J Physiol 586.18 (2008) pp 4341–4342

Increased bursting in layer 2/3 neurones of awake neocortex

David J. Margolis
Department of Neurophysiology, Brain Research Institute, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

Email: margolis@hifo.uzh.ch

A central goal of neuroscience is to relate the biophysical properties of neurones to network function and ultimately to behaviour. This requires knowledge of cellular mechanisms in intact circuits, where neurones retain their full complement of synaptic connections and can be stimulated with their natural sensory inputs. Major efforts have been made toward this goal in the last decade as *in vivo* electrical and optical recording techniques have become increasingly sophisticated (Kerr & Denk, 2008). One lingering caveat is the effect of anaesthesia, which profoundly influences brain state, making it difficult to extrapolate results to awake behaving conditions. Thus, a surge of recent studies have made a further push into the realm of awake behaving animals. Experiments that were once only fantasy are now being done: whole-cell patch recordings, 2-photon calcium imaging, voltage-sensitive dye imaging, sometimes in combination, and some are even possible in freely moving animals, without head restraint.

An important issue is whether cellular mechanisms that have been well characterized *in vitro* also occur in their natural context *in vivo*. Active biophysical properties, such as burst firing and dendritic spiking, are of particular interest because they are thought to endow neurones with important computational functions (London & Hausser, 2005). In cortical pyramidal cells, backpropagating action potentials (APs) interact with synaptic input in the apical dendrite to evoke dendritic calcium APs. Dendritic APs can then trigger high-frequency bursts of somatic APs, which are thought to play a privileged role in signal transmission by increasing information transfer to target neurones. However, recent *in vivo* experiments have found that cortical neurones fire only sparsely and rarely in bursts, even in response to sensory stimulation. Therefore it is still unclear how these potentially powerful cellular mechanisms contribute to network function *in vivo*.

One possibility is that active properties depend on brain state and emerge only in awake animals. To test this it is necessary to compare activity of identified cell types in anaesthetized and awake conditions. In a recent paper in *The Journal of Physiology*, de Kock & Sakmann (2008) recorded from neocortical layer 2/3 (L 2/3) and 5B neurones from primary somatosensory (barrel) cortex in anaesthetized and awake rats. They used juxtasomal recording (a.k.a. 'loose patch'), which enables recording of suprathreshold spiking activity followed by intracellular dye fills and morphological reconstruction. This technique has four advantages over other recording methods: (1) unequivocal single unit isolation due to a loose seal being formed on to an individual soma; (2) unbiased single unit recording of both silent and active cells, unlike metal electrodes which select for active cells; (3) minimal disruption because intracellular composition is undisturbed during recording; (4) ability to stain neurones after recording, allowing reconstruction of dendritic and axonal arbors. One limitation is that only spiking activity, no subthreshold information, is acquired.

The authors first observed that spontaneous firing rates in anaesthetized animals were low. L5B cells showed higher mean rates (∼3 Hz) than L2/3 cells (∼0.4 Hz). They also showed more frequent burst firing, with ∼15 % of APs occurring less than 10 ms apart (i.e. 100 Hz) compared to ∼2 % of APs for L2/3 cells. A level of 100 Hz was chosen as the cut-off for the definition of a burst because this was previously shown to be the critical frequency for AP backpropagation and induction of dendritic spikes in L5 cells. Upon principal whisker deflection, still only ∼1 % of APs were in bursts for L2/3 cells, and ∼17 % for L5B, indicating that burst occurrence does not change with sensory stimulation. In these conditions, the rare burst from L2/3 cells never contained more than two APs, whereas L5B bursts had up to six APs. AP amplitude adaptation, the successive shortening of APs during a burst, occurred strongly in L5B cells, more so than in L2/3. These experiments, using juxtasomal recording, provide confirmation of sparse firing as measured previously (by the authors and others) using whole-cell recordings and population calcium imaging. In the anaesthetized rat, barrel cortex is sparsely active, with L5B cells more bursty than L2/3 cells.

These results established a baseline for the critical experiment, comparison with awake conditions. Success rates of *post hoc* dye-fills were lower in unanaesthetized rats trained to tolerate head fixation, but still sufficient to measure ongoing activity from identified L2/3 and L5B neurones. Surprisingly, the authors found that mean firing rates were not different for either cell type during quiet wakefulness compared to anaesthetized conditions. However, the statistics had changed: now L2/3 neurones fired in bursts. Under anaesthesia, the distribution of interspike intervals (ISIs) for L2/3 cells was planted on the 10 ms (100 Hz) threshold; in awake animals ISIs routinely invaded this region. Now 15 % of APs occurred in bursts of higher frequency than 100 Hz (< 10 ms ISI; up from 2 %), nearly matching the burst occurrence of L5B cells. The number of APs per burst also increased. Whereas bursts in L2/3 cells in anaesthetized animals never contained more than two APs, events with up to six APs were encountered in awake animals, and on average, the number of APs per burst was similar for L2/3 and L5B cells (2.3 % of all bursts contained at least 3 APs, compared to 3.5 % for L5B cells). Contrariwise, L5B cells in awake animals fired bursts with the same occurrence and number of APs per burst as under anaesthesia. Within their bursts, however, AP amplitude adaptation was strongly reduced, with subsequent spikes maintaining larger peaks throughout the burst. Thus, in awake animals, burst strength increased in both L2/3 and L5B cells in specific ways for each cell type.

Overall, these results show convincingly that the firing properties of cortical neurones are state dependent and can change in a cell type-specific manner. Given that L2/3 neurones fire rarely both at rest and during sensory stimulation in anaesthetized animals, the emergence of bursts during wakefulness indicates the occurrence of a state-dependent mode switching. The enhanced bursting properties in both cell

DOI: 10.1113/jphysiol.2008.160341

types, without proportional changes in mean firing rates, suggests that neocortex tends toward a state of sparse bursting during quiet wakefulness in which bursts account for a relatively large proportion of APs. Since bursts are strong drivers of postsynaptic neurones at many central synapses, it will be interesting to investigate the consequences of sparse bursting for barrel cortex networks. Whether bursts play a role in active sensory processing during behaviour, and what stimulus parameters evoke bursts, will be important questions to answer.

It is not clear how bursts increased during wakefulness. The authors argue that neuromodulatory input from brainstem nuclei active during arousal could directly modulate active neuronal properties. Acetylcholine (ACh), for example, could modulate voltage-gated ion channels to lower the threshold or increase the duration of dendritic spikes. Enhanced occurrence or duration of dendritic spikes would increase their influence on neuronal output by causing additional high-frequency APs. Recent work by Larkum *et al.* (2007) found that dendritic spikes in L2/3 neurones *in vitro* are fairly brief and evoke only one additional somatic AP. Thus, neuromodulators would have to elicit dendritic spikes with increased duration in order to account for the increased incidence of bursts with three to six APs, as described in the de Kock & Sakmann (2008) paper. Anaesthesia can inhibit neuronal function, but as the authors point out, burst occurrence and the number of spikes per burst changed only in L2/3 cells, supporting the notion that direct neuromodulation might enable cell type-specific changes in burst firing in awake cortex.

Although the results have important implications for dendritic calcium electrogenesis, imaging experiments were not done and the authors relied upon previous work to infer the relationship between somatic APs and dendritic calcium signalling. The definition of a burst ($\geq$100 Hz) was based on the critical frequency in L5 cells for dendritic calcium electrogenesis. However, since the ISI distribution was continuous in the 5–15 ms range, a small change in the definition would strongly affect burst classification. Recent work found the critical frequency for L2/3 cells to be 130 Hz (Larkum *et al.* 2007), slightly higher than the 100 Hz cut-off used here. The proportion of events reflecting dendritic calcium spikes may therefore have been slightly overestimated. An ISI cut-off of 7.7 ms instead of 10 ms (130 Hz instead of 100 Hz) would yield a burst occurrence in L2/3 cells in awake cortex of $\sim$10–12 % instead of the reported $\sim$15 %.

Another limitation of the present study is that anaesthetized and awake recordings were done in different groups of animals. An even more convincing demonstration would be to compare activity in the same neurones before and after anaesthesia. This was accomplished recently using population calcium imaging to measure ongoing activity in L2/3 neurones in visual cortex of awake rats, then in the same neurones after induction of anaesthesia (Greenberg *et al.* 2008). Importantly, the results of Greenberg *et al.* (2008) confirmed the basic findings of the present study, including higher burst occurrence in L2/3 neurones in awake animals. In the future, a combination of electrical and optical approaches, both on the single-cell and population level, will allow a more detailed understanding of single-neurone computation in network function and behaviour.

References

de Kock CP & Sakmann B. (2008). High frequency action potential bursts $\geq$100 Hz) in L2/3 and L5B thick tufted neurons in anaesthetized and awake rat primary somatosensory cortex. *J Physiol* **586**, 3353–3364.

Greenberg DS, Houweling AR & Kerr JN. (2008). Population imaging of ongoing neuronal activity in the visual cortex of awake rats. *Nat Neurosci* **11**, 749–751.

Kerr JN & Denk W. (2008). Imaging *in vivo*: watching the brain in action. *Nat Rev Neurosci* **9**, 195–205.

Larkum ME, Waters J, Sakmann B & Helmchen F. (2007). Dendritic spikes in apical dendrites of neocortical layer 2/3 pyramidal neurons. *J Neurosci* **27**, 8999–9008.

London M & Hausser M. (2005). Dendritic computation. *Annu Rev Neurosci* **28**, 503–532.

Acknowledgements

I thank Fritjof Helmchen for encouragement and support, and members of the Helmchen and Gerber labs for helpful discussions.