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## Synaptic plasticity of local connections in rat motor cortex

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Mini review

**Abstract.** This paper reviews studies that investigated mechanisms of the induction of long-term synaptic efficacy increase in local horizontal connections in slices of adult rat motor cortex. Long-term potentiation (LTP) could be induced by electrical stimulation of afferents using theta burst stimulation (TBS) conditionally, when synaptic inhibition was transiently blocked by focal application of GABA<sub>A</sub> receptor antagonist. Robust, long-lasting enhancement of synaptic transmission in horizontal connections was induced by brief application of the potassium channel blocker, tetraethylammonium (TEA, 25 mM), to the incubation medium. This TEA-LTP could be blocked by nifedipine, a voltage-dependent calcium channel blocker. A transient exposure of slices to elevated extracellular calcium (5 mM) resulted in a long-lasting enhancement of responses, termed Ca-LTP, which could be blocked by the antagonist of NMDA receptors, APV. The induction of both TEA-LTP and Ca-LTP, could be prevented by inhibitors of the extracellular signal regulated kinase (ERK) cascade U0126 and PD 98059. A transient activation of the ERK, 15 min after application of TEA or elevated [Ca<sup>2+</sup>], was demonstrated using immunofluorescence. Both forms of plasticity could also be prevented by the inhibitor of cAMP-dependent protein kinases (PKA), Rp-cAMPS. These studies indicate the involvement of the ERK and PKA signaling mechanisms in synaptic plasticity of the motor cortex *in vitro*. Since LTP in horizontal connections of the motor cortex has previously been shown to be related to the acquisition of a motor skill, it is suggested that the ERK and PKA signaling pathways may be involved in motor learning.

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**Key words:** long-term potentiation; horizontal connections; extracellular signal regulated kinase; protein kinase A

## INTRODUCTION

It is widely accepted that reorganizations of neuronal circuitry form a basis of various form of learning and compensatory processes occurring after brain lesions. The extent of reorganizations in the adult brain is smaller than in the developing brain but within the cerebral cortex at least some systems remain plastic into adulthood. While adult neocortical plasticity has been studied by numerous investigators, particular attention has been paid to primary sensory cortices. However, it has also been demonstrated that adult primary motor cortex express a potential for plasticity (for review see Sanes and Donoghue 2000). Reorganizations of the motor cortex, involving the expansion of motor representations, have been shown to accompany motor learning in rats (Kleim et al. 1998, Remple et al. 2001), monkeys (Nudo et al. 1996) and humans (Cohen et al. 1993). Unmasking of pre-existing local excitatory connections by disinhibition (Jacobs and Donoghue 1991), forming new synapses (Kleim et al. 1996) and long-term potentiation (LTP) of synaptic transmission in local horizontal connections (Hess and Donoghue 1994) have been hypothesized to be involved in reorganizations of cortical motor maps, however, their cellular and molecular mechanisms still remain to be elucidated.

## LONG-TERM POTENTIATION IN MOTOR CORTICAL PLASTICITY

Conditions for the induction of LTP in local horizontal connections of the motor cortex have been studied in brain slices prepared from adult rats (Hess et al. 1996). Field potential and intracellular recordings demonstrated excitatory synaptic interactions across layers II/III that could be enhanced transiently by focal application of the GABA<sub>A</sub> antagonist, bicuculline, at the recording site. It has been found that LTP of responses evoked in the horizontal pathways could be induced by theta burst stimulation (TBS), but only when bicuculline was applied transiently at the recording site immediately before TBS. TBS delivery during focal bicuculline application increased field potential amplitudes by 25-35% and stable LTP could be recorded for at least 1-2 hours. In the absence of bicuculline TBS failed to induce LTP in horizontal connections but LTP could also be induced, without the need for focal disinhibition, by co-tetanization of vertical pathways simultaneously with horizontal activation. LTP in

horizontal connections was dependent on the activation of NMDA receptors.

To determine whether LTP of local horizontal connections of the motor cortex is involved in acquisition of skilled motor behavior, rats were trained in a skilled reaching task with one forepaw (Riout-Pedotti et al. 1998), which induces expansion of the forepaw representation in the motor cortex (Kleim et al. 1998, 2004). Rats were trained to reach through an opening in a wall of the cage with a single forepaw in order to retrieve small food pellets from a food box using a grasping motion. Training and subsequent practice lasted three or five successive days with one training session per day. By the final two days of training, rats achieved a performance of about 1.5 pellet retrievals per minute using one, preferred forepaw, with few errors in the reach, grasp or retrieval actions. On the next day, *ex vivo* brain slices containing a part of forepaw motor representation were prepared bilaterally. It has been found that the amplitude of field potentials in the forepaw region of the hemisphere contralateral to the trained paw (termed "trained" side) was significantly increased over a wide range of stimulation intensities relative to the opposite ("untrained") side. No differences were seen in the hindlimb region. The amount of LTP that could be induced by simultaneous focal application of bicuculline and theta burst stimulation in "trained" area was less than in controls, suggesting that the effect of training was at least partly due to LTP-like mechanisms. It has subsequently been shown that not only LTP was markedly reduced in the "trained" area but at the same time the potential for an opposite phenomenon, long-term depression (LTD) (Hess and Donoghue 1996) was enhanced (Riout-Pedotti et al. 2000).

An involvement of LTP in motor learning has originally been hypothesized on the basis of investigations of the projection from somatosensory to motor cortices in cats (Sakamoto et al. 1987). The present results are consistent with the use of LTP in local horizontal connections within rat motor cortex to strengthen excitatory synaptic transmission during motor skill training, at least in its initial phase of acquisition. With more prolonged training, lasting 7-10 days, formation of new synapses within expanded motor representations becomes evident, which may represent a correlate of the process of consolidation (Kleim et al. 2004). Recent work has suggested the involvement of LTP-like processes in the acquisition of motor skills in humans (Buetefisch et al. 2004, Ziemann et al. 2004).

## ERK SIGNALING IN MOTOR CORTICAL LONG-TERM POTENTIATION

The extracellular signal regulated kinase (ERK) pathway, a cascade consisting of three serially linked kinases sequentially phosphorylating each other, belongs to the family of mitogen-activated protein kinase (MAPK) pathways, which have originally been linked to processes of cellular proliferation, differentiation and development (for review see Seger and Krebs 1995). Subsequent work has implicated the ERK cascade of neurons in the formation of memory traces and synaptic plasticity (for reviews see Berman and Dudai 2004, Sweatt 2001, Thiels and Klann 2001, Thomas and Huganir 2004). It has been established that the ERK cas-

cade plays a key role in the induction of hippocampal LTP both *in vitro* (English and Sweatt 1996, 1997, Rosenblum et al. 2002) and *in vivo* (Davis et al. 2000). Specifically, the ERK cascade has been suggested to mediate the induction of protein synthesis-dependent late phase of LTP, *via* phosphorylation of the transcription factor cAMP response element binding protein (CREB) (for review see Adams et al. 2000b). Fewer studies addressed the involvement of the ERK pathway in neocortical plasticity. Visual stimulation after dark rearing induces a transient ERK activation in the visual cortex (Cancedda et al. 2003, Kaminska et al. 1999) and intracortical administration of the ERK pathway inhibitors to monocularly deprived rats prevents the shift in ocular dominance towards the nondeprived eye (Di Cristo et al. 2001). Moreover, pharmacological inhibi-

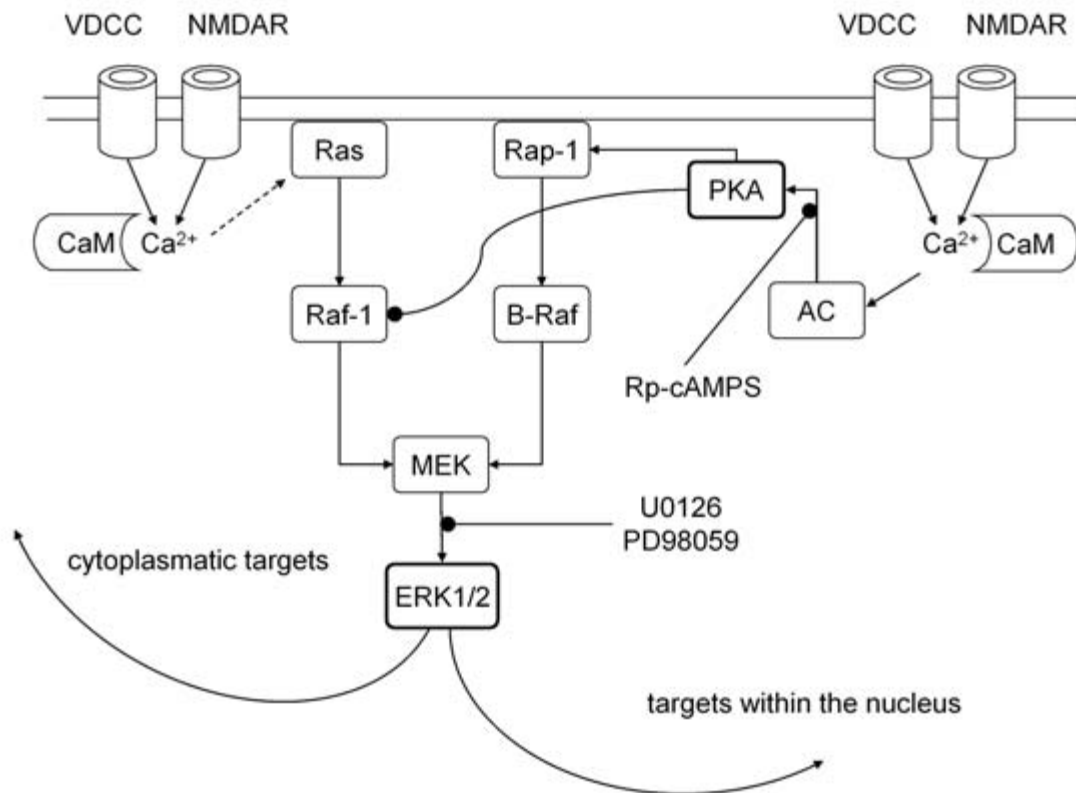


Fig. 1. Proposed mechanism of the induction of TEA-LTP and Ca-LTP in rat motor cortex. Calcium influx through NMDA receptors (NMDAR) or through voltage-dependent calcium channels (VDCC) may result in an increased level of Ras-GTP (Ras) through as yet undetermined mechanism and, subsequently, in the activation of protein kinases of the ERK cascade (Raf-1, MEK, ERK1/2). Activated ERK1/2 phosphorylates cytoplasmic and nuclear proteins. The induction of TEA-LTP and Ca-LTP could be blocked by MEK inhibitors (U0126, PD989059). Alternatively, MEK might be activated *via* calcium-calmodulin (CaM) activated adenyl cyclase(s) (AC) and the stimulation of protein kinase A (PKA) pathway involving small G-protein Rap-1 (Rap-1) and the protein kinase B-Raf (B-Raf). Activated PKA inhibits Ras/Raf-1 pathway. The involvement of the Ras/Raf-1 pathway in TEA-LTP and Ca-LTP is unlikely, since the induction of both is prevented by PKA inhibitor (Rp-cAMPS). Arrowheads and filled circles denote activation and inhibition, respectively.

tion of the ERK pathway prevents the induction of LTP in visual cortical slices (Di Cristo et al. 2001).

The involvement of the ERK cascade in motor cortical plasticity has been tested using two *in vitro* models of chemically-induced modifications of synaptic efficacy in brain slices containing a part of the motor cortex. Field potentials were evoked in horizontally-oriented intralaminar connections of layer II/III and layer V (Hess et al. 1994). Bath application of tetraethylammonium (TEA, 25 mM) a potassium channel blocker, for 11 minutes, induced stable long-lasting potentiation (TEA-LTP), which developed gradually over approx. 30 min after the beginning of TEA washout and could be recorded for at least 2 hours (Jagodzinski and Hess 2001). Intracellular recordings from presumed layer V pyramidal cells demonstrated that this effect was not due to persistent modifications of neuronal excitability. On the other hand, during TEA application and its washout, strong enhancement of excitability was evident, which led to the occurrence of  $\text{Ca}^{2+}$  spikes, a finding consistent with earlier studies on hippocampal preparations (Huang and Malenka 1993). The induction of TEA-LTP in the motor cortex was not dependent on NMDA receptors, since it could be evoked in the presence of D, L-2-amino-5-phosphonovalerate (APV), but it could be blocked by voltage-dependent calcium channel blocker, nifedipine. The second model of the induction of long-term increase in synaptic efficacy in motor cortical slices involved the exposure to transiently elevated concentration of extracellular calcium (5 mM for 10 minutes), which resulted in long-lasting potentiation of field potentials and intracellularly recorded excitatory postsynaptic potentials lasting at least 2 hours (Hess 2002). Similarly to TEA-LTP, this calcium-induced LTP (Ca-LTP) developed gradually during 20-30 min after termination of 5 mM  $\text{Ca}^{2+}$  application, but in contrast to TEA-LTP, Ca-LTP could be blocked by APV and thus it represents a form of NMDA receptor-dependent synaptic plasticity.

To test the effects of the blockade of the ERK cascade on TEA-LTP and Ca-LTP, slices were preincubated with MAP kinase kinase (MAPKK, MEK) inhibitors U0126 (20  $\mu\text{M}$ ) or PD 98059 (50  $\mu\text{M}$ ). These compounds inhibit the kinase acting upstream the two isoforms of the ERK kinase, ERK1 and ERK2 (see Fig. 1), also known as p44 MAPK and p42 MAPK, respectively. In the presence of MEK inhibitors, the induction of TEA-LTP and Ca-LTP was blocked (Grzegorzewska et al. submitted). While 1 hour after the induction of the

TEA-LTP in control incubation conditions, field potential amplitude was increased by 40-60%, at this time after the application of TEA in the presence of U0126 or PD 98059 the response amplitude was close to baseline values. Similarly, while in control experiments the application of 5 mM  $\text{Ca}^{2+}$  resulted in an increase of response amplitude by 30-40%, in the presence of U0126 or PD 98059 no lasting response amplitude occurred. Also, no short-term increases of the responses were evident after TEA or increased  $[\text{Ca}^{2+}]$  co-application with either of the two MEK inhibitors.

To demonstrate the activation of ERK1/2 by TEA and increased  $[\text{Ca}^{2+}]$ , motor cortical slices have been incubated in conditions identical as during electrophysiological experiments. Slices were collected immediately after termination of the LTP induction procedure (termed 0 min) as well as 15, 30 and 45 min later and kept frozen. Next, slices were homogenized and probed with an antibody that selectively recognizes dually-phosphorylated, active form of the ERK1/2 using the Western blotting method. It has been found that phosphoERK1/2 immunoreactivity was increased by 40-50% above baseline levels 15 min after termination of either TEA or increased  $[\text{Ca}^{2+}]$  perfusion. In slices collected at 0 min as well as 30 and 45 min later phosphoERK1/2 immunoreactivity was not different from baseline levels. This result is in agreement with the slow timecourse of the development of both TEA-LTP and Ca-LTP.

These findings are consistent with reports showing that both early and late phases of hippocampal LTP depend on the activation of the ERK cascade. It has been suggested that activated ERK1/2 modulates the early phase of LTP by phosphorylation of the membrane potassium channel  $\text{K}_{\text{v}}4.2$  (Adams et al. 2000a), and induces late LTP by regulating the phosphorylation of transcription factors and, consequently, the synthesis of new proteins (Sweatt 2001). In the CA1 area of the hippocampus both NMDA receptor-dependent and -independent forms of LTP have been shown to require the activation of ERK1/2 (English and Sweatt 1996, 1997, Kanterewicz et al. 2000). It has been suggested that calcium influx, either through NMDA receptors or voltage-dependent calcium channels results in an increase in the level of Ras-GTP, a low molecular weight G-protein, which results in the activation of Raf and, consequently, other protein kinases in the ERK cascade (Sweatt 2001, Thomas and Huganir 2004). A similar mechanism has been suggested to operate in the visual

cortex (Berardi et al. 2003). Transiently increased phosphoERK1/2 immunoreactivity accompanied the induction of NMDA-dependent and -independent forms of hippocampal LTP (Davis et al. 2000, Kanterewicz et al. 2000).

The activation of the ERK cascade may result from the action of a wide variety of signals. One of the pathways, which have been shown to converge on the ERK cascade, is related to the production of cAMP and the activation of cAMP-dependent protein kinase A (PKA). It has been shown that the late phase of hippocampal LTP and certain forms of hippocampus-dependent learning require the activity of the PKA (for reviews see Sweatt 2001, Waltereit and Weller 2003). PKA has been implicated in ocular dominance plasticity and LTP in the visual cortex (Berardi et al. 2003, Liu et al. 2003). To test the involvement of PKA on TEA-LTP and Ca-LTP, slices were preincubated with Rp-cAMPS (100  $\mu$ M), a specific inhibitor of activation by cAMP of cAMP-dependent protein kinases. In the presence of Rp-cAMPS the induction of both TEA-LTP and Ca-LTP was blocked (Grzegorzewska et al., submitted). The activity of adenylyl cyclase could be stimulated by  $Ca^{2+}$  ions and this mechanism has been implicated in late phase of hippocampal LTP (Wong et al. 1999). Interestingly, the Ras/Raf-1 pathway leading to the activation of ERK1/2 is inhibited by protein kinase A (Sweatt 2001, Waltereit and Weller 2003). Thus, since the induction of TEA-LTP and Ca-LTP in the presence of the PKA inhibitor is blocked, it is unlikely that the Ras/Raf-1 pathway is involved in these two forms of plasticity. As illustrated in Fig. 1, an alternative pathway of MEK activation involves a low molecular weight G-protein Rap-1 and protein kinase B-Raf.

## CONCLUSIONS

The mechanisms of NMDA receptor-dependent and -independent, chemically-induced synaptic modifications across horizontally connected neurons of rat motor cortex *in vitro* involve the ERK and PKA signaling cascades. Thus, motor cortex could be added to the list of brain areas, where a role of these systems in plastic processes has been established. The results support the general idea that the ERK system plays a key function in synaptic plasticity and memory. However, it remains to be established whether the ERK and PKA signaling pathways are indeed involved in motor learning under physiological conditions, which might be tested with

the intraperitoneal or intraventricular administration of MEK inhibitors before motor skill training. Since plastic reorganizations of human motor cortex have been implicated in recovery of motor function, for example in patients with stroke or multiple sclerosis (Liepert et al. 2000, Reddy et al. 2000), understanding of motor cortical plasticity mechanisms is of importance for elaboration of better strategies of rehabilitation.

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