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# Oxytocin attenuates deficits in social interaction but not recognition memory in a prenatal valproic acid-induced mouse model of autism

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ABSTRACT

Recent studies have reported that oxytocin ameliorates behavioral abnormalities in both animal models and individuals with autism spectrum disorders (ASD). However, the mechanisms underlying the ameliorating effects of oxytocin remain unclear. In this study, we examined the effects of intranasal oxytocin on impairments in social interaction and recognition memory in an ASD mouse model in which animals are prenatally exposed to valproic acid (VPA). We found that a single intranasal administration of oxytocin restored social interaction deficits for up to 2 h in mice prenatally exposed to VPA, but there was no effect on recognition memory impairments. Additionally, administration of oxytocin across 2 weeks improved prenatal VPA-induced social interaction deficits for at least 24 h. In contrast, there were no effects on the time spent sniffing in control mice. Immunohistochemical analysis revealed that intranasal administration of oxytocin increased c-Fos expression in the paraventricular nuclei (PVN), prefrontal cortex, and somatosensory cortex, but not the hippocampal CA1 and CA3 regions of VPA-exposed mice, suggesting the former regions may underlie the effects of oxytocin. These findings suggest that oxytocin attenuates social interaction deficits through the activation of higher cortical areas and the PVN in an ASD mouse model.

## 1. Introduction

Autism spectrum disorders (ASD) are neurodevelopmental disorders characterized by core symptoms including impairments in social behavior and communication (Geschwind and Levitt, 2007; Persico and Bourgeron, 2006). Individuals with ASD often also display other symptoms, such as cognitive impairment, hyperactivity, anxiety, and sensory abnormalities (Cervantes and Matson, 2015; Gadke et al., 2016; Joshi et al., 2010; Yeargin-Allsopp et al., 2003).

Although the etiology of ASD remains elusive, clinical research has indicated that doses of valproic acid (VPA) during pregnancy increases the incidence of autism in children (Christensen et al., 2013; Ornoy, 2009). We recently reported that prenatal VPA exposure at embryonic day 12.5 (E12.5) induces ASD-like behavioral abnormalities, including social interaction and recognition memory impairments, in male mice (Kataoka et al., 2013). Furthermore, we suggest that the brain regions responsible for social and recognition memory impairments are the cortical areas and hippocampus, respectively (Takuma et al., 2014).

Oxytocin, a neuropeptide synthesized in the paraventricular nucleus (PVN) and supraoptic nucleus of the hypothalamus (Lee et al., 2009), is known to play a critical role in a range of social behaviors, such as maternal behavior, pair bonding, sexual behavior, and social memory/recognition (Insel, 2010; Ross and Young, 2009). It has been suggested that alterations in the oxytocinergic system are associated with the pathogenesis of ASD. Genetic studies revealed that single nucleotide polymorphisms in the oxytocin receptor were a risk factor for ASD (Lerer et al., 2008; Liu et al., 2010; Wu et al., 2005). In animal studies, both oxytocin knockout mice (Lazzari et al., 2013; Winslow et al., 2000) and oxytocin receptor knockout mice (Pobbe et al., 2012a, 2012b; Sala et al., 2011; Takayanagi et al., 2005) display ASD-like behavioral abnormalities. Accordingly, recent animal studies reported that intraperitoneally (Peñagarikano et al., 2015; Teng et al., 2013, 2016) or subcutaneously (Meziane et al., 2015) administered oxytocin attenuated social behavioral deficits in animal models of ASD.

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Furthermore, recent clinical studies also showed that intranasal treatment with oxytocin improved impairments in socio-communication and social reciprocity in people with ASD (Watanabe et al., 2014, 2015). However, it remains unclear how oxytocin exerts these ameliorating effects on ASD-like behaviors, especially when administered intranasally.

In the present study, we examined the effects of intranasal oxytocin on social interaction deficits and recognition memory impairments in mice exposed prenatally to VPA. Furthermore, we analyzed c-Fos expression in the brain of oxytocin-treated ASD mice. In addition, we investigated the effect of repeated treatment with intranasal oxytocin.

## 2. Materials and methods

### 2.1. Animals

ICR (CD1) mice were purchased from Japan SLC Inc. (Hamamatsu, Japan) at 8 weeks of age. The mice were housed individually in plastic cages (28 × 17 × 12 cm) under a standard light/dark cycle (12-h light cycle starting at 8:00) at a constant temperature of 22 ± 1 °C. The animals had *ad libitum* access to food and water, and were handled in accordance with the guidelines established by the Animal Care and Use Committee of the Graduate Schools of Pharmaceutical Sciences and Dentistry, Osaka University, the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society, and the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Vaginal smear checks were conducted as previously reported (Hara et al., 2015, 2016). Vaginal smears were collected from all female mice every day, and stained using Giemsa solution. Female mice were mated with male mouse overnight when a vaginal smear exhibited proestrus or early estrus. The next day was defined as gestation day 0.

### 2.2. Drug administration

VPA and oxytocin were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Peptide Institute Inc. (Osaka, Japan), respectively. Both drugs were dissolved in 0.9% NaCl solution (Otsuka pharmaceutical Co., Ltd., Tokushima, Japan) before use. Administration of VPA was conducted as previously reported (Hara et al., 2012, 2015, 2016; Kataoka et al., 2013; Takuma et al., 2014). Either VPA (500 mg/kg) or saline was administered intraperitoneally to pregnant mice on E12.5 at a volume of 10 mL/kg body weight. All animals were returned to their home cages immediately after administration and left undisturbed until weaning of the offspring. Offspring were weaned, sexed, and caged in groups of 5–6 mice of the same sex at postnatal day 21. In this study, we used only male offspring to avoid sex differences that we reported in previous publications (Hara et al., 2012, 2015; Kataoka et al., 2013).

Oxytocin was administered intranasally to male offspring. For the single treatment experiment, oxytocin (50–200 μg/kg) or saline was intranasally administered at a volume of 0.2 mL/kg body weight. For the repeated treatment experiment, oxytocin (100 μg/kg) was intranasally administered once daily for 2 weeks, and behavioral analysis were carried out 24 h after the last administration. We used the same dose of oxytocin as was used in a previous study (Peñagarikano et al., 2015). Pedersen et al. (2011) have already reported that intranasal oxytocin administration for 2 weeks improved psychiatric symptoms and social cognition in patients with schizophrenia. In addition, we recently demonstrated that 2-week treatments with attention deficit/hyperactivity disorder drugs, methylphenidate and atomoxetine (Hara et al., 2016), and the atypical antipsychotic drugs, risperidone and aripiprazole (Hara et al., 2017), alleviated the behavioral and prefrontal dendritic spine deficits in VPA-exposed mice. Therefore, in this study, we decided to administer oxytocin for 2 weeks.

### 2.3. Social interaction test

The social interaction test was carried out according to our previous reports (Hara et al., 2016; Kataoka et al., 2013). Briefly, an intruder mouse was placed in the test cage after habituation of the test mouse to the same cage for 60 min. Over the total experimental period (20 min), the duration of face and ano-genital sniffs of the resident mouse to the intruder mouse was measured with a stopwatch. This test was carried out between 10:00–14:00.

### 2.4. Novel object recognition test

The novel object recognition test was carried out according to our previous reports (Hara et al., 2016; Takuma et al., 2014). In brief, after habituation to the experimental box under dim light conditions (30 lx) for 3 consecutive days, the test mouse was allowed to freely explore two novel objects (A and B) placed in the box for 10 min. Twenty-four hours after the training session, the retention session was conducted. In the retention session, object B was replaced with novel object C, and the mouse was allowed to move freely for 5 min in the same box. The exploration time for each object was measured with a stopwatch. The *discrimination index* (%) was the difference between the exploration time for the novel object plus that for the familiar object divided by total exploration time. This index was used to calculate values for recognition memory. This test was conducted between 10:00–14:00.

### 2.5. Immunohistochemistry

c-Fos immunostaining was conducted according to the protocol presented in our previous reports (Hara et al., 2015). In brief, 2 h after intranasal administration of oxytocin or saline, mice were perfused transcardially with saline, followed by a solution of 4% paraformaldehyde in phosphate-buffered saline (PBS). Serial 20-μm-thick coronal sections were cut using a cryostat (CM1520; Leica Microsystems GmbH, Wetzlar, Germany) at −20 °C. After treatment with citrate buffer and 0.3% $H_2O_2$, the sections were blocked with 5% goat serum for 1 h at room temperature. They were then incubated with anti-c-Fos rabbit polyclonal primary antibody (1:2000; SC-253, Santa Cruz Biotechnology, Dallas, TX, USA) overnight at 4 °C. The following day, the sections were incubated with biotinylated goat anti-rabbit IgG (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature. Subsequently, the sections were incubated with avidin-biotin-horseradish peroxidase complex (Vectastain ABC kit, Vector Laboratories) for 1 h, and then reacted with 3,3′-diaminobenzidine (DAB). Five sections per mouse were selected and c-Fos-positive nuclei were counted using an AxioImager.M2 microscope (Carl Zeiss, Jena, Germany).

### 2.6. Statistical analysis

All data are represented as the mean ± standard error of the mean (SEM). Data for behavioral tests were analyzed with two-way analysis of variance (ANOVA) followed by *post hoc* Bonferroni's or Tukey's multiple comparisons test. Data for the immunohistochemical analysis was analyzed by unpaired *t*-test. Statistical analyses of the experimental data were performed with Prism 5/6 for Windows/Mac (GraphPad Software, San Diego, CA, USA). The criterion for statistical significance was $P < 0.05$. Effect sizes for ANOVA and *t*-test were expressed as $\eta^2$ and Cohen's *d*, respectively. The effect size was classified as small (0.01 for $\eta^2$, 0.2 for Cohen's *d*), medium (0.06 for $\eta^2$, 0.5 for Cohen's *d*) and large (0.14 for $\eta^2$, 0.8 for Cohen's *d*).