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Contribution of GABA_A receptor subunits to attention and social behavior

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Abstract

Introduction: GABA dysfunction is associated with a number of psychiatric conditions including schizophrenia, autism and depression. Blocking cortical GABA_A receptors in rodents causes behavioral deficits, including impaired attention and sociability, that are consistent with the symptoms of these conditions. The subunit composition of GABA_A receptors is diverse and can affect receptor function. The current experiment examined the role of GABA_A receptors containing different α -subunits in social behavior and attention.

Methods: Male Sprague-Dawley rats were administered FG7142 (0.0–5.0 mg/kg; a non-selective GABA_A receptor inverse agonist), L-655,708 (0–1.0 mg/kg; a low efficacy inverse agonist at α_5 -containing GABA_A receptors), MRK-016 (0.0–2.0 mg/kg; a high efficacy inverse agonist at α_5 -containing GABA_A receptors), or L-838,417 (0.0–3.0 mg/kg; an antagonist at α_1 -containing receptors and a partial agonist at α_2 , α_3 , α_5 -containing GABA_A receptors) and either tested on the social interaction and social preference tests or the 5-choice serial reaction time task.

Results: FG7142 decreased social interactions and impaired attention. MRK-016 impaired attention but did not affect social behavior. Neither L-655,708 nor L-838,417 significantly affected either social behavior or attention.

Discussion: Systemic reduction in GABA_A receptor signaling decreased sociability and attention, a result consistent with past research demonstrating cortical GABA_A receptor blockade impairs social behavior and attention. Overall, the effects of the receptor subtype selective ligands were minimal; α_5 -containing GABA_A receptors may contribute to the attentional deficit but do not contribute to the decrease in sociability. Further research is needed to determine the GABA_A receptor subunits that contribute to social behavior and attention.

1.0 Introduction

Changes in cortical GABAergic neurotransmission have been observed in a number of psychiatric disorders including schizophrenia (e.g., [1]), autism (e.g., [2, 3]) and depression (e.g., [4, 5]). In schizophrenia, for example, GAD₆₇ is consistently found to be reduced in

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parvalbumin-containing interneurons [1, 6, 7] and GABA_A receptor subunits have been found to be differentially distributed [1, 9, 10]. In autism spectrum disorders, reductions in GABA_A receptor subunit binding have been observed in frontal cortical areas [2, 3], as have changes in the number of parvalbumin-containing interneurons [11]. In depression, reductions in GABA levels have most consistently been observed, with some, but not all, studies finding changes in GAD67 or GAD65 expression [4, 5]. The observation that GABA dysfunction is a common feature to a number of psychiatric conditions has led to the investigation into how GABA may be contributing to symptoms of these disorders.

Rodent models support the hypothesis that GABA dysfunction contributes to cognitive and social deficits observed in psychiatric conditions such as schizophrenia, autism and depression. Indeed, inhibition of cortical GABA_A receptors causes impairments in attention [12, 13, 14, 15], social behavior [16], set-shifting [17], working memory [18] and reward-related decision making [19]. These findings support a role for cortical GABA_A receptors contributing to the cognitive deficits observed in psychiatric illness. However, there is considerable diversity in GABA_A receptors and the specific subtype(s) of GABA_A receptors contributing to the aforementioned behavioral changes are not known.

GABA_A receptors are heteropentamers made from 19 known subunits (reviewed in [20, 21]). Most GABA_A receptors are made of two α subunits, two β subunits and a single γ subunit [20, 21] and those containing α_1 , α_2 , α_3 , and α_5 subunits show benzodiazepine sensitivity [20, 21]. Moreover, these benzodiazepine-sensitive subunits show distinct anatomical distributions and subcellular localizations: α_1 -containing receptors are the most prevalent and widely distributed in the brain and are primarily found in synaptic locations; α_2 and α_3 -containing receptors are primarily synaptic, have moderate expression and are found most abundantly in the cerebral cortex, hippocampus and amygdala; α_5 -containing receptors are largely extrasynaptic, have generally low expression and are most abundant in the hippocampus and cerebral cortex (reviewed in [22, 23, 24]). Furthermore, the functional roles of the different benzodiazepine-sensitive α subunits have begun to be assessed using genetic and pharmacological tools. The α_1 subunits contribute to the sedative and amnesic effects of benzodiazepines, α_2 subunits contribute to the anxiolytic effects and α_5 subunits contribute to the cognitive effects of benzodiazepines [22, 25, 26, 27, 28, 29, 30, 31].

Given that benzodiazepine-sensitive GABA_A receptors are the majority of GABA_A receptors in the brain [20, 21] and because previous work has shown that cortical GABA_A receptors regulate attention and social behavior [12, 13, 14, 15, 16], the aim of the current experiment was to begin to delineate the specific GABA_A receptor subunits to attention and social behavior. First, we confirmed that widespread reduction of GABA_A receptor signaling using FG7142, a nonselective inverse agonist at the benzodiazepine site on GABA_A receptors [32], impairs attention in the 5-choice serial reaction time task (5CSRTT) and social behaviors in a manner similar to that caused by intra-cortical injection of the GABA_A receptor antagonist bicuculline [13, 16] or the GABA synthesis inhibitor L-allylglycine [14, 16]. Next, we determined if ligands with relatively high selectivity at GABA_A receptors containing α_5 subunits had similar effects. GABA_A receptors containing α_5 subunits were focused on because these receptors have been demonstrated to affect performance on memory tasks [30, 31, 33, 34, 35, 36, 27; see 38 for review]. Two different drugs were tested: MRK-016, a high

efficacy inverse agonist at GABA_A receptors containing α_5 subunits, and L-655,708, a low efficacy inverse agonist at GABA_A receptors containing α_5 subunits [26, 34]. Finally, for comparison, the effects of L-838,417, an antagonist at α_1 -containing GABA_A receptors and a partial agonist at α_2 , α_3 and α_5 -containing receptor subunits [28], was also tested.

2. Methods

2.1 Animals

A total of one hundred and forty-seven male Sprague Rats were used in this experiment; rats were born at Oberlin College and derived from rats obtained from Hilltop Lab Animals, Inc. (Scottsdale, PA). All rats were maintained on a 14-h/10-h light-dark cycle (lights on at 0700h) and were group housed until approximately post-natal day (PND) 55. During this time rats were allowed free access to food (Purina Rat Chow) and water.

Eighty-eight rats were used in Experiment 1. Seventy-three rats were experimental rats; these rats were between 75–80 days of age at the time of the social interaction test. Fifteen rats were stimulus rats; these rats were between 50–60 days of age at the time of the social interaction test. All rats were housed in groups of 8 (in $\sim 66 \times 61 \times 30$ cm cages) until one week prior to the start of the social interaction test. At that time experimental rats were then housed singly (in $\sim 27 \times 48 \times 20$ cm cages) and stimulus rats were housed in groups of 2–3. Rats were allowed free access to food and water (except when noted below) while in their home cage.

Fifty-nine rats were used in Experiment 2; these rats were approximately 60 days of age at the start of training. Rats were pair-housed and food restricted to approximately 85% of their pre-feeding weight beginning 2-days before the start of training. Rats were fed after daily training sessions.

Experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals [39] and Oberlin College policies.

2.2 Drugs and Administration

All drugs were obtained from Tocris (Bristol, UK) and were dissolved to a volume of 1.0 ml/kg. FG7142 was dissolved in 20% Kolliphor (Sigma-Aldrich) to doses of 2.5 and 5.0 mg/mL and was administered IP 30 minutes before testing; doses based on Pehrson et al. [15] and Stuart et al [40]. MRK-016 was dissolved in 75% DMSO (Sigma-Aldrich) to the dose of 2.0 mg/kg and was administered SC 30 min prior to testing; dose based on Attack et al. [34]. L-655,708 was dissolved in 100% DMSO (Sigma-Aldrich) to doses of 0.5 and 1.0 mg/kg and was administered SC 10 min prior to testing (dose based on Attack et al. [35, 36]). L-838,417 was dissolved in 20% Kolliphor (Sigma-Aldrich) to doses of 1.5 and 3.0 mg/kg and was administered IP 30 min prior to testing; doses based on Mathiasen et al [41].

2.3 Experiment 1: Role of GABA_A receptors in Social Behaviors

The experimental timeline is depicted in Figure 1. Each rat was tested in every behavioral test and behavioral tests were always conducted in the same order. Prior to the social

interaction test, rats were assigned to a drug group; they received the same drug and dose throughout the experiment.

2.3.1. Social Interaction Test—The social interaction test, based on the one described by Lapiz-Bluhm et al. [42] and one that we have used previously [16], occurred over 4 days in an 80 × 80 × 30 cm black Plexiglas open field; the floor of the arena was covered in pine bedding material.

On days 1–3, rats were handled and then placed individually (experimental rats) or in pairs (stimulus rats) in the center of the open field. After 5 min, rats were removed from the open field and the bedding was mixed to redistribute odors that could affect social behavior. On day 4, the experimental rats were treated with the appropriate drug dose, returned to their home cage for the appropriate absorption period and then placed in the open field with a “stranger” stimulus rat for 5 min. The social interaction was videotaped for off-line analysis of the number of experimental rat initiated social interactions (sniffing, climbing on/under, chasing, wrestling) and the total time the experimental rat engaged in social behavior (based on procedures described in Lapiz-Bluhm et al., 2008; Paine et al., 2017).

2.3.2 Social Preference Test—Social preference was tested using a modified version of the 3-chamber social preference test described by Moy et al. [43] and we have used previously [16]. Social preference testing took place in the same Plexiglas open field used in the social interaction test and contained two acrylic restrainers (Harvard Apparatus) located in opposite corners. During habituation sessions the restrainers were empty; during the test session one restrainer held a “stranger” stimulus rat (social stimulus) and the other restrainer contained a white plastic rat (non-social stimulus).

On days 1–2, experimental rats were individually habituated to the arena containing the restrainers for 5 min; during habituation the restrainers were empty. On day 3, the experimental rat was placed in the arena for a 5 min test. During the test, one restrainer contained the stimulus rat and the other contained the inanimate, plastic rat. The test session was videotaped and the time the experimental rat spent exploring the restrainer containing the social stimulus and the time spent exploring the restrainer containing the non-social stimulus across the 5 min test was measured. These data were used to calculate a social preference index: $[(\text{social stimulus (sec)} - \text{non-social stimulus (sec)}) / (\text{social stimulus (sec)} + \text{non-social stimulus (sec)})] * 100$.

The stimulus rats were habituated to confinement in the restrainers over 3 days; on each day they were confined to the restrainer for 5 min. The acrylic restrainer was suitable for use for rats weighing up to 500 g (Harvard Apparatus); stimulus rats typically weighed ~300 g.

2.3.3 Sucrose Intake Test—The sucrose preference test was conducted over 3 consecutive days and consisted of 2 habituation days and a test day. On habituation day 1, rats were provided with 2 water bottles; one containing 1% sucrose and the other containing water. On habituation day 2 (24h after introduction), the left/right location of the sucrose and water bottles was reversed. On the test day (day 3), both water bottles were removed, and the rats were water deprived for 4h. After 4h, the 30-min test was administered. During the test,

the rats were provided with 2 water bottles: one contained 10% sucrose, the other contained water. At the end of the test, the volume of each liquid consumed was recorded and used to calculate the sucrose preference index [sucrose solution consumed (ml) – water consumed (ml)]/[sucrose solution consumed (ml) + water consumed (ml)].

2.3.4. Locomotor Activity—Because locomotor activity can affect the ability to interact socially, and because some drug manipulations can affect locomotor activity, locomotor activity was assessed using 60 × 60 × 30 cm activity chambers (Med-Associates) over 4 days. On days 1–3, rats were habituated to activity chamber for 30-min. On day 4, the rats were administered the appropriate drug dose, returned to their home cage for the absorption period, and then put in the activity chamber for the 30-min test. Med-Associates software was used to track the distance travelled (cm) by the rat during the sessions.

2.4 Experiment 2: Role of GABA_A receptors in Attention and Impulse Control

2.4.1 The Standard 5-Choice Serial Reaction Time Task (5CSRTT)—Rats were trained on the 5CSRTT as described previously [13, 44]. Sessions started with the delivery of 1 food pellet (45-mg, Bio-Serv, Frenchtown NJ); the first trial commenced upon pellet retrieval. A nose poke into the magazine initiated the first 5-sec inter-trial interval (ITI) and illumination of the house light. At the end of the ITI, a 1-sec light stimulus was presented at the rear of one of the five stimulus locations (apertures). Rats had up to 5 sec (limited hold) to make a response. A response in the illuminated aperture (correct response) triggered delivery of 1 food pellet and illumination of the magazine light, which remained illuminated for 5 sec following pellet delivery. Nose pokes in the remaining apertures were considered incorrect responses and triggered a 5-sec time-out (TO) during which the house light was extinguished. Similarly, failure to respond during the limited hold (i.e., an omission) triggered a 5-sec TO. The subsequent trial was automatically initiated at the end of the TO period or the limited hold (correct responses). Responses occurring during the ITI were considered premature responses and also triggered a 5-sec TO; the same trial was automatically re-started at the end of the TO period. Responses occurring during the TO period had no programmed consequences. Sessions ended after 90 trials or 30 min. Performance measures of interest were: % accuracy ((correct responses/[correct + incorrect responses])*100), % omissions ([omissions/trials completed]*100), premature responses, magazine entries, correct response latency (the time from the stimulus onset to a correct response) and reward latency (the time from a correct response to the collection of the food). Subjects were considered to have acquired the task when their accuracy was greater than 60% (chance performance in this test is 20%) and omissions were fewer than 20% for 5 consecutive days. Once rats reached criterion performance, they began drug testing. Half of the rats received the drug doses in an ascending order and half of the rats received the drug dose in a descending order. There was a minimum of two drug-free days between drug tests.

2.5 Statistical Tests

A blind rater scored the videos of the social interaction and social preference tests. Data were analyzed with either a one-way (Dose) or two-way (Dose X behavior/location/bottle/time) analysis of variance. Post-hoc analyses were conducted using an estimated marginal means procedure with a Bonferroni correction.

Data from the 5CSRTT (% accuracy, % omissions, premature responses, response latency) were analyzed using a within subjects one-way ANOVA (Dose) or one-sample t-tests. Post-hoc analyses were conducted using an estimated marginal means procedure with a Bonferroni correction.

3.0 Results

3.1 Experiment 1: *Role of GABA_A receptors in Social Behaviors*

3.1.1 Sociability Testing

3.1.1.1 FG7142: Twenty-one rats were tested with FG7142 in the sociability tests; $n = 7, 5$ and 9 in the $0.0, 2.5$ and 5.0 mg/kg groups respectively.

FG7142 significantly affected time engaged in the social interaction test ($F(2, 20) = 7.19, P < 0.05$; Figure 2A–B). Both doses of FG7142 significantly decreased the amount of time engaged in social interactions relative to vehicle (2.5 mg/kg, $P < 0.05$; 5.0 mg/kg, $P < 0.01$).

FG7142 significantly affected the number of social interactions engaged in ($F(2, 18) = 13.91, P < 0.05$); both doses of FG7142 significantly decreased the number of interactions engaged in relative to vehicle (2.5 mg/kg, $P < 0.05$; 5.0 mg/kg, $P < 0.01$). There was a trend for FG7142 to decrease the number of interactions in a behavior specific fashion ($F(6, 54) = 2.19, P = 0.06$). Post-hoc analysis revealed that high dose of FG7142 (5.0 mg/kg) decreased the number of chases ($P < 0.05$) and climbing bouts ($P < 0.05$) engaged in relative to vehicle. Both doses of FG7142 decreased the number of wrestling bouts (both $P < 0.01$).

In the social preference test, rats spent more time in the location with the stimulus rat than the non-stimulus rat regardless of dose of FG7142 administered ($F(1, 18) = 119.06, P < 0.01$; Figure 2C). There was no evidence that FG7142 affected the amount of time the experimental rats spent with the social and non-social stimuli ($F(2, 18) = 2.83, P > 0.05$), nor was there evidence that FG7142 affected the social preference index ($F(2, 20) = 1.13, P > 0.05$; Table 1).

3.1.1.2 L-655,701: Sixteen rats were tested with L-655,708 in the sociability tests; $n = 7$ and 9 in the 0.0 and 1.0 mg/kg groups, respectively.

L-655,708 did not affect the time rats engaged in social interactions ($t(14) = -1.52, P > 0.05$; Figure 2D–E). The frequency with which rats engaged in the different social behaviors differed across behaviors ($F(3, 42) = 32.51, P < 0.05$) with rats engaging in more sniffing behavior than all other behaviors ($P < 0.05$). This effect was not, however, affected by L-655,708 (no main effect or interaction, both $F < 1.30, P > 0.05$).

In the social preference test, all rats preferred to spend time in the location of the social stimulus compared with the location of the non-social stimulus ($F(1, 14) = 134.09, P < 0.05$; Figure 2F). L-655,708 did not affect the time spent in either location (no main effect or interaction, both $F < 1.52, P > 0.05$) or the social preference index ($t(14) = 1.47, P > 0.05$; Table 1).

3.1.1.3 MRK-016: Fourteen rats were tested with MRK-016 in the sociability tests; $n = 7$ and 7 in the 0 and 2.0 mg/kg drug groups, respectively.

MRK-016 did not affect the time engaged in social interactions ($t(12) = 1.04$, $P > 0.05$; Figure 2G–H). The total number of behaviors engaged in differed across categories ($F(3, 36) = 56.84$, $P < 0.05$); regardless of drug dose, all rats engaged in more sniffing bouts than any other behavior (all $P < 0.05$). There was no effect of MRK-016 dose on behavior ($F(1, 12) = 1.48$, $P > 0.05$) nor was there a dose X behavior interaction ($F(3, 36) = 0.73$, $P > 0.05$).

In the social preference test, all rats spent more time in the location of the social stimulus than the non-social stimulus ($F(1, 12) = 11.46$, $P < 0.05$; Figure 2I), but this was not affected by MRK-016 administration (neither the main effect or interaction, $F > 1.95$, $P < 0.05$). Furthermore, MRK-016 did not affect the social preference index ($t(12) = -1.39$, $P > 0.05$; Table 1).

3.1.1.4 L-838,417: Twenty-two rats were tested with L-838,417 in the sociability tests; $n = 8, 7, 7$ in the 0, 1.5 and 3.0 mg/kg drug groups, respectively.

L-838,417 did not affect the total time rats engaged in social interactions ($F(2, 19) = 0.26$, $P > 0.05$; Figure 2J–K). The number of behaviors engaged in differed across categories ($F(3, 57) = 158.44$); rats engaged in more bouts of sniffing than any other behavior (all $P < 0.05$). There was no effect of L-838,417 dose on social behavior ($F(2, 19) = 0.46$, $P > 0.05$) nor was there a dose X behavior interaction ($F(6, 57) = 1.35$, $P > 0.05$).

In the social preference test, all rats spent more time in the location of the social stimulus than the non-social stimulus ($F(1, 19) = 24.91$, $P < 0.05$; Figure 2L); this was not affected by L-838,417 dose (neither the main effect or interaction, $F < 1.95$, $P < 0.05$). Furthermore, L-838,417 did not affect the social preference index ($F(2, 19) = 1.78$, $P > 0.05$; Table 1).

3.1.2 Sucrose Preference Test

3.1.2.1 FG7142: The effects of FG7142 on sucrose preference are depicted in Figure 3A–B and Table 1. During habituation, all rats preferred to drink the 1% sucrose solution to water ($F(1, 18) = 12.20$, $P < 0.05$; Figure 3A); this was not affected by condition (no main effect of group or interaction, both $F < 1.99$, $P > 0.05$). Furthermore, there was no effect of FG7142 on the sucrose preference index ($F < 1.00$, $P > 0.05$; Table 1).

During the test session, all rats drank more 10% sucrose than water ($F(1, 18) = 5.76$, $P < 0.05$; Figure 3B), however the amount of each liquid consumed was affected by dose of FG7142 administered ($F(2, 18) = 5.73$, $P < 0.05$). Post-hoc analyses found that only rats administered vehicle preferred sucrose to water ($P < 0.01$), rats treated with either dose of FG7142 did not exhibit a similar preference ($P > 0.05$). Furthermore, FG7142 (5.0 mg/kg) decreased consumption of sucrose relative to vehicle ($P < 0.05$) but did not affect consumption of water ($P > 0.05$). Interestingly, FG7142 administration did not affect sucrose preference index scores ($F(2, 20) = 2.52$, $P > 0.05$; Table 1).

3.1.2.2 L-655,708: The effects of L-655,608 on sucrose preference are depicted in Figure 3C–D and Table 1. During habituation, all rats preferred to drink the 1% sucrose solution to water ($F(1, 14) = 26.15, P < 0.05$; Figure 3D); this was not affected by condition (no main effect of group or interaction, both $F < 2.34, P > 0.05$). Furthermore, there was no effect of L-655,708 on the sucrose preference index ($t(14) = -1.72, P > 0.05$; Table 1).

During the 30-min test, all rats preferred to drink the 10% sucrose solution to water ($F(1, 14) = 44.50, P < 0.05$; Figure 3E); this was not affected by condition (no main effect of group or interaction, both $F < 2.29, P > 0.05$). Despite this, there was a trend for L-655,708 to affect sucrose preference index ($t(14) = -2.01, P = 0.06$; Table 1).

3.1.2.3 MRK-016: The effects of MRK-016 on sucrose preference are depicted in Figure 3E–F and Table 1. During habituation, two bottles leaked (one for a rat in the vehicle group and one for a rat in the MRK-016 group); these data were excluded from the analysis of the habituation data. During habituation, the amount of each liquid consumed was dependent upon the MRK-016 dose rats would be treated with ($F(1, 10) = 5.19, P < 0.05$); post-hoc tests revealed that rats in the MRK-016 group consumed less water than rats in the vehicle group ($P = 0.05$); there was no difference in the consumption of the 1% sucrose solution ($P > 0.05$). This difference was confirmed when assessing the sucrose preference index; rats in the MRK-016 had a greater sucrose preference index ($t(10) = -2.52, P < 0.05$).

During the 30-min sucrose intake test, all rats drank more 10% sucrose than water ($F(1, 12) = 13.77, P < 0.05$), but this effect was not influenced by MRK-016 administration (both $F < 0.5, P > 0.05$). Moreover, MRK-016 did not affect the sucrose preference index during the 30-min test ($t(12) = 0.86, P > 0.05$).

3.1.2.4 L-838,417: The effects of L-838,417 on sucrose preference are depicted in Figure 3G–H and Table 1. During habituation, all rats preferred sucrose over water ($F(1, 180) = 16.68, P < 0.08$); this was not affected by L838,417 dose (neither a main effect or interaction, $F < 1.30, P > 0.05$). Furthermore, there was no effect of L-838,417 group on sucrose preference index ($F(2, 18) = 0.14, P > 0.05$).

One bottle leaked during the test condition; data from this animal was excluded from the statistical analysis. During the 30-min sucrose intake test, all rats drank more 10% sucrose than water ($F(1, 19) = 27.75, P < 0.05$; Figure 3K), but this effect was not influenced by L-838,417 dose nor was there a dose X bottle interaction (both $F < 0.5, P > 0.05$). Moreover, L-838,417 did not affect the sucrose preference index during the 30-min test ($F(2, 18) = 0.78, P > 0.05$; Table 1).

3.1.3 Locomotor Activity

3.1.3.1 FG7142: Effects of FG7142 on locomotor activity are depicted in Figure 4A–B. During the 30-min test, rats decreased their activity across blocks ($F(5, 90) = 78.30, P < 0.05$) but FG7142 did not affect overall activity ($F(2, 18) = 0.49, P > 0.05$) or activity across time ($F(10, 90) = 0.75, P > 0.05$). Moreover, FG7142 did not affect the % of distance travelled in the inner zone of the open field ($F(2, 18) = 0.74, P > 0.05$).

3.1.3.2 L-655,708: Effects of L-655,708 on locomotor activity are depicted in Figure 4C–D. Rats decreased their activity across the 30-min locomotor activity test ($F(5, 70) = 43.04$, $P < 0.05$); activity was highest in the first block compared to all other blocks (all $P < 0.05$). L-655,708 did not affect overall activity ($F(1, 14) = 0.11$, $P > 0.05$) or activity across time ($F(5, 70) = 1.13$, $P > 0.05$). Furthermore, L-655,708 did not affect % exploration of the inner zone of the activity chamber ($t(14) = 0.80$, $P > 0.05$).

3.1.3.3 MRK-016: Effects of MRK-016 on locomotor activity are depicted in Figure 4E–F. Rats decreased their activity across the 30-min locomotor activity test ($F(5, 60) = 51.28$, $P < 0.05$); activity was highest in the first block compared to all other blocks (all $P < 0.05$). MRK-016 did not affect overall activity ($F(1, 12) = 1.09$, $P > 0.05$) or activity across time ($F(5, 60) = 0.98$, $P > 0.05$). Interestingly, there was a trend towards rats treated with MRK-016 to exhibit decreased activity in the inner zone of the open field ($t(12) = 2.12$, $P = 0.06$).

3.1.3.4 L-838,417: Effects of L-838,417 on locomotor activity are depicted in Figure 4G–H. For technical reasons, three rats were unable to be tested with the 3.0 mg/kg dose of L-838,417, leaving a sample size of 4 in this group. Rats decreased their activity across the 30-min locomotor activity test ($F(5, 80) = 102.65$, $P < 0.05$); activity was highest in the first block compared to all other blocks (all $P < 0.05$). L-838,417 dose-dependently affected activity ($F(2, 16) = 4.50$, $P < 0.05$; Figure 3G); the L-838,417 (3.0 mg/kg) significantly decreased activity relative to vehicle ($P < 0.05$). There was not a dose X block interaction ($F(10, 80) = 1.70$, $P > 0.05$). There was no difference in the % activity in the inner zone of the open field ($F(2,16) = 0.36$, $P > 0.05$; Figure 3H).

Table 2. summarizes the effects of FG7142, MRK-016, L-655,7018 and L-838,417 on sociability testing.

3.2 Experiment 2: Role of GABA_A receptors in Attention and Impulse Control

3.2.1 FG7142—The data for FG7142 were analyzed using a one-way between-subjects ANOVA because only a subset of rats received the 2.5 mg/kg drug dose. In addition, two animals were excluded from the 5 mg/kg dose because they responded on less than 10% of the trials. Thus, data from $n=15$, 11 and 13 rats were analyzed for the 0, 2.5 and 5.0 mg/kg doses, respectively.

The effects of FG7142 on 5CSRTT performance are depicted in Figure 5A–C and Table 3. FG7142 dose-dependently affected accuracy ($F(2, 36) = 4.97$, $P < 0.05$), omissions ($F(2, 36) = 15.77$, $P < 0.05$) and correct response latency ($F(2, 36) = 5.37$, $P < 0.05$). Accuracy was significantly decreased by the 5.0 mg/kg dose ($P < 0.05$), omissions were significantly increased by the 2.5 ($P < 0.05$) and 5.0 mg/kg ($P < 0.01$) dose and the correct response latency was significantly longer following administration of the 5.0 mg/kg ($P < 0.05$) dose of FG7142. All other $F < 2.81$, $P > 0.05$.

3.2.2 L-655,708: Only a subset of animals were tested at all three doses; $n=15$, 11 and 15 rats were tested at the 0.0, 0.5 and 1.0 mg/kg doses, respectively. These data were analyzed using a between-subjects ANOVA.

The effects of L-655,708 on 5CSRTT performance are depicted in Figure 5D–F and Table 3. L-655,708 significantly affected omissions ($F(2,38) = 4.34, P < 0.05$); neither drug dose was significantly different from vehicle; rather, the low dose (0.5 mg/kg) was significantly different from the high dose (1.0 mg/kg) of L-655,708 ($P < 0.05$). All other $F < 2.73, P > 0.05$.

3.2.3 MRK-016: One animal was excluded from the 0.0 mg/kg dose because he never regained stability following administration of the 2.0 mg/kg dose and five animals were excluded from the 2.0 mg/kg dose because they responded on less than 10% of the trials during that test session. Thus, data from $n=15$ and 11 rats were analyzed for the 0.0 and 2.0 mg/kg doses, respectively.

The effects of MRK-016 on 5CSRTT performance are depicted in Figure 5G–I and Table 3. Independent samples t-tests revealed that MRK-016 significantly increased omissions ($t(24) = -4.60, P < 0.01$) and decreased premature responses ($t(24) = 2.14, P < 0.05$). There was also a tendency for rats treated with MRK-016 to take longer to respond correctly ($t(24) = -2.00, P = 0.06$). There were no other significant effects (all $t < 0.81, P > 0.05$).

3.2.4 L-838,417: A total of 12 rats were tested at each of the 0.0, 1.5 and 3.0 mg/kg doses of L-838,417. The effects of L-838,417 on 5CSRTT performance are depicted in Figure 5J–L and Table 3. L-838,417 did not affect performance on the 5CSRTT (all $F < 1.38$, all $P > 0.05$).

Table 4. summarizes the effects of FG7142, MRK-016, L-655,7018 and L-838,417 on 5CSRTT performance.

4.0 Discussion

Results from the current experiment suggest that GABA_A receptors, particularly GABA_A receptors containing α_5 subunits, impair attention as measured by the 5CSRTT. Similarly, GABA_A receptors mediate social behavior in the social interaction test, but the subunit specificity of this effect remains to be elucidated.

4.1 Role of GABA_A receptors in attention

Wide spread reduction in GABA_A receptor function using the benzodiazepine site inverse agonist FG7142 caused attentional deficits as measured by the 5CSRTT—there was a reduction in accuracy and an increase in omissions; these changes were accompanied by a slowing of the correct response latency. It is unlikely that changes in attentional performance are related to non-specific motor impairments because there was no change in premature responses in the 5CSRTT nor was there a change in locomotor activity in the open field. This result is consistent with past research finding that reducing GABA_A receptor function in either the prefrontal cortex [12, 13, 14, 15] or hippocampus [45] impairs attention.

Selective reduction of GABA_A receptors containing α_5 subunits also caused attentional deficits. MRK-016, the high efficacy inverse agonist, negatively affected 5CSRTT performance increasing omissions and decreasing premature responses; it also had a

tendency to increase the time to respond correctly. It is possible that this pattern of behavior reflects a slight motor deficit, which impaired the animals' ability to respond, resulting in increased omissions and decreased premature responses. However, a global motor deficit is unlikely because MRK-016 did not affect global locomotor activity in the open field. Alternatively, it is equally likely that the pattern of behavior reflects an attentional deficit paired with an improvement in impulse control. Increases in omissions, even in the absence of changes in accuracy, reflect attentional deficits because well-trained rats are likely to refrain from responding rather than simply "guessing" incorrectly [44, 46].

The impairment in attention following MRK-016 is consistent with past research finding that decreases in cortical GABA function impairs attention [12, 13, 14, 15] but is contrary to research finding improvements in cognition following administration of that drugs that act as inverse agonists at α_5 -containing GABA_A receptors [33, 34, 35, 47] and knock-out of GABA_A receptor α_5 subunits [37; for review see 38]. That said, there is a growing body of evidence suggesting that partial knock-out of α_5 subunits in the hippocampus or conditional knockout of α_5 subunits in the dentate gyrus impairs prepulse inhibition, latent inhibition, Morris water maze reversal learning, and spatial object location memory [25, 30, 31 48]. Indeed, it has been suggested that reductions in GABA_A receptor α_5 subunits may negatively impact performance on tasks with increased complexity [48]; the 5CSRTT could be considered a complex cognitive task. GABA_A receptors containing α_5 subunits are particularly important for maintaining tonic inhibition [22, 48] and a reduction in inhibition would be predicted to cause deficits in attention. Indeed, blockade of GABA_A receptors in specific brain areas with full competitive antagonists causes both pronounced disinhibition and attentional deficits [12, 13, 14, 15, 45].

It is unclear why L-655,708, the low efficacy inverse agonist at GABA_A receptors containing α_5 , subunits, did not affect 5CSRTT performance. On the one hand, it is possible that we simply did not administer a high enough dose of L-655,708 to affect 5CSRTT performance. The doses of L-655,708 (0.5–1.0 mg/kg) that we used are similar to the doses that have previously been found to improve performance in the Morris water maze [35]. Similarly, others found that L-655,708, at the dose range used in the current study, reversed deficits in social interaction caused by chronic stress [49] and learning deficits following administration of the NMDA receptor antagonist MK-801 [50]. Although we cannot rule out the possibility that a higher dose L-655,708 could have affected 5CSRTT performance, it seems unlikely.

Alternatively, the failure to find an effect of L-655,708 may be due to its pharmacology. Like MRK-016, L-655,708 shows a relatively high affinity for α_5 subunits compared to α_1 , α_2 or α_3 subunits [27, 34, 35, 36], however, L-655,708 has relatively low efficacy at these receptors [36]. In contrast, MRK-016 has a much higher efficacy at GABA_A receptors containing the α_5 subunit [34, 36]. Thus, the difference in efficacy at α_5 -containing GABA_A receptors is a likely explanation for the disparate behavioral profiles of the two drugs.

Finally, L-838,417, the antagonist at α_1 -containing GABA_A receptors and a partial agonist α_2 , α_3 and α_5 -containing receptors subunits, also did not affect performance on the 5CSRTT. Given that GABA_A receptors containing $\alpha_{2/3}$ subunits have not been as

consistently implicated in cognitive functions [22, 26, 38, 41], the failure to observe an effect in the 5CSRTT is consistent with this research.

4.2 Role of GABA_A receptors in sociability

Non-selective inhibition of GABA_A receptors with FG7142 reduced social behavior in the social interaction, but not social preference, test. Since FG7142 did not affect locomotor activity across time, it is unlikely that a non-specific motor impairment caused the reduction in social behavior in the social interaction test. Rather, it is possible that FG7142 reduced social behavior because it increased anxiety; increases in anxiety reduce social behavior in the social interaction test [52] and FG7142 is well known to cause anxiety [53, 54, 55]. However, we did not observe a reduction in the % distance travelled in the center of the open field, an indirect measure of anxiety [16, 56]. As such, it is unlikely the reduction in social behavior was caused entirely by increased anxiety. Curiously, FG7142 did not change social motivation as measured by the social preference test. Thus, the change in social behavior observed following FG7142 administration is specific to that encountered in the dyadic social interaction test; this test requires a form of social cognition not necessary for the social preference test. The effects of FG7142 are consistent with some of the effects of intra-cortical administration of the GABA_A receptor antagonist bicuculline on social behaviors [13, 16] and with others who have found disinhibition of specific brain areas reduces sociability [57].

Surprisingly, none of the subunit specific GABA_A receptor ligands affected social behavior. It is possible that the doses chosen were not sufficiently high to cause changes in sociability. That said, MRK-016 did tend to decrease distance travelled in the center of the open field, suggesting that it may increase anxiety and caused behavioral changes in the 5CSRTT. L-655,708, at the doses used in the current experiment, has been found to be effective in other behavioral tasks (see above). GABA_A receptors containing α_5 subunits have not been reported to affect social behaviors, so these results are not particularly surprising. In contrast, L-838,417 has previously been reportedly to have anxiolytic effects in a variety of behavioral tests [41, 58] including social interaction tests [59, 60]. It is possible that we did not observe changes in social interaction following L-838,417 administration because we tested rats under low anxiety conditions (e.g., rats were habituated to the testing environment and tested under low lighting). As a result, the ability to detect an anxiolytic effect of L-838,417 in the social interaction test may have been hampered by a “ceiling effect.” In contrast to the current experiment, L-838,417 was anxiolytic in the social interaction test under conditions that could be considered anxiety-provoking (i.e., unfamiliar testing environment [59]). Furthermore, L-838,417 decreased anxiety in a mouse strain that exhibits reduced social interaction [60]. Thus, it is feasible that L-838,417 only improves social interaction under conditions in which it is suppressed.

Alternatively, it is possible that we did not observe an anxiolytic effect because we did not test a sufficiently high dose of L-838,417. The higher dose used, 3 mg/kg, has previously been shown to occupy ~80% of receptors [61]. Moreover, others have found behavioral effects using doses between 0.5–3.0 mg/kg [28, 41, 59, 60]. Finally, we observed a reduction in motor function using the 3.0 mg/kg dose (given the small sample size, this result should

be taken with caution), and doses higher than that have been found by others to cause more profound motor deficits [41, 59, but see 62]. Thus, insufficient dosing is an unlikely explanation for the failure to find behavioral effects of L-838,417 on either 5CSRTT performance or social behavior.

It is possible that tolerance to the effects of the drugs limited our ability to detect drug effects. In the sociability experiment in particular, rats were repeatedly tested with a single dose of a specific drug. That said, we attempted to decrease the likelihood that tolerance would develop by ensuring that there were multiple drug-free days between each test session. Nevertheless, the effects of tolerance cannot be ruled out.

4.3 Conclusions

Consistent with past research investigating intra-cortical reductions of GABA function [12, 13, 14, 15], systemic reduction of GABA function via administration of the GABA_A receptor inverse agonist FG7142 impaired attention in the 5CSRTT and decreased social behavior in the social interaction test. This effect may be mediated, at least in part, by GABA_A receptors containing α_5 subunits: MRK-016, the high efficacy inverse agonist at α_5 -containing GABA_A receptors, decreased performance on the 5CSRTT. That said, we did not observe an effect of MRK-016 on social behavior nor was there an effect of L655,708 on either cognitive function or social behavior. Finally, α_1 antagonist and α_2 , α_3 , α_5 partial agonist, L-838,417 did not affect either 5CSRTT performance or social behavior. Thus, more research is needed to determine the subunit specificity of the GABA_A receptors that contribute to the attention and sociability deficits caused by reduced GABA.

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Highlights

- The role of GABA_A receptor subunits in attention and sociability was investigated
- FG7142 (non-selective GABA_A inverse agonist) decreased attention and sociability
- MRK-016 (α_5 high efficacy inverse agonist) decreased attention but not sociability
- L-655,708 (α_5 low efficacy inverse agonist) did not affect attention or sociability
- L-838,417 ($\alpha_{2,3,5}$ partial agonist) did not affect attention or sociability

Figure 1.

Experimental timeline. Rats underwent four behavioral tests over the course of ~3 weeks: the social interaction test, the sucrose preference test, the social preference test and finally a test of locomotor activity. Prior to each test, rats were habituated to the testing arena for several days. On the day of the drug test, rats were transferred to a holding room, administered drug, and then tested.

Figure 2.

Effects of FG7142 (A-C), L-655,708 (D-F), MRK-016 (G-I) and L-838,417 (J-L) on the time engaged in social behavior and total number of social behaviors in the social interaction test and social preference index. FG7142 reduced the time and total number of social behaviors; none of the other drugs affected social behavior. All rats, regardless of drug condition, preferred the social stimulus to the non-social stimulus. * $P < 0.05$, ** $P < 0.01$ relative to the 0.0 mg/kg dose. # $P < 0.05$ social versus non-social stimulus.

Non-specific GABA_A Receptor Inverse Agonist

Low Efficacy Inverse Agonist at GABA_A Receptors containing α_5 subunits

High Efficacy Inverse Agonist at GABA_A Receptors containing α_5 subunits

Partial Agonist at GABA_A receptors containing α_2 , α_3 , or α_5 subunits

Figure 3.

Effects of FG7142 (A-B), L-655,708 (C-D), MRK-016 (E-F) and L-838,417 (G-H) on consumption of water and sucrose during the habituation and test phases of the sucrose intake test. During habituation, all rats preferred the 1% sucrose solution to water but rats to be treated with 2.0 mg/kg MRK-016 drank less water than rats in the corresponding saline group. During the test session, FG7142 reduced sucrose consumption relative to vehicle but did not affect consumption of water. None of the other drugs affected sucrose intake during the test session. * $P < 0.05$ relative to vehicle, # $P < 0.05$ relative to water.

Non-specific Inverse Agonist GABA_A receptors

Low Efficacy Inverse Agonist at GABA_A Receptors containing α_5 subunits

High Efficacy Inverse Agonist at GABA_A Receptors containing α_5 subunits

Partial Agonist at GABA_A receptors containing α_2 , α_3 , or α_5 subunits

Figure 4.

Effects of FG7142 (A-B), L-655,708 (C-D), MRK-016 (E-F) and L-838,417 (G-H) on locomotor activity. All rats decreased activity across blocks. L-838,417 decreased total activity relative to vehicle and MRK-016 tended to decrease motor activity in the center of the open field. Neither of the other drugs affected locomotor activity. $^{\wedge}P < 0.10$ relative to vehicle, $*P < 0.05$ relative to vehicle.

Non-specific GABA_A Receptor Inverse Agonist

Low Efficacy Inverse Agonist at GABA_A Receptors containing α_5 subunits

High Efficacy Inverse Agonist at GABA_A Receptors containing α_5 subunits

Partial Agonist at GABA_A receptors containing α_2 , α_3 , or α_5 subunits

Figure 5.

Effects of FG7142 (A-C), L-655,708 (D-F), MRK-016 (G-I) and L-838,417 (J-L) on performance in the 5CSRTT. FG7142 decreased accuracy and increased omissions without affecting premature responses relative to vehicle. MRK-016 increased omissions and decreased premature responding relative to vehicle. Neither L-655,708 nor L-838-417 affected 5CSRTT performance relative to vehicle. However, the 1.0 mg/kg dose of L-655,708 lead to significantly more omissions than the 0.5 mg/kg dose. * $P < 0.05$, ** $P < 0.01$ relative to 0.0 mg/kg dose. # $P < 0.05$ low vs high dose of L-655,708.

Table 1.Effects of GABA_A receptor ligands on other sociability measures

Drug & Dose	Social Preference Index	Sucrose Preference Index (Habituation)	Sucrose Preference Index (Test)
FG7142			
0 mg/kg	46.74 ± 6.55	40.34 ± 16.69	44.49 ± 9.57
2.5 mg/kg	46.29 ± 8.96	29.80 ± 29.36	38.67 ± 21.75
5.0 mg/kg	58.75 ± 6.14	47.52 ± 16.55	0.86 ± 15.90
L-655,708			
0.0 mg/kg	65.49 ± 6.77	32.82 ± 10.04	53.19 ± 10.06
1.0 mg/kg	50.20 ± 5.33	57.12 ± 9.71	78.30 ± 5.10 [#]
MRK-016			
0.0 mg/kg	27.32 ± 21.54	44.11 ± 9.35	55.79 ± 15.07
2.0 mg/kg	60.09 ± 10.09	63.89 ± 7.13	43.59 ± 15.56
L-838,417			
0.0 mg/kg	71.49 ± 3.98	51.94 ± 17.57	85.20 ± 3.14
1.5 mg/kg	70.66 ± 5.09	37.98 ± 21.09	65.17 ± 18.67
3.0 mg/kg	39.25 ± 23.82	37.53 ± 20.41	57.71 ± 20.40

Note: During the 30-min test, there was a trend for L-655,708 to increase sucrose preference index ([#]*P* = 0.06).

Table 2.Summary of the effects of GABA_A receptor ligands on sociability

Drug (subunit)	Social Interaction	Social Preference	Sucrose Preference	Locomotion
FG7142 (non-selective)	↓	--	↓	--
MRK-016 (α_5)	--	--	--	↓ (% center)
L655,708 (α_5)	--	--	--	--
L838,417 ($\alpha_{2,3,5}$ partial agonist)	--	--	--	↓ (total activity)

Table 3.Effects of GABA_A receptor ligands on other 5CSRTT performance measures

Drug & Dose	Correct Latency (sec)	Reward Latency (sec)	Magazine Entries
FG7142			
0 mg/kg	0.75 ± 0.02	1.48 ± 0.07	205.5 ± 21.7
2.5 mg/kg	0.94 ± 0.08	1.57 ± 0.09	182.7 ± 19.3
5.0 mg/kg	1.13 ± 0.13*	1.79 ± 0.13	157.9 ± 18.2
L-655,708			
0.0 mg/kg	0.73 ± 0.32	1.87 ± 0.30	195.7 ± 22.2
0.5 mg/kg	0.69 ± 0.04	1.65 ± 0.24	205.8 ± 34.7
1.0 mg/kg	0.82 ± 0.05	1.49 ± 0.08	184.9 ± 20.1
MRK-016			
0.0 mg/kg	0.78 ± 0.04	1.82 ± 0.22	195.3 ± 23.6
2.0 mg/kg	0.91 ± 0.06 [#]	1.69 ± 0.07	213.3 ± 30.8
L-838,417			
0.0 mg/kg	0.78 ± 0.03	1.512 ± 0.11	246.0 ± 30.3
1.5 mg/kg	0.77 ± 0.04	1.63 ± 0.21	249.7 ± 33.3
3.0 mg/kg	0.79 ± 0.04	1.47 ± 0.18	288.2 ± 42.3

Note: FG7142 (5.0 mg/kg) increased response latencies relative to vehicle (* $P < 0.05$) and there was a trend for MRK-016 (2.0 mg/kg) to increase response latencies relative to vehicle ([#] $P = 0.06$).

Table 4.

Summary of the effects of GABA_A receptor ligands on attention and impulse control

Drug(Route)	Accuracy	Omissions	Premature Responses	Response Latency
FG7142 (non-selective)	↓	↑	--	↑
MRK-016 (α_5)	--	↑	↓	↑
L655,708 (α_5)	--	--	--	--
L838,417 ($\alpha_{2,3,5}$ partial agonist)	--	--	--	--