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Intact LTP and Fear Memory but Impaired Spatial Memory in Mice Lacking $Ca_v2.3$ (α_{1E}) Channel

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To investigate the functional roles of the $Ca_v2.3$ (α_{1E}) channel in hippocampal CA1 pyramidal neurons, we studied *in vitro* synaptic properties and *in vivo* behaviors of the $Ca_v2.3$ gene deficient mice. The $Ca_v2.3$ channel mRNA was identified in the hippocampal formation of the wild-type mouse by *in situ* hybridization. The basic excitatory synaptic transmission and long-term potentiation by theta-burst stimulation were intact in CA1 region of $Ca_v2.3-/-$ mice. We performed two forms of behavioral tests to examine the hippocampus-dependent function, i.e., emotional and spatial learning tests. The $Ca_v2.3-/-$ mice were able to establish and maintain fear memories. Although general improvement in the performance of Morris water maze test was seen in $Ca_v2.3-/-$ mice, they displayed an obvious impairment in the probe test. These results suggest that the $Ca_v2.3$ channel plays some role in formation of the accurate spatial memory but not of the fear memory.

Key Words: **$Ca_v2.3$; α_{1E}; Ca^{2+} channel; long-term potentiation; hippocampus; spatial learning and memory; Morris water maze test; fear conditioning.**

Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCC) plays crucial roles in intracellular signal transduction in neurons. The α_1 subunit of VDCC is responsible for determining functional properties of the various subclasses, which have been extensively characterized biochemically and pharmacologically (1). $Ca_v2.3$ (α_{1E}) gene encodes one of HVA types of VDCC, which is considered to correspond to the R-type channel (2–5). Although the functional properties of $Ca_v2.3$ channel are described as those between typical HVA and LVA classes of VDCCs (6), its role in the physiological functions has not been fully clarified so far, partly because of the limited availability of the pharmacological tool to dissect its activity. We have therefore generated the mouse strain lacking $Ca_v2.3$ channel and reported on an abnormal nociceptive behavior caused by the mutation (7). The $Ca_v2.3$ knockout mouse was also noticeable with its relatively short staying compared with wild-type mice in the center of the open-field, which may suggest some abnormal emotional state.

The limbic system, in particular hippocampus, is widely recognized for having central significance in certain aspects of CNS (central nervous system) functions such as emotional behaviors, learning and memory of spatial recognition (8, 9). The cellular mechanisms underlying these activities are accounted for by particular synaptic properties of this structure, in which long-term potentiation (LTP) is hypothesized as the substrate for enduring information storage. Studies on mice mutant for molecules related to the Ca^{2+} signaling, such as, NMDA-R1, αCaMKII, PKCγ, and CREB, have exhibited a distinct set of impairments in the hippocampal synaptic plasticity and in learning/memory (10). Although, in addition to NMDA-R, the VDCC is an essentially important source of Ca^{2+} influx for induction of LTP in particular conditions (11), involvement of their subtype remains controversial.

In this study, we, therefore, investigated contribution of $Ca_v2.3$ Ca channel to the synaptic physiology and plasticity in the CA1 pyramidal neurons using the

Abbreviations used: high-voltage activated, HVA; low-voltage activated, LVA; VDCC, voltage dependent calcium channel; long-term potentiation, LTP; theta burst stimulation, TBS; *N*-methyl-D-aspartate acid receptor, NMDA; α-calcium-calmodulin-dependent kinase II, αCaMKII; adenosine 3′5′-monophosphate-responsive element binding protein, CREB; cyclic AMP response element binding protein.

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gene-targeted mice. The $Ca_v2.3$ knockout mice were also examined for the fear conditioning and the Morris water maze test to estimate their ability of emotional and spatial learning.

MATERIALS AND METHODS

Animals. Wild-type ($Ca_v2.3+/+$), heterozygous mutant ($Ca_v2.3+/-$), and homozygous ($Ca_v2.3-/-$) mice were generated as described previously (7).

RNA in situ hybridization. *In situ* hybridization to frozen brain sections was performed as described (7, 12). To prepare riboprobes for mouse *cacnale* encoding $Ca_v2.3$ channel, a partial cDNA fragment (1 kb) corresponding to the cytoplasmic loop between the repeat II and III of the $Ca_v2.3$ was amplified by RT-PCR and cloned into pCRII vector (Invitrogen). The resulting plasmid was linearized and used for synthesis of DIG-labeled riboprobes.

Electrophysiology. Four to seven week-old mice were anesthetized with pentobarbital sodium (50 mg/kg) and were decapitated. Hippocampal slices (400 μm) were cut with a vibratome (VT1000S; Leica, Germany) and were placed in a holding chamber for at least 1 h in RT. A slice was then transferred to the recording chamber. The slice was maintained at 27 ± 1°C with a thermo-controller (DTC-200T, Dia Medical System Co., Japan) and continuously superfused with artificial cerebrospinal fluid (ACSF, 2.5 ml/min) that had been saturated with 95% O_2-5% CO_2 (pH 7.4). The composition of the ACSF is (in mM): 137 NaCl, 2.5 KCl, 0.58 NaH_2PO_4, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 21 $NaHCO_3$, and 10 glucose. For all experiments, (+)-bicuculline (5 μM; Sigma) was added to the perfusate in order to eliminate inhibitory transmission, and a cut was made between CA3 and CA1 region. Field excitatory postsynaptic potential (fEPSP) was recorded from the stratum radiatum with a glass pipette filled with 0.5 M NaCl (resistance 8–10 MΩ) using an amplifier (WPI, DAM 80, U.S.A.). fEPSPs were evoked by stimulation of Shaffer collaterals with a bipolar co-axial tungsten electrode placed on the stratum radiatum and stimulus was applied every 15 s. Stimulus strength initial EPSP slopes of 0.11–0.15 mV/ms, and LTP was induced by conditioning stimulation with the theta-burst stimulation (TBS) pattern: 5 trains at 10-s intervals of 10 bursts at 5 Hz, with each burst consisting of 4 stimuli at 100 Hz.

Behavioral studies. For behavioral studies, animals at the age of 7 to 13 weeks were tested between 10 AM and 6 PM. Naive adult mice were housed individually for at least 1 week before behavioral testing, and during this period they were handled for 1 min every day to reduce stress.

Contextual- and tone-dependent fear conditioning. Experimentation was set following the previous report (13). The small chamber (10 × 10 × 10 cm) with a stainless-steel rod floor (CL-MI; O'Hara, Tokyo, Japan) was placed in a sound-isolation box with a lamp inside. Scrambled shocks (1 s, 0.5 mA) were delivered to the grid floor. Behaviors of mice were recorded on videotapes. Freezing was defined as the absence of any locomotion during the continuous 2 s in recording frames, and the percentage of freezing was calculated per minute. Before experiments, the chamber was cleaned with 1% acetic acid. For tone-dependent fear conditioning test, mice were placed in the conditioning chamber for 3 min and then presented with 3 sets of tone and foot-shock repeated at 2-min intervals. Freezing responses were monitored during these conditioning and for additional 100 s after the last foot-shock. On the next day the mice were placed in a novel chamber. Freezing was scored for 3 min before the tone presentation and for subsequent 6 min in the presence of tone. For contextual fear conditioning test, mice were placed in the chamber for 3 min and then given 3 foot-shocks (1 s, 0.5 mA) at 1 min intervals. Freezing responses were monitored for 10 min. On the next day, freezing response was scored for 10 min in the same chamber as that used for the fear conditioning without foot-shocks.

Water maze test. Procedures were principally based on those of Morris and colleagues (14) and slightly modified following Tsien and colleagues (15). The training session consisted of the 3 days of hidden-platform test, and was followed by the 2 days of visible-platform test. A circular pool (100 cm diameter and 30 cm height) was filled with opaque water with white paint (22–24°C). On the first day, mice were allowed to get accustomed to shallow and then deep water without the platform. On the next day, a mouse was placed for 30 s on the platform (10 cm diameter and 1 cm beneath the water surface). Each mouse was given 2 sessions of 3 training trials per day with a 20 min interval between trials. The starting points for each mouse were chosen randomly, and the latency to reach the platform was recorded. If it failed to reach the platform within 60 s, the experimenter guided it to the platform, and the trial was assigned to a latency of 60 s. In the hidden platform test, the platform location was always in the same position for each mouse. The probe trial was run after the last hidden trial on the third day. Duration and the number of crossing over the platform position were counted for estimation of accuracy of spatial memory. In the visible platform test, the submerged platform was indicated with a black flag located at 4 cm high from the platform, and its location was varied randomly for each trial. Data were averaged over blocks of 3 trials. Swimming paths were recorded with a video camera connected to a digital tracking device and processed with a software for the water maze analysis (O'Hara, Tokyo, Japan), which was modified from the NIH Image program (developed at the U.S. National Institutes of Health and available at http://rsb.info.nih.gov/nih-image/).

We excluded the data from mice that stopped searching the platform and floated in the water for more than 100 s in total over the whole series of trials. There was a tendency that this type of abnormality was observed in the $Ca_v2.3-/-$ group.

Statistical analysis. All data are presented as mean ± SEM. Group differences were analyzed using the Tukey–Kramer test for multiple comparisons. Difference was considered significant at $P < 0.05$. All the experiments were performed in a blind manner.

RESULTS

Expression of $Ca_v2.3$ Channel mRNA

To test the possible involvement of this channel subtype in the hippocampal functions, the expression of $Ca_v2.3$ channel mRNA was examined using *in situ* hybridization with a probe for mouse *cacnale* gene in hippocampal sections from adult wild-type mouse. A large number of positive cells were identified in the pyramidal cell layers of CA1 and CA3, as well as in the granule cell layer of dentate gyrus (Fig. 1A), suggesting abundant expression of this channel in the hippocampus.

Synaptic Transmissions and LTP in CA1 Region in Hippocampus in $Ca_v2.3-/-$ Mice

Contribution of the Ca^{2+} channel encoded by $Ca_v2.3$ gene to synaptic transmission and plasticity was investigated by comparing the excitatory synaptic responses in CA1 pyramidal neurons between wild-type and knockout mice. fEPSPs were recorded from the stratum radiatum in CA1 in response to single electrical shock to the Schaffer collateral. The shape of fEPSP and input-output relation that was indicated by plotting an initial slope of fEPSP versus presynaptic fiber-volley amplitude were indistinguishable between

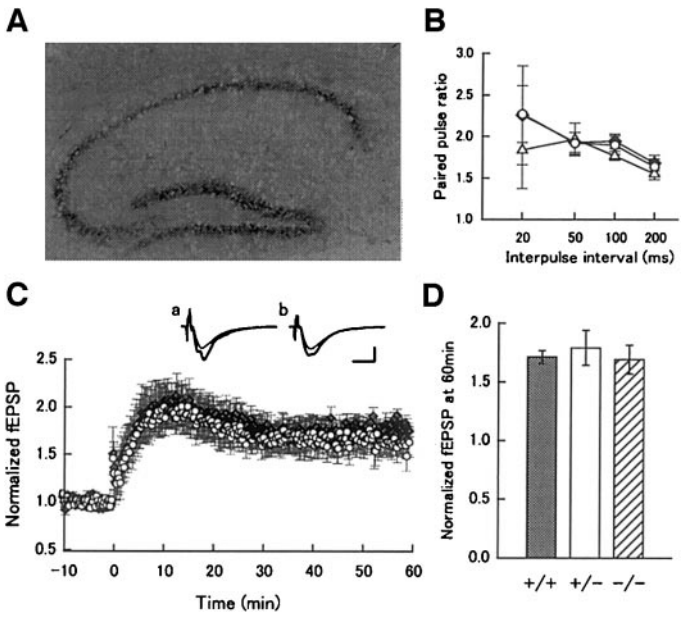

FIG. 1. Identification of $Ca_v2.3$ channel mRNA and expression of LTP in CA1 region in hippocampus. (A) $Ca_v2.3$ expression in the hippocampus. A sagittal section from adult wild-type mouse was *in situ* hybridized with a DIG-labeled *cacnale* riboprobe. High level expression was detected in the pyramidal cell layer of CA1–CA3 and granule cell layer of DG. (B) Magnitude of the paired pulse facilitation of fEPSP in CA1 region for each group ($Ca_v2.3+/+$, filled diamond, n = 10; $Ca_v2.3+/-$, open triangle, n = 8; $Ca_v2.3-/-$, open circle, n = 11). The averaged ratio of the second fEPSP slope to the first fEPSP slope was plotted against the interpulse intervals. (C) Robust LTP was induced by TBS to the Schäffer collateral-CA1 synapse in $Ca_v2.3-/-$. The averaged time course of LTP in $Ca_v2.3+/+$ (filled diamond, n = 13 slices from 5 mice) and $Ca_v2.3-/-$ (open circle, n = 13 slices from 7 mice) are shown. Each point represent the averaged fEPSP slope normalized to the baseline slope averaged for 10 min prior to conditioning. TBS was applied at time 0. Inset are the representative superimposed traces (average of 12 sweeps) of fEPSP obtained from $Ca_v2.3+/+$ (a) and $Ca_v2.3-/-$ (b) slices before (thin traces) and 60 min after conditioning (thick traces). Calibration bars indicate 0.2 mV and 10 ms. (D) Comparison of LTP maintenance by the three genotypes of mice. Apparent LTP was elicited in the slices from $Ca_v2.3-/-$ (hatched column, n = 13) as much as the other genotypes ($Ca_v2.3+/+$, dark column, n = 13; $Ca_v2.3+/-$, open column, n = 14).

$Ca_v2.3+/+$ and $Ca_v2.3-/-$ mice (data not shown). Response profile to the consecutive stimuli with a short interval is a useful index for characterizing the presynaptic terminal property for transmitter release. In Fig. 1B, the magnitude of paired-pulse facilitation was plotted as a function of the inter-pulse interval (IPI). Paired-pulse ratio (initial slope of the second fEPSP divided by that of the first) ranged in 1.6 to 2.3 over 20–200 ms of IPI, and there was no difference between the genotypes. Previous studies have revealed that LTP induced by theta-burst stimulation (TBS) to the Schaffer collateral-CA1 synapses was reduced by Ni^{2+} (25 μM) which is known as a preferential blocker of the R- and T-type Ca^{2+} channels in rat (16). Since these results suggest Ni^{2+}-sensitive Ca^{2+} influx possibly through the R-type channel may be involved in TBS-induced LTP, we tested if this synaptic plasticity is expressed at the Schaffer collateral-CA1 synapses from $Ca_v2.3-/-$ mouse. As shown in Fig. 1C, fEPSP slope increased to the twice as large as the control at around 10 min after conditioning stimulation with TBS to the same input, and the elevated response was maintained at least for 60 min. The magnitude of LTP measured at 60 min was 1.71 ± 0.06 in $Ca_v2.3+/+$ (n = 13 slices from 5 mice), 1.79 ± 0.15 in $Ca_v2.3+/-$ (n = 14 slices from 7 mice) and 1.69 ± 0.12 in $Ca_v2.3-/-$ (n = 13 slices from 7 mice), which shows no statistically significant difference between each other (Fig. 1D). The potentiation by this conditioning protocol was NMDA-R dependent because a specific blocker to this receptor, D-APV (100 μM), completely inhibited the established potentiation (0.98 ± 0.07 of the control at 30 min after the conditioning stimulus, n = 2).

Contextual Fear Conditioning

We have previously reported the behaviors indicating abnormal emotional states in $Ca_v2.3-/-$ mice. In order to determine if the deletion of $Ca_v2.3$ induces alterations in hippocampus-dependent behavior in the mutant mice, the fear conditioning test was performed by employing the contextual-fear conditioning as well as the tone-dependent fear conditioning, for which hippocampal and non-hippocampal contributions are considered to be responsible, respectively, as shown by lesion studies (17, 18). First, we studied the effect of deficiency of $Ca_v2.3$ channel on the hippocampus-dependent task. A conditioned freezing was characterized by an immobile, crouching posture during conditioning noxious stimuli or after transferring to the same context in the absence of the noxious stimuli (19). Compared with the wild-type animals, $Ca_v2.3+/-$ and $Ca_v2.3-/-$ mice displayed a comparable degree of freezing responses in the training phase with three foot shocks (Fig. 2A, a). Moreover, in the test phase on the next day, their freezing responses were intensified during 6 min after exposure to the recording chamber without foot-shocks in a similar way to that of the wild-type mice (Fig. 2A, b).

Tone-Dependent Fear Conditioning

In the training phase of tone-dependent fear conditioning (Fig. 2B, a), a significantly lower frequency of freezing was observed in $Ca_v2.3-/-$ mice (16.0 ± 6.6%, n = 5) than in the other groups during and for 40 s after the last foot-shock ($Ca_v2.3+/+$: 50.5 ± 7.6%, n = 10, p = 0.020, $Ca_v2.3+/-$: 44.9 ± 5.9%, n = 14, p = 0.043). However, the impairment of the freezing response in $Ca_v2.3-/-$ mice was restored by prolonged exposure for 1 min in the conditioning chamber. On the next day, all groups displayed similar degrees of freezing in a new context with the tone stimulus alone (Fig.

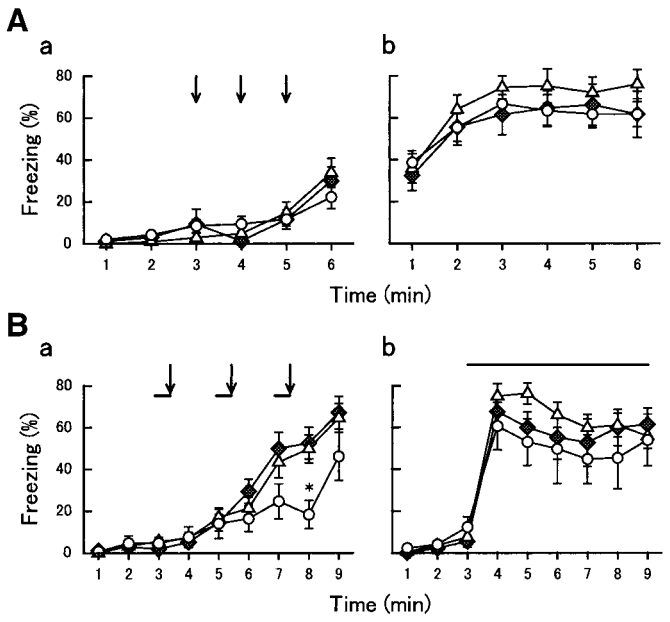

FIG. 2. Contextual- and tone-dependent fear conditioning. (A) In the contextual fear conditioning test, a comparable degree of freezing responses were displayed among all genotypes in the training phase (a) and the test phase (b). Three foot shocks were given at 3–5 min after the start of recording ($Ca_v2.3+/+$: n = 9; $Ca_v2.3+/-$: n = 10; and $Ca_v2.3-/-$: n = 15). (B) In the training phase of the tone-dependent fear conditioning (a), a significantly lowered level of tone-dependent learning was apparent in $Ca_v2.3-/-$ mice on the conditioning day (7–8 min) compared with between $Ca_v2.3+/+$ and $Ca_v2.3+/-$ mice. But this impairment of freezing responses was restored by longer exposure in conditioning chamber at 9 min. On the next day, all genotypes displayed a comparable degree of freezing in the test phase (b) with the tone in a novel context. Data from three genotypes were shown as $Ca_v2.3+/+$, filled diamond; $Ca_v2.3+/-$, open triangle; $Ca_v2.3-/-$, open circles. The arrow and solid line indicate the foot shock and the duration of tone stimulation, respectively.

2B, b). Thus, it is suggested that $Ca_v2.3-/-$ mice retain intact ability of both hippocampus-dependent and independent types of fear learning.

Spatial Learning and Memory in the Morris Water-Maze Test

Another examination of the behavior indicative of the hippocampal function is the Morris water-maze test, which has been frequently used to estimate the ability of spatial recognition and learning. During acquisition phase, $Ca_v2.3-/-$ mice showed slightly longer escape latencies than the other groups, but consistently improved their performance to reach the platform (Fig. 3A). In the summarized results, no significant differences in the latency at any session point were found among the three groups. To more precisely determine the participation of spatial-cue guided learning performance, the probe test was done after the end of the hidden test. In the absence of the platform, mice explored the learned area. In comparisons of the time spent in the target quadrant, $Ca_v2.3-/-$ mice displayed the lower score (17.1 ± 2.0 s: n = 10) than the other littermates ($Ca_v2.3+/+$: 27.5 ± 2.9 s, n = 17, p = 0.011 and $Ca_v2.3+/-$: 24.7 ± 1.4 s, n = 7, p = 0.045, Fig. 3B). The difference was also prominent for the crossing times over the target; $Ca_v2.3-/-$: 2.4 ± 1.4, compared with $Ca_v2.3+/+$: 8.1 ± 1.2 (p = 0.016), or with $Ca_v2.3+/-$: 7.6 ± 1.0 (p = 0.016) as shown in Fig. 3C. Furthermore, it was noticed that some mice swam near the wall along fixed circular path. This stereotypic swimming pattern is noted as *thigmotaxic* swimming, which has been regarded as an indication of the emotionally disturbed states (20). Mice from all the genotypes swam in the peripheral annulus in the free swimming, and early training sessions. As the training went on, wild-type mice changed their pathway more directing to the platform. However the $Ca_v2.3-/-$ mice tended to stay in the circular pattern in the late trials even though their path-length of swimming became shorter (Fig. 3D). This tendency was quantified from the last session by averaging the percentage of swimming time spent in the peripheral 10 cm annulus from the wall as indicated in Fig. 3E. There was a significant difference in this peripheral swimming time between the $Ca_v2.3-/-$ mice (35.7 ± 12.9%) and $Ca_v2.3+/+$ (10.1 ± 3.4%, p = 0.013) or $Ca_v2.3+/-$ mice (6.9 ± 2.0%, p = 0.002).

In the visible platform test, no significant differences in the performance were observed between the groups (Fig. 3F), excluding the possibility of any deficits in the motor or visual functions involved in the above tests.

DISCUSSION

In the present study, a high level expression of $Ca_v2.3$ channel was detected in the hippocampal formation of the wild-type mouse by *in situ* hybridization. This is consistent with the reports by Northern analysis of $Ca_v2.3$ mRNA in the adult rat hippocampus (21, 22) and by immunocytochemical staining with antibodies specific for $Ca_v2.3$ channels (21). We therefore focused on the effects of $Ca_v2.3$ channel deficit on hippocampal functions.

A variety of studies using animals with lesions or genetic disruptions have evidenced the close correlation between the hippocampal synaptic plasticity and the spatial and contextual learning/memory (10, 23). Our results from the $Ca_v2.3$ mutant mice, however, exhibited impaired spatial memory with the intact synaptic plasticity in CA1. Two considerations may be possible for this discrepancy. First, the level of potentiation is known to be strongly influenced by the temporal structure of conditioning protocol used for inducing LTP (24). In fact, αCaMKII-mutated mice displayed frequency-dependent characteristics, in which LTP was maintained by 100 Hz stimuli but not by 5–10 Hz (theta frequency) (25). Therefore, other

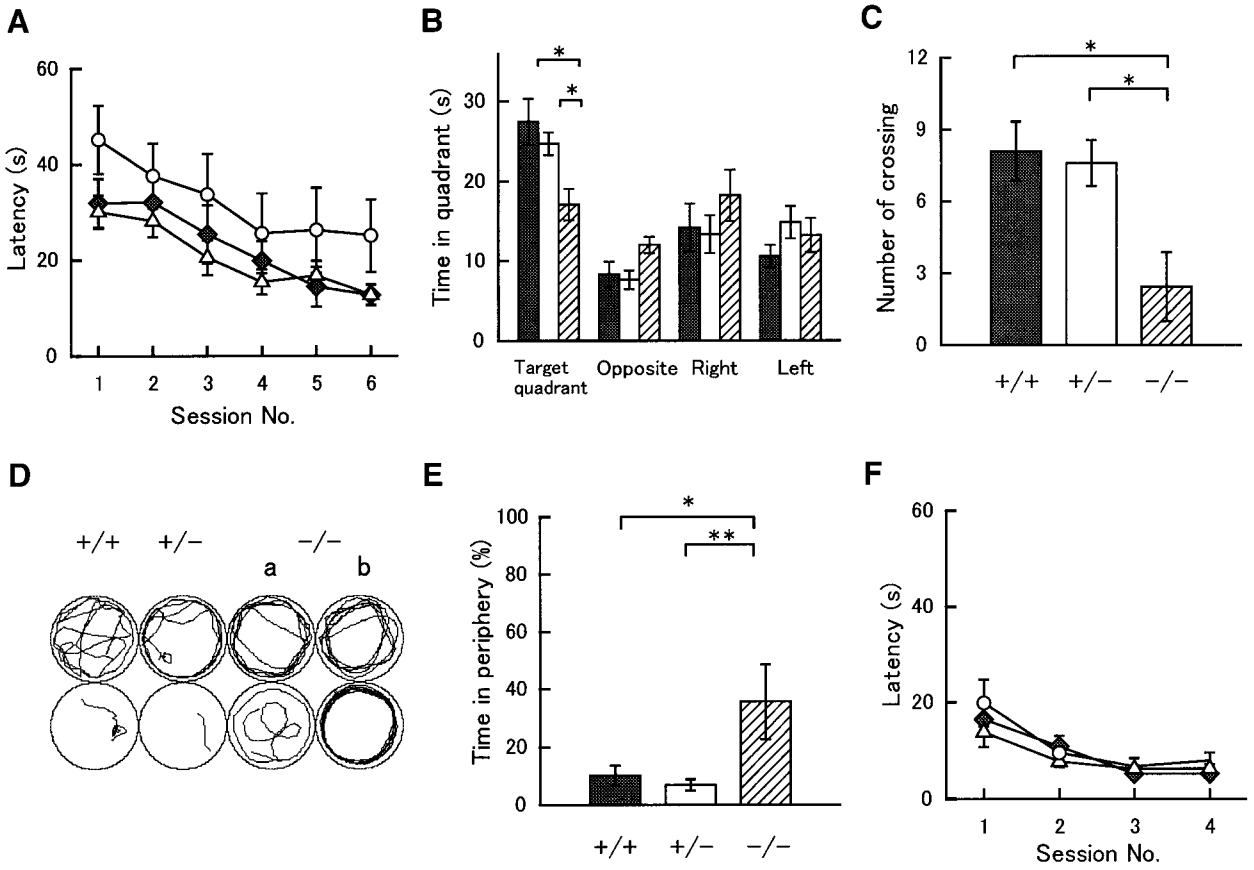

FIG. 3. Spatial learning and memory in the Morris water-maze test. (A) Escape latency in the hidden platform test. $Ca_v2.3-/-$ mice improved their performance during training although their latency was longer than the other genotypes at each session. (B) The average time spent in each quadrant during the probe trial. $Ca_v2.3-/-$ mice showed a tendency of decreased spatial preference for the target quadrant as compared with the other genotypes. (C) The number of crossings over the exact location of the former platform. $Ca_v2.3+/+$ and $Ca_v2.3+/-$ mice precisely crossed over the position more often than $Ca_v2.3-/-$ mice. (D) Examples of swimming paths in the first and the last trial in the hidden platform test of each genotype. $Ca_v2.3-/-$ mice swam longer distance in the last trial as shown in the lower panel of −/− a, b. Note also that the stereotypic circular swimming path was shown in the lower panel of −/− b. (E) Swimming time in the periphery of the pool. Time spent in the area of 10 cm inside from the wall was summated during the last three trials of the hidden platform test. $Ca_v2.3-/-$ mice swam predominantly at the peripheral zone for longer time (*$p < 0.05$, **$p < 0.01$). (F) Escape latency in the visible platform test. No significant differences between the genotypes were observed. $Ca_v2.3+/+$, filled column and filled diamonds, n = 10; $Ca_v2.3+/-$, open column and open triangles, n = 17; $Ca_v2.3-/-$, hatched column and open circles, n = 7.

patterns of conditioning stimulation might enable us to uncover defects in the plasticity in our mutants.

Second, the inferior performance in water maze test could be attributable to the abnormal emotional state in $Ca_v2.3$ channel deficient mice. In addition to the previous description (7), we observed in the present study the longer thigmotaxic swimming time in periphery of the pool, and as well a higher tendency to encounter the floating animals among $Ca_v2.3$ mutant mice than other genotypes. These behaviors are characteristics for the animal under stressful conditions in general. We may be able to interpret the results such that the $Ca_v2.3$ mutant mice were more susceptible to stress due to exposure to cold water, resulting in the deprived learning opportunity even though they have ability to learn. These emotional disturbances may be derived from the altered functions in other limbic structures such as parahippocampal areas, including the amygdala and hypothalamus, where the abundant expression of this channel protein or message was confirmed (21, our unpublished observation on data).

On the other hand, the mutant mice displayed fairly normal scores of emotional aspects assessed by fear memories which are associated with tone or context. The former form of fear memory is identified to link with amygdala function, and the latter one with the hippocampal activity (18). Thus, it may be suggested by these findings from the mutant mice deficient of the single molecule that there are multiple and independent mechanisms underlying spatial memory (water-maze test) and emotional memory (contextual fear) for hippocampal functions, as well as amygdala-mediated fear memory (tone-fear) and above-mentioned emotional abnormality (thigmotaxis).

At present, there are no mechanistic explanations for the impaired spatial memory of the $Ca_v2.3$ deficient mouse evidenced in this study. The basic presynaptic activity, as well as gross plasticity in CA1, was well maintained. We may therefore need to explore the functional alteration in other aspects of neuronal activities such as the dendritic integration of synaptic inputs, or correlated firings among neurons, particularly of the neurons tuned to specific location ("place cells", 26).

The $Ca_v2.3$ channel has been identified to conduct R-type current of HVA, which however indicated similarities to T-type current (LVA) by their moderate activation range and inactivation kinetics in contrast to the other HVA Ca^{2+} channels (2, 4, 6). The single channel recording study (27, 28) as well as imaging studies using Ca^{2+} indicator dyes (29) revealed that the medium-range activated, possibly R-type, and Ni^{2+}-sensitive, likely T- and R-type currents were preferentially identified in the distal dendrites of the CA1 pyramidal neurons of rats. These findings seem meaningful from the point of view of the dendritic integration. Dendritic Ca^{2+} spikes may boost distal synaptic inputs transferring to the soma, Ca^{2+} influx-triggered depolarization would determine the membrane excitability and induce burst firings, and a local rise in $[Ca^{2+}]_i$ near the site of input are definitely essential to the plasticity (30). In fact, in CA1 pyramidal neuron, the distally located synapses are remarkably effective for firing of the cells (31, 32).

Interestingly, related to the location of the R-type channels, the distal portion of the pyramidal neuron dendrites harbor rich innervation of adrenergic fibers (33). Björklund and colleagues (34) reported that mice over-expressing α_{2C}-adrenoceptor in CA1 region showed a remarkable thigmotaxis. This may suggest the possible functional linkage between R-type Ca^{2+} channel and the α_{2C}-adrenoceptor that is known to inhibit Ca^{2+} channels via $G_{i/o}$ (35). It may be therefore worth examining if the α_2-adrenoceptor agonist exacerbate the thigmotaxis in the present $Ca_v2.3$ channel deficient mice not as much as in the wild-type mice.

Another unanswered question raised by the present study is which VDCC is important for LTP expression. Considering the reported Ni^{2+} sensitivity of TBS-induced LTP in CA1 (16) together with robust LTP observed in the present study, it may be suggested that the VDCC contributing to TBS-LTP in the CA1 may be the T-type, or the R-type encoded by gene(s) other than $Ca_v2.3$, which was recently suggested by Wilson and colleagues (36).

In conclusion, we presented that the $Ca_v2.3$ channel was essential for the accurate spatial memory among the hippocampus-mediated functions in spite of the apparently normal synaptic plasticity in CA1. Further investigations are expected to clarify the roles of the $Ca_v2.3$ channel in the neuronal signaling with a causative relation to behavioral phenotypes. Our genetically modified mouse strain would provide a useful and sensitive means for detailed investigation of the physiological roles of the Ca^{2+} channel encoded by $Ca_v2.3$ genes and others.

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