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Oxytocin and Serotonin and their Roles in Pre-Pubertal Social Development in Syrian Golden Hamsters

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Oxytocin and Serotonin and their Roles in Pre-Pubertal Social Development in Syrian Golden Hamsters

by

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Thesis

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Dedication

This work is dedicated to Amy, whose compassion, loyalty, and stoicism helped make me the person I am today.

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Thank you to everyone who has supported me during my time in the Delville lab. Special thanks to Yvon Delville, who has patiently taught me all the various lab techniques and guided me through the research process. Thank you to Lina and Kristie who contributed a great deal to the execution of this study. Thank you to August, Steven, Patience, Nishat, Annette, and all the other wonderful undergraduates who have coded hours of video without complaint and sacrificed weekend time to help see this project through.

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Abstract

Oxytocin and Serotonin and their Roles in Pre-Pubertal Social Development in Syrian Golden Hamsters

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The pre-pubertal infancy stage of development is marked by numerous neurological and behavioral changes in Syrian Golden Hamsters. One of the most notable changes during this period is the onset of social behaviors including social playfighting. Social playfighting is initiated approximately two weeks postpartum and is completely abolished by mid-puberty. The neural mechanisms that underlie this behavioral change are not well understood, but previous research has identified both oxytocin (OT) and serotonin (5-HT) as possible regulators of this behavior. In the present study, immunohistochemical techniques were used to evaluate changing levels of OT and serotonin 5-HT in the developing brain with the hypothesis that both OT and 5-HT levels would increase and decrease simultaneously with the onset and decline of social playfighting. Additionally, it was predicted that injections of a 5-HT3 receptor agonist, m-Chlorophenylbiguanide hydrochloride (CBG), into hamster in late infancy would reinstated social playfighting behaviors. Contrary to the hypothesis, OT was found to continually increase in the fornix, lateral hypothalamus, nucleus accumbens, medial

preoptic area, and anterior hypothalamus, while 5-HT continually decreased in the lateral septal nucleus and medial preoptic area. CBG injections did not reinstate social playfighting behaviors, however a large stress effect was observed, potentially masking any other effect. Analysis of OT and 5-HT receptors during this developmental stage is necessary for a better understanding of this neural mechanism. Further research into this topic may have important implications for animal models of autism spectrum disorders.

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List of Abbreviations

The following is a list of abbreviations that will be used throughout this document:

5-HT	Serotonin
AH	Anterior hypothalamus
AVP	Vasopressin
BST	Bed nucleus of the stria terminalis
CBG	m-Chlorophenylbiguanide hydrochloride
IL	Infralimbic
LH	Lateral Hypothalamus
LSV	Lateral septal nucleus
MePD	Posterodorsal medial amygdala
MPOA	Medial preoptic area
NA	Nucleus accumbens
PrL	Prelimbic
VMLH	Ventromedial lateral hypothalamus

Introduction

Syrian golden hamsters (*Mesocricetus auratus*) undergo many developmental changes, both neurological and social, between birth and adulthood. Postnatal development can be divided into three distinct periods. In the first two weeks postpartum, pups are incapable of foraging for food and thermoregulation, depending completely on the mother to sustain them. As pups, hamsters huddle together, a behavior that is primarily driven by a need for thermoregulation. However, as pups enter the second infancy stage of development, huddling takes on a more social aspect, similar to huddles seen in rats (Siegel, 1985, pp. 310–312). Two weeks postpartum, infants display burst in locomotion and exploration outside the nest in search of food, which eventually evolves into the establishment of individual nest sites during the onset of puberty at approximately postnatal day 28 (P28) (Siegel, 1985, Chapter 13). This second developmental period continues for about two weeks, at which time juvenile hamsters enter puberty which extends from P28 – P60.

The work presented her focuses on the second stage of development. This infancy period is marked by many neurobiological and behavioral changes. As thermoregulation increases, time spent in huddles decreases (Roswell, 1961). Such stereotyped behaviors as flank marking also emerge during this time. Although the flank organs are primarily testosterone-driven, the marking behavior begins around P21 (Daly, 1976; Johnston, 1981). Although the affiliation with the mother declines during this period, other social interactions, most notably social play, begin taking place (Siegel, 1985, Chapter 13).

Infant hamsters begin engaging in playfighting around P15 and the behavior peaks around P21 then steadily declines (Yvon Delville, David, Taravosh-Lahn, & Wommack, 2003). Social playfighing is different from serious playfighting in that it is

rewarding for all participants, while serious fighting is used to establish dominance and subordinance (C. Ferris & Delville, 1994). In the Syrian Golden Hamster, infants perform playfighting, which gives way to the rough-and-tumble serious playfighting mid-puberty, and eventually develops into adult fighting (Yvon Delville et al., 2003). These behaviors can be distinguished by the target of attack. In play, infant hamsters tend to attack the face and cheek pouch areas; mid-puberty serious fighting is marked by an increase in side attacks, while the body targets of serious fighting are the belly and rump area (Yvon Delville et al., 2003; Pellis & Peills, 1988; Wommack & Delville, 2003). Figure 1 shows a representation of attack sites. It has been proposed that playfighting may play a sociosexual role. Studies have shown that investigation of the cheek area in adult hamsters occurs solely during sexual interaction, and not during aggressive resident/intruder interactions (Pellis & Peills, 1988).

The underlying neural mechanisms that regulate the onset and decline of playfighting and this developmental period are not well understood. Previous research has indicated that the presence of arginine-vasopressin (AVP) in the anterior hypothalamus inhibits offensive components of playfighting as well as offensive aggression in adult hamsters (S-Y Cheng, Taravosh-Lahn, & Delville, 2008; Shao-Ying Cheng & Delville, 2009; C. Ferris & Delville, 1994). Serotonin (5-HT) has been shown to modulate AVP in the central nervous system and AVP-mediated adult agonistic behaviors (C. Ferris & Delville, 1994). This indicates a possible role for 5HT in the onset and decline of infant playfighting behaviors. Evidence has indicated that low dose injections of fluoxetine, a selective serotonin reuptake inhibitor (SSRI), enhance offensive playfighting responses in juveniles, while high doses reduce them (Taravosh-Lahn, Bastida, & Delville, 2006), and recent research has indicated a role specifically for

the 5-HT3 receptor subtype in modulating juvenile aggressive behavior (Ricci, Grimes, & Melloni, 2004).

Another possible regulator of juvenile social play is the neuropeptide oxytocin (OT). Structurally, OT and AVP are very similar, differing by only two out of nine amino acids. It is widely supported that OT and AVP play a major role in regulation of adult social behavior across mammalian species. However, their roles in infant behavior have not been extensively studied. In adults, it is widely known that OT promotes prosocial and affiliative behaviors, especially in females. A recent study found that injections of an OT receptor antagonist inhibited juvenile social play in female rats (Bredewold, Smith, Dumais, & Veenema, 2014). OT has also been implicated in reducing social deficits in mouse models of the asocial component of autism spectrum disorders (Teng et al., 2013). Evidence has also been found for a role for OT and AVP in mediating early social environment-induced changes in social behaviors (Veenema, 2012).

The present study seeks to elucidate both a role for 5-HT and OT in the behavioral regulation of the development of infant playfighting. In the first experiment, immunohistochemical techniques were used to visualize OT fibers in juvenile hamster brains at P15, P18, P21, and P25. Based on previous data, it was predicted that OT promotes playfighting, and as such, the density of OT fibers would increase starting at P15, peak around P21, then steadily decline in a pattern mirrors the onset and decline of playfighting.

The second experiment used immunohistochemical techniques to quantify 5-HT in the brains of hamsters at P15, P21, and P27. To assess the effects of 5-HT on behavior, litters were injected with a 5-HT agonist during the decline of playfighting (P26), and playfighting behaviors were quantified. It was predicted that administration of the 5-HT agonist would reinstate playfighting.

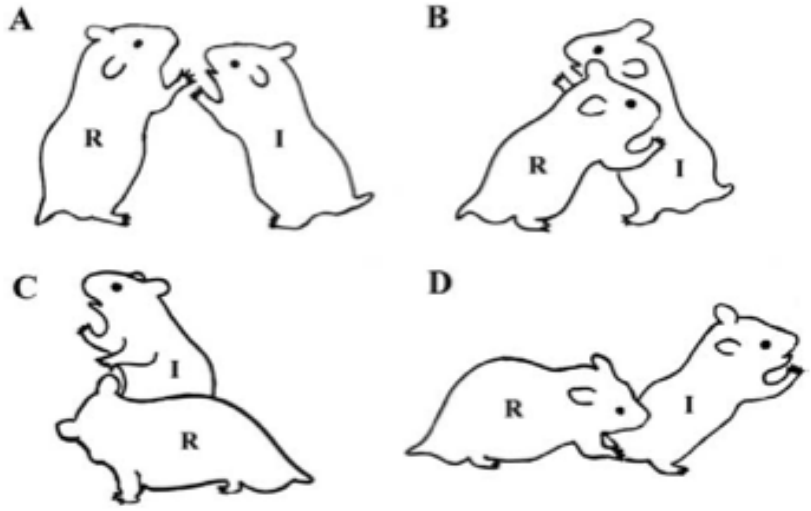

Figure 1: Representation of attack targets. A) Face B) flank C) belly D) rump (Delville, Taravosh-Lahn, & Wommack, 2003)

Method

ANIMALS

All experiments were performed using male Syrian Golden hamsters. Hamsters were raised in the laboratory colony from stock originating from Harlan (Indianapolis, IN). On postnatal day seven (P7), litters were culled to 6 pups (4 males and 2 females). Animals were kept on a reverse light/dark cycle with 14 hours of light and 10 hours of dark and had *ad libitum* access to food and water. All experimental procedures were performed during the active (dark) phase. The animal colony was kept in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and the studies were approved by the University of Texas at Austin's Institutional Animal Care and Use Committee.

OXYTOCIN

Oxytocin Immunohistochemistry

On postnatal day 15, 18, 21, or 28 (P15, P18, P21, P28) litters were sacrificed and their brains were removed and drop fixed in an immersion fixation consisting of 10% acrolein in potassium phosphate-buffered saline (KPBS). Four hours later, brains were transferred to a 20% sucrose in KPBS solution until slicing. Whole brains were sectioned at 40 um on a rotary microtome then stored at -20C in a cryoprotectant.

Oxytocin immunohistochemical procedures were as follows. Brain sections were rinsed in tris-buffered saline (TBS) and treated with 1%w/v s sodium borohydride in TBS to remove aldehydes. Sections were then washed in TBS and treated with 0.3% hydrogen peroxide to inactivate endogenous peroxidases. Sections were then washed in TBS several times then pre-incubated in a 20% normal donkey serum, 0.3% Triton X-100, and bovine serum albumin solution for an hour. Sections were then immediately placed in a

1:16000 guinea pig monoclonal primary antibody to oxytocin for incubation (Lot 050163-4, Peninsula Laboratories, San Carlos, CA) overnight. The next day, sections were washed and incubated in the secondary antibody, .05% biotin-sp-conjugated donkey anti-guinea pig (Lot 80977, Jackson ImmunoResearch Laboratories, West Grove, PA) in TBS. Sections were rinsed in TBS then incubated in an avidin peroxidase complex (Vectastain ABC Elite Kit, Vector Laboratories, Inc., Burlingame, CA). After another set of TBS washes, sections were stained with 3,3'-diaminobenzadine tetrahydrochloride (DAB) (Sigma-Aldrich) in TBS (1mg/2mL), which produced a brown staining of oxytocin fibers comparable to other studies (Kita, Yoshida, & Nishino, 2006). Sections were then mounted on slides and coverslipped with Permount (Fischer Scientific International, Pittsburgh, PA).

Data Analysis

Slides were viewed using a 20x objective on a Zeiss Axio Scope.A1 light microscope. Brain regions of interest were selected based on the presence of oxytocin fibers and named using a stereotaxic brain atlas (Paxinos & Watson, 1998). Regions identified were the lateral hypothalamus (LH), fornix (FX), medial preoptic area (MPOA), nucleus accumbens (NA) core, and anterior hypothalamus (AH). Oxytocin was quantified by measuring the density of stained oxytocin fibers. The area of each brain region covered by oxytocin fibers was calculated using the threshold function in ImageJ software (U.S. National Institutes of Mental Health, Bethesda, MD). All statistics were calculated using IBM SPSS Statistics Version 22 (IBM Corporation, New York, NY).

SEROTONIN

Serotonin Immunohistochemistry

On postnatal day 15, 21, or 27, litters were sacrificed and intracardially perfused with paraformaldehyde. Whole brains were removed and sectioned at 40 um on a rotary microtome and stored in cryoprotectant.

Brain sections were processed using similar techniques to those mentioned above, omitting the sodium borohydride treatment and using a 1:50000 rabbit monoclonal serotonin primary antibody (Immunostar, Hudson, WI) and a .5% goat anti-rabbit IgG (Vector) secondary antibody. Sections were labeled with nickel-conjugated DAB (1mg/2mL) which produced bluish black staining of varicosities as seen in previous studies (C F Ferris et al., 1997).

Behavior

On postnatal day 26 (P26), dams were removed from the home cage, and litters were recorded on a digital video camera (Sony Digital8 Handycam, Sony Corporation of America, New York) to assess playfighting behavior. Following video recording, litters were administered intraperitoneal injections of 1ml/kg m-Chlorophenylbiguanide hydrochloride (CBG) (n=5), a serotonin receptor subtype 3 (5-HT3) agonist or saline (n=5). Thirty minutes post-injection, behavior was again recorded for 20 minutes. Behavior recordings were viewed using VLC Media Player (VideoLAN Organization, Paris, France). Real-time recordings were made for both playfighting behavior and playfight duration and coded with EventCoder 1.0b10 software (donated by Dr. Michael Goldstein, Cornell University, Ithaca, NY). Descriptions of all behaviors coded can be seen in Table 1 and Table 2. Behaviors that were coded for duration were dyads, triads, dogpiles, and huddles. A dyad was defined as two pups engaged in a playfight. Up to

three dyads could occur simultaneously. Three pups engaged in a playfight was labeled a triad, and four pups engaged in a playfight was labeled dogpiling. A huddle was defined as two or more hamsters huddled tightly together for warmth. Behaviors that were coded for frequency were approaches, attacks, pins, and reversals. An approach was defined as one pup approaching another using the paws, usually directed at the face. An attack was when one pup attempted to initiate a playfight by jumping towards the face or body of another pup. A pin was when one pup pinned another by holding down its body, and a reversal was when pups in a pin switch which positions by flipping over.

Data Analysis

Slides were viewed using a 100x objective on a Nikon Optiphot-2 light microscope. Brain regions of interest were selected by the presence of serotonin varicosities and labeled using a stereotaxic brain atlas (Paxinos & Watson, 1998). Regions identified were the AH, bed nucleus of the stria terminalis (BST), infralimbic (IL) and prelimbic (PrL) cortex, lateral septal nucleus (LSV), MPOA, NA core and shell, ventromedial lateral hypothalamus (VMLH), and medial amygdala (MePD). Serotonin was quantified by calculating the average number of varicosities in each brain region using ImageJ software (U.S. National Institutes of Mental Health, Bethesda, MD). Frequency and duration behaviors were averaged for each time point. All statistics were calculated using IBM SPSS Statistics Version 22 (IBM Corporation, New York, NY).

Name	Description
First Dyad	One set of two pups engaged in a playfight
Second Dyad	Separate set of two pups playfighting simultaneously with another dyad
Third Dyad	A third set of two pups engaged in a playfight
First Triad	One set of three pups engaged in a playfight
Second Triad	A second set of thre pups playfighting simultaneously with another triad
Dogpiling	Four or more pups engaged in a playfight
Huddling	Two or more hamsters huddled tightly together for warmth

Table 1: Descriptions of behaviors coded for duration

Name	Description
Approach	One pup approaches another using the paws, usually at the face
Attack	A pup attempts to initiate playfighting by jumping towards the face or body of another
Pin	One pup pins the other by holding down its body; usually follows an attack
Reversal	Pups in a pin switch which is on top or bottom by flipping over

Table 2: Descriptions of behaviors coded for frequency

Results

OXYTOCIN

One-way Randomized Analyses of Variance (ANOVA) were performed to analyze the differences in oxytocin quantity in the fornix (FX), lateral hypothalamus (LH), fornix (FX) nucleus accumbens (NA), medial preoptic area (MPOA), and anterior hypothalamus (AH) between P15, P18, P21, and P28.

The omnibus ANOVA for the FX revealed a significant effect of age on oxytocin density, $F(3, 15) = 12.12$, $p < .001$ as shown if Figure 2. A Tukey's Honestly Significant Difference (HSD) post hoc test indicated oxytocin density was significantly greater between P15 ($M = 1.56$, $SD = 0.42$) and P21 ($M = 4.01$, $SD = 1.44$), $p = .016$; between P18 ($M = 2.43$, $SD = 1.51$) and P28 ($M = 5.74$, $SD = 0.41$), $p = .003$; and between P15 and P28, $p < .001$.

In the LH, the omnibus ANOVA was significant, $F(3, 16) = 5.005$, $p = .012$, as shown in Figure 2. Tukey's HSD indicated that oxytocin density was greater between P15 ($M = .50$, $SD = .18$) and P18 ($M = 1.72$, $SD = .78$), $p = .043$ and between P15 and P21 ($M = 2.17$, $SD = .96$), $p = .011$. Density at age P28 ($M = 1.46$, $SD = .61$) was not significantly different from any other group.

An ANOVA for the NA yielded a significant effect of age on oxytocin fiber density, $F(3, 15) = 24.53$, $p < .001$, as shown in Figure 2. Tukey's HSD revealed an increase in oxytocin density between P15 ($M = .83$, $SD = 0.42$) and P28 ($M = 6.09$, $SD = 1.31$), $p = .000017$; P18 ($M = .86$, $SD = 0.25$) and P28, $p < .001$; and P21 ($M = 2.24$, $SD = 1.58$) and P28, $p < .001$.

The analysis of the MPOA revealed a significant effect of age on oxytocin density, $F(3, 15) = 50.63$, $p < .001$, as shown in Figure 2. Tukey's HSD indicated that oxytocin density at P15 ($M = 0.43$, $SD = 0.45$) was less than at P18 ($M = 1.59$, $SD =$

0.40), $p = .001$; P21 ($M = 2.01$, $SD = 0.36$), $p < .001$; and P28 ($M = 3.68$, $SD = 0.27$), $p < .001$. Density was also less at P18 that at P28, $p < .001$, and density was less at P21 than P28, $p < .001$. No significant differences observed between P18 and P21.

In the AH, an omnibus ANOVA revealed a significant effect of age on oxytocin density, $F(3, 14) = 3.76$, $p = .04$, as shown in Figure 2. Tukey's HSD indicated that density was greater at P28 ($M = .50$, $SD = .08$) than P15 ($M = .36$, $SD = .05$), $p = .03$. No significant differences were seen between P18 ($M = .41$, $SD = .07$) or P21 ($M = .47$, $SD = .09$) and any other age.

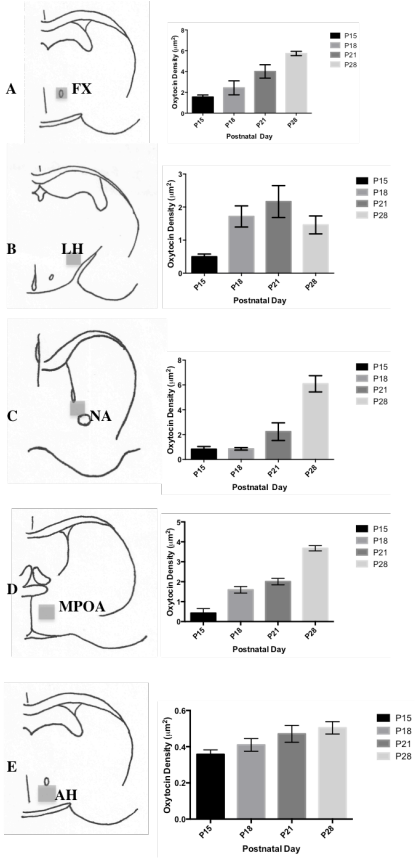

Figure 2: Oxytocin fiber densities across age. A) shows density in the fornix B) shows density in the LH C) shows density in the NA D) shows density in the MPOA and E) shows density in the AH. All bars represent mean±SEM.

SEROTONIN

Serotonin Varicosities

One-way ANOVAs were conducted to compare serotonin the average number of 5HT varicosities between P15, P21, and P27 for each brain region (AH, BST, Cortex(IL), Cortex(PrL), LSV, MePD, MPOA, NA (core), and NA (shell), VMLH). The ANOVA for the LSV revealed a significant effect of age on serotonin density, $F(2, 16) = 11.175$, $p = .001$ (Figure 3). A Tukey's HSD post hoc analysis indicated that serotonin density was less at P21 ($M = 225.90$, $SD = 41.51$) than P15 ($M = 302.17$, $SD = 36.35$), and density was less at P27 ($M = 212.00$, $SD = 20.55$) than P15. No differences were seen between P21 and P27 in the LSV.

In the MPOA, the ANOVA test revealed a significant effect of age on serotonin density, $F(2, 16) = 11.18$, $p = .001$ (Figure 4). Tukey's HSD showed a decrease in serotonin density between P15 ($M = 337.5$, $SD = 1.49$) and P21 ($M = 228.85$, $SD = 21.31$). Serotonin density also decreased between P15 and P27 ($M = 222.64$, $SD = 45.85$). No significant changes were seen between P21 and P27.

ANOVA tests did not yield significant results for the AH, $F(2, 16) = 0.38$, $p = .69$; BST, $F(2, 16) = 2.63$, $p = .10$; Cortex(IL), $F(2, 15) = .035$, $p = .97$; Cortex(PrL), $F(2, 15) = .066$, $p = .936$; MePD, $F(2, 15) = .054$, $p = .95$; NA(core), $F(2, 23) = .74$, $p = .49$, NA (shell), $F(2, 23) = .12$, $p = .89$; and VMLH, $F(2, 15) = 2.88$, $p = .09$.

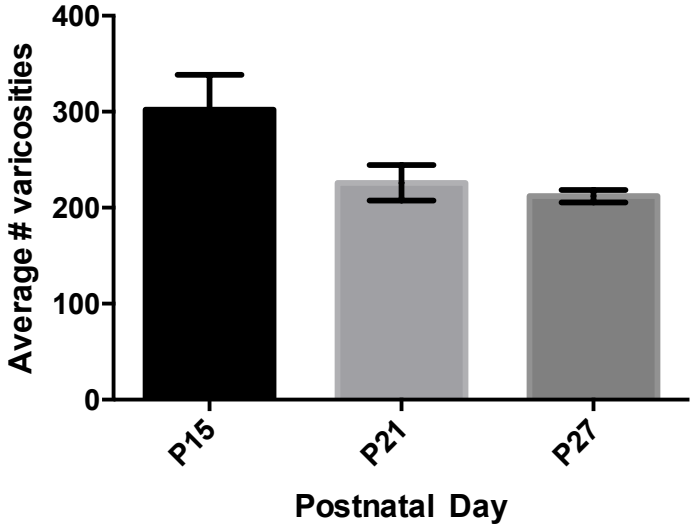

Figure 3: Serotonin varicosities in the LSV across age. Bars represent mean±SEM.

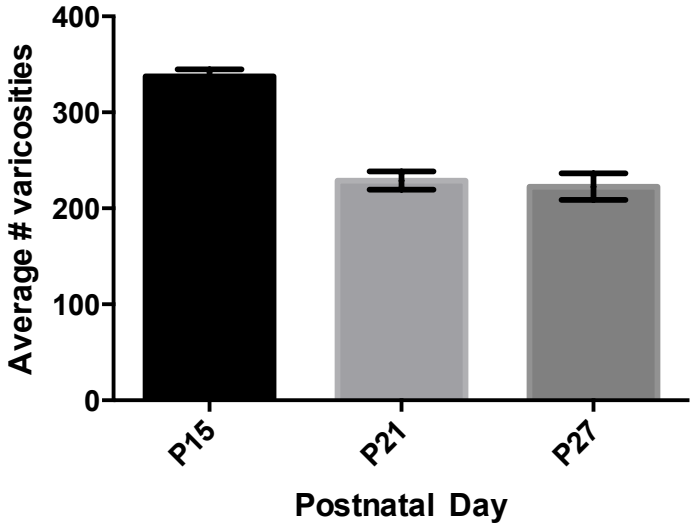

Figure 4: Serotonin varicosities in the MPOA across age. Bars represent mean±SEM.

Duration Behaviors

Two-way mixed ANOVAs were used for each behavior coded for duration (dyad, triad, dogpile, huddle, total). Each behavior had one within-groups variable (time relative

to injection) which had two levels of time relative to CBG injection (pre, post). Group was the between groups variable and also had two levels (control, experimental).

For dyads, A main effect of time was observed, $F(1, 7) = 19.18$, $p = .003$, indicating the number of seconds animals spent in dyads decreased between the pre-injection period ($M = 94.86$, $SD = 84.81$) and the post-injection period ($M = 27.58$, $SD = 58.82$), as seen in Figure 5. There was no main effect of group, $F(1, 7) = 2.13$, $p = .19$, No interaction was observed, $F(1, 7) = 1.5$, $p = .26$.

For triads, there was no main effect of time, $F(1, 7) = .45$, $p = .53$, or group, $F(1, 7) = 2.82$, $p = .14$. A significant interaction was not found, $F(1, 7) = .01$, $p = .92$.

The ANOVA test revealed no significant main effects of time, $F(1, 7) = 2.12$, $p = .19$ or group, $F(1, 7) = .19$, $p = .68$ on time spent in dogpiles, and no significant interaction was observed, $F(1, 7) = .19$, $p = .68$.

The ANOVA test for huddling indicated that there was no main effect of time, $F(1, 7) = 5.10$, $p = .059$, and no main effect of group, $F(1, 7) = .004$, $p = .952$. An interaction effect was not found, $F(1, 7) = .044$, $p = .84$.

The ANOVA test for total time spent in social interaction did not yield significant main effects of time, $F(1, 7) = 1.70$, $p = .23$, or group, $F(1, 7) = .83$, $p = .39$. A significant interaction was not observed, $F(1, 7) = .134$, $p = .73$.

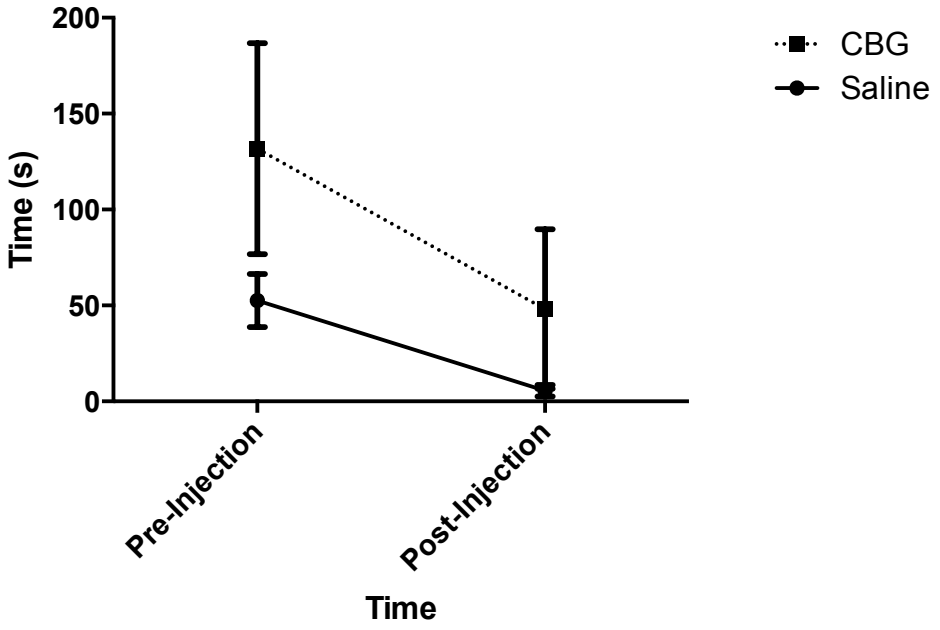

Figure 5: Time spent in dyads following injection with CBG or saline. Both saline-injected and CBG-injected animals show decreased playfighting post-injection. Error bars = ±SEM.

Frequency Behaviors

Wilcoxon Signed Ranks Tests were used to evaluate the frequency of both behaviors coded for frequency (approaches, attacks, pins, reversals, total frequency behaviors) and behaviors coded for duration (dyads, triads, dogpiles, huddles, and total duration behaviors) pre- and post-injection. Significant differences pre- and post-injection in the number of attacks, pins, total frequency behaviors, and dyads were found in the control groups (Figure 6).

The number of attacks were significantly greater in the control group pre-injection (*Mdn* = 7) than post-injection (*Mdn* = 1), $z = -2.06$, $p = .039$. The number of pins were significantly greater for the control group pre-injection (*Mdn* = 7) than post-injection (*Mdn* = 0), $z = -2.023$, $p = .043$. The total frequency of behaviors coded for frequency was greater in the pre-injection control animals (*Mdn* = 19) than the post-injection control

animals (*Mdn* = 6), z = -2.032, p = .042. The frequency of dyads was greater in pre-injection control animals (*Mdn* = 8) than post-injection control animals (*Mdn* = 1), z = -2.023, p = .043.

No significant differences were found in control animals pre- and post-injection for number of approaches, z = -1.76, p = .078; reversals, z = -1.00, p = .32; triads, z = -1.41, p = .16; huddles, z = -1.60, p = .11; dogpiles, z = -1,00, p = .32; or total behaviors coded for duration, z = -1.83, p = .07.

No significant differences were found in experimental animals pre- and pot-injection for number of approaches, z = -0.56, p = .58; attacks, z = -1.84, p = .07; pins, z = -1.60, p = .11; reversals, z = -0.45, p = .66; dyads, z = -1.83, p = .07; triads, z = -1.07, p = .29; dogpiles, z = -1.41, p = .16; total frequency behaviors, z = -1.83, p = .07; or total duration behaviors, z = -1.83, p = .07.

Mann-Whitney U Tests indicated that there were no significant differences between control and experimental groups for any groups, as can be seen in Table 3.

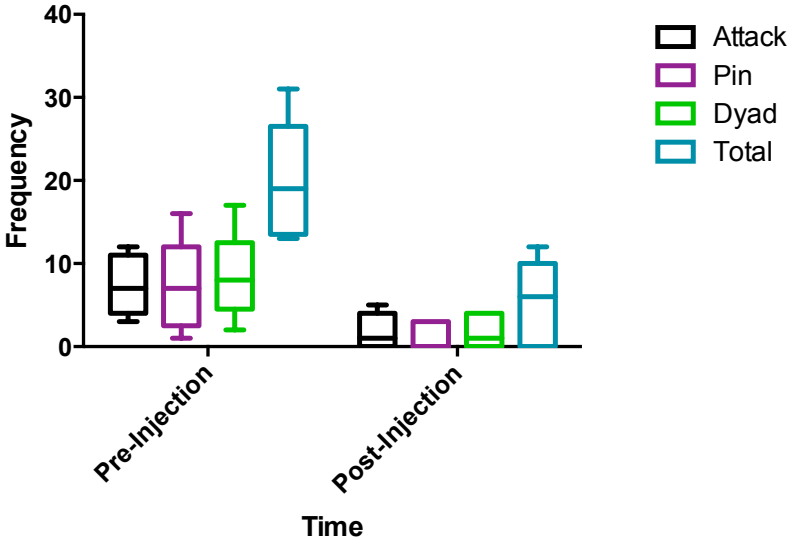

Figure 6: Median frequencies of behaviors pre- and post-saline injection. Bars represent medians with interquartile range.

Behavior	Mann-Whitney U	Wilcoxon W	Z	p-value
Approach Pre-Injection	7.500	17.500	-.643	.521
Attack Pre-Injection	8.500	18.500	-.369	.712
Pin Pre-Injection	6.500	21.500	-.861	.389
Reversal Pre-Injection	7.500	22.500	-1.118	.264
Total Frequency Behaviors Pre-Injection	8.000	23.000	-.490	.624
Approach Post-Injection	7.000	17.000	-.298	.766
Attack Post-Injection	6.500	16.500	-.447	.655
Pin Post-Injection	6.000	16.000	-.619	.536
Reversal Post-Injection	8.000	18.000	0.000	1.000
Total Frequency Behaviors Post-Injection	7.000	17.000	-.289	.773
Dyad Pre-Injection	7.000	22.000	-.738	.461
Triad Pre-Injection	4.500	19.500	-1.433	.152
Huddling Pre-Injection	9.500	24.500	-.169	.866
Dogpiling Pre-Injection	7.000	22.000	-.894	.371
Total Duration Behaviors FrequencyPre-Injection	5.500	20.500	-1.112	.266
Dyad Post-Injection	5.500	15.500	-.730	.465
Triad Post-Injection	6.000	16.000	-1.000	.317
Huddling Post-Injection	9.500	19.500	-.127	.899
Dogpiling Post-Injection	8.000	18.000	0.000	1.000
Total Duration Behavior Frequency Post-Injection	6.500	21.500	-.876	.381

Table 3: Mann-Whitney U test for all behaviors between control and experimental groups

Discussion

OXYTOCIN DATA

It has been well-documented that OT plays a role in rewarding social behavior, so it is unsurprising that OT fibers were found in the MPOA and NA, regions which have also been strongly linked to social behaviors (Keverne & Curley, 2004; Lee, Clancy, & Fleming, 1999). Previous research has indicated that chronic exposure to OT elicits increase nonsexual social behaviors in males (Witt, Winslow, & Insel, 1992). Taking this into account, it is unsurprising that OT fibers were found in the AH given that AVP in the AH of hamsters regulates adult agonistic behavior (Craig F. Ferris & Potegal, 1988). The limbic system, which includes the FX, is widely known to be involved in the regulation of emotions and social behaviors. Damage to the FX has been shown to modulate aggression in both hamsters in guinea pigs (Sainsbury & Jason, 1976; Sodetz & Bunnell, 1970). Additionally, AVP in the FX has been shown to modulate social memory, suggesting that the presence of OT in the FX may also be a regulator of social behaviors (Dantzer, Koob, Bluthé, & Le Moal, 1988). The lateral hypothalamus, known to contain AVP receptors and be active in adult offensive behaviors and juvenile playfighting, also showed increased levels of OT (Cheng et al., 2008; Ferris et al., 1997).

Consistent with the original hypothesis, OT levels appear to increase during the first week of the infancy period in all observed brain regions. However, contrary to the predicted decline of oxytocin levels following P21, OT appears to continue increasing from P21 to P28. This may indicate a possible role for OT in the onset of social playfighting, but other neural mechanisms must underlie the cessation of social playfighting. Unsurprisingly, the continuing increase in OT is similar to the increase seen in vasopressinergic systems (Delville, Mansour, Quan, Yules, & Ferris, 1994). However, the different populations of AVP neurons show differential developmental patterns.

While AVP binding sites in the paraventricular area and central nucleus of the amygdala gradually increase from P1 to P28, AVP binding sites in the BST increase in the first postnatal week, then decline somewhat between P18 and P28. Evidence has suggested that the location and density of OT binding sites is related to social organization in different mammalian species, so future studies investigating OT receptors may shed further light on the relationship between OT and playfighting behavior (Young, 1999).

SEROTONIN DATA

Varicosities

Contrary to the hypothesis that 5-HT levels would increase as social playfighitng increased then decrease with the decline in social playfighting, 5-HT levels appeared to decrease with age in the LSV and MPOA and remain consistent in all other observed brain regions. AVP in the MPOA and LSV has been implicated in agonistic behaviors in hamsters (Cooper, Karom, Huhman, & Albers, 2005; Delville, De Vries, Schwartz, & Ferris, 1998). The presence of 5-HT in these regions suggests that 5-HT may be interacting with AVP to induce behavioral changes. However, the decline in 5-HT coinciding with an increase in playfighting suggests that this interaction is not a main mechanism controlling social playfighting behavior. However, an investigation of 5-HT binding sites during the infancy period of development may lead to further understanding of the 5-HT and AVP relationship in controlling social behavior.

Previous studies have indicated a converse relationship exists between OT and 5-HT—injections of fluoxetine result in increased OT levels (Landry et al., 2005; Mennigen et al., 2008). In conflict with this, the present study found increasing levels of OT coinciding with decreasing levels of 5-HT. A possible explanation for these conflicting findings lies in AVP. The observed decreases in 5-HT were limited to specific

brain areas. AVP and OT show similar activation times and patterns. As AVP levels increase, this may cause an inhibitory effect on 5-HT in specific brain regions, like the MPOA and LSV. This would explain the decreasing levels of 5-HT. Previous studies have primarily focused on adult animals. OT levels of animals in the infancy stage may be controlled by a different receptor subtype, explaining the contradictory relationship seen between 5-HT and OT in adults and infants.

Behavior

Contrary to the hypothesized outcome, CBG injections did not reinstate social play in near-juvenile animals. However, the observed decrease in playfighting behaviors in the control groups post-injection suggest that a stress effect due to handling and injections may be masking any other effect. Although previous studies using CBG waited a comparable amount of time post-injection (Nakagawa, Ishima, & Takashima, 1998; Ricci et al., 2004), the infant animals used in this study are likely more susceptible to the stress of handling and injection than adults. Therefore, future studies should consider increasing the post-injection time period before behavioral observations.

Although the data do not support the original hypothesis, future investigation of the neural mechanisms underlying the social changes in the infancy period this may be crucial for developing an animal model for autism. Hamster social behavior, though evident in infancy disappears in the juvenile period and adulthood, and many of the asocial behaviors observed in hamsters are similar to the deficits in sociability seen in those with autism spectrum disorders (ASDs) (Teng et al., 2013). Numerous studies have identified both OT and 5-HT as modulators of ASD-like behavior, including the use of both oxytocin and selective serotonin reuptake inhibitors as potential treatments for ASDs (Dadds et al., 2013; Gregory et al., 2009; Kolevzon, Mathewson, & Hollander,

2006; Ritvo, 1970). Most animal models of ASD involve the use of adult animals, however ASD is primarily a disorder observed in adolescents and children. However, studies have indicated that fluoxetine elicited an inhibition of aggressive behavior in adults, but, depending on dose, either inhibited or enhanced behaviors in juveniles. This suggests that adult animal models of ASD may be appropriate for demonstrating the adolescent disorder.

CONCLUSIONS

The increase in OT and decrease in 5-HT seen in this study are contradictory to past findings. However, this difference may be attributed to both an interaction with AVP and a different predominant receptor subtype in infants versus adults. The increase in OT that coincides with the onset of social playfighting is observed in regions of the brain that have long been associated with social reward and behaviors, suggesting that OT may be contributing to the onset of playfighting. However, the decline in social playfighting does not occur with a simultaneous decline in OT levels, which indicates that another neural mechanism intervenes at this time to instate asocial adult behaviors. Further research into OT and 5-HT receptors during this developmental period is necessary in order to better understand the complex relationship between OT, AVP, and 5-HT in neural and behavioral development. Further investigation of this topic may play a critical role in developing an appropriate animal model for ASDs and expanding our knowledge of the neural underpinnings of the neural mechanisms underlying them, possibly leading to improved therapeutic treatments for ASDs.

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