Involvement of prefrontal AMPA receptors in encounter stimulation-induced hyperactivity in isolation-reared mice

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Abstract

We recently showed that social encounter stimulation induces hyperactivity in mice reared in social isolation from early life and this is associated with the transient activation of prefrontal dopaminergic and serotonergic systems. In the present study, we examined the effect of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist 2, 3-dioxo-6-nitro-1, 2, 3, 4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) on encounter-induced behavioural and neurochemical changes to study the role of the receptor in abnormal behaviours in isolation-reared mice. The encounter to an intruder mouse induced hyperactivity with transient increases in prefrontal dopamine and serotonin levels in isolation-reared mice. NBQX attenuated the encounter-induced hyperactivity and the associated neurochemical changes in isolation-reared mice. In addition, NBQX reduced aggressive behaviour and cognitive impairment in isolation-reared mice, but did not affect depressive-like behaviour or spontaneous hyper-locomotion in these animals. The AMPA receptor agonist (S)-AMPA increased prefrontal dopamine and serotonin release, and this effect was higher in isolation-reared mice than in the group-reared mice, suggesting higher prefrontal AMPA receptor activity in isolation-reared mice. Furthermore, isolation rearing increased the expression of AMPA receptor subunits (GluR1, GluR2 and GluR3) and GluR1 Ser845 phosphorylation in the prefrontal cortex, but not in the hippocampus or nucleus accumbens. Taken together, these results suggest that an increase in AMPA receptor activity in the prefrontal cortex contributes to some, but not all, abnormal behaviours in isolation-reared mice.

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Introduction

Rearing in social isolation from early life causes abnormal behaviours such as hyper-locomotion, aggressive behaviour, deficits in pre-pulse inhibition, cognitive impairments and depressive and anxiety-like behaviours in rodents (Sakaue et al., 2001; Ago et al., 2007, 2008, 2012; Fone and Porkess, 2008; Koda et al., 2008). Because aggressive behaviours and social interaction deficits in isolation-reared rodents are induced by exposure to an intruder, it is likely that an encounter with an intruder may elicit neurobiological changes that are responsible for subsequent abnormal behaviours, such as aggression and social interaction deficits. We recently found that isolation rearing enhances encounter stimulation-induced increases in c-Fos expression in the prefrontal cortex, dorsal raphe nucleus and ventral tegmental area, and causes encounter-induced increases in prefrontal dopamine (DA) and serotonin (5-HT) levels (Ago et al., 2013a). This suggests that isolation rearing enhances neuronal responses to encounter stimulation in these brain regions. Furthermore, pharmacological studies showed that the encounter-induced behavioural and neurochemical responses could be blocked by the metabotropic glutamate receptor 2/3 (mGluR2/3) agonist LY379268 (Ago et al., 2013a). In line with this finding, we showed that MSG0028, a selective mGluR2/3 agonist, reduces hyper-locomotion, aggressive behaviour and deficits in pre-pulse inhibition in isolation-reared male mice (Ago et al., 2012). Furthermore, Navarro et al. (2006, 2008, 2009) reported that the mGluR1 antagonist JNJ16259685, the mGluR5 antagonist MPEP and the mGluR7 positive allosteric modulator AMN082 reduced aggression in...
isolation-reared mice. These findings suggest that the glutamate system, including the metabotropic receptors, plays a key role in the abnormal behaviours exhibited by isolation-reared rodents.

Glutamate receptors are classified into the ionotropic and metabotropic receptors. While the metabotropic receptors are involved in abnormal behaviours in isolation-reared mice, it is not known whether the ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor also plays a role in the abnormal behaviours exhibited by these animals. In the present study, we examined the effect of the AMPA receptor antagonist 2, 3-dioxo-6-nitro-1, 2, 3, 4-tetrahydrobenzo [f]quinoxaline-7-sulfonamide (NBQX) on encounter-induced behavioural and neurochemical changes in isolation-reared mice. We also investigated the effect of isolation rearing on AMPA receptor expression in the prefrontal cortex, nucleus accumbens and hippocampus, as well as on prefrontal AMPA receptor function.

Method

Animals and drug treatments

The experimental procedures involving animals in this study were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals, approved by the Japanese Pharmacological Society. Every effort was made to minimize animal suffering, and to reduce the number of animals used. Three-week-old male ddY mice (Shimizu Laboratory Supplies Co., Ltd., Japan) were commercially purchased and divided equally and simultaneously into isolation and group-housed conditions. The mice in the isolation group were individually housed for 6 wk in wire-topped opaque polypropylene cages (24 × 17 × 12 cm), while the control group continued to be housed under normal group-housed conditions (five or six animals per cage) in same-sized wire-topped clear plastic cages (24 × 17 × 12 cm), with free access to food and water throughout the experiments. We used a total of 196 mice in the various experiments; different mice were used for each experiment. The following drugs were used: NBQX, (S)-AMPA (both Sigma, St. Louis, USA) and pentobarbital (Nacalai Tesque, Inc., Japan). All other commercially available chemicals used in the experiments were of the highest purity. NBQX was dissolved in saline (0.9% NaCl w/v). Pentobarbital was dissolved in sterile water containing 10% ethanol. The drugs were injected i.p. at 10 ml/kg. The behavioural and in vivo microdialysis studies, NBQX was administered i.p. 15 min before the test or social encounter. The condition for NBQX administration used here was selected referring to previous studies (Shimizu-Sasamata et al., 1996; Vekovischeva et al., 2007; Szewczyk et al., 2010).

Social encounter stimulation and behavioural analysis

The social encounter stimulation and behavioural analysis were performed as previously reported (Ago et al., 2013a). An isolation-reared mouse was placed in the large compartment of a novel clear Plexiglas cage (30 × 30 × 35 cm), which was divided by a mesh partition into smaller compartments. This allowed the animal to see, hear and smell, but not physically contact, the neighbour. After a 3-h habituation period, an unfamiliar 9-wk-old ddY mouse was introduced into the unoccupied small compartment as an intruder. The resident and intruder mice were allowed to interact through the partition for 20 min, and then the intruder mouse was removed. The behaviours of the resident mouse were videotaped, and its locomotor path was automatically analysed offline using the ANY-maze video tracking software (Stoelting Company, USA). The total time spent and locomotor activity (movement time) near the partition was used to assess the behavioural response of the resident mouse to the intruder. The interaction between the resident and intruder mouse (time spent smelling, putting one or two paws on the partition, and placing the nose into the holes toward the intruder) were also analysed.

In the preset study, we focused on the behaviour of the resident, not the intruder, mouse, since we found that the locomotor activity of the intruder did not affect the behaviour of the resident mouse.

In vivo microdialysis study

Each mouse was anaesthetised with sodium pentobarbital (40 mg/kg, i.p.) and stereotactically implanted with a guide cannula (one site per animal) for a dialysis probe (Eicom Corp., Japan) in the prefrontal cortex (A +1.9 mm, L −0.5 mm, V −3.8 mm, from the bregma) (Franklin and Paxinos, 1997). The cannula was cemented in place with dental acrylic, and the animal was kept warm and allowed to recover from anaesthesia. Post-operative analgesia was performed with a single injection of buprenorphine (0.1 mg/kg, i.p.) (Ago et al., 2008, 2011, 2013a). The active probe membrane length was 3 mm in the prefrontal cortex. Two days after the surgery, the probe was perfused with Ringer’s solution (147.2 mM NaCl, 4.0 mM KCl and 2.2 mM CaCl₂ [pH 6.0]; Fuso Pharmaceutical Industries, Osaka, Japan) at a constant flow rate of 2 μl/min. A stabilization period of 3 h (corresponding to the 3-h habituation period in the clear Plexiglas cage mentioned above) was established before the onset of the experiment. Microdialysis samples (20 μl) were collected every 10 min and injected immediately onto a high-performance liquid chromatography column for simultaneous assaying of DA and 5-HT, as previously reported (Ago et al., 2006, 2008). In the AMPA function experiment, extracellular monoamines were analyzed during a 30-min period using Ringer’s solution containing 10 μM (S)-AMPA, which was used to replace normal solution. After the experiments, Evans Blue dye was
micro-injected through the cannula to histologically verify the position of the probe, and only data from animals with correct probe placement were used in the analysis.

**Measurement of aggressive behaviour**

Two isolation-reared mice, pre-treated with the drug 15 min before the test, were placed in a neutral cage (24 × 17 × 12 cm), and their behaviours were videotaped over a 20-min period (Sakaue et al., 2001). In this experiment, we did not use group-reared mice because they did not show aggressive behaviour. Trained experimenters, blind to treatment, carried out the analysis. Two isolated mice are divided to win (more aggressive and less defensive to the opponent) and lose (less aggressive and more defensive) mice, and then the effect of NBQX on behaviours of each mouse was analysed separately. The following behaviours were measured: aggressive behaviour (biting, tail rattling, wrestling and lateral threats), walking, rearing, grooming and snifing.

**Novel object recognition test**

Novel object recognition test was performed as previously reported (Ago et al., 2013b). Mice were habituated to the test box (30 × 30 × 30 cm) for 10 min in the absence of objects over 3 d. During training trial on the following day, two identical objects were placed in the box, and the mouse was allowed to explore for 10 min. During test trial, 1-h after the training trial, one of the familiar objects was replaced with a novel object, and the mouse was allowed to explore for 5 min. The behaviours of mice in the training and test trial were videotaped. Exploratory preference was calculated by dividing the time spent exploring the novel object by the total time spent exploring either object.

**Measurement of spontaneous locomotor activity**

Locomotor activity was measured using a digital counter system with an infrared sensor (Supermex®, Muromachi Kikai Co., Ltd., Japan; Ago et al., 2009; Koda et al., 2010). Fifteen minutes after injection of NBQX, mice were placed individually in a novel clear plastic cage (24 × 17 × 12 cm), and then locomotor activity was recorded for 60 min.

**Forced swim test**

The forced swim test was performed as previously reported (Ago et al., 2008). Briefly, a mouse was placed in the individual acrylic cylinder (25 cm high × 19 cm in diameter) containing 25 ± 1 °C water at a depth of 13 cm, so that the mice could not support themselves by touching the bottom with their paws. The performance of the mice for 6 min in the swimming session was videotaped using a digital video camera for subsequent analysis. After the session, the mice were removed from the cylinders, dried with paper towels and placed under a warming lamp until completely dry, and then returned to their home cages. The total time of immobility was measured during 6 min in the swimming session by an observer blind to the treatment and rearing conditions.

**Western blot analysis**

Tissues were homogenised at 4 °C in lysis buffer (50 mM Tris-HCl [pH 7.4], 100 mM NaCl, 2 mM MgCl2, 1% NP-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4 and 5 mg/ml leupeptin) supplemented with a protease inhibitor cocktail (Sigma). The homogenate was incubated at 4 °C for 30 min and then centrifuged at 14000 g for 20 min at 4 °C, and the resulting supernatant was collected. Protein concentrations were determined using the BCA Protein Assay (Thermo Fisher Scientific, Rockford, IL, USA). Adequate amounts of protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then transferred electrophoretically to a hydrophobic polyvinylidene fluoride membrane. The blotted membranes were blocked for 1 h in 5% non-fat skim milk/TBS-T (20 mM Tris–HCl, 137 mM NaCl and 0.05% Tween-20; pH 7.4), and then incubated with primary antibodies: rabbit anti-GluR1 (1:500, overnight at 4 °C; Abcam, USA), rabbit anti-GluR2 (1:1000, overnight at 4 °C; Abcam, USA), rabbit anti-GluR3 (1:250, overnight at 4 °C; Millipore, USA), rabbit anti-Ser 845-phosphorylated GluR1 (1:1000, overnight at 4 °C; Acris, Germany) or mouse anti-β-actin (1:5000, 2 h at room temperature; Sigma). Membranes were then incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (1:2000, 1 h at room temperature; MP Biomedicals, USA) or mouse anti-β-actin (1:5000, 2 h at room temperature; Sigma). Membranes were then incubated with horseradish peroxidase–HRP:polyacrylamide gel electrophoresis, and then transferred electrophoretically to a hydrophobic polyvinylidene fluoride membrane. The blotted membranes were blocked for 1 h in 5% non-fat skim milk/TBS-T (20 mM Tris–HCl, 137 mM NaCl and 0.05% Tween-20; pH 7.4), and then incubated with primary antibodies: rabbit anti-GluR1 (1:500, overnight at 4 °C; Abcam, USA), rabbit anti-GluR2 (1:1000, overnight at 4 °C; Abcam, USA), rabbit anti-GluR3 (1:250, overnight at 4 °C; Millipore, USA), rabbit anti-Ser 845-phosphorylated GluR1 (1:1000, overnight at 4 °C; Acris, Germany) or mouse anti-β-actin (1:5000, 2 h at room temperature; Sigma). Membranes were then incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (1:1000, 1 h at room temperature; MP Biomedicals, USA) or mouse anti-β-actin (1:5000, 1 h at room temperature; MP Biomedicals) for β-actin. The immune complexes were visualised using ECL Plus Western Blotting Detection Reagents (GE Healthcare Life Sciences, USA) and quantified using a light-capture cooled CCD camera system for bio/chemiluminescence detection (AE-6981, Atto Co., Japan).

**Statistical analyses**

All data are expressed as the mean ± S.E.M. Data for the microdialysis experiment were calculated as percent change from dialysate baseline concentrations, with 100% defined as the average of three fractions before administration. Data were analyzed using two-way analysis of variance (ANOVA) with drug treatment as the inter-subject factor and repeated measures with time as the intra-subject factor. Data for social encounter stimulation-induced behaviours were analysed using one-way ANOVA followed by the Tukey-Kramer post hoc test. Data for aggressive behaviour were analysed using one-way ANOVA followed by the Dunnett post hoc test. Data for locomotor activity, novel object recognition test and forced swim test were analysed using two-way
ANOVA followed by the Tukey-Kramer post-hoc test. Data for AMPA receptor expression and phosphorylation were analysed by Student’s t-test. Statistical analyses were performed using a software package, Statview 5.0 J, for Apple Macintosh (SAS Institute Inc., USA). A value of \( p < 0.05 \) was considered statistically significant.

**Results**

**Effect of NBQX on encounter stimulation-induced hyperactivity**

Isolation-reared mice were hyperactive during the encounters, compared with group-reared mice. Locomotor activities (movement time) of isolation-reared mice in the area near the partition were significantly higher than those of group-reared mice. However, the total time spent in the area near the partition by the resident mouse and the total time the resident and intruder mice spent interacting did not differ between group-reared and isolation-reared animals. Next, we examined the effect of NBQX on encounter stimulation-induced hyperactivity in isolation-reared mice. Figure 1a shows representative locomotor paths of resident mice during the 20-min encounters. NBQX (10 mg/kg) inhibited encounter stimulation-induced hyperactivity \( (F_{2,12} = 7.846, p = 0.0066) \), but did not affect the total time spent in the area near the partition \( (F_{2,12} = 0.375, p = 0.6951) \) or the total time the resident and intruder mice spent interacting \( (F_{2,12} = 0.095, p = 0.9100) \) (Fig. 1b).

**Effect of NBQX on encounter stimulation-induced increases in prefrontal DA and 5-HT levels**

We examined the effect of NBQX on encounter stimulation-induced increases in prefrontal DA and 5-HT levels. NBQX (10 mg/kg) inhibited the encounter-induced increases in DA \( (F_{10,80} = 3.950, p = 0.0002) \) and 5-HT \( (F_{10,80} = 3.423, p = 0.0009) \) levels in isolation-reared mice. NBQX (10 mg/kg) did not affect basal extracellular
isolation-reared mice. NBQX (5, 10 mg/kg) significantly reduced the effect of NBQX on abnormal behaviours, which were examined in the prefrontal cortex may be responsible for abnormal behaviours.

**Effect of NBQX on isolation rearing-induced abnormal behaviours**

Encounter stimulation-induced neurochemical changes in the prefrontal cortex may be responsible for subsequent abnormal behaviours. Therefore, we next examined the effect of NBQX on abnormal behaviours, including aggressive behaviour, cognitive impairment, hyper-locomotion and depressive-like behaviour in isolation-reared mice. NBQX (5, 10 mg/kg) significantly inhibited abnormal behaviour in the ‘win’ (F(2,15) = 9.842, p = 0.0019) and ‘lose’ mice (F(2,15) = 10.995, p = 0.0011) (Fig. 3a). Furthermore, NBQX at 10 mg/kg decreased the walking time of the ‘win’, but not ‘lose’, mice (F(2,15) = 4.801, p = 0.0245 for the ‘win’ mice; F(2,15) = 2.067, p = 0.1611 for the ‘lose’ mice) (Fig. 3b). NBQX (5, 10 mg/kg) did not affect other non-aggressive behaviours such as rearing (F(2,15) = 0.021, p = 0.9796 for the ‘win’ mice; F(2,15) = 0.044, p = 0.9567 for the ‘lose’ mice), grooming (F(2,15) = 1.093, p = 0.3606 for the ‘win’ mice; F(2,15) = 0.549, p = 0.5885 for the ‘lose’ mice) and sniffing (F(2,15) = 0.141, p = 0.8693 for the ‘win’ mice; F(2,15) = 0.237, p = 0.7922 for the ‘lose’ mice) (Fig. 3c–e).

**Figures 4a, b** show the effect of NBQX on exploratory preference in the novel object recognition test. Since NBQX (10 mg/kg) decreased the exploration time of group- and isolation-reared mice (F(2,27) = 13.027, p = 0.0001 for group-reared mice; F(2,27) = 4.343, p = 0.0232 for isolation-reared mice) (Fig. 4a), the effect of NBQX was examined at 5 mg/kg (Fig. 4b). NBQX (5 mg/kg) alleviated cognitive impairment in the novel object recognition test in isolation-reared mice. Two-way ANOVA revealed the main significant effects of the rearing (F(1,36) = 5.259, p = 0.0278) and of the drug (F(1,36) = 5.603, p = 0.0234), and there was a significant interaction between rearing and drug (F(1,36) = 5.075, p = 0.0305). On the other hand, NBQX (5, 10 mg/kg) did not affect hyper-locomotion or depressive-like behaviour in the forced swim test (Figs. 4c, d). Two-way ANOVA revealed the main significant effects of the rearing (F(1,34) = 15.411, p = 0.0002 for locomotor activity; F(1,60) = 17.221, p = 0.0001 for forced swim test), but not of the drug (F(2,54) = 0.331, p = 0.7195 for locomotor activity; F(2,60) = 0.024, p = 0.9759 for forced swim test), and there was no significant interaction between rearing and drug (F(2,27) = 0.252, p = 0.7779 for locomotor activity; F(2,60) = 0.080, p = 0.9231 for forced swim test).

### AMPA receptor expression and phosphorylation

NBQX inhibited encounter stimulation-induced hyperactivity and increases in prefrontal DA and 5-HT levels. The finding shows that AMPA receptor activation plays a key role in encounter stimulation-induced behavioural and neurochemical changes we thus, examined the expression of GluR1, GluR2 and GluR3 AMPA receptor subunits in the prefrontal cortex, nucleus accumbens and hippocampus (Fig. 5). GluR1, GluR2 and GluR3 expression was increased in the prefrontal cortex of isolation-reared mice, but not in the nucleus accumbens or hippocampus. Previous studies have shown that Ser 845 phosphorylation of GluR1 enhances AMPA receptor function (Banke et al., 2000), and that Ser 845 phosphorylation increases delivery of GluR1-containing AMPA receptors to synaptic membrane (Ehlers, 2000). Therefore, to assess the expression of synaptic membrane-associated functional AMPA receptors in isolation-reared mice, we examined Ser 845 phosphorylation of GluR1. Ser 845 phosphorylation was increased in the prefrontal cortex of isolation-reared mice, but not in the nucleus accumbens or hippocampus of these animals.

### AMPA receptor function

GluR1, GluR2 and GluR3 expression and Ser 845 phosphorylation of GluR1 were increased in the prefrontal cortex of isolation-reared mice. This finding suggests that there may be a change in AMPA receptor function in the prefrontal cortex of isolation-reared mice. To assess whether there is indeed a change in AMPA receptor function in the prefrontal cortex of isolation-reared mice, we examined responses to (S)-AMPA, a selective AMPA receptor agonist, in the prefrontal cortex using in vivo microdialysis (Fig. 6). Previous studies have shown that local application of (S)-AMPA, a selective AMPA receptor agonist, increases DA and 5-HT levels in the prefrontal cortex of rodents, suggesting that prefrontal AMPA receptor activation may promote prefrontal DA and 5-HT release (Jedema and Moghddam, 1996; Amargós-Bosch et al., 2007). In the present study, the application of 10 μM (S)-AMPA in the prefrontal cortex increased local...
DA and 5-HT release in both isolation and group-reared mice. However, (S)-AMPA had a more robust effect in isolation-reared mice than in group-reared animals (Fig. 6). Repeated measures two-way ANOVA revealed significant main effects of rearing ($F_{1,8} = 34.821, p = 0.0004$ for DA; $F_{1,8} = 17.569, p = 0.0030$ for 5-HT) and time ($F_{5,40} = 41.336, p < 0.0001$ for DA; $F_{5,40} = 50.399, p < 0.0001$ for 5-HT). Furthermore, there was a significant interaction between rearing and time ($F_{5,40} = 18.285, p < 0.0001$ for DA; $F_{5,40} = 4.507, p = 0.0024$ for 5-HT). Baseline extracellular DA and 5-HT levels did not differ between the group and isolation-reared mice: the DA levels (the mean±S.E.M., $n = 5$) were $0.43±0.03$ pg/20 μl for group-reared mice and $0.44±0.04$ pg/20 μl for isolation-reared animals. The 5-HT levels (the mean±S.E.M., $n = 5$) were $0.61±0.02$ pg/20 μl for group-reared mice and $0.65±0.06$ pg/20 μl for isolation-reared animals.

**Discussion**

We recently showed that social encounter stimulation-induced hyperactivity is associated with transient increases in DA and 5-HT levels in the prefrontal cortex of isolation-reared mice (Ago et al., 2013a). Furthermore, our pharmacological study showed that agonists for 5-HT$_{1A}$, GABA$_{A}$ and mGluR2/3 receptors block the encounter-induced behavioural and neurochemical changes. The present study shows that the AMPA receptor antagonist NBQX also inhibits encounter-induced hyperactivity and the associated increases in prefrontal DA.
and 5-HT levels. This finding suggests that the AMPA receptor, like mGluR2/3, plays a role in encounter-induced hyperactivity and the transient increases in prefrontal DA and 5-HT levels. Furthermore, we found that the AMPA receptor antagonist NBQX also inhibits aggressive behaviour in isolation-reared mice. This is in agreement with previous studies using GluR1 subunit-deficient (Vekovischeva et al., 2004) and GluR2 subunit-deficient (Shimshek et al., 2006) mice showing that the AMPA receptor is involved in aggressive behaviour. Moreover, Vekovischeva et al. (2007) showed that an AMPA receptor antagonist inhibits aggressive behaviour in the resident-intruder paradigm. Collectively, these findings suggest that the AMPA receptor is involved in aggressive behaviour in isolation-reared mice.

In isolation-reared mice, encounter stimulation increases c-Fos expression in the prefrontal cortex, the dorsal raphe nucleus and the ventral tegmental area (Ago et al., 2013a). Thus, it is likely that activation of the ventral tegmental area and raphe nucleus results in increases in prefrontal DA and 5-HT release, respectively. In the present study, the increases in prefrontal DA and 5-HT release were blocked by NBQX, an AMPA receptor antagonist. Previous studies show that prefrontal AMPA receptors regulate the extracellular levels of DA and 5-HT (Jedema and Moghaddam, 1996; Tao et al., 1997; Wu et al., 2002). Taken together, these findings suggest that functional AMPA receptors are present in the prefrontal cortex. It is therefore likely that systemic NBQX blocks prefrontal AMPA receptors, thereby inhibiting the encounter-induced increases in DA and 5-HT release. However, we must acknowledge that other brain regions, in addition to the prefrontal cortex, may also mediate the effect of NBQX.
Our present results suggest that prefrontal neurochemical changes may underlie not only the encounter-induced hyperactivity, but also the aggressive behaviour, in isolation-reared mice. Furthermore, we found that NBQX alleviated isolation rearing-induced cognitive impairment in the novel object recognition test. However, NBQX did not inhibit hyper-locomotion or the depression-like behaviour in isolation-reared mice. These findings suggest that prefrontal amine systems play a key role in some, but not all, abnormal behaviours in isolation-reared animals. We recently demonstrated that the selective mGluR2/3 agonist MGS0028 inhibits hyper-locomotion in isolation-reared mice (Ago et al., 2012). This is in agreement with a previous observation in isolation-reared rats (Jones et al., 2010). Furthermore, we

Fig. 5. AMPA receptor subunit expression and Ser 845 phosphorylation of GluR1 in isolation-reared mice. AMPA receptor subunit expression and phosphorylation in the prefrontal cortex (a), the hippocampus (b) and the nucleus accumbens (c) are shown. Values are expressed as the mean±S.E.M. of 6 mice. *p<0.05 compared with group-reared mice.

Fig. 6. AMPA receptor function in the prefrontal cortex of isolation-reared mice. (S)-AMPA-induced increases in DA and 5-HT levels are shown. (S)-AMPA (10μM) was perfused into the prefrontal cortex via the dialysis probe for the time indicated by the horizontal bar. Values are expressed as the mean±S.E.M. of 5 mice.
observed that isolation rearing increases mGluR2/3 binding in the cortex and hippocampus (Kawasaki et al., 2011), and that MC50039, a selective mGluR2/3 antagonist, reduces the immobility time of isolation-reared mice in the forced swim test (Kawasaki et al., 2011). These observations suggest that isolation rearing perturbs the glutamate system, resulting in abnormal behaviours in isolation-reared rodents.

To clarify the role of the AMPA receptor in abnormal behaviours in isolation-reared mice, we examined whether isolation rearing alters the expression or function of the receptor. Isolation rearing increased GluR1, GluR2 and GluR3 expression, as well as Ser 845 phosphorylation of GluR1, in the prefrontal cortex, but not in the nucleus accumbens or hippocampus. This is in line with a previous report showing that isolation rearing increases prefrontal GluR3 mRNA levels in male rats (Levine et al., 2007). Because Ser 845 phosphorylation of GluR1 promotes the delivery of GluR1-containing AMPA receptors to synaptic membranes, the increase in Ser 845 phosphorylation may enhance AMPA receptor function. In the present study, we found that isolation rearing enhanced the (S)-AMPA-induced increases in DA and 5-HT levels in the prefrontal cortex. These results indicate that isolation rearing increases AMPA receptor activity in the prefrontal cortex, and suggest that the receptor plays a role in the abnormal behaviours exhibited by isolation-reared mice.

The activity of pyramidal neurons in the prefrontal cortex is controlled by excitatory inputs (which act through AMPA receptors) from other cortical areas, as well as the thalamus, hippocampus and amygdala (Kuroda et al., 1998; Groenewegen and Uylings, 2000; Van der Werf et al., 2002). Prefrontal pyramidal neurons project to the midbrain and control the activity of the ascending monoaminergic systems (i.e., dopaminergic neurons of the ventral tegmental area and serotonergic neurons of the dorsal raphe nucleus) (Aghajanian and Wang, 1977; Thierry et al., 1979; Hajós et al., 1998; Jodo et al., 1998; Celada et al., 2001; Martin-Ruiz et al., 2001; Puig et al., 2003). Dopaminergic neurons in the ventral tegmental area and serotonergic neurons in the dorsal raphe nucleus project to the prefrontal cortex. Encounter stimulation specifically activates the prefrontal DA and 5-HT systems. The enhanced activation of these systems can be attenuated by the AMPA receptor antagonist NBQX. It is likely that excitatory input via AMPA receptors plays a key role in the induction of abnormal behaviours in isolation-reared mice. The neuronal network underpinning the encounter-induced hyperactivity is schematically shown in Fig. 7.

In conclusion, the present study demonstrates that the AMPA receptor antagonist NBQX inhibits not only the encounter-induced hyperactivity and increases in prefrontal DA and 5-HT levels, but also inhibits aggressive behaviour and cognitive impairment, in isolation-reared mice. Furthermore, isolation rearing increases the expression of AMPA receptor subtypes and enhances the AMPA receptor agonist-induced release of DA and 5-HT in the prefrontal cortex. Collectively, these findings suggest that prefrontal AMPA receptors are involved in some, but not all, abnormal behaviours in isolation-reared mice.

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Statement of Interest
None.

References


