

Phenotyping of GluR1-Knockout Mice: Implications in the Treatment of Mood Disorders

Abstract

Introduction: Limitations of current mood disorder treatment options have led to the pursuit of therapeutic alternatives mediated through the glutamatergic system. Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors are comprised of four subunits known as GluR1-4. The aim of the present study was to evaluate the baseline behavioral phenotype of GluR1-knockout (KO). Ultimately, the goal is to explore the involvement of GluR1 in the treatment mechanisms of antidepressant and antimanic agents.

Materials and Methods: To evaluate the phenotype of GluR1-KO mice, a series of behavioral tests was conducted: the open field test (OFT), forced swim test (FST), tail suspension test (TST), elevated plus maze (EPM) test, novel object test (NOT), social interaction test (SIT), and saccharin preference test (SPT).

Results: GluR1-KO mice exhibited several key components of the manic-like phenotype, including hyperactivity, enhanced exploratory and risk-taking behaviors, and increased social initiation. GluR1-KO mice were not, however, found to exhibit enhanced hedonistic drive, a putative component of the manic-like phenotype in mice.

Discussion: The robustness and inconsistency of the baseline phenotype of GluR1-KO could confound further study by limiting the ability to differentiate among the KO, wild-type, and heterozygote groups. Our results suggest that although the suppression of *gria1*, the coding gene for GluR1, through homologous recombination did influence several mood-related behaviors, conventional KO techniques cannot be used to generate an ideal GluR1 model to study the mechanisms underlying antidepressant and antimanic agents. Further study, however, of the involvement of GluR1 in risk-taking behavior and the use of alternative methods of gene suppression could lead to novel therapeutic options for these serious illnesses.

Introduction

Annually, approximately seven percent of Americans suffer from mood disorders, including the two major forms, unipolar major depression and bipolar disorder. Unipolar major depression is the leading cause of disability worldwide, while bipolar disorder is ranked among the top ten (Department of Health and Human Services, 1999). Until recently, treatment research has focused on the regulation of monoamine neurotransmitters such as norepinephrine, serotonin, and dopamine. Limitations of current treatment options, however, including response latency, inconsistent outcome, and severe side effects (Alt et al., 2006), have led to the pursuit of novel therapeutic options mediated through the glutamatergic system (Maeng et al., 2007).

Three types of ionotropic glutamate receptors are expressed in the central nervous system (CNS): α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA), and kainate receptors (Pittenger et al., 2007). AMPA receptors mediate the majority of excitatory synaptic transmission in the CNS and are comprised of combinations of four subunits known as GluR1-4 (Du et al., 2007). GluR1 phosphorylation at Ser845 by cAMP-dependent protein kinase, or protein kinase A (PKA), is a critical step in the trafficking of AMPA receptors to the postsynaptic membrane; thus, GluR1 is a key regulator of AMPA receptor synaptic plasticity and thus glutamatergic neurotransmission (Esteban, 2003; Martínez-Turillas et al., 2007).

According to the neurogenesis hypothesis of depression, several interrelated pathways enhance the expression of brain-derived neurotrophic factor (BDNF), resulting in antidepressant effects. Chronic administration of several conventional antidepressants has been found to enhance BDNF mRNA levels in the rat hippocampus; conversely, suppression of BDNF expression in mice has been shown to reduce behavioral response to antidepressant treatment. Recent studies implicate AMPA receptor activity in the neurogenesis hypothesis. The AMPA receptor potentiator LY451646 has been found to increase BDNF expression and progenitor cell proliferation in the

adult rat hippocampus (Alt et al., 2006). A recent study of mice deficient in stargazin, a transmembrane AMPA receptor regulatory protein (TARP), provides further evidence for the link between AMPA receptor activity and BDNF expression. “Stargazer” mice, known to lack AMPA receptors in the cerebellar granule cells, were found to exhibit a 70% reduction in BDNF mRNA and protein levels in the cerebellum (Bleakman et al., 2007). These data suggest that both monoaminergic and glutamatergic pathways regulate the expression of BDNF and thus are important in the underlying pathophysiology of depression (Alt et al., 2006).

Other studies have explored the effects of antidepressant and antimanic treatment on GluR1 expression in mice. Du et al. (2004) found that the antimanic agents lithium and valproate reduce GluR1/2 membrane expression and PKA phosphorylation *in vitro*, while the antidepressant imipramine produced opposite effects. In a later study, Du et al. (2007) showed that the anticonvulsants lamotrigine and riluzole, known to possess antidepressant properties, enhance GluR1/2 membrane expression and PKA phosphorylation. Additionally, Maeng et al. (2007) found that in mice, the AMPA receptor antagonist NBQX reduces the antidepressant effects of ketamine, which was shown to decrease GluR1 phosphorylation *in vivo*. These results suggest that AMPA receptor potentiation is associated with antidepressant-like effects; conversely, AMPA receptor attenuation is linked to antimanic-like effects.

Knockout animals can serve as powerful tools in elucidating the intricate pathophysiology of mental disorders (Takao et al., 2007). The aim of the present study was to evaluate the baseline behavioral phenotype of GluR1-knockout (KO) mice. Ultimately, GluR1-KO mice could be used to explore the involvement of GluR1 in the mechanisms underlying antidepressant and antimanic agents. Based on data from previous studies, which suggest that AMPA receptor attenuation is associated with antimanic-like effects (Du et al., 2004), it was hypothesized that GluR1-KO mice would exhibit abnormal performance on a series of tests designed to model mood-related behaviors.

The behavioral data were used to assess the suitability of GluR1-KO mice in the study of mood disorder treatment mechanisms mediated by AMPA receptors.

Materials and Methods

Animals: GluR1-knockout (KO) mice (C57BL/6J, male), in which *gria1*, the coding gene for GluR1, was suppressed through homologous recombination, were obtained from Jackson Laboratory. GluR1 heterozygotes were generated by interbreeding wild-type and KO animals. All animals were housed in groups of four in a standard NIH facility in which a constant temperature of $22 \pm 1^\circ \text{C}$ and a 12-hour light/dark cycle were maintained. Each experimental group consisted of 9-10 animals. The second series of tests (EPM, NOT, SIT, & SPT) was conducted two weeks after the initial set of tests (OFT, FST, & TST) to prevent stress interference. All animal treatments, procedures, and care were approved by the NIMH Animal Care and Use Committee and were conducted according to NIH guidelines.

Genotyping: Tail tips were obtained from the animals in order to verify GluR1 genotypes. Tail tips were dissolved in tubes containing 250 μl DirectPCR Lysis reagent (Viagen) and 25 μl Proteinase K solution (Viagen). The tubes were then incubated overnight in a 55°C thermomixer. After remixing, the tubes were incubated at 85°C for 45 minutes to deactivate Proteinase K. Polymerase chain reaction (PCR) was performed using puReTaq Ready-To-Go PCR beads (Amersham) along with 5 μl of the DNA solution, 15 μl of pure water, and 2.5 μl of each of the three primers oIMR3364, oIMR3365, and oIMR3366 (Jackson Laboratory). Following gel electrophoresis, the gels were viewed under ultraviolet light to verify GluR1 genotypes.

Open Field Test (OFT): The OFT examines locomotion. Animals were introduced into the center of an enclosed, 50 x 50 cm arena. Movements were tracked for 30 minutes and analyzed using EthoVision (Noldus).

Forced Swim Test (FST): The FST, as described by Porsolt et al. (1977), examines despair behavior. Animals were introduced into a 50 cm high, transparent Plexiglas cylinder with a diameter of 20 cm. The cylinder was filled with tap water maintained at $23\pm 1^{\circ}\text{C}$ to a depth of 25 cm. Animal movements were recorded on videotape, and the duration of immobility (cessation of limb movement other than efforts to maintain buoyancy) during the last four minutes of the six-minute test was recorded *post hoc*.

Tail Suspension Test (TST): The TST examines despair behavior. Animals were suspended by their tails using masking tape. Movements were recorded on videotape, and the duration of immobility (complete cessation of limb movement) during the last four minutes of the six-minute test was recorded *post hoc*.

Elevated Plus Maze (EPM): The EPM examines risk-taking and exploratory behaviors as well as anxiety. Animals were introduced into a Plexiglas, plus-shaped maze elevated 50 cm above the ground and containing two closed arms and two open arms. It has been shown that wild-type animals prefer the darker, closed arms over the brighter, open arms (Shaltiel et al., 2007). The arms were 30×5 cm, and the walls of the closed arms were 40 cm high. Light intensity was maintained at 7 lux in the closed arms and 24 lux in the open arms. Animal movements were tracked for five minutes and analyzed using EthoVision (Noldus).

Novel Object Test (NOT): The NOT examines risk-taking and exploratory behaviors as well as anxiety. After a habituation period of 30 minutes in a 50 x 50 cm, enclosed arena, an inverted wire container with a diameter of 8 cm was introduced into the center of the arena. Animal movements were tracked for an additional 30 minutes and analyzed using EthoVision (Noldus).

Social Interaction Test (SIT): The SIT examines social behavior. Animals were housed individually in separate cages for one week prior to the SIT to ensure social isolation. Animals were habituated for 30 minutes in an enclosed, 50 x 50 cm arena in which an inverted wire

container with a diameter of 8 cm was placed at the center. After the habituation period, an unfamiliar social partner was introduced into the arena and enclosed within the container. Animal movements then were tracked for 30 minutes and analyzed using EthoVision (Noldus). The SIT also was recorded on videotape to allow for *post hoc* analysis of five social behaviors: front approach, side approach, climbing, tail rattling, and olfactory investigation (sniffing), as described by Shaltiel et al. (2007) and Moy et al. (2004). Only significant instances of each behavior (those lasting one second or longer) were recorded. Front approach was defined as a forward advance on the social partner without olfactory investigation. Side approach was defined as a cessation of movement tangential to the wire container. Only climbing around the circumference of the wire container was recorded. Tail-rattling was defined as a rapid agitation of the tail. Lastly, olfactory investigation was defined as any instance of sniffing, with or without approach.

Saccharin Preference Test (SPT): The SPT examines hedonistic behavior. Animals were housed individually in separate cages for this test. After a two-day habituation period during which the animals were given a free choice between two tubes of tap water, one tube was replaced with saccharin solution. The saccharin concentration was increased daily, and the positions of the tubes were reversed to eliminate side preferences. The saccharin concentrations of 0.05%, 0.1%, 0.2%, and 0.25% were tested. Tubes were weighed at 6 pm in the evening and at 10 am the following day to monitor overnight fluid consumption. Saccharin preference was defined as the proportion of saccharin solution consumed over total consumption.

Statistical Analyses: One-way parametric analysis of variance (ANOVA) and Tukey's *post hoc* test (where appropriate) were conducted using Prism 5 software (GraphPad). Statistical significance was set at $p < 0.05$.

Results

To evaluate the phenotype of GluR1-KO mice, a battery of behavioral tests designed to model mood-related behaviors was conducted. The results are summarized in Table 1.

Locomotion: Locomotion was evaluated using the open field test (OFT) and the elevated plus maze (EPM). GluR1-KO traveled farther in the OFT (Figure 3b), in the open arms of the EPM (Figure 2c), and in the entire EPM (Figure 3a). No significant differences in distance traveled, however, were found in the closed arms of the EPM (Figure 2d).

Immobility: GluR1-KO were not as immobile as GluR1 heterozygotes and wild-types in the forced swim test (FST) (Figure 1a) and in the tail suspension test (TST) (Figure 1b).

EPM Arm Preference: Compared to GluR1 heterozygotes and wild-types, GluR1-KO spent more time in the open arms (Figure 2a) and less time in the closed arms (Figure 2b). Surprisingly, they spent more time in the open arms than they did in the closed arms.

Center Preference: The novel object test (NOT) and the social interaction test (SIT) were conducted to determine whether GluR1-KO mice show abnormal responses to unfamiliar objects and social partners placed at the center of an enclosed arena. In both of these tests, GluR1-KO mice spent more time in the center of the arena than their control counterparts (Figures 4a & b).

Social Initiation: GluR1-KO mice exhibited greater instances of front approach, side approach, and olfactory investigation than GluR1 heterozygotes and wild-types (Figures 5a, b, & e). GluR1 heterozygotes showed more occurrences of climbing than GluR1-KO (Figure 5c). No significant differences, however, were found in tail rattling (Figure 5d).

Saccharin Preference: GluR1-KO showed less saccharin preference than wild-types did at the 0.05% concentration (Figure 6a). At the 0.1% concentration, however, GluR1 heterozygotes showed a stronger preference for saccharin solution than the wild-types (Figure 6b). No significant differences were found among the groups at the 0.2% and 0.25% concentrations (Figures 6c & d).

Table 1. Summary of Results

Test	Behavior	Result	ANOVA	Tukey's <i>Post Hoc</i> Test	Phenotype	Figure
Open Field Test (OFT)	Locomotion	KO>WT	F(2,27)= 38.36, p<0.0001	Tukey's HSD(2,9)= 11.35, p<0.0001	KO showed enhanced locomotion	3b
		KO>Het		Tukey's HSD(2,9)= 9.977, p<0.0001		
Forced Swim Test (FST)	Immobility	KO<WT	F(2, 27)= 28.33, p<0.0001	Tukey's HSD(2,9)= 9.841, p<0.0001	KO showed reduced immobility	1a
		KO<Het		Tukey's HSD(2,9)= 8.437, p<0.0001		
Tail Suspension Test (TST)	Immobility	KO<WT	F(2, 27)= 12.59, p<0.0001	Tukey's HSD(2,9)= 6.012, p<0.001	KO showed reduced immobility	1b
		KO<Het		Tukey's HSD(2,9)= 6.270, p<0.0001		
Elevated Plus Maze (EPM)	Open Arm Preference	KO>WT	F(2,27)= 16.38, p<0.0001	Tukey's HSD(2,9)= 6.819, p<0.0001	KO showed enhanced open arm preference	2a
		KO>Het		Tukey's HSD(2,9)= 7.186, p<0.0001		
	Closed Arm Preference	KO<WT	F(2,27)= 17.62, p<0.0001	Tukey's HSD(2,9)= 6.997, p<0.0001	KO showed reduced closed arm preference	2b
		KO<Het		Tukey's HSD(2,9)= 7.515, p<0.0001		
	Open Arm Locomotion	KO>WT	F(2,27)= 7.238, p<0.001	Tukey's HSD(2,9)= 4.571, p<0.001	KO showed enhanced open arm locomotion	2c
		KO>Het		Tukey's HSD(2,9)= 4.744, p<0.001		
	Closed Arm Locomotion	KO=WT	F(2,27)= 1.685, p>0.05	N/A	No significant differences	2d
		KO=Het		N/A		
	Overall Locomotion	KO>WT	F(2,27)= 8.407, p<0.001	Tukey's HSD(2,9)= 4.815, p<0.001	KO showed enhanced overall locomotion	3a
		KO>Het		Tukey's HSD(2,9)= 5.207, p<0.001		

Test	Behavior	Result	ANOVA	Tukey's <i>Post Hoc</i> Test	Phenotype	Figure
Novel Object Test (NOT)	Center Preference	KO>WT	F(2,27)= 5.980, p<0.001	Tukey's HSD(2,9)= 3.979, p<0.01	KO showed enhanced center preference	4a
		KO=Het		Tukey's HSD(2,9)= 0.3235, p>0.05		
Social Interaction Test (SIT)	Center Preference	KO>WT	F(2,26)= 35.30, p<0.0001	Tukey's HSD(2,8)= 11.88, p<0.0001	KO showed enhanced center preference	4b
		KO>Het		Tukey's HSD(2,8)= 6.560, p<0.0001		
	Front Approach	KO>WT	F(2,27)= 15.46, p<0.0001	Tukey's HSD(2,9)= 7.087, p<0.0001	KO showed greater instances of front approach	5a
		KO>Het		Tukey's HSD(2,9)= 6.497, p<0.0001		
	Side Approach	KO>WT	F(2,27)= 4.933, p<0.05	Tukey's HSD(2,9)= 3.602, p<0.05	KO showed greater instances of side approach	5b
		KO>Het		Tukey's HSD(2,9)= 4.052, p<0.05		
	Olfactory Investigation	KO>WT	F(2,27)= 11.09, p<0.0001	Tukey's HSD(2,9)= 6.460, p<0.0001	KO showed greater instances of olfactory investigation	5e
		KO>Het		Tukey's HSD(2,9)= 4.640, p<0.001		
	Climbing	KO=WT	F(2,27)= 11.51, p<0.0001	Tukey's HSD(2,9)= 2.190, p>0.05	Heterozygotes showed greater instances of climbing than KO	5c
		KO<Het		Tukey's HSD(2,9)= 6.658, p<0.0001		
	Tail Rattling	KO=WT	F(2,27)= 1.061, p>0.05	N/A	No significant differences	5d
		KO=Het		N/A		

Test	Concentration	Result	ANOVA	Tukey's <i>Post Hoc</i> Test	Phenotype	Figure
Saccharin Preference Test (SPT)	0.05%	KO<WT	F(2,27)= 3.776, p<0.05	Tukey's HSD(2,9)= 3.635, p<0.05	KO showed less saccharin preference than wild-types at the 0.05% concentration	6a
		KO=Het		Tukey's HSD(2,9)= 3.009, p>0.05		
	0.1%	KO=WT	F(2,27)= 4.865, p<0.05	Tukey's HSD(2,9)= 0.881, p>0.05	Heterozygotes showed enhanced saccharin preference compared to the wild-types	6b
		KO=Het		Tukey's HSD(2,9)= 3.303, p>0.05		
	0.2%	KO=WT	F(2,27)= 0.7662, p>0.05	N/A	No significant differences	6c
		KO=Het		N/A		
	0.25%	KO=WT	F(2,27)= 0.2966, p>0.05	N/A	No significant differences	6d
		KO=Het		N/A		

Figure 1. Immobility - Forced Swim Test (FST) & Tail Suspension Test (TST)

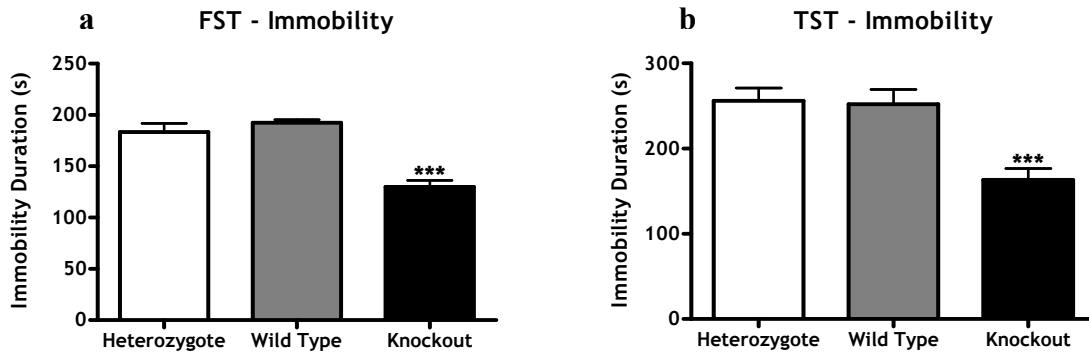


Figure 2. Locomotion & Arm Preference - Elevated Plus Maze (EPM)

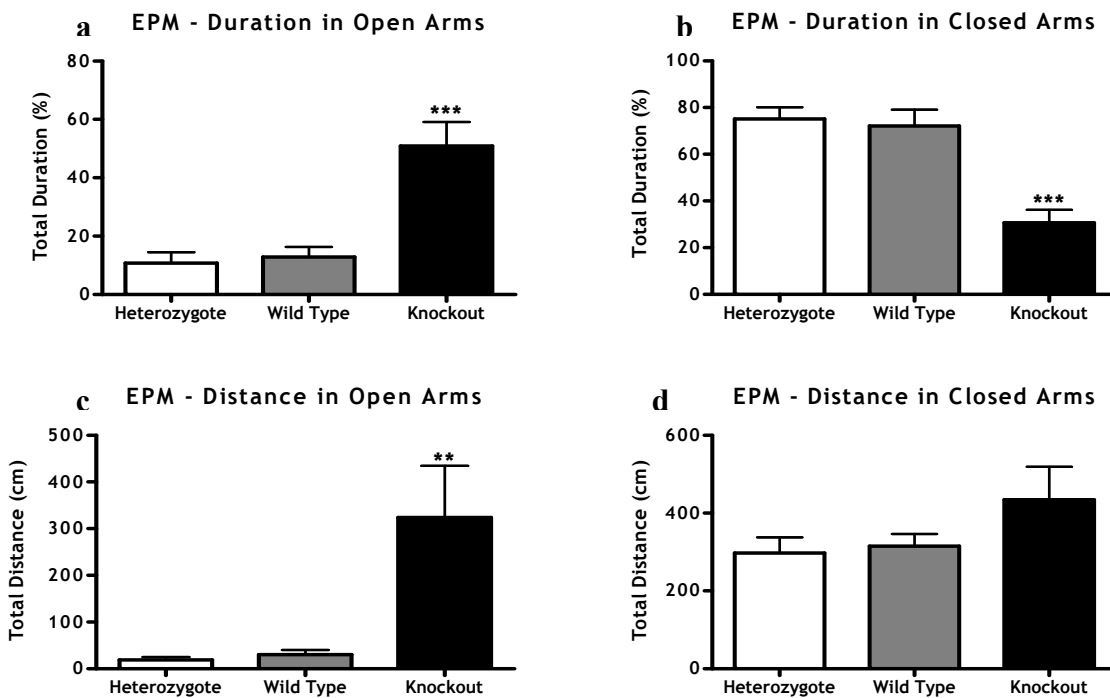
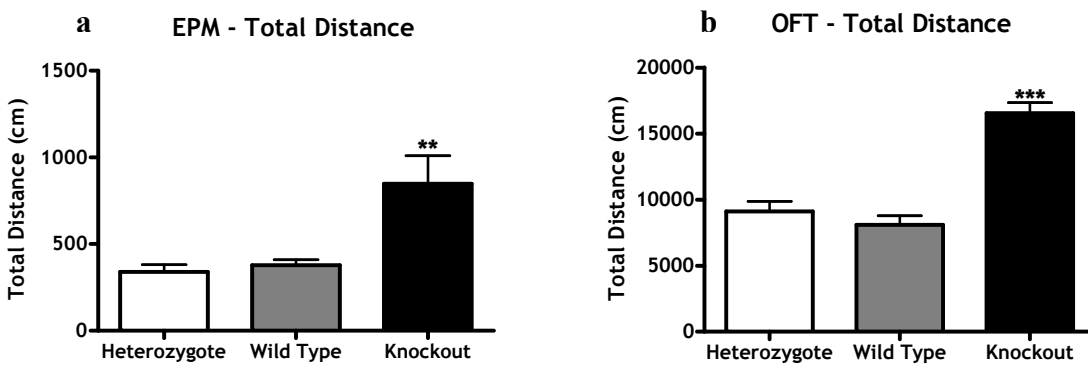


Figure 3. Distance - Elevated Plus Maze (EPM) & Open Field Test (OFT)



In all figures, * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$.

Figure 4. Center Preference - Novel Object Test (NOT) & Social Interaction Test (SIT)

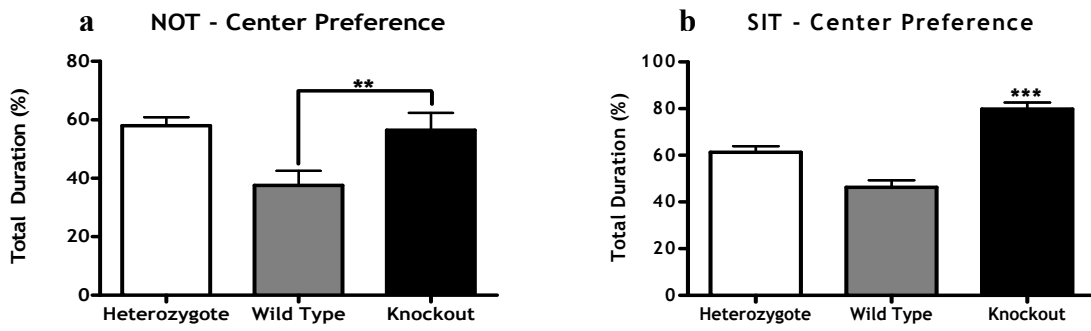
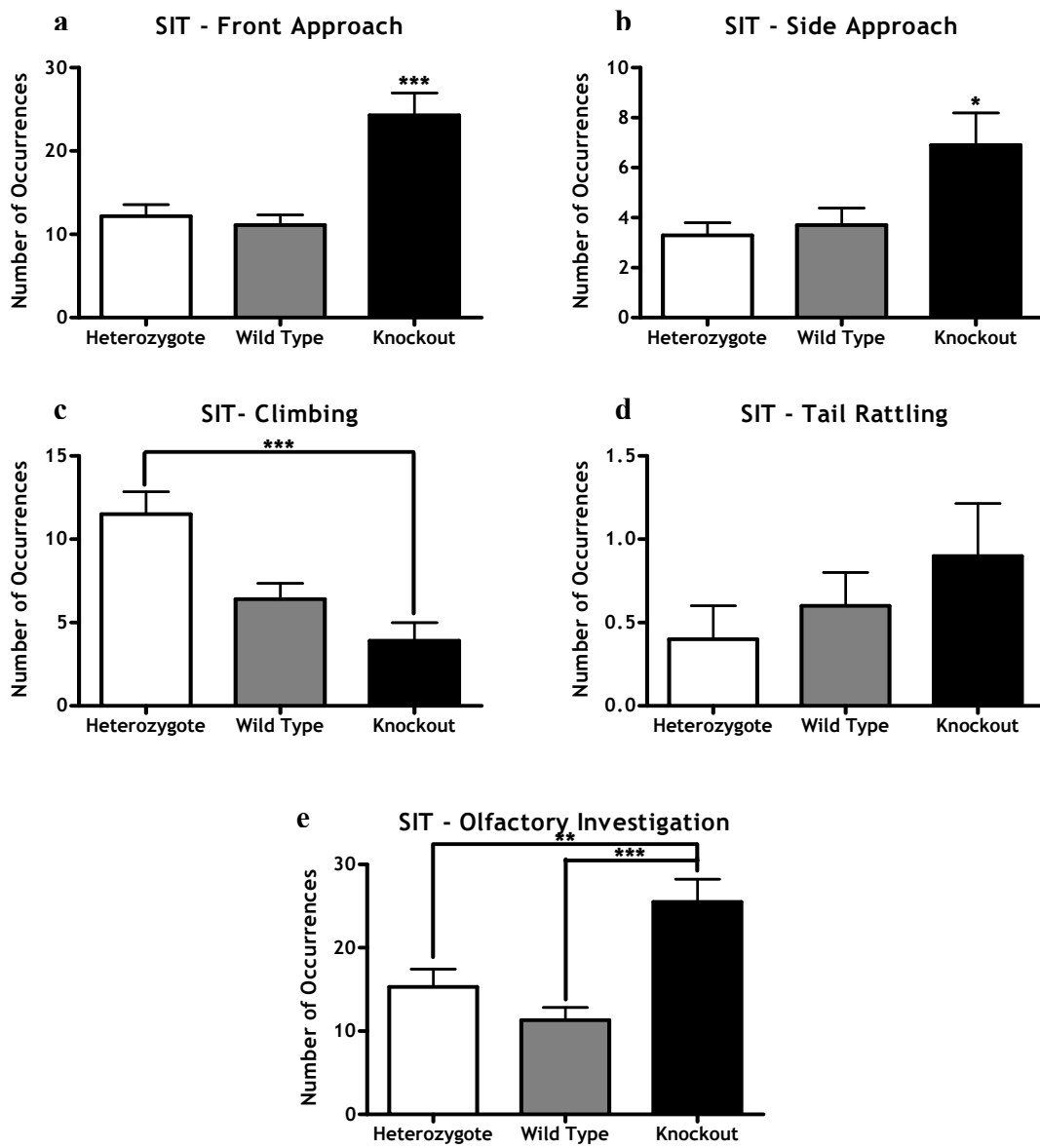
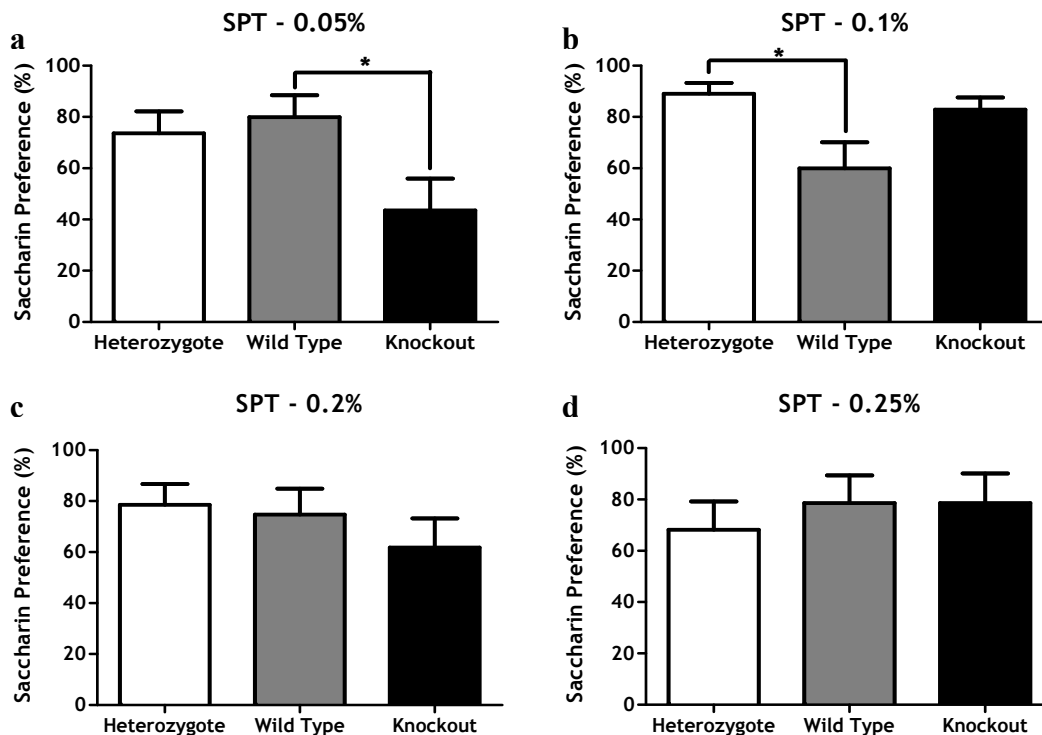


Figure 5. Social Initiation - Social Interaction Test (SIT)



In all figures, * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$.

Figure 6. Saccharin Preference - Saccharin Preference Test (SPT)



In all figures, * indicates $p < 0.05$, ** indicates $p < 0.01$, and *** indicates $p < 0.001$.

Discussion

The aim of the present study was to determine the baseline behavioral phenotype of GluR1-knockout (KO) mice. A series of behavioral tests was conducted to evaluate several mood-related behaviors in GluR1 KO, heterozygotes, and wild-types. Conclusions are summarized in Table 2.

Results from the forced swim test (FST) and the tail suspension test (TST) indicate that GluR1-KO showed less immobility than GluR1 heterozygotes and wild-types, suggesting reduced despair behavior (Porsolt, 1977). In the elevated plus maze (EPM) test and the open field test (OFT), GluR1 KO showed enhanced locomotion, suggesting that they are hyperactive. Enhanced open arm preference in the EPM test and increased center preference in the novel object test (NOT) and the social interaction test (SIT) suggest that GluR1-KO show enhanced risk-taking and exploratory behaviors. Increased risk-taking and exploratory behaviors also are associated with reduced anxiety (Boyce-Rustay & Holmes, 2006). The SIT also showed that GluR1-KO exhibit

enhanced social initiation behaviors including front approach, side approach, and olfactory investigation. GluR1-KO, however, exhibit fewer instances of climbing behavior and show no significant differences in tail rattling, a form of aggressive behavior (Shaltiel et al, 2007). Lastly, data from the saccharin preference test (SPT) were inconclusive and suggest that GluR1-KO do not show enhanced hedonistic drive.

Table 2. Summary of the GluR1-KO Phenotype

Test	GluR1-KO Behavior	GluR1-KO Phenotype
OFT	Enhanced locomotion	GluR1-KO mice exhibit hyperactivity, suggesting antidepressant-like effects (Einat, 2007b).
EPM	Enhanced open arm locomotion	
	No significant differences in closed arm locomotion	
	Enhanced overall locomotion	
FST	Reduced immobility	GluR1-KO mice show reduced despair behavior, suggesting antidepressant-like effects (Boyce-Rustay & Holmes, 2006).
TST		
EPM	Enhanced open arm preference	GluR1-KO mice exhibit enhanced risk-taking and exploratory behaviors, suggesting antidepressant-like effects (Boyce-Rustay & Holmes, 2006).
	Reduced closed arm preference	
NOT	Enhanced center preference	
SIT	Greater instances of olfactory investigation	
	Greater instances of front approach	
	Greater instances of side approach	
	No significant differences in tail rattling	GluR1-KO mice did not exhibit increased aggression, a component of the manic-like phenotype in mice (Einat, 2007b).
	GluR1 heterozygotes showed greater instances of climbing than wild-types did; no other significant differences	GluR1-KO mice did not exhibit increased distractibility and reduced concentration, components of the manic-like phenotype in mice (Einat, 2007b).
SPT	Reduced saccharin preference at the 0.05% concentration	GluR1-KO mice did not exhibit enhanced hedonistic drive, a component of the manic-like phenotype in mice (Einat, 2007a).
	GluR1 heterozygotes showed greater saccharin preference than wild-types did; no other significant differences	
	No significant differences at the 0.2% concentration	
	No significant differences at the 0.25% concentration	

Our locomotion data compare favorably to those from a recent study by Wiedholz et al. (2007), who showed that GluR1-KO exhibit hyperactivity in the OFT. In contrast to the findings of Wiedholz et al., however, who concluded that GluR1-KO do not exhibit significant differences in social behaviors, our results strongly show that GluR1 KO exhibit enhanced social initiation. Protocol differences may explain this inconsistency. In their protocol, Wiedholz et al. did not isolate their animals prior to the SIT, which would have promoted social unfamiliarity. Additionally, Wiedholz et al. did not immobilize the social partner (Wiedholz et al., 2007). It has been shown that the activity of the social partner significantly influences the behavior of the test subject (Panskepp, 1998). In our protocol, we enclosed the social partner in a wire container to address this issue.

Previously, GluR1-KO mice have been shown to exhibit dissociations in spatial working memory using the T-maze and Y-maze tests (Reisel et al., 2002). Spatial working memory defects in GluR1-KO conceivably could have influenced their performance on the EPM, but it cannot account fully for the preference for open arms over closed arms. In addition, hyperactivity, as shown in the OFT and the EPM, could have influenced the performance of the GluR1-KO in the FST and the TST, which are designed to measure despair behavior. Although hyperactivity is a key component of manic behavior, it does not necessarily imply mania itself (Einat, 2007b). Moreover, manic models that are based solely on hyperactivity may lack face validity, as hyperactivity is not present in all manic patients and can be caused by a variety of other conditions. Nevertheless, the majority of manic models can be influence heavily by locomotion; thus, a battery of tests generally is used to provide multiple assurances of validity (Einat, 2007b). Specifically, in our study, the SPT was chosen to assess the hedonistic component of mania in GluR1-KO mice because the SPT is not significantly influenced by hyperactivity.

Interestingly, GluR1-KO mice exhibited several key components of the manic-like phenotype, including hyperactivity, enhanced risk-taking and exploratory behaviors, and increased social initiation. GluR1-KO, however, were not found to exhibit enhanced hedonistic drive, a putative component of the manic-like phenotype in mice (Einat, 2007a). The robustness and inconsistency of the baseline phenotype of GluR1-KO could confound further study by limiting the ability to differentiate among KO, wild-types, and heterozygotes. Our results suggest that although the suppression of *gria1*, the coding gene for GluR1, through homologous recombination did influence several mood-related behaviors, conventional KO techniques cannot be used to generate an ideal GluR1 model to study the mechanisms underlying antidepressant and antimanic agents.

The GluR1-KO mice used in our study were created through homologous recombination at the embryonic stage; thus, developmental side effects, such as innately programmed, compensatory mechanisms, could have influenced the adult phenotype. Newer KO methods that have region-specific targeting capabilities, such as the *Cre-LoxP* approach (Metzger & Feil, 1999) and the *Flp-frt* technique (Ruff & Kieffer, 2007), could result in a better GluR1-KO model. The hippocampus and prefrontal cortex have been found to exhibit the greatest expression of AMPA receptors and are believed to be highly significant in the pathophysiology of mood disorders (Bleakman et al., 2007). Thus, these regions may be interesting targets for region-specific KO strategies. Gene knockdown techniques, such as RNA interference (RNAi), also could be used to selectively reduce (rather than completely suppress) GluR1 expression. Finally, the integration of *Cre-LoxP* with RNAi is an exciting possibility, offering both regional and temporal regulation of gene expression. Combining *Cre-LoxP* with RNAi would lessen developmental and brain region-specific complications in KO mice generated by conventional means (Gao & Zhang, 2007).

Our data show that GluR1-KO mice exhibit enhanced risk-taking behavior, suggesting that GluR1 may play an important role in risk-taking behavior. Excessive risk-taking is a key

component of mood disorders, especially bipolar disorder (Einat, 2007b). Specifically, excessive risk-taking has been shown to be a major cause of suicide in children and adolescents diagnosed with bipolar disorder (Papolos et al., 2005). Ramrakha et al. (2000) found that young adults diagnosed with manic episodes and depressive disorders exhibit increased occurrences of risky sexual behavior and thus have a greater prevalence of sexually transmitted diseases (Ramrakha et al., 2000). Further study of the role of GluR1 in risk-taking behavior could lead to alternative methods of mood disorder treatment as well as suicide prevention.

Our study presents a comprehensive baseline phenotype of GluR1-KO mice for the study of mood disorder treatment mechanisms. Recent studies have suggested that AMPA receptors may be key mediators underlying the pathophysiology and treatment of mood disorders. Data from our study show that GluR1-KO mice exhibit hyperactivity, enhanced risk-taking and exploratory behaviors, and increased social initiation. GluR1-KO mice were not, however, found to exhibit an increase in hedonistic drive compared to their heterozygous and wild-type counterparts. Our data suggest that although the suppression of *gria1*, the coding gene for GluR1, through homologous recombination did influence several mood-related behaviors, conventional KO methods cannot be used to generate an ideal GluR1-KO mouse model to study the involvement of GluR1 in the underlying pathophysiology of mood disorders. Further study, however, of the involvement of GluR1 in risk-taking behavior and the use of alternative methods of gene suppression could lead to novel therapeutic options for these serious illnesses.

References

- Alt, A., Nisenbaum, E., Bleakman, D., & Witkin, J. (2006). A role for AMPA receptors in mood disorders. *Biochemical Pharmacology*, *71*, 1273-1288.
- Bleakman, E., Alt, A., & Witkin, J. (2007). AMPA receptors in the therapeutic management of depression. *CNS & Neurological Disorders Drug Targets*, *6*, 117-126.

- Boyce-Rustay, J. & Holmes, A. (2006). Genetic inactivation of the NMDA receptor NR2A subunit has anxiolytic- and antidepressant-like effects in mice. *Neuropsychopharmacology*, *31*, 2405-2414.
- Department of Health and Human Services (1999). *Mental Health: A Report of the Surgeon General*. Rockville, MD: National Institute of Mental Health.
- Du, J., Gray, N., Falke, C., Chen, W., Yuan, P., Szabo, S., et al. (2004). Modulation of synaptic plasticity by antimanic agents: The role of AMPA glutamate receptor subunit 1 synaptic expression. *Journal of Neuroscience*, *24*, 6578-6589.
- Du, J., Suzuki, K., Wei, Y., Wang, Y., Blumenthal, R., Chen, Z., et al. (2007). The anticonvulsants lamotrigine, riluzole, and valproate differentially regulate AMPA receptor membrane localization: Relationship to clinical effects in mood disorders. *Neuropsychopharmacology*, *32*, 793-802.
- Einat, H. (2007a). Different behaviors and different strains: Potential new ways to model bipolar disorder. *Neuroscience & Biobehavioral Reviews*, In Press.
- Einat, H. (2007b). Establishment of a battery of simple models for facets of bipolar disorder: A practical approach to achieve increased validity, better screening, and possible insights into endophenotypes of disease. *Behavioral Genetics*, *37*, 244-255.
- Esteban, J. (2003). AMPA receptor trafficking: A road map for synaptic plasticity. *Molecular Interventions*, *3*, 375-385.
- Gao, X. & Zhang, P. (2007). Transgenic RNA interference in mice. *Physiology*, *22*, 161-166.
- Martínez-Turillas, R., Del Río, J., & Frechilla, D (2007). Neuronal proteins involved in synaptic targeting of AMPA receptors in rat hippocampus by antidepressant drugs. *Biochemical and Biophysical Research Communications*, *353*, 750-755.
- Maeng, S., Zarate, C. II, Du, J., Schloesser, R., McCammon, J., Chen, G., et al. (2007). Cellular mechanisms underlying the antidepressant effects of ketamine: Role of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biological Psychiatry*, In Press.
- Metzger, D. & Feil, R. (1999). Engineering the mouse genome by site-specific recombination. *Current Opinion in Biotechnology*, *10*, 470-476.

- Moy, S., Nadler, J., Perez, A., Barbaro, R., Johns, J., Magnuson, T., et al. (2004). Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes, Brain and Behavior*, 3, 287–302.
- Panksepp, J. (1998). *Affective Neuroscience: The Foundations of Human and Animal Emotions*. New York: Oxford University Press.
- Papoulos, D., Hennen, J., & Cockerham, M. (2005). Factors associated with parent-reported suicide threats by children and adolescents with community-diagnosed bipolar disorder. *Journal of Affective Disorders*, 86, 267-275.
- Pittenger, C., Sanacora, G., & Krystal, J. (2007). The NMDA receptor as a therapeutic target in major depressive disorder. *CNS & Neurological Disorders*, 6, 101-115.
- Porsolt, R., Bertin, A., & Jalfre, M. (1977). Behavioral despair in mice: a primary screening test for antidepressants. *Archives Internationales de Pharmacodynamie et de Thérapie*, 229, 327-36.
- Ramrakha, S., Caspi, A., Dickson, N., Moffitt T., & Paul, C. (2000). Psychiatric disorders and risky sexual behaviour in young adulthood: Cross sectional study in birth cohort. *BMJ*, 321, 263-266.
- Reisel, D., Bannerman, D., Schmitt, W., Deacon, R. J., Flint, J., Borchardt, T., et al. (2002). Spatial memory dissociations in mice lacking GluR1. *Nature Neuroscience*, 5, 868-873.
- Ruff, C. & Kieffer, B. (2007). Conditional gene targeting in the mouse nervous system: Insights into brain function and diseases. *Pharmacology & Therapeutics*, 113, 619-634.
- Shaltiel, G., Maeng, S., Rogawski, M., Gasior, M., Chen, G., & Manji, H. (2007). Evidence for glutamate receptor 6 involvement in mediating mood-related behaviors. Submitted.
- Takao, K., Yamasaki, N., & Miyakawa, T. (2007). Impact of brain-behavior phenotyping of genetically-engineered mice on research of neuropsychiatric disorders. *Neuroscience Research*, 58, 124-132.
- Wiedholz, L. M., Owens, W., Horton, R., Feyder, M., Karlsson, R., Hefner, K., et al. (2007). Mice lacking the AMPA GluR1 receptor exhibit hyperdopaminergia and ‘schizophrenia-related’ behaviors. *Molecular Psychiatry*, In Press.