calpain-mediated proteolysis as a regulatory mechanism capable of signaling in local fashion at synapses and to nuclear targets. Future work in understanding the regulatory role of calpain thus might concentrate not on definition of targets alone but also regulation of these targets. For example, the fusion protein controlling mGluR cleavage appears to be selectively directed to NMDA-receptor-activated calpain at the synapse by mechanisms that are not yet clear (Xu et al., 2007). Two important concepts that emerge as regulatory mechanisms involve control of substrate specificity by phosphorylation and of calpain activity itself by the balance with its endogenous inhibitor calpastatin (Wu and Lynch, 2006; Cuevas et al., 2003; Sawhney et al., 2006). In focal adhesion, activity of calpain and its targeting to specific substrates involves formation of a macromolecular complex involving calpain, ERK, Src, and the target integrins. Perhaps synapses use similar mechanisms for directing calpain to relevant substrates.

Finally, the present studies also raise the question of whether other regulatory proteases not yet discovered exist in the nervous system. Although NR1 of the NMDA receptor is not normally a calpain substrate in neurons, some studies have suggested that its C terminus can undergo nuclear translocation (Wu and Lynch, 2006; Bradley et al., 2006). Similarly, the C termini of various voltage-gated calcium channels can be cleaved and translocated to the nucleus (Gomez-Ospina et al., 2006; Kordasiewicz et al., 2006), where they can act as transcription factors (Gomez-Ospina et al., 2006). Such a process may be involved in the pathogenesis of spinocerebellar ataxia type 6. While it is suspected that calpain may be involved, direct evidence for a role for calpain is lacking. Consequently, the concept of proteolysis as a regulatory rather than destructive mechanism may exist even beyond the calpain system.

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Cortical Songs Revisited: A Lesson in Statistics

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Recordings from single neurons in the cortex have revealed precisely repeating patterns of synaptic events. These repeats are known as cortical "motifs" and have been suggested to reflect the precise replay of spatiotemporal firing sequences ("synfire" chains). In this issue of *Neuron*, Mokeichev et al. use compelling statistical analysis to show that, rather than being evidence of deterministic synfire chains, such cortical motifs are bound to appear by chance due to the natural dynamics of voltage fluctuations in neurons.

How do neurons encode information? Donald Hebb suggested that the cortex processes information through the *sequential* activation of neuronal assemblies (Hebb, 1949). Compared to a rate code, in which only the *num-* *ber* of spikes in a given interval counts, high quantities of information can be carried in the temporal *order* of

neuronal activation (Gautrais and Thorpe, 1998). A popular model using such a scheme is Abeles' synfire chains (Abeles, 1991). Under this formulation, neurons communicate information through the precisely timed order of spikes in successive sets of neurons. A prediction from this model is that one should be able to record groups of neurons repeatedly spiking in the same order with ms precision (Abeles, 1991). However, it has proven difficult to demonstrate that such repeating spike sequences occur above chance level and that the precise spike sequence carries information beyond that carried by the spike rate (Oram et al., 1999). Therefore, Ikegaya et al. (2004) generated much interest when they reported the occurrence of such precise repeats of sequential neuronal activation in the visual cortex in vitro and showed that these were reflected as precise repeats of synaptic events ("motifs") of 1–2 s duration in individual cells. On longer timescales, series of these sequential activations were found to be repeated, supersequences referred to as "cortical songs" (Ikegaya et al., 2004). Detection of the intracellular motifs provided an indirect method by which the authors could search for evidence of sequential activation of multiple neurons in vivo, using intracellular recordings from single neurons. Applying this method to intracellular recordings from the visual cortex of the anaesthetized cat, they found similar repeating motifs, suggesting that repeating sequential activations of neuronal assemblies occur in sensory cortex in vivo, in the absence of sensory input (Ikegaya et al., 2004). Now a new analysis suggests that these repeating motifs may occur by chance (Mokeichev et al., 2007).

Critical to the detection of significant repeats is the null hypothesis used. A standard statistical technique is to compare the actual data against a surrogate data set, often generated by some shuffling procedure. In this case, the aim was to test the null hypothesis that the fine temporal structure is generated by chance, i.e., that repeating motifs emerge stochastically. Ikegaya et al. (2004) identified

synaptic events, then shuffled the time intervals between them while preserving their order. With the in vitro data, synaptic events above a certain threshold could be clearly identified against the baseline. However, it is not obvious that this procedure would be equally appropriate for in vivo recordings. To obtain surrogate data with a statistical distribution similar to their in vivo physiological data, Mokeichev et al. (2007) used three different techniques (shuffling of short data segments in the time domain, phase randomization in the frequency domain, or generation of pseudorandom data by computer simulation). They concluded that, with each of these surrogate data sets, the intracellular motifs in the physiological data occur no more frequently than expected by chance. Admirably, after having reached this conclusion in recordings from barrel cortex in anaesthetized rat, they reanalyzed the traces from the original Ikegaya et al. report (on which the last author of the present article is a coauthor). Having verified that their method is capable of detecting events as rare as a single 1 s long motif repeating every minute on average, they then demonstrated that such motifs were not found above chance level in this original data set. Thus, the cortical motifs described by Ikegaya et al. (2004) can be explained by stochastic mechanisms within the constraints imposed by the natural dynamics of voltage fluctuations in neurons. Of course, a failure to detect statistically significant sequences does not prove that they do not occur. Although the authors found no evidence for the occurrence of 1 s long motifs in the anaesthetized animal, this does not rule out that similar motifs, possibly of shorter duration, might occur in awake animals. Nevertheless, this result is consistent with the way many neurophysiologists think about cortical network activity driven by stochastic spike generation and unreliable synapses.

But how does this result square with other reports that cortical spike sequences do repeat? Again, it depends on the null hypothesis. Whereas there is agreement that precisely timed spike sequences do occur, e.g.,

following sensory stimulation, there is evidence that they may be no more frequent than expected by chance, once the coarse temporal structure of evoked activity is taken into account (Oram et al., 1999). Similarly, it was reported that sequential activation regularly occurs during neocortical UP states, most precisely during the first 100 ms after UP state onset and gradually deteriorating thereafter (Luczak et al., 2007). As spike timing of individual neurons seems to be often locked to the onset of a stimulus or network event, it was recently suggested that ordered activation of neurons is more closely related to systematic control of spike latencies from such an event, rather than the sequential activation of a chain of neurons (Luczak et al., 2007). A latency-based mechanism could explain why repeats may not be reliably detected above chance level in intracellular recordings from individual neurons. In fact, it is to be expected that such a mechanism would include, and be robust to, stochastic variations in temporal structure, in a way that a chain of neuronal activation would not. On this basis, one may question how useful single-cell recordings in isolation can be in elucidating network mechanisms. It seems likely that simultaneous recordings from large numbers of neurons will be necessary to understand the spatiotemporal patterns of activity within the cortex. With recent developments in recording technology, the prospects are good that such data will become available (Csicsvari et al., 2003; Gobel et al., 2007; Kelly et al., 2007). A further challenge is to develop statistical techniques that can analyze such data sets (Abeles and Gat, 2001; Lee and Wilson, 2004). The techniques described by Mokeichev et al. (2007) provide an additional useful approach for future work on this topic.

In conclusion, this new study emphasizes the need for rigorous statistical analysis in interpreting electrophysiological data. Human observers might find it intuitively very unlikely that cortical motifs could occur by chance, but by taking the dynamics of neuronal events into account, this paper compellingly demonstrates that

such precise repeats are bound to emerge stochastically.

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Behavioral Flexibility and the Frontal Lobe

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Brain areas in the frontal lobe have been implicated in behavioral flexibility and control. The study by Johnston et al. in this issue of *Neuron* provides novel insights into the roles of the prefrontal and anterior cingulate cortices in controlling behavior.

Knowing which action is appropriate in particular circumstances is an essential element of successful behavior. For example, while punching (your opponent) in the boxing ring may lead to various rewards such as riches and fame, performing the same action on random passers-by in the street is unlikely to do so. Depending on the current task, an action can thus be beneficial or detrimental to achieving a defined behavioral goal such as reward maximization. Maintaining task-specific information and rapidly modifying it in response to environmental demands are considered to be hallmarks of primate behavior. In the laboratory, this kind of behavioral flexibility can be studied by training subjects on different tasks involving the same actions and then having them perform interleaved blocks of trials of each task while recording brain activity. In this issue of *Neuron*, Johnston et al. (2007)

have employed a prosaccade and an antisaccade task, during which monkeys had to either look toward or away from a briefly flashed peripheral target. Monkeys did not receive an explicit cue as to which task they were on but figured this out themselves by noticing which behaviors were rewarded during each block of trials. After performance of prosaccades for a number of trials, reward contingencies were switched at an unpredictable point in time and previously successful behaviors were now unsuccessful and vice versa. Behaviorally, monkeys were quick to shift from one task to the next and did so within a few trials.

How is this rapid switching accomplished, and how do monkeys manage to remember which task they are on over the course of each block? To answer these questions, Johnston et al. studied single-neuron activity (SUA) in the prefrontal (PF) and the

anterior cingulate (AC) cortex as monkeys were switching back and forth between these two tasks. They focused not on responses associated with peripheral flashes or saccadic eye movements during the task but instead on differences in preparatory or more commonly known as baseline activity between the two tasks. In the visual system, baseline activity changes have been associated with the maintenance of spatial attention (Luck et al., 1997). Allocation of attention over the course of a block of trials thus leads to an increase in baseline firing rate of neurons representing that region of space, and a visual stimulus presented in the attended region accordingly elicits overall more activity than one presented in an unattended region. By analogy, baseline changes are thought to be involved in maintaining and switching between task rules in the present study and in previous