Supporting Information

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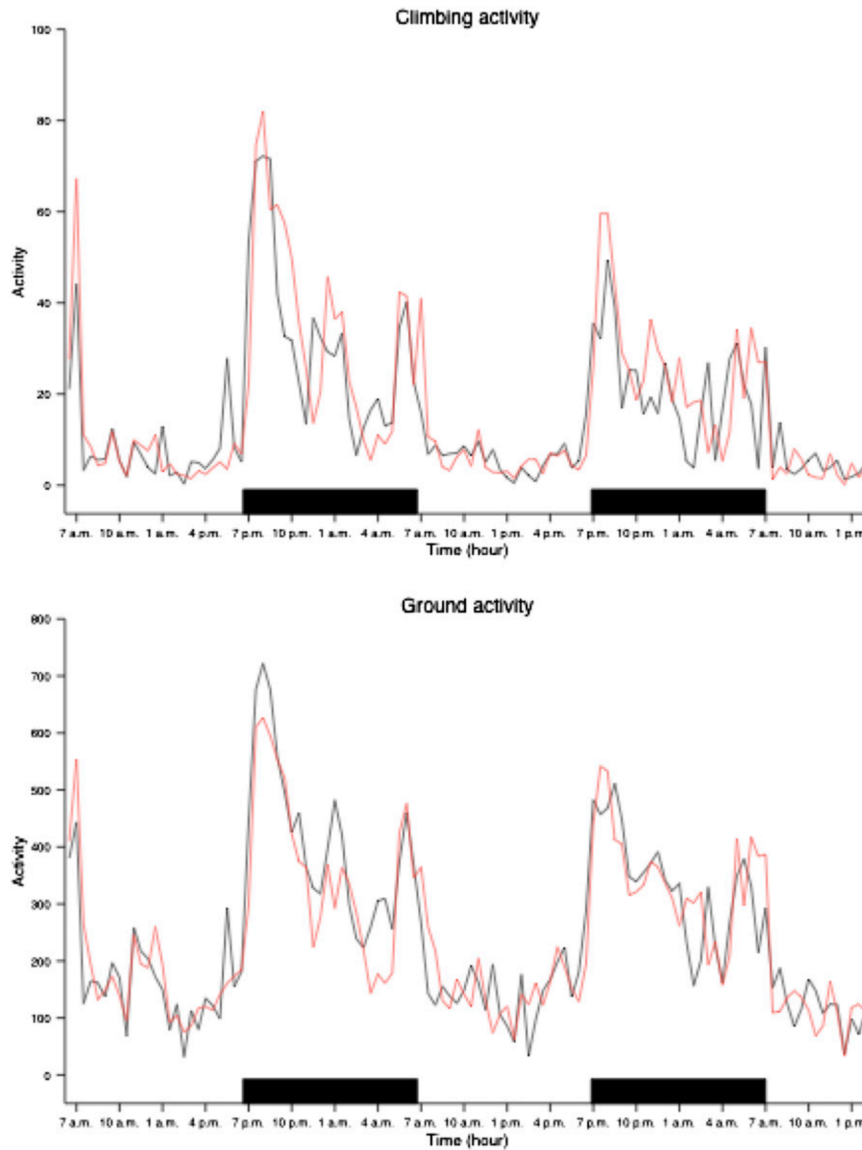

Fig. S1. Nycthemeral activity rhythm. Two day follow-up of the locomotor activity of WT (n = 16) and $Ca_V3.1$ KO animals (n = 12) are indicated by blue and red traces, respectively. Animals for experimental purpose were maintained on a 12 h day–12 h night cycle, and activity recording was started after 1 d adaptation to the new cage. No obvious alteration of the sleep cycle was observed. Furthermore, note the similar behaviors of the two genotypes: $Ca_V3.1$ gene deletion does not induce major changes of activity as detected by a number of motion sensors distributed in the cage, either rearing (upper traces) or the sum of all activity minus rearing plus all displacements on the floor of the cage (lower traces). Mean values are shown.

Fig. S2. Absence of effect of genetic or pharmacological inactivation of $Ca_V3.1$ function on basic motor footprint test and eye movements. (*A*) Analysis of footprint patterns (*Methods*). In WT and $Ca_V3.1^{-/-}$ mice (black and red, respectively), the distance between footprints of both the left and the right limbs and their lateral spreading were almost identical (respectively for WT and $Ca_V3.1^{-/-}$ mice, in cm: left limb stride, 7.3 ± 0.2 and 7.4 ± 0.2; right limbs stride, 7.4 ± 0.2 and 7.4 ± 0.2; limbs spreading, 2.7 ± 0.1 and 2.8 ± 0.1; at least P values > 0.35, 12 $Ca_v3.1^{-/-}$ and 16 WT). Deletion of $Ca_v3.1$ does not affect normal walking. (*B–D*) The baseline characteristics of compensatory eye movements. Compensatory eye movements evoked by sinusoidal visual and/or vestibular stimulation were analyzed in control mice (n = 9), $Ca_v3.1^{-/-}$ mice (n = 12), and mice injected with T-type antagonist (TTA)-P2 (n = 7). Neither the optokinetic reflex (OKR, *B*) nor the vestibulo-ocular reflex (VOR, *C*) were affected by manipulation of the $Ca_v3.1$ channel. Combined visual and vestibular stimulation, to generate the visually enhanced VOR (VVOR, *D*), revealed a slight impairment compared with controls in the $Ca_V3.1^{-/-}$ mice, but not in mice injected with TTA-P2.

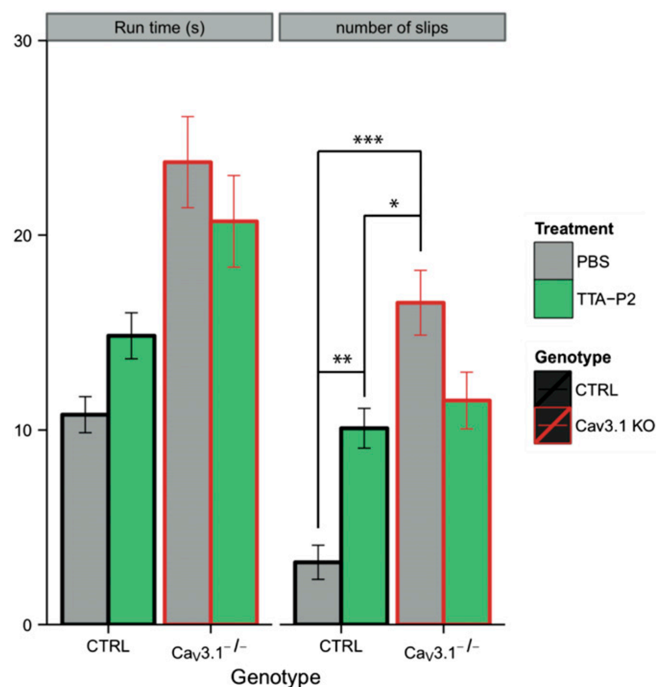

Fig. S3. Motor behavioral deficit after acute inactivation of T-type channels. To exclude compensatory effects that could have intervened during development, similar tests were carried after i.p. injection of TTA-P2 at 2 mg/kg. In parallel, control animals received an i.p. injection of phosphate buffered solution (PBS). The day before testing, the animals were trained on the elevated beam as described above. For i.p. injections, TTA-P2 was prepared as a 5 mM stock solution in DMSO to be diluted 10-fold in PBS pH 7.2 just before injection of 0.1 mL per 10 g of body weight (i.e., at 2 mg/kg). Such injections should result in ∼1–2 μM extracellular concentration and were assumed to be achieved during the 25 min necessary for a series of four successive behavioral tests. The experiment was started 120 min after injection by a 10 min observation of global locomotor activity, followed by three trials on an 9 mm diameter and 100-cm-long horizontal beam (on average 1 min per trial, latency, walk to the platform, and return to the cage). Each animal was successively examined for its walk on an elevated beam. The time to reach the platform and the number of errors increased in $Ca_v3.1^{-/-}$ mice, $F(1, 37) > 27.05$ ($P < 0.001$, repeated measures ANOVA). There is an effect of the treatment only in the number of slips; a Tukey post hoc analysis shows that there is a significant decrease of performance in TTA-P2–injected WT animals compared with PBS-injected WT animals ($P < 0.01$); in PBS-injected $Ca_v3.1^{-/-}$ compared with PBS-injected WT animals ($P < 0.001$), which is coherent with Fig. 3*A*; and in TTA-P2–injected WT mice compared with PBS-injected $Ca_v3.1^{-/-}$ mice ($P = 0.10$), which could be explained by the fact that TTA-P2 could block other T-type channels. All other paired-wise comparisons are not significant ($P > 0.05$). These results show that acute inactivation of T-type calcium channels with TTA-P2 produces a reduced motor performance. Values on figures are mean ± SEM. There are nine $Ca_v3.1^{-/-}$ mice injected with PBS, 11 $Ca_v3.1^{-/-}$ mice injected with TTA-P2, 10 WT mice injected with PBS, and 11 WT mice injected with TTA-P2.

Table S1. Paired-pulse ratio before and after induction protocol

Long-term plasticity	Control	TTA-P2	$Cav3.1^{-/-}$
Long-term potentiation			
Before induction	1.60 ± 0.10	1.68 ± 0.07	1.62 ± 0.13
After induction	1.75 ± 0.15	1.67 ± 0.05	1.77 ± 0.11
P value	0.31 (*n* = 8)	0.81 (*n* = 7)	0.16 (*n* = 7)
Long-term depression			
Before induction	1.83 ± 0.06	1.80 ± 0.07	1.88 ± 0.10
After induction	1.78 ± 0.08	1.84 ± 0.06	1.79 ± 0.07
P value	0.30 (*n* = 7)	0.68 (*n* = 7)	0.22 (*n* = 7)

Mean ± SEM.