Behavioral/Systems/Cognitive

A Novel Role for Extracellular Signal-Regulated Kinase in Maintaining Long-Term Memory-Relevant Excitability Changes

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Pyramidal neurons in the piriform cortex from olfactory-discrimination-trained rats show enhanced intrinsic neuronal excitability that lasts for several days after learning. Such enhanced intrinsic excitability is mediated by long-term reduction in the postburst afterhyperpolarization (AHP), which is generated by repetitive spike firing. AHP reduction is attributable to decreased conductance of a calcium-dependent potassium current, the sI_{AHP}. We have previously shown that such learning-induced AHP reduction is maintained by PKC activation. However, the molecular machinery underlying such long-lasting modulation of intrinsic excitability is yet to be fully described. Here we examine whether the extracellular signal-regulated kinase I/II (ERKI/II) pathway, which is known to be crucial in learning, memory, and synaptic plasticity processes, is instrumental for the long-term maintenance of learning-induced AHP reduction. PD98059 or UO126, which selectively block MEK, the upstream kinase of ERK, increased the AHP in neurons from trained rats but not in neurons from naive and pseudo-trained rats. Consequently, the differences in AHP amplitude and neuronal adaptation between neurons from trained rats and controls were abolished. This effect was not mediated by modulation of basic membrane properties. In accordance with its effect on neuronal excitability, the level of activated ERK in the membranal fraction was significantly higher in piriform cortex samples taken from trained rats. In addition, the PKC activator OAG (1-oleoyl-20acety-*sn*-glycerol), which was shown to reduce the AHP in neurons from control rats, had no effect on these neurons in the presence of PD98059. Our data show that ERK has a key role in maintaining long-lasting learning-induced enhancement of neuronal excitability.

Key words: olfactory learning; piriform cortex; neuronal excitability; pyramidal neurons; postburst AHP; ERK

Introduction

Learning-induced enhancement in neuronal excitability was shown after classical conditioning (Moyer et al., 1996; Thompson et al., 1996) and water-maze training (Oh et al., 2003) in hippocampal neurons and after olfactory-discrimination (OD) learning in piriform cortex neurons (for review, see Saar and Barkai, 2003). This enhanced excitability is manifested by reduced spike-frequency adaptation (Moyer et al., 1996; Saar et al., 2001) and lasts for several days after training completion (Saar and Barkai, 2003).

Neuronal adaptation is modulated by the postburst afterhyperpolarization (AHP), generated by potassium currents that develop after spike firing (Madison and Nicoll, 1984; Constanti and Sim, 1987; Schwindt et al., 1988; Saar et al., 2001). The postburst AHP is reduced after learning (Moyer et al., 1996; Saar et al., 1998). Indeed, several studies indicate that the learning-induced reduction in neuronal adaptation and in AHP amplitude results from reduction in a Ca^{2+}-dependent potassium current

(Sanchez-Andres and Alkon, 1991; Saar et al., 2001; Power et al., 2002). It was recently shown that only the apamin-insensitive portion of the postburst AHP is reduced after OD learning (Brosh et al., 2006), suggesting that learning modulates specifically the sI_{AHP}. Changes in the sI_{AHP} were also implicated in learning-related modifications in hippocampal neurons after spatial learning (Oh et al., 2003).

The identity of the channel that mediates the sI_{AHP} is yet unknown (Sah and Faber, 2002). This current is reduced by PKC-dependent activation of glutamate-mediated kainite receptors (Melyan et al., 2002, 2004). Accordingly, OD learning-induced postburst AHP reduction is mediated by persistent PKC activation (Seroussi et al., 2002). The mechanism by which such long-lasting PKC-dependent AHP reduction persists is yet to be described. Recently, it was shown that kainate receptor-mediated long-lasting AHP reduction in hippocampal neurons is dependent on MEK [mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase] activation (Grabauskas et al., 2007). Thus, one possibility is that PKC maintains its activity via another signal transduction pathway such as the MAP kinase.

A wide range of studies testify to the importance for ERK in memory formation across many species and brain areas (for review, see Adams and Sweatt, 2002). In a time frame of minutes and up to a few hours, ERK activation increases transiently after acquisition of fear conditioning (Atkins et al., 1998; Schafe et al.,

2000), taste learning (Berman et al., 1998; Belelovsky et al., 2005), classical conditioning (Crow et al., 1998), and water-maze training (Blum et al., 1999).

ERK is crucially involved in both short- and long-term modulation of synaptic transmission (English and Sweatt, 1996, 1997; Atkins et al., 1998; Rosenblum et al., 2000, 2002; Thomas and Huganir, 2004). Such short-term ERK-dependent modifications may be attributable to enhancement of intrinsic neuronal excitability by modulating the I_A potassium current (Sheng et al., 1992; Yuan et al., 2002).

The purpose of the present study was to examine the involvement of ERK in maintenance of long-lasting olfactory learning-induced enhancement of neuronal excitability and to test whether such long-term modulation is related to the effect of PKC on the postburst AHP.

Materials and Methods

Animal training
Subjects and apparatus. Age-matched young adult Sprague Dawley male rats were used. Before training, they were maintained on a 23.5 h water-deprivation schedule, with food available *ad libitum*. The OD training protocol was performed daily on each trained and pseudo-trained rat in a four-arm radial maze, as described previously (Saar et al., 1999, 2001), with commercial odors that are regularly used in the cosmetics and food industries.

Training. Olfactory training consisted of 20 trials per day for each rat as described previously (Saar et al., 2001). In short, in each trial the rat had to choose between two odors (positive and negative cues) presented simultaneously. Rats designated to the trained group were rewarded after choosing the positive cue. Rats in the pseudo-trained group were rewarded in a random manner, after choosing any odor. The criterion for learning was at least 80% positive-cue choices in the last 10 trials of a training day, as was used previously (Staubli et al., 1987; Saar et al., 1999, 2001). Rats in the naive group were water restricted but not exposed to the maze. Typically, two to three trained rats and two to three pseudo-trained rats were trained at the same training period, and all the rats in the trained group had to meet the criteria for the first pair of odors before all trained and pseudo-trained rats were exposed to a second pair of odors. Training for a new pair began only after training for the second pair was completed for all rats. Rats were trained to discriminate between three pairs of odors, to confirm that rule learning was achieved and to ensure that rats are always killed 3 d after the last training session. As described previously (Saar et al., 1999, 2001), rats indeed learned the second and third pairs of odors much faster than the first pair (7–8 d of training for the first pair and 1–2 d for the second and third pairs). A detailed description of OD learning efficacy was described previously (Zelcer et al., 2006).

Slice preparation, stimulation, and recording
Coronal brain slices (400 μm) were cut as described previously (Saar et al., 1998) and kept in oxygenated (95% O_2 plus 5% CO_2) normal saline Ringer's (NSR) solution (in mM: 124 NaCl, 3 KCl, 2 MgSO$_4$, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, 2 CaCl$_2$, and 10 glucose). Intracellular recordings were obtained from pyramidal cells in layer II of the piriform cortex, with 4 M K-acetate-filled sharp glass microelectrodes, at 35°C. Several piriform cortex slices were obtained from each rat.

AHPs were recorded within minutes after good recording conditions were established (resting potential of at least −65 mV and action potential amplitude of ≥80 mV). To standardize AHP recordings, neurons were depolarized to a holding potential of −60 mV by direct current application via the recording electrode. Postburst AHP amplitude was then measured after a 100 ms depolarizing current step with intensity that generates six action potentials. The AHP amplitude was determined from an average of eight consecutive measurements of AHP evoked in responses to stimuli applied once every 10 s.

The postburst AHP amplitude was measured from baseline to the peak of the hyperpolarizing voltage deflection that follows an evoked train of six action potentials (see Fig. 1). The stimulus intensity required to evoke six action potentials always remained constant throughout the recording

period of each neuron, before and after drug application. Such stability of the required stimulus intensity well coincides with the stability of basic membrane properties, such as the input resistance or resting potential of the neurons, which are not affected by the drugs [see Results and also Seroussi et al. (2002)].

The identity of the rat from which neurons were recorded (naive, trained, or pseudo-trained) was not known to the person conducting the experiments and measurements.

All drugs were applied into the perfusing Ringer's solution, at the following concentrations: MEK inhibitor PD98059, 38 μM; MEK inhibitor UO126, 30 μM; 1-oleoyl-20acety-*sn*-glycerol (OAG), 10 μM. Slices were exposed to drugs for at least 20 min before the recordings. In NSR solution and PD98059, several cells were recorded before and after drug application, whereas others were recorded under one condition only. All cells in OAG and UO126 were recorded before and after drug application.

Sample preparation
Preparation of membranal fraction. The protocol was adapted from Schilstrom et al. (2006). Piriform cortices from both hemispheres of each rat were homogenized in a Teflon/glass 1 ml tissue grinder and with 800 μl of homogenization buffer [10 mM Tris-HCl, pH 7.4, 2 mM EDTA, 2 mM EGTA, 0.5 mM DTT, 100 mM Na$_3$VO$_4$, 5 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 0.32 M sucrose, 100 mM PMSF, 1% phosphatase inhibitor mixture (Sigma, St. Louis, MO), and 1% protease inhibitor mixture (Sigma)]. Samples were sonicated and kept on ice for 30 min. An aliquot of the homogenized tissue (80 μl) was retained and mixed with 80 μl of SDS sample buffer (total fraction). The remaining material was centrifuged at 1000 × g for 3 min. The supernatant was centrifuged at 20,000 × g for 1 h. The pellet, containing the crude synatposomal membranes (membranal fraction), was resuspended in lysis buffer [10 mM Tris-HCl, pH 7.4, 2 mM EDTA, 2 mM EGTA, 0.5 mM DTT, 100 mM Na$_3$VO$_4$, 5 mM NaF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1% Triton, 1% phosphatase inhibitor mixture (Sigma), and 1% protease inhibitor mixture (Sigma)], whereas the cytosol and light membranes remained in the supernatant (cytosol fraction). SDS sample buffer was added to both the pellet and supernatant fractions. Samples were stored at −80°C until further use.

Western blot analysis
All samples were resolved by SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA) for immunoblotting. Blots were visualized with enhanced chemiluminescence (ECL Plus; Amersham Biosciences, Piscataway, NJ) and quantified using a CCD camera (XRS; Bio-Rad) and Quantity-One software. The ratio between phosphorylated ERK (pERK) and the total amount of the protein for each rat was calculated and normalized to the naive group average run on the same gel.

The following reagents were used: p44/42 MAP kinase antibody (rabbit polyclonal, 1:1000; Cell Signaling Technology, Beverly, MA), phospho-p44/42 MAP kinase (Thr202/Tyr204) antibody (1:500; Cell Signaling Technology), and goat anti-rabbit IgG (1:20,000; Jackson ImmunoResearch, West Grove, PA).

Statistical analysis
For all the electrophysiological measurements, between-groups comparison was done using one-way ANOVA for the three groups (naive, trained, and pseudo-trained), and *post hoc* multiple *t* tests were than applied to compare between each two groups. The effect of PD98059, UO126, and OAG on each of the three groups was evaluated by comparing the recordings from the same group, with and without the treatment, using independent *t* tests. Values throughout the text are presented as mean ± SD. Data in graphs are presented as mean ± SE.

Results
Recordings in neurons from trained and pseudo-trained rats were performed 3 d after the last training session, when learning-related reduction in AHP amplitude is most prominent (Saar and Barkai, 2003). In control saline Ringer's solution (NSR solution), the averaged postburst AHP in neurons from trained rats was significantly smaller compared with the averaged AHP amplitudes in neurons

from pseudo-trained and naive rats (Fig. 1C) (see also details below), confirming our previous reports (Saar et al., 1998, 2001; Brosh et al., 2006). As reported previously (Saar et al., 1998, 2001), resting membrane potential (naive: -79.3 ± 7.3 mV, $n = 34$; trained: -79.2 ± 7.0 mV, $n = 49$; pseudo-trained: -82.0 ± 7.4 mV, $n = 34$) and input resistance (naive: 33.4 ± 12.7 MΩ, $n = 24$; trained: 30.0 ± 8.4 MΩ, $n = 30$; pseudo-trained: 35.8 ± 10.9 MΩ, $n = 18$) did not differ between neurons from the three groups.

Learning-induced long-lasting AHP reduction is abolished by ERK inhibition

To examine whether the long-lasting reduction in postburst AHP amplitude is maintained by ERK activation, we applied the MEK inhibitors PD98059 (38 μM) and UO126 (30 μM). PD98059 significantly increased the AHP amplitude in neurons from trained rats only. In neurons from naive and pseudo-trained rats, the averaged AHP value was not affected by the inhibitor. In neurons from trained rats, the averaged AHP value was significantly increased from 5.57 ± 1.80 ($n = 48$) in NSR solution to 6.89 ± 2.51 ($n = 12$) in PD98059 ($p < 0.05$). For naive rats, the averaged values were 7.04 ± 1.89 ($n = 21$) in NSR solution and 6.34 ± 1.20 ($n = 11$) in PD98059. For pseudo-trained rats, the averaged values were 7.94 ± 1.72 ($n = 26$) in NSR solution and 7.18 ± 1.89 ($n = 13$) in PD98059. Two typical examples for the different effect of PD98059 on neurons from a pseudo-trained and a trained rat are shown in Figure 1, A and B. After PD98059 application, the difference in AHP amplitude between neurons from trained rats and controls was abolished (Fig. 1C). Similar results were obtained with the second specific MEK inhibitor, UO126. In neurons from trained rats, the averaged AHP value was significantly increased to 7.56 ± 1.38 ($n = 7$; $p < 0.05$). In neurons from naive rats, the averaged value in UO126 was to 7.81 ± 1.11 ($n = 5$), and in neurons from pseudo-trained rats, the averaged value in UO126 was 7.15 ± 1.48 ($n = 7$). These values were not different from the averaged values recorded in NSR solution (Fig. 1C).

MEK inhibitors reduce neuronal excitability in neurons from trained rats only

A direct consequence of the learning-induced AHP reduction is enhanced neuronal excitability. Such enhancement is manifested by increased frequency of repetitive firing in response to a prolonged depolarizing pulse applied via the recording electrode (Saar et al., 2001; Saar and Barkai, 2003). To examine whether ERK activation underlies the maintenance of such learning-induced neuronal excitability, we compared the effect of its in-
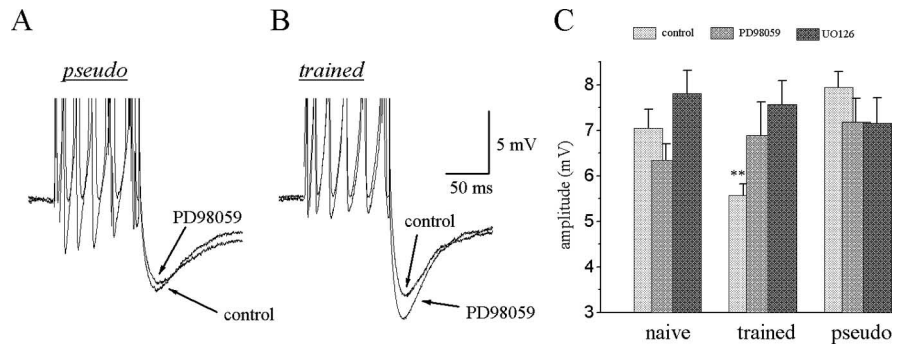

Figure 1. MEK inhibitors increase the AHP in neurons from trained rats only. AHP measurements in piriform cortex neurons are shown. Neurons were held at a membrane potential of -60 mV, and the postburst AHP was generated by a 100 ms depolarizing current step injection via the recording electrode, with intensity sufficient to generate a train of six action potentials. **A, B,** Although PD98059 application had no effect on a neuron from a pseudo-trained rat (**A**), it enhanced the AHP in a neuron from a trained rat (**B**). **C,** Averaged AHP amplitude in neurons from the three groups recorded in NSR solution, under PD98059, and under UO126. Averaged AHP amplitude in trained rats is significantly smaller compared with naive and pseudo-trained rats (******$p < 0.01$). This difference is abolished after PD98059 or UO126 application. AHP in NSR solution was measured in neurons from 9 naive rats, 15 trained rats, and 10 pseudo-trained rats. AHP in PD98059 was measured in neurons from five naive rats, eight trained rats, and six pseudo-trained rats. AHP in PD98059 was measured in neurons from four naive rats, five trained rats, and five pseudo-trained rats. Values represent mean $\pm$ SE.
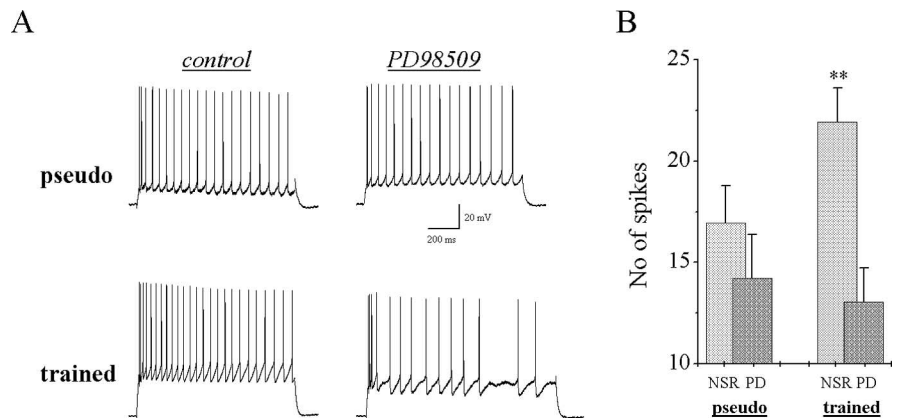

Figure 2. PD98059 reduced neuronal excitability in neurons from trained rats. **A,** Examples for the effect of PD98059 on repetitive spike firing in a neuron from a pseudo-trained rat and a neuron from a trained rat. With a stimulus intensity of $I_{th} \times 2$, a 1 s depolarizing pulse generates repetitive firing throughout the stimulation period. Although PD98059 has no effect on the pseudo-trained neuron, repetitive firing of the trained neuron is significantly suppressed by the same agent. **B,** Whereas PD98059 (PD) application has no effect on the number of spikes evoked in neurons from pseudo-trained rats, it significantly reduces the averaged number of spikes in neurons from trained rats (******$p < 0.001$). Consequently, the difference in spike numbers between neurons from the pseudo-trained and trained groups disappears in the presence of the ERK inhibitor. The number of spikes in NSR solution was measured in neurons from 8 pseudo-trained and 11 trained rats. The number of spikes in PD98059 was measured in neurons from five pseudo-trained and seven trained rats. Stimulus intensity was kept constant in each cell throughout the recording period. Values represent mean $\pm$ SE.

hibitor on the number of spikes generated by a 1 s depolarizing pulse, with a stimulus intensity of $I_{th} \times 2$.

As described previously (Saar et al., 2001), the averaged number of action potentials evoked in neurons from trained rats in NSR solution (21.9 ± 9.1; $n = 30$) was significantly ($p < 0.05$) higher compared with neurons from pseudo-trained rats (16.9 ± 7.7; $n = 17$). As shown in Figure 2, application of PD98059 significantly reduced ($p < 0.01$) the averaged number of spikes in neurons from trained rats (13.1 ± 7.0; $n = 18$) but had no effect on the averaged value in neurons from pseudo-trained rats (14.2 ± 7.7; $n = 13$). Similar results were obtained with UO126. In neurons from trained rats, the averaged number of spikes reduced to 17.0 ($n = 6$). In neurons from naive rats, the averaged value in UO126 was 17.4 ($n = 5$), and in neurons from pseudo-
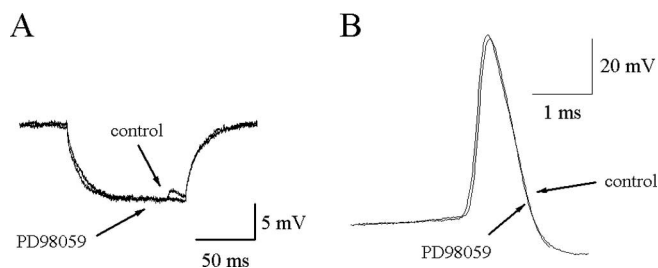
Figure 3. PD98059 does not affect basic membrane properties. **A**, An example of the lack of effect of PD98059 on resting membrane potential and input resistance in a neuron from a pseudo-trained rat. The resting potential was −80 mV; input resistance is measured by the voltage deflection in response to a 100 pulse of −0.3 nA, applied via the recording electrode. **B**, An example of lack of effect of PD98059 on the duration of the action potential in a neuron from a trained rat.

trained rats, the averaged value was 18.2 ($n = 8$). Thus, the difference in repetitive spike firing was also abolished by UO126.

The effects of MEK inhibition on neurons from trained rats are not the result of changes in basic neuronal properties

Such learning-induced modification of ERK inhibition on the postburst AHP may be the result of a direct effect on the currents underlying the postburst AHP, by modulating the properties of single action potentials via inhibition of the I_A potassium current (Yuan et al., 2002), or by a nonspecific effect on basic membrane properties. For example, a learning-induced increase in the averaged input resistance or the averaged width of single action potentials of neurons would result in enhanced postburst AHP without modifying its underlying currents directly. Our data show that such a nonspecific effect was not induced by PD98059 application.

Resting membrane potential in neurons from the three groups (naive: −79.5 ± 6.1 mV, $n = 19$; trained: −80.1 ± 7.5 mV, $n = 14$; pseudo-trained: −81.3 ± 6.5 mV, $n = 15$) and input resistance (naive: 36.7 ± 12.6 MΩ, $n = 16$; trained: 30.7 ± 11.9 MΩ, $n = 13$; pseudo-trained: 35.5 ± 10.6 MΩ, $n = 13$) were not affected by PD98059 application.

As reported previously (Saar et al., 1998; Brosh et al., 2006), the averaged action potential duration in NSR solution was similar in all groups (naive: 1.44 ± 0.24 ms, $n = 34$; trained: 1.51 ± 0.30, $n = 49$; pseudo-trained: 1.44 ± 0.20, $n = 34$). These values were not modified by PD98059 application (naive: 1.59 ± 0.37 ms, $n = 19$; trained: 1.54 ± 0.29, $n = 14$; pseudo-trained: 1.35 ± 0.24, $n = 15$) (Fig. 3).

OD learning induces long-term membranal ERK activation

Because learning-induced enhancement of neuronal excitability and the postburst AHP reduction are dependent of persistent ERK activation, we hypothesized that learning should be accompanied by an increased ERK phosphorylation. Using Western blot analysis, we measured the total amount of ERK and its phosphorylation state in the three experimental groups. We found that although the basal ERK protein level in the membranal fraction was not modified, the level of pERK significantly increased after learning (Fig. 4). Such increase in ERK phosphorylation was specific to the membranal fraction; no differences were observed between groups for the total homogenate fraction (total amount of ERK: naive, 1.00 ± 0.06; pseudo-trained, 1.02 ± 0.07; trained, 1.01 ± 0.07; pERK/ERK ratio: naive, 1.03 ± 0.09; pseudo-trained, 0.91 ± 0.1; trained, 1.04 ± 0.09) nor for the cytosolic fraction (total amount of ERK: naive, 1.00 ± 0.05; pseudo-
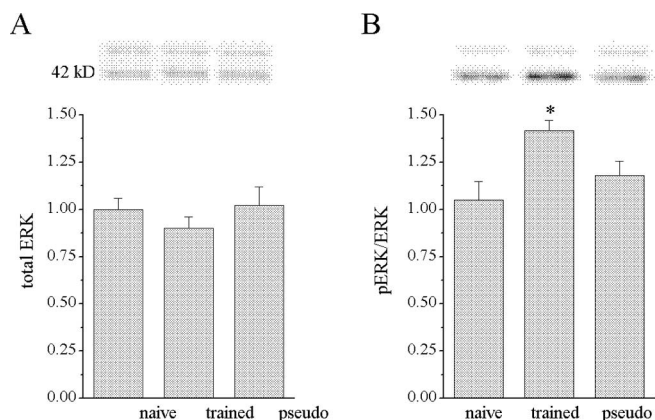

Figure 4. Membranal ERK is activated 3 d after learning. **A**, Protein expression level of ERKII is not modified after olfactory learning. The protein level is normalized to the average value obtained from the naive animals. Summarized data are presented as mean OD ± SE; $n = 9$ rats for each experimental group. Above are representative immunoblots for ERKI/II protein prepared from the piriform cortex of naive, pseudo-trained, and trained animals. **B**, After learning the level of pERKII is increased. Summarized data are presented as the ratio of pERKII/total ERKII; $n = 9$ rats for each experimental group. Above are representative immunoblots for pERKII prepared from piriform cortex of each of the three groups. Values represent mean ± SE.

trained, 1.01 ± 0.06; trained, 1.05 ± 0.06; pERK/ERK ratio: naive, 1.00 ± 0.05; pseudo-trained, 1.00 ± 0.08; trained, 1.08 ± 0.08). (For all the measurements, $n = 9$ rats in each group.)

These results also suggest that ERK activation is both correlated and necessary for the maintenance of learning-relevant enhancement in neuronal excitability.

PKC-induced AHP reduction is ERK dependent

Recordings performed in piriform cortex neurons after olfactory learning show that the PKC blocker GF-109203X increases the AHP in neurons from trained rats only, and that the PKC activator OAG reduced the AHP in neurons from control groups more than in neurons from trained rats (Seroussi et al., 2002). In particular, the PKC activator reduced the AHP in neurons from naive rats by 40% (Seroussi et al., 2002). We hypothesized that if PKC-induced reduction of the AHP is mediated via ERK activation, application of PD98059 should block its effect. As shown previously (Seroussi et al., 2002), the PKC activator OAG (10 μM) significantly reduces the postburst AHP in neurons from naive rats (from 7.2 ± 2.4 in control conditions to 5.1 ± 0.54 after OAG application; $n = 6$). An example of OAG effect on such a naive neuron is shown in Figure 5A. In sharp contrast, in the presence of the MEK inhibitor, the PKC activator did not have a significant effect of the averaged AHP amplitude in neurons from naive rats [6.20 ± 2.35 ($n = 8$) in PD98059 compared with 5.46 ± 1.36 in the same eight neurons after OAG application], and accordingly, it also did not affect the repetitive spike firing (Fig. 5).

Discussion

Previous studies have shown that olfactory learning-induced long-lasting enhancement of neuronal excitability is the result of reduction in the conductance of the acetylcholine-sensitive, calcium-dependent current, the sI_{AHP} (Saar et al., 2001; Saar and Barkai, 2003; Brosh et al., 2006). Here we show that ERK activation is both correlated and necessary for such long-term enhanced excitability.

ERK activation modifies neuronal excitability only after learning

Our data show that in piriform cortex layer II pyramidal neurons, the postburst AHP amplitude as well as neuronal adaptation are

subjected to modifications by ERK only after learning, when both the AHP amplitude and neuronal adaptation are reduced. The block of ERK activity results with complete abolishment of the learning-induced modifications in intrinsic neuronal properties. This robust effect suggests that persistent ERK activation is an obligatory condition for maintaining the neuron in its highly excitable state. Interestingly, before OD learning, ERK has no significant effect on the postburst AHP in piriform cortex neurons, although ERK is activated to a considerable level in control conditions (pERK/ERK ratio of ~1 in brains of control rats). This finding suggests that ERK activation is necessary but not sufficient for persistent AHP reduction.

We also show that such modifications in intrinsic neuronal excitability, which last for 3 d after rule learning, are mediated by membrane-specific, long-term ERK activation. The activity level of ERK in the total and cytosolic fractions, which is known to mediate many cellular events, is similar in all groups. This site-specific ERK activation, next to the cell membrane, supports the notion of its possible modulation of a channel(s) that mediates the ionic currents that underlie the learning-induced modifications.

Figure 5. PKC activation does not reduce the AHP amplitude in the presence of the ERK inhibitor. ***A***, An example of the effect of the PKC activator OAG on a neuron taken from a naive rat in control conditions (left trace) and the lack of such effect on another neuron from a naive rat in the presence of PD98059 (PD; right trace). ***B***, According to its lack of effect on the AHP, OAG does not enhance the firing rate of a naive neuron in the presence of PD.

ERK affects neuronal excitability by direct modulation of the postburst AHP

Increased neuronal excitability is frequently the result of phosphorylation-dependent modulation of ionic channels. The postburst AHP is affected by activation of PKC (Malenka et al., 1986; Agopyan and Agopyan, 1991; Pineda et al., 1995; Seroussi et al., 2002), protein kinase A (PKA) (Pedarzani and Storm, 1993; Pedarzani et al., 1998; Haug and Storm, 2000; Seroussi et al., 2002), and Ca^{2+}-calmodulin kinase II (Sah, 1996).

Although the effects of ERK on the postburst AHP and neuronal adaptation after learning imply that its primary role after learning is to maintain the reduced conductance of the sI_{AHP}, other potential effects on basic membranal properties such as input resistance or the duration of the single action potential would also be manifested by modulating repetitive firing and amplitude of calcium-dependent potassium currents. In particular, ERK phosphorylation was shown to affect the A-type potassium current (I_A) (Adams et al., 2000; Hu et al., 2003). The I_A current is primarily localized in the somas and dendrites (Sheng et al., 1992; Maletic-Savatic et al., 1995) and strongly affects the action poential duration (Kim and Chang, 2005).

Our data indicate that the basic membrane properties, such as resting potential, input resistance, and action potential width and amplitude, are not affected by ERK inhibition. Thus, we suggest that phosphorylation of the I_A channels by ERK is probably not the mechanism underling the learning-induced modifications in the postburst AHP, at least at the time point we examined (3 d after rule-learning acquisition).

ERK activation is required for PKC-induced modulation of neuronal excitability

We have previously shown that the maintenance of the olfactory learning-induced reduction of the AHP is dependent on PKC but not PKA activation (Seroussi et al., 2002). Here we show that the involvement of PKC in maintenance of the learning-induced neuronal excitability is mediated via ERK activation. The selec-
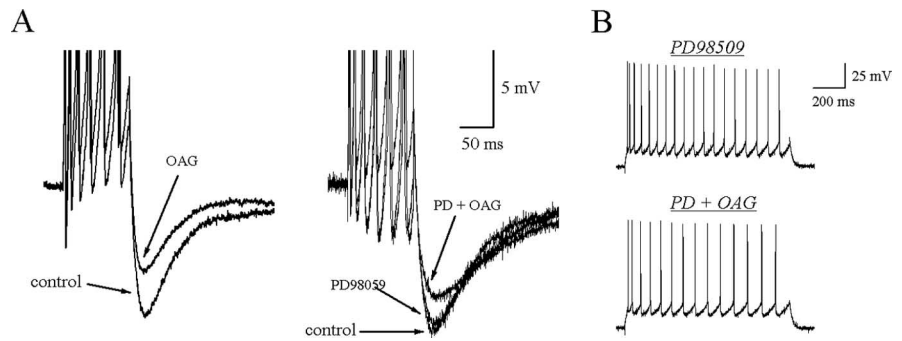
tive MEK inhibitor PD98059 was shown to inhibit up to 90% of ERK activation (Alessi et al., 1995), without affecting PKC activity (Blum et al., 1999). Here application of PD98059 to slices from control rats abolishes the typical reduction in AHP amplitude observed in these neurons when the PKC agonist OAG is applied. Thus, we suggest that the effect of PKC on learning-induced neuronal excitability is mediated by ERK activation. A similar sequence of events was suggested to describe the effect of PKC on the I_A potassium current (Hu et al., 2003).

Time-specific effects of ERK on learning and memory

Direct evidence in support of a role for ERK in memory has been accumulating rapidly over the past decade. Although the evidences stem from findings involving different learning types and brain areas, they all show a pattern of temporal ERK activation, initiated minutes to several hours after training completion (Atkins et al., 1998; Berman et al., 1998; Blum et al., 1999; Schafe et al., 2000; Belelovsky et al., 2005; Herry et al., 2006). The picture emerging from these previous studies is that at relatively early stages of memory formation and maintenance, ERKs modulate learning-relevant processes by affecting different substrates, which are yet to be identified.

Our study shows that at a later stage, days after learning completion, ERK is still activated at a specific cellular compartment and that such activation is necessary for maintaining learning-induced cellular modifications. However, at this time point its action is aimed directly or indirectly at a new target, the slow calcium-dependent potassium current.

In conclusion, our study shows that ERK activation is crucial for maintaining learning-induced cellular modifications for days after learning completion while having no effect on basal levels of cellular properties. Such activation is crucial for persistent reduction of the sI_{AHP}, which results with long-lasting enhancement of neuronal excitability. This enhanced excitability plays a key role in maintaining the cortical network in a "learning mode" in which memories can be stored rapidly and efficiently (Barkai and Hasselmo, 1994; Saar and Barkai, 2003).

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