Medial septal GABAergic projection neurons promote object exploration behavior and type 2 theta rhythm

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Contributed by Hee-Sup Shin, April 20, 2016 (sent for review February 16, 2016; reviewed by Jan Born, György Buzsáki, and Alcino J. Silva)

Exploratory drive is one of the most fundamental emotions, of all organisms, that are evoked by novelty stimulation. Exploratory behavior plays a fundamental role in motivation, learning, and well-being of organisms. Diverse exploratory behaviors have been described, although their heterogeneity is not certain because of the lack of solid experimental evidence for their distinction. Here we present results demonstrating that different neural mechanisms underlie different exploratory behaviors. Localized $Ca_v3.1$ knockdown in the medial septum (MS) selectively enhanced object exploration, whereas the null mutant (KO) mice showed enhanced-object exploration as well as open-field exploration. In MS knockdown mice, only type 2 hippocampal theta rhythm was enhanced, whereas both type 1 and type 2 theta rhythm were enhanced in KO mice. This selective effect was accompanied by markedly increased excitability of septo-hippocampal GABAergic projection neurons in the MS lacking T-type Ca^{2+} channels. Furthermore, optogenetic activation of the septo-hippocampal GABAergic pathway in WT mice also selectively enhanced object exploration behavior and type 2 theta rhythm, whereas inhibition of the same pathway decreased the behavior and the rhythm. These findings define object exploration distinguished from open-field exploration and reveal a critical role of T-type Ca^{2+} channels in the medial septal GABAergic projection neurons in this behavior.

$Ca_v3.1$ T-type $Ca2^+$ channel | exploratory behaviors | hippocampal theta rhythm | medial septum | septo-hippocampal GABAergic neurons

When confronted with an unfamiliar environment, or physical or social objects, animals often exhibit behavior patterns that can broadly be termed exploration, such as moving around the environment, touching or sniffing novel objects, and interacting with social stimuli (1). Social exploration involves complex processes that differ from those involved in the nonsocial exploration (2). Several distinctions were proposed to categorize the different forms of nonsocial exploratory behaviors from a motivational perspective (3). Behaviorally, two types of nonsocial exploration are observed in rodents and humans (3–5): object exploration and spatial or environmental exploration in the absence of objects. Object exploration is the behavior to explore discrete novel objects. This activity is elicited and sustained by the physical presence of an object. Several types of preference or "novelty" tests have been developed to investigate object exploration in rodents (3, 5–7). Environmental or spatial exploration in the absence of objects refers to the inquisitive activity of an animal in a new space, where the eliciting and sustaining stimulus is the "place" itself. Various forms of open-field tests have been used to investigate environmental or spatial exploration in rodents (3, 5, 8). Experimentally, however, the distinction can be less obvious because both can occur together (4, 7–9). Spatial exploration is suggested to be hippocampal-dependent (10)—although that is controversial (11)—whereas object exploration is suggested to be hippocampal-independent (12). Thus, it is still a matter of debate whether animal exploration belongs to a unitary category or not (9). To resolve this issue, neural definitions of these two previously proposed exploratory behaviors are needed.

Interestingly, the medial septum (MS), where $Ca_v3.1$ T-type Ca^{2+} channels are highly expressed (13), is suggested to be critical for exploratory behaviors (5, 14–16). Moreover, the MS is also the nodal point for ascending afferent systems involved in the generation of hippocampal theta rhythms, the largest synchronous oscillatory signals in the mammalian brain, which are implicated in diverse brain functions (17, 18). Although the heterogeneity of hippocampal theta rhythms has long been under debate (19), recent studies based on genetic mutations in mice and optogenetics provide strong support for theta rhythm heterogeneity (20–22). However, their exact behavioral correlates are still debated. $Ca_v3.1$ Ca^{2+} channels play an important role in diverse behaviors, as well as the generation of physiologic and pathophysiologic brain rhythms (23). Notably, T-type, low-threshold Ca^{2+} currents are assumed to be a candidate ionic mechanism of theta rhythm genesis (24), analogous to the role of T-type channels in the generation of oscillations in the reticular nucleus of the thalamus (25). Nevertheless the involvement of T-type Ca^{2+} channels in hippocampal theta rhythms or exploratory behavior has not been examined. Here, we analyzed global KO mice and mice with MS-specific inactivation of the $Ca_v3.1$ gene encoding T-type Ca^{2+} channels, focusing on finding the neural mechanism that control the exploratory behaviors. Using a combination of tools, we provide evidence that

Significance

Two different kinds of exploratory behavior, object and place, have been proposed. Their distinction has been debated, however, because of the lack of solid experimental evidence to support their heterogeneity. In this report, we show neural evidence for the heterogeneity of exploratory behaviors. Thus, we demonstrate that T-type Ca^{2+} channels in the septo-hippocampal GABAergic pathway play a specific role in control of exploratory behavior of novel objects. In addition, we show that type 2 but not type 1 hippocampal theta rhythm is associated with object exploration.

Author contributions: G.G., J.S., and H.-S.S. designed research; G.G., S.-W.K., A.K., A.P., D.-S.K., and T.M. performed research; Y.Y., J.K., and Y.-S.K. contributed new reagents/analytic tools; G.G., S.-W.K., A.K., A.P., D.-S.K., T.M., and M.W. analyzed data; and G.G., J.S., D.K., and H.-S.S. wrote the paper.

Reviewers: J.B., University of Tübingen; G.B., New York University Neuroscience Institute; and A.J.S., University of California, Los Angeles.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1605019113/-/DCSupplemental.

object and open field exploratory behaviors are processed differently in the brain. Furthermore, $Ca_v3.1$ T-type Ca^{2+} channels in the septo-hippocampal GABAergic projection neurons are critically involved in controlling object exploration through modulating hippocampal type 2 theta rhythm.

Results

Generalized Enhancement of Exploratory Behaviors in $Ca_v3.1^{-/-}$ Mice, Whereas Selective Increase in Object Exploration in MS $Ca_v3.1$ Knockdown Mice.

Generalized enhancement of exploratory behaviors in $Ca_v3.1^{-/-}$ mice. To explore the contribution of T-type Ca^{2+} channels in exploratory behaviors, we compared the object and open-field exploratory behavior pattern between $Ca_v3.1^{-/-}$ (KO) and WT littermates using published protocols, with some modifications (6, 26). Moreover, in the home cage, $Ca_v3.1^{-/-}$ mice were more active than their WT littermates, prompting us to more closely examine the exploratory behavior of the $Ca_v3.1^{-/-}$ mice. To evaluate object exploration behavior, mice exposed to novel objects in a familiar arena were monitored during a 20-min period (*SI Materials and Methods* and Fig. S1). We observed a significant increase in exploration of the novel objects by KO mice ($n = 10$) relative to their WT littermates ($n = 10$) (Fig. 1*A*) [two-way repeated-measures ANOVA (two-way rmANOVA), group effect, $F_{(1\ 18)} = 15.408$, $P \leq 0.001$]. Thus, the total time spent exploring the novel objects over 20 min was significantly greater for the KO mice (180.56 ± 31.08 s) than for the WT mice (41.11 ± 7.59 s; $P \leq 0.001$, Student's t test). To monitor the open-field exploration behavior, locomotor activity in a novel open-field arena was monitored during a 30-min period (*SI Materials and Methods*). KO ($n = 8$) mice exhibited increased locomotor activity relative to their WT littermates ($n = 10$), two-way rmANOVA, group effect [$F_{(1, 16)} = 13.654$, $P = 0.002$] (Fig. 1*B*). The total distance traveled over 30 min was much greater for the KO (6,045.8 ± 608.3 cm) than for the WT mice (3,450.2 ± 399.5 cm, $P = 0.002$, Student's t test).

Silencing of the $Ca_v3.1$ gene in the MS by short-hairpin RNA interference. It is known that the MS lesions affect open-field exploration as well as object exploration (14). To investigate whether the deletion of MS $Ca_v3.1$ channels was responsible for the enhanced exploratory behaviors in the $Ca_v3.1^{-/-}$ mice, we used short-hairpin (sh)RNA-mediated gene silencing to specifically knockdown $Ca_v3.1$ gene function in the MS, as described in the *SI Materials and Methods*. Postmortem examinations of the brains revealed that the mean percentage of $Ca_v3.1^{+}$ neurons in the MS was significantly reduced to 18.7 ± 3.9% that of the control) (Fig. S2) (**$P = 0.018$, Student's t test).

Selective enhancement of object exploration in MS $Ca_v3.1$ knockdown mice. Like the null mutant mice, the sh$Ca_v3.1$ mice ($n = 10$) exhibited significantly enhanced exploration of novel objects relative to the control virus-injected mice ($n = 8$) (Fig. 1*C*) [two-way rmANOVA, group effect, $F_{(1, 16)} = 5.895$, $P = 0.027$]. The total time spent exploring objects over 20 min was greater for the sh$Ca_v3.1$ mice (129.3 ± 35.3 s) than for the control shRNA mice (32.0 ± 6.0 s, *$P = 0.016$, Mann–Whitney rank-sum test). Surprisingly, however, the open-field exploration activity did not differ between the two groups of mice [two-way rmANOVA, group effect, $F_{(1, 16)} = 0.0344$, $P = 0.855$] (Fig. 1*D*). Thus, the total distance traveled over the 30-min period did not significantly differ between the sh$Ca_v3.1$ mice (2,833.6 ± 261.9 cm, $n = 10$) and the control shRNA mice (2,735.7 ± 493.9 cm, $n = 8$, $P = 0.855$ Student's t-test).

Generalized Enhancement of Theta Rhythms in $Ca_v3.1^{-/-}$ Mice, Whereas Selective Increase in Type 2 Theta Rhythm in MS $Ca_v3.1$ Knockdown Mice.

Generalized enhancement of theta rhythms in $Ca_v3.1^{-/-}$ mice. Because the MS is necessary for the generation of hippocampal theta rhythms (17, 18, 27), we decided to find out whether MS T-type Ca^{2+} channels have any role in theta rhythm genesis. Therefore, we recorded and compared the hippocampal local field potential (LFP) pattern between KO and WT littermates using published protocols and criteria (20, 21). To characterize type 2 theta rhythm in vivo in the mutant mice, we performed hippocampal LFP recordings of mice anesthetized with urethane (*SI Materials and Methods*). This procedure induces isolated type 2 theta rhythms mediated via muscarinic acetylcholine receptors (18, 20, 21). We previously reported that this urethane-induced type 2 theta rhythm is specifically absent, whereas the type 1 theta rhythm remains intact, in mice with mutations in *phospholipase Cβ1* (*PLC β1*), which is a signaling enzyme downstream of the M1, M3, and M5 muscarinic receptors (20). Interestingly, in the power spectral analysis, KO mice ($n = 6$) showed increased theta power compared with the WT littermates ($n = 5$) under urethane (Fig. 2 *A* and *C*, ***$P \leq 0.001$, Student's t test). To examine type 1 theta rhythms observed during locomotion in behaving KO mice, we recorded hippocampal electrical activity while the mice were running on a wheel (*SI Materials and Methods*). The LFP power was significantly increased in the theta band of 7–12 Hz in KO mice ($n = 6$) compared with WT littermates ($n = 5$, ***$P \leq 0.001$, Student's t-test) (Fig. 2 *B* and *D* and Fig. S3).

Selective increase in type 2 theta rhythm in the MS $Ca_v3.1$ knockdown mice. We next evaluated the theta rhythms of the mice with selective silencing of $Ca_v3.1$ function in MS neurons, to see whether they would replicate the phenotype of $Ca_v3.1^{-/-}$ mice. Type 2 theta rhythms recorded during urethane anesthesia was significantly increased in the sh$Ca_v3.1$ mice ($n = 10$) compared with the control shRNA mice ($n = 8$) (Fig. 2 *E* and *G*) ($P \leq 0.001$, Mann–Whitney rank-sum test). In contrast, however, type 1 theta rhythm observed during locomotion (i.e., running on a wheel) remained unchanged in the sh$Ca_v3.1$ mice (Fig. 2 *F* and *H*) ($P = 0.674$, Mann–Whitney rank-sum test).

The power of type 2 theta rhythm strongly correlated with the amount of object exploration. To assess whether the enhanced object exploration behavior in sh$Ca_v3.1$ mice was related to the increased type 2 theta power, a Pearson correlation analysis was performed on the combined behavioral and physiologic data from the sh$Ca_v3.1$ mice. Interestingly, the power of the type 2 theta rhythms recorded under urethane anesthesia was strongly correlated with the amount

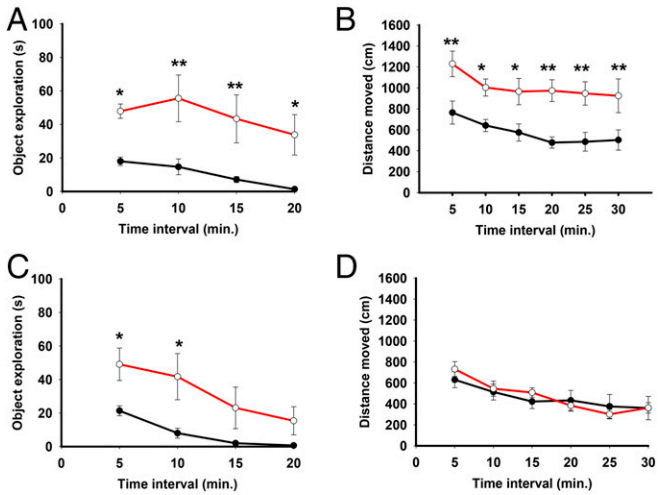

Fig. 1. Generalized enhancement of exploratory behaviors in $Ca_v3.1^{-/-}$ mice, whereas selective increase in object exploration in MS $Ca_v3.1$ knockdown mice. (*A*) Enhanced object exploration behavior by KO mice (WT, $n = 10$, black line and KO, $n = 10$, red line). (*B*) Enhanced open field exploration by KO mice (WT, $n = 10$, black line and KO, $n = 8$, red line). (*C*) Enhanced object exploration behavior by sh$Ca_v3.1$ mice. (*D*) No difference in open-field exploration between sh$Ca_v3.1$ and control shRNA mice (shRNA control, $n = 8$, black line and Sh$Ca_v3.1$, $n = 10$, red line). Data represent the mean ± SEM; *$P < 0.05$, **$P < 0.01$.

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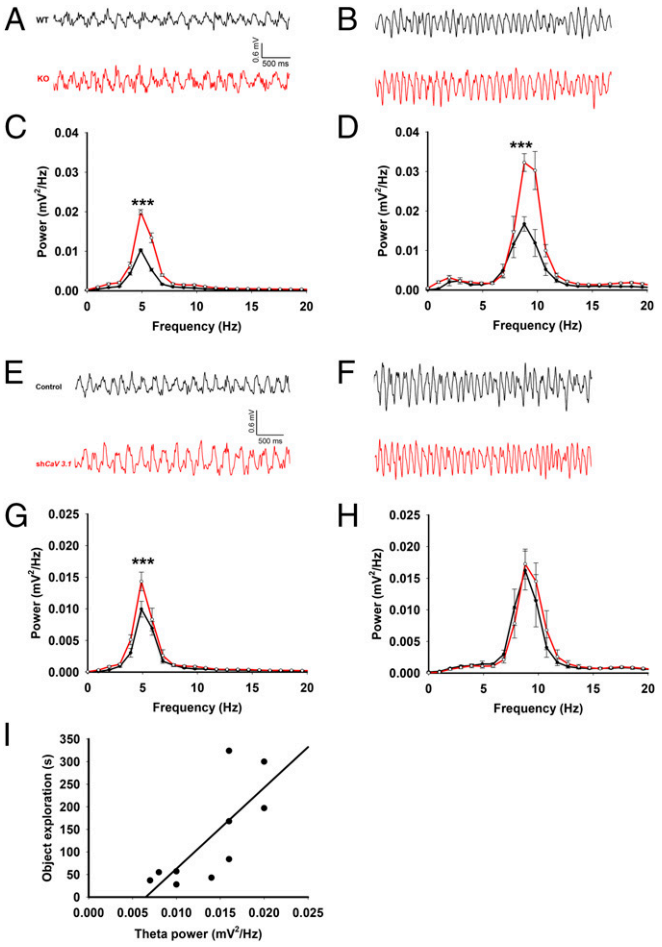

Fig. 2. Generalized enhancement of theta rhythms in $Ca_v3.1^{-/-}$ mice, whereas selective increase in type 2 theta rhythm in MS $Ca_v3.1$ knockdown mice. (*A* and *B*) Representative LFP waveforms under urethane anesthesia and during wheel running, respectively, in WT ($n = 5$, black color) and KO mice ($n = 6$, red color), and (*C* and *D*) the corresponding averaged power spectra. (*E* and *F*) Representative LFP waveforms under urethane anesthesia and during wheel-running, respectively, in shRNA control ($n = 8$, black color) and $shCa_v3.1$ mice ($n = 10$, red color), and (*G* and *H*) the corresponding averaged power spectra. (*I*) Positive correlation between type 2 theta power recorded under urethane anesthesia and the amount of novel object exploration behavior observed in $shCa_v3.1$ knockdown mice (*r*: Pearson correlation coefficient = 0.758; $P = 0.011$). Data represent the mean ± SEM; $^{***}P \leq 0.001$.

of object exploration among individual $shCa_v3.1$ mice (*r*: Pearson correlation coefficient = 0.758; $P = 0.011$) (Fig. 2*I*).

Deletion of $Ca_v3.1$ T-Type Ca^{2+} Channels Increased Excitability of Septo-Hippocampal GABAergic Projection Neurons. The two major neuronal types projecting from the MS to the hippocampus are cholinergic and GABAergic (28, 29). Among these two neuronal types, only the GABAergic neurons express $Ca_v3.1$ proteins (*SI Results* and Fig. S4), and therefore could be affected by the lack of $Ca_v3.1$ gene function. Thus, we focused our analysis on this neuronal population. First, we marked the septo-hippocampal GABAergic projection neurons in the MS via retrograde labeling (Fig. S5), and then examined their intrinsic firing properties in brain slices (*SI Materials and Methods*). Based on the responses to hyperpolarizing current pulses, we classified septo-hippocampal GABAergic neurons into two distinct types: those exhibiting low-threshold spikes (LTS^+) and those not exhibiting LTS (LTS^-). We found that only 10 of 18 GAD67 GABAergic projection neurons were LTS^+ in WT mice, indicating that about half of the GABAergic projection neurons in the MS did not express $Ca_v3.1$ channels. In contrast, all of the GABAergic projection neurons tested ($n = 13$) in the KO mice were LTS^- (Fig. 3*A*). To examine tonic firing activities of the GABAergic projection neurons, depolarizing currents (10-pA increments; 8 steps; 1-s duration) were injected into the cells from a holding potential of −60 mV. Tonic spike numbers were significantly higher in $Ca_v3.1^{-/-}$ septo-hippocampal $GAD67^+$ GABAergic neurons as a group compared with WT cells [$F_{(1, 29)} = 8.470$, $P = 0.007$, two-way rmANOVA] (Fig. 3 *B* and *C*). Interestingly, *K*-means clustering analysis revealed two subgroups of neurons in terms of firing properties within the KO cells: one subgroup with a higher tonic spike frequency compared with LTS^+ WT cells [$F_{(1, 98)} = 10.44$, $P = 0.006$, two-way rmANOVA] and LTS^- WT cells [$F_{(1, 77)} = 26.88$, $P = 0.0003$, two-way rmANOVA], and another subgroup that did not differ from LTS^+ WT cells [$F_{(1, 105)} = 0.03$, $P = 0.8562$, two-way rmANOVA] or LTS^- WT cells [$F_{(1, 84)} = 2.98$, $P = 0.1098$, two-way rmANOVA] (Fig. 3*D*). Importantly, there was no difference in

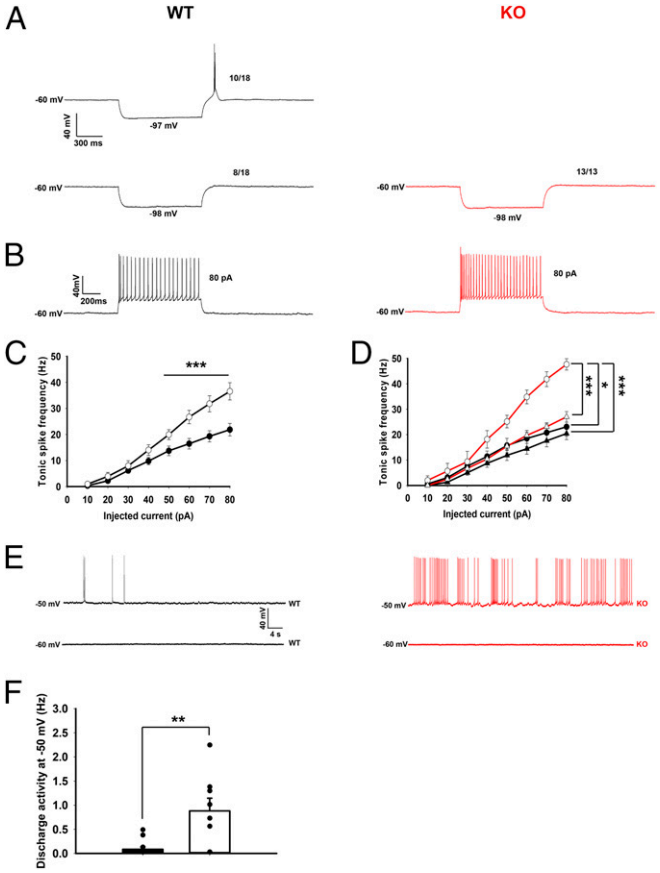

Fig. 3. Deletion of $Ca_v3.1$ T-type Ca^{2+} channels results in increased excitability in septo-hippocampal GABAergic projection neurons. (*A*) Representative traces of LTS^+ (*Upper Left*) and LTS^- projection neuron of a WT (*Lower Left*) and KO mouse (*Lower Right*) in response to negative step-current input. (*B*) Representative traces of WT (*Left*) and KO (*Right*) projection neurons response patterns to positive step-current input. The applied currents are indicated in each trace. (*C*) Enhanced tonic firing in KO septo-hippocampal GABAergic neurons (black circle, WT; white circle, KO). (*D*) No difference in tonic spike frequency (Hz) between LTS^+ ($n = 10$ cells, black circle) and LTS^- WT cells ($n = 8$ cells, black triangle). *K*-means clustering analysis revealed two groups of KO cells: one group of cells with high tonic spike frequency ($n = 6$ cells, ○) compared with LTS^+ and LTS^- WT, and another group ($n = 7$ cells, △) exhibiting no difference from LTS^+ or LTS^- WT cells. (*E*) Spontaneous firing patterns of the WT LTS^+ (*Left*) and KO LTS^- (*Right*) septo-hippocampal GAD67 GABAergic neurons at −50 mV and −60 mV. (*F*) Basal discharge activity of WT ($n = 11$ cells, black bar) and KO ($n = 8$ cells, white bar) neurons. Data represent the mean ± SEM; $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

the tonic spike frequency (Hz) between LTS$^+$ and LTS$^-$ neurons in the WT, [$F_{(1, 105)} = 0.85$, $P = 0.37$, two-way rmANOVA], suggesting that the high-frequency firing cells in the mutant MS emerged as a result of the deletion of the $Ca_v3.1$ gene (and thus of LTS) in the relevant neurons in WT mice. These results suggested that the spontaneous discharge activity of the septo-hippocampal GABAergic neurons might be altered by the $Ca_v3.1$ mutation. Thus, we recorded the discharge activity of GABAergic neurons in MS slices at −50 mV for 1 min. This experiment was based on previous reports that maintaining the membrane potential at around −50 mV generates spontaneous-like discharge activity in neurons (30). The discharge activity of septo-hippocampal GAD67$^+$ GABAergic neurons in WT mice was very low, 0.084 ± 0.049 Hz (Fig. 3 *E* and *F*), and there was no significant difference in the discharge activity between LTS$^+$ (0.075 ± 0.057 Hz, six cells) and LTS$^-$ cells (0.095 ± 0.091 Hz, five cells; $P = 0.849$, Student's *t* test) (Table S1). In contrast, KO septo-hippocampal GAD67$^+$ GABAergic neurons exhibited a much wider range of discharge activities, with many neurons showing greater discharge activity (0.881 ± 0.263 Hz, eight cells) than those in WT mice ($P = 0.003$, Student's *t* test) (Fig. 3*F*). Hyperexcitable cells, arbitrarily defined as those exhibiting a discharge activity greater than 0.5 Hz, were only observed in a subpopulation of septo-hippocampal GABAergic neurons in KO mice (five of eight cells), suggesting that they are those affected by the deletion of $Ca_v3.1$.

Optogenetic Modulation of Septo-Hippocampal GABAergic Fibers Selectively Modulated Object Exploration and Type 2 Theta Rhythm. To confirm whether the increased nonrhythmic drive of septo-hippocampal GABAergic neurons is in fact involved in the enhanced type 2 theta rhythms and increased object exploration, we used an optogenetic strategy to specifically stimulate or inhibit septo-hippocampal fibers in the dorsal fornix (*SI Materials and Methods*). We confirmed that parvalbumin$^+$ (Pv) cells colocalize with GAD67 septo-hippocampal neurons (*SI Materials and Methods* and Fig. S6). For stimulation experiments, we injected a Cre-dependent viral vector [channelrhodopsin-superfolder green fluorescent protein (ChR2-sfGFP)] into the MS of Pv::Cre transgenic mice (*SI Materials and Methods*) to selectively induce the expression of ChR2-sfGFP in Pv neurons in the MS. Postmortem histological analysis confirmed that the ChR2-sfGFP was abundantly expressed in the MS of the injected mice (Fig. S7). Furthermore, the ChR2-sfGFP was also observed in the dorsal fornix, through which the septo-hippocampal GABAergic fibers project from the MS to the hippocampus (31). The ChR2-sfGFP-expressing mice that received 10-Hz ($n = 9$) or 20-Hz ($n = 3$) optic stimulation in the dorsal fornix exhibited enhanced object exploration behavior relative to the nonstimulation group [$n = 10$; $F_{(2, 19)} = 8.192$, $P = 0.003$, two-way rmANOVA] (Fig. 4*A*). Interestingly, optogenetic stimulation enhanced the object exploration in the animals in a dose-dependent manner during the 20-min monitoring period (34.5 ± 5.8 s for 10-Hz group and 85.8 ± 33.0 s for 20-Hz group compared with 14.8 ± 3.3 s for the nonstimulation group, $^*P \leq 0.05$, one-way ANOVA, Dunn's method). In contrast, open-field exploration activity was not significantly different between the stimulation and nonstimulation groups [$F_{(2, 19)} = 0.483$, $P = 0.624$, two-way rmANOVA] (Fig. 4*B*). Thus, the total distance traveled in the open-field arena over the 30-min period did not differ significantly between the stimulation group (3,942.0 ± 512.5 cm for the 10-Hz group and 4,824.7 ± 556.2 cm for the 20-Hz group) and the nonstimulation group (3,762.34 ± 525.52 cm, $P = 0.314$, one-way ANOVA, Dunn's method). Under urethane anesthesia, hippocampal local field potentials spontaneously repeat the theta-off state and theta-on state in a period of tens of minutes. During the theta-off state, optic stimulation of septo-hippocampal GABAergic fibers using tonic pulse trains (10 and 20 Hz, pulse duration of 6.25 ms) not only did not induce theta rhythm, but also did not affect power spectral characteristics of LFPs measured during the theta-off state (Fig. S8). However, the same optic stimulation during the theta-on period induced an increase in type 2 theta power in the hippocampus compared with the control ($P \leq 0.05$, one-way ANOVA, Dunn's method) (Fig. 4 *C* and *E*). These results suggest that septo-hipopocampal GABAergic activity can neither induce theta rhythm nor modulate power spectral characteristics of LFPs during the theta-off state, but can modulate the power of theta rhythm during the theta-on state under urethane anesthesia. On the other hand, type 1 theta rhythms observed during locomotion (i.e., running on a wheel) were not affected by the same treatments ($P = 0.849$, one-way ANOVA, Dunn's method) (Fig. 4 *D* and *F*).

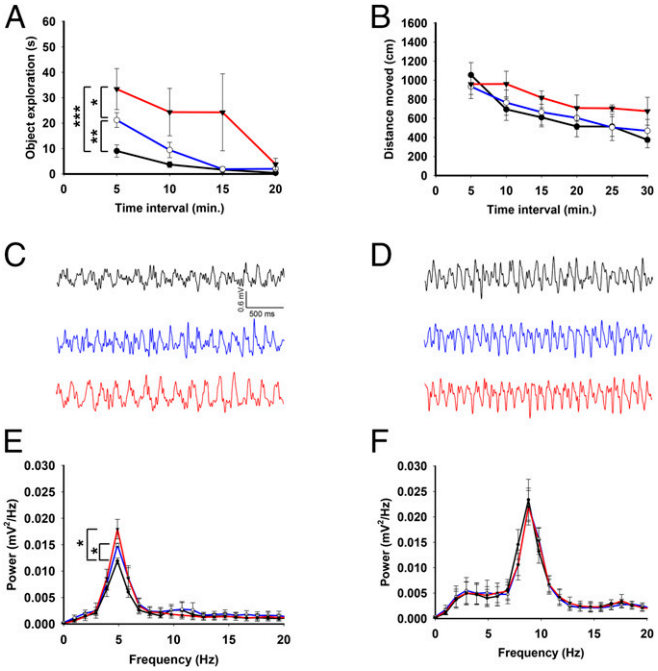

Fig. 4. Optogenetic stimulation of septo-hippocampal GABAergic fibers selectively increased object exploration and type 2 theta rhythm. (*A*) Enhanced object-exploration behavior by optic stimulation group [10 Hz ($n = 9$, blue line), 20 Hz ($n = 3$, red line), no-stimulation ($n = 10$, black line)]. (*B*) No difference in the open-field exploration among different groups. (*C* and *D*) Representative LFP waveforms, and (*E* and *F*) corresponding averaged power spectra recorded during urethane anesthesia's theta-on state and wheel running respectively in mice during no stimulation, and 10- or 20-Hz optic stimulation period ($n = 6$). Data represent the mean ± SEM; $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

For inhibition experiments, floxed Archaeodhopsin (AAV9.CBA.Flex.Arch-GFP.wPRE.SV40) virus was injected into the MS of B6 Pv::Cre transgenic mice (*SI Materials and Methods* and Fig. S9). In the inhibition experiments, we found that mice with photo inhibition of septo-hippocampal GABAergic fibers in the dorsal fornix significantly reduced exploration of novel objects relative to the control mice [control group ($n = 6$), photo-inhibition group ($n = 5$)] [$F_{(1, 9)} = 6.091$, $P = 0.036$, two-way rmANOVA] (Fig. 5*A*). Optogenetic inhibition reduced the total amount of object exploration during the 20-min monitoring period (161.88 ± 38.59 s for control group compared with 38.48 ± 17.600 s for the photo-inhibition group, $P = 0.036$, Student's *t* test). On the other hand, open-field exploration was not significantly different between the control and photo-inhibition groups [$F_{(1, 9)} = 2.131$, $P = 0.178$, two-way rmANOVA] (Fig. 5*B*). The total distance traveled over the 30-min period did not differ significantly between the photo-inhibition group (5,801.77 ± 803.85 cm) and the control group (4,146.16 ± 780.58 cm, $P = 0.178$, Student's *t* test). Under urethane anesthesia, photo inhibition of septo-hippocampal GABAergic fibers in the dorsal fornix (*SI Materials and Methods*) using continuous light application reduced the type 2 theta power in the hippocampus

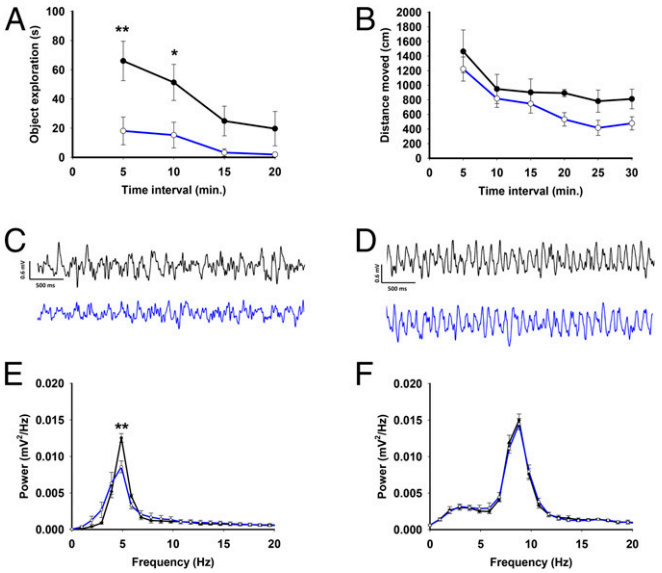

Fig. 5. Optogenetic inhibition of septo-hippocampal GABAergic fibers selectively decreased object-exploration behavior and type 2 theta rhythm. (*A*) Decreased object exploration behavior by photo-inhibition group [photo inhibition ($n = 5$, blue line), control ($n = 6$, black line)]. (*B*) No difference in open-field exploration between the two groups. (*C* and *D*) Representative LFP waveforms and (*E* and *F*) corresponding averaged power spectra recorded during urethane anesthesia's theta-on state and wheel-running, respectively, in mice during the period of no application of light (black trace) and photo-inhibition period (blue trace) ($n = 6$). Data represent the mean ± SEM; $*P \leq 0.05$, $**P \leq 0.01$.

compared with the control (no light application) ($n = 6, P \leq 0.01$, Student's t test) (Fig. 5 *C* and *E*). In contrast, type 1 theta rhythms observed during locomotion (i.e., running on a wheel) were not affected by the same treatments ($n = 6$, $P = 0.690$, Student's t test) (Fig. 5 *D* and *F*). The high level of exploration activity in control group seems as a result of the change in mouse genetic background. For the optogenetic inhibition of septo-hippocampal GABAergic fibers, we used PV::Cre mice in a B6 background, whereas for the rest of the experiments we used mice with F1 (B6x129) background.

Discussion

Two-Tiered Control of Exploratory Behaviors by $Ca_v3.1$ T-Type Ca^{2+} Channels. The environment to which an animal is exposed comprises two components: object and place (environment) itself. Animals exhibit two different types of exploratory behaviors toward objects and the place in the absence of objects: inspective exploration behavior for objects and inquisitive exploration behavior for the place (3–5). Although previous lesion studies demonstrated that the MS participates in both the object and the place (open-field) exploration behaviors (5, 14–16), whether distinct neural mechanisms mediate the two exploration behaviors is unknown. Here, our findings indicate that, even though $Ca_v3.1$ T-type Ca^{2+} channels are involved in the control of both forms of exploratory behaviors, as suggested by the phenotypes of global mutants, $Ca_v3.1$ T-type Ca^{2+} channels in the MS GABAergic neurons participate specifically in controlling object exploration behavior, without affecting open-field exploration as shown by the MS-specific $Ca_v3.1$ channel knockdown mice. Moreover, the selective stimulation or silencing of septo-hippocampal GABAergic pathway (i.e., axons) by optogenetic methods further confirmed the idea that this pathway is selective for object exploration behavior. Interestingly, a recent study using optogenetic stimulation of septo-hippocampal glutamergic neurons showed that those neurons specifically participate in initiation and control of locomotion activity (32). Taken together, these results suggest that although MS glutamergic projection neurons are involved in open-field exploration using locomotion, MS GABAergic projection neurons are involved in object exploration behavior.

Type 2 Theta Rhythm and Novel Object-Induced Behavior. One form of inquisitive behavior seen in rodents is a strong preference for novelty (3). In rodents, novel stimuli elicit a behavioral conflict between avoidance and exploration (1, 33). The novelty behavior paradigm is a method to elicit relatively robust approach behaviors in rodents when they encounter novel objects (6, 34, 35). Hippocampal theta rhythms are associated with diverse cognitive and behavioral functions in rodents and humans (17, 36, 37). The heterogeneity of hippocampal theta rhythms has long been under debate (19, 24). Recent studies based on genetic mutations in mice, however, provide strong support for theta rhythm heterogeneity (20, 21). Furthermore, recent experiments by Vandecasteel et al. demonstrated that optogenetic stimulation of cholinergic MS neurons selectively enhances cholinergic type 2 theta rhythm without affecting noncholinergic type 1 theta rhythm (22). In the present study, we revealed a neural mechanism involved in object exploration distinct from open-field exploration behavior. Our findings indicate that object exploration is strongly associated with type 2 theta rhythm. The restricted phenotypes, both in physiology and behavior, of the MS-specific $Ca_v3.1$ channel knockdown mice compared with the global mutants suggested that the increased power of the type 2 theta rhythm is linked to the enhanced object exploration behavior. In this regard, it is notable that a significant positive correlation exists between the power of the type 2 theta rhythm and the amount of object exploration behavior in animals with MS-specific knockdown of $Ca_v3.1$ (Fig. 2*I*). Furthermore, optogenetic modulations of septo-hippocampal GABAergic activity controlled both object exploration behavior and type-2 theta rhythm (Figs. 4 and 5). Taken together, our results suggest a possibility of a functional relationship between type 2 theta rhythm and object exploration behavior. Another interesting issue raised in our study is the relationship among $Ca_v3.1$ T-type Ca^{2+} channels, increased open-field exploration, and increased type 1 theta rhythm, in $Ca_v3.1^{-/-}$ mice (Figs. 1*B* and 2*D*). Interestingly, a recent study showed that septo-hippocampal circuit mediated by glutamatergic neurons can control the initiation and velocity of the locomotion as well as locomotion-associated hippocampal type 1 theta oscillations (32). On the other hand, in the present study the knockdown of $Ca_v3.1$ channel expression in MS neurons, including GABAergic and glutamatergic neurons, did not lead to increased type 1 theta rhythm or increased open-field exploration phenotypes of the global KO mice. These results show that the function of the glutamatergic neurons involved in the control of type 1 theta rhythms is not dependent on T-type Ca^{2+} channels. Additional studies will be required to better define the T-type Ca^{2+} channels' role in the open-field exploration and its association with type 1 theta rhythm.

Role of $Ca_v3.1$ T-Type Ca^{2+} Channels in Control of Hippocampal Theta Rhythms: Spatially Segregated Two-Tiered Roles. T-type Ca^{2+} channels, especially those in the thalamus, are responsible for many neuronal oscillations, including delta rhythms during nonrapid-eye movement sleep, sleep spindles, and spike and wave discharges during the absence of seizures (38). Thalamic relay cells fire in two distinct modes, burst or tonic, which are dictated by the state of low-threshold, voltage-gated, T-type Ca^{2+} channels (39, 40). Using $Ca_v3.1^{-/-}$ mice, we previously demonstrated that the deletion of $Ca_v3.1$ T-type currents results in the absence of LTS and an increase in tonic firing in periaqueductal GABAergic neurons (30), whereas enhanced expression of T-type currents decreases tonic firing activity in thalamic relay neurons (41). In the present study, using null-mutant and MS-specific knockdown of $Ca_v3.1$, we demonstrated that $Ca_v3.1$ T-type Ca^{2+} channels are involved in the

suppression of both type 2 and type 1 hippocampal theta rhythms, whereas the same channels in the MS control only the type 2 theta rhythm. Here, we found that within the MS, $Ca_v3.1$ is detected mainly in GABAergic neurons and is absent in cholinergic neurons. Furthermore, we found that a group of septo-hippocampal projecting GABAergic neurons in $Ca_v3.1^{-/-}$ mice exhibited increased tonic firing activity in the absence of LTS. The septo-hippocampal projecting GABAergic neurons inhibit local inhibitory GABAergic interneurons in the hippocampus, leading to disinhibition (28, 42). Therefore, we speculated that the increased tonic firing activity in the septo-hippocampal GABAergic neurons without $Ca_v3.1$ T-type Ca^{2+} channels might disinhibit the hippocampal pyramidal neurons, resulting in an enhanced type 2 theta rhythm. This prediction was confirmed in vivo by the optogenetic activation of axon bundles in the dorsal fornix. The optogenetically stimulated mice showed enhanced type 2 theta rhythms with intact type 1 theta rhythms, whereas the inhibition of the same pathway resulted in the reduction of type 2 theta rhythms with intact type 1 theta rhythms. These findings indicate that $Ca_v3.1$ channels have spatially segregated two-tiered roles in the control of theta rhythms.

Materials and Methods

Animal care and all experiments were conducted in accordance with the Institutional Review Board (IRB) of Institute for Basic Science (IBS), Korea for the ethical guidelines of Animal Care and Use. Detailed descriptions of study methods are provided in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Dr. Miwako Yamasaki, Dr. Cheongdahm Park, Dr. Daejong Jeon, Dr. Jungryun Lee, Dr. Il-hwan Choe, and Jong-Hyun Kim for technical and intellectual support. This work was supported by the grants from Institute for Basic Science (IBS), Korea (IBS-R001-D1-2016-a00).

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