Odor-intensity Coding in the Anterior Piriform Cortex

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Introduction

The primary olfactory cortex is defined as the region that receives direct fiber projection from the olfactory bulb (OB). The piriform cortex (PC) is the largest structure in the olfactory cortex. However, no 'point to point' anatomical connection between the OB and PC has been found (Haberly, 1998). In addition, no evidence that neurons with similar odorant preferences are located in a cluster in the PC has been reported. A recent study has demonstrated, however, that anterior PC neurons discriminate alkane odorants based on carbon chain length (Wilson, 2001). On the other hand, genetic tracing studies in mice have indicated that inputs from different odorant receptors are mapped onto overlapping clusters of neurons in the PC (Zou *et al.*, 2001). It has been suggested, thus, that the coding for each odor is spatially distributed in the PC (Haberly, 1998). Further, a recent mapping study of c-fos immunoreactivity in response to odorants suggested odor-specific spatial patterns of activity within the anterior PC (Illig and Haberly, 2003). It remains unclear, however, whether there is any spatial organization or clustering of the PC neurons with similar stimulus preferences.

Because the optical imaging method for detecting intrinsic signals has been used to monitor neural activity in the sensory cortices (Bonhoeffer and Grinvald, 1993; Yoshimura *et al.*, 2004), high resolution mapping of odor-induced activation is now possible. We explored the organization of olfactory information processing in the anterior PC using optical imaging and compared these data to unit activity.

Methods

Animal protocols used in this study complied with all pertinent institutional and Japanese Government regulations and every attempt was made to minimize the number of animals utilized. Twenty-nine and 11 guinea-pigs were used in the optical imaging and extracellular unit recording studies, respectively. Animals were anesthetized with Na-barbiturate. The skull overlying the anterior PC was thinned. The heart rate and rectal temperature were monitored continuously. The pulmonary rate and thorax movements were recorded. The animal's heads were fixed on the stereotaxic frame (Onoda *et al.*, 1984) and tilted until the camera field was as horizontal as possible. Intrinsic signals (absorption changes at 646 nm of light) in the anterior PC were recorded through silicone oil. The imaged area was $8.7 \times 6.5\ mm^2$ and contained 320×240 pixels. The intrinsic signal images were recorded at 500 μm below the cortical surface.

Amyl acetate (AA), *n*-butyl alcohol (butanol), butyraldehyde (butanal), diethyl ether (ether) and xylene were used as odor stimuli. Odorous vapor was delivered from the syringe by the push of each plunger, which was motor-driven and under the control of a pulse generator. Several odors were applied in order for 6 s at 5 min intervals to avoid habituation. Each trial consisted of a pairs of records with and without stimulation at 2.5 min intervals. For each record, 16 frames each with a 500 ms frame length were collected for 8 s.

Individual images from the 4th to the 15th frames with and without stimulation were averaged. The average image without stimulation was subtracted from that with stimulation and averaged across eight trials (a differential image). The statistically significant pixels in signal intensity were calculated by a *t*-test ($P < 0.05$). In this analysis, a Gaussian spatial filter was used (Tsunoda *et al.*, 2001).

Extracellular single unit activity was recorded using glass microelectrodes from the rostral and caudal anterior PC regions. Each odor stimulus with different concentrations was tested at least four times to determine statistically the concentration threshold of the unit.

After optical and/or electrical recording sessions, a glass pipette containing brilliant blue dye was inserted ~500 μm below the cortical surface into the center of each active zone or of each unit-recording region and the dye was deposited. The animal was perfused with 4% paraformaldehyde. Serial frontal sections at 40 μm thickness were analyzed histologically.

Results

Optical odor maps obtained from intrinsic signal imaging in the anterior PC

Odor-induced cortical active regions appeared in a narrow band beneath the rhinal sulcus over the lateral olfactory tract (LOT), which was defined as the dorsal part of the anterior PC (aPCd) in the rat (Haberly, 1998). Differential images demonstrated optical response to 1.0% AA but no response to mineral oil. Focusing depths were changed vertically from 0 to 800 μm deep to the cortical surface. Average depth profiles ($n = 6$) showed a great increase over 400 μm and a peak at 500 μm. Optical signals disappeared at 800 μm. A histological section showed a marking spot (~500 μm below the cortical surface) in layer II. These results indicate that active regions in the aPCd primarily originate in layer II.

Optical maps of the aPCd in response to changes in concentrations of AA

AA at 0.01 and 0.03% evoked a small number of active spots in the most rostral aPCd region. AA at 0.1% induced several clusters of active spots in the narrow band. Application of 0.3% caused large clusters of strong activation in the AON and rostral aPCd and then small clusters of activation spread until it reached the caudal end of the LOT. Application of 1.0% AA generated a long and narrow band of strong activation. It appeared that increasing concentrations increased the number of active spots which spread into the extent of the caudal aPCd, suggesting a rostro-caudal gradient in activation.

Optical maps of different odorants in the aPCd

The lowest concentration odors generated a small number of active spots in the narrow band. Their locations of active spots in response to different odorants overlapped slightly. Medium concentration odors generated active spots as far as the caudal region. The highest

concentration odors generated a large number of active spots in the whole aPCd. Locations of active spots obtained in response to different odorants with higher concentrations considerably overlapped. It appeared that increasing concentrations of various odors also showed a rostro-caudal gradient in activation in the aPCd.

A relation between a total area of cortical activation and odor concentrations

The relation between the total area and odor concentrations was examined. Total areas of the cortical activation were calculated from the number of the active spots. In general, the relation between the magnitude of the sensation felt and stimulus intensity is described by a power function. In log–log coordinates, the relation shows a straight line whose slope is the exponent in the power law. The average exponents of AA was 0.74 ± 0.14 (mean $\pm$ SE, $n = 3$). The exponents of butanal, ether and xylene were 0.53 ± 0.05 ($n = 3$), 0.57 ± 0.09 ($n = 3$) and 0.69 ± 0.11 ($n = 3$), respectively. It appeared that the relation between the total area of the cortical activation and concentrations might be described by a relatively linear power function within a certain concentration range.

Single-unit recordings of cortical neurons in response to changes in odor concentrations

The distribution of odor thresholds for cortical neurons recorded in rostral and caudal aPCd regions was examined. The mean concentration threshold for AA in the rostral units was $0.18 \pm 0.06\%$, whereas that in the caudal ones was $1.62 \pm 0.57\%$ (mean $\pm$ SE). The mean thresholds in rostral and caudal units for AA were significantly different (t-test, $P < 0.01$). The mean threshold for xylene was $0.27 \pm 0.14\%$ and $1.45 \pm 0.66\%$ in rostral and caudal units, respectively. The difference between the mean thresholds for xylene in rostral and caudal units was also significant (t-test, $P < 0.01$). From these results, thus, neurons with lower concentration threshold in the aPCd were dominant in the rostral region, whereas neurons with higher concentration threshold were prominent in the caudal region, suggesting that cortical neurons have a rostro-caudal gradient in mean thresholds for odorants.

Discussion

In this study, optical images of intrinsic signals from the aPCd in response to odor stimulation are mapped. The results showed that response patterns of individual odorants were mapped onto spatially overlapping locations of neural activity in the long narrow band, corresponding to the aPCd. Further, lower concentrations elicited activation in the rostral region of the aPCd, whereas higher ones generated caudally spreading activation and increasing concentrations lead to increasing the total active areas. Data obtained from the depth profile and histological reconstruction demonstrated that the optical signals primarily originate in layer II. Works with tracers and with unit recordings have shown that the superficially situated tufted cells, which project mainly to rostral regions of the PC (Mori, 1987), are more responsive (Schneider and Scott, 1983). These tufted cells have shorter latencies and lower thresholds for electrical stimulation of the olfactory nerve layer than mitral and internal tufted cells, which project axons further caudally to the PC (Schneider and Scott, 1983; Mori, 1987). Further, since a rostro-caudal gradient in afferent versus association fibres has been described (Haberly, 1998), the input is heaviest in the rostral region of the PC. These findings imply that central olfactory connections with a rostro-caudal gradient contribute into olfactory coding in the PC. The spatial representation of odor concentration, therefore, might be due to the spatially organized rostro-caudal gradient in axonal projections of bulbar output cells with the rostro-caudal gradient in their thresholds and spatial differences in summed inputs from bulbar cells would be occurred in the aPCd along a rostro-caudal axis.

Acknowledgements

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