Schizophrenia Bulletin vol. 00 no. 00 pp. 1–16, 2025
https://doi.org/10.1093/schbul/sbaf161
Advance Access publication Month 00, 0000

REGULAR ARTICLE

Sex-dependent Schizophrenia and Drug Reward Behavioral Phenotypes in Metabotropic Glutamate 5 Receptor Heterozygous and Homozygous Mice

Rossana Rosa Porto[1]; Fabian Kreilaus[1]; Anthony J. Hannan[2,3]; Rose Chesworth[1]; Tim Karl[1,*]

[1]School of Medicine, Western Sydney University, Campbelltown, NSW 2560, Australia; [2]Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, VIC 3052, Australia; [3]Department of Anatomy and Neuroscience, The University of Melbourne, Parkville, VIC 3052, Australia

*To whom correspondence should be addressed: Tim Karl, School of Medicine, Western Sydney University, Campbelltown, NSW 2560, Australia (t.karl@westernsydney.edu.au)

Background and Hypothesis: **The metabotropic glutamate 5 (mGlu5) receptor is a potential therapeutic target for psychiatric disorders, including schizophrenia and substance use disorders. Indeed, mGlu5 is expressed in forebrain regions (eg, striatum, prefrontal cortex), and mGlu5 modulates N-methyl-D-aspartate receptor function and second messenger signaling. Also, male mice lacking mGlu5 display schizophrenia-like and substance use-relevant behaviors. However, there are limited investigations of sex differences or gene-dose effects in this model.**

Study Designs: **We evaluated schizophrenia-relevant and cocaine reward-relevant behaviors in adult male and female mice with heterozygous (mGlu5 HET) and homozygous (mGlu5 HOMO) mGlu5 deletion and their wildtype-like (WT) littermates. We assessed locomotion and exploration, anxiety, sensorimotor gating, novel object recognition, fear conditioning, social interaction, cocaine sensitization, and cocaine-conditioned place preference. We also examined fear memory generalization.**

Study Results: **mGlu5 HOMO mice of both sexes showed hyperlocomotion and anxiolytic-like behavior in the open field, as well as enhanced cocaine sensitization and persistent cocaine place preference. When tested for memory generalization, mGlu5 HOMO mice exhibited greater *freezing* to a novel context, suggesting overgeneralization of fear in these mice. mGlu5 HOMO females showed reduced sensorimotor gating. mGlu5 HET mice of both sexes showed a largely similar phenotype to sex-matched WT controls.**

Conclusions: **Our data demonstrate schizophrenia- and drug use-relevant phenotypes in mGlu5 HOMO mice, some of which are sex-dependent. These phenotypes do not occur in mGlu5 HET males and females. We also show for the first time an overgeneralization phenotype in mGlu5 HOMO mice, which may be related to poor contextual discrimination.**

Key words: open field; cocaine; fear memory; generalization; prepulse inhibition; social interaction.

Introduction

The metabotropic glutamate 5 (mGlu5) receptor is a G-protein coupled receptor, which is a treatment target for several psychiatric disorders, including schizophrenia[1,2] and substance use disorder[3–5] because of its expression profile and function. mGlu5 is highly expressed in forebrain regions, including cortical, hippocampal, and striatal neurons,[6,7] and is involved in various cellular processes, including long-term depression (LTD) and long-term potentiation (LTP). It is also involved in the production of molecules implicated in the pathophysiology of psychiatric disorders.[8–11] Indeed, changes to mGlu5 protein levels are observed in substance use disorder, with reductions evident in nicotine and cocaine use disorder[12,13] and increased mGlu5 expression found in alcohol use disorder.[14] There are no global changes to *GRM5* gene or mGlu5 protein levels in the prefrontal cortex, striatum, or anterior cingulate in postmortem schizophrenia samples[15,16] (review: Matosin et al.[17]), but considering that mGlu5 can be differentially expressed across various cell types (eg, neurons, interneurons, astrocytes), it is possible that cell-type specific changes to mGlu5 have not been detected.[17] Furthermore, antipsychotic medication can also increase mGlu5 receptor availability in the orbitofrontal cortex in individuals with schizophrenia, complicating causal interpretations.[18]

Genetic mouse models have highlighted a critical role for mGlu5 receptors in behavioral phenotypes relevant to schizophrenia and substance use disorder. Homozygous deletion of mGlu5 in adult mice causes

schizophrenia-relevant behaviors, including hyperlocomotion and sensorimotor gating impairment,[19–22] as well as cognitive deficits, including a perseverative phenotype and impaired extinction learning in male mice.[23,24] Furthermore, mGlu5 homozygous deletion in male mice elevates motivation for cocaine, impairs extinction of methamphetamine seeking, and increases sensitivity to alcohol.[25–27] Both an increase[28] and decrease in anxiety-like behaviors[22] has been detected in male mice lacking mGlu5 receptors (see also Inta et al.[28]). Complex effects of mGlu5 deletion in male mice have also been reported for social behaviors, with mGlu5 homozygous deletion improving recognition of a novel mouse compared to a familiar mouse,[29] which is a well-established index of social recognition memory,[30] but also decreasing preference for a mouse compared to an empty chamber.[22]

Importantly, the preclinical mouse model research to date has largely been conducted in male mice[19,22,23,25–28] or studies have not considered sex as a biological variable.[20,21,24,31,32] Despite sex differences in the expression of schizophrenia-relevant glutamatergic and γ-aminobutyric acid (GABA)ergic genes in mGlu5 homozygous (mGlu5 HOMO) mice compared to wildtype-like (WT) controls,[33] the impact of sex on behavior in mGlu5 mutant mice has received very limited attention, with only 1 paper examining sex effects for locomotion and sensorimotor gating in mice with cortex-specific mGlu5 deletion.[31] This is a significant limitation as sex differences in schizophrenia symptoms, brain function, and treatment efficacy, as well as substance use symptoms, patterns of use, and relapse susceptibility are observed in individuals with schizophrenia and substance use.[34,35] Similarly, animal models of schizophrenia and substance use show sex-dependent behaviors.[36,37] Also, the impact of gene-dose on experimental outcomes has received limited attention (ie, homozygous vs heterozygous mGlu5 deletion), with most studies only examining effects of homozygous deletion of mGlu5 on behavior.[19–21,23–28,31,32] Interestingly, 1 study has shown that mGlu5 heterozygous deletion in male mice increases, but homozygous mGlu5 deletion decreases operant sensation seeking (ie, lever pressing for a compound audio-visual stimulus in an operant chamber).[38] Despite this, heterozygous deletion of mGlu5 in male mice does not alter locomotion, anxiety, or sociability parameters, where homozygous deletion causes hyperlocomotion, decreased anxiety, and decreased sociability compared to WT controls.[22] These preliminary investigations suggest some behavioral domains may be differentially altered by heterozygous or homozygous mGlu5 deletion, which we sought to clarify here.

Considering the lack of comprehensive investigation into whether sex and gene-dose modulate mGlu5-based behavioral phenotypes, here we evaluated sex differences and gene-dose effects on schizophrenia- and substance use-relevant behaviors in mGlu5 transgenic mice, including locomotion and exploration, short-term recognition memory, associative learning, and generalization of learning, social behavior, sensorimotor gating, and cocaine reward.

Materials and Methods

Animals

Male and female mGlu5 heterozygous mice were provided by Prof Anthony Hannan (Florey Institute of Neuroscience and Mental Health, VIC),[21,23] having been bred for >10 generations on a C57BL/6J genetic background. Experimental animals were bred using heterozygous breeding pairs at Australian BioResources (Moss Vale, Australia) on a C57BL/6J background. Homozygous mGlu5 **-/-** (mGlu5 HOMO), heterozygous mGlu5 +/- (mGlu5 HET), and wildtype-like control +/+ (WT) littermates were transported to the animal facility at Western Sydney University (School of Medicine, Campbelltown, Australia) at least 2 weeks before testing. Standard social interaction (SI) opponents were adult, sex-matched A/JArc mice. A/JArc mice were used because they are docile and submissive, and they initiate much less social behavior compared to C57BL/6J mice.[39] Importantly, there appears to be no influence of the strain of the opponent on social behaviour.[40]

Test animals were group housed (2-3 littermates/cage) in filter-top cages (Type 1284B; Tecniplast, Rydalmere, Australia) with a wire lid, which provided climbing opportunities. Home cages contained corn-cob bedding, crinkle-cut bedding (Crink-l'Nest, Kraft), and tissues for nesting. Mice were kept under a 12:12 h white light:red light schedule (white lights on 9 AM-9 PM/red lights on from 9 PM-9 AM), food and water *ad libitum*, with a temperature between 22 and 24 °C and humidity between 40% and 70% RH. Mice were between 5 and 6 months old at the start of experiments.

Research and animal care procedures were approved by the Western Sydney University Animal Care and Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (approval number: #A12850).

Behavioral Phenotyping

Animals were transported to test rooms 30 min prior to behavioral testing to allow animals to habituate. Experiments were performed within the first 6 h of the light phase with an inter-test interval of at least 48 h. Test equipment was cleaned after each animal with 80% v/v ethanol.

Two experimental cohorts were tested. The first cohort (baseline testing) was tested in the following tests, in this order: open field (OF), novel object recognition, SI, prepulse inhibition, fear conditioning (FC), and cocaine-conditioned place preference. A second cohort assessed generalization of FC. The sample sizes for each cohort were: Baseline testing: Female $n = 14$ WT, 8 mGlu5

HET, 12 mGlu5 HOMO; Male $n = 13$ WT, 10 mGlu5 HET, 10 mGlu5 HOMO. Generalization of FC: Female $n = 24$ WT, 28 mGlu5 HOMO; Male $n = 29$ WT, 13 mGlu5 HOMO. For generalization of FC, mice were allocated to 1 of 2 groups within each sex/genotype, reflecting the order in which they were exposed to different contexts (ie, context A then B [A-B] or context B then A [B-A]) but as these groups were not statistically different, the data were pooled across the groups, giving a higher N for this experiment.

Open Field. Locomotor activity, exploration, and anxiety-like behaviors were measured in the OF (methods[41,42]). Mice were placed into the right corner of the OF chamber (43 × 43 cm, ENV-515S; Activity Monitor, Med Associates, Fairfax, VT, USA) and allowed to explore the arena freely for 30 min. The arena was divided into a central and peripheral zones (software coordinates 3/3, 3/13, 13/3/, 13/13[43]; software settings[43]) with the central zone being a more aversive, anxiety-inducing area. Time, horizontal activity (distance traveled), and vertical activity (*rearing,* ie, exploration) in central and peripheral zones were measured by the chambers' infrared photo beams. The percentage of central to total distance traveled (percentage of centre distance) and time spent in the central area of the OF were taken as measures of anxiety.[41,42]

Novel Object Recognition Test (NORT). The distinction between familiar and unfamiliar objects is an index of recognition memory.[44] The NORT can assess short-term memory in rodents.[45] This is analogous to human declarative memory, which is impaired in individuals with schizophrenia.[45]

The protocol was adapted from our previous methods and ran over consecutive 2 days in a gray Perspex arena (35 × 35 × 30 cm).[41] On the first day, mice were allowed to explore the arena for 10 min, twice, with an intertrial interval of 2 h. On the second day, mice were placed in the arena with 2 identical objects (2 LEGO© elephants or 2 rectangular blocks of LEGO©) and allowed to explore the objects for 10 min. After a 15-min intertrial interval, mice were replaced in the arena with 1 familiar object and 1 novel object (LEGO© elephant + block of LEGO©) for 5 min. Object exploration was measured via *nosing* toward the objects (ie, when the mouse directed its nose to an object at a distance of 1-2 cm). We calculated the percentage of time spent *nosing* the novel object, compared to the time spent *nosing* novel + familiar objects. *Nosing* behavior was manually scored using ANY-maze™ (Stoelting, IL, USA).

Social Interaction. Social withdrawal is observed in schizophrenia patients[46,47] (review: Green et al. 2015[46]). To assess social behaviors in mice, test mice and adult, sex-matched A/JArc standard opponent mice were placed in opposite corners of an OF gray Perspex arena (35 × 35 × 30 cm) and allowed to explore the space and each other freely for 10 min (methods[41]). Frequency and duration of the active social behaviors were recorded manually using ANY-maze™ (Stoelting, IL, USA), including *nosing*, *anogenital sniffing*, *rearing*, *following*, and *crawling over*.

Prepulse Inhibition (PPI). Prepulse inhibition (PPI), an operational measure of sensorimotor gating, is the attenuation of the startle response by a non-startling stimulus (prepulse) presented 30-500 ms before a startle pulse.[48] PPI is impaired in schizophrenia patients.[49–51] Startle reactivity was measured using SR-LAB startle chambers (San Diego Instruments, San Diego, USA), where the startle response intensity of rodents (whole body flinch amplitude) was measured using a piezoelectric accelerometer. Methods have been published previously.[52]

Animals were habituated to the startle chambers and test enclosures twice a day for 10 min on 2 consecutive days with a 2-h intertrial interval. On the third day, the PPI test was conducted and consisted of a 5 min acclimatization period to a 70 dB background noise, followed by 97 trials presented in a pseudorandom order: 5 × 70 dB trials (background noise); 5 × 100 dB trials; 15 × 120 dB trials (for acoustic startle response, ASR) and 72 PPI trials comprising 6 sets of a prepulse of either 74, 82, or 86 dB presented 32, 64, 128, or 256 ms (variable interstimulus interval; ISI) prior to a startle pulse of 120 dB. The intertrial interval varied randomly between 10 and 20 s.

Responses to each trial were calculated as the average mean amplitude detected by the accelerometer. The startle response was calculated as the mean amplitude across all 15 startle trials, and percentage PPI (%PPI) was calculated as [(mean startle response (120 dB) – PPI response)/mean startle response (120 dB)] × 100.

Fear-Conditioning. Fear conditioning (FC) is a form of associative learning that is impaired in individuals with schizophrenia.[53–55] FC measures the association of a previously neutral stimulus with an aversive stimulus (eg, an electric foot shock) by the expression of *freezing* behavior, which is the absence of all movement except breathing. Methods were identical to our previous work.[42]

On the first day (ie, Conditioning), mice were placed in a test chamber (Med Associates; MED-VFC-SCT-M) with a vanilla scent cue and house lights on for 120 s. After this, an 80 dB conditioned stimulus (CS) was presented for 30 s, which co-terminated with a 0.4 mA 2 s foot shock (unconditioned stimulus, US). After an interstimulus interval of 120 s, the CS-US presentation was repeated, and the test concluded 120 s later. On the second day (Context test), mice were returned to the apparatus for 7 min with the vanilla scent cue and house lights on, and *freezing* was measured. On the third day (Cue test), mice were placed in an altered environment (ie, a black Perspex triangular insert added to the chamber, house lights on but no scent cue) for 9 min. After 120 s, the CS was continuously presented for 5 min, and the test concluded 120 s later.

Generalization of FC. In FC, we observed elevated *freezing* to a novel context in the absence of a fear-associated stimulus in mGlu5 HOMO mice. Thus, we performed another experiment in a different cohort of WT and mGlu5 HOMO mice of both sexes to assess generalization of hippocampal-dependent fear memory. Methods were based on published work,[56,57] and a schematic is provided in Figure 4A. For this experiment, we used 2 contexts in the FC chambers: Context A, which had a vanilla scent cue, house lights on, a rod floor, and stainless steel walls, and Context B, which had no scent cue, no house light, black Perspex triangular insert added to the chamber, and a gray PVC insert covering the rod floor.

On day 1, animals were placed into Context A for 3 min, after which a 2 s 0.4 mA shock occurred twice with an intertrial interval of 120 s. There was no auditory cue preceding the shock, unlike methods for FC. Mice were returned to the home cage 120 s later.

Mice were then randomly split into 2 groups: A-B and B-A, referring to the order which they were tested over the next 2 days, that is, Context A then B (A-B), or Context B then A (B-A). There were equal numbers of each sex and genotype in each group. Over the next 2 days, mice were either returned to context A then B, or B then A, and *freezing* measured for 7 min each day.

Group A-B allowed us to assess fear memory expression in the original context, followed by generalization to a novel context (replicating our findings from the Cue test), and B-A allowed us to assess generalization to a novel context without interference from expression of fear memory in the original context. However, as we did not detect differences in *freezing* between groups A-B and B-A, data from each group were pooled to assess *freezing* responses to each context.

Cocaine-Induced Conditioned Place Preference (CPP). Conditioned place preference measures the rewarding properties of a drug and the persistence of a drug-associated contextual memory.[58] The CPP apparatus was a modified OF arena (43.2 × 43.2 cm, Med Associates), with a black Perspex divider separating 2 equally sized compartments. A small gate (11 × 9 cm) allowed access to both compartments. Wall patterns distinguished the 2 compartments: white walls in the left compartment and black spots on a white background in the right compartment. The time spent in each compartment and general locomotor activity was recorded via horizontal infrared beams. CPP methods were similar to our laboratory's published work.[59,60]

Cocaine hydrochloride (National Measurements Institute, ACT, Australia) was dissolved in 0.9% saline. Vehicle injections were 0.9% saline. Injections were administered intraperitoneally (i.p.) at 10 mL/kg.[59–61]

On day 1 (habituation), mice were placed randomly in either compartment and allowed free access to the entire apparatus for 30 min. The time spent in each compartment during habituation was used to allocate drug pairings.[52,59–61] On days 2-4 (conditioning), mice received i.p. injections of saline or cocaine and immediately confined into 1 of the 2 conditioning compartments for 30 min. Saline conditioning was conducted in the morning (30 min after light phase onset), and cocaine conditioning was conducted in the afternoon, with 5 h separating the start of vehicle and cocaine conditioning sessions. The cocaine dose used was 20 mg/kg[27]. Locomotor data was collected to assess the development of locomotor sensitization for cocaine, which we measured as an increase in cocaine-induced locomotion compared to day 1 of cocaine administration, within sex and genotype.

At each CPP test, mice were placed in the vehicle-paired compartment and given free access to the entire CPP apparatus for 30 min. A preference score was calculated for habituation and test (time in drug zone – time in vehicle zone); a positive preference score indicated a preference for the drug-paired compartment.[52,59–61] Drug-free tests were conducted 1, 7, and 21 days postconditioning to assess persistent of place preference, similar to published data.[27]

Statistical Analysis

Data were analyzed using SPSS Statistics 29 (IBM, NY, USA). Four-, three-, and two-way repeated measures (RM) analysis of variance (ANOVA) were conducted, with between-subjects factors "sex" (male/female) and "genotype" (WT/mGlu5 HET/mGlu5 HOMO). As we detected sex main effects or interactions for most parameters, all data were split by sex, in line with previously published work in HOMO mice,[33] and Bonferroni post-hoc tests were used to identify genotype or day differences. Data is presented as mean ± standard error of the mean (SEM) and effect sizes (partial eta-squared, η^2), and differences were regarded as statistically significant if $P < .05$. For the conditioned place preference data, we compared effect sizes using 90% confidence intervals to ascertain if cocaine memory was stronger in mGlu5 HOMO or mGlu5 HET than WT, as all genotypes showed highly significant P-values (all P's $< .001$) and large effect sizes (all $\eta^2 > .14$).[62]

Exclusions: 1 WT male was excluded from the OF as the equipment stopped working 20 min into the test. 2 mice were excluded from the NORT analysis because they spent <10 s total time *nosing* both objects (1x WT male, 1x mGlu5 HOMO male). 1 mGlu5 HOMO male was excluded as an outlier from PPI because the acoustic startle response was >2 standard deviations from the mean.

Results

Locomotion, Exploration, and Anxiety

mGlu5 HOMO mice exhibited elevated locomotion [three-way RM ANOVA genotype main effect,

$F(2,60) = 21.65$, $P < .001$, $\eta^2 = .42$], which did not habituate across the test, unlike WT and mGlu5 HET mice [time x genotype, $F(10,300) = 14.37$, $P < .001$, $\eta^2 = .32$]. Female mice traveled further than males [sex, $F(1,30) = 5.4$, $P = .02$, $\eta^2 = .08$; no sex interactions]. Split by sex, we confirmed genotype effects in both sexes [males: $F(2,29) = 7.40$, $P = .003$, $\eta^2 = .34$; females: $F(2,31) = 17.24$, $P < .001$, $\eta^2 = .53$], as well as time x genotype interactions [males: $F(10,145) = 6.01$, $P < .001$, $\eta^2 = .29$; females: $F(10,155) = 8.97$, $P < .001$, $\eta^2 = .37$]. Post-hoc tests confirmed elevated locomotion in the latter half of the test in male and female mGlu5 HOMO mice compared to WT littermate controls (Figure 1A, 1B).

Rearing was reduced in mGlu5 HET and mGlu5 HOMO mice compared to WT mice [genotype, $F(2,60) = 12.86$, $P < .001$, $\eta^2 = .30$], and this was most pronounced in the first half of the test [time x genotype, $F(10,300) = 4.01$, $P < .001$, $\eta^2 = .12$]. Females *reared* less than males [sex, $F(1,60) = 18.81$, $P < .001$, $\eta^2 = .24$], and the increase in *rearing* across the test was less prominent in females compared to male mice [time x sex, $F(5,300) = 3.39$, $P = .005$, $\eta^2 = .05$] (Figure 1C, 1D). Split by sex, we confirmed genotype effects in both sexes [males: $F(2,29) = 7.31$, $P = .003$, $\eta^2 = .34$; females: $F(2,31) = 6.50$, $P = .004$, $\eta^2 = .30$], as well as time x genotype interactions [males: $F(10,145) = 2.02$, $P = .04$, $\eta^2 = .12$; females: $F(10,155) = 2.78$, $P = .003$, $\eta^2 = .15$]. Post-hoc tests demonstrated reduced *rearing* in male and female mGlu5 HOMO and mGlu5 HET mice compared to WT mice, largely in the first half of the test (Figure 1C, 1D).

When measuring anxiety-like behavior, we found that both mGlu5 HET and mGlu5 HOMO mice had a greater center distance percentage than WT littermates [genotype, $F(2,60) = 9.36$, $P < .001$, $\eta^2 = .24$]. Males also had a higher center distance percentage than females [sex, $F(1,60) = 4.73$, $P = .033$, $\eta^2 = .07$] (Figure 1E, 1F). Split by sex, we detected genotype effects in males [$F(2,29) = 3.81$, $P = .034$, $\eta^2 = .21$] and females [$F(2,31) = 6.499$, $P = .004$, $\eta^2 = .30$]. Post-hoc tests showed greater center distance percentage in mGlu5 HOMO males at the beginning of the test, and greater percentage of center distance in female mGlu5 HET and mGlu5 HOMO mice toward the end of the test compared to female WT controls (Figure 1E, 1F). Time in the center of the OF confirmed center distance percentage findings (Supplementary Table S1). Totals for each parameter above are presented in Supplementary Table S1.

Novel Object Recognition Memory

Using two-way ANOVA, there were no genotype or sex differences in object recognition memory [genotype, $F(2,61) = 1.42$, $P = .25$, $\eta^2 = .05$; sex, $F(1,61) = 1.71$, $P = .20$, $\eta^2 = .03$; Supplementary Table S1]. Split by sex, we did not detect any genotype effects (Supplementary Figure S1A-1B).

Social Behaviors

Total active SI time was unaffected by genotype or sex [two-way ANOVA, sex: $F(1,61) = 1.46$, $P = .23$, $\eta^2 = .02$; genotype: $F(2,61) = 1.02$, $P = .37$, $\eta^2 = .03$] (Supplementary Figure S2A-2B). However, analyzing the time spent in individual behaviors, we found a genotype main effect for time spent *crawling over* [$F(2,61) = 5.02$, $P = .01$, $\eta^2 = .14$] and *rearing* [$F(2,61) = 3.57$, $P = .03$, $\eta^2 = .11$]. Male mice *reared* for longer than female mice [sex main effect, $F(1,61) = 6.80$, $P = .01$, $\eta^2 = .10$; no interactions]. Split by sex, there was a genotype effect for *rearing* in male mice [$F(2,30) = 4.84$, $P = .016$, $\eta^2 = .24$] and for *crawling over* in female mice [$F(2,31) = 6.30$, $P = .005$, $\eta^2 = .29$]. Post hoc tests showed reduced time spent *crawling over* in mGlu5 HET and mGlu5 HOMO female mice compared to WT mice (Supplementary Figure S2A-2B), and reduced time spent *rearing* in male mGlu5 HOMO vs mGlu5 HET (Supplementary Figure S2A-2B).

Acoustic Startle Response and Prepulse Inhibition

The acoustic startle response increased with increasing startle pulse intensities in all groups [three-way RM ANOVA, startle intensity, $F(2,120) = 135.73$, $P < .001$, $\eta^2 = .69$; Figure 2A-2B]. Acoustic startle was also affected by genotype [$F(2,60) = 3.76$, $P = .03$, $\eta^2 = .11$], and this was most evident at higher startle pulse intensities (ie, 120 dB) [startle intensity x genotype, $F(4,120) = 4.18$, $P = .003$, $\eta^2 = .12$]. The startle response was lower in females compared to males, particularly at higher startle intensities [sex, $F(1,60) = 8.63$, $P = .005$, $\eta^2 = .13$; startle intensity × sex, $F(2,120) = 6.49$, $P = .002$, $\eta^2 = .10$]. Split by sex, there was a main effect of genotype in female mice [$F(2,31) = 3.39$, $P = .046$, $\eta^2 = .18$; no interactions] and a startle intensity × genotype interaction in males [$F(4,58) = 3.02$, $P = .03$, $\eta^2 = .17$; no genotype main effect]. While post-hoc tests failed to detect specific significant genotype differences at individual startle pulse intensities, examination of Figure 2A-B suggests overall reduced startle in male mGlu5 HOMO mice (vs WT) at 120 dB, and reduced startle in female mGlu5 HET and mGlu5 HOMO (vs WT) at 100 and 120 dB.

%PPI increased with increasing prepulse intensities [$F(2,120) = 478.45$, $P < .001$, $\eta^2 = .89$], and there was also a main effect of genotype [$F(2,60) = 5.25$, $P = .008$, $\eta^2 = .15$]. A sex x genotype interaction suggested that genotype differences in %PPI were sex-dependent [$F(2,60) = 3.92$, $P = .025$, $\eta^2 = .12$] (Figure 2C-2D). Split by sex, we confirmed that genotype differences in %PPI were only evident in female mice [females: $F(2,31) = 11.69$, $P < .001$, $\eta^2 = .43$; males: $F(2,29) = .26$, $P = .77$, $\eta^2 = .02$] and a prepulse intensity × genotype interaction in females suggested genotype differences were only apparent at higher prepulse intensities [$F(4,62) = 3.20$, $P = .02$, $\eta^2 = .17$]. Post-hoc tests confirmed female mGlu5 HOMO mice had lower %PPI

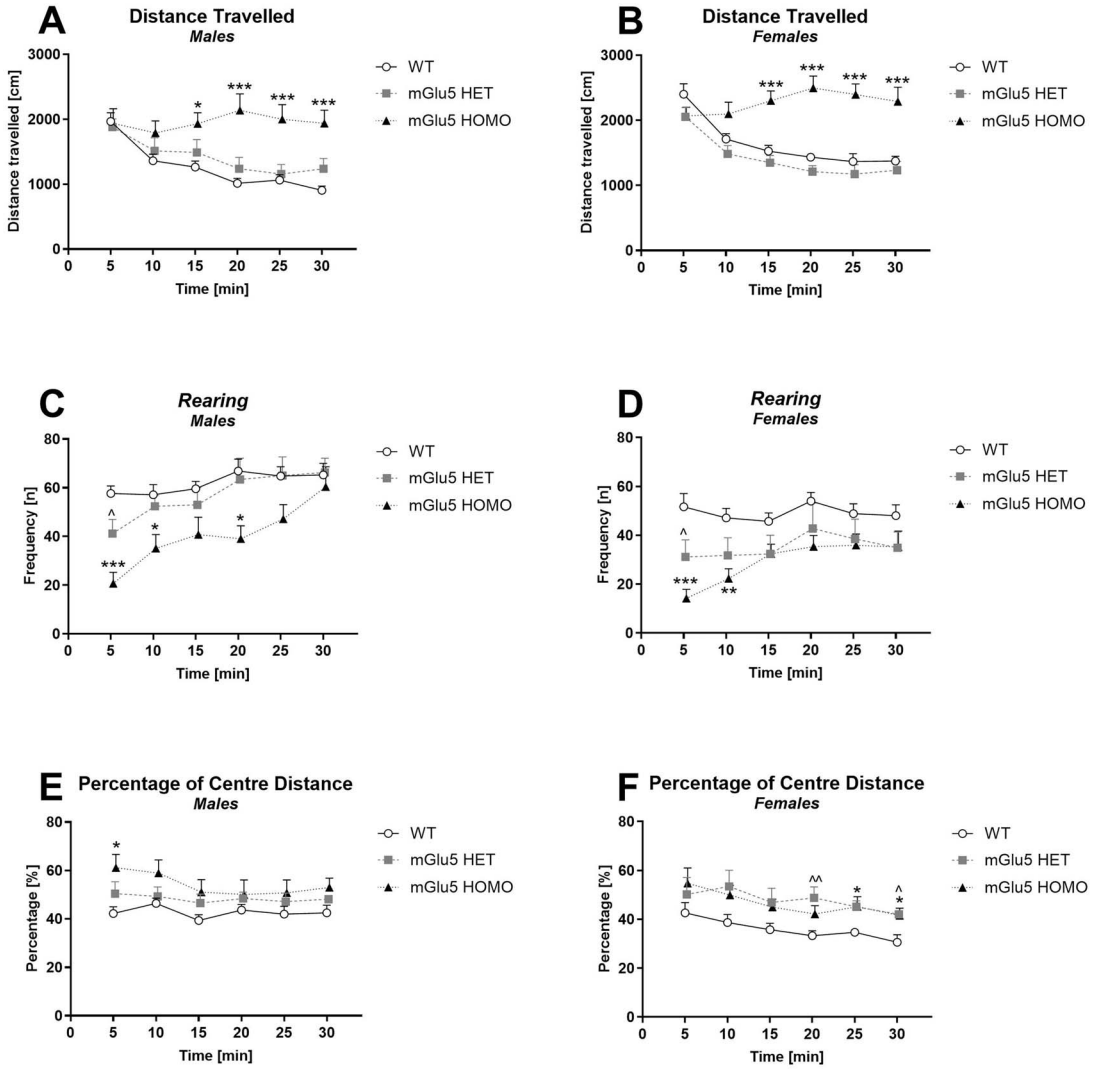

Figure 1. Open-Field Locomotion, Exploratory and Anxiolytic-Like Behaviors. Open-field behaviors in male (left) and female (right) WT, mGlu5 HET, and mGlu5 HOMO mice. (A-B) distance traveled [cm], (C-D) *rearing* frequency [n], and (E-F) centre distance percentage [%]. Data were analyzed using three-way RM ANOVA, then split by sex, followed by Bonferroni post-hoc tests. Sex effects were detected for distance traveled, percentage of centre distance, and *rearing*; a sex × time interaction was detected for *rearing*. Data presented as mean ± SEM. Bonferroni genotype effects compared to WT mice are indicated by "^" in mGlu5 HET mice ($^{P} < .05$, $^{^P} < .01$) and by asterisks in mGlu5 HOMO mice ($^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$). Abbreviations: mGlu5 HET: heterozygous deletion; mGlu5 HOMO: homozygous deletion; mGlu5: metabotropic glutamate 5 receptor; WT: wildtype-like.

than female WT mice at 82 and 86 dB prepulses (Figure 2C-2D).

Fear-Associated Memory

Mice of both genotypes and sexes learnt the association between tone and shock, evidenced by increased *freezing* during conditioning [three-way RM ANOVA, time, $F(6,366) = 77.26$, $P < .001$, $\eta^2 = .56$] although this was influenced by sex [time × sex, $F(6,366) = 3.04$, $P = .007$, $\eta^2 = .05$] and genotype [time × genotype, $F(12,366) = 5.88$, $P < .001$, $\eta^2 = .16$]. Split by sex, time x genotype interactions were detected in both sexes [males: $F(12,180) = 3.68$, $P < .001$, $\eta^2 = .20$; females: $F(12,186) = 3.10$, $P < .001$, $\eta^2 = .17$]. Post-hoc tests showed increased *freezing* in male and female mGlu5 HOMO mice vs WT in minute 3 of the test, immediately after the first shock presentation (Figure 3A-3B). Totals for conditioning are presented in Supplementary Table S1.

In the context test, all groups increased their *freezing* across the test [time, $F(6,366) = 5.18$, $P < .001$, $\eta^2 = .08$], but there was no influence of genotype or sex on *freezing* (Figure 3C-3D). Splitting by sex did not reveal any genotype effects or interactions. Totals for the context test are presented in Supplementary Table S1.

During the cue test, all groups increased their *freezing* across the test [time, $F(8,488) = 75.02$, $P < .001$, $\eta^2 = .55$; totals in Supplementary Table S1], but this was moderated by sex [time x sex, $F(8,488) = 2.16$, $P = .03$, $\eta^2 = .03$] and genotype [time × genotype, $F(16,488) = 2.24$, $P =$

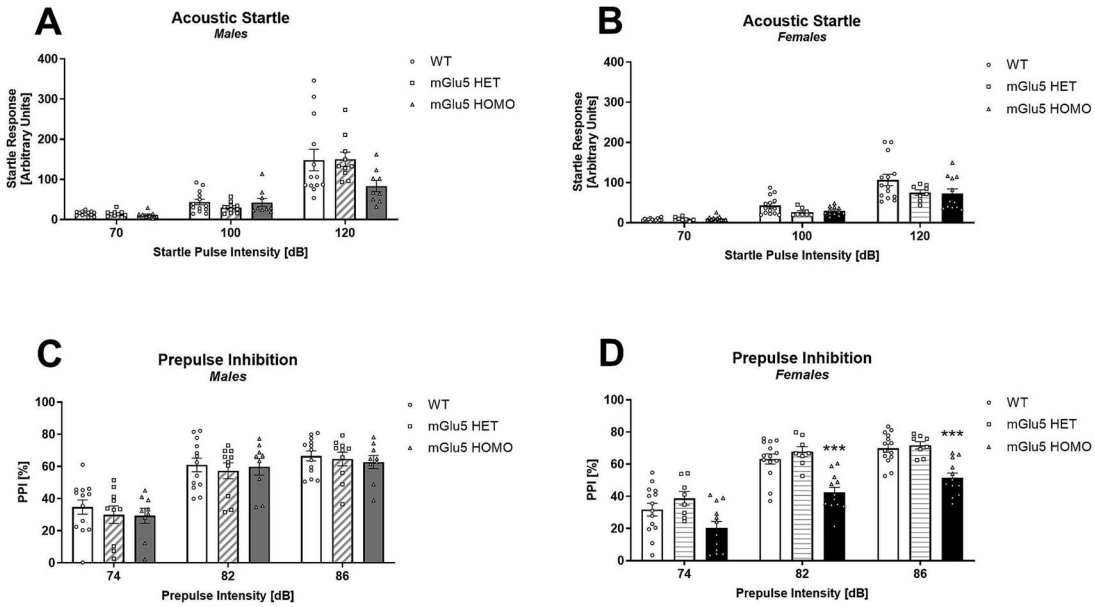

Figure 2. Acoustic Startle and Prepulse Inhibition. Acoustic startle and prepulse inhibition in male (left) and female (right) WT, mGlu5 HET, and mGlu5 HOMO mice. (A-B) acoustic startle response [arbitrary units] at 70, 100, and 120 dB, (C-D) prepulse inhibition [%] at 74, 82, and 86 dB. Data analyzed using three-way RM ANOVA and then split by sex, followed by Bonferroni post-hoc tests. Sex effects and a startle intensity × sex interaction were detected for acoustic startle response. A sex x genotype interaction was detected for prepulse inhibition. Data presented as mean ± SEM. Genotype effects within sex (vs WT) are indicated with asterisks (***$P < .001$). Abbreviations: mGlu5 HET: heterozygous deletion; mGlu5 HOMO: homozygous deletion; mGlu5: metabotropic glutamate 5 receptor; PPI: prepulse inhibition; WT: wildtype-like.

.004, $\eta^2 = .07$] (Figure 3E-3F). Split by sex, we did not detect any further time x genotype interactions. However, visual examination of Figure 3E-3F suggested increased *freezing* in male and female mGlu5 HOMO mice before and after the cue presentation. We hypothesized this could be due to increased generalization of fear memory to a novel context in mGlu5 HOMO mice. Thus, we conducted another experiment to examine fear generalization in a second cohort of male and female WT and mGlu5 HOMO mice.

Fear Memory Generalization

A schematic of fear memory generalization procedures is provided in Figure 4A. During conditioning, *freezing* increased across the test [three-way RM ANOVA, time, $F(6,432) = 53.92$, $P < .001$, $\eta^2 = .43$], and was different in mGlu5 HOMO mice compared to WTs [time x genotype, $F(6,432) = 21.32$, $P < .001$, $\eta^2 = .23$]. There were no sex effects or interactions, and when data were split by sex, we confirmed time x genotype interactions in male [$F(6,216) = 10.75$, $P < .001$, $\eta^2 = .23$] and female mice [$F(6,216) = 11.35$, $P < .001$, $\eta^2 = .24$]. Post hoc tests showed increased *freezing* in male and female mGlu5 HOMO mice vs WTs immediately after shock presentation, but reduced *freezing* in the final minute of the test (Figure 4B-4C).

In context A, which was the context that mice were conditioned in, *freezing* increased across the test [$F(6,432) = 2.83$, $P = .01$, $\eta^2 = .04$] (Figure 4D-4E), but was not different between genotypes or sexes, similar to our findings in the Context test in the baseline cohort (no main effects of genotype or sex, no interactions). Split by sex, there were no genotype main effects or interactions.

In context B, which was a novel context to assess fear generalization, *freezing* was elevated in mGlu5 HOMO mice of both sexes [genotype, $F(1,72) = 13.79$, $P < .001$, $\eta^2 = .16$], and genotype differences were most prominent at the beginning of the test [time x genotype, $F(6,432) = 5.24$, $P < .001$, $\eta^2 = .07$]. There was no effect of sex [$F(1,72) = 2.59$, $P = .1$, $\eta^2 = .04$] or time × sex interaction [$F(6,432) = 1.83$, $P = .09$, $\eta^2 = .03$]. Split by sex, we confirmed genotype effects in males [$F(1,36) = 4.56$, $P = .04$, $\eta^2 = .11$] and females [$F(1,36) = 9.63$, $P = .004$, $\eta^2 = .21$], and a time x genotype interaction in male mice only [$F(6,216) = 5.48$, $P < .001$, $\eta^2 = .13$; females: $F(6,216) = 1.68$, $P = .12$, $\eta^2 = .05$]. Post hoc tests showed greater *freezing* in mGlu5 HOMO male mice, which was most prominent in the first half of the test, whereas the genotype main effect in females suggests elevated *freezing* in female mGlu5 HOMO mice compared to WTs throughout the test (Figure 4F-G). Confirming this, a four-way RM ANOVA comparing *freezing* across the test in context A and B in male and female WT and mGlu5 HOMO mice showed a genotype × minutes × context × sex interaction [$F(6,432) = 3.28$, $P = .004$, $\eta^2 = .04$], and post hoc tests (not shown) demonstrated differences between context A and B in female mGlu5 HOMO from minutes 4-7 of the test, in female WT in minutes 1, 3, and 4, and in male WT in minutes

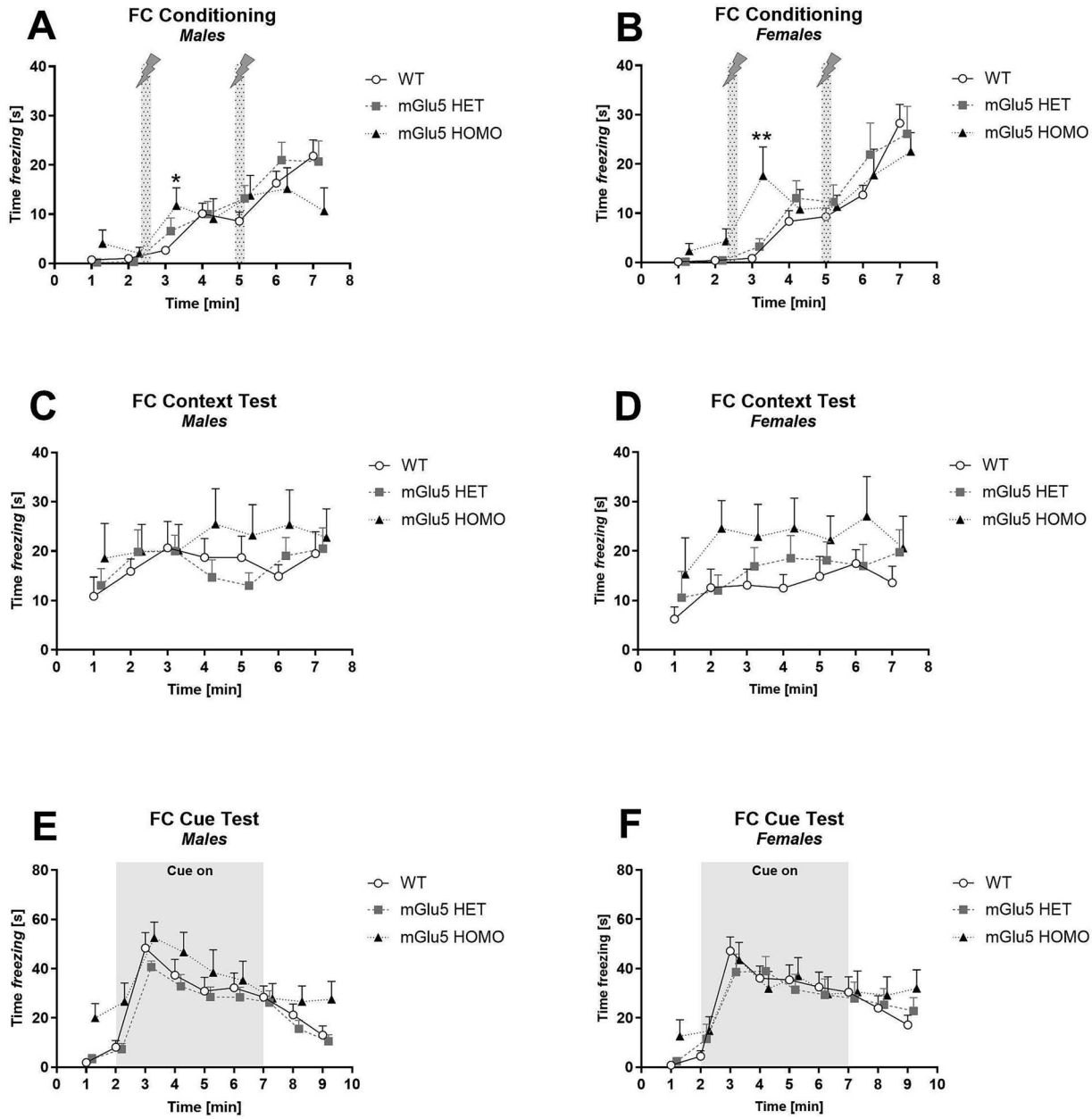

Figure 3. Fear Conditioning. *Freezing* [s] in male (left) and female (right) WT, mGlu5 HET, and mGlu5 HOMO mice during (A-B) conditioning, (C-D) context test, and (E-F) cue test. Data analyzed using three-way RM ANOVA, then split by sex, followed by Bonferroni post-hoc tests. A time × sex interaction was detected for conditioning and the cue test. Data presented as mean ± SEM. Post-hoc genotype effects indicated by asterisks (vs WT; HOMO: $^{*}P < .05$, $^{**}P < .01$). Abbreviations: FC: fear conditioning; mGlu5 HET: heterozygous deletion; mGlu5 HOMO: homozygous deletion; mGlu5: metabotropic glutamate 5 receptor; WT: wildtype-like.

1-3. This suggests that WT mice *freeze* less in context B at the beginning of the test, indicating that the novel context initially did not bring up the memory of the shocked context and confirming our novel test conditions worked, but that female mGlu5 HOMO mice were more susceptible to fear generalization in the latter part of the test.

Total *freezing* during generalization is presented in Supplementary Table S1.

Locomotor Sensitization

All mice sensitized to repeated cocaine across conditioning [days, $F(3,174) = 57.16$, $P < .001$, $\eta^2 = .50$]; this was influenced by genotype [days x genotype, $F(6,174) = 3.91$, $P = .001$, $\eta^2 = .12$]. Sex did not affect cocaine sensitization (no main effects of sex or interactions). Data were split by sex to confirm sensitization in all genotypes and sexes. Both males and female mice showed a significant increase in cocaine-locomotion across days [days × drug

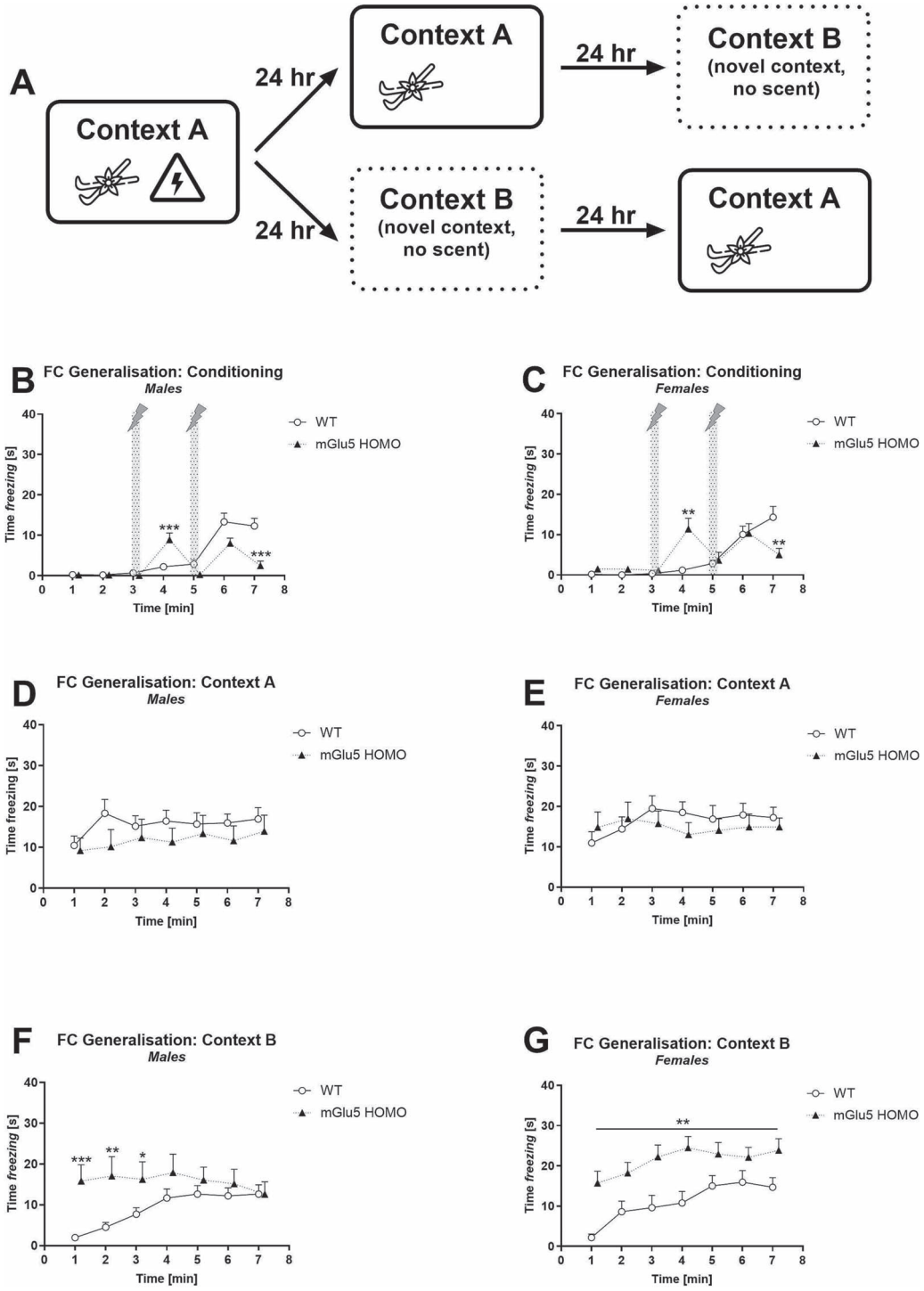

Figure 4. Fear Generalization. (A) Schematic of the fear generalization design: Mice were conditioned in context A, and then half of the mice were tested in the absence of shock first in context A (same context as conditioning) and then in the novel context B, and the other half were tested first in context B, and then in context A. Data for context A and B were pooled, irrespective of test order. *Freezing* [s] in male (left) and female (right) WT and mGlu5 HOMO mice during (B-C) conditioning (context A), (D-E) context A and (F-G) context B. Data analyzed using three-way RM ANOVA, then split by sex, followed by Bonferroni post-hoc tests. Data presented as mean ± SEM. Post-hoc genotype effects indicated by asterisks (vs WT: $^{*}P < .05$; $^{**}P < .01$, $^{***}P < .001$); in (G) a genotype main effect is indicated by asterisks (vs WT: $^{**}P < .01$). Abbreviations: FC: fear conditioning; mGlu5 HET: heterozygous deletion; mGlu5 HOMO: homozygous deletion; mGlu5: metabotropic glutamate 5 receptor; WT: wildtype-like.

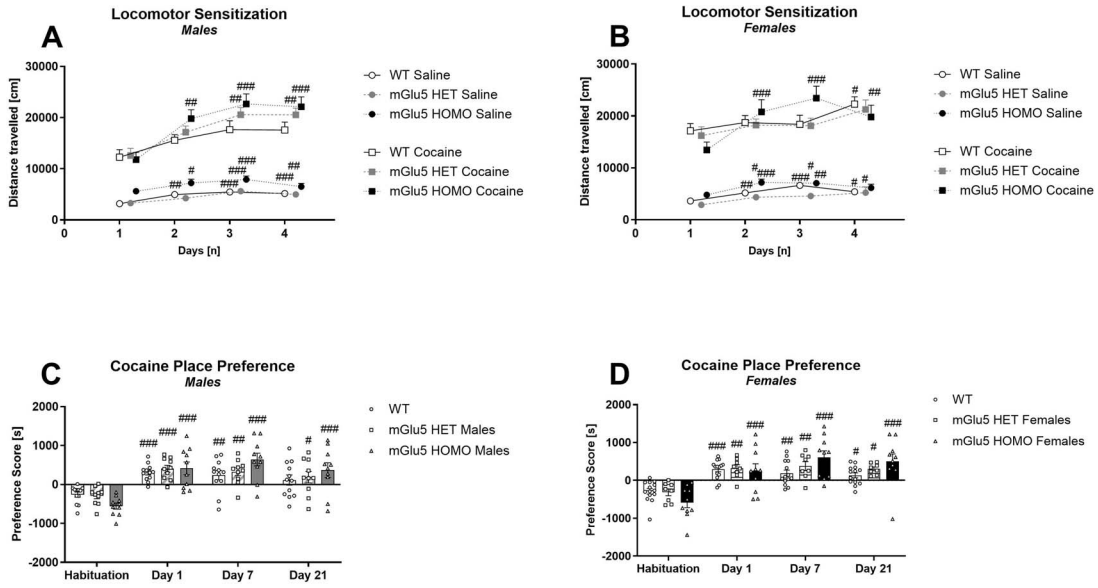

Figure 5. Development of Cocaine Locomotor Sensitization and Expression of Cocaine Conditioned Place Preference. (A-B) distance traveled [cm] under saline or 20 mg/kg cocaine in WT, mGlu5 HET, and mGlu5 KO mice during the 4 days of cocaine conditioning. (C-D) preference for the cocaine-paired environment expressed as a preference score [s] at habituation, day 1, day 7, and day 21 after conditioning in WT, mGlu5 HET, and mGlu5 HOMO mice. Males are shown on the left (A, C), females on the right (B, D). Data analyzed using three-way RM ANOVA and presented as mean ± SEM. Significant effects of day vs day 1 within drug treatment group for A and B, or vs habituation for C and D, are indicated by hash symbols ($^{\#}P < .05$, $^{\#\#}P < .01$, $^{\#\#\#}P < .001$). Abbreviations: mGlu5 HET: heterozygous deletion; mGlu5 HOMO: homozygous deletion; mGlu5: metabotropic glutamate 5 receptor; WT: wildtype-like.

in males: $F(3,87) = 15.67$, $P < .001$, $\eta^2 = .35$, females: $F(3,87) = 5.28$, $P = .002$, $\eta^2 = .15$], but genotype affected locomotor sensitization in female mice only [days x drug x genotype in females, $F(6,87) = 3.92$, $P = .002$, $\eta^2 = .21$; males: $F(3,87) = 1.29$, $P = .27$, $\eta^2 = .08$]. Post hoc tests demonstrated that male mGlu5 HET and mGlu5 HOMO mice sensitized to cocaine, showing increased locomotion under cocaine compared to day 1 (Figure 5A), and female WT and mGlu5 HOMO mice also showed sensitization (Figure 5B). All genotypes increased their saline-induced locomotion across conditioning (Figure 5A-5B).

Cocaine Place Preference

All mice increased their preference for the cocaine-paired environment compared to habituation [days, $F(3,174) = 79.18$, $P < .001$, $\eta^2 = .58$], and this was influenced by genotype [days x genotype, $F(6,174) = 5.80$, $P < .001$, $\eta^2 = .17$; Figure 5C; no effect of sex or interactions]. Split by sex, we confirmed the preference score was different among genotypes in both sexes [days × genotype in males: $F(6,87) = 2.60$, $P = .02$, $\eta^2 = .15$; females: $F(6,87) = 3.48$, $P = .004$, $\eta^2 = .19$]. Post-hoc tests comparing habituation to each test show that the preference score increased compared to habituation for all genotypes and sexes, except for male WT mice at day 21. Examination of Figure 5C-5D shows that the magnitude of change in the preference score between habituation to day 21 was greater in male mGlu5 HOMO mice than in male WT mice. We confirmed this using effect sizes: while there was a main effect of "day" in each group (all P's $< .001$), we found a greater effect size in male mGlu5 HOMO than WT mice, with no overlapping confidence interval (mGlu5 HOMO: $\eta^2 = 0.76$, 90% CI, 0.58, 0.82; mGlu5 HET: $\eta^2 = 0.50$, 90% CI, 0.21, 0.61; WT: $\eta^2 = 0.40$, 90% CI, 0.14, 0.52). However, while effect sizes were greater in female mGlu5 HOMO and mGlu5 HET than female WT mice, the overlapping confidence intervals suggest the effect of day was not different between the female genotypes (mGlu5 HOMO: $\eta^2 = 0.62$, 90% CI, 0.37, 0.71; mGlu5 HET: $\eta^2 = 0.65$, 90% CI, 0.36, 0.74; WT: $\eta^2 = 0.57$, 90% CI, 0.35, 0.66).

Discussion

We examined sex and gene-dose effects of mGlu5 deletion on schizophrenia-relevant and cocaine-induced behaviors. Some mGlu5 HOMO phenotypes were evident in both sexes, for example, hyperlocomotion and reduced *rearing* in the OF, decreased anxiety-like behavior, and overgeneralization of fear memory. However, cocaine-induced behaviors were more prominent in male mice, while sensorimotor gating deficits were evident only in female mGlu5 HOMO mice. Often, gene-dose effects were evident, as mGlu5 HET mice largely showed similar phenotypes to WT controls. Our data supports using both male and female mGlu5 HOMO mice as models of schizophrenia-induced behaviors, but perhaps only male mice for cocaine-induced behaviors, and highlights a novel fear generalization phenotype with potential

translational value for disorders characterized by pervasive fear memory across contexts.

The current study explored behavioral phenotypes previously reported for only male mGlu5 HOMO mice in female mGlu5 HOMO mice. We detected OF hyperlocomotion and reduced *rearing* in both sexes, extending similar to reports of hyperlocomotion in male mGlu5 HOMO mice,[63] and also when both sexes were collapsed.[20,21,32] While 1 study found no change to baseline locomotion in mGlu5 HOMO males, this may be due to those test mice being single-housed for 2 weeks prior to testing,[29] and mGlu5 HOMO mice are susceptible to changes in their environment.[21] It is likely the hyperlocomotive phenotype is driven by deletion of mGlu5 on GABAergic forebrain neurons, as in male and female mice, this produces a similar pattern of initial hypolocomotion in the OF, followed by increased locomotion as the test progresses.[64] We also detected reduced anxiety-like behavior in the OF in both sexes, broadening findings using only male mGlu5 HOMO mice.[22,38] Our findings extend current literature to show that when both sexes are analyzed, some phenotypes previously detected only in male mice are evident in both sexes. This is important, as females have often been excluded from behavioral experiments using mGlu5 HOMO mice, and our data support the use of both sexes for the detection of hyperlocomotion and anxiety-like behaviors.

We also found that mGlu5 HOMO mice of both sexes showed enhanced fear generalization compared to WT controls, as we detected elevated *freezing* to a context not associated with shock. While WT mice also increased their *freezing* across the session in the novel context (similar to published work[65]), which potentially indicates recognition of shared contextual features in the experimental room, *freezing* was nonetheless greater in mGlu5 HOMO of both sexes than WT controls. Our finding was specific to a novel context, in contrast to previous reports showing increased *freezing* of male mGlu5 HOMO mice to a shock-associated context.[24,66] Generalization of learning between contexts is an adaptive response, enabling learning from one context to inform a novel context; however, overgeneralization can be maladaptive, leading to inaccurate assumptions about the presence of stimuli/reinforcers in novel environments, and limiting the expression of appropriate behavioral responses within those environments.[67] We hypothesize that increased generalization in mGlu5 HOMO mice is distinct from previous reports of fear extinction impairments, which may be linked to perseverative behavior in mGlu5 HOMO mice.[23,24] Rather, generalization of fear learning in mGlu5 HOMO mice may represent a failure to discriminate between contextual stimuli.[67] Supporting this, male mGlu5 HOMO mice show impaired discrimination learning in touchscreen tasks.[23] Poor contextual discrimination may be driven by NMDA receptors, as mGlu5 regulates NMDA receptor signaling,[68] and NMDA activation reduces defensive behaviors, including *freezing* when presented with a non-reinforced stimulus.[69] Considering mGlu5 HOMO mice show developmental dysregulation of NMDA subunit signaling in the prefrontal cortex and hippocampus,[70] it is possible that this could disrupt the development of circuits controlling defensive behaviors in the presence of non-reinforced stimuli. Furthermore, NMDA NR1 receptor deletion in hippocampal dentate gyrus granule cells limits the ability of mice to distinguish between perceptually similar contexts[71] and, considering mGlu5 facilitates hippocampal serine phosphorylation of NR1,[72] it is possible that a reduction in hippocampal NR1 signaling limits differentiation between contexts, which prevents an NMDA-mediated reduction in *freezing* to a non-shocked context. Interestingly, overgeneralization of autobiographical memory can occur in individuals with schizophrenia,[73,74] and our data suggests that one mechanism for overgeneralization may relate to impaired mGlu5 function. Our findings support the growing literature implicating mGlu5 signaling in psychiatric disorders where overgeneralization is evident, including schizophrenia and post-traumatic stress disorder.[75–77] More globally, our data affirm a role for mGlu5 in psychiatric disorders characterized by cognitive impairment, including deficits in inhibition regulation and discrimination learning.

We detected some differences between fear conditioning and fear generalization cohorts. We found lower *freezing* in both genotypes in the fear generalization experiment compared to the fear conditioning experiment, and this may be due to stress resulting from previous behavioral test history.[78] Also, sex did not affect the fear generalization cohort. This may be due to protocol differences. In fear conditioning, we used an auditory tone and shock combination, whereas in fear generalization, we used only a shock, that is, no auditory tone, to mimic published methods.[56,57] Our team has shown that females *freeze* more in response to fear-associated auditory cues than males,[42,79] which may be because female animals can discriminate better than males for discrete cues that are predictive of shock.[80] However, in the absence of predictive auditory cues, sex differences in contextual fear conditioning are absent,[81] although sex differences are dependent on factors such as species, strain, and protocol in contextual and cued fear conditioning (review: Bauer et al.[82]).

Interestingly, we detected a cocaine-affected phenotype in male but not female mGlu5 HOMO mice. We replicated previous work showing greater cocaine environment memory in male mGlu5 HOMO mice compared to WT controls,[27,38] although we also observed locomotor sensitization in male mGlu5 HET and mGlu5 HOMO but not WT mice, in contrast to Bird et al.[27] Interestingly, place preference and locomotor sensitization in female mGlu5 HOMO were not different to WT mice, suggesting that the impact of mGlu5

signaling on drug-associated cues may be modulated by sex. Indeed, while the mGlu5 positive allosteric modulator CDPPB reduces alcohol cue-induced reinstatement in male rats, female rats are unaffected,[83] and parvalbumin-positive interneurons in the prefrontal cortex are more responsive to mGlu5 stimulation in male than female mice.[84] Also, female rats can be susceptible to the effect of mGlu5 receptor antagonism on cocaine seeking, based on estrous cycle.[85] Considering AMPA receptor subunit GluA1 surface expression changes across the course of the estrous cycle, which influences how glutamate targeting drugs reduce cocaine-seeking,[86] it is possible that mGlu5 deletion has a greater impact on cocaine-induced behaviors at different stages of the estrous cycle. Assessment of the estrous cycle in mGlu5 HOMO mice in future studies will illuminate whether female mGlu5 HOMO mice can model cocaine susceptibility.

Some behavioral phenotypes were only evident in female mGlu5 HOMO mice. We detected PPI deficits in female, but not male, mGlu5 HOMO mice. This contrasts with earlier studies showing reduced PPI but unaltered startle in male mGlu5 HOMO mice[19] or when both sexes were combined.[20,87] However, these protocols used lower prepulse intensities (69, 73, 77 dB) than the current study (74, 82, and 86 dB) and employed a fixed 100 ms interstimulus interval compared to our variable 32-256 ms interstimulus interval, and these factors can affect the detection of genotype effects in transgenic mouse lines.[88] Interestingly, in a quinpirole rat model of psychosis, females are more sensitive to improvement of PPI by the mGlu5 positive allosteric modulator CDPPB,[89] which may be linked to greater cortical and hippocampal mGlu5 receptor expression in female than male animals,[90] suggesting sex-dependent mGlu5 expression may influence PPI.

We also largely observed no change to social behaviors or object recognition in mGlu5 HOMO mice. This was not altogether surprising, as previous research has shown inconsistent social phenotypes[22,28] and object recognition phenotypes[91–93] in male mGlu5 HOMO mice. Features such as test modality, duration, objects used, and test construct can affect the expression of behaviors in social[94] and object recognition tests.[95] Our data highlights the importance of assessing social behavior using different behavioral tests (ie, social interaction, social preference test) before drawing conclusions about the social phenotypes of genetic mouse models, and future research can examine resident intruder and social dominance tests to further probe the social phenotype of mGlu5 HOMO mice. Similarly, as hippocampally-mediated spatial memory deficits have been found in mGlu5 HOMO mice,[20,21,27,66] examining novel object location may be relevant for this model.

Finally, we found limited differences between mGlu5 HET and WT mice in both sexes. There were transient reductions to exploratory *rearing* in male and female mGlu5 HET mice compared to WTs, and anxiolytic behavior in female mGlu5 HET mice, but all other behaviors were similar to sex-matched WT controls. The limited phenotype in mGlu5 HET mice may be due to several reasons, including gene expression thresholds, whereby a critical amount of gene expression is required to affect behavior, and one functional allele in mGlu5 HET mice is sufficient for this, or compensatory mechanisms that can be activated through the one functional allele but cannot be engaged in when no functional alleles are present. We conclude that there is limited benefit to assessing mGlu5 HET mice in schizophrenia and drug-reward behavioral paradigms, as they lack the face validity of the mGlu5 HOMO mouse model.

Overall, our findings correspond with the published literature on mGlu5 HET and mGlu5 HOMO mice. However, we greatly expanded on published reports by evaluating novel behavioral paradigms and assessing sex and gene-dose effects. Our findings support a more personalized, sex-stratified approach to schizophrenia and substance use disorder treatment, as some treatments show greater efficacy in women than men, for example, selective estrogen receptor modulators including raloxifene improve cognition in women with schizophrenia more than in men,[96,97] while others are worse, for example, disulfiram is less effective in women than men for cocaine dependence.[98] Future investigation of this genetic mouse model should include the consideration of sex differences, as this has often been neglected in past studies.

Acknowledgments

We would like to thank Ashleigh Deschamps, Nikola Mills, and Amy Woodley at Western Sydney University for animal care and husbandry. We would also like to thank Jerry Tanda for critical comments on the manuscript.

Author Contributions

AH provided the mouse model. R.R.P., R.C., and T.K. designed the research. R.R.P., F.K., and R.C. performed the research and completed the data analysis. R.R.P. and R.C. wrote the manuscript; R.C. revised the manuscript. All authors commented on the manuscript and approved the submission.

Supplementary Material

Supplementary material is available at https://academic.oup.com/schizophreniabulletin.

Funding

R.R.P. is supported by the Ainsworth Medical Research Innovation Fund (AMRIF) as well as Dementia Australia. T.K. and R.C. are supported by NSW Health

and the Ainsworth Medical Research Innovation Fund. T.K. is also supported by a Drug Development Grant from FightMND, R.C. is also supported by the Rebecca Cooper Medical Research Foundation (Project Grant PG2020883), and the Dementia Australia Research Foundation (Project Grant 2022).

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Matosin N, Fernandez-Enright F, Lum JS, Newell KA. Shifting towards a model of mGluR5 dysregulation in schizophrenia: consequences for future schizophrenia treatment. *Neuropharmacology*. 2017;115:73-91. https://doi.org/10.1016/j.neuropharm.2015.08.003
2. Dogra S, Conn PJ. Metabotropic glutamate receptors as emerging targets for the treatment of schizophrenia. *Mol Pharmacol*. 2022;101:275-285. https://doi.org/10.1124/molpharm.121.000460
3. Hamor PU, Knackstedt LA, Schwendt M. The role of metabotropic glutamate receptors in neurobehavioral effects associated with methamphetamine use. *Int Rev Neurobiol*. 2023;168:177-219. https://doi.org/10.1016/bs.irn.2022.10.005
4. Niedzielska-Andres E, Pomierny-Chamiolo L, Andres M, et al. Cocaine use disorder: a look at metabotropic glutamate receptors and glutamate transporters. *Pharmacol Ther*. 2021;221:107797. https://doi.org/10.1016/j.pharmthera.2020.107797
5. Kumar J, Ismail Z, Hatta NH, et al. Alcohol addiction-metabotropic glutamate receptor subtype 5 and its ligands: how they all come together? *Curr Drug Targets*. 2018;19:907-915. https://doi.org/10.2174/1389450118666170511144302
6. Romano C, Sesma MA, McDonald CT, O'Malley K, Van den Pol AN, Olney JW. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J Comp Neurol*. 1995;355:455-469. https://doi.org/10.1002/cne.903550310
7. Shigemoto R, Nomura S, Ohishi H, Sugihara H, Nakanishi S, Mizuno N. Immunohistochemical localization of a metabotropic glutamate receptor, mGluR5, in the rat brain. *Neurosci Lett*. 1993;163:53-57. https://doi.org/10.1016/0304-3940(93)90227-c
8. Abe T, Sugihara H, Nawa H, Shigemoto R, Mizuno N, Nakanishi S. Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca2+ signal transduction. *J Biol Chem*. 1992;267:13361-13368. https://doi.org/10.1016/S0021-9258(18)42219-3
9. Kumar V, Jong YJ, O'Malley KL. Activated nuclear metabotropic glutamate receptor mGlu5 couples to nuclear Gq/11 proteins to generate inositol 1,4,5-trisphosphate-mediated nuclear Ca2+ release. *J Biol Chem*. 2008;283:14072-14083. https://doi.org/10.1074/jbc.M708551200
10. Conn PJ, Pin JP. Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol*. 1997;37:205-237. https://doi.org/10.1146/annurev.pharmtox.37.1.205
11. Hagena H, Manahan-Vaughan D. mGlu5 acts as a switch for opposing forms of synaptic plasticity at mossy fiber-CA3 and commissural associational-CA3 synapses. *J Neurosci*. 2015;35:4999-5006. https://doi.org/10.1523/JNEUROSCI.3417-14.2015
12. Akkus F, Ametamey SM, Treyer V, et al. Marked global reduction in mGluR5 receptor binding in smokers and ex-smokers determined by [11C]ABP688 positron emission tomography. *Proc Natl Acad Sci USA*. 2013;110:737-742. https://doi.org/10.1073/pnas.1210984110
13. Milella MS, Marengo L, Larcher K, et al. Limbic system mGluR5 availability in cocaine dependent subjects: a high-resolution PET [(11)C]ABP688 study. *NeuroImage*. 2014;98:195-202. https://doi.org/10.1016/j.neuroimage.2014.04.061
14. Akkus F, Mihov Y, Treyer V, et al. Metabotropic glutamate receptor 5 binding in male patients with alcohol use disorder. *Transl Psychiatry*. 2018;8:17. https://doi.org/10.1038/s41398-017-0066-6
15. Matosin N, Frank E, Deng C, Huang XF, Newell KA. Metabotropic glutamate receptor 5 binding and protein expression in schizophrenia and following antipsychotic drug treatment. *Schizophr Res*. 2013;146:170-176. https://doi.org/10.1016/j.schres.2013.01.018
16. Matosin N, Fernandez-Enright F, Frank E, et al. Metabotropic glutamate receptor mGluR2/3 and mGluR5 binding in the anterior cingulate cortex in psychotic and nonpsychotic depression, bipolar disorder and schizophrenia: implications for novel mGluR-based therapeutics. *Journal of psychiatry & neuroscience : JPN*. 2014;39:407-416. https://doi.org/10.1503/jpn.130242
17. Matosin N, Newell KA, Quide Y, et al. Effects of common GRM5 genetic variants on cognition, hippocampal volume and mGluR5 protein levels in schizophrenia. *Brain Imaging Behav*. 2018;12:509-517. https://doi.org/10.1007/s11682-017-9712-0
18. Akkus F, Treyer V, Ametamey SM, Johayem A, Buck A, Hasler G. Metabotropic glutamate receptor 5 neuroimaging in schizophrenia. *Schizophr Res*. 2017;183:95-101. https://doi.org/10.1016/j.schres.2016.11.008
19. Brody SA, Dulawa SC, Conquet F, Geyer MA. Assessment of a prepulse inhibition deficit in a mutant mouse lacking mGlu5 receptors. *Mol Psychiatry*. 2004;9:35-41. https://doi.org/10.1038/sj.mp.4001404
20. Gray L, van den Buuse M, Scarr E, Dean B, Hannan AJ. Clozapine reverses schizophrenia-related behaviours in the metabotropic glutamate receptor 5 knockout mouse: association with N-methyl-D-aspartic acid receptor up-regulation. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*. 2009;12:45-60. https://doi.org/10.1017/S1461145708009085
21. Burrows EL, McOmish CE, Buret LS, Van den Buuse M, Hannan AJ. Environmental enrichment ameliorates behavioral impairments modeling schizophrenia in mice lacking metabotropic glutamate receptor 5. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2015;40:1947-1956. https://doi.org/10.1038/npp.2015.44
22. Xu J, Marshall JJ, Kraniotis S, Nomura T, Zhu Y, Contractor A. Genetic disruption of Grm5 causes complex alterations in motor activity, anxiety and social behaviors. *Behav Brain Res*. 2021;411:113378. https://doi.org/10.1016/j.bbr.2021.113378
23. Zeleznikow-Johnston AM, Renoir T, Churilov L, Li S, Burrows EL, Hannan AJ. Touchscreen testing reveals clinically relevant cognitive abnormalities in a mouse model of schizophrenia lacking metabotropic glutamate receptor 5. *Sci Rep*. 2018;8:16412. https://doi.org/10.1038/s41598-018-33929-3
24. Xu J, Zhu Y, Contractor A, Heinemann SF. mGluR5 has a critical role in inhibitory learning. *J Neurosci*. 2009;29, 12:3676-3684. https://doi.org/10.1523/JNEUROSCI.5716-08.2009

25. Chesworth R, Brown RM, Kim JH, Lawrence AJ. The metabotropic glutamate 5 receptor modulates extinction and reinstatement of methamphetamine-seeking in mice. *PLoS One*. 2013;8:e68371. https://doi.org/10.1371/journal.pone.0068371
26. Bird MK, Kirchhoff J, Djouma E, Lawrence AJ. Metabotropic glutamate 5 receptors regulate sensitivity to ethanol in mice. *The International Journal of Neuropsychopharmacology/Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum*. 2008;11:765-774. https://doi.org/10.1017/S1461145708008572
27. Bird MK, Lohmann P, West B, et al. The mGlu5 receptor regulates extinction of cocaine-driven behaviours. *Drug Alcohol Depend*. 2014;137:83-89. https://doi.org/10.1016/j.drugalcdep.2014.01.017
28. Inta D, Vogt MA, Luoni A, et al. Significant increase in anxiety during aging in mGlu5 receptor knockout mice. *Behav Brain Res*. 2013;241:27-31. https://doi.org/10.1016/j.bbr.2012.11.042
29. Ramos-Prats A, Kolldorfer J, Paolo E, Zeidler M, Schmid G, Ferraguti F. An appraisal of the influence of the metabotropic glutamate 5 (mGlu5) receptor on sociability and anxiety. *Front Mol Neurosci*. 2019;12:30. https://doi.org/10.3389/fnmol.2019.00030
30. Arakawa H. From multisensory assessment to functional interpretation of social behavioral phenotype in transgenic mouse models for autism spectrum disorders. *Frontiers in psychiatry*. 2020;11:592408. https://doi.org/10.3389/fpsyt.2020.592408
31. Jew CP, Wu CS, Sun H, et al. mGluR5 ablation in cortical glutamatergic neurons increases novelty-induced locomotion. *PLoS One*. 2013;8:e70415. https://doi.org/10.1371/journal.pone.0070415
32. Halberstadt AL, Lehmann-Masten VD, Geyer MA, Powell SB. Interactive effects of mGlu5 and 5-HT2A receptors on locomotor activity in mice. *Psychopharmacology.* 2011;215:81-92. https://doi.org/10.1007/s00213-010-2115-1
33. Luoni A, Gass P, Brambilla P, Ruggeri M, Riva MA, Inta D. Altered expression of schizophrenia-related genes in mice lacking mGlu5 receptors. *Eur Arch Psychiatry Clin Neurosci*. 2018;268:77-87. https://doi.org/10.1007/s00406-016-0728-z
34. Mendrek A, Mancini-Marie A. Sex/gender differences in the brain and cognition in schizophrenia. *Neurosci Biobehav Rev*. 2015;67:57-78. https://doi.org/10.1016/j.neubiorev.2015.10.013
35. McHugh RK, Votaw VR, Sugarman DE, Greenfield SF. Sex and gender differences in substance use disorders. *Clin Psychol Rev*. 2018;66:12, 12-23. https://doi.org/10.1016/j.cpr.2017.10.012
36. Hill RA. Sex differences in animal models of schizophrenia shed light on the underlying pathophysiology. *Neurosci Biobehav Rev*. 2016;67:41-56. https://doi.org/10.1016/j.neubiorev.2015.10.014
37. Maddern XJ, Walker LC, Anversa RG, Lawrence AJ, Campbell EJ. Understanding sex differences and the translational value of models of persistent substance use despite negative consequences. *Neurobiol Learn Mem*. 2024;213:107944. https://doi.org/10.1016/j.nlm.2024.107944
38. Olsen CM, Childs DS, Stanwood GD, Winder DG. Operant sensation seeking requires metabotropic glutamate receptor 5 (mGluR5). *PLoS One*. 2010;5:e15085. https://doi.org/10.1371/journal.pone.0015085
39. Barabas AJ, Soini HA, Novotny MV, et al. Compounds from plantar foot sweat, nesting material, and urine show strain patterns associated with agonistic and affiliative behaviors in group housed male mice, Mus musculus. *PLoS One*. 2021;16:e0251416. https://doi.org/10.1371/journal.pone.0251416
40. Arakawa H. Revisiting sociability: factors facilitating approach and avoidance during the three-chamber test. *Physiol Behav*. 2023;272:114373. https://doi.org/10.1016/j.physbeh.2023.114373
41. Kreilaus F, Chesworth R, Eapen V, Clarke R, Karl T. First behavioural assessment of a novel Immp2l knockdown mouse model with relevance for Gilles de la Tourette syndrome and autism spectrum disorder. *Behav Brain Res*. 2019;374:112057. https://doi.org/10.1016/j.bbr.2019.112057
42. Guerra S, Chesworth R, Weickert CS, Karl T. Effects of handling on the behavioural phenotype of the neuregulin 1 type III transgenic mouse model for schizophrenia. *Behav Brain Res*. 2021;405:113166. https://doi.org/10.1016/j.bbr.2021.113166
43. Karl T, Duffy L, Scimone A, Harvey RP, Schofield PR. Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. *Genes Brain Behav*. 2007;6:677-687. https://doi.org/10.1111/j.1601-183X.2006.00298.x
44. Leger M, Quiedeville A, Bouet V, et al. Object recognition test in mice. *Nat Protoc*. 2013;8:2531-2537. https://doi.org/10.1038/nprot.2013.155
45. Rajagopal L, Massey BW, Huang M, Oyamada Y, Meltzer HY. The novel object recognition test in rodents in relation to cognitive impairment in schizophrenia. *Curr Pharm Des*. 2014;20:5104-5114. https://doi.org/10.2174/1381612819666131216114240
46. Green MF, Horan WP, Lee J. Social cognition in schizophrenia. *Nat Rev Neurosci*. 2015;16:620-631. https://doi.org/10.1038/nrn4005
47. Bellack AS, Morrison RL, Wixted JT, Mueser KT. An analysis of social competence in schizophrenia. *Br J Psychiatry*. 1990;156:809-818. https://doi.org/10.1192/bjp.156.6.809
48. Swerdlow NR, Caine SB, Braff DL, Geyer MA. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. *J Psychopharmacol*. 1992;6:176-190. https://doi.org/10.1177/026988119200600210
49. Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology.* 2001;156:234-258. https://doi.org/10.1007/s002130100810
50. Swerdlow NR, Light GA, Sprock J, et al. Deficient prepulse inhibition in schizophrenia detected by the multi-site COGS. *Schizophr Res*. 2014;152:503-512. https://doi.org/10.1016/j.schres.2013.12.004
51. San-Martin R, Castro LA, Menezes PR, Fraga FJ, Simoes PW, Salum C. Meta-analysis of sensorimotor gating deficits in patients with schizophrenia evaluated by prepulse inhibition test. *Schizophr Bull*. 2020;46:1482-1497. https://doi.org/10.1093/schbul/sbaa059
52. Chesworth R, Rosa-Porto R, Yao S, Karl T. Sex-specific sensitivity to methamphetamine-induced schizophrenia-relevant behaviours in neuregulin 1 type III overexpressing mice. *J Psychopharmacol*. 2021;35:50-64. https://doi.org/10.1177/0269881120967870
53. Tuominen L, Romaniuk L, Milad MR, Goff DC, Hall J, Holt DJ. Impairment in acquisition of conditioned fear in schizophrenia. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology.* 2022;47:681-686. https://doi.org/10.1038/s41386-021-01193-1
54. Feola B, Moussa-Tooks AB, Sheffield JM, Heckers S, Woodward ND, Blackford JU. Threat responses in schizophrenia:

a negative valence systems framework. *Curr Psychiatry Rep*. 2024;26:9-25. https://doi.org/10.1007/s11920-023-01479-9

55. Holt DJ, Lebron-Milad K, Milad MR, et al. Extinction memory is impaired in schizophrenia. *Biol Psychiatry*. 2009;65:455-463. https://doi.org/10.1016/j.biopsych.2008.09.017
56. Fujinaka A, Li R, Hayashi M, et al. Effect of context exposure after fear learning on memory generalization in mice. *Mol. Brain.* 2016;9:2. https://doi.org/10.1186/s13041-015-0184-0
57. Bergstrom HC. Assaying fear memory discrimination and generalization: methods and concepts. *Curr Protoc Neurosci*. 2020;91:e89. https://doi.org/10.1002/cpns.89
58. Huston JP, Silva MA, Topic B, Muller CP. What's conditioned in conditioned place preference? *Trends Pharmacol Sci*. 2013;34:162-166. https://doi.org/10.1016/j.tips.2013.01.004
59. Chesworth R, Karl T. Cannabidiol (CBD) reduces cocaine-environment memory in mice. *Pharmacol Biochem Behav*. 2020;199:173065. https://doi.org/10.1016/j.pbb.2020.173065
60. Chesworth R, Visini G, Karl T. Impaired extinction of operant cocaine in a genetic mouse model of schizophrenia risk. *Psychopharmacology.* 2023;240:1531-1546. https://doi.org/10.1007/s00213-023-06386-8
61. Chesworth R, Yim HC-H, Watt G, El-Omar E, Karl T. Cannabidiol (CBD) facilitates cocaine extinction and ameliorates cocaine-induced changes to the gut microbiome in male C57BL/6JArc mice. *Prog Neuro-Psychopharmacol Biol Psychiatry*. 2024;133:111014. https://doi.org/10.1016/j.pnpbp.2024.111014
62. Lakens D. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for *t*-tests and ANOVAs. *Front Psychol*. 2013;4:863. https://doi.org/10.3389/fpsyg.2013.00863
63. Bird MK, Reid CA, Chen F, Tan HO, Petrou S, Lawrence AJ. Cocaine-mediated synaptic potentiation is absent in VTA neurons from mGlu5-deficient mice. *The International Journal of Neuropsychopharmacology/Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum*. 2010;13:133-141. https://doi.org/10.1017/S1461145709990162
64. Wu CS, Jew CP, Sun H, Ballester Rosado CJ, Lu HC. mGlu5 in GABAergic neurons modulates spontaneous and psychostimulant-induced locomotor activity. *Psychopharmacology.* 2020;237:345-361. https://doi.org/10.1007/s00213-019-05367-0
65. Radulovic J, Kammermeier J, Spiess J. Generalization of fear responses in C57BL/6N mice subjected to one-trial foreground contextual fear conditioning. *Behav Brain Res*. 1998;95:179-189. https://doi.org/10.1016/s0166-4328(98)00039-4
66. Lu YM, Jia Z, Janus C, et al. Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *J Neurosci*. 1997;17:5196-5205. https://doi.org/10.1523/JNEUROSCI.17-13-05196.1997
67. Asok A, Kandel ER, Rayman JB. The neurobiology of fear generalization. *Front Behav Neurosci*. 2019;12:329. https://doi.org/10.3389/fnbeh.2018.00329
68. Homayoun H, Stefani MR, Adams BW, Tamagan GD, Moghaddam B. Functional interaction between NMDA and mGlu5 receptors: effects on working memory, instrumental learning, motor behaviors, and dopamine release. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology.* 2004;29:1259-1269. https://doi.org/10.1038/sj.npp.1300417
69. Vieira PA, Corches A, Lovelace JW, Westbrook KB, Mendoza M, Korzus E. Prefrontal NMDA receptors expressed in excitatory neurons control fear discrimination and fear extinction. *Neurobiol Learn Mem*. 2015;119:52-62. https://doi.org/10.1016/j.nlm.2014.12.012
70. Imbriglio T, Verhaeghe R, Antenucci N, et al. Developmental up-regulation of NMDA receptors in the prefrontal cortex and hippocampus of mGlu5 receptor knock-out mice. *Mol. Brain.* 2021;14:77. https://doi.org/10.1186/s13041-021-00784-9
71. McHugh TJ, Jones MW, Quinn JJ, et al. Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. *Science.* 2007;317:94-99. https://doi.org/10.1126/science.1140263
72. Takagi N, Besshoh S, Morita H, Terao M, Takeo S, Tanonaka K. Metabotropic glutamate mGlu5 receptor-mediated serine phosphorylation of NMDA receptor subunit NR1 in hippocampal CA1 region after transient global ischemia in rats. *Eur J Pharmacol*. 2010;644:96-100. https://doi.org/10.1016/j.ejphar.2010.07.026
73. Nieto M, Latorre JM, Garcia-Rico MA, Hernandez-Viadel JV, Ros L, Ricarte JJ. Autobiographical memory specificity across life periods in people with schizophrenia. *J Clin Psychol*. 2019;75:1011-1021. https://doi.org/10.1002/jclp.22746
74. Zhang Y, Kuhn SK, Jobson L, Haque S. A review of autobiographical memory studies on patients with schizophrenia spectrum disorders. *BMC psychiatry*. 2019;19:361. https://doi.org/10.1186/s12888-019-2346-6
75. Schwendt M, Shallcross J, Hadad NA, et al. A novel rat model of comorbid PTSD and addiction reveals intersections between stress susceptibility and enhanced cocaine seeking with a role for mGlu5 receptors. *Transl Psychiatry*. 2018;8:209. https://doi.org/10.1038/s41398-018-0265-9
76. Shallcross J, Hamor P, Bechard AR, Romano M, Knackstedt L, Schwendt M. The divergent effects of CDPPB and cannabidiol on fear extinction and anxiety in a predator scent stress model of PTSD in rats. *Front Behav Neurosci*. 2019;13:91. https://doi.org/10.3389/fnbeh.2019.00091
77. Asch RH, Fowles K, Pietrzak RH, Taylor JR, Esterlis I. Examining mGlu5 receptor availability as a predictor of vulnerability to PTSD: an [(18)F]FPEB and PET study in male and female rats. *Chronic Stress (Thousand Oaks) Jan-Dec*. 2023;7:24705470231215001. https://doi.org/10.1177/24705470231215001
78. von Kortzfleisch VT, Kastner N, Prange L, Kaiser S, Sachser N, Richter SH. Have I been here before? Complex interactions of age and test experience modulate the results of behavioural tests. *Behav Brain Res*. 2019;367:143-148. https://doi.org/10.1016/j.bbr.2019.03.042
79. Guerra S, Chung R, Yerbury J, Karl T. Behavioural effects of cage systems on the G93A superoxide dismutase 1 transgenic mouse model for amyotrophic lateral sclerosis. *Genes Brain Behav*. 2021;20:e12735. https://doi.org/10.1111/gbb.12735
80. Day HLL, Reed MM, Stevenson CW. Sex differences in discriminating between cues predicting threat and safety. *Neurobiol Learn Mem*. 2016;133:196-203. https://doi.org/10.1016/j.nlm.2016.07.014
81. Matsuda S, Tohyama S, Mizutani A. Sex differences in the effects of adult short-term isolation rearing on contextual fear memory and extinction. *Neurosci Lett*. 2018;687:119-123. https://doi.org/10.1016/j.neulet.2018.09.030
82. Bauer EP. Sex differences in fear responses: neural circuits. *Neuropharmacology.* 2023;222:109298. https://doi.org/10.1016/j.neuropharm.2022.109298

83. Wills LJ, Schwartz B, McGuffin B, Gass JT. Combined stress and alcohol exposure: synergistic effects on alcohol-seeking behaviors and neuroinflammation. *J Pharmacol Exp Ther*. 2025;392:103386. https://doi.org/10.1016/j.jpet.2025.103386
84. Fabian CB, Jordan ND, Cole RH, et al. Parvalbumin interneuron mGlu(5) receptors govern sex differences in prefrontal cortex physiology and binge drinking. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*. 2024;49:1861-1871. https://doi.org/10.1038/s41386-024-01889-0
85. Corbett CM, Miller END, Loweth JA. mGlu5 inhibition in the basolateral amygdala prevents estrous cycle-dependent changes in cue-induced cocaine seeking. *Addict Neurosci*. 2023;5:100055. https://doi.org/10.1016/j.addicn.2022.100055
86. Bechard AR, Hamor PU, Schwendt M, Knackstedt LA. The effects of ceftriaxone on cue-primed reinstatement of cocaine-seeking in male and female rats: estrous cycle effects on behavior and protein expression in the nucleus accumbens. *Psychopharmacology*. 2018;235:837-848. https://doi.org/10.1007/s00213-017-4802-7
87. Chen HH, Stoker A, Markou A. The glutamatergic compounds sarcosine and N-acetylcysteine ameliorate prepulse inhibition deficits in metabotropic glutamate 5 receptor knockout mice. *Psychopharmacology*. 2010;209:343-350. https://doi.org/10.1007/s00213-010-1802-2
88. Karl T, Burne THJ, Van den Buuse M, Chesworth R. Do transmembrane domain neuregulin 1 mutant mice exhibit a reliable sensorimotor gating deficit? *Behav Brain Res*. 2011;223:336-341. https://doi.org/10.1016/j.bbr.2011.04.051
89. Cuozzo AM, Peeters LD, Ahmed CD, Wills LJ, Gass JT, Brown RW. Investigation of the roles of the adenosine a(2A) and metabotropic glutamate receptor type 5 (mGlu5) receptors in Prepulse inhibition and CREB Signaling in a heritable rodent model of psychosis. *Cells*. 2025;14:182. https://doi.org/10.3390/cells14030182
90. Wang Y, Ma Y, Hu J, et al. Prenatal chronic mild stress induces depression-like behavior and sex-specific changes in regional glutamate receptor expression patterns in adult rats. *Neuroscience*. 2015;301:363-374. https://doi.org/10.1016/j.neuroscience.2015.06.008
91. Mesic I, Guzman YF, Guedea AL, et al. Double dissociation of the roles of metabotropic glutamate receptor 5 and oxytocin receptor in discrete social behaviors. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*. 2015;40:2337-2346. https://doi.org/10.1038/npp.2015.81
92. Salomons AR, Pinzon NE, Boleij H, et al. Differential effects of diazepam and MPEP on habituation and neuro-behavioural processes in inbred mice. *Behav Brain Funct*. 2012;8:30. https://doi.org/10.1186/1744-9081-8-30
93. Horio M, Fujita Y, Hashimoto K. Therapeutic effects of metabotropic glutamate receptor 5 positive allosteric modulator CDPPB on phencyclidine-induced cognitive deficits in mice. *Fundam Clin Pharmacol*. 2013;27:483-488. https://doi.org/10.1111/j.1472-8206.2012.01045.x
94. Powell SB, Swerdlow NR. The relevance of animal models of social isolation and social motivation for understanding schizophrenia: review and future directions. *Schizophr Bull*. 2023;49:1112-1126. https://doi.org/10.1093/schbul/sbad098
95. Ennaceur A, Silva MAdS. Handbook of object novelty recognition. *Handbook of Behavioral Neuroscience*, 27. Elsevier, Academic Press, London, UK; 2018. https://doi.org/10.1016/B978-0-12-812012-5.00001-X.
96. Fernando P, Sommer IEC, Hasan A. Do we need sex-oriented clinical practice guidelines for the treatment of schizophrenia? *Curr Opin Psychiatry*. 2020;33:192-199. https://doi.org/10.1097/YCO.0000000000000597
97. Moniem I, Kafetzopoulos V. Sex differences in schizophrenia: symptomatology, treatment efficacy and adverse effects. *Frontiers in psychiatry*. 2025;16:1594334. https://doi.org/10.3389/fpsyt.2025.1594334
98. DeVito EE, Babuscio TA, Nich C, Ball SA, Carroll KM. Gender differences in clinical outcomes for cocaine dependence: randomized clinical trials of behavioral therapy and disulfiram. *Drug Alcohol Depend*. 2014;145:156-167. https://doi.org/10.1016/j.drugalcdep.2014.10.007

Schizophrenia Bulletin vol. 00 no. 00 pp. 1–16, 2025
https://doi.org/10.1093/schbul/sbaf161
Regular Article