stimulation, motor performance or motor learning. The current data raise the possibility that the bistability found in neurons in other brain regions[1] is also related to anesthesia, and they indicate that one should, in general, be careful in assigning important physiological roles to the phenomenon of bistability.

Note: Supplementary information is available on the Nature Neuroscience website.

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Loewenstein *et al.* reply:

We reported[1] that the membrane potential of Purkinje cells *in vivo* can exist in multiple stable states that correspond to firing and silent periods. Toggling between these states can be triggered by current injection or synaptic input, but the maintenance of the states is an intrinsic membrane property of these neurons[1–4]. Our experiments were done in rats and guinea pigs using ketamine-xylazine or barbiturate anesthesia, but similar behavior occurs in awake monkeys[5–7], frogs[8] and cats[9–11]. Schonewille *et al.* investigated Purkinje cell bistability in awake mice and concluded that membrane potential bistability is rare or absent in awake animals. However, in our view, their data is insufficient to support this conclusion.

Because intracellular recordings in awake animals are technically challenging, Schonewille *et al.* used extracellular recordings of spikes as an indirect measure of membrane potential. However, the extent to which the membrane potential of a Purkinje cell can be inferred from the firing pattern of simple spikes is questionable. For example, they report that in intracellular recordings from isoflurane-anesthetized mice, 57 ± 16% of transitions from down to up state were preceded by a complex spike, compared to only 9.3 ± 3.0% when the cell's state was determined by the timing of extracellularly recorded simple spikes under the same anesthesia conditions. Because the timing of complex spikes is determined unequivocally in both cases, the parsimonious conclusion is that although the timing of simple spikes recorded extracellularly matches the timing of simple spikes recorded intracellularly, the timing of transitions between states is not detected correctly from this analysis. Their most compelling indirect evidence against bistability is the statistically significant difference in the coefficient of variation, percentage of pausing time, and percentage of cells with a pause larger than a specific value between awake and anesthetized animals. However, a statistically significant difference in these same variables is also evident between ketamine-xylazine and isoflurane anesthetized animals, where in both cases Purkinje cells exhibit bistability (as confirmed in intracellular recordings). Therefore, these criteria do not seem sufficient to rule out bistability.

Additionally, Schonewille *et al.* analyzed the histogram of the logarithm of the inter–simple spike interval and found that only 5–10% of the cells recorded in the awake condition are bimodal. However, the same analysis applied to the firing pattern of Purkinje cells recorded extracellularly in anesthetized conditions gives a large percentage of unimodal cells, whereas the membrane potential of these neurons in the same conditions is mostly bimodal. This discrepancy between the firing pattern and the corresponding changes in membrane potential undermines the validity of the test used by Schonewille *et al.* Moreover, strong parallel fiber input could trigger occasional simple spikes from the down state, which renders all the above criteria irrelevant for determining membrane potential state.

Finally, there seem to be significant species differences between the dynamics of Purkinje cells in rats and guinea pigs compared to mice. For example, we reported that 24 of 24 Purkinje cells in ketamine-xylazine anesthetized rats were bimodal, whereas Schonewille *et al.* report that only 6 of 10 Purkinje cells are bimodal in mice using the same anesthesia. Furthermore, the percentage of time that the membrane potential was in the down state in mice was only 3 ± 2%, much less frequently than in rats (52 ± 4%). Thus, the generality of the Schonewille *et al.* results for other species is questionable.

Our results highlight a key biophysical feature of Purkinje cells—the ability to adopt two intrinsic states that can be sustained without persistent synaptic input—that provides a new framework for interpreting Purkinje cell activity patterns *in vivo*. Although it is plausible that the amount of time that Purkinje cells spend in the down state in awake animals is reduced compared to that in anesthetized animals or that occasional simple spikes are generated in the down state, the fundamental issue is that the biophysical mechanisms underlying bistability are likely to be present in Purkinje cells in awake animals, and could thus influence network function in the cerebellum. Further experiments in awake behaving animals, preferably using intracellular recording, are required to understand the role of membrane potential bistability in different species and under different behavioral conditions.

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