Simple spike activity predicts occurrence of complex spikes in cerebellar Purkinje cells

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Purkinje cells are the only output neurons in the cerebellar cortex, which plays a vital if poorly understood role in the coordination of movement. Purkinje cells show two forms of activity. Complex spikes (CS) occur at a low frequency and are driven by excitatory input from a single climbing fiber, whereas simple spikes (SS) occur at much higher frequency and are driven by the inputs from a much larger number of parallel fibers (Fig. 1). It is well established that the parallel fiber-Purkinje cell synapse can undergo long-term depression (LTD) in response to the coincident firing of both parallel and climbing fibers[1]. There is also some evidence that repetitive firing of parallel fibers alone can induce long-term potentiation (LTP) at the same synapses. Presumably, these opposing tendencies must be balanced in order to maintain an appropriate distribution of synaptic strengths, and the climbing fibers are likely to play an important role in controlling this balance.

Parallel fibers carry a broad range of signals, including proprioception from the periphery, efferent copy of the output of motor cortical areas, and inputs from sensory and motor association areas. The signals carried by climbing fibers, which originate only in the inferior olive, are far less well understood. Sensory stimuli may trigger climbing fiber activity (and thus CS), but they often also fire in close association with movement and have been suggested to have an instructive or error-corrective role[2,3]. However, CS occur even in quiescent animals, with an average firing frequency of around 1 Hz but with high variability. So Keating and Thach[4] reported that CS occur close to the start or end of movements, but that at other times they have no underlying periodicity. This apparently random CS activity in the absence of movement is a problem for many theories of cerebellar function; because the Purkinje cells continue to fire SS at a background rate of 30-70Hz, driven by parallel fiber input, it would be expected that random occurrences of CS would produce random depression of these parallel fiber-Purkinje cell synaptic weights. It is generally assumed that such changes would have little functional significance, averaging out over long time intervals because of the lack of consistent coupling with specific parallel fiber inputs, but this seems naïve. It is equally likely that the effect would be to specifically depress the most active synapses, and thus change the inputs most important for activating the Purkinje cell . Understanding the events that trigger complex spikes is therefore crucial for understanding the cerebellum.

However, these 'random' CS may in fact reflect subtle changes in cerebellar output. Given that parallel fiber activity is thought to induce LTP in the parallel fiber-Purkinje cell synapses[5], we presume that the ongoing activity of parallel fibers would, if unchecked, lead to an ever-increasing level of Purkinje cell SS firing. We, like Mauk[6,7], propose that CS activity in the absence of movement is not simply 'noise', but instead may be actively correcting this trend towards excessive potentiation. Mauk and colleagues suggested an "autocorrective" effect; because Purkinje cells are inhibitory, an increase in their firing rate would reduce the deep nuclear cell firing rate (Fig. 1), including that of inhibitory nuclear cells that project to the inferior olive[8]. Disinhibition of the olive would then cause a proportionate increase in CS activity, which would induce LTD and thereby counteract the tendency toward increasing potentiation. This combination of synaptic depression and potentiation would help maintain the cerebellar cortex within its operational range – Purkinje cells would neither fall silent due to LTD of their inputs nor be driven into excessive activity through LTP.

If this hypothesis were true, there should be a predictable relationship between increased SS activity driven by the potentiating parallel fiber input and subsequent corrective CS activity. This relationship should hold true even when the animal is quiescent and when the parallel fibers show no movement-related variations in firing rate. Furthermore, we can make some assumptions about the temporal relationship between these events. Miall and colleagues[9] have proposed that the cerebellum has a role in generating internal feedback signals that are used to adjust on-going motor commands. These internal predictive signals can be thought of as estimates of the sensory consequences of movement. To learn these predictions and keep them accurate, it is proposed that they are compared with real sensory feedback at or before the level of the inferior olive. The climbing fibers would signal discrep-

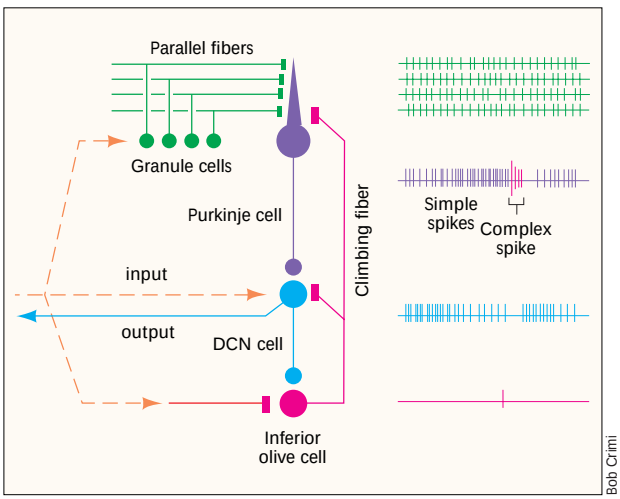

Fig. 1. A schematic diagram of the cerebellar cortico-nuclear-olivary circuit; the main connections are shown on the left, excluding interneurons. Spike activity in the cells is indicated on the right. Purkinje cells receive many excitatory parallel fiber inputs and a single, powerful, climbing fiber input from the inferior olive (IO). Each climbing fiber (CF) spike leads to a complex spike in the Purkinje cell. The parallel fiber-Purkinje cell synapses evoke simple spikes, and disply long term potentiation[5], as well as CF-driven long-term depression[1]. The Purkinje cells inhibit deep cerebellar nuclear cells (DCN), which in turn inhibit the inferior olive.

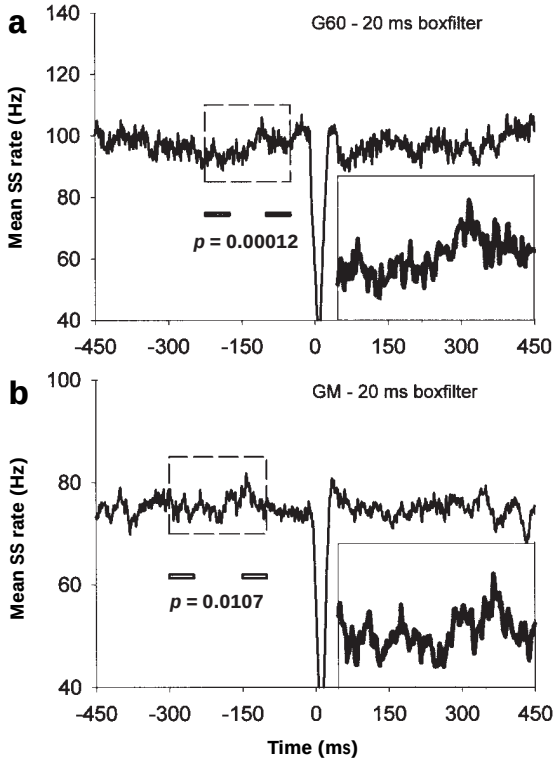

Fig. 2. Simple spike activity time locked to complex spikes. **(a)** This record is the mean response of 9 blocks of trials from one cell. Each block (6-65 trials per block, median 13) was averaged, and the average values used for statistical testing. For display purposes only, the average data from the 9 blocks have been averaged together, and smoothed with a 20 ms box-car filter. Also for display only an artifact at t = 0 has been removed before smoothing; this was due to the simple spike detector being triggered by the complex spikes. Horizontal bars indicate the pair of intervals with the most significant increase in SS activity preceding the CS; the area within the dashed box has been enlarged in the gray inset. **(b)** Averaged data from a group of 10 different Purkinje cells, in the same format (29-72 trials per cell, median 45).

ancies between the prediction and feedback. Because we propose that the SS activity represents a predictive signal – either a prediction of sensory feedback[9] or a predictive motor command[4] – we would expect a consistent delay between the increase in SS activity and the resulting CS, equivalent to the prediction interval. This is because to assess any prediction errors accurately, the predictions must be delayed to be in synchrony with the feedback. For visually guided movement, we estimate a prediction interval of about 150 ms, based on the visuo-motor feedback delay[10–12]. We can therefore predict, in the absence of other changing inputs, a rise in SS activity about 150 ms before CS activity. The amount of LTD generated by a single CS is small; thus, if its function is to correct an excess of SS, the system should require only a small *percentage* increase in the number of SS to induce a CS.

To test this hypothesis, we have analyzed the rate of SS activity at defined time intervals preceding a CS. We reexamined data previously recorded from Purkinje cells in the intermediate cerebellar cortex of two rhesus monkeys[4]. Details of the experimental methods are in the original reports[13,14]; briefly, the animals tracked a visual target with a cursor controlled by a manipulandum that was moved by the wrist. On each trial they were required to hold the cursor steadily at a start position for an interval of 1-2 s before making a discrete movement to capture the target. We have analyzed data only from these pauses between movements, so as to minimize any changes in SS activity related to visual cues or to the monkeys' movements. We selected CS that occurred in the middle of the pause, discarding those that occurred within 500 ms of either the start or the end of the pause period. We also discarded CS that occurred within less than 500 ms of another CS. After this selection, sufficient data were available from 11 cells that showed well-isolated CS. We aligned the records from these cells by their CS, in order to examine the average SS activity at defined intervals relative to the CS. To generate a control data set, we also collected 100 records from each cell aligned to the start of the instructed pause interval rather than to a CS. For one of the 11 cells, we had 9 separate blocks of data (6-65 trials per block, median 13), each recorded during the pause within 9 behaviorally different tasks[13,14]; we have treated these as 9 independent samples. For the remaining 10 cells, the data were collected from within a single task. All available trials for each cell (29-72 trials, median 45) were averaged and the mean firing rate for each cell calculated over a 50 or 100 ms interval either side of the test time-point of 150 ms before the CS ("pre-CS"). Our hypothesis was that there would be an increase in average SS firing at this point, so the mean firing rate in these two intervals was then compared with a one-tailed paired t-test.

For the 9 blocks from a single cell, the most significant increase in SS found was in comparing 225-175 ms pre-CS with 100-50 ms pre-CS (p = 0.00012, n = 9, Fig. 2a). Comparison of the same interval across the other 10 cells was not significant (p = 0.18, n = 10), but the adjacent comparison of 225-175 ms pre-CS with 150-100 ms pre-CS was significant (p = 0.0152). For these 10 cells, the most significant increase found was 300-250 pre-CS vs. 150-100 ms pre-CS (p = 0.0107, Fig. 2b). Every pair of intervals tested was also tested with data from the control set; none of 22 comparisons were statistically significant (p > 0.4). Hence the rise in SS preceding CS was not due to a task-related change in inputs. The group of 10 cells also showed a significant decrease in SS activity after the CS, presumably reflecting the induction of LTD (200-100 ms pre-CS vs. 100-200 ms post-CS, p = 0.0481).

In a contrasting hypothesis, Llinas, Welsh and colleagues[15,16] have suggested that activity across groups of climbing fibers is correlated with about a 10 Hz rhythm. Rhythmic CS activity could lead to an apparent increase in SS activity preceding each CS similar to that shown in Fig. 2 because changes in SS activity following one CS would appear to precede the next. But these same CS records have been exhaustively tested for any rhythmic behavior that might have resulted in time-locked changes in the SS and none were found[4].

In summary, we have observed a small but statistically significant increase in simple spike activity about 150 ms before apparently random complex spikes recorded during a pause between visually guided movements. This 150 ms interval is important. It is much longer than expected on the basis of axonal and synaptic delays from the Purkinje cells to the inferior olive and back again, but it is close to the estimated visuo-motor feedback delay[10–12]. If the Purkinje cell output represents a predictive signal and the CS represents an error

correction mechanism, we would expect the interval between the SS and the CS to depend on the delay between the movement and the feedback it causes; for these cells, which display activity related to the monkeys' visually guided movements, this interval is appropriate.

Such a subtle rise in SS activity may be undetectable within a single Purkinje cell on a trial-by-trial basis, but 15 or 20 Purkinje cells converge on each nuclear cell, and hence even a small change in SS across such a group of cells might have a significant effect on activity in the nucleus. There may be further convergence from the cerebellar nuclei to the inferior olive, and it is this combined input to the olive that would lead to a CS. Then, through LTD, the CS would down-regulate the average SS activity across a small number of Purkinje cells. So in this framework, the apparently random CS activity that occurs between movement trials may be correcting cerebellar errors that follow parallel fiber-Purkinje cell LTP.

Why would the olive correct for cerebellar errors even in the absence of movement? When examined from the viewpoint of the ascending cerebello-cerebral projection, the predictive nature of the Purkinje cell discharge may act as a feedforward motor correction, fine tuning ongoing processing in higher motor centers. Discrepancies between cerebellar predictions and sensory feedback could arise during movement because the cerebellar output is inappropriate for the current motor state of the animal or because of external disturbances. Of course, external perturbations may not be predictable, but this should not stop the cerebellum from trying to predict them. Exactly the same causes of discrepancy are present even without active movement. Thus there may be one neural mechanism, LTD of parallel fiber synapses driven by complex spikes, which seeks to maintain accuracy of the cerebellar output during active movement, during external perturbation, and during steady posture. With this finely honed output, the cerebellum can then contribute to the normal smooth and coordinated control of stable posture and of active movement.

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Sex, Lies And Virtual Reality

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Do male and female brains differ with respect to spatial processing? Gender differences in spatial abilities[1–4] as well as in cerebral organization[5,6] have led to suggestions that the sex of subjects should be taken into account in neuroscience studies[7]. Here we demonstrate gender differences in the response to a visuovestibular information conflict created by a deceptive virtual environment.

The visual system interacts strongly with the vestibular system, as both sensory modalities are directly related to spatial processing. When visual and vestibular signals conflict, the dominance of vision in perceiving spatial orientation might cause recalibration of the vestibular system. Alternatively, the brain might ignore vestibular inputs altogether. Differences in the response to conflicting visuovestibular information could characterize inherent gender-related spatial abilities and be related to the increased susceptibility of females to motion sickness[8].

The experiment used a virtual reality display to induce a twofold visuovestibular discrepancy for angular whole-body displacement. Twenty-six right-handed undergraduate students (13 males and 13 females) rotated themselves by 90° in a virtual visual room (Fig.1a) by controlling a rotating chair with a joystick. The speed of these rotations was controlled by the experimenter, and the subjects used the joystick as a start-stop button. A gain of 0.5 was introduced between the movements of the chair and the visual feedback given. To achieve a 90° rotation in the virtual room, subjects had to rotate the chair by 180°. Subjects performed 180-200 rotations, alternating to the left and right, during 45 min of this visuovestibular stimulation (VVS). The magnitude and the mean speed of rotations were the same for all subjects. Before and after exposure to VVS, passive whole-body angular displacements (45°, 90°, 135°, 180° to the left and to the right, triangular velocity profile, 11°/s^2) were administered in random order to blindfolded subjects to assess their turning perception in darkness. (See ref. 9 for details on the method.) Subjects gave their written informed consent to the study after the procedure had been explained and were paid for their time.

Vestibular stimulation in darkness elicited a sensation of whole-body rotation with a magnitude proportional to the angular stimuli. Estimates of imposed rotations were not significantly different in males and females prior to VVS. After exposure to conflicting VVS, decreases in turning estimates were significantly greater for males than for females ($F(1,24) = 7.96$, $p<0.01$) (Fig.1b), although some females did adapt more than some males. Interestingly, recalibration of vestibular inputs at the perceptual level occurred without awareness of the visual-vestibular information conflict.