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Disruption of GluA2 Phosphorylation Potentiates Stress Responsivity

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Abstract

Cocaine addiction is characterized by persistent craving and addicts frequently relapse even after long periods of abstinence. Exposure to stress can precipitate relapse in humans and rodents. Stress and drug use can lead to common alterations in synaptic plasticity and these commonalities may contribute to the ability of stress to elicit relapse. These common changes in synaptic plasticity are mediated, in part, by alterations in the trafficking and stabilization of AMPA receptors. Exposure to both cocaine and stress can lead to alterations in protein kinase C-mediated phosphorylation of GluA2 AMPA subunits and thus alter the trafficking of GluA2-containing AMPARs. However, it is not clear what role AMPAR trafficking plays in the interactions between stress and cocaine. The current study utilized a mouse with a point mutation within the GluA2 subunit c-terminus resulting in a disruption of PKC-mediated GluA2 phosphorylation to examine stress responsivity. Although no differences were seen in the response to a forced swim stress in naïve mice, GluA2 K882A knock-in mice exhibited an increased stress response following cocaine self-administration. Furthermore, we demonstrated that disrupting GluA2 phosphorylation increases vulnerability to stress-induced reinstatement of both cocaine seeking and cocaine-conditioned reward. Finally, GluA2 K882A knock-in mice exhibit an increased vulnerability to social defeat as indicated by increased social avoidance. Taken together these results indicate that disrupting GluA2 phosphorylation leads to increased responsivity to acute stress following cocaine exposure and increased vulnerability to chronic stress. These results highlight the GluA2 phosphorylation site as a novel target for the stress-related disorders.

Keywords

cocaine addiction; stress; reinstatement; AMPA trafficking; self-administration; conditioned place preference; social defeat

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1. Introduction

An estimated 1.5 million Americans per year combat cocaine addiction, however no efficacious treatment options exist and relapse rates remain high [1]. Exposure to stress is a potent trigger for cocaine relapse and thus, life stress is a critical factor in substance abuse treatment outcomes [2–4]. Therefore, treatments aimed at reducing stress could decrease relapse rates and aid in treatment success [5]. However, the mechanisms underlying the ability of stress to elicit relapse are not well understood.

Independently, both cocaine and stress have been shown to elicit functional alterations in glutamatergic synapses through trafficking of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [6–10]. The trafficking of AMPA receptors is regulated, in part, by phosphorylation, a post-translational modification that can modulate receptor expression, channel function and synaptic plasticity [11–14]. PKC-dependent phosphorylation of GluA2 AMPA subunits at Serine880 (S880) leads to dissociation of AMPARs with GRIP1, and association with PICK1, causing internalization of receptors [15, 16]. We have recently shown that disrupting the ability of PKC to phosphorylate GluA2 subunits potentiates cue-induced cocaine reinstatement via upregulation of GluA2-mediated AMPA transmission [17].

As both acute and chronic stress alter phosphorylation of GluA2 subunits and correspondingly affect surface expression of AMPARs [18–20], this may represent a mechanism by which stressful experiences can impact addiction-like behaviors. Furthermore, as cocaine experience has been shown to alter subsequent responses to stress and glutamatergic transmission plays a role in this sensitization [7], the trafficking of AMPARs may also be important for the effects of drug use on stress responsivity. Therefore, the goal of these studies was to determine the role of PKC-mediated phosphorylation of GluA2-containing AMPARs in stress responsivity following drug use. To accomplish this, we used a line of mice with a point mutation within the intracellular C terminus of the GluA2 AMPAR subunit, preventing the PKC-mediated phosphorylation of the S880 residue. We examined the response to a forced swim stress in both naïve and cocaine-experienced mice. Although often used as a model of depressive-like behavior, immobility in the forced swim test provides us with insight into stress coping strategies that is not possible with other acute stressors [21]. Additional studies examined stress-induced reinstatement of both cocaine seeking and cocaine conditioned reward. Finally, to characterize the role of AMPAR trafficking in response to a chronic stressor, we investigated social avoidance behaviors following chronic social defeat. The present results indicate that disrupting PKC-mediated phosphorylation of GluA2-containing AMPARs potentiates the response to acute stressors following cocaine experience and the response to chronic stress in the absence of cocaine.

2. Materials and Methods

2.1 Animals.

The current study utilized GluA2 K882A knock-in mice with a mutation of K882 within the intracellular C terminus of the GluA2 subunit (ablating the S/T-X-K/R kinase recognition motif) resulting in the prevention of PKC-mediated phosphorylation at S880A as described

previously [22]. Heterozygous GluA2 K882A KI mice on a C57bl/6 background were mated resulting in mutant and wildtype littermates. Mice (2–6 months old, 20–40g; age matched across group) were housed individually following catheterization surgery and during experimental paradigms. Male and female mice were included in all studies except for those involving social defeat. Statistical analyses of sex differences were performed but no significant differences were found. All animals were housed in a temperature and humidity controlled animal care facility with a 12 hr light/dark cycle (lights on a 7:00 A.M.). The Temple University Animal Care and Use Committee approved all procedures.

2.2 Drugs.

Cocaine was obtained from the National Institutes of Drug Abuse Drug Supply Program (Bethesda, MD) and dissolved in sterile 0.9% saline.

2.3 Forced swim stress.

Naïve wildtype and K882A KI mice were placed in plastic cylinders (23 cm tall x 14 cm-diameter) containing water (23–25°C), 10 cm deep for 6 min. A separate cohort of wildtype and K882A KI mice were tested following cocaine self-administration and extinction. Total immobility time per minute was scored by the ANY-Maze videotracking system (Wood Dale, IL) and confirmed with visual scoring by a trained observer.

2.4 Operant Food Training.

At 8 weeks of age, mice were single-housed, food restricted to approximately 90% of their free feeding weight, and began operant food training. The animals were first trained to exhibit an operant response for sucrose pellets. They were placed in operant chambers (Med-Associates) and learned to spin a wheel manipulandum to receive a sucrose pellet. A light and tone cue simultaneously occur with administration of pellet, followed by an 8s time-out with the house light off and no programmed consequences to wheel spins. The mice were limited to a maximum of 50 pellets during the 60 min operant session.

2.5 Jugular Catheterization Surgery.

Before surgery, mice were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine. An indwelling silastic catheter was inserted into the right jugular vein and sutured in place. The catheter was threaded subcutaneously over the shoulder blade and routed to a mesh back mount platform (Strategic Applications, Inc) to secure the placement. Following surgery, catheters were flushed daily with 0.1 mL of antibiotic (Timentin, 0.93 mg/ml) dissolved in heparinized saline and sealed with plastic obturators while not in use.

2.6 Cocaine Self-administration.

Mice were given 3–4 d to recover from surgery before the initiation of behavioral testing. The cocaine self-administration behavior was tested in 2-hour sessions (6 d per week) in the same chamber used for sucrose pellet self-administration. However, wheel responding now delivered an intravenous cocaine infusion (0.6 mg/kg/infusion), paired with the same compound cue, under the same fixed ratio schedule as food training, followed by an 8 s time-out. After 10 days of self-administration, cocaine-seeking behavior was extinguished

by replacing cocaine with 0.9% saline and removing the light and tone cues, previously paired with cocaine delivery. Daily 2 h extinction sessions occurred until animals performed <25% of their self-administration responding (average of last 3 days). Twenty-four hours after meeting this extinction criterion, animals underwent a stress-induced reinstatement session. Twenty minutes prior to the test, mice were exposed to a 6-min swim stress (see above). They were then placed in the operant chamber under the same conditions as extinction.

2.7 Conditioned Place Preference.

On day 1, mice were allowed to explore both sides of an unbiased 2 chambered CPP apparatus (20X20X20 cm) for 900 sec and time spent in each side was recorded. These data were used to separate animals into groups with approximately equal biases for each side. Beginning on day 2, animals received pairings for 8 days: cocaine (20mg/kg) on one side and saline on the other side. Drug-paired sides were randomized among all groups. Following conditioning, animals were all given a saline injection and allowed to explore freely between the two sides and time spent on each side was recorded. The Preference Score (time spent in drug paired side minus time in saline paired side) was calculated for each mouse. After the preference test, animals underwent extinction training during which saline was paired with both sides of the box for a total of 12 days. On the extinction test day, the time spent on each side was recorded. For the stress-induced reinstatement test, animals were exposed to a 10-min restraint stress immediately prior to being placed in the CPP apparatus and measuring time spent on each side.

2.8 Social Defeat Stress.

Wildtype and GluA2 K882A mice were submitted to social defeat stress for 15 consecutive days. Every day, each experimental mouse was introduced into the home cage of an unfamiliar resident for 5 min and was physically defeated. Resident aggressor mice were CD1 breeders selected for their attack latencies that were reliably shorter than 30 s upon 3 consecutive screening tests. After 5 min of physical interaction, intruders were housed in sensory contact with the aggressor through a perforated Plexiglas partition for 24 hours. Intruder mice were exposed to a new resident aggressor mouse each day. The non-defeated control animals were individually housed without any contact with aggressor mice. On Day 10 and Day 15 of defeat, interaction tests were carried out as previously described [23]. Briefly, both naïve male control mice and male mice exposed to the defeat procedure were allowed to habituate to and explore the testing arena: a white plastic open field (42 × 42 cm), which contained an empty removable wire mesh Plexiglas box (10 × 6.5 cm) located at one end of the field (“no-target” condition). After 2.5 minutes, the experimental mouse was removed from the arena and placed back into its home cage for approximately one min, while a CD1 male aggressor was placed inside the Plexiglas box. The experimental mouse was moved back in the arena and its trajectories were recorded for 2.5 min (“target” condition). A video tracking system was used to record and analyze the time spent in the “interaction zone” (8 cm perimeter surrounding the Plexiglas box) during the “no target” and “target” conditions, and time spent interacting was confirmed with visual scoring by a trained observer. In addition, the time spent in the corners, opposite to the location of the

box, and total locomotion were recorded. A different mouse was used on the first and second interaction tests.

2.9 Data Analysis.

All analyses were performed using GraphPad Prism 7.0 software package (GraphPad Software, San Diego, CA). Behavioral data were analyzed using two-tailed Student's t-tests (in cases of equal variances between the groups), two-tailed Welch's t-tests (in cases of unequal variance), or two-way ANOVA with Sidak's post hoc as appropriate. Statistical significance for all tests was set at $\alpha = 0.05$.

3. Results

3.1 Disruption of PKC-mediated phosphorylation of GluA2 increases vulnerability to stress following cocaine exposure

Cocaine-naïve GluA2 K882A KI and wildtype control mice were exposed to a 6-min forced swim stress. While both wildtype and K882A KI mice exhibited an increase in time spent immobile over the course of the 6-min stress exposure, no differences were seen between the genotypes (Fig. 1a; effect of time, $F(5, 35)=30.8$, $p < 0.0001$; effect of genotype, $F(1,13)=1.31$, $p=0.27$; Fig. 1b; $t(13)=1.15$, $p=0.27$; $N=70-8$). However, following 2 weeks of cocaine self-administration and extinction of cocaine responding, GluA2 K882A KI mice exhibit a sensitized response to the forced swim stress compared to the wildtype mice (Fig. 1c; effect of genotype, $F(1,7)=10.3$, $p=0.015$; interaction, $F(5,35)=2.85$, $p=0.03$; Fig. 1d; $t(7)=3.21$, $p=0.015$; $N=4-5$).

3.2 Disruption of PKC-mediated phosphorylation of GluA2 increases vulnerability to stress-induced reinstatement of cocaine seeking and conditioned preference

GluA2 K882A KI and wildtype control mice received 10 days of food self-administration sessions to acquire an operant response for food. As we have shown previously, the K882A mutation did not alter the ability of mice to acquire an operant response for food, nor alter their food intake or responding [17]. After ten days of food training, all the mice underwent jugular catheterization surgery and completed ten days of cocaine self-administration. The K882A mutation did not affect drug intake, responding for a cocaine reward, or extinction of cocaine seeking (Fig. 2a; effect of test, $F(1,13)=26.1$, $p=0.0002$; effect of genotype, $F(1,13)=0.385$, $p=0.55$; $N=6-9$). Twenty-four hours after the last extinction session, mice were exposed to a 6-min forced swim stress. Twenty minutes after the swim the animals were placed back into the operant chamber for a reinstatement session in which no cues or drug were presented. GluA2 K882A KI mice exhibited an increase in cocaine seeking following the stress exposure compared to wildtype littermates (Fig. 2b; $t(9.96)=3.49$, $p=0.0059$).

To determine whether the effects on stress-induced reinstatement were specific to reinstatement of cocaine seeking, we examined stress-induced reinstatement of cocaine conditioned place preference in an independent group of mice. Preconditioning day data revealed no initial bias in either wildtype or K882A mutant mice. On test day, both wildtype and K882A KI mice exhibited a significant preference for the cocaine-paired side of the

testing apparatus (Fig. 2c; effect of test, $F(1,22)=146$, $p<0.0001$; effect of genotype, $F(1,22)=0.100$, $p=0.75$; $N=10-14$). On the extinction test day none of the groups demonstrated a significant place preference. Twenty-four hours following the extinction test, mice were exposed to a 10-min restraint stress. Immediately after, they were placed in the conditioning apparatus and tested for preference. The GluA2 K882A mice spent significantly more time on the cocaine-paired side compared to the wildtype mice during this stress reinstatement test (Fig. 2d; $t(20)=2.20$, $p=0.039$). Furthermore, a two-way ANOVA revealed the GluA2 K882A mice exhibit significant reinstatement of responding following restraint stress compared to their extinction responding, while wildtype mice do not (Fig. 2; interaction, $F(1,20)=6.9$, $p=0.016$; Sidak's post-hoc test K882A extinction vs. reinstatement, $p=0.007$).

3.3 Disruption of PKC-mediated phosphorylation of GluA2 increases vulnerability to social defeat stress

To evaluate the impact of chronic social stress, we examined social interactions in wildtype and GluA2 K882A KI mice following a 10-day social defeat paradigm and compared them to control mice that were not defeated. No differences in social interaction behavior were seen between wildtype and K882A KI mice in the control (non-defeated) condition so these two groups were combined into a single control group for statistical purposes (Difference between no target and target animal conditions, WT: 10s; K882A KI: 9.2s). Following this 10 day defeat, GluA2 K882A KI mice spent less time within the interaction zone when the novel CD1 mouse was present compared to the control condition (Fig. 3a; effect of group, $F(2,16)=4.61$, $p=0.026$; effect of social interaction test condition, $F(1,16)=2.52$, $p=0.13$; interaction, $F(2,16)=4.57$, $p=0.027$; Sidak's *post hoc*, GluA2 K882A KI no target vs. target animal $p=0.0096$; $N=5-8$). Defeated K882A KI mice also spent significantly more time in the corner of the interaction chamber in the presence of a novel animal (Fig. 3b; effect of group, $F(2,16)=3.97$, $p=0.038$; effect of social interaction test condition, $F(1,16)=12.77$, $p=0.0025$; interaction, $F(2,16)=4.99$, $p=0.021$; Sidak's *post hoc*, GluA2 K882A KI no target vs. target animal $p=0.0003$). To determine whether these differences are related to the length of the social stressor, the same cohort of defeat mice was exposed to 5 additional days of defeat and again tested for social interaction. No differences were seen in the behavior of the controls between the first and second social interaction tests. Following 15 days of defeat, both wildtype and GluA2 K882A KI mice spent less time within the interaction zone when the novel CD1 mouse was present compared to the control condition (Fig. 3c; effect of group, $F(2,16)=30.7$, $p<0.0001$; effect of social interaction test condition, $F(1,16)=10.7$, $p=0.0051$; interaction, $F(2,16)=7.73$, $p=0.0049$; Sidak's *post hoc*, no target vs. target animal: Wildtype $p=0.013$, K882A $p=0.0078$). However, even after 15 days of defeat, only K882A KI mice spent more time in the corner when the target animal was present during the interaction test (Fig. 3d; effect of group, $F(2,16)=6.63$, $p=0.0087$; effect of social interaction test condition, $F(1,16)=16.3$, $p=0.0011$; interaction, $F(2,16)=5.38$, $p=0.017$; Sidak's *post hoc*, no target vs. target animal K882A $p=0.0002$).

4. Discussion

Tuning of excitatory transmission is an essential mechanism for activity-dependent alterations in neural signaling. This tuning occurs, in part, through the trafficking of glutamatergic AMPA receptors in and out of the synapse [24–26]. Both stress [18–20, 27] and cocaine experience [6, 17, 28–30] lead to changes in AMPA receptor trafficking, and the current data demonstrate that this trafficking is involved in the behavioral intersection between stress and cocaine. These results reveal that disrupting GluA2 phosphorylation affects responsivity to an acute stressor only after chronic exposure to cocaine. This increased responsivity to acute stress leads to an increased vulnerability to stress-induced reinstatement of both cocaine seeking and cocaine conditioned reward in our GluA2 phosphomutant mice. Furthermore, we found that impaired GluA2 phosphorylation enhances vulnerability to chronic social defeat stress, suggesting that AMPA receptor trafficking may be more important for behavioral outcomes after chronic manipulations rather than acute exposure.

We have previously shown that disrupting activity-dependent AMPA receptor trafficking, either by disrupting PKC-mediated GluA2-containing AMPA receptor internalization, knocking out a protein involved in the anchoring of GluA2-containing AMPA receptors, or by overexpressing GluA2-containing AMPA receptors, leads to increased cue-induced reinstatement behavior [17, 30]. Although seemingly opposing in their specific effects on GluA2-containing AMPARs, they all lead to a net increase in transmission in the nucleus accumbens [17, 30]. Increased AMPA transmission within the nucleus accumbens is seen following exposure to acute stress and manipulations that decrease glutamate overflow disrupt the ability of stress to potentiate the acquisition of drug self-administration [31]. As chronic cocaine use triggers the insertion of calcium-permeable AMPA receptors (CP-AMPARs) in the nucleus accumbens and our phosphomutant mice exhibit increased AMPA transmission independent of actions at CP-AMPARs, the combination of this mutation and cocaine experience could represent a two-hit model in which mutant mice are rendered even more sensitive to stress due to the increased glutamate transmission [32, 33].

It should be noted that the ability of stress to elicit locomotor sensitization to cocaine is driven by its actions on dopamine rather than its actions on glutamate [34]. Furthermore, manipulations that disrupt the ability of stress to increase acquisition of cocaine self-administration do not affect the ability of stress to alter AMPA transmission [31]. Together with the current results, this suggests that the mechanisms by which cocaine can sensitize responses to stress are mediated by different substrates than those responsible for the ability of stress to sensitize drug responses. This would also support the hypothesis that the ability of disrupting GluA2 phosphorylation to potentiate vulnerability to stress-induced reinstatement of cocaine seeking and cocaine conditioned reward was driven by the effects on stress responsivity.

The effects of cocaine experience on stress responsivity in the current study are mediated by both an increase in the response to stress in the K882A KI mice but also, in part, by a decrease in the response to stress in the wildtype mice. As cocaine exposure is stressful and can lead to increased anxiety [35–37], this suggests that wildtype animals have an adaptive

response to repeated stress that was not engaged in the K882A KI mice. Future work could be done to examine how repeated stress engages trafficking of GluA2-containing AMPARs.

In support of the idea that AMPAR trafficking plays a role in the response to chronic stress, the current results demonstrate that GluA2-containing AMPA receptors play a role in the susceptibility to social defeat. We have demonstrated an increase in susceptibility to social defeat in mice with increased accumbal AMPA transmission mediated by GluA2-containing AMPARs [17]. Susceptibility to social defeat stress is correlated with an increase in spine density within the nucleus accumbens [38], a structural change that is mediated by GluA2-containing AMPA receptors [39]. This structural plasticity is accompanied by an increase in the frequency of mEPSCs, indicating increased glutamate release into the nucleus accumbens [38]. This increased input activity seems to be mediated by projections from the ventral hippocampus to the nucleus accumbens as decreasing activity in this projection decreases susceptibility to social defeat [40]. While increases in presynaptic glutamate transmission are seen in vulnerable animals following social defeat, postsynaptic increases in AMPA transmission seem to confer resilience. Animals that are resilient to social defeat exhibit an increase in GluA2 mRNA and overexpression of GluA2 in the nucleus accumbens induces a resilient phenotype [41]. This ability of GluA2 overexpression to promote resilience may be mediated by a decrease in GluA2-lacking, CP-AMPARs, which seem to mediate susceptibility [41]. In the current model, our mice exhibit specific changes in GluA2-containing AMPARs without any alterations in CPAMPAR activity [17]. Therefore, it is possible that the differences between the current results and those seen following GluA2 overexpression are due to the different effects on CP-AMPARs. However, as the mice used in the study exhibit a global disruption in GluA2 phosphorylation, our effects on social defeat vulnerability may be due to actions outside of the nucleus accumbens.

Parallel to what is seen in the nucleus accumbens; social defeat stress leads to decreased AMPA transmission within the hippocampus [42]. Furthermore, social defeat stress leads to decreased hippocampal GluA2 surface expression and decreased spine density [43]. Chronic unpredictable stress, another model of chronic stress exposure, also leads to a decrease in hippocampal AMPA transmission and subunit expression [44]. Consistent with the idea that decreased AMPA activity in the hippocampus leads to depressive phenotypes, decreased expression of genes encoding AMPA receptor subunits has been found in the hippocampus of depressed human patients [45].

Although the hippocampus also plays a clear role in social defeat stress, it is unlikely the increased hippocampal AMPA transmission would lead to increased susceptibility. Therefore, it seems unlikely that the depressive-like phenotype seen in our phosphomutant mice is due to actions within the hippocampus.

In contrast to the hippocampus and nucleus accumbens, stress leads to increased glutamate-mediated plasticity in the amygdala. Chronic stress increases spine density within the basolateral amygdala and this increase in spine density leads to increased capacity for glutamatergic plasticity [46–48]. Additionally, chronic stress leads to an increase in the frequency of AMPAR-mediated mEPSCs in the basolateral amygdala as well as an increase in amygdala excitability [49–51]. These increases in AMPAR-mediated transmission may be

due to an increase in AMPAR surface expression [50]. Taken together, this might suggest that the increase in vulnerability to social defeat seen here may be due primarily to the disruption of GluA2 phosphorylation within the amygdala. However, site-specific manipulations of AMPA receptor trafficking are needed to determine this conclusively.

Taken together, the current results indicate that disrupting GluA2 phosphorylation leads to an increase in the ability of cocaine to potentiate the response to an acute stressor. Furthermore, GluA2 K882A knock-in leads to an increased vulnerability to social defeat stress. These results highlight the importance of AMPA receptor trafficking in psychiatric disease and suggest that pharmacotherapies aimed at augmenting GluA2 phosphorylation may be effective in the treatment of stress-related disorders and stress-induced drug relapse.

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Highlights

- Disruption of PKC-mediated phosphorylation of GluA2 potentiates response to swim stress in cocaine-experienced mice
- Disruption of PKC-mediated phosphorylation of GluA2 increases vulnerability to stress-induced reinstatement of cocaine seeking
- Disruption of PKC-mediated phosphorylation of GluA2 Increases vulnerability to stress-induced reinstatement of cocaine conditioned reward
- Disruption of PKC-mediated phosphorylation of GluA2 potentiates vulnerability to social defeat stress

a. Naive Mice **b. Cocaine-Experienced Mice**

Figure 1. Disrupting GluA2 phosphorylation increases response to forced swim stress following cocaine exposure.

Naïve K882A KI and wildtype mice show no difference in time spent immobile during forced swim stress (a,b). However, following cocaine self-administration, K882A KI mice spend significantly more time immobile during a forced swim stress compared to wildtype animals (c,d; * $p < .05$).

Figure 2. Disrupting GluA2 phosphorylation potentiates stress-induced reinstatement of cocaine seeking and conditioned place preference.

While K882A KI mice do not differ from wildtypes in their cocaine seeking or extinction behavior, they exhibit increased reinstatement of cocaine-seeking following a six-minute forced swim stressor (a,b; * $p < .05$). Similarly, K882A KI exhibit normal acquisition and extinction of cocaine-conditioned place preference (c). However, following a restraint stress exposure that does not elicit reinstatement in wildtype mice, K882A KI mice exhibit reinstatement of cocaine conditioned reward (d; * $p < .05$).

Figure 3. Disrupting GluA2 phosphorylation increases vulnerability to social defeat stress.

Following 10 days of social defeat, male K882A KI mice exhibit decreased time in the interaction zone and increased time in the corners compared to nondefeated control mice when exposed to a novel CD-1 mouse (a,b). Wildtype mice do not exhibit a significant decrease in social interaction following 10 days of defeat. However, after 5 additional days of defeat both wildtype and K882A KI mice spend less time in the interaction zone than nondefeated controls (c). However, even after 15 days of defeat, only the K882A KI mice spend more time in the corners during the interaction test (d). * $p < .05$; *** $p < .001$ compared to nondefeated controls