Learning-Induced Enhancement of Postsynaptic Potentials in Pyramidal Neurons

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Received 4 September 2001; accepted in final form 27 December 2001

Saar, Drorit, Yoram Grossman, and Edi Barkai. Learning-induced enhancement of postsynaptic potentials in pyramidal neurons. J Neurophysiol 87: 2358–2363, 2002; 10.1152/jn.00744.2001. We studied the effect of olfactory learning-induced modifications in piriform (olfactory) cortex pyramidal neurons on the propagation of postsynaptic potentials (PSPs). Rats were trained to distinguish between odors in pairs, in an olfactory discrimination task. Three days after training completion, PSPs were evoked in layer II pyramidal cells in piriform cortex brain slices by electrical stimulation of two pathways. Stimulation of layer Ia activated the afferent axons that originate from the olfactory bulb and terminate on the distal apical dendrites. We have previously shown that olfactory training is accompanied by enhanced synaptic transmission in the intrinsic pathway, but not in the afferent pathway at 3 days after training. Here we show that at this stage, in both pathways PSPs evoked in neurons from trained rats had significantly faster rise time measured at the soma compared with PSPs in neurons from pseudo-trained and naive rats. Activation of the slow afterhyperpolarization (AHP), which is generated by potassium channels probably located at the proximal region of both apical and basal dendrites, reduced the amplitude measured at the soma of the proximal intrinsic pathway PSPs more effectively than PSPs that were generated distally by the afferent fibers. Thus the amount of reduction by AHP was used as a measure for the relative distance of PSP-generating sites from the soma. In neurons from trained rats, despite the previously reported reduction in AHP amplitude, AHP conductance shunted the PSPs from both synaptic pathways more efficiently compared with neurons from the control rats. We suggest that in neurons from trained rats PSPs are electrotonically closer to the soma.

INTRODUCTION

Studies of the physiological basis of learning in the mammalian brain report on enhanced synaptic transmission observed in vivo (Rioult-Pedotti et al. 1998; Wilson and McNaughton 1994) and in vitro (McKernan and Shinnick-Gallagher 1997; Power et al. 1997; Rioult-Pedotti et al. 1998; Saar et al. 1999). Also, enhanced neuronal excitability, which results from reduction in the slow afterhyperpolarization (AHP) currents, has been reported in relation to classical and operant conditioning (Coulter et al. 1989; Moyer et al. 1996; Saar et al. 1998; Thompson et al. 1996).

We have previously shown that both modifications are present in pyramidal neurons in layer II of the rat piriform cortex after odor-discrimination learning. AHP in layer II neurons was reduced for 3 days after acquisition of the task (rule learning), due to long-lasting effect of acetylcholine (Saar et al. 2001). Enhanced synaptic transmission, indicated by reduced paired-pulse facilitation (PPF), was apparent in the intrinsic connections between 3 and 8 days posttraining, whereas the afferent connections showed increase in PPF during the 1st 2 days after training (Saar et al. 1999). Since these modifications were temporary, and since their magnitude did not increase with additional learned odors, we suggested that they underlie the state of enhanced odor-learning capability and not the formation of long-term memory for specific learned odors (Saar et al. 1998, 1999). Recently we also found learning-related increase in the number of dendritic spines along apical dendrites of these neurons, suggesting dynamic modifications at the postsynaptic dendritic membrane (Knafo et al. 2001).

The purpose of the present study was to explore whether the enhanced odor-learning state is associated with modifications in PSP propagation within dendrites.

METHODS

Animal training

Young adult water-deprived Sprague-Dawley male rats were trained with 20 trials per day as previously described (Saar et al. 1998). In short, the rat is positioned at the center of a four-arm maze. An electronic “start” command releases two streams of pressured air into two randomly picked arms: a stream with positive-cue odor into one of the arms, and one with negative-cue odor into another. The rat can choose to enter one of the arms to be rewarded at the far end with a drop of drinking water. Rats in the trained group are rewarded randomly. Naive rats are not exposed to the maze. A fan is operated between trials, to clear the air. Once all the trained rats have met the criterion for learning the first pair of odors (≥80% positive-cue choices in a day), on the next day both trained and pseudo-trained groups resumed training with a new pair of unfamiliar odors. Trained rats show increased capability to learn additional odors with the same training paradigm (rule-learning) (see Saar et al. 1998; Staubli et al. 1987). Rats were trained with at least two pairs of odors to ensure rule learning.

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Slice preparation, stimulation, and recording

Animals were killed 3 days after training completion, when learning-induced intrinsic and synaptic modifications are most apparent (Saar et al. 1998, 1999). Once the first rat was killed for brain slice recordings, the rest of the group was further trained, to assure a delay of 3 days from the last training session to physiological experiments. Thus rats were trained with two to four pairs of odors.

Experiments were done blind; the group affiliation of the rats (naive, trained, or pseudo-trained) was unknown to the person conducting the experiments and measurements. Rats were anesthetized with pentobarbital sodium (30 mg/kg), the brain was removed, and coronal brain slices of 400 μm were cut as previously described (Saar et al. 1998). Brain slices were kept in oxygenated (95% O₂–5% CO₂) normal slice Ringer solution (NSR) containing (in mM) 124 NaCl, 3 KCl, 2 MgSO₄, 1.25 NaH₂PO₄, 26 NaHCO₃, 2 CaCl₂, and 10 glucose.

Intracellular current-clamp recordings were performed using sharp microelectrodes filled with 4 M K-Acetate. The synaptic inputs to layer II pyramidal neurons in the piriform cortex are topographically segregated. Layer Ia contains afferent axons arriving from the olfactory bulb that terminate on the distal part of the apical dendrites, and layer Ib contains intracortical axons that terminate on the proximal part of the apical and basal dendrites (Johnson et al. 2000; Price 1973). Stimuli were applied at 0.1 Hz at layer Ia, to activate the afferent axons, or at layer Ib, to activate the intracortical axons (Fig. 1A).

PSP rise time

Stimulus intensity was adjusted to evoke PSP with amplitude of 10 mV. PSP rise time was measured from 10 to 90% of the maximal voltage deflection, in a digital average of five consecutive responses.

The typical synaptic response in pyramidal neurons in the piriform cortex is an initial excitatory postsynaptic potential (EPSP), followed by a GABAₐ-mediated fast inhibitory postsynaptic potential (IPSP), and a slow GABAₐ-mediated IPSP. Thus the rising phase of the EPSP is not contaminated by the later occurring IPSPs (Tseng and Haberly 1988).

Since we have previously shown, at 3 days after training, learning-induced enhanced synaptic transmission in the intrinsic, but not in the afferent axons (Saar et al. 1999), we first tested the responses to stimulations applied to the intrinsic axons in layer Ib. Recordings in some cells were lost before the responses to stimuli applied to the afferent axons in layer Ia could be obtained. Thus the number of recorded PSPs in response to layer Ib stimulation is larger than the number of PSPs recorded in response to layer Ia stimulations.

Effect of AHP conductance on EPSPs’ amplitude

To study the effect of AHP conductance on the amplitude of the PSP, PSPs were evoked at the time to coincide with the peak of the AHP conductance. The latter was determined as follows: the cell was depolarized with DC to attain membrane potential of −60 mV. Then, a 100-ms depolarizing step was applied, its intensity adjusted to generate six action potentials (Fig. 2A). Under these conditions, considerable AHP is apparent (Saar et al. 1998). The delay to the peak AHP was measured and was assumed to represent the time to the peak conductance involved in the process. Then the cell was allowed to return to its normal resting potential, and synaptic stimuli were applied at 0.1 Hz, with stimulus intensity adjusted to create PSPs of 10 mV. The stimulus delay was adjusted to coincide with the previously determined AHP peak (Fig. 2B). Then, PSPs were evoked simultaneously with the AHP (Fig. 2C, black line). To reveal the actual amplitude of the “shunted” PSP, responses to current steps alone (Fig. 2C, gray line) were digitally subtracted from the combined responses (Fig. 2C, black line). The result of the subtraction is presented in Fig. 2D. Both control and shunted PSP’s amplitude were determined from digital average of five consecutive responses.

The effect of AHP conductance on PSP amplitude (% reduction) was calculated as follows: \( \text{PSP}_{\text{control}} - \text{PSP}_{\text{shunted}} \div \text{PSP}_{\text{control}} \times 100 \).
Rise time in proximally originated PSPs is shorter than in distally originated PSPs

Resting membrane potential was held at −80 mV when synaptic potentials were evoked. In agreement with the theory of dendritc membrane cable properties, PSPs evoked by stimulation of axons in layer Ib, which terminate close to the cell soma, had significantly shorter rise time compared with PSPs activated by layer Ia axons, which terminate distally. This was clearly evident in all groups, when rise times of PSPs evoked by layer Ia axons were compared with rise times of PSPs evoked by layer Ib axons in neurons in which PSPs evoked by the two synaptic pathways were recorded (Fig. 1B).

PSP rise time in both pathways is reduced after training

We have previously examined PSP rate of rise in trained rats, 1–6 days after the last training session, and could not detect significant difference between trained and control groups. However, we also found that learning-induced cellular modifications are dynamic. Thus a change that is apparent at 3 days after training, but not before or after this time, may not be detected if data from different days are grouped together. Therefore in the present study PSPs were recorded at only 3 days after rule learning, when learning-induced intrinsic and synaptic modifications are most apparent (Saar et al. 1998, 1999). We found that the rise time in neurons from trained rats was significantly shorter in both the intrinsic (2.64 ± 0.58 ms, mean ± SD, n = 26) and the afferent (3.04 ± 0.49 ms, n = 17) pathways, compared with neurons from pseudo-trained (2.93 ± 0.62 ms, n = 53, P < 0.05 and 3.66 ± 0.98 ms, n = 43, P < 0.01, respectively) and naive rats (3.05 ± 0.51 ms, n = 34, P < 0.005 and 3.67 ± 0.63 ms, n = 22, P < 0.001, respectively). For both synaptic pathways, no significant difference was observed between the pseudo-trained and naive groups (Fig. 4A). Notably, the averaged ratio between rise times of PSPs evoked in the intrinsic fibers and in the afferent fibers was unchanged in trained rats (0.83 ± 0.2, n = 13) compared with naive (0.84 ± 0.15, n = 15) and pseudo-trained (0.86 ± 0.24, n = 36).

We also measured the stimulus intensity required to elicit a standard 10-mV PSP in both synaptic pathways. When stimulating in layer Ia, the averaged stimulus intensity required for generating such response showed tendency to decrease in neurons from trained rats. However, this decrease just fell short of statistical significance (Fig. 3A). When stimulating in layer Ib, the averaged intensity required to elicit the standard response was significantly smaller (P < 0.01) in neurons from trained rats compared with the control groups, which did not differ from each other (Fig. 3B). These results are in agreement with our previous observation that the ratio between field PSP and stimulus intensity in this pathway is increased 3 days after olfactory learning (Saar et al. 1999).

Since PSP rise time was modified in both synaptic pathways, we hypothesized a general change in membrane properties of the postsynaptic dendrite, which leads to reduction in its apparent electrotonic length.

### Statistical analysis

One-way ANOVA followed by post hoc Scheffe tests was used to statistically detect significant differences between the three neuronal populations (e.g., trained, pseudo-trained, and naive groups). Within-cell comparisons of PSP rise times in response to layer Ib and layer Ia stimulations were done using paired t-test.

### Results

Brain slices were taken from 16 naive rats, 10 pseudo-trained, and 15 trained rats, 3 days after rule learning. As previously reported (Saar et al. 1998, 1999, 2001), neurons from the three groups did not differ in their membrane resting potential, input resistance and time constant that were measured at the soma (Table 1).

**Table 1.** Passive membrane properties are not modified by olfactory learning

<table>
<thead>
<tr>
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<th>$V_{rest}$, mV</th>
<th>$R_{in}$, MΩ</th>
<th>$\tau$, ms</th>
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<tr>
<td>Naive</td>
<td>82.7 ± 4.1 (15)</td>
<td>40.8 ± 8.6 (11)</td>
<td>16.11 ± 5.2 (9)</td>
</tr>
<tr>
<td>Trained</td>
<td>82.8 ± 2.9 (16)</td>
<td>36.4 ± 6.8 (13)</td>
<td>16.0 ± 4.1 (13)</td>
</tr>
<tr>
<td>Pseudo</td>
<td>84.6 ± 3.8 (21)</td>
<td>35.7 ± 10.6 (16)</td>
<td>16.4 ± 4.6 (16)</td>
</tr>
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Values represent means ± SD; number of cells is in parentheses. Membrane resting potential ($V_{rest}$) was determined from the voltage change accompanying electrode withdrawal from the cell at the end of the recording. Input resistance ($R_{in}$) was determined by calculating the best linear regression fit to a voltage/current curve, constructed from voltage responses to 100-ms current pulses ranging between −0.3 and +0.3 nA. Membrane time constant was calculated from the rising phase of a 10-mV response to an intracellular depolarizing current step.
Shunting effect of AHP on PSPs increases after training in both pathways

Since activation of the slow AHP, which is generated by potassium channels probably located at the proximal region of both apical and basal dendrites, reduced the amplitude of proximally generated PSPs more effectively than the amplitude of distally generated PSPs, the amount of this reduction by AHP was used as an estimate of the relative electrotonic distance between the PSP-generation sites and the soma. AHP in neurons from trained rats was reduced by about 20%, in agreement with our previous reports (Saar et al. 2001). However, in these neurons AHP conductance shunted the PSPs from both synaptic pathways more efficiently compared with neurons from the control groups (Fig. 4B), suggesting a smaller electrotonic distance between the PSP-generating sites and the soma after training.

**DISCUSSION**

Our results show that after odor training, PSPs generated in layer II pyramidal neurons by activation of two different pathways have shorter rise time at the soma and are shunted more effectively by the slow AHP-generating conductance, even though the AHP amplitude is reduced (Saar et al. 1998). We have previously shown pathway-specific enhancement in synaptic transmission in the piriform cortex of odor-trained rats (Saar et al. 1999). Enhanced synaptic transmission, indicated by reduced paired-pulse facilitation (PPF), was apparent in the intrinsic connections, between 3 and 8 days posttraining, whereas the afferent connections showed increase in PPF during the 1st 2 days after training (Saar et al. 1999). The similar reduction in PSP rise time for both pathways at 3 days after training in the present experiments implies another general, probably postsynaptic modification.

**Reduced PSP rise time**

Several postsynaptic mechanisms can account for the reduced PSP rise time at the soma. The finding that the membrane time constant, as measured by voltage responses induced by current application to the cell bodies, is not modified by learning, suggests that the learning-induced changes in PSP kinetics result from modifications along the dendrite. One possible mechanism is a general change in glutamate receptors’ properties. However, the simplest explanation could be a reduction in electrotonic distance. According to the cable theory, PSPs at short electrotonic distance from the soma would have shorter rise time at the soma than similar inputs at a longer distance. Indeed, we show that PSP rise time is always shorter for more proximal inputs in layer II pyramidal neurons. A

**FIG. 3.** Stimulus intensity required to elicit 10-mV postsynaptic responses in neurons from the 3 groups. A: stimuli applied to the afferent axons in layer Ia. Values represent means ± SE; n, number of cells. B: stimuli applied to the intrinsic axons in layer Ib. Values represent means ± SE. The averaged stimulus intensity is significantly smaller in neurons from trained rats (*P < 0.05). n, number of neurons.

**AHP conductance reduces proximally originated PSPs more than distally originated PSPs**

Since AHP was also changed after training, it was important to examine how the postulated change in length constant of the dendrite may interact with the shunt affected by the AHP.

In neurons from all three groups, in responses to both synaptic pathways, PSPs that were generated at the time of AHP peak were shunted by the slow potassium conductance, and their amplitude at the soma was significantly reduced. Moreover, PSPs that were generated at the proximal dendrites, by stimulation of layer Ib axons, were significantly more reduced by the slow AHP (−11.9 ± 6.2%, n = 27 in pseudo-trained, −15.7 ± 6.3%, n = 22 in trained, and −12.0 ± 4.7%, n = 17 in naive) compared with PSPs generated by stimulation of layer Ia axons (−8.0 ± 4.6%, n = 23 in pseudo-trained, −12.7 ± 5.5%, n = 19 in trained, and −8.6 ± 6.9%, n = 16 in naive, P < 0.05).

Shunting effect of AHP on PSPs increases after training in both pathways

Since activation of the slow AHP, which is generated by potassium channels probably located at the proximal region of
nonselective reduction in postsynaptic electrotonic length can account for decreased PSP rise time at the soma for both the proximal and the distal inputs in neurons from trained rats. This effect would be independent of their modulation after training. Physical shortening of dendritic branches could in principle underlie reduction in the electrotonic length. However, our recent morphological study shows similar length for dendritic branches in neurons from trained, pseudo-trained and naive rats (Knafo et al. 2001). Alternatively, reduced electrotonic length can result from increased membrane resistance, effected by reduced activity of dendritic ion channels. Computational modeling indicate that block of voltage-gated dendritic potassium channels would reduce the leakage of axial synaptic current through the dendritic membrane, resulting in greater axial current reaching the soma (Wilson 1995). Indeed, it has been shown in neocortical pyramidal neurons that block of several types of K\(^+\) channels, including the Ca\(^{2+}\)-activated K\(^+\) channels (and also block of noninactivating Na\(^+\) current) enhance the amplitude of axial current transmitted to the soma during glutamate-evoked dendritic depolarization (Schwindt and Crill 1995, 1997). We have previously shown acetylcholine-induced long-lasting decrease in the Ca\(^{2+}\)-activated K\(^+\) conductance in neurons from trained rats (Saar et al. 2001). In addition, the small but statistically significant depolarization of resting membrane potential, and the loss of acetylcholine capability to further depolarize the membrane in neurons from trained rats (Saar et al. 2001), imply learning-related cholinergic-induced long-lasting reduction in the neurons’ leak current as well. We therefore suggest that ionic channels modulation after odor learning induces apparent shortening in the dendritic electrotonic length of pyramidal neurons in the piriform cortex.

AHP shunting of PSPs

Our results show that PSP reduction by AHP conductance is greater for proximal intrinsic inputs compared with the distal, afferent inputs. Since the AHP site of conductance change is located even more proximally on the apical dendrite (Sah and Bekkers 1996; Schwindt and Crill 1997), this result seems intuitively simple to interpret. However, Rall (1964) has shown in mathematical model of passive dendritic tree that inhibitory shunt located at the proximal segment and soma of the model neuron is more effective in reducing EPSP than identical shunt located at the middle of the dendrite, where the EPSP input is introduced. Thus the inhibitory effect depends not only on the location of the excitatory input, but also on a strategic location of the inhibition in relation to the excitatory input.

Another explanation for the stronger effect on proximally generated EPSPs may be related to their faster rise time, since according to the cable theory, the effective length constant of a homogeneous cylinder is inversely (and nonlinearly) related to the frequency of the voltage change (Johnston and Wu 1997). Similar reasoning can account for the increase in AHP-induced PSP shunting in both the intrinsic (enhanced) and the afferent (unchanged) synaptic pathways in neurons from trained rats, because rise time in both is decreased. However, one should bear in mind that the spread of the physiological PSP occurs in a nonhomogeneous cylinder in terms of membrane resistance. The training-related increased efficacy of the AHP shunting is associated with 20% decrease in AHP amplitude, which indicates even greater reduction in its conductance change. Therefore the small reduction in PSPs rise time may only partially account for the increase in shunting. Further study, with consideration of the recent finding that large fractions of the intrinsic fiber inputs terminate on the basal dendrite (Johnson et al. 2000) and that a significant part of the AHP conductance is also located there (Bekkers 2000), may be needed to fully understand the mechanism of enhanced PSP shunting.

Physiological significance of reduced electrotonic length

We have previously suggested that, during odor learning, the piriform cortex network becomes more susceptible to long-term synaptic modifications. Several cellular modifications were detected in neurons from trained rats: increased neuronal excitability due to reduction in AHP conductance (Saar et al. 1999, 2001), enhanced synaptic transmission (Saar et al. 1999), and strengthened synaptic connectivity (Knafo et al. 2001). The present study suggests that synaptic integration is enhanced also by improved PSPs propagation into the soma, thus facilitating action potential generation. However, during excessive activity PSPs will be more susceptible to the AHP shunting effect.

This work was supported by the Israel Science Foundation.

REFERENCES


