Synaptic diversity enables temporal coding of coincident multisensory inputs in single neurons

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The ability of the brain to rapidly process information from multiple pathways is critical for reliable execution of complex sensory-motor behaviors, yet the cellular mechanisms underlying a neuronal representation of multimodal stimuli are poorly understood. Here we explored the possibility that the physiological diversity of mossy fiber (MF) to granule cell (GC) synapses in the mouse vestibulocerebellum may contribute to the processing of coincident multisensory information at the level of individual GCs. We found that the strength and short-term dynamics of individual MF-GC synapses can act as biophysical signatures for primary vestibular, secondary vestibular and visual input pathways. Most GCs receive inputs from different modalities, which, when coactivated, produce enhanced GC firing rates and distinct first spike latencies. Thus, pathway-specific synaptic response properties permit temporal coding of correlated multisensory inputs by single GCs, thereby enriching sensory representation and facilitating pattern separation.

To represent and process information from complex natural events, the brain must integrate signals from multiple senses1, as well as those arising from self-generated actions2. Several studies have shown that convergence of functionally distinct inputs occurs at the level of single neurons in the neocortex3–5, superior colliculus6, striatum7 and cerebellum8. The primary neuronal computation reported for multimodal integration is to increase firing rate upon coincident cross-modal stimuli (either subadditively, additively or superadditively), thus enhancing saliency of a particular event1,9. Short-term synaptic dynamics can provide an additional nonlinearity that can contribute to neuronal computations of unisensory feature-selectivity10,11, but it remains to be determined how individual synaptic response properties could contribute to multimodal processing. Thus, a pertinent question is how the diversity of synaptic efficacy and dynamics, observed throughout the brain12–14, can be exploited to encode spike representations of multisensory information.

Cerebellar GCs are the most numerous neurons in the brain and relay rich contextual information from MFs to Purkinje cells (PCs) to fine tune motor behaviors with tens of milliseconds precision15. Theoretical models suggest that large divergent connectivity of single MFs to many GCs and the mixing of different input features onto individual synaptic response properties could contribute to temporal coding of correlated multisensory inputs by single GCs, thereby enriching sensory representation and facilitating pattern separation.

RESULTS

Diversity of MF-GC synaptic behavior

MF-GC synapses exhibit a striking diversity in strength and short-term dynamics across connections20,24. We investigated the in vitro functional properties of MF-GC synapses in the nodulus (lobule X), a region of the vestibulocerebellum in which the origins of MF projections have been well characterized25. This region receives projections primarily from the medial vestibular nucleus (MVe), nucleus prepositus hypoglossi (PrH; optokinetic26 and object motion8, referred to as “visual”) and vestibular ganglion (VG)25. Unitary AMPA receptor-mediated synaptic currents (EPSCs) were evoked from single MFs using a blind, minimal stimulation protocol20 (Fig. 1a). Unitary MF-GC EPSCs were highly heterogeneous across connections (Fig. 1b); initial mean amplitude varied from 10 to 290 pA, trial-to-trial variability of unitary inputs (coefficient of variation, CV) varied from 0.1 to 0.7 and the paired-pulse ratio (PPR) varied from 0.4 to 1.8 (n = 83 MF inputs; Supplementary Fig. 1a). Since we observed striking correlations between these EPSC metrics (Supplementary Fig. 1b), we performed a k-means clustering analysis (KMC), leading to the identification of five groups of inputs (Fig. 1c), which accounted for nearly 80% of the variance of EPSC metrics across the entire population of inputs (Supplementary Fig. 2). MF input groups were numbered in descending order according to the peak amplitude of their average EPSC, and the group-averaged traces are plotted in Figure 1d. The mean CV and PPR for each group were larger for smaller inputs.
Figure 1 Identification of MF-GC input types using k-means clustering analysis of EPSC properties. (a) Diagram of a parasagittal slice of cerebellar vermis, showing the vestibular region (lobule X) where we studied single MF-GC connections using extracellular stimulation and postsynaptic whole-cell voltage-clamp recording of unitary EPSCs. (b) Unitary EPSCs from individual trials (gray traces) and mean (black trace) from two example MF-GC synapses. Arrowheads indicate timing of stimulation. EPSC peak amplitude, coefficient of variation of the peak amplitude (CV) and paired-pulse ratio (PPR) were used for cluster analysis. (c) Plot of the two principal components (PrinC) calculated using the KMC algorithm on 276 inputs. The first principal component results from the negative correlation between peak amplitude versus CV and PPR. The second principal component results from a positive correlation between peak amplitude and PPR. Data points are experiments in which blind stimulation was performed (n = 212 inputs). Black circles are the cluster centers with an area proportional to the number of points inside the cluster; ellipse perimeters indicate 95% confidence intervals. The percentages of inputs in each cluster are indicated. Color code is used throughout all figures referring to input groups. (d) Evoked EPSCs across all cells averaged according to the KMC cluster to which they were assigned. We refer to each cluster as a group; numbers of inputs are 19, 58, 82, 57 and 43 for groups 1–5, respectively. (e) Summary plots of EPSC metrics according to group for all 259 inputs recorded in lobule X. Box plots represent quartiles (minimum, 25%, median, 75% and maximum values). Statistical comparisons between groups were performed using a Steel-Dwass all-pairs test. All comparisons were significant, *P < 0.0001, except amplitude between G4 and G5 (NS, P = 0.14).

(Fig. 1e), consistent with a potential source of diversity arising from the different properties of vesicular release of neurotransmitter.

Using multiple probability fluctuation analysis (Fig. 2a), we observed significant differences across groups in the number of release sites (N) and release probability per vesicle (P_r), but did not detect a difference in quantal size (Fig. 2b,c). Large inputs with low CV required both high P_r and N, while small inputs resulted from low P_r and low N. The PPR was not always correlated with P_r: notably, input groups 4 and 5 had low P_r, but only inputs from group 5 exhibited short-term facilitation. Thus we identified several functionally distinct classes of MF-GC synapse that differed primarily in their presynaptic properties, suggesting they might belong to distinct MF input pathways.

Input pathway–specific synaptic behavior
To examine whether different precerebellar projections comprise different functional classes of MF synapses, we identified single MFs for targeted stimulation in acute brain slices using various strategies of genetic expression of fluorescent proteins. Of the three principal cerebellar structures projecting to the vestibulocerebellum, we found that only the VG expressed GFP in a Thy1-mGFP transgenic mouse line (Supplementary Fig. 3). MVe and PrH neurons were fluorescently labeled by stereotaxic injection of an adeno-associated virus (AAV9) expressing turbo red fluorescent protein (TurboRFP; Supplementary Fig. 5). We then used simultaneous two-photon fluorescence imaging and Dodt contrast to selectively stimulate fluorescently labeled MFs (Supplementary Fig. 5). EPSCs evoked from VG MFs were large and exhibited a low CV and strong short-term depression, with 89% of the synaptic responses being classified (using KMC) as group 1 or 2 (Fig. 3a–d). EPSCs evoked from MVe and PrH MFs had small mean amplitudes, large CV and short-term dynamics ranging from little depression to strong facilitation (Fig. 3e–I). Eighty-eight percent of MF inputs from MVe were categorized as groups 3 or 5 (Fig. 3h), while 69% of PrH MF inputs were categorized as group 4 (Fig. 3i). The results of KMC were corroborated by statistical differences in the EPSC mean amplitude between VG versus MVe and VG versus PrH, and in CV between the different MF projections (Fig. 3m). The PPR of VG MF inputs was significantly smaller than that of either of the other projections types (Fig. 3m), but because MVe MFs conveyed both depressing and facilitating inputs (groups 3 and 5) we did not detect a difference in PPR as compared to MF inputs arising from PrH. These data demonstrate that visual, primary and secondary vestibular pathways use distinct classes of MF inputs with distinct synaptic behaviors.

Sensory-specific EPSC properties in vivo
To directly examine whether MF-GC synaptic properties differ according to the sensory modalities they convey, we recorded...
sensory-evoked EPSCs from GCs in the vestibulocerebellum (folliculus) in vivo from anesthetized mice. Visual pathways were stimulated using drifting gratings of different orientations and vestibular pathways were stimulated by whole-body rotation. Spontaneous EPSC rates were 9.1 ± 6.0 and 5.5 ± 5.2 Hz (mean and s.d.) for cells in which vestibular (n = 28) and visual inputs (n = 10) could be evoked, respectively. We found that EPSCs evoked by moving gratings in the preferred direction were smaller on average by a ratio of 1:1.7 than those evoked by vestibular stimulation and did not depress significantly (P = 0.72, Spearman’s rank-order stability test) during the stimulus presentation. The ratio of amplitudes of vestibular- versus visual-evoked responses was consistent with in vitro results provided that we pooled only MVe inputs as the vestibular population, since the folliculus does not receive primary vestibular afferents, and considered PrH as the visual population of MF inputs. PrH inputs were 1.4 times smaller than MVe inputs. Taken together, both in vitro and in vivo findings demonstrate that the MF-GC synaptic behavior is input pathway-specific and thus acts as a biophysical signature of input modality.

Convergence of multimodal inputs onto single GCs

To determine how input-specific synaptic properties might facilitate multimodal integration, we first examined whether single GCs were innervated by MF inputs carrying different sensory signals, which has been shown to occur in a cerebellum-like structure in weakly electric fish and in other regions of the rodent cerebellum. We performed stereotoxic viral injections in Thy1-mGFP mice (labeling VG) to express TurboRFP in either MVe or PrH MFs. Innervation patterns were then determined by labeling single GCs in acute slices using whole-cell loading of Alexa 594 and then examining whether dendrites of a single GC contacted GFP- and/or TurboRFP-positive MFs. We found that single GCs were frequently contacted by both VG and MVe MFs (27%; n = 43 labeled GCs; Group 4), as well as both VG and PrH MFs (15%; n = 35 GCs). These data indicate that mixed-modal innervation is a prominent wiring feature in the GC layer, allowing single GCs to integrate synaptic inputs carrying primary and secondary vestibular (VG versus MVe) modalities, as well as vestibular and visual modalities (VG versus PrH).

Because the functional properties of many synapses are target cell dependent, we next examined whether different MF-GC synapses impinging on the same GC preserve their pathway-specific synaptic behavior. Blind stimulation of two different MF inputs onto single GCs, followed by a post hoc KMC (Fig. 5e,f), indicated that most GCs received MF inputs belonging to at least two different input groups (n = 41 of 50 input pairs; Fig. 5g). Moreover, MF inputs characteristic of VG and MVe (that is groups 1 and 2 or groups 3 and 5) were often associated with the PrH input group 4 (29 of 50). These results corroborate the anatomical findings and demonstrate that the functional properties of MF-GC synapses are determined by the input pathway, that GCs are generally contacted by MFs originating from different input pathways, and that mixed innervation of single GCs often involves different sensory modalities.

GC integration of pathway-specific synaptic inputs

We next examined how GCs integrate the different MF input types. We performed blind stimulation of MF-GC synapses at different frequencies and monitored GC membrane voltage in whole-cell current-clamp mode. For these experiments, NMDA receptors were not blocked because they are known to participate in synaptic integration. We performed blind stimulation of MVe and PrH MFs from groups 1 and 2 produced robust GC firing for stimulation frequencies above 50 Hz, with short first-spike latencies. Regardless of frequency, the first EPSP of group 1 inputs exhibited a high probability of firing (39%, n = 7 cells). By contrast, MF inputs from groups 3 and 4 produced little, if any, GC firing for stimulation frequencies up to 200 Hz. Notably, group 5 MF inputs also produced robust firing, despite the small amplitude of
their initial EPSC. This was due to synaptic facilitation and temporal integration of EPSPs, which in turn resulted in longer first-spike latencies (Fig. 6a,c). The differences in EPSC amplitude and short-term dynamics are reminiscent of “driver” and “modulatory” synaptic input types in thalamus and cortex. From here on we will refer to inputs from groups 1, 2 and 5 as “driver inputs” because they induced GC firing alone. Inputs from groups 3 and 4 we will refer to as “supporting inputs,” rather than “modulatory inputs,” a term referring to inputs modulated by metabotropic glutamate receptors.

**GC output represents mixed-modal activation**

We next investigated whether simultaneous activation of different MF types could be transformed into distinct GC spike representations. Here we not only considered the coactivation of driver and supporting inputs, but also tried to mimic the spontaneous and stimulus-evoked MF firing rates observed in vivo. Each stimulation train was comprised of 10 initial pulses delivered at 50 Hz for primary vestibular inputs (groups 1 and 2), 10 Hz for secondary vestibular inputs (groups 3 and 5) and 10 Hz for inputs from the visual pathway (group 4), taken from a conservative estimate of the in vivo results above. Following the spontaneous firing period, sensory stimuli were mimicked by stimulating all MFs ten times at 100 Hz, a firing frequency in the range of saccadic eye movement–related activity in PrH. The stimulation onset for input groups 3, 4 and 5 was delayed by 3 ms to account for polysynaptic delays. Spontaneous MF firing dramatically reduced the ability of input groups 1 and 2 to engage GC firing because of synaptic depression, while the 10-Hz tonic MF firing did not appreciably change the impact of group 5 inputs (Fig. 6d). Vestibular driver inputs (groups 1, 2 and 5) were often found to co-innervate single GCs with...
Figure 4 Sensory-specific MF-GC synaptic properties recorded in vivo. (a) Example of current traces recorded from GCs in voltage clamp in vivo during the presentation of visual gratings of different orientations. Gray shaded area indicates the period when the gratings moved. (b) Visual motion-evoked EPSCs (gray) and average EPSC waveform (black) for the cell in a. (c) Polar plot showing the direction tuning of the cell shown in a. The arrow represents the visual-motion tuning vector. (d) Top, evoked EPSCs in GCs in response to an in vivo vestibular stimulation by vertical rotation about the combined roll/pitch axis (see Online Methods). Bottom, EPSC frequency modulation (black) follows the velocity stimulus waveform (orange). (e) Evoked EPSCs (gray) and average EPSC waveform (black) for the cell in d. (f) Left, vestibular (n = 28 inputs) and visual (n = 10) EPSCs have different peak amplitudes in vivo (**P = 0.04, Wilcoxon rank-sum test). Right, the same trend is observed in EPSC recordings from acute slices (in vitro) when stimulating MVe (n = 16 inputs) and PrH MFs (n = 13; *P = 0.03, Wilcoxon rank-sum test). Box plots represent quartiles (minimum, 25%, median, 75% and maximum values).

Visual supporting input (group 4, PrH). Simultaneous stimulation of driver and supporting inputs produced a robust enhancement of GC firing rate (Fig. 6d,e), reminiscent of the superadditive multisensory integration observed in the superior colliculus5. Moreover, the first spike latencies of GC responses from the onset of 100-Hz stimulation were markedly different across the different input combinations (Fig. 6d,f) and mirrored the firing onset delays of the individual driver inputs when stimulated without spontaneous activity (Fig. 6c). Combined activation of inputs from groups 1 and 4 produced spikes on the first or second synaptic stimulation with an average delay of 10 ms, whereas activation of groups 5 and 4 produced spikes on the third stimulus (average delay = 25 ms). Additionally, we recorded GC output in response to simultaneous stimulation of group 3 and 4 inputs. Neither input drove GC firing on its own; however, the combined activation of the two was sufficient to produce a few action potentials (Fig. 6e), albeit with longer latencies (44 ms) than those evoked in response to combined activation of group 5 and 4 inputs (Fig. 6f).

Figure 5 Multimodal convergence onto single GCs. (a) Double labeling of primary (1°) and secondary (2°) vestibular afferents, or primary vestibular and visual afferents. AAV9-TurboRFP (magenta) was injected in MVe or PrH of transgenic animals (Thy1-mGFP, green). (b) Example two-photon laser scanning microscopy images of fluorescent MFs in lobule X. (c) Example whole-cell patched GCs filled with Alexa 594 (white) that were found to be connected to VG and MVe or VG and PrH MFs (stars). (d) z-projection of three-dimensional reconstructions of the connected GCs and MFs shown in c. (e) Blind stimulation of two MF inputs in single GCs. Superimposition of two-photon laser scanning microscopy images of a GC filled with Alexa 594 (green) and an infrared Dodt contrast images showing the positions of the stimulation electrode (stars) where two different inputs were evoked. Other labeled GCs are from previous attempts. (f) Unitary EPSCs evoked from locations shown in e. KMC was used to identify input type. (g) Summary of MF input type pairs observed in single GCs. Thickness of connecting lines between groups indicates frequency with which a pair was observed; values are number of observations per pair. Circular arrows represent GCs where the two evoked inputs belonged to the same group.
To examine the effect of a wide range of combined MF stimulation frequencies on GCs output, we used an established synaptic and integrate-and-fire (sIAF) model. The synaptic parameters were adjusted to mirror the mean EPSC and short-term dynamics for each input group. First we verified that the model could replicate recorded single GC input-output (I/O) curves and first-spike latencies (Supplementary Fig. 7a–c). We then simulated coactivation of vestibular driver inputs (groups 1, 2 or 5) and visual supporting input (group 4, PrH) as in the experiments described in Figure 6. The simulations corroborated our experimental results: namely, that tonic firing activity induces steady-state synaptic depression of groups 1 and 2 inputs, that input combinations greatly increase GC responses and that first-spike latencies were combination specific (Supplementary Fig. 7d,e).

It is thought that a minimum of three active MF inputs are necessary to drive GC firing in the presence of tonic inhibition. Since 98% of inhibition is thought to be mediated by tonic activation of GABA_A receptors, we next examined the effect of tonic inhibition on coactivation of MF types using the sIAF model. Such experiments are difficult to implement in situ. The combination of synaptic depression and tonic inhibition prevented single driver inputs from evoking GC firing for MF stimulation rates below 175 Hz (Fig. 7a). However, when one driver and one supporting input were combined, GC output was strongly enhanced (as in Fig. 6d,e) and exhibited an increase in gain and dynamic range for input frequencies <200 Hz. But higher supporting input frequencies were required than in the case without tonic inhibition (Fig. 7a versus Supplementary Fig. 7f).

**Figure 6** Input-specific synaptic integration in single GCs. (a) Representative current-clamp traces showing GC voltage responses evoked by trains of 30 synaptic stimuli at 20, 50 and 100 Hz of each MF input type. Right column shows early EPSP summation expanded from boxed region, illustrating first spike delays relative to the onset of stimulation. (b) I/O relationships for MF types from groups 1–5 (n = 3, 7, 8, 4 and 7 inputs, respectively). Lines are fits to a Hill function. Error bars represent s.e.m. (c) First-spike latencies for each input group at different MF stimulation frequencies. Note the short first-spike delay for group 1 (G1) at all frequencies because of spike initiation on the first EPSP. Error bars represent s.e.m. (d) Voltage responses of single GCs evoked from individual and simultaneous stimulation of supporting (G4) and driver MF inputs (G1, 2 and 5). Early EPSP summation expanded from boxed region (right column) demonstrates combination-specific delays akin to those obtained with single driver MF input stimulation without baseline tonic activity (see a). (e) Comparison of GC firing rates when driven by single inputs (G1, 2, 5 and 3; n = 4, 3, 3 and 5 cells, respectively) versus when combined with input group 4 (or, in one case (square symbol), group 3). Red curve indicates a line with unity slope. (f) Summary plot of combination-specific first-spike latencies. Box plots represent quartiles (minimum, 25%, median, 75% and maximum values).
Thus, synaptic depression and tonic inhibition ensure that only coactivated inputs produce appreciable GC firing.

The simulations also predict that in the presence of tonic inhibition each input combination elicits a characteristic range of frequency-dependent firing delays, relative to the time of cross-modal stimulus presentation (Fig. 7b). Combinations of inputs from groups 1 and 4 produce spikes with delays from 6 to 28 ms, groups 2 and 4 from 14 to 44 ms and, finally, groups 5 and 4 from 24 to 74 ms. To examine the influence of a third input, we chose the other supporting input, group 3 (secondary vestibular), which were often combined with driver inputs (Fig. 5g). We also increased tonic inhibition by 30% to account for the increase in MF drive to Golgi cells. With three coactivated inputs, GCs uniquely represented the type of driver input by distinct output frequencies. Average output frequencies are indicated. (c) Simulation similar to that in b, except with coactivation of a third MF input (G3) and a 30% greater tonic inhibition. Combinations of three inputs produce distinct delays according to the type of driver input: red shaded area, G1; orange, G2; blue, G5.

We compared the decorrelation performance of the threshold model to that of the delay coding model. Overlapping pairs of input patterns of net synaptic input strengths were created using a correlated joint normal distribution with a mean and s.d. of 2±1 across N = 1,000,000 GCs. For a random subset of model GCs, inputs and their corresponding outputs were plotted as images in which pixel intensity is either input strength or output response for a particular GC (Fig. 8c,d). To determine the decorrelation performance of each model, we quantified the overlap between pairs of input and corresponding output patterns by calculating the Pearson product moment correlation coefficient r. We found that the I/O transformations resulting from both models were able to generate substantial decorrelation (that is, output r values were smaller than input values; Fig. 8c–e). However, for short delays, the decorrelation obtained with delay coding was substantially more pronounced than decorrelation achieved by thresholding (r = 0.61 versus 0.71 for delay 1 and threshold 5, respectively). Intuitively, this is explained by the jump in the GC I/O function, which enhances ‘contrast’ between active and inactive GCs compared to that in the continuous threshold model. Delay coding provided better pattern decorrelation for strongly correlated inputs (r > 0.5), but not when paired inputs were already dissimilar (Supplementary Fig. 8). With longer delays, the decorrelation performance became gradually more similar between delay coding and thresholding as the discontinuity in the delay coding model became less pronounced.

To estimate the influence of enhanced pattern decorrelation by delay coding, we quantified the pattern discriminability using an optimal linear classifier in the presence of output noise. Specifically, we asked how much noise could be added to the decorrelated patterns before the percentage of misclassifications exceeded a preset threshold (for example, 5%). We then calculated a signal-to-noise ratio (SNR). The delay coding model routinely detected small differences in patterns with lower SNRs than the thresholding model, suggesting improved discriminability. For difficult discriminations,
the delay coding model reached classification criterion at a threefold lower SNR than the threshold model (Fig. 8f). This measure of relative improvement was insensitive to the specific misclassification threshold chosen. Taken together, the experimental results and delay coding simulations show that variable GC spiking delays support enhanced decorrelation of input activity patterns relative to the standard thresholding model proposed by Marr and thus can better represent rich spatiotemporal patterns of MF activity.

**DISCUSSION**

Diversity in synaptic strength and short-term dynamics is ubiquitous across excitatory connections. Here we demonstrate that different MF input pathways exhibit signature synaptic behaviors that account for the functional diversity within a single anatomical class of inputs. In the case of the vestibulocerebellum, we found that different sensory modalities (primary vestibular, secondary vestibular and visual) converged onto single GCs and preserved their functional signatures. Integration of these inputs by single GCs resulted in distinct output firing representations of specific correlated multimodal input combinations. This temporal coding strategy represents a basis for single-neuron computation of multimodal information. Within the input layer of the cerebellar cortex, a delay coding strategy can also contribute to enhanced pattern separation, allowing a more robust classification of input activity patterns by PCs than originally predicted by Marr and Albus.

**Origin of MF-GC synaptic diversity**

Using variance-mean analysis of EPSCs, we established that the diversity in the MF-GC input pathways arises from differences in the presynaptic properties of vesicular release. Presynaptic mechanisms therefore ensure that each MF pathway is capable of maintaining its functional specificity, even when co-innervating single GCs. Because most short-term dynamics mechanisms are also presynaptic, the synaptic diversity of MF-GC synapses may be greater than what could be achieved by a purely postsynaptic mechanism. The molecular underpinnings of synaptic vesicle release diversity are as yet unknown. Nevertheless, synaptic diversity could be established by genetic, developmental or activity-dependent mechanisms. Consistent with the last possibility, long-term potentiation of MF-GC synapses is expressed presynaptically. Moreover, other long-term or homeostatic changes in release properties may result from the differences in the firing rates of primary versus secondary vestibular inputs.

It is possible that input-specific synaptic behaviors may be tuned to the inherent temporal response of the sensory modality they convey. Within the primary vestibular pathway, group 1 and 2 MFs could arise from regular and irregular vestibular nerve afferents, or from semicircular canal and otolith afferents. The large MF-GC EPSC amplitude and short-term depression of primary vestibular afferents may underlie the short, high-frequency bursts of single-unit GC recordings in the nodulus during initiation of whole-body movements. We speculate that PCs could use this information as a time stamp of the initiation of whole-body movement, since PCs have been shown to encode whole-body acceleration in the nodulus. Secondary vestibular afferents have been shown to linearly report self-motion velocity in the flocculus, which is consistent with the mean PPR (~1.0) observed in MVe MFs (Fig. 3m). Notably, despite the small initial synaptic response of group 5 MFs, the combination of input groups 5 and 4 was the most efficient at driving GC firing at low frequencies of MF activation (Fig. 7a). This was due to the synaptic facilitation of group 5 inputs and a decreased sensitivity of I/O relationships of non-depressing synapses.

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**Figure 8** Delay coding enhances pattern decorrelation. (a,b) Schematic diagrams of thresholding and delay coding models. Color code refers to open squares in c. In both models, GCs only generate output if their input exceeds a certain threshold (T). In the thresholding model, this output is proportional to the difference between input and threshold. In the delay-coding model, the output follows the input after a delay (D) that is shorter for stronger inputs. The I/O transformation at a fixed delay expresses a discontinuity in the GC output at the delay-dependent threshold. The resulting GC output exceeds that of the thresholding model for any suprathreshold input strength. (c) Left, an image map of MF inputs for two similar patterns. Each panel shows the same random subset of 144 cells. Cells are arranged by decreasing average input intensity from center to periphery. Pattern overlap as quantified by Pearson correlation r is indicated for each pair. (d) Same as c but with output patterns calculated using the delay-coding model. (e) Pattern decorrelation (r) in the large N limit as a function of the threshold. Delay coding achieves substantially stronger decorrelation (lower r values) than thresholding. (f) Relative improvement of the delay-coding model, estimated from the ratio of output SNRs required for reliable pattern discrimination by a linear classifier. The largest value graphed corresponds to input correlation r = 0.99.
to tonic inhibition\textsuperscript{23}. These results highlight the important influence of short-term dynamics in information processing.

**Multimodal processing by single GCs**

We found that GC responses to vestibular driver inputs were greatly enhanced when the visual supporting input was concurrently activated (Figs. 6 and 7). This phenomenon, termed multisensory enhancement, has been reported in superior colliculus\textsuperscript{6} and neocortex\textsuperscript{4} as a mechanism to enhance saliency of stimuli, as well as to generate a unified percept from inherently different psychophysical responses\textsuperscript{9}. In the cerebellum, our data suggest that GC multimodal integration supports both strategies for detecting correlations of sensory features\textsuperscript{38} in the complex sensory-motor environment (Figs. 6 and 7), similarly to what has been found in the weakly electric fish\textsuperscript{27}. The convergence of primary and secondary vestibular afferents onto single GCs could participate in cerebellar computations observed in monkeys, such as estimation of orientation relative to gravity\textsuperscript{39}, which require the integration of information from multiple vestibular channels (that is, semicircular canals and otoliths). Proprioceptive inputs originating from the spinal cord could also be integrated along with vestibular and visual inputs; however, we have not characterized their input properties and wiring.

The vestibulocerebellum is crucial for the elaborate computation of self motion through the integration of allocentric and egocentric cues (vestibular and optic flow versus proprioception and copies of motor commands) in both mice\textsuperscript{33} and monkeys\textsuperscript{8,40}. Fundamental to this computation is the ability of the cerebellum to predict and cancel the sensory consequences of self motion\textsuperscript{2,40}. By analogy to the circuit computation in the cerebellum-like structure of the electric fish\textsuperscript{41}, the multisensory consequences of expected motion are transmitted via MFs to GCs and then on to PCs, where synaptic weights can be tuned by supervised learning rules under the control of climbing fibers, such that the PC output can cancel expected sensory information\textsuperscript{2,33}. Indeed, biophysical models of PCs have shown that supervised learning rules allow PCs to encode learned patterns of GC synaptic activity in the temporal structure of their firing\textsuperscript{42}. Here we found that mixed input pathway convergence occurred in most vestibulocerebellar GCs (Fig. 5), with the pairing of one driver and two supporting inputs producing three distinct sets of GC response delays corresponding to each driver input. Thus, the network of delay-coding GCs could be responsible for relaying the temporal structure of the vestibular and/or visual reafference (self-generated sensory response), thereby allowing efficient sensory cancellation by PC activity.

**Synaptic diversity enhances pattern decorrelation**

Single GCs have been shown to integrate proprioceptive information and motor command copies\textsuperscript{27,28}. Our finding that GCs can be innervated by MFs carrying information from different senses reveals a more general wiring principle that is consistent with the long-standing theory of expansion recoding in the GC layer\textsuperscript{16,18} (except see ref. 22). We propose here that the input-specific synaptic behaviors and mixed innervation together contribute to a temporal coding scheme that effectively enhances the differences between output patterns in response to similar input patterns, thereby enhancing their discriminability relative to that of a threshold model (for example, the model of Albus\textsuperscript{16} or Marr\textsuperscript{18}). The spike onset delays represent distinct input combinations for coactivation of three or more MF inputs (Fig. 7c), consistent with recent theoretical findings that three or four synaptic inputs are optimal for sparse coding and information transmission\textsuperscript{17}. Our simple delay coding model provides an extra dimension for performing pattern decorrelation in the GC layer\textsuperscript{16,18,43}. Although it is difficult to predict how PCs will read out the different GC activity patterns, simple linear discriminators showed a several-fold improvement in the ability to classify similar patterns (Fig. 8f).

An alternative function of GC delay coding is to provide a repertoire of temporal waveforms to PCs in order to integrate and sculpt the temporal spike pattern of PC output\textsuperscript{41}. A scenario combining pattern decorrelation and temporal response sculpting could arise when two similar sensory contexts would require a PC to respond with two distinct temporal response waveforms. Our modeling suggests that this task would be facilitated by GC delay coding, but this remains to be confirmed experimentally. Nevertheless, spatio-temporal pattern decorrelation supports the notion that the GC network can present complex sensory contexts to PCs using variable temporal delays, which can be independently associated with the teaching signals conveyed by climbing fibers, as hypothesized for the timing-dependent learning mechanism necessary for predictive motor control\textsuperscript{34} and the cancellation of expected sensory information\textsuperscript{40,41}.

It has recently been shown that unipolar brush cells provide the late component of GC response delays necessary for canceling sensory consequences of self-generated stimulation in the cerebellum-like structure of the weakly electric fish\textsuperscript{41}. In mammals, expected sensory cancellation could take advantage of input-specific synaptic behaviors to provide GC response delays of up to 100 ms, while unipolar brush cells could provide the longer delays\textsuperscript{44}. Intrinsic differences in the temporal activation of MFs within an input pathway\textsuperscript{45,46} might also contribute to differential GC delays.

Expansion recoding and sparse representations of incoming inputs are common features of several sensory systems\textsuperscript{43}. As in the cerebellar cortex, sensory afferents diverge onto a cortical layer containing numerous neurons that convey a sparse representation of the sensory information to a second layer made of readout units. Given the known functional synaptic diversity of thalamocortical (visual\textsuperscript{13}, auditory\textsuperscript{14} or somatosensory\textsuperscript{14,47}) and intracortical projections\textsuperscript{14} and the cross-feature and cross-modal innervation of cortical\textsuperscript{3} and hippocampal neurons\textsuperscript{48}, input-specific delay coding could serve as a ubiquitous strategy for enhancing pattern separation of complex, time-varying input stimuli\textsuperscript{19}.

Finally, whether input-specific synaptic diversity constitutes a fundamental component of sensory processing (for example, feature extraction) for neurons is an area of active research\textsuperscript{49}. We speculate that the temporal coding strategies imparted by driver and supporting inputs in GCs may provide insight into how thalamic and cortical circuits might process type I (driver) inputs, which are strong inputs carrying the primary sensory information, and type II (modulatory) synaptic inputs\textsuperscript{14}. Input-specific synaptic diversity could also underpin feature selection, as exemplified by strong synaptic weights between recurrently connected layer 2/3 pyramidal neurons of the primary visual cortex with similar orientation preferences\textsuperscript{50}.

**METHODS**

Methods and any associated references are available in the online version of the paper.

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ONLINE METHODS
Cerebellar slice preparation and synaptic recordings. All animal experimental procedures were approved by the ethics committee for animal experimentation at the Pasteur Institute. Acute parasagittal slices (200 μm) of cerebellum were prepared from F1 and F2 mice (cross of BALB/c and C57BL/6), both males and females, between 30 and 60 days of age (mean = 46 ± 6), except for mice injected with viral constructs, which were between 60 and 90 d old (at least 2 weeks after injection). Transgenic mice expressing mGFP under the Thy1 promoter (line 17, gift from P Caroni, Friedrich Miescher Institute for Biomedical Research, Basel) were bred on a C57BL/6j background and maintained as heterozygotes. Mice were swiftly decapitated and the brain quickly removed and placed in ice-cold slicing solution containing (in mM) 2.5 KCl, 0.5 CaCl2, 1.25 NaH2PO4, 24 NaHCO3, 25 glucose and 230 sucrose. Slices were cut from the dissected cerebellar vermis using a vibrotome (Leica VT1200S) and directly transferred to an incubation chamber containing (in mM) 85 NaCl, 2.5 KCl, 0.5 CaCl2, 4 MgCl2, 1.25 NaH2PO4, 24 NaHCO3, 25 glucose and 75 sucrose, maintained at 32 °C for 30 min. Subsequently the slices were transferred to an external recording solution kept at room temperature and containing (in mM) 125 NaCl, 2.5 KCl, 1.5 CaCl2, 1.5 MgCl2, 1.25 NaH2PO4, 25 NaHCO3 and 25 glucose. All solutions were saturated with 95% O2 and 5% CO2.

Whole-cell patch-clamp recordings of cerebellar granule cells (GCs) were performed using a Multiclamp 700B amplifier (Axon Instruments) with fire-polished, thick-walled glass patch electrodes (1.5 mm OD, 0.75 mm ID, Sutter Instruments; 3–10 MΩ tip resistance) filled with the following K+-based solution (in mM): 110 KOH, 110 methanesulfonic acid, 6 NaOH, 4.6 MgCl2, 5 EGTA, 1.78 CaCl2, 2 Na-ATP, 0.3 Na-GTP (280 mM osm, pH 7.3). Cells with series resistances above 30 MΩ were discarded from analysis. Series resistance (24.6 ± 4.4 MΩ, mean and s.d., n = 259) was not corrected online. GC capacitance, estimated from the cancelation of the transient current response to a 5 mV step depolarization, was 3.5 ± 0.6 pF (mean and s.d., n = 259). A liquid junction potential of 6 mV (ref. 20) was used to correct all membrane voltage values (subtracted from measured value).

For voltage-clamp recordings, GCs were held at −87 mV and single mossy fiber transmission was evoked with 10 μs voltage pulses 5 V above threshold (Digitimer Ltd, Letchworth Garden City, UK) using a second patch pipette (typically with a tip resistance of 4–6 MΩ) filled with ACSF and placed in the GC layer (typically 50–100 μm) from the soma of the GC. Activation of a single MF was routinely confirmed by verifying that increasing the stimulus intensity did not evoke an increase in EPSC amplitude51. For blind stimulation, MFs were stimulated by trains of 30 stimulations at 10, 20, 50, 100 and 200 Hz were delivered to single MFs at 1-min intervals to prevent long-term changes in synaptic strength. AMPA receptor–mediated (AMPAR) EPSCs were isolated using the following drug mix: SR 95531 (2-(3-carboxypropyl)-3-amino-6-(4-methoxyphenyl)pyridazinium bromide, 10 μM) to block GABA<sub>A</sub>Rs, t-AP5 (d(-)-2-amino-5-phosphono pentanoic acid) (10 μM) and 7-chlorokynurenic acid (7-chloro-4-hydroxyquinoline-2-carboxylic acid) (20 μM) to block NMDA receptors (NMDARs), and strychnine (0.3 μM) to block glycine receptors (all drugs were purchased from Abcam, Cambridge, UK).

For current-clamp recordings, patch electrode tips were coated with wax. The resting membrane potential (V<sub>r</sub>) was maintained at −89 mV, if necessary, using small amounts of injected current (typically <10 pA). This potential corresponds to the average resting V<sub>r</sub> measured immediately following formation of the whole-cell patch-clamp (~89 ± 1.5 mV, n = 37 cells). EPSPs were recorded in the presence of GABA<sub>A</sub> and glycine receptor blockers but without NMDARs antagonists. Trains of 30 stimulations at 10, 20, 50, 100 and 200 Hz were delivered to single MFs at 1-min intervals to prevent long-term changes in synaptic efficacy52. Because the action potential (AP) threshold decreases rapidly after establishing whole-cell patch-clamp, we discarded recordings where the threshold changed by more than 3 mV. The mean AP threshold was −55 ± 0.5 mV (n = 26 cells). Although our resting V<sub>r</sub> and AP threshold values are lower than those reported in the literature, we find that the difference of potential between them is in the range reported12,13.

All recordings were performed at 35–37 °C, low-pass filtered at 10 kHz and digitized at 100 kHz using an analog-to-digital converter (model NI USB 6259, National Instruments, Austin, TX, USA) and acquired with Nclamp (http://www.neuromatic.thinkrandom.com/) running in Igor PRO (WaveMetrics, Lake Oswego, OR, USA).

Analysis of EPSCs and GC firing. Analysis was performed with Neuromatic in Igor PRO (v. 6.2). Traces were filtered offline at 4 kHz. Data points during the extracellular stimulus artifact were blanked and the capacitive response was subtracted using a double exponential fit to its decay. All EPSC traces were corrected for series resistance error using an algorithm written in Igor based on ref. 54. EPSCs were baseline-subtracted using the average value over a 1-ms window ending immediately before the stimulus. The mean peak amplitude and variance were measured from the average in a 100-μs window around the peak. Background variance was calculated from a 100-μs window reflected around the baseline region, then subtracted from the peak variance28. The CV was estimated from the square root of the baseline-subtracted variance divided by the mean peak amplitude and determined from a minimum of 30 consecutive traces. To correctly estimate the amplitude of the second EPSC, for calculating paired-pulse ratios (PPRs), we subtracted the averaged single EPSC trace from the averaged paired stimulation trace. The final PPR was estimated from the ratio EPSC<sub>2</sub>/EPSC<sub>1</sub>. PPRs were calculated from a minimum of 20 trials per pulse train. For EPSC traces displayed in figures, peak amplitude, CV and PPR values were calculated from the displayed traces (that is, trials with paired stimulation only).

GCs firing rates were measured in a time window starting from the onset of the first MF stimulation and ending 10 ms (twice the average membrane time constant measured from the decay of a voltage response to a 100-ms-long, 4-pA square pulse; n = 6 cells) after the last stimulus. AP threshold and onset of firing were measured at the first point of the AP upstroke.

Statistical tests and k-means cluster analysis. To detect potential differences between groups, we first used an analysis of variance. Subsequent statistical tests were performed using nonparametric methods. The Wilcoxon rank-sum test was used to compare EPSC metrics between two samples. For multiple comparisons, we used the nonparametric Steel-Dwass test that corrects for error rate. In the figures statistical significance (P < 0.05) is indicated by an asterisk or noted as nonsignificant (NS). All error bars are s.e.m. unless otherwise indicated. Box plots show minimum, median and maximum values, and the top and bottom of the box are first and third quartile.

An unsupervised k-means clustering (KMC) method (JMP software, SAS, NC, USA) was used to identify putative input groups within the population of inputs obtained from all experiments (n = 276 inputs). We used the EPSC mean amplitude, CV and PPR as metrics for the cluster analysis, as they yielded the strongest correlations. We chose to use the PPR between the second and first EPSCs because it better revealed short-term facilitation, which in a subset of synapses was rapidly counteracted by short-term depression in subsequent pulses (see Fig. 1d). In brief, the KMC algorithm uses an iterative refinement technique that alternates between assigning each observation to the closest mean and updating the means to be the centroids of the observations in one cluster25. Sets of correlated variables (our multidimensional data points) are converted to principal components and the principal component subspace is used as the cluster centroid subspace. Because the KMC results depend on the ordering of the input data set, we randomly shuffled our data points and repeated the analysis 100 times. The number of clusters (k means) is an input parameter and was determined using the elbow method, in which the proportion of variance explained by the number of clusters is plotted against the number of clusters for each individual EPSC metric (Supplementary Fig. 2). The plot allowed us to determine that five clusters was the minimum number needed to account for the maximum amount of variance across all three EPSC metrics.

Estimating synaptic quantal parameters with multiple probability fluctuation analysis. MF-GC EPSCs were consecutively recorded in 0.5/5, 1.5/1.5 and 8/0 mM of Ca<sup>2+</sup>/Mg<sup>2+</sup>. Some inputs recorded in nonvestibular regions of the vermal cerebellum (n = 17 out of 37 of all inputs) were added to the data to increase the sample size. Mean peak current amplitude (I) and background-corrected variance (σ<sup>2</sup>) were calculated from a minimum of 30 EPSCs during stable epochs (Spearman’s rank order stability test). The mean and variance at each [Ca<sup>2+</sup>]<sub>i</sub> were fit with a multimodal model56:

\[
\sigma^2_I = Q_0I - \frac{I^2}{N} + Q_1CV_{GQ}^2 + Q_2JCV_{GQ}^2
\]

where Q<sub>0</sub> is the mean quantal size, N is the number of release sites and CV<sub>GQ</sub> and CV<sub>QI</sub> are the coefficients of variation of intrasite and intersite quantal variability56. CV<sub>GQ</sub> is composed of CV<sub>QG</sub>, the quantal variability at single sites, and CV<sub>QI</sub>,...
the variability arising from stochastic release latency. As our data did not contain MF-GC connections with single synapses we could not estimate \( CV_{Q1} \), and we therefore used the value 0.26 from ref. 20. We calculated \( CV_{Q2} \) from the difference in relative variability between stimulus-aligned (0.51, \( n = 7 \)) and rise-time-aligned quantal currents (0.35) to be 0.17. The total quantal variance \( CV_{Q} \) can be calculated as follows:

\[
CV_{Q} = \sqrt{CV_{Q1}^2 + CV_{Q2}^2} = \sqrt{CV_{Q1}^2 + CV_{Q2}^2 + CV_{Q3}^2}
\]  

(2)

\( CV_{Q} \) was estimated from stimulus-aligned EPSCs (\( n = 37 \) inputs) to be 0.44. \( CV_{Q1} \) was calculated to be 0.31 from \( CV_{QS} \) and \( CV_{Q2} \). Thus \( CV_{Q1} \) was 0.32 in good agreement with previous estimates. Errors in estimating the variance were calculated using the following equation:

\[
\sigma_{\text{sample-variance}} = \frac{\sqrt{\sigma^2}}{\sqrt{n - 1}}
\]  

(3)

where \( \sigma^2 \) is the population variance and \( n \) is the number of observations. Errors in estimating the variance are represented by error bars in Figure 2. Multinomial fits to the variance-mean plots were weighted according to errors.

**GFP expression in Thy1-mGFP (line 17) mice.** Expression of GFP in vestibular ganglion (VG) neurons was verified in Thy1-mGFP (line 17) mice aged from postnatal day 7 to 10. The inner ear becomes too hard to extract the ganglion (\( n = 4 \) mice). Mice were swiftly decapitated and placed in ice-cold ACSF, and the temporal bone was dissected out using a stereo microscope (Leica Microsystems). The vestibular ganglion (VG), which is situated in the upper part of the outer end of the internal auditory meatus, was extracted by carefully breaking the bone around it using fine forceps and gently pulling it from the nerve end cut from the brainstem during the dissection. The VG was then put into a chamber under a confocal microscope and held in place with a brain slice harp.

To examine GFP expression in brainstem nuclei, 100-\( \mu \)m-thick coronal slices of cerebellum and brainstem were cut from fixed brains of adult Thy1-mGFP (line 17) mice (\( n = 5 \)). Simultaneous contrast and fluorescence images were acquired using the microscopy system described below to visualize neuronal somata and eventual GFP expression, respectively.

**Stereotoxic injections.** To fluorescently label different MF pathways, we performed stereotoxic injections of viral vectors expressing fluorescent protein into precerebellar nuclei. We used the following vectors: AAV2/9.JhSyn.ChrR2 (H134R), mCherry; WPRE, provided by K. Deisseroth (Stanford University) and produced by the INSERM UMR1089 platform (used in 9 of 16 recordings from MVe MFs; Fig. 3e–h), or an AAV9.JhSyn.TurboRFP.WPRE.RBG vector purchased from Penn Vector Core (Philadelphia, PA, USA). Mice between 40 and 50 d of age were deeply anesthetized before surgery with a mixture of hypnotic (ketamine 1.5%, Mérial) and analgesic (xylazine 0.05%, Bayer) anesthetics mixed in phosphate buffer and injected in the peritoneum. A local anesthetic (xylcaine 2% gel, Newpharma) was applied on top of the location of the cranial incision. The anesthetized mouse was then placed on a stereotoxic frame adaptor comprising adjustable ear bars and tooth holder. The skull was then perforated at the injection site using a surgical drill. Nuclei were identified using the Paxinos and Franklin mouse brain atlas. The injection of viral constructs in the MVe (120 nl; virus titer, 6.6 \( \times \) 10^{12} GC/ml; 6 mm caudal to bregma, lateral 1 mm, ventral 4.3 mm) or the PrH (60 nl; 6.48 mm caudal to bregma, lateral 0.37 mm, ventral 5.1 mm) was performed by slow infusion (200 nl/min) with steel needles (26G \( \times \) 30 mm and 36G \( \times \) 70 mm, Phymep) connected to a pump via a catheter and a Hamilton syringe. Injected mice were then kept for 2 to 4 weeks toallow transgene expression.

To verify that the location of stereotoxic injections was correct and that viral transduction did not spill out of the targeted nuclei, we examined fluorescent protein (FP) expression in 100-\( \mu \)m-thick coronal slices of fixed cerebellum and brainstem (Supplementary Fig. 4). The concentrations of viral vectors and the volume of injection were adjusted in order to restrict viral transduction to the targeted structures. Images were acquired with a confocal microscope (LSM700, Zeiss) equipped with an air objective (10×, 0.45 NA, Zeiss) and a laser (555 nm) and running Zen 2009 v.5.5 software (Zeiss). Nuclei were identified using the Paxinos and Franklin mouse brain atlas. In a subset of viral injections, we verified the nuclei targeting accuracy. In all cases where FP expression was observed in the brainstem (\( n = 4 \) of 7 and 3 of 4 mice for MVe and PrH, respectively), the targeted nuclei exclusively expressed the FP.

**Targeted stimulation of fluorescent mossy fibers guided by two-photon scanning microscopy.** GCs were identified and whole-cell patch-clamped using infrared Dodt-gradient contrast and a QCClick digital CCD camera (QImaging, Surrey, BC, Canada) mounted on an Ultima multiphoton microscopy system (Bruker Nano Surfaces Division, Middleton, WI, USA) that was mounted on an Olympus BX61WI1 microscope, equipped with a water-immersion objective (60×, 1.1 NA, Olympus Optical, Tokyo, Japan). Two-photon excitation was performed with a Ti-sapphire laser (Spectraphysics). To visualize GCs contacting GFP-labeled MFs, Alexa Fluor 594 (20 \( \mu \)M) was dialyzed via the patch electrode. Two-photon excitation of GFP and Alexa 594 was performed at 810 nm. To visualize GCs contacting MFs labeled with TurboRFP, we dialyzed them via the patch electrode with Alexa 488 (20 \( \mu \)M) and used an excitation wavelength of 1,000 nm. Simultaneous Dodt-gradient contrast and fluorescence imaging were used to position the stimulation pipette near a labeled axon whose MF connected to the patched GC. If colocalization of FP (MFs) and Alexa fluorescence (GC dendritic ‘claw’) was observed over several focal planes spanning at least 3 \( \mu \)m, we considered the MF to be anatomically connected to the GC. We then performed minimal stimulation as described for the blind stimulation protocol (see above). As expected for fluorescence-guided targeting of MFs, lower levels of voltage (typically 2–5 \( \mu \)V) were needed to evoke transmission than during blind stimulation. To insure that we were stimulating the intended MFs, we performed a set of experiments in which we carefully withdrew the stimulation pipette from the proximity of the axon until we lost transmission and repeated minimal stimulation by gradually adjusting voltage (Supplementary Fig. 5). The rationale is that the initial proximity to the stimulated axon would be demonstrated if stimulation voltage had to be consistently increased with further distance from the targeted axon. Our results showed that stimulus voltage was always positively correlated with distance from the targeted axon (\( n = 3 \) cells). Moreover, minimal stimulations (5 V above threshold) covered an area no more than 5 \( \mu \)m wide, demonstrating the spatial precision of our method. Because individual dendrites of GCs extend apart substantially further than 5 \( \mu \)m, this method is very unlikely to stimulate more than one MF contacting the same GC, and thus allows specific stimulation of the fluorescently labeled MF.

**Estimating MF pathway convergence onto single GCs.** To examine possible convergence of primary vestibular with secondary vestibular or PrH afferents in single GCs, we performed stereotoxic injections in Thy1-mGFP (line 17) mice (\( n = 6 \) and 5 for injections in MVe and PrH, respectively). Acute slices were then prepared and GCs were whole-cell patched and filled with a fluorescent dye to visualize dendritic claws contacting fluorescently labeled MFs. Because the emission spectra of Alexa 488 and GFP or of Alexa 594 and TurboRFP overlap, we used combined one- and two-photon imaging to separate spectrally the different fluorophores. GCs were filled with Alexa 594 and imaged using a confocal microscope and a 594-nm diode laser (Omicron Lasers), which did not excite the far-red RFP MFs expressing either GFP were imaged using two-photon excitation at 1,000 nm. Connections between GCs and fluorescent MFs were verified as described above. Three-dimensional reconstructions of GCs and connected MFs were performed with Fiji-TrackEM2.

**Synaptic behavior of different MF inputs converging on single GCs.** A blind minimal stimulation protocol was used to evoke synaptic transmission from two successive, independent MFs innervating the same GC. Input types were classified by KMC analysis. If the two inputs belonged to the same cluster, we compared their EPSCs mean amplitude, CV, PPR and waveform (10–90% rise time and weighted decay). In 6 of 8 cells, the mean difference between EPSC metrics was greater than 20%, and the two inputs were thus considered to come from different synapses. In the two other cases, inputs could not be differentiated with those metrics (mean differences < 10%), and they were therefore rejected and not analyzed further. The EPSC weighted decay was measured as the area under the peak-normalized trace inside a 20-ms window starting from the EPSC peak.

**Animals and surgical preparations for in vivo recordings.** All experiments were performed on 4- to 6-week-old C57BL/6 mice, of either sex, anesthetized with either a mix of ketamine and xylazine (100 mg/kg and 10 mg/kg body weight, respectively).
respectively) or a mix of fentanyl, midazolam and medetomidine (0.05 mg/kg, 5.0 mg/kg and 0.5 mg/kg, respectively), with anesthesia topped up throughout the experiment as required, indicated by the recovery of the toe-withdrawal reflex. We did not find significant differences between the two anesthetic conditions in mean EPSC peak amplitude: 23.3 ± 3.9 pA with ketamine/xylazine versus 29.3 ± 4.9 pA with fentanyl/midazolam/medetomidine (n = 16 and 12 vestibular recordings, respectively; P = 0.30) and frequency of spontaneous EPSCs: 9.4 ± 1.4 versus 8.7 ± 1.9 Hz (P = 0.36). Experiments performed with the two different anesthetic conditions were therefore pooled.

Mice were head-fixed with a holder consisting of ear bars and a mouth clamp (Narishige, modified) in the prone position, with the head angled nose-down by 32.6°. Mice were freely breathing and maintained at a body temperature of 37 °C with a heating blanket (FHC), controlled with a rectal thermometer. To access the flocculus, a small craniotomy was performed in the left petrosal bone over the paraflocculus. The dura mater was removed with fine forceps (Dumont no. 5). The brain was kept moist with phosphate-buffered saline (PBS, Sigma). All experiments were in compliance with UK Home Office regulations.

**In vivo electrophysiology.** Whole-cell patch-clamp recordings were performed from GCs in the left cerebellar flocculus using the blind patch-clamp technique as previously described. Recordings were performed in the flocculus rather than in the nodulus because the mechanical support provided by the cranium facilitates whole-cell recordings. Electrodes were pulled on a vertical electrode puller (Narishige) to show a resistance of 7–10 MΩ, which facilitates whole-cell recordings. Electrodes were advanced through the paraflocculus into the flocculus to a depth of 1,200 to 2,000 μm from the pial surface of the paraflocculus. GCs showed a capacitance of 3.6 ± 0.1 pF. Recordings with series resistances over 55 MΩ were discarded (average series resistance = 41.0 ± 1.2 MΩ).

Excitatory postsynaptic currents (EPSCs) were recorded in voltage-clamp mode at −70 mV, close to the reversal potential of Cl−, to isolate excitatory postsynaptic currents, with an Axon Multiclamp 700B amplifier (Molecular Devices), low-pass-filtered at 8 kHz, digitized at 33.3 kHz with an IT-C18 ADC/DAC board (HEKA), acquired with an Apple Power Mac computer running Igor Pro (Wavemetrics) with Neuromatic/Nclamp (http://www.neuromatic.thinkrandom.com/), and stored digitally for offline analysis.

**Vestibular stimulation.** For vestibular stimulation, the mouse and head holder were fixed on a custom-built, computer-controlled motion device (rotational stages by Newport) allowing separate and combined rotations about a horizontal and a vertical axis. The axes of rotation were centered between the vestibular organs. With the head tilted 32.6° nose-down and the anterior-posterior axis of the mouse oriented 45° to the axis of vertical rotation, horizontal rotation primarily stimulated the horizontal semicircular canals (SCCs), while vertical rotation stimulated almost exclusively the left anterior and right posterior SCCs (ref. 60). The mouse was rotated in the dark following a discontinuous compound sinusoidal position/velocity profile, controlled by custom-written routines in Igor Pro (WaveMetrics). For horizontal rotations, maximum velocities of 38°/s were presented. For reasons of recording stability, vertical rotations were usually limited to maximum velocities of 9°/s.

**Visual motion stimulation.** Visual stimuli were presented to the ipsilateral or contralateral eye on a LCD monitor (Dell) using an Apple Mac Mini running the psychophysics toolbox in Matlab, triggered by the electrophysiological recording software. The LCD monitor (screen size 17 inch diagonal) was positioned at a distance of 21 cm from the eye of the mouse at an angle of 45° to the animal’s anterior-posterior axis, thus covering approximately 79° in azimuth and 63° in elevation. Motion stimulation consisted of drifting black and white square-wave gratings at full screen contrast with a spatial frequency of 0.04 cycles/° moving with a temporal frequency of 2 cycles/°. Each trial consisted of eight blocks of equally spaced directions of motion, with stripes stationary for 1 s, drifting at constant speed for 1 s, and being stationary for 1 s before the orientation was changed by 225°.

**Analysis of in vivo data.** Data analysis was performed using Igor Pro with Neuromatic. EPSCs were detected using a −3 to −5 pA threshold criterion from the recorded traces after binomial smoothing over six points and subsequently confirmed by visual inspection. EPSCs were aligned on the event onset and baseline over a window of 1 ms ending 0.1 ms before the event onset. EPSC traces were corrected for series resistance error as described above for *in vitro* recordings. Peri-stimulus time histograms (PSTHs) were calculated for 100-ms time bins. To determine the presence of a vestibular response, the EPSC rate was tested for correlations to the stimulus position, velocity and acceleration. We did not analyze EPSCs of cells in which there was evidence of a second input, to avoid errors in estimating amplitudes from individual inputs due to imperfect input separation. Visual-motion-evoked responses were calculated from the average EPSC frequency over the period of visual-motion stimulation for each of the eight stimulus directions measured and normalized to the average baseline rate calculated from the eight 1 s intervals preceding the visual motion stimuli. Polar plots show these baseline-normalized responses according to their direction of stimulation. Visual-motion tuning vectors were calculated as the vector sum of these response vectors from all 8 directions measured, divided by 2, the number of stimulus directions per quadrant. For visually evoked EPSCs (preferred direction), stability of EPSCs amplitude over the stimulus epoch was examined using a Spearman’s rank-order stability test routine written in Neuromatic with a first-pass mean search window of 10 points, a second-pass window with fraction 0.5 and a significance level of 0.05.

**Numerical simulations of synaptic integration in GCs.** GC voltage responses to MF inputs were simulated as in ref. 23. Briefly, AMPAR and NMDAR conductances (G<sub>AMPAR</sub> and G<sub>NMDAR</sub>) were injected into an integrate-and-fire model written in Igor Pro. Membrane voltage (V) was calculated with the following differential equation:

\[-C_m V' = G_m (V - E_m) + G_{GABAR} (V - E_{GABAR}) + \sum G_{AMPAR,i} \Delta_{AMPAR,i} (t) (V - E_{AMPAR}) + G_{NMDAR,i} \Delta_{NMDAR,i} (t) m(V) (V - E_{NMDAR})\]

where V′ is the time derivative of V, C<sub>m</sub> is the membrane capacitance, G<sub>m</sub> and E<sub>m</sub> are the membrane leak conductance and its reversal potential, respectively, and i is the index for different sets of conductances representing different MFs. G<sub>AMPAR</sub> and G<sub>NMDAR</sub> are the AMPAR and NMDAR reversal potentials, Δ<sub>AMPAR</sub>(t) and Δ<sub>NMDAR</sub>(t) indicate the spike trains convolved with the unitary synaptic conductance waveforms A(t) and m(V) is the fraction of GluN2C-containing NMDARs not blocked by Mg<sup>2+</sup> (ref. 60). C<sub>m</sub> was set to 5 pF, as calculated from the membrane time constant (4.71 ± 0.7 ms) measured from the decay of voltage in response to a current step of 4 pA and 100 ms duration (n = 6 cells). We chose to use the estimation of C<sub>m</sub> from current-clamp recordings because the capacitance transient cancelation in voltage-clamp mode neglects slower components (for example, axonal compartments), which could contribute to the total capacitance. G<sub>m</sub> was set to 1.1 nS and E<sub>m</sub> to −89 mV, as measured from recorded cells (1.1 ± 0.4 nS and −82 ± 0.9 mV, n = 29). E<sub>AMPAR</sub> and E<sub>NMDAR</sub> were set to 0 mV. The action potential threshold was set to −55 mV, as measured (see above).

The time course of synaptic conductances was modeled with the following multiple-exponential equation:

\[A(t) = (1 - e^{-\tau_1 / \tau_2}) a_1 + d_1 e^{-\tau_1 / \tau_2} + d_2 e^{-\tau_3 / \tau_4} + d_3 e^{-\tau_3 / \tau_4} / \Delta_{a_1} / \Delta_{a_2} / \Delta_{a_3} / \Delta_{a_4}\]

where \(\tau_1\) is the synaptic event time, \(\tau_2\) is the rise time constant, \(\tau_3\) and \(\tau_4\) are the decay time constants, \(d_1\) and \(d_2\) are the percentage amplitudes of the different components and \(\Delta_{a_1}\) is a normalization scale factor.

Direct and spillover-mediated AMPAR conductance waveforms were adjusted to match the G<sub>AMPAR</sub> obtained from recorded AMPAR EPSCs divided by the driving force. The AMPAR spillover component peak amplitude was set to 0.34 of the direct component peak amplitude, as described in ref. 60. G<sub>NMDAR</sub> waveform parameters were taken from ref. 53 and the G<sub>NMDAR</sub> to G<sub>AMPAR</sub> ratio was adjusted so that simulated EPSPs fitted the recorded
AMPAR+NMDAR-mediated EPSPs. A $G_{\text{NMDAR}}/G_{\text{AMPAR}}$ ratio of 0.1 produced good fits of EPSPs for all different input groups, consistent with the low ratio measured in adult GCs.53. The unblocked $G_{\text{NMDAR}}$ was then calculated from the NMDAR $I-V$ relation in ref. 23.

Depression and facilitation of the AMPAR (direct and spillover) and NMDAR synaptic conductances were modeled as single exponential functions. We adjusted both the time constants ($\tau_D$ and $\tau_F$) and their amplitudes ($D$ and $F$) to fit the train of AMPAR conductances estimated from EPSCs for each group. $G_{\text{NMDAR}}$ dynamics were subsequently adjusted to reproduce summation of EPSPs recorded in current clamp. Parameters for model synaptic conductances and short-term dynamics of each input group are displayed in Supplementary Tables 1 and 2.

Tonic activation of GABA$_A$Rs was model as described in ref. 17.

Briefly, a constant inhibitory conductance of 438 pS and reversal potential of $-79$ mV were added when two MF inputs were simulated. For the three-MF-input condition, we increased the inhibitory conductance by 30% (to 573 pS) to mimic the network activity dependence of inhibition described in ref. 17.

### Simplified GC layer model for pattern decorrelation.

In the thresholding model the GC output is given by the formula

$$y_t = [x_t - k]_+$$

where $x_t$ and $y_t$ are the input and output of the $t$th GC, $k$ is the threshold and the subscript $"+"$ denotes half-wave rectification.18,61–64. In the delay coding model, the output replicates the input after a delay $d$ that depends on the strength of the input. The output as a function of the delay can thus be expressed by the formula

$$y_t(d) = \begin{cases} x_t, & x_t \geq k(d) \\ 0, & \text{else} \end{cases}$$

with a delay-dependent threshold $k(d)$. We used a range of delays denoted from 1 to 5, the threshold $k$ being set at 4 at delay 1 and reduced to 0 at delay 5 in equidistant steps.

Output pattern correlations in the large $N$ limit of the simplified GC model were calculated by numerical integration. Double integrals of the form

$$M_{\text{inn}}(k,l,p) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{(x-k)^{m_1}(y-l)^{m_2}}{n!m_1!2\pi\sqrt{1-\rho^2}} \frac{x^2 + y^2 - 2\rho xy}{2(1-\rho^2)}$$

were computed as described.64 Output correlation of the thresholding model was computed as $|M_{11}(k-2,k-2,0) - M_{11}(k-2,k-2,0)|/|M_{11}(k-2,k-2,0) - M_{11}(k-2,k-2,0)|$. Output correlation of the delay coding model was computed as $|C(k,d_2,2) - C(k,d_1,2)|/|C(k,d_2,2) - C(k,d_1,2)|$ with

$$C(k,l,p) = M_{11}(k-l,k-l,p) + 2kM_{10}(k-l,k-l,p) + k^2M_{00}(k-l,k-l,p)$$

### Calculation of minimum SNRs tolerated by linear classifier.

We assumed that the patterns to discriminate have equal mean and variance. Then, using elementary trigonometry and the fact that the scalar product of unit length vectors equals the cosine of the angle subtended by these vectors, the Euclidean distance between the patterns in units of pattern s.d. equals

$$d_{\text{decorrelator}} = \sqrt{2(1 - r_{\text{decorrelator}})}$$

where $\text{decorrelator}$ is either thresholding or delay coding.

Assuming isotropic noise, the maximum permissible noise variance equals

$$\frac{1}{2}f d_{\text{decorrelator}}^2$$

where $f$ depends on the level of acceptable misclassifications (if, for example, 5% misclassifications are just acceptable and noise is Gaussian then $f = 0.37$). The ratio of minimum required SNRs is thus $(1 - f_{\text{delay-coding}})/(1 - f_{\text{thresholding}})$.

A Supplementary Methods Checklist is available.

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