RESEARCH ARTICLE

Lipopolysaccharide-Induced Depressive-Like Behavior is Associated with α$_1$-Adrenoceptor Dependent Downregulation of the Membrane GluR1 Subunit in the Mouse Medial Prefrontal Cortex and Ventral Tegmental Area

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Abstract

Background: Chronic stress-induced depressive-like behavior is relevant to inflammatory immune activation. However, the neurobiological alterations in the brain following the central inflammatory immune activation remain elusive.
Methods: Therefore, we investigated the neurobiological alterations during depressive-like behavior induced in mice by systemic administration of lipopolysaccharide (LPS; 1.2 mg/kg administered twice at a 30-min interval via intraperitoneal injection).
Results: At 24 h after the second administration of LPS, an increased immobility time in the tail suspension test and the forced swimming test were observed, as well as reduced sucrose preference. Protein levels of the AMPA receptor GluR1 were significantly decreased at the plasma membrane in the medial prefrontal cortex (mPFC) and ventral tegmental area (VTA), while levels of the GluR2 were increased at the plasma membrane in the nucleus accumbens (NAc) at 24 h after LPS. However, total GluR1 and GluR2 protein levels in the mPFC, VTA, and NAc were not affected by LPS. Moreover, LPS facilitated release of noradrenaline in the mPFC and VTA, but not in the NAc. Consistently, systemic administration of prazosin, an α$_1$-adrenoceptor antagonist, blocked the LPS-induced downregulation of the membrane GluR1 subunit in both the mPFC and VTA and also blocked the upregulation of the membrane GluR2 subunit in the NAc. Intracerebroventricular administration of prazosin 30 min before LPS injection abrogated the LPS-induced depressive-like behaviors. In opposition, administration of propranolol, a β-adrenoceptor antagonist, did not affect the LPS-induced downregulation of GluR1, the upregulation of GluR2, or the depressive-like behavior.
Conclusions: These results suggest that LPS-activated α$_1$-adrenoceptor-induced downregulation of membrane GluR1 in the mPFC and VTA is associated with inflammation-induced depressive-like behavior.

Keywords: α$_1$-adrenoceptor, depressive-like behavior, immune system, neuronal plasticity, reward system

Introduction

Since the initial proposal of the cytokine hypothesis of depression (Maes et al., 1991; Smith, 1991), many clinical studies have provided the evidence to support this hypothesis (Maes et al., 1997; Maes, 1999; Kim et al., 2007; Simon et al., 2008). In particular, meta-analyses have revealed that elevations of interleukin (IL)-6 and tumor necrosis factor (TNF)-α in peripheral blood are reliable biomarkers of depression (Zorrilla et al., 2001; Dowlati et al., 2010). It has been demonstrated that long-term administration of interferon (IFN)-α in patients with hepatitis C increases depressive symptoms compared with control patients not given this therapy over the same duration (Majer et al., 2008; Raison et al., 2010; Felger et al., 2012). Furthermore, it has been suggested that there is considerable overlap in symptom expression between cytokine-induced depression and idiopathic depression in medically healthy subjects (Capuron et al., 2009). Recent studies have also demonstrated that administration either of the bacterial endotoxin lipopolysaccharide (LPS) or of proinflammatory cytokines can cause depressive symptoms in rodents (Frenois et al., 2007; Koo and Duman, 2008; O'Connor et al., 2009; Bay-Richter et al., 2011) and humans (Reichenberg et al., 2001). Consistent with these results, IL-6 knockout mice exhibit resistance to stress-induced development of depression-like behaviors (Chourbaji et al., 2006), and TNF-α receptor knockout mice exhibit an antidepressant phenotype (Simen et al., 2006).

Because monoamine reuptake inhibitors and monoamine oxidase inhibitors have been shown to be effective against major depression, depletion of monoamines has been suggested as a central mechanism of major depression (Ressler and Nemeroff, 2000; Morilak and Frazer, 2004). However, because the currently available antidepressants have a therapeutic lag time before they become effective, more complicated neurobiological alterations must be involved in the development of depression. Recently, dopamine-related neuroanatomical alterations in the reward system in major depression have also been suggested (Tremblay et al., 2005; Nestler and Carlezon, 2006). Bidirectional neural encoding of action in dopamine neurons of the nucleus accumbens (NAc) affects depressive-like behavior (Tye et al., 2013). Phasic activation of ventral tegmental area (VTA) neurons projecting to the NAc induces susceptibility to social-defeat stress. Furthermore, inhibition of the VTA-NAc projection induces resilience, whereas inhibition of the VTA projecting to the medial prefrontal cortex (mPFC) promotes susceptibility (Chaudhury et al., 2013). Therefore, the contribution of the reward system is important for understanding the development of major depression.

Peripheral administration of LPS increases the extracellular concentration of noradrenaline, serotonin (Linthorst et al., 1996), and dopamine in the brain (Lavicky and Dunn, 1995). However, the role of these elevated monoamine system components in the induction of depressive-like behavior remains unclear. A recent study suggested that LPS-induced depressive-like behavior is mediated by activation of the N-methyl-D-aspartate (NMDA) receptor (Walker et al., 2013). Furthermore, LPS impairs the induction of long-term potentiation (LTP) in the hippocampus (Kelly et al., 2003; Kavanagh et al., 2004). In addition, it has been reported that the NMDA receptor–independent LTP was also impaired by chronic administration of LPS in rats (Min et al., 2009). Therefore, it is possible that the alteration of synaptic strengthening induced by neuromodulators in the brain is involved in the mechanism of depressive symptoms.

In the present study, we found that downregulation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor GluR1 subunit at the plasma membrane in the mPFC and VTA was induced at 24h after LPS administration. Conversely, upregulation of the membrane GluR2 subunit was observed in the NAc at 24h after LPS administration. Because LPS induced both depressive-like behaviors and downregulation of the membrane GluR1 subunit in the mPFC and VTA, and because upregulation of the membrane GluR2 subunit in the NAc was abrogated by the administration of prazosin, an α_1-adrenoceptor antagonist, we suggest that locus coeruleus activity-dependent neuroplasticity is part of the mechanism of inflammation-induced depressive-like behavior.

Methods

Chemicals and Antibodies

Rabbit polyclonal antibodies against AMPA-GluR1 (1:1000) were purchased from Abcam. A mouse monoclonal antibody against AMPA-GluR2 (1:1000) and Anti-NMDAR1 (clone 1.17.2.6) were purchased from Millipore. A mouse monoclonal antibody against Na^+/K^+ ATPase α-1 was purchased from Novus Biologicals LLC. A rabbit polyclonal antibody against the postsynaptic density protein-95 (PSD-95; 1:200) was purchased from Santa Cruz Biotechnology, Inc. A rabbit monoclonal antibody against β-actin (D6A8) was purchased from Cell Signaling Technology. Lipopolysaccharides (L3129, serotype 0127:B8), Prazosin hydrochloride (P7791), and Propranolol hydrochloride (P0884) were purchased from Sigma-Aldrich.

Ethics Statement, Animal Care, and LPS Administration

This study was approved by the Animal Care Committee of Ohu University. All animal procedures were performed in accordance with the guidelines of the Animal Care Committee of Ohu University (see Supplementary Materials for additional information). Special care was taken to reduce animal distress, and to use the minimum number of animals needed for all studies. Adult CD1 mice (male; aged 7 weeks) were supplied by Charles River Laboratories Japan, Inc. All mice were housed at 25 ± 2 °C on a 12-hour light (07:00-19:00) to 12-hour dark (19:00-07:00) schedule with ad libitum access to food and water. Mice were handled individually every day for the 10 days before the behavioral test. The mice that were to have a guide cannula implanted into the skull were handled individually once daily for the 4 days before surgery, and then twice daily during the 6 days after recovery from anesthesia until the behavioral tests were performed. LPS was dissolved in sterile endotoxin-free isotonic saline and administered intraperitoneally (at a dose of 2.4 mg/kg, given as two injections of 1.2 mg/kg each, with 19:00 [first injection] and 19:30 [second injection]) on the first day. The guide cannula–implanted mice used for studying intracerebroventricular drug administration had a higher activity level than the naïve mice, even though they were handled before performing the behavioral tests. Therefore, we believe that higher doses of LPS than those used in the present study are required for the induction of depressive-like behaviors. Additionally, we ascertained that our dose of LPS is within the appropriate range for the evaluation of depressive-like behavior in naïve mice.

Behavioral Tests

All behavioral tests were performed during the first 4h of the dark phase of the light-dark schedule (23:00 on the first day

for the locomotor activity test, corresponding to 4 h post-LPS, and 19:00 on the second day for the locomotor activity test, tail suspension test [TST], and forced swimming test [FST]). Brain tissues were harvested at 23:00 on the first day (corresponding to 4 h post-LPS) and 19:00 on the second day (corresponding to 24 h post-LPS) from mice that performed the behavioral tests.

Measurement of Locomotor Activity

Mobility time was measured as locomotor activity for 5 min in an acrylic plastic box (294 × 294 × 297 mm) lit with 40 lux. The box was covered by non-reflective paper on the bottom and the four inside walls. Each individual mouse was placed in the center of the open field, and was recorded by digital video camera in the absence of the investigator. Moving time was measured, except for periods of rearing and excreting, and was analyzed after the test.

Tail Suspension Test

For the TST, each mouse was individually suspended by the tail from a horizontal bar located 42 cm above the bench top using adhesive tape placed approximately 2 cm from the tip of the tail. Each mouse was suspended for 6 min via a hanging hook connected from the horizontal bar, and was recorded with a digital video camera in the absence of the investigator. The immobility time during TST was measured after the experiments. All vertical movements over 1 cm and movements consisting of twisting of the body were not counted, but the encompass immobility was included as the immobility time in this paradigm.

Forced Swimming Test

For the FST, each mouse was placed for 6 min in a 3000 mL glass beaker (15.3 x 22.5 cm) containing 2250 mL of water at 24 ± 1° C. The water was changed between test sessions. Test sessions were recorded from the front of the beaker by digital video that was set at the same vertical height as the surface of the testing water, and were analyzed and scored by an investigator blinded to the allocated group. Immobility duration and latency were measured for the last 5 min of the FST. All vertical movement over 1 cm were excluded from the measurements of immobility.

Sucrose Preference Test

Sucrose preference was evaluated using a two-bottle free choice paradigm. Each mouse was habituated to a solution of 2% sucrose in tap water for 24 h in its home cage. To assess individual sucrose intake, the mouse was deprived of water for 15 h in its home cage before LPS injection. Immediately following saline or LPS injection, each tested mouse was placed in its home cage for 48 h with access to two identical drinking bottles, containing either the 2% sucrose solution or normal sterilized water. To avoid any side bias during the test, the locations of the two drinking bottles were switched after 12 h. In order to exclude any bias of the activity phase according to the light-dark cycle, three mice of each group were started at 07:00, and the other three mice of the same group were started at 19:00. In order to evaluate the extent of anhedonia-like symptoms, we considered the sucrose preference of mice during the first 24 h post-LPS to be the effect of sickness, and during the second 24 h period (24–48 h post-LPS) to be the effect of depression.

Tissue Harvesting and the Plasma Membrane Extraction

At 4 h and 24 h after LPS, mice were euthanized for preparation of the samples from the mPFC, NAc, and VTA for western blotting. The regions of interest in both hemispheres were adapted from the digital Paxinos mouse brain atlas (Paxinos, 2004;

Supplementary Figure S1A). The method for extraction of the plasma membrane fraction was performed as described previously (Ohtake et al., 2014). The plasma membrane fraction of each sample of interest was extracted using a commercially-available plasma membrane extraction kit (BioVision, Inc.).

The fidelity of these fractions was validated by the near-absence of the plasma membrane proteins (Na^+/K^+ ATPase) in the cytosolic fraction and the absence of the cytosolic protein (β-actin) in the plasma membrane fraction. To confirm whether the postsynaptic proteins were collected with the plasma membrane, the existence of postsynaptic density protein (PSD)-95 in the plasma membrane fraction and the near absence of PSD-95 in the cytosolic fractions were verified by immunoblotting with anti-PSD-95 antibody (Supplementary Figure S1B).

Western Blotting

Western blotting analysis was performed as described previously (Ohtake et al., 2014). Briefly, the protein extracts were mixed with sample buffer containing 50 mM Tris-HCl (pH 7.6), 2% SDS, 10% glycerol, 10 mM dithiothreitol, and 0.2% bromophenol blue and boiled at 94 °C for 5 min. The samples were separated by electrophoresis by Sodium Dodecyl Sulfate Poly-Acrylamide Gel Electrophoresis (SDS-PAGE) in 8–10% gels, and transferred onto polyvinylidene fluoride (PVDF) membranes. The blots were detected (PerkinElmer, Inc., Waltham, Massachusetts) and were visualized using the Versadoc imaging system (Model 5000, BioRad). The protein bands were analyzed using ImageJ software (Version 1.48a, Wayne Rasband).

In Vivo Microdialysis

Anesthetized animals were placed in a stereotaxic frame (Narishige Group, SR-6M). Three holes were made through the skull using a dentist drill. One hole was made for implantation of a steel guide cannula (AG-4, Eicom), and the other two holes were made in order to anchor the stabilizing screws. The stereotaxic coordinates (in mm) were as follows: for the mPFC, anteroposterior (AP): +1.6, lateral (L): -0.6, depth (DV): -1.0; for the NAc, AP: +1.3, L: -0.6, DV: -4.6; and for the VTA, AP: -3.05, L: -0.4, DV: -4.4. Coordinates refer to bregma and the dura surface. The cannula was held in position by dental cement (GC Unifast II, Tokyo, Japan) attached to the stabilizing screw. The dummy cannula (AD-4, Eicom) was inserted into the guide cannula and fixed with cap nuts (AC-1, Eicom) until the behavioral experiments. After the mice awoke from the anesthesia, they were each caged individually until the experiments. A dialysis probe (A-I-8-02, Eicom) was carefully inserted into the pre-implanted guide cannula without anesthesia, fixed by a cap-nut, and perfused at a constant rate of 1 µl/min with artificial cerebrospinal fluid containing 150 mM NaCl, 3 mM KCl, 1.4 mM $CaCl_2$, 0.8 mM $MgCl_2$, and 1.0 mM $NaHPO_4$, which was adjusted to pH 7.4 with 0.1% endotoxin-free bovine serum albumin containing 0.1 mM EDTA and 4 mM sodium metabisulfite to protect against oxidation. Dialysates were collected using an Eicom cannula swivel unit (SSU-20) attached to an injector and a 25 µl Hamilton syringe, every 30 min after the 90 min stabilization period allowed to achieve a steady state in the freely moving mouse. A polytetrafluoroethylene coiling tube (CT-20, Eicom) was used to infuse the drug, and the mouse was allowed to move freely in the test cage during sampling.

Measurement of Noradrenaline

Dialysate samples (30 µL) were assayed by a competitive enzyme-linked immunosorbent assay (ELISA; LDN Noradrenaline ELISA

kit, BA E-5200, Labor Diagnostika Nord in triplicate against standard curves of known dilution and positive and negative controls as appropriate. According to the manufacturer's instructions, the detection limits of this ELISA kit is 4 pg/ml. The minimum concentration of noradrenaline standard solution we measured was 0.15 pg/30 ml and the coefficient of variation was 9.5% (n=3). The coefficient of variations of the ELISA measured in this study from all concentrations of standard solutions, as a measure of intra-assay variation, were 4.4–9.5% (n=5).

Intracerebroventricular Administration

The intracerebroventricular administration was performed as described previously (Ohtake et al., 2014). The guide cannula (AG-4, Eicom) was implanted projecting to the lateral ventricle using stereotactic coordinates (bregma, AP: –0.25 mm, L: 1 mm, DV: 2.25 mm). A drug infusion probe was used as a dialysis probe, and the tip (the dialysis membrane part) was cut. The mice were administered a 5 µl intracerebroventricular infusion of either saline, 70 mM prazosin, or 400 mM propranolol 30 min before LPS injection under freely moving conditions in the test cage.

Statistical Analysis

Unpaired *t*-tests, a two-way (time × treatment) analysis of variance (ANOVA), and a one-way (treatment) ANOVA were performed as appropriate. After ANOVA was performed, Bonferroni post-hoc test was used for comparisons between two independent groups. The Wilcoxon signed ranks test was used for analyzing the data for noradrenaline release. All statistical analyses were performed with StatView (Version 5.0, SAS Institute Inc.) or ystat2013.xls (Igaku-Tosho) as appropriate. All data in the bar and line graphs indicate the mean ± SEM. The levels of significance for all analyses were set at $p < 0.01$ or $p < 0.05$.

Results

Assessment of Mouse Depressive-Like Behavior

Before the investigation of LPS-induced neurobiological alterations in the mouse brain, we confirmed the effects of LPS on sickness behavior and depressive-like behavior to determine if the concentration of LPS was adequate. We measured the locomotor activity at 4 and 24 h after LPS injection. A two-way ANOVA (time × treatment) on the mobility time in the open field test demonstrated a significant interaction (Figure 1A; $F_{(1,20)} = 72.8$, $p < 0.001$). Bonferroni post-hoc tests revealed a significant difference between 4 h post-LPS versus 4 h post-saline ($p < 0.001$), but the decreased locomotor activity had recovered by 24 h post-LPS ($p = 0.429$, 24 h post-LPS vs. 24 h post-saline). Furthermore, a significant decrease in body weight was observed at 24 h post-LPS (Figure 1B; unpaired *t*-test, $p = 0.0053$). We next examined the mice at 24 h post-saline or post-LPS for the assessment of LPS-induced depressive-like behavior in the TST and FST. The immobility time during the TST for the mice in the LPS group was significantly longer than for the mice in the control group (Figure 1C; unpaired *t*-test, $p = 0.023$). The immobility time during the FST for mice in the LPS group was also significantly longer than for the mice in the control group (Figure 1D, unpaired *t*-test, $p = 0.013$). In addition, LPS-injected mice showed a significantly lower preference for sucrose compared with the saline-injected mice at both 0–24 h (Figure 1E; unpaired *t*-test, $p = 0.004$) and 24–48 h post-LPS (Figure 1F; unpaired *t*-test, $p = 0.047$). These results

indicate that the concentration of LPS used in the present study is an appropriate range for the assessment of depressive-like behavior.

LPS-Induced Downregulation and Upregulation

It has previously been suggested that the mesolimbic dopamine system has reward-related (Schultz et al., 1997), hedonic (Chaudhury et al., 2013), and motivational components (Ikemoto and Panksepp, 1999; Wise, 2004). In addition to these components, the VTA dopaminergic neurons modulate the neural representation in the NAc of escape behavior from aversive stimuli (Roitman et al., 2008; Tye et al., 2013). Because our LPS-injected mice showed lower sucrose preferences and increased immobility times in the TST and FST, we investigated whether LPS modulates neuronal activity in the reward system. To investigate the effects of LPS at 4 and 24 h on neuronal network activity in the reward system, we measured the protein levels of the AMPA receptor GluR1, the GluR2 subunit, and the NMDA receptor NR1 subunit at the plasma membrane in the mPFC, NAc, and VTA regions. One-way ANOVA (with the factor treatment) revealed that LPS affected the protein level of the GluR1 subunit at the plasma membrane in the mPFC (Figure 2A and B, $F_{(2,9)} = 102.044$, $p < 0.0001$) and VTA (Figure 2O and P, $F_{(2,9)} = 13.338$, $p = 0.0020$). Bonferroni post hoc tests between individual groups in the mPFC and VTA indicated that there were significant differences between saline and 4 h post-LPS in the mPFC ($p < 0.0001$), between control and 24 h post-LPS in the mPFC ($p < 0.0001$), between saline and 4 h post-LPS in the VTA ($p = 0.0007$), and between saline and 24 h post-LPS in the VTA ($p = 0.0059$). The protein level of the NR1 subunit at the plasma membrane in the mPFC was also affected by LPS (Figure 2A and D, $F_{(2,9)} = 37.733$, $p < 0.0001$). Bonferroni post hoc tests revealed a significant reduction in NR1 subunits at the plasma membrane at 4 h post-LPS in the mPFC ($p < 0.0001$), but recovery to the control level by 24 h post-LPS ($p = 0.1378$ for saline vs. 24 h-post LPS). However, the effects of LPS on the protein level of NR1 at the plasma membrane in the VTA were not significant (Figure 2O and R, $F_{(2,9)} = 1.778$, $p = 0.2235$ for VTA). Contrary to the results for GluR1 and NR1, the protein level of the GluR2 subunit was affected only in the NAc (Figure 2A, C, H, J, O and Q; $F_{(2,9)} = 22.027$, $p = 0.0003$). Bonferroni tests revealed that, compared with the control, the protein level of the GluR2 subunit at the plasma membrane was significantly increased in the NAc at both 4 h and 24 h-post LPS ($p = 0.0001$ for 4 h-post LPS vs. control and $p = 0.010$ for 24 h-post LPS vs. control). The total protein levels of the GluR1 and NR1 subunits in the mPFC (and VTA (Figure 2A, E and G for mPFC and Figure 2O, S and U) were not affected by LPS. Moreover, the total GluR2 protein level in the NAc was also unaffected by LPS (Figure 2H and M). From these data, we believe that long-term downregulation of the protein level of the GluR1 subunit at the plasma membrane in the mPFC and VTA is induced by LPS. Moreover, long-term upregulation of the membrane GluR2 subunit in the NAc is induced by LPS.

LPS Facilitation of Noradrenaline Release

Because the neurobiological effects of LPS in the NAc were different from those in the mPFC and VTA, we hypothesized that the origin of neurotransmission to the mPFC and VTA after LPS are different from that of the NAc. The activity of the monoamine system in the brain is very important for
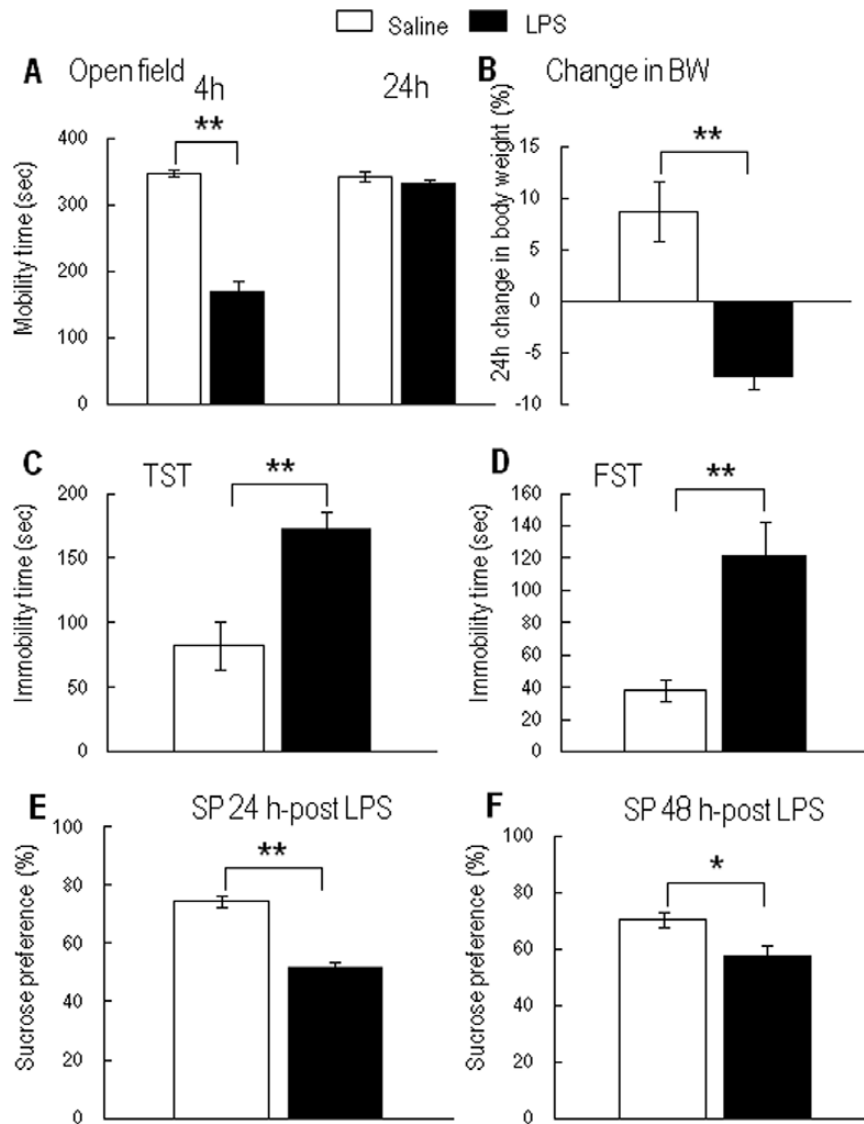
Figure 1. Mice were injected with LPS (1.2 mg/kg) twice with a 30 min interval. The locomotor activity was measured at either 4 or 24 h after LPS injection. The mobility time of mice in the open field was recorded over each 6 min period, and results are presented as mouse locomotor activity (A; control, n=6; LPS, n=6). Change in body weight at 24 h post-LPS was measured. The body weight of each mouse was measured at 5 min before and 24 h after LPS injection (B; control, n=6; LPS, n=6). The duration of immobility during the TST at 24 h post-LPS was recorded for 6 min (C; control, n=6; LPS, n=6). The duration of immobility during the FST at 24 h post-LPS was recorded each for 6 min (D; control, n=6; LPS, n=6). Sucrose preference was measured at 24 h post-LPS (E; control, n=6; LPS, n=6) and 24–48 h post-LPS (F; control, n=6; LPS, n=6) and the percentage of sucrose intake against total intake was calculated. All data are presented as means ± SEM. Statistically significant effects of LPS injection (**p < 0.01) are noted.

regulating mood and motivation (Ressler and Nemeroff, 2000; Morilak and Frazer, 2004). Dopamine neurons in the VTA project to neurons in the NAc and mPFC, and serotonergic neurons in the raphe nucleus project to the mPFC, NAc. and VTA. However, the primary source of adrenergic innervations to the NAc is the A2 region of the NTS, while the primary source of adrenergic innervations to the mPFC and the VTA is the locus coeruleus (LC; Mitrano et al., 2012). Therefore, we tested whether the effect of LPS on noradrenaline release in the mPFC, NAc, and VTA is different. Dialysates were discarded if the probe location was not in the correct area (mPFC, NAc, or VTA), and we verified the probe location histologically after each experiment (Figure 3A, C, and E). We injected LPS in freely moving mice 1.5 h after the insertion of the dialysis probe. As shown in Figure 3B and F, the amounts of dialysate noradrenaline in the mPFC and VTA were significantly increased at

30–60 min after LPS injection (Wilcoxon signed rank test; mPFC: $p = 0.0216$ for 30 min and $p = 0.0047$ for 60 min; VTA: $p = 0.0012$ for 30 min and $p = 0.0061$ for 60 min). However, the content of noradrenaline in the NAc was not affected by LPS (Figure 3D). These results indicate that LPS activates adrenergic neurons in the LC.

Effects of α₁- and β-Adrenoceptor Antagonists on LPS-Induced Downregulation and Upregulation

To investigate whether LPS-induced facilitation of noradrenaline release is involved in either the downregulation of the membrane GluR1 subunit in the mPFC and VTA or the upregulation of the membrane GluR2 subunit in the NAc at 24 h post-LPS, we administered prazosin (3.0 mg/kg), an α₁-adrenoceptor antagonist, or propranolol (2.0 mg/kg), a β-adrenergic
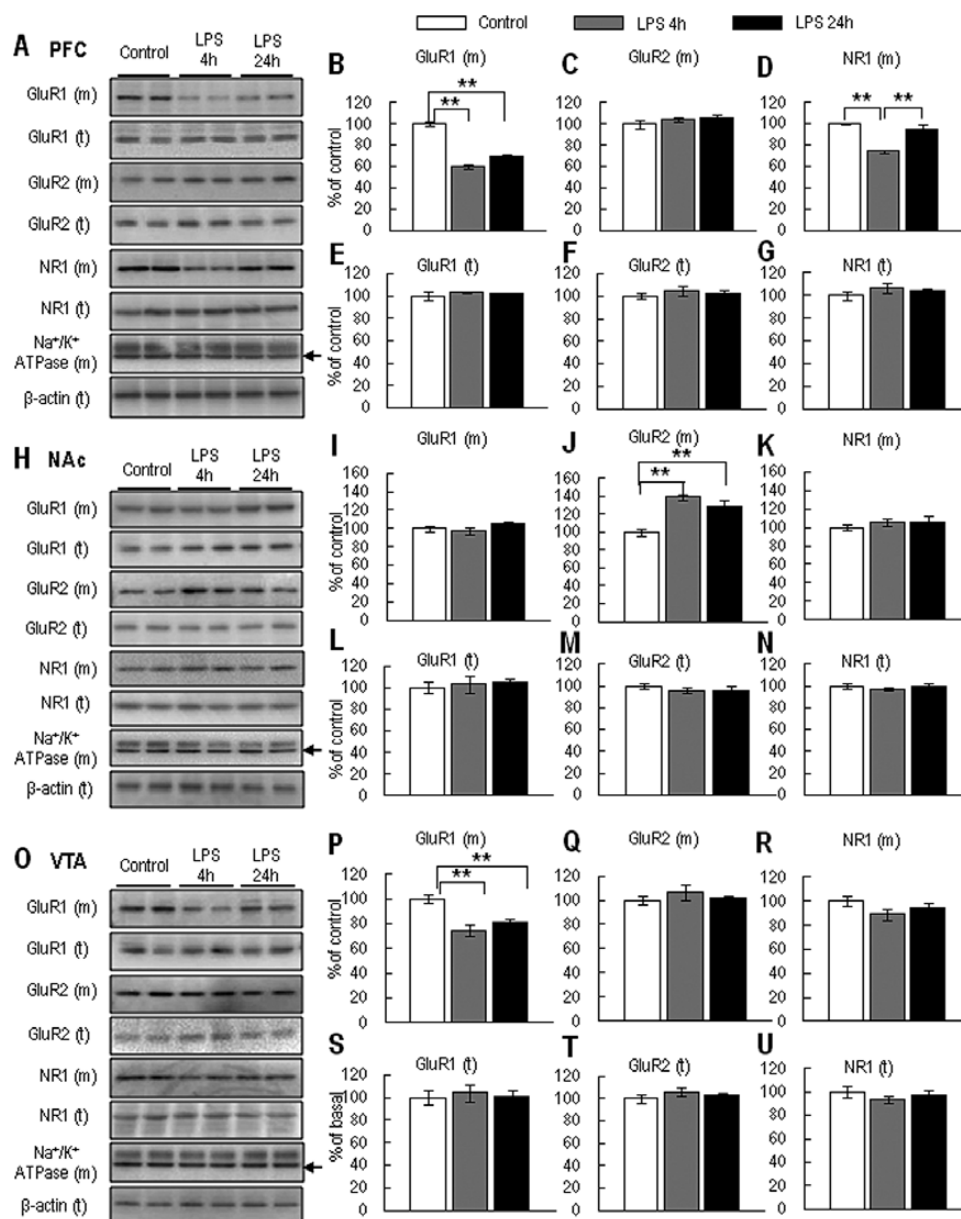
Figure 2. Representative western blot of the protein level of membrane and total GluR1, GluR2, and NR1 in mouse mPFC (A), NAc (H), and VTA (O) with at 4 h and 24 h post-LPS injection (1.2 mg/kg, twice at 30 min interval). Representative graphs of the protein levels of membrane GluR1 (B; control, n=4; LPS, n=4), GluR2 (C; control, n=4; LPS, n=4), and NR1 (D; control, n=4; LPS, n=4) in the mPFC; of the total protein level of GluR1 (E; control, n=4; LPS, n=4), GluR2 (F; control, n=4; LPS, n=4), and NR1 (G; control, n=4; LPS, n=4) in the mPFC; of the protein levels of membrane GluR1 (I; control, n=4; LPS, n=4), GluR2 (J; control, n=4; LPS, n=4), and NR1 (K; control, n=4; LPS, n=4) in the NAc; of the total protein levels of GluR1 (L; control, n=4; LPS, n=4), GluR2 (M; control, n=4; LPS, n=4), and NR1 (N; control, n=4; LPS, n=4) in the NAc; of the protein levels of membrane GluR1 (P; control, n=4; LPS, n=4), GluR2 (Q; control, n=4; LPS, n=4), and NR1 (R; control, n=4; LPS, n=4) in the VTA; and of the total protein levels of GluR1 (S; control, n=4; LPS, n=4), GluR2 (T; control, n=4; LPS, n=4), and NR1 (U; control, n=4; LPS, n=4) in the VTA. The graph indicates the percentage of control (saline) levels. All data are presented as mean ± SEM. Statistically significant effects of LPS injection (*p < 0.05, **p < 0.01) are noted.

antagonist, 30 min before the LPS injection. Neither prazosin nor propranolol affected the total protein levels of GluR1 and GluR2 in either the mPFC or the VTA (Figure 4A, D, and E for mPFC; Figure 4K, N, and O for VTA). However, one-way ANOVA of the protein level of GluR1 at the plasma membrane in the mPFC and the VTA showed a significant interaction (Figure 4A and B for mPFC, $F_{(3,16)}$ = 19.944, $p < 0.0001$; Figure 4K and L for VTA, $F_{(3,16)}$ = 22.234, $p < 0.0001$). The protein level of GluR1 at the plasma membrane at 24 h post-LPS in the mPFC and the VTA was abrogated by intracerebroventricular infusion of prazosin, but not propranolol (Bonferroni tests; in the mPFC, $p < 0.0001$ for LPS vs. control, $p = 0.1401$ for prazosin + LPS vs. control, and

$p < 0.0001$ for LPS vs. prazosin + LPS; in the VTA, $p < 0.0001$ for LPS vs. control, $p = 0.0689$ for prazosin + LPS vs. control, and $p < 0.0001$ for LPS vs. prazosin + LPS). Propranolol did not affect LPS-induced downregulation of the membrane GluR1 in either the mPFC or VTA at 24 h post-LPS (Bonferroni tests; in the mPFC, $p < 0.0001$ for LPS vs. control, $p = 0.0017$ for propranolol + LPS vs. control, and $p < 0.1630$ for LPS vs. propranolol + LPS; in the VTA, $p < 0.0001$ for LPS vs. control, $p = 0.0007$ for propranolol + LPS vs. control, and $p < 0.4475$ for LPS vs. propranolol + LPS). In addition, although the total GluR2 protein level in the NAc was also not affected by either prazosin or propranolol (Figure 4F and J), one-way ANOVA of the GluR2 protein level at the plasma membrane
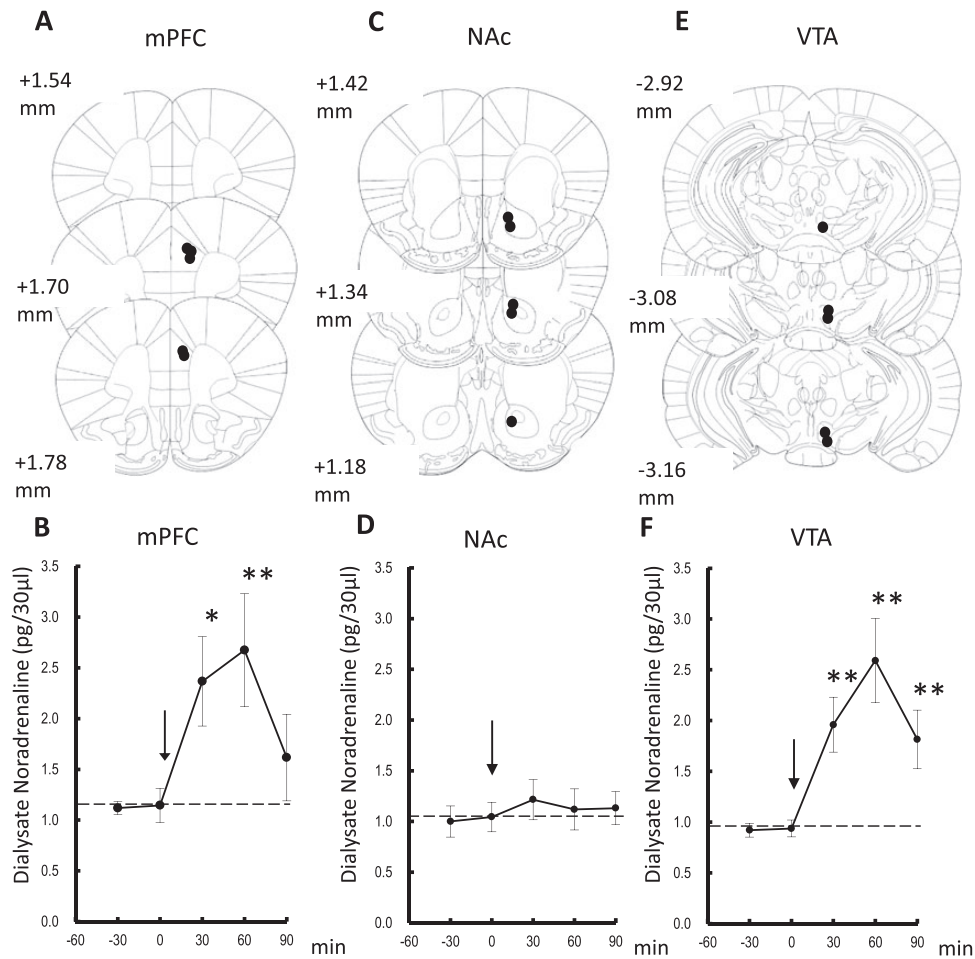
Figure 3. Schematic drawing of the histological reconstruction of coronal sections from the mPFC (A), the NAc (C), and the VTA (E) in the microdialysis studies. Each symbol represents the approximate microdialysis probe placement for the sampling of the artificial cerebrospinal fluid containing the noradrenaline. LPS-induced facilitation of noradrenaline release in the mPFC (B, n=5), the NAc (D, n=5) and the VTA (F, n=5). Drawings were adapted from the digital Paxinos and Franklin Mouse Brain Atlas; the numbers on the left upper side of the drawings indicate the distance (in millimeters) anterior (+) and posterior (-) to bregma. Mice were injected with LPS (1.2 mg/kg, twice at a 30 min interval) and microdialysate effluents were collected from the mPFC (B), NAc (D), and VTA (F) at 30 min intervals, starting at 30 min before LPS injection (i.e., at the end of the preceding 90 min stabilization period) and ending 90 min after the LPS injection. Values are the mean ± SEM. *p < 0.01 relative to content of noradrenaline (pg/30 μl) at 0 min are noted.

after LPS, prazosin, or propranolol showed a significant interaction (Figure 4F and H; $F_{(3,16)}$ = 7.638, p = 0.0022). Bonferroni tests revealed that prazosin blocked the LPS-induced upregulation of the membrane GluR2 in the NAc at 24 h post-LPS (p = 0.0031 for LPS vs. control, p = 0.8600 for prazosin + LPS vs. control, and p = 0.0021 for prazosin + LPS vs. LPS). However, propranolol did not affect the LPS-induced upregulation of the membrane GluR2 in the NAc. These results indicate that both downregulation of the membrane GluR1 in the mPFC and VTA and LPS-induced upregulation of membrane GluR2 in the NAc are mediated by α_1-adrenoceptor activation.

Effects of Intracerebroventricular Infusion of α_1- or β-Adrenoceptor Antagonists on LPS-Induced Depressive-Like Behavior

We next investigated whether the LPS-induced depressive-like behavior was dependent on α_1-adrenoceptor stimulation. To avoid any direct effect of prazosin-induced or propranolol-induced changes in blood pressure or heart rate on depressive-like behavior, we infused 5 μl intracerebroventricularly

with 70 mM prazosin or 400 mM propranolol at 30 min before LPS injection. Pretreatment with prazosin did not attenuate the LPS-induced reduction in locomotor activity at 4 h or the changes in body weight at 24 h after LPS (Figure 5A and B). However, a one-way ANOVA of the effect of antagonist pretreatment on the LPS-induced increase in immobility time during the TST and FST showed significant interactions (Figure 5C and D; for TST, $F_{(3,20)}$ = 7.078, p = 0.0020; for FST, $F_{(3,20)}$ = 8.281, p = 0.0009). Bonferroni tests revealed that the LPS-induced increase in immobility time during the TST was attenuated by pretreatment with prazosin, but not propranolol, as measured at 24 h post-LPS (p = 0.5797 for prazosin + LPS vs. control and p = 0.0010 for prazosin + LPS vs. LPS; p = 0.0191 for propranolol + LPS vs. control and p = 0.4775 for propranolol + LPS vs. LPS). LPS-induced increase in immobility time during the FST was also attenuated by pretreatment with prazosin, but not propranolol (p = 0.1514 for prazosin + LPS vs. control and p = 0.0259 for prazosin + LPS vs. LPS; p = 0.0004 for propranolol + LPS vs. control and p = 0.6954 for propranolol + LPS vs. LPS). Moreover, one-way ANOVA showed a significant effect of these antagonists on the LPS-induced decrease in sucrose preference during both the
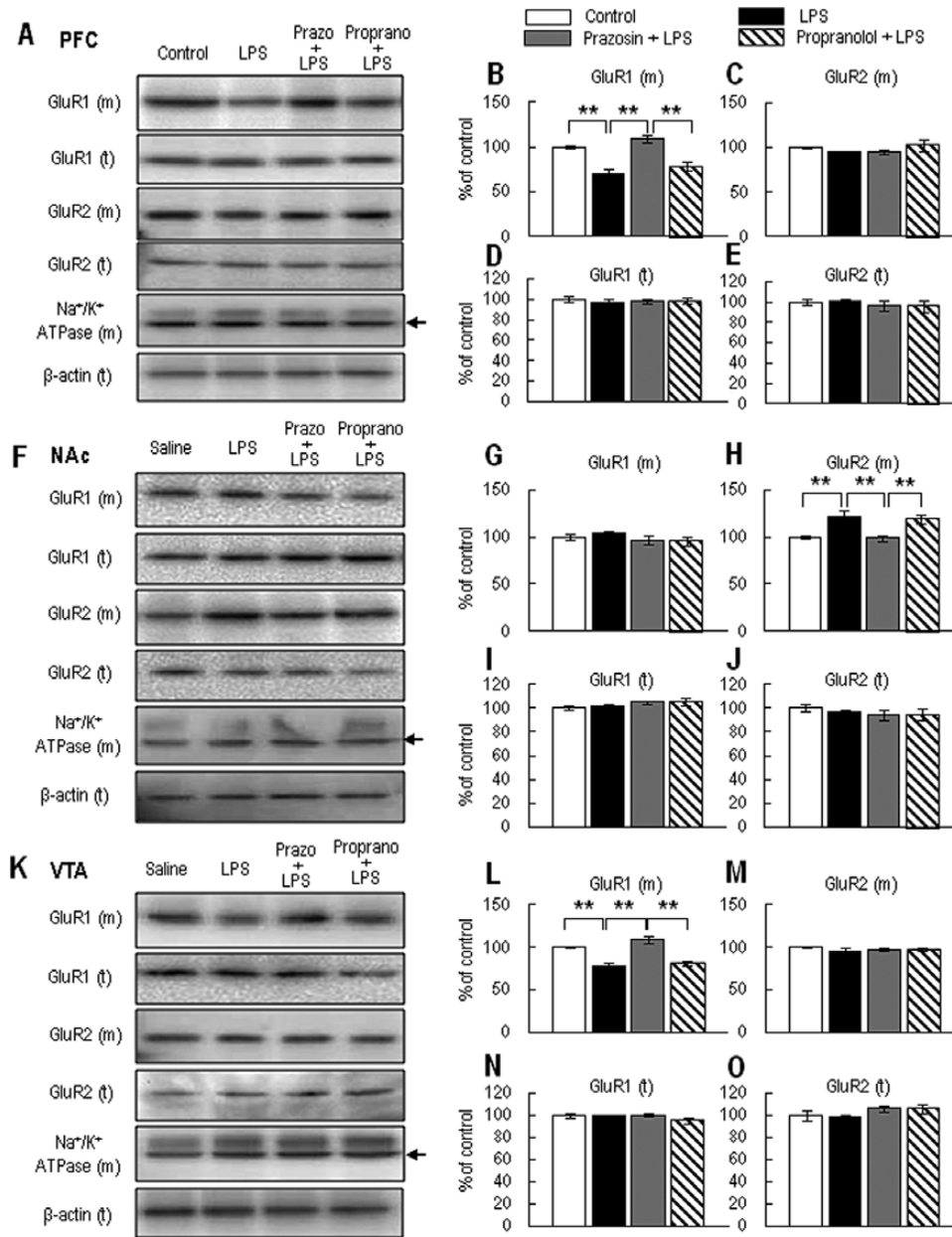
Figure 4. Effects of pretreatment with prazosin (Prazo) or propranolol (Proprano) on LPS-induced alterations of the GluR1 and GluR2 subunits protein levels at the plasma membrane fraction and total extracts of the mouse mPFC (A-E), NAc (F-J), and VTA (K-O) at 4 and 24 h post-LPS injection. Prazosin and propranolol were injected intracerebroventricularly at 30 min before LPS injection (1.2 mg/kg, twice at 30 min interval). The graph indicates the percentage of control (saline) levels. Representative graph for the protein level of membrane GluR1 (B; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) and GluR2 (C; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) in the mPFC; of the total protein levels of GluR1 (D; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) and GluR2 (E; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) in the mPFC; of the protein levels of membrane GluR1 (G; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) and GluR2 (H; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) in the NAc; of the total protein levels of GluR1 (I; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) and GluR2 (J; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) in the NAc; of the protein levels of membrane GluR1 (L; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) and GluR2 (M; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) in the VTA; and of the total protein level of GluR1 (N; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) and GluR2 (O; control, n=5; LPS, n=5; prazosin + LPS, n=5; propranolol + LPS, n=5) in the VTA. All data are presented as mean ± SEM. Statistically significant effects of LPS injection (*p < 0.01) are noted.

first 24 h post-LPS and 24–48 h post-LPS periods (Figure 5E and F; for 0–24 h, $F_{(3,20)}$ = 19.199, $p < 0.0001$; for 24–48 h, $F_{(3,20)}$ = 3.965, p = 0.0228). Bonferroni tests revealed that pretreatment with prazosin significantly increased sucrose preference at 24 h post-LPS (p = 0.0738 for prazosin + LPS vs. control; p = 0.0015 for prazosin + 24 h post LPS vs. LPS:) and at 48 h (p = 0.5706 for prazosin + LPS vs. control; p = 0.0328 for prazosin + LPS vs. LPS) post LPS.

However, propranolol did not affect the LPS-induced decease in sucrose preference at 24 h ($p < 0.0001$ for propranolol + LPS vs. control; p = 0.2987 for propranolol + LPS vs. LPS) and at 48 h (p = 0.0220 for propranolol + LPS vs. control; p = 0.7028 for propranolol + LPS vs. LPS). These results suggest that LPS-induced depressive-like behavior is associated with α_1-adrenoceptor activation.
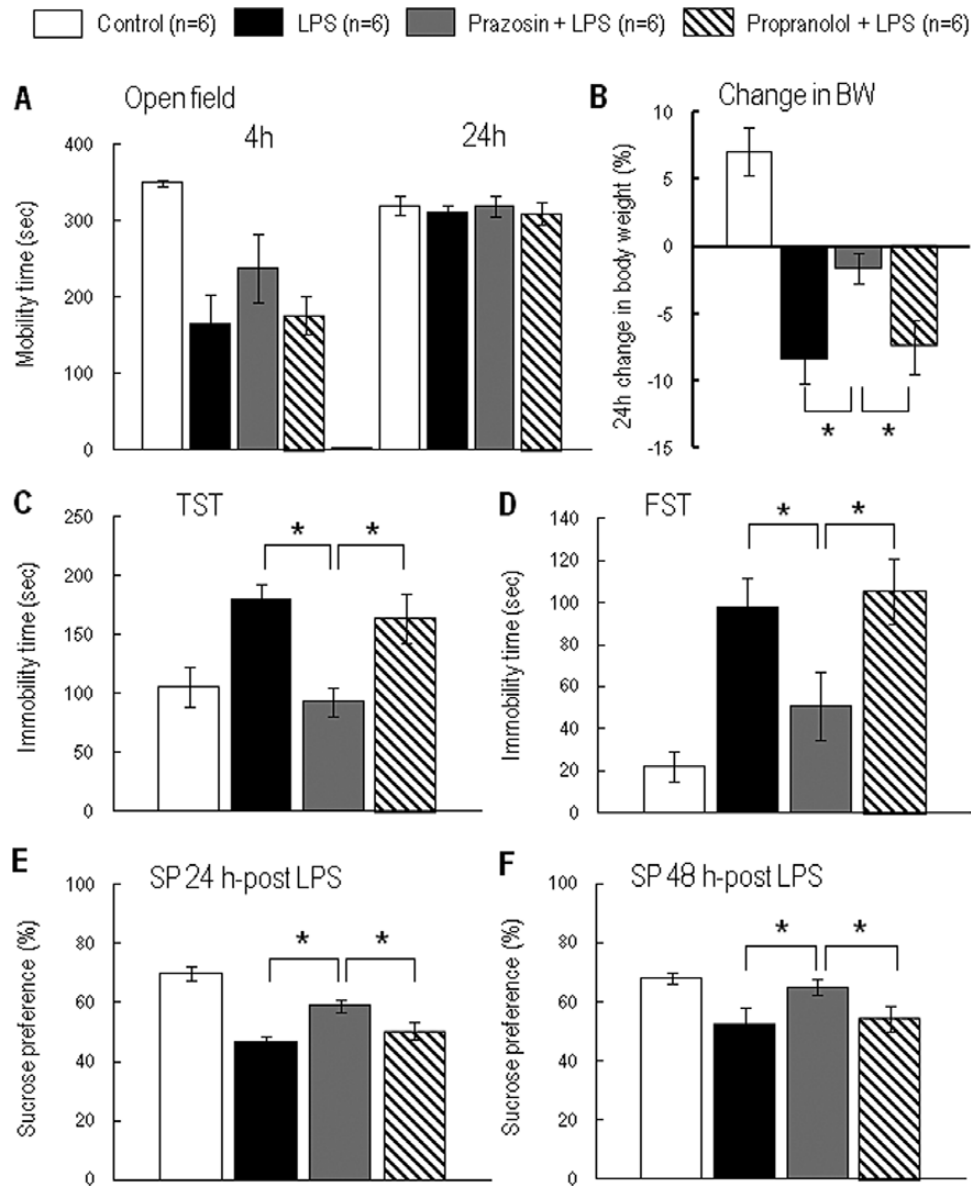
Figure 5. Prazosin or propranolol were injected intracerebroventricularly 30 min before the LPS injections (1.2 mg/kg, twice at a 30 min interval). (A) The locomotor activity was measured at either 4 or 24 h following the relevant injection. The mobility time in the open field was recorded every 6 min, and results are presented as mouse locomotor activity (control, n=6; LPS, n=6; prazosin + LPS, n=6; propranolol + LPS, n=6). (B) Changing body weight at 24 h post LPS was measured. The body weight of each mouse was measured at 5 min before and 24 h after LPS or saline injection (control, n=6; LPS, n=6; prazosin + LPS, n=6; propranolol + LPS, n=6). (C) The duration of immobility during the tail suspension test at 24 h post LPS was recorded for each 6 min test (control, n=6; LPS, n=6; prazosin + LPS, n=6; propranolol + LPS, n=6). (D) The duration of immobility during the forced swim test at 24 h post LPS or saline was recorded for each 6 min test (control, n=6; LPS, n=6; prazosin + LPS, n=6; propranolol + LPS, n=6). Sucrose preference was measured at the first 24 h post LPS (E; control, n=6; LPS, n=6; prazosin + LPS, n=6; propranolol + LPS, n=6) and at 24–48 h post LPS or saline (F; control, n=6; LPS, n=6; prazosin + LPS, n=6; propranolol + LPS, n=6), and the percentage of sucrose intake against total intake was presented. All data are presented as the mean ± SEM. Statistically significant effects of LPS injection (*p < 0.01) are noted.

Discussion

In the present study, we found that LPS facilitates noradrenaline release, which stimulates the α_1-adrenoceptor and induces GluR1 internalization in the mPFC and VTA. To our knowledge, this is the first demonstration that downregulation of the AMPA receptor GluR1 subunit at the plasma membrane in the reward system is associated with induction of depressive-like behaviors. Chronic unpredictable stress in animals can elicit symptoms similar to those in depressed human patients, such as anhedonia and decreased social interaction (Willner et al., 1987; Berton and Nestler, 2006). It has been demonstrated that

chronic social stress decreases the amount of mRNA for GluA1 AMPAR subunits in the CA1 region (Schmidt et al., 2010) and also decreases the number and function of AMPA receptors in specific synapses. In addition, stress-induced impairment of excitatory synapses has been described in the mPFC (Yuen et al., 2012) and NAc (Lim et al., 2012). From these reports, it has been suggested that decreased synaptic input in neuron is an underlying cause of depression (Kallarackal et al., 2013). By contrast, AMPA receptor potentiation has an antidepressant effect in mice with chronic mild stress (Farley et al., 2010). Therefore, from the results of our study, we suggest that LPS-induced downregulation of GluR1 protein levels at the plasma membrane results in

a decrease in the strengthening of excitatory synapses, which might contribute to the pathogenesis of depression.

Peripheral administration of LPS produces cognitive impairment with decreased hippocampal expression of brain-derived neurotrophic factor (BDNF) and its receptor, tyrosine kinase-B (Wu et al., 2007), which sustains the GluR1 subunit distribution within the synapses (Jourdi and Kabbaj, 2013). Internalization of the AMPA receptor GluR1 subunit is a central mechanism of long-term synaptic depression (Brown et al., 2005). Consistently, reduced BDNF content in platelets and decreased BDNF mRNA expression in peripheral blood lymphocytes and mononuclear cells have also been reported in patients with depression (Lee and Kim, 2009; Pandey et al., 2010). Conversely, intracerebroventricular BDNF infusion induced sustained antidepressant-like effects (Hoshaw et al., 2005). In addition, enhanced long-term synaptic depression (LTD) in an animal model of depression has been reported (Holderbach et al., 2007). A recent study suggested that a receptor coupled to the Gq/11 protein promotes LTD, but suppresses LTP; conversely, a Gs-coupled receptor promotes LTP, but suppresses LTD (Huang et al., 2012). The α_1-adrenoceptor is coupled to a Gq/11 protein, whereas all subtypes of β-adrenoceptors are coupled to Gs proteins. In the present study, prazosin, an α_1-adrenoceptor antagonist, blocked the downregulation of the membrane GluR1 subunit in the mPFC and VTA, whereas propranolol, a non-selective β-adrenoceptor antagonist, did not affect this downregulation of the membrane GluR1 subunit. Therefore, we suggest that α_1-adrenoceptor stimulation following LPS-induced facilitation of noradrenaline release induces downregulation of membrane GluR1 in the mPFC and VTA. Furthermore, these neuroplastic properties of the reward system are part of the mechanism of LPS-induced depressive-like behaviors.

Acute stress inhibits LTP at synapses from the hippocampus to the PFC in the rat (Dupin et al., 2006). Antidepressants can reverse the impairment in LTP induced by LPS treatment (Rocher et al., 2004). Moreover, nicotine exposure-induced depressive-like behavior is accompanied by dephosphorylation of serine residues at position 845 (Ser845) of the GluR1 in the hippocampus (Parameshwaran et al., 2012). These results imply that the LTD is involved in depressive-like behavior.

In the present study, upregulation of membrane GluR2 in the NAc was also accompanied by LPS-induced depressive-like behavior, and prazosin blocked membrane GluR2 upregulation in the NAc. However, adrenergic neurons in the LC have fewer innervations to the NAc. Because LPS did not facilitate noradrenaline release in the NAc in the present study, there is the possibility that LPS-induced upregulation of membrane GluR2 in the NAc is independent of α_1-adrenoceptor stimulation. It has been reported that activation of the dopamine D_2 receptor induces internalization of GluR2 (Zou et al., 2005). From our results, it seems possible that LPS-induced downregulation of the membrane GluR1 subunit in the VTA neurons might also reduce excitatory input to the dopaminergic neurons in the VTA, resulting in a reduction of dopamine release in the NAc. It has been reported that activation of the dopamine D_2 receptor induces internalization of GluR2 (Zou et al., 2005), and from the present study, it appears the internalization of the GluR2 subunit is relatively decreased because of the reduction in dopamine release, resulting in reduction of D_2 receptor stimulation in the NAc. However, the mechanisms and roles of GluR2 upregulation in the NAc in depressive symptoms remain unclear from our present work. Therefore, elucidating these observations in future studies might support our understanding of the mechanisms behind inflammation-induced depressive-like behavior.

The role of α-adrenoceptor activation in depression is controversial. One study suggested that prazosin does not affect the efficacy of antidepressants (Souza et al., 2013). Moreover, postsynaptic α_2-adrenoceptor stimulations amplify the effect of antidepressants, but antagonism of presynaptic α_2-adrenoceptor facilitates noradrenaline release, which potentiates the effects of antidepressants (Zhang et al., 2009). By contrast, some studies have suggested that chronic treatment with antidepressants leads to desensitization of α_1-adrenoceptors in the brain, indicating that insensitivity of α_1-adrenoceptors is involved in improvement of major depression (Subhash et al., 2003; Ramakrishna and Subhash, 2010). Recently, 6-fluoronorepinephrine, a fully selective α-adrenoceptor agonist, has been shown to produce antidepressant effects by acting primarily as an α_2-adrenoceptor agonist; this antidepressant effect of 6-fluoronorepinephrine is mediated though inhibition of LC neuronal activity and the enhancement of NAc activity (Stone et al., 2011). Although the mechanism behind the development of major depression is not entirely the same as the antagonism of their antidepressant effects, our results serve as an important reference for developing a novel treatment for preventing the progression of depressive disorders.

Supplementary Material

For supplementary material accompanying this paper, visit http://www.ijnp.oxfordjournals.org/

Acknowledgments

Statement of Interest

None.

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