Elimination of climbing fiber instructive signals during motor learning

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Supplementary Figure 1

b. a. Climbing fibers Simple spikes Ipsi NOS 2 NOS 2 Contra 50 SOH per Monkey L Monkey E 20/40 44/49 Lead Lag Lead Lag Contra Ipsi head head

C. Learning



Neural responses and changes in VOR gain elicited by the x2T/x0BG training stimulus. Climbing fiber responses (a), Purkinje cell simple spike responses (b), and behavioral changes (c) associated with the x2T/x0BG training stimulus. Neural responses significantly different from zero are indicated by black symbols, and the number of significant neurons out of the total recorded is noted on each polar plot.

In Monkey L (circles), the climbing fiber response to the $\times 2T/\times 0BG$ stimulus was not statistically different from the response to the $\times 0T/\times 2BG$ stimulus (P > 0.05, paired t-test; Table 1). Nevertheless, $\times 2T/\times 0BG$ training induced a learned increase in VOR gain in Monkey L, in contrast to the decrease in VOR gain induced by $\times 0T/\times 2BG$ training (Fig. 4b). Thus, stimuli that induced similar climbing fiber responses could induce opposite changes in VOR gain.

x2T/x2BG	x0T/x0BG	x0T/x2BG	
Head Ipsi Contra 10 °/s	Ipsi Contra	Ipsi Contra	
1 s			
	and the first		

Spike trains in individual climbing fibers during training.

Rasters showing the responses in 15 climbing fibers in Monkey L (a) and 24 climbing fibers in Monkey E (b) during 35-40 cycles of the x2T/x2BG, x0T/x0BG, and x0T/x2BG training stimuli, aligned on the onset of ipsiversive head movement. Each tick represents the occurrence of a spike in the climbing fiber, measured as a complex spike in its Purkinje cell target. All recordings were from horizontal gaze velocity Purkinje cells (HGVPs).

x2T/x2BG	x0T/x0BG	x0T/x2BG
Head Ipsi Contra 10 °/s	Ipsi Contra	Ipsi Contra
1s		
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Average firing rate of climbing fibers did not vary across training stimuli.

Each set of connected points represents the average firing rate of a single climbing fiber during $\times 2T/\times 2BG$, $\times 0T/\times 0BG$, and $\times 0T/\times 2BG$ training stimuli, plus spontaneous activity. The training stimuli did not affect the average firing rate (P > 0.05, ANOVA).



Most of the learning occurs early in the training session.

Changes in VOR gain after 30 minutes of training in Monkey L, and 15 and 30 minutes in Monkey E. Each point represents the change in VOR gain at the indicated time points during a single replication of a behavioral training session. Solid bars represent the median change in VOR gain at the indicated time points, and dotted lines represent median changes in VOR gain during the entire training session (2 hrs for Monkey L and 1 hr for Monkey E; as shown in Figure 4). Most of the changes in VOR gain occurred early in the training session.



Climbing fiber responses in non-HGVPs during $\times 0T/\times 0BG$ and $\times 0T/\times 2BG$ training. Polar plots showing the climbing fiber responses recorded in Purkinje cells that were sensitive to head and/or eye velocity, but did not fulfill the criteria for classification as an HGVP. Individual climbing fibers with a response significantly different from zero are shown in black. Neither $\times 0T/\times 0BG$ nor $\times 0T/\times 2BG$ training elicited a significant response in this population of climbing fibers (P > 0.05, one sample t-test).



Monkey L ● "gain decrease" signal Monkey E ♦ "gain increase" signal

Climbing fibers with "gain decrease" and "gain increase" responses during x0T/x2BG training had similar responses during standard training stimuli.

The climbing fiber responses to x0T/x0BG, x0T/x2BG and x2T/x2BG training stimuli are plotted, with positive values representing an increase in firing rate during ipsiversive head velocity, and negative values representing an increase in firing during contraversive head velocity. 39 of the 45 climbing fibers shown in **Fig. 2** were recorded for all three stimuli, and were included in this plot and the analysis in **Supplementary Table 1**. Each climbing fiber was categorized according to whether its response during x0T/x2BG training was in the "gain decrease" (grey) direction or "gain increase" (black) direction. An increase in firing during contraversive head velocity was categorized as "gain decrease", since this is what is observed during standard x0T/x0BG training, which decreases VOR gain. An increase in firing during ipsiversive head velocity was categorized as "gain increase", since this is what is observed during standard x2T/x2BG training, which increases VOR gain. Climbing fibers with "gain decrease" and "gain increase" responses during x0T/x2BG training had similar responses during the standard training stimuli.



Climbing fiber and Purkinje cell simple spike responses to $\times 0.5T/\times 1BG$, $\times 1.5T/\times 0.5BG$ and $\times 1T/\times 0BG$ training stimuli.

Polar plots showing the climbing fiber (a) and Purkinje cell simple spike responses (b) of individual neurons to the $\times 0.5T/\times 1BG$ and $\times 1.5T/\times 0.5BG$ training stimuli (Monkey E) and the $\times 1T/\times 0BG$ training stimulus (Monkeys L and E, see Methods for detailed description of stimuli). Neural responses significantly different from zero are indicated in black symbols, and the number of significant neurons out of the total recorded is noted on each polar plot.

Supplementary Table 1

Manhard	"gain de	crease" (8)	"gain inc	rease" (7)	P-value
Monkey L	Mean	SEM	Mean	SEM	
Climbing fiber respo	onse (spike	es per s)			
x0T/x0BG	-0.64	0.07	-0.77	0.06	0.15
x2T/x2BG	0.58	0.05	0.76	0.10	0.08
x2T/x0BG	0.14	0.06	0.10	0.09	0.70
x1T/x0BG	-0.54	0.09	-0.50	0.11	0.79
Climbing fiber mean	n firing rate	e (spikes per s	3)		
x0T/x0BG	0.86	0.10	0.96	0.12	0.51
x2T/x2BG	0.92	0.10	0.87	0.11	0.76
x0T/x2BG	0.82	0.07	0.85	0.09	0.80
x2T/x0BG	0.90	0.10	0.81	0.13	0.55
x1T/x0BG	0.76	0.06	0.89	0.08	0.20
Purkinje cell simple	spike sens	sitivity (spikes	s per s per	°/s)	
Eye velocity	1.30	0.18	0.71	0.25	0.05
Head velocity	0.97	0.16	0.66	0.19	0.19
Cell location (cm)					
A/P	-0.25	0.43	-0.14	0.32	0.84
M/L	0.07	0.46	-0.43	0.26	0.35
Monkey E	"gain deo Mean	crease" (13) SEM	"gain inc Mean	rease" (11) SEM	<i>P</i> -value
Monkey E	"gain deo Mean	crease" (13) SEM	"gain inc Mean	rease" (11) SEM	<i>P</i> -value
Monkey E Climbing fiber response	"gain deo Mean onse (spike	crease" (13) SEM es per s) 0.10	"gain inc Mean	rease" (11) SEM	P-value
Monkey E Climbing fiber response x0T/x0BG x2T/x2BG	"gain deo Mean onse (spike -0.82 0.76	erease" (13) SEM <u>es per s)</u> 0.10 0.08	"gain inc Mean -0.76 1.01	rease" (11) SEM 0.13 0.16	<i>P</i> -value
Monkey E Climbing fiber response x0T/x0BG x2T/x2BG x2T/x0BG	"gain deo Mean onse (spike -0.82 0.76 0.16	erease" (13) SEM es per s) 0.10 0.08 0.10	"gain inc Mean -0.76 1.01 0.25	rease" (11) SEM 0.13 0.16 0.08	<i>P</i> -value
Monkey E Climbing fiber respo x0T/x0BG x2T/x2BG x2T/x0BG x1T/x0BG	"gain deo Mean onse (spike -0.82 0.76 0.16 -0.53	erease" (13) SEM 0.10 0.08 0.10 0.09	"gain inc Mean -0.76 1.01 0.25 -0.57	rease" (11) SEM 0.13 0.16 0.08 0.24	<i>P</i> -value 0.69 0.14 0.47 0.82
Monkey E <u>Climbing fiber respo</u> x0T/x0BG x2T/x2BG x2T/x0BG x1T/x0BG Climbing fiber mean	"gain deo <u>Mean</u> onse (spike -0.82 0.76 0.16 -0.53	erease" (13) <u>SEM</u> <u>es per s)</u> 0.10 0.08 0.10 0.09 0.09	"gain inc Mean -0.76 1.01 0.25 -0.57	rease" (11) SEM 0.13 0.16 0.08 0.24	<i>P</i> -value 0.69 0.14 0.47 0.82
Monkey E Climbing fiber response x0T/x0BG x2T/x2BG x2T/x0BG x1T/x0BG Climbing fiber mean	"gain deo Mean -0.82 0.76 0.16 -0.53 n firing rate	erease" (13) <u>SEM</u> <u>es per s)</u> 0.10 0.08 0.10 0.09 <u>e (spikes per s</u>) 0.27	"gain inc Mean -0.76 1.01 0.25 -0.57	rease" (11) SEM 0.13 0.16 0.08 0.24	<i>P</i> -value 0.69 0.14 0.47 0.82
Monkey E <u>Climbing fiber respo</u> ×0T/x0BG ×2T/x2BG ×2T/x0BG ×1T/x0BG <u>Climbing fiber mean</u> ×0T/x0BG	"gain deo Mean -0.82 0.76 0.16 -0.53 n firing rate 0.99	erease" (13) <u>SEM</u> 0.10 0.08 0.10 0.09 e (spikes per s 0.07 2.22	"gain inc <u>Mean</u> -0.76 1.01 0.25 -0.57 3) 1.06	rease" (11) SEM 0.13 0.16 0.08 0.24 0.06	<i>P</i> -value 0.69 0.14 0.47 0.82 0.48
Monkey E Climbing fiber response ×0T/x0BG ×2T/x2BG ×2T/x0BG ×1T/x0BG Climbing fiber mean ×0T/x0BG ×2T/x2BG	"gain deo <u>Mean</u> onse (spike -0.82 0.76 0.16 -0.53 n firing rate 0.99 1.02	erease" (13) <u>SEM</u> 0.10 0.08 0.10 0.09 e (spikes per s) 0.07 0.06 2.20	"gain inc <u>Mean</u> -0.76 1.01 0.25 -0.57 5) 1.06 1.19	rease" (11) SEM 0.13 0.16 0.08 0.24 0.06 0.10 0.25	<i>P</i> -value 0.69 0.14 0.47 0.82 0.48 0.14
Monkey E Climbing fiber response ×0T/×0BG ×2T/×2BG ×2T/×0BG ×1T/×0BG Climbing fiber mean ×0T/×0BG ×2T/×2BG ×0T/×2BG	"gain deo Mean onse (spike -0.82 0.76 0.16 -0.53 n firing rate 0.99 1.02 0.92	erease" (13) <u>SEM</u> 0.10 0.08 0.10 0.09 e (spikes per s) 0.07 0.06 0.06 0.06 0.06	"gain inc Mean -0.76 1.01 0.25 -0.57 5) 1.06 1.19 0.94	rease" (11) SEM 0.13 0.16 0.08 0.24 0.06 0.10 0.05 0.23	<i>P</i> -value 0.69 0.14 0.47 0.82 0.48 0.14 0.76
Monkey E Climbing fiber response ×0T/×0BG ×2T/×2BG ×2T/×0BG Climbing fiber mean ×0T/×0BG ×2T/×2BG ×2T/×2BG ×2T/×2BG ×2T/×2BG ×2T/×0BG	"gain deo Mean onse (spike -0.82 0.76 0.16 -0.53 n firing rate 0.99 1.02 0.92 0.90	erease" (13) <u>SEM</u> 0.10 0.08 0.10 0.09 9 (spikes per s) 0.07 0.06 0.06 0.06 0.06 0.06	"gain inc Mean -0.76 1.01 0.25 -0.57 (5) 1.06 1.19 0.94 1.02	rease" (11) SEM 0.13 0.16 0.08 0.24 0.06 0.10 0.05 0.08 0.10	P-value 0.69 0.14 0.47 0.82 0.48 0.14 0.76 0.21
Monkey E <u>Climbing fiber respo</u> ×0T/×0BG ×2T/×2BG ×2T/×0BG ×1T/×0BG <u>Climbing fiber mean</u> ×0T/×0BG ×2T/×2BG ×0T/×2BG ×2T/×0BG ×1T/×0BG	"gain deo Mean onse (spike -0.82 0.76 0.16 -0.53 n firing rate 0.99 1.02 0.92 0.90 0.85	erease" (13) <u>SEM</u> <u>as per s)</u> 0.10 0.08 0.10 0.09 e (spikes per s) 0.07 0.06 0.06 0.06 0.06	"gain inc Mean -0.76 1.01 0.25 -0.57 3) 1.06 1.19 0.94 1.02 1.01	rease" (11) SEM 0.13 0.16 0.08 0.24 0.06 0.10 0.05 0.08 0.10	P-value 0.69 0.14 0.47 0.82 0.48 0.14 0.76 0.21 0.12
Monkey E Climbing fiber response ×0T/x0BG ×2T/x2BG ×2T/x0BG ×1T/x0BG Climbing fiber mean ×0T/x0BG ×2T/x2BG ×0T/x2BG ×2T/x0BG ×2T/x0BG Purkinje cell simple	"gain deo Mean -0.82 0.76 0.16 -0.53 n firing rate 0.99 1.02 0.92 0.90 0.85 spike sens	erease" (13) <u>SEM</u> 0.10 0.08 0.10 0.09 0.09 0.07 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06	"gain inc Mean -0.76 1.01 0.25 -0.57 3) 1.06 1.19 0.94 1.02 1.01 s per s per	rease" (11) SEM 0.13 0.16 0.08 0.24 0.06 0.10 0.05 0.08 0.10 ² /s)	P-value 0.69 0.14 0.47 0.82 0.48 0.14 0.76 0.21 0.12
Monkey E Climbing fiber response ×0T/x0BG ×2T/x2BG ×2T/x0BG Climbing fiber mean ×0T/x0BG Climbing fiber mean ×0T/x0BG ×2T/x2BG ×2T/x2BG ×2T/x0BG ×2T/x0BG ×2T/x0BG ×2T/x0BG ×2T/x0BG ×2T/x0BG ×2T/x0BG ×2T/x0BG	"gain deo Mean onse (spike -0.82 0.76 0.16 -0.53 n firing rate 0.99 1.02 0.92 0.92 0.90 0.85 spike sens 1.38	crease" (13) <u>SEM</u> 0.10 0.08 0.10 0.09 (spikes per s) 0.07 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.18	"gain inc Mean -0.76 1.01 0.25 -0.57 3) 1.06 1.19 0.94 1.02 1.01 5 per s per 1.67	rease" (11) SEM 0.13 0.16 0.08 0.24 0.06 0.10 0.05 0.08 0.10 ⁵ /s) 0.39	P-value 0.69 0.14 0.47 0.82 0.48 0.14 0.76 0.21 0.12 0.18
Monkey E Climbing fiber response ×0T/×0BG ×2T/×2BG ×2T/×0BG Climbing fiber mean ×0T/×0BG ×2T/×2BG ×0T/×2BG ×2T/×2BG ×2T/×2BG ×2T/×0BG Purkinje cell simple Eye velocity Head velocity	"gain deo Mean onse (spike -0.82 0.76 0.16 -0.53 n firing rate 0.99 1.02 0.99 1.02 0.90 0.85 spike sens 1.38 1.09	erease" (13) <u>SEM</u> <u>as per s)</u> 0.10 0.08 0.10 0.09 <u>a (spikes per s)</u> 0.07 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.18 0.15	"gain inc Mean -0.76 1.01 0.25 -0.57 5) 1.06 1.19 0.94 1.02 1.01 5 per s per 1.67 1.62	rease" (11) <u>SEM</u> 0.13 0.16 0.08 0.24 0.06 0.10 0.05 0.08 0.10 <u>0.05</u> 0.08 0.10 <u>0.05</u> 0.08 0.10 <u>0.39</u> 0.33	P-value 0.69 0.14 0.47 0.82 0.48 0.14 0.76 0.21 0.12 0.18 0.49
Monkey E Climbing fiber response ×0T/×0BG ×2T/×2BG ×2T/×0BG ×1T/×0BG Climbing fiber mean ×0T/×0BG ×2T/×2BG ×0T/×2BG ×2T/×2BG ×0T/×2BG ×2T/×0BG Purkinje cell simple Eye velocity Head velocity Cell location (cm)	"gain deo Mean onse (spike -0.82 0.76 0.16 -0.53 n firing rate 0.99 1.02 0.92 0.90 0.85 spike sens 1.38 1.09	erease" (13) <u>SEM</u> <u>as per s)</u> 0.10 0.08 0.10 0.09 <u>a (spikes per s)</u> 0.07 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.18 0.15	"gain inc Mean -0.76 1.01 0.25 -0.57 3) 1.06 1.19 0.94 1.02 1.01 5 per s per 1.67 1.62	rease" (11) SEM 0.13 0.16 0.08 0.24 0.06 0.10 0.05 0.08 0.10 ^{2/s)} 0.39 0.33	<i>P</i> -value 0.69 0.14 0.47 0.82 0.48 0.14 0.76 0.21 0.12 0.18 0.49
Monkey E Climbing fiber response ×0T/x0BG ×2T/x2BG ×2T/x0BG ×1T/x0BG Climbing fiber mean ×0T/x0BG ×2T/x2BG ×2T/x2BG ×2T/x0BG ×2T/x0BG ×2T/x0BG Purkinje cell simple Eye velocity Head velocity Cell location (cm) A/P	"gain deo <u>Mean</u> onse (spike -0.82 0.76 0.16 -0.53 n firing rate 0.99 1.02 0.92 0.90 0.85 spike sens 1.38 1.09 0.24	erease" (13) <u>SEM</u> 0.10 0.08 0.10 0.09 e (spikes per s) 0.07 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.06 0.18 0.15 0.31	"gain inc Mean -0.76 1.01 0.25 -0.57 3) 1.06 1.19 0.94 1.02 1.01 s per s per 1.67 1.62 -0.22	rease" (11) <u>SEM</u> 0.13 0.16 0.08 0.24 0.06 0.10 0.05 0.08 0.10 <u>5/s)</u> 0.39 0.30	P-value 0.69 0.14 0.47 0.82 0.48 0.14 0.76 0.21 0.12 0.18 0.49 0.27

Climbing fibers carrying "gain decrease" versus "gain increase" climbing fiber signals during ×0T/×2BG training did not differ in any other property. Climbing fibers carrying "gain decrease" versus "gain increase" signals during ×0T/×2BG training were categorized as described in **Supplementary Figure 6**. Responses of the two groups of climbing fibers to other training stimuli, and their average firing rate, sensitivity to eye and head velocity, and anatomical location were compared using t-tests. Sensitivity to eye velocity was measured during smooth pursuit eye movements; sensitivity to head velocity was measured during VOR cancellation (see Methods). Anterior-posterior (A-P) and medial-lateral (M-L) locations were measured relative to the mean of the full population of neurons recorded. No significant differences were found.