

- S.P., Ferguson-Smith, A.C., and Cavaille, J. (2004). A large imprinted microRNA gene cluster at the mouse Dlk1-Gtl2 domain. *Genome Res.* 14, 1741-1748.
- Seitz, H., Youngson, N., Lin, S.P., Dalbert, S., Paulsen, M., Bachelier, J.P., Ferguson-Smith, A.C., and Cavaille, J. (2003). Imprinted microRNA genes transcribed antisense to a reciprocally imprinted retrotransposon-like gene. *Nat. Genet.* 34, 261-262.
  - Lin, S.P., Youngson, N., Takada, S., Seitz, H., Reik, W., Paulsen, M., Cavaille, J., and Ferguson-Smith, A.C. (2003). Asymmetric regulation of imprinting on the maternal and paternal chromosomes at the Dlk1-Gtl2 imprinted cluster on mouse chromosome 12. *Nat. Genet.* 35, 97-102.
  - Cockett, N.E., Jackson, S.P., Shay, T.L., Farnir, F., Berghmans, S., Snowden, G.D., Nielsen, D.M., and Georges, M. (1996). Polar overdominance at the ovine callipyge locus. *Science* 273, 236-238.
  - Davis, E., Jensen, C.H., Schroder, H.D., Farnir, F., Shay-Hadfield, T., Kliem, A., Cockett, N., Georges, M., and Charlier, C. (2004). Ectopic expression of DLK1 protein in skeletal muscle of padumal heterozygotes causes the callipyge phenotype. *Curr. Biol.* 14, 1858-1862.
  - Charlier, C., Segers, K., Karim, L., Shay, T., Gyapay, G., Cockett, N., and Georges, M. (2001). The callipyge mutation enhances the expression of coregulated imprinted genes in cis without affecting their imprinting status. *Nat. Genet.* 27, 367-369.
  - Tang, G. (2005). siRNA and miRNA: an insight into RISCs. *Trends Biochem. Sci.* 30, 106-114.
  - Wilkins, J.F., and Haig, D. (2003). What good is genomic imprinting: the function of parent-specific gene expression. *Nat. Rev. Genet.* 4, 359-368.
  - Li, Y.M., Franklin, G., Cui, H.M., Svensson, K., He, X.B., Adam, G., Ohlsson, R., and Pfeifer, S. (1998). The H19 transcript is associated with polysomes and may regulate IGF2 expression in trans. *J. Biol. Chem.* 273, 28247-28252.
  - Fitzpatrick, G.V., Soloway, P.D., and Higgins, M.J. (2002). Regional loss of imprinting and growth deficiency in mice with a targeted deletion of KvDMR1. *Nat. Genet.* 32, 426-431.
  - Sleutels, F., Zwart, R., and Barlow, D.P. (2002). The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature* 415, 810-813.
  - Volpe, T.A., Kidner, C., Hall, I.M., Teng, G., Grewal, S.I., and Martienssen, R.A. (2002). Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. *Science* 297, 1833-1837.
  - Lewis, A., Mitsuya, K., Umlauf, D., Smith, P., Dean, W., Walter, J., Higgins, M., Feil, R., and Reik, W. (2004). Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. *Nat. Genet.* 36, 1291-1295.
  - Neumann, B., Kubicka, P., and Barlow, D.P. (1995). Characteristics of imprinted genes. *Nat. Genet.* 9, 12-13.
  - Kanellopoulou, C., Muljo, S.A., Kung, A.L., Ganesan, S., Drapkin, R., Jenuwein, T., Livingston, D.M., and Rajewsky, K. (2005). Dicer-deficient mouse embryonic stem cells are defective in differentiation and centromeric silencing. *Genes Dev.* 19, 489-501.

Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Babraham Research Campus, Cambridge CB2 4AT, UK.

DOI: 10.1016/j.cub.2005.04.003

## Neuronal Networks: Flip-Flops in the Brain

Neuronal activity can rapidly flip-flop between stable states. Although these semi-stable states can be generated through interactions of neuronal networks, it is now known that they can also occur *in vivo* through intrinsic ionic currents.

David A. McCormick

When asked how the brain works, most neuroscientists would respond that the brain is a large, web-like structure in which neurons gather information from other neurons, make a decision to discharge or not, and then pass this information onto other cells; magically, somehow, through this large interaction of neuronal elements, information is extracted, decisions are made, and responses are executed. Activity is imagined to flow through the neural net with the spatiotemporal path determining the outcome of the particular computation, be it a thought, perception, feeling or action. Although this is certainly true at some level within the nervous system, there are several complicating factors. One of these is the presence of spontaneous, or persistent, activity that can rapidly flip between stable states.

Neurons and neuronal networks generate spontaneous activity, even after isolation from external inputs, such as when a cell or a network is placed *in vitro* [1-3]. Far from being random noise, this spontaneous activity provides the context under which the brain operates. It determines which cells are responsive, and just how responsive they are. Just as context dramatically influences perception — for example, bumping into your bank teller at the grocery store can be a surreal experience — the background ‘context’ of the brain, which is largely represented by the state of ongoing activity, provides a strong modulating influence on exactly how information is processed and interpreted [4]. The questions then arise: how is this spontaneous activity generated? How does it influence neuronal responsiveness? And how does it rapidly undergo state changes?

Two leading models of on-going activity generation have been put forward: one is based on reverberation within recurrent neuronal networks, and the other on maintained depolarization through ionic currents intrinsic to the persistently discharging neuron [5,6]. The evidence in favor of recurrent networks being largely responsible for the generation of on-going or persistent activity in the waking brain is extensive — neurons are massively interconnected with tens of thousands of inputs and outputs, many of which are themselves spontaneously active, and intracellular recordings in cortical neurons, for example, confirm the presence of a large, ongoing synaptic barrage that maintains a depolarized state [3].

Nearly all of the evidence for the generation of tonic activity through intrinsic ionic mechanisms has been obtained *in vitro*, typically under conditions whose relevance to the natural, waking state is questionable (the persistent intrinsic activity only occurs in slices that are bathed in drugs of various sorts) [6]. A clear example of persistent activity generated *in vivo* through intrinsic mechanisms, and the ability to modulate this persistent activity with afferent inputs, has been largely lacking.

A recent paper by Loewenstein *et al.* [7] changes this situation.

Purkinje cells, the output neurons of the cerebellar cortex, discharge at high rates (20–100 Hz) in awake, behaving animals. This ongoing discharge provides a background that is smoothly increased or decreased in precise relation to movement and behavior [8]. Is this background discharge generated through intrinsic mechanisms or through synaptic inputs? Purkinje cells receive two main excitatory inputs: a massive synaptic convergence of approximately 100,000 inputs from cerebellar granule cells, and a massive, strong input from typically one inferior olivary cell. Interestingly, removal of these, and all other, synaptic inputs does not stop the maintained discharge of Purkinje cells.

Detailed analyses revealed that this ongoing discharge is generated through persistent currents, of which Na<sup>+</sup> is the major player [1,9]. Purkinje cells contain biophysical adaptations that allow them to fire steadily at high frequencies. One of these is a very short spike refractory period, which results from a rapid blocking of the Na<sup>+</sup> pore — thereby preventing channel inactivation — by a segment of the β4 subunit of the channel [10]. But this unusual mechanism comes with an additional consequence. As the blocking particle removes itself from the channel upon repolarization of the membrane potential, the channel conducts Na<sup>+</sup> once again and, as a population, this ‘resurgent’ current can generate a persistent depolarization and high frequency discharge [10].

Purkinje cells then can exhibit two stable states: hyperpolarized and quiescent, or depolarized and tonically discharging, purely through their intrinsic membrane properties (Figure 1A). Loewenstein *et al.* [7] have demonstrated that these two states can be observed *in vivo* during anesthesia, and that activation of climbing fibers can trigger the transition between states. Thus, Purkinje cells can act as the neuronal equivalent of an electronic ‘flip-flop’, with

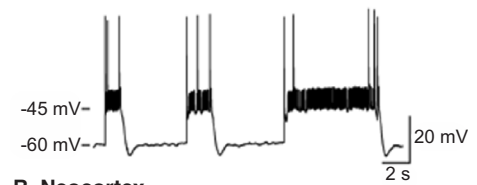
Figure 1. Examples of bistable behavior in single cells and local networks.

(A) Presumed intradendritic recording from a cerebellar Purkinje cell *in vivo*. The small spikes are somatic Na<sup>+</sup> action potentials. Note the spontaneous transitions between active and inactive states associated with large events in the dendrites. (B) Intracellular recording in a cortical interneuron during state transitions between Down and Up states, initiated by activation of afferent pathways. (C) Schematic of attractor dynamics in a bistable system. The active state is maintained through re-entrant excitation, either from depolarizing currents or recurrent excitatory pathways, balanced with inhibitory influences such as hyperpolarizing currents or recurrent inhibitory pathways. Transitions between states can be brought about through the activation of intrinsic ionic currents or synaptic pathways. (A) adapted from [7] and (B) from [2].

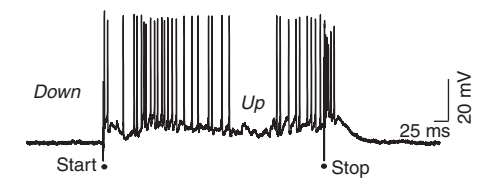
climbing fibers signaling state transitions. This suggests that if one were able to look down upon the flattened, large cortical sheet of the cerebellum, one would see Purkinje cells flashing on and off during behavior, as if they were the twinkling lights of a Christmas tree. Such a scenario, however, remains to be demonstrated, as state transitions in Purkinje cells following natural climbing fiber activation have only been clearly observed in non-behaving preparations, such as in anesthetized or immobilized animals.

Neocortical pyramidal cells can also generate bistable behavior, although they do so through an entirely different mechanism. During slow wave sleep, under anesthesia or even in the dish, neurons of the neocortex spontaneously, and in response to afferent inputs, jump between a quiescent ‘Down’ state and a tonically active ‘Up’ state (Figure 1B). The transition to waking results in what appears to be a maintained ‘Up-like’ state [3]. These rapid transitions occur through neuronal network mechanisms. The neocortex, in contrast to the cerebellar cortex, is massively interconnected with

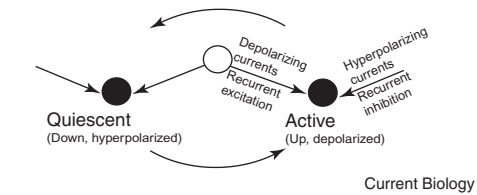
### A Cerebellar Purkinje



### B Neocortex



### C Attractor dynamics



recurrent excitatory collaterals. This feedback excitation results in an explosive tendency for the cortex to re-excite itself. But the activation of feedback excitation also results in an immediate and strong response from local inhibitory interneurons that control and tune the activity generated. The neocortex thus operates through a precisely adjusted balance between excitation and inhibition in the local network [2].

Do Up and Down states occur during waking behavior? Although frank Up and Down states of sleep and anesthesia have not been clearly shown in the waking neocortex, cells do exhibit rapid state changes that exhibit strong similarities. For example, cells in parts of the cerebral cortex associated with ‘working memory’ rapidly transit from a low firing rate to a high firing rate when an animal is asked to retain information [11], or when the animal’s ‘spot-light’ of attention is moved to a new location [12]. At the other end of the spectrum, local dendritic branches of cortical cells are also capable of rapidly switching between semi-stable states, such as through the activation of voltage-dependent Ca<sup>2+</sup> currents or ‘NMDA-spikes’

[13,14], and a recent model of memory is based upon multistable states in synapses [15].

What could be the role of neuronal flip-flops? In computers, these devices are commonly used for storage of a bit of information. A similar role is possible in the nervous system, though using an entire cell or local network of cells to store a simple piece of information seems a waste. Multistable states in cells and neural networks may play other roles, such as bringing cells or entire networks 'on-line' in a behaviorally appropriate manner. The combination of synaptic, dendritic, neuronal and network flip-flops could provide a powerful range of states to guide and influence neuronal processing. Multiple stable states in neurons and networks can be used to perform complicated calculations such as integration and gain modulation in a robust and stable manner [16]. These rapid modifications in excitability and activity are useful for keeping track of information such as the positions of the eyes or head, or for coupling the rapidly changing sensory world to the appropriate motor responses [17], making decisions based upon accumulated evidence [18], and enhancing signal detection through attentional mechanisms [19,20].

Computation in the brain is not simply a matter of gathering

influences from synaptic inputs and integrating these into a decision to spike. Spontaneous activity, generated both through intrinsic and network mechanisms, provides the context under which content is interpreted. Without context, all is lost.

#### References

1. Llinas, R., and Sugimori, M. (1980). Electrophysiological properties of *in vitro* Purkinje cell somata in mammalian cerebellar slices. *J. Physiol.* 305, 171–195.
2. Shu, Y., Hasenstaub, A., and McCormick, D.A. (2003). Turning on and off recurrent balanced cortical activity. *Nature* 423, 288–293.
3. Steriade, M., Timofeev, I., and Grenier, F. (2001). Natural waking and sleep states: a view from inside neocortical neurons. *J. Neurophysiol.* 85, 1969–1985.
4. Shu, Y., Hasenstaub, A., Badoual, M., Bal, T., and McCormick, D.A. (2003). Barrages of synaptic activity control the gain and sensitivity of cortical neurons. *J. Neurosci.* 23, 10388–10401.
5. Wang, X.J. (2001). Synaptic reverberation underlying mnemonic persistent activity. *Trends Neurosci.* 24, 455–463.
6. Major, G., and Tank, D. (2004). Persistent neural activity: prevalence and mechanisms. *Curr. Opin. Neurobiol.* 14, 675–684.
7. Loewenstein, Y., Mahon, S., Chadderton, P., Kitamura, K., Sompolinsky, H., Yarom, Y., and Hausser, M. (2005). Bistability of cerebellar Purkinje cells modulated by sensory stimulation. *Nat. Neurosci.* 8, 202–211.
8. Thach, W.T. (1970). Discharge of cerebellar neurons related to two maintained postures and two prompt movements. II. Purkinje cell output and input. *J. Neurophysiol.* 33, 537–547.
9. Llinas, R., and Sugimori, M. (1980). Electrophysiological properties of *in vitro* Purkinje cell dendrites in mammalian cerebellar slices. *J. Physiol.* 305, 197–213.
10. Grieco, T.M., Malhotra, J.D., Chen, C., Isom, L.L., and Raman, I.M. (2005). Open-channel block by the cytoplasmic tail of sodium channel beta4 as a mechanism for resurgent sodium current. *Neuron* 45, 233–244.
11. Goldman-Rakic, P.S. (1995). Cellular basis of working memory. *Neuron* 14, 477–485.
12. Lebedev, M.A., Messinger, A., Kralik, J.D., and Wise, S.P. (2004). Representation of attended versus remembered locations in prefrontal cortex. *PLoS Biol.* 2, e365.
13. Loewenstein, Y., and Sompolinsky, H. (2003). Temporal integration by calcium dynamics in a model neuron. *Nat. Neurosci.* 6, 961–967.
14. Schiller, J., and Schiller, Y. (2001). NMDA receptor-mediated dendritic spikes and coincident signal amplification. *Curr. Opin. Neurobiol.* 11, 343–348.
15. Fusi, S., Drew, P.J., and Abbott, L.F. (2005). Cascade models of synaptically stored memories. *Neuron* 45, 599–611.
16. Koulakov, A.A., Raghavachari, S., Kepecs, A., and Lisman, J.E. (2002). Model for a robust neural integrator. *Nat. Neurosci.* 5, 775–782.
17. Salinas, E., and Sejnowski, T.J. (2001). Gain modulation in the central nervous system: where behavior, neurophysiology, and computation meet. *Neuroscientist* 7, 430–440.
18. Wang, X.J. (2002). Probabilistic decision making by slow reverberation in cortical circuits. *Neuron* 36, 955–968.
19. Chance, F.S., Abbott, L.F., and Reyes, A.D. (2002). Gain modulation from background synaptic input. *Neuron* 35, 773–782.
20. McCormick, D.A., Shu, Y., Hasenstaub, A., Sanchez-Vives, M., Badoual, M., and Bal, T. (2003). Persistent cortical activity: mechanisms of generation and effects on neuronal excitability. *Cereb. Cortex* 13, 1219–1231.

Department of Neurobiology, Kavli Center for Neuroscience, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510, USA.  
E-mail: david.mccormick@yale.edu

DOI: 10.1016/j.cub.2005.04.009

## Animal Phylogeny: Fatal Attraction

Phylogenetic analyses of hundreds of genes from model animals have placed flies closer to vertebrates than to nematodes; recent work suggests this may be due to an artefact known as long branch attraction.

Maximilian J. Telford<sup>1</sup> and Richard R. Copley<sup>2</sup>

The traditional view of animal evolution is one of gradually increasing complexity. The earliest-branching flatworms lack the body cavity known as a coelom, which is a characteristic feature of the two traditional groups of 'higher' animals: deuterostomes, including echinoderms and chordates, and

protostomes, such as annelids, molluscs and arthropods. Between these two extremes, according to the traditional view, lie the pseudocoelomate worms such as the nematodes, the body cavities of which lack the refinements of a true coelom. This hierarchical view was shaken in the mid 1990s by a phylogenetic study of small subunit ribosomal (r)RNA genes [1]. This work elevated the acoelomate

flatworms to a close relationship with the coelomate annelids and molluscs, in a group called the Lophotrochozoa, and pseudocoelomate nematodes moved close to the coelomate arthropods, creating a group called the Ecdysozoa.

Opposing the 'new animal phylogeny', as this new scheme has been called [2], are several analyses [3–5] of huge numbers of genes — close to 800 in the most recent [6] — sampled from the few animals with completely sequenced genomes: fruitfly, nematode and various vertebrates. These multigene analyses are unanimous in grouping coelomate arthropods and vertebrates to the exclusion of the pseudocoelomate