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Sex-dependent effects of social isolation on the regulation of arginine-vasopressin (AVP) V1a, oxytocin (OT) and serotonin (5HT) 1a receptor binding and aggression

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ABSTRACT

It is widely held that social isolation produces higher rates of mortality and morbidity and has deleterious effects on an individual's sociality. Relatedly, it is widely observed that socially isolated adult rodents display significantly higher levels of aggression when placed in a social situation than do their conspecifics living in social groups. In the following study, we investigated the effects of social isolation on several neurochemical signals that play key roles in the regulation of social behavior in adults. More specifically, we examined the effects of social isolation on vasopressin (AVP) V1a, oxytocin (OT) and serotonin (5-HT)1a receptor binding within the neural circuit controlling social behavior. Male and female Syrian hamsters were housed individually or with two other hamsters for four weeks and were then tested with a same-sex nonaggressive intruder in a neutral arena for 5 min. Social isolation significantly increased aggression in both males and females and altered receptor binding in several brain regions in a sex-dependent manner. For example, V1a receptor binding was greater in socially isolated males in the anterior hypothalamus than it was in any other group. Taken together, these data provide substantial new support for the proposition that the social environment can have a significant impact on the structural and neurochemical mechanisms regulating social behavior and that the amount and type of social interactions can produce differential effects on the circuit regulating social behavior in a sex-dependent manner.

1. Introduction

There is considerable evidence that the quality and quantity of social relationships in adulthood can have significant effects on an individual's health. Indeed, there are data showing that humans and animals with limited social relationships, or who are socially isolated, as adults have higher rates of mortality and morbidity than do those with adaptive social relationships (House et al., 1988; Cacioppo and Hawkley, 2009). The mechanisms underlying how social isolation influences social behavior, and ultimately health, are not well understood but likely involve changes within a variety of neural structures such as the lateral septum, extended amygdala, midbrain, preoptic area, and hypothalamus.

The impact of social isolation on the expression of many different social behaviors has been documented across diverse groups of species. One of the more pronounced effects of social isolation on subsequent social behavior is its ability to increase an individual's aggressiveness (Brain et al., 1971; Brain, 1972; Stevenson and Rillich, 2013; Scotti et al., 2015; Oliveira et al., 2019). Another effect of social isolation is that it can increase circulating levels of corticoids (Dronjak et al., 2004), suggesting that social isolation is stressful; however, while social isolation has been found to increase circulating corticoids in some studies, these "stress-like" effects have not been observed in others (Ross et al., 2017). Indeed, the effects of social isolation on physiology and behavior may be species- and sex-related (Cacioppo et al., 2015).

The present study was conducted in Syrian hamsters because they

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are an excellent animal model for preclinical studies relevant to the understanding of primate social behavior. Although the limited amount of field data available suggests that hamsters are not a gregarious species, hamsters display a sophisticated array of social behaviors and skills. Like primates, and unlike many other laboratory rodents, both male and female hamsters establish hierarchical dominance relationships (Drickamer and Vandenbergh, 1973; Drickamer et al., 1973), display rich social, agonistic (Albers et al., 2002), and communicative behaviors (Johnston, 1985), and are capable of complex social discriminations such as the ability to use multiple sensory modalities to discriminate among individuals as well as to identify kin (Todrank et al., 1988; Heth et al., 1998).

Arginine-vasopressin (AVP), oxytocin (OT) and serotonin (5-HT) have been demonstrated to play a significant role in the expression of many different social behaviors in a wide range of species (Terranova et al., 2017; Kelly and Wilson, 2019; Smith et al., 2019). For example, in hamsters, aggression and dominance are regulated by AVP, OT and 5-HT in a sex-dependent manner (Ferris et al., 1997; Gutzler et al., 2010; Terranova et al., 2016). There is also evidence that social experience, including social isolation, can influence key elements of these neurochemical systems (Albers et al., 2006; Albers, 2012; Oliveira et al., 2019). In prairie voles, for example, social isolation can alter the number of OT immunoreactive cells and the number of V1a and OT receptors in specific brain areas, and these effects can be sex-dependent (Grippo et al., 2007; Hiura and Ophir, 2018).

The purpose of the present study was to investigate the effects of social isolation on several neurochemical systems that play a fundamental role in the regulation of social behavior in hamsters. More specifically, we examined whether social isolation results in changes in the number of AVP V1a, OT and 5-HT1a receptors within several regions that are critical in modulating a wide range of social behaviors (Olivier, 2015; Caldwell and Albers, 2016; Johnson and Young, 2017). These studies were conducted in both males and females because there is increasing evidence that AVP, OT and 5-HT have substantial, but very different, roles in the regulation of competitive aggression in males and females (Harmon et al., 2002; Takahashi et al., 2011; Terranova et al., 2016).

2. Methods

2.1. Animals

Adult male and female Syrian hamsters (120–130 g) were purchased from Charles River Laboratories (Wilmington, MA) and were housed in polycarbonate cages (23 × 43 × 20 cm) with wire lids, corncob bedding and cotton nesting material. Chow (LabDiet 5001, Purina Mills, Gray Summit, MO) and tap water were available ad libitum, and the colony room was maintained on a 14:10 light:dark cycle as is customary in this species to maintain gonadal patency. Hamsters were housed singly or in groups of three same sex individuals for four weeks before behavioral testing began. All procedures were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for the Care of Use of Laboratory Animals.

2.2. Estrous cycle monitoring

On each of the 8 days prior to the start of behavioral testing, the estrous cycle was monitored in all females by examining vaginal discharge. Regular estrous cycles are characterized by post-ovulatory vaginal discharge on the day of estrus which occurs every fourth day of the cycle. Male hamsters were also handled in a manner similar to the females on each of these days to control for any effects of handling.

2.3. Behavioral testing and scoring

Each hamster was placed in a clean cage containing fresh corncob bedding. A same-sex, nonaggressive, smaller hamster (intruder) was immediately placed into the same cage. All female hamsters were tested on diestrus 1 of their estrous cycle. All behaviors emitted during the 5 min social encounters were scored as described previously (Albers et al., 2002). Briefly, the total duration of four classes of behaviors were scored during the test session: (1) social behavior (stretch, approach, sniff, nose touching, and flank marking); (2) non-social behavior (locomotion, exploration, grooming, nesting, feeding, and sleeping); (3) submissive/defensive behaviors (flight, avoidance, tail up, upright, side defense, full submissive posture, stretch attend, head flag, attempted escape from cage); and (4) aggressive behaviors (upright and side offense, chase and attack, including bite).

2.4. Autoradiography

Immediately after behavior testing, hamsters were lightly anesthetized with isoflurane and decapitated. Brains were collected and frozen in dry ice. They were stored at −80 °C until they were cut into 20 μm coronal sections using a cryostat. Sections were thaw-mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA) and stored at −80 °C until further processing.

2.4.1. V1a and OT receptors

V1a receptor binding was determined with the I^{125}-labeled linear V1aR antagonist [^{125}I]-Phenylacetyl-D-Tyr(Me)-Phe-Gln-Asn-Arg-Pro-Arg-Tyr-NH (Perkin-Elmer, Boston, MA). OT receptor binding was determined with the I^{125}-labeled ornithine vasotocin analog Vasotocin, d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Orn^8,[^{125}I]Tyr9-NH2] (Perkin-Elmer). For both assays, the tissue was allowed to thaw and dry. It was then fixed in 0.1% paraformaldehyde for 2 min. Slides were then rinsed twice for 10 min each in buffer (50 mM Tris, pH 7.4) and were then incubated in tracer buffer (0.35 mM bacitracin, Sigma-Aldrich, St. Louis, MO; 0.015 mM bovine serum albumin, Sigma-Aldrich, St. Louis, MO; 50 mM I^{125} linear V1aR antagonist or 50 mM I^{125} vasotocin analog) for 1 h. Slides were then rinsed twice for 5 min each and then for 35 min with agitation in buffer (50 mM Tris, 21 mM MgCl). All incubations and washes were performed at room temperature. Finally, the slides were dipped in 4 °C deionized water and allowed to dry. The slides and either an I^{125} or a C^{14} standard calibration strip (American Radiolabeled Chemicals, St. Louis, MO) were loaded into autoradiography cassettes and exposed to film (Kodak, Rochester, NY) for 3 days (V1a) or 7 days (OT) at room temperature.

2.4.2. 5-HT1a receptors

5-HT1a receptor binding was determined with the H^3-labeled 5-HT1a receptor full agonist 8-Hydroxy-DPAT,[Propyl-2,3-ring-1,2,3-^{3}H] (Perkin Elmer). The tissue was allowed to thaw and dry. Slides were then incubated in buffer (50 mM Tris, 120 mM NaCl, 4 mM $CaCl_2$, pH 7.4) for 15 min, followed by a 1 h incubation in tracer buffer (50 mM Tris, 6 μM H^3 5-HT1a agonist). Slides were then rinsed twice for 10 min each in 4 °C buffer and then dipped in 4 °C deionized water and allowed to dry. The slides and an H^3 standard calibration strip (American Radiolabeled Chemicals) were loaded into autoradiography cassettes and exposed to film for 14 weeks.

2.4.3. Autoradiography analysis

Densitometry analysis was performed using Scion Image software (NIH, Bethesda, MD) and a lightbox (Imaging Research, Inc., Ontario, Canada) attached to a camera (Panasonic, Newark, NJ). Standard curves were created using the H^3 microscales on the standard calibration strip. For each brain area of interest, three tissue sections located 60 μm apart were analyzed. With the exception of the PVN, a 0.35 mm^2 box was placed over the center of each brain area (Tables 1–3), and the

Table 1
P-values for isolation, sex and interaction effects on V1a receptor densities.

Brain area	Isolation effect	Sex effect	Interaction effect
LS	$p = 0.246$	$p = 0.273$	$p = 0.614$
BNST	$p = 0.014$[a]	$p = 0.001$[a]	$p = 0.830$
MPOA	$p = 0.413$	$p = 0.005$[a]	$p = 0.075$
AH	$p = 0.031$[a]	$p = 0.000$[a]	$p = 0.044$[a]
LH	$p = 0.383$	$p = 0.975$	$p = 0.610$
CeA	$p = 0.704$	$p = 0.313$	$p = 0.213$
HC	$p = 0.661$	$p = 0.661$	$p = 0.954$
PVN	$p = 0.602$	$p = 0.922$	$p = 0.204$
PAG	$p = 0.105$	$p = 0.094$	$p = 0.450$
DRN	$p = 0.300$	$p = 0.141$	$p = 0.044$[a]

[a] Indicates significant effect.

Table 2
P-values for isolation, sex and interaction effects on OT receptor densities.

Brain area	Isolation effect	Sex effect	Interaction effect
LS	$p = 0.793$	$p = 0.067$	$p = 0.439$
BNST	$p = 0.882$	$p = 0.306$	$p = 0.940$
MPOA	$p = 0.900$	$p = 0.445$	$p = 0.121$
AH	$p = 0.773$	$p = 0.074$	$p = 0.464$
LH	$p = 0.919$	$p = 0.017$[a]	$p = 0.763$
CeA	$p = 0.618$	$p = 0.533$	$p = 0.239$
HC	$p = 0.541$	$p = 0.168$	$p = 0.149$
BLA	$p = 0.133$	$p = 0.251$	$p = 0.185$
PVN	$p = 0.600$	$p = 0.000$[a]	$p = 0.186$
PAG	$p = 0.082$	$p = 0.363$	$p = 0.991$
DRN	$p = 0.283$	$p = 0.024$[a]	$p = 0.017$[a]

[a] Indicates significant effect.

Table 3
P-values for isolation, sex and interaction effects on 5HT1a receptor densities.

Brain area	Isolation effect	Sex effect	Interaction effect
LS	$p = 0.628$	$p = 0.043$[a]	$p = 0.377$
BNST	$p = 0.144$	$p = 0.000$[a]	$p = 0.012$[a]
MPOA	$p = 0.377$	$p = 0.000$[a]	$p = 0.158$
AH	$p = 0.394$	$p = 0.000$[a]	$p = 0.600$
LH	$p = 0.114$	$p = 0.589$	$p = 0.077$
HC	$p = 0.235$	$p = 0.383$	$p = 0.426$
PVN	$p = 0.168$	$p = 0.113$	$p = 0.536$
PAG	$p = 0.102$	$p = 0.345$	$p = 0.099$
DRN	$p = 0.750$	$p = 0.890$	$p = 0.540$

[a] Indicates significant effect.

optical density was recorded. A 0.26 mm^2 box was used to analyze the paraventricular nucleus (PVN). Background binding was subtracted from this measurement. Optical densities were calculated as disintegrating units per min per mg tissue (dpm/mg). Optical densities for each region of interest were averaged within each animal and then averaged within groups. All data were stored and analyzed with Microsoft Excel, Version 14.7.1 and Statistical Package for the Social Sciences (SPSS), Version 23. A two-way analysis of variance (ANOVA) and Tukey post hoc tests were performed to determine if there were differences between male and female and group versus single housed hamsters.

3. Results

3.1. Behavioral studies

Male and female hamsters that were socially isolated for four weeks were more aggressive than were hamsters that were housed socially [$F(1, 73) = 15.96$, $p < 0.05$; $\eta_p^2 = 0.19$; Fig. 1A]. Levels of aggression were similar in socially isolated males and females. Socially housed female hamsters displayed half as much aggression as socially isolated females while socially housed males displayed little or no aggression. As a result, overall, female hamsters were found to be significantly more aggressive than were male hamsters [$F(1, 73) = 4.36$, $p < 0.05$; $\eta_p^2 = 0.06$]. Socially housed male and female hamsters displayed significantly more nonsocial behaviors than did isolated hamsters [$F(1, 73) = 123.26$, $p < 0.05$; $\eta_p^2 = 0.64$; Fig. 1B]. In addition, socially

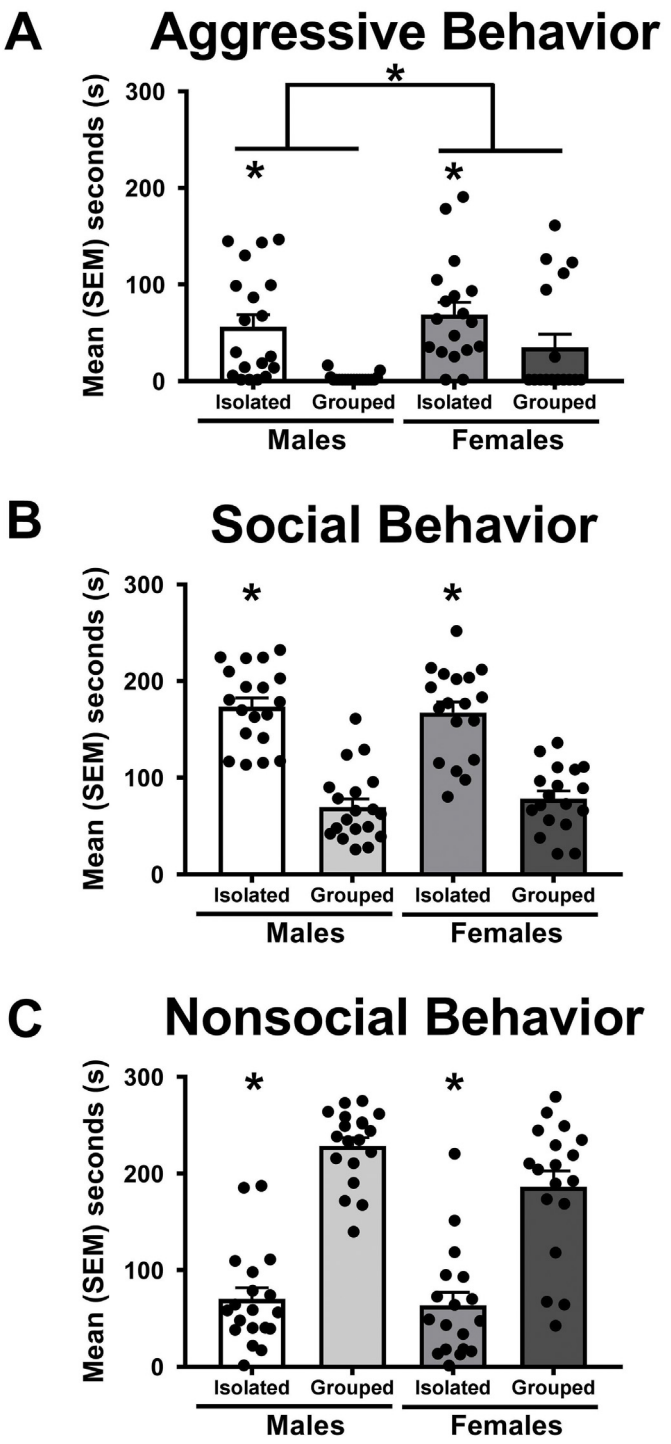

Fig. 1. Differences in social behaviors between isolated vs group housed hamsters. Mean ± SEM and black dots indicate data from individual hamsters. A. Female hamsters were more aggressive than male hamsters, and socially isolated hamsters were more aggressive than socially housed hamsters. B. Socially housed animals displayed more nonsocial behaviors compared to isolated animals. C. Isolated animals displayed more social behaviors than did socially housed animals. * $p < 0.05$.

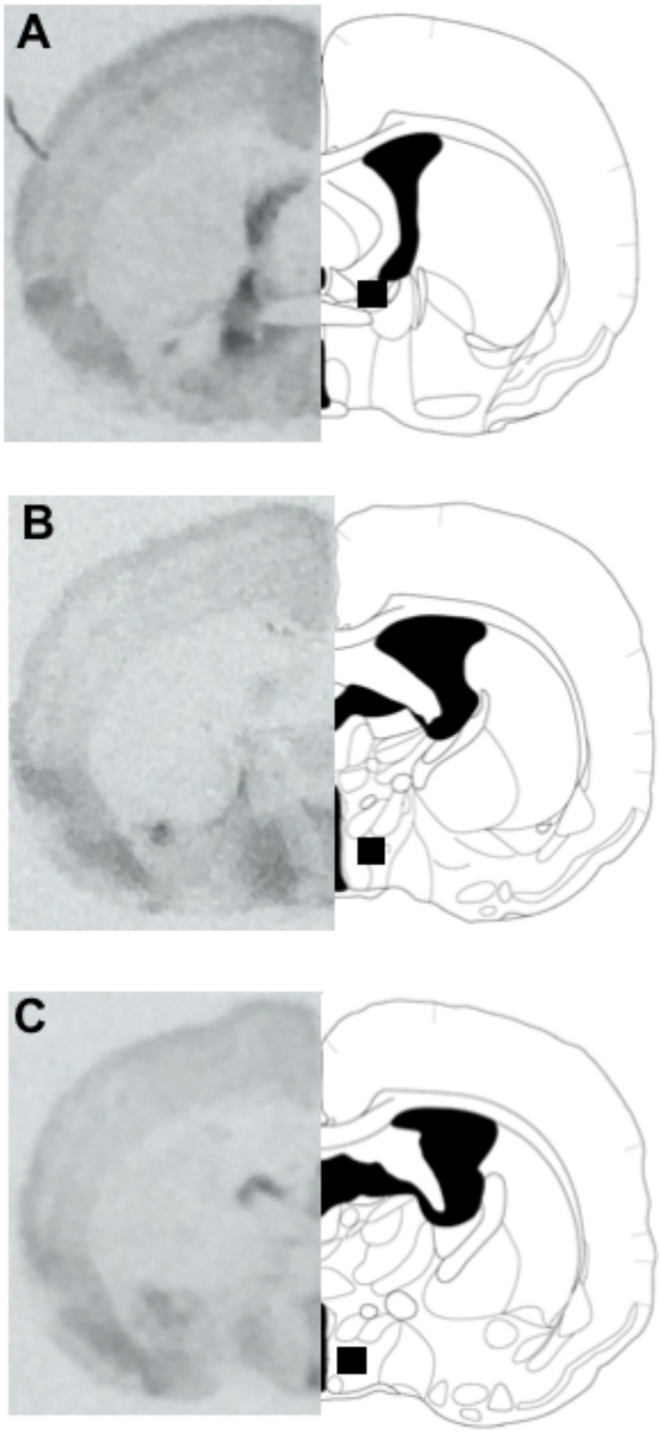

Fig. 2. Representative autoradiograms illustrating receptor binding and brain sites where the binding was quantified (adapted from Morin and Wood (2001)). A. BNST. B. MPOA. C. AH.

isolated male and female hamsters displayed more social behavior than did individuals that were socially housed [$F(1, 73) = 109.45$, $p < 0.05$; $\eta_\rho^2 = 0.61$; Fig. 1C] as well as more flank marking $F(1,70) = 8.12$, $p < 0.05$, $\eta_p{}^2 = 0.10$.

3.2. V1a receptor binding

The anatomical distribution of V1a binding was similar to that seen in previous studies conducted in male and female hamsters (Dubois-Dauphin et al., 1990; Ferris et al., 1993; Johnson et al., 1995). V1a binding sites were detectable throughout key regions of the circuitry controlling social behavior (Fig. 2). Particularly high V1a binding was found in the lateral septum (LS), bed nucleus of the stria terminalis (BNST) and central amygdala (CeA). Lower to moderate V1a binding was observed in the anterior hypothalamus (AH), medial preoptic area (MPOA), the lateral hypothalamus (LH) and the Ca1 region of the hippocampus (HC). In contrast, little to no V1a binding was identified in the basolateral amygdala (BLA) (Table 1). Perhaps the most interesting finding was that there was a significant interaction between sex and housing conditions in V1a binding within the anterior hypothalamus (AH) [$F(1, 68) = 4.22$, $p < 0.05$; $\eta_\rho^2 = 0.06$; Fig. 3A]. V1a binding was greater in isolated males than in group housed males while no difference in V1a binding was observed between isolated females and group housed females. The higher levels of V1a binding in isolated males led to significantly greater V1a binding in males compared to females in the AH. Significantly higher V1a binding was seen in the MPOA of males compared to females [$F(1, 60) = 8.69$, $p < 0.05$; $\eta_\rho^2 = 0.13$; Fig. 3B]. In the BNST, socially isolated males and females displayed significantly lower V1a binding than did socially housed hamsters [$F(1, 70) = 6.44$, $p < 0.05$; $\eta_\rho^2 = 0.09$; Fig. 3C]. There was also a significant overall sex difference in V1a binding with females having significantly higher levels of V1a binding than did males in the BNST [$F(1, 70) = 11.18$, $p < 0.05$; $\eta_\rho^2 = 0.14$; Fig. 3C]. In the DRN, there was a significant interaction between social isolation and sex with socially isolated males exhibiting less V1a binding than did all other groups [$F(1, 42) = 4.32$, $p < 0.05$; $\eta_\rho^2 = 0.09$; Fig. 3D]. There were no other significant effects of sex or housing condition on V1a binding in any of the other regions examined (Table 1).

3.3. Oxytocin receptor (OTR) binding

The overall distribution of OTR binding was similar to that reported previously in male and female hamsters (Dubois-Dauphin et al., 1992). Like previous work, the present study identified high levels of OTR binding within elements of the circuitry controlling social behavior such as the BNST and CeA. In contrast to the prior study where no OTR binding was found in the hypothalamus, the present results found moderate OTR binding in the AH. Females had greater OTR binding than did males in the LH [$F(1, 32) = 6.47$, $p < 0.05$; $\eta_\rho^2 = 0.18$; Fig. 4A], but males had greater OTR binding than did females in the PVN [$F(1, 27) = 20.51$, $p < 0.05$; $\eta_\rho^2 = 0.46$; Fig. 4B]. There was also a significant effect of sex [$F(1, 22) = 6.17$, $p < 0.05$; $\eta_\rho^2 = 0.28$; Fig. 4C] and a significant interaction [$F(1, 22) = 7.09$, $p < 0.05$; $\eta_\rho^2 = 0.31$; Fig. 4C] between sex and social isolation in the DRN. There were no other significant effects of sex or housing condition on OTR binding in any of the other regions examined (Table 2).

3.4. Serotonin 1a receptor binding

There are only limited data on 5-HT1a binding in Syrian hamsters (Duncan et al., 1999). In the present study, 5-HT1a binding was detectable in most brain areas examined with the exception of the CeA or BLA. In general, the pattern of 5HT-1a binding was similar to that seen previously in male rats (Pazos and Palacios, 1985). Males had higher 5-HT1a binding in the MPOA [$F(1, 28) = 16.77$, $p < 0.05$; $\eta_\rho^2 = 0.40$; Fig. 5A], the AH [$F(1, 24) = 25.87$, $p < 0.05$; $\eta_\rho^2 = 0.55$; Fig. 5B], and the LS [$F(1, 31) = 4.51$, $p < 0.05$; $\eta_\rho^2 = 0.14$; Fig. 5C] than did females. In addition, there was a significant interaction between sex and housing condition in the BNST such that isolated males had greater 5-HT1a binding compared to socially housed males as well as socially isolated and socially housed females [$F(1, 32) = 7.24$, $p < 0.05$; $\eta_\rho^2 = 0.20$; Fig. 5D]. There were no significant effects of sex or housing condition on 5HT1a receptor density in the other regions examined (Table 3).

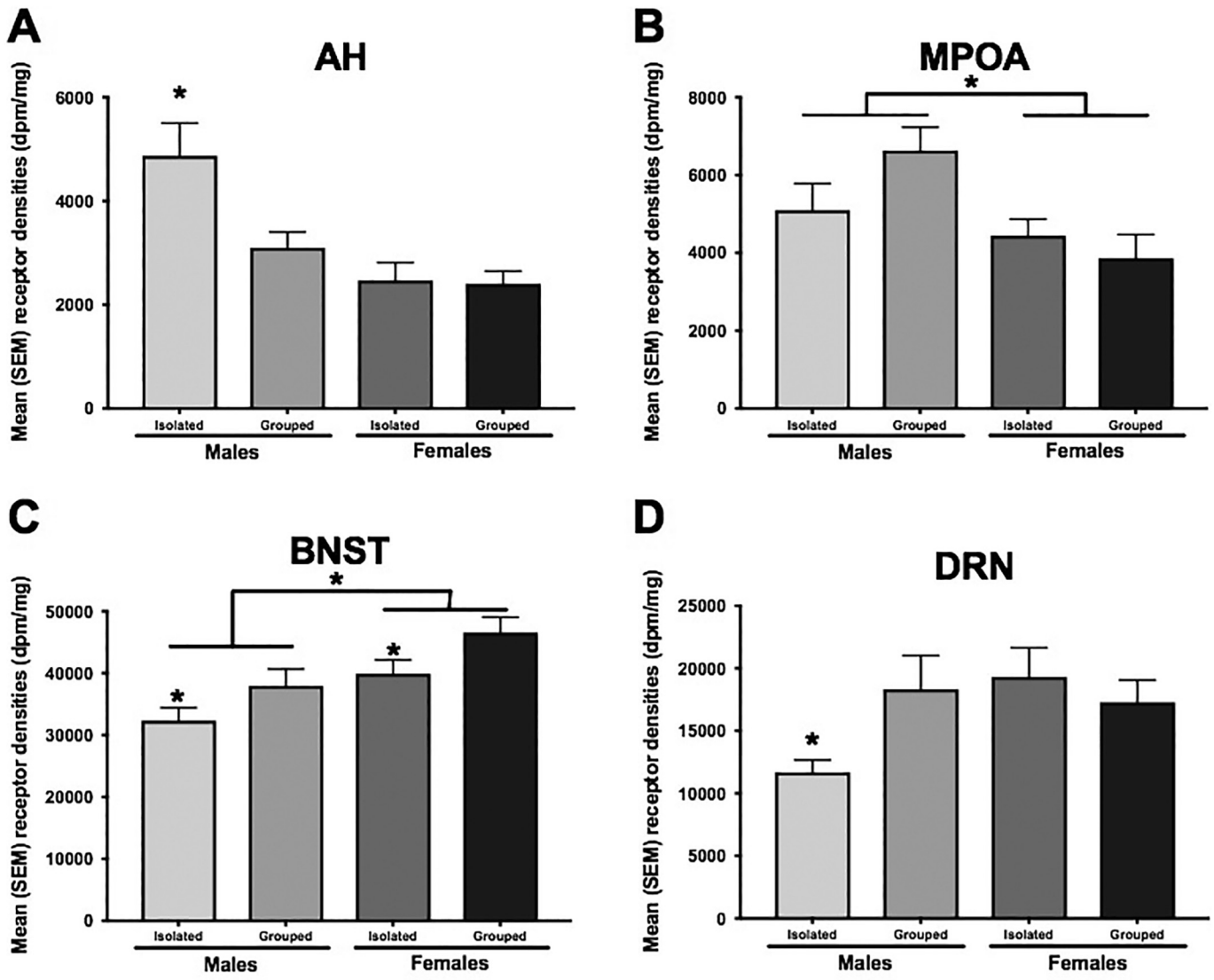

Fig. 3. A. Isolated males had a greater V1a receptor binding in the AH compared to isolated females and socially housed males and females. B. Males had a greater receptor binding compared to females in the MPOA. C. In the BNST, males had a greater V1a receptor binding compared to females and socially housed animals had a greater V1a receptor binding than did isolated animals. * $p < 0.05$.

4. Discussion

As expected, socially isolated adult males and females displayed significantly more aggression than did hamsters housed in social groups. Interestingly, however, social housing reduced aggression only by about 50% in females while aggression was almost completely eliminated in socially housed males. Recent studies in male and female rats found that social isolation for seven weeks significantly increased aggression in both sexes by a similar amount when compared to socially housed rats (Oliveira et al., 2019). In the present study, socially housed male and female hamsters spent significantly more time expressing nonsocial behaviors than did socially isolated hamsters, and hamsters housed in social isolation spent significantly more time socially interacting than did hamsters housed in groups. The differences in the time spent socially interacting between the socially isolated and socially housed hamsters may be the result of differences in the rewarding properties of those interactions. Other studies have found that rodents housed in social isolation find brief social interactions more rewarding than do rodents housed in social groups (Douglas et al., 2004; Matthews et al., 2005).

Four weeks of social isolation resulted in alterations in the density of V1a, OT and 5-HT1a receptor binding within specific brain regions involved in the regulation of social behavior when compared with the receptor binding observed in socially housed hamsters. The effects of housing conditions were observed most prominently on V1a receptor binding. In the BNST, social isolation appeared to reduce the number of V1a receptors in both male and female hamsters. In contrast, in the AH social isolation appeared to increase the number of V1a receptors, although this effect occurred primarily in males. Comparison of the present data with a previous study on the effects of social isolation and social interaction in male hamsters suggests that the amount as well as the nature of the social interaction can have differential effects on the expression of aggression and V1a receptor binding (Albers et al., 2006). In the earlier study, social experience was manipulated in a very different way than in the present study. Instead of simply housing hamsters in groups as in the present study, social experience was provided giving singly housed male hamsters social interactions with a small, nonaggressive male for 30 min/week for three weeks. Aggression in the males that engaged in social interaction for 30 min/week for three weeks was then significantly lower when compared to hamsters in complete social isolation for three weeks. Interestingly, however, although aggression was significantly higher in the socially isolated

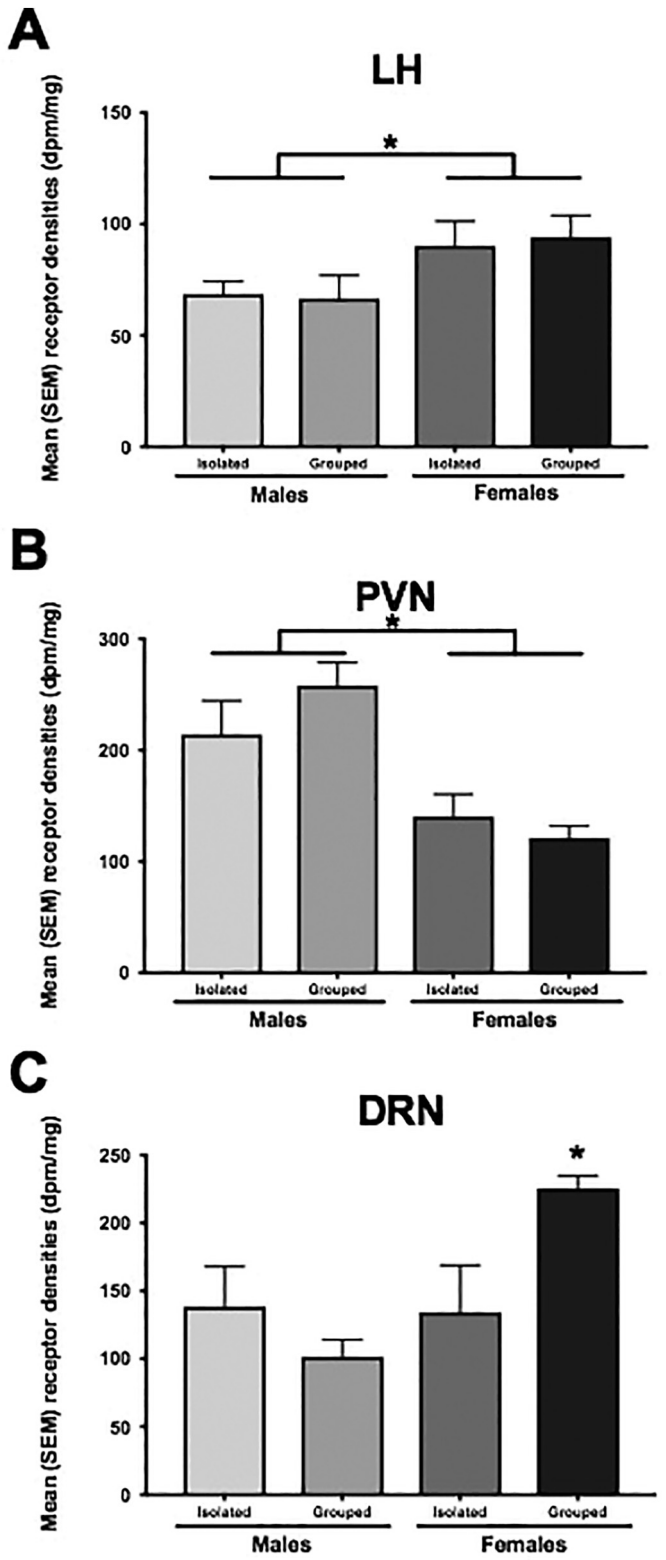

Fig. 4. A. Females had a greater OT receptor binding compared to males in the LH, but B. males had a greater binding than females in the PVN. * $p < 0.05$.

group, when hamsters were provided social interactions for 30 min/week for three weeks, a substantial amount of aggression was found to occur. Thus, the amount and type of social interaction appears to determine the extent to which aggressiveness is reduced by social interaction.

The amount and type of social interaction also appears to have differential effects on V1a receptor binding. In the present study, males and females housed socially for four weeks exhibited significantly more V1a receptor binding in the BNST than did hamsters that were socially isolated. In contrast, no differences were observed in V1a receptor binding in the BNST in the previous study wherein male hamsters were allowed to socially interact for 90 min over three weeks (Albers et al., 2006). In the previous study, however, this brief amount of social interaction resulted in higher V1a receptor binding in the PVN and LH and lower V1a binding in the CeA compared with males housed in total social isolation. No similar, site-specific effects on V1a receptor binding between social isolation and social housing were observed in the present study. Interestingly, V1a receptor binding was significantly lower in the AH of the socially housed group in the present study as well as in the previous study where only 90 min of social interaction was provided. Thus, the inhibitory effects of social stimulation on V1a receptor number in the AH appears to be quite robust and reproducible over a variety of conditions. The higher V1a receptors seen in socially isolated males may explain the higher levels of aggression observed as the result of social isolation. Indeed, injection of AVP into the AH significantly increases aggression in socially isolated males and in males trained to fight but not in males that are socially housed (Ferris et al., 1997; Huhman et al., 1998; Caldwell and Albers, 2004).

The effects of social experience on OTR and 5-HT1a receptor binding were more limited than that seen for V1a binding. The only effects of social experience on OTR binding were in the DRN where socially housed females displayed greater binding than did socially housed and socially isolated males and socially isolated females. This is in contrast to other rodents, such as rats, where socially housed females have higher OTR binding in the posterior BNST and nucleus accumbens (NAc) compared with isolated females (Oliveira et al., 2019). In terms of 5-HT1a receptor binding, the only effects of social experience were seen in greater 5-HT1a binding in the BNST in socially isolated males compared to all other groups.

The data of the present study add to the increasing body of evidence that the social environment can alter the functioning of neurochemical circuits containing AVP, OT or 5-HT1a in a variety of species. For example, in female rats, social housing results in higher OTR binding in the posterior BNST and nucleus accumbens compared with isolated females (Oliveira et al., 2019), and in prairie voles social isolation significantly increases OT receptor mRNA in the hypothalamus (Pournajafi-Nazarloo et al., 2013). Socially isolated male and female rats have lower levels of V1a receptor binding in the LH and dentate gyrus, although no differences were observed in several other brain regions (Oliveira et al., 2019). Social isolation also modulates 5-HT neurotransmission in a variety of brain sites (Malick and Barnett, 1976; Bibancos et al., 2007; Sargin et al., 2016; Liu et al., 2019). Social experiences other than the withdrawal of social interaction (i.e., social isolation) can also have substantial effects on the expression of these circuits. For instance, pair bonding in male prairie voles results in higher V1a receptor binding in the AH and MPOA than that seen in sexually naïve males that are not in a pair bond (Winslow et al., 1993; Gobrogge et al., 2009). The larger number of V1a receptors in the AH may also explain the higher levels of aggression (i.e., mate guarding) seen in pair-bonded male voles compared to males that are not in a pair bond. Indeed, how social stimuli regulate the expression of V1a and other receptors likely differs across species. Even within species, however, high levels of plasticity in the expression these receptors have been observed in response to different social conditions at a variety of development stages (Prounis et al., 2015; Hiura and Ophir, 2018; Prounis et al., 2018). The structural changes that can occur in the neural circuitry regulating social behavior in response to dynamic changes in the social environment may tune these circuits in order to provide more adaptive behavior in response to new social challenges.

In the present study, sex differences in V1a, OT and 5-HT1a receptor binding were observed in several different brain regions. For example, V1a and 5-HT1a binding was greater in the AH and MPOA in males

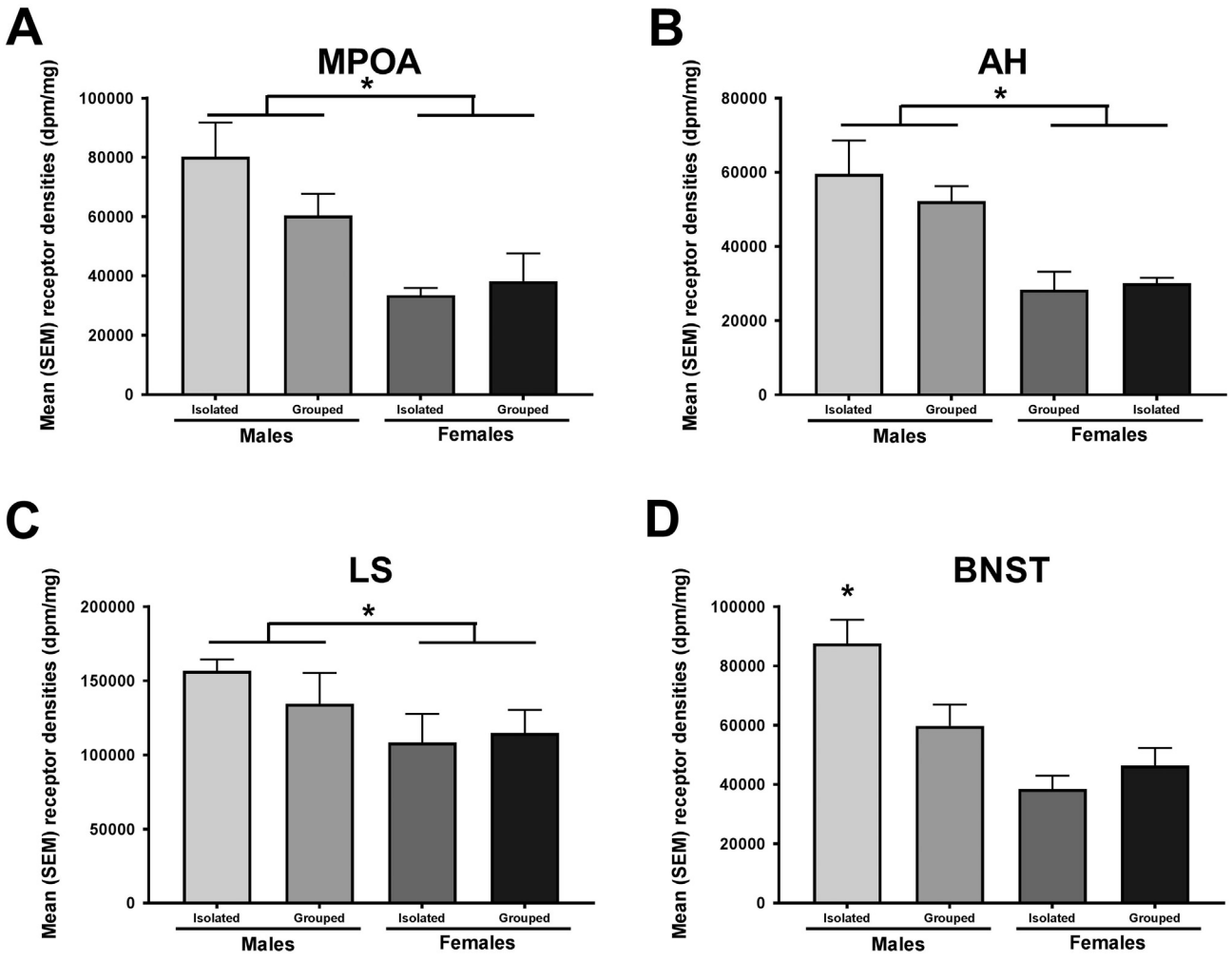

Fig. 5. Males had a greater 5HT1a receptor binding compared to females in the A. MPOA, B. the AH and C. the LS D. Isolated males had a greater 5HT1a receptor binding compared to isolated females and socially housed males and females. * $p < 0.05$.

compared to females. Previous studies in rodents have also identified sex differences in V1a, OT and 5-HT1a receptors in various brain sites (Fischette et al., 1983; Zhang et al., 1999; Albers, 2015; Dumais and Veenema, 2016), although the brain regions wherein sex differences in the number of these receptors are observed differs across species. The present study also demonstrates that the effects of social experience on receptor number can be sex-dependent. Because gonadal hormones can alter V1a, OT and 5-HT1a receptor binding, sex differences in the circulating levels of these hormones could underlie at least some of the sex differences (Johnson, 1992; Johnson et al., 1995; Simon et al., 1998). In the present study all males and females were gonadally intact and, to reduce variability and the effects of hormonal cyclicity, all females were studied during the diestrous phase of the estrous cycle.

Many of the brain sites wherein we observed the effects of social isolation and sex on receptor binding are part of the circuitry involved in controlling aggression. While this complex circuitry is not well understood, particularly in females, the present data may provide some clues as to how aggression is regulated (Delville et al., 2000; Terranova et al., 2017). The AH is a critical site for the control of aggression in males and females, and AVP- and 5-HT containing projections into the region are involved in aggression and dominance (Terranova et al., 2016). In males, the AH is likely involved in mediating at least some of the aggression-promoting effects of social isolation through an up-regulation of V1a receptors in this region. Another relevant finding is that V1a receptor binding was significantly lower in the DRN in isolated males compared to all other groups. Activation of V1a receptors in the DRN increases the activity of 5-HT neurons through a glutamate-dependent mechanism (Rood and Beck, 2014). Because the DRN provides about half of the 5-HT innervation to the AH and a reduction in 5-HT release in the AH would increase aggression, the decrease in V1a receptor binding in the DRN by social isolation could contribute to the aggression-promoting effects of social isolation (van de Kar and Lorens, 1979; Delville et al., 2000). Another interesting finding from the present study was that social isolation significantly reduced V1a receptor binding in the BNST. Although AVP has been found to increase male aggression in several brain sites, AVP release in the BNST in male rats is negatively correlated with aggression (Veenema et al., 2010). Thus, if activation of V1a receptors by AVP in the BNST reduces aggression, then the reduction in V1a receptor binding in the BNST could contribute to the increase in aggressiveness of males and females housed in social isolation.

In socially housed females, OTR binding in the DRN was higher than in socially isolated females or males. Consistent with these data, studies in female rats have found that knocking down OTRs in the DRN increases female aggression toward a male intruder (Grieb et al., 2018). Other studies have found that activation of OTRs can increase the release of 5-HT within the raphe, which can in turn inhibit the activity of 5-HT neurons (Yoshida et al., 2009). Given the substantial 5-HT containing projections from the DRN to the AH (van de Kar and Lorens, 1979; Delville et al., 2000), increased OTR binding in the DRN might decrease 5-HT output from the DRN to the AH, thereby decreasing aggression. These data suggest that the lower OTR binding in the DRN

may play a role in reducing aggression in socially housed females.

In conclusion, the mechanisms underlying the ability of social experience to alter an individual's subsequent social behavior are not well understood. The present study, along with other data, demonstrates that the amount and type of social interactions can produce differential effects on the circuit regulating social behavior in a sex-dependent manner. Indeed, the present data illustrate the plasticity that can occur in three of the major receptors that have key roles in the regulation of social behavior in response to social experience, V1a, OT and 5HT1a receptors.

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