Homeostatic plasticity mechanisms in mouse V1

Megumi Kaneko and Michael P. Stryker

Center for Integrative Neuroscience and Department of Physiology, University of California, San Francisco, CA 94143-0444, USA

Mechanisms thought of as homeostatic must exist to maintain neuronal activity in the brain within the dynamic range in which neurons can signal. Several distinct mechanisms have been demonstrated experimentally. Three mechanisms that act to restore levels of activity in the primary visual cortex of mice after occlusion and restoration of vision in one eye, which give rise to the phenomenon of ocular dominance plasticity, are discussed. The existence of different mechanisms raises the issue of how these mechanisms operate together to converge on the same set points of activity.

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1. Introduction

The fact that the neocortex remains in a narrow range of its possible states of activity between seizure and coma is a cause for celebration, but the processes that maintain it in the face of Hebbian plasticity mechanisms that cause strong inputs to each neuron to grow ever stronger are still obscure. The maintenance of a steady average level of activity, averaged over long times, is referred to loosely as homeostasis. The idea of homeostasis has a long history in physiology, dating at least from the work of Claude Bernard more than 150 years ago on the stable regulation of the milieu intérieur and its elaboration by Walter Canon, who coined the term homeostasis [1,2]. A homeostat senses and measures some variable, compares its value with a set point, and engages effectors to bring the variable back to its set point, a form of negative feedback. In many cases of physiological homeostasis, there are several levels of effectors that are engaged at different degrees of deviation from the set point, such as sweating and panting for temperature regulation.

The concept of homeostasis can be relatively straightforward for a simple system, like a thermostat that engages a furnace to keep a house warm in the winter, with the only complex issue being the strategy to prevent oscillation. In a densely coupled network like the brain, however, where neurons excite and inhibit one another, homeostatic regulation may be much more complex. The existence of Hebbian plasticity, in which inputs that are effective in driving a post-synaptic cell rapidly become stronger and stronger, makes the requirement for some form of homeostatic plasticity all the more urgent. The positive feedback of a purely Hebbian system would cause synapses either to become completely ineffective or to reach saturating strength.

We pose below some of the questions about homeostatic plasticity that must be answered in order to understand its operation in the brain and its interaction with Hebbian plasticity.

First, we must ask what aspect of neural activity is being maintained? Is it some average spike firing frequency of the entire network, or does each cell have its own activity set point [3]? If it were the latter, many simple neural circuits would appear to have great difficulty. If, for example, cell A excites itself and inhibits cell B and cell B excites itself and inhibits cell A, such a neural flip-flop has only two seemingly stable states, with either cell A or cell B fully active and the other cell silent. How then would a cell-by-cell homeostasis work? A homeostatic process that acted to reduce the activity of the active cell and increase that of the inactive cell would...
merely cause the simple network to switch states, back and forth, with a period that reflected the time course of the homeostatic mechanism. The existence of persistent states would require a cell-by-cell mechanism of homeostasis that has a very slow time course.

The neural flip-flop is only the most extreme case. For any typical neural circuit, one has to ask how the homeostatic set point of each cell in the circuit is reconciled with those of the neurons that it excites or inhibits. However, once established, perhaps by a slow plasticity process during development, a complexly interconnected circuit with all neurons at their activity set points would exert a powerful drive to restore any individual member’s activity to the set point if external inputs caused it to deviate from that level of activity.

Other modes of operation in the nervous system also have difficulty with anything but a very long time-constant for cell-by-cell homeostatic regulation of firing rates. Sparse coding in sensory areas means that many sensory neurons fire only rarely, and not at all until the animal is exposed to an appropriate stimulus; neurons in areas CA1 and CA3 of the hippocampus may fail entirely to fire in many environments during waking [4]; and the spinal motor neurons innervating the most powerful collection of muscle fibres may not fire for days at a time, until the most powerful muscle contraction is called for [5]. The homeostatic machineries of different cells may have different time courses, but it is hard to believe that any operate over long enough periods to account for homeostasis in cells with the most sparse firing rates.

If firing rates are maintained for the network as a whole, or over some region, rather than on a cell-by-cell basis, one has to ask how the aggregate firing rate is measured and how the effectors operate to influence each of the cells in the network. Indeed, perhaps it is not firing rate itself that is maintained but some correlate, the maintenance of which happens also to keep firing rates stable.

Second, one has to ask what, exactly, does the homeostat measure? The rate of action potentials averaged over some time is an abstract concept, but homeostatic mechanisms in the brain must measure something concrete. Firing action potentials has numerous effects on neurons. For example, the calcium entering through NMDA receptors acts through CaM-kinase and other pathways to produce Hebbian, synapse-specific long-term potentiation or depression. To understand homeostatic plasticity, we must determine the cellular indicator of activity that is sensed.

The large number of cell types in the brain, or within many small regions of the brain, raises the possibility of multiple homeostatic mechanisms operating to maintain distinct levels of activity in different cell types. They may use the same sensor but have different set points, or may instead have completely different sets of sensors and effectors, as well as different set points. A multiplicity of mechanisms for synaptic homeostasis is evident in invertebrate systems [6].

It is also possible that multiple different homeostatic mechanisms operate in individual cells. If this is the case, then there must be some mechanism for harmonizing their set points if they are not to be continuously engaging their effectors to create a steady state of opposite-directed plasticity.

2. Three homeostatic mechanisms in mouse V1
We present below three examples in which distinct phenomena operate to regulate neuronal firing rates. We conclude that the regulation of neuronal firing is achieved by multiple mechanisms acting at a range of levels, from the molecular to the rewiring of neural circuits. Whether these constitute a homeostat, strictly speaking, is a matter for discussion, but all have the desired effect of restoring firing rates to a level at which they can convey information.

All of these examples take advantage of plasticity in the responses of neurons in the primary visual cortex (V1) of the mouse to visual stimulation. Mice, like species studied earlier, have a brief critical period in early life during which occlusion of vision in one eye for several days while the other eye is allowed to see causes a rapid reduction of visual responses to the deprived eye (measured, of course, with good optics in both eyes) [7]. This manipulation is referred to as monocular deprivation (MD), and the change in response is referred to as ocular dominance plasticity (ODP). In the mouse, the inputs serving the contralateral eye are much more numerous and stronger than those of the ipsilateral eye, producing visual responses that are 50% greater [8]. MD experiments conventionally deprive the contralateral eye in order to produce the biggest effects. Over the first 2–3 days of MD during the critical period, responses to visual stimulation of an occluded contralateral eye dramatically decrease. During this initial period, the responses to the open eye do not change, but after that time they increase dramatically, becoming as strong as or stronger than those now driven through the deprived eye [8]. If such MD persists for some weeks beyond the critical period, the reduction of deprived-eye response becomes long lasting, and visual responses to that eye do not recover more than about half way to normal levels [9, 10].

MD begun after the critical period does not have the same effect. The changes in response produced by adult MD are much slower, much smaller, and are qualitatively different [11], with the most prominent feature being an increase in the response to the open eye rather than a reduction in the closed-eye response [11–14].

Many aspects of these phenomena have been studied in species other than mice, and anatomical changes that accompany and may be responsible for the changes in response, some of which are specific to particular cortical laminae, have been described (reviewed in [8]). Changes in the type and number of synaptic receptors have been shown to underlie changes in response (also reviewed in [8]), but here our focus is on selected upstream mechanisms that are required for homeostatic regulation of visual responses. The existence of additional such mechanisms, outlined at this meeting by Turrigiano [3], Fox [15], Chen [16] and others, only adds to the problem of how their actions are reconciled.

3. Homeostatic synaptic scaling, TNFα, and the regulation of open-eye responses after monocular deprivation
The first example of homeostatic regulation concerns the events that follow the first days of MD during the critical period. After the response to the contralateral eye is reduced in the first 2–3 days of MD, neither eye drives V1 as well as the contralateral eye did prior to deprivation [17]. The prompt reduction in cortical responses to the dominant, deprived eye, which is thought to be due to a loss of anatomical inputs serving that eye, causes a large overall reduction in
cortical activity. This reduction in cortical activity appears to stimulate an upregulation of cortical responses to the remaining inputs. Indeed, a hypothetical homeostatic compensation for reduced cortical activity provides an explanation not only for the dramatic increase in responses to the open eye after 2–3 days of MD but also for the surprising fact that even the deprived-eye responses start to increase to some extent at the same time. The hypothesis that the increase in open-eye response resulted from homeostatic compensation for the reduction of the powerful drive from the deprived contralateral eye remained an attractive but idle speculation until a means was found to block an essential step in the mechanism of homeostatic regulation.

Stellwagen & Malenka [18] studied a model of homeostatic regulation in vitro, measuring the change in spontaneous synaptic events in hippocampal slices and cultures that were subjected to several days of blockade of activity or synaptic transmission. Normally, such a transient blockade causes a dramatic increase in the size (but no change in the frequency) of the spontaneous synaptic events, referred to as homeostatic synaptic scaling. In tissue from mice that lacked tumour necrosis factor alpha (TNFα), no increase was found, suggesting that this form of homeostatic regulation requires signalling by TNFα. Similar results were obtained in tissue from wild-type mice in which soluble TNF receptors were used to compromise TNFα signalling. These findings provided compelling evidence that one form of homeostatic plasticity requires TNFα signalling.

We studied the course of ODP during the critical period in TNFα−/− mice in vivo [17]. The initial reduction of deprived-eye responses produced by MD during the critical period was entirely normal in these mutant animals. But there was no subsequent increase whatsoever in the open-eye responses. Tissue from the binocular zone of visual cortex at the height of the critical period showed the same dependence on TNFα signalling for the production of homeostatic synaptic scaling of miniature synaptic potentials in vitro as was previously found in the hippocampal cultures. These findings established that TNFα-dependent homeostatic synaptic scaling is responsible for one phase of ODP in vivo (figure 1).

Many reports had observed little or no plasticity in the monocular zone of V1 during the critical period: one week of MD did not reduce monocular zone responses to the sole, deprived eye that drives it, certainly not to the same extent as in the binocular zone. This finding was interpreted as evidence that competition between the pathways serving the two eyes was necessary for plasticity. However, using longitudinal measurements of visual responses we found that deprived-eye responses in the monocular zone of wild-type mice were reduced as much as in the binocular zone during the first 2–3 days of MD, but the responses returned to normal levels by the end of a week of continued MD. This finding suggested that the apparent lack of plasticity in the monocular zone was due to the same homeostatic synaptic scaling that takes place in the binocular zone. To test this idea, we measured the effect of MD in the monocular zone of TNFα−/− mice, which lack homeostatic synaptic scaling. Indeed, unlike in WT mice, responses to the deprived eye did not recover after the first few days of MD, and were even smaller at the end of the week than they had been mid-week.

Homeostatic synaptic scaling therefore plays a crucial role in regulation of responses during MD, a prominent form of cortical plasticity. The morphological correlates of this form of plasticity are not known.

4. Brain-derived neurotrophic factor and the recovery of deprived-eye responses after ending monocular deprivation

The effects of MD can be reversed and normal responses to the two eyes rapidly restored if the deprived eye is re-opened during the critical period. It has been thought that the restoration is due to the regrowth of synaptic inputs serving the deprived eye that were lost during the initial stage of MD. Consistent with this notion is the fact that such rapid and full restoration does not take place if MD is maintained past the close of the critical period. The loss of rapid plasticity in response to deprivation initiated after the critical period is mirrored by the failure of recovery.

In nearly pure neuronal cultures studied in vitro, the formation of new synapses and the strengthening of existing ones requires signalling by brain-derived neurotrophic factor (BDNF). Food et al. [19] showed that MD reduces the number of excitatory inputs to cultured neurons and that BDNF injection rescued them to levels comparable to those of non-deprived cultures. This rescue supports the idea that deprivation reduces growth of inputs and that BDNF stimulates growth of those inputs. In the same study, deprivation reduced the coupling of synaptic inputs to tonic currents in cultured neurons. These findings suggest that deprivation reduces the availability of inputs to mature neurons, which then become hyperpolarized and less likely to generate action potentials in response to natural stimuli. Whether or not this is true in vivo remains to be seen.

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factor (BDNF) on its principal receptor, TrkB [19]. To determine whether the recovery of responses after the cessation of MD to the re-opened, formerly deprived eye requires BDNF-TrkB signalling, we used a chemical-genetic approach. We studied recovery in mice in which TrkB receptors had been engineered to be susceptible to a small-molecule ‘Shokat’ inhibitor [20]. Application of the inhibitor in these mice completely blocked the recovery of deprived-eye responses, as well as the apparently homeostatic reduction in the responses to the open eye [21]. These findings provide strong evidence that BDNF secretion mediates an essential step in this recovery (figure 2).

Figure 2. Changes in responses of mouse V1 to visual stimulation before and after recovery from 5 days of monocular deprivation in the critical period with (filled circles, experimental) and without (open circles, vehicle control) administration of inhibitor of TrkB neurotrophin signalling in Shokat-inhibitor mice. Triangular symbols show data from wild-type control mice receiving the inhibitor. Panel (a) shows that blockade of BDNF-TrkB signalling prevents normal recovery of responses to deprived eye; panel (b) shows that restoration of normal open-eye responses is also blocked. Data from Kaneko et al. [21].

Figure 3. Selective recovery of responses in mouse V1 to visual stimuli presented during locomotion following five months of monocular visual deprivation. Top displays experiment timeline, showing MD extending through and beyond the critical period followed by a three week period of recovery after opening of the deprived eye. During recovery, the mouse received selective visual stimulation for 4 h d−1 while running on a styrofoam ball floating on air. Arrows indicate days on which V1 responses to the formerly deprived eye were measured using intrinsic signal imaging. Visual stimuli were contrast-modulated noise patterns (red symbols), moving bars of various orientations ( purple symbols), or a uniform grey screen (blue symbols). Each mouse viewed only one of the two visual stimuli during locomotion but responses to both dynamic stimuli were measured. Panel (a) shows enhanced recovery of responses to the noise pattern without recovery greater than control to moving bars. Panel (b) shows the converse. Data from Kaneko & Stryker [10].

5. Regulation of the activity of inhibitory neurons in the maintenance of excitatory activity

After long-term MD through the critical period and extending several months, neurons in the visual cortex of mice become much less responsive to visual stimulation through the deprived eye [8]. Just as in human deprivation-amblyopes, these responses do not recover to normal levels even three weeks after re-opening the deprived eye. However, we recently discovered a remarkable phenomenon of adult plasticity: viewing high-contrast stimuli for 4 h per day while the cortex was placed in a high-gain state by locomotion caused responses to those specific stimuli to recover to normal levels in less than a week [10] (figure 3).
This plasticity phenomenon has a number of striking features, none more so than its stimulus-specificity. But the feature relevant to the present discussion is the answer to the question of how visual responses can return to normal levels following the loss of at least half of the excitatory input from the dorsolateral geniculate nucleus, the major source of visual input to V1 [22]. What change in the circuitry of V1 permits such a recovery? To answer this question, we made extracellular recordings using multi-site silicon microelectrodes that capture waveforms that distinguish between narrow-spiking (presumed inhibitory) and broad-spiking (presumably mostly excitatory) neurons [23]. In recordings made immediately following prolonged MD, visual responses were greatly reduced in both the broad- and narrow-spiking neurons, but the reduction in the narrow-spiking presumed inhibitory neurons was much greater [10] (figure 4). Visual responses in the broad-spiking cells were reduced by 50%, while those in the narrow-spiking cells were reduced by more than 80%. Spontaneous activity recorded in the absence of a visual stimulus was reduced by more than 75% in the narrow-spiking cells but was increased by more than 200% in the broad-spiking cells. After recovery, these differences moderated slightly for responses to the specific stimulus that recovered, but the differences between narrow-spiking and broad-spiking cells, particularly in spontaneous activity, were still vast. Visual responses in the broad-spiking cells were almost fully restored to the level of normal non-deprived animals, while those in narrow-spiking cells recovered only 25–30%. Spontaneous activity in broad-spiking cells recovered by approximately 75% while that in narrow-spiking cells remained depressed [10].

These opposite changes in spontaneous activity between broad- and narrow-spiking cells suggest a homeostatic mechanism that alters excitatory–inhibitory balance to maintain cortical activity during prolonged deprivation, which may provide an environment that allows meagre deprived-eye excitatory pathways of the cortical circuit to drive activity upon re-opening.

6. Multiple mechanisms for homeostasis of cortical activity

The three examples from the primary visual cortex of the mouse reveal that multiple mechanisms act, sometimes in concert, to restore the activity of the principal excitatory neurons to something like a homeostatic set point. The mechanisms in these examples are completely different from one another: one (TNFα) a glial-derived signal required for homeostatic synaptic scaling and operating in vivo, the second a neurotrophic factor secreted by neurons and important for the maintenance and growth of synapses, and the third a circuit adjustment that dramatically alters the balance of activity in excitatory versus inhibitory pathways. We know from genetic and chemical-genetic studies that the first two operate independently of each other [17,21], but we do not know whether either is involved in the third mechanism, and we do not know the extent to which the first two mechanisms operate past the time of the critical period at the end of the first month of life.

These are only three of what are likely to be many more mechanisms, some of which were evident at this meeting (Turrigiano [3], Fox [15], Chen [16] and others). The interaction of specific ones of these mechanisms with conventional NMDA-receptor dependent Hebbian plasticity has been examined both theoretically and in experiments (for example, [24]), but most have been studied as isolated phenomena. The tools needed for further study are in many cases in hand. How it is that all of these mechanisms permit a stable level of activity in the normal healthy brain remains a major question for neuroscience, one that merits great attention in the years to come.

Ethics. All experiments were conducted following the U.S. NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco.

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References