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SOCIAL EXPERIENCE AND SEX-DEPENDENT REGULATION OF AGGRESSION IN THE LATERAL SEPTUM BY EXTRASYNAPTIC δ GABA_A RECEPTORS

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Abstract

Understanding the neurobiological mechanisms mediating dominance and competitive aggression is essential to understanding the development and treatment of various psychiatric disorders. Previous research suggests that these mechanisms are both sexually differentiated and influenced substantially by social experience. In numerous species, GABA_A receptors in the lateral septum have been shown to play a significant role in aggression in males. However, very little is known about the role of these GABA_A receptors in female aggression, the role of social experience on GABA_A receptor mediated aggression, or the roles of different GABA_A subtypes in regulating aggression. Thus, in the following set of experiments we determined the role of social experience in modulating GABA_A receptor induced aggression in both male and female Syrian hamsters, with a particular focus on the GABA_A receptor subtype mediating these effects. Activation of GABA_A receptors in the dorsal lateral septum increased aggression in both males and females. Social housing, however, significantly decreased the ability of GABA_A receptor activation to induce aggression in males but not females. No significant differences were observed in the effects of GABA_A receptor activation in dominant and subordinate group housed hamsters. Finally, examination of potential GABA_A receptor subtype specificity revealed that social housing decreased the ratio of extrasynaptic to synaptic subunit GABA_A receptor mRNA expression in the anterior dorsal lateral septum. While activation of extrasynaptic, but not synaptic GABA_A receptors in the dorsal lateral septum increased aggression. These data suggest that social experience can have profound effects on the neuronal mechanisms mediating aggression, especially in males, and that δ extrasynaptic GABA_A receptors may be an important therapeutic target in disorders characterized by high levels of aggression.

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Keywords

Social behavior; social isolation; agonistic behavior; sex differences; synaptic γ GABA_A receptors; dominance relationships

1. Introduction

Dominance and competitive aggression play a critical role in the development and maintenance of social relationships in nearly all mammalian species. Relatively little is known, however, about the neurobiological mechanisms underlying dominance and aggression in females. Emerging evidence indicates that these mechanisms can regulate dominance and aggression in radically different ways in males and females (Terranova et al., 2016). Perhaps this should not be surprising because social behavior is evolutionarily ancient and evolved in males and females in response to very different selective pressures. Understanding the sex differences in the neurobiological mechanisms regulating social behaviors is clinically important because it is likely that these differences underlie the sex differences in the prevalence of psychiatric disorders (Young & Pfaff, 2014).

Social isolation can have detrimental effects on social behavior and mental health (Suomi, Harlow, & Kimball, 1971), while social interaction and strong social networks can have beneficial effects on health and increase resilience to stress (Morrison et al., 2014; Snyder-Mackler et al., 2016). Very little is known, however, about how social experience modulates the neural circuits controlling social behavior. One of the most robust effects of social isolation is a substantial increase in aggression in both males and females (Brain, 1972a; Chang, Hsiao, Chen, Yu, & Gean, 2015; Tremblay, 2014). In the present study, we investigate potential effects of social experience (i.e. isolated vs. socially housed) and the quality of social experience (i.e. dominant vs. subordinate) on the neural mechanisms regulating aggression in both males and females.

Syrian hamsters are an outstanding animal model for the investigation of the neurobiology of dominance and aggression (Terranova et al., 2016). Unlike laboratory mice and rats, females as well as male hamsters rapidly establish stable hierarchical dominance relationships like those seen in primates (Drickamer & Vandenberg, 1973; Drickamer, Vandenberg, & Colby, 1973). Hamsters also engage in numerous social behaviors that are essential for developing and maintaining social relationships such as social recognition, social reward, social avoidance, and social communication (Albers, 2015; Huhman et al., 2003). Much is also known about the neural mechanisms and the hormones controlling aggression in hamsters (Albers, 2012; Albers & Bamshad, 1998; Harmon, Moore, Huhman, & Albers, 2002).

The lateral septum (LS) has long been recognized as a structure involved in the control of a variety of social behaviors including aggression (Bredewold, Schiavo, van der Hart, Verreij, & Veenema, 2015; Nelson & Trainor, 2007). The LS is reciprocally connected to a number of structures that form a neural circuit controlling aggression that includes the medial amygdala, bed nucleus of the stria terminalis, anterior hypothalamus and ventrolateral hypothalamus (Delville, De Vries, & Ferris, 2000). Several lines of evidence suggest that

GABA in the lateral septum influences various forms of aggression in several different species (DaVanzo, Chamberlain, & McConnaughey, 1986; G. Lee & Gammie, 2009; Potegal, Perumal, Barkai, Cannova, & Blau, 1982; Potegal, Yoburn, & Glusman, 1983; Simler, Puglisi-Allegra, & Mandel, 1982). More recently, activation of GABA_A receptors in the lateral septum has been found to induce high levels of aggression in male Syrian hamsters (McDonald, Markham, Norvelle, Albers, & Huhman, 2012).

GABA_A receptors are a family of heteropentameric receptors composed from 19 identified subunits (Sieghart & Sperk, 2002; Sigel & Steinmann, 2012). The most well studied GABA_A receptors are those containing the $\gamma 2$ subunit ($\gamma 2$ GABA_A receptors) (Belelli et al., 2009; Farrant & Nusser, 2005) that are found in synaptic regions, have a low GABA binding affinity, and mediate a transient phasic current. More recently, however, GABA_A receptors containing the δ subunit (δ GABA_A receptors) have been identified in extrasynaptic regions. δ GABA_A have a high GABA binding affinity, are found on dendrites, somas, and axons, and mediate a persistent tonic current (Albers, Walton, Gamble, McNeill, & Hummer, 2017; Brown, Kerby, Bonnert, Whiting, & Wafford, 2002; V. Lee & Maguire, 2014). Thus, in the following set of experiments we determined the role of social experience in modulating GABA_A receptor induced aggression in both male and female hamsters, with a particular focus on the GABA_A receptor subtype mediating these effects.

2. Materials and Methods

2.1 Subjects

Male and female Syrian hamsters (120–130 g) 11 weeks old were purchased from Charles River Laboratory (Wilmington, MA) and were housed on a reverse light-dark cycle (14L: 10D). All hamsters (housed singly or in groups) were housed in solid-bottom polycarbonate cages (43 cm × 22 cm × 20 cm) containing corncob bedding and cotton nesting material (Neslets; Ancare, Bellmore, NY). Food and water were available *ad libitum*. All females were examined daily to determine stage of the estrous cycle. Hamsters were gently restrained while vaginal secretion was examined. Only two out of the 47 female subjects displayed an abnormal estrous cycle, and they were excluded from statistical analysis (approximately <5%). Males were also examined in the same frequency as females to control for handling effects. Animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Georgia State University Institutional Animal Care and Use Committee.

2.2 Housing & Stereotaxic Surgery

Upon arrival hamsters were housed socially (3–4 same sex hamsters per cage) or singly. Three weeks after arrival hamsters were anesthetized with isoflurane (induced at 5% and maintained at 2–4%) and a 4 mm 26-gauge cannula was implanted unilaterally aimed at the LS (from bregma; anteroposterior (AP) +1.7 mm; mediolateral (ML) +1.5 mm; dorsoventral (DV) –3.5 mm; 10° angle). Guide cannula were secured to the skull with screws, 11 mm wound clips and dental adhesive. Dummy caps were inserted to prevent clogging. All hamsters were injected subcutaneously with the analgesic agent ketoprofen (5 mg/kg) and were allowed to recover for at least 1 week prior to behavioral testing.

2.3 Microinjections

Early in the dark phase of the light:dark cycle, microinjections were administered with a 1 μ l Hamilton syringe using a 12 mm, 32-gauge needle that extended an additional 1.2 mm beyond cannula to a final depth of 4.7 mm below skull surface. Drug was delivered in a volume of 50 or 200 nl at a rate of 0.400 μ l min^{-1} unless noted otherwise. Pilot studies revealed no behavioral differences between bilateral versus unilateral injections in the lateral septum. The needle was left in place for an additional 30 seconds to allow diffusion away from the tip of the injection cannula. Microinjections were completed in a counterbalanced order with treatments spaced at least 4 days apart. The drugs used were muscimol (non-selective GABA_A receptor agonist) (Sigma-Aldrich) dissolved in sterile saline to a final concentration of 25mM and 5mM (McDonald et al., 2012; Potegal et al., 1983); 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridine-3-ol (THIP: gaboxadol, a selective δ subunit containing GABA_A receptor agonist) (Sigma-Aldrich) dissolved in sterile saline to a final concentration of 25mM (Depaulis & Vergnes, 1983, 1985; Ehlen & Paul, 2009); chlordiazepoxide (CDP, a selective γ_2 subunit containing GABA_A receptor agonist) (Grace Discovery Sciences) dissolved in sterile saline to a final concentration of 2mM, 25mM and 200mM (Frye, McCown, & Breese, 1983; G. Lee & Gammie, 2009), and Ro15–4513 (a selective γ_2 subunit containing GABA_A receptor antagonist) (Tocris Bioscience) dissolved in 0.1% dimethylsulfoxide (DMSO) saline solution to a final concentration of 40 μ M (Dar, 2001; McElroy, Zakaria, Glass, & Prosser, 2009; Melon & Boehm, 2011; Saldivar-Gonzalez, Gomez, Martinez-Lomeli, & Arias, 2000). For all experiments utilizing Ro15–4513 all drugs were dissolved in sterile saline and 0.1% DMSO as the vehicle. The concentrations of all drugs administered were based on the concentrations used in previous studies that were found effective in altering behavior in hamsters or other rodents.

2.4 Social Behavior Testing

Ten min following microinjection, hamsters were placed into a neutral arena (43 cm $\times$ 22 cm $\times$ 20 cm) with a novel non-aggressive smaller same-sex stimulus hamster (100–110 g). Test sessions lasted five min and were recorded using the Noldus Observer system. The videos were later scored by an experimenter blind to the treatment groups. All behavioral tests were performed during the first three hours of the dark phase of the light cycle. Females were tested on their first day of diestrus (Drickamer & Vandenberg, 1973). The duration of aggression and the number of attacks were scored in all hamsters (Drickamer & Vandenberg, 1973; Drickamer et al., 1973; Gray, Norvelle, Larkin, & Huhman, 2015). The duration of aggression was a conglomerate of behaviors in which the subject hamster either pins (subject is positioned on top of stimulus and restrains locomotion of stimulus), bites/attacks (opened mouth with head pushed into stimulus animal's flank or abdomen and elicits an audible vocalization from stimulus animal) or intensely pursues or chases fleeing stimulus hamster. In experiment 2A, the number of times aggression was initiated (Aggression Bouts), the latency to the initial onset of aggression (Latency to Aggression), the latency to the initial attack (Latency to Attacks), the amount of time each hamster spent approaching and sniffing the other hamster (Social Investigation Duration) and the duration of time each hamster spent not engaging in any form of social behavior (Non Social Duration) were also recorded.

2.5 Histology

Within 24 hours of the final behavioral test hamsters were killed with a lethal dose of sodium pentobarbital (0.2 ml, i.p. Henry Schein Animal Health, Dublin, OH) and 50 or 200 nl of India ink was microinjected through the guide cannula to mark the injection site. Brains were extracted and submerged in 10% formalin for at least 24 hours at 4° C. Brains were sectioned at 40 µm with a cryostat and collected onto superfrost plus slides. Tissues were stained with neutral red. Cannula placements were classified as hits if ink was seen within the dorsal or intermediate lateral septum, referenced to the hamster stereotaxic atlas (Morin & Wood, 2001).

2.6 Quantitative Real-Time Polymerase Chain Reaction

Hamsters were given a lethal dose of sodium pentobarbital, decapitated, and brains were rapidly removed and flash frozen by submerging in isopentane on dry ice. Brains were collected during the first three hours of the dark phase of the light cycle. After flash freezing, brains were faced in the coronal plane in an RNase free cryostat and 1.00 mm bilateral punches were collected from the lateral septum and medial septum. Four separate bilateral punches were collected for the lateral septum: anterior dorsal, anterior ventral, posterior dorsal and posterior ventral (Figure 5a). Samples were kept in an –80°C freezer until RNA isolation. For RNA isolation samples were homogenized in 1.0 ml Trizol (Ambion) using a sterile pestle, vortexed, and left at room temperature for 5 min. RNA was then washed twice with 200 µl chloroform and centrifuged at 12,000 g for 15 min at 4°C. After collection of aqueous phase, to facilitate RNA precipitation 2 µl of glycoblue was added and mixed by flicking tube. RNA was precipitated with 500 µl of 100% isopropanol and pellet collected following centrifugation at 12,000 g for 15 min at 4°C. Supernate was carefully drawn off and pellet washed twice with 1.0 ml of 75% ethanol and 25% RNase-free water. Pellet was finally air dried for 10–15 min, resuspended in 15 µl of RNase-free water, and RNA concentration was determined using a NanoDrop 2000. Samples were then stored in –80°C freezer until cDNA synthesis. For cDNA synthesis, 150 ng of total RNA was then reverse transcribed into cDNA using Cloned AMV First-Strand cDNA Synthesis Kit (Invitrogen) following the manufacturer's protocol. In brief, RNA and random-hexamer primer were denatured by incubating at 65°C for 5 min, and then following addition of the master reaction mix, tubes were transferred to a thermal cycler and heated to 25°C for 10 min, then 50°C for 45 min and finally the reaction was terminated by incubating at 85°C for 5 min. For RT-qPCR, cDNA was diluted 1:5 in deionized water and each well was composed of 10 µl buffer, 1 µl cDNA, 1 µl gene of interest assay (probes and primers), 1 µl housekeeping gene (18s) assay and 7 µl deionized water. Relative gene expression was quantified using an ABI 7500 FAST Real-Time system using Taqman Universal PCR master mix and the following universal two-step RT-PCR cycling conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. The following primer/probe sets from Applied Biosystems were used: δ GABA_A (ABI Mm0126603_g1), γ ₂GABA_A (ABI Rn00788325_m1), and 18s (4319413E). Relative gene expression for each sample run in duplicate was calculated by comparing to a relative standard curve and then standardized to 18s rRNA expression. Relative cDNA standards were generated using pooled hippocampal RNA extracts. Only average quantity values with standard deviations less than 0.12 were

included. Quantity values beyond 2.0 standard deviations from the overall mean of all samples were also excluded from data analysis as outliers.

2.7 Experimental Design

Experiment 1—The goal of this experiment was to determine the subregion(s) of the septum where activation of GABA_A increased aggression. In order to conserve animal usage this study was conducted only in males. 46 males were housed singly for 3 weeks prior to stereotaxic surgery. One week following surgery hamsters were injected with 50 nl of saline or 5mM muscimol in the dorsal LS (n=9), ventral LS (n=6), medial septum (n=10), or lateral ventricle (n=7). Ten minutes after injection hamsters were tested for social behavior with a same-sex stimulus hamster. Each hamster received both saline and muscimol injections in a counter-balanced manner. Testing occurred 4 days apart.

Experiment 2—The goal of this experiment was to confirm that activation of GABA_A receptors in the dorsal LS increased aggression in females as well as male hamsters and to determine if social experience influences aggression induced by GABA_A receptors. Part A) 18 males and 23 females were either housed socially (3–4 per cage) or singly for 3 weeks prior to stereotaxic surgery. One week following surgery hamsters were injected with 200 nl of saline or 5mM muscimol in the dorsal LS 10 min prior to the social behavior test. Injections were counter-balanced and testing occurred 4 days apart (Figure 1). Part B) The hamsters were also later injected with 200 nl of 25mM muscimol in the LS 10 min prior to social behavior test. This testing occurred 4 days after second drug injection (either saline or 5mM muscimol). Part C) Because group-housed male and female hamsters likely form dominance hierarchies in their home cages, another experiment was conducted to determine if muscimol had different effects on aggression in dominant versus subordinate hamsters. The dominant and subordinate hamsters in each cage were identified by comparing the composite aggression score (aggression duration) during a baseline aggression test (highest scores were considered to be the dominant hamster). The experiment was conducted as described in Part A above comparing dominant males (n=4), subordinate males (n=11), dominant females (n=5) and subordinate females (n=11).

Experiment 3—The goal of this experiment was to examine whether there are sex and/or housing differences in the expression of δ GABA_A and γ_2 GABA_A receptor subunit mRNA. 16 males and 16 females were either socially (3–4 per cage) or single housed for 4 weeks. δ GABA_A and γ_2 GABA_A receptor subunit mRNA expression was analyzed by RT-qPCR (aLSd, aLSv, pLSd, pLSv, MS).

Experiment 4—Because no sex differences were observed in δ or γ_2 subunit GABA_A mRNA expression in the previous experiment only males were used in the following experiment in order to conserve animal usage. 12 males were housed singly for 3 weeks prior to stereotaxic surgery. One week following surgery, hamsters were injected with 200 nl of saline, 5mM muscimol, 25mM THIP or 25mM CDP in the dorsal LS 10 min prior to being tested for social behavior. Each subject received each of the four drug treatments; injections were counter-balanced and testing occurred 4 days apart.

Experiment 5—Because no sex differences were observed in δ or γ_2 subunit GABA_A mRNA expression in experiment 3 only males were used in the following two experiments in order to conserve animal usage. 5 males were housed singly and were injected with 200 nl of saline, 2mM CDP or 200mM CDP in the dorsal LS prior to social behavior test. Injections were counter-balanced and testing occurred 4 days apart.

Experiment 6—12 males were housed singly and were injected with 200 nl of vehicle (saline + 0.1%DMSO), 25mM THIP, 40 μ M Ro15–4513 or a 1:1 cocktail of 50mM THIP + 80 μ M Ro15–4513 into the dorsal LS. Injections were counter-balanced and testing occurred 4 days apart.

2.8 Data Analysis

All data are presented as mean $\pm$ standard error of the mean. For the first experiment, hamsters were categorized based on site of injection, and the change in the duration of aggression and the number of attacks (muscimol 5mM – saline condition) were analyzed using a one-way analysis of variance (ANOVA). Paired sample t-tests were also used to compare muscimol versus saline for the duration of aggression and the number of attacks for each brain region. For Experiment 2A, a 2×2 ANOVA was used to confirm main effects of sex and housing as well as their interactions on the duration of aggression in saline-treated hamsters. Post hoc comparisons were carried out using Tukey's HSD. A 2×2 ANOVA was used to examine the main effects of sex and housing as well as their interactions on the change in the duration of aggression and the number of attacks (muscimol 5mM – saline condition). Post hoc comparisons were carried out using Tukey's HSD. Paired sample t-tests were also employed to compare muscimol versus saline on the duration of aggression, the number of attacks, number of aggression bouts, the latency to aggression, the latency to attack, social investigation duration and non-social duration for each sex and housing condition. For experiment 2B, one-way ANOVAs were utilized to examine the effect of high concentration of muscimol (25mM) on the duration of aggression and the number of attacks for each treatment condition. For experiment 2C, a 2×2 ANOVA was used to examine the main effects of sex and hierarchy status as well as their interaction on the change in the duration of aggression and the number of attacks. Post hoc comparisons were carried out using Tukey's HSD. Paired sample t-tests were also employed to compare muscimol versus saline on the duration of aggression and the number of attacks for each sex and hierarchy condition. Finally, a $2 \times 2 \times 3$ ANOVA and one-way ANOVAs were utilized to examine the effect of high concentration of muscimol (25mM) on the duration of aggression and the number of attacks for each treatment group. For Experiment 3, a 2×2 ANOVA was used to examine the main effects of sex and housing on δ GABA_A and γ_2 GABA_A receptor expression in the lateral septum. All pharmacological GABA_A receptor subtype specificity experiments (4–6) were analyzed using a one-way repeated measures ANOVA followed by pairwise comparisons using Tukey's HSD. Data were examined to determine if the assumptions of parametric statistical tests were met. When assumptions were violated, data were log or cube root transformed. All analyses were completed with the use of SPSS statistical software (SAS Institute, 1990). All tests were two-tailed and results were considered statistically significant if $p < 0.05$. Animals were not included in statistical

analysis if their injection sites were not within the boundaries of the dorsal or intermediate lateral septum.

3. Results

3.1 Experiment 1: Localization of sites within the septum where aggression is induced by activation of GABA_A receptors

To determine where activation of GABA_A receptors in the septum stimulates aggression, male hamsters were injected with 50 nl of 5mM muscimol or saline in a counter-balanced order in various sub-regions of the septum or surrounding brain regions (Fig 2a). Injections of muscimol into the dorsal LS significantly increased the duration of aggression ($p=0.006$ Fig 2b) and the number of attacks ($p=0.030$ Fig 2c) compared with saline. However, neither injections into the medial septum, ventral LS nor lateral ventricle increased the duration of aggression or the number of attacks, respectively, compared with saline ($p>0.05$). Injections into the dorsal LS yielded the greatest increase in the duration of aggression (main effect: $p=0.013$, $F(3,31) = 4.303$; Fig 2d) and the number of attacks (main effect: $p=0.237$, $F(3,31) = 1.496$; Fig 2e) compared with other brain regions.

Experiment 2: Effect of social experience on spontaneous aggression and aggression induced by activation of GABA_A receptors in males and females

To determine if social experience influences aggressive behavior, male and female hamsters were housed singly or in groups (3–4) for four weeks before behavior testing. Social housing decreased the duration of aggression in both males and females ($p=0.000$, $F(1,26) = 34.434$). Isolated males had a longer duration of aggression than socially housed males ($p=0.006$ Fig. 3) and socially isolated females had a longer duration of aggression than socially housed females ($p=0.000$ Fig. 3). Furthermore, although there was a main effect of sex on the duration of aggression ($p=0.019$, $F(1,26) = 6.204$), socially housed males and females did not differ in the duration of aggression ($p>0.05$) and isolated males and females did not differ in the duration of aggression ($p>0.05$). Also, there was similar amount of variability in the duration of aggression in socially housed and single housed hamsters (Levene's Test of Equality of Error Variance: $p>0.05$) suggesting that it was unlikely that the formation of dominance hierarchies in the group housed hamsters was responsible for their lower duration of aggression (Fig. 3).

To examine if social experience influences aggression induced by GABA_A receptors, male and female hamsters were housed singly or in groups for 4 weeks and then injected with either saline or 5mM muscimol in a counter-balanced order (Part A). Muscimol (5mM) increased the duration of aggression in isolated males ($p=0.004$, $n=8$, Fig. 4b), isolated females ($p=0.018$, $n=7$, Fig. 4b) and socially housed females ($p=0.042$, $n=8$, Fig. 4b), but not socially housed males ($p>0.05$, $n=7$, Fig. 4b). Muscimol also increased the number of attacks in isolated males ($p=0.038$, $n=8$, Fig. 4c), isolated females ($p=0.049$, $n=7$, Fig. 4c), and socially housed females ($p=0.046$, $n=8$, Fig. 4c), but not socially housed males ($p>0.05$, $n=7$, Fig. 4c). Thus, both sex and housing interacted such that socially isolated males had an increase in the duration of aggression (interaction: $p=0.024$, $F(1,26) = 5.773$; $p=0.024$ Fig. 4d) and in the number of attacks (interaction: $p=0.075$, $F(1,26) = 3.428$; $p=0.030$ Fig. 4e)

induced by GABA_A receptor activation compared with socially housed males, but there were no differences in the change of the duration of aggression or the number of attacks ($p>0.05$) between socially housed and isolated females.

To determine if a higher concentration of muscimol is needed to increase aggression in socially housed males, all subjects were injected with 25mM muscimol and examined for social behavior (Part B). 25mM muscimol did not increase the duration of aggression nor the number of attacks in isolated males ($p>0.05$ Fig 4f, 4g), socially housed males ($p>0.05$ Fig 4f, 4g), isolated females ($p>0.05$ Fig 4f, 4g), nor socially housed females ($p>0.05$ Fig 4f, 4g).

Histology: Hamsters were included in the study only if the tip of the injection cannula was located within the dorsal or intermediate LS. Eight hamsters were removed due to injection cannula tips located in the lateral ventricle, medial septum or corpus callosum, one guide cannula fell off before second drug injection and two female hamsters were removed due to abnormal estrous cycles (Figure 4a).

The effects of muscimol on additional measures of aggression and social interaction can be seen in Figure 5. Treatment of muscimol (5mM) decreased the number of aggressive bouts in isolated males ($p=0.043$) and isolated females ($p=0.041$), but had no effect in socially housed males nor socially housed females ($p>0.05$, interaction: $p=0.024$, $F(1,54) = 60.421$, Fig. 5a). Injections of muscimol (5mM) into the lateral septum decreased the latency to initiate aggressive behavior in isolated females ($p=0.024$), socially housed females ($p=0.049$) and isolated males just missed significance ($p=0.105$, interaction: $p=0.059$, $F(1,54) = 3.709$, Fig. 5b). Muscimol treatment had no effect on the latency to initiate aggression in socially housed males ($p>0.05$). Females were also quicker to attack stimulus hamsters compared to males ($p<0.001$, $F(1,54) = 20.699$, Fig. 5c) and isolated hamsters were also quicker to attack stimulus hamsters compared to socially housed hamsters ($p=0.008$, $F(1,54) = 7.691$, Fig. 5c). Interestingly though, post hoc comparisons revealed that muscimol (5mM) decreased the latency to attacks only in socially housed females ($p=0.043$), muscimol had no effect on the latency to attacks in isolated nor socially housed males, nor isolated females ($p>0.05$).

In relation to non-aggressive descriptive behaviors, muscimol treatment decreased social investigation duration in isolated females ($p=0.027$), socially housed females ($p=0.044$), and isolated males ($p=0.073$) just missed significance, but had no effect in socially housed males ($p>0.05$; $p=0.004$, $F(1,54) = 9.357$, Fig. 5d). Finally, no effects of drug treatment were observed on the time spent displaying non-social behaviors when analyzing isolated and socially housed male and female groups independently ($p>0.05$, Fig. 5e).

To determine if social status altered the effects of muscimol on aggression the dominant and subordinate hamsters in each group housed cage were identified by comparing the duration of aggression during a baseline aggression test (hamsters with the longest duration of aggression in each group housed cage were considered dominant while all other subjects in the cage were considered subordinate). Thus, the experiment was conducted as described in Part A above comparing dominant males ($n=4$), subordinant males ($n=11$), dominant

females (n=5) and subordinate females (n=11). As can be seen in Figure 6, The effects of muscimol on aggression in dominant and subordinate males and females was very similar. Dominant subjects spent more time displaying aggression towards stimulus hamsters compared to subordinate subjects ($p=0.001$, $F(1,54) = 11.299$). Independent of social status, there was a trend for females treated with muscimol (5mM) to display an increase in aggression duration compared to saline treated females (interaction: $p=0.099$, $F(1,54) = 2.820$), but no effect was observed for males injected with muscimol (5mM) ($p>0.05$, Fig. 6a) compared to saline injections. Social status had no effect on aggression duration in saline treatment condition versus muscimol 5mM treatment condition ($p>0.05$). Dominant subjects also displayed a greater number of attacks compared to subordinate subjects ($p=0.013$, $F(1,54) = 6.664$). Similar to aggression duration, females injected with muscimol (5mM) displayed an increase in the number of attacks compared to females injected with saline (dominant females, $p=0.011$; subordinate females, $p=0.077$; interaction: $p=0.011$, $F(1,54) = 6.907$, Fig. 6b), but no effect was observed in males injected with muscimol (5mM) ($p>0.05$) compared to males injected with saline. Social status had no effect on the number of attacks in saline treatment condition versus muscimol 5mM treatment condition ($p>0.05$).

Thus, when investigating the potential of social status to regulate the ability of GABA_A receptor activation in the lateral septum (muscimol 5mM – saline treatment condition) to increase aggressive behavior; social status had no effect on muscimol (5mM) induced change in aggression duration ($p>0.05$, Fig. 6c). In other words, dominant versus subordinate status had no effect on muscimol (5mM) induced changes in aggression duration. However, there was a trend for socially housed females to display a greater change in aggression duration following muscimol (5mM) treatment compared to socially housed males ($p=0.086$, $F(1,27) = 3.101$, Fig. 6c) independent of social status. Likewise, social status had no effect on muscimol (5mM) induced change in the number of attacks ($p>0.05$, Fig. 6d). In other words, dominant versus subordinate status had no effect on muscimol (5mM) induced changes in the number of attacks. However, socially housed females did display a greater increase in the number of attacks induced by muscimol (5mM) treatment compared to socially housed males ($p=0.016$, $F(1,27) = 6.623$, Fig. 6d) independent of social status.

Finally, to determine if a higher concentration of muscimol has social status specific effects on aggression, all subjects were injected with 25mM muscimol. Muscimol (25mM) did not increase the duration of aggression in dominant males, subordinate males, dominant females, nor subordinate females ($p>0.05$, Fig. 6e) compared to saline injections and muscimol (25mM) had no effect on the number of attacks in dominant males, subordinate males, dominant females, nor subordinate females ($p>0.05$, Fig. 6f) compared to saline injections.

3.2 Experiment 3: Effects of social experience on the expression of δ GABA_A and $\gamma 2$ GABA_A subunit receptor mRNA in the lateral septum in males and females

Because GABA_A receptors have different physiological properties depending on their subunit composition, in the next set of experiments we examined if δ -containing GABA_A or $\gamma 2$ -containing GABA_A receptors in the lateral septum regulate aggressive behavior. Because

social experience dramatically influences spontaneous aggression and aggression stimulated by activation of GABA_A receptors in the septum, we examined if social experience alters δ GABA_A and γ_2 GABA_A receptor mRNA expression in the lateral septum. Male and female hamsters were housed in groups or housed singly for 4 weeks and mRNA expression in the septum for the δ and γ_2 GABA_A subunits were analyzed by RT-qPCR (Figure 7a). No differences in sex or housing in δ or γ_2 subunit GABA_A mRNA expression were observed ($p > 0.05$; Data not shown). However, in the anterior dorsal lateral septum social housing decreased the ratio of the relative mRNA expression of δ to γ_2 subunit GABA_A receptors ($F(1,21) = 4.305$, $p=0.050$; Figure 7b). Trends towards significance in the anterior ventral lateral septum ($F(1,23) = 2.764$, $p=0.110$) for social housing to decrease the ratio of the relative mRNA expression of δ to γ_2 subunit GABA_A receptors were also observed (Figure 7d). However, no differences were observed for the posterior dorsal nor posterior ventral lateral septum (Figure 7c and e). Thus, social housing decreases the ratio of δ to γ_2 subunit GABA_A receptors within specific subregions of the septum.

3.3 Experiment 4–6: Effect of GABA_A receptor subtype specific agonists and antagonists in the LS on aggression

Because social housing reduces aggression and decreases the ratio of δ to γ_2 subunit GABA_A receptors in the septum, we next examined if selective activation of δ GABA_A versus γ_2 GABA_A receptors in the lateral septum induces aggression. Male hamsters were housed singly for 4 weeks before behavioral testing and injected with either saline, muscimol, THIP (a selective δ subunit containing GABA_A receptor agonist) or CDP (a selective γ_2 subunit containing GABA_A receptor agonist) in a counter-balanced manner. Muscimol increased the duration of aggression (main effect: $p=0.000$, $F(3,29) = 8.198$; $p=0.012$ Fig 8b) and the number of attacks (main effect: $p=0.006$, $F(3,29) = 5.130$; $p=0.031$ Fig 8c) compared with saline, and THIP increased the duration of aggression ($p=0.007$ Fig 8b) while the number of attacks just missed significance ($p=0.095$ Fig 8c) compared with saline controls. CDP had no effect on the duration of aggression ($p=1.000$ Fig 8b) nor the number of attacks ($p=1.000$ Fig 8c). To determine if high or low concentrations of CDP increase aggression, male hamsters were injected with either 5mM or 200mM CDP or saline. CDP had no effect on the duration of aggression ($p=0.968$, $F(2,12) = 0.032$ Fig 8d) or on the number of attacks ($p=0.725$, $F(2,12) = 0.330$ Fig 8e).

To further determine that the increase in aggression induced by THIP was the result of activation of GABA_A receptors containing the δ subunit and not the γ_2 subunit, hamsters were injected with either THIP, Ro15–4513 (a highly selective γ_2 GABA_A antagonist), a cocktail of THIP + Ro15–4513 or vehicle. THIP increased the duration of aggression (main effect: $p=0.003$, $F(3,36) = 5.602$; $p=0.005$ Fig 8f) and the number of attacks (main effect: $p=0.001$, $F(3,36) = 6.957$; $p=0.005$ Fig 8g) compared with saline controls, and a cocktail of THIP and Ro15–4513 increased the duration of aggression ($p=0.042$ Fig 8f) and the number of attacks ($p=0.003$ Fig 8g) compared with saline controls. Ro15–4513 had no effect on the duration of aggression ($p=0.868$ Fig 8f) or on the number of attacks ($p=0.657$ Fig 8g).

Histology: Three hamsters were removed from analysis due to injection sites located in the lateral ventricle or corpus callosum, one guide cannula was dislodged before the third drug

injection and one before the fourth drug injection in Experiment 4 (Figure 8a). No hamsters were excluded in Experiment 5 (Figure 8a). Two hamsters were removed due to injection sites located in the lateral ventricle or corpus callosum in Experiment 6 (Figure 8a).

4. Discussion

These data confirm a previous report that activation of GABA_A receptors by injection of muscimol into the septum can stimulate offensive aggression in male hamsters housed in social isolation (McDonald et al., 2012). In this prior work, bilateral injections of muscimol significantly increased aggression in males tested under territorial conditions (i.e., home cage), while in the present study unilateral injections of muscimol were also highly effective in inducing aggression when tested in non-territorial conditions (i.e., neutral arena). The present data also extend this prior work by demonstrating that the aggression inducing effects of activation of GABA_A receptors within the septum are restricted to the dorsal lateral septum. One limitation of this finding is that this localization study was only conducted in males so it remains possible that there are sex differences in the septal regions where activation of GABA_A receptors stimulate aggression. Another limitation is that dose-response studies were not conducted so it is possible that other concentrations of muscimol could have induced aggression in other septal regions. It is noteworthy, however, that muscimol injected into the dorsal lateral septum was effective in stimulating aggression in both males and females. Although social isolation increases aggression in both males and females the present data indicate that there are substantial sex differences in the effects of social experience on the ability of GABA_A receptor activation to stimulate aggression. In females, activation of GABA_A receptors significantly increased aggression whether the hamsters were socially isolated or housed socially (whether dominant or subordinate). In males, GABA_A receptor activation increased aggression only in the socially isolated group. Activation of GABA_A receptors in the lateral septum did not increase aggressive behavior in either dominant nor subordinate socially housed male hamsters even when high concentrations of muscimol (i.e., 25 mM) were administered. Muscimol also decreased the number of aggressive bouts in singly housed males and females but not in group housed hamsters. Analysis of the distribution of δ and $\gamma 2$ subunit GABA_A receptor mRNA expression throughout the septum identified no significant sex differences or effects of social housing. Social isolation did, however, significantly increase the ratio of δ to $\gamma 2$ subunit GABA_A receptor mRNA expression in the anterior dorsal LS. Finally, the ability of muscimol to induce aggression by its actions in the dorsal lateral septum appears to be mediated by extrasynaptic δ GABA_A and not synaptic $\gamma 2$ GABA_A receptors.

The lateral septum is very heterogeneous with afferent and efferent projections from and to a variety of structures (e.g. olfactory bulb, amygdala, thalamus ect.) (Risold & Swanson, 1997a, 1997b; Swanson & Cowan, 1979). There are substantial reciprocal connections between the lateral septum and the hypothalamus. Various subregions of the lateral septum (e.g. ventral lateral septum) topographically project to specific subregions of the hypothalamus (e.g. ventral lateral and anterior hypothalamus) (Risold & Swanson, 1997b). The dorsal hippocampus also has reciprocal connections with the lateral septum, and this hippocampus-lateral septum-hypothalamus circuitry may play a role in the integration and translation of memory and motivational processing (Leroy et al., 2018). For example,

activation of hippocampal projections to the lateral septum, results in decreased inhibitory input by the lateral septum to the ventromedial hypothalamus (disinhibition) which increases aggression in male mice (Leroy et al., 2018).

Several lines of evidence indicate an important role for septal GABA in the regulation of aggression (McDonald et al., 2012; Potegal et al., 1983; Simler et al., 1982). For example, the higher levels of aggression seen in response to adolescent anabolic steroid treatment in hamsters is associated with changes in glutamic acid decarboxylase (GAD₆₅) levels in various brain regions including the lateral septum (Grimes, Ricci, & Melloni, 2003). There is also evidence to suggest that GABA_A receptors in the septum are involved in other forms of aggression, i.e. maternal aggression (G. Lee & Gammie, 2009). In addition to GABA_A receptors, the lateral septum also contains a large number of vasopressin V1a receptors. Activation of V1a receptors in the septum plays a substantial role in a form of social communication (i.e., flank marking) that has been linked the aggression and dominance in Syrian hamsters (Irvin, Szot, Dorsa, Potegal, & Ferris, 1990). The expression of flank marking by the activation of V1aRs in the lateral septum is dependent on septal interactions with hypothalamic structures (Ferris, Delville, Irvin, & Potegal, 1994). Thus, the lateral septum serves as an important structure in the neural circuitry integrating neural signals from various other brain structures as they relate to the formation and maintenance of dominance relationships.

The present data indicate that social experience can have powerful, but sex-dependent effect on whether activation of GABA_A receptors can induce aggression. Other studies have also found that experience can modulate GABAergic activity (Alvarez et al., 2016; Primus & Kellogg, 1991). Interestingly, the present study suggests that social experience has similar effects in males and females on the modulation of the ratio of synaptic and extrasynaptic GABA_A receptors, even though social experience (i.e., social housing versus social isolation) alters the ability of muscimol to induce aggression only in males. It is also somewhat surprising that social status had no effect on the ability of GABA_A activation in the lateral septum induce aggression in males or females. While the functional significance of the inability of GABA_A activation to stimulate aggression in socially housed males it not clear, it does reinforce the idea that aggression is mediated by circuits that function very differently in males and females (De Vries, 2004; McCann, Sinkiewicz, Rosenhauer, Beach, & Huhman, 2018; Terranova et al., 2016). Perhaps the sex differences in the effects of muscimol in the septum are mediated by the dense projections from the septum to sexually differentiated circuits in the hypothalamus (e.g., anterior hypothalamus). Perhaps understanding the sex differences in how social experience alters the ability of GABA_A receptors to induce aggression in males and females will provide insight into the significant sex differences in the development and prevalence of psychiatric disorders (Han et al., 2012; Mohler, 2015; Sesarini et al., 2014).

The present study as well as previous work indicates that male and female hamsters housed in social isolation are significantly more aggressive than hamsters housed in groups (Brain, 1972b; Wise, 1974). The factors responsible for this increase in aggression are not well understood. One possibility is that social isolation serves as a stressor that promotes aggressiveness. Indeed, social isolation can produce some stress-like effects in some species

(Locci & Pinna, 2018). In Syrian hamsters, while there is some evidence that group housing might be stressful (see Ross et al., 2017 for a discussion), male and female hamsters adapt quite well to both group housing and single housing and display no differences in cortisol levels and only minor changes in stress-related measures (Ross et al., 2017). It is also interesting that muscimol increases the number of aggressive bouts in both males and females that are singly housed but not in group housed hamsters.

These data represent some of the first evidence that δ extrasynaptic GABA_A receptors play a central role in the regulation of social behavior. Interestingly, one previous study found that aggression was not altered in virgin or postpartum δ GABA_A null female mice, although abnormal maternal behavior was exhibited in mice lacking the δ GABA_A subunit (Maguire & Mody, 2008). There is also evidence that δ GABA_A subunit expression occurs at higher levels in the hippocampus of socially isolated compared with socially housed male rats (Serra et al., 2006). Beyond these studies, however, little is known about the effects of the social environment on the expression of extrasynaptic δ GABA_A receptors or their role in the regulation of social behavior. This is likely due to the fact that only recently have δ extrasynaptic GABA_A receptors, along with other non-traditional GABA_A receptors been identified and characterized. Although our data suggests that social experience modulates the ratio of δ extrasynaptic and γ 2 synaptic GABA_A receptor mRNA, it remains important to examine the receptor protein itself because mRNA expression does not always correlate with protein expression of these receptors (Walton, McNeill, Oliver, & Albers, 2017). For example, it is possible that there might be sex differences in the receptor proteins despite their absence in mRNA expression. Furthermore, caution should also be used in interpreting the data from the use of subtype specific GABA_A receptor agonists and antagonists because these studies were conducted only in males and because of the sometimes-controversial pharmacological properties and selectivity of the drugs used (Albers et al., 2017; Hancher et al., 2006; Mortensen, Ebert, Wafford, & Smart, 2010; Wallner, Hancher, & Olsen, 2014). For example, benzodiazepines such as CDP are positive modulators at γ 2 subunit containing GABA_A receptors and their effects may be influence by GABA tone and Ro 15-4513 has been considered a partial inverse agonist that may have effects on GABA_A receptors that do not contain γ 2 subunits. Nevertheless, these data indicate that social experience can have profound effects on social behavior and the neuronal mechanisms mediating aggression, especially in males and also suggest that δ extrasynaptic GABA_A receptors may be an important therapeutic target (Brickley & Mody, 2012).

5. Conclusions

These data demonstrate that activation of GABA_A receptors specifically within the dorsal lateral septum, but not other subregions of the septum increases aggression in male and female hamsters. In males, but not females, social housing, significantly decreased the ability of GABA_A receptor activation to induce aggression. Social housing decreased the ratio of extrasynaptic to synaptic subunit GABA_A receptor mRNA expression in the anterior dorsal lateral septum and activation of extrasynaptic, but not synaptic GABA_A receptors in the dorsal lateral septum increased aggression. We conclude that social experience can have profound effects on the neuronal mechanisms mediating aggression, especially in males, and

that δ extrasynaptic GABA_A receptors may be an important therapeutic target for disorders characterized by high levels of aggression.

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Abbreviations

GABA_AR	GABA _A receptor
dLS	dorsal lateral septum
adLS	anterior dorsal lateral septum
CDP	chlordiazepoxide
THIP	gaboxadol
MS	medial septum

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Figure 1:

Experimental design for Experiments 2 and 3. Male and female hamsters were either socially housed or socially isolated for three weeks. In Experiment 2, they were anesthetized and a cannula was stereotactically aimed at the lateral septum and given one week to recover from surgery. Ten minutes after receiving either saline or muscimol, hamsters were placed in a neutral arena with a same-sex smaller (100–110g) stimulus hamster for five minutes. Subsequent drug injections and behavior testing occurred no less than four days apart. In Experiment 3, after four weeks of social or single housing, tissue samples from the septum were collected and processed for δ and γ_2 GABA_A receptor mRNA expression.

Figure 2:

Exp. 1: Effect of muscimol injected into the septum and surrounding brain regions on aggression. (a) Coronal sections illustrating the sites of injections. Dark circles indicate a more than 50 sec increase in the duration of aggression in hamsters injected with muscimol 5mM compared with those injected with saline. Light circles indicate no increase or a decrease in the duration of aggression in hamsters injected with muscimol 5mM compared to those injected with saline. Muscimol (grey bars) in the dorsal LS, but not ventral LS, medial septum nor lateral ventricle increased the duration of aggression (b) and the number of attacks (c) compared to saline controls (black bars). Muscimol injections into the dorsal LS yield the greatest increase in the duration of aggression (d) and in the number of attacks (e) compared with the other sites examined (* indicates $p < 0.05$). The greatest increase in aggression was found with injections into the dorsal lateral septum. (f) Representative image of an injection tract (blue dye) into the dorsal lateral septum. LSd, dorsal lateral septum; LSi, intermediate lateral septum; LSv, ventral lateral septum; MS, medial septum; LV, lateral ventricle; TS, triangular septal nucleus. (dorsal LS $n=9$, ventral LS $n=6$, medial septum $n=10$ and lateral ventricle $n=7$).

Figure 3:

Effect of social housing and sex on aggression. Social isolation increased the duration of aggression in both male and female hamsters injected with saline in the dorsal LS (* indicates $p < 0.05$). (Single Housed Males $n=8$, Socially Housed Males $n=7$, Single Housed Females $n=7$, Socially Housed Females $n=8$).

Figure 4:

(a) Coronal sections illustrating the sites of injections. Shape indicate experimental group: circle, isolated males; triangle, socially housed males; diamond, isolated females; pentagon, socially housed females. Closed shapes indicate injections into dorsal or intermediate lateral septum, classified as hits; open shapes indicate missed injection sites. LSd, dorsal lateral septum; LSi, intermediate lateral septum; LSv, ventral lateral septum; MS, medial septum; TS, triangular septal nucleus. Exp. 2 Part A (b-e): Effect of social housing and sex on aggression induced by GABA_A receptor activation in the dorsal lateral septum. Muscimol 5mM (grey bars) injected into the dorsal lateral septum increased the duration of aggression (b) and the number of attacks (c) in isolated and socially housed females, isolated males, but not in socially housed males when compared with groups injected with saline (black bars). Social housing significantly decreased the change in the duration of aggression (d) and the change in the number of attacks (e) in males, but not females ($p < 0.05$). Part B (f-g): Effect of a high concentration (25mM) of muscimol injected into the dorsal lateral septum on aggression. 25mM muscimol (dark grey bars) did not increase the duration of aggression (f) or the number of attacks (g) in socially housed or isolated males or females ($p < 0.05$). (* indicates $p < 0.05$). (Single Housed Males $n=8$, Socially Housed Males $n=7$, Single Housed Females $n=7$, Socially Housed Females $n=8$).

Figure 5:

Effect of GABA_A receptor activation in the dorsal lateral septum on addition measures of aggression and social behaviors. (a) Muscimol 5mM (grey bars) injected into the dorsal lateral septum decreased the number of aggressive bouts in isolated males and isolated females, but not in socially housed males nor socially housed females ($p > 0.05$) compared to saline injections (black bars). (b) Muscimol 5mM decreased the latency to aggression in isolated and socially housed females. Muscimol 5mM to decrease the latency to aggression just missed significance in isolated males ($p = 0.105$), but had no effect on the latency to aggression in socially housed males ($p > 0.05$). (c) Muscimol 5mM decreased the latency to attacks in socially housed females, but had no effect in isolated nor socially housed males, nor isolated females ($p > 0.05$). (d) Muscimol 5mM decreased social investigation duration in isolated and socially housed females, but not socially housed males ($p > 0.05$). The ability for muscimol 5mM to decrease social investigation duration in isolated males just missed significance ($p = 0.073$). (e) Muscimol 5mM had no effect on the time spent displaying non-social behaviors in isolated and socially housed males and females ($p > 0.05$) (* indicates $p < 0.05$). (Single Housed Males $n = 8$, Socially Housed Males $n = 7$, Single Housed Females $n = 7$, Socially Housed Females $n = 8$).

Figure 6:

Effect of social status on GABA_A receptor activation induced aggression. (a) There were no differences between dominant versus subordinate males nor between dominant versus subordinate females in muscimol 5mM in the lateral septum induced aggression duration ($p > 0.05$). (b) There were no differences between dominant versus subordinate males nor between dominant versus subordinate females in muscimol 5mM in the lateral septum induced number of attacks ($p > 0.05$). However, dominant females injected with muscimol 5mM showed an increase in the number of attacks compared to saline injections and subordinate females injected with muscimol 5 mM showed a trend ($p = 0.077$) to display an increase in the number of attacks compared to saline injections. (c) Females trend to display a greater change in aggression duration induced by muscimol 5mM compared to males independent of social status ($p = 0.086$). (d) Females also displayed a greater change in the

number of attacks induced by muscimol 5mM compared to males independent of social status. (e) High concentration of muscimol 25mM (dark grey bars) had no effect on aggression duration compared to saline treated dominant males, subordinate males, dominant females, nor subordinate females ($p>0.05$). (f) High concentration of muscimol 25mM had no effect on the number of attacks compared to saline treated dominant males, subordinate males, dominant females, nor subordinate females ($p>0.05$) (* indicates $p < 0.05$). (Dominant Males $n=4$, Subordinant Males $n=11$, Dominant Females $n=5$, Subordinate Females $n=11$).

Figure 7:

Effect of social housing and sex on δ and γ_2 subunit GABA_A receptor mRNA expression in the septum. (a) Coronal sections illustrating where 1.00 mm bilateral punches were collected from the lateral septum and medial septum (light grey circle). Four separate bilateral punches were collected for the lateral septum: anterior dorsal (light grey striped), anterior ventral (dark grey striped), posterior dorsal (open circle) and posterior ventral (dark grey circle). No differences in δ nor γ_2 subunit GABA_A receptor mRNA expression were observed in the anterior dorsal LS, anterior ventral LS, medial septum, posterior dorsal LS, and posterior ventral LS (data not reported). However, social housing decreased the ratio of δ to γ_2 subunit GABA_A receptor mRNA expression in the anterior dorsal LS ($p < 0.05$; b), and similar trends were observed in the anterior ventral LS ($p = 0.110$; d). No differences in the ratio of δ to γ_2 were observed in the posterior dorsal LS (c) and posterior ventral LS (e). Social experience regulates the ratio of δ to γ_2 subunit GABA_A receptor mRNA expression. (* indicates $p < 0.05$). (Anterior dorsal LS: Socially Housed Males $n = 5$, Socially Housed Females $n = 7$, Single Housed Males $n = 7$, Single Housed Females $n = 6$). (Anterior ventral LS: Socially Housed Males $n = 7$, Socially Housed Females $n = 7$, Single Housed Males $n = 6$, Single Housed Females $n = 7$). (Posterior dorsal LS: Socially Housed Males $n = 6$, Socially Housed Females $n = 5$, Single Housed Males $n = 7$, Single Housed Females $n = 8$). (Posterior ventral LS: Socially Housed Males $n = 5$, Socially Housed Females $n = 5$, Single Housed Males $n = 3$, Single Housed Females $n = 6$).

Figure 8:

Effect of selective agonists and antagonist that act on GABA_A receptors containing the δ subunit that are found extrasynaptically or on GABA_A receptors containing the γ_2 subunit that are found synaptically in the lateral septum on aggression. (a) Coronal sections illustrating the sites of injections. Shape indicates experiment: circle, Experiment 4; triangle, Experiment 5; diamond, Experiment 6. Closed shapes indicate injections into the dorsal or the intermediate lateral septum, classified as hits; open shapes indicate missed injection sites. LSd, dorsal lateral septum; LSi, intermediate lateral septum; LSv, ventral lateral septum; MS, medial septum; TS, triangular septal nucleus. Exp. 4: Effect of selective GABA_A agonists in the lateral septum on aggression in males. Muscimol (non-selective GABA_A agonist) or THIP (extrasynaptic δ GABA_A agonist) injected into the LS increased the duration of aggression (b) and the number of attacks (c) compared with saline and CDP injections (synaptic γ_2 GABA_A agonist). (* indicates $p < 0.05$; # indicates $p < 0.10$). Exp. 5: Effect of a selective synaptic γ_2 GABA_A receptor agonist on aggression. High (200mM) and low (2mM) concentrations of CDP had no effect on the duration of aggression (d) or the number of attacks (e) compared with saline controls ($p < 0.05$). Exp. 6: Effect of a selective synaptic γ_2 GABA_A antagonist on aggression. THIP or a cocktail of THIP and Ro15–4513 (synaptic γ_2 GABA_A antagonist) increased the duration of aggression (f) and the number of attacks (g) compared with saline and Ro15–4513 injections. (* Indicates a significant

difference compared with saline; $p < 0.05$). (Exp. 4 $n = 7$ per group, Exp. 5 $n = 5$ per group, Exp. 6 $n = 10$ per group).

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