Cellular/Molecular

Mitogen-Activated Protein Kinase-Mediated Reinforcement of Hippocampal Early Long-Term Depression by the Type IV-Specific Phosphodiesterase Inhibitor Rolipram and Its Effect on Synaptic Tagging

Sheeja Navakkode, Sreedharan Sajikumar, and Julietta Uta Frey
Department of Neurophysiology, Leibniz Institute for Neurobiology, D-39118 Magdeburg, Germany

Rolipram, a selective inhibitor of cAMP-specific phosphodiesterase 4 (PDE4), has been shown to reinforce an early form of long-term potentiation (LTP) to a long-lasting LTP (late LTP). Furthermore, it was shown that the effects of rolipram-mediated reinforcement of LTP interacts with processes of synaptic tagging (Navakkode et al., 2004). Here we show in CA1 hippocampal slices from adult rats *in vitro* that rolipram also converted an early form of long-term depression (LTD) that normally decays within 2–3 h, to a long-lasting LTD (late LTD) if rolipram was applied during LTD-induction. Rolipram-reinforced LTD (RLTD) was NMDA receptor- and protein synthesis-dependent. Furthermore, it was dependent on the synergistic coactivation of dopaminergic D_1 and D_5 receptors. This let us speculate that RLTD resembles electrically induced, conventional CA1 late LTD, which is characterized by heterosynaptic processes and synaptic tagging. We therefore asked whether synaptic tagging occurs during RLTD. We found that early LTD in an S1 synaptic input was transformed into late LTD if early LTD was induced in a second independent S2 synaptic pathway during the inhibition of PDE by rolipram, supporting the interaction of processes of synaptic tagging during RLTD. Furthermore, application of PD 98059 ($2'$-amino-$3'$-methoxyflavone) or U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene), specific inhibitors of mitogen-activated protein kinases (MAPKs), prevented RLTD, suggesting a pivotal role of MAPK activation for RLTD. This MAPK activation was triggered during RLTD by the synergistic interaction of NMDA receptor- and D_1 and D_5 receptor-mediated Rap/B-Raf pathways, but not by the Ras/Raf-1 pathway in adult hippocampal CA1 neurons, as shown by the use of the pathway-specific inhibitors manumycin (Ras/Raf-1) and lethal toxin 82 (Rap/B-Raf).

Key words: hippocampus; long-term potentiation; neuromodulation; protein synthesis; reinforcement; long-term depression; memory formation

Introduction

Activity-dependent changes in synaptic strength are thought to underlie learning and memory. Most attention has been given to long-term potentiation (LTP), in which brief high-frequency stimulation results in a long-lasting increase in synaptic efficacy (Bliss and Lomo, 1973). However, distinct patterns of low-frequency stimulation (LFS) can result in a long-term depression (LTD) of synaptic efficacy (Mulkey and Malenka, 1992; Bear and Malenka, 1994; Linden and Connor, 1995; Sajikumar and Frey, 2003). It has been reported that late LTP as well as late LTD in the hippocampal CA1 region from adult rats require the coactivation of the NMDA receptor (NMDAR), as well as dopaminergic D_1 and D_5 receptors and protein synthesis (Frey et al., 1988, 1990,

1991a,b, 1993; Matthies et al., 1990; Dudek and Bear, 1992; Huang and Kandel, 1995; Manahan-Vaughan, 2000; Sajikumar and Frey, 2003, 2004). The activation of the cAMP/cAMP-dependent protein kinase (PKA) pathway through the synergistic action of NMDAR and dopaminergic D_1 and D_5 receptor activation is crucial for the development of the protein synthesis-dependent late LTP as well as late LTD in CA1 neurons from adult rats (Frey et al., 1990, 1991b, 1993; Matthies et al., 1990; Matthies and Reymann, 1993; Huang and Kandel 1995; Abel et al., 1997; Morozov et al., 2003; Nguyen and Woo, 2003; Fischer et al., 2004; Sajikumar and Frey, 2004). Furthermore, LTP as well as LTD are characterized by late-associative properties involving processes of "synaptic tagging" (Frey and Morris, 1997, 1998; Kauderer and Kandel, 2000; Navakkode et al., 2004) and "synaptic cross-tagging" (Sajikumar and Frey, 2004; Sajikumar et al., 2005). Recently, a number of studies have shown that mitogen-activated protein kinases (MAPKs) play an important role in inducing the maintenance of long-lasting forms of synaptic plasticity and thus may regulate both local protein synthesis and the expression of genes whose products are required to stabilize LTP or LTD over prolonged periods (English and Sweatt, 1997; Otani

et al., 1999; Roberson et al., 1999; Davis et al., 2000; Matsuo et al., 2000; Rosenblum et al., 2000; Watabe et al., 2000; Dudek and Fields, 2001; Rosenblum et al., 2002; Kelleher et al., 2004a,b).

Although all of these data fit in a common scheme, there are many differences in how these data were obtained that may confound simple models. For example, the age and species of the investigated animals have varied, and, as we have mentioned previously (Frey and Morris, 1998; Sajikumar and Frey, 2004), it is important to differentiate between the possible different intracellular pathways crucial for information processing in juvenile versus adult animals or in rats versus mice, etc. For example, in juvenile tissue or in mice, a Ca^{2+}/calmodulin-dependent pathway of cAMP/PKA activation as well as Ras/Raf-1-mediated activation of MAPKs were described (Chetkovich and Sweatt, 1993; Morozov et al., 2003). In contrast, in adult rats, the activation of cAMP/PKA results by D_1 and D_5 receptor activation in a synergistic action with NMDAR activation (Frey et al., 1990, 1991b, 1993; Huang and Kandel, 1995; Frey and Morris, 1998; O'Carroll and Morris, 2004; Sajikumar and Frey, 2004). We have focused here on processes in adult rats, and thus we were interested to know which of the above pathways are used in the adult animal.

We describe that early LTD can be transformed into late LTD by the activation of the cAMP/PKA pathway using rolipram, an inhibitor of cAMP-degradation by phosphodiesterase 4B (PDE4B). It is known that rolipram is an inhibitor selective for the Ca^{2+}/calmodulin-independent and cAMP-specific isozyme of PDE4 (Beavo, 1988). Therefore, we also investigated whether rolipram-reinforced LTD (RLTD), i.e., the expression of a protein synthesis-dependent stage of LTD, requires MAPKs. Furthermore, we studied the involvement of D_1 and D_5 receptor coactivation for RLTD and properties of synaptic tagging during RLTD.

Materials and Methods

Experimental procedures. One hundred twenty transverse hippocampal slices (400 μm) were prepared from 120 seven-week-old male Wistar rats as described previously (Frey and Morris, 1997, 1998; Sajikumar and Frey, 2004). Slices were incubated within an interface chamber at a temperature of 32°C. [A modified Krebs'–Ringer's solution containing the following (in mM): 124 NaCl, 4.9 KCl, 1.2 KH_2PO_4, 2.0 $MgSO_4$, 2.0 $CaCl_2$, 24.6 $NaHCO_3$, and 10 D-glucose was used as artificial CSF (ACSF); carbogen consumption, 32 L/h.] In all experiments, two monopolar lacquer-coated stainless steel electrodes were positioned within the stratum radiatum of the CA1 region for stimulation of two independent synaptic inputs, S1 and S2. For recording of the field EPSP and the population spike, two electrodes were placed in the CA1 dendritic and cell body layer of a single neuronal population, respectively (see Fig. 1*A*). Slices were preincubated for at least 4 h before recording the baseline, a period that is critical for a stable long-term recording (Sajikumar and Frey, 2004). After the preincubation period, the test stimulation strength was determined for each input to elicit a population spike of 40% of its maximal amplitude. For stimulation, biphasic constant-current pulses (impulse duration, 0.1 ms/half-wave) were used. Late LTD was induced using strong low-frequency stimulation (SLFS) of 900 bursts (one burst consisted of three stimuli at 20 Hz; interburst interval, = 1 s; i.e., $f = 1$ Hz; stimulus duration, 0.2 ms/half-wave; total number of stimuli, 2700). This stimulation pattern produced a stable LTD *in vitro* for at least 8 h (Sajikumar and Frey, 2004). In experiments in which a weaker induction of LTD was induced, a transient early LTD was induced using weak low-frequency stimulation (WLFS) consisting of 900 pulses (1 Hz; impulse duration, 0.2 ms/half-wave; total number of stimuli, 900). The population spike amplitude and the slope of the field EPSP were monitored on-line. The time course of the population spike resembled that of the field EPSP. Thus, only the time course of the field EPSP is described in detail and presented in the figures.

Baseline was recorded for a minimum of 1 h before LTD induction

(four 0.2 Hz biphasic constant-current pulses every 15 min, averaged on-line). Four 0.2 Hz biphasic constant-current pulses (0.1 ms/polarity) were used for testing, 21, 25, and 30 min after LFS, and then every 15 min.

Rolipram (Tocris Cookson, Bristol, UK), a type IV phosphodiesterase inhibitor, was used at a concentration of 0.1 μM (Dym et al., 2002) dissolved in ACSF and 0.1% dimethylsulfoxide. [0.1% DMSO had no effect on control recordings (Navakkode et al., 2004).] aminophosphonopentanoic acid (AP-5; Sigma, St. Louis, MO) was used at a concentration of 50 μM (dissolved in ACSF) to block the NMDA receptor. Anisomycin (Sigma), a reversible protein synthesis inhibitor, was used at a concentration of 25 μM (a concentration that blocked at least 85% of incorporation of [^{3}H]leucine into hippocampal slices) (Frey et al., 1991a). Emetine (Tocris Cookson) was used at a concentration of 20 μM (dissolved in ACSF and 0.1% DMSO). The selective dopaminergic D_1 and D_5 receptor antagonist R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH23390) was used at a concentration of 0.1 μM (dissolved in ACSF) (Frey et al., 1991b) (Tocris Cookson). The mitogen-activated/extracellular-signal regulated kinase (MEK) inhibitor 1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene (U0126; Promega, Madison, WI) was used at a concentration of 1 μM (dissolved in 0.1% DMSO) (Davies et al., 2000); 2′-amino-3′-methoxyflavone (PD 98059) (Calbiochem, La Jolla, CA) was used at a concentration of 1 μM (dissolved in 0.1% DMSO) (Davies et al., 2000). Manumycin-A (*Streptomyces parvulus*; Calbiochem), a selective inhibitor of the farnesyltransferase, was used at a concentration of 2 μM (Murray and O'Connor, 2004) (dissolved in 0.02% DMSO). Lethal toxin-82 (LT-82; *Clostridium sordelli*) was used at a concentration of 200 ng/ml (Murray and O'Connor, 2004) (donated by Prof. Michel Popoff, Institut Pasteur, Paris, France).

Statistics. The average values of the population spike (millivolts) and slope function of the field EPSP (millivolts per millisecond) per time point were analyzed by the Wilcoxon signed rank test when compared within one group or the Mann–Whitney U test when data were compared between groups ($p < 0.05$ considered significantly different).

Results

Rolipram-induced reinforcement of early LTD

In a first control set of experiments, we have induced late LTD in an S1 synaptic input by the application of an SLFS, which resulted in robust late LTD of the field EPSP in S1 for at least 6 h (Fig. 1*B*, filled circles). A separate S2 synaptic control pathway (Fig. 1*B*, open circles) remained stable at baseline values. A statistically significant difference between S1 and S2 was observed up to 6 h after SLFS induction in S1. This late LTD was protein synthesis-dependent (Sajikumar and Frey, 2003, 2004). In contrast, induction of early LTD by WLFS in synaptic input S1 (Fig. 1*C*, filled circles) resulted in a transient early form of LTD with a duration of 2–3 h before returning to baseline values, at which the potentials remained stable for the time of recording. A control S2 input (Fig. 1*C*, open circles) remained stable at baseline levels for the entire experimental session (statistically significant difference between S1 and S2 for 2 h after WLFS to S1). This type of early LTD is protein synthesis-independent (data not shown). Application of rolipram (0.1 μM) 30 min before until 30 min after the induction of early LTD in input S1 converted the transient form of early LTD into a statistically significantly late form with a duration of up to 6 h (Fig. 1*D*, filled circles). Control responses from S2 remained stable at baseline levels (Fig. 1*D*, open circles). Analysis of the input–output relationship before the application of rolipram and WLFS and 6 h after LTD induction revealed no differences when compared with rolipram-untreated slices (data not shown). A comparable and similar EPSP–spike relationship was observed in the LTD-induced input. We then investigated whether the PDE inhibitor has to be present during the induction of early LTD to be effective in generating RLTD, or whether it is sufficient to apply rolipram at any time after WLFS. As shown in

Figure 1 *E*, filled circles, rolipram application 25 min after the induction of early LTD in S1 was unable to induce RLTD in that input. The control S2 input again remained stable throughout the recorded period of 6 h (Fig. 1 *E*, open circles).

Properties of rolipram-reinforced LTD
We investigated whether NMDA receptor activation is necessary for RLTD. After recording the baseline for 50 min, AP-5 (50 μM) was bath-applied for 10 min before being coapplied with rolipram for another 60 min (Fig. 1 *F*). When WLFS was applied to S1 (filled circles) in presence of AP-5 together with rolipram, the induction of early LTD as well as RLTD was prevented. Then we studied whether protein synthesis is required for the reinforcement of early into late LTD. Application of 25 μM anisomycin (Fig. 1 *G*) or 20 μM emetine (Fig. 1 *H*) in a similar manner as in Figure 1 *F* prevented any lasting form of LTD in S1 (filled circles). Potentials in the control input remained stable (Fig. 1 *G*,*H*, open circles). Thus, reinforcement of early LTD by rolipram was both NMDAR- and protein synthesis-dependent.

Rolipram-reinforced LTD and synaptic tagging
Subsequently, we studied whether the protein synthesis-dependent RLTD was characterized by processes of synaptic tagging because only those inputs that received WLFS, i.e., in which early LTD was induced, expressed RLTD. RLTD was therefore input-specific and dependent on protein synthesis. We have reported earlier that the inductions of conventional early LTD in two inputs, S1 and S2, do not interfere with each other. However, if instead of early LTD a late form was induced in one of the inputs within a distinct interval, then the weakly stimulated, normally transient early LTD input showed late LTD. This process was mediated by mechanisms of synaptic tagging (Sajikumar and Frey, 2004). For investigating the processes of synaptic tagging during RLTD, early LTD was induced in synaptic input S1 after 25 min of rolipram (0.1 μM) was applied (a point at which it was ineffective in affecting the first input) (Fig. 1 *E*) for 1 h. Fifty-five minutes after induction of early LTD in S1 (filled circles), early LTD was induced in input S2 (open circles), but now it was under the influence of rolipram. Paradoxically, in both inputs, a late form of LTD was observed (Fig. 2 *A*), which supports our hypothesis that synaptic tagging takes place during RLTD.

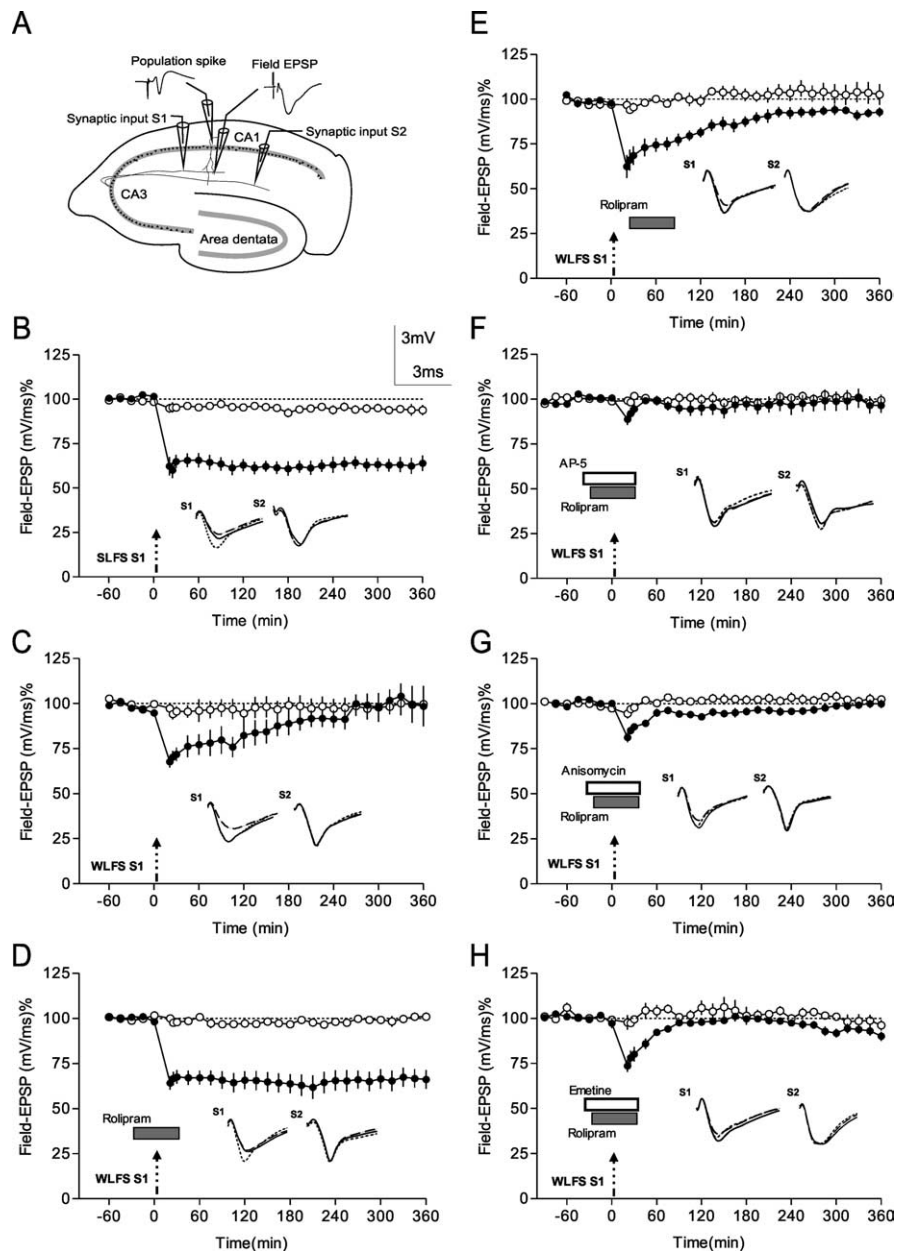In the next series of experiments, we

Figure 1. Properties of rolipram-reinforced early LTD. *A*, Transversal hippocampal slice showing the positioning of the electrodes. The two independent S1 and S2 inputs to the same neuronal population and the recording sites for the population spike amplitude and the field EPSP, as well as analog recording traces as representative examples of them, are shown. *B*, Induction of late LTD in S1 (filled circles) using a repeated LFS protocol (i.e., SLFS; dashed arrow) resulted in late LTD, which is significantly different for the 6 h investigated when compared with a control input S2 (*p* < 0.05, *U* test; *n* = 10). Control stimulation of S2 revealed relatively stable potentials for the time course investigated (open circles). The analog examples given in represent potentials 30 min before (dotted line), 30 min after (dashed line), and 6 h after the induction of the event (here after induction of SLFS in S1; solid line) in input S1 and S2, respectively. Calibration: 3 mV, 3 ms (valid for all single analog examples presented). *C*, A WLFS protocol (dashed arrow) was used, which elicited transient early LTD of the field EPSP with a duration of 185–210 min (185 min when compared with control input S2; *U* test; and 210 min when compared with its baseline before WLFS; Wilcoxon test; *p* < 0.05; *n* = 7). *D*, Transformation of early into late LTD by rolipram. Rolipram (0.1 μM) was applied 30 min before WLFS of S1 (filled circles), which normally would induce early LTD (washout of the drug, 30 min after WLFS; open circles; *n* = 8). *E*, Same experiment as in *D*, with the exception that rolipram was applied 25 min after LTD and elapsed for 1 h (*n* = 7). *F*, Influence of the NMDA receptor blocker AP-5 on RLTD. After recording a baseline for 50 min, AP-5 (50 μM) was applied for 10 min before being coapplied with rolipram for another 1 h. WLFS in the presence of AP-5 prevented the early LTD (filled circles); the control recording of S2 remained stable during the recorded period (open circles; *n* = 7). *G*, Same as in *F*, but instead of AP-5, the reversible protein synthesis inhibitor anisomycin (25 μM) was applied 10 min before coapplication with rolipram for an additional 1 h (*n* = 7). *H*, Effect of a structurally different protein synthesis inhibitor, emetine (20 μM), on RLTD. Analog traces always represent typical field EPSPs 30 min before (dotted line) and 30 min (dashed line) and 6 h (solid line) after WLFS of input S1 (*n* = 4). Dashed arrows indicate the time point of SLFS or WLFS of the corresponding synaptic input.
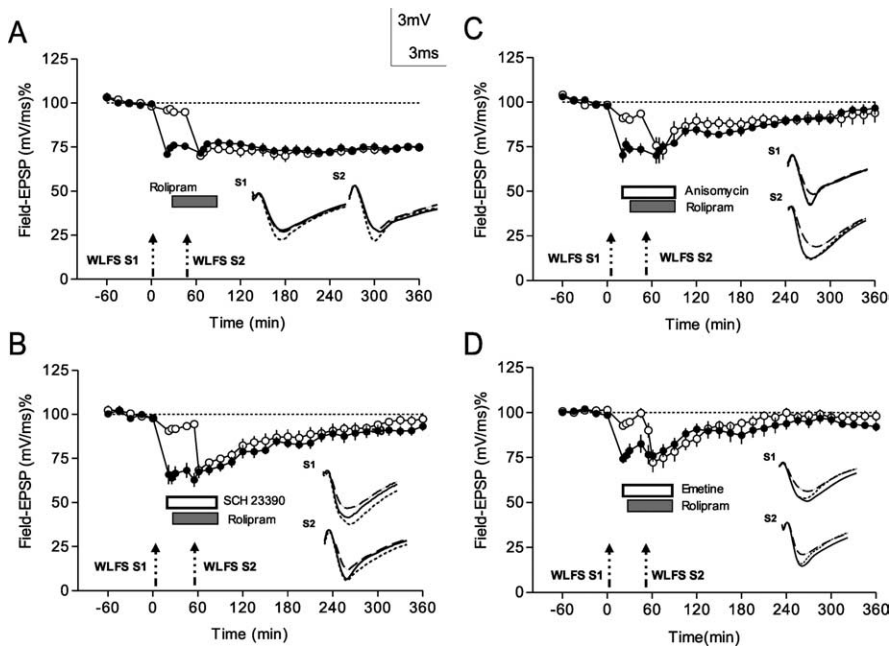
Figure 2. PDE inhibition by rolipram and synaptic tagging. *A*, Transformation of early into late LTD in S1 (filled circles) by subsequent induction of RLTD in S2 55 min later (open circles; $n = 7$). WLFS was induced in S1, and 25 min after WLFS of S1, rolipram was added to the bath medium for 1 h. *B*, Same experiment as in *A* with the exception of the additional application of D_1 and D_5 receptor antagonist SCH23390 (0.1 μM) 15 min after WLFS of S1 ($n = 9$). Here, synaptic tagging by RLTD is prevented. *C*, *D*, The experiment presented in *B*, was repeated using anisomycin (25 μM; $n = 8$) and emetine (20 μM; $n = 8$); protein synthesis inhibition prevents synaptic tagging in RLTD. Analog traces always represent typical field EPSPs 30 min before (dotted line) and 30 min (dashed line) and 6 h (solid line) after WLFS of input S1 or in cases in which WLFS was delivered to S2. The dashed arrows indicate the time point of the WLFS of the corresponding synaptic input.

investigated whether synaptic tagging in LTD required the heterosynaptic activation of glutamatergic and dopaminergic D_1 and D_5 receptors. The experiment described in Figure 2*A* was repeated, except that shortly before application of rolipram, the D_1 and D_5 receptor-specific antagonist SCH23390 (0.1 μM) was now applied (Fig. 2*B*). SCH23390 prevented synaptic tagging and the rolipram-induced transformation of early into late LTD in both inputs S1 and S2. Thus, the action of rolipram in the hippocampal CA1 of adult rats was dependent on D_1 and D_5 receptor activation. In a similar manner, coapplication of the protein synthesis inhibitors anisomycin and emetine with rolipram also prevented processes of synaptic tagging induced by rolipram and the transformation from early into late LTD (Fig. 2*C*,*D*).

Reinforcement of early LTD by rolipram through MAPK activation

Zhang et al. (2004) have found in behavioral experiments a functional link between MAPK/extracellular regulated kinase (ERK) and cAMP signaling pathways in the mediation of long-term memory, in which PDE4 is likely to be involved. Moreover, ERK activation is necessary to induce the persistent maintenance and expression of NMDA receptor-dependent LTD in area CA1 of the adult hippocampus *in vivo* (Thiels et al., 1996). Is MAPK activation also essential for RLTD? As shown in Figure 3, *C* and *D*, the MEK inhibitors U0126 (1 μM) and PD98059 (1 μM) effectively blocked RLTD, which suggests that MAPK mediates RLTD processes. In each set of experiments, a baseline was recorded for 50 min, and then one of the MEK inhibitors, either U0126 (1 μM) (Fig. 3*C*) or PD98059 (1 μM) (Fig. 3*D*) was applied for 10 min before coapplying with rolipram for another 60 min. The MEK inhibitors prevented RLTD without affecting early LTD in S1

when WLFS was applied 30 min after application of rolipram to S1 (Fig. 3*C*,*D*, filled circles). The control S2 inputs in the two sets of experiments remained stable throughout the recordings (Fig. 3*C*,*D*, open circles).

These experiments showed that MEK activation is essential for RLTD. The question then arose with regard to which signaling cascade is involved in the activation of MEK. First, we investigated the Ras-mediated cascade. Application of manumycin-A, a Ras inhibitor (2 μM), 10 min before coapplication with rolipram for 30 min before early LTD did not prevent the expression of RLTD, i.e., the transformation of early into late LTD in input S1 (Fig. 3*A*, filled circles), excluding the Ras signaling cascade as a candidate for the activation of MEK during RLTD (Zhu et al., 2002). The next series of experiments was conducted to examine whether the Rap-mediated pathway of activating MEK may take place in RLTD. LT-82 (200 ng/ml), an inhibitor of the Rap1/B-Raf pathway, was applied in a similar manner as described in Figure 3*A*. In contrast to manumycin-A, LT-82 blocked RLTD (Fig. 3*B*, filled circles). We can conclude that the prevention of RLTD was due to Rap1/B-Raf inhibition. In both cases, the control input S2 remained stable at baseline levels throughout the recordings (Fig. 3*A*,*B*, open circles).

Discussion

The type IV-specific PDE inhibitor rolipram can reinforce a normally transient early LTD into a long-lasting form of LTD with a duration of at least 6 h, which we named RLTD. It was previously shown that rolipram can also reinforce a transient early LTP into late LTP in mice or in rat hippocampal slices *in vitro* (Barad et al., 1998; Navakkode et al., 2004). Thus, our data now revealed similar effects of rolipram in both forms of plasticity in CA1 from adult rats (Fig. 1*D*). We also showed that this reinforcement of early LTD by rolipram was dependent on the activation of NMDARs and protein synthesis (Fig. 1*F–H*). Hippocampal LTD, similar to persistent hippocampal LTP, appears to require protein synthesis from local mRNA and for its prolonged maintenance and probably also *de novo* mRNA synthesis (Frey and Morris, 1998; Kauderer and Kandel, 2000; Sajikumar and Frey, 2003; Kelleher et al., 2004a; Blitzer et al., 2005; Smith et al., 2005; Sutton and Schuman, 2005). Therefore, a mechanism must exist that transduces the local translational process to a transcriptional one.

The MAPK/ERK cascade is thought to participate in such a signal transduction process during LTP (Giovannini et al., 2001). Our current results suggest that MAPK/ERK-regulated processes during LTD in adult rats are mediated by D_1 and D_5 receptor-mediated cAMP/PKA-dependent processes. Thus, the cAMP/PKA-mediated activation of p42/44MAPKs may be an important pathway in regulating local protein synthesis, as well as gene expression during functional plasticity and memory formation. However, the specific pathways by which cAMP/PKA are coupled to p42/44MAPK are not yet fully understood. In recent reports,

Costa et al. (2002) and Morozov et al. (2003) discussed distinct pools of p42/44MAPKs during different forms of LTP with their specific signaling pathways. One pathway for LTP maintenance involves the binding of the Raf family proteins (Raf-1, A-Raf, and B-Raf) by activated Ras (Marshall, 1995). The major function of Ras in activating Raf is to relocate Raf to the cell membrane, where it is activated by a yet unknown mechanism and then in turn activates MEKs. In addition the NMDAR/PKC-dependent Ras/Raf-1 pathway of MAPK/ERK activation is one of the most important pathways for maintaining hippocampal CA1 LTP (English and Sweatt, 1997). However, it remains unclear which pathway of MAPK/ERK activation is used for the prolonged, protein synthesis-dependent late LTD in CA1. In the dentate gyrus, the Ras pathway seems also to be important for LTD (Murray et al., 2004). However, in CA1, we showed that the Ras inhibitor manumycin-A had no effect on RLTD. In contrast, inhibitors of the Rap1 pathway, i.e., LT-82 and the MEK inhibitors (U0126 and PD98059), did block RLTD (Fig. 3B–D), indicating that MAPK activation is not mediated via Ras but Rap1 signaling. Rap1 exerts a dual regulation of p42/p44 MAPK by either inhibiting Raf1 or activating B-Raf. In addition, Rap1 can also antagonize Ras (Altschuler and Ribeiro-Neto,

Figure 3. MAPK pathway during RLTD. **A**, Effect of Ras inhibition by manumycin-A on RLTD (n = 7). After recording a baseline for 50 min, manumycin-A (2 μM) was applied for 10 min before being coapplied with rolipram for another 1 h. WLFS in S1 in the presence of manumycin-A had no effect on RLTD (filled circles); control potentials from S2 remained stable (open circles). **B**, Effect of LT-82 (n = 7) on RLTD. This is the same experiment as in Figure 2C but with the presence of a Rap inhibitor, LT-82 (200 ng/ml), which prevents the RLTD (filled circles). **C**, Influence of the MEK inhibitor U0126 on RLTD (n = 7). After recording a baseline for 50 min, U0126 (1 μM) was applied for 10 min before being coapplied with rolipram for another 1 h. WLFS in S1 in the presence of MEK inhibitor U0126 prevents RLTD (filled circles); control recording of S2 (open circles) remained stable during the recorded period. **D**, PD98059 (1 μM; n = 7) confirmed the results obtained in **C**. Analog traces always represent typical field EPSPs 30 min before (dotted line) and 30 min (dashed line) and 6 h (solid line) after WLFS of input S1 or in cases in which WLFS was delivered to S2. Dashed arrows indicate the time point of the WLFS of the corresponding synaptic input.

1998). Because Ras is anchored to the plasma membrane, whereas Rap is anchored to the membrane of endosomal compartments (Kim et al., 1990; Pizon et al., 1994; Resh, 1996), Rap1 and Ras may regulate distinct and different pools of MAPK (see above), resulting in different functions (supplemental material, available at www.jneurosci.org).

If RLTD is mechanistically similar to conventional late LTD, it should also be characterized by processes of synaptic tagging. If so, then for a protein synthesis-dependent stage, synapse-specific tags must be set in the activated synapse population that then can capture plasticity-related proteins (PRPs) whose synthesis took place nonspecifically in response to the activated synapses. We have shown that the induction of early LTD in one S1 synaptic input can be input-specifically reinforced by rolipram to a late LTD, which is dependent on D_1 and D_5 receptor activation as well as protein synthesis (Fig. 2A–D), supporting synaptic tagging during RLTD (Sajikumar and Frey, 2004).

Although we provide evidence that the D_1/D_5–cAMP/PKA–Rap1/B-Raf-mediated pathway of MAPK/ERK activation seems to be one of the effective pathways during RLTD, many open questions remain. For instance, although rolipram seems specifically to induce late LTD, we cannot fully exclude that other additional mechanisms may take place during conventional, electrically induced LTD, other than those described here for RLTD. The requirement of a coactivation of NMDAR and D_1 and D_5 receptors for RLTD also remains unclear. We suggest that through NMDAR interactions the stimulated input was tagged to interact in an input-specific manner with the PRPs, allowing the synapse population to express synapse-specific late LTD.
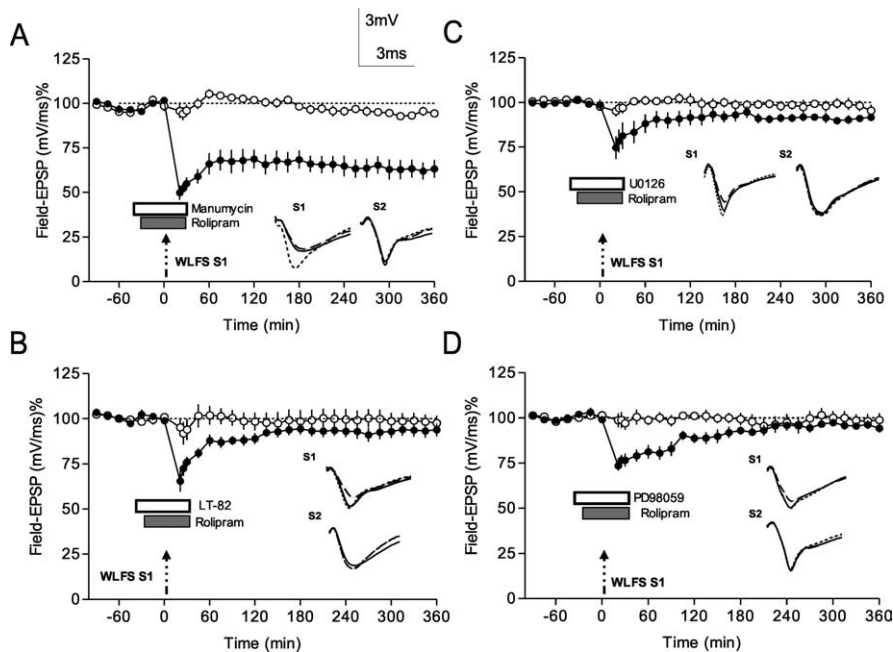
In addition, our results contribute to our understanding of how the synthesis of PRPs, i.e., the prerequisite for a cellular long-lasting memory trace, might be regulated via the activation of modulatory, nonglutamatergic inputs if this activation occurs within a distinct time window around the induction of normally transient plastic phenomena such as early LTP and early LTD at a glutamatergic input (Matthies et al., 1990; Frey and Morris, 1998; Frey, 2001; Sajikumar and Frey, 2004; Korz and Frey, 2005). The nature of the PRPs is only beginning to be identified. We had suggested that the synthesis of both process-specific and -nonspecific PRPs occurs during the induction of either late LTP or late LTD (Kelleher et al., 2004a; Sajikumar and Frey, 2004; Sajikumar et al., 2005). Recently, we identified PKMζ as the first LTP-specific PRP (Sajikumar et al., 2005). Furthermore, we have suggested that PDE4B3 could represent a process-nonspecific PRP that regulates the synthesis of secondary PRPs maintaining either LTP or LTD (Ahmed and Frey, 2003, 2005; Navakkode et al., 2004; Sajikumar and Frey, 2004; Sajikumar et al., 2005). In addition, such regulatory PRPs might be involved in gene expression, resulting in a local pool of mRNA whose products are specific for either LTP or LTD and which guarantee a prolonged maintenance of LTP or LTD beyond 8 h (Kelleher et al., 2002a,b). Our data here provide more evidence for a specific role of PDEs in the regulation of processes required for the prolonged maintenance of hippocampal CA1 LTD.

Our results further support the requirement of heterosynaptic activation of processes that are critical for the expression of a long-lasting memory trace. We have shown for hippocampal CA1 RLTD of adult rats a specific role of D_1 and D_5 receptor

activation, which is in line with earlier findings from our laboratory demonstrating an important role of dopaminergic coactivation for the modulation of transient into lasting forms of LTP and LTD in rat hippocampal CA1 neurons from adult rats (Frey et al., 1990, 1991b, 1993; Matthies et al., 1990; Frey and Morris 1998; Sajikumar and Frey, 2004). Because these results were confirmed by a number of other laboratories (Chen et al., 1995; Huang and Kandel, 1995; O'Carroll and Morris, 2004), we hope that the focus of research in synaptic plasticity will, in addition to glutamatergic mechanisms, also include the modulatory processes required for the consolidation of a memory trace at the cellular and network levels in the adult brain.

References

Abel T, Nguyen PV, Barad M, Deuel TAS, Kandel ER (1997) Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell 88:615–626.

Ahmed T, Frey JU (2003) Expression of the specific type IV phosphodiesterase gene PDE4B3 during different phases of long-term potentiation in single hippocampal slices of rats in vitro. Neuroscience 117:627–638.

Ahmed T, Frey JU (2005) Phosphodiesterase 4B (PDE4B) and cAMP-level regulation within different tissue fractions of rat hippocampal slices during long-term potentiation in vitro. Brain Res 1041:212–222.

Altschuler DL, Ribeiro-Neto F (1998) Mitogenic and oncogenic properties of the small G protein Rap1b. Proc Natl Acad Sci USA 95:7475–7479.

Barad M, Bourtchouladze R, Winder DG, Golan H, Kandel E (1998) Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. Proc Natl Acad Sci USA 95:15020–15025.

Bear MF, Malenka RC (1994) Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 4:389–399.

Beavo JA (1988) Multiple isozymes of cyclic nucleotide phosphodiesterase. Adv Second Messenger Phosphoprotein Res 22:1–38.

Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol (Lond) 232:331–356.

Blitzer RD, Iyengar R, Landau EM (2005) Postsynaptic signaling networks: cellular cogwheels underlying long-term plasticity. Biol Psychiatry 57:113–119.

Chen Z, Fujii S, Ito K, Kato H, Kaneko K, Miyakawa H (1995) Activation of dopamine D1 receptors enhances long-term depression of synaptic transmission induced by low frequency stimulation in rat hippocampal CA1 neurons. Neurosci Lett 188:195–198.

Chetkovich DM, Sweatt JD (1993) NMDA receptor activation increases cyclic AMP in area CA1 of the hippocampus via calcium/calmodulin stimulation of adenylyl cyclase. J Neurochem 61:1933–1942.

Costa RM, Federov NB, Kogan JH, Murphy GG, Stern J, Ohno M, Kucherlapati R, Jacks T, Silva AJ (2002) Mechanism for the learning deficits in a mouse model of neurofibromatosis type 1. Nature 415:526–530.

Davies SP, Reddy H, Caivano M, Cohen P (2000) Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J 351:95–105.

Davis S, Vanhoutte P, Pagés C, Caboche J, Laroche S (2000) The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. J Neurosci 20:4563–4572.

Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci USA 89:4363–4367.

Dudek SM, Fields RD (2001) Mitogen-activated protein kinase/extracellular signal-regulated kinase activation in somatodendritic compartments: roles of action potentials, frequency, and mode of calcium entry. J Neurosci 21:RC122(1–5).

Dym O, Xenarios I, Ke H, Colicelli J (2002) Molecular docking of competitive phosphodiesterase inhibitors. Mol Pharmacol 61:20–25.

English JD, Sweatt JD (1997) A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. J Biol Chem 272:19103–19106.

Fischer QS, Beaver CJ, Yang Y, Rao Y, Jakobsdottir KB, Storm DR, McKnight GS, Daw NW (2004) Requirement for the RIIβ isoform of PKA, but not

calcium-stimulated adenylyl cyclase, in visual cortical plasticity. J Neurosci 24:9049–9058.

Frey JU (2001) Long-lasting hippocampal plasticity: cellular model for memory consolidation? In: Cell polarity and subcellular RNA localization (Richter D, ed), pp 27–40. Berlin: Springer.

Frey S, Schweigert C, Krug M, Lossner B (1991a) Long-term potentiation induced changes in protein synthesis of hippocampal subfields of freely moving rats: time-course. Biomed Biochim Acta 50:1231–1240.

Frey U, Morris RG (1997) Synaptic tagging and long-term potentiation. Nature 385:533–536.

Frey U, Morris RG (1998) Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. Trends Neurosci 21:181–188.

Frey U, Krug M, Reymann KG, Matthies H (1988) Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. Brain Res 452:57–65.

Frey U, Schroeder H, Matthies H (1990) Dopaminergic antagonists prevent long-term maintenance of posttetanic LTP in the CA1 region of rat hippocampal slices. Brain Res 522:69–75.

Frey U, Matthies H, Reymann KG (1991b) The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region in vitro. Neurosci Lett 129:111–114.

Frey U, Huang YY, Kandel ER (1993) Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. Science 260:1661–1664.

Giovannini MG, Blitzer RD, Wong T, Asoma K, Tsokas P, Morrison JH, Iyengar R, Landau EM (2001) Mitogen-activated protein kinase regulates early phosphorylation and delayed expression of Ca²⁺/calmodulin-dependent protein kinase II in long-term potentiation. J Neurosci 21:7053–7062.

Huang YY, Kandel ER (1995) D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. Proc Natl Acad Sci USA 92:2446–2450.

Kauderer BS, Kandel ER (2000) Capture of a protein synthesis-dependent component of long-term depression. Proc Natl Acad Sci USA 97:13342–13347.

Kelleher III RJ, Govindarajan A, Jung HY, Kang H, Tonegawa S (2004a) Translational control by MAPK signaling in long-term synaptic plasticity and memory. Cell 116:467–479.

Kelleher III RJ, Govindarajan A, Tonegawa S (2004b) Translational regulatory mechanisms in persistent forms of synaptic plasticity. Neuron 44:59–73.

Kim S, Mizoguchi A, Kikuchi A, Takai Y (1990) Tissue and subcellular distributions of the smg-21/rap1/Krev-1 proteins which are partly distinct from those of c-ras p21s. Mol Cell Biol 10:2645–2652.

Korz V, Frey JU (2005) Emotional and cognitive reinforcement of rat hippocampal long-term potentiation by different learning paradigms. Neuron Glia Biol 1:253–261.

Linden DJ, Connor JA (1995) Long-term synaptic depression. Annu Rev Neurosci 18:319–357.

Manahan-Vaughan D (2000) Group III metabotropic glutamate receptors modulate long-term depression in the hippocampal CA1 region of two rat strains in vivo. Neuropharmacology 39:1952–1958.

Marshall M (1995) Interactions between Ras and Raf: key regulatory proteins in cellular transformation. Mol Reprod Dev 42:493–499.

Matsuo R, Murayama A, Saitoh Y, Sakaki Y, Inokuchi K (2000) Identification and cataloging of genes induced by long-lasting long-term potentiation in awake rats. J Neurochem 74:2239–2249.

Matthies H, Reymann KG (1993) Protein kinase A inhibitors prevent the maintenance of hippocampal long-term potentiation. NeuroReport 4:712–714.

Matthies H, Frey U, Reymann K, Krug M, Jork R, Schroeder H (1990) Different mechanisms and multiple stages of LTP. Adv Exp Med Biol 268:359–368.

Morozov A, Muzzio IA, Bourtchouladze R, Van Strien N, Lapidus K, Yin D, Winder DG, Adams JP, Sweatt JD, Kandel ER (2003) Rap1 couples cAMP signaling to a distinct pool of p42/44MAPK regulating excitability, synaptic plasticity, learning, and memory. Neuron 39:309–325.

Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

Murray HJ, O'Connor JJ (2004) A role for monomeric G-proteins in synaptic plasticity in the rat dentate gyrus in vitro. Brain Res 1000:85–91.

Navakkode S, Sajikumar S, Frey JU (2004) The type IV-specific phosphodiesterase inhibitor rolipram and its effect on hippocampal long-term potentiation and synaptic tagging. J Neurosci 24:7740–7744.

Nguyen PV, Woo NH (2003) Regulation of hippocampal synaptic plasticity by cyclic AMP-dependent protein kinases. Prog Neurobiol 71:401–437.

O'Carroll CM, Morris RG (2004) Heterosynaptic co-activation of glutamatergic and dopaminergic afferents is required to induce persistent long-term potentiation. Neuropharmacology 47:324–332.

Otani S, Auclair N, Desce JM, Roisin MP, Crepel F (1999) Dopamine receptors and groups I and II mGluRs cooperate for long-term depression induction in rat prefrontal cortex through converging postsynaptic activation of MAP kinases. J Neurosci 19:9788–9802.

Pizon V, Desjardins M, Bucci C, Parton RG, Zerial M (1994) Association of Rap1a and Rap1b proteins with late endocytic/phagocytic compartments and Rap2a with the Golgi complex. J Cell Sci 107:1661–1670.

Resh MD (1996) Regulation of cellular signalling by fatty acid acylation and prenylation of signal transduction proteins. Cell Signal 8:403–412.

Roberson ED, English JD, Adams JP, Selcher JC, Kondratick C, Sweatt JD (1999) The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. J Neurosci 19:4337–4348.

Rosenblum K, Futter M, Jones M, Hulme EC, Bliss TVP (2000) ERKI/II regulation by the muscarinic acetylcholine receptors in neurons. J Neurosci 20:977–985.

Rosenblum K, Futter M, Voss K, Erent M, Skehel PA, French P, Obosi L, Jones MW, Bliss TV (2002) The role of extracellular regulated kinases I/II in late-phase long-term potentiation. J Neurosci 22:5432–5441.

Sajikumar S, Frey JU (2003) Anisomycin inhibits the late maintenance of long-term depression in rat hippocampal slices in vitro. Neurosci Lett 338:147–150.

Sajikumar S, Frey JU (2004) Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. Neurobiol Learn Mem 82:12–25.

Sajikumar S, Navakkode S, Sacktor T, Frey JU (2005) Synaptic tagging and cross-tagging: the role of protein kinase Mζ in maintaining long-term potentiation, but not long-term depression. J Neurosci 25:5750–5756.

Smith WB, Starck SR, Roberts RW, Schuman EM (2005) Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. Neuron 45:765–779.

Sutton MA, Schuman EM (2005) Local translational control in dendrites and its role in long-term synaptic plasticity. J Neurobiol 64:116–131.

Sweatt JD (2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. J Neurochem 76:1–10.

Thiels E, Xie X, Yeckel MF, Barrionuevo G, Berger TW (1996) NMDA receptor-dependent LTD in different subfields of hippocampus in vivo and in vitro. Hippocampus 6:43–51.

Waltereit R, Weller M (2003) Signaling from cAMP/PKA to MAPK and synaptic plasticity. Mol Neurobiol 27:99–106.

Watabe AM, Zaki PA, O'Dell TJ (2000) Coactivation of β-adrenergic and cholinergic receptors enhances the induction of long-term potentiation and synergistically activates mitogen-activated protein kinase in the hippocampal CA1 region. J Neurosci 20:5924–5931.

Zhang HT, Zhao Y, Huang Y, Dorairaj NR, Chandler LJ, O'Donnell JM (2004) Inhibition of the phosphodiesterase 4 (PDE4) enzyme reverses memory deficits produced by infusion of the MEK inhibitor U0126 into the CA1 subregion of the rat hippocampus. Neuropsychopharmacology 29:1432–1439.

Zhu JJ, Qin Y, Zhao M, Van Aelst L, Malinow R (2002) Ras and Rap control AMPA receptor trafficking during synaptic plasticity. Cell 110:443–455.