LETTERS

## Isochrony in the olivocerebellar system underlies complex spike synchrony

In a recent issue of *The Journal of Physiology* Baker & Edgley (2006) published results which they interpreted as showing a non-uniform olivocerebellar conduction time in the rat. Baker and Edgley used the climbing fibre reflex to measure conduction time to different points along a folial wall. This reflex is initiated by electrical stimulation of the cerebellar white matter, which triggers antidromic spikes in olivocerebellar axons that invade the inferior olive (IO). Current generated by these spikes spreads to neighbouring IO neurones via the gap junctions that electrically couple IO neurones (Llinás *et al.* 1974). This spreading excitation can trigger orthodromic spikes that return to the cerebellar cortex and trigger Purkinje cell complex spikes (CSs). Baker and Edgley found that climbing fibre reflex latency varied with recording electrode depth, and inferred from this that conduction time between the IO and cerebellar cortex varies systematically with cortical location. This study conflicts with results by us and others that indicate a near uniform olivocerebellar conduction time in rats and turtles (Sugihara *et al.* 1993; Lang & Rosenbluth, 2003; Ariel, 2005). Below we discuss several troubling issues with this new study, but first we would like to make the point that spontaneous CS synchrony patterns almost necessitate a uniform olivocerebellar conduction time.

Synchronous (on a millisecond time scale) CS activity has been demonstrated in several species (Sasaki *et al.* 1989; Sugihara *et al.* 1993; Wylie *et al.* 1995; De Zeeuw *et al.* 1997; Lang *et al.* 1999; Marshall *et al.* 2004). Although in many studies recordings were obtained only from the apex of crus 2a, where the olivocerebellar path length is similar to all cells, and thus conduction velocity is not a major issue, in other studies, recordings were made from areas to which olivocerebellar path lengths almost certainly are not identical. For example, recordings from the apex of crus 2a and along its folial wall to a depth of 2 mm show that significant levels of synchronization occur between CSs of cells at these locations (Sugihara *et al.* 1993). Most importantly, neither the level of synchrony nor the time lag of the correlogram peak varies with the position of the cell along the folial wall. In other studies, recordings were obtained from multiple lobules simultaneously, and synchronous CS activity was found to occur between cells located on different lobules: crus 2a and vermis lobule 9 (De Zeeuw *et al.* 1996); crus 1 and 2a, 2a and 2b, 2a and vermis 6c (Yamamoto *et al.* 2001); and floccular and nodular regions (Wylie *et al.* 1995).

In order for CS synchrony to occur among these various regions in the absence of a matching of conduction velocity to olivocerebellar axonal length, either the path lengths to these lobules must be identical or activity in the IO subregions projecting to these various lobules must be phase lagged to account for the differences in conduction time. Tracing results indicate that the first possibility is not true (Sugihara *et al.* 1993). The second possibility is also *a priori* unlikely, and recordings from IO neurones typically show precisely synchronized activity (Llinás & Yarom, 1986; Long *et al.* 2002). Thus, by themselves these findings on spontaneous CS synchrony almost force the conclusion that the conduction velocities of olivocerebellar axons vary with axonal length so as to preserve a constant conduction time between the IO and cerebellar cortex.

Conversely, the impact of a conduction velocity tuning mechanism on olivocerebellar conduction time, and its importance for CS synchrony were shown recently (Lang & Rosenbluth, 2003). In this study, olivocerebellar conduction time was measured in juvenile rats in which full myelination of the olivocerebellar pathway has not yet occurred, and in myelin-deficient mutant rats. In both cases, the absence of myelination was associated with widely varying conduction times to different regions of the cerebellar cortex (~10 ms variation), and a reduction of CS synchrony. These results show that the differing distances to the various parts of the cerebellar cortex can, and do, in the absence of a tuning mechanism, generate significant conduction time differences. The reduction of these timing differences with myelination from a 10-ms to a $< 1$ ms time scale provides further strong evidence that the conduction velocity of an olivocerebellar axon is specifically tuned to its length.

Given the above, how can we explain the results of Baker and Edgley? First, we note that at least one significant data point appears either to have been left out of the analyses or plotted incorrectly. Specifically, their Fig. 2 shows the reflex responses of a cell that is 2.02 mm deep. These responses have a latency of 8.4 ms, but in their Figs 3 and 4 no such data point is plotted. This point was likely misplotted because there appear to be 33 points plotted in Fig. 4, and the total number of recorded cells is listed as 33. Because of this error, the listed regression line and significance levels are likely not correct. This error is also significant, because if correctly plotted, this data point fits with the idea of a relatively uniform conduction time.

A second troubling issue is the very short latencies (~4 ms) of some of Baker and Edgley's 'reflex responses'. The one way conduction time for the olivocerebellar system in the rat is ~4 ms (Sugihara *et al.* 1993). Therefore, reflex responses cannot be shorter than this time. Moreover, given that there is also a significant antidromic travel time, and time is required for spread of activity through the IO, reflex responses should be at least double the one-way conduction time. The shortest responses reported by Baker and Edgley are only 4.3 ms, and thus are physically impossible for a reflex response. Moreover, many other responses are significantly less than 8 ms, and thus are also unlikely to be reflex responses. Note that Baker and Edgley cite our work as stating that the rat olivocerebellar conduction time ranges from 2 to 5 ms, but nowhere in our papers are such short conduction times described. We do mention a hypothetical conduction time of 2.34 ms, but that value was calculated for the shortest axons assuming no velocity tuning. However, experimentally no such values were found.

In short, latency considerations make it likely that at least some of the responses observed by Baker and Edgley are direct axonal responses rather than climbing fibre reflex responses. The description of their responses is consistent with this possibility. Baker and Edgley describe their 'climbing fibre reflex' responses as being 'consistently evoked' with a 'small amount of jitter'

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that averaged 0.32 ± 0.21 ms. This sub-millisecond jitter is more consistent with a direct response than a reflex response. In our experience the latency of the reflex response for a single cell typically varies by several milliseconds. Moreover, reflex responses are typically evoked very irregularly, in contrast to direct axonal responses. These differences in the two responses are not surprising, as the direct response depends on secure axonal transmission, whereas the reflex response depends on the state of olivary neurones, which is very much in flux.

A third problematic issue with Baker and Edgley's conclusion is that it requires an intrafolial conduction velocity of 0.6 m $s^{-1}$. This value would necessitate a radical slowing of the action potential, and is inconsistent with the known relationship of axonal diameter and conduction velocity. Given a conduction time of ∼4 ms and path lengths of 9–17 mm, olivocerebellar axons have conduction velocities on the order of 2.4–4.2 $ms^{-1}$ (Sugihara *et al.* 1993). While these values for olivocerebellar conduction velocity were estimated from the measured axonal lengths and conduction times (the latter of which is being disputed Baker and Edgley), they are consistent with the conduction velocity–axonal diameter relationship determined for myelinated mammalian axons (Hursh, 1939; Rushton, 1951), and thus are likely to be valid. Conduction velocity in myelinated nerves is proportional to axonal diameter (Johnston & Wu, 1995); thus, a slowing down of an olivocerebellar action potential to 0.6 $ms^{-1}$ would require a 4- to 7-fold reduction in axonal diameter. Climbing fibres have a 1–2 $\mu$m diameter in the restiform body (Van Der Want *et al.* 1989). Thus, if such a slowing as required by Baker and Edgley occurred, climbing fibres ascending in the folial white matter typically would be 0.27 $\mu$m in diameter, with some as thin as 0.14 $\mu$m. In actuality, even smaller diameters would be necessary, because if there truly was a slowing of the velocity in the intrafolial portion of the axon, the more proximal axon would have a somewhat higher conduction velocity, producing an even greater ratio of velocities, and therefore, diameter reduction. There is no evidence for this required dramatic reduction in axonal diameter. Instead, measurements in the restiform body, cerebellar white matter, and granule cell layer indicate that olivary axons maintain a similar diameter throughout their course, and only demyelinate as they reach the Purkinje cell (Palay & Chan-Palay, 1974; Van Der Want *et al.* 1989).

Finally, Baker and Edgley make a critical assumption that the time from antidromic invasion of the IO to orthodromic spike initiation is constant. This assumption is likely false and so provides a further possible explanation for their results. First, we note that Baker and Edgley state that they 'were unable to target activated units in the deepest parts of the fissures' because of the narrowness of the reflex response distribution. This statement implies that their recording electrode was crossing zebrin-defined zones as it descended, either because it was moving along an axis perpendicular to the parasagittal plane defined by their stimulus electrode, or because the zebrin-defined compartments shift 200–300 $\mu$m toward the midline as they descend toward the bottom of the folia (Fig. 1*D* of Sugihara & Shinoda, 2004). This in turn raises the possibility that at different depths the climbing fibre reflex pathway through the IO was different, and as we show below, almost certainly was shorter for the deeper responses, which would lead to a shorter latency and hence the observed relationship.

Given the zonal pattern in lobules VI and VII, where the stimulus electrode was placed, it is likely that fibres from zebrin compartment a+, which is much wider than other compartments in these lobules, were stimulated, and thus the antidromic volley would have entered the IO subdivision called medial accessory olive subnucleus c (MAOc). In contrast, in lobule VIII, where the recordings were obtained, compartments 1+, 1− and 2+, from medial to lateral, receive axons from IO regions MAOa, MAOb, and MAOc, respectively. Because of the compartmental shift mentioned above, it is likely that the recording electrode started in compartments 1+ or 1− at the apex of the folia, and then as it descended either crossed from 1+ → 1− → 2+ or from 1− → 2+. In either case, the intra-IO portion of the climbing fibre reflex would shorten because of the positional relationship of MAOa, MAOb and MAOc. That is, when the electrode shifts from compartment 1+ to 1−, the reflex pathway in the IO shortens from MAOc → MAOb → MAOa to MAOc → MAOb, and when the electrode moves from compartment 1− to 2+, the IO pathway shortens from MAOc → MAOb to MAOc only. Moreover, even within MAOc, areas innervating the lateral and medial parts of compartment 2+ in lobule VIII are different: the lateral area is closer to the area that innervates compartment a+ in lobules VIb-c and VII (Fig. 5 of Sugihara & Shinoda, 2004). Thus, in all cases, the intra-IO pathway, and hence the latency for the reflex response, becomes shorter as the electrode moves deeper.

In sum, the above issues raise questions about Baker and Edgley's conclusion that there is a non-uniform olivocerebellar conduction time based on variations in climbing fibre reflex response latencies. We have offered plausible alternative explanations for these variations. Moreover, a uniform olivocerebellar conduction time is consistent with the existence of synchronous CS activity between different cerebellar lobules and down folial walls, whereas it is difficult to explain such synchrony if there was no velocity tuning mechanism.

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