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Research report

Over-expression of the GluN2B subunit in the forebrain facilitates the acquisition of morphine-related positive and aversive memory in rats

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HIGHLIGHTS

- Over-expression of GluN2B subunits facilitated the acquisition of morphine CPP.
- GluN2B transgenic rats showed more vulnerability to naloxone-induced CPA.
- Over-expression of GluN2B subunits had no effect on natural reward-induced CPP.

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ABSTRACT

GluN2B-containing *N*-methyl-D-aspartate (NMDA) receptors in the brain are known to have an important role in drug-associated learning and memory. Selective blockage of GluN2B-containing NMDA receptors (GluN2B-NMDARs) has been shown to impair morphine-induced conditioned place preference (CPP) without affecting natural reward-induced CPP. In the present study, GluN2B transgenic rats with over-expressed GluN2B-subunits in the forebrain were used to assess the susceptibility to CPP induced by morphine and natural rewards as well as to naloxone-induced conditioned place aversion (CPA). The results showed that GluN2B transgenic rats exhibited a relatively higher susceptibility to morphine-induced CPP and naloxone-induced CPA than their wild-type littermates did, while they retained the similar sensitivity as wild-type rats to CPP induced by natural reinforcers (food and sucrose). These findings suggest that increased level of GluN2B-NMDARs in forebrain facilitates formation of drug-related memory, but not that associated with natural rewards. GluN2B-NMDARs might be a potential target for the treatment of drug abuse.

1. Introduction

Relapse is thought to be the most intractable problem in the treatment of drug abuse. Drug-associated memory can persist throughout the lifetime of a patient and is a major contributor to relapse after abstinence. Therefore, the elimination of drug-associated memory is considered to be key to the treatment of drug addiction. Additionally, studies have demonstrated that natural reward- and addictive drug-induced learning and memory share some common neural circuits [1–3].

The *N*-methyl-D-aspartate (NMDA) receptor is a heteromeric complex composed of obligatory GluN1 and functional GluN2 subunits [4]. NMDA receptors (NMDARs), which are essential to normal learning and memory [5,6], have also been found to be deeply involved in addictive memory [7,8]. The predominant NMDARs in the brain area that are associated with addiction are the GluN2A-containing and GluN2B-containing NMDA receptors (GluN2B-NMDARs). The expression of GluN2A and GluN2B subunits markedly change over development [9,10] as the composition of the NMDAR in the synaptic area changes from being composed of predominantly GluN2B subunits to mostly GluN2A subunits during the course of postnatal development [11–13]. Interestingly, GluN2B-NMDAR levels increase following exposure to addictive drugs, such as cocaine or morphine [14–16], while synaptic transmission is enhanced. Recently, Dong and Nestler proposed a neural rejuvenation hypothesis of drug addiction [17]. According to this hypothesis, drugs of abuse could awaken and then utilize the highly efficient plasticity mechanisms that are normally associated with brain development within the reward circuitry to produce abnormally robust and stable memories that are related to addiction.

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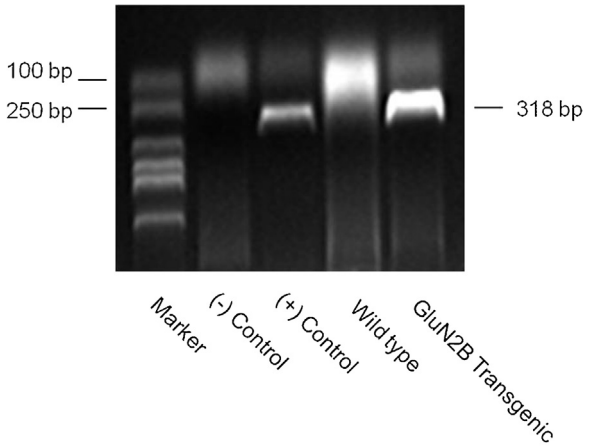

Fig. 1. Genotyping of transgenic rats offsprings by PCR. From left to right: marker, negative control, positive control, wild-type and transgenic rats.

Therefore, drugs that are able to reverse this shift toward increased GluN2B-NMDAR levels may reduce drug-associated memories.

An ideal approach for the treatment of drug abuse would be to weaken addictive memory, but not natural reward-related memory, to maintain the patient's quality of life. Our previous study showed that the selective GluN2B-NMDAR antagonist ifenprodil could inhibit morphine-induced conditioned place preference (CPP) without affecting the preference induced by natural rewards, such as food and social interactions [18]. In the present study, we adopted another strategy to further evaluate the possibility of regarding GluN2B-NMDARs as a therapeutic target for erasing morphine-related memory. We used GluN2B-subunit over-expressing rats, which showed enhanced spatial reference memory and working memory [19], to validate whether the over-expression of GluN2B subunits would selectively facilitate the acquisition of drug-associated memory without affecting natural reward-related memory. In addition to drug-related appetitive motivation, the aversive affective consequences of opiate withdrawal also contributed to drug relapse [20]. Therefore, naloxone-induced conditioned place aversion (CPA) was used to measure drug-associated aversive memory in GluN2B transgenic rats.

2. Materials and methods

2.1. Production and basic characterization of GluN2B transgenic rats

The GluN2B transgenic Long-Evans rat line was kindly provided by Dr. Joe Z. Tsien. The forebrain-specific CaMKII promoter was used to drive GluN2B transgene expression in the rat hippocampus and cortex [19]. The detailed procedures were described in a previous study [19]. The genotypes of all offsprings were analyzed by extracting DNA from tail samples and polymerase chain reaction (PCR). The 5′ and 3′ primers for detecting the GluN2B transgene SV40 polyA sequence were 5′-GCT AGA GGA TCT TTG TGA AGG AAC C-3′ and 5′-GGA AAG TCC TTG GGG TCT TCT ACC T-3′, respectively. DNA from tail samples was amplified over 35 cycles (5 min, 94 °C; 45 s, 94 °C; 45 s, 55 °C; 50 s, 72 °C) on a thermal cycler. Afterwards, the products were resolved using ethidium bromide-stained 1% agarose gels and visualized under u.v. light (Fig. 1). The amount of GluN2B proteins in the rat hippocampus and cortex was detected using western blots. A significant increase in the level of GluN2B protein was also observed (data not shown).

All experiments were performed on male GluN2B transgenic rats and their littermates, which weighed 180–200 g at the beginning of the experiment. Four rats per cage were housed in a thermo-regulated room (22 ± 1 °C) with a 12 h: 12 h light-dark cycle and had access to food and water *ad libitum*. Different groups were used for each experiment, with 113 total rats (Table 1). All experimental procedures were approved by the Animal Use Committee of Peking University Health Science Center and were carefully designed to minimize the number of animals used and their suffering.

Table 1
Grouping of the experimental animals.

Experiment	Morphine-induced CPP			
Group	Tg-MOR	Tg-SAL	WT-MOR	WT-SAL
N	12	6	10	6
Experiment	Naloxone-induced CPA			
Group	Tg-NLX	Tg-SAL	WT-NLX	WT-SAL
N	10	6	9	6
Experiment	Food-induced CPP			
Group	Tg-Food	Tg-Control	WT-Food	WT-Control
N	6	6	6	6
Experiment	Sucrose-induced CPP			
Group	Tg-Sucrose	Tg-Water	WT-Sucrose	WT-Water
N	6	6	6	6

WT: wild type; Tg: transgenic; SAL: saline; MOR: morphine; NLX: naloxone. Tg-MOR means transgenic rats received morphine conditioning. The same logic for Tg-SAL, Tg-NLX, WT-MOR, WT-NLX and WT-SAL.

2.2. CPP conditioning procedure

2.2.1. Apparatus

Place conditioning was conducted in a three-chambered CPP apparatus. Two black conditioning chambers, which contained distinct visual and tactile cues (A and C, $280 \times 220 \times 225\,mm^3$), were separated by a small gray center choice chamber B ($135 \times 220 \times 225\,mm^3$) that was accessed through guillotine doors. Chamber A had four light-emitting diodes (LEDs) that formed a square on the wall and a stainless-steel mesh floor ($130 \times 130\,mm^2$), while chamber C had four LEDs that formed a triangle on the wall and a stainless-steel rod floor (130 mm apart). Chamber B had gray walls and a plain floor (Fig. 2). Fourteen photobeams were placed across the chambers and were 47.5 mm apart. Using a computer interface, the time spent in each chamber was recorded by infrared beam crossings.

2.2.2. Pre-conditioning test

On day 0, the rats were placed in chamber B with the guillotine doors removed to allow them to explore the entire apparatus freely for 15 min.

2.2.3. Conditioning with morphine

Thirty four rats were used in the morphine-induced CPP (morphine CPP) experiment. Animals were allowed two training sessions per day (at 8:30 a.m. and 3:30 p.m.). During conditioning, transgenic and wild-type rats of the morphine-induced CPP group (Tg-MOR group, n = 12; WT-MOR group, n = 10) received an intraperitoneal (i.p.) injection of saline (2 ml/kg) and were confined in the saline-paired chamber (A or C, counterbalanced within groups) for 45 min. Seven hours later, 4 mg/kg of morphine (Pharmaceutical Factory of Qinghai, China) was administered before the morphine CPP rats were confined in the other chamber (C or A, morphine-paired chamber) for 45 min. Transgenic and wild-type rats of the saline CPP group (Tg/WT-SAL, n = 6) were injected with saline before being confined to both chambers (A and C). To investigate the progress of the acquisition of CPP, we alternately

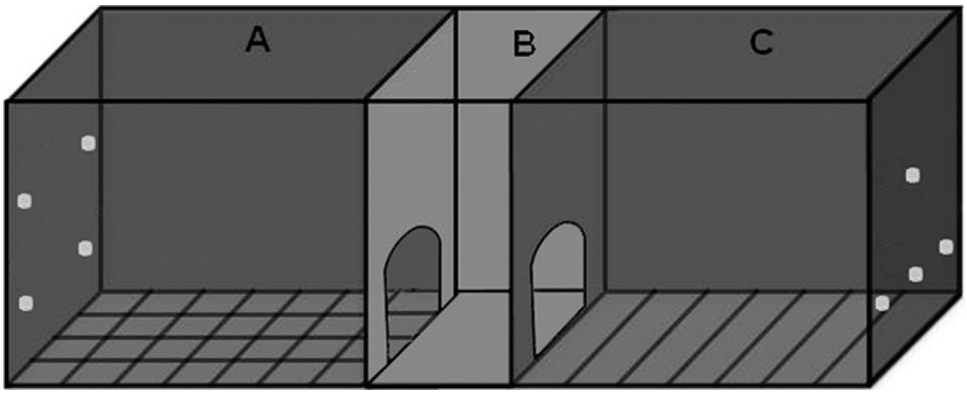

Fig. 2. Diagrams of the CPP apparatus.

performed conditioning and testing. Rats had four pairs of 45 min conditioning sessions on days 1, 3, 5 and 7 and were tested on days 2, 4, 6 and 8 (Fig. 3A).

2.2.4. Post-conditioning test

On the testing day, all animals were placed in chamber B with the guillotine doors removed to allow access to all compartments for 15 min.

2.2.5. Conditioning with naloxone

The naloxone-induced CPA experiment was performed in 31 rats. On the conditioning session, rats in the naloxone-induced CPA group (Tg-NLX, n = 10; WT-NLX, n = 9) were given 10 mg/kg morphine (i.p.) and then returned to their home cages. Twenty-four hours later, rats were intraperitoneally treated with 1 mg/kg naloxone (Sigma, USA) 5 min before being placed in the naloxone-paired chamber (A or C, counterbalanced within groups) for 30 min. On the next day, rats were given saline (24 h after saline injection) and placed in the other chamber (C or A, saline-paired chamber) for 30 min. Rats in the control groups (Tg/WT-SAL, n = 6) were given saline before being placed in chamber A and C. Post-conditioning tests were performed after each pair of conditionings (Fig. 4A).

2.2.6. Conditioning with food

During this experiment, all the 24 rats were deprived of food for 23 h per day. For the first eight days, rats were adapted to the feeding procedure. The rats were placed individually for 1 h into the 'feeding cages' (plastic cages that measured 275 × 215 × 130 mm^3, with a bottle of tap water and sawdust bedding). At the end of the eight-day period, the rats retained no less than 80% of their original body weight.

During the food conditioning sessions, the rats of the food-induced CPP group (Tg/WT-Food, n = 6) were placed in the 'feeding cages' with food for 1 h and were then transferred into the food-paired chamber (A or C, counterbalanced within groups) for 20 min. During the non-food conditioning sessions, rats were placed in the 'feeding cages' without food for 1 h and then transferred into the non-food-paired chamber (C or A) for 20 min. The rats in the control groups (Tg/WT-Control, n = 6) were given nothing before being confined in chamber A and C. They were fed once daily (1 h), at least three hours after conditioning or test (between 6:00 p.m. and 9:00 p.m.). The rats had three pairs of conditioning sessions (twice a day) on days 1, 3 and 5 and were tested on days 2, 4 and 6 (Fig. 5A).

2.2.7. Conditioning with sucrose

There were 24 rats used in the sucrose-induced CPP experiment. The day before the pre-conditioning test, rats were given one bottle containing a 20% sucrose solution and another bottle containing water to habituate them to drinking sucrose. In the sucrose conditioning sessions, each rat of the sucrose-induced CPP group (Tg/WT-Sucrose, n = 6) was given one bottle containing a sucrose solution and one bottle containing water. Fifteen minutes later, the rats were placed into the sucrose-paired chamber (A or C, counterbalanced within groups) for 20 min. In the non-sucrose conditioning sessions, each rat was given two bottles containing water for 15 min before being placed in the other chamber (C or A, water-paired chamber) for 20 min. The rats in the control group (Tg/WT-Water, n = 6) were given two bottles containing water before being placed in the apparatus. Rats had four pairs of conditioning sessions (twice a day) on days 1, 3, 5 and 7 and were tested on days 2, 4, 6 and 8 (Fig. 6A).

2.3. Data analysis

The CPP score represents the index of the place preference for each rat and was calculated by dividing the time spent in the morphine/food/sucrose-paired compartment by the time spent in both conditioning compartments during the CPP test. The CPA score was defined as the ratio of the time spent in the naloxone-paired chamber divided by the total time spent in both the naloxone and saline-paired chamber during the CPA testing. Data were processed by commercially available software GraphPad Prism 5.0. The results are presented as the mean ± SEM. The results of the CPP and CPA assays were analyzed using two-way ANOVA followed by the Bonferroni *post hoc* test. Statistical significance was at $p < 0.05$.

3. Results

3.1. GluN2B transgenic rats showed a higher sensitivity to morphine CPP induction than the wild-type rats

As depicted in Fig. 3, GluN2B transgenic rats showed a significant preference for the morphine-paired chamber after two sessions of morphine conditioning [Fig. 3B, Tg-SAL (n = 6) *vs* Tg-MOR (n = 12): two-way ANOVA, treatment (saline conditioning, morphine conditioning): $F_{(1, 80)} = 22.56$, $p < 0.0001$; day (pretest, test 1, 2, 3 and 4): $F_{(4, 80)} = 1.44$, $p = 0.2286$; day × treatment: $F_{(4, 80)} = 4.56$, $p = 0.0023$; Bonferroni *post hoc* test, $p < 0.01$, $p < 0.05$, $p < 0.001$, compared with Tg-SAL in Test 2, 3 and 4, respectively], while the wild-type rats showed a significant preference only after four sessions of training [WT-SAL (n = 6) *vs* WT-MOR (n = 10): two-way ANOVA, treatment: $F_{(1, 70)} = 11.06$, $p = 0.0014$; day: $F_{(4, 70)} = 0.47$, $p = 0.7553$; day × treatment: $F_{(4, 70)} = 2.10$, $p = 0.0904$; Bonferroni *post hoc* test, $p < 0.01$, compared with WT-SAL in Test 4]. There was a significant difference between the genotypes with respect to the speed of morphine CPP induction [Fig. 3C, n = 10-12; two-way

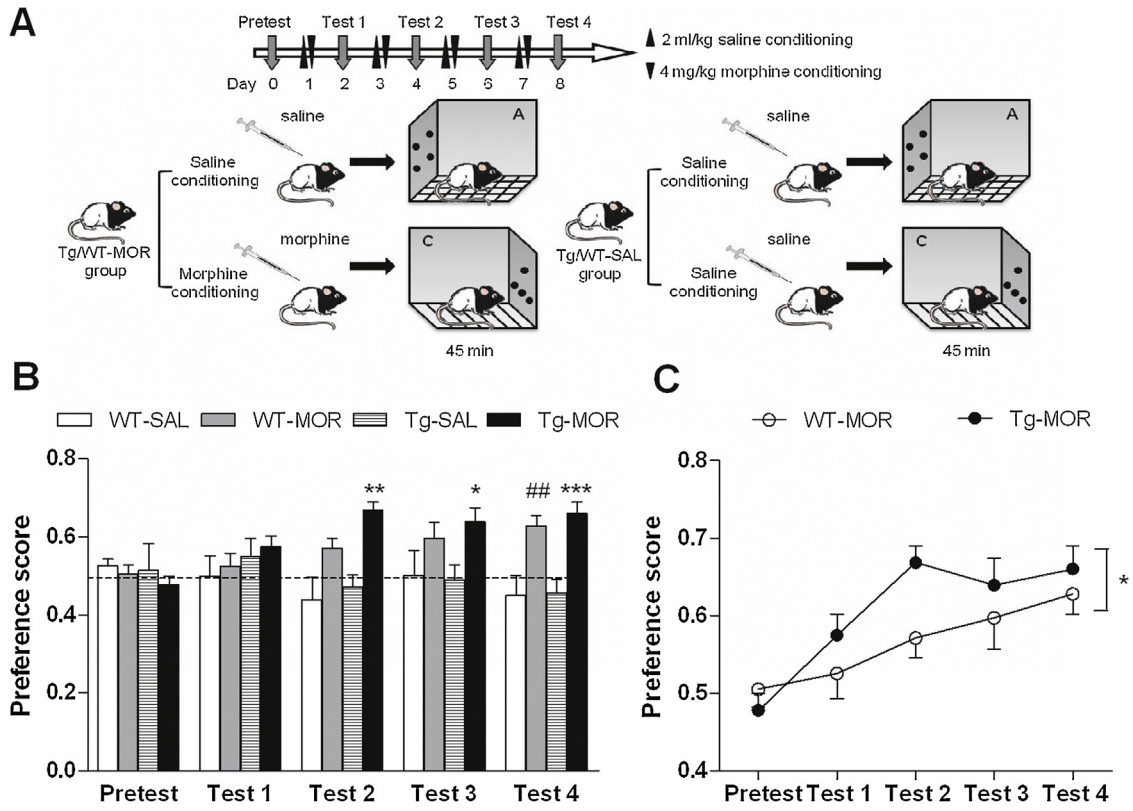

Fig. 3. GluN2B transgenic rats showed a high sensitivity to morphine CPP induction. (A) A schematic presentation of the experimental procedures. (B) Development of morphine-induced CPP in two genotype groups. The Tg-MOR group showed a significant preference for the morphine-paired chamber in Test 2, while the WT-MOR rats expressed CPP in Test 4. *, ##, **, ***$p < 0.05, 0.01, 0.001$, compared with their saline CPP control groups, respectively. n = 6–12. (C) Speed of morphine CPP induction in two genotype groups. *$p < 0.05$ between genotypes, n = 10–12. WT: wild type; Tg: transgenic; SAL: saline; MOR: morphine. Tg-MOR means transgenic rats received morphine conditioning. The same logic for Tg-SAL, WT-MOR and WT-SAL. The horizontal dotted line represents a score of 0.5.

ANOVA, genotype (WT and Tg): $F_{(1, 100)} = 4.48$, $p = 0.0367$; day: $F_{(4, 100)} = 9.46$, $p < 0.0001$; genotype × day: $F_{(4, 100)} = 1.20$, $p = 0.3171$].

3.2. GluN2B transgenic rats showed more vulnerability to naloxone-induced CPA than the wild-type rats

As depicted in Fig. 4, the GluN2B transgenic rats showed significant aversion after two sessions of naloxone conditioning [Fig. 4B, Tg-NLX (n = 10) *vs* Tg-SAL (n = 6): two-way ANOVA, treatment (saline conditioning, naloxone conditioning): $F_{(1, 56)} = 8.90$, $p = 0.0042$; day (pretest, test 1, 2 and 3): $F_{(3, 56)} = 1.80$, $p = 0.1569$; day × treatment: $F_{(3, 56)} = 5.67$, $p = 0.0018$; Bonferroni *post hoc* test, $p < 0.05$, $p < 0.001$, compared with Tg-SAL in Test 2 and 3, respectively], while their wild-type littermates showed significant aversion only after three sessions of training [Fig. 4B, WT-NLX (n = 9) *vs* WT-SAL (n = 6): two-way ANOVA, treatment: $F_{(1, 52)} = 8.93$, $p = 0.0043$; day: $F_{(3, 52)} = 2.84$, $p = 0.0468$; day × treatment: $F_{(3, 52)} = 3.00$, $p = 0.0390$; Bonferroni *post hoc* test, $p < 0.05$, compared with pretest]. The GluN2B transgenic rats were quicker to acquire the naloxone-induced CPA than their wild-type littermates [Fig. 4C, two-way ANOVA, genotype (WT and Tg): $F_{(1, 68)} = 3.31$, $p = 0.0732$; day: $F_{(3, 68)} = 11.47$, $p < 0.0001$; genotype × day: $F_{(3, 68)} = 2.79$, $p = 0.0470$]. The control groups (WT-SAL and Tg-SAL) never showed an aversive effect after training (Fig. 4).

3.3. GluN2B transgenic rats were not more susceptible to food-induced CPP than wild-type rats

Fig. 5 shows the profile of food-induced CPP in GluN2B transgenic rats and their wild-type littermates. An eight-day period of food deprivation (23 h per day) was sufficient to adapt the rats to only 1 h of feeding per day (Fig. 5B). There was no difference in the consumption of food between the two genotypes of rats (Fig. 5C). As shown in Fig. 5D, both of the genotypes displayed a significant preference for the food-related compartment after three sessions of training [n = 6; For WT-Control *vs* WT-Food, two-way ANOVA, treatment (saline conditioning, food conditioning): $F_{(1, 40)} = 4.74$, $p = 0.0354$; day (pretest, test 1, 2 and 3): $F_{(3, 40)} = 4.92$, $p = 0.0053$; day × treatment: $F_{(3, 40)} = 4.25$, $p = 0.0107$; Bonferroni *post hoc* test, $p < 0.01$, compared with WT-Control in Test 3. For Tg-Control *vs* Tg-Food, two-way ANOVA, treatment: $F_{(1, 40)} = 2.83$, $p = 0.1001$; day: $F_{(3, 40)} = 3.75$, $p = 0.0182$; day × treatment: $F_{(3, 40)} = 2.92$, $p = 0.0455$; Bonferroni *post hoc* test, $p < 0.01$ compared with Tg-Control in Test 3], which suggests a similar ability for developing the food-induced CPP between the two genotypes. Although both of transgenic and wild-type rats needed three trainings to acquire food-induced CPP, surprisingly, a significant effect of genotype was observed [Fig. 5E, two-way ANOVA, genotype (WT and Tg): $F_{(1, 40)} = 6.11$, $p = 0.0178$; day: $F_{(3, 40)} = 13.17$, $p < 0.0001$; genotype × day: $F_{(3, 40)} = 0.95$, $p = 0.4270$], showing wild-type rats got higher preference score than the transgenic rats.

3.4. Response to sucrose-induced CPP was similar between the two genotypes of rats

The results of sucrose-induced CPP are shown in Fig. 6. All rats appeared to prefer the sucrose solution to water [the intake of sucrose was significantly higher than water, For Tg-Water *vs* Tg-Sucrose, two-way ANOVA, treatment (water and sucrose): F

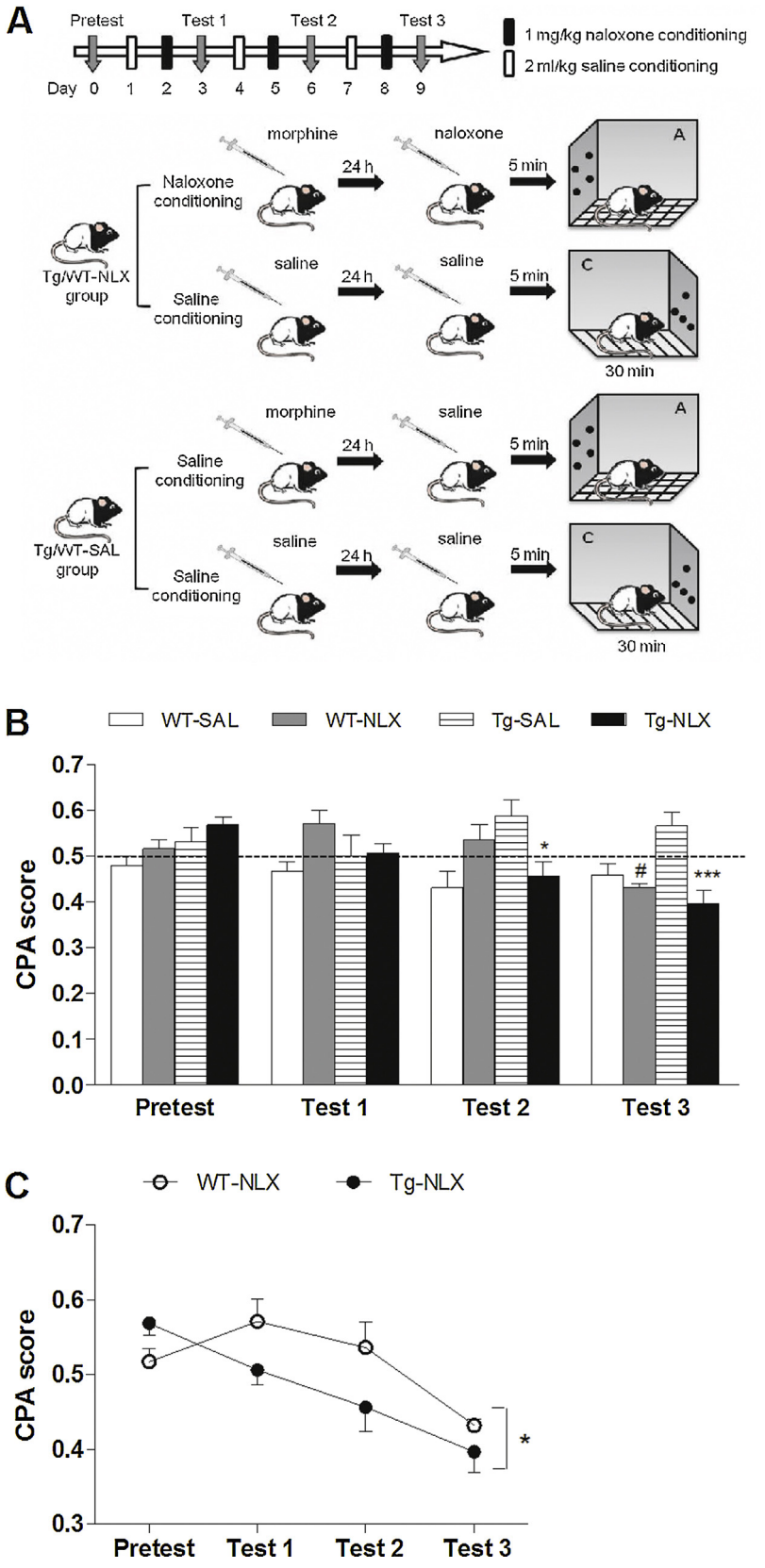

Fig. 4. GluN2B transgenic rats showed more vulnerability to naloxone-induced CPA. (A) A schematic presentation of the experimental procedures. (B) Development of naloxone-induced CPA in the two genotype groups. The Tg-NLX group showed a significant aversion for the naloxone-paired chamber in Test 2, while the WT-NLX rats expressed CPA in Test 3. #$p < 0.05$, compared with pretest; *$p < 0.05$, ***$p < 0.001$, compared with Tg-SAL groups in Tests 2 and 3, respectively. n = 6–10 (C) Speed of CPA induction in two genotype groups. *$p < 0.05$ between genotypes, n = 9–10. WT: wild type; Tg: transgenic; SAL: saline; NLX: naloxone. Tg-NLX means that transgenic rats received naloxone conditioning. The same logic for Tg-SAL, WT-NLX and WT-SAL. The horizontal dotted line represents a score of 0.5.

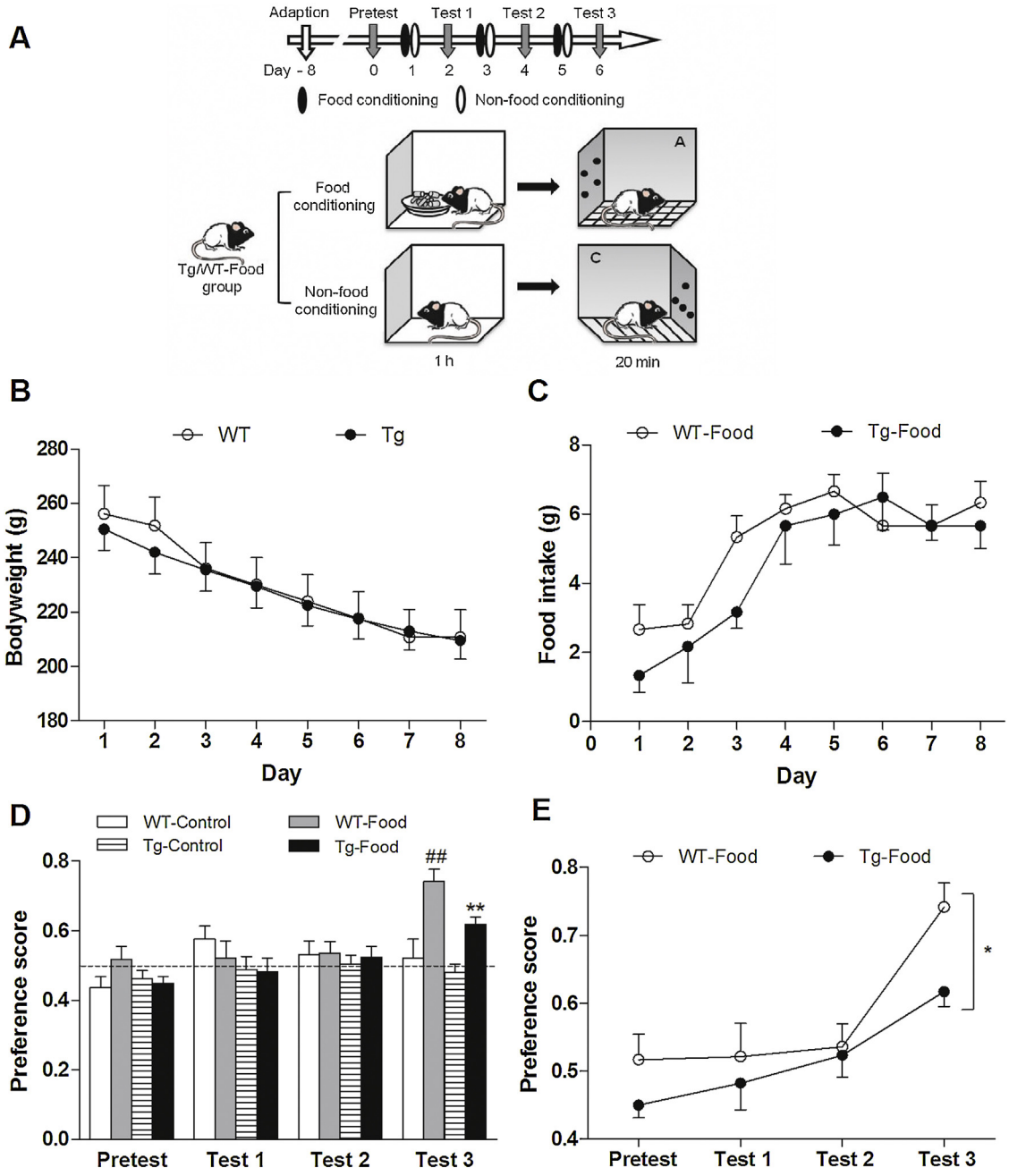

Fig. 5. GluN2B transgenic rats were not more susceptible to food-induced CPP than wild-type rats. (A) A schematic presentation of the experimental procedures. (B) There was no significant difference of bodyweight between genotypes. (C) Food intake over an eight-day period of daily food deprivation for 23 h. The amount of food consumed became stable by day six. There was no significant difference in food intake between transgenic and wild-type rats conditioned with food. (D) Development of food-induced CPP. Both of the genotypes displayed a significant preference for the food-related compartment in Test 3. ##, $^{**}p < 0.01$, compared with their control groups, respectively, n = 6. The horizontal dotted line represents a score of 0.5. (E) Speed of food CPP induction in two genotype groups. $^{*}p < 0.05$ between genotypes.

$_{(1, 50)} = 8.56$, $p = 0.0051$; for WT-Water *vs* WT-Sucrose, two-way ANOVA, treatment (water and sucrose): $F_{(1, 50)} = 10.56$, $p = 0.0021$]. There was no difference in the intake of sucrose between the two genotypes of rats (Fig. 6B). As shown in Fig. 6C, sucrose-induced CPP appeared after four sessions of training in GluN2B transgenic rats and wild-type rats [n = 6; for WT-Water *vs* WT-Sucrose, two-way ANOVA, day × treatment: $F_{(4, 50)} = 2.61$, $p = 0.0466$; Bonferroni *post hoc* test, $p < 0.05$, compared with WT-Water in Test 4. For Tg-Water *vs* Tg-Sucrose, two-way ANOVA, day × treatment: $F_{(4, 50)} = 2.58$, $p = 0.0487$; Bonferroni *post hoc* test, $p < 0.05$ compared with Tg-Water in Test 4]. There was no significant difference in the acquisition of sucrose-induced CPP between the two genotypes of the rats [Fig. 6D, two-way ANOVA, genotype (WT and Tg): $F_{(1, 50)} = 0.21$, $p = 0.6466$; day: $F_{(4, 50)} = 8.77$, $p < 0.0001$; genotype × day: $F_{(4, 50)} = 0.42$, $p = 0.7911$].

4. Discussion

Drug addiction is intimately related to pathological learning and memory. Various strategies have been used to study the key role of GluN2B-NMDARs in mediating addiction-related learning and memory. The commonly used procedures include the measurement of receptor abundance during the drug-related learn-

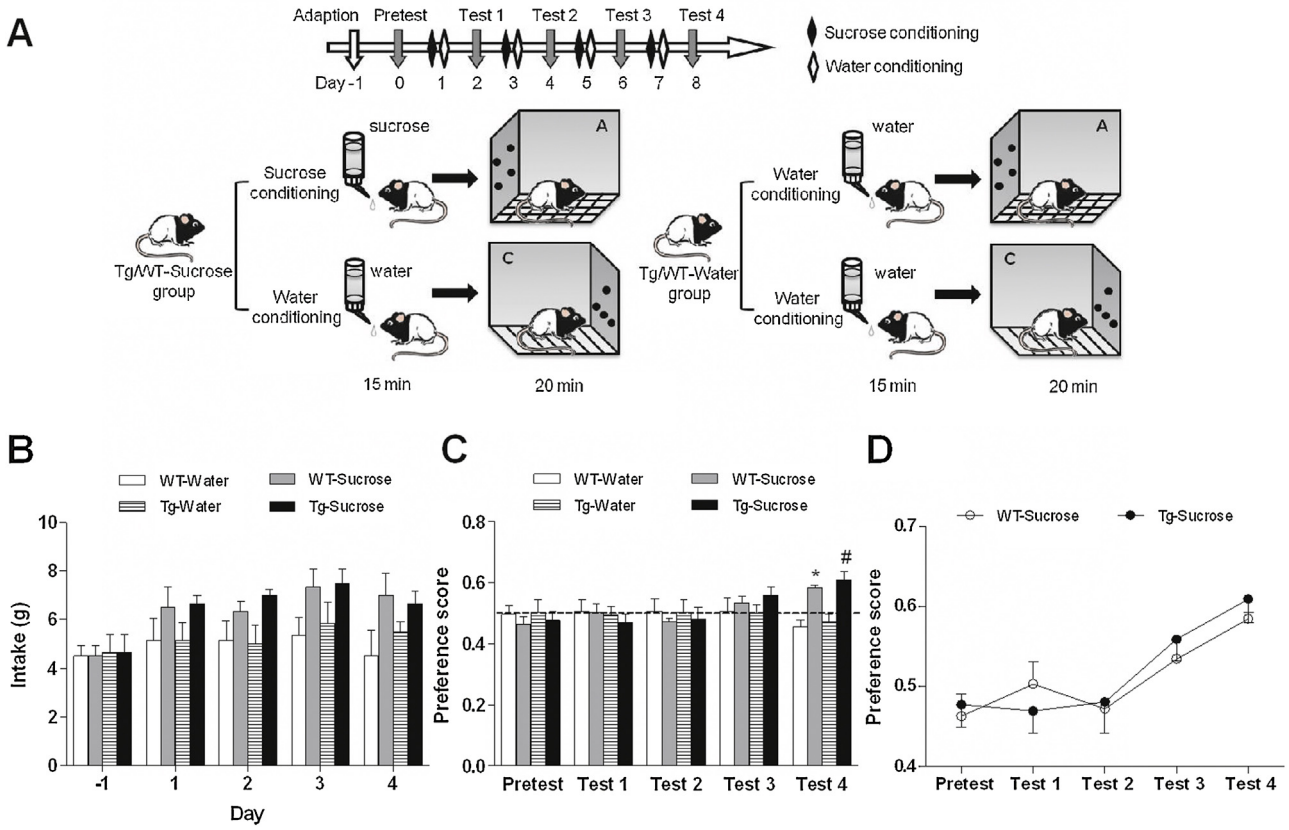

Fig. 6. No significant difference was found between two genotypes in the acquisition of sucrose-induced CPP. (A) A schematic presentation of the experimental procedures. (B) Sucrose and water intake of the first adaptation (day −1) and the sucrose-conditioning days (days 1, 3, 5 and 7). There was no significant difference in sucrose intake between the genotypes. (C) Development of sucrose-induced CPP. Both genotypes displayed a significant preference for the sucrose-related compartment in Test 4. *, # $p < 0.05$, compared with their control groups, respectively, n = 6. The horizontal dotted line represents a score of 0.5. (D) Speed of sucrose CPP induction in two genotype groups.

ing/memory process, and observing the deficit of addictive learning and memory after GluN2B-NMDAR deactivation.

Narita et al. found that the GluN2B subunit protein levels were specifically up-regulated in the limbic forebrain of morphine-conditioned mice [21]. In our previous work, an increase in the level of the GluN2B protein was observed in the hippocampus and nucleus accumbens (NAc) of morphine CPP rats, but not in rats with food-induced CPP. Further study revealed that this change in GluN2B subunit expression was induced by the combined action of morphine with the morphine-paired context, rather than the simple pharmacological effect of morphine because morphine injection in the home cage did not induce a similar change as that observed in the CPP paradigm [18]. GluN2B-NMDARs could be functionally blocked by antibodies or subunit selective receptor antagonists. Intracerebroventricular injection of an antibody against GluN2B subunits has been shown to abolish morphine-induced CPP in mice [21]. Likewise, microinjection of the selective GluN2B-NMDAR antagonist ifenprodil into the hippocampus or NAc inhibited the acquisition of morphine-induced CPP in rats [18].

Another strategy is to increase the abundance of GluN2B subunits in the brain and observe its effect on drug-related learning and memory. In GluN2B transgenic mice that over-express GluN2B-NMDAR in their forebrain, Tang et al. found that the hippocampal synapse showed significantly stronger NMDAR-mediated field EPSPs [22]. The over-expression of the GluN2B subunit in rats led to larger LTP in hippocampal slices than in wild-type rats [19]. Both transgenic mice and rats displayed an enhanced spatial learning and memory ability. In the present study, we demonstrated that GluN2B transgenic rats showed a significantly increased sensitivity to the morphine-induced CPP paradigm, as indicated by needing fewer conditioning to establish morphine-induced CPP.

A negative motivational state that is caused by the aversive effect of drug withdrawal has a key role in relapse. We wondered if GluN2B-NMDARs are also involved in the aversive memory that is associated with drug withdrawal. Compared with the wild-type rats, the GluN2B transgenic rats showed a significantly higher sensitivity to naloxone-induced CPA, thereby indicating that GluN2B subunits participated in drug-associated aversive memory. Blair et al. reported that injecting a selective GluN2B-NMDAR antagonist, ifenprodil, into the amygdala prevented the acquisition of auditory fear conditioning in rat [23], thereby suggesting that GluN2B-NMDAR activity in the amygdala is necessary for the association between aversive stimuli and the related environment. The crucial role of glutamate receptors has been observed in the motivational component of withdrawal during the acute morphine dependence. The NMDAR antagonist MK-801 significantly attenuated naloxone-induced CPA following microinjection into the NAc [24]. In line with the previous studies, our results further highlight the importance of GluN2B-NMDAR in naloxone-induced CPA.

Because the GluN2B subunit is involved in both drug-induced appetitive and aversive learning and memory, it may be regarded as a potential target to antagonizing drug-related memory. However, an ideal therapy for drug abuse should selectively interfere with the drug-associated responses while preserving natural biologically significant appetitive behaviors, thereby ensuring the quality of the patient's life [25]. Thus, this study exploited the food- and sucrose-induced CPP model to detect whether over-expression of GluN2B subunits promote the natural reward-related memory.

Concerning the possible differences that exist between two genotypes on natural reinforcement, we found that (a) there was no significant difference regarding the consumption of food during the period of food deprivation as well as the consumption of sucrose during sucrose-induced CPP training between wild-type and GluN2B transgenic rats; (b) transgenic rats were not more sensitive to food- or sucrose-induced CPP, thereby suggesting that the over-expression of the GluN2B subunit in the forebrain does not boost the basic requirement and desire for natural reward. These results were in line with those of a previous report that showed the NMDAR channel blocker memantine inhibited the expression of morphine-induced CPP, without affecting food-induced CPP [25]. Furthermore, the systemic administration of ACPC, a functional NMDAR antagonist, blocked morphine- and cocaine-induced CPP, but had no effect on the acquisition of place preferences that were conditioned with natural reinforcers (e.g., food, sucrose, social interaction and novelty) [26].

The mechanism by which GluN2B transgenic rats demonstrate high sensitivity to drug (morphine or naloxone)-induced contextual learning, but not to natural reinforcers (food and sucrose), is currently unknown. Activation of the dopamine (DA) D1 receptor is believed to selectively enhance the surface expression and function of GluN2B-NMDARs in prefrontal and striatum neurons [27–30]. Studies have shown that the neural circuits that are involved in drug- and natural-reinforcement overlap extensively. Both drug- and natural-reinforcements increase DA release in the NAc [31–36]; however, the most elevated DA concentration was highest during the self-administration of cocaine compared with sucrose [37]. The magnitude and duration of the changes in the excitatory transmission have also been suggested to be related to the type of reward [38]. Compared with food, cocaine self-administration produced the most robust increase in the ratio of AMPAR- to NMDAR-dependent currents in the ventrolateral bed nucleus of the stria terminalis [39]. The synaptic function of DA neurons in the ventral tegmental area was persistently enhanced by cocaine-seeking behavior, while the natural reward could only induce a transient synaptic potentiation [40]. Therefore, we proposed that the DA release that is induced by food- or sucrose-induced CPP expression could not reach the level that was required to up-regulate the surface expression and function of GluN2B-NMDARs and could not mediate persistent synaptic enhancement. This hypothesis could also explain why the GluN2B-NMDAR antagonist had no effect on the food-induced CPP [18] and why the GluN2B transgenic rats showed no difference in the natural reinforcer (food and sucrose)-associated memory compared with wild-type rats.

Conversely, brain areas that are activated by natural reward-related stimuli (e.g., food, sex) do not show a complete overlap with those that are activated by addictive drug-associated stimuli [41]. Carelli et al. found that cocaine could activate a population of neurons in the NAc that is generally not responsive during water and food reinforcement. These findings provide evidence that there are separate neural circuits in the NAc that process information about drug (cocaine) versus food and water reward [42].

One of the differences between drugs and natural reinforcers is the lack of feedback from the periphery for drugs, which may circumvent control from the hypothalamus in the development of reinforcement circuits [43]. Furthermore, natural reinforcers usually have exteroceptive visual, gustatory, and/or olfactory features, which are processed in brain areas that may be not involved in drug-induced CPP [26].

There were some weaknesses in this study. For example, because the regions in which GluN2B subunits were over-expressed included the whole forebrain, it is hard to determine the roles of GluN2B subunits in specific brain regions. Further studies are needed to compare the change in dopamine release that is induced by drug or natural stimuli in both transgenic and wild-type rats.

In conclusion, rats with over-expressed GluN2B-subunits in the forebrain showed significantly higher sensitivity to positive reinforcement of addictive drugs and aversion of drug withdrawal compared with their wild-type littermates. However, GluN2B transgenic rats did not promote the susceptibility in acquiring CPP that was conditioned by food/sucrose as wild-type rats. These findings suggest that GluN2B subunits facilitate drug-associated appetitive and aversive learning and memory, but not the processes that are induced by natural reinforcers, such as food/sucrose. These results provide new evidence for identifying GluN2B-NMDARs as a potential target for antagonizing addictive memory.

Conflict of interest

The authors declare that they have no financial relationship with the organization that sponsored the research. No conflict of interest exits in this manuscript.

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