GABAB_1 receptor subunit isoforms differentially regulate stress resilience

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Stressful life events increase the susceptibility to developing psychiatric disorders such as depression; however, many individuals are resilient to such negative effects of stress. Determining the neurobiology underlying this resilience is instrumental to the development of novel and more effective treatments for stress-related psychiatric disorders. GABA receptors are emerging therapeutic targets for the treatment of stress-related disorders such as depression. These receptors are predominantly expressed as heterodimers of GABA_1a subunit with either a GABA_1b or a GABA_1g subunit. Here we show that mice lacking the GABA_1b subunit isoform are more resilient to both early-life stress and chronic psychosocial stress in adulthood, whereas mice lacking GABA_1g receptors are more susceptible to stress-induced anhedonia and social avoidance compared with wild-type mice. In addition, increased hippocampal expression of the GABA_1b receptor subunit is associated with a depression-like phenotype in the helpless H/Rouen genetic mouse model of depression. Stress resilience in GABA_1b−/− mice is coupled with increased proliferation and survival of newborn cells in the adult ventral hippocampus and increased stress-induced c-Fos activation in the hippocampus following early-life stress. Taken together, the data suggest that GABA_1b receptor subunit isoforms differentially regulate the deleterious effects of stress and, thus, may be important therapeutic targets for the treatment of depression.

Although chronic and/or severe stress is a risk factor for the development of several different psychiatric disorders including depression and anxiety, many individuals remain resilient to such negative effects of stress. The mechanisms underlying this resilience are not yet fully understood, although it is thought to involve a complex interplay between several environmental factors such as social support and biological and genetic risk factors (1, 2). Currently, there is an impetus to determine the neural substrates underlying stress resilience and susceptibility, as these are poised to be key novel targets for the development of more effective treatments for depression and anxiety disorders.

Accumulating evidence suggests that GABA_1 receptors may be important therapeutic targets for the treatment of stress-related psychiatric disorders such as anxiety and depression (3–5). Functional GABA_1 receptors are composed of heterodimers of GABA_1a and GABA_1b subunits (6). Interestingly, the GABA_1b subunit is expressed as different isoforms, and in the brain the predominant isoforms are GABA_1b_1 and GABA_1b_2 (6). Mice deficient in GABA_1b_1 and GABA_1b_2 exhibit differential cognitive and conditioned fear responses, indicating a potential role for these isoforms in psychiatric illness (7–10). Recent postmortem brain studies report alterations in the expression of GABA_1 receptor subunits in depression (4, 11), and clinical studies suggest that neurophysiology deficits in GABA_1 receptors may play a role in major depression (12) and the antidepressant response (13). In addition, mice lacking functional GABA_1 receptors exhibit an antidepressant-like phenotype and increased anxiety (14, 15), and pharmacological blockade of these receptors induces antidepressant-like behavior (16–18). GABA_1 receptor antagonists have also recently been shown to increase cell proliferation in the adult hippocampus (16), which is an important regulator of stress- and antidepressant-related neuroplasticity. However, the specific role of GABA_1 receptor isoforms in stress sensitivity is unclear. Therefore, we assessed the susceptibility and resilience to stress during either early life (maternal separation) or adulthood (psychosocial stress) in GABA_1b_1−/− and GABA_1b_2−/− mice.

Results

GABA_1b_1−/− Mice Are Resilient Whereas GABA_1b_2−/− Mice Are More Susceptible to Social Avoidance Behavior Following Social Defeat Stress. In the social interaction test (Fig. 1C), there was a significant effect of social defeat (SD) stress [F(1,56) = 15.357, P < 0.001] and genotype [F(2,56) = 12.843, P < 0.001] on social avoidance behavior. SD stress decreased time spent in the interaction zone in the presence of a social target in WT (P =
The effects of SD stress or maternal separation on stress resilience were studied in male and female mice. In male GABA<sub>(B1a)</sub>−/− mice, social defeat stress (SDS) reduced immobility in both the forced swimming test (FST) and tail suspension test (TST) irrespective of MS. Male GABA<sub>(B1b)</sub>−/− mice exhibited reduced immobility in both the FST and TST irrespective of MS. In female mice, the level of maternal care that a pup receives can program stress sensitivity later in adulthood (19). To exclude the possibility that the resilient phenotype of female GABA<sub>(B1a)</sub>−/− mice is due to increased locomotor activity, because activity in the open field did not differ across any of the female MS groups (Fig. 1G). In contrast, female GABA<sub>(B1b)</sub>−/− mice were hyperactive in the open field (Fig. 1G; genotype effect [F(2,55) = 15.984, P < 0.001]). Both NMS mice and GABA<sub>(B1b)</sub>−/− mice displayed decreased immobility compared with NMS WT mice. However, following MS, only GABA<sub>(B1a)</sub>−/− mice maintained this reduced immobility (P < 0.001), thus suggesting that female GABA<sub>(B1b)</sub>−/− mice are more resilient to the effects of early-life stress. The reduced immobility in MS GABA<sub>(B1b)</sub>−/− female mice is unlikely due to increased locomotor activity, because activity in the open field did not differ across any of the female MS groups (Fig. 1G). In contrast, female GABA<sub>(B1a)</sub>−/− mice were hyperactive in the open field (Fig. 1G; genotype effect [F(2,55) = 8.949, P < 0.0001]); thus we cannot exclude the possibility that this contributed to reduced immobility under nonseparated conditions. Male GABA<sub>(B1b)</sub>−/− mice exhibited reduced immobility in both the FST and TST irrespective of MS (Fig. S2 C and E), but this effect may have been confounded by increased locomotor activity (Fig. S2D). Interestingly, NMS GABA<sub>(B1a)</sub>−/− mice exhibited differential phenotypes in the FST and TST irrespective of sex whereby immobility was increased in the FST but decreased in the TST (Fig. 1F and Fig. S2 C, E, and F).

Stress Resilience in GABA<sub>(B1a)</sub>−/− Mice Is Not Associated with Higher Levels of Maternal Care or Significant Alterations in the Stress-Induced Corticosterone Response. The level of maternal care that a pup receives can program stress sensitivity later in adulthood (19). To exclude the possibility that the resilient phenotype of GABA<sub>(B1a)</sub>−/− mice is due to increased maternal care, we measured high maternal care behaviors (Fig. S3A) and time spent off-nest (Fig. S3B) in WT, GABA<sub>(B1a)</sub>−/−, and GABA<sub>(B1b)</sub>−/− dams of both nonseparated and separated pups on postnatal day 7 (PND 7). GABA<sub>(B1b)</sub>−/− dams did not provide more maternal care. On the contrary, GABA<sub>(B1a)</sub>−/− dams provided higher maternal care than GABA<sub>(B1b)</sub>−/− and WT dams, irrespective of whether their pups had undergone MS (Fig. S3).

Stress-induced effects on plasma corticosterone diverged across genotypes and sexes. Interestingly, in males (Fig. S3C), stress-induced corticosterone levels were attenuated in GABA<sub>(B1a)</sub>−/− mice and enhanced in GABA<sub>(B1b)</sub>−/− mice. In females (Fig. S3D), there were no genotype differences. However, MS had no effect on this parameter in any genotype or sex.

Anxiety Levels in Adulthood Are Unaffected by Early-Life Stress and GABA<sub>(B1a)</sub> Receptor Subunit Isoforms. GABA<sub>(B1a)</sub> receptors have been reported to play a role in innate anxiety (14, 15). However, the contribution of specific GABA<sub>(B1a)</sub> isoforms to this effect is less obvious (20). Here, neither MS nor GABA<sub>(B1a)</sub> receptor subunit isoform disruption nor MS coupled with GABA<sub>(B1a)</sub> receptor disruption had a significant effect on anxiety. The forced swimming test (FST) and tail suspension test (TST) were used to examine the effects of MS and GABA<sub>(B1a)</sub> receptor subunit disruption on antidepressant-like behavior. In the FST in female mice (Fig. 1F), there was a significant effect of genotype [F(2,55) = 15.984, P < 0.001]. Both NMS mice and GABA<sub>(B1a)</sub>−/− mice exhibited differential phenotypes in the FST and TST irrespective of sex whereby immobility was increased in the FST but decreased in the TST (Fig. 1F and Fig. S2 C, E, and F).
Stress-Induced Neural Activation Is Enhanced in GABA<sub>B</sub>(1b)<sup>−/−</sup> Mice. Toward identifying the neural circuitry underlying the differential stress susceptibility between GABA<sub>B</sub>(1a)<sup>−/−</sup> and GABA<sub>B</sub>(1b)<sup>−/−</sup> mice, we measured the expression of c-Fos, an established marker of neural activation, in response to acute restraint stress in adult WT, GABA<sub>B</sub>(1a)<sup>−/−</sup> and GABA<sub>B</sub>(1b)<sup>−/−</sup> mice, with and without prior MS. Restraint stress was induced by placing mice in ventilated tubes for a period of 2 h, and animals were killed 2 h after cessation of restraint. The number of c-Fos-positive cells was measured in stress-related brain areas including the hippocampus, nucleus accumbens (NAcc), dorsal raphe nucleus (DRN), paraventricular nucleus (PVN), ventral tegmental area (VTA), medial prefrontal cortex, and amygdala. The PVN is known to be activated by restraint stress (21). Therefore, to evaluate the validity of our restraint stress paradigm, we first confirmed that it induced c-Fos activation in the PVN of the hypothalamus of WT mice that did not undergo early-life stress (Fig. S5F). All data including statistical analysis are summarized in Table S1.

One of the most striking observations was that in several areas of the hippocampus, the number of c-Fos-positive cells in response to acute stress was significantly increased in GABA<sub>B</sub>(1b)<sup>−/−</sup> mice compared with WT and GABA<sub>B</sub>(1a)<sup>−/−</sup> mice. Importantly, the hippocampus is a key brain area involved in regulation of the stress response (22, 23). The enhanced stress-induced c-Fos activation was most apparent in the dorsal and ventral dentate gyrus and ventral CA3 (Fig. 2 and Table S1), and this effect occurred to the same extent in both NMS and MS GABA<sub>B</sub>(1b)<sup>−/−</sup> mice. Interestingly, NMS GABA<sub>B</sub>(1a)<sup>−/−</sup> mice exhibited decreased stress-induced c-Fos in the ventral CA3 and this effect was not apparent in MS GABA<sub>B</sub>(1a)<sup>−/−</sup> mice (Fig. 2 and Table S1), thus further supporting the hypothesis that GABA<sub>A</sub> receptors in the hippocampus might be important in the differential response to stress. A similar but weaker pattern of effects was also observed in the PVN and the DRN whereby GABA<sub>B</sub>(1b)<sup>−/−</sup> mice enhanced stress-induced neural activation irrespective of prior MS, although statistical differences were generally restricted to comparisons with GABA<sub>B</sub>(1a)<sup>−/−</sup> and not WT mice (Table S1). Of all the regions investigated, the NAcc was the only area where stress-induced c-Fos expression was differentially regulated in GABA<sub>B</sub>(1b)<sup>−/−</sup> mice by prior MS (Fig. 2 and Table S1). Specifically, although there were no statistically significant genotype differences under NMS conditions, MS significantly increased stress-induced c-Fos expression in the NAcc of GABA<sub>B</sub>(1b)<sup>−/−</sup> mice compared with WT (P < 0.01) and MS GABA<sub>B</sub>(1a)<sup>−/−</sup> (P < 0.01) mice. Interestingly, NMS GABA<sub>B</sub>(1a)<sup>−/−</sup> mice exhibited decreased stress-induced c-Fos activation in the VTA, and this effect was not apparent in MS GABA<sub>B</sub>(1a)<sup>−/−</sup> mice (Table S1).

GABA<sub>B</sub>(1b) mRNA Expression Is Increased in the Hippocampus of a Genetic Model of Depression, the Helpless H/Rouen Mouse. Given our data showing that the GABA<sub>B</sub>(1b) receptor isoform regulates stress resilience and increased stress-induced neural activation in the hippocampus, it was thus of interest to investigate whether a well-characterized model of depression, helpless H/Rouen mice (24), would exhibit alterations in hippocampal expression of this subunit. GABA<sub>B</sub>(1b) mRNA was increased in the dentate gyrus (t = 7.591, df = 10, P < 0.001), CA1 (t = 6.377, df = 10, P < 0.001), and CA3 (t = 6.674, df = 10, P < 0.001) of helpless H/Rouen (H) mice compared with nonhelpless (NH) controls (Fig. 3 E–H). GABA<sub>B</sub>(1a) mRNA expression did not differ between the two groups (Fig. 3 A–D).

GABA<sub>B</sub>(1b)<sup>−/−</sup> Mice Exhibit Increased Proliferation of Newly Born Cells in the Ventral Hippocampus. Adult hippocampal neurogenesis is thought to play important roles in the stress response, anhedonia, antidepressant action, and regulation of the stress response by antidepressants (25–27). We previously reported that pharmacological inhibition of the GABA<sub>B</sub> receptor increases hippocampal cell proliferation and induces antidepressant-like behavior (16). This finding along with the present study, which demonstrates increased expression of GABA<sub>B</sub>(1b) mRNA in the hippocampus of a mouse model of depression and enhanced stress-induced activation of the hippocampus in GABA<sub>B</sub>(1b)<sup>−/−</sup> mice,
suggest that increased adult hippocampal neurogenesis may be a potential mechanism underlying the stress-resilient and antidepressant-like phenotype of GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice. Emerging evidence suggests that the effects of stress and antidepressant treatments on adult hippocampal neurogenesis might occur preferentially in the ventral (vHi) rather than the dorsal hippocampus (dHi) (28, 29), and we previously reported that chronic treatment with a GABA<sub>1</sub> receptor antagonist increases cell proliferation in the vHi but not dHi (16). This is interesting in light of the preferential roles of the dHi in spatial learning and memory and the vHi in the regulation of the stress response and anxiety (30). Therefore, we determined whether the stress-resilient and antidepressant-like phenotype of GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice was accompanied by increased proliferation and survival of newly born cells and whether such effects occurred preferentially in the vHi rather than in the dHi.

The effects of MS on cell proliferation in the subgranular zone (SGZ) and granule cell layer (GL) are illustrated in Fig. 4 B and C, respectively (representative photographs are in Fig. S6; the experimental design is in Fig. 4A). In the SGZ, there was a significant effect of genotype [F(1,27) = 8.160, P < 0.01]. Specifically, NMS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice displayed increased cell proliferation in the SGZ compared with the corresponding WT group (P < 0.05). Cell proliferation in MS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice was not significantly different from that of WT mice, although it should also be noted that cell proliferation in MS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice was not significantly different from that in NMS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice (P = 0.272). Upon segregation of the hippocampus into dorsal and ventral regions, it became apparent that increased hippocampal cell proliferation in NMS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice was restricted to the ventral SGZ (genotype [F(1,25) = 15.16, P < 0.001]; genotype-stress interaction [F(1,25) = 3.813, P = 0.062]; post hoc: NMS WT vs. NMS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup>, P < 0.01) but that this effect was prevented by MS. Cell proliferation in the SGZ of the dHi did not differ between any of the groups. In the GL, neither genotype, stress, nor stress x genotype affected cell proliferation. However, upon segregation of the GL, there was an effect of genotype [F(1,24) = 7.726, P < 0.05] in the ventral GL. Specifically, NMS but not MS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice displayed an increased number of Ki67-positive cells (P < 0.05) compared with their corresponding WT counterparts. Cell proliferation in the GL of the dHi was not different between any of the groups.

GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> Mice Exhibit Increased Survival of Newly Born Cells and Are Resistant to MS-Induced Decreases in the Survival of Newly Born Cells in the Ventral Hippocampus. The effects of MS on the survival of newly born cells in the SGZ and GL are illustrated in Fig. 4 D and E, respectively (representative photographs are in Fig. S7). In the SGZ there was a significant effect of genotype [F(1,36) = 5.246, P < 0.05]. Post hoc analysis revealed increased survival of newly born cells in MS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice compared with MS WT mice (P < 0.05), although this difference was not observed in NMS mice (P = 0.219). Interestingly, upon segregation of the hippocampus into the dHi and vHi, NMS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice exhibited increased survival of newly born cells compared with NMS WT mice specifically in the dorsal but not ventral SGZ. However, under stress conditions, this increased survival of newly born cells in GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice shifted from the dHi to the vHi [MS WT vs. MS GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup>, P < 0.01] (two-way ANOVA dorsal SGZ: genotype [F(1,36) = 5.671, P < 0.05]; two-way ANOVA ventral SGZ: genotype [F(1,34) = 5.546, P < 0.05]).

Surviving newly born neurons generated in the SGZ migrate to the GL, where they mature to become integrated into the neural circuitry of the hippocampus. Therefore, we also determined the number of surviving newly born cells in the GL of these mice (Fig. 4E). Whereas the survival of newly born cells in the whole GL was unaffected, segregation of the GL into dorsal and ventral regions revealed interesting effects. In the vHi, MS decreased the survival of newly born cells in the GL of WT mice (P < 0.05) but not in GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice, thus suggesting that GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> mice are resilient to stress-induced reductions in the survival of newly born cells (genotype [F(1,34) = 3.403, P = 0.07]; genotype-stress interaction [F(1,34) = 4.350, P < 0.05]). In contrast, there were no group differences in the survival of newly born cells in the dHi, thus suggesting a preferential effect of both MS and the protective effects of GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> deletion in the vHi.

GABA<sub>B<sub>1b</sub></sub><sup>−/−</sup> but Not GABA<sub>B<sub>1a</sub></sub><sup>−/−</sup> Mice Exhibit Increased Adult Hippocampal Neurogenesis. Experiments using doublecortin (DCX) immunohistochemistry in female mice confirmed that increased neurogenesis is restricted to GABA<sub>B<sub>1a</sub></sub><sup>−/−</sup> mice (P < 0.05) and that GABA<sub>B<sub>1a</sub></sub><sup>−/−</sup> mice were not different from WT mice (Fig. S8, SI Materials and Methods, and SI Results). Although a similar pattern of effects was observed in the dHi and vHi of NMS and MS mice, statistically significant effects were observed only in the dHi. Variability in these data might be a function of the limited sample number available for analysis (n = 6 vs. n = 10 in the cytogenesis study), estrus cycle regulation of neurogenesis in females (males were used in the cytogenesis study), and variation in the age or maturation state of individual DCX-positive cells, which future studies could investigate using appropriate immunohistochemical analyses.

Discussion

Understanding the molecular mechanisms underlying stress susceptibility and resilience is a key step toward identifying novel targets that could be exploited in the development of new, more effective treatments for stress-related psychiatric disorders, including depression. Here we show that the GABA<sub>1</sub> receptor may be one such target. Specifically, mice lacking the GABA<sub>B<sub>1b</sub></sub> subunit are resilient to both stress-induced anhedonia and...
psychosocial stress-induced social withdrawal and exhibit antidepressant-like behavior, whereas GABA<sub>B(1a)</sub><sup>−/−</sup> mice are more susceptible to stress-induced anhedonia and psychosocial stress-induced social withdrawal. These findings have important implications for the pathophysiology and treatment of depression. Furthermore, they support the contention that GABA<sub>B</sub> receptor subunit isoforms play differential roles in mediating behavioral responses (7–9).

GABA<sub>B(1a)</sub><sup>−/−</sup> mice have previously been shown to exhibit sleep disturbances (6), cognitive impairments (7–9), and a reduced threshold for fear generalization (10). Here we show that GABA<sub>B(1a)</sub><sup>−/−</sup> mice are more susceptible to stress-induced anhedonia. We cannot rule out that the increased maternal care behavior provided by GABA<sub>B(1a)</sub><sup>−/−</sup> dams may have contributed to this phenotype. Future studies examining the phenotype of GABA<sub>B(1a)</sub><sup>−/−</sup> mice following cross-fostering with WT mice would give further insight. Interestingly, a recent small post-mortem study reported that GABA<sub>B(1a)</sub> mRNA expression is decreased in the dentate gyrus of depressed individuals (4), whereas antidepressant-increased increases in GABA<sub>B(1a)</sub> mRNA expression have been reported in the rat hippocampus (31).

Taken together, this suggests that the GABA<sub>B(1a)</sub> receptor subunit may play a role in depression and antidepressant action. On the other hand, data from GABA<sub>B(1b)</sub><sup>−/−</sup> mice point to a novel role for this isoform in stress resilience. Previously, these mice have been shown to exhibit impaired fear conditioning (10) and cognitive deficits, including impairments in spatial working memory and extinction of aversive taste memory (7, 8). Here we show that GABA<sub>B(1b)</sub><sup>−/−</sup> mice are stress-resilient and that the hippocampus is a key area that is differentially activated in GABA<sub>B(1b)</sub><sup>−/−</sup> and GABA<sub>B(1a)</sub><sup>−/−</sup> mice in response to stress. Moreover, in another model of depression, helpless H/Rouen mice, hippocampal GABA<sub>B(1b)</sub> mRNA expression was increased compared with nonhelpless counterparts. This suggests that increased hippocampal GABA<sub>B(1b)</sub> mRNA expression is associated with a depression-like phenotype and that reducing its expression may have antidepressant-like effects. Indeed, we observed that compared with both WT and GABA<sub>B(1a)</sub><sup>−/−</sup> mice, GABA<sub>B(1b)</sub> mice were resilient to psychosocial stress-induced social avoidance and the anhedonic effects of early-life stress in the female urine sniffing test. We also observed that GABA<sub>B(1b)</sub> mice exhibited antidepressant-like behavior in the forced swim test, although this effect should be interpreted with caution given their hyperactive phenotype. Importantly, GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> proteins are up-regulated 29% and 15% in CA1 of GABA<sub>B(1b)</sub> and GABA<sub>B(1a)</sub><sup>−/−</sup> mice, respectively (9), and thus it cannot be ruled out that their differential phenotypes are due to compensatory up-regulation of the other subunit.

Given the focus on the role of adult hippocampal neurogenesis in stress-related disorders (32) and the fact that we recently showed that chronic treatment with a GABA<sub>B</sub> receptor antagonist increases hippocampal cell proliferation (16), we thus assessed whether the stress-resilient phenotype of GABA<sub>B(1b)</sub><sup>−/−</sup> mice is accompanied by changes in adult hippocampal cytoogenesis and neurogenesis. Indeed, GABA<sub>B(1b)</sub><sup>−/−</sup> mice exhibited increased proliferation and survival of newly born cells in the adult hippocampus, and these effects occurred predominantly in the ventral hippocampus, particularly under stress conditions. The vHi is thought to play a preferential role in the modulation of the stress response and anxiety, whereas the dHi is thought to play a preferential role in spatial learning and memory (30). Intriguingly, emerging evidence suggests that stress and antidepressant-like treatments may exert their effects on adult hippocampal neurogenesis preferentially in the vHi rather than in the dHi (28, 29). Moreover, analogous findings have been reported in humans, with antidepressants increasing neurogenesis in the anterior hippocampus (33). In addition to increases in the survival of newly born cells, GABA<sub>B(1b)</sub><sup>−/−</sup> mice were also resistant to stress-induced decreases in the survival of newly born cells. The regulation of adult hippocampal neurogenesis by GABA<sub>B</sub> receptors has remained largely unexplored, but we previously reported that chronic treatment with a GABA<sub>B</sub> receptor antagonist that has antidepressant-like behavioral effects increases cell proliferation specifically in the dHi (16). Similarly, it was recently reported that the GABA<sub>B</sub> receptor is expressed on neural stem cells, and that mice lacking both GABA<sub>B(1a)</sub> isoforms exhibit increased proliferation and accelerated neuronal differentiation (34). Taken together, it is clear that GABA<sub>B</sub> receptors modulate adult hippocampal neurogenesis and that these effects are coupled with alterations in antidepressant-like behavior and stress resilience, with specific GABA<sub>B(1)</sub> receptor subunit isoforms playing differential roles in these behavioral effects. Future studies investigating neurogenesis-dependent behaviors such as pattern separation, or antidepressant-induced decreases in anxiety in the novelty-suppressed feeding test (27), will give further insight into the role of the GABA<sub>B(1)</sub> neurogenesis relationship in behavior, although it is also important to note that differences in innate anxiety were not observed in other tests (20).

Although our mechanistic studies focused on the hippocampus, it is important to note that GABA<sub>B(1b)</sub><sup>−/−</sup> mice also exhibited enhanced stress-induced activation of some other brain regions, although these effects were less robust. Interestingly, the nucleus accumbens was the only region that was differentially affected in NMS and MS GABA<sub>B(1b)</sub><sup>−/−</sup> mice by restraint stress. GABA<sub>B</sub> interactions at the level of the NAcc are well-described in the context of drug addiction (35) and may play a role in stress-induced anhedonia (18). Previous studies have reported serotonin–GABA<sub>B</sub> receptor interactions (17, 36), and here we show that stress-induced c-Fos activation was increased in the dorsal raphe nucleus in both NMS and MS GABA<sub>B(1b)</sub><sup>−/−</sup> mice. This is interesting in light of recent data showing that the activity of GABAergic neurons in the DRN regulates resilience to SD stress (37) and that the antidepressant fluoxetine suppresses GABA<sub>B</sub> receptor activity in the DRN (38).

The molecular mechanisms underlying phenotypic differences between GABA<sub>B(1a)</sub><sup>−/−</sup> and GABA<sub>B(1b)</sub><sup>−/−</sup> mice are not yet known, but differences in cellular localization, physiological properties, and ontogenic expression of these subunits have been reported previously (1). Unlike the GABA<sub>B(1b)</sub><sup>−/−</sup> mice, GABA<sub>B(1a)</sub> can localize to axons via Sushi domains, which also increase surface stability of GABA<sub>B(1a,2)</sub> receptors (6, 39). In dendrites, GABA<sub>B(1a)</sub> localizes to glutamatergic terminals for heteroreceptor function whereas GABA<sub>B(1b)</sub> localizes to spines opposing glutamate release sites, thus affecting pre- or postsynaptic inhibition (6). Interestingly, GABA<sub>B(1a)</sub> and GABA<sub>B(1b)</sub> are preferentially expressed in the developing and adult brain, respectively (3), and thus GABA<sub>B(1b)</sub> levels are relatively lower during the plastic period of brain development. In parallel, the present data suggest that inhibiting GABA<sub>B(1b)</sub> expression facilitates plasticity in the form of neurogenesis. Future studies characterizing the roles of these subunits will give further insight into the mechanisms underlying the differential phenotypes observed here.

In conclusion, GABA<sub>B(1)</sub> receptor subunit isoforms differentially regulate resilience to stress-induced anhedonia, with reductions in GABA<sub>B(1)</sub> receptors associated with resilience whereas reductions in GABA<sub>B(1a)</sub> receptors are associated with increased susceptibility. These effects were coupled with alterations in stress-induced neural activity of reward pathways and adult hippocampal neurogenesis, and are further supported by alterations in the expression of GABA<sub>B(1)</sub> Subunit isoforms in a genetic mouse model of depression and in response to antidepressant treatment (31) as well as recent postmortem findings in the human hippocampus (4). Taken together, these data further support the concept that the GABA<sub>B</sub> receptor may play a crucial role in the pathophysiology and treatment of stress-related disorders.
Materials and Methods

Animals. WT, GABA<sub>B1a</sub>−/−, and GABA<sub>B1b</sub>−/− mice were bred at University College Cork. Helpless HR/ROuen and nonhelpless mice were bred at Centre de Recherche en Neurosciences de Lyon INSEMR U1028-CNRS 5292, Lyon, France, as described previously (24). Experiments were conducted in accordance with the European Community Council Directive (86/609/EEC) and approved by the Animal Experimentation Ethics Committee of University College Cork. See SI Materials and Methods for details.

Social Defeat Stress. The effects of a 10-d SD stress (40) on saccharin preference and social interaction in WT, GABA<sub>B1a</sub>−/−, and GABA<sub>B1b</sub>−/− mice were examined. See Fig. 1A and SI Materials and Methods for details.

Maternal Separation Behavioral Experiment. Pups underwent unpredictable maternal separation combined with unpredictable maternal stress from postnatal day 1 to 14 as well as a battery of behavioral tests conducted in the following order (Fig. 1A): ultrasonic vocalizations, stress-induced hyperthermia (SH), open field (OF), tail suspension test, elevated plus maze (EPM), saccharin preference test (SPT)/female urine sniffing test (FUST), and forced swim test. Anhedonia was assessed in females using the SPT and in males using the FUST. Maternal care behaviors were assessed 2–3 h after separation. See SI Materials and Methods for details.

In Situ Hybridization. In situ hybridization was conducted as previously described (41) (probes complementary to GABA<sub>B1a</sub> mRNA [595–636 bp, National Center for Biotechnology Information (NCBI) Nucleotide Database reference no. NM_019439.3] and GABA<sub>B1b</sub> mRNA [39–82 bp, NCBI Nucleotide Database reference no. AF120253]). See SI Materials and Methods for details.

Immunohistochemistry. c-Fos and doublecortin immunohistochemistry was conducted in female mice as previously described (21), BrdU and Ki67 immunohistochemistry was conducted in male mice as previously described (28). See Fig. 4A and SI Materials and Methods for details.

Statistical Analysis. Data were analyzed using two-way ANOVA and Fisher’s least significant difference (LSD) post hoc test with the exception of USV (one-way ANOVA and Fisher’s LSD post hoc test), and FUST and in situ hybridization data (unpaired Student t test). P < 0.05 was the criterion for statistical significance.

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1. Feder A, Nestler EJ, Charney DS (2009) Psychobiology and molecular genetics of resilience. Nat Rev Neurosci 10(6):446–457.
2. Russo SJ, Murrrough JW, Han MH, Charney DS, Nestler EJ (2012) Neurobiology of resilience. Nat Neurosci 15(11):1475–1484.
3. Cryan JF, Kaumann K (2005) Don’t worry ’B happy: A role for GABA(B) receptors in anxiety and depression. Trends Pharmacol Sci 26(1):38–43.
4. Ghose S, Winter MK, McCarson KE, Tamminga CA, Enna SJ (2011) The GABA<sub>B</sub> receptor as a target for antidepressant drug action. Br J Pharmacol 162(1):1–17.
5. Pilk A, Nowak G (2005) GABAergic hypotheses of anxiety and depression: Focus on GABA-B receptors. Drugs Today (Barc) 41(11):755–766.
6. Gassmann M, Bettler B (2012) Regulation of neuronal GABA(B) receptor functions by subunit composition. Nat Rev Neurosci 13(6):380–394.
7. Jacobson LH, Kelly PH, Bettler B, Kaumann K, Cryan JF (2007) Specific roles of GABA (B1) receptor isoforms in cognition. Memory and anxiety.
8. Jacobson LH, Kelly PH, Bettler B, Kaumann K, Cryan JF (2006) GABA(B1) receptor isoforms differentially mediate the acquisition and extinction of aversive taste memories. J Neurosci 26(34):8880–8883.
9. Vigo R, et al. (2006) Differential compartmentalization and distinct functions of GABAB receptor variants. Neuron 50(6):589–601.
10. Shaban H, et al. (2006) Generalization of amygdala LTP and conditioned fear in the absence of presynaptic inhibition. Nat Neurosci 9(8):1028–1035.
11. Klempan TA, et al. (2009) Altered expression of genes involved in ATP biosynthesis and the GABAergic neurotransmitter in the ventral hippocampal cortex of suicides with and without major depression. Mol Psychiatry 14(2):175–189.
12. Levinson AJ, et al. (2010) Evidence of cortical inhibitory deficits in major depressive disorder. Biol Psychiatry 67(5):458–464.
13. Croarkin PE, et al. (2014) Evidence for pretreatment LICI deficits among depressed children and adolescents with nonresponse to fluoxetine. Neuropsychopharmacology 39(8):1259–1269.
14. Mombereau C, et al. (2004) Genetic and pharmacological evidence of a role for GABA (B) receptors in the modulation of anxiety- and antidepressant-like behavior. Neurouropsychopharmacology 29(6):1050–1062.
15. Mombereau C, et al. (2005) Altered anxiety and depression-related behaviour in mice lacking GABA<sub>B2</sub> receptor subunits. Neuroreport 16(3):307–310.
16. Felice D, O’Leary OF, Pizzo RC, Cryan JF (2012) Blockade of the GABA<sub>B</sub> receptor increases neurogenesis in the ventral but not dorsal adult hippocampus: Relevance to antidepressant action. Neuropharmacology 63B:1380–1388.
17. Slattery DA, Desrauyl S, Cryan JF (2005) GABA<sub>B</sub> receptor antagonist-mediated antidepressant-like behavior is serotonin-dependent. J Pharmacol Exp Ther 312(1):290–296.
18. Novak G, et al. (2006) Antidepressant-like activity of CGP 36742 and CGP 51176, selective GABA<sub>B</sub> receptor antagonists, in rodents. Br J Pharmacol 149(5):581–590.
19. Henningken K, et al. (2012) Low maternal care exacerbates adult stress susceptibility in the chronic mild stress rat model of depression. Behav Pharmacol 23(7):735–743.
20. Jacobson LH, Bettler B, Kaumann K, Cryan JF (2007) Behavioral evaluation of mice deficient in GABA(B1) receptor isoforms in tests of unconditioned anxiety. Psycho-pharmacology (Berl) 190(4):541–553.
21. O’Mahony CM, Sweeney FF, Daly E, Dinan TG, Cryan JF (2010) Restraint stress-induced brain activation patterns in two strains of mice differing in their anxiety behaviour. Behav Brain Res 213(2):148–154.