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GABA_A receptor deficits cause HPA axis hyperactivity and antidepressant drug sensitivity reminiscent of melancholic forms of depression

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Abstract

BACKGROUND: GABA_A receptor deficits that are induced by global or forebrain-specific heterozygous inactivation of the $\gamma 2$ subunit gene in mouse embryos result in behavior indicative of trait anxiety and depressive states. By contrast, a comparable deficit that is delayed to adolescence is without these behavioral consequences. Here we characterized $\gamma 2$ -deficient mice with respect to HPA axis abnormalities and antidepressant drug responses.

METHODS: We analyzed the behavioral responses of $\gamma 2^{+/-}$ mice to desipramine and fluoxetine in Novelty Suppressed Feeding (NSFT), Forced Swim (FST), Tail Suspension (TST) and Sucrose Consumption (SCT) tests, as well as GABA_A receptor deficit- and antidepressant drug treatment-induced alterations in serum corticosterone.

RESULTS: Baseline corticosterone concentrations of adult $\gamma 2$ -deficient mice were elevated independent of whether the genetic lesion was induced during embryogenesis or delayed to adolescence. However, the manifestation of anxious-depressive behavior in different $\gamma 2$ -deficient mouse lines was correlated with early onset HPA axis hyperactivity during postnatal development. Chronic but not subchronic treatment of $\gamma 2^{+/-}$ mice with fluoxetine or desipramine normalized the anxiety-like behavior in the NSFT. Moreover, desipramine had antidepressant-like effects in that it

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normalized HPA axis function and depression-related behavior of $\gamma 2^{+/-}$ mice in the FST, TST and SCT. By contrast, fluoxetine was ineffective as an antidepressant and failed to normalize HPA axis function.

CONCLUSIONS: Developmental deficits in GABAergic inhibition in the forebrain cause behavioral and endocrine abnormalities and selective antidepressant drug responsiveness indicative of anxious-depressive disorders such as melancholic depression, which are frequently characterized by HPA axis hyperactivity and greater efficacy of desipramine vs. fluoxetine.

Keywords

anxious depression; anhedonia; fluoxetine; desipramine; corticosterone; stress

Introduction

Anxiety and mood disorders represent broadly defined psychiatric syndromes that exhibit extensive comorbidity and overlapping genetic origins, yet their etiology is ill defined and they exhibit heterogeneous and poorly predictable responsiveness to different types of anxiolytic and antidepressant drugs (1-4). Patients who suffer from anxious depression generally respond more poorly to antidepressant medications than patients suffering from depression alone (5). Stress is a major vulnerability factor for both anxiety and mood disorders, especially in early life (6-8). Moreover, increased secretion of glucocorticoids and aberrant function of the hypothalamic–pituitary–adrenal (HPA) axis are well-replicated findings in a prominent subset of patients suffering from severe forms of depressive disorders, especially melancholic depression (9-12).

GABA type A receptors (GABA_ARs) are the principal receptors mediating neural inhibition in the brain, and altered expression or function of these receptors or GABA in patients is increasingly implicated in the etiology of anxiety and depressive disorders (13-20). However, a causative relationship between GABAergic dysfunction and depression is not yet established. Studies in rodents indicate that GABAergic transmission is highly sensitive to stressful situations, including moderate early life stress and chronic stress in adulthood used to induce anxiety and depressive-like phenotypes (21-24). GABAergic dysfunction associated with anxiety and mood disorders therefore might be secondary to stress-induced HPA hyperactivity. Alternatively, the HPA axis is itself subject to GABAergic inhibition at multiple levels (25,26), suggesting that GABAergic deficits might independently contribute to HPA axis hyperactivity associated with depression.

Mice that are heterozygous for the $\gamma 2$ subunit of GABA_ARs ($\gamma 2^{+/-}$) exhibit a modest functional deficit in GABA_ARs, as evidenced by unaltered GABA_AR numbers but loss of GABA_AR benzodiazepine binding sites in 6 to 35% of GABA_ARs, depending on brain region, and they recapitulate behavioral, pharmacological and cognitive alterations reminiscent of anxiety disorders (27). This phenotype of $\gamma 2^{+/-}$ mice includes increased behavioral passivity under stressful conditions, as expected of an animal model of mood disorders (28). Conditional heterozygous knockout of the $\gamma 2$ subunit selectively in glutamatergic neurons of the embryonic telencephalon (Emx1Cre X $\gamma 2^{+/-}$ = ‘early’ $\gamma 2^{+/-}$ mice) reproduces the behavioral phenotype of global $\gamma 2^{+/-}$ mice, whereas a comparable GABA_AR deficit delayed to the fourth postnatal week (CaMKIICre2834 X $\gamma 2^{+/-}$ = ‘late’ $\gamma 2^{+/-}$ mice) is without corresponding behavioral consequences (28,29). These data suggest that the behavioral abnormalities of $\gamma 2^{+/-}$ mice are caused by a developmental deficit in GABAergic transmission in the telencephalon.

In light of the HPA axis dysfunction often associated with major depressive disorder in humans, we investigated corticosterone (Cort) serum levels and behavioral and endocrine

responses of $\gamma 2$ -deficient mice to antidepressants representative of serotonin (5-HT) and norepinephrine (NE) selective reuptake inhibitors (SSRIs and NSRIs), respectively. We show that GABA_AR $\gamma 2^{+/-}$ mice exhibit constitutively elevated serum Cort levels and selective antidepressant-like behavioral and endocrine responsiveness to desipramine compared to fluoxetine, a phenotype that is reminiscent of melancholic depression.

Methods and Materials

Animals

Experiments were performed in accordance with NIH guidelines, and protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Pennsylvania State University. Experimental $\gamma 2^{+/-}$ and wild-type mice were produced as littermates by mating $\gamma 2^{+/-}$ and WT mice. CaMKIICre2834 X $\gamma 2^{+/-}$ and Emx1Cre X $\gamma 2^{+/-}$ mice and the respective $\gamma 2^{+/-}$, CaMKIICre2834 and Emx1Cre littermate controls were generated as offspring of hemizygous Cre transgenic and $\gamma 2^{+/-}$ mice (27-29). Experiments were performed with female mice and in the holding room unless indicated otherwise. For further details and additional Methods see Supplement 1.

Drug treatments

Fluoxetine (Mallinckrodt, Hazelwood, MO) and desipramine (Sandoz, Princeton, NJ) were dosed as detailed in Supplement 1.

Behavioral assessments

Behavioral testing was performed at 12-14 (chronic drug treatment) or 9 weeks of age (subchronic), at least 72 h after the last cage change and starting 1 h after the start of the dark phase. Genotypes were coded such that the experimenter was unaware of the genotype. Comparisons between different groups were between littermates, starting with the novelty suppressed feeding test (NSFT) followed by the forced swim test (FST) and/or tail suspension test (TST), one week apart. The behavior was video recorded under red light for subsequent off-line analyses. In the NSFT (30), mice were deprived of food for 18 h preceding placement into the corner of a plastic box (50 × 50 × 20 cm) containing 3 cm of bedding and a pellet of food placed on a white paper nesting square (6 × 6 × 0.5 cm) in the center of the cage. Each test lasted 5 min and the measure of interest (chewing) was scored when the mouse was sitting on its haunches and biting with use of its forepaws. In the FST (31), mice were placed into a plastic bucket 19 cm in diameter and 27 cm deep and filled to 18 cm with 24-26°C water and videotaped for 6 min. We recorded the real time spent swimming until the first floating episode and the cumulative time spent immobile during the final 4 min, using a 5 s interval sampling method. In the TST (32,33), the mice were suspended individually by their tails from a stainless steel rod (affixed with adhesive tape) that was positioned 30 cm above the floor of a test apparatus (50 × 50 × 45 cm) consisting of two 25 cm wide compartments separated by an opaque PVC board. Two mice were tested side-by-side and videotaped for 6 min. The time to first immobility and the total immobility time were recorded. For the Sucrose Consumption Test (SCT), drug and vehicle treated mice were singly housed starting on the 24th day of drug treatment and trained to drink sucrose (10%) from the 25th to the 27th day. Water consumption from calibrated drinking tubes was then quantitated over the following three days. Finally, the mice were reexposed to sucrose for 4 days and 24 h consumption was quantitated on the last day.

Quantitation of serum Cort

Trunk blood of adult and post weaning mice was collected from the opened body cavity following cervical dislocation 3 h before the end of the light phase. The blood of preweaning

mice was collected by decapitation. To avoid order effects, all animals in a cage were euthanized at once within less than one minute. The serum was collected using Amber tubes with serum separator (BD, Franklin Lakes, NJ) and Cort was quantified using the Correlate EIA Corticosterone Enzyme Immunoassay Kit (Assay Designs, MI).

Statistical analyses

were conducted using Minitab15 (Minitab Inc., State College, PA). ANOVAs were used for analyses of genotype X treatment interactions and multiple comparisons of group means, followed by Fisher's Least Significant Difference or Dunnett's test for *post hoc* analyses. Experiments performed on different days were treated as blocks and the blocking effect treated as random factor. Outliers that differed from the mean by > 2 SD were removed from ANOVAs if necessary to satisfy normality assumptions. Simple comparisons of two group means were by two sample two-tailed *t*-tests.

Results

Increased anxiety-like behavior in $\gamma 2^{+/-}$ mice is ameliorated by chronic antidepressant treatment

The behavioral phenotype of $\gamma 2^{+/-}$ compared to WT mice includes an increased mean latency to feed in the NSFT (Fig. 1A, vehicle treatment), as shown previously (28). This test is thought to have predictive validity for antidepressant drug activity in humans, although ethologically the behavioral measure is anxiety-related. Moreover, the NSFT is among the few paradigms that elicit behavioral changes selectively in response to chronic but not acute or subchronic antidepressant drug treatment (34). Compared to vehicle treatment, both fluoxetine (20 mg/L in drinking water, *ad lib* for 28 days) and desipramine (480 mg/L in drinking water, *ad lib* for 28 days) produced anxiolytic-like effects in $\gamma 2^{+/-}$ mice and effectively normalized the increased latency to feed of $\gamma 2^{+/-}$ mice to WT levels [for statistics see Figure legends; a complete list of ANOVAs and 2-way ANOVAs for all behavioral tests is provided in Tables S1-S3 in Supplement 1]. No significant treatment effects were evident in WT mice. Analysis of genotype X treatment interactions confirmed that $\gamma 2^{+/-}$ mice were sensitivity to chronic treatment with desipramine, in contrast to WT mice ($p < 0.05$). A clear corresponding trend was also evident for fluoxetine ($p = 0.08$). Unlike chronic treatment, subchronic treatment with either drug for five days failed to reduce latency to feed in $\gamma 2^{+/-}$ mice (Fig. 1B). Moreover, while subchronic treatment with desipramine had no effect, subchronic fluoxetine further increased the latency to feed in $\gamma 2^{+/-}$ mice, consistent with anxiogenic effects and elevated Cort levels reported in patients during initial treatment with fluoxetine (35).

The $\gamma 2^{+/-}$ mice exhibit depression-related behavior and selective antidepressant drug responses to desipramine but not fluoxetine

In the FST, $\gamma 2^{+/-}$ mice demonstrated a phenotype indicative of behavioral despair, as suggested by the reduced mean time spent swimming until the first immobility episode, and the increased time spent immobile compared to WT littermates (Fig. 2A, C, vehicle; Table S2 in Supplement 1), consistent with previous results (28). Chronic treatment with desipramine had antidepressant-like effects in that it dose-dependently reversed the behavior of $\gamma 2^{+/-}$ mice to that of vehicle- or desipramine-treated WT littermates. Specifically, desipramine (240 or 480 mg/L, in drinking water for 35 days) compared to vehicle treatment dose-dependently increased the time to first immobility and reduced the total time spent immobile of $\gamma 2^{+/-}$ mice (Fig. 2A). No behavioral effects were evident with a low dose of desipramine at 120 mg/L (Fig. 2A). When analyzed in the TST (Fig. 2B), chronic desipramine (240 mg/L, 42 days) reduced the immobility of $\gamma 2^{+/-}$ mice analogous to results from the FST. Similar to the NSFT, the behavior of WT mice was unaffected by chronic

desipramine treatment in both the FST and TST, and genotype X treatment ANOVAs of FST data indicated increased sensitivity to desipramine of $\gamma 2^{+/-}$ vs. WT mice ($p < 0.01$ for both parameters).

Chronic fluoxetine had anxiolytic-like effects in $\gamma 2^{+/-}$ mice in the NSFT, similar to desipramine (see above). By contrast in the FST and TST, the behavioral effects of fluoxetine were markedly different from those of desipramine. Chronic fluoxetine compared to vehicle paradoxically reduced the latency to first immobility in WT mice in the FST and failed to ameliorate the behavioral immobility of $\gamma 2^{+/-}$ mice with respect to all parameters evaluated in these two tests (Fig. 2C,D). Drug treatment had no effect on body weight [mean weights following treatment with desipramine for six weeks @ 480 mg/L and 120 mg/L, 26.1 and 26.3 g vs. vehicle 26.8 g, $n = 16$; fluoxetine @ 20 mg/L, 23.1 g vs. vehicle 23.3 g, $n = 23, 24$, $p > 0.05$ for all pairwise comparisons], indicating that the drug effects were not simply due to altered calorie intake or body weight. To test whether lack of a behavioral response of $\gamma 2^{+/-}$ mice to fluoxetine was a peculiar feature of the 129X1/SvJ strain background of $\gamma 2^{+/-}$ mice, we crossbred $\gamma 2^{+/-}$ 129X1/SvJ and wildtype C57BL/6 mice to generate $\gamma 2^{+/-}$ mice on a filial generation 1 (F1) hybrid background. Similar to $\gamma 2^{+/-}$ 129X1/SvJ mice, $\gamma 2^{+/-}$ (F1) C57BL/6:129X1/SvJ mice lacked antidepressant-like responses to fluoxetine in the FST, while maintaining an anxiolytic-like response in the NSFT (Figure S1 in Supplement 1). Thus, the lack of antidepressant-like responses of $\gamma 2^{+/-}$ mice to fluoxetine is not limited to the 129X1/SvJ strain.

To substantiate the depression-related phenotype and selective responsiveness to antidepressant drugs of $\gamma 2^{+/-}$ mice we employed a variation of the sucrose consumption test (SCT) to assess anhedonia-like behavior (Fig. 3A, Table S3 in Supplement 1) (36), a core symptom of clinical depression (37). Vehicle-treated $\gamma 2^{+/-}$ mice consumed less sucrose than WT controls (Fig. 3B, Table S2 in Supplement 1), consistent with an anhedonia-like phenotype of $\gamma 2^{+/-}$ mice. Chronic treatment with desipramine (28+ days) increased the sucrose consumption of both $\gamma 2^{+/-}$ and WT mice, indicating an antidepressant-like drug effect independent of genotype. By contrast, fluoxetine had an anhedonia-like depressive effect on WT but not $\gamma 2^{+/-}$ mice, reminiscent of the genotype-specific detrimental effects of this drug in the FST. The water consumption of drug-treated $\gamma 2^{+/-}$ and WT mice analyzed by pairwise comparison of groups was indistinguishable from vehicle controls. However, desipramine resulted in a genotype-independent reduction in water consumption (Fig. 3C). Importantly, normalization of sucrose consumption to water consumption reproduced the data seen without normalization. The data indicate that anhedonia-like behavior of $\gamma 2^{+/-}$ mice is reversed by desipramine but not fluoxetine. Unlike in the NSFT, FST and TST, the SCT showed antidepressant-like effects of desipramine in WT mice.

The phenotype of $\gamma 2^{+/-}$ mice is not associated with changes in brain concentrations of 5-HT or NE or serotonergic innervation

The monoamine hypothesis of depression suggests that altered serotonergic and/or noradrenergic transmission contributes to the etiology of mood disorders. Noradrenergic and serotonergic neurons are known to be subject to GABAergic control (38,39), suggesting that perhaps $\gamma 2^{+/-}$ mice have alterations in these neurotransmitter systems. In particular, an excess of extracellular serotonin might contribute to increased emotionality as suggested by analysis of serotonin transporter KO mice (40,41). However, the levels of 5-HT, its metabolite 5-HIAA and NE in 9-week-old $\gamma 2^{+/-}$ vs. WT mice were unaltered in extracts of the six brain areas analyzed (Figure S2A-C and Table S4 in Supplement 1). Moreover, the immunoreactivity of serotonergic axons at 3 and 9 weeks of age was unchanged except for a modest and transient yet significant reduction of serotonin axon density in the primary motor cortex (M1) of 3-week-old $\gamma 2^{+/-}$ vs. WT mice ($-26 \pm 8\%$) that was no longer evident in 9-week-old mice (Figure S2D-I in Supplement 1) (for representative images see Figures S3 and

S4, for statistical analyses see Table S5; all in Supplement 1). Therefore, altered behavior of $\gamma 2^{+/-}$ mice does not involve overt genotype-related changes in serotonergic or noradrenergic neurotransmitter levels or serotonergic innervation of the forebrain.

GABA_ARs regulate Cort levels

HPA axis dysfunction represents the most common neuroendocrine abnormality of major depressive disorder (10). To assess whether $\gamma 2^{+/-}$ mice exhibit altered HPA axis activity we measured serum Cort levels before and at different time points after a 5 min forced swim stressor (Fig. 4A, Table S6 in Supplement 1). The baseline Cort levels of $\gamma 2^{+/-}$ vs. WT mice were elevated approximately two-fold, both before the stressor and after a one-hour period of recovery. By contrast, stress-induced Cort concentrations at 7.5, 12.5 and 30 min after the end of the stressor were unaltered in $\gamma 2^{+/-}$ compared to WT mice, indicating that the response to and recovery from acute physical stress remained intact. The genotype-dependent differences in basal Cort were seen in both male and female mice (B). The data indicate that $\gamma 2^{+/-}$ mice represent an animal model of depressive disorders associated with constitutive HPA axis hyperactivity.

We previously showed that the behavioral phenotype of $\gamma 2^{+/-}$ mice is replicated in conditional ‘early’ $\gamma 2^{+/-}$ mice (Emx1Cre x $\gamma 2^{+/-}$), which exhibit a GABA_AR deficit induced at approximately embryonic day 10 that is restricted to glutamatergic neurons of the telencephalic forebrain (28). By contrast, the developmentally delayed forebrain-specific deficit of ‘late’ $\gamma 2^{+/-}$ mice (CaMKIICre 2834 x $\gamma 2^{+/-}$) induced between the fourth and fifth postnatal week does not affect anxiety and depression-related behavior, suggesting selective vulnerability to GABAergic deficits during embryonic or postnatal development (28,29). Here, we here examined whether differences in anxiety- and depression-related behavior of the two forebrain-specific $\gamma 2$ -deficient mouse lines were correlated with different deficits in HPA axis function. Baseline Cort concentrations assessed at 8 weeks of age were elevated approximately two-fold in both ‘early’ and ‘late’ $\gamma 2$ -deficient mice compared to respective pseudo WT ($\gamma 2^{+/+}$) controls, and this was evident in both behaviorally naïve animals and after recovery from stress, and in both female (Fig. 4C) and male mice (not shown). Therefore, HPA axis hyperactivity assessed in adulthood is independent of the developmental time course of the GABA_AR deficit. The fact that Emx1Cre-mediated recombination is highly restricted to the telencephalic forebrain and absent in the hypothalamus (42), indicates that HPA axis hyperactivity is caused by a primary GABA_AR deficit in the telencephalic forebrain rather than the HPA axis.

The above experiments suggested that two-fold elevated baseline Cort alone is insufficient to induce the anxious depressive phenotype of ‘early’ or global $\gamma 2^{+/-}$ mice. Alternatively, differences in behavior between ‘early’ or global and ‘late’ GABA_AR-deficient mice might reflect the different developmental time courses of HPA axis deficits. Consistent with this latter idea, Cort concentrations of global $\gamma 2^{+/-}$ mice were normal at P17 (3 days before weaning) and markedly elevated at postnatal day (P) 23 (3 days after weaning) compared to littermate WT controls, while ‘late’ GABA_AR-deficient mice remained indistinguishable from littermate controls until at least P23 (Fig. 4D). Thus, HPA axis hyperactivity that develops around the time of weaning correlates with heightened emotional behavior of $\gamma 2$ -deficient mice in adulthood, whereas developmentally delayed HPA axis hyperactivity of ‘late’ GABA_AR-deficient mice is without anxiety- and depression-related behavioral consequences.

Antidepressant efficacy of desipramine in $\gamma 2^{+/-}$ mice is associated with normalizing effects on HPA axis function

Remission from depressive disorders in response to antidepressant therapy is typically associated with normalization of HPA axis activity (43,44). Consistent with antidepressant effects in $\gamma 2^{+/-}$ mice, desipramine (28 days, 240 mg/L in drinking water) effectively reversed the increased Cort concentrations of $\gamma 2^{+/-}$ mice to WT levels, while it had no effect on WT mice (Fig. 5A). In stark contrast, fluoxetine (20 mg/L for 28 days) increased the serum Cort concentrations in WT mice and had no effect on $\gamma 2^{+/-}$ mice (Fig. 5B). These divergent effects of desipramine vs. fluoxetine on Cort levels are consistent with their different behavioral effects in the FST and SCT (Figs. 2, 3), and further suggest that fluoxetine is ineffective as an antidepressant in $\gamma 2^{+/-}$ mice. Moreover, the data indicate that the anxiolytic-like behavioral effects of fluoxetine in $\gamma 2^{+/-}$ mice in the NSFT occur despite continuously elevated Cort concentrations.

Discussion

In this study we demonstrate that GABA_AR $\gamma 2^{+/-}$ mice represent a novel animal model of anxious depression characterized by heightened emotionality and anhedonia, selective antidepressant drug responsiveness and endocrine abnormalities reminiscent of melancholic forms of depression.

First, we showed that GABA_AR $\gamma 2^{+/-}$ mice exhibit selective behavioral responses to desipramine and fluoxetine with anxiolytic and/or antidepressant-like responses in mutant but not WT mice. This indicates greater sensitivity of the GABA_AR-deficient brain state to the therapeutic effects of these currently used antidepressants, and that modest GABA_AR deficits can cause alterations in serotonergic and noradrenergic transmission that are predicted based on the monoamine hypothesis of depression. GABA_AR deficit-induced changes in antidepressant drug sensitivity of $\gamma 2^{+/-}$ mice are likely to be postsynaptic, and consistent with an altered set point of G-protein coupled receptor signaling observed in brain of depressed patients (45). Lack of behavioral responses of WT mice (129SvJ/X1 strain) to fluoxetine and desipramine seen in most tests is at odds with anxiolytic and antidepressant-like drug effects seen in other laboratories and may reflect different drug sensitivities of different inbred strains of mice (46,47). However, our findings are consistent with a recent large meta-analysis of clinical studies showing that true beneficial drug effects of antidepressant medications are significant compared to placebo only in relatively severe cases of major depressive disorder (48).

Second, we found that fluoxetine differed from desipramine in that it lacked normalizing effects in $\gamma 2^{+/-}$ mice in the FST, TST and SCT, whereas both drugs reversed the mutant phenotype in the NSFT. Ethologically, the FST/TST and SCT are thought to assess depression-related behavioral despair and anhedonia, respectively, while the NSFT measures anxiety-related behavioral inhibition. Thus, desipramine showed both anxiolytic and antidepressant effects, whereas fluoxetine was merely anxiolytic. The qualitatively lesser response of $\gamma 2^{+/-}$ mice to fluoxetine than desipramine is reminiscent of severe subtypes of anxious depressive disorders including melancholic depression, which tend show greater responsiveness to tricyclic antidepressants (TCAs) than SSRIs (49-56). Importantly, there is rapidly accumulating evidence for reduced GABAergic transmission in depressed patients and these deficits are most pronounced in patients suffering from treatment resistant and melancholic subtypes of depression (14,17,20,57). Thus, the results of this study establish $\gamma 2^{+/-}$ mice as a genetically defined animal model of mood disorders with construct, predictive, and discriminating validity for selective anxiolytic and antidepressant drug effects indicative of melancholic and treatment-resistant subtypes of depression.

A third key finding entails that the anxious-depressive phenotype and poor responsiveness to fluoxetine of $\gamma 2^{+/-}$ mice was associated with a constitutive increase in Cort. Elevated basal activity of the HPA axis has been proposed as a characteristic of melancholic depression and, consistent with our findings in mice, linked to poor responsiveness to fluoxetine in patients (49,58,59). However, Cort concentrations of $\gamma 2$ -deficient mice are elevated even if the GABA_AR deficit was delayed to adolescence, while the behavioral alterations are dependent on induction of the GABA_AR deficit during development (28). These findings indicate that the elevated Cort concentrations alone are insufficient to cause the behavioral changes of $\gamma 2$ -deficient mice and may represent an epiphenomenon unrelated to anxious depressive-like behavioral outcomes. However, the manifestation of altered behavior of different $\gamma 2$ -deficient mouse strains correlates with an earlier developmental onset of HPA axis hyperactivity (Fig. 4D). Thus, consistent with the notion that the vulnerability to stress is elevated in the immature brain (6-8), HPA axis hyperactivity in young $\gamma 2^{+/-}$ mice may contribute to the perturbation of brain development that leads to anxious depressive behavior. Cort. concentrations of $\gamma 2$ -deficient mice were elevated even in Emx1Cre x $\gamma 2^{+/-}$ mice. Cre-driven recombination in these mice is limited largely to the telencephalon and absent in the hypothalamus (42), indicating that the primary GABA_AR deficit of $\gamma 2$ -deficient mice causing elevated Cort levels was extra-hypothalamic. Moreover, glucocorticoids are known to negatively affect expression of GABA_ARs in the forebrain, including particularly the frontal cortex and ventral hippocampus (21-23). Thus, GABA_AR deficits in the telencephalic forebrain may be both a cause for, and a consequence of, HPA axis hyperactivity, a feature that may initiate a positive feedback loop that amplifies GABAergic deficits in the limbic forebrain, with HPA axis hyperactivity as an amplifying link.

A fourth important finding to emerge from this study is that the anxiolytic- and antidepressant-like behavioral effects of desipramine in the $\gamma 2^{+/-}$ model are associated with HPA axis normalizing effects, whereas the selective anxiolytic-like effects of fluoxetine occurred without reductions in Cort. Consistent with our findings in $\gamma 2^{+/-}$ mice, fluoxetine and TCAs are known to exhibit different effects on HPA axis function in animal models and patients. Fluoxetine increases Cort concentrations in animal models (60) and fails to normalize HPA axis activity in patients (49,50,61). In contrast, desipramine and other TCAs normalize HPA axis function in animal models (60,62,63) and patients (64), and this drug effect is associated with remission from depression (64,65). Reboxetine, which blocks the reuptake of norepinephrine similar to desipramine, has complex brain region-specific effects on expression of glutamic acid decarboxylase 67, a principal enzyme involved in the synthesis of GABA (66). Thus, mechanisms of norepinephrine reuptake inhibitors might involve modulation of GABAergic transmission.

Characterization of $\gamma 2^{+/-}$ mice by fear conditioning has revealed a heightened sensitivity and attentional bias towards negative associations (27), reminiscent of corresponding cognitive deficits in melancholic depressed patients (67,68). In addition, $\gamma 2^{+/-}$ mice exhibit selectively reduced survival of adult-born hippocampal granule cells (28), reminiscent of corresponding deficits in chronic stress-induced animal models of depression (69), which may be related to hippocampal volume reductions observed in depressed patients (70). In sum, the GABA_AR $\gamma 2^{+/-}$ mouse model includes behavioral, cognitive, cellular, and endocrine dimensions as well as antidepressant drug response characteristics expected of an animal model of melancholic depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. The $\gamma 2^{+/-}$ mice show increased anxiety-related behavior that is sensitive to chronic desipramine and fluoxetine

A, Separate groups of mice were subject to 28-day chronic treatment with vehicle, desipramine or fluoxetine, respectively, followed by analysis in the NSFT at 12 weeks of age. Vehicle-treated $\gamma 2^{+/-}$ vs. WT mice showed increased latency to feed ($n = 8-16$, $p < 0.05$ in both experiments, ANOVA, Dunnett's test), consistent with previous results (28). Chronic treatment of $\gamma 2^{+/-}$ mice with fluoxetine (20 mg/L in drinking water, left panel) or desipramine (480 mg/L in drinking water, right) had anxiolytic like effects in that both drugs reduced the latency to initiate feeding compared to vehicle controls (fluoxetine, $n = 16$, $p < 0.01$; desipramine, $n = 10-11$, $p < 0.01$; ANOVA, Dunnett's test). By contrast, drug treatment had no effect on WT mice ($n = 8-13$, $p > 0.05$ for either drug). **B**, Subchronic fluoxetine (5 days, 20 mg/L) vs. vehicle increased the mean latency of $\gamma 2^{+/-}$ mice to initiate feeding ($n = 14-16$, $p < 0.05$, ANOVA, Dunnett's test) and thus had an anxiogenic-like effect. By contrast, subchronic treatment of $\gamma 2^{+/-}$ mice with desipramine (480 mg/L) vs. vehicle had no effect on the mean latency to initiate feeding ($n = 13-16$, $p > 0.05$, ANOVA, Dunnett's test). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 2. The $\gamma 2^{+/-}$ mice show antidepressant-like responses to desipramine but not fluoxetine in the FST and TST

A-D, Antidepressant drug- or vehicle-treated $\gamma 2^{+/-}$ and WT mice were subjected to the FST (A, C) and TST (B, D) at 13 and 14 weeks of age, corresponding to drug treatment for 35 and 42 days, respectively. (A) In the FST, vehicle-treated $\gamma 2^{+/-}$ vs. WT mice started to float sooner and spent more time immobile ($n = 26-29$, mean time to first immobility, $p < 0.001$; immobility, $p < 0.001$, ANOVA, Fisher's test). Desipramine (240 and 480 mg/L) vs. vehicle increased the time to first immobility ($p < 0.05$ and $p < 0.001$, resp.) and reduced the total immobility of $\gamma 2^{+/-}$ mice [$n = 14-29$, $p < 0.01$ (240 mg/ml), $p < 0.001$ (480 mg/ml)]. No significant drug effects were observed in $\gamma 2^{+/-}$ mice treated with 120 mg/L desipramine, nor with any desipramine concentration in WT mice. (B) In the TST, desipramine (240 mg/L) vs. vehicle reduced the immobility of $\gamma 2^{+/-}$ mice [$t(19) = 2.26$, $n = 10-11$, $p < 0.05$] but had no significant effect on WT mice [$t(23) = 0.98$, $n = 13$, $p(\text{ns})$]. Simple comparison of vehicle-treated $\gamma 2^{+/-}$ and WT mice revealed a tendency towards greater immobility in $\gamma 2^{+/-}$ mice [$t(21) = 1.82$, $p = 0.08$]. (C, D) Fluoxetine (20 mg/L, 35 days) paradoxically reduced the time to first immobility of WT mice in the FST (C) ($n = 12-14$, $p < 0.001$, ANOVA, Fisher's test) and lacked antidepressant like effects, irrespective of test, in $\gamma 2^{+/-}$ mice. Vehicle-treated $\gamma 2^{+/-}$ vs. WT littermates analyzed in parallel showed the depressive-like phenotype also evident in ($p < 0.01$)(A, B).

Figure 3. The $\gamma 2^{+/-}$ mice show antidepressant-like responses to desipramine but not fluoxetine in the SCT

A. Time course of experiment: Antidepressant drug treatment was initiated on Day 1 (8 weeks of age) and was switched to daily p.o. injections starting on the 22nd day (fluoxetine, 4 mg/kg; desipramine, 30 mg/kg). Vehicle treated mice were handled identically but injected with water. The mice were singly housed starting on the 24th day of drug treatment and trained to drink sucrose (10%) for three days starting on the 25th day. Water consumption was measured over a period of three days from the 28th to the 30th day, and sucrose consumption was measured over 22 h on the last day of a four-day sucrose consumption period. **B.** Sucrose consumption: Vehicle-treated $\gamma 2^{+/-}$ mice consumed less sucrose than WT littermates ($n = 13-15$; $p < 0.05$, Fisher's test), indicating an anhedonia-like phenotype of $\gamma 2^{+/-}$ mice. Chronic treatment with desipramine had antidepressant-like effects in that it increased the sucrose consumption of both WT and $\gamma 2^{+/-}$ mice ($n = 11-15$; $p < 0.01$ for both comparisons). By contrast, fluoxetine reduced the sucrose consumption of WT mice ($n = 14-15$; $p < 0.05$) and had no effect on $\gamma 2^{+/-}$ mice. Similar genotype and treatment effects but with lower levels of significance were evident for sucrose consumption on day 31-33 (not shown). **C.** Water consumption assessed by pairwise comparisons of vehicle and drug treated groups was unaltered ($p > 0.05$ for all comparisons). However, desipramine treated mice showed a genotype independent reduction in water consumption ($p < 0.05$). **D.** Normalization of sucrose consumption data in (A) for water consumption in (B) reproduced the anhedonia like phenotype and accentuated the antidepressant drug effects observed without normalization.

Figure 4. Cort levels are elevated in $\gamma 2$ subunit-deficient mice

A, B, Serum Cort levels in 8-week old female (A, B) and male (B) $\gamma 2^{+/-}$ and WT controls were measured at baseline and 7.5, 12.5, 30 or 60 min after a 5-min forced swim stressor. Cort levels of $\gamma 2^{+/-}$ vs. WT littermates were elevated both in behaviorally naïve animals (baseline) and after 60 min recovery from a 5-min stressor, and this effect was seen in both females and males ($n = 9$, $p < 0.001$ for all comparisons). Stress-induced Cort levels 7.5, 12.5 and 30 min after the end of the stressor were not different between genotypes ($n = 3$, $p > 0.05$ for all comparisons). **C,** Cort concentrations at 8 weeks of age of both Emx1Cre X $\gamma 2^{+/-}$ mice with an 'early' deficit and CaMKIICre2834 X $\gamma 2^{+/-}$ with a 'late' deficit were elevated compared to respective littermate $\gamma 2^{+/-}$ controls, both at baseline and after recovery from stress and in both male and female mice ($n = 3-6$, $p < 0.001$ for all comparisons, data shown for females only). **D,** Cort concentrations of $\gamma 2^{+/-}$ mice compared to WT littermates (mixed sex) were unaltered at P14 ($n = 8, 12$) and P17 ($n = 6, 10$; $p > 0.05$ in both cases) but markedly increased two days after weaning at P23 ($n = 8$, $p < 0.05$). By contrast, CaMKIICre2834 X $\gamma 2^{+/-}$ remained indistinguishable from littermate controls (CaMKIICre2834 and $\gamma 2^{+/-}$) until at least P23 ($n = 7, 9$, $p = 0.95$). Values represent means $\pm$ SEMs (two sample two-tailed t -tests). For numerical values see Table S6 in Supplement 1.

Figure 5. HPA axis function of $\gamma 2^{+/-}$ mice is normalized by desipramine but not fluoxetine

A. Desipramine (240 mg/L, 28 days) vs. vehicle reduced the elevated Cort levels of $\gamma 2^{+/-}$ mice ($p < 0.05$), while WT mice were unaffected ($n = 5-7$, $p > 0.05$, ANOVA, Dunnett test). **B.** Fluoxetine (20 mg/L, 28 days) increased Cort levels of WT mice ($n = 9$, $p < 0.05$, ANOVA, Dunnett's test) and had no effect in $\gamma 2^{+/-}$ mice ($n = 9$, $p > 0.05$). Basal Cort levels were elevated in vehicle-treated $\gamma 2^{+/-}$ vs. WT mice ($n = 9$, $p < 0.001$) as evident by a strong tendency also in (A) ($p = 0.07$). Veh, vehicle; Des, desipramine; FLX, fluoxetine.