Brief Communication

Olfactory learning-induced enhancement of the predisposition for LTP induction

Yaniv Cohen,¹ Sonya Avramoav,¹ Edi Barkai,^{1,2,3} and Mouna Maroun¹¹Department of Biology, Faculty of Natural Sciences, University of Haifa, Haifa 31905, Israel; ²Department of Neurobiology, University of Haifa, Haifa 31905, Israel

Learning of a particularly difficult olfactory-discrimination (OD) task results in acquisition of rule learning. This enhancement in learning capability is accompanied by the long-term enhancement of synaptic connectivity between piriform cortex pyramidal neurons. In this study we examined whether olfactory rule learning would modify the predisposition to induce long-term potentiation (LTP) in the pathway projecting from the piriform cortex to the olfactory bulb. We report that OD learning was associated with enhancement in the predisposition to induce LTP. This learning-related effect may be affected by process generation of new granule cells located in the olfactory bulb.

Learning of a particularly difficult olfactory-discrimination (OD) training paradigm results in acquisition of rule learning, manifested in a dramatic enhancement of learning capability. OD rule learning is accompanied by a series of profound physiological and morphological modifications in piriform cortex (PC) pyramidal neurons. In particular, intrinsic neuronal excitability is increased (for review, see Saar and Barkai 2003), synaptic transmission is enhanced (Saar et al. 1999, 2002), and the number of dendritic spines is increased (Knafo et al. 2001). Such OD learning-related modifications are spread throughout the neuronal ensemble (Saar et al. 1999; Knafo et al. 2001). Complex OD learning-induced cellular modifications are not confined to the piriform cortex. OD learning is also accompanied by long-term enhancement of synaptic transmission of the descending pathway to the piriform cortex from the orbitofrontal cortex and the ascending input to the piriform cortex from the olfactory bulb (OB) (Cohen et al. 2008).

Olfactory learning-related activity (Martin et al. 2007; Kay and Beshel 2010) and experience-induced plasticity has been shown to occur also within the OB (Wilson and Sullivan 1994; Fletcher and Wilson 2003). The olfactory bulb has been suggested to have a specific role in enhancing olfactory discrimination and learning (Shimshak et al. 2005). Learning-induced changes within the piriform cortex seem to not only modify the responses of the cortical neuron directly but also, via the extensive cortical feedback to the olfactory bulb (Haberly and Price 1978), to shape subsequent mitral and tufted cell response patterns (Murphy et al. 1999; Ghazanfar et al. 2001). Olfactory learning has been shown to modulate the activity in the OB in a manner that suggests that the PC–OB connectivity has been modified (Ravel et al. 2003; Martin et al. 2004, 2006). Piriform cortex excitatory synaptic inputs into the olfactory bulb terminate upon inhibitory interneurons (Migliore et al. 2010), particularly on granule cells (Gao and Strowbridge 2009). These synaptic inputs can be potentiated by stimulating the cortical feedback connections (Gao and Strowbridge 2009). We have previously reported that following OD learning, the predisposition to induce long-term potentiation (LTP) in the anaesthetized animal by stimulating the ascending pathway from the OB to PC is reduced. These results are in

accordance with our previous reported data that also in vitro, OD reduced the ability to induce LTP and increased the predisposition to induce long-term depression (LTD) in the PC (Lebel et al. 2001).

Interestingly, olfactory learning was also shown to affect the survival of newly generated inhibitory neurons in the adult OB (Mouret et al. 2008; Lazarini and Lledo 2011). Such new born neurons are GABAergic neurons of the granule cell layer (Gheusi et al. 2000). Excitatory synaptic connections onto these adult born granule cells can be potentiated shortly after their arrival to the bulb (Nissant et al. 2009).

Here we examine whether OD learning affects the ability to induce long-lasting modulation in the strength of connectivity between the piriform cortex and the olfactory bulb and modifies the susceptibility for activity-dependent synaptic plasticity in this descending pathway.

Age-matched young adult Sprague Dawley male rats (6–8 wk of age) were used. Before training, they were maintained on a 23.5 h water-deprivation schedule, with food available ad libitum. OD training protocol was performed with commercial odors that are used regularly in the cosmetics and food industry. Olfactory training consisted of 20 trials per day for each rat as described previously (Saar et al. 1999). Briefly, 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. The criterion for learning was at least 80% positive cue choices in the last 10 trials of a training day. Rats in the pseudotrained group were rewarded in a random manner in 50% of the trials, regardless of the odor they chose. Rats in the naive group were water deprived but not exposed to the maze. Rats were trained to discriminate between two pairs of odors to confirm that rule learning was achieved. As described previously (Saar et al. 1999), rats indeed learned the second pair of odors much faster than the first pair (7–8 d of training for the first pair and 1–2 d for the second pair). In vivo recordings were performed 3 d after training completion, when most cellular modifications are present (Saar et al. 1999, 2002; Knafo et al. 2001). All experiments were done blind; the identity of the rat from which neurons were recorded (naive, trained, or pseudotrained) was not known to the person conducting the experiments and measurements.

Rats were anesthetized (with 40% urethane, 5% chloral hydrate in saline, and 0.5 mL/100 g, i.p.) and placed in a stereotaxic frame with body temperature maintained at 37 ± 0.5°C.

³Corresponding author.E-mail ebarkai@research.haifa.ac.il.Article is online at <http://www.learnmem.org/cgi/doi/10.1101/lm.223191>.

Figure 1. Monosynaptic response in the granule cells layer. The location of the recording electrode in the granule cell layer in the OB and depth profiles of synaptic potential recorded in the olfactory bulb in response to stimuli applied in the descending pathway from the piriform cortex. At all recorded depths, fast monosynaptic responses are recorded with the amplitude increasing as the recording electrode nears the granule cell bodies layer. Each trace is an average of five synaptic responses evoked at 0.1 Hz.

The procedure was performed in strict accordance with the Haifa University regulations and guidelines of the National Institutes of Health.

A recording microelectrode (glass, tip diameter of 2–5 μm , filled with 2 M NaCl, resistance of 1–4 M Ω) was slowly lowered into the left OB (8.2–8.5 mm anterior to bregma; 1.5 mm lateral; 4 mm below the surface of the bulb).

For stimulating, a bipolar 125- μm stimulating electrode was implanted in the anterior PC. Electrode position was 3.7 mm anterior to bregma, 3.4–3.5 mm lateral, and 5.2 mm below the pial surface (Fig. 1). Evoked responses were digitized (10 kHz) and analyzed using the Cambridge Electronic Design 1401+ and its Spike 4 software. Off-line measurements were made of the amplitude of field post-synaptic potential (fPSP) using the average of five successive responses to a given stimulation intensity. Test stimuli (monopolar pulses, 100- μsec duration) were delivered at 0.1 Hz. After positioning the electrodes, the rats were left for 30 min before commencing the experiment.

Input–output (I–O) functions for stimulus intensity vs. fPSP magnitude were recorded in response to increasing intensities of stimulation from 0.3 to 1 mA applied at 0.1 Hz. I–O curves were constructed by using the average of five successive responses to a given stimulation intensity vs. the entire range of intensities sampled. Only those experiments having stable baselines and stable I–O curves over multiple trials were included in the analysis.

LTP was induced by applying theta burst stimulation (TBS) to the anterior piriform cortex (APC) (three sets of 10 trains; each train consisted of 10 pulses at 100 Hz; intertrain interval, 200 msec; interest interval, 1 min). Field potentials were recorded from the OB for 60 min after the TBS to the APC. LTP was measured as an increase in fPSP amplitude. LTP was applied at stimulus intensity that evoked a fPSP with an amplitude of 1 mV.

After the electrophysiological testing, marking lesions were made by passing anodal currents (10 mA for 3 sec; 10 mA for 2 min) through the recording electrode located in the OB. The rats' brains were removed, post-fixed for three nights in formaldehyde (10%), and sectioned (120 μm) on a sledge microtome. The sections were then mounted on

gelatin-coated slides, stained with cresyl violet, dehydrated, and cover-slipped. Following this procedure, the electrode tract and lesion locations were identifiable under a light microscope. The location of the recording in the OB is shown in Figure 1.

For all the electrophysiological measurements, a between-groups comparison was done using one-way ANOVA for the three groups (naive, trained, and pseudotrained), and post-hoc multiple *t*-tests were then applied to compare between each two groups (trained, pseudotrained, and naive).

We first examined the responses to stimulations applied to the PC at different vertical positions of the recording electrode (Fig. 1). As the responses in the OB to stimulation of the lateral olfactory tract are well documented (Stripling and Galupo 2008), we used these responses to determine whether the recording electrode is positioned to record synaptic responses in the granule cell layer. We found a typical mono synaptic response with a delay of 4.5–5.5 msec, with amplitude increasing gradually with the recording electrode nearing the cell bodies layer (Fig. 1).

We showed previously that the strength of the ascending synaptic pathway between the OB and the PC is enhanced after learning (Cohen et al. 2008). We thus examined whether such enhancement is reciprocated by similar learning-induced modifications in the descending synaptic connections between these two structures. Input–output functions for stimulus intensities vs. fPSP amplitudes were obtained by recording the responses to a range of increasing intensities of stimulation in rats taken from the three groups (naive, pseudotrained, and trained). The amplitude of the field potential increased linearly with the increase in stimulus intensity in the three groups (Fig. 2A). The averaged amplitudes in the trained groups were similar to those recorded in the two control groups (Fig. 2B). These data support the hypothesis that basal synaptic connectivity between the OFC and the APC is not modified after learning.

Following OD learning the predisposition for LTP is reduced in the ascending OB–PC pathway (Cohen et al. 2008), as well as in the association fibers interconnecting the pyramidal neurons within the PC (Lebel et al. 2001; Quinlan et al. 2004). Here we examined whether a similar effect occurs also in the descending PC–OB pathway. The stimulus intensity was adjusted to evoke a fPSP with an amplitude of ~ 1 mV. Subsequently, repetitive theta burst stimulation was applied. Interestingly, such stimulation induced LTP in trained rats with a significantly ($P < 0.05$) higher

Figure 2. Strength of synaptic connectivity in the PC–OB pathway is not modified after learning. (A) A series of synaptic responses increasing monotonically as stimulus intensity is gradually enhanced for 0.3–1.0 mV. Each trace is an average of five synaptic responses evoked at 0.1 Hz. Example is taken from a naive animal. (B) Analysis of the input–output function of the synaptic response in the PC–OB pathway. The averaged amplitudes of the synaptic response, representing activation of the centrifugal fibers, is not modified after learning throughout the range of different stimulus intensities. (N, number of rats; values represent mean $\pm$ SE.)

Figure 3. Learning-induced modification in predisposition for LTP induction. (A) Example of LTP induction in PC-OB pathway. Traces before and 50 min after repetitive stimulation applications are superimposed. Although TBS induced only moderate LTP in the naive rat, significant LTP is observed in the trained rat. Each trace is an average of five synaptic responses evoked at 0.1 Hz. (B) In the APC-OB pathway, a mild LTP following TBS is observed in the naive and the pseudotrained groups, whereas a much more pronounced potentiation was recorded in the trained group. (*N*, number of rats; values represent mean $\pm$ SE.)

averaged amplitude ($42.1 \pm 6.95\%$, $n = 7$) than in naive ($21.0 \pm 1.5\%$, $n = 9$) and pseudotrained rats ($24.5 \pm 3.9\%$, $n = 6$) rats (Fig. 3A,B).

Experience-dependent regulation of synaptic strength has long been hypothesized to be the physiological basis of learning and memory. Accordingly, an increase in synaptic strength accompanies learning in vivo (Rogan et al. 1997; Cohen et al. 2008) and persists in brain slices (McKernan and Shinnick-Gallagher 1997; Power et al. 1997; Rioult-Pedotti et al. 1998; Saar et al. 2002). Learning-induced potentiation of synaptic strength is accompanied by an increase in the threshold for further synaptic enhancements (Rioult-Pedotti et al. 2000; Brun et al. 2001; Lebel et al. 2001). Here we show that OD learning is accompanied by enhanced predisposition for LTP induction in the centrifugal synaptic inputs from the piriform cortex to the olfactory bulb. Thus, reciprocal bulb-cortical connections undergo unique long-term modifications after learning. Although the ascending connections are strengthened by learning to point where they cannot be further potentiated (Cohen et al. 2008), the descending connections show the same response to low frequency stimulation and are more prone to be strengthened by intense activity.

Such a unique effect of learning, namely enhancing the predisposition to induce LTP, could result from the process generation of new granule cells located in the olfactory bulb. These granule cells, the primary target of monosynaptic inputs from the PC (Migliore et al. 2010), may be potentiated by stimulating the ascending inputs from the PC (Gao and Strowbridge 2009). In particular, synaptic inputs into new born neurons are transiently more readily potentiated. As olfactory learning increases the survival of new born neuron at a particular time window (Mouret et al. 2008), learning-induced increased predisposition for LTP induction in the PC-OB pathway may be the result of such readiness for potentiation synaptic connections.

The olfactory bulb has been suggested to have a role in olfactory discrimination and learning (Shimshak et al. 2005). Our data suggest that learning-induced cellular modifications are not only intrinsic to the olfactory bulb, but may also be shaped by descending synaptic inputs from the piriform cortex. Thus, olfactory learning is not simply divided between different stations of the olfactory pathways, but requires a combined and correlated activation of the different brain regions, such as the olfactory bulb, the piriform cortex, and the orbitofrontal cortex (Cohen et al. 2008).

Acknowledgments

We thank Dr. Hiram Gurden and Dr. Claire Martin (UMR8165 Imagerie et Modélisation en Neurobiologie et Cancérologie, CNRS/Université Paris XI/Paris VII, Bat 440, Campus d'Orsay, Orsay, France) for the valuable help and discussions. This work was supported by research grants from the Israel Science Foundation (to E.B. and M.M.).

References

- Brun VH, Ytterbo K, Morris RG, Moser MB, Moser EI. 2001. Retrograde amnesia for spatial memory induced by NMDA receptor-mediated long-term potentiation. *J Neurosci* **21**: 356–362.
- Cohen Y, Reuveni I, Barkai E, Maroun M. 2008. Olfactory learning-induced long-lasting enhancement of descending and ascending synaptic transmission to the piriform cortex. *J Neurosci* **28**: 6664–6669.
- Fletcher ML, Wilson DA. 2003. Olfactory bulb mitral/tufted cell plasticity: Odorant-specific tuning reflects prior odorant exposure. *J Neurosci* **23**: 6946–6955.
- Gao Y, Strowbridge BW. 2009. Long-term plasticity of excitatory inputs to granule cells in the rat olfactory bulb. *Nat Neurosci* **12**: 731–733.
- Ghazanfar AA, Krupa DJ, Nicolelis MAL. 2001. Role of cortical feedback in the receptive field structure and nonlinear response properties of somatosensory thalamic neurons. *Exp Brain Res* **141**: 88–100.
- Gheusi G, Cremer H, McLean H, Chazal G, Vincent JD, Lledo P-M. 2000. Importance of newly generated neurons in the adult OB for odor discrimination. *Proc Natl Acad Sci* **97**: 1823–1828.
- Haberly LB, Price JL. 1978. Association and commissural fiber systems of the olfactory cortex of the rat. *J Comp Neurol* **178**: 711–740.
- Kay LM, Beshel J. 2010. A beta oscillation network in the rat olfactory system during a 2-alternative choice odor discrimination task. *J Neurophysiol* **104**: 829–839.
- Knafo S, Grossman Y, Barkai E, Benshalom G. 2001. Olfactory learning is associated with increased spine density along apical dendrites of pyramidal neurons in the rat piriform cortex. *Eur J Neurosci* **13**: 633–638.
- Lazarini F, Lledo PM. 2011. Is adult neurogenesis essential for olfaction? *Trends Neurosci* **1**: 20–30.
- Lebel D, Grossman Y, Barkai E. 2001. Olfactory learning modifies predisposition for long-term potentiation and long-term depression induction in the rat piriform (olfactory) cortex. *Cereb Cortex* **11**: 485–489.
- Martin C, Gervais R, Hugues E, Messaoudi B, Ravel N. 2004. Learning modulation of odor-induced oscillatory responses in the rat olfactory bulb: A correlate of odor recognition? *J Neurosci* **24**: 389–397.
- Martin C, Gervais R, Messaoudi B, Ravel N. 2006. Learning-induced oscillatory activities correlated to odour recognition: A network activity. *Eur J Neurosci* **23**: 1801–1810.
- Martin C, Beshel J, Kay LM. 2007. An olfacto-hippocampal network is dynamically involved in odor-discrimination learning. *J Neurophysiol* **98**: 2196–2205.
- McKernan MG, Shinnick-Gallagher P. 1997. Fear conditioning induces a lasting potentiation of synaptic currents in vitro. *Nature* **390**: 607–611.
- Migliore M, Hines ML, McTavish TS, Shepherd GM. 2010. Functional roles of distributed synaptic clusters in the mitral-granule cell network of the olfactory bulb. *Front Integr Neurosci* **4**: 122. doi: 10.3389/fnint.2010.00122.
- Mouret A, Gheusi G, Gabellec MM, Chaumont F, Olivo-Marin JC, Lledo PM. 2008. Learning and survival of newly generated neurons: When time matters. *J Neurosci* **28**: 11511–11516.
- Murphy PC, Duckett SG, Sillito AM. 1999. Feedback connections to the lateral geniculate nucleus and cortical response properties. *Science* **286**: 1552–1554.
- Nissant A, Bardy C, Katagiri H, Murray K, Lledo PM. 2009. Adult neurogenesis promotes synaptic plasticity in the olfactory bulb. *Nat Neurosci* **12**: 728–730.
- Power JM, Thompson LT, Moyer JR, Disterhoft JF. 1997. Enhanced synaptic transmission in CA1 hippocampus after eyeblink conditioning. *J Neurophysiol* **78**: 1184–1187.

- Quinlan E, Lebel D, Brosh I, Barkai E. 2004. A molecular mechanism for stabilization of learning-induced synaptic modifications. *Neuron* **41**: 185–192.
- Ravel N, Chabaud P, Martin C, Gaveau V, Hugues E, Tallon-Baudry C, Bertrand O, Gervais R. 2003. Olfactory learning modifies the expression of odour-induced oscillatory responses in the gamma (60–90 Hz) and beta (15–40 Hz) bands in the rat olfactory bulb. *Eur J Neurosci* **17**: 350–358.
- Riout-Pedotti MS, Friedman D, Hess G, Donoghue JP. 1998. Strengthening of horizontal cortical connections following skill learning. *Nat Neurosci* **1**: 230–234.
- Riout-Pedotti MS, Friedman D, Donoghue JP. 2000. Learning-induced LTP in neocortex. *Science* **290**: 533–536.
- Rogan MT, Stäubli UV, LeDoux JE. 1997. Fear conditioning induces associative long-term potentiation in the amygdala. *Nature* **390**: 604–607.
- Saar D, Barkai E. 2003. Long-term modifications in intrinsic neuronal properties and rule learning in rats. *Eur J Neurosci* **17**: 2727–2734.
- Saar D, Grossman Y, Barkai E. 1999. Reduced synaptic facilitation between pyramidal neurons in the piriform cortex after odor learning. *J Neurosci* **19**: 8616–8622.
- Saar D, Grossman Y, Barkai E. 2002. Learning-induced enhancement of postsynaptic potentials in pyramidal neurons. *J Neurophysiol* **87**: 2358–2363.
- Shimshek DR, Bus T, Kim J, Mihaljevic A, Mack V, Seeburg PH, Sprengel R, Schaefer AT. 2005. Enhanced odor discrimination and impaired olfactory memory by spatially controlled switch of AMPA receptors. *PLoS Biol* **3**: e354. doi: 10.1371/journal.pbio.0030354.
- Stripling JS, Galupo MP. 2008. Differential potentiation of early and late components evoked in olfactory cortex by stimulation of cortical association fibers. *Brain Res* **1246**: 70–79.
- Wilson DA, Sullivan RM. 1994. Neurobiology of associative learning in the neonate: Early olfactory learning. *Behav Neural Biol* **61**: 1–18.

Received April 2, 2011; accepted in revised form June 28, 2011.

Olfactory learning-induced enhancement of the predisposition for LTP induction

Yaniv Cohen, Sonya Avramoav, Edi Barkai, et al.

Learn. Mem. 2011, **18**:

Access the most recent version at doi:[10.1101/lm.2231911](https://doi.org/10.1101/lm.2231911)

References

This article cites 31 articles, 9 of which can be accessed free at:
<http://learnmem.cshlp.org/content/18/9/594.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).