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Central Olfactory Pathway in Response to Olfactory Stimulation in Rats Detected by Magnetic Resonance Imaging

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Introduction

The olfactory system is known to have important roles in neuroendocrine regulation, emotional responses, reproductive/maternal functions, aggression, food selection and the recognition of conspecifics, predators and prey, etc. (Shipley *et al.*, 1995). Especially in the case of food/fluid selection and intake, olfaction plays very important roles in combination with taste and texture. The pathway of the olfactory system has a unique characteristic relative to other sensory systems in that the signals go directly to the primary sensory cortical areas and limbic system without involvement of the brain stem. The limbic system is related to emotion, learning and memory. Therefore, odors can affect the preference for food and its memory. So far, there is little direct evidence demonstrating the activation of higher-order olfactory centers during olfactory stimulation except for activation in the olfactory bulb (OB) (Yang *et al.*, 1998; Xu *et al.*, 2000, 2003). In the first part of the present study (experiment I), brain activation areas in response to olfactory stimulation were investigated in anesthetized rats using functional magnetic resonance imaging (fMRI).

In the second part of the present study (experiment II), neural projections in higher-order olfactory centers, especially projections from several olfactory centers to the medial frontal cortex (MFC), were investigated using manganese-enhanced MRI (MEMRI), a newly developed *in vivo* neural tracing technique (Pautler *et al.*, 1998, 2003; Van der Linden *et al.*, 2002). The first part of the study suggested that the MFC, which is a part of the limbic system and not considered one of the olfactory centers (Shipley *et al.*, 1995), responded well to olfactory stimulation to a similar extent and with response patterns similar to the primary olfactory cortical areas, i.e. the piriform cortex, olfactory tubercle, etc. (Kondoh *et al.*, 2002, 2003). Injection of Mn^{2+}, a biological calcium analogue and paramagnetic tract tracing agent, allows highlighting specific brain areas that are active in MEMRI.

Materials and methods

Animals

Male Wistar rats (Charles River Japan, Inc.), weighing 300–450 g, were used. All procedures in the present study were approved by the Institutional Animal Care and Use Committee and adhered to the Declaration of Helsinki and the *Guiding Principles in the Care and Use of Animals* (DHEW Publication, NIH86-23).

Experiment I (fMRI)

Eight essential oils (peppermint, true lavender, petitgrain, tea tree, cinnamon, lemon, roman chamomile or ginger) and three standard odorants of the T&T olfactometer [odorants A (β-phenylethyl alcohol), C (isovaleric acid) and E (skatol)] were used as odorants. Blood oxygenation level-dependent (BOLD) fMRI was performed on a SMIS MRI system consisted of a 4.7 T/400 horizontal superconducting magnet equipped with actively shielded gradient coils (54 mT/m, 26 cm i.d.). Rats were anesthetized with urethane (1.0 g/kg, i.p.) and their heads were fixed in a non-magnetic stereotaxic apparatus specially designed for MRI. A home-made volume coil (60 mm diameter) was used for both transmission and reception of radio frequency. Body temperature was controlled at 37°C by circulating water. One of the odorants was positioned on a paper 5–7 cm apart from the nose of the rat. BOLD images were acquired at three separate coronal slices [+7, +2 and –3 mm to the bregma, which include the OB, the frontal cortex (FC) and the hypothalamus, respectively] by using a T_2^*-weighted gradient echo pulse sequence with the following parameters: repetition time = 90 ms, echo time = 15 ms, field of view = 38.4 × 38.4 mm, acquisition matrix = 128 × 64, slice thickness = 2.0 mm, number of slices = 3, one average. Three planes of images were collected every 6 s for the total of 10 min. Olfactory stimulation was made 3 min after the start of image collection. Intertrial intervals between stimuli were set at 20–30 min. A two-dimensional Gaussian filter was applied to each image and the averaged signal intensity of 3 × 3 pixels was used for subsequent data analysis by home-made image-analysis software. Statistical comparison of control periods to olfactory stimulus periods was carried out using Student's *t*-test.

Experiment II (MEMRI)

Mn^{2+} is a paramagnetic MRI contrast agent, causing positive contrast enhancement in tissue where it has accumulated. Each rat was anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and mounted in a stereotaxic apparatus. Pressure injection of $MnCl_2$ (100 mM, 0.2–0.5 μl) was made into one of several sites in the olfactory pathway by a syringe pump through glass micropipette (tip diameter, 20–40 μm) connected to a Hamilton microsyringe. The sites injected (left side) were the OB, the lateral olfactory tract, the lateral hypothalamic area (LHA), the basolateral nucleus of the amygdala, the mediodorsal nucleus of the thalamus (MD) and the CA1 region of the hippocampus. After surgery, rats were returned to their cages to recover. At 8 and 24 h after the injection of $MnCl_2$, rats were re-anesthetized with 1.5% isoflurane in a mixture of 70% N_2O/30% O_2 gas and their heads were fixed in a non-magnetic stereotaxic apparatus. T_1-weighted multislice spin-echo images were acquired with the following parameters: repetition time = 500 ms, echo time = 20 ms, field of view = 38.4 × 38.4 mm, matrix = 128 × 128, slice thickness = 1.0 mm, slice gap between center-to-center of consecutive slices = 1.5 mm, number of slices = 6–10, one average. Data were processed by home-made image-analysis software.

Results

Experiment I (fMRI)

Responses of higher-order olfactory areas to odors were observed by fMRI in rats. Responses to essential oils were observed most prominently in the OB, the ventral FC (including the piriform cortex, olfactory tubercle and medial forebrain bundle), the MFC (including the anterior cingulate cortex, infralimbic cortex, prelimbic cortex, dorsal peduncular cortex, tenia tecta, septal nucleus and nucleus of the diagonal band), the LHA and the MD. Positive or negative BOLD signal changes were observed in the OB and FCs, whereas positive BOLD signal changes were observed in the LHA and MD. Responsiveness to odors was the highest in the OB and decreased significantly in the posterior slice, including the LHA and the MD. Responsiveness in the LHA and MD to essential oils were high, but those to standard odorants of the T&T olfactometer (odorants A, C and E) were not. Differences in activation sites among essential oils or standard odorants were not clear.

Experiment II (MEMRI)

Injection of Mn^{2+} into the OB clearly enhanced T_1-weighted MR signals in the ipsilateral side of the anterior olfactory cortex, the lateral olfactory tract, the piriform cortex and the orbital cortex, but not in the MFC. Injection of Mn^{2+} into the lateral olfactory tract enhanced the signals in the OB, the anterior olfactory cortex, the lateral entorhinal cortex, the LHA and the substantia nigra, but not in the MFC. Hyperintensity in the MFC was observed by injection of Mn^{2+} into the amygdala, the MD and the CA1 region of the hippocampus.

Discussion

The present study clearly demonstrated the direct evidence of activation in higher-order structures such as the ventral FC, MFC, LHA and MD, during olfactory stimulation in rats. Activation of these areas may lead to changes in autonomic and endocrine systems, learning and memory, emotion and in behavior such as food intake. Interestingly, the MFC which is not considered an olfactory cortical structure (Shipley *et al.*, 1995) responded strongly to ordors. Neurons in the MFC project directly to the nucleus of the solitary tract (Terreberry and Neafsey, 1983) and its electrical stimulation produces changes in heart rate, blood pressure and gastric motility (Buchanan and Powell, 1982; Hurley-Gius and Neafsey, 1986). As proposed by Neafsey *et al.* (1986), the MFC is a 'visceral motor cortex' so that olfactory input may regulate autonomic or visceral functions through activation of the MFC. The present study also demonstrated that the MFC receives inputs from higher-order olfactory centers (i.e. the amygdala, the MD and the CA1 of the hippocampus) but not from the OB and the primary olfactory cortical areas. MEMRI is a useful mapping technique to visualize neural connections in the olfactory pathway in rats. This technique would also be very useful for mapping other central pathways in future experiments.

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