Attenuated inhibition of medium spiny neurons participates in the pathogenesis of childhood depression

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Abstract

Accumulating evidence suggests that the nucleus accumbens, which is involved in mechanisms of reward and addiction, plays a role in the pathogenesis of depression and in the action of antidepressants. In the current study, intraperitoneal injection of nomifensine, a dopamine reuptake inhibitor, decreased depression-like behaviors in the Wistar Kyoto rat model of depression in the sucrose-preference and forced swim tests. Nomifensine also reduced membrane excitability in medium spiny neurons in the core of the nucleus accumbens in the childhood Wistar Kyoto rats as evaluated by electrophysiological recording. In addition, the expression of dopamine D2-like receptor mRNA was downregulated in the nucleus accumbens, striatum and hippocampus of nomifensine-treated childhood Wistar Kyoto rats. These experimental findings indicate that impaired inhibition of medium spiny neurons, mediated by dopamine D2-like receptors, may be involved in the formation of depression-like behavior in childhood Wistar Kyoto rats, and that nomifensine can alleviate depressive behaviors by reducing medium spiny neuron membrane excitation.

Key Words: nerve regeneration; brain injury; neurophysiology; MSNs; dopamine D2-like receptors; childhood depression; Wistar Kyoto rats; nucleus accumbens; excitatory inhibition; neural plasticity; nomifensine; NSFC grant; neural regeneration

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Introduction

Wistar Kyoto (WKY) rats have been reported to display a series of depression-like behaviors, such as significantly lower social play behavior (Malkesman et al., 2006), anhedonia in the saccharin preference test (Malkesman et al., 2005) and elevated forced swimming test immobility (Malkesman and Weller, 2009) compared with control Wistar rats. In the open field test, childhood WKY rats show severe anxiety-like behaviors compared with Wistar rats (Malkesman et al., 2005). Studies examining basal levels of monoamines and their metabolites in childhood WKY rats suggest that serotonergic and dopaminergic signaling is perturbed in some regions of the limbic system (De La Garza and Mahoney, 2004; Malkesman et al., 2009; Nagasawa et al., 2012). Adult WKY rats have extensively been used to study the neurobiological basis of depression and to evaluate treatment strategies (Braw et al., 2006; El Yacoubi and Vaugeois, 2007; Malkesman et al., 2007; Braw et al., 2009; Jeannotte et al., 2009; Carr et al., 2010; O'Mahony et al., 2010; Scholl et al., 2010; Tizabi et al., 2010; Pajer et al., 2012). These studies indicate that childhood WKY rats can serve as a suitable animal model of depression as well.

Pharmacotherapeutic studies of antidepressant treatment show that adult WKY rats exhibit a lowered response to acute or chronic imipramine treatment compared with Brown Norway or Sprague-Dawley rats in the forced swimming test (Lahmame et al., 1997). Adult WKY rats are also resistant to fluoxetine (a typical SSRI antidepressant drug) in the forced swimming test, although the drug has anxiolytic-like effects in the elevated plus-maze test 24 hours after treatment (Griebel et al., 1999).

Abnormal levels of monoamines, including norepinephrine, dopamine and serotonin, have been considered to contribute to depressive behaviors in WKY rats (Scholl et al., 2010; Lemos et al., 2011). WKY rats exhibit significantly reduced immobility and increased swimming time in the forced swimming test after chronic desipramine (a norepinephrine uptake blocker) and nomifensine (a norepinephrine and dopamine uptake blocker) treatment, and particularly elevated activity in the open field test after nomifensine treatment (Tajani-Butt et al., 2003; Getachew et al., 2010). Furthermore, the 5-HT2c receptor agonist WAY-163909 produces rapid onset antidepressant-like effects in WKY rats (Rosenzweig-Lipson et al., 2007).

The mesolimbic dopamine system, which plays a critical role in the rewarding effects of food, sex and drug abuse, has been considered to have a major role in the development of depressive symptoms as well (Nestler and Carlezon, 2006; Chen et al., 2010; Lammel et al., 2013). The ventral tegmental area and the nucleus accumbens (NAc) are the
most important anatomical substrates of the dopamine system (Koob and Le Moal, 2001; Juvenile et al., 2011; Trainor, 2011). Interrupting dopaminergic transmission in the ventral tegmental area-NAc pathway modulates depression-like behaviors in animal models (Nieoullon and Coquerel, 2003; Rada et al., 2003; Femenia et al., 2012). The antidepressant effect of the mesolimbic dopamine system has been studied extensively (Gershon et al., 2007; Chiba et al., 2010; D’Aquila et al., 2010). Several pharmacological experiments have shown that antidepressant treatments increase dopamine D2 receptor or/and D3 receptor binding activity in the region of the NAc (Lammers et al., 2000; Dziedzicka-Wasylewska et al., 2002; Gershon et al., 2007).

Therefore, to investigate the possible involvement of the NAc in childhood depression, we measured the membrane excitability of medium spiny neurons (MSNs) in the core of the NAc in WKY rats by using the patch clamp recording technique. We also examined the effects of chronic antidepressant treatment on depression-like behaviors and membrane excitability of MSNs in WKY rats. We provide evidence that dopamine D2-like receptor-mediated regulation of MSN excitability in the core of the NAc plays an important role in the pathogenesis of depression.

Materials and Methods

Animals

Male childhood Wistar rats (n = 20) and WKY (n = 20) rats (aged 21 days; Shanghai Laboratory Animal Center, Chinese Academy Sciences, Shanghai, China; license No. SYXX (Hu) 2007-0005) were used in this study. Wistar rats, as the outbred progenitor strain from which WKY rats are derived, are a proper control for WKY rats. All rats were housed individually in a 12-hour light/dark cycle room (lights on from 12:00 a.m. to 12:00 p.m.) with continuous access to food and water throughout the study. Room temperature was maintained at 25 ± 1°C, relative humidity 60%. All experiments were carried out in accordance with the Guidelines of the Bioethics Committee of Bio-X Institutes of Shanghai Jiao Tong University in China.

The 40 rats in this study were divided into six groups. Wistar rats (n = 6) and WKY rats (n = 6) were used for evaluating depression-like behavior. Among these, one Wistar rat used in the sucrose preference test was excluded due to bottle leakage (this rat drowned in the forced swim test). The antidepressant effect of nomifensine (an isoquinoline derivative that prevents dopamine reuptake into synaptosomes) treatment was measured and compared among four groups: Wistar saline (n = 6), Wistar nomifensine (n = 6), WKY saline (n = 8) and WKY nomifensine (n = 8). Rats in the Wistar nomifensine and WKY nomifensine groups were treated daily with intraperitoneal injection of 10 mg/kg nomifensine for 12 days. The vehicle groups (Wistar saline group and WKY saline group) were treated daily with 0.9% NaCl for 12 days. All 39 rats were used for electrophysiological recordings and real-time quantitative RT-PCR analysis.

Drugs

Nomifensine (Powder, 087K4064; Sigma-Aldrich, St. Louis, MO, USA) was dissolved in saline to the final concentration of 1 mg/mL.

Nomifensine treatment

Nomifensine (10 mg/kg; Sigma-Aldrich) solution was intraperitoneally injected into rats starting at the age of 21 days, once per day for 12 days. The rats in the vehicle groups were treated daily with 0.9% NaCl (Sangon Biotech, Shanghai, China) for 12 days.

Sucrose preference test

Before the sucrose preference test, all rats were habituated for at least 4 days to two bottles of water, and were housed individually (Bolanos et al., 2003). The animals were not previously deprived of water or food. The sucrose preference test was performed on the last day of drug treatment. During the test, each animal (32 days old) was presented with a bottle (60 mL) of 1% (g/mL) sucrose (Sangon Biotech) solution and a bottle of water. The animals had the opportunity to drink as much of the solutions as they wanted during 24 hours. We switched the order of the left/right bottles at 12 hours to avoid position preferences. The weight of the bottles before the experiment and after 24 hours was measured. The total consumption of water and sucrose in grams was used to calculate the preference as follows: consumed sucrose/(consumed water + consumed sucrose) (g/g) × 100%. Sucrose was dissolved in tap water. Food was available throughout the experiment.

Open field test

After the sucrose preference test was complete, the open field test was performed to assess exploratory activity and depressive behaviors (Miyakawa et al., 2001). Briefly, the apparatus was a gray isolation square (40 cm × 40 cm × 45 cm; Shanghai Mobile Datum Information Technology Co., Ltd., Shanghai, China). Each rat (33-day-old) was placed in the center of the field and adapted for 10 seconds, and the distance of spontaneous movement and the number of rearings were recorded for 5 minutes. After each test, the arena was cleaned with 75% alcohol solution and dried with a hair dryer.

Forced swim test

The forced swim test was utilized to measure depressive behaviors (Porsolt et al., 1978). Rats (34–35 days old) were subjected to two swim sessions for 2 consecutive days. On the first day, each rat was placed in a vertical Plexiglas cylinder (30 cm diameter and 45 cm high; Shanghai Mobile Datum Information Technology Co., Ltd.) filled with water (25 ± 1°C) to a depth of 35 cm. After 5 minutes, the rat was removed from the water and dried with towels and a hair dryer. The test sessions (5 minutes) were conducted 24 hours later in the same cylinder and recorded using a video camera in a room dimly illuminated. The total duration of immobility was used to evaluate the level of depression. The video recordings were analyzed by behavioral software (Shanghai Mobile Datum Information Technology Co., Ltd.), and reconfirmed by an observer with a stopwatch. The immobility measure was defined as follows: the lack of motion of the whole body, except for small movements necessary to keep the animal’s head above the water. Tank water was cleaned after each trial.
Electrophysiological recordings
The experimental conditions (e.g., control or nomifensine-treated rats) were unknown to the experimenters to avoid bias. Rats (33–35 days old; without any previous behavioral testing) were decapitated under inhalation halothane anesthesia, and the brain was immediately removed and immersed in ice-cold artificial cerebrospinal fluid (119 mmol/L NaCl, 2.5 mmol/L KCl, 1.3 mmol/L MgSO$_4$, 2.5 mmol/L CaCl$_2$, 1.0 mmol/L Na$_2$HPO$_4$, 26.2 mmol/L NaHCO$_3$, and 11 mmol/L glucose; pH 7.4; 295–305 mOsm/L).

All of these reagents were purchased from Sangon Biotech, Shanghai, China. Artificial cerebrospinal fluid was continuously oxygenated with 95% O$_2$ and 5% CO$_2$. Coronal slices (300-µm thickness) containing the NAc were cut using a Vibratome tissue slicer (Vibratome 1000, Vibratome Company, St. Louis, MO, USA). Before the experiments, slices were maintained in the holding chamber for at least 1 hour at 25°C.

Whole cell current-clamp recordings were carried out at 25°C. For recordings, slices were transferred to a submerged chamber at a flow rate of 2.5 mL/min. Artificial cerebrospinal fluid containing 1 mmol/L kynurenic acid (058K1517; Sigma) and 100 µmol/L picrotoxin (7A91015; Tocris, Bristol, UK) was used to block ionotropic glutamate and GABA$_A$ (γ-aminobutyric acid type A) receptors. Patch clamp recording pipettes (3–5 MΩ) were filled with internal solution (in mmol/L): K$^+$-glucuronate 120 mmol/L, HEPES 10 mmol/L, KCl 20 mmol/L, MgCl$_2$ 2 mmol/L, Na$_2$-ATP 3 mmol/L, Na$_2$GTP 0.3 mmol/L and bicynit 0.1% (g/mL) (pH 7.2, 290–310 mOsm). All reagents were purchased from Sigma. MSNs in the core of the NAc were visualized with differential interference contrast microscopy (Olympus Corporation, Tokyo, Japan) and identified by their unique electrophysiological properties (Kreitzer, 2009). Voltage signals were amplified and digitized using DigiData 1440A and pClamp 700B (Molecular Devices Corporation, Sunnyvale, CA, USA).

To examine the effects of the selective D2 receptor agonist quinpirole (10 µmol/L; 068K4603; Sigma) on the membrane excitability of MSNs, the number of action potentials and the threshold intracellular injection current to evoke the action potential were measured. The step-depolarizing currents ranged from 0 pA to 500 pA with 50 pA increments, lasting for 400 ms, and the inter-pulse interval was 20 seconds. The MSNs were recorded under a holding membrane potential of ~80 mV. Series and input resistances were monitored throughout each experiment. The cells were excluded from data analysis if more than a 25% change in series resistance occurred during the experiment.

Real-time quantitative RT-PCR
Rats were decapitated 24 hours under inhalation of halothane anesthesia after the last nomifensine treatment, and their brains were rapidly removed. Based on the atlas of Paxinos and Watson (Paxinos et al., 1985), tissue punches of the NAc, hippocampus and striatum were dissected and stored at ~80°C. Total RNA was extracted from tissues using Trizol reagent (Life Technologies Corporation, St. Louis, MO, USA) and reverse-transcribed. The first cDNA strand was generated by reverse transcribing 1.5 µg of total RNA (20 µL reaction volume) using MMLV reverse transcriptase (Epizentrum, Wisconsin, USA) at 37°C for 60 minutes and heated at 95°C for 5 minutes (Gene Amp PCR System 9700, Applied Biosystems, Foster, CA, USA). Quantitative real-time PCR analysis was performed using Rotor-Gene 3000 Real-time PCR system (Corbett Research, Cambridge, UK). The following primers were used:

| Gene  | Sequence (5’→3’) |
|-------|-----------------|
| Drd2  | Sense: GGT AAT GCC GTG GTG TGT C  |
|       | Antisense: GGT CTG TAT TGT TGA GTC CGA AG |
| Drd3  | Sense: GCT CCA TCT CCA ACC CTG A  |
|       | Antisense: TAT TGT TTG TCT TCT ATG TGC TCC |
| Dat1  | Sense: GGT TTG GAG TGC TGA TTG CC  |
|       | Antisense: AGA CGA CGA AGC CAG AGG AG |

The brain tissues from each group were assembled from three to six rats, and each quantitative real-time PCR analysis was repeated three times, and the data are presented as a ratio normalized to the expression of the GAPDH housekeeping gene. The average value was calculated as the mean. $C_{\text{in}(\text{DRO})}$ = 19.59; $C_{\text{in}(\text{DRD3})}$ = 21.58; $C_{\text{in}(\text{DAT})}$ = 19.66; $C_{\text{in}(\text{GAPDH})}$ = 20.48.

Statistical analysis
Data are presented as mean ± SEM. Statistical analysis was performed using SPSS 16.0 software (SPSS, Chicago, IL, USA), and significance was determined at P < 0.05. Group differences in the sucrose preferences test were analyzed with independent samples t-test. We analyzed within group differences in sucrose preference using the one sample t-test. Results of the open field test and forced swim test were analyzed with independent sample t-test. Non-normally distributed data were analyzed using the Mann-Whitney rank sum test. Comparisons of drug-induced changes in current-response (evoked spike) curves were carried out with repeated-measures analysis of variance. In addition, post hoc comparisons were carried out using the Student-Newman-Keuls test. The threshold current was analyzed with independent sample t-test.

Results
Comparison of depression-like behaviors between childhood Wistar and WKY rats
When all rats were 32 days old and were habituated for at least 4 days to the two bottles of water, depressive behavior in Wistar and WKY rats was evaluated using a series of behavioral tests, including sucrose preference, open field and forced swim tests. Wistar rats significantly preferred sucrose solution over water (vs. 50%; P < 0.005; Figure 1A). In contrast, the WKY rats did not show such a preference. In the open field test, compared with Wistar rats, the distance traveled and number of rearings were significantly decreased in WKY rats (P < 0.005 or P < 0.05; Figure 1B and C). In the forced swim test, WKY rats showed significantly increased immobility time compared with Wistar rats (P < 0.005; Figure 1D).
Figure 1 Depression-like behavior in childhood Wistar and Wistar Kyoto (WKY) rats.
Data are presented as mean ± SEM. Group differences in the sucrose preferences test were analyzed by one sample t-test. Results of the open field test and forced swim test were analyzed by independent sample t-test.
(A) In the sucrose preference test, childhood Wistar rats (n = 5) showed a significant sucrose preference, while childhood WKY rats (n = 6) had no preference. ##P < 0.005, vs. 50%.
(B) In the open field test, childhood WKY rats (n = 6) showed a significantly shorter distance compared with childhood Wistar rats (n = 6). ##P < 0.005, vs. Wistar rats. (C) In the open field test, childhood WKY rats (n = 6) showed significantly decreased rearing times compared with childhood Wistar rats (n = 6). #P < 0.05, vs. Wistar rats. (D) In the forced swim test, childhood WKY rats (n = 6) exhibited a significantly longer immobility time compared with childhood Wistar rats (n = 5). ##P < 0.005, vs. Wistar rats.

Figure 2 Depression-like behaviors in childhood Wistar and Wistar Kyoto (WKY) rats after nomifensine treatment.
Data are presented as mean ± SEM. Group differences in sucrose preferences test were analyzed by one sample t-test (compared with 50%). Results of the open field test and forced swim test were analyzed by independent sample t-test.
(A) In the sucrose preference test, childhood Wistar rats showed no change in the sucrose preference test after nomifensine treatment. In contrast, nomifensine-treated, but not saline-treated, WKY rats displayed a significant preference for sucrose solution. #P < 0.05, ##P < 0.005, vs. 50%. The open circles and open triangles represent saline-injected Wistar and WKY rats, respectively, and the closed circles and closed triangles represent nomifensine-treated Wistar and WKY rats, respectively.
(B) In the open field test, nomifensine treatment did not change traveling distance or (C) rearing times in Wistar or WKY rats. (D) In the forced swim test, nomifensine treatment significantly decreased immobility time. The white rectangle and the striped white rectangle represent saline-treated Wistar and WKY rats, respectively, and the grey rectangle and striped grey rectangle represent nomifensine-treated Wistar and WKY rats, respectively. Wistar saline group, n = 6; Wistar nomifensine group, n = 6; WKY saline group, n = 8; WKY nomifensine group, n = 8; ##P < 0.005, vs. WKY saline group.
Antidepressant effects of chronic nomifensine treatment in childhood WKY rats

At 21 days of age, chronic nomifensine treatment was given to Wistar and WKY rats. The effects of nomifensine on depression-like behaviors in these animals are presented in Figure 2. As shown in Figure 2A, nomifensine-treated WKY rats preferred sucrose solution over water more than expected randomly (50%) (one sample t-test, \( P < 0.05 \)). In contrast, WKY rats treated with saline did not show such a preference (\( P = 0.115 \)). Wistar rats treated with nomifensine or saline preferred the sucrose solution over the water more than expected randomly (50%) (saline-treated Wistar rats, one-sample t-test, \( P \leq 0.001 \); nomifensine-treated Wistar rats, one-sample t-test, \( P < 0.05 \)). In the open field test, neither Wistar rats nor WKY rats treated with nomifensine displayed significant differences from the control groups treated with saline in either distance or rearing (Figure 2B and C). In the forced swim test, nomifensine-treated WKY rats were significantly more active than WKY rats treated with saline; the immobility time was significantly shorter (Figure 2D, \( P < 0.01 \)). Wistar rats showed no significant difference.

D2-like receptor-mediated inhibition of membrane excitability was impaired in MSNs in WKY rats

The effect of the dopamine D2-like receptor agonist quinpirole on membrane excitability in MSNs was analyzed in WKY and Wistar rats at 35 days of age. All MSNs within the core region with a stabilized resting membrane potential between −85 and −60 mV were recorded (O’Donnell and Grace, 1993). Depolarizing current steps were used to evoke repetitive action potential firing (Figure 3A). There were no statistical differences in the numbers of action potentials (Figure 3B: (a) Wistar rats, \( n = 13 \); WKY rats, \( n = 9 \); repeated-measures analysis of variance, \( F_{(1,20)} = 0.386, P > 0.05 \)) or in the threshold current for induction of action potentials (Figure 3B: (b) Mann-Whitney rank sum test, \( P > 0.05 \)) between Wistar and WKY rats. In Wistar rats, bath application of quinpirole (10 \( \mu \)mol/L) significantly decreased the number of action potentials (Figure 3C: (a) before quinpirole vs. after quinpirole, \( n = 13 \), repeated-measures analysis of variance, \( F_{(1,12)} = 17.153, P < 0.05 \)) and increased the threshold current for induction of action potentials (Figure 3C: (b) before quinpirole vs. after quinpirole, \( n = 13 \), Mann-Whitney rank sum test, \( P < 0.005 \)). In contrast, in WKY rats, quinpirole did not change the number of action potentials (Figure 3D, left: before quinpirole vs. after quinpirole, \( n = 9 \), repeated-measures analysis of variance, \( F_{(1,8)} = 0.401, P > 0.05 \)), but significantly increased the threshold current for induction of action potentials (Figure 3D, right: before quinpirole vs. after quinpirole, \( n = 9 \), Mann-Whitney rank sum test, \( P < 0.05 \)).

Nomifensine treatment decreased membrane excitability of MSNs in WKY rats

When rats were 35 days old, chronic nomifensine treatment was terminated. Based on the preceding experiments, we surmised that the membrane excitability of MSNs in the NAc may have been altered by nomifensine treatment. Therefore, after nomifensine treatment, we further examined the membrane excitability of MSNs within the NAc core. As shown in Figure 4A, the number of action potentials was significantly decreased in nomifensine-treated WKY rats (WKY rats, \( n = 9 \); nomifensine-treated WKY rats, \( n = 7 \); post hoc test, at current intensities of 250, 300, 350 and 400 pA, \( P < 0.05 \)) and the threshold current for induction of action potentials was significantly increased (WKY rats, \( n = 9 \); nomifensine-treated WKY rats, \( n = 7 \); Mann-Whitney rank sum test, \( P < 0.05 \)). This suggests that the membrane excitability of MSNs was inhibited by chronic nomifensine administration. Bath application of quinpirole did not change either the number of action potentials (Figure 4B: before quinpirole vs. after quinpirole, \( n = 7 \), repeated-measures analysis of variance, \( F_{(1,12)} = 0.668, P = 0.70 \)) or the threshold current for induction of action potentials (before quinpirole vs. after quinpirole, \( n = 7 \), Mann-Whitney rank sum test, \( P = 0.378 \)).

Dopamine D2 receptor mRNA expression after chronic nomifensine administration in WKY rats

As shown in Figure 5A, in the NAc region, mRNA expression of the dopamine active transporter (DAT) was significantly decreased in nomifensine-treated WKY rats compared with Wistar rats or WKY rats treated with saline, while mRNA levels of dopamine receptors D2 and D3 (DRD2 and DRD3) were not changed. In the hippocampus, mRNA expression of DRD2 was increased in nomifensine-treated WKY rats compared with Wistar rats or saline-treated WKY rats, while DAT mRNA expression was decreased (Figure 5B). In the striatum, mRNA levels of DRD3 and DAT were decreased in nomifensine-treated WKY rats compared with saline-treated WKY rats (Figure 5C). DRD2 mRNA expression was up-regulated to varying degrees in the NAc, hippocampus and striatum of MKY rats after nomifensine treatment.

Discussion

Chronic nomifensine administration has antidepressant effects in childhood WKY rats

The WKY rat has been widely used to study the neurobiological basis of human depression and to determine the efficacy of treatment strategies (Malkesman et al., 2006; Ricart et al., 2011; Rosenfeld and Weller, 2012; Vinod et al., 2012). Childhood WKY rats show many depressive symptoms that mimic those in humans, such as longer immobility time in the forced swim test, less social play, anhedonia and anxiety-like symptoms (Malkesman and Weller, 2009; O’Mahony et al., 2011; Overstreet, 2012), as well as abnormal levels of monoamines, including dopamine and noradrenalin, in the brain (Krishnan and Nestler, 2008; Aan het Rot et al., 2008; Andrus et al., 2012). Our previous studies showed that chronic nomifensine treatment has antidepressant effects in adult WKY rats (data not shown). It has been reported by several studies that some brain regions, including the NAc, in WKY rats display low dopamine concentration and reduced metabolite turnover compared with control Wistar rats (De La Garza and Mahoney, 2004). In the current study, we demonstrated that nomifensine treatment for 12 consecutive days significantly alleviated depressive behavior in childhood WKY rats in the sucrose preference and forced swim tests. However, the antidepressant effect was not significant in the open field test. One possible reason for this lack of effect in the open field test is the hypersensitivity to stress.
Figure 3 Inhibitory effect of D2-like receptor activation on membrane excitability in medium spiny neurons (MSNs).

Data are presented as mean ± SEM. Comparisons of the drug-induced alterations in the current-response (evoked spikes) curves were carried out with repeated-measures analysis of variance. In addition, post hoc comparisons were carried out using the Student-Newman-Keuls test. The threshold current was analyzed by independent sample t-test. (A) The protocol for evoking action potentials and representative traces of evoked action potentials in MSNs. Representative traces showing action potentials evoked by injection of depolarizing current pulses of 400 pA. (B) a, current-evoked spike-response curves showing that the numbers of evoked action potentials were not different between childhood Wistar and WKY rats (repeated-measures analysis of variance, F(1,20) = 0.386, P > 0.05). b, the threshold depolarizing current for induction of action potentials was similar in Wistar and WKY rats (Mann-Whitney rank sum test, P > 0.05). (C) a, in Wistar MSNs, the number of evoked action potentials was significantly reduced at each current intensity after applying quinpirole (10 µmol/L; n = 13, repeated-measures analysis of variance, F(1,24) = 17.153, *P < 0.05). b, the threshold depolarizing current for inducing action potentials was significantly increased after quinpirole application (Mann-Whitney rank sum test, ##P < 0.005, vs before quinpirole). (D) a, in WKY MSNs, the number of evoked action potentials was unchanged after applying quinpirole (10 µmol/L; n = 9, repeated-measures analysis of variance, F(1,16) = 0.401, P > 0.05). b, the threshold depolarizing current for inducing action potentials was significantly increased after quinpirole application (Mann-Whitney rank sum test, *P < 0.05, vs before quinpirole). Wistar rats, n = 13; WKY rats, n = 9.
displayed by WKY rats. It has been shown that WKY rats exhibit excessive neuroendocrine and behavioral responses to stress and are especially prone to develop stress-induced depressive disorder (Lopez-Rubalcava and Lucki, 2000; Lei and Tejani-Butt, 2010; Lemos et al., 2011; Sterley et al., 2011; Xu et al., 2011; Nagasawa et al., 2012). In the forced swim test, the WKY rats were subjected to strong stress during the first day of swim practice. Nomifensine treatment may help to enhance the ability of WKY rats to resist stress-induced depression on the following day of formal testing. Nonetheless, the improvement in depressive behavior by nomifensine suggests that the dopaminergic system plays a major role in the pathogenesis of depression in childhood WKY rats.

**Nomifensine may mediate its antidepressive effects by decreasing membrane excitability in MSNs**

Dopamine receptors are divided into two categories based on sequence homology and pharmacological properties. DRD1 and DRD5 are classified as “D1-like”, while DRD2, DRD3 and DRD4 receptors are considered “D2-like” (Jaber et al., 1996). Studies have shown that chronic antidepressant treatment with drugs such as mianserin, imipramine and amitriptyline can increase D2-like binding activity in the NAc of rats. Moreover, the antidepressant effects can be blocked by D2-like receptor antagonists, and D2-like agonists also have antidepressant effects (Gershon et al., 2007; Hirvonen et al., 2011; Tsuchimine et al., 2012; Zurawek et al., 2013). Imaging studies have shown that DRD2 density is increased in the basal ganglia of depressive patients (D’Haenen H and Bossuyt, 1994; Marchand and Yurgelun-Todd, 2010). Collectively, these studies suggest that D2-like receptors play an important role in the pathogenesis of depression. In the current study, we found that the excitability of MSNs was modulated by D2-like receptor signaling, and that nomifen-
Figure 5 The mRNA levels of dopamine receptor D2 (DRD2), dopamine receptor D3 (DRD3) and dopamine active transporter (DAT) in saline-treated Wistar rats, saline-treated WKY rats and nomifensine-treated WKY rats.

Data are expressed as the ratio of target gene expression to GAPDH gene expression. (A) In the NAc region, mRNA expression of DAT was decreased in nomifensine-treated WKY rats compared with Wistar rats or saline-treated WKY rats, while mRNA levels of DRD2 and DRD3 were not changed. (B) In the hippocampus, mRNA expression of DRD2 was increased in nomifensine-treated WKY rats compared with Wistar rats or saline-treated WKY rats, while mRNA expression of DAT was decreased. mRNA expression of DRD3 was not changed among the three groups. (C) In the striatum, mRNA expression of DRD2 was increased in nomifensine-treated WKY rats compared with saline-treated WKY rats, while mRNA levels of DRD3 and DAT were decreased. WKY: Wistar Kyoto.
sine treatment inhibits the excitability of MSNs. This indicates that the regulation of MSN excitability by D2-like receptor signaling in the core of the NAc plays a key role in the pathogenesis of depression.

**DAT, DRD2 and DRD3 mRNA levels are regulated by nomifensine treatment**

In the NAC, DRD2 and DRD3 mRNA levels were not different among Wistar, WKY control and nomifensine-treated WKY rats, while DAT mRNA levels in nomifensine-treated WKY rats were decreased significantly. These data indicate that the changes in membrane excitability in MSNs may not be due to changes in expression levels of D2-like dopamine receptor or its binding capacity to dopamine, but result from changes in downstream signaling. It has been found that D2-like receptor signaling regulates the excitability of MSNs in the NAC by modulating activities of K+ and Na+ channels. Therefore, it is possible that the properties and/or regulation of K+ and Na+ channels play a major role in the pathogenesis of childhood depression. In the striatum, DRD2 mRNA levels were decreased in WKY rats compared with Wistar rats, and nomifensine treatment increased the expression of DRD2 mRNA, suggesting that dopamine D2-like receptor signaling in the striatum may also contribute to nomifensine's antidepressive effects in WKY rats.

Childhood depression has been of great concern recently, although it was nearly completely ignored until recently (Zalsman et al., 2006; Bhatia and Bhatia, 2007; Eiland et al., 2012). Early-onset episodes of depression strongly and persistently impair psychosocial function during development (Puig-Antich et al., 1993; Juvenile et al., 2009; Jonsson et al., 2011; Weir et al., 2012). In the current study, we investigated the molecular pathogenesis of childhood depression by using a putative genetic animal model (WKY rats). Because WKY rats are a relatively new animal model of childhood depression, and because nomifensine also alters norepinephrinergic signaling by inhibiting reuptake of the neurotransmitter in the central nervous system, more studies are required to more fully elucidate the molecular biological basis of this disorder. For example, the impact of dopamine on other subregions (e.g., hippocampus, prefrontal cortex, ventral tegmental area) and the role of norepinephrinergic signaling in childhood WKY rats needs to be clarified for a better understanding of the pathogenesis of this major neurological disorder.

**Author contributions:** Liu DD and Li ST designed the study. Liu DD, Hu LH, Zhang JQ and Zhang P conducted experiments. Liu DD and Li ST analyzed the data and wrote the manuscript. All authors approved the final version of the manuscript. 

**Conflicts of interest:** None declared.

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