Time Course of Odorant-Induced Activation in the Human Primary Olfactory Cortex

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Sobel, Noam, Vivek Prabhakaran, Zuo Zhao, John E. Desmond, Gary H. Glover, Edith V. Sullivan, and John D. E. Gabrieli. Time course of odorant-induced activation in the human primary olfactory cortex. J. Neurophysiol. 83: 537–551, 2000. Paradoxically, attempts to visualize odorant-induced functional magnetic resonance imaging (fMRI) activation in the human have yielded activations in secondary olfactory regions but not in the primary olfactory cortex-piriform cortex. We show that odorant-induced activation in primary olfactory cortex was not previously made evident with fMRI because of the unique time course of activity in this region: in primary olfactory cortex, odorants induced a strong early transient increase in signal amplitude that then habituated within 30–40 s of odorant presence. This time course of activation seen here in the primary olfactory cortex of the human is almost identical to that recorded electrophysiologically in the piriform cortex of the rat. Mapping activation with analyses that are sensitive to both this transient increase in signal amplitude, and temporal-variance, enabled us to use fMRI to consistently visualize odorant-induced activation in the human primary olfactory cortex. The combination of continued accurate odorant detection at the behavioral level despite primary olfactory cortex habituation at the physiological level suggests that the functional neuroanatomy of the olfactory response may change throughout prolonged olfactory stimulation.

INTRODUCTION

Functional magnetic resonance imaging (fMRI) is a method used to visualize changes in blood flow as an indirect measure of neural activity. The main paradigm of neural coding holds that sensory input is encoded by overall changes in rate of neural activity. The main paradigm of neural coding holds that sensory input is encoded by overall changes in rate of neural activity. However, several attempts to visualize odorant-induced fMRI activation in POC have yielded, at best, only weak activations (e.g., Koizuka et al. 1994; Sobel et al. 1998a, 1999a; Yousem et al. 1997). One possible reason for this lack in fMRI activation may lie in the specific technical complications of obtaining homogeneous fMRI signal in the ventral temporal region (Yang et al. 1997). There are two additional potential reasons for the lack in fMRI activation that relate to the temporal dynamics of odorant-induced activity in POC.

1) Odorant-induced neural activity in POC does not induce an overall local increase in blood flow. This is plausible considering at least two different suggested activity-patterns in POC.

First, electrical recordings have shown that different piriform neurons may respond to odorants by either exclusively increasing activity, exclusively decreasing activity, or a complex combination of both (Nemitz and Goldberg 1983; Tanabe et al. 1975; Wilson 1998a). The spatial resolution of fMRI may summate such simultaneous neighboring increases and decreases in activity to result in net zero change in overall blood-flow.

Second, the classic model of neural encoding—rate encoding—suggests that information is encoded within the mean of overall activity. Alternative models of neural encoding, such as temporal encoding, suggest that information is in the precise timing of individual spikes (Friston 1997; Sejnnowski 1995; Shallen and Newsome 1994). Findings from the olfactory systems of locusts and honeybees (MacLeod et al. 1998; Stopfer et al. 1997; Wehr and Laurent 1996), as well as recordings in mammalian olfactory bulb and POC (reviewed in Bhalla and Bower 1997; Ketchum and Haberly 1991), together suggest that olfactory information may be encoded temporally rather than rate encoded (e.g., Hopfield 1995). If odorants change the timing of individual spikes in POC but not the overall rate of activity, then odorants would not induce an overall increase in blood flow, and no change in the amplitude of the fMRI signal would be recorded.

2) Odorant-induced neural activity in POC does induce an increase in blood flow, but the time course of the increase differs from the time course of odorant stimulation. Electrophysiological and optical recordings show prolonged habituation patterns in POC neurons after an initial excitatory response (Haberly 1973a,b; Litaudon et al. 1997; Scholfield 1978; Wilson 1998a). Habituation here refers to a reduction in physiological response that may or may not alter behavioral performance. In fMRI block design experiments, the Pearson statistic is commonly used to cross-correlate the time course of activation with the time course of recurrent epochs of experimental
(e.g., odorant) and control (e.g., no-odorant) conditions (Fig. 1). If odorants induce only a transient increase in activation amplitude that then does not follow the time course of continued odorant presentations (due to habituation), this transient would not satisfy the criteria of the Pearson statistic that is sensitive to the shape of the activation time course.

To test the preceding possibilities, we examined the time course of odorant-induced activation in the POC of eight subjects.

METHODS

Subjects

Participants were four men and four women; all were right-handed and ranged in age from 20 to 39 (mean age 25). Each scanning session lasted ~2 h. The study was approved by the Stanford University Institutional Review Board, and all subjects signed informed consent.

Odorants and odorant generation

Methods of air dilution olfactometry were modified to accommodate the MRI environment (for methods in detail, see Sobel et al. 1997). In brief, the system enabled switching from odorant to no-odorant conditions in ~500 ms. The alternation from odorant to no-odorant conditions produced no auditory, visual, tactile, or thermal cues regarding the alteration between conditions. The odorants used were vanillin, propionic acid, and valeric acid, all diluted in double-distilled deionized water, and decanoic acid diluted in odorless mineral oil. Odorant concentration was preset individually for each subject at the lowest concentration that still enabled >90% accuracy in detection throughout the duration of the scan (Sobel et al. 1997). Whereas vanillin and decanoic acid are pure olfactants (Doty et al. 1978), propionic acid and valeric acid have a strong trigeminal component (Doty et al. 1978; Kendal-Reed et al. 1998). Each subject was scanned six times, once with each odorant, and twice in scans used as a reference for analyzing sniff-induced activation, as described later.

Note that we are careful to use the term odorant rather than odor. This is because we believe that in these experiments, and under natural circumstances—in the olfactory system, there is no such condition as no odor. When we are not generating an odorant, there are always other odors surrounding the nose. These may be odors of the facial mask, odors of the room, or odors of the subject himself/herself. Thus the olfactory system is always comparing with a baseline odor over which we can introduce an odorant.

Task design

Alternating half blocks of diluent with odorant versus diluent only were generated (Fig. 1). Eight such 40-s half blocks, for a total duration of 320 s constituted a single scan. During a scan, a line of script reading, “Sniff and respond, is there an odor? Press the right button for yes or the left button for no” was projected to the subject once every 8 s. Subjects sniffed and then responded by using the right index finger only to press one of two buttons. The number of sniffs and button presses thus was balanced over the odorant and the no-odorant conditions and constituted a constant baseline. The only difference between the half blocks was in the presence or absence of the odorant. Sniff duration was held constant by instructing the subjects to maintain the inhalation of the sniff for the duration of the projected message that was set to 800 ms. Response accuracy was recorded on a computer that controlled the olfactometer determining stimulus presence and triggered the scanner, thus maintaining synchronization between the task, stimulus presentation, and data acquisition. Only scans in which odorant detection accuracy was >90% were completed. Scans in which detection accuracy dropped <90% were terminated at that point and restarted after odorant concentration adjustments (Sobel et al. 1997).

Data acquisition

Each subject was accommodated with a custom-built bite-bar to prevent head motion. Imaging was performed using a 1.5 T whole-body MRI scanner (GE Signa, Rev. 5.5 Echospeed). For functional imaging, two 5-in diam local receive coils were used for signal reception. A T2*-sensitive gradient echo spiral sequence (Glover and Lai 1998), which is relatively insensitive to cardiac pulsatility motion artifacts was employed with parameters of repetition time (TR) = 720 ms, echo time (TE) = 40 ms, flip angle = 65°. Spatial resolution was set by a 153 × 153 voxel matrix covering a 42 × 42 cm field of view resulting in an in-plane resolution of 2.75 × 2.75 mm. Four interleaves were collected for each frame, with total acquisition time of 2.88 s per frame; 115 frames were acquired for a total scan duration of 331 s.

Eight 4-mm-thick slices with a 2-mm interslice gap were acquired at an oblique plane traversing from the frontal pole to the temporal pole [typically 30° clockwise to the anterior commissure to posterior commissure (AC-PC) plane; Fig. 2]. This slice orientation was chosen so as to maximize the volume of olfactory cortex within the acquisition (Sobel et al. 1997). The experimental sequence automatically
initiated 12 s after scanning onset, allowing the first four frames to be discarded from the analysis. This eliminated transients arising before the achievement of dynamic equilibrium. T1-weighted flow compensated spin-warp anatomy images (TR = 500 ms, minimum TE) were acquired at the same plane as a substrate on which to overlay functional data. For each subject, an additional whole brain acquisition of T1-weighted flow compensated spin-warp anatomy images was collected in the coronal plane to later assist in the validation of localization of cortical regions.

Analysis of functional data

Image reconstruction was performed off-line on a Sun SparcStation. A gridding algorithm was employed to resample the raw data into a Cartesian matrix before processing with two-dimensional fast Fourier transform. Motion artifacts were assessed (Friston et al. 1996) and corrected (Woods et al. 1992).

Pearson statistic

Analysis was performed using standard methods (Desmond et al. 1995, 1997a; Friston et al. 1994, 1996). Once individual images were reconstructed, the time series of each voxel was correlated with a reference waveform and transformed into a Fisher's Z score map, SPM[Z] (Friston et al. 1994). The waveform was calculated by convolving a square-wave representing the time course of the alternating conditions (odorant/no-odorant) with a data-derived estimate of the hemodynamic response function. The frequency of the square-wave in these experiments was 4 cycles/320 s = 0.0125 Hz. The correlation (cc) was calculated by

\[ cc = \frac{\sum_{j=1}^{n} (Y_j - \bar{Y})(\delta_j - \bar{\delta})}{\sqrt{\sum_{j=1}^{n} (Y_j - \bar{Y})^2 \sum_{j=1}^{n} (\delta_j - \bar{\delta})^2}} \]

Where \( Y_j \) = value of the \( j \)th image in a given voxel and \( \delta_j \) = the reference function. SPM[Z] map averaging and subject-by-subject-based region of interest (ROI) analysis were then used to analyze patterns of functional activation across subjects.

Kolmogorov-Smirnov statistic

Analysis was performed using standard methods (Aguirre et al. 1998). All images obtained during odorant blocks were combined to form the task condition and all images obtained during the no-odorant blocks were combined to form the control condition. The nonparametric Kolmogorov-Smirnov (KS) two-sample test was then applied to these two conditions (d1 and d2) (Siegel and Castellan 1988). The maximum distance (\( D \)) between the cumulative probability distributions \( [S_d(y)] \) of the two conditions was then computed for each voxel by

\[ D = \max |S_{d1}(Y_j) - S_{d2}(Y_j)| \]

Where \( j = \) index of time point and \( Y = \) value of time point.

To test the significance of the difference between the \( D \) values, the distribution was transformed to a chi-squared distribution with \( df = 2 \) (Siegel and Castellan 1988) as follows

\[ X^2 = 4D^2i_1i_2 \frac{i_1i_2}{i_1 + i_2} \]

Where \( i_{12} \) = sample sizes of odorant and no-odorant conditions.

Simulations have shown that in contrast to the Pearson statistic analysis described above, this analysis is highly sensitive to changes in the variance of two distributions of fMRI time series (Zhao et al. 1997).

Localization and making composite images

For accurately localizing activations, centroids of maximum activity were converted to the coronal plane acquisition of each subject using a cross-reference program (Desmond et al. 1997b). Regions were then identified using the atlas of Mai, Assheuer, and Paxinos (1997). This atlas was preferred over the commonly used Talairach and Tournoux (1998) atlas because it gives far more detailed anatomy of the olfactory regions. To prevent confusion, note that in contrast to the latter, the convention used in the Mai, Assheuer, and Paxinos atlas (and in this paper) for coronal slices is negative numbers for slices anterior to the anterior commissure (AC), and positive numbers for slices posterior to the AC.

Composite images were made by first creating an outline of each oblique section using a T1-weighted anatomy image of a representative subject to form a template for that slice. Then each subject's functional map (containing either Pearson Z scores or KS scores) at each section was transformed into the region specified by the template, as described by Desmond et al. (1998), using the following steps: translating, scaling, and rotating the functional map to match the centroid and dimensions of the template; defining a matching set of points around the perimeter of the functional map and that of the template; creating a grid of points from the perimeter points of the functional map and a corresponding grid on the template such that a one-to-one mapping existed for the grid points in each set; and mapping the values from the grid points of the functional image to the grid points of the template. The resulting averaged functional activation maps then were intensity thresholded, and each slice was subjected to a cluster analysis procedure (Xiong et al. 1995) to correct for multiple statistical comparisons, using a spatial extent threshold that yielded a \( P < 0.05 \) significance level over the entire composite image. The composite image that is obtained through this process inherently contains a loss in spatial resolution in comparison to the single-subject SPM[Z], KS, and ROI-based analysis. Thus to faithfully represent the spatial resolution of the composite, rather than present it overlaid on the template subject or line drawing, the composite is presented overlaid on similarly composited T1 anatomy images of all subjects. Furthermore all statistical comparisons were made on data obtained from the individual delineated ROIs, thus the composite images serve primarily for visual presentation of the data.

RESULTS

Odorant-induced activation revealed by the Pearson statistic

All 4 odorants induced consistent increases in activation in all eight subjects. Figure 3 is a composite image of activation induced by the odorant vanillin in all eight subjects. Table 1 lists the regions significantly activated by all four odorants in all eight subjects. In addition to the activations previously reported in olfactory imaging studies (Dade et al. 1998; Kettenmann et al. 1997; Levy et al. 1997, 1998; Malaspina et al. 1998; Sobel et al. 1997, 1998a,b, 1999a; Youssef et al. 1997, 1999a,b; Zald and Pardo 1997; Zald et al. 1998) such as the lateral orbital gyri, superior temporal gyrus, cingulate gyrus, peri-insular region, anterior medial thalamus, amygdala, and inferior and middle frontal gyri, a consistent activation also was witnessed deep and caudal to the area of the piriform (slice 5) (Fig. 4). The anatomic resolution of the acquisition did not permit demarcation of specific nuclei within this area, but the activation is centered at the expected location of the anterior olfactory nucleus, and borders the olfactory tubercle. As in previous studies, the Pearson analysis revealed only occasional inconsistent increases in amplitude of fMRI signal in the piriform and olfactory ventral temporal regions after stimulation with odorants.
Sniff-induced activation revealed by the Pearson statistic

Previously, we have shown that sniffs induce fMRI activation in the ventral temporal region (Sobel et al. 1998a). Several controls indicated that sniff-induced activation was related to the sensation of air flow in the nostrils (Sobel et al. 1998a). To test whether here too sniffing was activating the ventral temporal regions, we reanalyzed the same data, but rather than correlate the fMRI activation time series with the frequency of sniffing (0.125 Hz), we correlated activation with the frequency of activity was that of odorant presence (0.0125 Hz), we correlated activation with Odorant-induced activation revealed by the KS statistic

In contrast to the Pearson-correlation-based analysis that is sensitive to only the first moment of the fMRI signal (signal amplitude), the KS statistic is sensitive to all moments of the fMRI signal (including signal variance). Considering that the analysis of the time series in the sniff region revealed that odorants increase the variance of fMRI signal in the piriform cortex, we expected that using the KS statistic (which is sensitive to variance) to reanalyze the data may enable visualization of odorant-induced activation in the ventral temporal regions.

As predicted, the KS statistic revealed significant activation...
Assheuer, and Paxinos atlas (1997), and thus the 50-mm of the activation patterns were lateralized. As lateralization patterns are out of the scope of this report, we will address this topic separately in future publication.

Anteriole from the anterior commisure in the coronal plane. Also note that these are centroids of activation, in some structures, e.g., cingulate gyrus, activation possible that odorants were in fact increasing the amplitude of variation only, at least one additional explanation had to be considered: as noted in the introduction, patterns of rapid habituation have been recorded in piriform neurons. Thus it is possible that odorants were in fact increasing the amplitude of signal in the piriform, but that this increase was short-lived and hence did not closely follow the time course of the task. Whereas the previously used Pearson statistic is very sensitive to the shape of the signal time course, the KS statistic compares the cumulative distributions of the activation related to the task and control conditions and is far less sensitive to the shape of the time course. Thus the increased variance may not be the only activation parameter that helped satisfy the KS but not the Pearson statistic.

To test why the KS test was distinguishing 8% of the sniff-activated voxels as also odorant activated, we analyzed the time course of activation within the KS odorant activations. A functional ROI was constructed of the voxels in the ventral temporal region that were activated significantly by odorants in the KS test. The signal time course in this region revealed a steep rise in activation during the first odorant block, which then was followed by very small increases in activation during the following three odorant blocks (Fig. 9). The early transient persisted for ~30–40 s, through which activation returned to baseline level. In these voxels there was an overall odorant-induced increase in both amplitude [0.92% increase, F(1, 7) = 14.84, P < 0.006] and variance [8.5% increase, F(1, 7) = 18.57, P = 0.003] of activation (Fig. 7). Throughout the scans, a short-lived peak also was seen in the signal at every point of transition in condition from odorant to no-odorant and vice versa.

To test if the overall increases in amplitude and variance in
the KS region were dependent on the initial increase only, we reanalyzed the data following exclusion of the first block (i.e., 80 s). In the remaining 240 s of the experiment, there was only an insignificant increase in amplitude [0.3% increase, \( F(1,7) = 0.56, P = 0.48 \)] and variance [1.8% increase, \( F(1,7) = 0.4, P = 0.54 \)] in the odorant versus the no-odorant conditions. This analysis showed that the overall increases in amplitude and variance seen in the KS odorant-induced activations were largely dependent on the sharp increases during the initial block of the experiment (Fig. 9).

To test if the variance effect previously witnessed in the sniff region may have been carried exclusively by the KS odorant activated voxels within this region, we reanalyzed the sniff region excluding the KS odorant voxels (i.e., excluding 8% of the sniff region). The odorant-induced increase in variance in this region remained significant after this exclusion [\( F(1,7) = 14.97, P = 0.006 \)]. This analysis showed that information regarding odorant presence was available in the sniff region (as an increase in variance) even in those voxels in which there was no significant early transient increase in amplitude.

To test if the increase in variance in the sniff region was also largely dependent on the initial block, even though the increase in amplitude was not significant in this block (in the sniff region), we reanalyzed the data in the sniff-region after exclusion of the first block (i.e., 80 s.). In the remaining 240 s of the experiment, there was only an insignificant increase in variance [0.4% increase, \( F(1,7) = 0.7, P = 0.42 \)] in the odorant versus the no-odorant conditions. This analysis showed that the overall odorant-induced increase in variance seen in the sniff-induced region was largely dependent on the sharp increases during the initial block of the experiment.

The preceding analysis show that our success in showing piriform activation was largely dependent on the initial response obtained during the first odorant block. This raises the concern that the initial increase in activation may have been...
nonolfactory in origin. It is possible that the early increase in vigilance and attention associated with the beginning of any sensory task is in fact the source of the early activation transient witnessed here. This concern was negated partially by the methods, in that a period of 10 s in which no odorants were presented was scanned at the beginning of each experiment to achieve dynamic equilibrium, and further by the results, in that the activation occurred specifically in the olfactory regions and not randomly throughout the brain. That said, and to further control for this concern, two subjects were retested at a later date in which the order of odorant and no-odorant conditions was reversed such that the scans began with a no-odorant block. In these scans we expected the transient to occur only in the second block of the experiment. As predicted, in the KS region, a transient sharp increase in activation occurred in these scans only 40 s in to the experiment. This control showed that the transient was related to the onset of odorant presence and not to the onset of the experiment (Fig. 10).

Odorant-induced activation revealed by the Pearson statistic using a hemodynamic response function that considers habituation of the response

Using the KS statistic we successfully visualized odorant-induced activation in primary olfactory regions. Considering, however, that there are some statistical concerns regarding the

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**FIG. 5.** Magnification of ventral temporal area from composite images of all 8 subjects. A: increases in fMRI activation induced by sniffing, regardless of odorant presence. Significant activation is seen in the olfactory ventral temporal areas including the piriform. B: increases in activation induced by presence of the odorant vanillin using the Pearson statistic. No significant activation seen. C: increases in activation induced by presence of the odorant vanillin using the Kolmogorov-Smirnov (KS) statistic. Significant activation is seen in the olfactory ventral temporal areas including the piriform. D: increases in activation induced by presence of the odorant vanillin using the Pearson statistic and a reference waveform that takes into consideration the habituation of the response (Fig. 10). Significant activation is seen in the olfactory ventral temporal areas including the piriform. Note that all 4 analyses were performed here on the same data.
use of the KS statistic in fMRI (Aguirre et al. 1998), we now sought to use our knowledge of the habituation to visualize this activation with the more commonly used Pearson statistic. To this end, we repeated the initial analysis, substituting the boxcar hemodynamic reference wave form with a new hemodynamic reference wave form that takes the expected habituation into account.
into consideration (Fig. 11). This reference waveform was constructed based on responses recorded in the piriform cortex of the rat (Wilson 1998a), as well as on the data obtained here. The resulting expected waveform consists of an exponential habituation of the response within each odorant block, as well as an habituating component across the entire scan. Reanalysis of the data using the Pearson statistic and this reference waveform revealed extensive odorant-induced activation in the primary olfactory regions (Fig. 5D). Activation was robust in the AON, the olfactory tubercle, the periamygdaloid cortex, the entorhinal cortex including the uncus, and the frontal and temporal portions of piriform cortex, extending in to what traditionally has been considered agranular insular cortex [for the right side, centered at x = 24, y = -1, z = -11, extending rostrally up to y = -4, and caudally up to y = 2 (using the atlas of Mai et al. 1997)]. To assure that the sniffing frequency (0.125 Hz) was not being picked up in this analysis, it was repeated with a high-pass filter of 0.025 Hz (using SPM96, Wellcome Department of Cognitive Neurology). This reanalysis yielded identical results. We conclude here that we found use of the Pearson statistic (in SPM96) combined with the habituating reference wave-form (Fig. 11), the best way to visualize odorant-induced activation in primary olfactory cortex when using a box-car stimulation paradigm.

FIG. 7. Percentage change of amplitude and variance in different regions of interest (ROIs). Bars are SE. In the sniff-delineated region, there is no significant odorant-induced change in amplitude, but there is a significant increase in variance. This effect did not occur in a control region that consisted of an identical number of voxels in the surrounding regions that were not activated by sniffing. In the subset of voxels in the sniff region, that showed odorant-induced activation in the KS test (KS region), there were significant odorant-induced increases in both amplitude and variance.

FIG. 8. Magnification of the ventral temporal area from composite images of all 8 subjects with all 4 odorants. Whereas the Pearson statistic revealed only a small ventral temporal activation in only 1 of the odorant conditions (decanoic acid), the KS statistic revealed significant activation in the ventral temporal region of all 8 subjects for all 4 odorants. Note the reproducibility of this activation across all 4 odorants as highlighted by the green arrows. Activations are in POC, occurring in part in the piriform cortex and spanning medially in to the periamygdaloid cortex.
DISCUSSION

We have shown with fMRI that odorants induce a sharp increase in POC activation, which then rapidly habituates despite continued odorant presentation and detection. Rapid habituation has been recorded electrophysiologically in anesthetized rat POC (Haberly 1973a,b; Nemitz and Goldberg 1983; Wilson 1998a), with a time course strikingly similar to that seen here in humans. In rats, a 50-s continuous odorant...
stimulation led to a strong multiunit increase in piriform cortex activity that decreased to baseline within 25–35 s (Wilson 1998a). Here, a similar increase in activity was seen that decreased to baseline within 30–40 s. Repeated 2-s odorant stimuli in rats (30 s interstimulus interval) induced complete habituation within 5–10 stimuli (Wilson 1998a). Here, if one was to consider each sniff as a separate odorant presentation, habituation also occurred after about five presentations (the contribution, if any, of anesthesia to this habituation in animals is unclear) (McCollum et al. 1991; Schoenbaum and Eichenbaum 1995). Finally, the transient odorant-induced increase in activation occurred here in only a portion of POC. Restriction of a similar transient response to only a portion of POC was also evident in frogs (Duchamp-Viret et al. 1996).

Whereas the Pearson statistic did not reveal odorant-induced activation in POC, the KS statistic did. Two distinctive aspects of the KS statistic made this possible: comparison of cumulative distributions rather than time-course shape, which enabled the early transient to carry significant weight, and sensitivity to signal variance. What was the source of the increased signal variance in the odorant condition? Although a full consideration of the possible relations between neural response properties and fMRI signal is beyond the scope of this manuscript (e.g., Shulman and Rothman 1998), we offer a working hypothesis. In POC neurons, odorants can induce rapid short-lived increases in activity followed by a period of habituation (Haberly 1973a,b; Nemitz and Goldberg 1983; Wilson 1998a). The increases in activity can include both depolarization and hyperpolarization in sometimes complicated temporal sequences, and the habituation involves reductions in both of these phases of response. Depolarization and hyperpolarization, however, can both increase fMRI signal. Thus increased variance of fMRI signal during the odorant condition may have occurred because a given fMRI sample point taken in the odorant condition fell on either the early increase in activity (above baseline) or the ensuing habituation and decrease in activity (below baseline). In turn, in the no-odorant condition, all sampling points fell on the baseline. Therefore whereas the mean of the sampling points in the odorant and no-odorant conditions may be equal, the variance would be higher in the odorant in comparison to the no-odorant condition.

The preceding model suggests that there are scenarios of neural-response-induced blood flow where information is represented in changes of fMRI signal variance. One may argue that the observed odorant-induced increase in variance was only a reflection of the odorant-induced increases in amplitude during the first 40 s of the experiment. Yet the increase in variance in the cumulative odorant conditions versus the no-odorant conditions was significant, whereas the changes in amplitude were insignificant (in the sniff region; Fig. 7). In fact, for two of the four odorants used, the significant increase in variance persisted in spite of an insignificant decrease in amplitude (in the sniff region). This dissociation of a decrease...
in amplitude accompanied by an increase in variance, strongly suggests that measures of temporal variance should not be overlooked as a measure of potentially important information in fMRI. One may further argue that the Pearson statistic would have been sufficient to visualize odorant-induced activation in POC if we would have started off by using the appropriate reference wave-form that takes habituation into account (as indeed we did in the final set of analyses). Yet it was use of the KS statistic that was necessary to delineate the KS region where the habituating time course was found in the first place.

Now that odorant-induced fMRI activation can be recorded in POC, what can we learn from this activation regarding olfactory processing? In the olfactory system, odorants are first transduced at the olfactory epithelium. Olfactory information then is projected to the olfactory bulb, and via the olfactory tract, on to POC (reviewed in Shepherd 1991). The odorant-induced dynamics of neural activity in POC have been studied and modeled extensively (Eichenbaum et al. 1991; Freeman 1991; Haberly 1985; Haberly and Bower 1989; Hasselmo et al. 1990; Wilson and Bower 1992), but the way in which odorants are encoded in POC remains unknown.

The following is a working hypothesis for what may be occurring in POC as reflected in our fMRI findings. Adrian (1942) showed in the hedgehog that sniffing induces a high-amplitude oscillation in POC. This oscillation, typically in the 40- to 60-Hz gamma range, occurs regardless of odorant presence and has been witnessed in many species (Bressler and Freeman 1980; Domino and Ueki 1960; Ketchum and Haberly 1991). Evidence supporting the persistence of this phenomenon in humans first was obtained electrophysiologically at the level of the olfactory bulb (Hughes et al. 1969), and later with fMRI at the level of POC (Sobel et al. 1998a). In humans, sniffs induce increases in the amplitude of fMRI signal in the ventral temporal region, regardless of odorant presence. This effect was replicated in the current study, where it was evident in all eight subjects in all 32 scans. Thus the sniff-induced increases in fMRI signal amplitude may globally reflect the summated underlying oscillatory gamma wave, and the region delineated by sniffing, or olfactory exploration, may in fact be POC [i.e., piriform cortex + surrounding cortex and nuclei (Haberly 1985)]. The sniff-induced activation may in part represent information regarding the sniff itself, i.e., sniff duration, sniff air-flow-rate, and sniff volume, being made available to olfactory cortex. This information is necessary for computation of an accurate olfactory percept (Sobel et al. 1999b,c; Teghtsoonian et al. 1978).

The question remains as to how odorant information then is encoded within the sniff-induced oscillation. The initial transient increase in odorant-induced signal amplitude suggests that odorants may increase the overall rate of activity in POC. But whereas the increase in signal amplitude was significant only in the first block of the experiment, subjects continued to perform at >90% accuracy throughout the 320 s of the task. In other words, subjects were still accurately performing the task of odorant detection when there was no significant odorant-induced increase in POC signal amplitude.

Habituation of the fMRI signal from POC may reflect one of, or a combination of, several mechanisms. Habituation could have reflected plasticity within POC itself. Practice in detecting the identical odorant may have led to increased neural efficiency as the task progressed, which may have in turn been reflected in decreased blood flow. A similar mechanism has been suggested in an fMRI study of a verbal task in which practice eliminated an initial difference between two conditions (Raichle 1987; Raichle et al. 1994). Presumably here odorants continued to increase the rate of activity in POC, but practice made the neural encoding so efficient that the fMRI signal became too weak to measure a reliable difference (0.3% increase, P = 0.48 in the continued odorant presentations).

The preceding mechanism suggests an alteration in the efficiency, but not scheme, of encoding for the continued versus the initial response. An alternative mechanism suggests that the relative function of primary and secondary olfactory cortices shifted in the continued versus initial response. Although the piriform cortex habituated, the olfactory system continued to respond to the odorant. This was reflected in the time course of activation in the lateral orbitalfrontal gyrus (Fig. 6) that revealed continued response to odorant presence throughout the task. This dissociation between rapid habituation in the POC and sustained activation in secondary olfactory cortex suggests that the functional anatomy of the response may be altered with experience and that olfactory detection may proceed with lesser POC involvement. This dissociation may represent the functional significance of the olfactory bulb projections to insular and prefrontal regions that do not traverse the piriform cortex (Cinelli et al. 1987; Shipley and Adamek 1984). In other words, the continued response in secondary olfactory cortex may depend on a direct input from the olfactory bulb rather that on input that traverses POC. The preceding scenario suggests that POC is functioning as a change-detector in the pattern of olfactory input. Once a pattern of activity was set at the initial odorant presentation, each additional sampling (sniff) of the olfactory environment is either “same” or “different.” If the sample is “same,” then activity in POC is maintained at a low level, but if the sample is “different,” a sharp increase in activity is evident. In contrast, secondary olfactory cortex continues with a large response to continued “same” odorant presentations.

One may argue that reductions in POC activation reflect habituation of inputs to the POC from the olfactory bulb. Some peripheral habituation is indeed likely to have been occurring, but POC habituation occurs independently of peripheral habituation (Wilson 1998a) and may be the result of intrinsic POC mechanisms (Wilson 1998b). Furthermore the continued accurate behavioral response seen here suggests that any peripheral habituation was not a complete habituation.

Whereas previous fMRI studies of olfaction failed to show consistent robust odorant-induced POC activation (e.g., Sobel et al. 1998a, 1999a; Yousem et al. 1997), such activation was reported in a study using positron emission tomography (PET) (Zatorre et al. 1992). One possible reason for this discrepancy may be that the spatial resolution of PET did not permit distinguishing the AON activation (Fig. 4) from piriform cortex activation. Regardless, however, of the latter possibility, the findings here may resolve the apparent discrepancy between the PET and fMRI findings. The PET study used a statistical analysis that compares cumulative distributions. Thus like in the KS analysis here, the early transient response in the piriform cortex would be sufficient to induce a significant effect in the PET design. Perhaps most importantly, the PET study used eight different odorants within a given scan,
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