motivational control, form a specialized integrative unit within the mesocorticolimbic dopamine circuit (63) and that striatal MORs are specifically expressed in striosomal compartments (7). Further, Dlx-MOR mice also showed enhanced motivation to gain palatable food. This observation strengthens the heroin SA data, and opens the possibility that MORs in forebrain GABAergic neurons play a general role in motivational behaviors that perhaps extends to most forms of reward.

Finally, Dlx-MOR mice represent a unique genetic model, which allows dissociation of rewarding and motivational properties of opiates within the highly complex reward brain networks (64). Also remarkably, modifications of information flow between functional networks detected in live Dlx-MOR mice further support the notion of altered VTA dialogue with dorsal but not ventral striatum. These mutant mice therefore provide support to distinguish liking and wanting components of addiction (65), and position MOR as a player in both aspects. Intriguingly, increased performance of mutant mice to obtain heroin suggests that activation of MOR in forebrain GABAergic neurons normally limits motivation for heroin. We propose that, within corticomesolimbic circuitry, VTA-MORs mediate rewarding properties of opiates as previously established, whereas striatal MORs moderate incentive to gain the appetitive stimulus. The latter MOR function may contribute to homeostatic mechanisms, which possibly minimize transition from recreational to compulsive drug use and reduce abuse liability of drug reward. Our study therefore opens the way to circuit mapping of opioid mechanisms underlying motivation and decision-making (40, 66).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Nagina Mahmood and Christine Colley for their assistance with the microdialysis histology and the RNAscope experiment, respectively, Grégoire Maroteaux for help in statistical analyses and Maria Osikowicz for her help with figures. We thank the Mouse Clinical Institute and the animal core facility at the Institut de Génétique et de Biologie Moléculaire et Cellulaire for technical support (Illkirch, France), as well as Aude Villemain at the Douglas Research Centre (Montreal, Qc, Canada). This work was supported by the Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, and Université de Strasbourg. HLK was supported by an MRC/ESRC interdisciplinary studentship and the British Pharmacological Society” also work supported by Genaddict grant European Commission (Contract Number: LSHMCT2004-005166). We would also like to thank the US National Institutes of Health (National Institute of Drug Addiction, grant #05010 and National Institute on Alcohol Abuse and Alcoholism, grant #16658), the Canada Fund for Innovation and the Canada Research Chairs for financial support. Electrophysiological experiments were funded by the Intramural Programs of National Institute on Alcohol Abuse and Alcoholism and National Institute of Neurological Disorders and Stroke (#ZIA-AA000421) to VAA, Japan Society for Promotion of Science to AM. SA studies were supported by the Intramural Programs of National Institute on Alcohol Abuse and Alcoholism (NIAAA) and National Institute of Neurological Disorders and Stroke Grant ZIA-AA000421, the DG Research of the European Commission FP7 (#HEALTH-2013-602891), the Spanish ‘RETICS-Instituto de Salud Carlos III’ (#RD12/0028/0023), the Spanish ‘Ministerio de Economía y Competitividad’ (#SAF-2014-59648P), the ‘Plan Nacional Sobre Drogas’ (#PNSD-2013-5068) and the Catalan Government ‘AGAU’ (#2014-SGR-1547) and ‘ICREA-Acadèmia’ (2015) to RM. Part of the work was supported by DFG Excellence Cluster EXC-1086 “BrainLinks-BrainTools”.

PC and BLK designed the experiments; PC performed and analyzed c-Fos immunoreactivity, qRT-PCR and behavioral assays, except for self-administration procedures that were designed, performed and analyzed by EMG and RM; OG, AMati contributed to Dlx-MOR mice production and characterization; HLK and AB performed and analyzed autoradiography binding assay with I K; AMats performed and analyzed electrophysiology with VAA; AR and PC performed RNAscope assay. AEM, TB and LAH designed the rsfMRI study and acquired and analyzed the

rsfMRI data, DE and JH assisted in rsfMRI data analysis and study design. TN and ED performed the directional analysis of the rsfMRI data. PC and LM performed heroin microdialysis and AG helped for analysis. SBH helped for statistical analysis of behavioral data; PR performed and analyzed the cocaine microdialysis experiment. PC and BLK wrote the paper. All of the authors discussed the results and commented on the manuscript.

References

1. Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, et al. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. *Nature*. 1996; 383:819–823. [PubMed: 8893006]
2. Charbogne P, Kieffer BL, Befort K. 15 years of genetic approaches in vivo for addiction research: Opioid receptor and peptide gene knockout in mouse models of drug abuse. *Neuropharmacology*. 2014; 76(Pt B):204–217. [PubMed: 24035914]
3. Moles A, Kieffer BL, D'Amato FR. Deficit in attachment behavior in mice lacking the mu-opioid receptor gene. *Science*. 2004; 304:1983–1986. [PubMed: 15218152]
4. Le Merrer J, Becker JA, Befort K, Kieffer BL. Reward processing by the opioid system in the brain. *Physiol Rev*. 2009; 89:1379–1412. [PubMed: 19789384]
5. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. *Nat Rev Neurosci*. 2013; 14:609–625. [PubMed: 23942470]
6. Lobo MK, Karsten SL, Gray M, Geschwind DH, Yang XW. FACS-array profiling of striatal projection neuron subtypes in juvenile and adult mouse brains. *Nat Neurosci*. 2006; 9:443–452. [PubMed: 16491081]
7. Erbs E, Faget L, Scherrer G, Matifas A, Filliol D, Vonesch JL, et al. A mu-delta opioid receptor brain atlas reveals neuronal co-occurrence in subcortical networks. *Brain Struct Funct*. 2015; 220:677–702. [PubMed: 24623156]
8. Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neurosci*. 1992; 12:483–488. [PubMed: 1346804]
9. Kudo T, Konno K, Uchigashima M, Yanagawa Y, Sora I, Minami M, et al. GABAergic neurons in the ventral tegmental area receive dual GABA/enkephalin-mediated inhibitory inputs from the bed nucleus of the stria terminalis. *Eur J Neurosci*. 2014; 39:1796–1809. [PubMed: 24580812]
10. Lowe JD, Bailey CP. Functional selectivity and time-dependence of mu-opioid receptor desensitization at nerve terminals in the mouse ventral tegmental area. *Br J Pharmacol*. 2015; 172:469–481. [PubMed: 24467517]
11. Miura M, Saino-Saito S, Masuda M, Kobayashi K, Aosaki T. Compartment-specific modulation of GABAergic synaptic transmission by mu-opioid receptor in the mouse striatum with green fluorescent protein-expressing dopamine islands. *J Neurosci*. 2007; 27:9721–9728. [PubMed: 17804632]
12. Fields HL, Margolis EB. Understanding opioid reward. *Trends Neurosci*. 2015; 38:217–225. [PubMed: 25637939]
13. Wise RA, Rompre PP. Brain dopamine and reward. *Annu Rev Psychol*. 1989; 40:191–225. [PubMed: 2648975]
14. Matsui A, Jarvie BC, Robinson BG, Hentges ST, Williams JT. Separate GABA afferents to dopamine neurons mediate acute action of opioids, development of tolerance, and expression of withdrawal. *Neuron*. 2014; 82:1346–1356. [PubMed: 24857021]
15. Lammel S, Lim BK, Ran C, Huang KW, Betley MJ, Tye KM, et al. Input-specific control of reward and aversion in the ventral tegmental area. *Nature*. 2012; 491:212–217. [PubMed: 23064228]
16. Tan KR, Yvon C, Turiault M, Mirzabekov JJ, Doehner J, Labouebe G, et al. GABA neurons of the VTA drive conditioned place aversion. *Neuron*. 2012; 73:1173–1183. [PubMed: 22445344]
17. Pettit H, Ettenberg A, Bloom F, Koob G. Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology*. 1984; 84:167–173. [PubMed: 6438676]
18. Weibel R, Reiss D, Karchewski L, Gardon O, Matifas A, Filliol D, et al. Mu opioid receptors on primary afferent nav1.8 neurons contribute to opiate-induced analgesia: insight from conditional knockout mice. *PLoS One*. 2013; 8:e74706. [PubMed: 24069332]

19. Monory K, Massa F, Egertova M, Eder M, Blandzun H, Westenbroek R, et al. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron*. 2006; 51:455–466. [PubMed: 16908411]
20. Chu Sin Chung P, Keyworth HL, Martin-Garcia E, Charbogne P, Darcq E, Bailey A, et al. A novel anxiogenic role for the delta opioid receptor expressed in GABAergic forebrain neurons. *Biol Psychiatry*. 2015; 77:404–415. [PubMed: 25444168]
21. Befort K, Filliol D, Darcq E, Gbate A, Matifas A, Lardenois A, et al. Gene expression is altered in the lateral hypothalamus upon activation of the mu opioid receptor. *Ann N Y Acad Sci*. 2008; 1129:175–184. [PubMed: 18591478]
22. Slowe SJ, Simonin F, Kieffer B, Kitchen I. Quantitative autoradiography of mu-,delta- and kappa1 opioid receptors in kappa-opioid receptor knockout mice. *Brain Res*. 1999; 818:335–345. [PubMed: 10082819]
23. (!!! INVALID CITATION !!! (11)).
24. Mechling AE, Hubner NS, Lee HL, Hennig J, von Elverfeldt D, Harsan LA. Fine-grained mapping of mouse brain functional connectivity with resting-state fMRI. *Neuroimage*. 2014; 96:203–215. [PubMed: 24718287]
25. Mechling AEAT, Lee H-L, Bienert T, Reiser M, Ben Hamida S, Darcq E, Hennig J, v Elverfeldt D, Kieffer BL*, Harsan L-AA*. Deletion of the mu opioid receptor gene in mice reshapes the reward-aversion connectome. *Proceedings of the National Academy of Sciences*. in press.
26. Berrendero F, Castane A, Ledent C, Parmentier M, Maldonado R, Valverde O. Increase of morphine withdrawal in mice lacking A2a receptors and no changes in CB1/A2a double knockout mice. *Eur J Neurosci*. 2003; 17:315–324. [PubMed: 12542668]
27. Contet C, Filliol D, Matifas A, Kieffer BL. Morphine-induced analgesic tolerance, locomotor sensitization and physical dependence do not require modification of mu opioid receptor, cdk5 and adenylate cyclase activity. *Neuropharmacology*. 2008; 54:475–486. [PubMed: 18082850]
28. Tzschentke TM, Schmidt WJ. Morphine-induced catalepsy is augmented by NMDA receptor antagonists, but is partially attenuated by an AMPA receptor antagonist. *Eur J Pharmacol*. 1996; 295:137–146. [PubMed: 8720577]
29. Le Merrer J, Plaza-Zabala A, Del Boca C, Matifas A, Maldonado R, Kieffer BL. Deletion of the delta opioid receptor gene impairs place conditioning but preserves morphine reinforcement. *Biol Psychiatry*. 2011; 69:700–703. [PubMed: 21168121]
30. Martin-Garcia E, Barbano MF, Galeote L, Maldonado R. New operant model of nicotine-seeking behaviour in mice. *Int J Neuropsychopharmacol*. 2009; 12:343–356. [PubMed: 18752721]
31. Burokas A, Gutierrez-Cuesta J, Martin-Garcia E, Maldonado R. Operant model of frustrated expected reward in mice. *Addict Biol*. 2012; 17:770–782. [PubMed: 22264360]
32. Barnett L, Seth AK. The MVGC multivariate Granger causality toolbox: a new approach to Granger-causal inference. *J Neurosci Methods*. 2014; 223:50–68. [PubMed: 24200508]
33. Zhan Y, Paolicelli RC, Sforzini F, Weinhard L, Bolasco G, Pagani F, et al. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nat Neurosci*. 2014; 17:400–406. [PubMed: 24487234]
34. Contarino A, Picetti R, Matthes HW, Koob GF, Kieffer BL, Gold LH. Lack of reward and locomotor stimulation induced by heroin in mu-opioid receptor-deficient mice. *Eur J Pharmacol*. 2002; 446:103–109. [PubMed: 12098591]
35. Serrano A, Aguilar MA, Manzanedo C, Rodriguez-Arias M, Minarro J. Effects of DA D1 and D2 antagonists on the sensitisation to the motor effects of morphine in mice. *Prog Neuropsychopharmacol Biol Psychiatry*. 2002; 26:1263–1271. [PubMed: 12502012]
36. VanderWende C, Spoerlein MT. Morphine-induced catalepsy in mice. Modification by drugs acting on neurotransmitter systems. *Neuropharmacology*. 1979; 18:633–637. [PubMed: 40155]
37. Park Y, Ho IK, Fan LW, Loh HH, Ko KH. Region specific increase of dopamine receptor D1/D2 mRNA expression in the brain of mu-opioid receptor knockout mice. *Brain Res*. 2001; 894:311–315. [PubMed: 11251207]
38. Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, et al. Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology*. 2004; 47(Suppl 1):227–241. [PubMed: 15464140]

39. Castro DC, Berridge KC. Opioid hedonic hotspot in nucleus accumbens shell: mu, delta, and kappa maps for enhancement of sweetness “liking” and “wanting”. *J Neurosci.* 2014; 34:4239–4250. [PubMed: 24647944]
40. Koob GF, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacology.* 2010; 35:217–238. [PubMed: 19710631]
41. Cui Y, Ostlund SB, James AS, Park CS, Ge W, Roberts KW, et al. Targeted expression of mu-opioid receptors in a subset of striatal direct-pathway neurons restores opiate reward. *Nat Neurosci.* 2014; 17:254–261. [PubMed: 24413699]
42. Kupchik YM, Scofield MD, Rice KC, Cheng K, Roques BP, Kalivas PW. Cocaine dysregulates opioid gating of GABA neurotransmission in the ventral pallidum. *J Neurosci.* 2014; 34:1057–1066. [PubMed: 24431463]
43. Jabourian M, Venance L, Bourgoin S, Ozon S, Perez S, Godeheu G, et al. Functional mu opioid receptors are expressed in cholinergic interneurons of the rat dorsal striatum: territorial specificity and diurnal variation. *Eur J Neurosci.* 2005; 21:3301–3309. [PubMed: 16026468]
44. Svingos AL, Colago EE, Pickel VM. Vesicular acetylcholine transporter in the rat nucleus accumbens shell: subcellular distribution and association with mu-opioid receptors. *Synapse.* 2001; 40:184–192. [PubMed: 11304756]
45. O'Donnell P, Grace AA. Synaptic interactions among excitatory afferents to nucleus accumbens neurons: hippocampal gating of prefrontal cortical input. *J Neurosci.* 1995; 15:3622–3639. [PubMed: 7751934]
46. Sora I, Takahashi N, Funada M, Ujike H, Revay RS, Donovan DM, et al. Opiate receptor knockout mice define mu receptor roles in endogenous nociceptive responses and morphine-induced analgesia. *Proc Natl Acad Sci U S A.* 1997; 94:1544–1549. [PubMed: 9037090]
47. Pasternak GW. Opiate pharmacology and relief of pain. *J Clin Oncol.* 2014; 32:1655–1661. [PubMed: 24799496]
48. Navratilova E, Morimura K, Xie JY, Atcherley CW, Ossipov MH, Porreca F. Positive emotions and brain reward circuits in chronic pain. *J Comp Neurol.* 2016; 524:1646–1652. [PubMed: 26788716]
49. Lee M, Manders TR, Eberle SE, Su C, D'Amour J, Yang R, et al. Activation of corticostriatal circuitry relieves chronic neuropathic pain. *J Neurosci.* 2015; 35:5247–5259. [PubMed: 25834050]
50. Frenois F, Cador M, Caille S, Stinus L, Le Moine C. Neural correlates of the motivational and somatic components of naloxone-precipitated morphine withdrawal. *Eur J Neurosci.* 2002; 16:1377–1389. [PubMed: 12405997]
51. Williams JT, Christie MJ, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. *Physiol Rev.* 2001; 81:299–343. [PubMed: 11152760]
52. Zhu Y, Wienecke CF, Nachtrab G, Chen X. A thalamic input to the nucleus accumbens mediates opiate dependence. *Nature.* 2016; 530:219–222. [PubMed: 26840481]
53. Bailey A, Metaxas A, Al-Hasani R, Keyworth HL, Forster DM, Kitchen I. Mouse strain differences in locomotor, sensitisation and rewarding effect of heroin; association with alterations in MOP-r activation and dopamine transporter binding. *Eur J Neurosci.* 2010; 31:742–753. [PubMed: 20384817]
54. Leite-Morris KA, Fukudome EY, Shoeb MH, Kaplan GB. GABA(B) receptor activation in the ventral tegmental area inhibits the acquisition and expression of opiate-induced motor sensitization. *J Pharmacol Exp Ther.* 2004; 308:667–678. [PubMed: 14610238]
55. Barghon R, Protais P, Colboc O, Costentin J. Hypokinesia in mice and catalepsy in rats elicited by morphine associated with antidopaminergic agents, including atypical neuroleptics. *Neurosci Lett.* 1981; 27:69–73. [PubMed: 6120489]
56. Kuribara H. Modification of morphine sensitization by opioid and dopamine receptor antagonists: evaluation by studying ambulation in mice. *Eur J Pharmacol.* 1995; 275:251–258. [PubMed: 7768292]
57. Rodriguez-Arias M, Broseta I, Aguilar MA, Minarro J. Lack of specific effects of selective D(1) and D(2) dopamine antagonists vs. risperidone on morphine-induced hyperactivity. *Pharmacol Biochem Behav.* 2000; 66:189–197. [PubMed: 10837860]

58. Becker A, Grecksch G, Brodemann R, Kraus J, Peters B, Schroeder H, et al. Morphine self-administration in mu-opioid receptor-deficient mice. *Naunyn Schmiedebergs Arch Pharmacol*. 2000; 361:584–589. [PubMed: 10882032]
59. Sora I, Elmer G, Funada M, Pieper J, Li XF, Hall FS, et al. Mu opiate receptor gene dose effects on different morphine actions: evidence for differential in vivo mu receptor reserve. *Neuropsychopharmacology*. 2001; 25:41–54. [PubMed: 11377918]
60. Bozarth MA, Wise RA. Intracranial self-administration of morphine into the ventral tegmental area in rats. *Life Sci*. 1981; 28:551–555. [PubMed: 7207031]
61. Devine DP, Wise RA. Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. *J Neurosci*. 1994; 14:1978–1984. [PubMed: 8158252]
62. Fields HL, Hjelmstad GO, Margolis EB, Nicola SM. Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. *Annu Rev Neurosci*. 2007; 30:289–316. [PubMed: 17376009]
63. Crittenden JR, Tillberg PW, Riad MH, Shima Y, Gerfen CR, Curry J, et al. Striosome-dendron bouquets highlight a unique striatonigral circuit targeting dopamine-containing neurons. *Proc Natl Acad Sci U S A*. 2016; 113:11318–11323. [PubMed: 27647894]
64. Ikemoto S. Brain reward circuitry beyond the mesolimbic dopamine system: a neurobiological theory. *Neurosci Biobehav Rev*. 2010; 35:129–150. [PubMed: 20149820]
65. Berridge KC, Robinson TE, Aldridge JW. Dissecting components of reward: ‘liking’, ‘wanting’, and learning. *Curr Opin Pharmacol*. 2009; 9:65–73. [PubMed: 19162544]
66. Olmstead MC, Ouagazzal AM, Kieffer BL. Mu and delta opioid receptors oppositely regulate motor impulsivity in the signaled nose poke task. *PLoS One*. 2009; 4:e4410. [PubMed: 19198656]

Figure 1.

Dlx-MOR mice lack MORs mainly in striatal MSNs. **(A)** Quantification of triple *in situ* hybridization in mouse striatal sections (details in Supplemental Figure S1) shows major *Oprm1* mRNA co-localization with *Drd1*, *Drd2* or both in NAc and DS. **(B)** Quantitative RT-PCR analysis of *Oprm1* mRNA in microdissected brain regions from Ctl, conditional knockout Dlx-MOR and total knockout CMV-MOR mice reveals complete *Oprm1* deletion specifically in DS and NAc of Dlx-MOR mice. Transcript levels in mutants are expressed relative to Ctl (dotted line, n=3 to 4, in triplicates) and shown in rostrocaudal order. **(C)** Quantitative RT-PCR analysis of *Oprd1*, *Oprk1*, *Penk* and *pDyn* mRNAs in NAc (above) and DS (below) shows no change in Dlx-MOR mice (n=3 to 4, in triplicates). **(D)** Representative [³H]DAMGO (MOR agonist) binding autoradiograms of brain sections from Ctl and Dlx-MOR mice reveals complete receptor deletion at the level of striatum (bregma 1.1) but not in a more posterior section (Hb, bregma -1.46). Color bar, relative densities in fmol/mg tissue with non-specific binding at background level. **(E)** Quantification of [³H]DAMGO binding in areas with sufficiently high signal (≥ 60 fmoles/mg tissue) from the forebrain to spinal cord (n=3 to 4) confirms significant MOR decrease in the NAc and VP. MOR density is also lower in DS and VTA, although this is not significant. **(F, G)** Slice VTA electrophysiology (n=6 to 12). **(F)** In GABA interneurons (yellow), DAMGO (1 μM)

decreased evoked GABAA IPSCs (eIPSCs) in slices from Ctl but not Dlx-MOR mice, while N₆-CPA (A1 receptor agonist, 1μM) reduced eIPSCs in the two groups. **(G)** In dopamine neurons (blue), DAMGO (1μM) decreased GABAA eIPSCs similarly for Ctl and Dlx-MOR, while N₆-CPA had no significant effect. **(H)** Schematic representation of the MOR deletion within NAc/VTA circuitry of Dlx-MOR mice, as indicated by RNA, protein and electrophysiological analysis. Data are represented as mean + SEM. Blue star, significant genotype effect; one star, $p<0.05$; three stars, $p<0.001$. Serpentine, MOR. Amy, Amygdala; DS, dorsal striatum; DRN, dorsal raphe nucleus; EP, endopiriform nucleus; Hb, habenula (MHb, medial); LH, lateral hypothalamus; NAc, nucleus accumbens (NAcc, core; NAcsh, shell); PAG, periaqueductal gray; PFC, prefrontal cortex; RMTg, rostromedial tegmental area; SC, spinal cord (LI/II, lamina I/II); SN, substantia nigra; Th, thalamus (ThCL, central lateral; ThCM, central medial; ThIMD, intermediodorsal); VP, ventral pallidum; VTA, ventral tegmental area.

Figure 2.

Neuronal activity is modified in Dlx-MOR mice. **(A, B)** Heroin-induced neuronal activation. c-Fos immunohistochemistry (IHC) was performed on brain sections from animals perfused 2h after saline or heroin (10 mg/kg, ip) administration. **(A)** Representative images illustrate c-Fos IHC in the NAcsh, DLS and VTA of Ctl and mutant mice. **(B)** Quantification of c-Fos IHC, expressed as c-Fos-positive cells/mm² (bilateral, 4–7 sections/animal, n=5 to 6), shows a treatment effect for Ctl mice in all three regions. For Dlx-MOR mice, heroin-induced increase of c-Fos IHC was detected in VTA but not in NAcsh and DLS, indicating strongest modification in the striatum. **(C, D, E)** Whole-brain functional connectivity in live mice using resting-state functional MRI. High resolution spatial independent component analysis followed by partial correlation in time domain and graph theory identifies functional connectivity (FC) hubs displaying above-mean normalized connectivity strength and diversity, which differ across genotypes. **(C)** Focus on NAc-VTA FC shows two hubs covering NAc (left) detectable in Ctl mice (normalized strength>0.041 and/or diversity>0.966) but not Dlx-MOR mice (normalized strength<0.047 and/or diversity<0.940) and, conversely, two hubs with anatomical correspondence to VTA (right) detected in Dlx-MOR mice (normalized strength>0.047 and/or diversity>0.940) but not Ctl mice (normalized strength<0.041 and/or diversity<0.966). Thus, NAc nodes lose, while VTA nodes gain their hub status, indicating modified relay function between these two brain nodes. (n=10 to 12). **(D, E)** Seed-based Granger causality analysis considering 6 MOR-enriched regions within reward circuitry shows distinct information flow in Ctl and Dlx-

MOR mice (see full analysis in Supplemental Figure S2). **(D)** Identification of functional dominant or bi-directionality across NAc, DS and VTA is represented in matrices for Ctl and Dlx-mice. Brain regions on the horizontal axis are sources, and regions on the vertical axis are destination of the information flow and the number of subjects showing a significant directionality (by 100 permutations) is indicated. Mean conditional Granger causality (C-GC) values represent intensity of information flow for each connection (gradient grey scale). The dominant directionality is determined by comparing both number of significant subjects and mean C-GC for each connection (see Methods). **(E)** Scheme summarizing dominant or bi-directionality in Ctl and Dlx-MOR mice for the 3 seed regions of interest in this study (orange arrows) shows that VTA-DS connectivity is reversed in mutants, consistent with abolished heroin-induced locomotor activation and altered heroin SA, whereas VTA-NAc directional connectivity is unchanged concordant with maintained heroin CPP and heroin-induced DA release in the NAc (see Figure 4). Data are represented as mean + SEM. Black star, significant treatment effect; blue star, significant genotype effect. Two stars, $p < 0.01$; three stars, $p < 0.001$. DS, dorsal striatum; DLS, dorsolateral striatum; Hb, habenula; NAcsh, nucleus accumbens shell; PFC, prefrontal cortex; PAG, periaqueductal gray; VTA, ventral tegmental area. Scale bar = 100 μ m.

Figure 3.

Dlx-MOR mice show modified motor responses to heroin. Morphine analgesia was investigated in tail immersion [52°C (A)] test, tail flick (B) and hot plate (C) tests. (A, B) Acute thermal nociception in spinal reflex responses is unchanged in mutant mice (cumulative dose-response, ip injections, $n=12$) (C) Also, the three parameters measured in the hot plate test upon ip morphine administration (2 or 5 mg/kg, $n=5$ to 9) reveal treatment but no genotype effects. (D) Physical dependence was induced by repeated injections of ascending doses of morphine (10–100 mg/kg, ip, twice daily, 6 days). Scoring of naloxone-precipitated (1 mg/kg, sc) withdrawal signs shows no genotype difference in the expression of morphine physical dependence ($n=9$ to 15) (global score) (details in Supplemental Figure S3). (E) Dose-dependent heroin-induced locomotor activation in Ctl but not Dlx-MOR mice is shown (mg/kg ip, 2h-session, $n=4$ to 32). (F) Sensitization to the heroin locomotor effect (10 mg/kg ip) develops in Ctl mice only (5X 2h-sessions, $n=12$ to 32). (G) Heroin-induced catalepsy, expressed as latency to paw withdrawal in the bar test, develops in both Ctl and Dlx-MOR mice, and is enhanced in Dlx-MOR mice at 10 mg/kg ($n=8$ to 27). Data are represented as mean + or – SEM. Symbols: (A, B) black stars, significant difference to baseline; (C, D, E, G) black stars, significant difference to saline treatment; (E, G) blue star, significant genotype effect; (F) black stars, significant difference to all other groups; # significant time effect session 1 vs session 5 for Ctl 10. One symbol, $p<0.05$; two symbols, $p<0.01$; three symbols, $p<0.001$.

Figure 4.

Dlx-MOR mice show enhanced motivation for heroin and palatable food. **(A)** CPP to heroin (mg/kg sc, $n=4$ to 21), established over 6 conditioning sessions, does not differ between Ctl and Dlx-MOR mice (significant session X treatment interaction). Preference is expressed as % time spent in the drug-paired compartment during pre- (left) and post- (right) conditioning sessions. **(B)** DA microdialysis and concurrent locomotor activation. Heroin (10 mg/kg ip, injected after 120 min, recording during 320 min) increases extracellular DA above baseline in the NAc (Supplemental Figure S4) of Dlx-MOR and Ctl mice, with no genotype effect ($n=9$ to 13) (left). AUC ([DA] ng/mL, inset) analysis confirms no difference between Dlx-MOR and Ctl. Simultaneous to DA increase, heroin stimulates locomotion in Ctl but not Dlx-MOR mice (right). **(C–E)** Operant responding to heroin ($n=11$ to 18). **(C)** Both Dlx-MOR and Ctl mice acquire and increase heroin SA with decreasing doses (20 successive 1h-daily sessions, FR1 schedule of reinforcement). SA levels (mean number of infusions/session) are higher in the Dlx-MOR group. Heroin SA is significantly higher at 2 doses (dose-response analysis, inset). **(D)** Dlx-MOR mice achieve a higher breaking point in a 3h-PR session (0.0125 mg/kg/inf). **(E)** After extinction (1h-daily sessions for 13 days), Dlx-MOR but not Ctl mice reinstate heroin SA (0.006 mg/kg/inf) upon cue presentation (CIR). **(F, G)** Operant responding to chocolate-flavored pellets ($n=20$ Ctl, 13 Dlx-MOR). **(F)** Acquisition and maintenance of active nose-poking for chocolate reinforcement is shown (mean number of nose-pokes during 10 days FR1 and 5 days FR5 schedule of reinforcement, 1h-daily sessions). In both FR1 and FR5 sessions, Dlx-MOR mice show higher operant responding than Ctl mice. **(G)** Breaking-point for chocolate-flavored pellets is higher in mutant mice (5h PR session). Data are represented as mean + or - SEM. Black star, significant treatment effect; blue star, significant genotype effect. #, significant time

effect. One symbol, $p<0.05$; two symbols, $p<0.01$; three symbols, $p<0.001$. AUC, area under the curve; CIR, cue-induced reinstatement; FR, fixed ratio; PR, progressive ratio. See Tables 1 and 2 for statistical analysis of SA experiments.

Table 1

Statistical analysis of heroin self-administration data. Three-way ANOVA.

	Acquisition 0.1 mg/kg/inf		Acquisition 0.05 mg/kg/inf		Acquisition 0.025 mg/kg/inf		Acquisition 0.0125 mg/kg/inf		Acquisition 0.006 mg/kg/inf	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
Genotype	$F_{(1,26)} = 3.61$	0.0685	$F_{(1,27)} = 2.55$	0.122	$F_{(1,2)} = 5.44$	0.0274	$F_{(1,27)} = 2.81$	0.105	$F_{(1,27)} = 1.92$	0.127
Hole	$F_{(1,26)} = 20.40$	<0.001	$F_{(1,27)} = 6.27$	0.0187	$F_{(1,2)} = 10.08$	0.00373	$F_{(1,27)} = 8.64$	0.00667	$F_{(1,27)} = 73.78$	0.00341
Day	$F_{(3,78)} = 8.73$	<0.001	$F_{(2,54)} = 0.04$	0.961	$F_{(2,54)} = 0.19$	0.824	$F_{(5,135)} = 1.53$	0.184	$F_{(3,81)} = 25.50$	0.00513
Genotype × Hole	$F_{(1,26)} = 2.63$	0.117	$F_{(1,27)} = 2.05$	0.164	$F_{(1,2)} = 4.75$	0.0381	$F_{(1,27)} = 1.91$	0.179	$F_{(1,27)} = 1.94$	0.201
Genotype × Day	$F_{(3,78)} = 0.36$	0.779	$F_{(2,54)} = 0.43$	0.654	$F_{(2,54)} = 0.02$	0.976	$F_{(5,135)} = 0.72$	0.610	$F_{(3,81)} = 1.80$	0.791
Hole × Day	$F_{(3,78)} = 1.58$	0.202	$F_{(2,54)} = 0.07$	0.937	$F_{(2,54)} = 0.28$	0.760	$F_{(5,135)} = 1.10$	0.365	$F_{(3,81)} = 24.30$	0.0213
Genotype × Hole × Day	$F_{(3,78)} = 7.14$	0.025	$F_{(2,54)} = 0.58$	0.564	$F_{(2,54)} = 0.01$	0.993	$F_{(5,135)} = 1.34$	0.252	$F_{(3,81)} = 1.91$	0.737

Three-way ANOVA with genotype as between-subjects factor and repeated measures in the factors day/experimental phase and hole (active/inactive). See Methods and Materials for details.

Table 2

Statistical analysis of chocolate-flavored pellet self-administration data. Three-way ANOVA

	Acquisition FR1		Acquisition FR5	
	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
Genotype	$F_{(1,31)} = 2.87$	0.101	$F_{(1,31)} = 6.88$	0.0134
Hole	$F_{(1,31)} = 122.15$	<0.001	$F_{(1,31)} = 259.91$	<0.001
Day	$F_{(9,279)} = 31.40$	<0.001	$F_{(4,124)} = 0.81$	0.524
Genotype × Hole	$F_{(1,31)} = 2.79$	0.105	$F_{(1,31)} = 6.80$	0.0139
Genotype × Day	$F_{(9,279)} = 1.75$	0.0772	$F_{(4,124)} = 1.55$	0.193
Hole × Day	$F_{(9,279)} = 34.46$	<0.001	$F_{(4,124)} = 0.78$	0.542
Genotype × Hole × Day	$F_{(9,279)} = 1.83$	0.0629	$F_{(4,124)} = 1.56$	0.188

Three-way ANOVA with genotype as between-subjects factor and repeated measures in the factors day and hole (active/inactive). See Methods and Materials for details.