Optical Imaging of Odor Preference Memory in the Rat Olfactory Bulb

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Yuan, Qi, Carolyn W. Harley, John H. McLean, and Thomas Knöpfel. Optical imaging of odor preference memory in the rat olfactory bulb. J Neurophysiol 87: 3156–3159, 2002; 10.1152/jn.00917.2001. Early olfactory preference learning in rat pups occurs when novel odors are paired with reinforcing tactile stimulation that activate the noradrenergic locus coeruleus. Pairing of odor and a noradrenergic agonist in the olfactory bulb is both necessary and sufficient for odor preference learning. This suggests the memory change occurs in the olfactory bulb. Previous electrophysiological experiments demonstrated that odor preference training induces an increase in the field excitatory postsynaptic potential to olfactory nerve input and an alteration, after training, in glomerular [14C]2-deoxyglucose uptake and in single-unit responses of principal cells. We investigate here whether, 24 h after olfactory preference training, there is an alteration in intrinsic optical signals at the glomerular level. Six-day-old rat pups were trained, as previously, for a peppermint odor preference. Trained pups and control littermates were subjected to imaging of odor-induced intrinsic optical signals 1 day after the training session. Trained pups exhibited significantly larger responses to the peppermint compared with untrained littermates previously exposed to the same odor. The response of trained pups to a control odor (amyl acetate) was, however, not significantly different from that of untrained littermates. These observations demonstrate that odor preference memory can be read-out by optical imaging techniques.

INTRODUCTION

Neonate rats rapidly (1-trial learning) form a preference to an odor that is paired with a reinforcing tactile stimulus (Sullivan and Leon 1987) that activates the locus coeruleus (Nakamura et al. 1987) or that is paired with the beta adrenergic agonist, isoproterenol (Sullivan et al. 1989). Several lines of evidence suggest that the olfactory bulb (OB) itself is sufficient to mediate this early odor-preference learning: activation of beta receptors locally in the bulb, concomitant with peppermint odor presentation, is both necessary (Sullivan et al. 1989) and sufficient (Sullivan et al. 2000) for odor-preference learning to occur. Previous work has shown that odors induce focal uptake of [14C]2-deoxyglucose (2-DG) within the glomerular layer of the OB and that focal 2-DG uptake in the glomerular layer increases after early odor-preference learning (Johnson and Leon 1996; Sullivan et al. 2001). Effective odor-preference training protocols increase cAMP response element binding protein (CREB) phosphorylation (McLean et al. 1999, Yuan et al. 2000b) in the bulb and selectively increase the field excitatory postsynaptic potential (EPSP) to olfactory nerve stimulation (Yuan et al. 2000b). Both the N-methyl-D-aspartate (NMDA) and AMPA components of the olfactory nerve field EPSP are enhanced. Furthermore, odor conditioning enhances single-unit responses in mitral-tufted cells in areas that exhibit enhanced 2-DG labeling following exposure to a previously conditioned odor (Wilson and Leon 1988).

Recent advances in optical imaging have facilitated our understanding of the spatial representation of odors in the OB (Belluscio and Katz 2001; Meister and Bonhoeffer 2001; Rubin and Katz 1999; Uchida et al. 2000). Responses to odors can be measured directly by optical recording of intrinsic signals from the dorsal surface of the OB (Belluscio and Katz 2001; Meister and Bonhoeffer 2001; Rubin and Katz 1999; Uchida et al. 2000). Representations of odorants within the OB can be visualized at the level of glomeruli. The patterns of odor-induced optical signals are similar among different animals (Belluscio and Katz 2001).

Intrinsic optical signals are due to activity-dependent hemodynamic changes and light scattering (Malonek et al. 1997, Meister and Bonhoeffer 2001). Intrinsic signal imaging enables in vivo recording and multiple manipulations on anesthetized animals. Therefore it may serve as a useful tool to explore training-dependent changes in stimulus-induced patterns of neuronal activity. In this study, we investigated the feasibility of using intrinsic signal imaging to detect training-dependent changes within the OB 24 h after conditioned odor-preference training. We performed intrinsic optical imaging on the OBs of both trained and control 1-wk-old rat pups. An enhanced optical signal was observed in trained animals to the trained odor. The result demonstrated that intrinsic signal imaging could monitor training induced changes in neuronal activity.

METHODS

Odor-preference training

Eighteen Sprague-Dawley rat pups from five litters were used in this study. The procedure for conditioning has been previously de-
scribed in detail (McLean et al. 1993, 1999). Briefly, on postnatal day 6 (PND6, the day of birth was considered PND 0), rat pups were removed from the dam and put on fresh bedding 10 min before odor exposure. In one group, pups were placed on peppermint-scented bedding (0.3 ml peppermint/500 ml bedding) and stroked vigorously on the hind region using a sable brush every other 30 s for 30 s over a 10-min period (odor + stroking). In another group, the pups were only exposed to the peppermint bedding without being stroked (odor only). Immediately after training or odor exposure, the pups were returned to the dams. Previous studies (McLean et al. 1993; Price et al. 1998; Sullivan and Leon 1987; Sullivan et al. 1989, 1991) have shown that rat pups subjected to the preceding conditioning procedure develop a predictable odor preference for the odor used.

**Optical imaging**

Rat pups were subjected to optical imaging the day after training. Rats were anesthetized with a 2.25 g/kg intraperitoneal injection of 20% urethan. Anesthetized rat pups were placed in a stereotaxic frame, and the bone overlying the dorsal surface of the olfactory bulbs was carefully thinned until the blood vessels underneath the bone were visible (Rubin and Katz 1999; Uchida et al. 2000).

The stereotaxic frame with the anesthetized rat pups was mounted below optics consisting of a ×1 objective and a ×1.6 projection lens. Odorants were diluted in glycerol and delivered by computer-controlled pressure pulses into a stream of fresh air blowing over the rat’s nose (Fig. 1A). The bulbs were illuminated with red light (630 nm) via two light guides positioned lateral to the objective (Rubin and Katz 1999; Uchida et al. 2000). The light was focused just below the blood vessels at the level of the glomeruli. Images (640 × 480 pixel) were acquired by a cooled CCD system (Sensicam, PCO Computer Optics GmbH, Germany) under control of Axon Imaging Workbench software (Axon Instruments, Foster City, CA) at a frame rate of 2 Hz. Different odor and no-odor recordings were interleaved and repeated 5–10 times. Odors were presented for 4 s with a 60-s intertrial interval. Time series of images were averaged (n = 5 to 10), and responses were expressed as odor-induced fractional change in reflected light intensity (ΔR/R; see Fig. 1B). Thresholding (Rubin and Katz 1999; Uchida et al. 2000) or spatial filtering techniques (Meister and Bonhoeffer 2001) were not applied to avoid any interference between these data transformations and data quantification. Data processing and analysis were performed using Origin software (OriginLab) and custom-made software written in Interactive Data Language (IDL5.4, Research Systems). The experimental protocol was approved by the Experimental Animal Committee of the RIKEN Institute.

**RESULTS**

Figure 1A shows a schematic illustration of the experimental design for imaging of OB responses to amyl acetate and peppermint. The dorsal surface of the OB was imaged and reflected light was sampled from the mediorostral, laterorostral, mediocaudal, and laterocaudal quadrants. As shown in Fig. 1B application of peppermint (10%) for 4 s induced a transient change in light reflectance after a delay of about 3 s. Peak amplitudes of these responses amounted to 0.2% up to 1% of the baseline light intensity. Signal sizes of the four

![Fig. 1. A: schematic illustration showing the experimental design for imaging of olfactory bulb (OB) responses to 2 different odors (amyl acetate, AA, and peppermint, PP). The dorsal surface of the OB was imaged and reflected light was sampled from the mediorostral, laterorostral, mediocaudal, and laterocaudal quadrants. B: responses obtained with application of peppermint (10%) for 4 s. Individual traces were obtained from the 4 quadrants indicated in A.](http://jn.physiology.org/)

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quadrants did not differ significantly, and therefore signals from the four quadrants were averaged in subsequent analysis.

The preceding experimental design was then applied using odor-trained and control littermate pups (Fig. 2A). Control animals exhibited amyl acetate and peppermint-induced intrinsic optical signals of comparable peak amplitudes (0.41 ± 0.07, and 0.35 ± 0.11%, respectively; mean ± SE). Trained animals, however, exhibited larger signals to the trained odor (peppermint, 0.99 ± 0.23%) as compared with the control odor (amyl acetate, 0.52 ± 0.15%) applied to the same animals (Fig. 2B). Trained animals also responded with significantly larger intrinsic signals to the trained odor than did control littermates to the same odor (Fig. 2B). Furthermore, odor preference training significantly enhanced the ratio between the responses induced by peppermint and amyl acetate (Fig. 2C).

DISCUSSION

In the present study, we investigated whether odor-preference memory can be accessed by imaging of intrinsic signals at the level of the glomeruli and found that this was the case. This outcome is consistent with the earlier reports of enhanced 2-DG uptake at the glomerular level in the OB following peppermint-preference training. It has been established that odor-induced intrinsic signals imaged from the OB involve “global,” i.e., spatially less confined components as well as components that can resolve single glomeruli (Meister and Bonhoeffer 2001). The present odor-induced responses were seen over the dorsal surface of the OB, i.e., at the global level, and only occasionally more localized response patterns emerged (not shown). There are several reasons we might expect primarily global signals in these experiments. The first is the age of the subjects. Intrinsic optical signals in the somatosensory barrel fields of rats less than 7 wk of age are more diffuse than those in adults (Yazawa et al. 2001). This is attributed to horizontal interactions. Similarly only diffuse intrinsic optical signals are seen initially in the visual cortex of young ferrets when orientation maps are studied and there is considerable individual variation in the development of the more specific patterns (Chapman et al. 1996). Thus the olfactory maps in week-old rat pups may be more diffuse than in older rats even though glomerular organization has already been developed at this age (Bailey et al. 1999). On the other hand, the same concentration of peppermint used here produces discrete 2-DG spots in week-old pups (Sullivan and Leon 1987). 2-DG peppermint representations are, however, less sensitive to odorant concentration than optical signals appear to be (Carmi and Leon 1991). Signals for amyl acetate, for example, have been measured at similar concentrations with both methods (Rubin and Katz 1999; Stewart et al. 1979), and focal patterns are more discrete for higher concentrations with 2-DG (Stewart et al. 1999). In addition, increased 2-DG uptake over the entire glomerular layer, as well as enhanced focal uptake, occurs following peppermint preference learning even in older pups (Johnson and Leon 1996). It is unlikely the global increases seen here are due to respiratory changes to the learned odor because previous studies have found no change in respiration with peppermint preference learning (Sullivan et al. 1988). Finally, in 19-day-old pups, 2-DG and c-fos foci following extended odor preference training are primarily in the midlateral bulb (Johnson et al. 1995; Woo et al. 1987) that was not sampled here. In week-old pups 2-DG (Sullivan and Leon 1987; Yuan et al. 2000a) and pCREB (McLean et al. 1999) images show dorsolateral foci as well. Thus a portion of the focal peppermint representation was included in the present study although visualization of the midlateral bulb might have increased the probability of capturing a focal response.

These data are consistent with the evidence from earlier experiments showing an increase in the field EPSP to olfactory nerve input in pups of the same age that receive learning effective training conditions (Yuan et al. 2000b). The intrinsic signal change at the level of the glomeruli 24 h later in the present study may indicate that the synaptic modification seen during acquisition conditions is sustained.

Creation of an olfactory preference in the rat pup may therefore
be intimately related to an increase in synaptic strength at the level of the glomeruli. Such a hypothesis is consistent with the recent report of a Drosophila mutation that concomitantly produces an increase in glomerular synapses and the appearance of a behavioral preference for a normally neutral odor (Acebes and Ferris 2001). Transduction of the odor is not altered. Other evidence supporting a special role for the glomerular layer in odor preference learning is the report of increased glomerular size (Woo et al. 1987) (as in the Drosophila model) and of increased numbers of juxtaglomerular cells (Woo and Leon 1991) following peppermint preference training.

Future studies might examine glomerular intrinsic signal changes at a longer interval after training to assess focal alterations and to ask if the generalized response seen here is enduring as reported for 2-DG. Within-pup analysis of optical signals in an acquisition paradigm might permit an assessment of training-induced changes in discrete foci when they occur. This was precluded in the present between-group study due to the variability in the occurrence of discrete signals.

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REFERENCES


