SUPPLEMENTARY METHODS

Subjects
Twelve subjects (6 women and 6 men, mean age 29.8 ± 4.8) participated in the main experiment, and 8 in the control experiment, all after giving informed consent to procedures approved by the UC Berkeley Committee for the Protection of Human Subjects.

Odorants and olfactometry
Odorants were delivered by a computer-controlled air-dilution olfactometer that has been described in detail\textsuperscript{1}. This olfactometer switches between odorant presence and absence in less than 2 ms, with no non-olfactory cues as to the alteration. The olfactometer also provides an ongoing real-time measurement and recording of airflow in the nostrils. The five odorants that were used were citral, PEA, eugenol, propionic acid, and limonene, all at suprathreshold concentrations. The no-odorant condition was the identical condition in all scans. Out of the 264 "no odorant" trials conducted within the "task detection" condition in this study, only 2 false positives were generated. This demonstrates that the no-odorant condition was indeed perceived as such.

Experimental design
This study used an event-related design (Figure 1). There were four trial types, each presented 22 times across five 685-second long functional scans, using an inter-trial-interval of 35 seconds. Three of the trial types ("task detection - odorant"; "task detection - no-odorant"; "inhalation") were randomly mixed within four of the scans, and the fourth trial type ("inhalation2") was the only trial type in the fifth scan. "Inhalation2" trials were identical in all respects to "inhalation" trials, but occurred repeatedly throughout a scan, not interleaved with other trial types. The temporal order of the five different functional scans was counter-balanced across subjects.

Subjects received through earphones task instructions generated by digitally recorded voice. Each trial began with an auditory primer for "task detection" or "task inhalation". In "task detection" subjects were to take one sniff at the tone and determine whether an odorant was present or not. Odorants were present on half of these trials (hence "odorant" and "no-odorant" trials). The 5 odorants were randomly presented across the "odorant" trials in order to minimize adaptation. In "task inhalation" subjects were to take one sniff, but they knew in advance that an odorant would never be present during "task inhalation". Thus, the only difference between sniffs in "task inhalation", and the "no-odorant" sniffs of "task detection" were that in the latter condition subjects were exploring for the presence of odor. In other words, the only difference was the attentional setting. Subjects were instructed to try and maintain a constant sniff pattern across conditions. Psychophysical piloting suggested that switching between "task inhalation" and "task detection" within an event-related design is subjectively not easy. For this reason the fifth functional scan consisted of "task inhalation" only, and was called "inhalation2".

Control study
Each experiment began with a passive (no task) block-design olfaction scan. This scan was later used to functionally restrict the POC ROI. This scan was followed by 5 scans containing an event-related design, where subjects performed either task olfaction or task audition (ISI = 35). The sensory content of these two tasks was identical. In both tasks subjects sniffed an odorant (one of three concentrations, randomized across trials, equal
across tasks) for the duration of a tone (one of three pitches, randomized across trials, equal across tasks). The only difference between task olfaction and task audition was in an auditory primer that preceded the task (containing the words, “Task Olfaction” or "Task Audition"), and in the question following the task ("Rate Intensity" or "Rate the Pitch"). Both the concentration of the medium intensity odorant, and the degree of change in pitch, were individually adjusted for each subject such that performance was at ~75% accuracy, thus equating the difficulty, or effort, of the two tasks.

**Imaging parameters**

[All the raw MR data will be made publicly available on our website following publication]. The experiment was conducted on a 4T Varian Inova magnet. A costume-built full-head receive coil was used for signal reception. A T2* sensitive echo planer sequence was employed with parameters of TR = 500 ms, TE = 28 ms, flip angle = 20°. The Spatial resolution was set by a 64 x 64 voxel matrix covering a 19.2 cm x 19.2 cm field of view, resulting in a functional in-plane resolution of 3 mm and through-plane resolution of 3.5 mm. Two interleaves were collected for each frame, with total acquisition time of 1000 ms per frame. The interleaves were interpolated during image reconstruction resulting in an effective temporal resolution of 500 ms per frame. Fifteen frames were collected before task onset at the beginning of each scan in order to achieve dynamic equilibrium. Eight 3.5 mm thick slices were acquired at an oblique plane traversing from the frontal pole to the temporal pole (typically 30° clockwise to the AC-PC plane). This slice orientation was chosen so as to cover the entire primary olfactory cortex while minimizing partial voluming artifacts. In order to prevent head motion, a custom-formed bite bar was fit to the individual dental impression of each subject. This bite bar was also fit with a pyrolitic graphite implant that dramatically reduced ventral temporal susceptibility artifacts. Full brain T1-weighted flow compensated spin-warp anatomy images (TR = 500 ms, minimum TE, isotropic 0.875 mm voxels) were acquired as a substrate on which to overlay functional data.

**Imaging analysis**

Data were analyzed using MrVista (previously known as mrLoadRet) (http://white.stanford.edu/software/)

Following initial coregistration and motion estimation and correction, two of the 12 subjects showed evidence of significant head movement and were excluded from further analysis. We then set out to define piriform cortex.

**Defining piriform cortex**

Piriform cortex is defined cytoarchitecturally, and its exact borders cannot be delineated based on the MR image alone. Here we combined a structural and functional restriction in order to define the region of interest. As seen in Figure 1, we first outlined the expected piriform based on an atlas that is particularly detailed in this respect, and then functionally restricted this region to only voxels that responded hemodynamically to the odorant condition (p < 0.01). In the control study, we similarly restricted to voxels activated by odorants in the independently conducted passive block-design scan.

The analysis above, while corresponding to the methods used by others to analyze fMRI data, is a parametric analysis and relies on the assumption that the shape of the hemodynamic response in a given condition is relatively constant from trial to trial. Furthermore, the ANOVA also assumes that the distribution of peak responses are
normal and that the noise at the peak is independent of its magnitude. Neither of these assumptions are generally true in fMRI data\textsuperscript{10}, although most practitioners assume that the departures from them are small and tolerable\textsuperscript{11}. To address the possibility that the assumptions in our analysis may have biased our result, we also pursued an alternative and parallel analysis using nonparametric bootstrapping, a modern resampling technique that makes no such distributional or parametric assumptions\textsuperscript{12}, and has recently been applied to fMRI data\textsuperscript{13}. In brief, bootstrapping involves resampling the data with replacement and computing the statistic of interest for each replicate. By repeatedly resampling and computing statistics in this manner, one can form probability distribution for the statistic of interest from which one can deduce statistical significance or effect size. We bootstrapped the data using the following procedure. We first formed bootstrap replicates as follows. For each subject, we resampled with replacement from the trials within each condition and within each scan, forming a new set of trials of the same size as the original. Then across the complete replicate data set, we averaged all the time series across subjects within each condition, and measured the maximum value of the average response in a liberal window around the expected location of the peak for that condition (we used a peak ranging from 2 secs after trial onset to 10 seconds after trial onset). For each statistical test, we calculated 1000 bootstrap replicates. To test whether two conditions differed significantly from one another amounts to calculating the confidence intervals for the null hypothesis (difference=0) based on the bootstrap distribution for the difference of the two conditions\textsuperscript{12}.

The results from the bootstrap analysis are presented throughout this manuscript in parallel with the parametric analysis, and in general the two analyses were in good agreement, suggesting that the data were largely well modeled by the condition specific hemodynamic response functions. It is noteworthy that since we used the odorant condition to define our region of interest, the odorant condition is treated as a reference condition on which we do not make statistical inferences.

**Functionally defining additional regions of interest**

Whereas piriform cortex was defined using anatomical features and subsequently restricted to a functional subregion, we wanted to examine the response in other regions as well. Towards this exploratory end, we formed a composite statistical parametric map of the data for all subjects by computing activation maps within each subject’s inplane images, and then aligning these images to the same brain volume. In each subject, for each voxel, we calculated 4 parametric maps, one for each condition. Each voxel in each condition represented a beta parameter obtained by regressing the response for all trials of that type against a standard hemodynamic response function. Each of these statistical parametric maps was then spatially smoothed (3-D Gaussian kernel, FWHM=8 mm) and transferred into the same brain volume to which the inplanes had been aligned using a 9 parameter affine transform\textsuperscript{14}. Only voxels in the volume for which we had data from all subjects were included in the analysis. For each voxel in the volume we calculated a student’s T statistic, testing for difference from zero. This produced a random effects statistical parametric map of the significance level of each voxel\textsuperscript{11}. The statistical parametric map for response to the odorant condition was used with a threshold value of \(p<0.001\) to functionally define regions of interest. These ROIs were then transformed back into the individual subjects’ inplane coordinates and were analyzed in the same manner as the piriform ROIs.
References