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α2δ-1 Protein drives opioid-induced conditioned reward and synaptic NMDA receptor hyperactivity in the nucleus accumbens

Daozhong Jin[1], **Hong Chen**[1], **Shao-Rui Chen**[1,#], **Hui-Lin Pan**[1,#]

[1]Center for Neuroscience and Pain Research, Department of Anesthesiology and Perioperative Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

Abstract

Glutamate NMDA receptors (NMDARs) in the nucleus accumbens (NAc) are critically involved in drug dependence and reward. α2δ-1 is a newly discovered NMDAR-interacting protein that promotes synaptic trafficking of NMDARs independently of its conventional role as a calcium channel subunit. However, it is unclear how repeated opioid exposure affects synaptic NMDAR activity and α2δ-1–NMDAR interaction in the NAc. In this study, whole-cell patch-clamp recordings showed that repeated treatment with morphine in mice markedly increased the NMDAR-mediated frequency of miniature excitatory postsynaptic currents (mEPSCs) and amplitude of puff NMDAR currents in medium spiny neurons in the NAc core region. Morphine treatment significantly increased the physical interaction of α2δ-1 with GluN1 and their synaptic trafficking in the NAc. In *Cacna2d1* knockout mice, repeated treatment with morphine failed to increase the frequency of mEPSCs and amplitude of puff NMDAR currents in the NAc core. Furthermore, inhibition of α2δ-1 with gabapentin or disruption of the α2δ-1–NMDAR interaction with the α2δ-1 C terminus–interfering peptide blocked the morphine-elevated frequency of mEPSCs and amplitude of puff NMDAR currents in the NAc core. Correspondingly, systemically administered gabapentin, *Cacna2d1* ablation, or microinjection of the α2δ-1 C terminus–interfering peptide into the NAc core attenuated morphine-induced conditioned place preference and locomotor sensitization. Our study reveals that repeated morphine exposure strengthens presynaptic and postsynaptic NMDAR activity in the NAc via α2δ-1. The α2δ-1–bound NMDARs in the NAc have a key function in the rewarding effect of opioids and could be targeted for treating opioid use disorder and addiction.

Graphical Abstract

[#]Correspondence: Hui-Lin Pan, MD, PhD or Shao-Rui Chen, MD, Department of Anesthesiology and Perioperative Medicine, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030, USA. huilinpan@mdanderson.org, schen@mdanderson.org.

Author Contributions

D.J., H.C., and S.-R.C. collected data. D.J. and S.-R.C. performed data analysis. D.J. drafted the manuscript. H.-L.P. and S.-R.C. conceived the study and finalized the manuscript.

Conflict of Interest

The authors are employees of The University of Texas System, which currently holds a patent for targeting α2δ-1–bound glutamate receptors for treating diseases and disorders.

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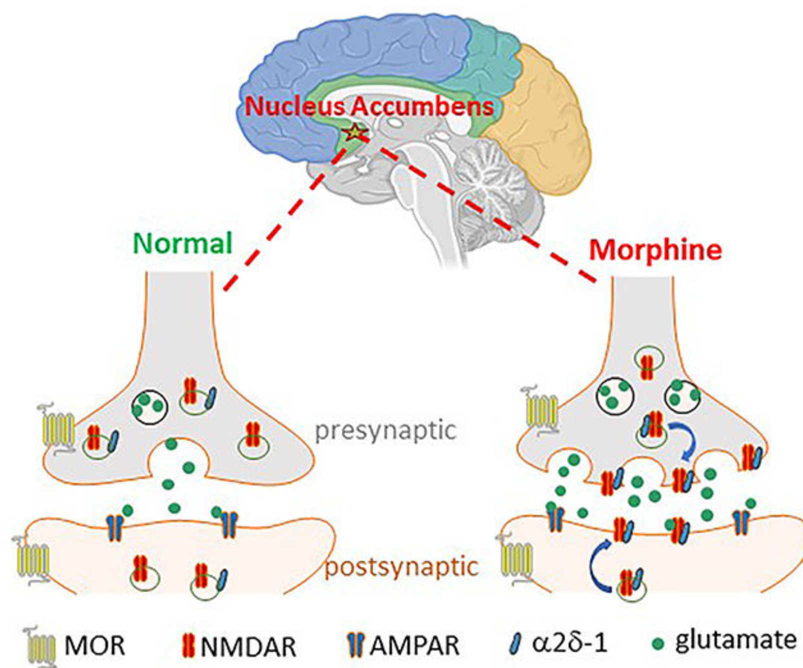

Glutamate NMDA receptors (NMDARs) in the nucleus accumbens (NAc), a key brain region involved in drug addiction, are critically involved in opioid dependence and reward. Under normal conditions, NMDARs are not associated with the α2δ-1 protein in the NAc. Repeated treatment with morphine increases the α2δ-1–NMDAR interaction and their synaptic trafficking in the NAc, leading to increased presynaptic and postsynaptic NMDAR activity. As a result, glutamatergic synaptic transmission is potentiated in the NAc, which promotes the formation of opioid reward memory. Thus, the α2δ-1–coupled NMDAR could be targeted for treating opioid use disorder and addiction.

Keywords

electrophysiology; gabapentinoid; glutamatergic transmission; opiate; pregabalin; synaptic plasticity

INTRODUCTION

Opioid misuse and addiction have become a serious national crisis that impacts public health and imposes an enormous societal cost. There is an urgent need to define the underlying molecular mechanism and develop effective treatments for opioid use disorders. Opioid drugs have a high potential for abuse because they produce highly rewarding effects that can result in addiction. Opioids induce functional changes in key areas of the brain that make individuals more susceptible to compulsive use, conditioned response to drug cues/contexts, and relapses (Scofield et al. 2016; Lüscher & Malenka 2011). Although dopamine plays a role in opioid addiction, some rewarding effects of opioids are mediated by dopamine-independent mechanisms (Koob & Le Moal 2001). The nucleus accumbens (NAc), a major component of the ventral striatum, integrates dopaminergic and glutamatergic inputs to

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mediate rewarding and aversive properties of psychostimulants and opioids (Scofield et al. 2016; Volkow et al. 2019). Glutamatergic synaptic plasticity in the NAc may play an important role in addiction-related behaviors (Scofield et al. 2016; Vega-Villar et al. 2019; Kalivas 2009; Russell et al. 2016). In this regard, repeated treatment with morphine potentiates NMDA receptor (NMDAR) phosphorylation in the NAc (Anderson et al. 2015), and microinjection of NMDAR antagonists into the NAc diminishes the acquisition and expression of morphine-induced conditioned place preference (CPP) (Bespalov & Zvartau 1996; Ma et al. 2006; Wu et al. 2012), a commonly used means of studying the rewarding effect of opioids in animal models (Mucha & Iversen 1984; Tzschentke 1998). Multiple brain regions, especially the basolateral amygdala, medial prefrontal cortex, ventral hippocampus, and ventral tegmental area, provide excitatory glutamatergic input to NAc neurons (Beckley et al. 2013; Perez & Lodge 2018; Stuber et al. 2011; Taylor et al. 2014). In addition, functional connectivity between the NAc and other brain regions is altered in opioid-dependent patients (Upadhyay et al. 2010; Zou et al. 2015). However, little is known about changes in synaptic NMDAR activity in the NAc associated with the rewarding effect of opioids.

α2δ-1 (encoded by the *Cacna2d1* gene) is previously known as a subunit of voltage-gated Ca^{2+} channels and is expressed at excitatory synapses throughout the mammalian brain, including the NAc (Cole et al. 2005; Taylor & Garrido 2008). α2δ-1 is also the therapeutic target of gabapentinoids (Gee et al. 1996; Marais et al. 2001), which are used clinically for treating neuropathic pain conditions. Repeated cocaine treatment increases the α2δ-1 protein level in the rat NAc, and injection of gabapentin into the NAc reduces cocaine-induced reward (Spencer et al. 2014). Because psychostimulants and opioids have distinct mechanisms of actions and may engage different brain circuits to promote addictive behaviors, it is important to determine the role of α2δ-1 in the NAc in the rewarding effect of opioids. Recent studies indicate that α2δ-1, via its C terminus (an intrinsically disordered protein region), physically interacts with NMDARs in the spinal cord and brain to enhance synaptic NMDAR activity associated with learning and memory, chronic pain, neurogenic hypertension, and stroke (Chen et al. 2019; Chen et al. 2018; Ma et al. 2018b; Luo et al. 2018; Zhou et al. 2018). At present, it is unclear how opioid exposure affects the α2δ-1–NMDAR association in the NAc and whether α2δ-1–bound NMDARs play a role in the rewarding and reinforcing effects of opioids.

Therefore, in this study, we determined how repeated treatment with morphine alters synaptic NMDAR activity in the NAc and whether α2δ-1–bound NMDARs play a role in the rewarding effect of morphine. We showed for the first time that repeated treatment with morphine increases the activity of both presynaptic and postsynaptic NMDARs in the NAc through α2δ-1. We also demonstrated that α2δ-1–bound NMDARs in the NAc are highly involved in morphine-induced expression of CPP and locomotor sensitization. Our study provides important new information on how NMDAR-dependent synaptic plasticity contributes to the rewarding effect of opioids.

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MATERIALS AND METHODS

Animals

This study was not pre-registered. The Institutional Animal Care and Use Committee of The University of Texas MD Anderson Cancer Center (Houston, TX) approved all experimental procedures and protocols (Approval #1174-RN02). The Guide for the Care and Use of Laboratory Animals of the National Institutes of Health was followed throughout the study. Wild-type (WT) and *Cacna2d1* knockout (KO) mice with a C57BL/6 genetic background (Stock #6900, Medical Research Council; Oxfordshire, UK) were used in the experiments. The generation of conventional *Cacna2d1* KO mice was described previously (Fuller-Bicer et al. 2009). The *Cacna2d1*$^{+/-}$ heterozygous mice were bred to produce C*acna2d1*$^{-/-}$ (KO) mice and *Cacna2d1*$^{+/+}$ (WT) littermates, which were confirmed by genotyping using ear biopsies. Both male and female mice (8 to 10 weeks of age, 20 – 25 g) were used for final experiments. Data from males and females were pooled because we found no sex difference in electrophysiological and behavioral data. Mice were housed in the animal facility at a controlled temperature (25°C) and lighting (12-h light/dark cycles). Animals had free access to rodent food and tap water. All final studies were conducted between 8:00 am and 5:00 pm. The timeline of behavioral, biochemical, and electrophysiological experiments is shown in Fig. 1.

Conditioned place preference and locomotor activity

Conditioned place preference (CPP) was performed in a two-chamber apparatus (Huang et al. 2022). The two chambers, separated by a door, were the same size but differed in their floors and walls. One chamber had white walls and a wire mesh floor, whereas the other chamber had black walls and a bar floor. The CPP test involved three phases: preconditioning, conditioning, and postconditioning (Fig. 1). In the preconditioning phase (days 1–4), mice were placed into the CPP apparatus with free access to the two chambers for 15 min every day. On day 4, the time spent in each chamber and locomotor activity (assessed by measuring the distance traveled based on consecutive breaking of adjacent photobeams) in both chambers were automatically recorded using ANY-maze video-tracking software (Stoelting) and a Photobeam Activity System (San Diego Instruments). All selected animals had similar locomotor activity before starting drug treatment.

In the conditioning phase (days 4–9), the animals received intraperitoneal (*i.p.*) injection of saline every day and were immediately placed into the assigned conditioning chamber for 45 min. The door between chambers was kept closed. Four hours later, the mice were injected intraperitoneally (*i.p.*) with 10 mg/kg of morphine (catalog #0641-6127-25, West Ward Pharmaceuticals, Eatontown, NJ). The mice were then placed into the opposite paired chamber for another 45 min. For testing the effect of gabapentin on morphine CPP, another group of mice was injected with gabapentin (60 mg/kg, *i.p.*; catalog #60142-96-3, MilliporeSigma, St. Louis, MO) or the same volume of vehicle 5 min prior to each morphine treatment. Repeated daily treatment with 10 mg/kg of morphine is known to reliably induce CPP in mice (Popik et al. 2003; Sun et al. 2020b; McKendrick et al. 2020).

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In the postconditioning phase (i.e., a withdrawal/extinction period), each mouse experienced an CPP assessment session 2 days and 9 days after the last conditioning session. The mice were placed in the drug-paired chamber with free access to both chambers (open door) and were allowed to explore freely for 15 min. The time spent in each chamber and the total locomotor activity were recorded. Because animals tend to show a preference for contexts that are associated with positive reinforcers (e.g., opioid drugs), time spent in the drug-associated context is considered to be a measure of the presence and potency of the reinforcing effects of opioids (Mucha & Iversen 1984; Tzschentke 1998).

Cannula implantation in the NAc core and microinjection

Mice were anesthetized by continuous inhalation of 2% isoflurane and placed onto a stereotaxic device (Stoelting). An incision was made along the midline, and the area surrounding the bregma was cleaned. In accordance with the mouse brain atlas (Paxinos & Franklin 2011), the stereotaxic coordinates used for the NAc core were 1.5 mm anterior to the bregma, ± 0.8 mm lateral to the sagittal suture, and 4.4 mm ventral from the top of the skull. Two holes were drilled into the skull at these stereotaxic coordinates. A stainless-steel double guide cannula (26-gauge, RWD Life Science Inc, Dover, DE) was bilaterally implanted 0.8 mm above the target region. The cannula was secured with jewelers' screws and dental acrylic cement. After the cement was completely hardened, a stainless-steel double dummy cannula was used to occlude the guide cannula (Sun et al. 2020a). The incision site was treated with 0.5% bupivacaine on the first 3 days after surgery to minimize postoperative pain. Animals were individually housed and allowed to recover for 7 days before behavioral tests (Fig. 1).

For NAc microinjection, a double injector was inserted 4.4 mm ventral from the top of the skull. A Tat-fused α2δ-1 peptide mimicking the C-terminal domain of α2δ-1 (VSGLNPSLWSIFGLQFILLWLVSGSRHYLW; α2δ-1CT peptide) or a Tat-fused scrambled control peptide (FGLGWQPWSLSFYLVWSGLILSVLHLIRSN) was injected bilaterally into the NAc (2 μg in 0.1 μl each side). These peptides were synthesized by Bio Basic Inc. (Markham, ON, Canada) and were dissolved in normal saline immediately before use. At the completion of the experiments, mice were decapitated under isoflurane anesthesia, and the brains were quickly removed. Coronal sections (40 μm thick) were cut to assess the placement of the cannula tips. The microinjection sites in the NAc core were confirmed with a fluorescent dye (FluoSpheres; catalog #F8810, Thermo Fisher Scientific, Waltham, MA) added to the injection solution. Three mice were excluded from data analysis because of incorrect microinjection sites.

Brain slice preparation and electrophysiological recordings

Brain slice preparation and recordings were performed similarly to our previously described methods (Ma et al. 2018a; Zhou et al. 2018; Finnegan et al. 2004). Mice were deeply anesthetized with 3% isoflurane, and the brain tissue was rapidly removed and immediately placed in ice-cold sucrose artificial cerebrospinal fluid pre-saturated with 95% O_2 and 5% CO_2. The sucrose-based artificial cerebrospinal fluid contained (in mM) 206 sucrose, 2.8 KCl, 1.2 $MgCl_2$, 2.0 MgSO4, 1.0 $CaCl_2$, 1.25 NaH_2PO_4, 26.0 $NaHCO_3$, and 10.0 glucose. The brain region containing the ventral aspect of the basal ganglia was trimmed between −1

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to +1.8 mm relative to the bregma (Matthews et al. 2004; Schotanus & Chergui 2008) and then glued to the stage of a vibratome (Leica, Wetzlar, Germany). Transverse slices (300 μm thick) of the brain tissues were cut in ice-cold sucrose-based artificial cerebrospinal fluid. Four sections that contained the NAc were kept, and each section was cut at the middle line to separate the left and right sides. The NAc-containing tissue sections were then incubated in Krebs solution oxygenated with 95% O_2 and 5% CO_2 at 34°C for at least 1 h before being transferred to the recording chamber. The Krebs solution contained (in mM) 117.0 NaCl, 3.6 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 11.0 glucose, and 25.0 $NaHCO_3$. The brain slices in the recording chamber were perfused with Krebs solution at 3.0 ml/min at 34°C.

The brain slices were visualized on an upright fixed-stage microscope with differential interference contrast/infrared illumination. The medium spiny neurons in the NAc core region were visualized and selected for recording. Miniature excitatory postsynaptic currents (mEPSCs) were recorded using whole-cell voltage-clamp mode at a holding potential of −60 mV (Zhou et al. 2018; Chen et al. 2014). The impedance of the glass electrode was 4 to 6 MΩ when the pipette was filled with an internal solution containing (in mM) 135.0 potassium gluconate, 2.0 $MgCl_2$, 0.5 $CaCl_2$, 5.0 KCl, 5.0 HEPES, 5.0 EGTA, 5.0 ATP-Mg, 0.5 Na-GTP, and 10 QX314 (280–300 mOsm adjusted to pH 7.25 with 1.0 M KOH). mEPSCs were recorded in the presence of Krebs solution and 0.5 μM tetrodotoxin (Finnegan et al. 2004). At the holding potential of −60 mV, postsynaptic NMDAR currents were blocked by Mg^{2+} in the extracellular solution (Chen et al. 2018).

Postsynaptic NMDAR currents were recorded by puff application of 100 μM NMDA directly onto the medium spiny neurons at a 30° angle using a positive pressure system (Toohey Company, Fairfield, NJ). The internal recording solution contained (in mM) 135.0 potassium gluconate, 2.0 $MgCl_2$, 0.5 $CaCl_2$, 5.0 KCl, 5.0 HEPES, 5.0 EGTA, 5.0 ATP-Mg, 0.5 Na-GTP, and 10 QX314 (280–300 mOsm, adjusted to pH 7.25 with 1.0 M KOH). Because NMDARs are blocked by Mg^{2+} at the negative holding potential and are co-activated by glycine, puff NMDA–elicited currents were recorded in the Mg^{2+}-free extracellular solution and in the presence of 10 μM glycine (Luo et al. 2018; Zhou et al. 2021a). The distance was 150 μm between the tip of the puff pipette and the recorded neurons. All signals were filtered at 1 to 2 kHz and digitized at 10 kHz using an amplifier (MultiClamp 700B; Molecular Devices, San Jose, CA).

Tetrodotoxin and D-2-amino-5-phosphonopentanoate (AP5, catalog #HB0225) were purchased from Hello Bio (Princeton, NJ). NMDA (catalog #M3262) was obtained from MilliporeSigma. All drugs were prepared in artificial cerebrospinal fluid before the recordings and delivered via syringe pumps to reach their final concentrations in the recording chamber.

Synaptosome preparation and coimmunoprecipitation

Mice were deeply anesthetized with 3% isoflurane, and the brain tissue was rapidly removed. The NAc was cut on ice and put in liquid nitrogen before being stored in −80°C freezer. Synaptosomes were isolated from the NAc as described previously (Jin et al. 2013; Huang et al. 2020). The NAc tissues were homogenized in ice-cold homogenization buffer containing 0.32 M sucrose, 10 mM HEPES, 2 mM EDTA (pH, 7.4), and a protease inhibitor

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cocktail (catalog #P8340, MilliporeSigma). The homogenate was centrifuged at 800 *g* for 10 min at 4°C. The supernatant was then centrifuged at 13,000 *g* for 20 min to obtain the crude synaptosomes. The synaptosomal pellets were solubilized in RIPA lysis buffer with a protease inhibitor cocktail for 1 h on ice and then centrifuged at 10,000 *g* for 15 min at 4°C. The synaptosomal fraction in the supernatant was used for further immunoblotting analysis.

NAc tissues were homogenized using immunoprecipitation lysis buffer (catalog #87788, Thermo Fisher Scientific) in the presence of a proteinase inhibitor cocktail (MilliporeSigma). The homogenate was centrifuged at 10,000 *g* for 10 min at 4°C. The protein amount of supernatants was quantified, and the same quantity of proteins from each group was incubated at 4°C overnight with protein G beads (catalog #16-266, MilliporeSigma) and rabbit anti-GluN1 antibody (1:500, catalog #G8913, MilliporeSigma) or normal rabbit IgG (catalog #12-371, MilliporeSigma). All samples were washed three times with immunoprecipitation lysis buffer. The beads were then incubated with 1 × NuPage loading buffer (catalog #NP0008, Thermo Fisher Scientific) with additional 100 mM DTT (catalog #P2325, Thermo Fisher Scientific) and 1% sodium dodecyl sulfate (SDS) for 10 min and boiled for 5 min. The eluted proteins were used for immunoblotting analysis. The amount of immunoprecipitated α2δ-1 was normalized to that in the input.

Immunoblotting

Immunoblotting was performed as described previously (Chen et al. 2019; Zhang et al. 2021). Briefly, proteins in synaptosomes and from coimmunoprecipitation were separated on SDS NuPAGE 4 to 12% Bis-Tris gels (catalog #NP0336BOX, Thermo Fisher Scientific) and were transferred to polyvinylidene fluoride membranes. Membranes were incubated with primary antibodies overnight at 4°C. This was followed by incubation with horseradish peroxidase-conjugated secondary antibodies (1:10,000, anti-rabbit, catalog #7074; anti-mouse, catalog #7076; Cell Signaling Technology, Danvers, MA) for 1 h at 22°C. For coimmunoprecipitation, HRP-conjugated TrueBlot secondary antibodies were used (1:10,000, anti-rabbit, catalog #18-8816-31; anti-mouse, catalog #18-8817-31; Rockland Immunochemicals, Limerick, PA). A complete wash was given after antibody incubation. Immunoblots were developed with an ECL kit (Thermo Fisher Scientific). The protein band was visualized using an Odyssey Fc Imager (LI-COR Biosciences, Lincoln, NE) and quantified with ImageJ software. The primary antibodies used were rabbit anti-GluN1 (1:1000, catalog #G8913, MilliporeSigma), mouse anti–α2δ-1 (1:2000, catalog #SC-271697, Santa Cruz Biotechnology), mouse anti-β-tubulin (1:10,000, catalog #32-2600, Thermo Fisher Scientific), and mouse anti-PSD95 (1:10,000, catalog #MABN1190, MilliporeSigma). The specificity of these primary antibodies has been validated previously (Chen et al. 2018; Chen et al. 2019; Luo et al. 2018). For protein quantification, the protein band intensity of GluN1 and α2δ-1 was normalized to that of PSD95 or β-tubulin.

Experimental design and statistical analysis

Data are expressed as means ± SEM. The sample sizes used in the study were based on our previous experience with similar studies (Deng et al. 2019; Finnegan et al. 2004; Zhou et al. 2018) and were similar to those generally employed in the field. The animals were assigned (1:1 allocation) to the control and treatment groups without using a randomization

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method. No animal died during final experiments, and no test for outlier in the data was conducted. The investigators performing the behavioral (D.J.) and electrophysiological (H.C. and S.-R.C.) experiments were blinded to the drug treatment and genotypes. For electrophysiological recordings, four to six mice were used in each group, and only one neuron was recorded from each NAc slice. The frequency and amplitude of mEPSCs were analyzed off-line using the peak detection MiniAnalysis program (Synaptosoft, Leonia, NJ). Puff NMDA currents were quantified by averaging the amplitude of currents elicited by three consecutive puff applications of NMDA using Clampfit 10.0 software (Molecular Devices). Cell capacitance, input resistance, and baseline holding current were monitored. The recording was discontinued if these parameters changed >15%. The CPP score was calculated as the time spent in the drug-paired chamber in the postconditioning phase minus the preconditioning time spent in the chamber (Huang et al. 2022). A total of 12 mice spending more than 550 s of the total time (900 s) in either chamber before preconditioning was excluded from further testing. Two-tailed Student *t* tests were used to determine the differences between two groups, and one-way analysis of variance (ANOVA) followed by Tukey *post hoc* tests were used to compare more than two groups. Data sets in the present study were normally distributed according to the Shapiro–Wilk test. All statistical analyses were performed using Prism software (version 9; GraphPad Software Inc.). *P* values of less than 0.05 were considered to be statistically significant.

RESULTS

Repeated morphine exposure potentiates synaptic NMDAR activity in the NAc

Many studies have shown that blocking NMDARs in the NAc inhibits the rewarding and reinforcing effects of opioids in animal models (Bespalov & Zvartau 1996; Ma et al. 2006; Wu et al. 2012). Biochemical studies also suggest that repeated opioid administration enhances NMDAR function in the NAc (Anderson et al. 2015; Murray et al. 2007). However, intracellular recordings with sharp electrodes showed that treatment with morphine pellets reduces evoked NMDAR-mediated EPSCs in the NAc core (Martin et al. 1999). To resolve these seemly paradoxical findings, we used whole-cell patch-clamp recordings to determine how repeated morphine exposure affects synaptic NMDAR activity in the NAc core region. We treated WT mice with vehicle or morphine (10 mg/kg/day, *i.p.*) for 6 days. Systemic injection of this dose of morphine produces profound analgesic effects (Sun et al. 2019; Jin et al. 2022), and repeated daily administration of this dose of morphine consistently induces CPP in mice (Popik et al. 2003; Sun et al. 2020b; McKendrick et al. 2020). Brain slices containing the NAc were obtained from mice on the 2nd day after the last treatment with morphine or vehicle (Fig. 1). We first recorded mEPSCs, which reflect quantal release of glutamate from presynaptic terminals (Sulzer & Pothos 2000; Huang et al. 2020). The baseline frequency of mEPSCs in medium spiny neurons in the NAc core was significantly higher in morphine-treated mice than in vehicle-treated mice [$F(5,84) = 5.928$, $P = 0.0091$, n = 15 neurons per group], whereas the baseline amplitude of mEPSCs did not differ significantly between the two groups (Fig. 2A,B). We assessed NMDAR-mediated changes in the mEPSC frequency and amplitude using AP5, a specific NMDAR antagonist. Bath application of 50 μM AP5 for 6 min markedly reduced the frequency of mEPSCs in medium spiny neurons in the NAc core from morphine-treated mice, but not vehicle-treated

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mice (Fig. 2A,B). Bath application of AP5 did not change the amplitude of mEPSCs in either groups.

To determine whether repeated treatment with morphine changes the postsynaptic NMDAR activity in the NAc core, we recorded NMDAR currents elicited by puff application of 100 μM NMDA directly onto the recorded medium spiny neurons (Zhou et al. 2018). The amplitude of puff NMDAR currents in the NAc core was significantly greater in morphine-treated mice than in vehicle-treated mice [$t(11) = 3.096$, $P = 0.0102$; n = 12 neurons per group; Fig. 1C,D]. These findings suggest that repeated morphine exposure increases the activity of both presynaptic and postsynaptic NMDARs in the NAc.

Repeated treatment with morphine increases the coupling and synaptic trafficking of α2δ-1 and NMDARs in the NAc

In the brain and spinal cord, α2δ-1 interacts directly with NMDARs to regulate their synaptic trafficking (Chen et al. 2018; Zhou et al. 2018; Ma et al. 2018a). Sustained exposure to many psychostimulants, such as nicotine, ethanol, methamphetamine, and cocaine, increases α2δ-1 protein levels in the frontal cortex and NAc (Spencer et al. 2014; Hayashida et al. 2005; Katsura et al. 2006; Kurokawa et al. 2011). We thus determined whether repeated treatment with morphine affects α2δ-1 expression and the α2δ-1 interaction with NMDARs in the NAc. We treated wild-type (WT) mice with vehicle or morphine (10 mg/kg/day, *i.p.*) for 6 days and obtained their NAc tissues 2 days after the last treatment. Immunoblotting showed that α2δ-1 protein levels (input) in the NAc were similar between morphine-treated and vehicle-treated mice (n = 9 mice per group, Fig. 3A,B). In contrast, the protein level of GluN1, an obligatory subunit of NMDARs (Traynelis et al. 2010), in the NAc was significantly greater in morphine-treated mice than in vehicle-treated mice [$t(16) = 3.666$, $P = 0.0021$, n = 9 mice per group; Fig. 3A,B]. Furthermore, coimmunoprecipitation assays showed that treatment with morphine profoundly increased the amount of α2δ-1–GluN1 protein complexes in the NAc compared with that in the vehicle-treated group [$t(10) = 4.077$, $P = 0.0022$, n = 6 mice per group; Fig. 3A,B].

Next, we isolated synaptosomes from the NAc of vehicle-treated and morphine-treated WT mice to quantify the amount of α2δ-1 and GluN1 proteins. Immunoblotting using NAc synaptosomes showed that both GluN1 [$t(10) = 2.374$, $P = 0.039$] and α2δ-1 [$t(10) = 10.04$, $P < 0.001$] protein levels were much higher in morphine-treated mice than in vehicle-treated mice (n = 6 mice per group; Fig. 3C,D). These data indicate that repetitive morphine exposure increases α2δ-1 interaction with NMDARs and their synaptic trafficking in the NAc.

α2δ-1 is essential for synaptic NMDAR activity in the NAc core augmented by morphine treatment

Because repeated treatment with morphine increased the α2δ-1–NMDAR interaction and NMDAR synaptic trafficking in the NAc, we determined whether α2δ-1 is involved in morphine-induced potentiation of synaptic NMDAR activity in the NAc. We treated the NAc slices obtained from morphine-treated mice with 100 μM gabapentin, an α2δ-1 inhibitory ligand (Marais et al. 2001; Fuller-Bicer et al. 2009), for 30 min before electrophysiological

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recordings. Gabapentinoids and α2δ-1 KO have no effect on basal NMDAR activity or EPSC currents in various brain regions, including the hippocampus, dorsomedial striatum, and NAc, under normal conditions (Luo et al. 2018; Ma et al. 2018a; Spencer et al. 2014; Zhou et al. 2018). Treatment with gabapentin normalized the baseline frequency of mEPSCs of medium spiny neurons in the NAc core from morphine-treated mice (n = 16 neurons, Fig. 4A,B) to a similar level as in vehicle-treated mice (Fig. 2A,B). Subsequent bath application of AP5 (50 μM) had no further effect on the frequency of mEPSCs in brain slices pretreated with gabapentin (n = 16 neurons, Fig. 4A,B). In addition, treatment with gabapentin significantly reduced the amplitude of currents elicited by puff application of 100 μM NMDA onto medium spiny neurons in the NAc core from morphine-treated mice to a similar level as in vehicle-treated mice [$t(11) = 4.027$, $P = 0.0020$, n = 12 neurons per group; Figs. 2C,D and 4C,D].

Because gabapentin binds to both α2δ-1 and α2δ-2 proteins (Gee et al. 1996; Marais et al. 2001; Fuller-Bicer et al. 2009), we then validated the role of α2δ-1 in morphine-induced enhancement of NMDAR activity in the NAc using *Cacna2d1* KO mice subjected to repeated treatment with morphine (10 mg/kg/day, *i.p.*) for 6 days. Genetic *Cacna2d1* ablation reduced the increased baseline frequency of mEPSCs of medium spiny neurons in the NAc core from morphine-treated mice (n = 16 neurons per group, Fig. 4A,B) to a similar level as in vehicle-treated WT mice (Fig. 2A,B). Subsequent bath application of AP5 produced no further effect on the frequency of mEPSCs of medium spiny neurons from the *Cacna2d1* KO mice (n = 16 neurons; Fig. 4A,B). In addition, the amplitude of currents elicited by puff application of 100 μM NMDA onto medium spiny neurons in the NAc core was significantly lower in morphine-treated *Cacna2d1* KO mice than in morphine-treated WT mice [$t(11) = 3.843$, $P = 0.0027$, n = 12 neurons per group; Figs. 2C,D and 4C,D]. Together, these results strongly suggest that α2δ-1 is required for morphine-induced hyperactivity of presynaptic and postsynaptic NMDARs in the NAc.

Repeated morphine treatment potentiates glutamatergic input to the NAc via α2δ-1–bound NMDARs

We next determined the role of α2δ-1–bound NMDARs in morphine-induced potentiation of glutamatergic input to the NAc. We treated the NAc slices from morphine-treated WT mice (10 mg/kg/day for 6 days, *i.p.*) with 1 μM Tat-fused α2δ-1CT peptide or 1 μM Tat-fused scrambled control peptide for 30 min immediately before whole-cell recordings. The cell-penetrating Tat-fused α2δ-1CT peptide mimics the C-terminal domain of α2δ-1 to uncouple the α2δ-1–NMDAR association (Chen et al. 2018; Huang et al. 2020). Treatment with α2δ-1CT peptide, but not the control peptide, reversed the baseline frequency of mEPSCs of medium spiny neurons in the NAc core that had already increased in morphine-treated WT mice (n = 15 neurons per group; Fig. 5A,B). Subsequent bath application of 50 μM AP5 had no effect on the mEPSC frequency in these α2δ-1CT peptide-treated NAc neurons, but it still significantly reduced the frequency of mEPSCs in the control peptide–treated neurons (Fig. 5A,B).

When we examined the effect of α2δ-1CT peptide on postsynaptic NMDAR activity, we also found that α2δ-1CT peptide significantly decreased the amplitude of NMDA puff

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currents compared with control peptide-treated NAc neurons [$t(12) = 3.128$, $P = 0.0087$, n = 13 neurons; Fig. 5C,D]. Together, these findings suggest that α2δ-1–bound NMDARs, at both presynaptic and postsynaptic sites, are essential for morphine-induced potentiation of glutamatergic input to the NAc.

α2δ-1 Mediates morphine-induced CPP and locomotor sensitization

Having demonstrated the important role of α2δ-1 in morphine-induced glutamatergic synaptic plasticity in the NAc, we then determined whether α2δ-1 plays a role in the rewarding effect of morphine by measuring morphine-induced expression of CPP and locomotor hyperactivity. CPP is an incentive-driven behavior, and if animals treated with morphine treatment exhibit morphine preference or "rewarding", they will likely spend more time in the chamber paired with morphine, thus producing CPP (Mucha & Iversen 1984; Tzschentke 1998). Locomotor sensitization is another common and robust behavioral alteration in rodents whereby the animal becomes significantly more hyperactive following exposure to addictive drugs (Brady & Holtzman 1981; Perreau-Lenz et al. 2017). We measured the expression of morphine-induced CPP and locomotor activity in a drug-free state 2 days after conditioning with morphine. Furthermore, to assess the memory associated with opioid reward after a prolonged extinction/abstinence period, CPP and locomotor activity were tested again 9 days after conditioning with morphine. Compared with the vehicle group (n = 6 mice), conditioning with morphine (10 mg/kg/day for 6 days, *i.p.,* n = 9 mice) markedly increased the CPP score and locomotor activity on the 2nd day and the 9th day after the last morphine treatment (Fig. 6A,B). The CPP score decreased when tested on the 9th day compared to the 2nd day. However, the locomotor activity was increased on the 9th day, showing a strong abstinence response (Fig. 6A,B).

We next treated WT mice with gabapentin (60 mg/kg, *i.p.*) 5 min prior to daily conditioning with morphine (10 mg/kg/day, *i.p.*) for 6 days (Fig. 1). Compared with the vehicle group, treatment with gabapentin significantly decreased the CPP score and locomotor activity elevated by conditioning with morphine when tested on the 2nd day [$F(3,32) = 27.80$, $P < 0.001$ for CPP; $F(3,32) = 107.8$, $P < 0.001$ for locomotor activity] and the 9th day [$F(3,32) = 27.80$, $P < 0.001$ for CPP; $F(3,32) = 107.8$, $P < 0.001$ for locomotor activity] (n = 9 mice per group, Fig. 6A). In contrast, treatment with gabapentin alone had no significant effect on the CPP score or locomotor activity in WT mice (n = 6 mice; Fig. 6B), which is consistent with previous findings in rats (Andrews et al. 2001).

In addition, we used *Cacna2d1* KO mice to ascertain the role of α2δ-1 in the rewarding effect of morphine. As expected, the CPP score and locomotor activity were significantly decreased in *Cacna2d1* KO mice compared with WT littermate control mice on the 2nd day [$F(3,40) = 24.61$, $P < 0.001$ for CPP; $F(3,40) = 47.98$, $P < 0.001$ for locomotor activity] and the 9th day [$F(3,40) = 24.61$, $P < 0.001$ for CPP; $F(3,40) = 47.98$, $P < 0.001$ for locomotor activity] after conditioning with morphine (n = 11 mice per group; Fig. 6C). Together, these results indicate that α2δ-1 is an important contributor to the rewarding effect of morphine.

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Disrupting α2δ-1–bound NMDARs in the NAc inhibits morphine-induced expression of CPP and locomotor sensitization

Lastly, we determined specifically whether α2δ-1–bound NMDARs in the NAc play a role in morphine-induced CPP and locomotor sensitization. We microinjected bilaterally a Tat-fused α2δ-1CT peptide or a Tat-fused scrambled control peptide (2 μg in 0.1 μl each side), via implanted cannulas, into the NAc core in WT mice 30 min prior to each morphine injection (10 mg/kg, *i.p.*) in a 6-day conditioning period. Compared with the control peptide group, concurrent treatment with α2δ-1CT peptide significantly decreased the CPP score and locomotor activity when tested on the 2nd day [$F(3,24) = 12.02$, $P < 0.001$ for CPP; $F(3,24) = 31.78$, $P < 0.001$ for locomotor activity] and the 9th day [$F(3,24) = 12.02$, $P = 0.0074$ for CPP; $F(3,24) = 31.78$, $P < 0.001$ for locomotor activity] after conditioning with morphine (n = 7 mice per group; Fig. 7). These findings suggest that α2δ-1–bound NMDARs in the NAc mediate the associative learning and memory of contextual cues paired with morphine treatment.

DISCUSSION

The primary finding of our study is that chronic morphine exposure, which consistently induces CPP and locomotor sensitization, augments NMDAR-mediated glutamatergic input to NAc neurons. The NAc, a major input structure of the basal ganglia, integrates information from cortical and limbic regions to mediate goal-directed behaviors (Kalivas & Volkow 2011; Kalivas & O'Brien 2008; McFarland et al. 2003). Heroin exposure can induce long-term potentiation in the NAc, and blocking NMDARs or AMPA receptors in the NAc attenuates heroin-seeking behaviors (Shen et al. 2011; LaLumiere & Kalivas 2008). Also, the expression of NMDAR subunits is increased in the NAc in morphine-dependent rats (Murray et al. 2007), and blocking NMDARs in the NAc inhibits opioid-seeking behaviors in animals (Bespalov & Zvartau 1996; Ma et al. 2006; Wu et al. 2012). Paradoxically, sharp-electrode recordings showed that evoked NMDAR-mediated EPSCs are reduced in the NAc core 4–6 days after implanting morphine pellets (75 mg) in rats (Martin et al. 1999). However, implanting morphine pellets does not induce CPP in rats (Smith & Aston-Jones 2014). Also, it is difficult to compare evoked EPSCs between different groups owing to the uncertainty of afferent pathways stimulated in thin brain slices. In addition, the high impedance of sharp electrodes results in large leak currents and makes voltage-clamp difficult. Because the low resistance of patch-clamp electrodes prevents current leakage and enables more accurate voltage clamp and analysis of medium spiny neurons in the NAc, whole-cell patch-clamp is a preferred configuration and was used in our brain slice recordings.

By recording AP5-sensitive presynaptic release of glutamate, we found that presynaptic NMDARs in the NAc are functionally quiescent under normal conditions, which is similar to findings in many other brain regions and the spinal cord (Chen et al. 2019; Ma et al. 2018a; Zhou et al. 2018). Strikingly, we showed that repeated treatment with morphine not only increased postsynaptic NMDAR activity but also induced tonic activation of presynaptic NMDARs in the NAc. Interestingly, even brief stimulation of μ-opioid receptors can lead to long-term potentiation mediated by presynaptic NMDARs in the spinal cord

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(Zhou et al. 2010). Furthermore, repeated treatment with morphine mainly potentiates presynaptic NMDAR activity in the spinal dorsal horn, which is a key mechanism responsible for opioid-induced hyperalgesia and tolerance (Zhao et al. 2012; Deng et al. 2019). The integrative function of synaptic NMDARs derives from their unique biophysical properties, including the Mg^{2+} block of NMDARs at resting membrane potentials, the high permeability to Ca^{2+}, and the slow kinetics of NMDAR-mediated synaptic potentials allowing for temporal summation (Traynelis et al. 2010). As a result, synaptic NMDAR plasticity represents a dominant mechanism for the fine-tuning of information encoding and storing in the CNS. The importance of NMDARs in the NAc in forming conditioned associations related to opioid exposure is consistent with the critical function of NMDARs in learning and memory in general. Thus, the aberrant hyperactivity of synaptic NMDARs can potentiate excitatory glutamatergic input from the medial prefrontal cortex, hippocampus, and basolateral amygdala to the NAc during the development of motivational learning associated with the rewarding effect of opioids.

Another salient finding of our study is that α2δ-1 is required for synaptic NMDAR hyperactivity in the NAc induced by repeated morphine exposure. We showed that systemic treatment with morphine potentiated the physical association of α2δ-1 with NMDARs and their synaptic trafficking in the NAc. Inhibition of α2δ-1 with gabapentin or α2δ-1 KO abolished morphine-induced hyperactivity of NMDARs at both presynaptic and postsynaptic sites in the NAc. In addition, uncoupling the α2δ-1–NMDAR interaction with α2δ-1CT peptide completely blocked morphine-induced potentiation of presynaptic and postsynaptic NMDAR activity in the NAc. These findings provide strong evidence indicating that α2δ-1–bound NMDARs are essential for augmented glutamatergic synaptic transmission in the NAc caused by repeated morphine exposure. Chronic treatment with morphine causes α2δ-1 upregulation in the frontal cortex and limbic forebrain in mice (Shibasaki et al. 2009). We found that although repeated treatment with morphine had no effect on α2δ-1 protein levels in the NAc, it potentiated the α2δ-1 interaction with NMDARs in the NAc. It is unclear how chronic morphine exposure promotes the association of α2δ-1 with NMDARs in the NAc. Treatment with morphine increases protein kinase C (PKC) activity in the NAc (Narita et al. 2002), and inhibiting PKC translocation in the NAc reduces morphine-induced CPP (Ping et al. 2012). We previously showed that α2δ-1 binds predominantly to phosphorylated NMDARs and that PKC activation increases NMDAR synaptic trafficking and activity only in the presence of α2δ-1 (Zhou et al. 2021b). Thus, repeated morphine treatment likely potentiates synaptic trafficking and activity of NMDARs in the NAc by inducing PKC activation and NMDAR phosphorylation, which can subsequently form a protein complex with α2δ-1.

Our study also reveals that α2δ-1–bound NMDARs in the NAc are key substrates for the reward memory conditioned with morphine. Opioid addiction development generally involves learning and memories that are associated with opioid exposure and related environmental cues with the positive emotion of reward. Opioid-induced behavioral sensitization is an associative learning process in which repetitive administration of opioids in a distinct environment produces enhanced behavioral and locomotor effects as a consequence of past exposure (Robinson & Kolb 2004; Koob & Le Moal 2001). Learned associations between drug-related cues and drug reward can lead to cravings that

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are powerful triggers of continued opioid use or relapse (Milton & Everitt 2012). As opioid reward is paired with specific cues and contexts, these environmental stimuli are linked to motivational significance through cortical glutamatergic input to the NAc (Kalivas & Volkow 2011). The CPP assay, which incorporates Pavlovian learning, memory, and motivated behaviors, is extensively used for studying the learning that associates drug use with the surrounding environment (Tzschentke 1998; Andrews et al. 2001; Bespalov & Zvartau 1996; Ping et al. 2012). In the present study, we found that systemic injection of gabapentin or α2δ-1 genetic KO remarkably inhibited morphine-induced expression of CPP and locomotor sensitization. In addition, we showed that disrupting α2δ-1–NMDAR interaction locally in the NAc similarly reduced CPP and locomotor sensitization 2 and 9 days after conditioning with morphine. Thus, α2δ-1–bound NMDARs in the NAc are critically important for the development of memories of relapse-provoking reward cues during the opioid abstinence period.

It has been shown that microinjection of gabapentin into the NAc core attenuates cocaine-induced addictive behaviors in rats (Spencer et al. 2014). Also, systemic treatment with gabapentin inhibits morphine-induced behavioral sensitization and CPP in mice (Shibasaki et al. 2009). However, these previous studies have attributed the gabapentin effect to inhibition of voltage-gated Ca^{2+} channels or thrombospondins. It should be noted that gabapentinoids have no effect on Ca^{2+} channel activity (Rock et al. 1993; Schumacher et al. 1998; Chen et al. 2018) or Ca^{2+} channel–mediated neurotransmitter release at presynaptic terminals (Brown & Randall 2005; Huang et al. 2022). Furthermore, α2δ-1 interacts with voltage-gated Ca^{2+} channels and thrombospondins via its VWA domain near the N terminus (Taylor & Harris 2020), whereas α2δ-1 forms a protein complex with NMDARs via its C-terminal domain (Chen et al. 2018). Because α2δ-1CT peptide, which specifically targets the α2δ-1–NMDAR interaction site (Chen et al. 2018; Luo et al. 2018), largely attenuated the rewarding effect of morphine, our findings suggest that α2δ-1–bound NMDARs in the NAc are a critical substrate for the development of cue memories of opioid reward. This α2δ-1–mediated NMDAR hyperactivity could maintain increased glutamatergic synaptic strength in the NAc, facilitating the formation and storage of associations between place or context and drug reward.

In conclusion, our findings provide new evidence linking α2δ-1 to increased synaptic NMDAR activity in the NAc and the rewarding effect of opioids. This information not only advances our understanding of synaptic and molecular mechanisms of opioid reward but also suggests new targets for treating opioid use disorders. Gabapentinoids and α2δ-1CT interfering peptides do not affect physiological α2δ-1–free NMDARs (Zhou et al. 2021b; Chen et al. 2018) and could therefore avoid the adverse effects associated with the use of general NMDAR antagonists. Thus, gabapentinoids could be repurposed for treating patients with opioid dependence and addiction. Consistent with this notion, initial studies suggest that gabapentinoids are promising in reducing opioid dependence in humans (Salehi et al. 2011; Kheirabadi et al. 2008). In addition, normal α2δ-2 activity in the brain is associated with many physiological functions (Brill et al. 2004; Ivanov et al. 2004; Wang et al. 2013). Unlike gabapentinoids, which inhibit both α2δ-1 and α2δ-2 (Gee et al. 1996; Marais et al. 2001; Fuller-Bicer et al. 2009), α2δ-1CT peptide specifically targets α2δ-1–bound NMDARs and could be further developed for clinical applications.

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List of Abbreviations:

AP5 D-2-amino-5-phosphonopentanoate

CPP conditioned place preference

KO knockout

mEPSC miniature excitatory postsynaptic current

NMDAR N-methyl-D-aspartate receptor

NAc nucleus accumbens

WT wild-type

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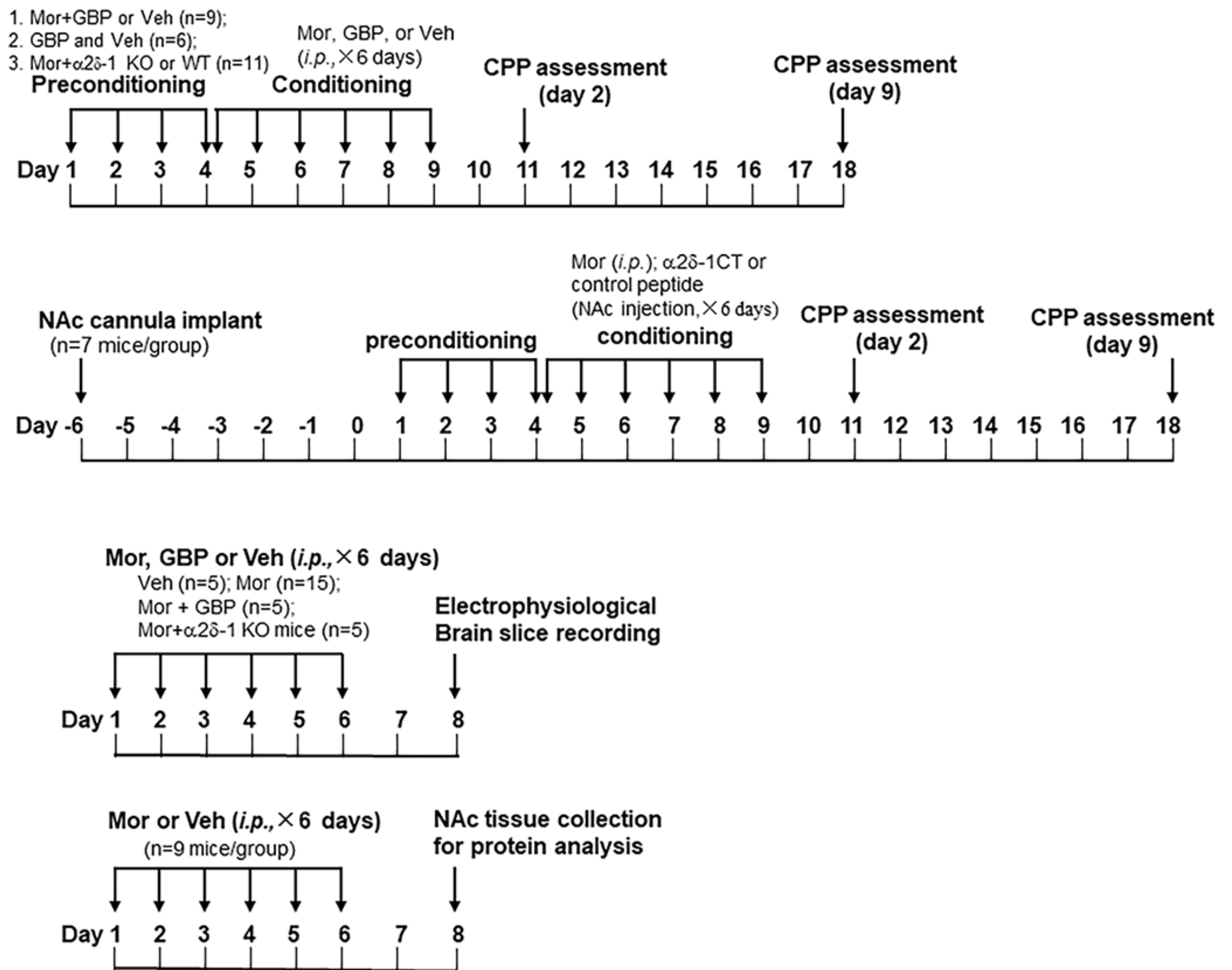

Fig. 1. Timelines of animal experiments.

Flowchart diagrams show the timeline of experimental procedures used for behavioral tests, biochemical assays, and electrophysiological recordings. The number of animals used in the experiments was indicated in parenthesis. GBP, gabapentin; Mor, morphine; Veh, vehicle.

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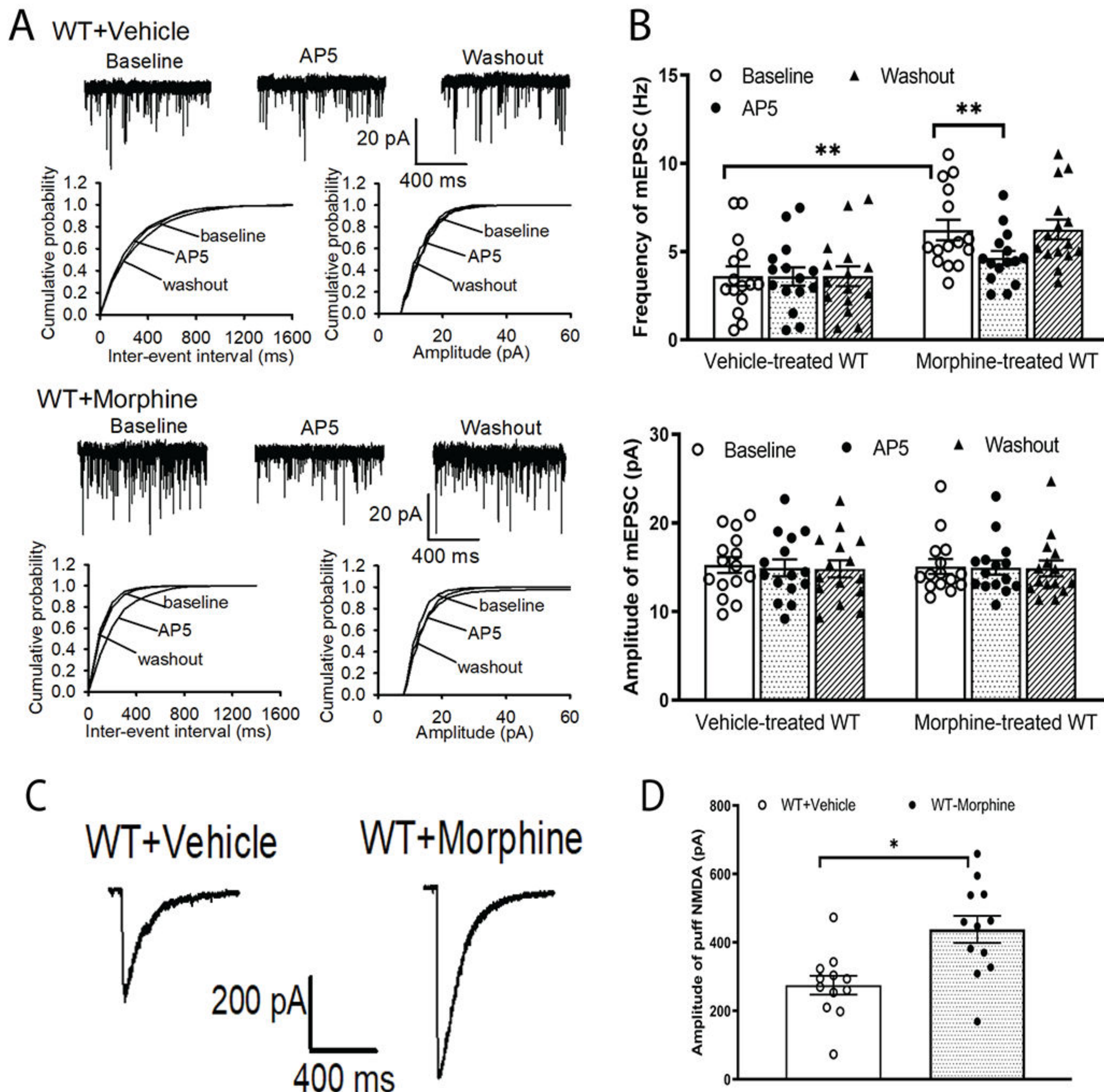

Fig. 2. Repeated morphine exposure increases presynaptic and postsynaptic NMDAR activity in the nucleus accumbens.

A and **B**, Representative current traces and cumulative plots (**A**) and mean changes (**B**) of mEPSCs of medium spiny neurons in the NAc core before (baseline), with (AP5), and after (washout) bath application of 50 μM AP5. Recordings were performed in brain slices from WT mice treated with vehicle or morphine (n = 15 neurons from 6 mice per group). **C** and **D**, Representative current traces (**C**) and mean changes (**D**) show NMDAR currents elicited by puff application of 100 μM NMDA onto medium spiny neurons in the NAc core from vehicle-treated or morphine-treated mice (n = 12 neurons from 5 mice per group). Data are

expressed as means ± SEM. *$P < 0.05$, **$P < 0.01$ (one-way ANOVA followed by Tukey *post hoc* test in **B**; two-tailed Student *t* test in **D**).

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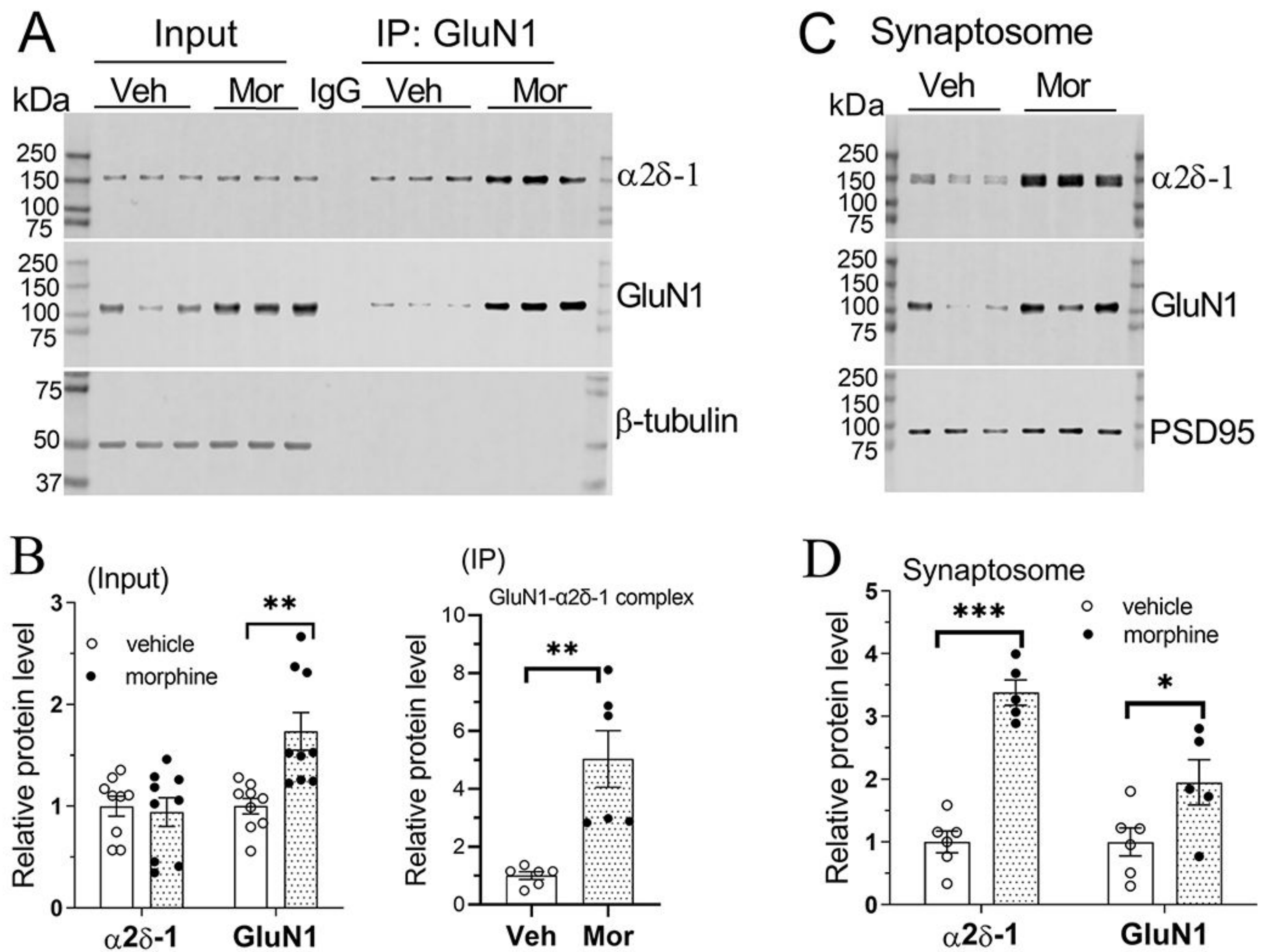

Fig. 3. Chronic morphine exposure increases α2δ-1–NMDAR interaction and their synaptic targeting in the nucleus accumbens.

A and **B**, Representative blotting images (**A**) and quantification (**B**) show the protein levels of α2δ-1 and GluN1 in the input (whole-tissue lysates, n = 9 mice per group) and immunoprecipitated (IP, n = 6 mice per group) by an anti-GluN1 antibody in NAc tissues. β-tubulin was used as a loading control for tissue lysates. WT mice were treated with morphine (Mor, 10 mg/kg, *i.p.*) or vehicle (Veh) daily for 6 days, and NAc tissues were collected on the 2nd day after the last injection. **C** and **D**, Representative blotting images (**C**) and quantification (**D**) show the protein levels of α2δ-1 and GluN1 in synaptosomes isolated from NAc tissues (n = 6 mice per group). PSD95, a synaptic protein, was used as a loading control for synaptosome samples. Data are expressed as means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (two-tailed Student *t* test).

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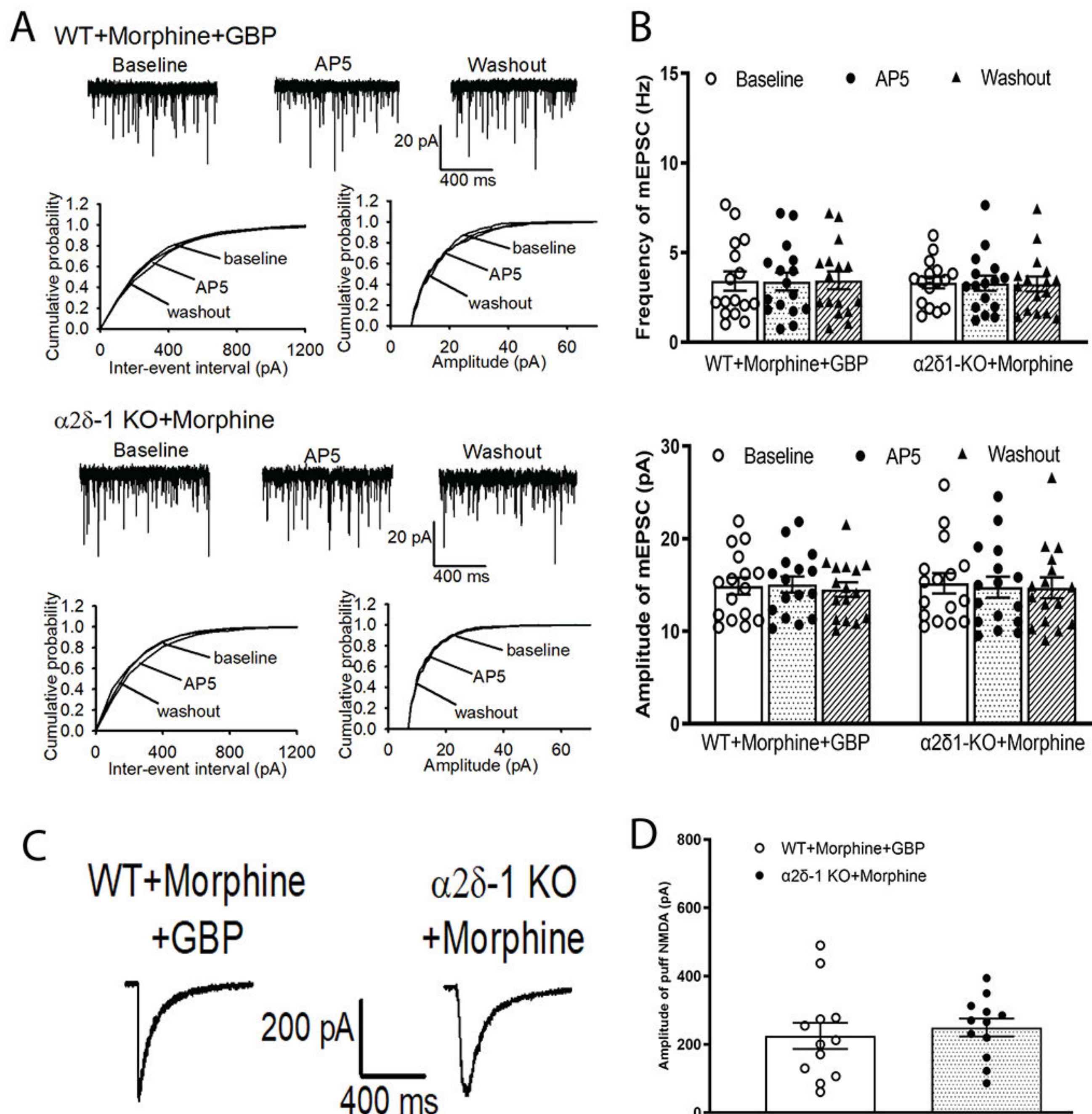

Fig. 4. Repeated morphine exposure increases synaptic NMDAR activity in the nucleus accumbens through α2δ-1.

A and **B**, Representative current traces and cumulative plots (**A**) and mean changes (**B**) of mEPSCs of medium spiny neurons in the NAc core before (baseline), with (AP5), and after (washout) bath application of 50 μM AP5. Recordings were performed using brain slices from morphine-treated WT mice incubated with 50 μM gabapentin (GBP) for 30 min and from morphine-treated *Cacna2d1*/α2δ-1 KO mice (n = 16 neurons from 5 mice per group). **C** and **D**, Representative current traces (**C**) and mean changes (**D**) show NMDAR currents

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elicited by puff application of 100 μM NMDA onto medium spiny neurons in the NAc core from morphine-treated WT mice incubated with 50 μM gabapentin for 30 min and from morphine-treated α2δ-1 KO mice (n = 12 neurons from 4 mice per group). Data are expressed as means ± SEM.

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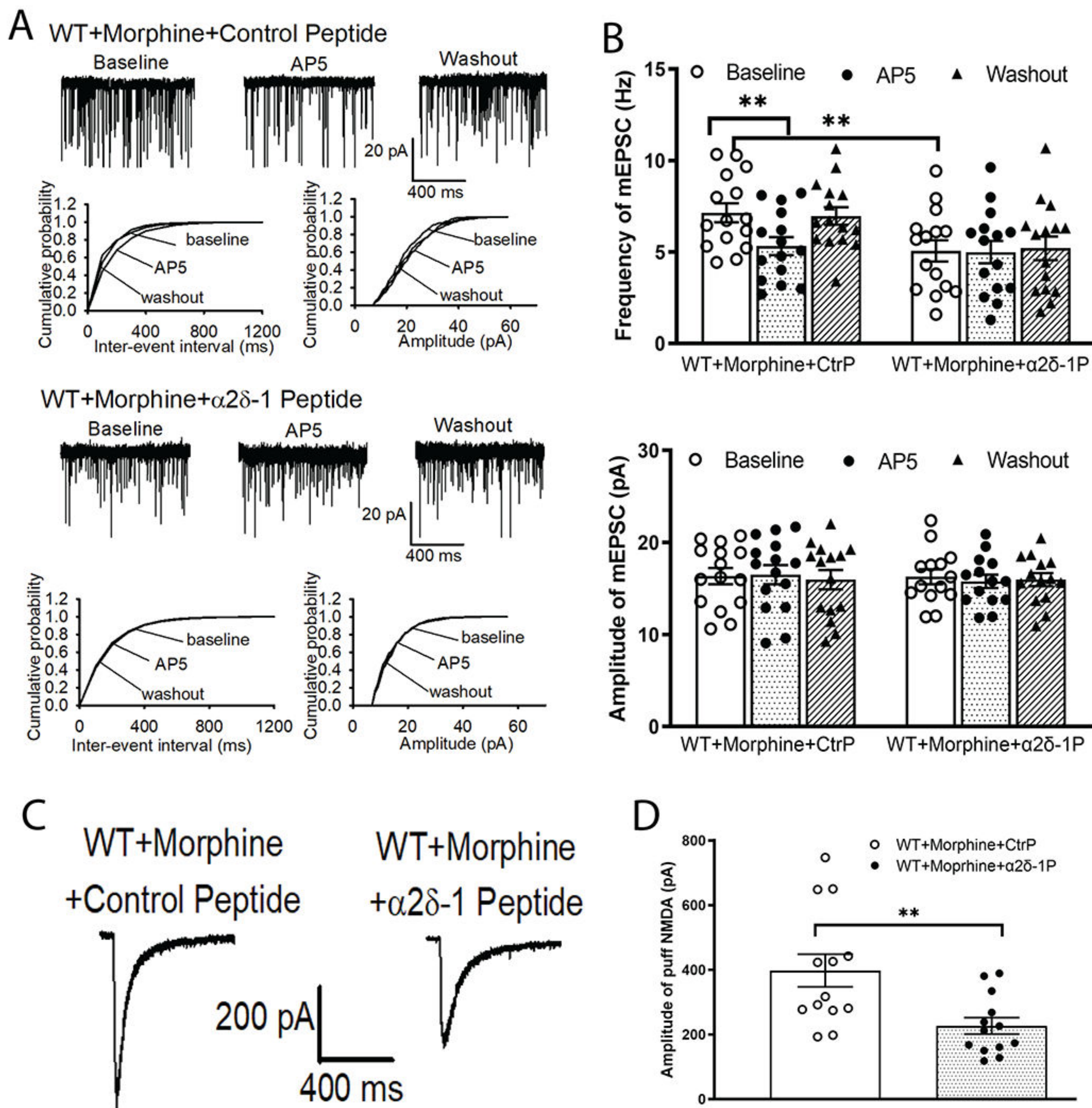

Fig. 5. Repeated morphine exposure increases glutamatergic input to the nucleus accumbens via α2δ-1–bound NMDARs.

A and **B**, Representative current traces and cumulative plots (**A**) and mean changes (**B**) of mEPSCs of neurons in the NAc core before (baseline), with (AP5), and after (washout) bath application of 50 μM AP5. Recordings were performed using brain slices from morphine-treated WT mice incubated with 1 μM α2δ-1CT peptide (α2δ-1P) or 1 μM control peptide (CtrP, n = 15 neurons from 5 mice per group) for 30 min. **C** and **D**, Representative current traces (**C**) and mean changes (**D**) show NMDAR currents elicited by puff application of 100

μM NMDA onto neurons in the NAc core from morphine-treated WT mice incubated with 1 μM α2δ-1CT peptide or 1 μM control peptide (n = 13 neurons from 4 mice per group). Data are expressed as means ± SEM. **$P < 0.01$ (one-way ANOVA followed by Tukey *post hoc* test in B; two-tailed Student *t* test in D).

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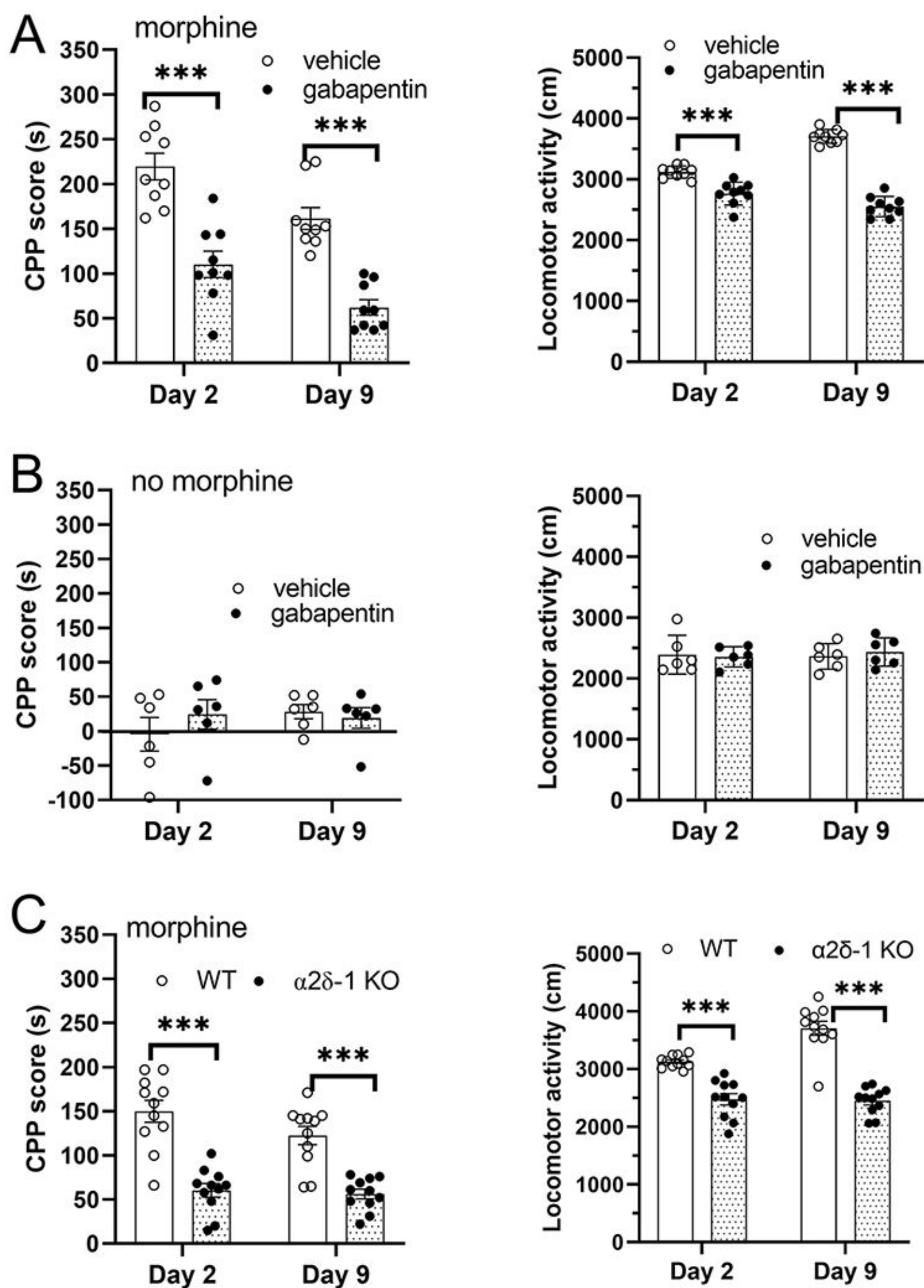

Fig. 6. α2δ-1 inhibition or genetic ablation reduces morphine-induced expression of CPP and locomotor sensitization.

A, CPP and locomotor activity tested 2 and 9 days after conditioning with morphine for 6 days in WT mice cotreated with gabapentin (60 mg/kg, *i.p.*) or vehicle (n = 9 mice per group). **B,** CPP and locomotor activity tested 2 and 9 days in WT mice after conditioning with gabapentin (60 mg/kg, *i.p.*) alone or vehicle for 6 days (n = 6 mice per group). **C,** CPP and locomotor activity tested 2 and 9 days after conditioning with morphine for 6 days in *Cacna2d1*/α2δ-1 KO and WT littermate control mice (n = 11 mice per group). Data are

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expressed as means ± SEM. ***$P < 0.001$ (one-way ANOVA followed by Tukey *post hoc* test).

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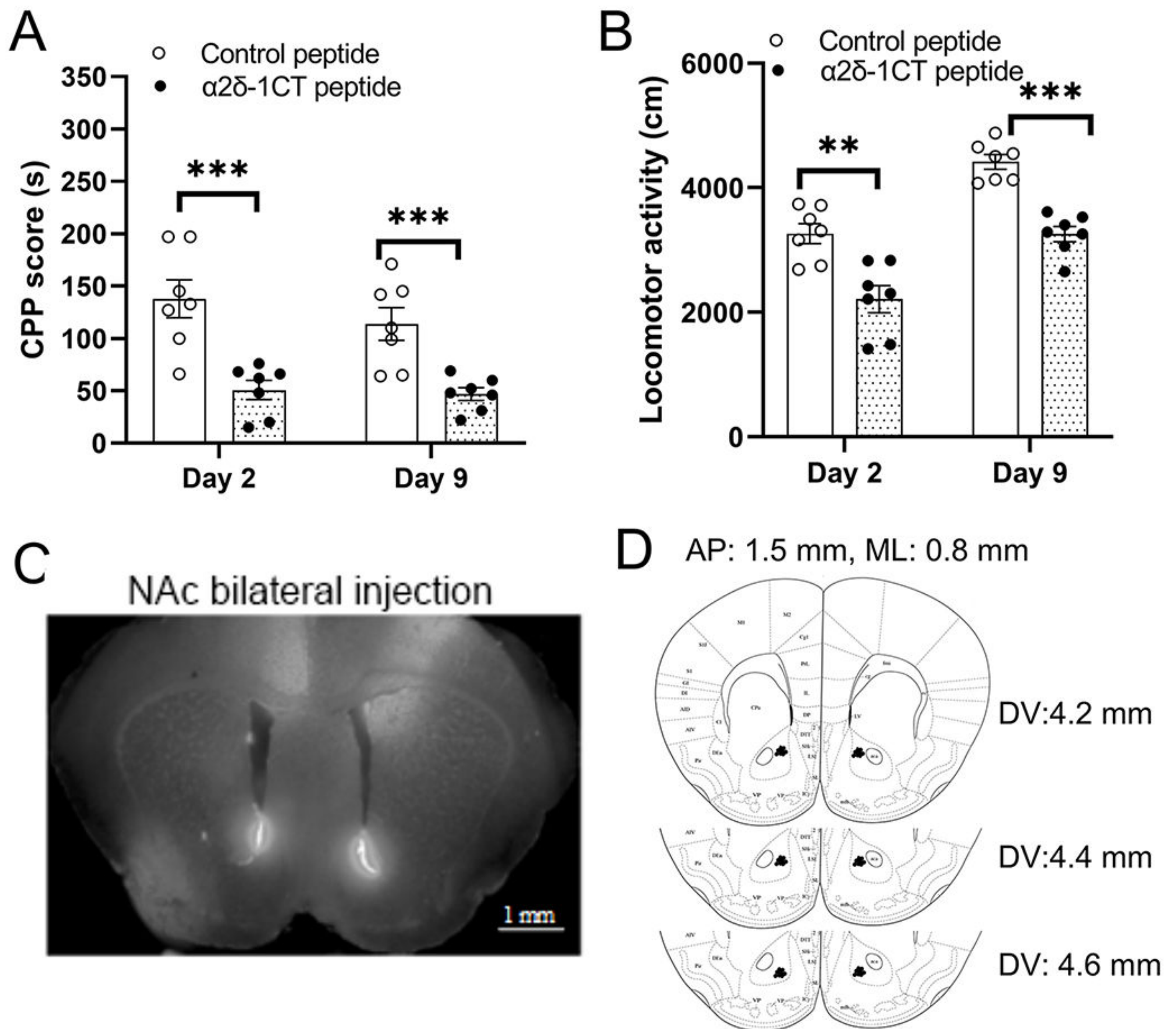

Fig. 7. α2δ-1–bound NMDARs in the nucleus accumbens mediate morphine-induced expression of CPP and locomotor sensitization.

A and **B,** CPP (**A**) and locomotor activity (**B**) tested 2 and 9 days after conditioning with morphine for 6 days in WT mice microinjected with α2δ-1CT peptide (2 μg) or control peptide (2 μg) into the NAc (n = 7 mice per group). Data are expressed as means ± SEM. $**P < 0.01$, $***P < 0.001$ (one-way ANOVA followed by Tukey *post hoc* test). **C** and **D**, Representative tissue section images (**C**) and schematic drawings (**D**) showing the sites of microinjection cannulas placed in the NAc core. AP, anteroposterior; ML, mediolateral; DV, dorsoventral.