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Effects of chronic oxytocin on attention to dynamic facial expressions in infant macaques

Lisa A. Parr[a,b,c,*], Jenna M. Brooks[a], Trina Jonesteller[a], Shannon Moss[a], James O. Jordano[d], Thomas R. Heitz[a]

[a] Yerkes National Primate Research Center, 954 Gatewood Rd., Atlanta, GA, 30329, USA
[b] Department of Psychiatry and Behavioral Science, Emory University, Atlanta, GA, 30322, USA
[c] Center for Translational Social Neuroscience, Emory University, Atlanta, GA, 30329, USA
[d] Department of Biology, Emory University, Atlanta, GA, 30322, USA

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ABSTRACT

Studies in a variety of species have reported enhanced prosocial effects after an acute administration of the neuromodulating hormone, oxytocin (OT). Although the exact mechanisms underlying these effects are not fully understood, there is broad interest in developing OT into a treatment for social deficits. Only a few studies, however, have examined the effects of OT if given repeatedly during early development, the period when early intervention is likely to have the greatest benefits for reversing the progression towards social impairment. Those studies, exclusively in rodents, report mixed results. Some have shown enhancement of prosocial behavior, including increased social exploration, but others have shown anti-social effects, including increased aggression. In the present study, infant rhesus macaques were treated with a high-frequency (3× per week) or low-frequency (1× per week) dose of intranasal oxytocin (IN-OT) or placebo (IN-saline) between two and six months of age, after which their reactions to dynamic facial expressions (neutral, lipsmacking and threats) were measured. Results showed that IN-OT, compared to placebo, increased the time monkeys spent viewing the expression videos, but selectively reduced attention to the eyes in neutral faces in a dose dependent manner. The mechanism for this non-prosocial effect may be that repeated IN-OT administration down-regulates the expression of OT receptors in brain regions important for regulating social attention. Consequently, our results raise questions about the efficacy of implementing chronic IN-OT as a pharmacotherapy for the treatment of social deficits, particularly if given early in development. More work is needed, not only to identify optimal treatment schedules, but also to understand how IN-OT exerts its influences on the brain and behavior.

1. Introduction

Numerous studies in humans and nonhuman primates have reported that an acute dose of intranasal oxytocin (IN-OT) improves various aspects of social cognition and prosocial behavior, including interpreting emotion from faces, enhancing trust, increasing social memory, enhancing social reward, and modulating social attention (Chang et al., 2012; Ebitz et al., 2013; Guastella and MacLeod, 2012; Parr et al., 2013; Parr, 2014; Shamay-Tsoory and Abu-Akel, 2016). This has led to great excitement over the past decade that IN-OT may be an effective pharmacotherapy for enhancing prosocial functions in individuals with disorders characterized by social impairments, like autism spectrum disorders (ASD). However, there is still much to be learned about exactly how IN-OT is influencing behavior and where in the brain it might be acting (Churchland and Winkielman, 2012). In addition to these concerns, the majority of studies have measured the behavioral effects of OT after a single, acute administration in adults (mostly male). Understanding the impact of OT if given repeatedly during early development is of particular importance because many social disorders in humans, like ASD, are developmental in nature. Thus, the greatest potential to reverse or suspend the progression towards social impairment would be associated with early, repeated intervention.

Research has shown that the oxytocinergic system is functional early in development and has important organizing effects on the brain and behavior (Hammock, 2015; Miller and Caldwell, 2015). Early manipulation of the OT system, either through per-

* Corresponding author at: 954 Gatewood Rd., Atlanta, GA, 30329, USA.
E-mail address: lparr@emory.edu (L.A. Parr).

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turbations of early-life experience (for reviews, Alves et al., 2015; Veenema, 2012) or exogenous administration can, therefore, have long-lasting effects on social behavior and brain function (Bales and Perkeybile, 2012; Hammock, 2015). Several studies have now begun to explore the effects of chronic OT administration early in development and the results are often contradictory to the broad prosocial functions described in acute studies. Bales (Bales et al., 2013) gave IN-OT to male and female prairie voles (21 days of age) and measured its effects on social interaction into adulthood (42 days of age). Males that received a low (0.08 IU/kg) or medium dose (0.80 IU/kg) of OT were less likely to form their species-typical partner bonds, whereas no differences were found in those receiving a high OT dose (8.00 IU/kg) (Bales et al., 2013). In captive pigs, IN-OT (50 μg) or placebo was given over the first three days of life and social responsiveness was tested post-weaning (17 days of age). The pigs given IN-OT showed more aggression towards peers and were less interested in social interactions compared to placebo subjects (Rault et al., 2013). Finally, adult male mice received two doses of IN-OT (0.15 IU and 0.30 IU) or placebo per day for 7–21 days. Individuals given IN-OT, regardless of the dose, showed a reduction in social investigation, in addition to a reduction in oxytocin receptor (OXTR) expression throughout the brain (Huang et al., 2014). In contrast, an acute administration of IN-OT in the same species increased social behavior directed towards a novel female, but not a novel male. These studies suggest that chronic overstimulation of the oxytocinergic system in healthy animals, particularly early in development, may have unwanted and detrimental effects on social behavior.

The rhesus monkey is an excellent species in which to study the effects of chronic OT on the development of social behavior. Rhesus monkeys live in large, highly social groups in which mothers form strong, protracted bonds with their infants. They have large brains that are highly homologous with humans and individuals display advanced social cognitive skills, including sensitivity to a diverse range of facial expressions (Hinde and Rowell, 1962). Moreover, monkeys develop much faster than humans (often reported as 4× as fast, Boothe et al., 1982), providing an opportunity for longitudinal behavioral studies on a much faster time scale than is possible in humans. Although there are a growing number of studies on the effects of acute IN-OT in adult monkeys (Chang et al., 2012; Dal Monte et al., 2014a; Ebitz et al., 2013; Landman et al., 2014; Parr et al., 2013; Parr, 2014), only one study to date has examined the effects of acute IN-OT administration in infant monkeys. Simpson et al. (2014) gave a 25 IU dose of IN-OT to 28 infant rhesus monkeys between 7 and 14 days of age. They found that IN-OT increased the facial gestures made by the infant monkeys in response to the same behavior displayed by an experimenter, e,g., facial mimicry. While these results suggest that IN-OT enhances prosocial behavior in infant monkeys, it should be noted that these monkeys were removed from their mothers at birth and nursery reared, and studies show that manipulation of the mother-infant relationship can have adverse consequences on the development of the OT system in a variety of species (e.g., see Hammock, 2015; Veenema, 2012).

The present study is the first to report on the effects of chronic IN-OT administration in 24 mother-reared infant rhesus macaques treated three times per week with either a high-frequency or low-frequency dose of IN-OT, or IN-saline between 2 and 6 months of age. Infants' viewing behavior was then measured at 6 months of age in response to videos of conspecific facial expressions. If chronic IN-OT enhances prosocial behavior in infant monkeys, we expect that IN-OT will increase the time monkeys spend looking at the eyes in all facial expressions, consistent with previous findings (Dal Monte et al., 2014a; Guastella et al., 2008; Ebitz et al., 2013), and this will be greater when the eyes and heads are directed back at the viewer, rather than averted. Moreover, we hypothesize that IN-OT will reduce the aversive quality of the threat expressions (Parr et al., 2013), resulting in longer viewing times for these expressions after IN-OT treatment compared to placebo. We expect these effects to occur in a dose dependent manner.

2. Methods

2.1. Subjects

Twenty-four, male infant rhesus macaques (*Macaca mulatta*) served as the subjects for this study. The study focused on males due to the increased prevalence of developmental social impairments, e.g., autism, in males compared to females. All infants were healthy, full-term, >450 g, offspring born into large social groups (~50–100 individuals) at the Yerkes National Primate Center field station (Lawrenceville, GA). Mothers consisted of both primiparous (N = 3) and multiparous (N = 21) females of all ranks. The infants were mother-reared and remained living in their social groups during the course of this study.

2.2. Treatment groups and dosing

The infants were assigned to one of three treatment groups at birth (placebo, high-frequency OT and low-frequency OT), balancing the group assignments for their mother's rank, e.g., high (alpha or beta family), middle, or low (bottom two families). Due to the limited number of infant male subjects available for this study, maternal parity was unable to be balanced across treatment group. Subjects were dosed three times per week, where the placebo group received 3 doses of saline, the high-frequency OT group received 3 doses of OT, and the low-frequency group received 1 dose of OT and 2 doses of saline. Each treatment group was color coded and the weekly doses prepared in three separate vials, labelled 1–3, to be given each week. In this way, research staff remained blind as to which vial contained placebo and which contained OT. Each OT vial contained 0.12 ml of concentrated OT (Oxytocin acetate salt, Sigma-Aldrich, 0.821 mg/ml), while each placebo vial contained 0.12 ml of saline and these were stored at −80 °C until the day of use. All vials were prepared by individuals not involved in either administering the doses to subjects, or the behavioral testing. Prior to use, research staff blind to both treatment condition and the contents of each vial thawed and then diluted each vial with 4 ml of sterile saline, so that it could be administered in aerosol form using a pediatric nebulizer. Subjects were nebulized for four minutes, which aerosolizes 2 ml of fluid keeping 2 ml in reserve to insure a steady stream of aerosol. Thus, each dose delivered approximately 0.049 mg of OT, equivalent to 24 IU (1.71 μg/IU). These procedures have been shown to successfully deliver IN-OT to the central and peripheral nervous systems in both monkeys and humans (Chang et al., 2012; Dal Monte et al., 2014b; Freeman et al., 2016; Modi et al., 2014; Striepens et al., 2013).

Dosing began when the infants were two months of age. Using established protocols, researchers entered the social group and isolated the mother-infant pair, moving them to the indoor enclosure (e.g., see Muschinski et al., 2016). The pair was then boxed and moved to a smaller testing cage where the infant was removed from its mother's ventrum. While one experimenter gently restrained the infant, another placed a mask connected to the nebulizer over the infant's face so as not to obstruct breathing (see Fig. 1). With the mask in place, the nebulizer was turned on and the infant passively breathed the aerosolized dose for four cumulative minutes (Modi et al., 2014). These procedures were used until the infants were 16 weeks of age, when it became more difficult to manually restrain them. After this time, all infants were dosed in a custom-made 'dosing box' that contained a clear front panel and several port openings for the nebulizers (see Fig. 1). Once inside the dosing box,