ing gene misexpression to one rhombomere, the registration between neural tube and branchial arches can be systematically disrupted, overriding the normal segregation of branchiomeric segments. These results demonstrate that a classical homeotic transformation (*23*), in terms of gene expression, neuronal migration, and axon targeting, can be induced in a defined neuronal population by the ectopic expression of a single *Hox* gene. Thus, in vertebrates, as in flies, the role of *Hox* genes is to confer positional identity on individual elements of a meristic series.

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24. We are grateful to S. Hughes for the pCla12Nco, RCAS(BP)A, RCAN(BP)A, and RCAS(BP)B vectors; J. Gilthorpe for the RCAN(BP)B vector; and R. Krumlauf for the mouse *Hoxb1* cDNA. We thank V. Prince and A. Graham for the chick *Hoxb1* and *GATA2* in situ probes, respectively, and M. Capecchi and N. Manley for the mouse antibody to Hoxb1. The BEN and QCPN monoclonal antibodies developed by N. Le Douarin and O. Pourquié and B. Carlson and J. Carlson, respectively, were obtained from the Developmental Studies Bank maintained by the University of Iowa, Department of Biological Sciences, under contracts N01-HD-7-3263 from the National Institute of Child Health and Human Development. The Wellcome Trust and the Medical Research Council supported this work. R.J.T.W. is a recipient of a Wellcome Career Development Award.

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Odor Response Properties of Rat Olfactory Receptor Neurons

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Molecular biology studies of olfaction have identified a multigene family of molecular receptors that are likely to be involved in odor transduction mechanisms. However, because previous functional data on peripheral coding were mainly collected from inferior vertebrates, it has been difficult to document the degree of specificity of odor interaction mechanisms. As a matter of fact, studies of the functional expression of olfactory receptors have not demonstrated the low or high specificity of olfactory receptors. In this study, the selectivity of olfactory receptor neurons was investigated in the rat at the cellular level under physiological conditions by unitary extracellular recordings. Individual olfactory receptor neurons were broadly responsive to qualitatively distinct odor compounds. We conclude that peripheral coding is based on activated arrays of olfactory receptor cells with overlapping tuning profiles.

The molecular receptive range (*1*) of olfactory receptor neurons (ORNs) is defined by the specificity of their olfactory receptors (ORs) regarding the structure of odor molecules and by the number and diversity of ORs expressed by the same neuron. Evidence has been provided that ORNs can express only one receptor subtype (*2*), so that ORNs may have a narrowly tuned specificity (*3*, *4*). Furthermore, the expression of ORs has been shown to be spatially segregated, and such an organization was proposed as defining the chemotopy of the olfactory mucosa (*5*). The rules governing the projection of ORNs to the olfactory bulb apparently support the spatial ORNs' segregation (*6*, *7*). If one takes these assumptions and results literally, the qualitative discrimination of odor molecules follows the coding scheme of a "labeled line system" (*8*) that is an extreme version of the one ORN–one OR hypothesis (*2*). In contrast, if broad tuning is assigned to ORs (*7*), this hypothesis is consistent with functional data on the qualitative tuning of ORNs in vivo. However these data have been mainly collected in amphibians, where individual ORNs respond to structurally different odor molecules (*9*, *10*). One may envisage that there is a real gap between molecular data obtained in mammals and cellular data obtained in amphibians that may be ascribed to phylogenetic evolution, if one assumes that ORNs become more and more selective. Such an explanation is in disagreement with the broad responsivity of mammalian olfactory bulb mitral cells (*11*), and our knowledge of the response properties of individual ORNs in intact mammals is limited (*12*). Thus, the question of the range of the chemical receptive field of individual ORNs was addressed by means of classical extracellular recording techniques in anesthetized rats.

Individual ORNs were recorded in vivo in freely breathing (n = 19) or tracheotomized (n = 16) rats (*13*). Ninety ORNs were recorded, generally in the endoturbinate II, during periods ranging from 20 min to 2 hours. The electro-olfactogram (EOG) was simultaneously recorded as close as possible to the single-unit record-

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ing site. EOG is a transepithelial potential resulting from the summed activity of numerous ORNs, which provides direct and global information on both the intensity of the ORNs' response and the number of responding neurons. Sixteen pure odor compounds were used as stimuli. They were selected from those previously tested in the frog according to their effectiveness and their molecular structure (*10*). They were chosen as members of the qualitative groups established through several studies by Duchamp and collaborators and belong mainly to the terpene, camphor, aromatic, and straight-chained ketone groups (*14*). Ethyl vanillin that had never been tested in the frog in vivo was added to get information on the IP3 transduction pathway (*15*). Stimuli were odor pulses of 2-s duration delivered at 200 ml/min. They were applied directly near the surface of the turbinate with a dynamic multistage olfactometer (*16*), which ensured a precise control of the concentration range and allowed 12 discrete concentrations to be delivered. The compounds were delivered at concentrations ranging from saturated vapor pressure (SV) to SV/562. Depending on their SV values, the lowest and highest concentrations were between 3×10^{-8} mol/liter and 5×10^{-7} mol/liter and between 2×10^{-5} mol/liter and 3×10^{-4} mol/liter, respectively.

In rats, ORNs were spontaneously active. About 40% of them fired spontaneously at more than 100 spikes per minute, which is a high rate when compared with rates reported in amphibians (*10*). Furthermore, they were highly responsive. Eighty-three percent of neurons were excited by one odor at least. When all odor tests were considered ($n = 540$), 53.5% induced excitatory responses, 5% induced suppressive responses, and only 41.5% did not evoke a response. According to the nature of the stimulus, the percentages of excitation induced by our odor set varied from 40 to 60%. Only ethyl vanillin was clearly different, with 15%. The same cells could respond to some odors by excitation and to others by inhibition. The excitatory and inhibitory response types were not associated with peculiar odors. Nevertheless, the excitatory response type clearly predominated in peripheral odor coding, each ORN being on average excited by four odors out of our whole odor set (*17*). The ORNs qualitative response spectra were poorly selective. Among the ORNs tested with the whole odor set, many of them were excited by several odors and some of them were even excited by all 16 odorants. The selectivity regarding odor subset 1 (*17*) is illustrated in Table 1, where ORNs are distributed as a function of the numbers of odors to which they responded with excitatory responses. More than 30% of ORNs responded to the six odorants of this subset. It is important to notice that these odorants are members of four distinct qualitative groups (*9*, *10*): Camphor for the camphor group, limonene for the terpene group, anisole and acetophenone for the aromatic group, and isoamyl acetate and methylamyl ketone for the straight-chained ketone group. Lastly, whenever a cell responded to no odor of subset 1, it never responded to any odor of the whole odor set. Taken together, the present results and previous data collected in the frog lead us to propose the odors of the subset 1 as representative of the qualitative olfactory space.

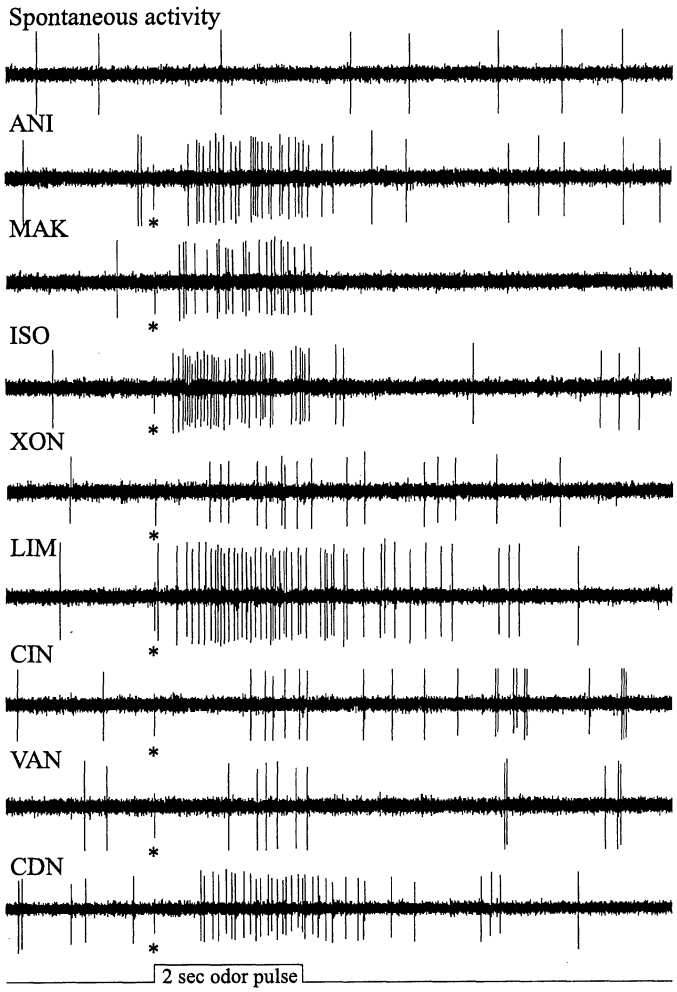

Fig. 1. Spontaneous activity (upper trace) and representative responses of ORN50 (illustrated from raw data stored in computer files) to different stimuli (lower traces). This ORN was tested with all the odors of subsets 1 and 2 (*17*). All induced excitatory responses. Responses to ACE, CAM, and CYM are not shown because their thresholds were not estimated. ORN50's thresholds were widely distributed over the available concentration range. For ANI, MAK, and ISO, sustained responses were observed for the lowest concentration allowed by the olfactometer (SV/562). Their thresholds were thus lower than 10^{-7} mol/liter. For XON, the threshold was SV/100 (2.5×10^{-6} mol/liter). Thresholds were SV/10 for LIM, CIN, and VAN and SV/5 for CDN. *, stimulation artifact.

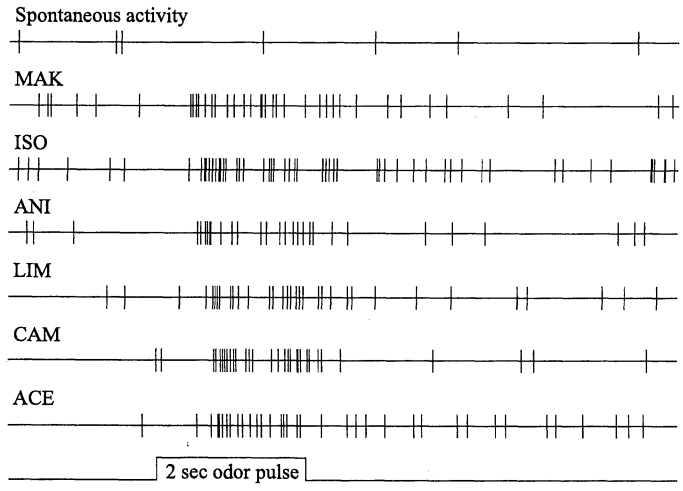

Fig. 2. Spontaneous activity (upper trace) and responses (lower traces) of ORN19 to different stimuli delivered at the same concentration in terms of ratio of the SV of the different compounds (SV/562). Only odors that induced a response are shown. This ORN displayed sustained discharges at the lowest concentration that can be delivered by the olfactometer (from 2.5×10^{-8} mol/liter to 5.2×10^{-7} mol/liter, according to the value of the SV/562 of each compound). Its response threshold was thus overpassed for all these stimuli. This figure indicates further that broad qualitative fields can be observed even at low concentrations; that is, that one may induce ORN responses to several different odorants without working at high concentrations.

Twenty-one cells were tested with several concentrations of each odorant, and their response thresholds were estimated. We observed that when repeated stimulation was applied, both the EOG amplitude and the single cell response patterns were reproducible (*18*). About 50% of ORNs were observed to reach their response thresholds at concentrations higher than SV/10 (10^{-6} mol/liter to 10^{-5} mol/liter), whereas 32% showed supraliminary responses at the lowest available concentration, SV/562 (10^{-7} mol/liter to 10^{-8} mol/liter). Some ORNs responded to different odors, with thresholds dispersed over a wide concentration range (Fig. 1), whereas others responded with thresholds that were grouped in a narrow concentration range (Fig. 2). Increasing the concentration led us to describe the dynamics of ORN functioning (Fig. 3). For most neurons, bursts became more sustained and appeared earlier with an increase of odorant concentration. The responses evoked by the highest concentrations often consisted of an early high-frequency and long-duration burst or of a decremental initial burst followed by an incremental high-frequency burst and sustained rebound. The delay between the two response phases increased with concentration. Such a relation between the response pattern and the odorant concentration suggested that ORNs did not work at saturation, but within a dynamic phase of their excitability. Simultaneous recordings by the EOG supported this assertion, because EOG amplitudes increased with concentration, mirroring the recruitment dynamics of ORNs that participated in odor coding.

This study presents functional evidence that rat ORNs have broadly tuned chemical receptive fields. Their selectivity and sensitivity are in agreement with those of mitral cells previously reported in the same animal species (*11*). Rat ORNs tended to display a broader qualitative profile and a lower sensibility than those of the frog (*19*). Our finding that one ORN could display different sensitivities to different odors is consistent with a calcium imaging study that reports that single cells respond to additional odorants when concentrations are increased (*20*).

How can the the molecular biology of OR proteins be interpreted to take into account our functional results? Two molecular studies that have addressed the chemical tuning of odor receptors contain some divergent results. In insect Sf9 cells transfected with the OR5 receptor, Raming and co-workers (*21*) show that several odor molecules increase IP3 responses, and they conclude that OR5 receptors are rather poorly selective. In contrast, Zhao and colleagues (*4*) report, for the first time in rat ORNs, that increasing the expression of a single gene leads to greater responsiveness to octanal and other compounds with a very similar molecular structure. They conclude that the recombinant virus drove the expression of a gene coding for a selective OR.

Here the molecular receptive range of ORNs was identified in a biological preparation that was as close as possible to physiological conditions. If the hypothesis that each ORN expresses one OR is true, the fact that most ORNs responded to several distinct odor molecules demonstrates that ORs are broadly responsive. Another possibility could be that each ORN would expresses several ORs of a given subfamily (*22*), so that its qualitative response spectrum is the sum of the individual receptive fields of its ORs. We found neurons that displayed differential sensitivity to different odors, which suggests that, at the level of a single neuron, not only the categories of ORs and their specificity may differ but also their number and their affinity for odor molecules.

At the level of a single neuron, in terms of olfactory quality coding, our data are in agreement with those previously obtained in the frog. In contrast, they disprove the extreme concept that individual ORNs express only a single OR and respond to only one odor (*8*). Our electrophysiological data add to those of a recent study combining calcium imaging and single-cell reverse transcription polymerase chain reaction, which indicates that ORs are broadly tuned with various odors and, conversely, that various odors can be recognized by the same OR (*23*). Thus, we conclude that in vertebrates at the cellular level, the odor receptor binding process results in activation of ORN arrays with partially overlapping tuning profiles.

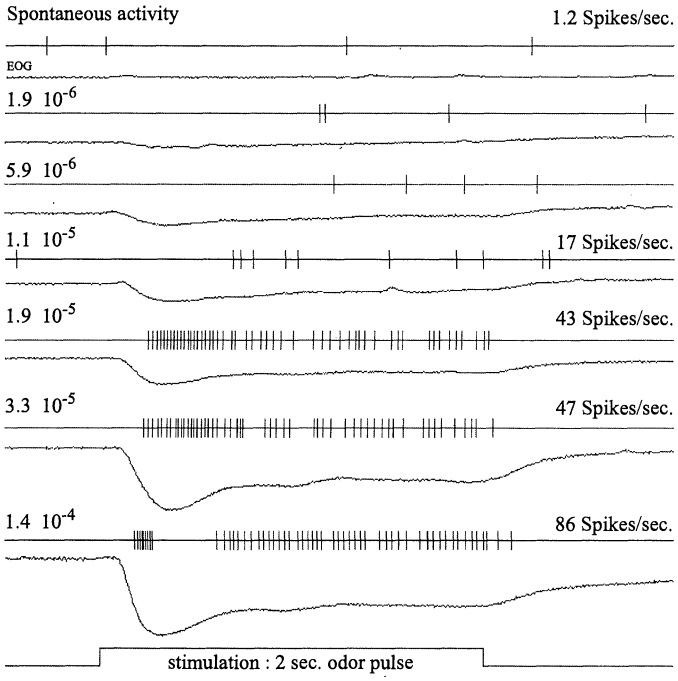

Fig. 3. Spontaneous (upper pair of traces) and odor-evoked (lower pairs of traces) EOGs and single-unit responses of ORN55 to increasing concentrations of LIM. This ORN had a low spontaneous firing frequency (about 1.2 spikes per second on the period shown). The lowest concentration shown (1.9×10^{-6} mol/liter) induced a small EOG but did not significantly modify the ORN firing activity. Increasing the concentration to 5.9×10^{-6} mol/liter (SV/20) induced a larger EOG and an ORN response characterized by a rhythmic discharge composed of four spikes. Thus, the response threshold of this ORN is between 1.9×10^{-6} mol/liter and 5.9×10^{-6} mol/liter. Increasing the concentration enhanced the ORN firing activity, which became a sustained tonic response pattern and then an initial high-frequency burst of activity followed by a silence and a rebound. EOG amplitude evolved in parallel: It was very small for 1.9×10^{-6} mol/liter and increased gradually, mirroring the global recruitment of the ORNs situated within the recording field of the electrode. Recordings also show that whereas the ORN burst discharge frequency increased with concentration, the latency of this discharge shortened with respect to the beginning of the odor pulse and thus appeared earlier and earlier with respect to the EOG kinetics. The concentration range used in this study overlaps the dynamic range of rat ORNs.

Table 1. Distribution of responding ORNs according to their selectivity to odor subset 1.

% responding to one-odor stimulus	% responding to two-odor stimulus	% responding to three-odor stimulus	% responding to four-odor stimulus	% responding to five-odor stimulus	% responding to six-odor stimulus
12	17	10	7	22	32

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13. Surgical methods were as follows: All experiments were performed according to animal care guidelines. Adult Wistar rats (250 to 300 g) were anesthetized by an intraperitoneal injection of Equithesine (a mixture of pentobarbital sodium and chloral hydrate) at an initial dose of 3 ml per kilogram of body weight (ml/kg). Anesthetic was then supplemented as necessary to maintain a deep level of anesthesia. Rectal temperature was maintained at 37° ± 0.5°C by a homeothermic blanket (Harvard Apparatus, USA), and the surgical wounds of the animals were regularly infiltrated with 2% Procaine. For recordings, anesthetized animals were secured in a sterotaxic apparatus. Recordings were performed in the endoturbinate II. Access to the olfactory mucosa was gained by removing the nasal bones and then gently slipping aside the dorsal recess underlying these bones. Recording procedures were as follows: Single-unit action potentials were recorded with metal-filled glass micropipettes (3 to 7 megohm), and the EOG was recorded with glass micropipettes 50 μm in diameter filled with saline solution. The recorded signals were led through conventional amplifiers. Spike signals were filtered between 300 and 3000 Hz. Data were stored on a Data Tape Recorder (Biologic, France). During the experiment, the single-unit nature of the recording was controlled online by triggering the recorded cell near the background noise. The activity was monitored on a storage oscilloscope. This allowed us to control the characteristics of the polyphasic spike of the cell that was studied in order to ensure that the same cell was recorded throughout all the experimental procedures. The single-unit activity and EOG signal were sampled offline at 15 kHz and 200 Hz, respectively, by means of a CED-1401 data acquisition system (Cambridge Electronic Design, UK) connected to a computer. Spikes were first detected by the waveform signal crossing a trigger level and then by visual inspection of the consistency of the shape of the sorted spikes on the computer screen.
14. Studies of the qualitative discrimination properties of ORNs in the frog (*9*, *11*) have shown that the concept of the odor group has a fundamental meaning that is related to the structure of olfactory molecules.
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22. In in situ hybridization experiments, K. J. Ressler, S. L. Sullivan, and L. B. Buck [*Cell* **73**, 597 (1993)] have used antisense probes to hybridize OR subfamilies. They observed a regional distribution of these subfamilies in the olfactory mucosa and hypothesized that each subfamily would code for ORs that would have identical or similar odor specificities. In contrast, our results suggest that a given OR subfamily may code for ORs expressing different odor-binding properties. This hypothesis does not call the importance of the regionalization of ORs subfamilies into question, because belonging to a subfamily would permit a precise targeting of epithelial zones to the olfactory bulb.
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Regulation of Transcription by a Protein Methyltransferase

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The p160 family of coactivators, SRC-1, GRIP1/TIF2, and p/CIP, mediate transcriptional activation by nuclear hormone receptors. Coactivator-associated arginine methyltransferase 1 (CARM1), a previously unidentified protein that binds to the carboxyl-terminal region of p160 coactivators, enhanced transcriptional activation by nuclear receptors, but only when GRIP1 or SRC-1a was coexpressed. Thus, CARM1 functions as a secondary coactivator through its association with p160 coactivators. CARM1 can methylate histone H3 in vitro, and a mutation in the putative *S*-adenosylmethionine binding domain of CARM1 substantially reduced both methyltransferase and coactivator activities. Thus, coactivator-mediated methylation of proteins in the transcription machinery may contribute to transcriptional regulation.

Nuclear hormone receptors (NRs) are a related group of hormone-regulated transcriptional activators that includes the receptors for steroid and thyroid hormones, retinoic acid, and vitamin D (*1*). Transcriptional activation by NRs is mediated by the NR (or p160) coactivators, a family of three related 160-kD proteins that includes SRC-1, GRIP1/TIF2, and pCIP/RAC3/ACTR/AIB1/TRAM1 (*2*). Transcriptional coactivators locally modify chromatin structure and help to recruit an RNA polymerase II transcription initiation complex to the gene promoter (*2*, *3*). The COOH-terminal AF-2 activation functions of NRs bind to p160 coactivators at multiple NR box motifs containing the sequence LXXLL (where L is leucine and X is any amino acid), located in the central region of the p160 polypeptide (*2*) (Fig. 1). The NH_2-terminal AF-1 activation functions of some NRs bind the COOH-terminal region of p160 coactivators (*4*–*6*). The activating signal received from the DNA-bound NRs by the p160 coactivator is transmitted to the transcription machinery by activation domains AD1 and AD2 of the p160 coactivators. AD1 binds CREB binding protein (CBP) or the CBP-related protein p300, which help to activate transcription because they contain a histone acetyltransferase (HAT) activity to modify chromatin structure; CBP and p300 also associate with other coactivators like p/CAF, which also contains HAT activity, and bind components of the basal transcription machinery (*7*, *8*). We recently demonstrated that AD2, a second putative AD located in the COOH-terminal region of p160 proteins (*7*, *9*), plays an important role in p160 coactivator function (*6*). To understand the mechanism of downstream signaling by AD2, we sought to identify proteins that interact physically and functionally with this domain.

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