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$GABA_AR$ Plasticity during Pregnancy: Relevance to Postpartum Depression

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SUMMARY

Fluctuating neurosteroid levels over the ovarian cycle modulate neuronal excitability through effects on $GABA_A$ receptors ($GABA_ARs$). The large increase in progesterone-derived neurosteroids during pregnancy and their precipitous decline at parturition may have considerable effects on $GABA_ARs$ during pregnancy and postpartum. Here we show a significant decrease in tonic and phasic inhibitions in pregnant mice, mediated by a downregulation of $GABA_AR$ δ and γ2 subunits, respectively, which rebounds immediately postpartum. Mice which do not exhibit $GABA_AR$ δ subunit regulation throughout pregnancy (*Gabrd*$^{+/-}$ and *Gabrd*$^{-/-}$) exhibit depression-like and abnormal maternal behaviors, resulting in reduced pup survival. These abnormal postpartum behaviors were ameliorated in *Gabrd*$^{+/-}$ mice by a $GABA_AR$ δ-subunit-selective agonist, THIP. We suggest that *Gabrd*$^{+/-}$ and *Gabrd*$^{-/-}$ mice constitute a mouse model of postpartum depression that may be useful for evaluating potential therapeutic interventions.

INTRODUCTION

$GABA_A$ receptors ($GABA_ARs$) are one of the principal targets of action for neuroactive metabolites of steroid hormones, or neurosteroids (Herd et al., 2007). Neurosteroids are synthesized de novo from cholesterol or converted from steroid precursors in the central nervous system (CNS) (Stoffel-Wagner, 2001). Altered neurosteroid levels are associated with debilitating psychiatric and neurological disorders, including premenstrual dysphoric disorder (PMDD), premenstrual syndrome (PMS), catamenial epilepsy, menstrual migraine, postpartum depression, and anxiety (Backstrom et al., 2003). However, the pathogenesis of these ailments remains unclear, mainly due to the lack of useful animal models to study such complex disorders.

Numerous studies have investigated the changes in neurosteroid levels associated with anxiety and depression in women (for review see Longone et al. [2008]). Decreased neurosteroid levels have been discovered in drug-naive patients with major depression, and antidepressants have been shown to increase neurosteroid levels (Romeo et al., 1998; Uzunova et al., 1998), which is thought to account for the therapeutic benefits of these drugs (Pinna et al., 2006). The GABAergic system has long been implicated in the pathophysiology of various psychiatric disorders due to the evidence of decreased benzodiazepine binding in the brains of patients with panic disorder (Bremner et al., 2000b; Malizia et al., 1998), posttraumatic stress disorder (Bremner et al., 2000a), and generalized anxiety disorder (Tiihonen et al., 1997). In addition, selectively increasing ambient levels of GABA in the extracellular space of the CNS is therapeutic for the treatment of anxiety disorders (Pollack et al., 1998; Vermetten and Bremner, 2002; Zwanzger et al., 2001), while experimentally, it increases tonic inhibition mediated by extrasynaptic δ-subunit-containing $GABA_ARs$ (Nusser and Mody, 2002).

The pathophysiology of postpartum mood disorders is thought to be triggered by the rapid decline in the levels of reproductive hormones following pregnancy. However, administration and withdrawal of exogenous steroid hormones, designed to mimic the hormonal changes during pregnancy, result in symptoms of depression only in women with a history of postpartum depression (Bloch et al., 2000), suggesting that women must be predisposed to postpartum depression. The reason for this predisposition is unknown, but it is reasonable to assume that a preferred target of neurosteroids, the δ-subunit-containing $GABA_ARs$, might be involved in this disorder.

Alterations in δ-subunit-containing $GABA_ARs$ occur as hormone levels fluctuate during the ovarian cycle (Griffiths and Lovick, 2005; Maguire et al., 2005) and may also take place during pregnancy and postpartum. Indeed, changes in GABAergic function during pregnancy have been inferred from binding studies (Concas et al., 1998; Majewska et al., 1989) and $GABA_AR$ mRNA studies (Concas et al., 1998; Follesa et al., 1998). However, the functional consequences of these changes remain unclear. The objective of our study was to identify functional changes in $GABA_ARs$ during pregnancy and the postpartum period, and to find possible behavioral correlates in mice.

RESULTS

Changes in $GABA_AR$ Expression during Pregnancy

Potential changes in $GABA_ARs$ during pregnancy were identified by western blot analysis of total hippocampal membrane protein, since this region has previously been shown to exhibit neurosteroid-sensitive plasticity (Maguire and Mody, 2007; Maguire et al., 2005) and is thought to be involved in the presentation of mood disorders (Tsetsenis et al., 2007). $GABA_AR$ δ subunit expression

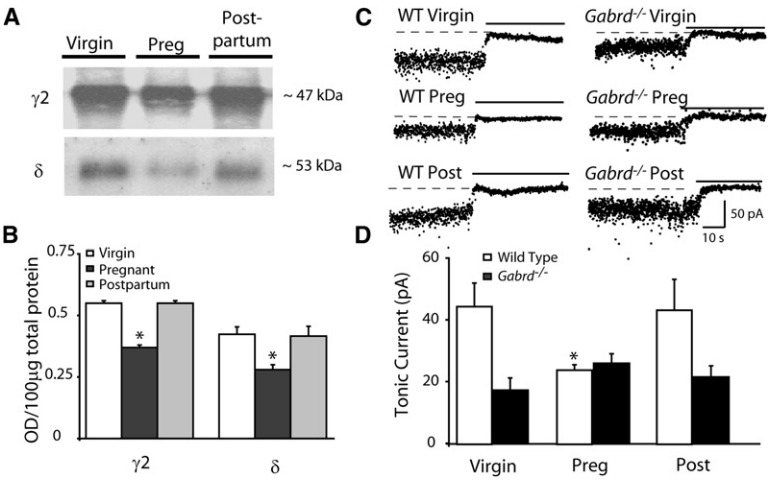

Figure 1. Alterations in GABA$_A$Rs during Pregnancy

(A) Representative western immunoblots of total hippocampal membrane protein from virgin, pregnant (d18), and postpartum (48 hr) mice. (B) Histograms of the average optical density (OD) of GABA$_A$R δ and γ2 subunit expression in virgin, pregnant, and postpartum mice. (C) Representative whole-cell patch-clamp recordings of tonic inhibition from DGGCs in virgin, pregnant (d18), and postpartum (48 hr) wild-type and *Gabrd*$^{-/-}$ mice before and after addition of >100 μM SR95531 (black bars; to reveal the amount of tonic current). (D) The average tonic current recorded in DGGCs was significantly decreased in wild-type pregnant mice compared with that from virgin wild-type and postpartum wild-type mice. There were no significant changes in tonic currents between virgin *Gabrd*$^{-/-}$ mice, pregnant *Gabrd*$^{-/-}$ mice, or postpartum *Gabrd*$^{-/-}$ mice.

in hippocampal membrane fractions was significantly decreased in mice at day 18 (d18) of pregnancy (0.28 ± 0.02 optical density [OD]/100 μg of total protein) compared with expression in virgin (0.42 ± 0.03 OD/100 μg of total protein) and postpartum (0.42 ± 0.05 OD/100 μg of total protein) mice (Figures 1A and 1B; n = 6; $p < 0.05$, one-way ANOVA). At d18 of pregnancy, GABA$_A$R γ2 subunit expression was also decreased (0.37 ± 0.01 OD/100 μg of total protein) compared with expression in virgin (0.55 ± 0.01 OD/100 μg of total protein) and postpartum (0.55 ± 0.01 OD/100 μg of total protein) mice (Figures 1A and 1B; n = 6; $p < 0.05$, one-way ANOVA). These findings indicate a considerable decrease in the expression of GABA$_A$Rs underlying both tonic and phasic inhibitions during pregnancy, which rebounds to control levels immediately postpartum.

Changes in GABAergic Inhibition during Pregnancy

To determine the functional consequences of GABA$_A$R plasticity during pregnancy, whole-cell patch-clamp recordings were performed on dentate gyrus granule cells (DGGCs) from wild-type mice and mice lacking GABA$_A$R δ subunits, *Gabrd*$^{-/-}$ mice, since the tonic inhibition in DGGCs is predominantly mediated by δ-subunit-containing GABA$_A$Rs. Tonic inhibition in DGGCs was significantly decreased at d18 of pregnancy in wild-type mice (19.7 ± 3.6 pA) compared with that of virgin (39.8 ± 4.4 pA) and postpartum (40.4 ± 7.1 pA) mice (Figures 1C and 1D; n = 14 mice, n = 47 cells; $p < 0.05$, one-way ANOVA). Tonic inhibition in virgin *Gabrd*$^{-/-}$ mice (17.1 ± 3.9 pA) was significantly decreased compared with that of virgin wild-type mice (39.8 ± 4.4 pA), but *Gabrd*$^{-/-}$ mice do not exhibit changes in tonic inhibition throughout pregnancy and postpartum (virgin: 17.1 ± 3.9 pA; pregnant: 25.6 ± 3.0 pA; postpartum: 21.3 ± 3.5 pA; Figures 1C and 1D; n = 12 mice, n = 36 cells; $p > 0.05$, one-way ANOVA). The average peak amplitudes of spontaneous inhibitory postsynaptic currents (sIPSCs) were also decreased in DGGCs of wild-type pregnant mice (45.4 ± 3.9 pA) compared with those of virgin (76.4 ± 5.5 pA) or postpartum (69.5 ± 6.9 pA) mice (Figure 2; n = 14 mice, n = 41 cells; $p < 0.05$, one-way ANOVA). The peak sIPSC amplitudes were also decreased during pregnancy in *Gabrd*$^{-/-}$ mice (45.5 ± 4.8 pA) compared with those of virgin *Gabrd*$^{-/-}$ mice (62.1 ± 4.6 pA) and postpartum *Gabrd*$^{-/-}$ mice (58.8 ± 8.0 pA) (Figure 2; n = 12 mice, n = 48 cells; $p < 0.05$, one-way ANOVA). There were no changes in the frequency or decay time constants of sIPSCs between any of the groups. Similar to sIPSCs, the peak amplitude of miniature inhibitory postsynaptic currents (mIPSCs) was significantly decreased in wild-type mice at d18 pregnancy compared with that of control and postpartum mice (Figure 2E; n = 12 mice, n = 36 cells; $p < 0.05$, one-way ANOVA). Nonstationary fluctuation analysis, albeit limited by averaging across receptors at different synapses, and its peak-scaled version that is unable to give an accurate estimate of P_o, can still be used to obtain a reasonable estimate of the average unitary conductance of the channels underlying mIPSCs (De Koninck and Mody, 1994). This analysis revealed no difference between the aggregate mean conductance of synaptic GABA$_A$Rs from control mice (30.9 ± 2.7 pS) or pregnant mice (31.2 ± 2.2 pS; Figure 2F; n = 12 mice, n = 36 cells; $p < 0.05$ by Student's t test), suggesting that the change in mIPSC size likely results from a reduced number of receptors, consistent with the changes in GABA$_A$R γ2 subunit expression observed during pregnancy (Figures 1A and 1B).

Abnormal Postpartum Behaviors in Mice with Deficiencies in δ-Subunit-Containing GABA$_A$Rs

To determine the behavioral impact of GABA$_A$R regulation during pregnancy and postpartum, postpartum behaviors were analyzed in mice deficient in GABA$_A$R δ subunits (*Gabrd*$^{-/-}$ and *Gabrd*$^{+/-}$ mice). We used the Porsolt forced swim test to assess behaviors sensitive to antidepressants that can thus be categorized in mice as depression-like. Consistent with an increase in depression-like behaviors, *Gabrd*$^{-/-}$ mice exhibited a decreased latency to immobility (45.3 ± 2.5 s) and an increased amount of total time spent immobile during the 6 min test (197.8 ± 9.1 s) compared with virgin *Gabrd*$^{-/-}$ mice (latency: 68.1 ± 6.3 s; immobility time: 156.4 ± 18.0 s), virgin wild-type mice (latency: 65.3 ± 7.0 s; immobility time: 157.1 ± 18.5 s), and postpartum wild-type mice (latency: 69.8 ± 10.1 s; immobility time: 98.0 ± 9.0 s; Figure 3; n = 4–8 mice per experimental group; $p < 0.05$, one-way ANOVA). We also performed the sucrose preference test to assess anhedonia, another indication of depression-like

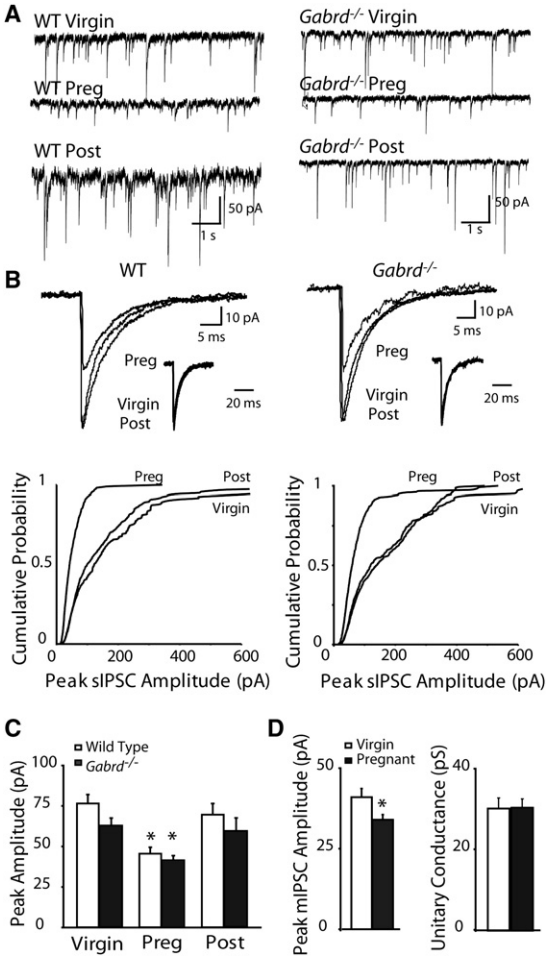

Figure 2. Decreased IPSCs during Pregnancy

(A) Representative whole-cell patch-clamp recordings from DGGC from wild-type and *Gabrd*$^{-/-}$ mice. (B) Superimposed averages reveal a decrease in the amplitudes of sIPSCs in both wild-type and *Gabrd*$^{-/-}$ mice during pregnancy. Peak-scaled sIPSCs show no change in kinetics in wild-type and *Gabrd*$^{-/-}$ mice. The decreased peak amplitude can be appreciated in the cumulative probability plots of an equal number of representative sIPSCs from wild-type (n = 700 per group) and *Gabrd*$^{-/-}$ (n = 1000 per group) virgin, pregnant (d18), and postpartum mice. (C) The average amplitudes of sIPSCs are decreased in both pregnant wild-type and *Gabrd*$^{-/-}$ mice. (D) The average mIPSC amplitude is decreased in pregnant wild-type mice compared with virgin mice. There is no change in the unitary conductance of receptors, as determined by nonstationary fluctuation analysis, between wild-type pregnant and virgin mice.

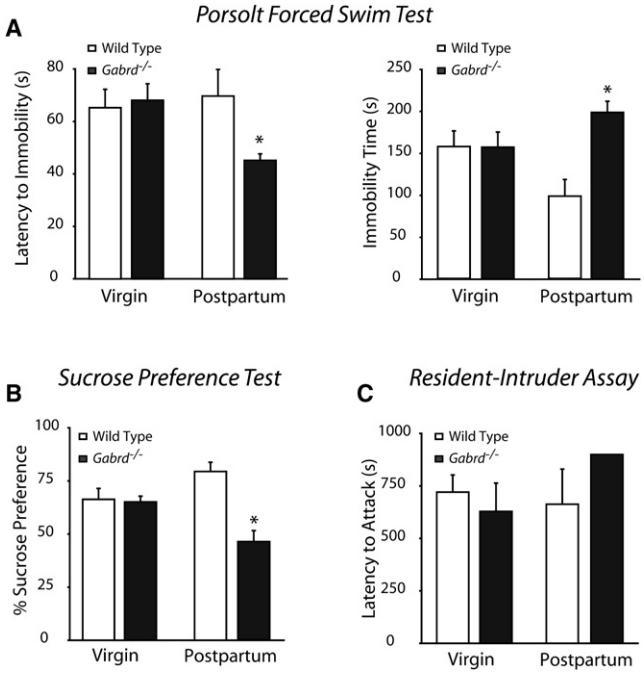

Figure 3. Behavioral Abnormalities in *Gabrd*$^{+/-}$ and *Gabrd*$^{-/-}$ Mice Postpartum

(A) *Gabrd*$^{-/-}$ mice exhibit a decreased latency to immobility and an increase in the total immobility time during a 6 min Porsolt forced swim test. (B) Over a 48 hr period, virgin wild-type, postpartum wild-type, and virgin *Gabrd*$^{-/-}$ mice consumed more 2% sucrose solution than water in the sucrose preference test. Postpartum *Gabrd*$^{-/-}$ mice did not prefer the 2% sucrose solution. (C) Virgin and postpartum wild-type and *Gabrd*$^{-/-}$ mice had a similar latency to attack in the resident-intruder assay.

behavior in mice. Mice were given access to two water bottles, one containing water and one containing a 2% sucrose solution, and the amount of each consumed over a 48 hr period was measured in virgin and postpartum mice of each genotype. Virgin mice from each genotype consumed equivalent volumes of water (23.3 ± 0.5 ml, wild-type, and 23.0 ± 1.7 ml, *Gabrd*$^{-/-}$) over a 48 hr period and exhibited a preference for the 2% sucrose solution. Of the total volume consumed (100%) by virgin mice, sucrose solution preference was 66.4% ± 5.2% (wild-type) and 65.2% ± 2.7% (*Gabrd*$^{-/-}$) (Figure 3B). The wild-type and *Gabrd*$^{-/-}$ postpartum mice drank more fluid than virgin mice: 32.8 ± 7.5 ml (wild-type) and 33.0 ± 6.2 ml (*Gabrd*$^{-/-}$). Similar to the virgin mice of both genotypes, the postpartum wild-type mice exhibited a preference for the 2% sucrose solution (79.4% ± 4.4%) (Figure 3B). In contrast, postpartum *Gabrd*$^{-/-}$ mice did not exhibit a preference for the 2% sucrose solution, consuming nearly equivalent amounts of water (53.6% ± 5.2%) and sucrose (46.4% ± 5.2%) (Figure 3B; n = 4–6 mice per experimental group; $p < 0.05$, one-way ANOVA), suggesting that *Gabrd*$^{-/-}$ mice exhibit anhedonia only in the postpartum period.

To assess whether *Gabrd*$^{-/-}$ mice exhibit signs of aggression postpartum, we performed the resident-intruder assay. Virgin and postpartum wild-type and *Gabrd*$^{-/-}$ mice were housed individually either beginning at d12 of pregnancy until 48 hr postpartum or for 2 consecutive weeks (for virgin mice). An intruder mouse was then introduced into the homecage of the resident and aggression levels were assessed in the residents over 15 min (900 s) by measuring the latency to attack the intruder. There were no significant differences in the latency to attack for wild-type virgin (720.6 ± 81.9 s), wild-type postpartum (663.0 ± 167.3 s), *Gabrd*$^{-/-}$ virgin (628.6 ± 134.9 s), and *Gabrd*$^{-/-}$ postpartum mice (900.0 ± 0.0 s) (Figure 3C; n = 4–8 for each experimental group; $p > 0.05$, one-way ANOVA). According to these findings, *Gabrd*$^{-/-}$ mice are not more

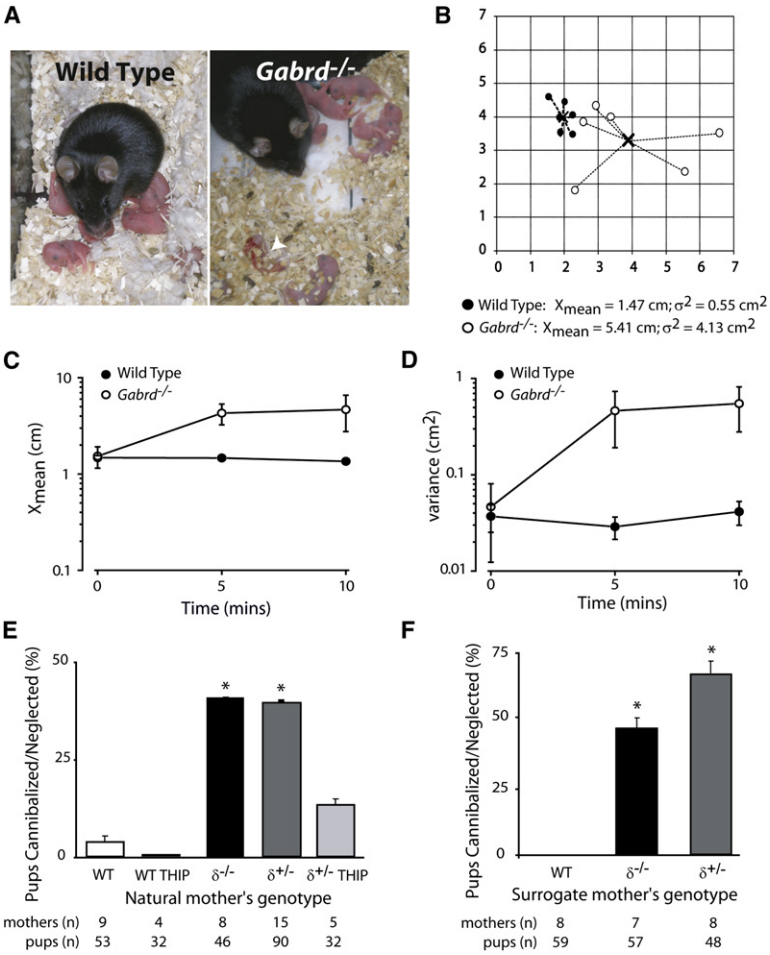

Figure 4. Abnormal Maternal Behaviors in *Gabrd*$^{-/-}$ Mice

(A) Representative photographs of nests built by wild-type and *Gabrd*$^{-/-}$ mothers. *Gabrd*$^{-/-}$ mothers fail to build a proper nest and keep the pups further away than the wild-type mother does. A partially cannibalized pup is indicated by an arrowhead. (B) Dispersion of the pups by their respective mothers is shown as the average distance of the pups from the centroid (X_{mean}) and the variance of the distance (σ^2) from the centroid for wild-type and *Gabrd*$^{-/-}$ pups. The values are shown for a representative wild-type litter (black circles) and *Gabrd*$^{-/-}$ litter (white circles) after 10 min. The summarized data for X_{mean} and σ^2 for all the wild-type litters and *Gabrd*$^{-/-}$ litters over a 10 min period are shown in (C) and (D), respectively. (E) Decreased survival of pups born to *Gabrd*$^{+/-}$ and *Gabrd*$^{-/-}$ mice is reduced by enhancing GABA$_A$R δ-subunit-mediated inhibition by including THIP in the drinking water. (F) Pups born to *Gabrd*$^{+/-}$ and *Gabrd*$^{-/-}$ mice but reared by a surrogate wild-type mother immediately following birth had an increase in survival compared with wild-type pups reared by *Gabrd*$^{+/-}$ and *Gabrd*$^{-/-}$ surrogate mothers immediately following birth.

aggressive postpartum. In response to the stress of the presence of the intruder, we also observed more digging, burrowing, and circling, considered to reflect anxiety (Hebb et al., 2004; Maldonado and Navarro, 2001; Njung'e and Handley, 1991). In *Gabrd*$^{-/-}$ postpartum mice during the 15 min tests, this type of behavior lasted longer (456.0 ± 49.7 s) than that in virgin *Gabrd*$^{-/-}$ mice (249.3 ± 64.2 s), virgin wild-type mice (13 ± 9.9 s), or postpartum wild-type mice (66.5 ± 29.9 s) ($p < 0.05$, one-way ANOVA; n = 4 for each group; Figure 3C). Taken together, all behavioral tests are consistent with *Gabrd*$^{-/-}$ mice exhibiting depression-like and anxiety-like behaviors during the postpartum period.

Abnormal Maternal Behavior during the Postpartum Period in Mice with Deficiencies in δ-Subunit-Containing GABA$_A$Rs

To further investigate the impact of the behavioral deficits observed in *Gabrd*$^{-/-}$ postpartum mice, we analyzed the maternal behavior of postpartum wild-type and *Gabrd*$^{-/-}$ mice. Compared with wild-type dams, *Gabrd*$^{-/-}$ mothers exhibited abnormal postpartum behaviors, such as the inability to build a proper nest, evidenced by the lack of a prominent wall surrounding the nest and the lack of bedding between the pups and the cage floor (Figure 4A), and the existence of an increased distance from their pups (Figures 4A–4D). The latter was measured by removing the mother from the homecage for 10 s, then replacing her into the homecage and measuring the distance of the pups from the mother over the following 10 min. The scatter of the pups was quantified as the mean distance (X_{mean}) of each pup from the litter centroid over time and the variance of the distance (σ^2) from the centroid. An example of the pup scatter for representative wild-type and *Gabrd*$^{-/-}$ litters 10 min after returning the mother to the homecage is shown in Figure 4B, demonstrating that the pups from *Gabrd*$^{-/-}$ mothers are more dispersed throughout the cage with an average X_{mean} for *Gabrd*$^{-/-}$ pups of 1.53 ± 0.38 cm, 4.29 ± 1.04 cm, and 4.67 ± 1.91 cm at 0, 5, and 10 min, respectively, and 1.48 ± 0.08 cm, 1.47 ± 0.08 cm, and 1.35 ± 0.08 cm for wild-type pups (Figure 4C). The variance of this distance is profoundly increased in *Gabrd*$^{-/-}$ litters, with 0.45 ± 0.33 cm^2, 4.44 ± 2.61 cm^2, and 5.27 ± 2.59 cm^2 at 0, 5, and 10 min, respectively, compared with wild-type litters at 0.36 ± 0.11 cm^2, 0.28 ± 0.07 cm^2, and 0.40 ± 0.11 cm^2 (Figure 4D; n = 4 for each group; $p < 0.05$ by one-way ANOVA), suggesting that the pups are scattered randomly throughout the cage in *Gabrd*$^{-/-}$ litters.

We also noted a significant decrease in the survival rate of pups born to *Gabrd*$^{-/-}$ and *Gabrd*$^{+/-}$ mothers compared with that of wild-type. The mean litter size was equivalent at the time of delivery for wild-type (5.89 ± 0.08 pups; n = 9 mothers; n = 53 pups), *Gabrd*$^{+/-}$ (6.00 ± 0.06 pups; n = 15 mothers; n = 90 pups), and *Gabrd*$^{-/-}$ (5.75 ± 0.11 pups; n = 8 mothers; n = 46 pups) mothers. However, the percentage of pup survival per litter born to *Gabrd*$^{-/-}$ mothers (58.7% ± 2.6%; n = 8 mothers; n = 46 pups) or *Gabrd*$^{+/-}$ mothers (60.0% ± 2.0%; n = 15 mothers; n = 90 pups), was significantly decreased compared with the survival per litter of wild-type mothers (96.2% ± 0.9%; n = 9 mothers; n = 53 pups; $p < 0.05$, one-way ANOVA). An

increased percentage of pups born to *Gabrd*$^{-/-}$ (41.3% ± 0.3%; n = 8 mothers; n = 46 pups) and *Gabrd*$^{+/-}$ (40.0% ± 0.3%; n = 15 mothers; n = 90 pups) mothers died due to neglect or cannibalism compared with pups born to wild-type mothers (3.8% ± 0.9%; n = 9 mothers; n = 53 pups; Figure 4E; $p < 0.05$, one-way ANOVA). Therefore, the decreased mean litter size originally reported in the first study describing *Gabrd*$^{-/-}$ mice (Mihalek et al., 1999) was probably due to cannibalism of the pups, which may have gone unnoticed if not observed immediately after delivery, as most of the cannibalism occurred within the first 24 hr.

To rule out the possibility that increased mortality of pups born to *Gabrd*$^{-/-}$ and *Gabrd*$^{+/-}$ mothers may have been triggered by an inherent defect in *Gabrd*$^{-/-}$ pups, we performed crossfostering experiments. Pup mortality was increased in wild-type pups crossfostered by surrogate *Gabrd*$^{-/-}$ (41.4% ± 1.2%; n = 7 litters; n = 57 pups) or *Gabrd*$^{+/-}$ (66.7% ± 1.5%; n = 8 litters; n = 48 pups) mothers due to neglect or cannibalism compared with crossfostered *Gabrd*$^{-/-}$ pups reared by surrogate wild-type mothers (0.0% ± 0.0%; n = 9 litters; n = 53 pups; Figure 4F; $p < 0.05$, one-way ANOVA), suggesting that the abnormal maternal behavior underlies the increased mortality of pups born to *Gabrd*$^{-/-}$ and *Gabrd*$^{+/-}$ mothers rather than some deficit in the pups. These data demonstrate that mice with defects in GABA$_A$R δ subunit expression exhibit behavioral abnormalities in the postpartum period. Thus, restoring GABA$_A$R δ subunit function may ameliorate these behavioral deficits in the postpartum period. We did this by administering a nonsedative dose of THIP (a GABA$_A$R δ-subunit-preferring agonist) in the drinking water of wild-type and *Gabrd*$^{+/-}$ mice, resulting in a dose of 82.8 ± 4.6 mg/kg over 24 hr, equivalent to the range of the 4–6 mg one-time administration employed in previous studies (Winsky-Sommerer et al., 2007). THIP treatment did not sedate or otherwise affect the maternal behaviors of wild-type mothers as evident from the low pup mortality rates (0.0% ± 0.0%; n = 4 litters; 32 offspring). However, this dose of THIP given to *Gabrd*$^{+/-}$ mothers significantly decreased pup mortality from maternal neglect or cannibalism (15.6% ± 0.6%; n = 5 litters; 32 offspring) compared with mortality of pups from untreated mothers (40.0% ± 0.3%; n = 15 litters; 90 offspring) (Figure 4E; $p < 0.05$, one-way ANOVA). Therefore, the defects in tonic GABAergic inhibition mediated by δ-subunit-containing GABA$_A$Rs must play an important role in the behavioral deficits in *Gabrd*$^{-/-}$ and *Gabrd*$^{+/-}$ postpartum mice. Pharmacologically enhancing the inhibition mediated by these receptors may be a useful therapeutic approach to alleviating abnormal postpartum behaviors.

DISCUSSION

Our study identified a mouse model, GABA$_A$R δ-subunit-deficient mice, that exhibits depression-like and anxiety-like behaviors during the postpartum period, associated with abnormal pup care and decreased pup survival. These behavioral deficits in *Gabrd*$^{-/-}$ mice are consistent with comorbidity of anxiety (Ross et al., 2003), but not aggression, in the human condition of postpartum depression. The onset of depression-like behaviors in *Gabrd*$^{-/-}$ mice is restricted to the postpartum period (Figure 3), suggesting that this is not merely a model of major depression but rather a specific model of postpartum depression. An in-depth search of the published literature does not reveal any mouse phenotypes with abnormal postpartum behavior associated with depression-like behaviors. For example, pup survival is decreased in oxytocin knockout mice due to abnormal milk ejection (Nishimori et al., 1996). Estrogen receptor-alpha knockout mice exhibit aggressive-like behavior and deficits in pup-induced maternal behavior but do not exhibit depression-like behaviors (Ogawa et al., 1998). 5HT1B receptor knockout mice exhibit abnormal maternal behavior that has been attributed to defects in ultrasonic vocalizations by the pups unrelated to depression (Weller et al., 2003). Further, a search of the Jackson Laboratory's Mouse Behavioral Database yielded 29 strains exhibiting "abnormal maternal behavior;" however, none of these strains exhibit depression-like behaviors as an associated phenotype. Similarly, mouse strains in the Jackson lab database exhibiting "depression-like" phenotypes do not associate with abnormal postpartum behavior. It is important to note that the mild epileptic phenotype of *Gabrd*$^{-/-}$ mice does not account for the maternal behavior abnormalities since *Gabrd*$^{+/-}$ mice with no overt epileptic phenotype still display the abnormal postpartum behaviors common to *Gabrd*$^{-/-}$ mice. Moreover, numerous other mice with epileptic phenotypes do not exhibit behavioral abnormalities exclusive to the postpartum period such as those observed in *Gabrd*$^{-/-}$ and *Gabrd*$^{+/-}$ mice. *Gabrd*$^{-/-}$ and *Gabrd*$^{+/-}$ mice therefore represent a unique mouse model exhibiting depression-like behaviors restricted to the postpartum period and associated with abnormal maternal behavior.

Our study reveals a potential functional mechanism for abnormal postpartum behavior resulting from dysfunction in GABA$_A$R regulation during pregnancy and postpartum. As neurosteroid levels increase tremendously during pregnancy, brain mechanisms must have evolved to decrease the sensitivity of gravid mammals to neurosteroids. The quick postpartum reversion of GABA$_A$R expression and inhibition to control levels in wild-type mice indicates that the rapid return of neurosteroids to pre-pregnancy levels after parturition is followed by a commensurate adjustment in the number of functional GABA$_A$Rs. Inability to properly regulate GABA$_A$Rs during pregnancy and postpartum, as in *Gabrd*$^{-/-}$ and *Gabrd*$^{+/-}$ mice, is associated with depression-like and abnormal maternal behaviors, which may be highly relevant to the human condition of postpartum depression. During the highly vulnerable postpartum period, failure of tonic inhibition to match the lowered neurosteroid levels after parturition, as in *Gabrd*$^{-/-}$ and *Gabrd*$^{+/-}$ mice, may precipitate mood disorders associated with the postpartum period. Our data are consistent with the idea that the pathophysiology of postpartum depression may be related to the inability to properly regulate GABAergic inhibition during pregnancy and postpartum. Since defects in other GABA$_A$R subunit genes, such as those in *Gabrg2*$^{+/-}$, *Gabra1*$^{-/-}$, *Gabra4*$^{-/-}$, and *Gabra6*$^{-/-}$ mice, do not seem to be associated with abnormal postpartum behaviors, the plasticity of δ-subunit-containing GABA$_A$Rs may be particularly important in regulating neuronal excitability. This idea is further supported by the beneficial effect of alleviating abnormal postpartum behaviors and increasing pup survival in *Gabrd*$^{+/-}$ mice by specifically enhancing the function of δ-subunit-containing GABA$_A$Rs by pharmacological means using THIP. The GABA$_A$R δ subunit may be a promising target

for therapeutic intervention in the treatment of postpartum depression.

Our study provides important clues for the pathogenesis of postpartum depression and provides a useful mouse model for the disease. This model will foster further insights into the mechanisms of postpartum depression and will provide much needed therapeutic potential for the large number of women suffering from mood disorders during early motherhood.

EXPERIMENTAL PROCEDURES

Western Blot Analysis

Western blot analysis was carried out as previously described (Maguire et al., 2005). One hundred micrograms of total hippocampal membrane protein was subjected to SDS-PAGE, transferred to a nitrocellulose membrane (Amersham), blocked in 10% nonfat milk, and probed with a polyclonal antibody, anti-GABA$_A$R δ (1:5000, a gift from Dr. W. Sieghart) or anti-GABA$_A$R γ2 (1:10,000, NOVUS). The blots were incubated with peroxidase-labeled anti-rabbit IgG (1:2000, Vector Laboratories), and immunoreactive proteins were visualized using enhanced chemiluminescence (Amersham). OD measurements were determined using the NIH Image J software.

Whole-Cell Recordings

Whole-cell patch-clamp recordings were performed on DGGCs at approximately 34°C as previously described (Maguire et al., 2005). The slices were perfused with normal ACSF (nACSF) (126 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1–2 mM $MgCl_2$, 1.25 mM $NaHPO_4$, 26 mM $NaHCO_3$, and 10–25 mM D-glucose, bubbled with 95% O_2 and 5% CO_2 [pH = 7.3–7.4]) containing 3 mM kynurenic acid and 5 μM GABA (Sigma, St. Louis, MO). Intracellular recording solution containing 140 mM CsCl, 1 mM $MgCl_2$, 10 mM HEPES, and 4 mM Na-ATP (pH = 7.25, 280–290 milliosmoles) and electrodes with DC resistance of 2–5 MΩ were used for all recordings. mIPSCs were recorded in ACSF containing 50 μM $CdCl_2$. Data analysis was performed as previously described (Maguire et al., 2005; Stell et al., 2003).

Nonstationary Fluctuation Analysis

mIPSCs were low-pass filtered at 1 kHz and sampled at 10 kHz. Large amplitude events with rapid 10%–90% rise times were selected to ensure that the mIPSCs were from a population of synapses with similar kinetics (De Koninck and Mody, 1994). Traces containing multiple events were discarded. The selected events were peak aligned and the average variance of the current around the mean current (I_m) was determined. The variance (σ^2) was plotted against I_m and binned (25 bins), and the relationship $\sigma^2 = iI_m - I_m/N$ was fit (least-squares simplex method) to the data points to obtain i, the unitary current, and N, the number of channels responsible for generating the mIPSC.

Animal Handling, Breeding, THIP Administration, and Crossfostering

Wild-type (C57Bl6), *Gabrd*$^{-/-}$, and *Gabrd*$^{+/-}$ mice on C57Bl6 background were bred with like stud males for 24 hr and subsequently isolated in separate cages for timed pregnancies. THIP (10 mg of THIP in 50 ml of water) was administered in the drinking water after establishing that wild-type and *Gabrd*$^{+/-}$ mothers drank the same amount of water during after parturition (wild-type: 9.6 ± 2.1 ml/day; *Gabrd*$^{+/-}$: 10.4 ± 1.6 ml/day). The drinking water was replaced without any disruption of the homecage. A separate set of experiments was carried out in which a surrogate *Gabrd*$^{-/-}$ or *Gabrd*$^{+/-}$ mother (which gave birth to her own pups within the past 24 hr) was placed in the homecage of pups immediately born to a wild-type mother. The wild-type mother then became the surrogate to the *Gabrd*$^{-/-}$ or *Gabrd*$^{+/-}$ pups. The mothers were swapped rather than the pups to limit the disruption of the pups from their homecage. The number of surviving pups and the cause of death (neglect versus cannibalism) was documented.

Porsolt Forced Swim Test

The Porsolt forced swim test was carried out by placing each mouse individually in a glass cylinder (21 cm × 12 cm), filled to 9 cm with room-temperature water (22°C–25°C). The latency to immobility was recorded and the total duration of immobility throughout the 6 min forced swimming test was measured. The mouse was considered immobile when it ceased swimming and remained floating motionless, except for infrequent movements to maintain afloat.

Aggression- and Anxiety-Related Behavior Assay

Forty-eight hours after parturition, aggressive behavior was monitored during 15 min exposures to a given wild-type male intruder mouse that had been group-housed (four mice per cage) and matched with resident mice for body weight. Briefly, an intruder male was introduced into the homecage of the isolated female for 15 min and the latency to attack and the total number of attacks were quantified. In addition, the time spent engaging in anxiety-related behaviors such as digging (dig, kick dig, push dig), burrowing, and circling were measured for both groups. The pups were removed from the cage 3 min before the onset of testing to avoid the possibility of injury to the pups, which does not alter the aggression of the mother (Svare et al., 1981).

Pup Dispersal Test

Three days after giving birth, the lactating female was removed from the homecage briefly (about 10 s) and then reintroduced to the homecage. The pups remained undisturbed. The behavior of the mother was videotaped and the location of the pups identified throughout the 10 min recording. Subsequently, pup location was determined at times 0, 5 min, and 10 min by calculating the x and y coordinates for each pup. The centroid (mean of all x and all y values) was determined for each litter at each time point. The mean distance from the centroid for each pup (X_{mean}) and the variance (σ^2) was calculated for each litter at each time point, and the averages were compared between genotypes.

Statistics

Statistical significance was set at $p < 0.05$. The Student's t test was used for comparison between two experimental groups. A one-way ANOVA was used for comparison of data sets with more than two groups.

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