Evaluating the Effects of Subchronic Oxytocin Administration on Social Behaviors in Juvenile Mice

by
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Administration on Social Behaviors in Juvenile Mice

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Abstract

Previous research has demonstrated the importance of social interactions in typical development and growth. Recent research demonstrates oxytocin's role in prosocial behaviors, and oxytocin has been proposed as a pharmacological treatment for certain social deficits. The purpose of this study is to explore the effects of sub-chronic oxytocin administration on social behaviors in male and female, juvenile mice. Mice underwent housing and drug manipulations to test the effects of sub-chronic oxytocin administration, isolation housing, and sex on social behaviors evaluated using a three-chamber assay developed for determining sociability and the preference for social novelty. Results indicate sub-chronic oxytocin administration induces sex-specific changes in social behavior with an interaction between housing condition, drug treatment, and sex. These results suggest that there is a complex relationship existing between these variables that should be considered further in the prescription and use of oxytocin to treat social symptoms in clinical populations.

Table of Contents

List of Tables

List of Figures

Introduction

Early socialization and life experiences create an immense impact on behavior and development through the lifespan (Ricceri, Moles, & Crawley, 2007). Social behavior impairments are key features of multiple neurodevelopmental disorders including autism spectrum disorder (ASD). The American Psychiatric Association (APA, 2016) defines ASD as a social disorder characterized by persistent deficits in communication and interaction in social situations. A sex bias is observed in ASD with males being more than three times as likely to have the disorder than females (Halladay et al., 2015). The phenotype for ASD is complex and variable and may also include repetitive, stereotyped behaviors in humans (Bodfish, Symons, Parker, & Lewis, 2000; Insel, 2001). Explanations of the social symptoms of autism are numerous, including genetic vulnerability and deficits in assigning social salience or a lack of social motivation (Gordon et al., 2013; Insel, 2001). Few effective pharmacological treatments for ASD exist, and most do not address the pervasive social deficits (Gordon et al., 2013). As recent research has shown oxytocin's importance in prosocial behavior, the ability of oxytocin as a drug to improve social symptoms is currently being investigated (Preti et al., 2014). However, the current literature remains divided on oxytocin's effectiveness (Tachibana et al., 2013).

Oxytocin is a hormone acting as a neurotransmitter and neuromodulator fueling sexual behavior, parenting behaviors, and the development of social attachments (Insel, 2001). Oxytocin administration in adults with Asperger syndrome and adults with ASD increased levels of eye contact and social approach (Andari et al., 2010). Intranasal oxytocin increases activity in brain areas key for social behaviors and improved social

responsiveness in ASD patients (Gordon et al., 2013; Yatawara, Einfeld, Hickie, Davenport, & Guastella, 2016). Oxytocin administration in individuals with ASD also increases the ability to process context information relevant to social interaction and then apply that information (Andari et al., 2010). Another study utilizing intranasal oxytocin administration in preadolescent boys with ASD shows that long-term use is safe, tolerable, and may improve the quality of communication and social interaction (Tachibana et al., 2013; Teng et al., 2016). In a double-blind placebo-controlled study, oxytocin administration in adults with ASD and adults with Asperger's disorder helped to facilitate the retention of social information (Hollander et al., 2007). Despite this work, there remains controversy over the effectiveness of oxytocin as other studies have found no therapeutic benefit or even negative social behaviors as a result of oxytocin administration (Shamay-Tsoory & Abu-Akel, 2016; Tachibana et al., 2013).

Animal models can be used to explore the potential reasons behind these conflicting results. Specifically, mice have often been used in this research due to many advantages including the ability to control genetics and environment, the ability to manipulate uniformly across subjects, and the animal's natural tendency to engage in complex social behaviors (Moy et al., 2007). Using animal models, it has been found that oxytocin may affect these social behaviors in a multitude of different ways, such as facilitating social learning and memory, selectively influencing sociability, and guiding social motivation (Andari et al., 2010; Ferguson et al., 2000; Gordon et al., 2013; Teng et al., 2016). Oxytocin may act in these situations by reinforcing social interactions and regulating attention to social cues (Hung et al., 2017; Shamay-Tsoory & Abu-Akel, 2016). Oxytocin has been shown to increase levels of social interaction in C57BL/6J

mice, but dose effects and effect timelines appear differential within the sexes (Teng et al., 2016; Zhang et al., 2015). By genetically altering mice to create non-functioning oxytocin genes, researchers have found that these oxytocin-knockouts have lower social approach and the inability to use the same social cues as normal mice (Winslow & Insel, 2002). Administering oxytocin in small doses to these mice has been shown in some cases to rescue the social deficits and social functioning to near normal levels (Teng et al., 2013; Sobota et al., 2015; Winslow & Insel, 2002). Even oxytocin administration provided in utero can affect future social behavior through the oxytocin system (Takayanagi et al., 2005). One common theme that has emerged in research is that the role of oxytocin in moderating social behavior is modulated by several factors including time point of administration, dose and number of doses, sex, and context. The current study uses C57BL/6J mice to further investigate factors that may influence the effectiveness of oxytocin in increasing prosocial behaviors with the goal of better understanding the mixed outcomes of oxytocin administration in clinical populations.

The C57BL/6J mouse is a specific inbred strain of mouse with moderately high levels of social behaviors and known sex differences in social behaviors making them ideal for exploring sex effects on social behaviors following oxytocin treatment (Moy et al., 2004). C57BL/6J mice score high on social tests, especially in areas of general sociability and in the preference for social novelty (Moy et al., 2007; Nadler et al., 2004). These results exist across the lifespan (Moy et al., 2004). Research with C57BL/6J has shown sex differences in baseline activity that parallel differences across sexes in those with ASD (Zhang et al., 2015). C57BL/6J is also frequently chosen as it is one of a few

strains to exhibit complexities of social behavior comparable to those of human social behavior (Bolivar, Walters, & Phoenix, 2007).

Many with ASD often report feelings of social isolation due to their social symptoms, often causing anxiety as well; because of this, another important factor to consider in the effectiveness of oxytocin treatment is environment (Orsmond et al., 2013; White et al., 2009). One way of modeling this social isolation in mice is to house the mice in isolation for a period of time (Koike et al., 2009; Ricceri et al., 2007). Mice and rats are highly social animals, and isolating them can induce several key social deficits and anxiety (Ago et al., 2007; Koike et al., 2009; Li et al., 2017). The long-term behavioral deficiencies seen in many neurodevelopmental disorders often parallel to those results seen in socially deprived lab rodents (Toth et al., 2008). In rats, isolation has also been shown to decrease exploring while increasing hyperactivity (Arakawa, 2005; Del Arco et al., 2004; Guo, Wu, Liu, Yang, & Chen, 2004). Isolating mice during the juvenile period takes away opportunities for mice to have key social experiences that may strongly affect the development of typical social behaviors (Koike et al., 2009; Tanaka et al., 2019). Mice isolated past weaning throughout the juvenile period show significantly altered behavioral changes including anxiety-like behavior in most tests (Voikar et al., 2005). Effects of isolation are differential in adult rodents than juveniles which has been suggested to point to a critical period for socialization that cannot be reversed (Arakawa, 2005; Bloomberg et al., 1994; Branchi et al., 2006; Garcia-Pardo et al., 2015; Sadler & Bailey, 2016).

Social behavior in the current study is measured by the Crawley Three-Chamber task. This task allows a mouse to seek out social interaction with another mouse while

restraining the non-experimental (stimulus) mouse. This allows the measurement of the experimental mouse's social behavior while reducing reactivity to the stimulus mouse's movements. Additionally, the Three-Chamber Task also allows for the experimental mouse to select familiar or novel social stimuli — testing not only the sociability of the mouse, but also its preference for social novelty (Crawley, 2004).

The current study aims to illustrate the effects of subchronic oxytocin administration, social isolation, and sex on sociability and preference for social novelty in C57BL/6J in juvenile mice. By doing so, we can further explore the potential of oxytocin as a treatment for social behavior impairments.

Methods

Seventy-five C57BL/6J (#000664) mice were purchased from Jackson Laboratories at 21 days of age. After a one-week habituation period, juvenile mice were randomly placed in either isolation housing or group hosing (the typical housing condition) with 4 other sex-matched mice for three weeks. During the last week of housing, each mouse received an intraperitoneal (i.p.) injection of either saline or oxytocin (1 mg/kg) every other day for one week (4 injections with 48 hours between injections). One area of disagreement in the field is whether oxytocin crosses the blood brain barrier following i.p. injection. Single i.p. injections have failed to illicit changes in the central nervous system; however, the subchronic schedule of oxytocin administration used in the current study has been previously shown to impact the central nervous system (Estes et al., 2019). Varying the sex (two levels), housing condition (two levels), and drug administration (two levels) yielded eight groups (Table 1).

Table 1. Experimental Groups of Subject Mice

Group	Sex	Housing	Drug	N
1	Male	Group	Saline	10
2	Female	Group	Saline	10
3	Male	Group	Oxytocin	9
4	Female	Group	Oxytocin	11
5	Male	Individually	Saline	10
6	Female	Individually	Saline	9
7	Male	Individually	Oxytocin	7
8	Female	Individually	Oxytocin	9

Immediately after the final injection, researchers used the Crawley Three-Chamber Task to evaluate sociability and preference for social novelty (Moy et al., 2004). The task was conducted in a clear, three-chambered plexiglass box with passages to allow the mouse to explore each chamber (left, center, and right). The arena measures 24” x 12” with each chamber measuring 8”. The task consists of three ten-minute phases. In each phase, the subject mouse was initially introduced in the center chamber. Phase one of the assay acted as an acclimation period for the subject mouse to habituate to the three-chamber box. During phase one, two empty, inverted, stainless-steel wire pencil cups were kept in each side chamber (left and right), and no social stimuli (mice) were presented. Phase two of the assay acted as a period to measure sociability of the subject mouse. Researchers added a trained conspecific mouse under one of the wired pencil

cups as a social stimulus. Stimulus mice were trained prior to the experiment by placing them in the inverted pencil cup two times a day for three days. This ensured that the stimulus mouse was calm during the experiment and would not affect the subject mouse's behavior. The pencil cups were used to allow the subject mouse to see, smell, and interact with conspecific mice at the subject mouse's choosing. Due to the social nature of mice, typical mice are expected to explore the chamber with the social stimulus more than the empty chamber. Phase three of the assay acted as a period to measure the preference for social novelty in subject mice. Researchers added a second conspecific mouse to the previously empty chamber under a wire pencil cup to allow the subject mouse to choose between the, now, familiar mouse from phase two or the novel mouse just introduced (Yang et al., 2011; see Figure 1). Due to the general preference of novelty in mice, typical mice are expected to seek out the novel social stimulus as compared to the familiar one. All phases and trials were recorded for data coding.

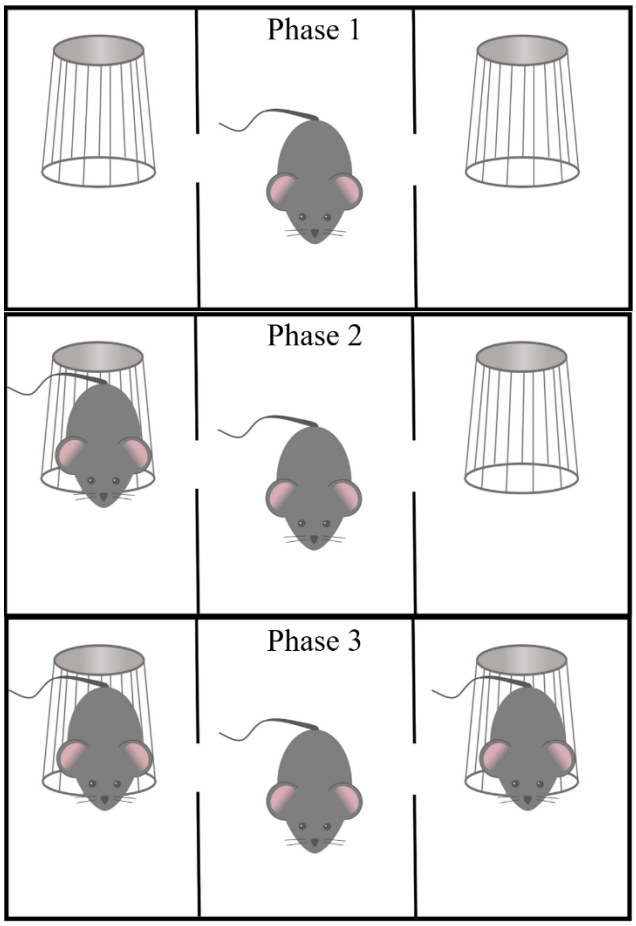

Figure 1. Visualization of Three Chamber Assay

Video recordings were hand coded by trained coders. As published previously, coders rated and recorded information such as number of entries in each chamber, time spent in each chamber and the time spent sniffing each stimulus mouse (Kaidanovich-

Beilin et al., 2011; Yang et al., 2011). These behaviors were chosen to help elucidate the sociability and preference for social novelty of the experimental mice.

Results

All data were entered into SPSS for analysis. Data from each phase of the Three-Chamber Task were analyzed separately. Since no social stimulus was presented in Phase 1, entries into chambers and time in chambers was analyzed to determine baseline differences in locomotion and side preference within the arena. A one-way ANOVA was used to determine the effect of group on the total number of chamber entries (a measurement of locomotion). No differences in baseline locomotion were observed between groups which indicated no hyperactivity in any of the groups ($F(6, 60) = 0.506$, $p = 0.80$). Paired t-tests were used to determine if any group spent more time in one side chamber as compared to the other. All t-tests were nonsignificant ($p > 0.05$) except that of the Male/Saline/Group Housing group ($t(9) = 2.54$, $p = 0.03$). This might indicate a side preference for this group, which served as a control group for the housing and drug conditions. However, the results of this test appear to be driven by an outlier. The data from this mouse were not excluded from the following analyses, however, as this was the only outlying data point for this subject. Overall, no side bias was observed during the first phase.

During the second phase, time in chambers and time spent sniffing were compared across and within groups. Each group showed a preference for the side containing the social stimulus as compared to the empty stimulus demonstrated by increased time spent in the chamber containing the social stimulus with the exception of the Female/Saline/Group Housing group that neared significance (Female/Saline/Group:

t(9) = 2.16, p = 0.05; Male/Saline/Group*: t(9) = 3.10, p = 0.013;* Female/Saline/Isolated: *t(8) = 3.36, p = 0.01*; Male/Saline/Isolated: *t(9) = 6.38, p < 0.001*; Female/Oxytocin/Group: *t(9) = 3.58, p = 0.006*; Male/Oxytocin/Group: *t(8) = 5.17, p = 0.001*; Female/Oxytocin/Isolated: *t(8) = 3.59, p = 0.007*; Male/Oxytocin/Isolated: *t(7) = 8.97, p < 0.001*). This indicates that each group had typical sociability and social isolation did not impair sociability (See Figure 2). When comparing the amount of time spent in the chamber with the social stimulus and the amount of time spent sniffing the social stimulus across groups, no group differences were found (*F(7, 67) = 1.47, p = 0.19, F(7, 67) = 0.86, p = 0.539*, respectively). Likewise, separate three-way ANOVAs found no main effects for sex, drug treatment, or housing condition on time spent in social chamber (*F(7, 67) = 1.47, p = 0.19*) or on time spent sniffing social stimulus (*F(7, 67) = 0.86, p = 0.54*).

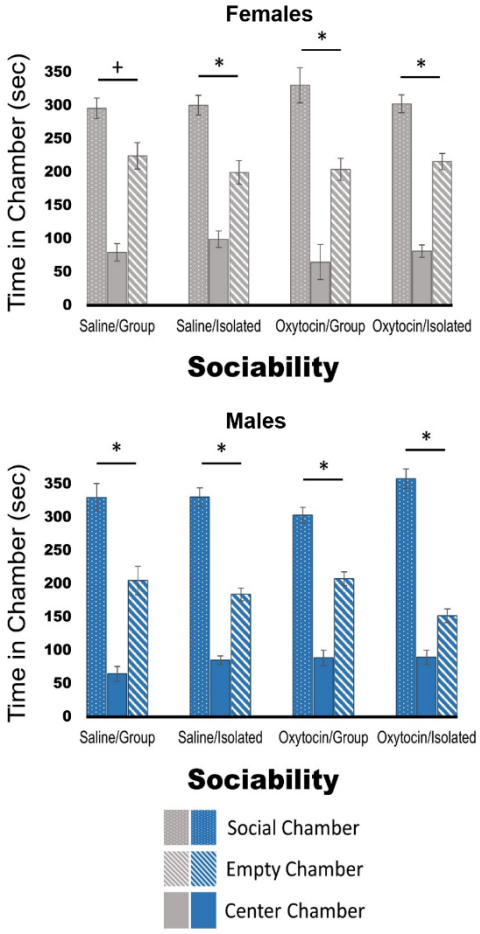

Figure 2. Phase 2 Results from Three-Chamber Task

During the third phase, time spent in the chamber with the novel social stimulus as compared with the familiar social stimulus and the time spent sniffing the novel social stimulus as compared with the familiar social stimulus were analyzed between and across groups (See Figure 3). A factorial ANOVA indicated that no main effects existed for sex, drug treatment, or housing condition for time spent in the chamber with the novel social stimulus (Sex: $F(1, 67) = 0.49, p = 0.61$; Drug: $F(1, 67) = 0.01, p = 0.94$; Housing: $F(1, 67) = 2.71, p = 0.53$). Likewise, no interactions were found to be significant (Sex*Drug:

F(1, 67) = 0.79, p = 0.54; Sex*Housing: *F(1, 67) = 2.16, p = 0.38*; Drug*Housing*: F(1, 67) = 0.38, p = 0.65*; Sex*Drug*Housing: *F(1, 67) = 0.41, p = 0.52)*. Similarly, no main effects or interactions were found for time spent sniffing the novel stimulus (Sex: *F(1, 67) = 0.79, p = 0.38;* Drug: *F(1, 67) = 1.01, p = 0.31*; Housing*: F(1, 67) = 1.56, p = 0.22*; Sex*Drug: *F(1, 67) = 2.23, p = 0.13*; Sex*Housing: *F(1, 67) = 0.96, p = 0.33*; Drug*Housing*: F(1, 67) = 1.45, p = 0.23*; Sex*Drug*Housing: *F(1, 67) = 2.12, p = 0.15*). However, unexpectedly, a significant three-way interaction was found for time spent in the chamber with the familiar stimulus (Sex*Drug*Housing*: F(1, 67) = 5.43, p = 0.02*) and for time spent sniffing the familiar stimulus (Sex*Drug*Housing: *F(1, 67) = 7.67, p = 0.007*). Pairwise comparisons identified specific group differences shown in Figure 3 *(p < 0.05*). In groups treated with saline, no differences were observed for male or female mice in the amount of time spent sniffing the familiar or novel social stimulus suggesting that the C57BL/6J mice did not display a preference for social novelty with or without the housing manipulation. However, both male and female mice in the oxytocin administration conditions display preference for social novelty. To better explain the significant three-way interactions, pairwise comparisons were conducted between the remaining groups. The social isolation housing condition did not have significant effects on the female mice, but decreased sniffing time of the familiar stimulus in the saline treated male groups (*p < 0.05*) indicating that social isolation increased social stimulus differentiation and preference for social novelty in males. In males, oxytocin increased sniffing across groups receiving the group housing condition (*p < 0.05*) indicating increased sociability without differentiating between novel and familiar social stimuli. In females, oxytocin decreased sniffing time of the familiar social stimulus (*p < 0.05*)

indicating increased social stimulus differentiation and preference for social novelty in female mice treated with oxytocin.

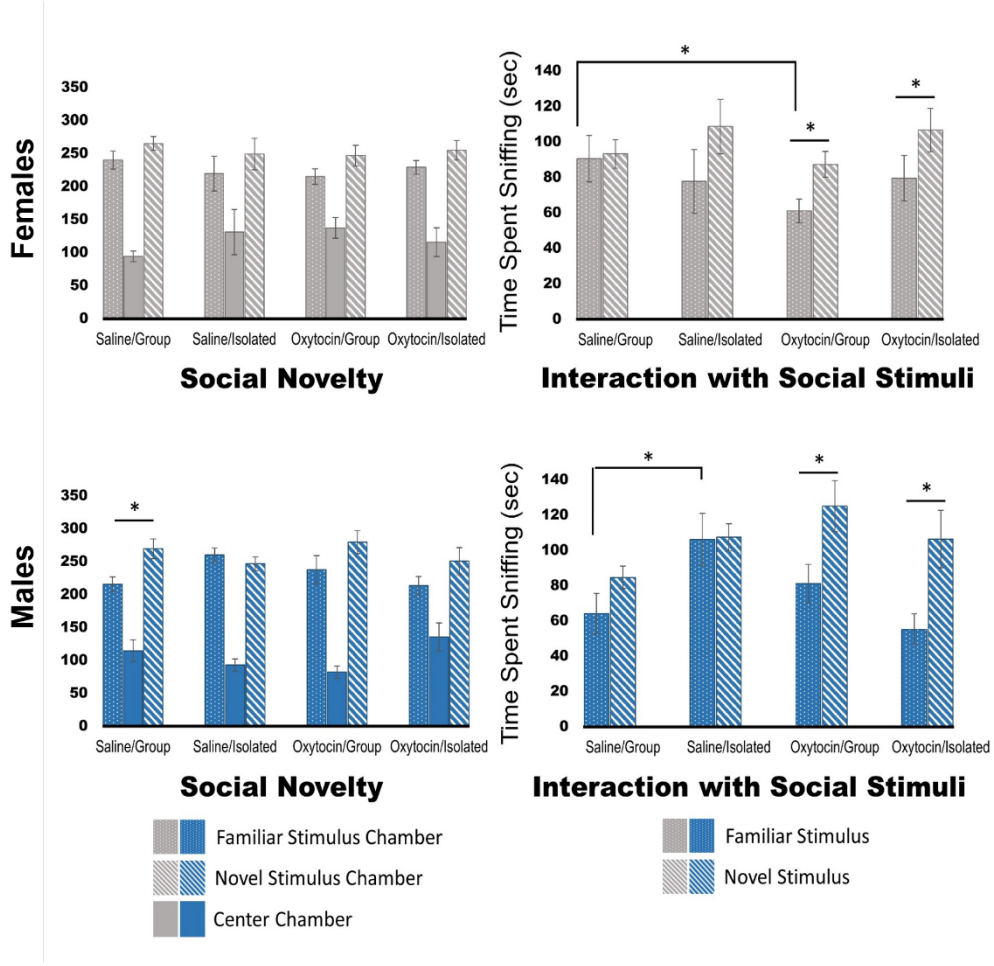

Figure 3. Phase 3 Results from Three-Chamber Task

Discussion

This study used mice to model social behaviors, as mice models have many advantages including easy manipulation, large levels of experimental control, and complex social behaviors that are comparable to humans. We chose specifically to use

the C57BL/6J strain due to their moderate levels of social behavior, sex differences that parallel those in ASD, and life-long social behaviors illustrated by the Three-Chamber Task. These mice were either group housed as a control or housed in isolation to replicate the social isolation felt by many with ASD. Because of the growing research indicating oxytocin's involvement in prosocial behavior, we chose to explore oxytocin administration's effects on social behaviors. We used the Three-Chamber Task to measure sociability and the preference for social novelty following these manipulations. The goal of this study was to detect whether sex and environmental stress factors may impact the effectiveness of subchronic oxytocin.

Regarding phase 1, the habituation period, there were no differences expected and no differences were found. Phase 2, the test for sociability, found expected differences in all mice groups which suggests that neither environmental stress nor oxytocin administration altered the sociability levels. This could potentially be due to a ceiling effect on oxytocin as social withdraw increases sociability. Phase 3, the test for preference for social novelty, showed several differential effects. These interactions suggest that aspects of social behavior such as social memory or social cognition are affected by sex and stress differentially following oxytocin. The differential effects found highlight the complex relationships between oxytocin and social behaviors.

The current study did have some limitations. The use of multiple coders may have increased variability in the data. Additionally, social behavior is known to be particularly variable, and additional mice could be added to the groups to increase statistical conclusion validity. The potential ceiling effect in phase 2 represents another confound in this study. In phase 3, even control groups have no social novelty effects; although,

recent research documents similar results in the C57BL/6J mouse strain (Pearson et al., 2010). Future research could expand on the current study by including additional outcome measures and timepoints of observation. Anxiety measures are needed to fully understand the effect of oxytocin as recent research indicates that it may increase anxiety in some clinical populations. Mice could also be observed in free interaction with a social stimulus following the same manipulations to determine how the subject mouse would respond and whether social approach observed was friendly or aggressive. Using other doses and routes of administration would also help to better understand the effect of subchronic i.p. oxytocin administration in mice.

The present study identified sex and social stress like isolation as factors that influence the effect of oxytocin on social behavior. Overall, the present study has highlighted the importance of further understanding the effects of subchronic oxytocin in conjunction with other variables to clarify these complex relationships and further help clinical populations.

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