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Supplemental Data

Selective Engagement of Plasticity Mechanisms for Motor Memory Storage

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Additional Text

Changes in VOR Phase Accompanying Changes in VOR Gain

Throughout all experiments, we measured the dynamics of the vestibulo-ocular reflex, as reflected by the phase of the VOR. VOR phase is a measure of the timing of peak eye velocity relative to peak head velocity (see Methods). Data on 24-hour retention of changes in VOR phase induced by 30-minute training sessions were consistent with those for retention of changes in VOR gain (Fig. 1C, 1D). Thirty minutes of gain-down training induced large and significant increases in phase lead ($p < 0.005$), however, the induction and retention of these phase changes were not different between wild-type and CaMKIV knockout mice (Fig. S1B; $p > 0.05$ at 30 min; $p > 0.85$ at 24 hours; t-test). During 30 minutes of gain-up training, the changes in VOR phase were small, and not significant (Fig. S1A; $p > 0.05$, paired t-test), as reported previously (Boyden and Raymond, 2003). However, with three, 30-min gain-up training sessions (separated by 24-hr retention periods in darkness), wild type mice exhibit a significant decrease in phase lead (Boyden and Raymond, 2003). CaMKIV knockout mice were significantly impaired in the retention of this phase lead after the second 24-hour retention period (Fig. S1C; $p < 0.05$; t-test). In contrast, after the third retention period, the learned changes in VOR phase in CaMKIV knockout mice were similar to those in wild-type mice ($p > 0.40$). Thus, whereas the memory for the gain changes associated with gain-up training were enduringly impaired by the deletion of CaMKIV, memory for the phase change was relatively intact with extended training. This dissociation between retention of changes in gain and phase during extended gain-up training demonstrates that even for a single learning task, different components of the motor memory can rely differentially upon a particular molecular mechanism.

We also examined the phases of the baseline sensory and motor performance measures. The phases of the VOR, OKR, and tracking of the gain-down stimulus were similar between knockouts and wild-types (Fig. S1D; $p > 0.1$ for VOR and OKR, $p > 0.05$ for tracking gain-down). For tracking of the gain-up training stimulus, there was a statistically significant ($p < 0.05$) but extremely small difference in phase between knockout and wild-type mice, which had tracking phase lags of $2.3^{o} \pm 0.33$ vs. $3.6^{o} \pm 0.35$ respectively.

Analysis of Memory Retention Using ANOVA

We performed a two-factor ANOVA on the 24-hour memory retention data for VOR gain increases, with genotype and frequency as the two factors. For this analysis, the data were log-transformed to facilitate the statistical comparison of ratios (data normalized to pre-training baseline). Neither factor alone was significant ($F_{3,166} = 1.01$, $p > 0.35$ for a main effect of frequency, $F_{1,166} = 0.02$, $p > 0.85$ for a main effect of genotype), but there was a significant interaction between genotype and frequency ($F = 2.90$, $p < 0.04$). This indicates that the effect of genotype on memory varies, depending on the frequency at which the experiment is performed. Thus, using three different multiple-hypothesis tests on the data from the gain-up memory retention experiments (Bonferroni corrected or uncorrected t-tests (see main text) and ANOVA), we were able to verify that the deficit in the CaMKIV knockout genotype was frequency-selective.

Two-Hour Retention of Gain Changes Is Normal in the CaMKIV Knockout

In Purkinje cell culture, cerebellar LTD is lost within 2 hours after induction, in the CaMKIV knockout. To investigate whether this timecourse would be reflected in the timecourse of forgetting for gain-up training, we measured memory retention 2 hours after 30 minutes of 1 Hz gain-up training. VOR memories thus assessed were intact in the CaMKIV knockout, compared to wild-type ($p > 0.45$; n = 8 KO, n = 9 WT). This result suggests that the kinetics of LTD expression differ between culture and *in vivo*. One explanation for a difference in expression kinetics is the difference in induction kinetics between stimulation-induced plasticity in culture and learning-induced plasticity *in vivo*. In intact animals, learning is induced over an extended period of time (30 minutes), whereas LTD *in vitro* is induced with brief, high-intensity postsynaptic AMPA and mGlurR1 activation and cellular depolarization (Ho et al., 2000). The temporally distributed nature of learning likely results in a memory that lasts longer than plasticity induced in culture with brief stimulation.

No Dependence of Gain-Down Learning and Memory on Training Stimulus Frequency

Since the impairment in gain-up memory in the CaMKIV knockouts varied with the stimulus frequency used for training and testing, we tested gain-down learning and memory over the same range of training frequencies. We found no difference between wild-type and CaMKIV knockout mice, either in gain-down learning (Fig. S2A, $p > 0.15$, 0.5 Hz; $p > 0.75$, 0.66 Hz, $p > 0.90$, 2 Hz) or gain-down 24-hour memory (Fig. S2B, $p > 0.80$, 0.5 Hz; $p > 0.55$, 0.66 Hz; $p > 0.85$, 2 Hz). This confirms that gain-down memories are spared in the CaMKIV knockout, across a range of training stimulus frequencies.

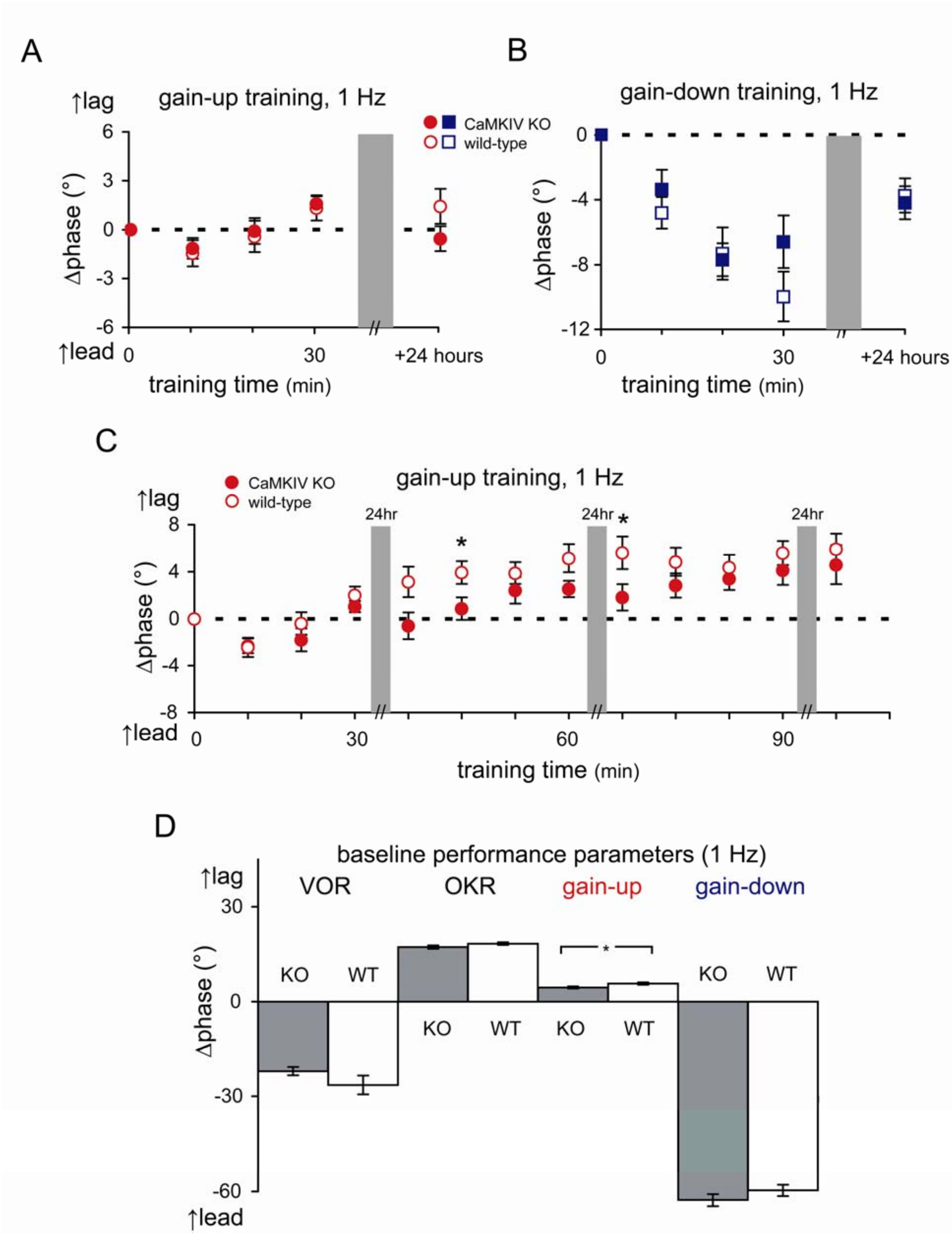
A
gain-up training, 1 Hz
CaMKIV KO
wild-type
↑lag
↑lead
Δphase (°)
training time (min)
+24 hours
B
gain-down training, 1 Hz
Δphase (°)
training time (min)
+24 hours
C
gain-up training, 1 Hz
CaMKIV KO
wild-type
24hr
↑lag
↑lead
Δphase (°)
training time (min)
D
baseline performance parameters (1 Hz)
VOR
OKR
gain-up
gain-down
KO
WT
↑lag
↑lead
Δphase (°)

Figure S1. Baseline and adapted values of eye movement phase.

(A) Changes in VOR phase accompanying the increase in VOR gain shown in Figure 2C. Filled symbols represent CaMKIV knockout mice, and open symbols represent wild-type mice.

(B) Changes in VOR phase accompanying the decrease in VOR gain shown in Figure 2D.

(C) Changes in VOR phase accompanying the changes in VOR gain shown in Figure 3. Asterisks indicate significant differences ($p < 0.05$) between wild-type and knockout mice, for the time points indicated.

(D) Baseline phase values for the VOR, OKR, and retinal slip during tracking of gain-up and gain-down stimuli, expressed relative to perfect tracking performance (filled bars: n = 30 knockout mice; open bars: n = 30 wild-type).

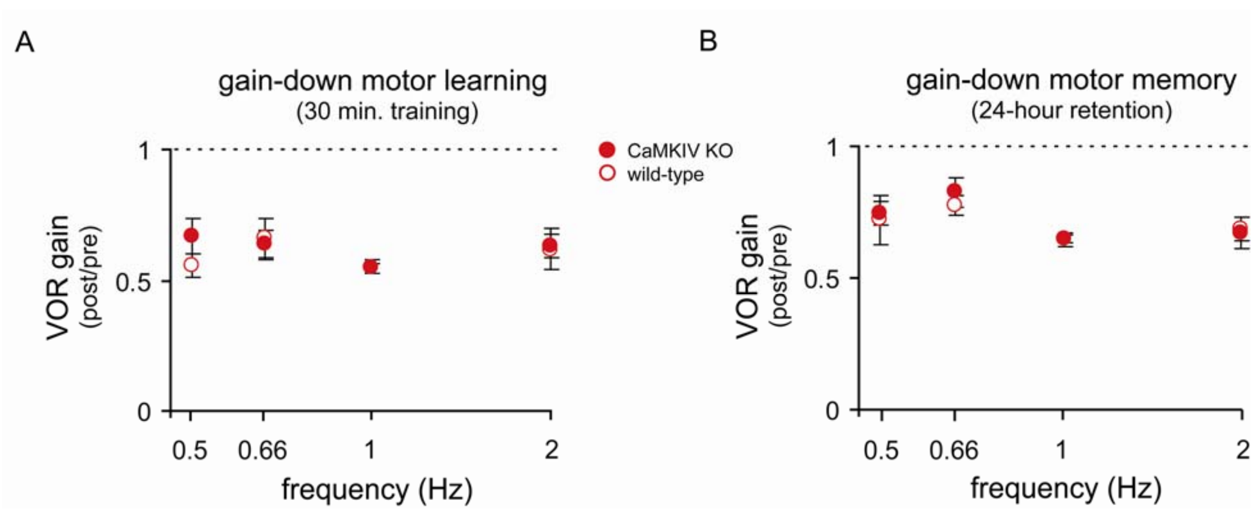

Figure S2. CaMKIV-independence of the memory for a decrease in VOR gain, across training/testing stimulus frequency.

(A) Changes in VOR gain induced by 30 minutes of gain-down training with 0.5, 0.66, 1, or 2 Hz rotational stimuli in CaMKIV knockout (filled symbols; n = 5 for 0.5 Hz, n = 6 for 0.66 Hz, n = 20 for 1 Hz, n = 6 for 2 Hz) and wild-type (open symbols; n = 5 for 0.5 Hz, n = 5 for 0.66 Hz, n = 20 for 1 Hz, n = 5 for 2 Hz) mice.

(B) Motor memory retention 24 hours after gain-down training with 0.5, 0.66, 1, or 2 Hz stimuli.