http://dx.doi.org/10.5607/en.2015.24.2.126
Exp Neurobiol. 2015 Jun;24(2):126-132.
pISSN 1226-2560 • eISSN 2093-8144

Original Article

Experimental Neurobiology

Aberrant Thalamocortical Synchrony Associated with Behavioral Manifestations in *Git1*$^{-/-}$ Mice

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Cross-talk between the thalamus and cortex has been implicated in attention but its pathogenic role in attention-deficit/hyperactivity disorder (ADHD) remains unknown. Here, I demonstrate that *Git1*$^{-/-}$ mice, previously proposed as an animal model for ADHD, show abnormal theta oscillation in the thalamus. Multi-electrode recordings revealed that *Git1*$^{-/-}$ mice have hyper-synchrony of neural activities between the thalamus and cortex. The abnormal thalamic oscillation and thalamocortical synchrony in *Git1*$^{-/-}$ mice were markedly reduced by amphetamine. In addition, ethosuximide ameliorates abnormal thalamic oscillation and ADHD-like hyperactivity shown in *Git1*$^{-/-}$ mice. My study suggests critical roles of GIT1 and thalamocortical neural circuitry in ADHD.

Key words: ADHD, GIT1, Thalamic oscillation, Coherence

INTRODUCTION

Among overwhelming amount of sensory signals from all sensory organs, brain selectively processes relevant sensory information at the expense of others. This process of allocating limited processing resources to relevant information is referred to as attention, which has been the focus of various studies because of its importance in human cognition. Thalamus is a brain region that relays sensory and motor information to the cortex and regulates cognitive processes including consciousness, attention, wakefulness, and sleep [1-7]. Cross-talk between the thalamus and the cortex has been implicated in attention since all sensory information is relayed to the cortex via thalamus [8].

Attention-deficit/hyperactivity disorder (ADHD) is a prevalent psychiatric disorder that affects 5~10% of school-age children worldwide and frequently persists into adulthood [9]. Because of its high prevalence and lifelong impairment, massive researches have been conducted to delineate neural correlates of the disorder. Though several lines of evidences imply the association between abnormalities in thalamus and ADHD [10-12], the pathogenic role of the thalamus in ADHD is still elusive.

GIT1 (G protein-coupled receptor kinase-interacting protein-1) is a multifunctional signaling adaptor associated with ADHD [13]. *Git1*$^{-/-}$ mice show ADHD-like hyperactivity and abnormal theta oscillations in prefrontal cortex (PFC) that are also detected in ADHD-affected individuals [14]. Unlike other animal models showing dopamine deficiency, however, *Git1*$^{-/-}$ mice show normal level of tyrosine hydroxylase which is critical for dopamine synthesis [13]. This implies that there might be other mechanism(s) responsible for the ADHD-like phenotypes shown in *Git1*$^{-/-}$ mice.

Here, I report that the thalamus of *Git1*$^{-/-}$ mice exhibits abnormally enhanced 3-Hz oscillations. These abnormal thalamic oscillations were observed with enhanced thalamocortical synchrony, both of which were ameliorated by amphetamine. Ethosuximide,

Received February 11, 2015, Revised March 2, 2015,
Accepted March 2, 2015

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an antiepileptic medication that modulates thalamic discharges by blocking T-type calcium channels, normalized 3-Hz thalamic rhythms, cortical theta rhythms, and behavioral hyperactivity in these mice. My results implicate enhanced 3-Hz thalamic rhythms in the development and treatment of ADHD-like phenotypes in *Git1*$^{-/-}$ mice, and suggest a novel therapeutic potential of ethosuximide in ADHD.

MATERIALS AND METHODS

Git1$^{-/-}$ *mice*

Git1$^{-/-}$ mice have been generated as described previously [13]. Mice at 2~4 months of age were used for all behavioral assays and LFP/EEG recordings. Experiments were done in accordance with the guidelines of the Animal Welfare Committee of KAIST, Korea.

Drug treatment

Amphetamine (U.S. Pharmacopia) and ethosuximide (Tokyo Chemical Industry) were dissolved in 0.9% saline and distilled water to final concentrations of 1.2 g/L and 60 g/L, respectively. Solutions for injection were filtered with Minisart filter (0.2 μm; Sartorius Stedim Biotech). WT and *Git1*$^{-/-}$ mice received intraperitoneal injection of amphetamine (4 mg/kg), ethosuximide (200 mg/kg), or the same volume of saline.

Electrode implantation and LFP recordings

Mice were anesthetized by ketamine (Yuhan Corporation). For depth recording, a parylene-coated tungsten electrode (0.005 in, 2 MΩ, A-M Systems, Inc.) was implanted into the ventrobasal or mediodorsal nucleus of the thalamus with grounding electrodes over the cerebellum. LFP recordings were performed 1 week after the implantation. LFP activities (sampled at 200 Hz) of freely moving mice were recorded for 1 h using the NACGather program (Theta Burst Corp.). Amphetamine was delivered intraperitoneally to mice 20 min after basal LFP recording. LFP activity was recorded for 1 h briefly after the injection. LFP recordings were analyzed by Matlab, using EEGLAB and custom-written coding.

Open field test

The size of the open field box was 40×40×40 cm, and the center zone line was located 6.5 cm apart from the edge. Mice were placed in the center of the chamber in the beginning of the assay, and spontaneous locomotion activity was observed and recorded for 60 min in an open field chamber, briefly after injection. The results were analyzed by Ethovision 3.1 program (Noldus). The total distance moved was obtained by summing the movements made during 10~60 min. All behavioral assays were performed in a blind manner.

Object recognition test

The apparatus used in the open field tests was used for object recognition tests. During the sample phase, mice were allowed to explore two identical objects for 10 min. Objects were put in the center of the chamber, and mice were first put in the chamber facing the wall. Exploration time for each object was measured. In the test phase, performed 24 h later, one of the two objects was replaced with a new one, and exploration time for each object was measured. All objects were pre-tested to confirm that there was no difference in object preference using C57BL/6 wild-type mice and C57BL/6-129/SV/Jae hybrid wild-type mice.

RESULTS

Enhanced 3-Hz rhythms in the Git1$^{-/-}$ *thalamus and their normalization by amphetamine*

As previous studies have identified close connection between ventrobasal thalamus and prefrontal cortex during attention task [15, 16], I hypothesized that abnormal theta oscillation in the PFC of *Git1*$^{-/-}$ mice might be associated with the abnormal activity of the ventrobasal thalamus.

Thus, I examined the functional properties of the ventrobasal thalamus of *Git1*$^{-/-}$ mice by measuring local field potentials (LFPs). Notably, power spectral density analysis of LFP revealed that 3-Hz rhythms were increased and 7~10-Hz rhythms were decreased in *Git1*$^{-/-}$ mice (Fig. 1A and B), whereas the high-frequency rhythms (>10 Hz) were unaffected (data not shown). I speculated that these enhanced thalamic 3-Hz rhythms may be associated with the ADHD-like phenotypes observed in *Git1*$^{-/-}$ mice [13]. Therefore, I tested whether amphetamine, which has been shown to rescue EEG theta rhythms and hyperactivity in *Git1*$^{-/-}$ mice [13], could affect these thalamic rhythms. Indeed, I found that amphetamine normalized 3-Hz rhythms but not the 7~10-Hz rhythms in *Git1*$^{-/-}$ mice (Fig. 1C~E). In contrast, amphetamine treatment caused a marked increase in 3-Hz rhythms in wild-type mice (Fig. 1C, D and F). These changes, which are analogous to the opposing effects of amphetamine on hyperactivity in *Git1*$^{-/-}$ mice (suppression) and wild-type mice (induction) [13], suggest that 3-Hz thalamic rhythms may be associated with the development of abnormally enhanced theta EEG rhythms and hyperactivity in *Git1*$^{-/-}$ mice.

Enhanced thalamocortical synchrony of abnormal rhythms in Git1$^{-/-}$ *mice*

Abnormal theta rhythms in the human cortex are thought to be coupled with those in the thalamus through the thalamocortical

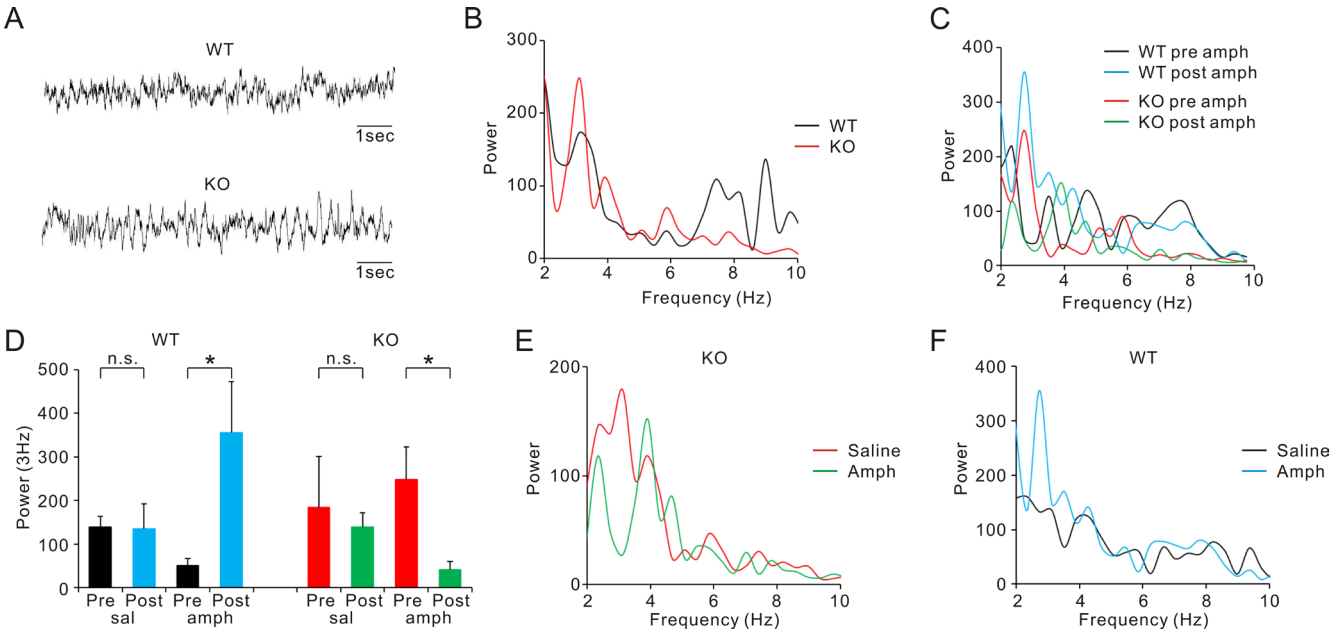

Fig. 1. Enhanced 3-Hz thalamic rhythms in *Git1*$^{-/-}$ mice are normalized by amphetamine. (A and B) Local field potential (LFP) in the 3-Hz frequency range is enhanced in the ventrobasal thalamus of *Git1*$^{-/-}$ mice, while LFP in 7~10 Hz range is decreased, as shown by the power spectral density (30~40 min) (B). Representative traces are shown in (A). n=5 (WT), 4 (KO). (C and D) Amphetamine (amph) rescues enhanced 3-Hz thalamic rhythms in *Git1*$^{-/-}$ mice, but enhances these rhythms in wild-type mice. n=7 (WT), 6 (KO). (E) Wild-type mice exhibit increased 3-Hz thalamic oscillations upon amphetamine treatment, while (F) enhanced thalamic oscillation in 3-Hz range of *Git1*$^{-/-}$ mice are alleviated by the same drug. n=7 (WT), 6 (KO). *$p < 0.05$, n.s., not significant; Student's t-test.

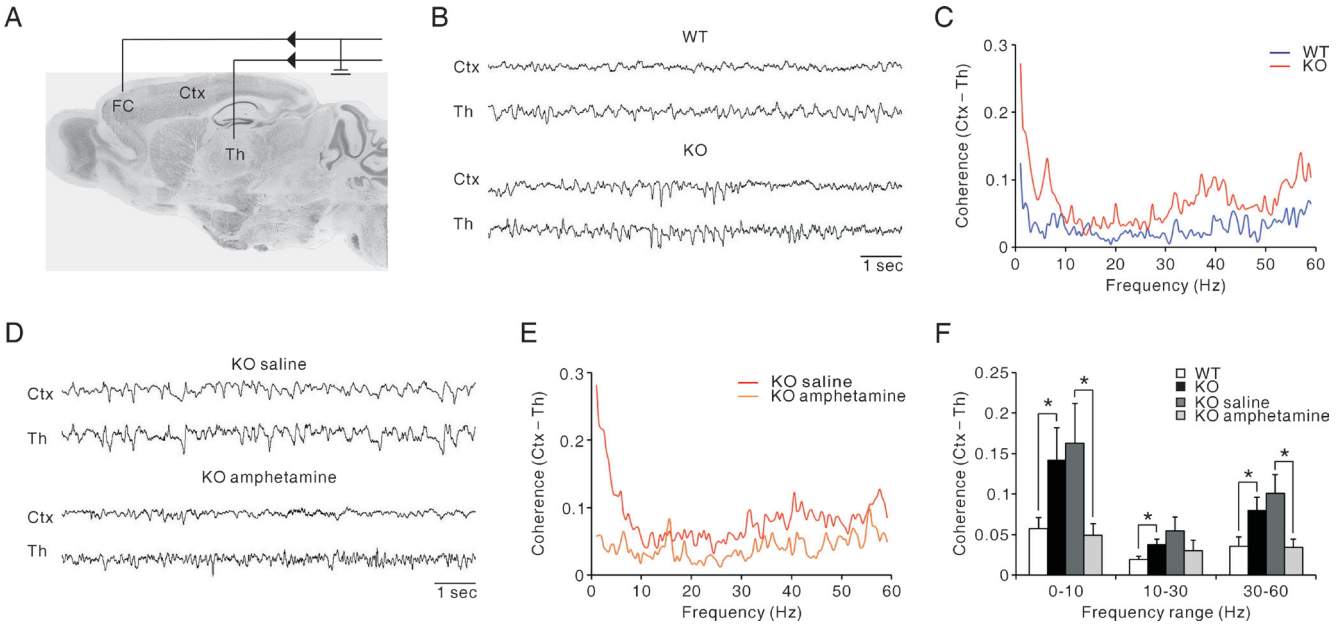

Fig. 2. Enhanced coherence between the *Git1*$^{-/-}$ thalamus and cortex in theta- and gamma-ranges is reduced by amphetamine. (A) Neuronal activities in the frontal cortex (FC) and mediodorsal nuclei of the thalamus (Th) were simultaneously recorded. Ctx, cortex. (B and C) Enhanced coherence between *Git1*$^{-/-}$ thalamus and cortex in theta and gamma ranges, as shown by representative traces (B) and coherence plot (C; 10~20 min). (D to F) Suppression of enhanced thalamocortical coherence in *Git1*$^{-/-}$ mice by amphetamine, as quantified in three frequency ranges (3~10, 10~30, and 30~60 Hz). n=4 (WT and KO). *$p < 0.05$, Student's t-test.

http://dx.doi.org/10.5607/en.2015.24.2.126

pathway, a process that has been termed thalamocortical dysrhythmia (TCD) [17]. To this end, I simultaneously measured cortical EEG rhythms and thalamic activities (LFP) in WT and *Git1*$^{-/-}$ mice (Fig. 2A). Indeed, enhanced theta rhythm was observed in the mediodorsal nucleus of thalamus in addition to the cortex (Fig. 2B), and theta rhythms in these two regions were highly synchronized (Fig. 2C). Enhanced coherence was also observed in the gamma range (30~60 Hz), a brain rhythm

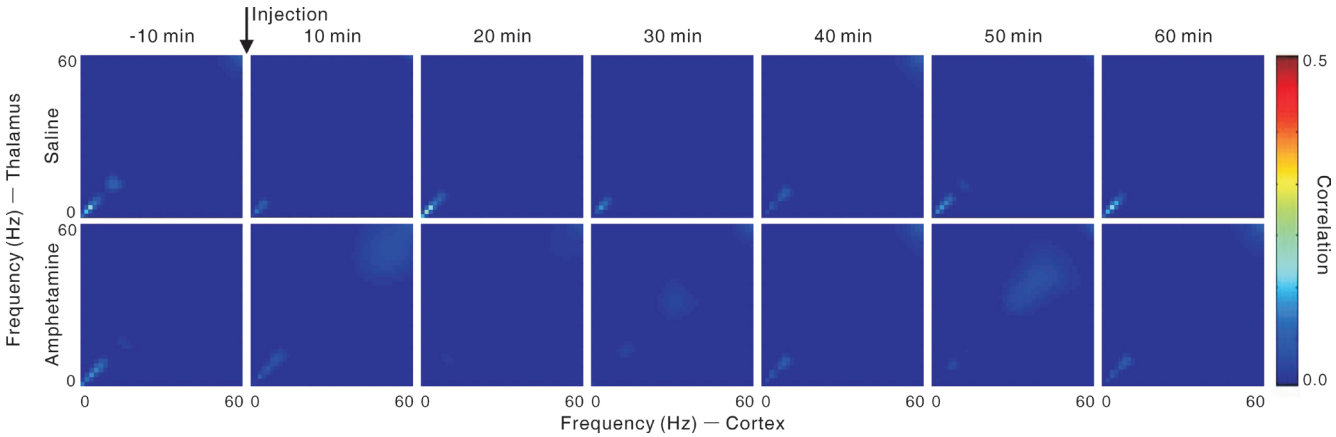

Fig. 3. Amphetamine-induced reduction in the thalamocortical coherence begins to weaken in *Git1*$^{-/-}$ mice ~40 min after amphetamine treatment, as indicated by time-dependent changes in the correlation between cortical EEG and thalamic LFP. The diagonal lines in the bottom left corner of the panels indicate high iso-frequency correlation in the theta range that is sensitive to amphetamine treatment.

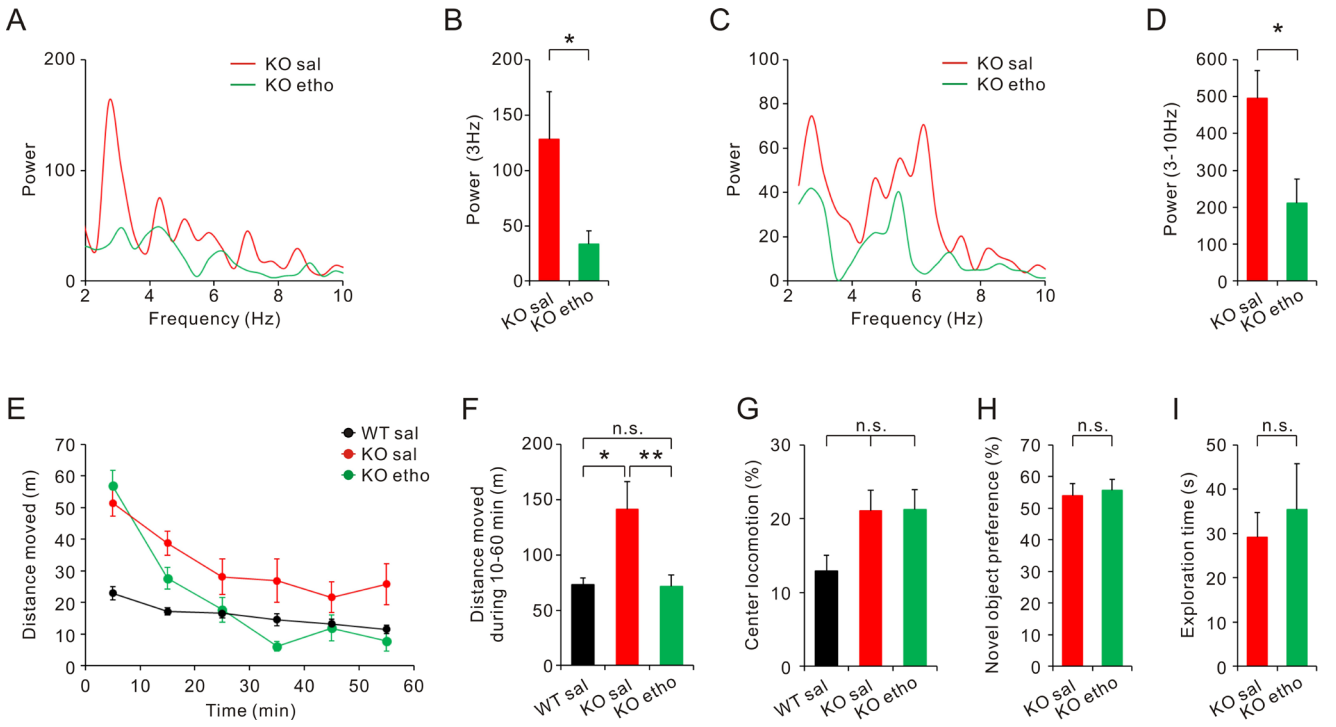

Fig. 4. Ethosuximide normalizes enhanced 3-Hz thalamic oscillations, enhanced cortical theta EEG rhythms, and hyperactivity in *Git1*$^{-/-}$ mice. (A and B) Ethosuximide (etho) but not saline (sal) normalizes enhanced 3-Hz thalamic rhythms in *Git1*$^{-/-}$ mice. n=4 (KO sal), 4 (KO etho). *$p < 0.05$; Student's t-test. (C and D) Ethosuximide reduces cortical theta EEG rhythms in *Git1*$^{-/-}$ mice. (E~G) Ethosuximide normalizes hyperactivity in *Git1*$^{-/-}$ mice in an open field arena, without affecting the time spent in the center region. n=8 (WT sal), 8 (KO sal), 10 (KO etho). *$p < 0.05$, **$p < 0.01$, n.s., not significant; one-way ANOVA. (H and I) Ethosuximide does not rescue impaired recognition memory in *Git1*$^{-/-}$ mice. Saline-treated *Git1*$^{-/-}$ mice (KO sal) and ethosuximide-treated *Git1*$^{-/-}$ mice (KO etho) spent comparable amounts of time exploring two identical objects in the sample phase of the object recognition test (I). n=9 (KO sal), 6 (KO etho). n.s., not significant; Student's t-test.

implicated in attention and memory [18], similar to observations in human TCD [17].

Increased coherence between the *Git1*$^{-/-}$ cortex and thalamus in the theta and gamma ranges was markedly reduced by amphetamine (not saline) treatment (Fig. 2D~F). These results collectively suggest that the amphetamine-induced reduction in cortical theta rhythms is associated with the amphetamine-mediated reduction of thalamo-cortical coherent activities. Amphetamine-induced reduction in thalamocortical coherence in *Git1*$^{-/-}$ mice began to weaken at ~40 min after amphetamine treatment (Fig. 3).

Ethosuximide normalizes 3-Hz thalamic rhythms, cortical theta EEG rhythms, and hyperactivity in Git1*$^{-/-}$ *mice

Inhibitory inputs into the thalamus facilitate hyperpolarization of thalamic neurons, which is required for the recovery of T-type calcium channels from inactivation and low-threshold spikes generation. The latter is a neuronal burst activity closely related to thalamic rhythms that are conveyed to other brain regions, including cortex [19]. Ethosuximide, which is mainly used as an antiepileptic agent, is a well-known T-type calcium channel blocker and suppressant of thalamic bursts [20]. Here, I tested whether ethosuximide could suppress 3-Hz rhythms in the *Git1*$^{-/-}$ thalamus.

Similar to the effects of amphetamine, ethosuximide normalized 3-Hz thalamic rhythms in *Git1*$^{-/-}$ mice (Fig. 4A and B). In addition, it normalized cortical EEG rhythms in the theta range (3~10 Hz) (Fig. 4C and D). Behaviorally, ethosuximide treatment of *Git1*$^{-/-}$ mice rescued hyperactivity in the open-field assay (Fig. 4E and F), but had no effect on the time spent in the center region, which is a measure of anxiety-like behavior (Fig. 4G). These results, together with the amphetamine-mediated rescue of aberrant 3-Hz rhythms, cortical theta EEG rhythms, and hyperactivity [13], strongly suggest that enhanced 3-Hz thalamic rhythms are associated with ADHD-like cortical theta rhythms and hyperactivity in *Git1*$^{-/-}$ mice.

Unlike the reported rescue of novel-object recognition memory in *Git1*$^{-/-}$ mice by amphetamine [13], ethosuximide did not normalize recognition memory in the novel-object-recognition test (Fig. 4H and I). This result implies that the suppression of enhanced 3-Hz thalamic rhythms is not sufficient to normalize impaired recognition memory in *Git1*$^{-/-}$ mice.

DISCUSSION

I herein provide evidence suggesting that enhanced 3-Hz rhythms in the thalamus may contribute to enhanced cortical theta EEG rhythms and hyperactivity in *Git1*$^{-/-}$ mice. In line with this hypothesis, amphetamine, which normalizes cortical theta EEG rhythms and hyperactivity in *Git1*$^{-/-}$ mice, was found to suppress the enhanced 3-Hz thalamic rhythms in these mice. Moreover, amphetamine increases 3-Hz thalamic rhythms in wild-type mice, analogous to its ability to induce hyperactivity in these mice [13]. Lastly, ethosuximide, a blocker of T-type calcium channels and thalamic burst outputs [20-22], normalizes 3-Hz thalamic rhythms, cortical theta EEG rhythms, and hyperactivity in *Git1*$^{-/-}$ mice. Collectively, these results strongly suggest that enhanced 3-Hz thalamic rhythms might be neural correlates of ADHD-relevant phenotypes in *Git1*$^{-/-}$ mice.

The 3-Hz rhythms in the *Git1*$^{-/-}$ thalamus are similar in frequency to the spike-wave discharges generated during epilepsy [20]. However, *Git1*$^{-/-}$ mice do not exhibit epileptogenic activities in both thalamic LFP and cortical EEG readings, and show no sign of convulsion. Moreover, the 3-Hz thalamic rhythms were measured in moving *Git1*$^{-/-}$ mice, ruling out the possibility of absence seizure.

The circuit-level mechanism for enhanced 3-Hz rhythms in the thalamus of *Git1*$^{-/-}$ mice would be an interesting topic to address in the future. Given that T-type calcium channels are activated by neuronal hyperpolarization, one possibility is that increased inhibition of thalamic neurons and subsequent deinactivation of T-type calcium channels may contribute to the enhanced 3-Hz thalamic rhythms. Thalamus is known to receive inhibitory inputs directly from the thalamic reticular nucleus (TRN) [23]. This input is thought to contribute to the generation of low-frequency brain rhythms including sleep spindles and spike-and-wave discharges [23-25].

Another source of inhibitory inputs to the thalamus comes from endopeduncular nucleus (analogous to the internal globus pallidus in primates), which is negatively regulated by the globus pallidus directly or indirectly via subthalamic nucleus [26, 27]. Inhibitory projections from the endopeduncular nucleus to the thalamus significantly influence firing rates and rhythmic activities of the ventrolateral and intralaminar thalamic nuclei [26]. Globus pallidus also modulates thalamus via its direct and inhibitory modulation of TRN neurons [28]. Amphetamine may indirectly regulate GABAergic neurons, as psychostimulants suppress the stimulatory action of the locus coeruleus onto the TRN [29].

Finally, I found that ethosuximide failed to restore novel object recognition while correcting hyperactivity in *Git1*$^{-/-}$ mice. This contrasts with the amphetamine-dependent normalization of both hyperactivity and novel object recognition in this mouse model [13], suggesting that the hyperactivity and learning/memory phenotypes of *Git1*$^{-/-}$ mice may arise via different mechanisms.

http://dx.doi.org/10.5607/en.2015.24.2.126

In summary, my data indicate that 3-Hz thalamic rhythms are associated with cortical theta EEG rhythms and hyperactivity in *Git1*$^{-/-}$ mice, as well as their amphetamine-mediated recovery. My data also suggest that ethosuximide has a novel therapeutic potential in the treatment of ADHD. Future studies are needed to explore direct associations among thalamic 3-Hz rhythms, cortical theta EEG rhythms, and behavioral hyperactivity in *Git1*$^{-/-}$ mice.

ACKNOWLEDGEMENTS

This research was supported by Kyungpook National University Research Fund, 2013.

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