Neuropharmacological effects of brexpiprazole on animal models of cognitive impairment

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I. GENERAL INTRODUCTION

Cognitive deficits in patients of psychiatric disorders including schizophrenia and major depressive disorder are recognized as a serious social problem in recent years. Especially in schizophrenia, it is thought that cognitive deficits are not epiphenomena arising from positive and negative symptoms but one of the main symptoms of schizophrenia. The Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) developed by the US National Institute of Mental Health (NIMH) defined that there are several main cognitive domains easily impaired by schizophrenia such as attention/vigilance, working memory, verbal learning and memory, visual learning and memory, reasoning and problem solving, speed of processing and social cognition (Table 1).

<table>
<thead>
<tr>
<th>Cognitive domains</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>attention/vigilance</td>
<td>ability to response appropriately to series of stimuli appeared quickly</td>
</tr>
<tr>
<td></td>
<td>ex) reading book, watching movie</td>
</tr>
<tr>
<td>working memory</td>
<td>ability to memorize information for short-time (about 5-20 seconds)</td>
</tr>
<tr>
<td></td>
<td>and process it</td>
</tr>
<tr>
<td></td>
<td>ex) memorizing phone number newly taught</td>
</tr>
<tr>
<td>verbal learning</td>
<td>ability to memorize verbal information for long-time (a few minutes- a few years)</td>
</tr>
<tr>
<td>verbal memory</td>
<td>ex) memorizing someone’s request to buy in the store</td>
</tr>
<tr>
<td>visual learning</td>
<td>ability to memorize visual information for long-time (a few minutes- a few years)</td>
</tr>
<tr>
<td>visual memory</td>
<td>ex) memorizing someone’s request to buy in the store</td>
</tr>
</tbody>
</table>
Table 1. Cognitive domains impaired in patients with schizophrenia

<table>
<thead>
<tr>
<th>Cognitive Domain</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reasoning</td>
<td>Ability to carry out a project effectively</td>
</tr>
<tr>
<td>Problem solving</td>
<td>Getting workplace in time despite of changing time of the usual bus</td>
</tr>
<tr>
<td>Speed of processing</td>
<td>Ability to process the information exactly and respond it quickly</td>
</tr>
<tr>
<td></td>
<td>Ex) Serving a customer with touch panel operation</td>
</tr>
<tr>
<td>Social cognition</td>
<td>Ability to process and memorize social information such as expressions, emotions and meaning of social interaction</td>
</tr>
<tr>
<td></td>
<td>Ex) Memorizing someone’s face or ability of recognizing someone’s feeling from his/her expression</td>
</tr>
</tbody>
</table>

These cognitive domains are necessary for working. Therefore cognitive deficits constitute a major cause of low employment rate in patients of psychiatric disorders (Mueser et al, 2002). Social isolation by disemployment might decrease patient’s motivation, and has possibility to result in worsening of symptoms. Additionally, cognitive deficits cause a decrease in the drug adherence rate of patients and might prevent improvement of symptoms (Patterson et al, 2002). Treatment for cognitive deficits is needed for not only improvement of cognitive deficits itself, but also improvement of other symptoms of psychiatric disorders. There is no antipsychotic drug having enough therapeutic effect for cognitive deficits despite of challenging for development of novel drugs. Development of effective drugs for cognitive deficits is critically-needed by psychiatric patients.
Brexpiprazole, 7-{4-[4-(1-benzothiophen-4-yl)piperazin-1-yl]butoxy} quinolin-2(1H)-one (Figure 1), is a novel serotonin-dopamine activity modulator developed within recent years. Brexpiprazole has higher serotonin 5-HT_{1A} receptor and dopamine D_2 receptor binding affinity than an atypical antipsychotic drug, aripiprazole (Figure 1, Table 2). Brexpiprazole has a slightly higher binding affinity for h5-HT_{1A} receptors than hD_2 receptors, whereas aripiprazole has reverse effect (Table 2).

Additionally, the inhibitory activity for hD_{2L} receptor on brexpiprazole was a little weaker than that on aripiprazole which indicates that the intrinsic activity for D_2 on brexpiprazole is slightly lower than that on aripiprazole (Figure 2). As brexpiprazole has lower intrinsic activity at D_2 receptor compared with aripiprazole, it can expect to have fewer adverse effects by D_2 receptor agonism and antagonism than aripiprazole. It is also an antagonist at 5-HT_{2A} receptors and adrenergic α_{1B/2C} receptors (Maeda et al., 2014a). The 5-HT_{1A} receptor agonism is focused on the ability for improvement of several clinical and non-clinical reports.
**Figure 1.** Chemical structure of brexpiprazole and aripiprazole

![Chemical structures of brexpiprazole and aripiprazole](image)

<table>
<thead>
<tr>
<th>Human Receptor</th>
<th>Brexpiprazole (nM)</th>
<th>Aripiprazole (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hD&lt;sub&gt;2L&lt;/sub&gt;</td>
<td>0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>hD&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>h5-HT&lt;sub&gt;1A&lt;/sub&gt;</td>
<td>0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>h5-HT&lt;sub&gt;2A&lt;/sub&gt;</td>
<td>0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>h5-HT&lt;sub&gt;2B&lt;/sub&gt;</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ha&lt;sub&gt;1B-adrenergic&lt;/sub&gt;</td>
<td>0.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ha&lt;sub&gt;2C-adrenergic&lt;/sub&gt;</td>
<td>0.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>hH&lt;sub&gt;1&lt;/sub&gt;</td>
<td>19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>hM&lt;sub&gt;1&lt;/sub&gt;</td>
<td>67% at 10 μM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6780&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data from Maeda et al., 2014a.
<sup>b</sup>Data from Shapiro et al., 2003.
Table 2. Binding affinities for cloned human receptors in vitro (Maeda et al. 2014a)
Data are calculated by nonlinear regression analysis using data from three assays performed in
duplicate or triplicate and expressed as mean values.

Figure 2. Partial agonist activity of brexpiprazole and reference drugs on human
cloned D2L receptors in vitro (Maeda et al. 2014a)
Concentration-response curves are shown for brexpiprazole and aripiprazole on forskolin induced
cAMP accumulation in hD2L receptor–expressing CHO cells. Data are mean 6 S.D. of three assays
performed in duplicate. Cyclic AMP accumulation was normalized to the percentage of
forskolin-induced cAMP accumulation (set at 100%).

The research in animal model reflecting clinical cognitive deficits is critically important
to evaluate the effects of drugs. However, there is no unified classification whether
which animal model for testing cognitive deficits match which domains in listed in
Table 1. Multiple testing for one drug can resolve this problem.
In the present study, we investigated the therapeutic effects of a novel antipsychotic drug brexpiprazole on social recognition impaired by the N-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine, and cognitive deficits in mice after administration of the NMDA receptor antagonist phencyclidine (PCP). We divided our study into the following two chapters.

CHAPTER 1

We investigated the effects of brexpiprazole, olanzapine and risperidone, on dizocilpine-induced social recognition impairment, in mice. Furthermore, we also investigated the effect of brexpiprazole, olanzapine, and risperidone on social recognition, in untreated mice. Moreover, we investigated the role of serotonin 5-HT\textsubscript{1A} receptor on the effect of brexpiprazole on dizocilpine-induced social recognition impairment.

CHAPTER 2

Using the novel object recognition test (NORT), we investigated the effects of subsequent subchronic brexpiprazole on PCP-induced cognitive deficits in mice. Furthermore, we investigated the role of 5-HT\textsubscript{1A} receptor on the effect of brexpiprazole in this model.

REFERENCES


II. CHAPTER 1

Improvement of dizocilpine-induced social recognition deficits in mice by brexpiprazole, a novel serotonin-dopamine active modulator

1. ABSTRACT

Cognitive impairment, including impaired social cognition, is largely responsible for the deterioration in social life suffered by patients with psychiatric disorders, such as schizophrenia and major depressive disorder (MDD). Brexpiprazole (7-{4-[4-(1-benzothiophen-4-yl)piperazin-1-yl]butoxy}quinolin-2(1H)-one), a novel serotonin-dopamine activity modulator, was developed to offer efficacious and tolerable therapy for different psychiatric disorders, including schizophrenia and adjunctive treatment of MDD. In this study, we investigated whether brexpiprazole could improve social recognition deficits (one of social cognition deficits) in mice, after administration of the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (dizocilpine). Dosing with dizocilpine (0.1 mg/kg) induced significant impairment of social recognition in mice. Brexpiprazole (0.01, 0.03, 0.1 mg/kg, p.o.) significantly ameliorated dizocilpine-induced social recognition deficits, without sedation or a reduction of exploratory behavior. In addition, brexpiprazole alone had no effect on social recognition in untreated control mice. By contrast, neither risperidone (0.03 mg/kg, p.o.) nor olanzapine (0.03 mg/kg, p.o.) altered dizocilpine-induced social recognition deficits. Finally, the effect of brexpiprazole on dizocilpine-induced social recognition deficits was antagonized by WAY-100,635, a selective serotonin 5-HT$_{1A}$ antagonist. These results suggest that brexpiprazole could improve dizocilpine-induced social recognition deficits via 5-HT$_{1A}$ receptor activation in mice. Therefore,
brexpiprazole may confer a beneficial effect on social cognition deficits in patients with psychiatric disorders.

2. INTRODUCTION

Cognitive impairment describes a diverse range of deficits, seen in psychiatric disorders. Of these, impaired social cognition greatly hampers everyday life, resulting in poor work productivity or underemployment of patients with schizophrenia and major depressive disorder (MDD) (Tandberg et al, 2011; Tse et al, 2013; Horan et al, 2012; Lo and Siu, 2014). It is generally accepted that reducing social cognitive dysfunction is an important factor in assisting psychiatric patients to make healthy adjustments in their social lives (Tandberg et al, 2011; Tse et al, 2013; Horan et al, 2012; Trapp et al, 2013). Current reports suggest that training of social cognition may help to improve functional outcome in patients with schizophrenia (Henderson, 2013). To complement this, the development of novel drugs to improve social cognition deficits in patients with schizophrenia is also imperative.

Social recognition testing is designed to measure the propensity of a mouse to make contact with a novel rather than familiar mouse. This testing therefore represents a cognitive model that reflects innate ability to communicate with others (Moy et al, 2004; van der Kooij and Sandi, 2012). Social recognition test in rodents is also one of the assays for evaluating social cognition in humans (Millan and Bales, 2013). Based on the N-methyl-D-aspartate (NMDA) hypofunction hypothesis of schizophrenia (Hashimoto, 2006; 2014; Hashimoto et al, 2013), the NMDA receptor antagonist, (+)-MK-801 (dizocilpine), is widely used to induce schizophrenia-like behavioral abnormalities, including positive and negative symptoms and cognitive deficits in

Brexpiprazole, 7-{4-[4-(1-benzothiophen-4-yl)piperazin-1-yl]butoxy} quinolin-2(1H)-one, is a novel serotonin-dopamine activity modulator with high affinity for serotonin, dopamine and noradrenaline receptors (Maeda et al, 2014a). It is a partial agonist at serotonin 5-HT₁A and dopamine D₂ receptors, with a relatively equal potency, and an antagonist at 5-HT₂A receptors and adrenergic α₁B/2C receptors. Brexpiprazole is currently under clinical evaluation and expected to show efficacy and tolerability when used as therapy for different psychiatric disorders, including schizophrenia and adjunctive treatment for MDD. Very recently, brexpiprazole was shown to improve the NMDA receptor antagonist phencyclidine-induced cognitive deficits in the novel object recognition test in rodents (Maeda et al, 2014b; Yoshimi et al, 2014). To date, the effect of brexpiprazole on social recognition has not been investigated. In this study, we evaluated the effects of brexpiprazole on dizocilpine-induced social recognition deficits in mice.

3. EXPERIMENTAL PROCEDURES

3.1. Animals

Male C57BL/6NCrSlc mice (Japan SLC Inc., Shizuoka, Japan) aged between 4 and 5 weeks old were selected as stranger mice, while animals between 8 and 10 weeks old were used for this study. All mice were housed in groups of five per cage, in a room maintained at 23 ± 2 °C and 60 ± 10 % humidity, with a 12/12 hour light/dark cycle.
(lights on at 7:00 a.m.). The mice were given free access to food and water. Animal care and use were conducted in accordance with the Institutional Guidelines for Animal Care and Use (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan).

3.2. Drugs

(+)-MK-801 hydrogen maleate (dizocilpine) was purchased from Sigma-Aldrich Co., Ltd (Tokyo, Japan). Brexpiprazole, risperidone, olanzapine and WAY-100,635 were synthesized at Otsuka Pharmaceutical Co., Ltd (Tokyo, Japan). Dizocilpine was dissolved in saline and administered intraperitoneally (i.p.) at 10 ml/kg, twice daily, on the day before and 30 minutes prior to sociability testing. Brexpiprazole, risperidone, olanzapine and WAY-100,635 were dissolved in 5% (w/v) gum Arabic and administered orally (p.o.), at 10 ml/kg, 1 hour prior to sociability testing. The doses of dizocilpine (0.1 mg/kg) and WAY-100,635 (1.0 mg/kg) were selected from previously published reports in mice (Hagiwara et al., 2008; Hashimoto et al., 2009; Takatsu et al., 2011; Yoshimi et al., 2014; Zhang et al., 2007). The doses of antipsychotic drugs were selected based on doses that did not impact locomotion (data not shown).

3.3. Apparatus

The test apparatus consisted of a rectangular, three-chambered box, and a lid with an attached infrared video camera (O’Hara & Co., Tokyo, Japan). The apparatus was 610W x 400D x 220H mm, and the dividing walls were made from clear Plexiglass, with small square openings (3 x 5 cm) allowing access into each chamber. The stranger mouse was enclosed in a small, round wire cage (diameter, 10 cm; height, 12 cm), allowing olfactory, visual, auditory, and tactile contact, but no deep contact. Using a CCD camera, measures were taken of the amount of time spent around the wire cage. The total distance traveled was calculated based on traced mice movement and
presented as the locomotor activity in this study. All data were computerized. Activity was monitored and analyzed using applications based on the public domain NIH Image or Image J program (developed by Wayne Rasband at the U.S. National Institute of Mental Health and available on the Internet at http://rsb.info.nih.gov/nih-image/) (O’Hara & Co., Tokyo, Japan).

3.4. Behavioral procedures

The measurement of sociability and social recognition was performed using the same procedure described in previous reports (Moy et al., 2007; Nakatani et al., 2009; Matsuo et al., 2009; Riedel et al., 2009). In this study, we focused on a narrowly defined set of parameters.

3.4.1. Habituation

Mice were randomly assigned to groups. Mice were first placed in the middle compartment of the apparatus and allowed to explore freely for 6 minutes. All other compartments were empty during this habituation period.

3.4.2. Sociability test

An unfamiliar male (stranger) that had no prior contact with the mouse was placed in one of the side chambers. After the habituation period, the unfamiliar male juvenile mouse (stranger 1) was placed inside the round wire cage, in one of the side compartments (randomly selected and counterbalanced for each group). The opposite compartment was empty. The mice were able to freely explore all three compartments of the apparatus for 6 minutes. The time spent around cages (stranger 1 or empty) was calculated as direct contact.

3.4.3. Social recognition test

After sociability testing, a novel unfamiliar juvenile mouse (stranger 2) was
placed inside a new round wire cage and introduced to the ‘empty compartment’ of the apparatus. Mice were confined to the central compartment and were able to explore freely in all compartments for 10 min.

The recognition index (RI) was calculated using parameters measured during social recognition sessions and was defined as the quotient of the time the mouse spent around the novel juvenile (stranger 2) divided by the sum of the time spent around the familiar (stranger 1) and novel (stranger 2) juvenile mice.

3.5. Statistical analyses

All statistical analyses were performed using Prism 5 (GraphPad Software Inc., La Jolla, USA). Data are presented as mean ± standard error of the mean (S.E.M.). To determine the effects of drug treatment, one-way ANOVA, followed by the post hoc Bonferroni test (for RI and total distance) or a two-tailed paired t-test (for RI and time spent around cage), was used. P values of less than 0.05 were considered statistically significant.

4. RESULTS

4.1 Effects of brexpiprazole on dizocilpine-induced social recognition deficits

Mice in all groups spent significantly more time around stranger 1, than the empty area in sociability sessions (data not shown). A one-way ANOVA on RI in social recognition session showed a significant effect of treatment ($F_{(4, 56)} = 5.83, p < 0.001$)(Figure 1A). Saline-treated control mice had a significant preference for spending more time with the novel unfamiliar stranger 2, in comparison with familiar stranger 1, in the social recognition session (Figure 1B). Treatment with dizocilpine (0.1 mg/kg, i.p.) significantly decreased the total time spent around stranger 2 in social
recognition sessions. A paired t-test showed a significant (p < 0.01) impairment of RI in the dizocilpine-treated group. Mice spent the same amount of time interacting with stranger 1 and stranger 2 mice (Figure 1A, and 1B). The total distance moved (exploratory behavior), indicative of locomotor activity, was significantly increased by dizocilpine administration (Figure 1C).

Next, we examined whether brexpiprazole attenuated dizocilpine-induced social recognition deficits in mice. Neither dizocilpine (0.1 mg/kg, i.p.) nor brexpiprazole (0.01, 0.03 and 0.1 mg/kg, p.o) altered the amount of time spent around stranger 1 mice in sociability sessions (data not shown). However, at all three doses of brexpiprazole, mice treated with brexpiprazole showed a significant preference for spending more time with novel unfamiliar stranger 2 mice, compared with familiar stranger 1 mice in social recognition sessions (Figure 1B), and additionally, impaired RI was significantly restored by treatment with brexpiprazole at the same doses used in sociability testing (Figure 1A). Furthermore, brexpiprazole (0.01, 0.03 and 0.1 mg/kg) did not temper the increased locomotor activity induced by dizocilpine in social recognition sessions (Figure 1C).

4.2 Brexpiprazole alone did not affect social recognition or exploratory behavior

We examined the effect of brexpiprazole on social recognition in untreated control mice. Treatment with brexpiprazole (0.01, 0.03 and 0.1 mg/kg) had no effect on the time spent around stranger 1 mice in either sociability (data not shown) or social recognition sessions (Figure 1D, and 1E). Brexpiprazole did not alter RI in social recognition sessions (Figure 1D), nor did it have an effect on exploratory behavior during either session (Figure 1F).
Figure 1. Effects of brexpiprazole on dizocilpine-induced social recognition deficits

Dizocilpine (0.1 mg/kg, i.p.) or saline (10 ml/kg, i.p.) was administered twice daily on the day before and 30 minutes prior to sociability testing. Brexpiprazole (0.01, 0.03, 0.1 mg/kg, p.o.) or vehicle (5% (w/v) gum Arabic, p.o.) was administrated 1 hour prior to sociability testing. (A) Social recognition was measured by the recognition index (RI), that is, the quotient of time the subject mouse spent around the novel juvenile (stranger 2) mouse divided by the sum of the time spent around the familiar (stranger 1) and novel juvenile (stranger 2) mice during social recognition sessions. (B) Duration of time spent around each stranger during the social recognition sessions and, (C) exploratory behavior during social recognition sessions. Values are the mean ± S.E.M (n = 11-15). *p < 0.05, **p < 0.01, ***p < 0.001 compared with dizocilpine + vehicle group (A and C, one-way ANOVA, followed by post hoc Bonferroni test). **p < 0.01, ***p < 0.001 compared with stranger 1 mice (B, two-tailed paired t-test). Brexpiprazole (0.01, 0.03, 0.1 mg/kg, p.o.) or vehicle (5% (w/v) gum Arabic, p.o.) was administrated 1 hour prior to sociability testing. The effects of brexpiprazole on the RI (D), duration of time spent around each stranger mouse (E), and exploratory behavior (F), during social recognition sessions. Values are the mean ± S.E.M (n = 7-14). *p < 0.05, **p < 0.01, ***p < 0.001 compared with stranger 1 (E, two-tailed paired t-test). N.S.: not significant.
4.3 Risperidone and olanzapine had no effect on dizocilpine-induced social recognition deficits

We examined whether the atypical antipsychotic drugs, risperidone and olanzapine, affected dizocilpine-induced social recognition deficits in mice. A one-way ANOVA on RI in social recognition session showed a significant effect of treatment ($F_{(2, 31)} = 4.60, p < 0.05$) (Figure 2A). *Pot-hoc* analysis showed that risperidone (0.03 mg/kg, p.o.) did not ameliorate dizocilpine-induced social recognition deficits (Figure 2A, 2B), and dizocilpine-induced hyperlocomotion in the recognition sessions (Figure 2C). When administered alone, risperidone (0.03 mg/kg, p.o.) significantly decreased both RI and locomotor activity in untreated control mice (Figure 2D, 2E, and 2F).
A one-way ANOVA on RI in social recognition session showed a significant effect of treatment \( F(2, 27) = 7.72, p < 0.01 \) (Figure 3A). Post-hoc analysis showed that olanzapine (0.03 mg/kg, p.o.) did not affect time spent around stranger 2 mice, nor did it reverse dizocilpine-induced impairment of the RI (Figure 3A, and 3B). Furthermore, olanzapine had no effect on dizocilpine-induced hyperlocomotion in the recognition sessions (Figure 3C). Olanzapine (0.03 mg/kg, p.o.) alone was incapable of altering RI, the time spent around stranger 2 mice, or exploratory behavior in untreated control mice (Figure 3D, 3E, and 3F).
Figure 3. Effect of olanzapine on dizocilpine-induced social recognition deficits

Dizocilpine (0.1 mg/kg, i.p.) or saline (10 ml/kg, i.p.) administered twice daily on the day before and 30 minutes prior to sociability testing. Olanzapine (0.03 mg/kg, p.o.) or vehicle (5% (w/v) gum Arabic, p.o.) was administrated 1 hour prior to sociability testing. The effects of olanzapine on RI (A), duration of time spent around each stranger mouse (B) and exploratory behavior (C) during social recognition sessions. Values are the mean ± S.E.M (n=10). **p < 0.01, ***p < 0.001 compared with vehicle-treated control group for (A) and (C) (one-way ANOVA, followed by post hoc Bonferroni test). ***p < 0.001 compared with stranger 1 mice for (B) (two-tailed paired t-test). Olanzapine (0.03 mg/kg, p.o.) or vehicle (5% (w/v) gum Arabic, p.o.) was administrated 1 hour prior to sociability testing. The effects of olanzapine on RI (D), duration of time spent around each stranger mouse (E) and exploratory behavior (F) during social recognition sessions. Values are the mean ± S.E.M (n=7). **p < 0.01, ***p < 0.001 compared with stranger 1 mice for (E) (two-tailed paired t-test). N.S.: not significant.
4.4 Role of the 5-HT$_{1A}$ receptor in brexpiprazole's effects on dizocilpine-induced social recognition deficits

To investigate the function of serotonin 5-HT$_{1A}$ receptors in the pharmacological effect of brexpiprazole on dizocilpine-induced social recognition deficits, we examined the effect of WAY-100,635, a selective 5-HT$_{1A}$ receptor antagonist. A one-way ANOVA on RI in social recognition session showed a significant effect of treatment ($F_{(4, 57)} = 7.82, p < 0.0001$). Post-hoc analysis showed that WAY-100,635 (1.0 mg/kg, p.o.) significantly antagonized the effect of brexpiprazole on dizocilpine-induced social recognition deficits (Figure 4A, and 4B). Treatment with WAY-100,635 alone did not alter RI and locomotion in dizocilpine-treated mice (Figure 4A, and 4C). Furthermore, treatment with WAY-100,635 alone did not affect RI, the time spent around stranger 2 mice, or exploratory behavior in untreated control mice (Figure 4D, 4E and 4F).
Dizocilpine (0.1 mg/kg, i.p.) or saline (10 ml/kg, i.p.) was administered twice daily on the day before and 30 minutes prior to sociability testing. Brexpiprazole (Brex: 0.03 mg/kg, p.o.), WAY-100,635 (WAY: 1 mg/kg, p.o.), or vehicle (5% (w/v) gum Arabic, p.o.) was administrated 1 hour prior to sociability testing. The effects of brexpiprazole on RI (A), duration of time spent around each stranger mouse (B) and exploratory behavior (C) during social recognition sessions. Values are the mean ± S.E.M (n = 11-13). *p < 0.05, ***p < 0.001 compared with dizocilpine plus vehicle group, ###p < 0.001 compared with the dizocilpine plus brexpiprazole-treated group (A, one-way ANOVA, followed by post hoc Bonferroni test). *p < 0.05, ***p < 0.001 compared with stranger 1 mice (B, two-tailed paired t-test). N.S.: not significant. WAY-100,635 (WAY: 1.0 mg/kg, p.o.), or vehicle (5% (w/v) gum Arabic, p.o.) was administrated 1 hour prior to sociability testing. The effects of WAY-100,635 on RI (D), duration of time spent around each stranger mouse (E) and exploratory behavior (F) during social recognition sessions were shown. Values are the mean ± S.E.M (n = 7). ***p < 0.001 compared with stranger 1 mice (E, two-tailed paired t-test). N.S.: not significant.
5. DISCUSSION

The two major findings of this study are that the novel serotonin-dopamine activity modulator brexpiprazole, but not risperidone or olanzapine, improved social recognition deficits in mice after the administration of dizocilpine, and that 5-HT$_{1A}$ receptors are critical to this beneficial effect. Antipsychotic drugs, such as risperidone and olanzapine, are known to show limited efficacy in cognitive impairment, including social recognition in schizophrenia and depression (Sergi et al., 2007; Krakowski and Czobor, 2011; Suzuki et al., 2011; Remberk et al., 2012), despite improving various cognitive functions in rodent disease models (Wang et al., 2007; Gumuslu et al., 2013; Mutlu et al., 2011; 2012; Wolff and Leander, 2003). It is of considerable clinical importance to find new ways of improving social recognition deficits in patients with psychiatric diseases, such as schizophrenia and MDD, because of their huge negative impact on the social functioning of patients (Tandberg et al., 2011; Tse et al., 2013; Horan et al., 2012). There are noted discrepancies in antipsychotic drug effects between rodents and humans, increasing the need for further detailed clinical studies on the effects of brexpiprazole on social recognition in patients with psychiatric diseases.

Social recognition testing is a paradigm capable of evaluating social recognition, based on the propensity of an individual mouse to spend more time with an unfamiliar mouse than with a familiar mouse (Riedel et al., 2009; Nakatani et al., 2009). In other cognitive tests performed in rodents, mice discriminate objects by vision in the novel objective recognition test, by hearing in the fear conditioning test and by odor in the social transmission food preference test (Hashimoto et al., 2005; 2007; Boix-Trelis et al., 2007; Amann et al., 2010). However, since social recognition testing incorporates various factors such as visual, auditory and olfactory function and some tactile stimuli
needed for mouse sociability, this test provides a comprehensive evaluation of
cognition in rodents, compared with other cognitive tests (Nadler et al, 2004; Moy et al,
2004; 2007). Therefore, the social recognition test in mice may represent a translational
rodent model for human social cognition, since the model utilizes multiple aspects of
mice socialization.

Here, we evaluated social recognition using an automated three-chambered
apparatus system. This apparatus is capable of measuring the time and distance a subject
mouse covers in approaching a novel mouse, thereby evaluating the subject’s
motivation to interact with the novel mouse. Furthermore, this system can also measure
exploratory behavior by producing a representative trace of locomotor activity. This
means that effects on locomotion, such as, sedation by a drug, can be easily detected.
Since stranger mice were isolated individually in a wire cage, the subject mouse could
interact with a stranger mouse without attack or aversive stimuli. This makes the social
recognition test in mice a potential tool for assessing the efficacy of new drugs on social
recognition.

We found that the 5-HT1A antagonist, WAY-100,635, reversed the effects of
brexpiprazole on dizocilpine-induced social recognition deficits, indicating that 5-HT1A
receptors are critical to brexpiprazole’s mechanism of action. A report suggests that
5-HT1A receptor agonism is related to social recognition in rats (Millan et al, 2004). In
addition, it is reported that 5-HT1A receptors are important in the action of other
antipsychotic drugs (perospirone, aripiprazole, blonanserin), when tested in a
phencyclidine-induced cognitive deficits model (Hagiwara et al, 2008; Nagai et al,
2009; Horiguchi and Meltzer, 2012; 2013). In contrast, WAY-100,635 could alleviate
cognitive deficits in monkeys after administration of dizocilpine (Harder and Ridley,
suggested pro-cognitive effect of 5-HT$_{1A}$ receptor antagonist. However, in this study, WAY-100,635 alone was no effect in social recognition in both dizocilpine-treated and vehicle-treated control mice. Further studies of the effects of 5-HT$_{1A}$ receptor agonists and antagonists on social recognition are needed. Previously, Sumiyoshi et al. (2001a; 2001b; 2007) reported that treatment with tandospirone (or buspirone), 5-HT$_{1A}$ receptor agonists, improved cognition including executive function and verbal learning in patients with schizophrenia, indicating a key role of 5-HT$_{1A}$ receptor in cognition in patients (Meltzer and Sumiyoshi, 2008; Sumiyoshi et al, 2013). Taken together, it seems that brexpiprazole can ameliorate cognitive deficits associated with NMDA receptor dysfunction, via its 5-HT$_{1A}$ agonistic activity. Further studies are needed to clarify the effects of other antipsychotic drugs with 5-HT$_{1A}$ agonistic activity, on social cognition in patients.

Previous reports demonstrated that 5-HT$_{1A}$ receptor antagonists impair sociability in rodents, and that 5-HT$_{1A}$ receptor agonists improved sociability in the social interaction in rodents (File and Seth, 2003; Bruins Slot et al, 2005; Snigdha and Neil, 2008; Gould et al, 2011). These findings suggest an important role of 5-HT$_{1A}$ receptors in social behaviors. A recent study showed that the rewarding properties of social interaction in mice require the coordinated activity of oxytocin and 5-HT$_{1B}$ receptor in the nucleus accumbens, which are implicated in the social cognitive dysfunction in patients with a number of psychiatric diseases (Dölen et al, 2013). Since brexpiprazole has a moderate affinity at 5-HT$_{1B}$ receptor (Ki = 32 nM) (Maeda et al, 2014a), it is possible that 5-HT$_{1B}$ receptor may, in part, be involved in the mechanisms of action of brexpiprazole on social recognition deficits. Nonetheless, further studies on the role of 5-HT$_{1B}$ receptors in the mechanisms of action of brexpiprazole would be needed to
confirm this hypothesis.

In conclusion, brexiprazole ameliorated social recognition deficits in mice after administration of dizocilpine, and the 5-HT$_{1A}$ receptor antagonist WAY-100,635 reversed the effects of brexiprazole in this model. Our results imply that brexiprazole could potentially serve as a therapeutic drug to treat social cognitive deficits in patients with schizophrenia and MDD.
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III. CHAPTER 2

Effects of brexpiprazole, a novel serotonin-dopamine activity modulator, on phencyclidine-induced cognitive deficits in mice: a role for serotonin 5-HT$_{1A}$ receptors.

1. ABSTRACT

Brexpiprazole, a serotonin-dopamine activity modulator, is currently being tested in clinical trials as a new therapy for a number of neuropsychiatric diseases, including schizophrenia and major depressive disorder. Accumulating evidence suggests that 5-hydroxytryptamine (5-HT)$_{1A}$ receptors play a role in cognition. This study was undertaken to examine whether brexpiprazole, a novel drug with 5-HT$_{1A}$ receptor partial agonism, could improve cognitive deficits in mice, induced by repeated administration of the N-methyl-D-aspartate (NMDA) receptor antagonist, phencyclidine (PCP).

Subsequent subchronic (14 days) oral administration of brexpiprazole (0.3, 1, or 3.0 mg/kg/day) significantly attenuated PCP (10 mg/kg/day for 10 days)-induced cognitive deficits in mice, in a dose-dependent manner. The effects of brexpiprazole (3.0 mg/kg) were significantly antagonized by co-administration of the selective 5-HT$_{1A}$ receptor antagonist, WAY-100,635 (1.0 mg/kg), although WAY-100,635 alone was not effective in this model. These findings suggest that brexpiprazole can ameliorate PCP-induced cognitive deficits in mice via 5-HT$_{1A}$ receptors. Therefore, brexpiprazole could ameliorate cognitive deficits as seen in schizophrenia and other neuropsychiatric diseases.
2. INTRODUCTION

Cognitive deficits are a core feature in people with schizophrenia, leading to both vocational and social disabilities (Coyle and Tsai, 2004; Freedman, 2003; Green et al, 2004; Niitsu et al, 2012; Sumiyoshi et al, 2013; Yoshida et al, 2012b). Current evidence suggests that dysfunctional glutamatergic neurotransmission, via N-methyl-D-aspartate (NMDA) receptors is instrumental in the pathophysiology of schizophrenia (Coyle and Tsai, 2004; Hashimoto et al, 2004; 2005b; 2013; Heresco-levy and Javitt, 1998; Krystal et al, 1999; Javitt and Zukin, 1991; Javitt et al, 2012). Antagonists at the NMDA receptor, such as phencyclidine (PCP), are known to induce schizophrenia-like symptoms, including cognitive deficits in healthy subjects (Javitt and Zukin, 1991). This has led to the use of sub-chronic administration of PCP, as an animal model of cognitive deficits in schizophrenia (Fujita et al, 2008; Hagiwara et al, 2008; Hashimoto et al, 2005a; 2006; 2007a; 2007b; Kunitachi et al, 2009; Javitt et al, 201; Jentsch and Roth, 1999; Tanibuchi et al, 2009). In the novel object recognition test (NORT), PCP (10 mg/kg/day for 10 days)-induced cognitive deficits were significantly ameliorated by subsequent, subchronic (14 days) doses of clozapine, but not haloperidol (Hashimoto et al, 2005a). These findings suggest that this reversal of PCP-induced cognitive deficits as measured by the NORT, may represent a potential animal model of atypical antipsychotic activity, in the amelioration of schizophrenia related cognitive deficits (Hagiwara et al., 2008; Hashimoto et al, 2005a; 2006; 2007a; 2007b; 2007c; Tanibuchi et al, 2009).

Several lines of evidence show that 5-hydroxytryptamine (5-HT)\textsubscript{1A} receptors play a role in the pathophysiology of neuropsychiatric diseases including schizophrenia, and that 5-HT\textsubscript{1A} receptors are important targets for emotion and cognition (Bantick et al,
Early evidence comes from findings that the density of 5-HT$_{1A}$ receptor binding is altered in the hippocampus and cerebral cortex of postmortem brains from schizophrenia patients (Burnet et al., 1996; Gurevich and Joyce, 1997; Joyce et al., 1993; Lopez-Figueroa et al., 2004). A positron emission tomography study demonstrated increased cortical 5-HT$_{1A}$ receptor binding in drug-naive patients with schizophrenia (Borg, 2008; Tauscher et al., 2002). In contrast, other studies found no changes in 5-HT$_{1A}$ receptor density between schizophrenia and control samples (Bantick et al., 2004; Borg, 2008; Frankle et al., 2006).

Second, atypical antipsychotic drugs, such as clozapine, ziprasidone, aripiprazole, and quetiapine are all partial 5-HT$_{1A}$ receptor agonists, a property which may be relevant for their therapeutic actions in schizophrenia (Jordan et al., 2002; Newman-Tancredi et al., 2001; Rollema et al., 2000; Sprouse et al., 1999). Third, Sumiyoshi and colleagues (2001a; 2001b) reported that adjunctive treatment with tandospirone, a selective 5-HT$_{1A}$ receptor agonist, induced improvements in some types of memory function, as well as cognitive performance in schizophrenia. Taken together, findings to date suggest 5-HT$_{1A}$ receptor agonists as potential therapeutic drugs for treating cognitive deficits in schizophrenia (Meltzer and Sumiyoshi, 2008; Newman-Tancredi, 2010; Yoshida et al., 2012b; Sumiyoshi et al., 2013).

Brexipiprazole, 7-\{4-\{4-\{(1-benzothiophen-4-yl)piperazin-1-yl\}butoxy\}quinolin-2(1H)-one (Figure 1), is a potent partial agonist at human 5-HT$_{1A}$ (Ki = 0.12 nM), and dopamine D$_{2L}$ (Ki = 0.3 nM), and an antagonist at 5-HT$_{2A}$ receptors (Ki = 0.47 nM)(Maeda et al., 2014).

Considering the high affinity of brexipiprazole for 5-HT$_{1A}$ receptors, it would be of interest to study the effects of brexipiprazole on PCP-induced cognitive deficits in mice.
In the present study, using the NORT, we examined the effects of subchronic treatment (14 days) with brexpiprazole on cognitive deficits in mice, induced after repeated administration of PCP. Furthermore, we examined the role of 5-HT$_{1A}$ receptors in the action of brexpiprazole on the PCP-induced cognitive deficit model.

3. EXPERIMENTAL PROCEDURES

3.1. Animals

Male ICR mice (6 weeks old) weighing 25–30 g were purchased from SLC Japan (Hamamatsu, Shizuoka, Japan). The mice were housed in clear polycarbonate cages (22.5×33.8×14.0 cm) in groups of 5 or 6 individuals under a controlled 12/12-h light–dark cycle (light from 7:00 AM to 7:00 PM), with the room temperature kept at 23 ± 1°C and humidity at 55 ± 5%. The mice were given free access to water and food pellets specifically designed for mice. The experimental procedure was approved by the Animal Care and Use Committee of Chiba University Graduate School of Medicine.

3.2. Drugs

PCP hydrochloride was synthesized in our laboratory at Chiba University. Brexpiprazole was provided from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). WAY-100,635 maleate, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexanecarboxamide maleate salt), was purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Other drugs were purchased from commercial sources.
3.3. Drug administration

Saline (10 ml/kg/day) or PCP (10 mg/kg/day expressed as a hydrochloride salt) was administered subcutaneously (s.c.) for 10 days (once daily on days 1-5, 8-12) with no treatment on days 6, 7, 13, and 14 (Figure 5). The treatment schedule was designed in the manner of a previous PCP-induced cognitive deficit model (Hashimoto et al, 2005a; 2006; 2007a; 2007b; 2007c). After the final administration of saline or PCP, vehicle (10 ml/kg/day; 0.5% methylcellulose), brexiprazole (0.3, 1, or 3.0 mg/kg/day), brexiprazole (3.0 mg/kg/day) plus WAY-100,635 (1 mg/kg/day), or WAY-100,635 (1 mg/kg/day) alone was administered orally for 14 consecutive days (once daily on days 15-28) (Figure 5). The dose (1 mg/kg) chosen for WAY-100,635 was selected because this dose was found to attenuate PCP-induced cognitive deficits via 5-HT1A receptor mechanisms in vivo (Hagiwara et al, 2008).
3.4. Novel object recognition test (NORT)

The NORT was administered as previously reported (Hashimoto et al., 2005a; 2006; 2007a; 2007b; 2007c). As shown in Figure 5, the training session of NORT was carried out 1 day (day 29) after the final administration of vehicle, brexpiprazole, or WAY-100,635. The apparatus for this task consisted of a black open-field box (50.8×50.8×25.4 cm). Before the test, mice were habituated to the box for 3 days. During the training session, two objects (various objects were used that differed with respect to shape and color, but that were similar in size) were placed in the box at a 35.5-cm distance from each other, and in a symmetrical fashion, and each animal was
allowed to explore the interior of the box for 10 min (5 min x 2). The animals were considered to be investigating the object when the head of the animal was either facing the object and was located within an inch of the object, or if any part of the body, except for the tail, was touching the object. The time that the mice spent exploring each object was recorded. After the training session, the mice were immediately returned to their home cages, and the box and objects were cleaned with 75% ethanol to avoid any possible pheromonal cues. The retention test session was carried out 1 day after the respective training sessions. During each retention test session, each mouse was placed back into the same box it had previously encountered, but in which one of the two objects used during training had been replaced by a novel object. The mice were then allowed to freely explore the interior for 5 min, and the time spent exploring each object was recorded. Throughout the experiments, the objects were used in a counter-balanced manner in terms of their physical complexity. In order to measure memory performance, a preference index was used, i.e., the ratio of the amount of time the mouse spent exploring any one of the two objects (training session) or the novel object (retention session) to the total time spent exploring both objects.

3.5. Statistical analysis

The data are expressed as mean ± S.E.M. Statistical analysis was performed using one-way analysis of variance (ANOVA) and the post hoc Bonferroni test. The P values of less than 0.05 were considered statistically significant.
4. RESULTS

4.1. Effects of brexiprazole on PCP-induced cognitive deficits in mice

In the NORT, repeated administration of PCP (10 mg/kg/day for 10 days) led to cognitive deficits in mice, consistent with previous reports (Hashimoto et al, 2005a; 2006; 2007a; 2007b). During the training session, there were no differences in exploratory preferences among the eight groups of mice (F (7, 96) = 0.444, p=0.872) (Figure 6A). Subsequent subchronic (14 days) administration of brexiprazole significantly attenuated PCP-induced cognitive deficits in mice, in a dose-dependent manner. However, during the test session, ANOVA analysis revealed significant differences in the exploratory preferences among the eight groups (F (7, 96) = 15.52, p < 0.001) (Figure 6B). Post hoc Bonferroni test results indicated that subchronic dosing with brexiprazole increased exploratory preferences in the PCP-treated group, in a dose dependent manner (Figure 6B). In addition, the effect of brexiprazole (3.0 mg/kg) on PCP-induced cognitive deficits was significantly antagonized by co-administration of the selective 5-HT1A receptor antagonist, WAY-100,635 (1.0 mg/kg/day) (Figure 6B). Moreover, a 14 day dosing with WAY-100,635 (1.0 mg/kg/day) alone did not reverse PCP-induced cognitive deficits in mice (Figure 6B). Neither did a 14 day dosing with brexiprazole (3.0 mg/kg/day) alone alter exploratory preferences in control (saline-treated) mice (Figure 6B).
**Figure 6. Effects of subchronic administration of brexpiprazole on PCP-induced cognitive deficits in mice**

Treatments were performed as shown in Figure 2. On days 29 and 30, the NORT was performed. Values are the mean ± S.E.M (n = 10-16). N.S.: not significant, Brex: brexpiprazole, WAY: WAY-100,635. **P < 0.01, ***P < 0.001 compared with the PCP plus vehicle-treated group. ###P < 0.001 compared with the PCP plus brexpiprazole (3.0 mg/kg)-treated group.
DISCUSSION

This study found that brexpiprazole was able to ameliorate PCP-induced cognitive deficits in mice, via 5-HT$_{1A}$ receptors. Previously, we reported that PCP-induced cognitive deficits as demonstrated on the NORT could be improved by subsequent subchronic (14 days) administration of clozapine, but not haloperidol. This suggested that the reversal of these deficits may represent a potential animal model of atypical antipsychotic activity, for use in the ongoing investigations aimed at restoring cognitive deficits in schizophrenia (Hashimoto et al., 2005a). Given the role of 5-HT$_{1A}$ receptors in cognition (Bantick et al., 2001; Meltzer, 1999; Ogren et al., 2008; Yoshida et al., 2012b; Sumiyoshi et al., 2013), it is likely that brexpiprazole confers a beneficial effect on cognitive deficits in patients with neuropsychiatric diseases, such as schizophrenia.

In an earlier report, we found that repeated dosing with PCP (10 mg/kg/day for 10 days) significantly reduced the density of 5-HT$_{1A}$ receptors in mouse hippocampus, although the precise mechanisms underlying this action are currently unknown (Hagiwara et al., 2008). Furthermore, we found that repeated PCP administration significantly altered the response to 8-OH-DPAT (8-hydroxy-2-(dipropylamino)tetralin hydrobromide: a 5-HT$_{1A}$ agonist)-induced hypothermia in mice (Hagiwara et al., 2008). We also reported that perospirone, an antipsychotic drug with 5-HT$_{1A}$ receptor agonism, could attenuate PCP-induced cognitive deficits via 5-HT$_{1A}$ receptors (Hagiwara et al., 2008). Together, these findings imply that altered 5-HT$_{1A}$ receptor function resulting from repeated PCP treatment may add to the dysregulation of glutamatergic neurotransmission in the brain.

Sumiyoshi et al. (2001a; 2001b) reported that adjunctive treatment with the
5-HT$_{1A}$ receptor agonist, tandospirone, improves certain types of memory function, as well as cognitive performance in patients with schizophrenia. In a later study, Sumiyoshi et al. (2007) also reported that adjunctive treatment with buspirone, a widely available 5-HT$_{1A}$ receptor agonist, significantly enhanced attention compared with the placebo control group, highlighting a possible benefit for buspirone augmentation of atypical antipsychotic drugs, to enhance attention. These findings suggest that 5-HT$_{1A}$ receptor agonists, including brexpiprazole, may be useful for treating cognitive deficits in schizophrenia.

Cognitive deficits are well documented in patients with other neuropsychiatric diseases, including major depressive disorder (Porter et al., 2003; Hindmarch and Hashimoto, 2010; Hasselbalch et al., 2011; Yoshida et al., 2012a), bipolar disorder (Harvey et al., 2010; Bourne et al., 2013), and substance abuse (Dean et al., 2013). In some ways, rodent NORT performance is analogous to human declarative (episodic) memory, one of the seven cognitive domains impaired in patients with schizophrenia (Rajagopal et al., 2014). It is therefore possible that brexpiprazole could be an equally useful therapeutic drug in these patients.

In conclusion, our findings showed that PCP-induced cognitive deficits in mice could be improved by subchronic treatment with brexpiprazole. Therefore, brexpiprazole which acts as a 5-HT$_{1A}$ receptor partial agonist, could potentially serve as a therapeutic drug for the treatment of cognitive deficits in patients with schizophrenia and other neuropsychiatric diseases.
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IV. CONCLUSION

Brexpiprazole improved social recognition deficits impaired by dizocilpine in social recognition test. This effect was antagonized by 5-HT1A receptor antagonist WAY-100,635, suggesting that brexpiprazole can improve social recognition deficits impaired by N-methyl-D-aspartate antagonist via 5-HT1A agonistic activity. Additionally, brexpiprazole improved cognitive deficits impaired by PCP in NORT. This effect was also antagonized by WAY-100,635, suggesting that brexpiprazole can improve cognitive deficits impaired by N-methyl-D-aspartate antagonist throughout 5-HT1A agonistic activity. Taken together, these studies suggest that brexpiprazole has ability to improve cognitive deficits in animal models of psychiatric disorders. Therefore, brexpiprazole would have beneficial effects for cognitive deficits in the patients with a number of psychiatric disorders.
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