Neuropsychiatric Phenotypes Produced by GABA Reduction in Mouse Cortex and Hippocampus

Stefan M Kolata1,8, Kazuhiro Nakao1,2,8, Vivek Jeevakumar2, Emily L Farmer-Alroth2, Yuko Fujita3, Aundrea F Bartley4, Sunny Zhihong Jiang1, Gregory R Rompala1,9, Robert E Sorge5, Dennisse V Jimenez6,10, Keri Martinovich5, Yolanda Mateo4, Kenji Hashimoto3, Lynn E Dobrunz4 and Kazu Nakazawa*,1,2

1Unit on Genetics of Cognition and Behavior, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA; 2Department of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL, USA; 3Division of Clinical Neuroscience, Chiba University Center for Forensic Mental Health, Chiba, Japan; 4Department of Neurobiology, University of Alabama at Birmingham, Birmingham, AL, USA; 5Department of Psychology, University of Alabama at Birmingham, Birmingham, AL, USA; 6Lieber Institute for Brain Development and Department of Psychiatry and Behavioral Science and Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA; 7National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA

Whereas cortical GAD67 reduction and subsequent GABA level decrease are consistently observed in schizophrenia and depression, it remains unclear how these GABAergic abnormalities contribute to specific symptoms. We modeled cortical GAD67 reduction in mice, in which the Gad1 gene is genetically ablated from ~50% of cortical and hippocampal interneurons. Mutant mice showed a reduction of tissue GABA in the hippocampus and cortex including mPFC, and exhibited a cluster of effort-based behavior deficits including decreased home-cage wheel running and increased immobility in both tail suspension and forced swim tests. Since saccharine preference, progressive ratio responding to food, and learned helplessness task were normal, such avolition-like behavior could not be explained by anhedonia or behavioral despair. In line with the prevailing view that dopamine in anterior cingulate cortex (ACC) plays a role in evaluating effort cost for engaging in actions, we found that tail-suspension triggered dopamine release in ACC of controls, which was severely attenuated in the mutant mice. Conversely, ACC dopamine release by progressive ratio responding to reward, during which animals were allowed to effortlessly perform the nose-poking, was not affected in mutants. These results suggest that cortical GABA reduction preferentially impairs the effort-based behavior which requires much effort with little benefit, through a deficit of ACC dopamine release triggered by high-effort cost behavior, but not by reward-seeking behavior. Collectively, a subset of negative symptoms with a reduced willingness to expend costly effort, often observed in patients with schizophrenia and depression, may be attributed to cortical GABA level reduction.

Neuropsychopharmacology (2018) 43, 1445–1456; doi:10.1038/npp.2017.296; published online 24 January 2018

INTRODUCTION

Corticolimbic GABAergic dysfunction is implicated in the etiology of schizophrenia, depression, and related neuropsychiatric diseases (Benes and Berretta, 2001; Lewis et al, 2005; Nakazawa et al, 2012). For example, the level of Gad1 mRNA encoding GAD67 (glutamic acid decarboxylase-67) falls below detectable levels in approximately 50% of parvalbumin (PV)-positive prefrontal cortex (PFC) interneurons in schizophrenia (Hashimoto et al, 2003). This has led to the hypothesis that GAD67 dysfunction, particularly in PV-negative cortical interneurons, may be implicated in development of schizophrenia (Lewis et al, 2005). Moreover, several conditional Gad1 deletion mouse lines have been tested for schizophrenia-related phenotypes with mixed results. For instance, a PV-neuron/Gad1 homozygous knockout mouse line (Georgiev et al, 2016) was spontaneously hyperactive but showed normal sensorimotor gating and spatial working memory, and had increased mRNAs for Pvalb, Bdnf, Kcns3 and Gad2. Since these mRNA are unaltered or decreased in patients with schizophrenia, it was suggested that PV neuron-selective GAD67 loss is not an upstream cause of schizophrenia (Georgiev et al, 2016). A conditional Gad1 heterozygous knockout mouse line was also generated by crossing the floxed-Gad1 line to a BAC (bacterial artificial chromosome)-Cre line, in which Cre is expressed not only in PV-positive interneurons but also in subsets of somatostatin- and calretinin-positive interneurons (Fujihara et al, 2015). This heterozygous KO mutant line
showed MK-801 induced hyperlocomotion and sensorimotor deficits with a reduction in PV levels; conclusively demonstrating that haploinsufficiency of the Gad1 gene in a subset of GABA neurons is sufficient to recapitulate schizophrenia-related phenotypes. In a third model, microRNA-mediated GAD67 knockdown mice exhibited sensorimotor deficits, as well as increased novelty-seeking and reduced fear extinction (Brown et al., 2015). The phenotypic discrepancies among these Gad1 mutant mice are difficult to reconcile. However, one possibility is that it could be due to an insufficient reduction in cortical GABA in these mutant lines, or it could be attributed to unforeseen subcortical deficits of the GABA system due to the brain-wide manipulations.

In order to explore the impact of cortical GABA reduction following Gad1 reduction, we genetically eliminated the Gad1 gene from approximately 50% of cortical and hippocampal interneurons; a majority (70%) of which are PV-positive. This was accomplished by crossing a loxP-flanked Gad1 mouse line (Chattopadhyaya et al., 2007) to a previously characterized Ppp1r2-Cre driver line (Belforte et al., 2010), in which Cre recombinase expression is largely confined to GABA neurons of the cortex and hippocampus. In this way we directly addressed which symptoms or phenotypes may be attributed to the lowered GABA levels in the cortex and hippocampus following GAD67 deficits.

**MATERIALS AND METHODS**

Conditional Gad1 homozygous knockout mutant mouse line was created by crossing a loxP-flanked Gad1 line (Chattopadhyaya et al., 2007) to a Ppp1r2-Cre driver line (Belforte et al., 2010). Measurement of tissue GABA and glutamate content was conducted as previously described (Fujita et al., 2016). Slice physiology was conducted in acute medial PFC (mPFC) slices and hippocampal slices (Li et al., 2017). In vivo awake brain microdialysis was performed in anterior cingulate cortex (ACC) or nucleus accumbens (NAC) of freely moving mice using concentric microdialysis probes (CMA/7, CMA/Microdialysis, Solna, Sweden) (Tejeda et al., 2013). Briefly, dialysate samples were collected in a microdialysis test chamber three times (20-min bin each) prior to injecting with d-amphetamine (2.5 mg/kg; i.p.), before tail hanging, or before placing the animal in the operant conditioning chamber. Four additional samples (20-min bin each) were collected in the same chamber during and after amphetamine injection or tail hanging for 6 min. Dialysate samples were also collected from the animal in the operant chamber during progressive ratio (PR) schedule of nose-poking (20-min bin each, 80-min total). For dopamine and serotonin (5-HT) quantification, HPLC with electrochemical detection (Eicom HTEC-500 HPLC/EC System, Kyoto, Japan) with an PP-ODS2 column (Eicom) run in 20-μl samples with a mobile phase flow rate of 500 μl/min and an applied potential of +400 mV vs Ag/AgCl was used. To assess the epileptiform activity, in vivo local field potential (LFP) recordings from sensory cortex and hippocampus were conducted during a 10-min immobility session following animal’s cessation of exploratory behavior in a high-walled small circular box (10-cm in diameter) as previously described (Jinde et al., 2009). Male or female mutants and their floxed-littermate controls of 10–12 weeks of age, which were single-housed for at least 1 week prior to testing, were subjected to a behavioral test battery. Experimental procedures including the behavioral tests, drugs, and statistics are described in detail in Supplementary Information. All experimental procedures were in accordance with National Research Council guidelines for the care and use of laboratory animals, and were approved by the Institutional Animal Care and Use Committee at NIMH and at University of Alabama at Birmingham.

**RESULTS**

Gad1 Ablation Leads to Reduced GABA Level and Altered GABA Transmission in mPFC and Hippocampus

Homozygous Gad1loxP/loxP;Ppp1r2-cre/+;Gad1loxP/loxP, hereafter simply mutant) were viable and fertile, with no gross morphological abnormalities revealed by Nissl staining (data not shown). We first assessed the extent of genetic Gad1 ablation by crossing the conditional Gad1 KO mutants further to a floxed-EYFP line (Figure 1a). Immunoreactivity of GAD67 encoded by Gad1 was absent in nearly all the Cre-targeted YFP-positive cells in the cortex and hippocampus of the mutant mice at 10 weeks old (see Figure 1b). Tissue homogenate GAD67 levels in somatosensory cortex were also reduced to roughly 50% (Figure 1c), which is consistent with the evidence that ~50% of cortical interneurons express Cre recombinase (Belforte et al., 2010). On the other hand, protein level of GAD65, the enzyme responsible for a minority of GABA synthesis, were increased in somatosensory cortex of the mutants (Figure 1c), presumably due to compensation. Nonetheless, tissue GABA levels, measured by HPLC, were reduced in mPFC, frontal cortex (a frontal portion of neocortex), and hippocampus of the mutant mice at 11–13 weeks old (Figure 1d). In contrast, no GABA level reduction was detected in striatum or cerebellum where Cre expression is negligible (Belforte et al., 2010). Since no change in tissue L-glutamate levels was detected in any tissues examined, the GABA/glutamate ratio was also diminished in mPFC, frontal cortex, and hippocampus of the mutant mice (Supplementary Figure S1A).

Next, we compared the GABAergic synaptic transmission to pyramidal neurons in the layer 2/3 of ACC by applying electric stimulation of different intensities (from 50 μA to 400 μA) ex vivo. The stimulation delivered in the vicinity of the patched neuron in the presence of glutamate blockers (CNQX and D-APV) produced an inhibitory postsynaptic current (IPSC) response which initially increased linearly until approaching a plateau in both groups (Figure 1e). However, the response in the mutants was smaller at all stimulus intensities, suggesting impaired efficiency of GABAergic synaptic transmission following Gad1 knockout. We also investigated whether the Gad1 knockout causes changes in the excitability of layer 2/3 pyramidal neurons in ACC. By injecting a series of hyper- and depolarizing current steps into the soma of the recorded neuron from resting membrane potential, action potentials were evoked and the membrane properties were measured. All cells recorded showed regular spiking and adapting action potentials. Threshold, action potential half-width and amplitude, fast-AHP (after hyperpolarization), input resistance and membrane time constant did not vary significantly between

Neuropsychopharmacology
Figure 1  Outline of transgenic approach and validation of Gad1 genetic ablation. (a) Schematic diagram showing generation of the conditional knockout of Gad1, Ppp1r2-CreT2;Gad1loxPloxP referred to as mutant and of the triple transgenic mouse that allows visualization of Cre-targeted cells by enhanced YFP (EYFP), Ppp1r2-CreloxP; Rosa26, loxP-Stop-loxP–EYFPloxP, referred to as YFP-mutant. (b) Double immunolabeling in the mPFC of GAD67 (red) and YFP (green) in mPFC of 10-week-old mice showing the YFP cells positive for GAD67. The table shows %YFP cells positive for GAD67 (out of 135 cells in the controls; 132 cells in the mutants in mPFC) in both genotypes demonstrating GAD67 deletion virtually all YFP-positive cells in mutant mice. (c) Representative western blot bands and their quantification (in graph) showing that GAD67 protein is reduced in somatosensory cortical tissue homogenates of 10-week-old mutants (t(10) = 3.09, p < 0.01) while GAD65 is increased (t(11) = 3.04, *p < 0.01). (d) Tissue GABA content in the control floxed mice (white, n = 14) and mutant mice (black, n = 13). GABA level is lower in mPFC (t(25) = 2.69, *p = 0.0013), frontal cortex (FC, t(25) = 4.07, *p = 0.0004) and hippocampus (Hipp, t(25) = 2.09, *p = 0.046), but not in striatum (t(25) = 0.12, p = 0.91) or cerebellum (Cere, t(25) = 1.03, p = 0.31). (e) Gad1 ablation in GABAergic interneurons reduces the amplitude of evoked IPSCs in the anterior cingulate cortex (ACC). Plot of evoked IPSC amplitudes in slices from control and mutant mice (two-way ANOVA, F(1,88) = 64.5 for genotype main effect, *p < 0.001). Inset: Representative traces from control and mutant groups showing the difference in amplitudes at the same stimulation intensity. (f) Pyramidal neurons in mutant mPFC are more excitable compared to controls. Summary graph of the firing frequency of pyramidal cells with the mutant group exhibiting significantly higher number of action potentials in response to somatic current injection (two-way ANOVA, F(1,187) = 27.1 for genotype main effect, *p < 0.001). Inset: Representative recordings showing the firing patterns of pyramidal neurons, for a current injection of 100 pA. All Data are mean ± SEM; animal number or cell number n is indicated in parentheses or plot bars."
Mutants exhibited a subset of negative symptom-like behavioral deficits. (a) Locomotor behavior in the open field did not differ between the genotype overall (F(1,23) = 3.33, p = 0.57) but there the mutant mice displayed novelty induced hyperlocomotion in the first 5 min (post-hoc planned comparisons, F(1.14) = 5.18, p < 0.05). (b) Mutant mice spent significantly less time in the center of the open field (F(1,23) = 13.74, p < 0.01). (c) Mutant mice spent less total time in the light side of the light/dark box (t(11) = 3.7, p < 0.01). (d) Overall social interaction time with a novel mouse in the sociability test was significantly reduced in the mutants (F(1,19) = 8.143, p < 0.01) and there was a significant interaction between genotype × novel/empty cage interaction (F(1,19) = 57, p < 0.01). (e) Over 8-min period, mutant mice spent significantly less time interacting with the sexually receptive female mice (F(1.19) = 17, p < 0.01). (f) Mutant mice differed significantly in their motivated wheel-running behavior (genotype × time interaction: F(1,120) = 2.68, p < 0.01) and their peak wheel running activity was attenuated (post-hoc planned comparisons, F(1,15) = 1.23, p < 0.02). (g) No difference in rotarod performance was observed between mutant (Mut) and control (Ctrl) mice (12–19 weeks of age), repeated measures ANOVA F(1.36) = 0.47, p = 0.50. (h) Male mutants showed increased immobility duration in tail suspension, t(17) = 4.51, p = 0.0003. (i) Male mutants showed increased immobility duration in forced swim chamber (t(14) = -2.77, p = 0.015). (j) No genotypic differences in the number of escapes in the learned helpless task (t(23) = 0.56, p = 0.58). (k) No genotypic differences in their preference for saccharin sweetened water at 0.01% (t(22) = 0.48, p = 0.64) and 0.03% (t(16) = 0.02, p = 0.98) solution. (l) No genotypic difference in breakpoint achieved in PR session following the acquisition training in fixed ratio 1 (FR1) and FR5 schedule of reinforcement to obtain chocolate pellets. t(16) = 0.14, p = 0.89. (m) Mutant mice showed increased weight gain (F(1,16) = 3.7, p < 0.05). (n) In the 48-h homecage scan, mutants showed increased feeding time during their first light cycle (600–1800 hours) (t(12) = 1.8, p < 0.05). Data are mean ± SEM; number n is indicated in parentheses or plot bars.

Avoidance of Center/light Arena Exploration, Social and Sexual Withdrawal and Higher Immobility during Effortful Behaviors

To assess the impact of cortical GABA reduction on murine behavior, we subjected male mice to a behavioral test battery. In a novel open field, the mutants displayed elevated, novelty-induced activity relative to the controls during the first 5 min (Figure 2a); however subsequently, the mutants spent less time in the center of the open field (Figure 2b). The mutant mice also spent less total time in the light side of light/dark box (Figure 2c), suggesting an increased anxiety or decreased risk-taking. In the three-chamber sociability test, the mutants showed a decrease in time spent exploring the stranger mouse cage vs the empty cage, which is indicative of social withdrawal (Nadler et al, 2004) (Figure 2d). The male mutant mice also spent less time interacting with super-ovulated female mice over an 8-min period (Figure 2e). Similarly, male mutants at 15-week-old showed reduced mating efficiency with super-ovulated female B6 wild-type mice (4/24 mutants mated vs 17/17 controls, judged by copulatory plug formation on the following day). In the voluntary wheel running behavior, the peak wheel running activity of the mutants was reduced (Figure 2f). This is unlikely to be caused by the motor dysfunction, because mutants’ voluntary locomotion (Figure 2a) and motor coordination in the rotarod test (Figure 2g) were normal. Similarly, the mutant mice used less nestlet material when tested in an overnight nest-building test (control: n = 10, unused nestlet (out of 1.9 g), avg. = 0; mutant: n = 10, avg. = 1.2(±0.5), data not shown). These results suggested that the mutants show increased socially/sexual anxiety and/or are less willing to engage in effortful behavior.

To further assess if the mutants were impaired in effortful behavior engagement, immobility was measured in the tail suspension (Figure 2h) and forced swim tests (Figure 2i). The mutants showed heightened immobility in the both tasks compared to the controls. However, no genotypic differences were observed in the learned helplessness test, in which animals are exposed to unavoidable shock and later are trained to escape from the shock (Shirayama et al, 2002) (Figure 2j). These results suggest that the mutant mice do not display a depressive-like phenotype. We further tested a sweet-taste preference in male mice that were placed in individual cages and offered choice between 0.01% or 0.03% saccharin or water for 24 h (Figure 2k). Both control and mutant mice drank more saccharin than water, and there was no difference between genotypes. Using a different cohort of animals, mice were trained to respond on a nose-poke apertures of an operant conditioning chamber, with fixed ratio (FR1 and FR5) schedule of reinforcement, and all the animals of both genotypes reached the acquisition criteria (see Supplementary Method). Subsequently, the mice were required to respond on a progressive ratio (PR) schedule of reinforcement (Richardson and Roberts, 1996) for food. We found that the average break points (Figure 2l), the total nose-poke number (Supplementary Figure S4A), and average rate of nose-poking during the session (Supplementary Figure S4B) were all comparable between the genotypes. The results were consistent with a recent report showing that reducing mPFC GABA transmission exerts little effect on PR responding to sucrose reward in rats (Piantadosi, 2016). These findings also suggested that mutant mice are normal in hedonic (saccharine preference test) and reward reinforcement (PR responding) behavior, but exhibit effort avoidance when reward provided is negligible, such as in the forced swim test, tail suspension test, and spontaneous wheel running. Interestingly, the mutant mice showed increased body weight gain after 8 weeks of age (Figure 2m), and in the 48-h-homecage scan, they showed increased feeding time during their expected sleep (light) cycle at 10–12 weeks of age (Figure 2n), despite no other significant activity changes over a 24-h period (Supplementary Figure S4C).

No Deficits in Cognitive or Positive Symptom-like Phenotypes of Schizophrenia

Prepulse inhibition (PPI) is consistently impaired in patients with schizophrenia and is considered a read-out of impaired sensorimotor gating (Braff et al, 2001). In 10–12 week-old mutant mice, there was neither a deficit in the degree of PPI...
(Figure 3a) nor an alteration in the startle reflex responses (Figure 3b). In the continuous alteration Y-maze task, the mutants showed no difference in either the number of alternations (Figure 3c) or in the total number of arm entries (avg. control = 30.9 (±2.06); avg. mutant = 35.8 (±2.44); p > 0.05, data not shown), which is suggestive of no spatial working memory impairments. In the NMDA antagonist MK-801-induced hyperlocomotion test, a model psychotomimetic response (van den Buuse, 2010), the mutants showed no differences in the extent of locomotor hyperactivity with a moderate dose (0.3 mg/kg) of MK-801 (Figure 3d). Amphetamine-induced hyperlocomotion, another measure of psychotomimetic response (van den Buuse, 2010), was also comparable to the control mice (Figure 3e). Indeed, in vivo awake microdialysis revealed no genotypic difference in an extracellular dopamine release in NAC lateral shell in response
to systemic amphetamine treatment (Figure 3f and Supplementary Figure S5B), whereas patients with schizophrenia are known to increase striatal dopamine by amphetamine administration (Laruelle et al., 1999). Baseline dopamine levels in NAC were also unaltered in the mutants (control: 0.83 nm ± 0.04; mutant: 0.76 nm ± 0.1; t(9) = 0.4; p = 0.69). Taken together, these results suggested that male mutant mice do not display cognitive or positive symptom-like phenotypes of schizophrenia, consistent with the conclusion reported for PV-neuron/Gad1 KO mice (Georgiev et al., 2016).

**Female Mutant Mouse with Less Epileptiform Discharges Shared Behavioral Deficits**

LFP recordings were conducted simultaneously in somatosensory cortex and hippocampus of mutant male and female mice and control mice while they were in an immobile state in a small circular enclosure (10 cm diameter). Male mutants (n = 5), but not floxed-Gad1 control male mice (n = 5, see Supplementary Figure S6), at 10 weeks of age showed the presence of interictal discharges, the frequency of which became progressively worse with age (Figure 4a and b). The epileptiform discharges were mostly of a larger magnitude in the hippocampus than those in the cortex, and the onset of the discharges was synchronized across the electrodes (Figure 4a). Unexpectedly, these epileptiform discharges were barely detected in female mutant mice (n = 5) until 15 weeks of age. After recording in the immobile state, the mice were forced to explore in a low-walled linear track (length, 72 cm; width, 8 cm). During the exploratory behavior, epileptiform discharges were not observed in male (n = 5) or female (n = 5) mutant mice, even at 15 weeks of age (data not shown).

To evaluate whether the observed male mutants’ behavioral changes in Figure 2 are affected by their epileptiform activity, we subjected the female mutant mice at 10–12 weeks old to the behavioral tests because at this age they lacked the interictal discharges. Similar to the male mice, the female mutants showed avoidance of risk area exploration (Figure 4c), social withdrawal (Figure 4d), higher immobility in both tail suspension (Figure 4e) and forced swim tests (Figure 4f), decreased voluntary wheel running (Figure 4g), and body weight gain (Figure 4h). They also showed nest building impairments (control: n = 8, avg: unused nestlet (out of 1.9 g) = 6; mutant: n = 8, avg = 1.1(±0.39)). These results indicated that behavioral phenotypes in male mice are shared with females, and are thereby unlikely to be attributed to males’ epileptiform activity.

**ACC Dopamine Release Impaired in Tail Suspension but Normal in PR Responding**

Among mutant behavioral phenotypes, the most puzzling finding was a heightened immobility in the tail suspension and forced swim tests, despite that effort-related motivation was normal which was assessed by the PR nose-poking. Actually, prefrontal GABA transmission blockade in rats has been suggested to alter the cost/benefit decision making (Paine et al., 2015; Piantadosi et al., 2016), while the PR responding task was minimally affected (Piantadosi et al., 2016). Furthermore, ACC dopamine has been suggested to play a major role in effort allocation (Bailey et al., 2016; Kurniawan et al., 2011; Miller et al., 2013; Salamone et al., 2016). 5-HT also appeared to play a role in overcoming effort cost (Meyniel et al., 2016). We sought to examine whether ACC dopamine level could be altered, concordant with the behavioral deficit. First, we measured extracellular levels of dopamine and 5-HT in the ACC before and after the tail suspension by in vivo awake brain microdialysis (Supplementary Figure S5A). Prior to tail suspension, baseline levels of dopamine and 5-HT of the mutant mice were both comparable to the controls. Hanging control mice, regardless of gender, by the tail for 6 min significantly increased extracellular dopamine in ACC by ~70% at least for 80 min followed by a gradual decline (Figure 5a). In contrast, hanging-triggered dopamine release was robustly impaired in the ACC of mutant mice. In the same dialysis samples collected, 5-HT level also modestly increased in both genotypes by tail hanging, suggesting 5-HT release is normal in the mutants (Figure 5b). Higher immobility of the mutants compared to controls was confirmed in this cohort of animals (Figure 5c). Per-animal analysis revealed a significant inverse correlation between immobility (%) and increased ACC dopamine fold-changes in combined genotypes (Figure 5d). Indeed, mutants with impaired dopamine release all showed higher immobility. Interestingly, no robust increase in dopamine (Supplementary Figure S7A) or 5-HT (Supplementary Figure S7B) concentrations was observed in the NAC (see Supplementary Figure S5) during tail suspension.

Tonic dopamine release in mPFC may also be triggered by cognitive demand (Karakuyu et al., 2007; Stefani and Moghaddam, 2006), aversion (Mantz et al., 1989; Pignatelli and Bonci, 2015) and stress (Holly and Miczek, 2016). We evaluated the ACC dopamine release during PR responding task in which the mutants showed comparable PR breakpoints to the controls (Supplementary Figure S7D). Simultaneous in vivo microdialysis experiment (Supplementary Figure S5A) revealed a robust reinforcement-triggered dopamine release in ACC of these mutant mice (Figure 5e and Supplementary Figure S7C). We also assessed the ACC dopamine release induced by tail pinch for 1 min. This stressful and noxious stimulus also elicited the ACC dopamine release in the mutants (Figure 5f). Therefore, cortical GABA reduction-mediated dopamine release deficits appeared to occur in a particular circuitry in ACC by which effortful behavior triggers dopamine release, such as in the tail suspension.

**DISCUSSION**

We examined whether cortical GABA level reductions could precipitate psychiatric-like phenotypes in mice, in which Gad1 was genetically ablated in ~50% of cortical GABA neurons. The mutant mice grew normally but demonstrated a cluster of effort-based behavior deficits, including decreased home-cage wheel running and increased immobility in both tail suspension and forced swim tests. In vivo microdialysis from awake behaving animals revealed that tail suspension triggered the extracellular release of dopamine in ACC of the control mice. However, with increased immobility, the mutant mice showed a robust deficit in effort-triggered dopamine release in AAC. Interestingly, the mutants had normal release of dopamine in ACC during PR
responding, a classical reward reinforcement task. These results suggest a novel association from cortical GABA reduction to impaired high-effort cost behavior, but not to reward-seeking behavior, through ACC dopamine deficiency. These results also shed light on the role of cortical GABA reduction in a subset of negative symptom-like phenotypes of major psychiatric disorders.

Of particular significance in the present study is that cortical GABA reduction results in a deficit in effort-triggered dopamine release in the ACC during the tail suspension test. The ACC is known to be critically involved in effort-based decision making (Bailey et al, 2016; Kurniawan et al, 2011; Miller et al, 2013; Salamone et al, 2016). For example, lesions or inactivation of the ACC results avoidance of previously preferred high-effort options, leading to a higher likelihood of choosing low-effort options (Floresco and Ghods-Sharifi, 2007; Hosking et al, 2014; Rudebeck et al, 2006; Walton et al, 2003; Walton et al, 2009). In particular, ACC dopamine appears to modulate the willingness of animals to exert physical effort, because dopamine depletion via 6-hydroxydopamine lesions (Schweimer et al, 2005) (but see Walton et al, 2005) or dopamine receptor blockade (Aly-Mahmoud et al, 2017; Schweimer and Hauber, 2006; Wang et al, 2017) in ACC resulted in effort avoidance during cost-benefit decision making. Consistent with such previous reports, we found, in
Figure 4  Female mutants at 10–12 weeks of age, with negligible epileptiform activity, shared similar magnitude of behavioral deficits. (a) Representative example of LFPs from primary somatosensory (S1) cortex and hippocampal areas CA1 and CA3 of 10-week-old mutant male mouse while the animal was held immobile in a small chamber (10 cm in diameter). Mutant mice displayed interictal discharges which were synchronized across cortical and hippocampal electrodes, with the higher amplitudes in the ripple-carrying hippocampal electrodes. (b) The number of mono-spikes detected in S1 cortex of 10-week-old male mutant mice was higher than 10-week-old female mutant and 10-week-old control male mice during the 10-min immobility period (F(1,16) = 8.56, p = 0.0029). The number of poly-spikes in 10- and 15-week-old male mutant mice was also much higher than 10-week-old mutant female mice during 10-min immobility period (F(1,16) = 7.94, p = 0.004). Repeated measures ANOVA, **p < 0.01. (c) Female mutant mice spent less time in the light side of the light/dark chamber than did their female floxed-control mice (t(12) = 3.6, *p < 0.01). (d) Social interaction time with a novel mouse (Stranger) in the sociability test was significantly reduced in female mutant mice (post-hoc planned comparisons: F(1,12) = 6.2, *p < 0.05). (e) Female mutants showed increased immobility duration in tail suspension (t(16) = −4.01, *p = 0.001). (f) Female mutants showed increased immobility duration in forced swim chamber (t(17) = −2.76, *p = 0.013). (g) Female mutants differed from the female controls in home-cage wheel-running behavior (genotype × time: F(1,120) = 2.1, p < 0.05). (h) Female mutant mice were heavier than female controls at 13–15 weeks old (*p < 0.05), while they tended to weigh more already at 10–12 weeks of age (p = 0.07). Data are mean ± SEM; n is indicated in parentheses or plot bars.
control mice that tail hanging increases dopamine concentration in the ACC. In addition, there was an inverse correlation observed between the degree of immobility and ACC dopamine fold-increase. Notably, all the mutant mice tested exhibited high immobility scores in the tail suspension test, and showed little increase in ACC dopamine in the same test. These results suggested that the effort required to escape from tail hanging is associated with ACC dopamine increase. Since PR responding to food, a classic measure of effort-related motivation, and ACC dopamine release during this
task was both comparable to that in the control mice, ACC dopamine appears to not always be responsible for all types of cost-benefit decision making, as previously reviewed (Walton et al, 2007). Indeed, ACC dopamine receptor inactivation has recently been shown to impair a rat nose-poking task that requires large effort with less reward, but the similar nose-poke task that allowed the animal to effortlessly perform nose-poking to obtain reward was not impaired (Wang et al, 2017). A plausible idea to explain these results is that GABA reduction preferentially impairs the effort-based behavior which requires considerable effort with little benefit, through ACC dopamine release deficit in ACC. In other words, the ACC dopamine release mechanism would be disturbed by cortical GABA reduction when the release is triggered by effortful behavior with little reward, such as tail suspension. Since such low-benefit high-cost task is inherently stressful, it is plausible that the ACC dopamine release deficit by tail suspension could rather be due to altered stress sensitivity of the mutants. Actually, stress is known to increase in ACC dopamine release (Holly and Miczek, 2016). However, normal ACC dopamine release triggered by tail pinch (ie, aversive stressful stimulus) in the mutants might preclude this possibility. Conversely, despite GABA reduction, ACC dopamine release would be unaffected when triggered presumably by reward-prediction or reward-seeking in PR responding task. Accordingly, it is conceivable that multiple dopamine release mechanisms may exist in the ACC and that GABA reduction following Gad67 KO would impair the release mechanism triggered by high-effort cost behavior, but not by reward-seeking behavior. Further research is warranted to decipher the mechanism underlying GABA level-dependent cortical dopamine release.

In the present study, the impact of Gad1 ablation appeared after young adulthood, as evidenced by the development of epileptiform discharges in male mutants after 10 weeks of age which gradually increased with age. This slow development could be due to the compensatory increase in GAD65 protein, which is also reported in other Gad1 KO mouse models (Fujihara et al, 2015; Georgiev et al, 2016). These aberrant LFP wave-forms in the cortex could conceivably underlie the behavioral deficits seen in the mutants and this possibility cannot be totally discounted. However, this interpretation is not entirely parsimonious with the data as female mutants before 13 weeks of age did not show such epileptiform activity but still showed similar behavioral deficits as male mutant mice. It is puzzling that the female mutants appear to be more resilient to epileptiform activity. This mutant mouse line could be a useful model to study the differential role of sex hormones in epilepsy as testosterone has been suggested to exacerbate temporal lobe epilepsy (Mejias-Aponte et al, 2002).

A somewhat puzzling finding in this study was that the spatial Y-maze alternation task to assess spatial working memory was normal in mutants. GABAergic transmission deficits are often linked to cognitive deficits, including working memory (Tse et al, 2015). Association between GABA content in dorsolateral PFC and working memory load, but not its maintenance, has been demonstrated in human subjects (Yoon et al, 2016). The mPFC GABA_A receptor blockade with bicuculline has also been shown to cause an increase in choice latency in the eight-arm spatial working memory task; however, choice accuracy was normal (Enomoto et al, 2011). Perhaps, testing with such highly elaborate rodent cognitive tasks may uncover the mutant cognitive phenotype.

A limitation of the present study is that GABA reduction in other brain regions may also impact on the behavioral phenotypes, because GABA reduction is not limited to the ACC. For example, both orbitofrontal cortex and prelimbic cortex may differentially affect a part of the cost–benefit analysis process, in particular, reward-learning and reward-valuation processes, which allows motivation to energize behavior (Bailey et al, 2016; Barch et al, 2016). Indeed, prelimbic cortex lesion in mice has been shown to decrease the breakpoints in the PR schedule (Gourley et al, 2010). Although normal PR responding by our mutants suggests that involvement of prelimbic GABA reduction in effort avoidance is unlikely, further studies are warranted to determine the role of GABA in each brain area involved in effort-based behavior.

Our results may have clinical implications in the elucidation of the role of reduced GABA levels in neuropsychiatric disorders, particularly a subset of negative symptoms. Although lower cortical GABA levels have been reported in several neuropsychiatric disorders, including schizophrenia and depression (Egerton et al, 2017; Schur et al, 2016), it remains to be determined whether cortical GABA reduction produces common deficits across the current diagnostic categories or whether it is uniquely associated with different facets of each disorder. Since the reduced effort allocation has also been reported in both schizophrenia and depression (Barch et al, 2016; Green et al, 2015; Strauss et al, 2014), it is tempting to predict that cortical GABA reduction in schizophrenia and depression produces common deficits in effort-based behavior through the decreased GABA levels in the ACC. In addition, in our mutant mice there were also changes in the circadian feeding pattern, which may be analogous to the night time eating syndrome sometimes observed in major depression (Gallant et al, 2012) and insomnia (Plante et al, 2012). Thus, these phenotypic domains elicited by lowered GABA levels are reminiscent of a subset of negative symptoms observed in human psychiatric disorders, which are not pathognomonic of schizophrenia but also appears frequently in depression.

FUNDING AND DISCLOSURE

This work was supported by the Intramural Research Program at the National Institute of Mental Health, National Institutes of Health (ZIA MH002895) and was funded in part by grants from the National Institute of Mental Health K22 MH099164 and R01 MH110681 (to K Nakazawa). The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Richard D. Palmeter for providing the floxed-Gad1 mouse strain.

AUTHOR CONTRIBUTIONS

SMK, KNakao and KNakazawa were responsible for study design. SMK conducted immunocytochemistry and western
blots. YF and KH performed amino acid measurement by HPLC. VJ, AFB, and LED conducted electrophysiological experiments in slice and analyzed the data. KNakao and YM were responsible for in vivo microdialysis experiments. KNakao performed in vivo LFP recording in mice. SMK, KNakao, ELF-A, ZJ, GRR, DVJ, KM and KNakazawa performed behavioral characterization and analyzed the data. RES contributed to reinforcement learning test. KNakazawa, SMK and KNakao wrote the manuscript.

REFERENCES

Aly-Mahmoud M, Carlier P, Salam SA, Hourai Selmani M, Moftaf MZ, Esclapez M et al (2017). Role of anterior cingulate cortex in confers schizophrenia-like phenotypes. *Nat Neurosci* 13: 76–83.

Benes FM, Berretta S (2001). GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology* 25: 1–27.

Braff DL, Geyer MA, Swerdlow NR (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berlin)* 156: 234–258.

Brown JA, Ramikie TS, Schmidt MJ, Baldi R, Garbett K, Everheart MG et al (2015). Inhibition of parvalbumin-expressing interneurons results in complex behavioral changes. *Mol Psychiatry* 20: 1499–1507.

Chattopadhyaya B, Di Cristo G, Wu CZ, Knott G, Kuhlman S, Fu Y et al (2007). GAD67-mediated GABA synthesis and signaling regulates inhibitory synaptic innervation in the visual cortex. *Neuron* 54: 889–903.

Egerton A, Modinos G, Ferrera D, McGuire P (2017). Neuroimaging studies of GABA in schizophrenia: a systematic review with meta-analysis. *Transl Psychiatry* 7: e1147.

Enomoto T, Tse MT, Floresco SB (2011). Reducing prefrontal gamma-aminobutyric acid activity induces cognitive, behavioral, and dopaminergic abnormalities that resemble schizophrenia. *Biol Psychiatry* 69: 432–441.

Floresco SB, Ghods-Sharif S (2007). Amygdala-prefrontal cortical circuitry regulates effort-based decision making. *Cereb Cortex* 17: 251–260.

Fujihara K, Miwa H, Kakizaki T, Kaneko R, Mikuni M, Tanahira C et al (2015). Glutamate decarboxylase 67 deficiency in a subset of GABAergic neurons induces schizophrenia-related phenotypes. *Neuropsychopharmacology* 40: 2475–2486.

Fujita Y, Ishima T, Hashimoto K (2016). Supplementation with D-serine prevents the onset of cognitive deficits in adult offspring after maternal immune activation. *Sci Rep* 6: 37261.

Gallant AR, Lundgren J, Drapeau V (2012). The night-eating syndrome and obesity, *Obes Rev* 13: 528–536.

Georgiev D, Yoshiterra T, Kawabata R, Matsubara T, Tsubomoto M, Minabe Y et al (2016). Cortical gene expression after a conditional knockout of 67 kDa glutamic acid decarboxylase in parvalbumin neurons. *Schizophr Bull* 42: 992–1002.

Gourley SL, Lee AS, Howell JL, Pittenger C, Taylor JR (2010). Dissociable regulation of instrumental action within mouse prefrontal cortex. *Eur J Neurosci* 32: 1726–1734.

Green MF, Horan WP, Barch DM, Gold JM (2015). Effort-based decision making: a novel approach for assessing motivation in schizophrenia. *Schizophr Bull* 41: 1035–1044.

Hashimoto T, Volk DW, Eggan SM, Mirnics K, Pierri JN, Sun Z et al (2003). Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J Neurosci* 23: 6315–6326.

Holly EN, Miczek KA (2016). Ventral tegmental area dopamine revisited: effects of acute and repeated stress. *Psychopharmacology (Berlin)* 233: 163–186.

Hosking JG, Cocker PJ, Winstanley CA (2014). Dissociable contributions of anterior cingulate cortex and basolateral amygdala on a rodent cost/benefit decision-making task of cognitive effort. *Neuropsychopharmacology* 39: 1558–1567.

Jinde S, Belforte JE, Yamamoto J, Wilson MA, Tonegawa S, Nakazawa K (2009). Lack of kainic acid-induced gamma oscillations predicts subsequent CA1 excitotoxic cell death. *Eur J Neurosci* 30: 1036–1055.

Karakuyu D, Herold C, Gunturkun O, Diekamp B (2007). Differential increase of extracellular dopamine and serotonin in the ‘prefrontal cortex’ and striatum of pigeons during working memory. *Eur J Neurosci* 26: 2293–2302.

Kurniawan JT, Guitart-Masip M, Dolan RJ (2011). Dopamine and effort-based decision making. *Front Neurosci* 5: 81. https://doi.org/10.3389/fnins.2011.00081.

Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R (1999). Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol Psychiatry* 46: 56–72.

Lewis DA, Hashimoto T, Volk DW (2005). Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci* 6: 312–324.

Li Q, Bartley AF, Dobrunz LE (2017). Endogenously released neuropeptide Y suppresses hippocampal short-term facilitation and is impaired by stress-induced anxiety. *J Neurosci* 37: 23–37.

Mantz J, Thierry AM, Glowinski J (1989). Effect of noxious tail pinch on the discharge rate of mesocortical and mesolimbic dopamine neurons: selective activation of the mesocortical system. *Brain Res* 476: 377–381.

Mejias-Aponte CA, Jimenez-Rivera CA, Segarra AC (2002). Sex differences in models of temporal lobe epilepsy: role of testosterone. *Brain Research* 944: 210–218.

Meynelt F, Goodwin GM, Deakin JW, Klinge C, MacFadyen C, Milligan H et al (2016). A specific role for serotonin in overcoming effort cost. *Elife* 5: pii: e17282; https://doi.org/10.7554/eLife.17282.

Miller MA, Thome A, Cowen SL (2013). Intersection of effort and risk: ethological and neurobiological perspectives. *Front Neurosci* 7: 208. https://doi.org/10.3389/fnins.2013.00208.

Nadler JJ, Muy SS, Dold G, Trang D, Simmons N, Perez A et al (2004). Automated apparatus for quantification of social approach behaviors in mice. *Genes Brain Behav* 3: 303–314.

Nakazawa K, Zsirös V, Jiang Z, Nakao K, Kolata S, Zhang S et al (2012). GABAergic interneuron origin of schizophrenia pathophysiology. *Neuropsychopharmacology* 62: 1574–1583.

Paine TA, O’Harra A, Plaut B, Lowes DC (2015). Effects of disrupting medial prefrontal cortex GABA transmission on decision-making in a rodent gambling task. *Psychopharmacology (Berlin)* 232: 1755–1765.

Piantadosi PT, Khayambashi S, Schluter MG, Kutarna A, Floresco SB (2016). Perturbations in reward-related decision-making induced by reduced prefrontal cortical GABA transmission: Relevance for psychiatric disorders. *Neuropsychopharmacology* 101: 279–290.

Pignatelli M, Bonci A (2015). Role of dopamine neurons in reward and aversion: a synaptic plasticity perspective. *Neuron* 86: 1145–1157.
Plante DT, Jensen JE, Schoerning L, Winkelmann JW (2012). Reduced gamma-aminobutyric acid in occipital and anterior cingulate cortices in primary insomnia: a link to major depressive disorder. Neuropsychopharmacology 37: 1548–1557.

Richardson NR, Roberts DC (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods 66: 1–11.

Rudebeck PH, Walton ME, Smyth AN, Bannerman DM, Rushworth MF (2006). Separate neural pathways process different decision costs. Nat Neurosci 9: 1161–1168.

Salamone JD, Yohn SE, Lopez-Cruz L, San Miguel N, Correa M (2016). Activity-dependent modulation of local neurotransmission and conditioned place aversion. Science 353(6305): 1312–1315.

Schur RR, Draisma LW, Wijnen JP, Boks MP, Koevoets MG, Joels M et al. (2016). Brain GABA levels across psychiatric disorders: A systematic literature review and meta-analysis of (1) H-MRS studies. Hum Brain Mapp 37: 3337–3352.

Schweimer J, Hauber W (2006). Dopamine D1 receptors in the anterior cingulate cortex regulate effort-based decision making. Learn Mem 13: 777–782.

Schweimer J, Saß S, Hauber W (2005). Involvement of catecholamine neurotransmission in the rat anterior cingulate in effort-related decision making. Behav Neurosci 119: 1687–1692.

Shirayama Y, Chen A. C.-H., Nakagawa S, Russell DS, Duman RS (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 22: 3251–3261.

Stefani MR, Moghaddam B (2006). Rule learning and reward contingency are associated with dissociable patterns of dopamine activation in the rat prefrontal cortex, nucleus accumbens, and dorsal striatum. J Neurosci 26: 8810–8818.

Strauss GP, Waltz JA, Gold JM (2014). A review of reward processing and motivational impairment in schizophrenia. Schizophr Bull 40(Suppl 2): S107–S116.

Tejeda HA, Counotte DS, Oh E, Ramamoorthy S, Schultz-Kuszak KN, Backman CM et al. (2013). Prefrontal cortical kappa-opioid receptor modulation of local neurotransmission and conditioned place aversion. Neuropsychopharmacology 38: 1770–1779.

Tse MT, Piantadosi PT, Floresco SB (2015). Prefrontal cortical gamma-aminobutyric acid transmission and cognitive function: drawing links to schizophrenia from preclinical research. Biol Psychiatry 77: 929–939.

van den Buuse M (2010). Modeling the positive symptoms of schizophrenia in genetically modified mice: pharmacology and methodology aspects. Schizophr Bull 36: 246–270.

Walton ME, Bannerman DM, Altersescu K, Rushworth MF (2003). Functional specialization within medial frontal cortex of the anterior cingulate for evaluating effort-related decisions. J Neurosci 23: 6475–6479.

Walton ME, Croxson PL, Rushworth MF, Bannerman DM (2005). The mesocortical dopamine projection to anterior cingulate cortex plays no role in guiding effort-related decisions. Behav Neurosci 119: 323–328.

Walton ME, Groves J, Jennings KA, Croxson PL, Sharp T, Rushworth MF et al. (2009). Comparing the role of the anterior cingulate cortex and 6-hydroxydopamine nucleus accumbens lesions on operant effort-based decision making. Eur J Neurosci 29: 1678–1691.

Walton ME, Rudebeck PH, Bannerman DM, Rushworth MF (2007). Calculating the cost of acting in frontal cortex. Ann NY Acad Sci 1104: 340–356.

Wang S, Hu SH, Shi Y, Li BM (2017). The roles of the anterior cingulate cortex and its dopamine receptors in self-paced cost-benefit decision making in rats. Learn Behav 45: 89–99.

Yoon JH, Grandelis A, Maddock RJ (2016). Dorsolateral prefrontal cortex GABA concentration in humans predicts working memory load processing capacity. J Neurosci 36: 11788–11794.

© The Author(s) 2018

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)