Antidepressant-like effects of an AMPA receptor potentiator under a chronic mild stress paradigm

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Abstract

Enhancement of AMPA receptor (AMPAR) function has emerged as a novel strategy for treatment of depression. Nevertheless, studies on AMPAR function in chronic animal models used to predict antidepressant efficacy are surprisingly lacking. We investigated the role of AMPARs in antidepressant action in an unpredictable chronic mild stress (UCMS) model in BALB/c mice. After 3 wk of UCMS, BALB/c mice developed a number of depressive-like behaviours that were successfully prevented by fluoxetine (20 mg/kg) administration. The AMPAR potentiator LY392098 [N-2-(4-(3-thienyl)phenyl)propyl 2-propanesulfonamide] (5 mg/kg), when administered alone, functioned like classic antidepressants by reducing weight loss, fur deterioration and immobility in the tail suspension test. However, LY392098 did not restore sucrose preference and did not reduce anxiety (marble-burying) in stressed mice. In the same protocol, the AMPAR antagonist GYKI (10 mg/kg) reversed most, but not all, of the antidepressant-like actions of fluoxetine. Thus, the antidepressant-like effects of LY392098 were fully predicted by the AMPAR dependence of effects demonstrated for fluoxetine. Our results demonstrate that, in the UCMS paradigm, AMPAR activation exhibits antidepressant-like activity that relates preferentially to specific depressive-like responses and that those specific responses can be defined by their regulation by AMPAR modulation under conditions of stress.

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Introduction

AMPA receptor (AMPAR) activation is currently considered as one of the most promising new approaches for new antidepressant therapies as the balance between glutamate and GABA in general is becoming increasingly relevant in the field of depression.

Direct evidence comes from preclinical models of antidepressant activity. Specifically, AMPAR potentiators such as LY392098 are active in both the forced swim test (FST) and the tail suspension test (TST), two behavioural despair models that are used to screen and identify compounds with antidepressant activity (Bai et al. 2001; Li et al. 2001). There is also an increasing body of proof-of-concept evidence suggesting a role for AMPARs in depression and in the actions of antidepressant drugs. AMPAR potentiators result in neurobiological adaptations similar to those produced by antidepressants that are currently used in the clinical setting, including BDNF induction and increases in hippocampal progenitor cell proliferation (reviewed in Alt et al. 2006a; Bleakman et al. 2007; Skolnick, 2002). Data from studies using AMPA antagonists have further implicated a role for...
AMPAR activation in mediating antidepressant-like actions of a number of either clinically used or experimental compounds. Thus, the AMPA antagonists NBQX and GYKI 52466 reduced the effects of lithium in the FST and/or TST (Gould et al. 2008) and GYKI 53655, another AMPA antagonist, prevented the effects of LY392098 in the FST and TST (Li et al. 2001). NBQX also prevented both antidepressant-like effects in the TST and serotonin increases in the medial prefrontal cortex induced by MGS0039 an mGluR4/5 receptor antagonist with antidepressant potential (Karasawa et al. 2005). NBQX also prevented antidepressant-like effects of the NMDA receptor antagonists ketamine, MK801 and Ro25-6981, an NR2B antagonist (Maeng et al. 2008). Given the clinical validation of ketamine (Zarate et al. 2006) and NR2B receptor blockade (CP101,606) (Preskorn et al. 2008) as effective antidepressants for treatment-resistant depression, identification of AMPAR potentiation as a potential mechanism for these effects (Maeng et al. 2008) is significant. Additional preclinical support comes from the finding that AMPAR subunit 1 (GluR-A) knockout mice show increased learned helplessness (Chourbaji et al. 2008).

However, only one study has previously evaluated this mechanism in antidepressant-detecting assays that require subchronic dosing (Knapp et al. 2002). Although models such as the FST and TST are widely used as tests of antidepressant efficacy, additional appreciation of the potential value of AMPAR potentiation in animal models of depression should be gained. Chronic mild stress models are considered of high face, construct and predictive validity. In these models prolonged exposure to uncontrollable and unpredictable stressors results in depressive-like behaviours that can be prevented or reversed by chronic but not acute antidepressant treatment (Griebel et al. 2002; Mineur et al. 2006; Surget et al. 2008).

In this paper, we provide the first evidence showing that an AMPAR potentiator can relieve some effects engendered by chronic mild stress. Specifically we examined (i) the possible antidepressant profile of the AMPAR potentiator LY392098 and (ii) the role of AMPAR activation in the antidepressant-like actions of the reference antidepressant fluoxetine.

**Materials and methods**

**Animals**

Male BALB/c mice (aged 6 wk; n = 10 per group) were purchased from Charles River (France). This strain was chosen because BALB/c mice are particularly sensitive to stress, and respond in a reproducible manner to unpredictable chronic mild stress (UCMS) protocols. Experiments started after a 2-wk acclimation period; at the beginning of experiments mice were aged 8 wk. During the 2-wk acclimation period all mice were housed under standard conditions and had access to rodent pellet food and water available ad libitum. All experiments were performed in accordance with the European Communities Council Directive (86/809/EEC) regarding the care and use of animals for experimental procedures and approved by the Comité d’éthique INSERM/UPMC/CNRS.

**Drugs**

GYKI 52466 was purchased from Tocris (USA). Fluoxetine and LY392098 [N-2-(4-(3-thienyl)phenyl)-propyl 2-propanesulfonamide] were synthesized by Eli Lilly and Company (USA). GYKI 52466 [10 mg/kg in acidified saline; a dose comparable to that used by Gould et al. 2008 (5 mg/kg) and Le Merrer & Stephens, 2006 (5–10 mg/kg)], fluoxetine (20 mg/kg in 5% DMSO, 5% cremor, 90% saline; Crozatier et al. 2007; Moutsimilli et al. 2005, 2008) and LY392098 (5 mg/kg in saline; Li et al. 2001, 2003) were injected intraperitoneally at a volume of 10 ml/kg. A number of studies from our group in rats and mice have shown that there are no behavioural effects upon intraperitoneal injection of 5% DMSO, 5% cremor, 90% saline vehicle in TST, locomotion and anxiety measures in mice (Crozatier et al. 2007; Herzog et al. 2008, Tzavara et al. 2003, 2006). In addition, previous chronic administration studies from our group have shown that there is no difference in biochemical (Moutsimilli et al. 2005, 2008) and behavioural (not shown) outcomes upon repeated administration of different vehicles (5% DMSO, 5% cremor, 90% saline vs. acidified saline vs. saline) in mice; therefore, vehicle values were pooled and vehicles were treated as a single experimental group in this study.

**Chronic mild stress protocol and behavioural tests**

After a 2-wk acclimation period mice were subjected to a 3-wk mild stress protocol. Mice subjected to the UCMS protocol were singly housed. Stressors were applied twice a day for a 3-h period and overnight in a randomized order; vehicle or drugs were injected daily between 12:00 and 14:00 hours (Fig. 1a). Stressors typically included wet bedding, tilted cages, forced swim stress (for 6 min), paired housing, food and water deprivation during confinement in
A 15 cm × 30 cm wired cage, crowding; overnight stressors included tilted cages, wet bedding, unpredictable illumination and reversed light–dark cycle. Control (non-stressed) mice were held in a room next to that of the stressed mice and were group-housed (n = 4 per cage).

During this period the animal’s weight and fur condition were measured every 3 d. At the end of the chronic stress protocol the emotional state of the animals was evaluated in the marble-burying test, TST, locomotor activity and the sucrose preference tests; the tests were conducted throughout a 3-d period during which mice continued to receive injections and stressors (Fig. 1b).

Fur condition rating

The score of the animal’s coat state was calculated as the sum of seven scores, one score for each of seven parts of the body (Surget et al. 2008) with some modifications. Namely, the state of each part was rated on a 3-point scale (3, very good; 2, fair; 1, poor).

TST

Behavioural despair was investigated in the TST as previously described (Crozatier et al. 2007) using an automated TST apparatus (Bioseb, France). Mice were individually suspended by their tail using a paper adhesive tape placed 1 cm from the tip of the tail and immobility (in seconds) was automatically recorded during the 6-min test period. In one TST experiment (Fig. 2a) one of the mice was observed climbing on its tail, this animal was excluded from the statistical analysis.

Marble-burying test

The marble-burying test (Li et al. 2006) was performed in cages 10 × 15 × 20 cm filled up to the 7.5 cm mark with sawdust on top of which were placed 12 marbles evenly spaced in four rows and 3 columns. The number of marbles buried was counted for every minute up to the first 10 min and then for every 5 min up to 30 min. Buried marbles were defined as being buried by at least three quarters of bedding.
Sucrose preference test

For the sucrose preference test mice were first habituated to drink from two graduated pipettes one filled with water, and the other with sucrose solution for 3 d, the side of the sucrose pipette being alternated each day. On day 4 the two pipettes were presented again; however, one was filled with water and the other with 4% sucrose.

Locomotor activity

Horizontal activity (ambulations) was assessed in transparent activity cages (20 × 15 × 25 cm), with
automatic monitoring of photocell beam breaks (Imetronic, France). The mice were placed into the recording chamber at 19:00 hours and locomotor activity was recorded for a 1-h period.

**Experiments**

**Expt 1**

In this experiment we sought to assess the potential antidepressant profile of the AMPA potentiator LY392098, by examining its ability to reverse physical and behavioural alterations induced by chronic stress. The experimental groups compared were (i) control non-stressed, non-treated mice; (ii) stressed mice treated with vehicle; (iii) stressed mice treated with LY392098 (5 mg/kg).

Physical (weight and fur condition) measurements and behavioural testing were conducted as above.

To ensure that our results are not due to a generalization of action of LY392098, the possible effects of repeated treatment with the AMPA potentiator were assessed in non-stressed mice, in a second set of mice. These were control mice that were group-housed (n=4 per cage) and administered with vehicle or LY392098, daily between 12:00 and 14:00 hours for a 3-wk period. Physical (weight and fur condition) measurements and behavioural testing were conducted exactly as for stressed mice as described above and as depicted in Fig. 1b, with the difference that no stress was ever applied.

**Expt 2**

In this experiment we sought to assess the ability of the AMPAR antagonist GYKI52466 to reverse antidepressant effects of fluoxetine in the chronic mild stress protocol.

For this, during the 3-wk stress period mice were randomly assigned to one of the following treatment groups: (i) vehicle; (ii) GYKI52466 (10 mg/kg); (iii) fluoxetine (20 mg/kg); (iv) GYKI52466 (10 mg/kg) in combination with fluoxetine (20 mg/kg).

Physical (weight and fur condition) measurements and behavioural testing were conducted as above.

As for expt 1, in order to ensure that our results are not due to a generalized action of fluoxetine, GYKI52466 or their combination, the possible effects of repeated treatment with the above compounds were assessed in non-stressed mice, in a second set of mice. These were control mice group-housed (n=4 per cage) and were administered with vehicle, fluoxetine, GYKI52466 or their combination daily between 12:00 and 14:00 hours for a 3-wk period.

**Statistics**

Data are presented as mean ± S.E. of n=10 mice per group.

**Expt 1**

Statistical analysis was performed by one-way ANOVA (three groups: non-stressed, stressed + vehicle, stressed + LY392098) for weight loss, fur condition, TST, locomotor activity and sucrose preference and by repeated-measures (time) one-way (three groups: non-stressed, stressed + vehicle, stressed + LY392098) ANOVA for marble-burying. When one-way ANOVA revealed significant group effects Duncan’s post-hoc test was utilized to compare between the three groups.

To analyse the effects of repeated treatment with the AMPA potentiator in non-stressed mice we utilized t test comparisons between vehicle-treated and LY392098-treated mice.

**Expt 2**

Statistical analysis was performed by two-way [treatment 1 (vehicle or GYKI52466)×treatment 2 (vehicle or fluoxetine)] ANOVA for weight loss, fur condition, TST, locomotor activity and sucrose preference and by repeated-measures (time) two-way (treatment 1×treatment 2) ANOVA for marble-burying. When two-way ANOVA revealed significant main or interaction effects Duncan’s post-hoc test was utilized to compare between groups. Statistical analysis was done separately for stressed and non-stressed mice.

All statistical analyses were performed with Statistica software (SysStat, Germany).

**Results**

**Expt 1**

For immobility in the TST one-way ANOVA revealed a statistically significant difference between groups [non-stressed, stressed + vehicle, stressed + LY392098; F(2,26)=3.66, p<0.05]. Stressed mice treated with LY392098 displayed more attempts to escape in the TST compared to vehicle-treated stressed mice (p=0.017, Duncan’s post-hoc test; Fig. 2a).

For fur condition, one-way ANOVA revealed a statistically significant difference between groups
[non-stressed, stressed + vehicle, stressed +LY392098; \( F(2, 27) = 56, p < 0.001 \)]. In stressed mice treated with vehicle, fur was markedly degraded compared to non-stressed mice \( (p = 0.0006, \text{Duncan's post-hoc test}; \text{Fig. 2b}) \), whereas in LY392098-treated stressed mice fur degradation was prevented \( (p = 0.0015, \text{Duncan's post-hoc test}; \text{Fig. 2b}) \).

For weight loss, one-way ANOVA revealed a statistically significant difference between groups [non-stressed, stressed + vehicle, stressed +LY392098; \( F(2, 27) = 4.7, p < 0.05 \)]. Stress resulted in weight loss in vehicle-treated mice \( (p = 0.0093, \text{Duncan's post-hoc test}; \text{Fig. 2c}) \), whereas weight evolution for LY392098-treated stressed mice did not differ from that of non-stressed mice \( (p = 0.54, \text{Duncan's post-hoc test}; \text{Fig. 2c}) \).

For sucrose preference, one-way ANOVA revealed a statistically significant difference between groups [non-stressed, stressed + vehicle, stressed +LY392098; \( F(2, 27) = 3.5, p < 0.05 \)]. Stress resulted in a marked loss of sucrose preference in vehicle-treated mice \( (p = 0.023, \text{Duncan's post-hoc test}; \text{Fig. 2d}) \). Loss of sucrose preference was attenuated in LY392098-exposed mice but this effect did not reach statistical significance \( (p = 0.45, \text{Duncan's post-hoc test}; \text{Fig. 2d}) \).

For marble-burying, repeated-measures (time) one-way ANOVA revealed a significant interaction for time \( \times \) group \( [F(26, 351) = 1.74, p < 0.05] \). Duncan’s post-hoc test showed that stressed + vehicle and stressed + LY392098 mice buried more marbles than non-stressed mice \( (p = 0.014 \text{ and } p = 0.011 \text{ for stressed + vehicle and stressed + LY392098, respectively, vs. non-stressed for the last time-point); there was no difference between stressed + vehicle and stressed + LY392098 mice \( (p = 1 \text{ for the last time-point}; \text{Fig. 2e}) \).

For locomotor activity one-way ANOVA revealed no statistically significant difference across the groups tested (Fig. 2f). This suggests that the reduction of immobility in the TST seen with repeated LY392098 in stressed mice is not due to a non-specific generalized effect on locomotor activity.

To ensure that our results were not due to a generalized action of LY392098 that was not specific to the control of stress-induced behavioural signs, the possible effects of repeated treatment with the AMPA potentiator were assessed in non-stressed mice. There was no effect of repeated LY392098 on any of the parameters tested in non-stressed mice (Fig. 3).

### Expt 2

For immobility in the TST, two-way ANOVA revealed a statistically significant interaction for treatment \( 1 \times \) treatment \( 2 \) (AMPA antagonist or vehicle) \( \times \) treatment \( 2 \) (fluoxetine or vehicle) \( [F(1,36) = 5.96, p < 0.05] \). Duncan’s post-hoc test revealed that fluoxetine prevents stress-induced increases in immobility in the TST \( (p = 0.038 \text{ for vehicle + vehicle vs. vehicle + fluoxetine); this antidepressant-like effect is blocked by co-administration of GYKI52466 \( (p = 0.044 \text{ for vehicle + fluoxetine vs. GYKI52466 + fluoxetine; } p = 1 \text{ for vehicle + vehicle vs. GYKI52466 + fluoxetine). GYKI52466 administered alone did not affect stress-induced increases in immobility in the TST \( (p = 0.23 \text{ for GYKI52466 + vehicle vs. vehicle + vehicle}) (\text{Fig. 4a}) \).

For fur condition, two-way ANOVA revealed a statistically significant interaction for treatment \( 1 \times \) treatment \( 2 \) \( [F(1,36) = 7.19, p < 0.05] \). Duncan’s post-hoc test showed that fluoxetine prevents stress-induced fur degradation \( (p = 0.0008 \text{ for vehicle + fluoxetine vs. vehicle + vehicle); this antidepressant-like effect is blocked by co-administration of GYKI52466 \( (p = 0.0015 \text{ for vehicle + fluoxetine vs. GYKI52466 + fluoxetine). In contrast, GYKI52466 administered alone has no effect on this behavioural parameter \( (p = 0.79 \text{ for GYKI52466 + vehicle vs. vehicle + vehicle}) (\text{Fig. 4b}) \).

For weight-loss, two-way ANOVA revealed a statistically significant interaction for treatment \( 1 \times \) treatment \( 2 \) \( [F(1,36) = 5.9, p < 0.05] \). Duncan’s post-hoc test showed that fluoxetine prevents stress induced reduction in body weight \( (p = 0.008 \text{ for vehicle + fluoxetine vs. vehicle + vehicle); this antidepressant-like effect is blocked by co-administration of GYKI52466 \( (p = 0.005 \text{ for vehicle + fluoxetine vs. GYKI52466 + fluoxetine). GYKI52466 administered alone has no effect \( (p = 0.79 \text{ for GYKI52466 + vehicle vs. vehicle + vehicle}) \text{ (Fig. 4c)} \).

For sucrose preference, two-way ANOVA did not show a statistically significant interaction for treatment \( 1 \times \) treatment \( 2 \), but revealed a significant main effect of fluoxetine \( [F(1,36) = 8, p < 0.01] \). Fluoxetine prevents stress-induced loss of sucrose preference \( (p = 0.044, \text{Duncan's post-hoc test}) \). However, fluoxetine + GYKI52466-treated mice do not differ from fluoxetine + vehicle-treated mice \( (p = 0.65 \text{ (Fig. 4d)}) \).

For marble-burying, repeated-measures (time) two-way ANOVA revealed a significant interaction for time \( \times \) treatment \( 1 \times \) treatment \( 2 \) \( [F(13, 468) = 3.577, p < 0.001] \). Duncan’s post-hoc test showed that fluoxetine-treated mice buried less marbles that saline-treated mice \( (p = 0.032 \text{ for vehicle + fluoxetine vs. vehicle + vehicle for the last time-point); this effect was accentuated in mice treated with fluoxetine + GYKI52466 \( (p = 0.0073 \text{ for GYKI52466 + fluoxetine vs. vehicle + fluoxetine for the last time-point}).
GYKI52466 alone had no effect \((p=0.94\) for GYKI52466 + vehicle vs. vehicle for the last time-point\) (Fig. 4e).

For locomotor activity, two-way ANOVA revealed no statistically significant difference among the groups tested (Fig. 4f). This suggests that the effects on immobility in the TST seen with fluoxetine, GYKI52466, or their combination in stressed mice, are not due to non-specific generalized effects on locomotor activity.

To ensure that our results are not due to a generalized action of fluoxetine, GYKI52466 or their combination, the possible effects of repeated treatment with the above compounds were assessed in non-stressed mice. None of the treatments affected fur condition, weight evolution, sucrose preference, or locomotor activity in non-stressed mice.

For the TST, two-way ANOVA revealed a statistically significant effect of treatment 2 (fluoxetine vs. vehicle) \([F(1, 36)=10.6, p<0.01]\); but not for interaction between treatment 1 (AMPA antagonist or vehicle) x treatment 2 (fluoxetine or vehicle). Duncan’s post-hoc test showed that fluoxetine reduces immobility in the TST \((p=0.0043\) for vehicle + fluoxetine vs. vehicle + vehicle); this effect is only partially affected by co-administration of GYKI52466 \((p=0.32\) for GYKI52466 + fluoxetine vs. vehicle + fluoxetine and \(p=0.039\) for0 GYKI52466 + fluoxetine vs. GYKI52466 + vehicle). GYKI52466 administered alone did not affect immobility in the TST \((p=0.83\) for GYKI52466 + vehicle vs. vehicle + vehicle) (Fig. 5a).

For marble-burying, repeated-measures (time) two-way ANOVA revealed a significant interaction for treatment 2 (fluoxetine vs. vehicle) \([F(13, 468)=5.4, p<0.001]\). Duncan’s post-hoc test showed that fluoxetine-treated non-stressed mice buried less marbles than saline-treated mice \((p=0.033\) for vehicle + fluoxetine vs. vehicle + vehicle for the last time-point);
Fig. 4. Effects of the AMPA antagonist GYKI52466 on the antidepressant effects of fluoxetine in the unpredictable chronic mild stress in the Balb/C mouse. (a) The AMPA antagonist GYKI52466 blocks the antidepressant effects of fluoxetine in the TST. Stressed mice treated with fluoxetine + GYKI did not differ from vehicle-treated stressed mice and displayed increased immobility compared to mice treated with fluoxetine alone (* p<0.05 compared to stress, * p<0.05 compared to stress-fluoxetine, two-way ANOVA and Duncan’s post-hoc test; n=10 per group). (b) The AMPA antagonist GYKI52466 blocks the antidepressant effects of fluoxetine in the fur condition. Stressed mice treated with fluoxetine + GYKI did not differ from vehicle-treated stressed mice and displayed greater coat deterioration compared to mice treated with fluoxetine alone (* p<0.05 compared to stress, # p<0.05 compared to stress-fluoxetine, two-way ANOVA and Duncan’s post-hoc test; n=10 per group). (c) The AMPA antagonist GYKI52466 blocks the antidepressant effects of fluoxetine in weight loss. Stressed mice treated with fluoxetine + GYKI did not differ from vehicle-treated stressed mice and displayed reduced weight gain compared to mice treated with fluoxetine alone (* p<0.05 compared to stress, * p<0.05 compared to stress-fluoxetine, two-way ANOVA and Duncan’s post-hoc test; n=10 per group). (d) The AMPA antagonist GYKI52466 does not block the antidepressant effects of fluoxetine in sucrose preference. Stressed mice treated with fluoxetine + GYKI did not differ from fluoxetine-treated stressed mice (* p<0.05 compared to stress-fluoxetine, repeated-measures two-way ANOVA and Duncan’s post-hoc test; n=10 per group). (e) The AMPA antagonist GYKI52466 potentiates the anxiolytic effects of fluoxetine in the marble-burying test. Stressed mice treated with fluoxetine + GYKI buried even fewer marbles than fluoxetine-treated stressed mice (* p<0.05 compared to stress, * p<0.05 compared to stress-fluoxetine, repeated-measures two-way ANOVA and Duncan’s post-hoc test; n=10 per group). (f) No difference in locomotor activity between any of the groups.
this effect was maintained in mice treated with fluoxetine + GYKI52466 ($p = 0.83$ for GYKI52466 + fluoxetine vs. vehicle + fluoxetine and $p = 0.033$ for GYKI52466 + fluoxetine vs. GYKI52644 + vehicle for the last time-point). GYKI52466 alone had no effect ($p = 0.83$ for GYKI52466 + vehicle vs. vehicle + vehicle for the last time-point) (Fig. 5c).

**Discussion**

This study assessed the role of AMPAR function in a chronic model relevant to depression and antidepressant reversal under conditions of chronic mild stress. We used a chronic mild stress protocol, in which BALB/c mice were subjected to a sequence of unpredictable stressors for 3 wk. In this study, after 3 wk of unpredictable stress we observed marked trends for increased helplessness and increased anxiety-like signs, as well as anhedonia in BALB/c mice. We also observed a progressive physical degradation, manifested as a marked deterioration of the state of the coat of the stressed animals that is attributed to reduced grooming. Growth, measured as body weight gain, was reduced in stressed BALB/c mice.
compared to non-stressed controls. These behavioural changes resulting from stress are comparable to those reported in the literature (Griebel et al. 2002; Mineur et al. 2006; Surget et al. 2008). Effects of a positive allosteric modulator of AMPARs was then compared to that of fluoxetine for its ability to prevent stress-induced behavioural deterioration. Further, an AMPAR antagonist was used to prevent effects of the SSRI antidepressant fluoxetine to ascertain which behavioural changes induced by chronic mild stress were responsive to AMPA-mediated changes.

The AMPAR potentiator LY392098 was administered daily throughout the 3-wk period in mice submitted to stress. The dose of LY392098 (5 mg/kg) was selected to be in the range of doses effective in acute tests of antidepressant activity (Li et al. 2001, 2003). LY392098 administration resulted in a less pronounced depressive-like phenotype in the mice when impacted by chronic stress than vehicle-treated mice. It should be noted that repeated LY392098 administration did not elicit any effect in non-stressed BALB/c mice, suggesting that the effects observed in stressed mice are specific to stress-induced behavioural alterations. Namely, depressive-like signs were markedly attenuated in stressed BALB/c mice receiving LY392098 compared to vehicle-receiving BALB/c controls. AMPAR potentiator-treated mice did not lose as much weight as vehicle controls, their coat was healthier and their attempts to escape in the TST were enhanced. These antidepressant-like effects were also not due to a non-specific effect of LY392098 on general activity, since LY392098 did not affect locomotion as measured in an actimeter. Thus, the effects of LY392098 were similar to those seen with different classes of compounds with antidepressant activity. Indeed, clinically useful antidepressants such as fluoxetine and imipramine, as well as putative antidepressant compounds with novel mechanisms of action (e.g. CRF1 antagonist SSR125543, or the vasopressin antagonist SSR149415) (Griebel et al. 2002; Surget et al. 2008) prevent stress-induced depressive-like behaviours in chronic stress models. However, LY392098 did not reduce anxiety-like behaviours as measured by the latency to bury marbles in stressed BALB/c mice. This is unsurprising since AMPAR antagonists were shown to display anxiolytic potential in rodents (Alt et al. 2006b). The fact that the AMPA/kainate blockade mediates anxiolytic-like effects whereas AMPA potentiation induces antidepressant-like effects, illustrates the idea that different molecular strategies might be needed for treating distinct symptom clusters of depressive disorders. LY392098, at the dose used, did not restore sucrose preference to a statistically significant level in stressed BALB/c mice. The role of AMPAR in perception of positive emotional valence and reward is unclear, and AMPA effects appear to be dependent on the region and the GluR subunit studied (Todtenkopf et al. 2006). Further experiments with different behavioural paradigms are needed to study the effects of AMPA potentiators in rodent models of depression.

In a parallel experiment the AMPA antagonist GYKI52466 was used to investigate whether AMPAR activation mediates antidepressant-like effects of fluoxetine in chronic mild stress. Fluoxetine, administered at 20 mg/kg throughout the chronic stress, prevented or alleviated the expression of depressive-like physical signs and behaviours. Similar results have been reported in the literature, as discussed above (Griebel et al. 2002; Mineur et al. 2006; Surget et al. 2008). Co-administration of the AMPAR antagonist GYKI52466, at 10 mg/kg, reversed most, but not all, of the antidepressant actions of fluoxetine, whereas GYKI52466 alone had no effect in any of the physical or behavioural changes induced by chronic mild stress in BALB/c mice. In particular, GYKI52466 prevented the effects of fluoxetine in the TST, in fur deterioration and in weight loss. In contrast, GYKI52466 did not alter the effects of fluoxetine on sucrose preference and even potentiated the effects of fluoxetine in the marble-burying test. Chronic antidepressant administration was shown to modulate neuronal gluta-matergic protein expression (Moutsimilli et al. 2005; Tordera et al. 2005) and several lines of evidence have suggested a role of AMPAR in mediating the effects of some classes of classical antidepressants. Fluoxetine has been found to alter AMPAR phosphorylation in a manner that is expected to increase AMPAR signalling (Svenningsson et al. 2002), and similar effects were seen with serotonergic compounds that may have antidepressant activity (Svenningsson et al. 2007a), while the effects of tianeptine in the TST are not seen in phosphomutant GluR1 mice (Svenningsson et al. 2007b). Increased expression of GluR1 has also been correlated with enhanced activity of antidepressants (Crozatier et al. 2007) in the same manner that LY392098 at low doses was shown to enhance antidepressant potency (Li et al. 2003). Furthermore, chronic administration of antidepressants was shown to increase GluR1 mRNA expression (Svenningsson et al. 2002), as well as AMPAR expression and synaptic targeting (Du et al. 2007; Martinez-Turrillas et al. 2007; Tan et al. 2006). Results of the present study are consistent with these biochemical findings since AMPAR activation was necessary for the full expression of...
the antidepressant-like effects of fluoxetine on a host of parameters in mice exposed to UCMS.

Although based upon limited parametric analysis (e.g. one dose of fluoxetine), the data comparing the behaviours/signs for which subchronic fluoxetine demonstrated AMPAR-dependent modifications to those behaviours/signs affected by LY392098 reveals critical new information on the AMPAR hypothesis of mood disorders (Alt et al. 2006a). AMPAR dependence on the effects of fluoxetine were demonstrated in the TST, fur deterioration, and body weight measures. Similarly, LY392098 was effective in preventing the effects of stress on these measures. Conversely, fluoxetine did not show AMPAR-dependent effects in the marble-burying or sucrose preference assays, the two assays for which LY392098 did not significantly modify stress-induced behavioural changes. These data provide at least two important conclusions. (1) Subchronic fluoxetine-induced antidepressant-like effects under these conditions are dependent upon AMPARs for some, but not all, behavioural signs and (2) the antidepressant-like efficacy of the AMPAR potentiator LY392098 is consistent with this mechanism of action; activity against behaviours driven by AMPAR modulation and lack of efficacy against those in which AMPAR modulation is less relevant.

Overall the data presented here show that the AMPA potentiator LY392098 exhibits antidepressant-like activity in key stress-induced behavioural alterations in the chronic mild stress protocol in mice. However, it should be noted that depression is not a homogenous disease but rather embraces a constellation of symptoms and diagnostic criteria. It has been proposed that different symptom clusters may distinguish between different types of depression. Thus, the prevalence of anhedonia and irritability in contrast to the prevalence of fatigue and impulsivity (reviewed in Gold & Chrousos, 2002) might be relevant to the need for different treatment options for different aspects/subtypes of mood disorders. A lack of effect on a standard measure of hedonic response in rodents, confirmed by the lack of effect of the AMPAR antagonist on the effects of fluoxetine on anti-anhedonic activity (sucrose consumption), suggests a partial antidepressant-like activity that is also supported by a lack of non-specific motor effects in stressed as well as control animals. AMPAR-mediated antidepressant effects preferentially prevent specific depressive-like responses, such as immobility in the TST, coat degradation, and weight loss, that may reflect a motivational component of emotionality. It is suggested that enhancement of AMPAR function could constitute a targeted psychotherapeutic approach for relevant subtypes of depression.

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Statement of Interest
J.M.W. is an employee of Eli Lilly and Company. E.T.T. is a former employee of Eli Lilly and Company.

References
Griebel G, Simiand J, Steinberg R, Jung M, et al. (2002). 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylyphenyl)ethyl]-5-methyl-2-propynyl]-1,3-thiazol-2-amino hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor(1) receptor antagonist. II. Characterization


