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Supporting Online Material

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Cerebellum Shapes Hippocampal Spatial Code

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Spatial representation is an active process that requires complex multimodal integration from a large interacting network of cortical and subcortical structures. We sought to determine the role of cerebellar protein kinase C (PKC)–dependent plasticity in spatial navigation by recording the activity of hippocampal place cells in transgenic L7PKCI mice with selective disruption of PKC-dependent plasticity at parallel fiber–Purkinje cell synapses. Place cell properties were exclusively impaired when L7PKCI mice had to rely on self-motion cues. The behavioral consequence of such a deficit is evidenced here by selectively impaired navigation capabilities during a path integration task. Together, these results suggest that cerebellar PKC-dependent mechanisms are involved in processing self-motion signals essential to the shaping of hippocampal spatial representation.

It is well established that rodents build an internal cognitive map to navigate in their environment. A key neural substrate enabling such representation is the hippocampus, which contains CA1 and CA3 pyramidal cells described as place cells. Each place cell fires for a restricted region (the place field) of the environment (*1*, *2*). Both external cues and self-motion cues (i.e., vestibular, proprioceptive, and optic flow cues) control place cell firing (*3*, *4*), which suggests the involvement of a large network of cortical and subcortical structures interacting with the hippocampus for navigation. Determining the functional architecture of such a network is thus essential to our understanding of how the hippocampal place cell code is generated. The medial entorhinal cortex, a key relay structure between neocortical areas and the hippocampus, contains grid cells with regularly spaced multiple firing fields (*5*), which integrate self-motion information and participate in path integration (*4*, *6*, *7*).

The cerebellum has also been shown to be essential to the processing of self-motion information: Cerebellar Purkinje cells respond to vestibular signals by transforming head-centered vestibular afferent information into Earth-reference self-motion and spatial orientation signals (*8*, *9*), and electrophysiological investigations suggest that the cerebellum and the hippocampus can be functionally connected during eyeblink conditioning (*10*, *11*). However, it is still unknown whether such an interaction is functionally relevant in navigation, and a mechanism that might underlie such a process has not been identified.

In the transgenic mouse strain L7PKCI, the pseudosubstrate protein kinase C inhibitor (PKCI) is selectively expressed in Purkinje cells under the control of the *pcp-2* (L7) gene promoter (*12*). This results in an impaired long-term depression (LTD) at cerebellar parallel fiber–Purkinje cell synapses. Such a plasticity mechanism has been proposed to work as an error-based (anti-Hebbian) learning process (*13*, *14*) during conditioning tasks (*15*) and in optimization of motor response during navigation (*16*).

A total of 506 dorsal CA1 hippocampal cells were recorded. A subset of 150 place cells was further analyzed in six L7PKCI mice and five wild-type littermate control mice. Relative to wild-type mice, L7PKCI mice had a significantly lower proportion of place cells [L7PKCI, $n = 53/218$ (24.3%); wild type, $n = 97/288$ (33.7%); $\chi^2 = 5.2$, df = 1, $P < 0.025$]. Neural activity was sampled as the mice freely explored a circular arena containing a salient cue (a card with a bottle attached to it), in standard sessions (S1 and S2) and involving cue manipulation in subsequent sessions (S3 and S4). A last session (S5) similar to sessions S1 and S2 was run to determine whether we could restore the initial firing pattern irrespective of the changes in cell firing observed during the cue manipulation sessions (Fig. 1A) (*17*).

After recording in the standard sessions, we used two distinct environmental manipulations, cue removal and cue conflict, in which mice are forced to use self-motion cues. In the cue removal condition, the arena was in the dark and the cue

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was removed. A control condition was also performed with the cue still present in darkness (Fig. 1B). In the cue conflict condition, we used a protocol previously developed in rats, in which the external cue was rotated 180° in the absence (hidden rotation) or in the presence (visible rotation) of the animal, therefore producing a conflict between visual and self-motion information (*18*). During the conflict, rats maintain place field stability relative to the standard session, thus suggesting the dominant use of self-motion cues (*18*).

The basic firing properties of place cells in the light condition were unaffected in L7PKCI mice (table S1). In addition, place field stability (measured as a correlation between two similar light sessions) was higher in L7PKCI mice (0.78 ± 0.05) than in wild-type mice (0.60 ± 0.03) (t_{146} = 3.0,

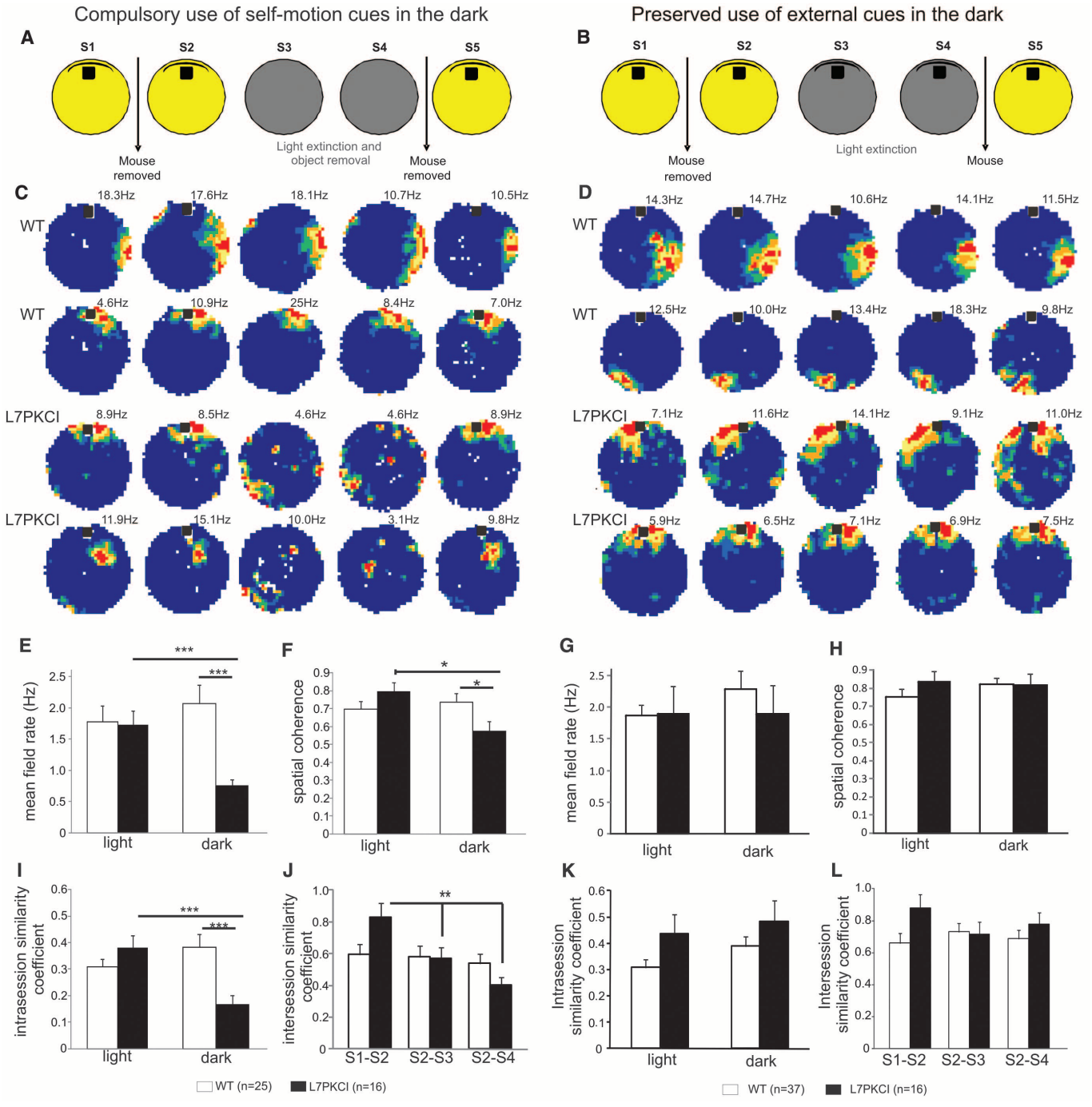

Fig. 1. The compulsory use of self-motion cues affects hippocampal place cell properties in L7PKCI mice. (**A** and **B**) Schematic diagram of the protocol used to assess the effect of self-motion stimulation on place cell properties. After two consecutive standard sessions (S1 and S2), light was turned off (S3 and S4) and objects were either removed (A) or maintained (B) in the arena. S5 was similar to S1 and S2. (**C** and **D**) Examples of color-coded rate maps showing firing activity of wild-type (WT) and L7PKCI single CA1 pyramidal cells over the five consecutive sessions; color coding ranges from blue (silent) to red (peak activity). Peak firing rates are indicated for each rate map. (**E** to **H**) Analysis of place cell characteristics shows that the suppression of external cue inputs significantly alters both the mean field rate (E) and spatial coherence (F) in L7PKCI mice specifically, whereas the suppression of the visual cue alone has no effect [(G) and (H)]. (**I** to **L**) Place field stability, as measured within (I) or across (J) sessions, is affected in L7PKCI mice after suppression of all external cues, but not after suppression of the visual cue alone [(K) and (L)]. *P < 0.05, **P < 0.01, ***P < 0.001 with a Newman-Keuls post hoc analysis. Error bars represent SEM.

$P = 0.003$). In sharp contrast, several firing parameters were strongly affected in the dark sessions after cue removal in L7PKCI mice (Fig. 1, C, E, and F, and figs. S1A and S2). The mean field rate, peak firing rate, and overall mean firing rate declined during the dark sessions in L7PKCI mice but not in wild-type mice (mean field rate, $F_{1,39} = 11.5$, $P = 0.002$; peak firing rate, $F_{1,39} = 12.1$, $P = 0.001$; overall mean firing rate, $F_{1,39} = 8.4$, $P = 0.006$) (Fig. 1E and fig. S2). The field spatial coherence was also found to be decreased in the dark ($F_{1,39} = 11.2$, $P = 0.002$) (Fig. 1F). Finally, place field stability in L7PKCI mice was markedly affected by the dark condition: Whereas wild-type mice maintained place field stability throughout the dark sessions, place cells of L7PKCI mice showed a progressive decrease of within-session stability ($F_{1,39} = 15.6$, $P = 0.0003$) as well as between-session stability ($F_{2,78} = 6.2$, $P = 0.003$) (Fig. 1, I and J). These results indicate that place field stability in L7PKCI mice gradually decreased over sessions in the dark (post hoc analysis; $P < 0.01$ between S1-S2 and S2-S3 correlations, $P < 0.001$ between S1-S2 and S2-S4 correlations) (Fig. 1J).

By contrast, place cell firing properties and place field stability were restored in session S5 (fig. S3). In most instances, all simultaneously recorded cells behaved homogeneously (i.e., all fields were either stable or remapped together). The modification of place cell properties of L7PKCI mice was not due to an impaired exploratory activity in the dark, because wild-type and L7PKCI mice displayed similar speed (2.09 ± 0.08 cm/s versus 1.97 ± 0.05 cm/s, $t_{19} = 1.7$, $P > 0.05$, *t* test) and similar traveled distance (14.24 ± 0.59 cm versus 13.10 ± 0.35 cm, $t_{19} = 1.4$, $P > 0.05$, *t* test) (Table 1). When the cue was available in the dark, the firing parameters and place field stability were not affected in L7PKCI mice (Fig. 1B and fig. S1B) ($P > 0.05$ for all parameters analyzed).

These results suggest that in the dark and in the absence of the cue, the place cell system of L7PKCI mice failed to use self-motion information to maintain stable place fields. Consistent with this finding, the mice were able to maintain stable place fields when they could update their position by using the cue. As a consequence, the number of place fields away from the object (>20 cm) was drastically reduced in L7PKCI mice relative to wild-type mice [L7PKCI, $n = 1/16$ cells (6%); wild type, $n = 20/37$ cells (46%); $\chi^2 = 10.67$, df = 1, $P = 0.0011$]. The relative power of the hippocampal theta band (5 to 10 Hz) was similar in L7PKCI and wild-type mice ($F_{1,21} = 1.72$, $P > 0.05$) in both light and dark cue removal conditions ($F_{1,21} = 3.86$, $P > 0.05$), which suggests that alteration of path integration was not caused by a modification of theta rhythm (fig. S4).

To further investigate the respective influence of self-motion and external information on spatial firing pattern in L7PKCI mice, we conducted a conflict condition protocol (Fig. 2A). After two standard sessions, a 180° hidden rotation of the cue resulted in similar rotation of the place fields in both wild-type and L7PKCI mice (Fig. 2, B

Table 1. General sensory-motor abilities of WT and L7PKCI mice in the dark (means ± SEM). No significant differences between WT and L7PKCI mice were revealed by the different sensory-motor tasks (*t* test, $P > 0.05$ for all parameters) assessed in the dark (i.e., using primarily the vestibular system).

Task	Measure	L7PKCI ($n = 7$)	WT ($n = 6$)	Mann-Whitney *P*
Spontaneous locomotor activity in the dark	Speed (cm/s)	1.97 ± 0.05	2.09 ± 0.08	0.10
	Distance traveled (cm)	13.10 ± 0.35	14.24 ± 0.59	0.09
	Rearing frequency (number/min)	4.29 ± 0.73	6.20 ± 0.73	0.18
Dynamic balance in the dark	Falling latency (s)	180	180	—
	Distance traveled (cm)	629 ± 77	542 ± 51	0.45
Static balance in the dark	Falling latency (s)	144 ± 18	105 ± 17	0.10
Motor coordination in the dark (rotarod)	5 rpm walking time (s)	141 ± 21	138 ± 12	0.45
	10 rpm walking time (s)	141 ± 28	157 ± 17	0.63

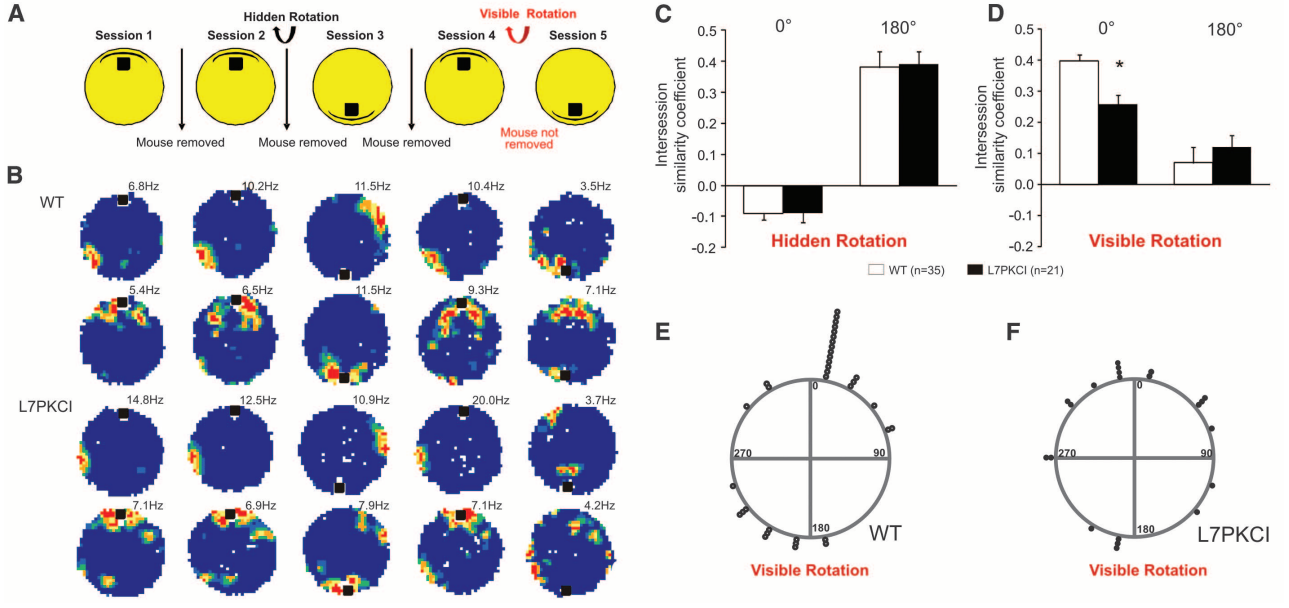

Fig. 2. Field locations are not efficiently controlled by self-motion cues in L7PKCI mice. (**A**) Schematic diagram illustrating the protocol used to assess the effect on place cell firing of a 180° rotation of the cue in the absence (hidden rotation) or presence (visible rotation) of the mouse in the arena. (**B**) Color-coded rate maps showing firing activity of WT and L7PKCI single CA1 pyramidal cells over the five consecutive sessions. (**C** and **D**) Histograms showing the intersession similarity coefficient score associated to a 0° or 180° field rotation after a hidden (C) or a visible (D) rotation of the cue. Field stability significantly decreased in L7PKCI after a visible rotation of the cue (D). (**E** and **F**) Polar distribution of the place field rotation angles after the visible rotation in WT mice (E) and L7PKCI mice (F). *$P < 0.05$, Student *t* test. Error bars represent SEM.

and C, and fig. S1C), indicating that the cue efficiently controlled place cell activity. Visible rotation of the cue was then performed, producing a conflict between external and self-motion sensory information. During the conflict, 63% of place cells in wild-type mice maintained their place field stability relative to the previous session (0° ± 30° rotation), which suggests that the mice resolved the conflict by relying on self-motion cues (*18*) (Fig. 2, B, D, and E, and fig. S1C). The distribution of place field rotation angles after the visible rotation was therefore concentrated around the same position (Fig. 2E; $Z = 13.53$, $P < 0.001$, Rayleigh test). In contrast, a majority of place cells in L7PKCI mice exhibited remapping at a different location, leading to a homogeneous distribution of place field rotation angles (Fig. 2, B, D, and F; $Z = 1.55$, $P = 0.2$, Rayleigh test): 30% of place fields remained stable, suggesting a control by self-motion cues; 20% exhibited a 180° ± 30° rotation, suggesting a control by the external cue; and the remaining 50% rotated at various angles. As a result, field stability (as measured by intersession similarity coefficient at a rotation angle of 0°) between sessions S4 and S5 was significantly lower in L7PKCI mice than in wild-type mice (Fig. 2D; $t_{54} = -2.0$, $P < 0.05$). The inability to maintain stable place fields in L7PKCI mice strengthens the idea of a deficit in the use of self-motion cues.

We next examined the ability of L7PKCI mice to navigate in the dark (i.e., using self-motion cues). L7PKCI mice were trained to find an escape platform at a constant location with a constant departure point in the water maze (Fig. 3A). Path optimization was analyzed in light and dark conditions (*17*) (table S3). In the light, L7PKCI mice learned to reach the platform as rapidly and accurately as their control littermates (Fig. 3, B and C). Both groups increased the use of direct trajectories across training sessions and decreased other nonoptimal trajectories (Fig. 3, D and E). In sharp contrast, navigation performance in the dark was impaired in L7PKCI mice (Fig. 3, B and C). Escape latencies and heading were significantly greater in L7PKCI mice than in wild-type mice (genotype effect, $F_{1,28} = 4.98$, $P = 0.034$, and $F_{1,28} = 9.63$, $P = 0.004$, respectively), even though there was no difference in swimming speed ($F_{1,28} = 2.01$, $P = 0.2$), circling ($F_{4,112} = 0.56$, $P = 0.7$) (fig. S5), or other behavioral parameters that could interfere with navigation (Table 1 and table S2; $P > 0.05$) (*19*). Thus, assessment of navigation abilities in the dark demonstrates impaired path integration performances in the L7PKCI mice.

The trajectories of the mutant mice were less efficient than those of their control littermates in darkness, as highlighted by the differences in the type of trajectory used (Fig. 3, D and E). The importance of the dark context on the deficit exhibited here by the L7PKCI mice was reinforced by the absence of significant genotype effect observed during a control trial that took place in the light condition during dark session 3 (D3T1 in Fig. 3, B and C) (table S3) (*17*). Accordingly, comparing this trial with the mean of the last trial (L5) in the light condition revealed no significant differences. This indicates that the disturbed trajectories displayed by transgenic mice in the dark cannot be attributed either to a deficit in the use of task rules, or to altered motivation.

The fundamental finding of our study is that mice lacking PKC-dependent cerebellar LTD showed disrupted hippocampal place cell properties and impaired goal-directed navigation in conditions in which self-motion information must be predominantly used. We previously suggested a role of PKC-dependent mechanisms in the linkage between the spatial context and the motor response characterized by the animal's trajectory (*16*, *20*). Here, we demonstrate an additional and complementary role of PKC-dependent cerebellar LTD in self-motion–based hippocampal representation and path integration. Although the cerebellum is classically viewed as a motor structure, a growing body of evidence indicates that cerebellar circuitry is well suited to act as an adaptive filter of sensory information (*21*–*23*). In particular, vestibular information is combined with proprioceptive inputs in the cerebellar fastigial nucleus to generate appropriate internal estimates of the animal's self-motion (*24*). In addition, cerebellar Purkinje cells from lobules IX and X transform vestibular head-centered signals into self-motion and spatial orientation signals relative to the external world (*8*, *9*). It thus appears that, beyond its role in motor adaptation during navigation (*16*, *20*), cerebellar LTD contributes to the representation of the relation of the body to the external world, thereby shaping hippocampal spatial representation.

Recent data show a clear contribution of the vestibular system to hippocampal-dependent spatial memory (*25*, *26*) as well as to spatial firing of

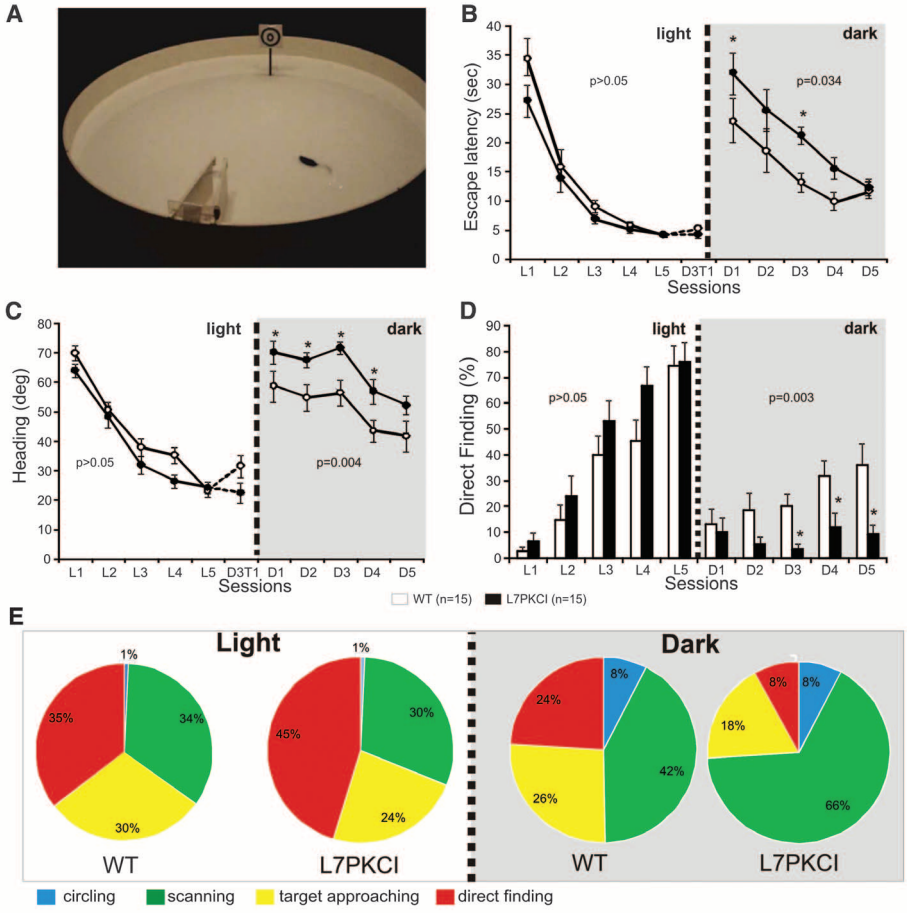

Fig. 3. Inactivation of PKC-dependent cerebellar LTD deteriorates path integration. (**A**) Design of the experimental space developed to evaluate navigation abilities using self-motion cues. (**B** and **C**) Quantification of escape latencies (B) and heading (C) in WT and L7PKCI mice during both light and dark conditions. In the light condition, WT and L7PKCI mice improved their performances significantly over sessions without genotype effect. In the dark condition, both groups improved their performance over time, but the performance of LKPCI mice was significantly poorer than that of their control littermates. (**D** and **E**) Swim path analyses during both light and dark conditions. The direct trajectory was significantly impaired in L7PKCI mice during the dark condition (D). L7PKCI mice cannot perform optimal trajectories during path integration, as highlighted by the differences between WT and L7PKCI mice in the type of trajectory used in the dark but not in the light condition (E). The *P* values indicated in (B) to (D) correspond to the genotype effect. *$P < 0.05$ with Newman-Keuls post hoc analysis. Error bars represent SEM.

hippocampal neurons (*27*). However, examination of the vestibular-associated motor activity of L7PKCI mice in both light and dark conditions revealed no deficit. Our data do not suggest a vestibular implication underlying the observed alterations of place cell firing and navigation in L7PCKI mice. Rather, they demonstrate that cerebellar LTD is also involved in processing self-motion cues. The cerebellum may therefore contribute to two major circuits crucial for the representation of space in the hippocampal system. The first is the retrosplenial cortex, which is closely associated with vestibular function (*27*). The second is the parietal cortex, which integrates self-motion and external information and receives input from the deep cerebellar nuclei (*28*, *29*). Our study demonstrates the crucial role of PKC-dependent cerebellar LTD in the preprocessing of self-motion information required for optimal hippocampal representation. This process appears to be essential for path integration.

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Supporting Online Material

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Activity-Dependent Long-Term Depression of Electrical Synapses

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Use-dependent forms of synaptic plasticity have been extensively characterized at chemical synapses, but a relationship between natural activity and strength at electrical synapses remains elusive. The thalamic reticular nucleus (TRN), a brain area rich in gap-junctional (electrical) synapses, regulates cortical attention to the sensory surround and participates in shifts between arousal states; plasticity of electrical synapses may be a key mechanism underlying these processes. We observed long-term depression resulting from coordinated burst firing in pairs of coupled TRN neurons. Changes in gap-junctional communication were asymmetrical, indicating that regulation of connectivity depends on the direction of use. Modification of electrical synapses resulting from activity in coupled neurons is likely to be a widespread and powerful mechanism for dynamic reorganization of electrically coupled neuronal networks.

The thalamic reticular nucleus (TRN) is a shell comprising a homogenous population of parvalbumin (PV)–positive γ-aminobutyric acid (GABA)–releasing (GABAergic) neurons surrounding the dorsal thalamus (*1*, *2*). These cells provide powerful inhibition to thalamocortical relay neurons (*3*) upon integration of their corticothalamic and thalamocortical inputs. In addition to its proposed role in focusing the neural spotlight of attention (*4*, *5*), the TRN is strongly involved in regulating states of arousal (*6*, *7*) by means of alternation between burst and tonic modes of firing. Burst firing in the TRN is a prominent component of sleep spindles (*8*, *9*) and absence seizures (*9*, *10*), both of which are marked by dramatic changes in cortical attention and behavioral responsiveness to sensory input.

In central mammalian neurons, electrical (gap-junctional) synapses appear all over the brain (*11*, *12*) and mainly couple GABAergic neurons of similar subtype (*13*–*15*). Electrical synapses contribute to synchrony in coupled networks (*11*, *16*–*21*), although computational studies suggest that the precise role of gap junctions in synchrony can be complex (*22*–*24*).

Cells in the TRN are densely and powerfully connected by electrical synapses (*17*, *18*) that persist into adulthood (*25*) and, as in other areas, participate in its synchronous activity (*18*). The experimentally isolated TRN generates spindle rhythms in the absence of other inputs (*26*), suggesting that electrical synapses are likely to be key players in TRN synchrony and in behavioral switching between firing states.

Activity-dependent forms of plasticity have been extensively described at excitatory (glutamatergic) chemical synapses (*27*, *28*) and, to a lesser extent, at inhibitory (GABAergic) chemical synapses (*29*–*31*). Although the issue has received far less attention than plasticity of chemical synapses, modifications of electrical synapses have been documented in a handful of reports (*32*, *33*). Because electrical synapses are likely to play a major role in coordinating TRN activity, we sought to investigate the effects of natural forms of activity in coupled neurons on the strength of the electrical synapses between them.

We recorded from pairs of gap junction–coupled TRN neurons (Fig. 1A) within conventional thalamocortical brain slices (*34*). To measure electrical synaptic strength, we delivered hyperpolarizing current injections into one neuron (cell 1) while recording voltage (V) responses in both neurons, which were maintained at a baseline $V_m = -65$ mV (Fig. 1B). Using these deflections, we determined the coupling coefficient $\mathbf{cc_{12}} = \Delta V_{cell\ 2}/\Delta V_{cell\ 1}$, and from injecting current into cell 2, similarly determined $\mathbf{cc_{21}} = \Delta V_{cell\ 1}/\Delta V_{cell\ 2}$. We also calculated coupling conductance $\mathbf{G_C}$ (*34*) in each direction. From a total of 313 paired recordings of coupled TRN neurons, we found an average **cc** of 0.12 ± 0.08 and $\mathbf{G_C}$ of 0.80 ± 0.63 nS (mean ± SD) (Fig. 1C), which is in line with the values for previous reports in TRN (*17*, *18*, *33*)

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