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Related functions of mGlu4 and mGlu8

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Abstract

Metabotropic glutamate receptors modulate glutamatergic and GABAergic neurotransmission. Our previous pharmacological data indicate that metabotropic receptor 4 (mGlu4) and metabotropic receptor 8 (mGlu8) might have related and overlapping functions. We explored this by analyzing the behavioral phenotypes of mice deficient in either (mGlu4^{-/-} or mGlu8^{-/-}) or both receptors (mGlu4/8^{-/-}). Our analysis focused on measures of anxiety in the open field and elevated zero maze, sensorimotor function on the rotorod, and fear conditioning, as mGlu4 and/or mGlu8 were shown to affect performance in these tests. mGlu8^{-/-} mice weighed more than mGlu4/8^{-/-} mice. In the open field, mGlu4^{-/-} mice showed lower levels of anxiety than mGlu8^{-/-} and mGlu4/8^{-/-} mice. In the elevated zero maze, mGlu4^{-/-} mice showed lower levels of anxiety than wild-type, mGlu8^{-/-} and mGlu4/8^{-/-} mice. In the open field, but not elevated zero maze, mGlu4^{-/-} mice showed lower activity levels than wild-type, mGlu8^{-/-} and mGlu4/8^{-/-} mice. mGlu4/8^{-/-} female mice showed less contextual freezing than wild-type and mGlu4^{-/-} female mice and there was a trend toward less freezing in male mGlu4/8^{-/-} than wild-type male mice. There were no genotype differences in cued fear conditioning. There were significant negative correlations between body weight and fall latency on the rotorod in wild-type, mGlu8^{-/-} and mGlu4/8^{-/-}, but not mGlu4^{-/-}, mice. These data suggest related functions of mGlu4 and mGlu8 in behavioral performance.

Keywords

mGlu4; mGlu8; open field; zero maze; fear conditioning; rotorod; body weight

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Conflict of Interest

No competing financial conflicts exist.

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1. Introduction

Ionotropic glutamate receptors mediate the fast actions of the excitatory neurotransmitter glutamate. In contrast, metabotropic glutamate receptors (mGluRs) modulate glutamatergic and GABAergic neurotransmission (Conn and Pin, 1997; Pin and Duvoisin, 1995). With the exception of mGlu6, which is only expressed in the retina, group III receptors (mGlu4, mGlu7, and mGlu8) are generally located presynaptically and regulate neurotransmitter release (Cartmell and Schoepp, 2000).

mGluRs are targets for therapies aimed at treating anxiety disorders (Niswender and Conn, 2010; Swanson et al., 2005). For example, acute pharmacological modulation using the mGlu8 positive allosteric modulator (PAM) AZ12216052 (Duvoisin et al., 2010a) in wild-type mice reduced these measures of anxiety (Duvoisin et al., 2010a). We have shown that mGlu-induced depression of transmission by AZ12216052 in the bed nucleus of the stria terminalis (BNST) is mediated by mGlu8, but not mGlu4 (Gosnell et al., 2011). However, AZ12216052 also reduced measures of anxiety in mGlu8^{-/-} mice (Duvoisin et al., 2011). This indicates that another receptor is involved in the regulation of anxiety measures by AZ12216052. This receptor is likely mGlu4. mGlu4 was positive in a drug screen assay that identified AZ12216052. In addition, the mGlu4 allosteric agonist VU0155041 reduced measures of anxiety in wild-type mice (Duvoisin et al., 2011). This is consistent with other studies showing the role of mGlu4 in the regulation of anxiety (Wieroska et al., 2010). These data indicate that mGlu4 and mGlu8 might have related functions. For example, mGlu4 might compensate for mGlu8 deficiency and deficiency of both receptors might result in a more pronounced phenotype than deficiency of either receptor alone. In this study, we explored these possibilities by analyzing the behavioral phenotypes of mice deficient in either or both receptors. Our analysis focused on measures of anxiety in the open field and elevated zero maze, sensorimotor function on the rotarod, and fear conditioning, as mGlu4 and/or mGlu8 were shown to affect performance in these tests (Davis et al., 2012; Duvoisin et al., 2010a; Duvoisin et al., 2011; Duvoisin et al., 2010b; Duvoisin et al., 2005). We selected 12-month-old mice, as the behavioral phenotype of mGlu4^{-/-} was more profound at 12 than 6 months of age (Davis et al., 2012).

2. Materials and methods

2.1. Animals

C57Bl6/J wild-type mice and mGlu4^{-/-} mice from Jackson Laboratory (stock #003619) and mGlu8^{-/-} mice were bred to generate the mice for the current study. Experimentally naïve 12-month-old female and male mice were used. All tested mice were littermates originating from the same breeding pairs. The total number of mice was 72, with 34 males (wild-type mice $n = 10$, mGlu4^{-/-} $n = 10$, mGlu8^{-/-} $n = 8$, mGlu4/8^{-/-} $n = 6$) and 38 females (wild-type mice $n = 13$, mGlu4^{-/-} $n = 8$, mGlu8^{-/-} $n = 8$, mGlu4/8^{-/-} $n = 9$). Animals were group housed in standard shoebox cages until three days prior to the start of behavioral testing. Then they were singly housed for the duration of the experiment. The mice were maintained on a 12:12 light/dark schedule, while standard laboratory chow (PicoLab Rodent diet 20, # 5053; PMI Nutrition International, St. Louis, MO, USA) and water was provided *ad libitum*. Experimenters were blinded to the genotype of the animals. All procedures were conducted in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee at Oregon Health & Science University. Mice were weighed and subsequently tested in exploratory activity and measures of anxiety in the open field (day 1) and elevated zero maze (day 2), for sensorimotor function on the rotarod (days 3–5), and for contextual and cued fear conditioning (days 8–9). Due to technical problems, we did not have body weight records for 2 wild-type mice, 3 mGlu4^{-/-} mice, 2 mGlu8^{-/-} mice, and 2 mGlu4/8^{-/-} mice.

2.2. Open Field

Exploratory and anxiety-like behaviors were first assessed in the open-field. The light intensity in the center of the open field was 100 lux. Mice were placed into a square arena (40.6 × 40.6 cm) and allowed to explore for 10 minutes. They were tested between 10 am and 2 pm. Behavioral performance was tracked and scored using an automated video system (Ethovision 7.0 XT, Noldus, Sterling VA). Exploratory behavior was analyzed using total distance moved (cm). Time spent in the more anxiety-provoking center of the open field was analyzed as well.

2.3. Elevated Zero Maze

Measures of anxiety were also assessed in the elevated zero-maze. The enclosure (Kinder Scientific, Poway, CA) consisted of four sections (6 cm wide), alternating between open and closed sections. Mice were placed into an open area of the maze and allowed to explore for 10 minutes. The mice were tested between 10 am and 2 pm. An automated photobeam detection method (Kinder Motor-Monitor software, Kinder Scientific, Poway, CA) was used to track mouse movements: distance moved (cm), time spent in the open and closed areas as well as crossings between the open and closed areas were analyzed.

2.4. Rotarod

Sensorimotor performance was assessed on a rotarod. Mice were placed on an elevated rotating rod (diameter: 3 cm, elevated: 45 cm, Rotamex-5, Columbus Instruments, Columbus, OH, USA), initially rotating at 5.0 rpm. The rod accelerated 1.0 rpm every 3 seconds. A line of photobeams beneath the rod recorded the latency to fall (seconds). Each mouse received three trials per day, with no delay between trials, on three consecutive days.

2.5. Fear Conditioning

During Pavlovian fear conditioning mice learn to associate a conditioned stimulus (CS, e.g. the environmental context, or a discrete cue) with a mild foot-shock (unconditioned stimulus, US). CS-US pairings are preceded by a short habituation period, during which a baseline measure of locomotor activity is analyzed. Contextual fear conditioning is considered to involve complex polymodal processing, and to be hippocampus- and amygdala-dependent, while fear conditioning to a modality-specific cue (e.g. a tone) is considered to be hippocampus independent (Phillips and LeDoux, 1992). Freezing, defined as immobility with the exception of respiration, is considered a post-exposure fear response (Fanselow, 1994), and is a widely used indicator of conditioned fear.

Mice were trained and tested using a Med Associates mouse fear conditioning system and VideoFreeze automated scoring system (Med Associates, St. Albans, Vermont), previously described in detail and validated against traditional hand scoring methods (Anagnostaras et al., 2010). On day 1, the mice were placed inside a dark fear-conditioning chamber. Chamber lights (at 100 lux) turned on at zero seconds, followed by a 120-second habituation period and a subsequent 10-second (2800 Hz, 80 dB) tone (cue). A 2-second 0.35 mA foot-shock was administered at 128 seconds, co-terminating with the tone at 130 seconds. After a 20-second inter-stimulus-interval the 10-second tone, which co-terminated with the 2-second shock, was repeated. After the second shock, lights remained on for an additional 20 seconds, for a total trial length of four minutes and 30 seconds. On day 2, hippocampus dependent associative learning was assessed during re-exposure to the training environment for 600 seconds. Three hours later, mice were exposed to a modified environment (scented with vanilla extract, cleaned with 10% isopropanol instead of 0.5% glacial acetic acid, novel floor texture covering the shock-grid, and rounded walls). They were allowed to habituate for 180 seconds, and then exposed to the cue for a second period of 180 seconds.

Associative learning was measured as the percent time spent freezing in response to the contextual environment or the tone. Immediate acquisition of conditioned fear was measured following CS-US pairings. Motion during shock (proprietary index, Med Associates) was measured to account for potential differences in response to the shock during training.

2.6. Statistical Analysis

Data are reported as means $\pm$ the standard error of the mean (SEM) and were analyzed using SPSS 16.0 (IBM, Armonk, NY, USA) and GraphPad Prism 5 (San Diego, CA) software. Results were considered significant at an α level of 0.05. Two-way ANOVAs were conducted with genotype and sex as between subject factors, followed by a Tukey post hoc test when appropriate. Correlations were assessed using the Pearson Test.

3. Results

3.1. Rotorod and body weights

A two-way ANOVA was conducted to examine the effect of sex and genotype on body weight of mice. There was a significant main effect of sex ($F(1, 62) = 45.279, p < 0.001$) and genotype ($F(1, 62) = 2.858, p = 0.045$) (Fig. 1A), but no significant interaction between the two independent variables ($F(1, 62) = 1.312, p = 0.280$). As expected, males (36.6 ± 0.9 g) were heavier than females (29.2 ± 0.7 g). Post-hoc comparisons using the Tukey HSD Test indicated that the mGlu8^{-/-} mice (35.8 ± 1.3 g) weighed more than mGlu4^{-/-} mice (31.5 ± 1.1 g, $p < 0.05$) and mGlu4/8^{-/-} mice (31.2 ± 2.4 g, $p < 0.05$) but only marginally more than wild-type mice (32.1 ± 1.1 g, $p = 0.068$).

For rotorod performance, there was a sex difference ($F(1, 71) = 21.985, p < 0.001$) showing better performance of females than males (mean fall latency; females: 31.1 ± 1.6 s; males: 21.0 ± 1.3 s). In addition, there was a trend towards an effect of genotype ($F(1, 71) = 2.667, p = 0.055$) with a better performance in wild-type (36.0 ± 2.5) than mGlu4^{-/-} (27.4 ± 3.9) mice ($p < 0.05$). There were significant negative correlations between body weight and fall latency on the rotorod in wild-type ($r^2 = 0.5205, p = 0.0002, n = 21$ data points, Fig. 1B), mGlu8^{-/-} ($r^2 = 0.6449, p = 0.0005, n = 14$ data points, Fig. 1D) and mGlu4/8^{-/-} ($r^2 = 0.5615, p = 0.0032, n = 13$ data points, Fig. 1E) mice. In contrast, in mGlu4^{-/-} mice there was no correlation between body weight and fall latency on the rotorod (Fig. 1C).

3.2. Open field and elevated zero maze

There was an effect of genotype on time spent in the center of the open field ($F(1, 70) = 6.023, p = 0.001$, Fig. 2A). mGlu4^{-/-} spent more time in the center of the open field than mGlu8^{-/-} ($p = 0.024$) and mGlu4/8^{-/-} ($p = 0.001$) mice. There were no significant differences between wild-type and mutant mice. There was also an effect of genotype on total distance moved in the open field ($F(1, 70) = 4.259, p = 0.008$, Fig. 2B) and a trend towards a sex difference ($F(1, 70) = 3.588, p = 0.063$). In the open field, mGlu4^{-/-} showed lower activity levels than wild-type ($p = 0.047$), mGlu8^{-/-} ($p = 0.016$) and mGlu4/8^{-/-} ($p = 0.013$) mice (Fig. 2B).

In the open field, there was also an effect of genotype on entries into the center ($F(1, 70) = 3.944, p = 0.012$, not shown). mGlu4^{-/-} mice entered the center of the open field more than mGlu4/8^{-/-} mice ($p = 0.018$) and there was a trend towards wild-type mice entering the center of the open field more than mGlu4/8^{-/-} mice ($p = 0.058$). In addition, there was an effect of genotype on distance moved in the center of the open field ($F(1, 70) = 2.984, p = 0.038$, not shown). mGlu4^{-/-} mice moved more in the center of the open field more than mGlu4/8^{-/-} mice ($p = 0.033$).

Consistent with the open field data, there was an effect of genotype on time spent in the open areas of the elevated zero maze ($F(1, 70) = 8.575, p < 0.001$, Fig. 2C). mGlu4^{-/-} mice spent more time in the open areas of the elevated zero maze than wild-type ($p = 0.020$), mGlu8^{-/-} ($p = 0.009$) and mGlu4/8^{-/-} ($p < 0.001$) mice. In contrast, there were no genotype differences in activity levels in the elevated zero maze (Fig. 2D). While mGlu4^{-/-} mice showed reduced measures of anxiety in the elevated zero maze, there were no genotype differences in activity in this maze, so genotype differences in measures of anxiety are seen in the absence of genotype differences in activity levels.

3.3. Fear conditioning

For baseline motion prior to the first tone, there were effects of sex ($F(1, 67) = 8.702, p = 0.005$), and a genotype x sex interaction ($F(1, 67) = 4.410, p = 0.007$), and a trend towards a genotype effect ($F(1, 67) = 2.280, p = 0.089$). Therefore, the female and male data were analyzed separately. There was an effect of genotype on baseline motion in female mice ($F(1, 33) = 3.320, p = 0.033$) and a trend towards a genotype differences in baseline motion on day prior to the first tone in male mice ($F(1, 33) = 2.841, p = 0.054$) (Fig. 3A). mGlu4^{-/-} female mice moved less than mGlu4/8^{-/-} mice (Fig. 3A, $p = 0.035$), and there was a trend towards less activity than wild-type mice ($p = 0.053$). In addition, there was an effect of genotype to the velocity during the shock (average of the two shocks) ($F(1, 33) = 8.391, p < 0.001$, not shown) in female mice. Wild-type ($p < 0.01$) and mGlu8^{-/-} ($p < 0.001$) mice showed a lower response to shock than mGlu4/8^{-/-} mice and there was a trend towards a lower response to shock than mGlu4^{-/-} mice ($p = 0.056$). There was also an effect of genotype to the velocity during the shock (average of the two shocks) ($F(1, 33) = 3.254, p = 0.035$) in male mice. mGlu8^{-/-} and ($p < 0.05$) wild-type ($p < 0.05$) mice showed a lower response to shock than mGlu4^{-/-} mice.

On day 2, the mice were first tested for contextual fear conditioning. There were effects of genotype on contextual freezing in female mice ($F(1, 33) = 5.426, p = 0.004$, Fig. 3B). mGlu4/8^{-/-} mice showed less freezing than wild-type ($p = 0.011$), mGlu4^{-/-} mice ($p = 0.005$) and there was a trend towards less freezing than in mGlu8^{-/-} mice ($p = 0.054$). There was also an effect of genotype on contextual freezing in male mice ($F(1, 33) = 3.369, p = 0.031$, Fig. 3B). mGlu8^{-/-} mice ($p = 0.061$) and mGlu4/8^{-/-} mice ($p = 0.052$) showed trends towards less freezing than wild-type mice.

In contrast to contextual freezing, there was no effect of genotype on cued freezing in female or male mice (Fig. 3C).

4. Discussion

In this study, we behaviorally analyzed wild-type, mGlu4^{-/-}, mGlu8^{-/-}, and mGlu4/8^{-/-} mice. In the open field and elevated zero maze, measures of anxiety in mGlu4/8^{-/-} mice were more like those in mGlu8^{-/-} than mGlu4^{-/-} mice. In the open field, activity levels of mGlu4/8^{-/-} mice were more like those of mGlu8^{-/-} than mGlu4^{-/-} mice. These data indicate related but opposite roles for mGlu4 and mGlu8 in regulation of anxiety in the open field and elevated zero maze and activity in the open field. Compared to mGlu4 deficiency, mGlu8 deficiency is dominant in affecting measures of anxiety and distance moved in the open field and measures of anxiety in the elevated zero maze. In addition, in male mice the contextual freezing levels in mGlu4/8^{-/-} were more similar to those of mGlu8^{-/-} than mGlu4^{-/-} mice. Thus, there was a dominant effect of mGlu8 deficiency on contextual fear conditioning in male mice. In contrast, compared to mGlu8 deficiency, mGlu4 deficiency was dominant in affecting body weights in female mice and response to shock in the fear conditioning test in female and male mice. Finally, there were significant negative correlations between body weight and fall latency on the rotarod in wild-type, mGlu8^{-/-},

and mGlu4/8^{-/-}, but not in mGlu4^{-/-}, mice. Together, these data suggest related functions mGlu4 and mGlu8 in behavioral performance.

In the open field and elevated zero maze, mGlu4^{-/-} mice showed lower levels of anxiety and spent more time in the center of the open field and open areas of the elevated zero maze than mGlu8^{-/-} and mGlu4/8^{-/-} mice. In addition, in the elevated zero maze, mGlu4^{-/-} mice showed lower levels of anxiety than wild-type mice. Because mGlu4^{-/-} mice showed lower activity levels than wild-type, mGlu8^{-/-}, and mGlu4/8^{-/-} mice in the open field but not elevated zero maze, differences in activity levels alone cannot account for the genotype differences seen in measures of anxiety. In addition, mGlu4^{-/-} female mice showed higher contextual fear conditioning than mGlu4/8^{-/-} female mice, a trend towards higher contextual fear conditioning than mGlu8^{-/-} female mice, and no differences in cued fear conditioning. Contextual fear conditioning is considered to be hippocampus- and amygdala-dependent, while cued fear conditioning is considered to be amygdala-dependent but hippocampus independent (Phillips and LeDoux, 1992). Since performance in anxiety tests such as the elevated zero maze is hippocampus-independent (Deacon et al., 2002), these data indicate that in mGlu4^{-/-} female mice generalized anxiety did not contribute to the enhanced contextual fear conditioning.

Compared to mGlu4 deficiency, mGlu8 deficiency was dominant in affecting measures of anxiety in the open field and the elevated zero maze. Age-related changes in middle-aged mice might be important for revealing the relationship between mGlu4 and mGlu8 with regard to the anxiety phenotype. Such changes might include brain levels of apolipoprotein E, a protein important in cholesterol transport and metabolism and increased in middle-aged animals (Landfield et al., 1978). Mice deficient in apoE (*ApoE*^{-/-}) show increased measures of anxiety (Bongers et al., 2004; Raber et al., 2000). ApoE might partially mediate the effects of mGlu8 deficiency on measures of anxiety. While acute pharmacological stimulation with the mGlu8 agonist DCPG reduces measures of anxiety in wild-type and *ApoE*^{-/-} mice (Duvoisin et al., 2010a), a lower dose of DCPG is effective in wild-type than in *ApoE*^{-/-} mice (Duvoisin et al., 2010a).

In wild-type, mGlu8^{-/-}, and mGlu4/8^{-/-} mice, there were significant negative correlations between body weight and ability to stay on the rotating rod. mGlu8^{-/-} mice weighed significantly more than mGlu4/8^{-/-} mice but only marginally more than mGlu4^{-/-} mice and not more than wild-type mice. These data indicate that, rather than group differences in body weight, mGlu8 deficiency is required for the relationship between body weight and sensorimotor function while mGlu4 deficiency is not sufficient. Pro melanin-concentrating hormone (PMCH) might be required for these correlations in the absence of mGlu8. We observed that PMCH mRNA levels were lower in the amygdala of mGlu8^{-/-} than wild-type mice and that compared to vehicle-treated genotype-matched mice, AZ12216052 increased PMCH mRNA levels in the amygdala of wild-type mice (unpublished observations). The amygdala, important in the regulation of anxiety, might be important for the effects of mGlu8 deficiency on regulation of anxiety. Systemic DCPG administration induced the expression of the immediate early gene c-Fos in the central nucleus of the amygdala of wild-type, but not mGlu8^{-/-}, mice (Linden et al., 2003).

Acute administration of the mGluR4 allosteric agonist VU 0155041, the mGlu8 agonist (S)-3,4,-dicarboxyphenylglycine (DCPG), or the mGlu8 PAM AZ12216052 reduces measures of anxiety in wild-type mice (Duvoisin et al., 2010a; Duvoisin et al., 2011). However, in the current study, mGlu4^{-/-} or mGlu8^{-/-} mice did not show higher measures of anxiety than wild-type mice. Thus, additional genetic changes during brain development and/or adulthood might contribute to these non-opposing results between acute pharmacological stimulation and long-term genetic deficiency.

In summary, the data of the current study suggest related but sometimes opposing functions of mGlu4 and mGlu8 in behavioral performance. Especially since mGlu8 agonists might also stimulate mGlu4, these distinct functions might be important for the evaluation of the clinical efficacy of compounds targeting mGlu4 or mGlu8 in disorders of the nervous system. Therefore, future studies are warranted to assess the relevant neurocircuitry that might be shared by mGlu4 and mGlu8, for example by using immunohistochemistry for immediate early genes induced by neuronal activity.

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Highlights

- mGlu4^{-/-} show lower levels of anxiety than mGlu8^{-/-} and mGlu4/8^{-/-} mice.
- In the open field, mGlu4^{-/-} show less activity than mGlu8^{-/-} and mGlu4/8^{-/-} mice.
- mGlu4/8^{-/-} mice show less contextual freezing than mGlu4^{-/-} and mGlu8^{-/-} mice.

Fig. 1.

Effects of genotype on body weights and genotype-dependent correlations of body weights with rotarod performance. A. Males were heavier than females and mGlu8^{-/-} mice weighed significantly more than mGlu4/8^{-/-} mice and marginally more than mGlu4^{-/-} mice. $*p < 0.001$. B. Negative correlation between body weight and fall latency on the rotarod in wild-type mice (B), in mGlu8^{-/-} mice (D), in mGlu4/8^{-/-} mice (E), but not in mGlu4^{-/-} mice (C). $n = 34$ males (wild-type mice $n = 10$, mGlu4^{-/-} $n = 10$, mGlu8^{-/-} $n = 8$, mGlu4/8^{-/-} $n = 6$) and 38 females (wild-type mice $n = 13$, mGlu4^{-/-} $n = 8$, mGlu8^{-/-} $n = 8$, mGlu4/8^{-/-} $n = 9$).

Fig. 2.

A. mGlu4^{-/-} mice spent more time in the center of the open field than mGlu8^{-/-} and mGlu4/8^{-/-} mice. * $p < 0.05$ versus mGlu8^{-/-} and $p = 0.001$ versus mGlu4/8^{-/-} mice. B. mGlu4^{-/-} showed lower activity levels than wild-type, mGlu8^{-/-}, and mGlu4/8^{-/-} mice. # $p < 0.05$ versus wild-type, mGlu8^{-/-}, and mGlu4/8^{-/-} mice. C. mGlu4^{-/-} mice spent more time in the open areas of the elevated zero maze than wild-type, mGlu8^{-/-}, and mGlu4/8^{-/-} ($p < 0.001$) mice. ^O $p < 0.05$ versus wild-type mice, $p = 0.009$ versus mGlu8^{-/-} mice, and $p < 0.001$ versus mGlu4/8^{-/-} mice. D. No genotype differences in activity levels in the elevated zero maze. $n = 34$ males (wild-type mice $n = 10$, mGlu4^{-/-} $n = 10$, mGlu8^{-/-} $n = 8$, mGlu4/8^{-/-} $n = 6$) and 38 females (wild-type mice $n = 13$, mGlu4^{-/-} $n = 8$, mGlu8^{-/-} $n = 8$, mGlu4/8^{-/-} $n = 9$).

Fig. 3.

A. Effect of genotype on baseline motion in female mice and a trend towards an effect of genotype on baseline motion in male mice. mGlu4^{-/-} female mice moved less than mGlu4/8^{-/-} and there was a trend towards less activity than wild-type mice. * $p < 0.05$ versus mGlu4/8^{-/-} and $p = 0.054$ versus wild-type mice. B. In female mice, mGlu4/8^{-/-} mice showed less freezing than wild-type and mGlu4^{-/-} mice and there was a trend towards less freezing than in mGlu8^{-/-} mice. ^o $p < 0.05$ versus wild-type mice, $p = 0.005$ versus mGlu4^{-/-} mice, and $p = 0.054$ versus mGlu8^{-/-} mice. In male mice, mGlu8^{-/-} mice and mGlu4/8^{-/-} mice showed trends towards lower freezing than seen in wild-type mice. # $p = 0.061$ versus mGlu8^{-/-} mice and $p = 0.052$ versus mGlu4/8^{-/-} mice. C. No genotype differences in cued fear conditioning. $n = 34$ males (wild-type mice $n = 10$, mGlu4^{-/-} $n = 10$, mGlu8^{-/-} $n = 8$, mGlu4/8^{-/-} $n = 6$) and 38 females (wild-type mice $n = 13$, mGlu4^{-/-} $n = 8$, mGlu8^{-/-} $n = 8$, mGlu4/8^{-/-} $n = 9$).