Correspondence Between Odorant-Evoked Patterns of Receptor Neuron Input and Intrinsic Optical Signals in the Mouse Olfactory Bulb

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Wachowiak, Matt and Lawrence B. Cohen. Correspondence between odorant-evoked patterns of receptor neuron input and intrinsic optical signals in the mouse olfactory bulb. J Neurophysiol 89: 1623–1639, 2003. First published October 23, 2002; 10.1152/jn.00747.2002. We compared odorant-evoked patterns of receptor neuron input to the mouse olfactory bulb, imaged with a calcium-sensitive dye, with those of intrinsic optical signals imaged from the same preparations. Both methods yielded patterns of glomerular activity that showed a strong concentration dependence, a loosely organized chemotopy, and involved widely distributed glomeruli. Presynaptic calcium and intrinsic signals showed similar odorant concentration thresholds. Intrinsic signal foci were larger than their corresponding calcium signals, and input to multiple adjacent glomeruli often appeared as a single intrinsic focus. Nonetheless, at near-threshold concentrations, the correspondence between the glomerular calcium and intrinsic signals averaged 75%, with a 71% correspondence between the most strongly activated glomeruli. The correspondence between strongly activated glomeruli decreased as odorant concentration increased, dropping to 51% at 5- to 15-fold higher concentrations. Intrinsic signal foci often saturated at lower concentrations than the calcium signal, implying a smaller dynamic range, and suprathreshold concentrations could recruit strong intrinsic signals in areas showing little or no calcium signal. These differences were such that, at suprathreshold concentrations, the chemotopy of calcium and intrinsic signal response maps often differed. These results suggest that intrinsic optical signals closely reflect receptor neuron input to glomeruli at low odorant concentrations but reflect additional processes at higher concentrations (activation of second-order neurons, centrifugal input, or constraints on the coupling between neuronal activity and hemodynamic changes). Intrinsic signals that are not associated with receptor neuron input have the potential to impact the interpretation of spatial coding strategies in the olfactory bulb.

INTRODUCTION

Olfactory stimuli evoke spatially organized patterns of neural activity in the vertebrate olfactory bulb. While other parameters are likely also important, spatial activity patterns have long been hypothesized to play an important role in encoding olfactory information (Adrian 1953; Kauer 1991; Shepherd 1994). Many studies have provided experimental evidence in support of this hypothesis and have explored the relationship between spatial patterns of olfactory bulb activity and features of olfactory stimuli (Friedrich and Korsching 1997; Imamura et al. 1992; Johnson et al. 2002; Linster et al. 2001; Rubin and Katz 1999; Stewart et al. 1979). The initial mapping of olfactory stimuli across the spatial dimension of the olfactory bulb arises from the precise convergence of receptor neurons expressing the same odorant receptor onto only a few olfactory bulb glomeruli in stereotyped locations (Bozza et al. 2002; Strotmann et al. 2000; Vassar et al. 1994). This spatial map of receptor neuron activation may be altered by synaptic interactions within glomeruli, between neurons in adjacent glomeruli, or between neurons in subglomerular layers, as olfactory input is transferred to higher-order neurons in the bulb.

Spatial patterns of odorant-evoked activity in the mammalian olfactory bulb have been visualized using a variety of different activity markers. These include 2-deoxyglucose metabolism (Johnson et al. 2002; Stewart et al. 1979), c-fos expression (Guthrie and Gall 1995; Schaefer et al. 2001), fMRI signals (Xu et al. 2000), and three optical measures of neural activity (Meister and Bonhoeffer 2001; Rubin and Katz 1999; Spors and Grinvald 2002; Wachowiak and Cohen 2001). Despite significant differences in the methodology of these approaches, findings common to all of these studies have emerged as important features of how odorants are represented in the mammalian olfactory bulb. First, odorants preferentially activate glomeruli in distinct subregions of the olfactory bulb, with structurally similar odorants often activating glomeruli in the same region, thus defining a regional chemotopy in the mapping of odorant identity. Second, odorants evoke activity in multiple glomeruli, such that odorant identity may be uniquely encoded by the combination and relative amount by which glomeruli are activated. Third, low concentrations of odorants activate small numbers of glomeruli, while higher concentrations activate more glomeruli. Thus both the chemical structure and the concentration of an odorant may be encoded by spatial patterns of olfactory bulb activity (Friedrich and Korsching 1997; Rubin and Katz 1999; Stewart et al. 1979; Wachowiak and Cohen 2001).

Other features of the relationship between olfactory stimuli and spatial patterns of olfactory bulb activity differ depending...
on the imaging method used. For example, 2-deoxyglucose and intrinsic optical imaging studies have both reported that structural features of an odorant, such as carbon chain length, are mapped in an ordered way within an activated subregion (Belluscio and Katz 2001; Johnson et al. 1999; Meister and Bonhoeffer 2001; Uchida et al. 2000), while such mapping has not been observed in studies imaging calcium influx into receptor neuron presynaptic terminals (Fried et al. 2002; Wachowiak and Cohen 2001). Different imaging methods also report differences in the number and distribution of glomeruli involved in the representation of a particular odorant. For example, several intrinsic imaging studies have reported that the six-carbon aldehyde hexanal activates glomeruli only within an anterior region of the dorsal olfactory bulb (Belluscio and Katz 2001; Meister and Bonhoeffer 2001; Uchida et al. 2000), while presynaptic calcium imaging studies have reported that hexanal can evoke receptor neuron input to glomeruli distributed widely across the dorsal surface (Fried et al. 2002; Wachowiak and Cohen 2001).

Differences in the spatial representations of odorants observed by different imaging methods could reflect real changes in the representations due to stimulus adaptation and/or synaptic processing. For example, patterns of 2-deoxyglucose uptake and c-fos expression reflect neuronal activity integrated over many minutes (Guthrie and Gall 1995; Johnson et al. 2002; Sallaz and Jourdan 1993; Stewart et al. 1979), while presynaptic calcium imaging reflects receptor neuron activity integrated over several hundred milliseconds (Fried et al. 2002; Wachowiak and Cohen 2001). Furthermore, 2-deoxyglucose, intrinsic, and fMRI imaging methods both depend on largely metabolic measures of activity in undefined neuronal populations (Lancet et al. 1982; Meister and Bonhoeffer et al. 2001; Rubin and Katz 1999; Xu et al. 2000), while calcium imaging specifically reflects activity in the presynaptic terminals of receptor neurons (Friedrich and Korsching 1997; Wachowiak and Cohen 1999, 2001). Alternatively, the differences in odorant representations obtained with different methods could simply reflect differences in the activity threshold, signal-to-noise ratio, or dynamic range of the methods. To date, no attempt has been made to directly compare odorant representations visualized by different imaging methods.

In this study, we compare odorant representations as reflected by calcium signals measured from the presynaptic terminals of receptor neurons and by intrinsic optical signals. These two signals can be easily recorded in the same preparation and in response to the same durations of odorant stimulation. In addition, while the neuronal basis for the presynaptic calcium signal is reasonably well defined (Friedrich and Korsching 1997; O’Donovan et al. 1993; Wachowiak and Cohen 1999, 2001), the origins of the intrinsic optical signal are less well understood and probably only indirectly reflect neuronal activity (Bonhoeffer and Grinvald 1996; Meister and Bonhoeffer 2001). Thus we hoped to learn the extent to which the intrinsic optical signal reflects how odorants are represented in terms of receptor neuron input, as opposed to postsynaptic activity. This information is important in interpreting imaged patterns of olfactory bulb activity and in understanding the strategies by which spatially organized activity patterns represent olfactory information at different synaptic levels in the olfactory bulb.

METHODS

Dye loading

Experiments were performed on C57/Bl6 mice, 8–12 wk of age. Olfactory receptor neurons were loaded in vivo with Calcium Green-1 dextran, 10 kD m.w. (12 animals) or Oregon Green BA PT A dextran, 10 kD m.w. (4 animals; see Figs. 5 and 6) (both from Molecular Probes, Eugene, OR) as described previously (Wachowiak and Cohen 2001). No differences were observed in the fluorescence signals measured with the two dyes. Mice were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg) and 2 μl of 0.25% Triton X-100 was perfused into the nasal cavity using a custom-made cannula (Cohen ‘Code Red II’ Stainer, MW Plastic Works, New Haven, CT). After 60 s, 2 μl of dye solution (4%, in mouse ringer) was perfused. Perfusion was repeated at 5-s intervals until a total volume of 4–8 μl had been injected. Mice recovered from anesthesia and were held for 4–8 days before imaging.

Imaging

In 12 preparations, mice were anesthetized with pentobarbital (50 mg/kg ip), and atropine (5 mg/kg) was injected subcutaneously. Two tubes were inserted into the trachea to allow control of odorant access to the nasal cavity (see following text). The mice breathed freely through the lower tracheotomy tube. Heart rate was maintained at 400–500 beats per minute by periodic injection of pentobarbital. In four preparations, mice were anesthetized with a mixture of medetomidine (0.5 mg/kg ip), diazepam (5 mg/kg ip), and fentanyl (0.01 mg/kg ip) and were freely breathing through the nose for the duration of the experiment. We observed no differences in the spatial patterns of optical signals in tracheotomized and freely breathing mice (see RESULTS). Mice were secured in a stereotaxic headholder and the skin overlying the dorsal skull was retracted. Local anesthetic (1% bupivacaine) was applied to all incisions. The bone overlying the olfactory bulbs was thinned to a thickness of 100–200 μm and Ringer solution and a coverslip were placed at 5-s intervals until a total volume of 25 mm was perfused. In vivo TTX application, the bone overlying the dorsal surface was removed prior to imaging. In these preparations, dexmedethasone (10 mg/kg) was injected subcutaneously at the start of the dissection to reduce brain swelling. Saline and a coverslip surrounded by gel foam was placed over the bulb. TTX (10–50 μm, Sigma-Aldrich, St. Louis, MO) was applied directly to the dorsal surface and left under the coverslip. All procedures were approved by the Yale University and Marine Biological Laboratory Animal Care and Use Committees.

For calcium imaging, the dorsal surface of one olfactory bulb was illuminated with 480 ± 25 nm light using a 150 W Xenon arc lamp (Opti-Quip, New York), a 515-nm long-pass dichroic mirror, and fluorescence emission above 530 nm was collected. For intrinsic imaging, the preparation was illuminated with light from a tungsten halogen lamp passed through a 630 ± 15 nm interference filter and transmitted to the preparation via fiber optic guides. Reflected light was collected using the same imaging system as for fluorescence. The intensity of the 630-nm reflected light was adjusted to be similar to the calcium dye fluorescence by adjusting the voltage on the halogen lamp power supply. Images were acquired and digitized with a 80 × 80 pixel CCD camera (NeuroCCD-SM, RedShirtImaging LLC, Fairfield, CT) at 125 Hz. Fluorescence signals were time-binned to 31.25 Hz frame rate before storing to disk; intrinsic signals were time-binned to 15 Hz. Optical signals were imaged using a 10.5 × 0.2 n.a. objective (1.7 × 1.7 mm field of view; spatial resolution, 22 μm2 per pixel assuming no scattering or out-of-focus signals) or a 14 ×, 0.4 n.a. objective (1.3 × 1.3 mm field; 16.5 μm2 per pixel resolution).

Odorant presentation

Odorants were obtained from Sigma or Fluka (all 95–99% pure). Odorants were diluted from saturated vapor with cleaned, desiccated
air using a flow dilution olfactometer described previously (Lam et al. 2000). Odorant concentrations are reported as percentage dilution from saturated vapor and as molar vapor concentrations. Vapor concentrations were calculated using the vapor pressure equation and appropriate coefficients, both provided by Yaws (1994). The accuracy and stability of the flow dilution system over the range used was confirmed with a photoionization detector. Dedicated lines for each odorant avoided cross-contamination.

Several of the odorants (2-hexanone, hexanal, octanal, benzaldehyde, and hexyl acetate) were screened for contaminants using a gas chromatograph/mass spectrometer, with temperatures and retention times chosen to cover components with vapor pressures as low as approximately 0.01 that of hexanal. No contaminants were detected within this range. Additionally, to check for oxidation of hexanal into hexanoic acid (Arctander 1994), hexanal vapor, as emitted from the olfactometer, was collected in methanol and screened by GC–MS. Contamination of the hexanal vapor with hexanoic acid was detectable only after >4 days storage of odorant in the olfactometer reservoir. To avoid this contamination, aliphatic aldehyde odorants (hexanal and octanal) were replaced in the olfactometer every 3 days or less. Stock solutions of aldehydes were stored under nitrogen.

In experiments using pentobarbital anesthesia, an artificial sniff paradigm controlled odorant access to the nasal cavity (Wachowiak and Cohen 2001). Only the naris on the side being imaged was left open. Square pulses of negative pressure (60–75 ml/min flow rate, 150 ms duration, 3.3 Hz) were applied to the upper tracheotomy tube. Sniffing was maintained throughout the experiment, with brief rest periods every several minutes. Cleaned, humidified air was continuously blown over the nares to prevent drying and was switched off during odorant presentation. In the experiments using medetomidine anesthesia, the animal was freely breathing at approximately 2 Hz. The olfactometer delivered square-shaped odorant pulses (2–6 s duration) at a flow rate of 300 ml/min. We waited a minimum of 45 s between trials. Repeated presentations of the same odorant at this interstimulus interval evoked similar-amplitude signals.

Data processing and analysis

While odorant-evoked signals were detected in single trials (see Fig. 1 of Wachowiak and Cohen 2001), we typically collected, then averaged, responses of four to eight consecutive odorant presentations to improve the signal-to-noise ratio and to obtain a measure of trial-to-trial variability. Individual trials were saved to disk and occasional trials with widespread artifactual signals (primarily due to movement) were discarded before averaging. A primary source of extrinsic noise was associated with respiration and/or heartbeat. Pixels receiving light from areas outside of the bulb were removed from the dataset (omitted) prior to analysis. After averaging, data from each pixel were temporally filtered. Except in Fig. 1, fluorescence signals were temporally filtered with a 1-Hz low-pass Gaussian and a 0.017-Hz high-pass digital RC filter (both filters have a low sharpness). Intrinsic signals were temporally filtered with a 0.5-Hz low-pass Gaussian and no high-pass filtering. To correct for unequal labeling of glomeruli and for uneven illumination, the signal from each pixel was divided by its resting fluorescence or reflectance obtained at the beginning of each trial. Spatial maps of response amplitudes were constructed from each pixel’s signal by subtracting the temporal average of a time window just preceding the stimulus from a temporal average centered near the peak of the response. The time windows were the same for all pixels in a given trial. Additional details regarding the choice of time windows used for averaging are described under RESULTS. Although different in order, the division by resting intensity and subtraction of a temporally averaged signal from baseline is equivalent to the operation used to construct response maps in recent intrinsic imaging studies of the olfactory bulb (Belluscio and Katz 2001; Meister and Bonhoeffer 2001; Rubin and Katz 1999). Additional spatial filtering or thresholding of the response maps was performed as described under RESULTS.

Two methods were used for measuring response amplitudes from individual glomeruli in the response maps. In the first, response amplitudes were measured by averaging the signal in four to six adjacent pixels in the center of a glomerulus. In the second, amplitudes were measured by fitting a one-dimensional Gaussian function to a profile (2–4 pixels wide) of the signal through a glomerulus. The first method gives a measure of signal amplitude relative to the resting fluorescence or reflectance, while the second method gives a measure of the amplitude relative to the local background signal.

In determining threshold and saturation concentrations from concentration–response function measurements, the threshold was defined as the lowest concentration tested that evoked a signal above background (glomeruli activated at the lowest concentrations tested were assigned a threshold equal to that concentration). Saturation concentration was defined as the concentration after which further concentration increases caused no increase in response amplitude (glomeruli showing no saturation were assigned a value equal to the highest concentration tested).

The correlation between maps of fluorescence and intrinsic signals was measured by identifying glomeruli showing optical signals above a given threshold level. We identified activated glomeruli according to criteria described previously (Wachowiak and Cohen 2001). The criteria included their appearance in multiple trials, a fit to a Gaussian function with an amplitude more than five times the noise in adjacent pixels, and a width similar to that of previously measured glomeruli. This approach required some judgment to be made in determining whether to count a signal as a glomerulus. However, we found that identifying glomeruli was preferable to computing the correlation of signal amplitudes across the entire image, because activated glomeruli occupied only a small fraction of all the pixels in the image. Thus small-amplitude signals representing noise or scattered light dominated the calculated correlations. Attempts to eliminate nonglomerular signals from the correlations by thresholding and/or spatial filtering also required judgments to be made about which signals represented glomeruli, to evaluate the filter. In some cases, intrinsic signal foci were clearly larger than a single glomerulus (see Figs. 1E, 3A, and 6A). For the purposes of the correlation analysis, these signals were counted as one glomerulus if only one or no glomeruli appeared in that location in the corresponding calcium signal maps but were counted as multiple glomeruli if multiple glomeruli appeared in the calcium maps.

For display in the figures, the maps of response amplitudes were smoothed slightly using a $3 \times 3$ pixel kernel with the center pixel given a weight of 6. The pixel resolution of these maps was then increased by a factor of two by linear interpolation between pixels. In most figures, response maps were normalized to the maximum signal amplitude for that trial and, except as noted, clipped at zero amplitude (i.e., “negative”-going signals were clipped to zero; we did not detect reproducible “negative”-going signals). Data processing and display was performed with NeuroPlex software (RedShirtImaging LLC, Fairfield, CT) and with custom software written in IDL (Research Systems, Boulder, CO) and LabVIEW (National Instruments, Austin, TX).

RESULTS

We imaged odorant-evoked calcium signals, as well as intrinsic optical signals, from the dorsal olfactory bulbs of 16 mice, 4–8 days after loading olfactory receptor neurons with Calcium Green-1 dextran (see METHODS). The artificial sniff paradigm ($n = 12$ animals) resulted in faster onset kinetics for the calcium signal than seen in freely breathing animals ($n = 4$) and also increased the consistency of evoked response amplitudes over time. However, we observed no differences in
the spatial or slow temporal character of either of the optical signals with the different anesthetic and odorant presentation regimens. These data were thus pooled in further analyses.

Initial comparison of receptor neuron input and intrinsic optical signals

General features of the kinetics and spatial organization of the calcium and intrinsic signals are illustrated in Fig. 1. Figure 1A, top left, shows a fluorescence image of the dorsal olfactory bulb from a Calcium Green dextran-loaded animal, revealing many labeled glomeruli. Labeling is brightest anterolaterally, where overlying receptor axons partially obscure individual glomeruli. Major blood vessels appear dark. An image of the same region viewed with 630 nm reflected light shows much less contrast (Fig. 1A, bottom left).

Figure 1B shows the time course of the changes in fluo-
calcium signal) and reflectance (intrinsic signal) evoked by a 4-s odorant presentation, measured from three different glomeruli. Odorant stimulation evokes a rapid increase in fluorescence (time to half-maximum = 245 ± 10 ms, mean ± SE, n = 9 odorant responses measured in 7 preparations with exceptional signal-to-noise ratios), reflecting the activation of receptor neuron populations converging onto these glomeruli. As noted earlier (Wachowiak and Cohen 2001), the precise kinetics of this calcium signal varied somewhat across glomeruli and for different odorants. However, the calcium signal most often consisted of a phasic and a tonic component, with the tonic component returning to baseline approximately 1 s after the end of odorant stimulation (Fig. 1B). In contrast, the odorant-evoked intrinsic signal appeared as a decrease in reflectance at 630 nm and was much slower. The time to half-minimum of the intrinsic signal was 1560 ± 140 ms (n = 9 odorant responses in 7 preparations, same odorants, preparations, and glomeruli as for calcium signal measurements). The intrinsic signal kinetics were less complex and showed little or no variation across glomeruli or for different odorants (not shown). The intrinsic signal returned to baseline slowly (Fig. 1B), even after a brief stimulus, with a recovery time typically outlasting our recordings (>8 s). The amplitude of the intrinsic signal was generally smaller than that of the calcium signal, ranging from approximately 0.1 to 2% ΔI/I, versus 0.1 to 6% ΔF/F for the calcium signal. However, because the lower contrast in the reflectance image resulted in less noise from respiration and heartbeat, the signal-to-noise ratios of the fluorescence and intrinsic signals were similar (Fig. 1B).

We recorded intrinsic signals from five animals that were not loaded with Calcium Green dextran. In addition, in two animals we loaded receptor neurons on one side only and recorded intrinsic signals on the loaded and unloaded sides. In all cases, the intrinsic signals recorded from Calcium Green dextran-loaded preparations were similar in amplitude, kinetics, odorant concentration thresholds, and spatial organization (described in the following text) to those measured from unloaded preparations. Thus loading receptor neurons with Calcium Green dextran does not significantly alter odorant responsiveness as measured using intrinsic signals.

Figure 1C shows spatial maps of the evoked calcium and intrinsic signals obtained at different times after odorant onset. The time window used for constructing the response maps is indicated by the gray columns in Fig. 1B. Because of the slower kinetics, maps of the intrinsic signal were constructed by integrating across a longer time window (1,280 ms) than for the calcium signal (320 ms). The maps in Fig. 1C are normalized to their own maxima and minima and use the same linear gray scale, such that fluorescence increases in the calcium signal appear light and reflectance decreases in the intrinsic signal appear dark. The calcium signal reaches a peak amplitude approximately 600 ms after stimulus onset and contains numerous foci corresponding to individual glomeruli. A diffuse, small-amplitude change in fluorescence is also present (see spot 3 trace in Fig. 1B), reflecting scattered or out-of-focus light and/or small amounts of calcium influx into receptor neuron axons distal to their entrance into glomeruli. The onset kinetics of the small-amplitude, diffuse calcium signals were identical to those of the glomerular calcium signals. At later times (>2 s poststimulus onset), we observed a decrease in fluorescence overlying some, but not all, major blood vessels (Fig. 1C, top right frames). In two preparations, we loaded receptor neurons with fluorescein dextran (10 kD) and imaged fluorescence changes evoked by odorant stimulation. In both preparations, odorants evoked no fluorescence increases but did elicit fluorescence decreases over blood vessels (not shown).

Despite the slower kinetics, the spatial organization of the intrinsic signal appears roughly similar to that of the presynaptic calcium signal (Fig. 1C, bottom). The intrinsic signal map is poorly defined at the time of peak calcium signal amplitude (approximately 640 ms after stimulus onset) but, at approximately 3 s after stimulus onset, contains both focal and diffuse components (Fig. 1C, bottom right and middle frames). Focal intrinsic signals appear as dark, roughly circular areas while the diffuse component appears as a gray background. Many of the intrinsic signal foci correspond to glomeruli present in the calcium signal maps. Comparison of the diameter of the glomerular calcium signals with their corresponding intrinsic signal foci, measured from multiple preparations, revealed that

**FIG. 1.** Time course of odorant-evoked calcium and intrinsic optical signals in the mouse olfactory bulb. A: Top: resting fluorescence image showing Calcium Green-1 dextran labeling of olfactory receptor neuron axons innervating the dorsal mouse olfactory bulb. Many labeled glomeruli and blood vessels are visible. Brightly fluorescent axon fibers overlay glomeruli anterior and lateral. Bottom: image of the same preparation illuminated with 630 nm light. The image shows little contrast between blood vessels, bulb tissue, and surrounding bone. Spots 1–3 indicate glomeruli for which time courses are shown in B. The glomerulus at spot 1 is not distinct because of fluorescence from overlying axons. B: time course of the fluorescence (calcium; 480 nm) and reflectance (intrinsic; 630 nm) signals evoked by 2-hexanone, measured from the three glomeruli indicated in A. Each trace is a spatial average of 6 pixels and 4 (480 nm) or 8 (630 nm) successive odorant presentations. Traces are low-pass—filtered at 2 Hz. The downward drift in the fluorescence traces is due to dye bleaching. This drift was removed with a high-pass filter (see METHODS) when constructing the maps in C. Note that the vertical scale is different for the fluorescence and reflectance traces. Horizontal dashed lines indicate the baseline for the intrinsic signal. Vertical dashed lines (i–iv) indicate the time points used for constructing the maps in C; shaded boxes with asterisks show the time window used to generate the maps. C: spatial maps of evoked changes in fluorescence (top) and reflectance (bottom), relative to resting levels, at different times after odorant onset. i–iv, the times shown in A. Each map is normalized to its own maximum and minimum. t, center time of the window used for each map. i–iv correspond to the glomeruli and traces in A and B. Asterisk: maps corresponding to the shaded boxes in B and also indicate the approximate time windows used in generating response maps for all subsequent figures and analyses. Fluorescence maps are generated by integrating over a shorter time window than the intrinsic signal map. Arrows indicate blood vessels that appear as late fluorescence and reflectance decreases in the calcium and intrinsic signal maps. Arrowheads indicate a blood vessel that appears as a late reflectance increase in the intrinsic signal map but shows no change in fluorescence in the calcium signal map. Dashed boxes indicate the locations of the insets shown in D. The maps reveal short-latency fluorescence increases in multiple glomeruli (top), and longer-latency reflectance decreases that are both focal and widespread in character (bottom). D: maps of the anterior lateral quadrant of the fluorescence and reflectance maps shown in C. Each map is normalized to its own maximum or minimum and clipped at zero signal amplitude. Several adjacent glomeruli can be distinguished in the fluorescence map (left). In the reflectance map (right) these glomeruli appear as a single large intrinsic signal focus.
intrinsic signal foci were approximately 45% larger in diameter (approximately 70% larger in area) than the glomerular calcium signals (width at half-maximum = 85 ± 3 μm (calcium signal) versus 122 ± 6 μm (intrinsic signal); n = 46). We also compared the kinetics of the focal and diffuse intrinsic signals by measuring the time to half-minimum at intrinsic signal foci corresponding to glomeruli activated by receptor neuron input and at locations showing no detectable receptor input. The time to half-minimum of the focal signals was slightly but significantly faster than that of the diffuse signals (1.6 ± 0.1 s vs 1.9 ± 0.2 s; P = 0.03, paired t-test, n = 9).

As with the fluorescence signal, blood vessel–associated changes in reflectance appear at later times after stimulation. Some blood vessels appear as a relative darkening while others show a late brightening (Fig. 1C, right frames). Interestingly, vessels that appear dark in the intrinsic signal maps also appear dark in the fluorescence maps (Fig. 1C, arrows), while those showing a brightening do not appear at all in the fluorescence maps (Fig. 1C, arrowheads). The intrinsic signal maps became slightly more diffuse with time, especially in response to higher odorant concentrations. We found that an integration time window of approximately 1.2 s, centered at 2.5–3.5 s after stimulus onset, resulted in maps with the most well-defined focal components and an adequate signal-to-noise ratio. For maps of the presynaptic calcium signal we used a time window of 320–640 ms, centered at 500–800 ms after odor onset.

To test whether any component of the optical signals might reflect odorant-evoked input to nondorsal regions of the olfactory bulb, for example, via long-range synaptic interactions, we applied TTX (10–50 μM) to the area of the dorsal bulb imaged in our optical recordings in three preparations. TTX eliminated all of the odorant-evoked calcium signal, indicating that receptor neuron input to the dorsal bulb was blocked. TTX also eliminated all focal intrinsic signals. The diffuse component of the intrinsic signal was not eliminated but was reduced to 39 ± 5% of its original amplitude (n = 3). The kinetics of the diffuse component were unchanged after TTX application. This result suggests that, in addition to the focal intrinsic signals, approximately 60% of the diffuse intrinsic signal component is driven by receptor neuron input to proximate (i.e., dorsal) regions of the bulb, while the remainder of the diffuse signal results from receptor neuron input to areas outside the imaged dorsal region.

The maps in Fig. 1C show a general correspondence between glomeruli receiving receptor neuron input and intrinsic signal foci. However, differences in the two maps are also apparent. For example, the glomerulus at spot 2 shows a clear calcium signal in response to 2-hexanone but does not appear in the intrinsic signal map. Also, activation of several adjacent anterolateral glomeruli can be resolved in the calcium signal maps; these glomeruli appear as a single, large focus in the intrinsic signal map (Fig. 1D). Figure 2A shows calcium and intrinsic signal maps in response to increasing concentrations of a different odorant, benzaldehyde, imaged from the same preparation as in Fig. 1. Increasing benzaldehyde concentration recruits receptor neuron input to numerous, widely distributed glomeruli and also recruits additional intrinsic signal foci. At the same time, increasing concentration increases the relative amplitude of the diffuse intrinsic signal, which appears as an increasingly gray background (Fig. 2A, bottom frames).

Differences in the onset kinetics of the focal and diffuse intrinsic signals were too small to use as a means for separating the two components. Instead, as described previously (Meister and Bonhoeffer 2001), we found that subtracting a spatially blurred version of the response map from the original image and thresholding the maps at zero signal amplitude was effective at removing most of the diffuse component from the response maps. We performed the same operation for the maps of the calcium signal.

Figure 2B shows the same data as in Fig. 2A after low-frequency subtraction and after inverting the gray scale for the calcium signal to match that of the intrinsic signal maps. The majority of glomeruli activated by receptor neuron input show corresponding focal intrinsic signals. Even glomeruli showing relatively low levels of receptor neuron input appear as intrinsic signal foci (Fig. 2B, open arrowhead). However, as in Fig. 1, differences between the calcium and intrinsic signal response maps are apparent. For example, the response to 0.13% saturated vapor (s.v.) benzaldehyde (left-most maps) reveals two intrinsic signal foci with no corresponding calcium signal (Fig. 2B, left frames, arrows). At higher benzaldehyde concentrations, however, these glomeruli show both calcium and intrinsic signals, suggesting that the threshold for detection of the two signal types may differ slightly. In contrast, the filled arrowheads in Fig. 2B mark two glomeruli that show a clear calcium signal in response to 0.4% s.v. and higher concentrations of benzaldehyde but show no corresponding focal intrinsic signal at any concentration. A third difference apparent in the maps of Fig. 2B is that the glomeruli showing the highest-amplitude calcium signals are sometimes different from the glomeruli showing the highest-amplitude intrinsic signals (see 2% s.v. and 5.6% s.v. response maps).

We investigated the relationship between receptor neuron input and intrinsic signals more systematically using two approaches. First, we compared concentration–response functions of the calcium and intrinsic signals for individual glomeruli. Second, we compared the spatial organization of maps of the calcium and intrinsic signal responses obtained in response to different odorants presented at near-threshold and suprathreshold concentrations.

Concentration–response functions of receptor neuron input and intrinsic signal foci

We compared concentration–response functions of the calcium and intrinsic signal in 15 glomeruli from five preparations. An example from one preparation is shown in Fig. 3. A common effect of increasing odorant concentration was a reduction in the definition of intrinsic signal foci (also apparent in Figs. 1 and 2). Figure 3A shows responses to low and high concentrations of acetophenone. The calcium signal (Fig. 3A, top maps) reveals numerous glomeruli in the caudal–lateral bulb receiving receptor neuron input. Activation of individual glomeruli can be easily resolved at both low (0.4% s.v./0.1 μM) and high (5.6% s.v./1.4 μM) concentrations (Fig. 3A, top inset). However, while this region also shows a strong intrinsic signal, individual glomeruli are poorly resolved at the high concentration (Fig. 3A, bottom inset).

Figure 3B shows concentration–response functions for five glomeruli activated by acetophenone, where response amplitudes were measured relative to the surrounding diffuse signal from unthresholded, low-frequency–subtracted maps (see
FIG. 2. Correspondence between spatial maps of odorant-evoked calcium (fluorescence) and intrinsic optical signals (reflectance). A: maps of fluorescence (top) and reflectance (bottom) changes evoked by increasing concentrations of benzaldehyde, imaged from the same preparation as in Fig. 1. Maps were made from the approximate time windows indicated by the shaded boxes in Fig. 1B. Fluorescence maps show intensity increases; reflectance maps show intensity decreases. Each map is normalized to its own maximum or minimum and clipped at zero signal amplitude and at 90% of the maximum signal change. The magnitude of the signal change is indicated below each map. Benzaldehyde evokes fluorescence increases in numerous glomeruli (top), with corresponding focal decreases in reflectance (bottom). Benzaldehyde also evokes a diffuse reflectance decrease (gray background, lower maps) which increases in relative magnitude with increasing odorant concentration. The approximate border of the dorsal surface of the olfactory bulb is drawn in gray. B: same data as in A after subtraction of the low-frequency components of the response maps (details in text). Maps are clipped at zero. The gray scale of the fluorescence maps is inverted to facilitate comparison to the reflectance maps. The vertical and horizontal lines indicate the approximate anterior–posterior and medial–lateral meridians of the dorsal bulb. Most glomeruli activated by receptor neuron input (top maps) appear as focal intrinsic signals (bottom maps), although often not as distinctly. Open arrowhead indicates a weakly activated glomerulus that appears in both the fluorescence and reflectance maps. Closed arrowheads indicate two glomeruli that appear in the fluorescence maps at dilutions of 0.4% s.v. and higher, but not the reflectance maps. Arrows indicate two glomeruli that are apparent at 0.13% s.v. and higher in the reflectance maps but are only apparent at 0.4% s.v. and higher in the fluorescence maps.
METHODS. Calcium signals reflecting receptor neuron input to four of the glomeruli (1, 2, 3, and 5) were detectable at the lowest concentration tested (0.016 μM). The signal in glomerulus 2 increased in amplitude over the entire tested range (0.016–1.4 μM), while the remaining glomeruli appeared to saturate at concentrations below 1.4 μM. By comparison, the lowest concentration of acetophenone (0.016 μM) evoked a focal intrinsic signal in only one of five glomeruli (Fig. 3B, glomerulus 1). Glomerulus 5 failed to appear as a focal intrinsic signal at any concentration. Finally, the amplitude of the focal intrinsic signals in glomeruli 2 and 4 saturated at lower concentrations than did the calcium signals. In fact, the focal intrinsic signal in glomerulus 2 could not be distinguished from the surrounding diffuse signal at the highest concentration (1.4 μM/5.6% s.v.; Figs. 3A, right frames and 3B). Measurement of the absolute fluorescence and intrinsic signal amplitudes, relative to the resting light levels (see METHODS), increased across all concentrations for all five glomeruli (Fig. 3C), indicating that the saturation observed in particular glomeruli did not reflect saturation of the optically recorded response as a whole. Thus concentration–response functions for receptor neuron input to a glomerulus and the intrinsic signal activation specific to that glomerulus often differed.

We measured concentration–response functions and com-

![FIG. 3. Concentration–response relationships for calcium and intrinsic signals in glomeruli. A: calcium and intrinsic signal response maps for near-threshold (0.4% s.v., 0.1 μM) and high (5.6% s.v., 1.4 μM) concentrations of acetophenone. For this and all subsequent figures, the maps were low-frequency subtracted, normalized, and clipped, and the outline and meridians of the dorsal olfactory bulb are shown as in Fig. 2. At these concentrations, acetophenone activates numerous lateral glomeruli. Inset: (5.6% response maps) response in the caudal–lateral quadrant of the bulb, rescaled to show the spatial definition of signals in that region. At least 7 nearby glomeruli remain distinct in the calcium signal map (top), but lose definition in the intrinsic signal map (bottom). The amplitude of the intrinsic signal for these glomeruli, relative to the surrounding signal, is thus reduced at high acetophenone concentrations. Numbers indicate glomeruli for which concentration–response functions were measured. Arrow indicates a glomerulus recruited by 5.6% acetophenone in both the calcium and intrinsic signal maps. B: concentration–response functions of the calcium (left) and intrinsic (right) signals for the 5 glomeruli indicated in A. Response amplitudes are measured relative to the local background from low-frequency–subtracted, unthresholded maps (see METHODS for details). Some glomeruli show continuous increases in signal amplitude over much or all of the concentration range while others show saturation at lower concentrations. The intrinsic signal in glomeruli 2 and 4 saturates at a lower concentration than does the calcium signal. The intrinsic signal in glomerulus 5 is never distinguishable from background. C: concentration–response functions for the same glomeruli, with response amplitudes measured relative to resting light intensities in unsubtracted images. Both the calcium and intrinsic signals increase in magnitude across the entire concentration range, indicating that the overall responsiveness of the system does not saturate at the highest concentrations.]

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pared the threshold and saturation concentrations for 15 glomeruli in the five preparations tested (see METHODS). We included only glomeruli tested across a concentration range of at least two log units, and only those for which both a calcium and an intrinsic signal could be detected (i.e., glomerulus 5 in Fig. 3 was excluded). The results are shown in Fig. 4. Threshold concentrations for detecting calcium and intrinsic signals were similar, with glomeruli showing threshold differences of <0.3 log units in 10 of 17 concentration series. The detection threshold was slightly (<1 log unit) lower for the calcium signal than for the intrinsic signal in five of the remaining seven cases. In contrast, saturation concentrations were different by more than 0.3 log units in the majority of cases (12 of 17), and the magnitude of these differences could be large in cases in which the intrinsic signal saturated earlier than the calcium signal. The intrinsic signal saturation concentration was more than 0.5 log unit higher than that for the calcium signal in only one case but was more than 0.5 log unit lower in five cases. These results indicate that the dynamic range of the intrinsic signal associated with a glomerulus has a tendency to be compressed relative to that of the calcium signal.

**Correspondence between relative maps of receptor neuron input and intrinsic signals**

We next compared how presynaptic calcium and intrinsic signals represent odorants in terms of spatial patterns of activity across glomeruli. We compared pairs of calcium and intrinsic signal maps evoked by near-threshold and suprathreshold odorant concentrations. At near-threshold concentrations (Fig. 5), calcium and intrinsic signal maps were similar. Figure 5A shows examples from four odorant response pairs, imaged from two different preparations. Most glomeruli activated in the calcium signal maps (Fig 5A, top row) show a corresponding intrinsic signal focus. As in Fig. 2, even weakly activated glomeruli show both calcium and intrinsic signals (Fig. 5A, red arrows).

We measured the correspondence between calcium and intrinsic signal responses by comparing glomeruli activated above two arbitrary threshold levels (Fig. 5, B and C). The criteria used to identify glomeruli are described under METHODS. Figure 5B shows the correspondence between glomeruli showing signal amplitudes >10% of the maximum signal amplitude measured for each map. This threshold level results in the inclusion of most activated glomeruli, some of which do not appear distinct as displayed in Fig. 5A. Weakly activated glomeruli were distinguished from background by rescaling the response maps and screening for the identifying criteria described under METHODS. At this 10% threshold level, the correspondence between the response maps in Fig. 5, indicated by the red circles in Fig. 5B, was high. Across all preparations tested, 80% of all glomeruli receiving receptor neuron input showing a corresponding intrinsic signal focus ($R_{in} = 0.80$) and 71% of all intrinsic signal foci were matched with a receptor neuron input signal ($R_{int} = 0.71$; see Table 1). By comparison, calcium or intrinsic signal maps acquired in response to repeated presentations of an odorant showed a 92% correspondence (Table 1).

The green and blue circles in Fig. 5B mark glomeruli showing a calcium signal but no corresponding intrinsic signal above the 10% cutoff (green) or vice versa (blue). In approximately half of these cases, a glomerular signal was apparent in one map but not the other (Fig. 5A, solid blue and green arrowheads). In the remaining cases, the glomerular signal was apparent in both maps but was below the 10% threshold level in one of the maps (Fig. 5A, open arrowheads). Thus the actual correspondence between glomeruli receiving receptor neuron input and those showing a focal intrinsic signal is likely somewhat higher than the 80 and 71% values in Table 1.

To evaluate the similarity in the relative amplitudes of the calcium and intrinsic signals across glomeruli, we measured the correspondence between the most strongly activated glomeruli in the calcium and intrinsic signal maps using a signal threshold of 50% maximal signal amplitude. For the maps in Fig. 5C, this correspondence is somewhat less than for the 10% cutoff level (fewer red circles and smaller $R$ values in Fig. 5C). Across all preparations tested, however, the correspondence
FIG. 5. Correspondence between presynaptic calcium and focal intrinsic signals evoked by near-threshold odorant concentrations. A: low frequency–subtracted maps of responses to near-threshold concentrations of 4 odorants, imaged from 2 different preparations (preparation 1, two left maps; preparation 2, two right maps). For preparation 1, the anterior portion of the bulb (indicated by the asterisk) was not imaged. For all odorants there is a close correspondence between glomeruli activated by receptor neuron input and focal intrinsic signals. Red arrows indicate weakly activated glomeruli appearing in both the fluorescence and intrinsic signal maps. Solid arrowheads (acetophenone and hexanal maps) indicate glomeruli present in the calcium but not the intrinsic signal maps (green) or vice versa (blue). Open arrowheads (benzaldehyde, acetophenone, and hexanal maps) indicate glomeruli in which a focal signal is present in both maps but falls below the 10% threshold cutoff in either the calcium (green) or intrinsic signal (blue) maps. These glomeruli therefore appear as unmatched in the overlays in B. B: correspondence between glomeruli showing signal amplitudes >10% of the maximum response (measured separately for each map). Green circles, glomeruli appearing only in the calcium signal maps. Blue circles, glomeruli appearing only in the intrinsic signal maps. Red circles, glomeruli activated above threshold in both maps. The correspondence ratios ($R_{Ca}$, $R_{int}$) for glomeruli in the calcium and intrinsic signal responses is given below each map (see Table 1 legend for explanation). Glomeruli were chosen according to criteria described under METHODS. The correspondence between activated glomeruli is high (many red circles). C: correspondence between glomeruli showing signal amplitudes >50% of the maximum response. Overlays are the same as in B. For 2-hexanone and benzaldehyde, the correspondence between the glomeruli activated above the 50% threshold is less than the correspondence between glomeruli activated above the 10% threshold. For acetophenone and hexanal, the correspondence is similar.
Correlations are based on numbers of glomeruli activated above a given signal threshold (10% max, 50% max) or on the correlation between the four to six most strongly activated glomeruli (4–6 strongest). Values are means ± SE. Glomeruli were counted according to criteria described under METHODS. $R_{\text{Ca}}$, the fraction of all glomeruli showing a calcium signal that had a corresponding intrinsic signal focus, $R_{\text{int}}$, the fraction of all intrinsic signal foci with a matching calcium signal. Repeats, the correlation between repeated odorant presentations imaged with the same signal type, $n$, the number of response pairs. Correlations that are significantly lower at suprathreshold than at near-threshold concentrations, * $P < 0.05$; ** $P < 0.01$.

was still relatively high ($R_{\text{Ca}} = 0.75$, $R_{\text{int}} = 0.66$, Table 1). Thus, at near-threshold odorant concentrations, spatial patterns of intrinsic optical signals are both qualitatively and, roughly, quantitatively similar to those of receptor neuron input to dorsal glomeruli.

At higher odorant concentrations, the correspondence between calcium and intrinsic signal response patterns decreased. Figure 6A shows responses to suprathreshold concentrations of the same odors from the same preparations as in Fig. 5. Despite the fact that suprathreshold concentrations evoke calcium and intrinsic signals in more glomeruli than at near-threshold concentrations, there appear to be more focal intrinsic signals with no corresponding calcium signal and vice versa (Fig. 6A, solid blue and green arrowheads). As a result, the correspondence between response maps at the 10% threshold level is less (compare Figs. 5B and 6B). Across all preparations, the correspondence between the calcium and intrinsic signal maps was significantly lower for suprathreshold odorant concentrations than for near-threshold concentrations (Table 1). Nonetheless, the average correspondence between calcium and intrinsic signal foci remained at approximately 70% (average of $R_{\text{Ca}}$ and $R_{\text{int}}$, Table 1).

In contrast, the correspondence between glomeruli activated above the 50% cutoff level dropped substantially at suprathreshold concentrations (compare Figs. 5C and 6C). The average correspondence between the most strongly activated glomeruli in each response map was only 51% (average of $R_{\text{Ca}}$ and $R_{\text{int}}$, Table 1). Thus suprathreshold response maps of calcium and intrinsic signals differed significantly in their relative patterns of signal amplitudes across glomeruli. This effect was seen in all preparations tested, independent of odorant. Similar low correspondence between response pairs was seen when the four to six most strongly activated glomeruli in each map were compared (Table 1). In fact, in Fig. 6A, with the exception of the acetophenone response, the four to six glomeruli showing the strongest receptor neuron input are almost all different from those showing the strongest intrinsic signals.

**Topography of receptor neuron input and intrinsic signal response patterns**

The general topography of an odorant-evoked response pattern across the dorsal surface of the olfactory bulb was characteristic for a particular odorant, as reported previously for both receptor neuron input and intrinsic signal responses (Uchida et al. 2000; Wachowiak and Cohen 2001). In Fig. 5A, for example, 2-hexanone, acetophenone, and benzaldehyde elicit maximal receptor neuron input to caudal–lateral glomeruli, while hexanal evokes strong input to several anterior–medial glomeruli. This topography is not sharply defined, however—note that benzaldehyde activates two medial glomeruli and hexanal strongly activates a single caudal glomerulus (Fig. 5A). The topographic organization of the intrinsic signal maps in Fig. 5A is similar to that of the calcium signal maps. At suprathreshold odorant concentrations, however, the topography of the response maps differs (Fig. 6A). For example, the glomeruli showing the strongest calcium signal in response to hexanal are still located anteromedially, but the strongest intrinsic signals have shifted laterally. Similar differences in the topography of the calcium and intrinsic signal responses can be seen for 2-hexanone and benzaldehyde (Fig. 6A).

To assess the difference in the topography of odorant representations by calcium and intrinsic signals, we thresholded response maps at 50 or 80% of the maximum signal amplitude and compared the positions of the centroids of the remaining strong signals for each pair of calcium and intrinsic signal maps. As a control, we performed the same analysis for repeated presentations of the same odorant. The results are shown in Table 2. At near-threshold concentrations, the difference in centroid position of the strongest calcium and intrinsic signals was not significantly greater than for repeat odorant presentations ($P = 0.09, 50\%$ threshold; Table 2), but there was a highly significant difference at suprathreshold concentrations ($P = 0.007, 50\%$ threshold; Table 2). In fact, at the 80\% threshold level, which isolates the few most strongly activated glomeruli, the mean difference in centroids of the calcium and intrinsic signals was $510\,\mu\text{m}$ at suprathreshold concentrations compared with $272\,\mu\text{m}$ at near-threshold concentrations ($P < 0.001$). Further, only 3 of 16 response pairs showed centroid position differences of greater than $400\,\mu\text{m}$ at near-threshold concentrations compared with 9 of 14 response pairs tested at suprathreshold concentrations.

The centroid analysis is a relatively insensitive measure of changes in topography of distributed odorant responses because even the most strongly activated glomeruli in the maps can be distributed widely across the dorsal surface (see Fig. 6A, acetophenone and hexanal intrinsic signal maps). This analysis also only reflects changes in the topography of response patterns across a relatively small change in concentration (5-to 20-fold). Figure 7A shows changes in the topography of the

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**Table 1. Correspondence between activated glomeruli in calcium and intrinsic signal maps**

<table>
<thead>
<tr>
<th>Signal Threshold</th>
<th>Near-threshold Concentration</th>
<th>Suprathreshold Concentration</th>
<th>Repeats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_{\text{Ca}}$ $R_{\text{int}}$</td>
<td>$R_{\text{Ca}}$ $R_{\text{int}}$ $\text{Repeats}$</td>
<td></td>
</tr>
<tr>
<td>10% max</td>
<td>$0.80 \pm 0.03$ $0.71 \pm 0.05$</td>
<td>$0.69 \pm 0.03^*$ $0.73 \pm 0.04$</td>
<td>$0.92 \pm 0.08$</td>
</tr>
<tr>
<td>50% max</td>
<td>$0.75 \pm 0.05$ $0.66 \pm 0.05$</td>
<td>$0.58 \pm 0.06$ $0.44 \pm 0.05^*$</td>
<td>$0.87 \pm 0.06$</td>
</tr>
<tr>
<td>4–6 strongest</td>
<td>$0.62 \pm 0.04$ $0.62 \pm 0.04$</td>
<td>$0.39 \pm 0.07^<em>$ $0.39 \pm 0.07^</em>$</td>
<td>$0.72 \pm 0.08$</td>
</tr>
<tr>
<td>$n$</td>
<td>18</td>
<td>18</td>
<td>16</td>
</tr>
</tbody>
</table>

Correlation between calcium and intrinsic signal maps imaged at near-threshold (0.1–0.4% s.v.) and suprathreshold odorant concentrations (1–2% s.v.).
intrinsic signal response evoked by increasing concentrations of hexanal across a larger range (approximately 300-fold increase). The change in the topography of the response is primarily due to the recruitment of large-amplitude signals laterally (Fig. 7A, solid arrowheads), an effect observed frequently (see also Fig. 6A). We also observed that some intrinsic signal foci activated at low concentrations could saturate and become indistinguishable from background (disappear) at higher concentrations (Fig. 7A and B, arrows). Blood vessel artifacts, sometimes focal in appearance, also became more apparent at higher odorant concentrations (Fig. 7A, open arrowhead).

The lateral shift in topography and the disappearance of glomeruli were rarely observed for the calcium signal. Figure
TABLE 2. Effect of concentration on topography of calcium and intrinsic signal maps

<table>
<thead>
<tr>
<th>Signal Threshold</th>
<th>Difference in Centroid Position (μm)</th>
<th>Repeats</th>
<th>Near-threshold</th>
<th>Suprathreshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% max</td>
<td>134 ± 24</td>
<td>226 ± 36</td>
<td>362 ± 50**</td>
<td></td>
</tr>
<tr>
<td>80% max</td>
<td>156 ± 41</td>
<td>273 ± 45</td>
<td>510 ± 67***</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>16</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

Differences in centroid position of the strongest calcium and intrinsic signals evoked by near-threshold and suprathreshold odorant concentrations. Response maps were low frequency–subtracted and thresholded at 50 and 80% of the maximum signal amplitude. Differences in centroid position between calcium and intrinsic signal maps were compared to differences between responses to repeated odorant presentations imaged with the same signal type (Repeats). Values are means ± SE. Differences in centroid position that are significantly greater than for repeat presentations, **P < 0.01; ***P < 0.001. n, number of odorant response maps.

7B shows calcium and intrinsic signal responses to a hexanal concentration series in a different preparation. Again, higher concentrations of hexanal recruit strong intrinsic signals laterally (Fig. 7B, solid arrowheads). The highest concentration of hexanal also elicits a strong blood vessel artifact medially, with no corresponding calcium signal (Fig. 7B, open arrowheads). By contrast, while the calcium signal shows recruitment of input to glomeruli with increasing concentration, the region of strongest receptor neuron input remains more anterior and medial at all concentrations. In fact, the strongest lateral intrinsic signal focus has no corresponding calcium signal and is larger than a single glomerulus. Figure 7B also shows that some intrinsic signal foci disappear at high concentrations, while calcium signals in these glomeruli remain (Fig. 7B, arrows).

DISCUSSION

Local and distant determinants of the odorant-evoked intrinsic optical signal

Several recent studies have used intrinsic optical signals to characterize the representation of odorants across the dorsal olfactory bulb (Belluscio and Katz 2001; Meister and Bonhoeffer 2001; Rubin and Katz 1999; Uchida et al. 2000). All have shown that odorant-evoked intrinsic signals consist of a widespread, diffuse component as well as focal signals that appear to correspond to individual glomeruli. However, the biophysical basis for the intrinsic optical signal in the olfactory bulb, or in other brain areas, is not simply defined. The signal may arise from changes in blood flow in response to increased neuronal activity, changes in blood oxygenation levels, changes in light scattering associated with activation of presynaptic or postsynaptic neurons, or a combination of these effects (for review see Bonhoeffer and Grinvald 1996). Furthermore, the diffuse and focal components of the intrinsic signal in the olfactory bulb might arise from different sources (Meister and Bonhoeffer 2001). In the present study, focal intrinsic signals showed slower, and less complex, kinetics than did calcium-sensitive dye measurements of receptor neuron input and were also larger in area. Thus it seems unlikely that the intrinsic signal is a simple measure of receptor neuron activation. However, the generally close correspondence between the presence of presynaptic calcium and intrinsic signal foci, especially at low odorant concentrations, indicates that focal intrinsic signals in the olfactory bulb are strongly driven by receptor neuron input.

In contrast, the diffuse component of the intrinsic signal was present even in bulb regions receiving no detectable receptor neuron input. Indeed, approximately 40% of the diffuse signal persisted after eliminating receptor neuron input to the dorsal bulb with TTX. The onset kinetics of the diffuse component were slightly slower than those of the focal signals, indicating a possibly different origin. The “long-distance” component of the diffuse signal could be driven by an increase in blood flow to the entire olfactory bulb, a lateral spread of activity in postsynaptic bulbar neurons via mitral cell secondary dendrites, or via centrifugal input (McLean and Shipley 1987).

Receptor neuron activation of focal intrinsic signals

Lateral synaptic interactions between glomeruli could alter glomerular representations of odorants at the presynaptic versus the postsynaptic level. For example, periglomerular interneurons appear to receive input primarily from a single glomerulus but can send axons to several nearby glomeruli (Pinching and Powell 1971). These lateral interactions could inhibit the activation of glomeruli receiving receptor neuron input or activate glomeruli not receiving receptor neuron input. Because it is likely that the intrinsic optical signal in the bulb is at least partially dependent on postsynaptic activity (M. Meister, personal communication; unpublished observations), lateral interactions between glomeruli should be reflected as differences in the glomerular calcium and intrinsic optical signals observed in this study.

However, we found a generally close correspondence between glomeruli receiving receptor neuron input and those showing a focal intrinsic signal. For response maps evoked by near-threshold odorant concentrations, the correspondence between glomeruli showing a presynaptic calcium signal and a focal intrinsic signal was between 70 and 80% (Table 1). Because the correlation between repeated presentations of the same odorant using the same signal type was only approximately 90% (Table 1), a significant portion (one-third to one-half) of the 20–30% of nonmatching glomerular signals are likely due to our imposing an arbitrary threshold in counting activated glomeruli and to slight variations in response amplitude across trials. Many of the remaining cases of nonmatching glomerular signals at low concentrations appeared due to slight differences in activation or detection threshold, so that these “missing” signals became apparent at higher odorant concentrations (see open arrowheads in Fig. 2B, for example). Only in a minority of cases did we observe clear calcium signals in glomeruli with no corresponding focal intrinsic signals at any odorant concentration (see solid arrowheads in Fig. 2B and glomerulus 5 in Fig. 3). We did, however, observe recruitment of intrinsic signal foci in areas showing little or no presynaptic calcium signal as odorant concentration increased (see Figs. 6A and 7A and B). These intrinsic signals were most often in the anterior and lateral regions of the olfactory bulb and were larger than a single glomerulus. Because these regions correspond to areas with a high density of receptor axon fibers innervating other bulb regions, these “ectopic” intrinsic signals could be driven by action potentials in axons of passage, rather than activation of glomeruli via postsynaptic, lateral interactions. The generally high correspondence between glomeruli...
receiving receptor neuron input and those showing a focal intrinsic signal suggests that lateral synaptic interactions may play a relatively minor role in shaping spatial patterns of neuronal activity within the glomerular layer of the olfactory bulb.

The concentration–response functions of presynaptic calcium and focal intrinsic signals, measured for the same glomerulus, could differ, with the dynamic range of the focal intrinsic signal often being compressed relative to that of the calcium signal. If the intrinsic signal is driven primarily by postsynaptic neuronal activity, these differences might reflect intraglomerular processing of receptor neuron input. Examples of intraglomerular processing pathways include self-inhibition of periglomerular neurons, mediated by a GABA-ergic path-

**FIG. 7.** Changes in topography of intrinsic signal maps, but not calcium signal maps, evoked by increasing odorant concentration. A: low frequency–subtracted intrinsic signal maps evoked by increasing concentrations of hexanal. Higher hexanal concentrations recruit strong intrinsic signals in lateral and progressively caudal positions. Solid arrowheads indicate recruited intrinsic signal foci. Arrows indicate intrinsic signal foci, activated at low hexanal concentrations, which disappear at higher concentrations. Open arrowhead indicates an intrinsic signal focus, resembling a glomerulus, that is likely a blood vessel artifact due to its location at vessel branching point (an intrinsic signal also appears in the vessels). B: low-frequency–subtracted maps of calcium (top) and intrinsic signals (bottom) evoked by a hexanal concentration series in a different preparation from A. Increasing concentration recruits calcium and intrinsic signals in multiple glomeruli. The glomeruli showing the strongest calcium signal remain clustered in the anteromedial bulb at all concentrations (top). The strongest intrinsic signals shift from anteromedial to lateral at higher concentrations (bottom). Solid arrowheads indicate focal intrinsic signals, recruited by increasing concentrations of hexanal, which show no corresponding glomerular calcium signal. These intrinsic signal foci are larger than a single glomerulus. Arrow points to a glomerulus that is recruited in response to 0.13% s.v. (0.5 μM) hexanal in the calcium and intrinsic signal maps. This glomerulus remains in the calcium signal map at all higher concentrations, but the corresponding intrinsic signal disappears at 5.6% s.v. (22.7 μM) hexanal. Open arrowheads indicate strong medial intrinsic signals that overlie a medial blood vessel (the vessel was apparent from the resting fluorescence).
way (Smith and Jahr 2002), intraglomerular excitation, mediated by glutamate release from mitral cell primary dendrites (Schoppa and Westbrook 2001), and nonlinear excitation of mitral cell dendrites (Mori and Takagi 1975; Mori et al. 1982). Alternatively, the smaller dynamic range of intrinsic signal foci might simply reflect a limited ability of this signal to track increases in the level of glomerular activation. In any case, our results suggest that the concentration–response functions of intrinsic signal foci do not simply reflect odorant-receptor interactions, as proposed previously (Meister and Bonhoeffer 2001).

Earlier intrinsic imaging studies have reported fewer active glomeruli than seen with presynaptic calcium imaging. For example, Belluscio and Katz (2001) reported that aliphatic aldehydes activated four to six glomeruli on the dorsal olfactory bulb of the mouse, while calcium imaging studies typically reveal four to five times that number for similar concentrations of the same odorants (Fried et al. 2002; Wachowiak and Cohen 2001). A possible explanation for this result is that small levels of receptor neuron input do not contribute significantly to glomerular activation. However, we measured similar thresholds for the detection of calcium and intrinsic signals in a glomerulus (Fig. 4). Smaller numbers of intrinsic signal foci might also reflect the fact that adjacent glomeruli activated by receptor neuron input often appear as a single intrinsic signal focus (Figs. 1D and 3A). Despite this effect, however, we detected similar numbers of activated glomeruli using the calcium and intrinsic signals at suprathreshold concentrations. Thus differences in the numbers of intrinsic signal foci detected in this and earlier studies may be largely due to differences in methodology. These differences include an anesthetic regimen with a less stable baseline reflectance (Meister and Bonhoeffer 2001; Rubin and Katz 1999), longer or later integration times that can result in more diffuse maps (Belluscio and Katz 2001; Meister and Bonhoeffer 2001), longer and/or slower odorant presentation (Belluscio and Katz 2001; Rubin and Katz 1999; Uchida et al. 2000), and thresholding of the intrinsic signal (Belluscio and Katz 2001; Luo and Katz 2001).

**Spatial organization of distributed odorant representations**

We found that patterns of receptor neuron input to glomeruli were similar to those of intrinsic signal activity at near-threshold concentrations but diverged with increasing odorant concentrations. As discussed above, this result was apparently due to differences in the concentration–response functions of calcium and intrinsic signals (Figs. 3 and 4), the recruitment of high-amplitude intrinsic signals in areas showing weak or, in some cases, no receptor neuron input (Figs. 6A and 7B), and the disappearance of some intrinsic signal foci at suprathreshold odorant concentrations (Fig. 7). Intrinsic signal response maps diverged from maps of receptor neuron input not only in the particular glomeruli that were most strongly activated, but in their overall topography (Table 2). We often observed changes in the topography of intrinsic signal response maps with increases in odorant concentration, but less frequently saw such changes in the topography of receptor neuron responses (Fig. 7). These differences are significant given the evidence that the spatial representation of odorants involves regionalized activity in the olfactory bulb (Johnson and Leon 2000; Johnson et al. 2002; Uchida et al. 2000; Wachowiak et al. 2001).

Earlier intrinsic imaging studies have characterized the chemotopy of odorant representations on the dorsal olfactory bulb in response to brief odorant presentations. These studies identified an anteromedial domain, activated by aldehydes and organic acids, and a posterolateral domain, activated by ketones, phenols, and alcohols (Meister and Bonhoeffer 2001; Uchida et al. 2000). Glomeruli located in different domains were reported to have nonoverlapping odorant response profiles (Uchida et al. 2000), suggesting a sharply defined chemotopy. In the present study, however, we obtained somewhat different results for both calcium and intrinsic signals. We found that near-threshold odorant concentrations activated glomeruli within their associated domains, but that suprathreshold concentrations activated glomeruli in both domains (e.g., acetophenone and hexanal response maps in Fig. 6A) (see also Wachowiak and Cohen 2001; Fried et al. 2002). Thus glomeruli in different domains can have overlapping odorant response profiles. As described above for counts of the numbers of activated glomeruli, methodological differences that result in a smaller signal-to-noise ratio, as well as thresholding of the intrinsic signal, could result in an artificially narrow response profile for a particular intrinsic signal focus and, consequently, a sharper chemotopy than observed in the present study.

Effects of odorant concentration on the broad topography of intrinsic signal responses have been reported previously for only a few odorants. Rubin and Katz (1999) showed that glomeruli recruited by increasing concentrations of amyl acetate were distributed across much of the bulb, with a significant change in the topography of the response (see Fig. 5 of Rubin and Katz 1999). In contrast, Uchida et al. (2000) reported that the topography of responses to aldehydes did not change with concentration. While the reasons for these differences remain unclear, our results suggest that controlling for effects of odorant concentration is important when characterizing how odorants are encoded in terms of spatial patterns of activity. We find that intrinsic optical signals least accurately reflect patterns of receptor neuron input at suprathreshold odorant concentrations (approximately 1% or higher of saturated vapor for most odorants). Previous intrinsic imaging studies have typically used comparable concentrations when characterizing odorant representations (Belluscio and Katz 2001; Luo and Katz 2001; Rubin and Katz 1999; Uchida et al. 2000). At these concentrations, odorants evoke input to many glomeruli and may activate additional metabolic processes that affect the appearance of intrinsic signals. The appearance of strong intrinsic signals in areas receiving no apparent receptor input could be driven by high-frequency action potential firing in anterolateral receptor neuron axon bundles (Fig. 7) or trigeminal input from the nasal epithelium to the olfactory bulb (Schaefer et al. 2002). The disappearance of intrinsic signal foci in isolated, moderately activated glomeruli at suprathreshold concentrations (Fig. 7) could reflect constraints on the coupling between neural activity and the hemodynamic changes underlying the intrinsic signal. In addition, some studies have characterized odorant representations on the dorsal bulb using only the largest-amplitude intrinsic signals (Belluscio and Katz 2001; Luo and Katz 2001), which, in this study, show the largest differences from maps of receptor neuron input. Our results suggest that intrinsic signals most accurately reflect the spatial organization of receptor neuron input at near-threshold odorant concentrations (0.1–0.4% saturated va-
por; typically <1 μM vapor concentration for the odorants used in this study) and when the locations of all apparent signal foci are mapped. Whether the most strongly activated glomeruli play a more important role in encoding odorant information than do moderately activated glomeruli is unknown.

Differences between intrinsic signal maps and maps of receptor neuron input might reflect real changes in the representation of odorants by postsynaptic processing. However, they may also reflect differences in the dynamic range, spatial, and/or temporal resolution of the different signal types. Identifying the primary neuronal determinants of intrinsic signal foci is therefore important in understanding the extent to which synaptic processing alters spatially organized representations of odorants in the glomerular layer. Our comparison of how odorants are represented using these two measures of activity does, however, suggest common features that are likely to be important components of the strategy for encoding odorant information early in the olfactory pathway. These features include a strong concentration dependence of glomerular activation patterns, a loosely organized chemotopy, and involvement of widely distributed glomeruli in representing all but the lowest concentrations of odorants. It is worth noting that these features are compatible with coding strategies that also use temporally organized activity to encode odorant information (Friedrich and Laurent 2001; Kashiwadani et al. 1999; Spors and Grinvald 2002). Imaging or electrophysiological techniques that focus on spatial and temporal patterns of mitral cell activation will be important in determining whether these features remain a part of the strategy for encoding odorant information at synaptic levels beyond the glomerular layer.

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