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The nature and mechanisms of plasticity

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Cortical map plasticity

It is now well established that the functional organization of the cerebral cortex is plastic, that is, changes in organization occur throughout life in response to normal as well as abnormal experience. The potential for reorganization has been demonstrated in both sensory and motor areas of adult cortex, either as a consequence of trauma, pathological changes, manipulation of sensory experience, or learning. These changes can only be evaluated with reference to an extensive experimental base that has identified a repeatable representation pattern (e.g. somatotopy, tonotopy, or retinotopy), for which change can be detected. While the scope of changes are often at the edge of our technical capabilities to assess, there are striking examples of significant and rapid change (for reviews, see Sanes & Donoghue, 2000; Buonomano & Merzenich, 1998). There is an overwhelming belief that modifications in cortical organization emerge through changes in synaptic efficacy within the cortex and elsewhere in the nervous system. Further, these changes are have been closely linked to the phenomena called long-term potentiation (LTP) and long-term depression (LTD). This review deals mainly with the changes that have been detected in the motor cortex and their link to synaptic modification. Some of the most convincing evidence that learning and practice influences cortical organization and that learning operates through LTP/D-mediated mechanisms has come through work in the motor cortex. This work is also of profound significance to the medical community because it implies that the impaired or damaged motor cortex can be restructured through appropriate physical rehabilitation schemes or through pharmacological means that alter mechanisms accounting for LTP/D.

Functional topography of the primary motor cortex (MI) can be modified by peripheral or central injury, electrical stimulation, pharmacological manipulations, or experience. Behaviourally or experimentally induced reorganization of MI output maps are characterized by shifts in borders between different motor representations. For example, MI representations undergo rapid reorganization within hours of peripheral nerve lesions (Sanes et al., 1988, 1990; Donoghue et al., 1990). Following transection of the peripheral facial motor nerve to the whiskers in rats, movements of the forelimb can be evoked by stimulation of the former MI whisker representation (Donoghue et al., 1990; Fig. 1.1, see colour plate section), indicating that cortex dedicated to the control of one set of muscles can be switched rapidly to process information for another set. It is further evident that sensory nerve damage can alter motor maps (Huntley, 1997; Keller et al., 1996). In these cases, the cortical territories adjacent to the functionally silent areas expanded into the cortical zone that previously represented output to the vibrissa as a result of the nerve lesion. Similar changes in cortical output maps can be induced with prolonged changes in limb positions (Sanes et al., 1992; Sanes & Donoghue, 1997), supporting the conclusion that sensory feedback is important in shaping MI representations. Very recently, a doubling of forelimb motor representation has been shown as a result of repeated seizure activity that is also accompanied by increased synaptic strength within adult rat MI (Teskey et al., 2002), indicating that activity drives the form of representations. The expanded areas do not have to represent new areas of forelimb motor cortex; rather they have undergone some functional changes that lead to facilitated induction of forelimb movement in areas in which they could not be induced previously.

MI is also a site where reorganization occurs during the acquisition or practice of motor skills. In monkeys, skilled finger use expanded the digit representation in MI (Nudo et al., 1996), and learning a new visuomotor task altered the output representation of wrist muscles (Sanes & Donoghue, 1997). Skill learning-induced changes in MI were also detected on the single cell level in primates (Gandolfo et al., 2000). Monkeys learned to adapt their reaching movements to externally applied force fields. The firing rate and the tuning of individually recorded cells in MI changed during the adaptation period to the new force field. A group of these cells (the memory cells) retained the newly acquired activity pattern even after the force field

was turned off and the monkey's hand trajectory returned to control condition. Other memory cells that normally were untuned became tuned with acquisition of the new skill and remained tuned after turning off the force-field. These data provide evidence for single-cell plasticity in MI. In humans, MI representations also appear to enlarge or rearrange during motor learning (Grafton et al., 1992; Pascual-Leone et al., 1994; Karni et al., 1995; Muellbacher et al., 2001). Further, a role of MI in early motor consolidation (Muellbacher et al., 2002) and in motor memory (Karni et al., 1995) has been demonstrated in humans.

In rats, learning a skilled but not an unskilled reaching task leads to a significant increase in the mean area of the wrist and digit representations at the expense of the size of the shoulder representation, demonstrating that training-induced map reorganization is characterized by an expansion of 'trained' into 'untrained' representations without an overall increase in map size (Kleim et al., 1998). These results indicate that representational map plasticity is driven by skill acquisition, learning, or practice of a newly acquired action, but not by simple repetitive motor activity (Plautz et al., 2000; Classen et al., 1998), which suggests that only specific patterns of activity are capable of producing functional MI plasticity.

Plasticity substrate

Cortical networks appear to contain an anatomical substrate that is well suited to provide a flexible framework for a multitude of representations. Horizontal (also called lateral) fibres form a dense network of short- and long-range connections within the cortex. They spread tangentially along cortical layers and form a diffuse, but extensive, intrinsic pathway that provide excitatory connections across wide areas of cortex. In primary visual cortex these fibres have precisely patterned terminations, but in motor cortex they appear to be largely unpatterned. This diffuse organization could make it possible to couple wide extents of cortex; synaptic plasticity would allow for the functional patterning of these connections. The most extensive intracortical pathways travel through layer II/III and form a broad projection system. The functions of these horizontal projections in MI have remained obscure until recently. Evidence for a role of horizontal connections in shaping the properties of adult cortical neurons originated from a series

of experiments in the visual cortex, which linked horizontal connections to receptive field dynamics (Gilbert et al., 1996).

Experimental studies in the rat support the conclusion that intrinsic horizontal connections spanning MI are a substrate for motor cortical map plasticity (Donoghue et al., 1996). Motor representations can be modified by pharmacological adjustments of the balance between excitation and inhibition within MI, suggesting that occult representations can be revealed by unmasking existing horizontal pathways (Jacobs & Donoghue, 1991). The role of horizontal connections in supporting MI representations is also suggested by the patterns of reorganization that occur after nerve lesions. Facial nerve lesions result in rapid MI reorganization at sites with strong horizontal connections between forelimb and whisker representation, while reorganization is not evident at sites with sparse horizontal connections (Huntley, 1997). The masking of horizontal excitatory connections by feed-forward inhibition has been demonstrated even more directly in vitro using cortical slice preparations containing MI. Local application of bicuculline enhances excitatory responses of horizontal connections in MI (Hess & Donoghue, 1994); in these preparations concerns about localization of drug application or stimulation site are reduced by much better control than in the in vivo situation. Most convincingly, these effects can be observed in slices in which subcortical and deep layer connections have been cut away. This evidence strongly supports the idea that intrinsic horizontal pathways form a substrate for motor cortex plasticity. However, MI plasticity also requires a mechanism inherent to horizontal connections in order to modify maps.

Plasticity mechanisms

Evidence for candidate mechanisms to support cortical plasticity on the population level as well as on the cellular level have been proposed and evaluated. Mechanisms that support rapid plasticity are uncovering latent or existing connections, activating existing but silent synapses, activity-dependent synaptic plasticity, or generalized excitability changes in postsynaptic neurons. Morphological changes such as neurogenesis, synaptogenesis or synaptic remodelling require time for full expression and therefore, might rather be involved in providing new space for further changes. Evidence exists for the operation of most of these mechanisms during development, with learning

or response to injury. Moreover, these mechanisms are not mutually exclusive; different mechanisms could operate simultaneously or in some serial order.

Uncovering or unmasking of pre-existing connections in MI (Jacobs & Donoghue, 1991; Huntley, 1997) could serve as a mechanism for rapid (early) plasticity as a response to manipulations of sensory inputs (Kaas, 1991; Merzenich & Shameshima, 1993) or motor outputs (Sanes et al., 1990; Donoghue et al., 1996) of cortical representational maps. As discussed above, a change in the balance between excitation and inhibition can also lead to rapid map plasticity, if such changes persist (Jacobs & Donoghue, 1991). An alternative or additional mechanism for rapid plasticity is the activation of existing but silent synapses. Silent synapses are connections between neurons displaying no AMPA-mediated glutamate responses (e.g. Liao et al., 1995; Isaac et al., 1995); presynaptic transmitter release would not result in a rapid potential shift in the target neuron. The 'awakening' of silent synapses by insertion of postsynaptic AMPA receptors (Liao et al., 1999; Gomperts et al., 1998; Nusser et al., 1998; Petralia et al., 1999) is a proposed mechanism to account for rapid increases in synaptic efficacy that have been observed experimentally. Silent synapses have been implicated in brain plasticity of both young and mature animals (Atwood & Wojtowicz, 1999). There is convincing evidence for the occurrence of silent synapses in the developing nervous system (e.g. Durand et al., 1996; Wu et al., 1996; Liao et al., 1995; Isaac et al., 1995, 1997; Malenka & Nicoll, 1997, 1999; Malenka, 1998; Malinow, 1998; Rumpel et al., 1998), but as maturation progresses, silent synapses become rare (Nusser et al., 1998; He et al., 1998) and are presumably replaced by active ones. Although there is little evidence for the existence of silent synapses in the mature nervous system, their presence remains an open question. If present, the unmasking of silent synapses could support functional reorganization.

The most widely studied mechanism to support representational plasticity is long-term potentiation (LTP) (Bliss & Lomo, 1973), but it remains controversial (Shors & Matzel, 1997, for an extensive review), especially as a critical link between behavioural change and synaptic function. In the hippocampal cortex, neocortex and amygdala evidence for a possible role of LTP in learning and memory has accumulated over the past 30 years; population measures indicate that LTP and LTD operate during learning to modify synaptic efficacy (Martin et al., 2000). Certain forms of learning lead to an enhancement

of synaptic responses in a variety of brain structures (Moser et al., 1993; Rogan et al., 1997; McKernan & Shinnick-Gallagher, 1997; Rioult-Pedotti et al., 1998). Recently, LTP has been demonstrated to be involved in learning new motor skills (Rioult-Pedotti et al., 2000) and provides compelling evidence for LTP to be a mechanism involved in natural learning. A great deal of effort has been devoted to the question as to whether LTP is a mechanism of memory storage (Miller & Mayford, 1999). Long-lasting LTP in the hippocampus decays within weeks of its induction and can parallel memory loss (Thompson et al., 1996; Castro et al., 1989; Villareal et al., 2002). If this were true for the motor cortex, one would expect that discontinuing skill training would lead to synaptic weakening and possibly declining skill performance. Results, however, indicate that increased synaptic efficacy with initial skill learning as well as skill performance is maintained (Rioult-Pedotti & Donoghue, 2002). Learning effects seem to persist for a longer time in MI than in the hippocampus, which is consistent with results from Trepel & Racine (1998), indicating that neocortical LTP lasts longer than hippocampal LTP. The appeal of LTP as a mechanism of learning and memory is that it is activity dependent and specific to the active synapses and their target neurons.

Excitability changes represent another way to change coupling between neurons, but this is less specific than LTP-like mechanisms. A generalized long-lasting increase in excitability of postsynaptic neurons in MI has been demonstrated to be involved in classical conditioning (Brons & Woody, 1980; Baranyi et al., 1991; Woody, 1986; Aou et al., 1992). In the hippocampus, trace eye blink conditioning leads to a transient increase in CA1 excitability within a time window of 1 hour to 7 days with a peak effect at 24 hours and therefore might represent a mechanism that enables consolidation of a learned behaviour (Moyer et al., 1996). A change in postsynaptic excitability would be less specific than LTP/D because it alters the effectiveness of all synapses to a neuron.

The mechanisms described up to this point rely on modifications of existing synapses that are readily available within the substantial horizontal intracortical plexus. Experience could also produce new connections through synaptogenesis or neurogenesis. Such processes, however, require more time for full expression and therefore might be involved in creating new space for subsequent learning rather than being involved in ongoing information encoding.

The traditional view of adult primate neocortex was the structural stability and inability of neurogenesis and synapse formation that seemed to occur only during development. Such structural plasticity, however, is found in adult lower vertebrates (Alvarez-Buylla & Lois, 1995), in the olfactory bulb (Rousselot et al., 1995; Doetsch et al., 1997), and in the hippocampus, even in primates (Altman & Das, 1965; Gould et al., 1997, 1999a–c; Kornack & Rakic, 1999), and in humans (Eriksson et al., 1998). The traditional view of a structurally stable neocortex has recently been challenged by Gould et al., (1999d). Newly generated neurons were detected in neocortex of adult primates that were exposed to the DNA marker BrdU (bromodeoxyuridine). New neurons were added in regions of the association cortex, areas that are involved in learning and memory (Miller et al., 1996). Adult neurogenesis in the hippocampus is increased by training on associative learning tasks that require the hippocampus (Gould et al., 1999c), indicating that hippocampus-dependent learning may affect adult-generated neurons.

The formation of new synapses or the remodelling of existing synapses has long been believed to be involved in cellular mechanisms of learning and memory (for review, see Geinisman, 2000; Klintsova & Greenough, 1999; Bailey & Kandel, 1993). Motor skill learning has been shown to increase the number of synapses per neuron in the motor cortex (Kleim et al., 1996) and the cerebellum (Black et al., 1990; Kleim et al., 1997, 1998). Like learning, exposure to a complex environment results in a larger number of synapses per neuron (Turner & Greenough, 1985), increases in spine density (Moser et al., 1997) and changes in spine morphology (Comery et al., 1996; Jones et al., 1997). However, Bourgeois et al. (1999) found no ultrastructural changes in synaptic density despite continuous acquisition of long-term memories over the entire period of adulthood in macaque monkeys, indicating that the formation of long-term memories following learning may not necessarily involve a net synaptogenesis.

Whether the induction of LTP, the most viable current memory model, induces synaptogenesis or synaptic remodelling also remains controversial. Using stereological techniques Sorra & Harris (1998) could not show any change in synapse number. In contrast to these results, new synapses were detected 30–60 minutes following LTP induction in hippocampal slice cultures using the two photon imaging technique (Engert & Bonhoeffer, 1999; Maletic-Savatic et al., 1999; Toni et al., 1999) indicating that synaptogenesis

might be involved in synaptic modification. It remains to be proven that such processes also take place during acquisition of new behaviours.

Plasticity of MI horizontal connections (in vitro)

Mechanisms of synaptic modification are more easily studied in slice preparations than in intact animals. An in vitro approach allows local connections to be evaluated directly under controlled conditions using intracellular- as well as extracellular population measures. Extracellular field potentials (FP), which reflect the concerted synaptic activity of groups of fibres, can be readily evoked in MI horizontal connections (Hess & Donoghue, 1994) (Fig. 1.2(*c*)). In neocortex, the amplitude of FPs reflects a monosynaptic current sink, which can be used to measure the strength of excitatory synaptic responses for a population of neurons (Aroniadou & Keller, 1995). Thus the FP amplitude correlates with intracellular excitatory postsynaptic potentials (EPSP) (Fig. 1.2(*c*); Hess et al., 1996). Pharmacological manipulations revealed that horizontal excitatory connections are mainly glutamatergic (Keller, 1993; Hess & Donoghue, 1994), with larger, fast AMPA and slower, low amplitude NMDA components. The strength of excitation is also regulated by feed-forward inhibition. The MI slice preparation is useful in that the same region can be repeatedly localized. To study horizontal connections in MI, stimulation and recording electrodes are placed on the surface of coronal slices containing MI (Fig. 1.2(*a*), see colour plate section). Most in vitro studies in MI have examined layer II/III horizontal connections within the region of the MI forelimb area. Stimulation of the superficial layers is more restricted to horizontal connections than in deeper layers, which contain a more complex mix of vertical, extrinsic connections as well as other intrinsic connections. The placement of stimulation and recording electrodes in the MI forelimb region has been verified by labelling layer V corticospinal neurons using fast blue injections into the cervical spinal cord. (Fig. 1.2(*b*), see colour plate section).

Using slice preparations it has been possible to test for the ability of horizontal connections to be modified and to search for the mechanisms that support modification. Studies in the hippocampus and in other cortical areas suggested that activity-dependent processes leading to long-term potentiation (LTP) and long-term depression (LTD) are likely candidates for plasticity in MI. LTP, discovered in the hippocampus (Bliss & Lomo, 1973)

a structure known to be critical for learning, is rapidly induced, and shows long-lasting increases in synaptic strength as a response to short bursts of coinciding activity at specific synapses, all useful features for a natural memory mechanism (Hebb, 1949). Classical forms of LTP, and variants, have also been documented in the amygdala (Clugnet & LeDoux, 1990; Marren, 1999; Martin et al., 2000) and neocortex (Artola & Singer, 1987; Iriki et al., 1989; Kirkwood et al., 1996; Trepel & Racine, 1998) and specifically in MI (Baranyi & Feher, 1978, 1981; Baranyi et al., 1991; Aroniadou & Keller, 1995; Castro-Alamancos et al., 1995; Hess et al., 1996; Rioult-Pedotti et al., 1998). Most forms of LTP are glutamatergic and depend on the activation of voltage-dependent NMDA receptors.

The potential for LTP of layer II/III intrinsic horizontal pathways in MI has been established (Castro-Alamancos et al., 1995; Hess & Donoghue, 1996; Hess et al., 1996). This activity-dependent synaptic modification is NMDA receptor dependent, pathway specific and long lasting (Hess et al., 1996) and thus resembles classical LTP. LTP is normally induced by high frequency stimulation or theta burst stimulation where several high frequency bursts are delivered in short succession. In the adult MI, similar stimulation patterns alone did not lead to an increase in synaptic strength as in the hippocampus and other cortical areas. LTP was only induced when inhibition was reduced transiently by local application of bicuculline, a GABA antagonist, prior to theta burst stimulation (Chen et al., 1994; Hess et al., 1996) or by concomitant stimulation of vertical and horizontal inputs (Hess et al., 1996). These findings suggest that local, GABA-mediated inhibition plays a critical role in cortex in regulating the potential for LTP induction, though maintenance of LTP does not require the sustained reduction of inhibition.

Partially because of theoretical considerations, it has been recognized that, if there is a mechanism for activity-dependent increases in synaptic strength, there should also be a mechanism to decrease synaptic strength in order to keep synaptic weights constant and to prevent runaway potentiation leading to synapse saturation. Therefore, individual synapses need to be capable of bidirectional modification, a strengthening and weakening, to avoid saturation effects. Mild but repetitive stimulation of synaptic inputs leads to long-term depression (LTD), a lasting activity-dependent decrease in synaptic efficacy. LTD was first discovered in the hippocampus by Lynch et al. (1977; e.g. Levi & Steward, 1979; Thiels et al., 1994; Dudek & Bear, 1992) and later in

other brain structures including the amygdala (Li et al., 1998) and neocortex (Artola et al., 1990; Kirkwood & Bear, 1994). As its LTP counterpart, LTD is long lasting and may be NMDA receptor dependent or independent. In MI, LTD depends on the activation of NMDA receptors, and, unlike LTP, LTD is readily induced in MI horizontal pathways by low frequency stimulation without additional manipulations (Hess et al., 1996).

In summary, then, MI horizontal connections meet important conditions for reorganizing motor representation patterns: they strengthen and weaken, based on established activity-dependent synaptic modification processes, and they interconnect widespread sets of neurons through their lateral-spreading connections.

MI's direct role in motor learning and memory

Motor skill learning and its trace in MI

The presence of this connectional substrate and activity-dependent synaptic modification mechanism provides strong support for the conclusion that operations within motor cortical circuitry are important for learning. Learning enhances synaptic responses in the hippocampus (Moser et al., 1993; Power et al., 1997), the amygdala (Rogan et al., 1997; McKernan & Shinnick-Gallagher, 1997), and the piriform cortex (Roman et al., 1999; Saar et al., 1999). Does motor learning lead to a similar enhancement in MI? There is now compelling evidence that motor skill learning involves LTP-mediated synaptic plasticity in MI, providing an important link between behavioural change, synaptic modification and LTP. In this novel model, evidence for synaptic change and mechanisms of change were examined in motor cortex slices (Rioult-Pedotti et al., 1998). Rats learned to reach, with their preferred forelimb, through a small aperture in a food box and grasp single food pellets (Fig. 1.3 left, see colour plate section). The rats acquired the skill and improved performance over 5 training days (Fig. 1.3 right, see colour plate section).

Because the reach and grasp task is quantifiable, improvement in behaviour can be directly associated with changes in synaptic strength observed in slices prepared after learning (Fig. 1.4, see colour plate section). Layer II/III intracortical connections were markedly enhanced only in the trained MI that related to the forelimb used in the task. The opposite (ipsilateral) MI for each animal showed no change and served as an important control