Title: Oscillations in the olfactory bulb carry information about odorant history

Running head: Presentation history dependent oscillatory response

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While odorant evoked oscillations in the vertebrate olfactory bulb have been studied extensively, information about their possible cognitive role has been missing. Using voltage sensitive dye imaging we show that repeated odorant presentations with interstimulus intervals of 2-12 seconds had dramatic and diverse effects on the three oscillations that occur in the turtle olfactory bulb. Two of the oscillations are strikingly depressed in response to the second stimulation even of a new odorant was presented. The third oscillation is enhanced if the odorant is the same but suppressed if the odorant is new. The effects suggest that the oscillations carry information about odorant novelty and consistency.
Introduction

E. D. Adrian (Adrian, 1942) discovered oscillations that appear in the mammalian vertebrate olfactory bulb during and after odorant presentations. Subsequently it has been shown that odorants evoke more or less complex oscillatory patterning across many animal species (e.g., Adrian, 1950; Hughes and Mazurkowski, 1962; Laurent and Naraghi 1994; MacLeod et al., 1998; Teyke and Gelperin, 1999). However, the relevance and function of the oscillations is not clear. In other sensory systems synchronous activity of neurons may be used by the brain as the mechanism underlying binding of different features of sensory information (Whittington et al. 1996; Singer, 1999; Von der Malsburg, 1999, Engel et al., 2001; Fries et al., 2001). Thus far, there are no results in vertebrates that have directly linked the olfactory bulb oscillations to a specific cognitive role. While there is evidence that the oscillations have a role in the differentiation of similar odors in locusts (Stopfer et al., 1997) and slugs (Teyke and Gelperin, 1999), they do not appear to have such a role in moths (Christensen et al, 2003), zebrafish (Friedrich et al 2004), rats (Fletcher et al, 2005; Lowry et al, 2005), or rabbits (Adrian, 1950; Di Prisco and Freeman, 1985).

In earlier experiments we used optical imaging with voltage sensitive dyes to monitor the odor evoked oscillations in the turtle olfactory bulb (Lam et al., 2000; Lam et al., 2003). We found that odorants evoke a slow depolarization and three independent oscillations in the turtle bulb. The three oscillations, rostral, middle and caudal, differ in their amplitude, location, frequency, duration, and latency (e.g. Figure 1A). In contrast to the results in locusts and slugs, but similar to the result in moths, fish, rats and rabbits, the oscillations in the turtle appear to be independent of odor quality or concentration (over a substantial range of concentrations) (Lam et al., 2000, 2003).

Here we describe dramatic alterations in the oscillatory pattern that occur on repeated odorant presentations. The changes to two of the oscillations are odorant independent; in contrast, the change observed in the third oscillation depends on whether the odorant in the two presentations is the same or different. Thus, oscillations may have more than one role during odorant processing in vertebrates; the middle oscillation could be used as an indicator of the continuous presence of an odorant while the rostral and caudal oscillations may be part of the mechanism for alerting the nervous system to a newly present odorant or have a role in odor accommodation.

MATERIALS AND METHODS
Odorant delivery

The design of the olfactometer was copied from Kauer and Moulton (1974) with minor modifications (Lam et al., 2000). Cleaned and desiccated carrier air and laboratory air saturated with odorant vapor were injected into and mixed in the inner tube of a double-barrel odor applicator. The flow rate of the carrier air was set to 300 ml/min. The outer tube of the applicator was normally under suction at 1500 ml/min to ensure that odorant does not leave the tube between the odorant applications. At a command pulse to a solenoid valve (South Bend Controls, South Bend, IN), this suction was turned off to release a pulse of diluted odorant. An additional solenoid valve was placed between the syringe pump and the odorant containers to allow for fast switching between the odorants during a trial. To draw the odorant through the nasal cavity...
suction (300 ml/min) through tubing inserted into the pharyngeal opening (see below) was activated 500-1000 ms before the odorant presentation and was maintained for 15 s after the second odorant presentation. In a few animals drawing room air alone through the nose elicited an oscillation of intermediate frequency (8 Hz) and very long duration (Lam, et al 2000). Recordings with this oscillation were not included in this paper. The minimum interval between trials was 60 seconds. We used 1 or 2 second odor pulses. Previous results indicated that longer odorant pulses did not substantially affect the amplitude or duration of the oscillatory response (Y-w. Lam, L.B. Cohen, M. Wachowiak, and M. Zochowski, unpublished observations).

**Surgery and Staining**

Oscillations were measured in experiments on 28 box turtles, *Terepene sp.* Not all animals were used for the statistical calculations either because too few trials were recorded or because only one or two of the three oscillations had signal-to-noise ratios large enough to make meaningful measurements. Experiments with signal-to-noise ratios of less than three were not analyzed. The composition of turtle saline that was used for dye dilution as well as washing the bulb during the experiment is as follows (in mM): NaCl 96.5, KCl 2.6, MgCl₂ 2.0, NaHCO₃ 31.5, CaCl₂ 4.0, dextrose 10 (all from Sigma, St. Louis, MO). The saline was bubbled with 95% O₂ / 5% CO₂ resulting in a pH of 7.0-7.2.

The turtles were partially immobilized with d-tubocurarine (1.5mg/kg, Sigma) and anesthetized by placing them on ice 2hr before as well as during the surgery. Local anesthetic (1% bupivicane, Sigma) was applied around the location of the craniotomy. A segment of polyethylene tubing (outer diameter 2mm, inner diameter 1mm) was inserted into the outlet of the nasal cavity in the roof of the mouth and fixed in place by Krazy Glue and epoxy. The dorsal surface of both olfactory bulbs was exposed by craniotomy of the overlying scull and removal of the dura and arachnoid matter.

**Voltage sensitive dye measurements:** We used the fluorescent styryl dye, RH414 (Grinvald et al 1994; Molecular Probes, Eugene, OR; concentration 0.05-0.1 mg/ml in saline). The dye was applied directly to the exposed bulbs for 60 minutes; the solution was replaced every 15min. After staining the remaining dye solution was removed and 2% agarose (Sigma) was placed on top of the bulbs. A coverslip was placed on top of the agarose to ensure a flat imaging surface. All of the results come from single-trial measurements; signal averaging could not be used because the oscillations were not precisely time-linked to the odorant pulse.

**Calcium dye measurements:** Olfactory receptor neurons were labeled with Calcium Green-1 dextran, 10 kD m.w. (Molecular Probes) by injecting 20 µl of 2% dye solution dissolved in 0.1 M NaCl plus 0.5% Triton-X 100 into each naris. After staining, the animals were held at room temperature for 5-18 days before the measurements. Similar surgical procedures were performed, except that the bone covering the olfactory bulb was only thinned and the imaging was performed through the thinned bone.

Before starting the measurements the turtle was kept at room temperature for 1h. The upper jaw was clamped to a metal bar with a plastic strap to stabilize the head during the optical recordings. The turtles were not anesthetized during the recordings. All experimental procedures were approved by the Yale University and the Marine Biological Laboratory Institutional Animal Care and Use Committees.

**Imaging**
For the voltage sensitive dye measurements the preparation was illuminated using a 100W tungsten bulb. The light was heat filtered and passed through a 520±45 nm incident light filter. The fluorescence emission above 610 nm was collected via a MacroScope (4X magnification, RedShirtImaging, LLC, Fairfield, CT) onto a 464-element photodiode array (NeuroPDA, RedShirtImaging). Each pixel of the photodiode received light from an area of the image plane of approximately 170\(\mu\)m\(^2\). The average recorded light intensity is \(10^8\) photons/msec/pixel; the amplifier filtering was set to 0.5-125Hz. For the Calcium dye measurements the preparation was illuminated with the same illumination source and a 480±25 nm incident light filter. The fluorescence emission above 530nm was collected and measured with the photodiode array. The estimated recorded light intensity is \(5 \times 10^7\) photons/msec/pixel; the same amplifier filtering was used.

**Data Analysis**

NeuroPlex software (RedShirtImaging) was used for much of the data analysis and display. We applied digital filtering using high and low pass Gaussian (5-30Hz) filters. The results in Figures 1, 2, 4, and 6 come from the outputs of individual pixels or the average of 10-20 neighboring pixels in the photodiode array.

**Identification of the three oscillations.** One parameter we used to distinguish the three oscillations was location on the olfactory bulb. Here we benefited from the fact that optical measurements have a spatial resolution that is substantially better than LFP-EEG measurements (Zochowski et al, 2000). Without this improved spatial resolution it would have been difficult to determine that repeated presentations had differential effects on the three oscillations. In almost all preparations we found pixels in rostral locations that had only the rostral oscillation. However, in only about half of the preparations could we find locations that had isolated middle and/or caudal oscillations. In the remaining animals the middle and caudal oscillations overlapped in location. In these instances the two oscillations were identified by their latency, frequency, duration, and response to repeated stimulus presentation.

**Changes in power of the observed oscillations in repeated presentations.** Two data fragments of the same duration (one for each presentation) containing one of the three observed oscillations were chosen. The power spectrum was calculated and the relative change of the power, \(C\), at the peak frequency of the oscillation was calculated according to equation:

\[
C = \frac{P_2 - P_1}{P_1 + P_2},
\]

where \(P_1\) and \(P_2\) are the powers of the same oscillation after the first and second odorant presentation, respectively. The measure \(C\) takes values between −1 and 1. An increased oscillation will yield positive values of \(C\), while a decrease will result in negative values. This measure was used because it avoids infinite values that would occur if we calculated simple percentage changes from results like those illustrated in Figure 4. In the instances where the caudal oscillation appeared only as a high frequency oscillation, we calculated the change of power based on the power of high frequency peak on the second presentation. The total number of trials observed (T) is indicated in the brackets in Table 1 and Figure 3.

**RESULTS**
Many animals in natural settings sample the odorant several times (e.g. repeated sniffs or movements of the antenna) to facilitate recognition or to monitor changes in a time varying odor scene (e.g. Youngentob et al, 1987; Goldman and Patek, 2002). The aim of the present study was to elucidate possible roles of the odor evoked oscillations in the olfactory bulb of the turtle by determining whether changes in oscillations occur in response to multiple odorant presentations. We used odorant presentations of 1 or 2 seconds, short compared to the respiratory rate of resting box turtles which ranges from 0.01 to 0.2 Hz (Landberg et al, 2003). In other experiments similar oscillations were measured in response to 0.5 second odorant presentations (data not shown).

We made voltage-sensitive dye measurements of odor evoked responses in the turtle bulb using a 464 element photodiode array camera. Changes in dye fluorescence provide a fast, presumably linear, measure of the population averaged membrane potential changes (Ross et al., 1977; Grinvald et al., 1988; Zochowski et al.; 2000) of the neural membranes imaged onto each pixel. In the experiments reported here we presented trains of two or more stimuli with an odorant concentration between 0.3% and 10% of saturation and an inter-stimulus interval between 2s and 10s.

The oscillatory response to the second presentation of the same odorant changed dramatically. Figure 1 A-B illustrates a typical result: 1) In response to the second odor presentation the rostral oscillation was greatly diminished and often undetectable, 2) the size of the caudal oscillation was also decreased substantially, and, in addition, its frequency in response to the second odorant pulse was twice that found in the first response, and finally 3) the size and duration of the middle oscillation (the oscillatory response with the shortest latency) was increased. The responses to the third and subsequent odorant stimuli were similar to the response to the second stimulus.

The three oscillations occur over relatively large areas ( ~ 1 mm²) of the turtle bulb (Lam, et al., 2000, 2003). While figures 1, 2, and 4 illustrate results from individual pixels in the photodiode array; the results shown for the effects of repeated odorant presentation were similar for all pixels exhibiting an oscillation.

The time course of the change in oscillatory power (C; see Methods) as a function of inter-stimulus interval (ISI) is shown in Figure 5. The time course of the increase in the middle oscillation to identical odorants is similar to the time course of the decline when different odorants were presented. The effect of the first stimulus on the succeeding response declined substantially for all three oscillations when the ISI was 12 seconds. When much longer interstimulus intervals (>30 seconds) were used, the response to the second odorant presentation was similar to the response to the first.

The data presented in the figures come from experiments where the odorants isoamyl acetate, hexanal, and cineole were used, similar results were obtained with the carvone, hexanone, and n-amyl acetate.

We found that the dramatic decreases in the rostral and caudal oscillations do not depend on the odorant that is presented, similar changes occur irrespective of whether the odorants in the two presentations are the same or different (e.g. Figure 2, top and bottom sections). The rostral oscillation (top section) to the second odorant presentation was substantially reduced or completely abolished at both odorant concentrations illustrated, 1.7% and 10%, independent of whether the second odorant was the same or different. Similarly, the low frequency component of the caudal
oscillation (bottom section) was greatly reduced in response to the second presentation at both odorant concentrations and for all four presentation pairs.

However, for the middle oscillation the situation was very different - if the odorant at the second presentation was the same as at the first, the middle oscillation increased in size and/or duration. In contrast, if the odorant presented on the second stimulation was different, the middle oscillation was significantly reduced or undetectable (e.g. Figure 2, middle section). The change in middle oscillation did not depend on the identity of the odorant itself but on its relation (i.e. same or different) to the odorant presented in the first stimulus.

Similar changes to those illustrated in Figures 1 and 2 were also observed for odorant concentrations of 0.3% of saturated vapor (data not shown) and thus these changes appear to be independent of odorant concentration over the range we tested.

The changes of the oscillatory response were relatively consistent across animals. To quantify the observed changes in the oscillatory response we measured the amplitude of the signals on sections of the recordings in 175 trials from 20 preparations. These preparations were selected for their large oscillatory response and included the experiments in Figures 1 and 2. The labeled horizontal lines in Figure 1A-B indicate the sections of that trial used to determine the power at the peak frequency of the oscillation from Fourier transforms. A measure of the change in power, C ± SEM, at the peak frequency of the observed oscillations, is plotted in Figure 3. The measure, C, takes values between –1 and 1. An increased oscillation will yield positive values of C, while a decrease will result in negative values. The mean reduction of the power of rostral and caudal oscillations in the second presentation is large and the reductions are independent of whether the odorants in the two trials are the same or different. In contrast the direction of the change in the middle oscillation is strikingly dependent on whether the odorant presented in the two trials was the same or different. Similar histograms are obtained when the results for three different odorants used in the first presentation are plotted independently (Figure 3 B,C,D).

The direction of the change (increase or decrease) for all of the trials in the preparations we studied is summarized in Table 1. In some preparations the middle oscillation could not be detected in response to the first odorant presentation but was clearly seen in the second presentation of the same odorant. An example of this kind of result is shown in Figure 4. Instances of this sort were included in the groups indicated by asterisks in Table 1. The number of trials used for each percentage is indicated by the T values in parentheses.

One source for changes in the response to multiple presentations might be adaptation of the olfactory receptor neuron input to the bulb. We examined this hypothesis by imaging the increases of calcium concentration in the presynaptic terminals of the receptor neurons in the glomeruli (Wachowiak and Cohen, 2001; Wachowiak et al., 2002) in response to repeated odorant presentations. The Calcium Green-1 signals from two locations in the dorsal bulb where we found the largest response to the two odorants, cineole and isoamyl acetate, are shown in Figure 6. The calcium increase, as expected, was on average smaller in response to the second stimulation than to the first. This decline in response may in part be due to receptor adaptation from odorant that is not cleared from the olfactory epithelium between trials. However, the calcium increase after the second odorant presentation was somewhat
larger when a different odorant was presented than when the same odorant was applied twice (Figures 6 and 7). Thus, because receptor adaptation is odorant dependent, it could only partially explain the decline of the rostral and caudal oscillations that are independent of odorant (Figures 2 and 3). Furthermore, the observed changes in the amplitude of the middle oscillations are inversely correlated to the changes in the input to the bulb. Clearly, the changes in the oscillations in response to the second presentation must also reflect internal processing in the bulb or feedback from higher brain regions.

**DISCUSSION**

The odor evoked oscillatory responses in the turtle change dramatically on repeated odorant presentations when the second presentation occurs within a time window of 2-12s. The rostral and caudal oscillations are greatly reduced on the second presentation irrespective of the configuration of the odorant presentations. However, the middle oscillation increases if the odorant delivered at the second presentation is the same as the one presented previously, or conversely, it is diminished if the presented odorants are different. Moreover, we find that the changes in the middle oscillation cannot be explained by a decreased input to the bulb, because adaptation of the input is substantially reduced when different odorants are delivered at consecutive odorant presentations.

**Mechanisms responsible for the changes in response that occur on repeated presentations.**

Multiple mechanisms might account for the observed changes in the oscillatory response(s). The reduction of the rostral and caudal responses on the second odorant pulse could be partially due to the decreased input to the bulb. However the decreased input is smaller than the reduction of oscillations and the decrease in input is somewhat dependent on whether the odorant is the same or different (Figure 7) while the decrease in rostral and caudal oscillation amplitudes is independent of odorant (Figure 3).

The olfactory bulb receives several kinds of feedback from higher centers (Scott and Harrison, 1991; Pinching and Powell, 1972). Extrinsic afferents primarily from the anterior olfactory nucleus synapse on periglomerular cells and, to lesser extent, on mitral/tufted cell dendrites (Luskin and Price, 1983; Macrides and Davis, 1983). Longer feedback loops involve the commissural projection system, which includes topographically organized mitral/tufted cell projection to the anterior olfactory nucleus whose cells in turn project to a region of the contralateral bulb homotopic to the region of the ipsilateral bulb from which they received the input (Davis et al, 1978; Schoenfeld and Macrides, 1984; Scott, 1985). Corresponding regions of the two bulbs thus indirectly receive feedback from each other. Those projections terminate in the granule as well as glomerular layer (Luskin and Price, 1983; Macrides and Davis, 1983) and are predominantly excitatory thereby causing an inhibitory effect. This feedback mechanism can be powerful. In preliminary experiments we found that conditioning of the olfactory bulb oscillations by prior stimulation of the contralateral (with respect to the imaged bulb) nares results in large changes in oscillations similar to those illustrated in this paper in response to repeated odorant presentations. (B. Singer, S. Kim, and M. Zochowski, in preparation).
A third major source of feedback is piriform cortex. Electrophysiological evidence suggests that piriform cortex projections also make excitatory synapses onto granule cells and their stimulation inhibits mitral/tufted cells (Nakashima et al, 1978).

The reduction of the middle oscillation after the presentation of a different odorant as the second stimulus might be due to lateral presynaptic inhibition known to exist in the turtle (Wachowiak and Cohen, 1999) and mammals (Shepherd and Greer, 1998) or negative feedback from higher cortical regions as described above. The increase in the middle oscillation after presentation of the same odorant as the second stimulation could be due to long lasting self-excitation of the mitral/tufted cells (Salin et al, 2001; Schoppa and Westbrook, 2001; Schoppa and Urban, 2003) or could point to the existence of extrinsic excitatory cortical feedback.

 Interruption of the lateral olfactory tract inhibits beta band oscillations in the bulb while leaving the gamma band oscillations unchanged (Neville and Haberly, 2003). This result suggests an important role of feedback from piriform cortex in modulation of the observed bulbar oscillations. In separate work we have used computer simulations (S. Kim, B. Singer, M. Zochowski, 2005, submitted) to indicate that the period doubling transition from fast caudal (14Hz) to slow caudal oscillation (7Hz) could be mediated through lateral olfactory tract feedback from cortical regions.

**Comparison with olfactory bulb oscillations in other animals.**

**Increased oscillations following repeated presentations.** We found that the middle oscillation in turtle increased in amplitude with repeated presentations if the same odorant was presented. Similar results were obtained in earlier measurements on locusts (Laurent and Naraghi, 1994; Stopfer and Laurent, 1997) and for some odorants in moths (Heinbockel et al, 1998). In agreement with Stopfer and Laurent (1997) we found that the over-all levels of input to the bulb (Figure 6) declined on repeated odorant presentations while there was a significant increase of the oscillatory response in the middle oscillation. In contrast, a substantial difference between our findings and those in insects is that two of the oscillations (rostral and caudal) dramatically declined on repeated odorant presentations, independent of the odorants applied.

**Localization of the oscillations.** All three oscillations in the turtle olfactory bulb cover substantial areas of the bulb, ~1 mm$^2$, which encompass hundreds of individual glomeruli (Lam, et al, 2000, 2003). Similarly, the oscillations in the locust appear to be identical over the whole mushroom body (Laurent and Naraghi, 1994). In dramatic contrast, oscillations in the moth are apparently localized to individual glomeruli (Okada and Kanzaki, 2001, Christensen, et al, 2003) and in dramatic contrast, oscillations in the moth are apparently localized to individual glomeruli (Okada and Kanzaki, 2001, Christensen, et al, 2003)

**Odorant dependence.** All three oscillations in the turtle occur after relatively long (500-1000 msec) latencies (Lam et al 2000). These latencies are much longer than recent reports of the time required for olfactory discrimination in the rat (Uchida et al, 2003; Abraham, et al 2004). However, other measurements of sniff durations in discrimination experiments were closer to one second (e.g. Youngentob et al, 1987; Slotnick and Schellinck, 2002). In addition, it is commonly observed that mammals will repeatedly sniff for several seconds at objects of interest. Thus the long latencies for the turtle oscillations probably cannot be used to rule out a role in odor discrimination.

In the locust, the oscillations can be different for different odorants and it is proposed that odorant quality is encoded in the spike activity of neuronal assemblies that is phase locked to the oscillations (Laurent and Davidowitz, 1994, Stopfer at al
1997). In contrast, the three oscillations in the turtle olfactory bulb can be very similar for odorants that have qualitatively different input maps to the bulb (Lam et al, 2003). Similarly, in the moth, zebrafish, rat, and rabbit, different odorants elicit similar oscillations (Christensen, et al, 2003; Friedrich et al 2004; Lowry et al, 2005; Adrian, 1950; Di Prisco and Freeman, 1985). Furthermore young rats can discriminate odorants quite well in the absence of measurable β or γ oscillations (Fletcher et al, 2005). Thus Adrian's (1942) original conjecture that the oscillations in mammals were involved in odorant recognition appears to be incorrect.

**State Dependence.** Ravel et al., (2003) and Martin et al., (2004) found that the sniffing of odorants decreased the γ band oscillation and increased the β band oscillation in the rat olfactory bulb. In a related observation, Kay (2003) found that sniffing decreased the low frequency (~40-60 Hz) γ band power but increased the high frequency (~70-100 Hz) power. These findings are similar to our results in that they suggest that the oscillations in mammals subserve higher order functions.

Thus it seems reasonable to conclude that the oscillations have different roles in different olfactory systems. Oscillations appear to be a neurophysiological mechanism that is adopted for different purposes by different animals. However, it remains possible that in any particular instance oscillations might be an epiphenomenon of network activity and have no cognitive function. Evidence concerning this possibility awaits a better understanding of olfactory processing.

In conclusion, our results suggest roles for the oscillatory response in turtles. One possible role of the oscillations is to provide an assessment of the novelty of the odorant. The increase in the middle oscillation that occurs in response in repeated presentation of the same odorant is blocked if the odorant is changed. This kind of response would be useful for recognition of odor quality consistency in while navigating in a complex, time-varying odorant plume. On the other hand, the dramatic (odorant independent) decreases in rostral and caudal oscillation may be part of the mechanism for odor accommodation or for alerting the nervous system to an important change in the odor environment. Consistent with this role is the finding that the rostral and caudal oscillation are elicited by relatively high odorant concentrations in the rabbit and turtle (Adrian, 1950; Lam et al, 2000) as are γ oscillations in the rat (Lowry et al 2005).

**Acknowledgements**

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REFERENCES


FIGURE CAPTIONS:
Figure 1. Changes in the three oscillatory patterns to the second presentation of the odorant. The three traces are taken from the locations shown approximately on the image at the top (trace numbers correspond to the numbered boxes). The missing segment (grey) is part of the inter-stimulus interval. The odorant for both presentations was isoamyl acetate at 10% of saturation (1s presentation, 4s inter-stimulus interval). The timing of the odorant presentation is indicated by the thin line under the bottom trace.
A: Response to the first odorant presentation. Three oscillations can be identified that, in addition to location, differ in amplitude, frequency, latency, and duration: rostral (top trace, ~14Hz frequency, 700ms latency), middle (middle trace, ~14Hz frequency, 500ms latency) and caudal oscillation (middle and bottom traces, ~7Hz frequency, 1000ms latency).
B: Response to the second odorant presentation. The rostral oscillation was dramatically reduced in size, the middle oscillation increased in size and duration, and the low frequency component of the caudal oscillation disappeared. The vertical calibration bar on the right in this and subsequent figures indicates the change in fluorescence, ΔF, divided by the resting fluorescence, F. The largest voltage-sensitive dye signals were fractional changes of about 1 part in 1000. The signals were digitally filtered to include a bandwidth of 5-30Hz. The power of the six oscillatory responses are: $P_R^1 = 4800$, $P_R^2 = 700$, $P_M^1 = 1000$, $P_M^2 = 1700$, $P_C^1 = 1500$, $P_C^2 = 1000$ (where subscript denotes oscillatory response and superscript the odorant presentation number) and thus the relative change of power, C, is: $C_R = -0.74$, $C_M = 0.26$, $C_C = -0.71$.

Figure 2. Modification of the oscillatory responses after the second odorant presentation as a function of changes in odorant identity. The effect of the second odorant presentation was odorant dependent for the middle oscillation but independent of odorant for the rostral and caudal oscillations. Data is presented for two odorant concentrations, 1.7% (left) and 10% of saturated vapor (right). The changes of oscillatory responses are grouped by the oscillation type, rostral (top), middle (middle), and caudal (bottom). The odorants were presented for 1s with a 4s inter-stimulus interval. The timing of the odorant presentations is indicated by the lines below each trace. The signals were digitally filtered to include a bandwidth of 5-30Hz.

Figure 3. Mean relative change of the power, C (+ SEM), of the oscillations after a second stimulation with the same (dark gray) or a different (light gray) odorant. For both the same and different odorants there was a dramatic and significant decrease
of the power of the rostral and caudal oscillations. In contrast, the middle oscillation increased when the same odorant was presented as the second stimulus and decreased if animal was exposed to a different odorant. A. tabulated over all responses in all experiments. B. tabulated over experiments in which isoamyl acetate was the first odorant. C. tabulated over experiments in which cineole was the first odorant. D. tabulated over experiments in which hexanal was the first odorant. The measure, C, was chosen because it avoids infinite values that would occur if we used simple percentage changes on results like those illustrated in Figure 4. The corresponding simple percentage changes were 74% and 76% and 38% and 44% for the rostral and caudal oscillations. The statistics were performed on results from 20 animals. The number of trials T used to calculate the increase or decrease of the specific oscillatory response is indicated in the bar.

**Figure 4. Example of the changes observed in the middle oscillation.** In this example the middle oscillation was not observed in response to the first stimulus but was present after the second stimulation when the odorant was the same (top) while it did not appear after the second stimulation if the odorant was different (bottom). These preparations were included in Table 1 in the section labeled with asterisks. The timing of the odorant presentations is indicated by the lines below each trace. The signals were filtered to include a bandwidth of 5-20Hz.

**Figure 5. Change in power between the first and second odorant response as a function of the inter-stimulus interval (ISI).** The ISI is taken as the time from the start of first odorant presentation to the start of second presentation. A) Presentation of same odorants. B) Presentation of different odorants. The opposite effect for the middle oscillation peaks in both cases at 5s. The amplitudes have not fully recovered at an interval of 12 seconds.

**Figure 6. Changes of the receptor input to the turtle olfactory bulb as indicated by calcium increases in the presynaptic terminals of the olfactory receptor neurons.** The calcium increase was relatively larger to the second odorant presentation if the odorant was different. The odorants were isoamyl acetate and cineole (1.7% saturation, 1s presentation, 4s inter-stimulus interval). The largest calcium dye signals were fractional changes of about 1 part in 100. The timing of the odorant presentations is indicated by the lines below each trace. The signals are filtered to include 0.5-5 Hz. One of the traces has an artifact after the response to the second presentation; a dashed line was used to indicate the artifact.

**Figure 7. Decrease in glomerular calcium signal in response to the second odorant presentation.** Darker gray denotes instances when the same odorant was presented as the second stimulus. Lighter gray are instances when a different odorant was presented as the second stimulus. The reduction is smaller when different odorant is presented at the second stimulus. The mean values ± SEM are shown at two locations: location 1 – the maximum cineole response, location 2 – the maximum isoamyl acetate response (T=26). The values were calculated as a ratio of the peak...
response to the second odorant presentation to the peak response to the first presentation. ISI = 5s.

TABLE CAPTION:
Table 1. Summary of the results from all preparations. The table shows the percentage of trials with each response type. The majority of comparisons were made using isoamyl acetate and cineole at concentrations of 0.3%-10% of saturated vapor. A few of the experiments were carried out with carvone, hexanal, and hexanone as odorants. The total number trials for a given odorant combination is indicated in the brackets. In some preparations the middle oscillation could not be detected in response to the first odorant presentation but was clearly seen in the second presentation of the same odorant. An example of this kind of result is shown in Figure 4. Instances of this sort were included in the groups indicated by asterisks in the table.
The response to the second odorant presentation is different.

A. 1st odorant presentation

B. 2nd odorant presentation

Figure 1
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<td>cin</td>
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<tr>
<td>iaa</td>
<td>cin</td>
</tr>
</tbody>
</table>

| Middle oscillation   | 1st odorant presentation       |
|                      | 2nd odorant presentation       |
| iaa                  | iaa                           |
| cin                  | iaa                           |
| cin                  | iaa                           |
| cin                  | iaa                           |
| iaa                  | cin                           |
| iaa                  | cin                           |
| iaa                  | cin                           |
| iaa                  | cin                           |
| iaa                  | cin                           |

| Mostly Caudal oscillations | 1st odorant presentation | 2nd odorant presentation |
|                            | iaa                      | iaa                      |
|                            | hex                      | iaa                      |
|                            | cin                      | iaa                      |
|                            | cin                      | iaa                      |
|                            | iaa                      | cin                      |

Figure 2
Figure 3
Middle oscillation

10% isoamyl acetate

10% cineole

10% isoamyl acetate

$\Delta F/F = 2 \times 10^{-4}$

1 sec

27406

27407

Figure 4
Figure 5
Calcium signals from the olfactory receptor neuron terminals

Figure 6
<table>
<thead>
<tr>
<th>Odorant pair</th>
<th>Fraction of observed change in the response at the second presentation (smaller/bigger)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rostral oscillation</td>
</tr>
<tr>
<td>Same</td>
<td>98%/2% (T=242)</td>
</tr>
<tr>
<td>Different</td>
<td>98%/2% (T=121)</td>
</tr>
</tbody>
</table>

Table 1