Olfactory Fingerprints for Major Histocompatibility Complex-Determined Body Odors

Michele L. Schaefer,^{1,2,3} David A. Young,⁴ and Diego Restrepo^{1,2,3}

¹Neuroscience Program, ²Rocky Mountain Taste and Smell Center, ³Department of Cellular and Structural Biology, and ⁴Department of Preventive Medicine and Biometrics, Biometrics Section, University of Colorado Health Sciences Center, Denver, Colorado 80262

Recognition of individual body odors is analogous to human face recognition in that it provides information about identity. Individual body odors determined by differences at the major histocompatibility complex (MHC or H-2) have been shown to influence mate choice, pregnancy block, and maternal behavior in mice. Unfortunately, the mechanism and extent of the main olfactory bulb (MOB) and accessory olfactory bulb (AOB) involvement in the discrimination of animals according to H-2-type has remained ambiguous. Here we study the neuronal activation patterns evoked in the MOB in different individuals on exposure to these complex, biologically meaningful sensory stimuli. We demonstrate that body odors from H-2 disparate

mice evoke overlapping but distinct maps of neuronal activation in the MOB. The spatial patterns of odor-evoked activity are sufficient to be used like fingerprints to predict H-2 identity using a novel computer algorithm. These results provide functional evidence for discrimination of H-2-determined body odors in the MOB, but do not preclude a role for the AOB. These data further our understanding of the neural strategies used to decode socially relevant odors.

Key words: major histocompatibility complex; congenic mice; olfactory; urine; coding; recognition; c-fos; complex odors; mapping

Individual body odors are of particular importance and provide information about gender, individual identity, reproductive status, and health (Brown, 1995). Of particular interest are the genetic differences at the major histocompatibility complex (MHC) locus known to impart unique body scents on individuals within a given species, including in humans (Yamaguchi et al., 1981; Boyse et al., 1982; Yamazaki et al., 1983a, 1986, 1988, 1990, 1994, 1999, 2000; Beauchamp et al., 1985; Boyse et al., 1987; Porter, 2000). These odors play a key role in mating preference, pregnancy block, maternal recognition, and kin recognition (Yamazaki et al., 1983b, 1988, 2000). In addition, it has been hypothesized that MHC functions to minimize genome-wide inbreeding (Potts et al., 1991; Apanius et al., 1997; Penn and Potts, 1998). The sensory system or systems and neural strategies used to accomplish these important biological tasks remain unclear. Identification of different spatiotemporal representations for MHC-determined body odors within a sensory system [e.g., the main olfactory bulb (MOB) and/or accessory olfactory bulb (AOB)] would support a role for the system in encoding these odors. We have focused on detailing the response of the MOB in mice exposed to odors from mice differing only at the MHC locus.

Investigation on how odorants are encoded in the mammalian MOB—the first central processing center—has indicated that

different odors activate distinct spatial activity patterns or “odor maps” (Sharp et al., 1975; Guthrie and Gall, 1995a; Johnson et al., 1998; Rubin and Katz, 1999; Uchida et al., 2000; Xu et al., 2000). Thus, a hypothesis has developed that odor maps are part of the neural basis for perceptual discrimination of odor quality and intensity (Xu et al., 2000). However, this hypothesis was primarily developed from work involving monomolecular odors. Little is known about odors produced by mixtures of dissimilar chemical components. If a simple odor can activate up to 5% of all glomeruli in the MOB, what percentage of glomeruli is activated by a complex mixture such as urine (Teicher et al., 1980; Astic and Saucier, 1982; Astic and Cattarelli, 1982; Johnson et al., 1999)? More than eighty compounds have been identified in mouse urine vapor (Miyashita and Robinson, 1980; Singer et al., 1997). These compounds are representative of nearly all of the common chemical classes such as aldehydes, alcohols, ketones, esters, ethers, aromatics, and acids. It is also unclear whether odor maps will differ when two distinct but similar complex odors (odor mixtures composed largely of the same components but that differ by a small fraction) are compared. For example, do urine odors from congenic mice that differ only with the MHC-haplotype of the individual (Singh et al., 1987; Singer et al., 1997) evoke distinct activity patterns? Alternatively, are the odor maps of these complex odors significantly redundant so that they cannot be resolved through spatial patterns alone and require temporal cues to distinguish them (Fleming et al., 1979; Di Prisco and Freeman, 1985; Wilson and Leon, 1988; Kashiwadani et al., 1999; Keverne, 1999; Laurent, 1999). Because a detailed understanding of the neural representation of complex odors is lacking, we addressed this more directly here. Specifically, our goal was to characterize the spatial activity patterns evoked by different H-2 body odors.

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Correspondence should be addressed to Dr. Diego Restrepo, Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, 4200 East Ninth Avenue, Room 4505 SOM, Denver, CO 80262. E-mail: diego.restrepo@uchsc.edu.

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MATERIALS AND METHODS

Odor exposure. Donor male urine was collected while applying gentle abdominal pressure and stored at -20°C until needed. Female odor recipients ranged in age from 12 to 20 weeks, a period of time during which the olfactory bulb changes little, if at all (Pomeroy et al., 1990). Vaginal swabs were used to determine state of estrus. Nonestrous female mice BALB/C (H-2^d) were placed in a 5 l glass jar and exposed to humidified fresh air for 40 min at 3 l/min. The fresh air controls were exposed for an additional 30 min. Experimental animals then were exposed to age-matched male urine odor from either of two different H-2 haplotypes (B6.AKR:H-2^k or C57BL6:H-2^b). Several dilutions of urine were used. The bulk of the exposures were performed using a 20% v/v of urine (1:5 dilution). This dilution was chosen on the basis of a paradigm used in a behavioral discrimination assay (Yamazaki et al., 1999). Odors were delivered for 3 min at 5 min intervals over a 30 min period. All procedures were done in compliance with standards of the University of Colorado Health Sciences Center Animal Care and Use Committee.

Measurement of odor-evoked activity. Mice were immediately killed after odor exposure, perfused with 4% paraformaldehyde, and the olfactory bulbs were harvested. Transverse sections (18 μm) of the OB were cut on a cryostat and collected onto glass slides. *In situ* hybridization analysis of *c-fos* mRNA expression was performed on every fourth section for a total of 36 sections, representative of nearly the entire length of the bulb. Sections were processed for colorimetric *in situ* hybridization localization of *c-fos* mRNA using a digoxigenin (DIG)-labeled riboprobe and alkaline phosphatase (Roche Molecular Biochemicals, Indianapolis, IN) as described elsewhere (Guthrie and Gall, 1995a). The antisense *c-fos* cRNA was transcribed from a mouse recombinant cDNA clone to generate a 535 base transcript corresponding to positions 1842–1944 and 2061–2493 of the mouse *c-fos* gene (MUSFOS). Increases in *c-fos* mRNA were measured in the juxtaglomerular cells (periglomerular and external tufted cells) surrounding glomeruli (Guthrie et al., 1993, 1995a, 1995b). Glomeruli were scored as positive when an arc of labeled juxtaglomerular cells spanning either 180° in any orientation or two 90° arcs spanning any region not abutting the external plexiform layer were identified.

Mapping the patterns of odor-evoked activity. The coordinates for each positive glomerulus are given in rostrocaudal distance and radial angle around a section. Anatomical landmarks determine the origins for the radial measurements. The first section is defined by the point at which complete mitral cell and external plexiform layers can be identified (Johnson and Leon, 1996; Johnson et al., 1998, 1999). The rostrocaudal distance in our maps is offset by 432 μm posterior to the first section. The 0 – 180° axis was drawn parallel to the more ventral aspect of the subependymal layer. For the rostral sections, the origin was taken as 1/3 the distance from the dorsal to the ventral mitral cell layer. At the level of the AOB, the origin is defined as the point just ventral to the AOB. Past the AOB the origin is placed at the granular cusp of the MOB.

To estimate the accuracy of our mapping procedure, we mapped P2 glomeruli in P2-IRES tau lacZ mice (Mombaerts et al., 1996). Our analysis indicates that biological and mapping variability in angle and rostrocaudal distance are of the order of $\pm 6^{\circ}$ and $\pm 153 \mu\text{m}$ and $\pm 4^{\circ}$ and $\pm 98 \mu\text{m}$ for the lateral and medial glomeruli, respectively (M. L. Schaefer and D. Restrepo, unpublished observations). It should be mentioned that the natural biological variability in glomerular position contributes to our estimate significantly. Some animals have double P2 glomeruli that can be separated by up to five glomerular widths ($\sim 425 \mu\text{m}$) (Royal and Key, 1999; Strotmann et al., 2000). Thus, our data are within the range of what would be expected for biological variability alone.

Binning and smoothing glomerular activation maps. The number of positive glomeruli is presented as uncorrected “raw” values. By processing every fourth section, we are sampling every 72 μm . Because the mean diameter of a glomerulus is 85 μm , this would mean that we undersample the smallest glomeruli (miss scoring some) and oversample the largest glomeruli (score some more than once) (Royet et al., 1988). Also, a small number of glomeruli lying anterior and posterior to our first and last sections would not be sampled. All of the positive events (glomeruli) for each section are arrayed into bins of 10° and 72 μm . These data are then summated using a kernel of three. Summating with a kernel of three results in averaging over areas comprising approximately three SDs for mapping a single glomerulus. Summation was used to decrease error because of biological and mapping variability (Schaefer and Restrepo, unpublished observations) (Strotmann et al., 2000). The kernelled data are then visualized as a color contour plot constructed in Microcal Origin

6.0. A self-extractable compressed file containing the program, ToMatrix, to transform and analyze glomerular activation data can be downloaded from the Restrepo Lab homepage at <http://www.uchsc.edu/ctrinst/rmtsc/restrepo/> by clicking on the biomedical information and tools tab.

Statistical analysis. As described above, all datasets consisted of rectangular arrays of bins each containing the number of positive glomeruli within a 10° and 72 μm interval. To estimate central tendency and fractional variability, the average and normalized SD were calculated binwise. Normalized SD was computed as SD divided by average value. To determine the statistical significance of differences among spatial patterns, we used a binwise Mann–Whitney *U* test (Siegel, 1956; Johnson and Leon, 1996). For each bin the Mann–Whitney *p* value was calculated by sorting and estimation of *U* for all values within nine adjacent bins. To determine which average pattern best fit an unknown pattern, we performed a least squares fit by minimizing the function:

$$\text{SSQ} = \sum_i^n (g_i - \alpha G_i),$$

where *n* is the total number of bins, *g_i* is the number of glomeruli in the *i*th bin in the unknown pattern, *G_i* is the number of glomeruli in the *i*th bin in the average pattern, and α is an arbitrary scaling variable adjusted to obtain the minimum SSQ. This was performed independently for H-2^k and H-2^b representations. The smallest sum of squares was used as the criterion to classify the unknown as H-2^k or H-2^b. Many of the methods used in calculations were taken from Numerical Recipes in C (indicated within the ToMatrix program) (Press et al., 1992).

RESULTS

Mapping *c-fos* glomerular activation patterns

The MOB in mice consist of ~ 1800 glomeruli in each bulb (Royet et al., 1988). Glomeruli are spherical modules of neuropil, within which several thousand olfactory receptor neurons converge onto the output neurons of the MOB ($\geq 5000:1$), and thus are thought to be functional units of processing, similar to cortical barrels and columns (Xu et al., 2000). Odor-induced neural activity in the MOB can be measured by mapping increases in *c-fos* mRNA in the glomerular layer. An example of a representative serial section from a BALB/C (H-2^d haplotype) female mouse exposed to urine odor from a B6.AKR male (H-2^k haplotype) is shown in Figure 1. Increases in *c-fos* mRNA were measured in the juxtaglomerular cells surrounding glomeruli (Guthrie and Gall, 1995b) (Fig. 1*B,C*). Three *c-fos* cRNA-positive glomeruli are shown in Figure 1*B*, and two of these are shown at higher magnification in Figure 1*C*. The coordinates for each positive glomerulus are given in degrees as measured by radial angle around a section and rostrocaudal distance. *c-fos* cRNA hybridization in the glomerular layer was mapped throughout the bulb in this manner on exposure to urine odor. The number of positive glomeruli per unit area then was determined, and the resulting arrays were smoothed and plotted as color contour charts of glomerular activation (Materials and Methods, Figs. 2–4).

Urine odors evoke simple and stereotypic glomerular activation patterns

Studies in mammals (rat, rabbit, and tree shrew) have demonstrated that patterns of glomerular activation are stereotyped between animals of a particular species (Skeen, 1977; Astic and Saucier, 1982; Guthrie et al., 1993; Johnson and Leon, 2000b). To determine whether urine elicits consistent spatial patterns between mice, we compared the activity maps of four individual female mice (H-2^d) exposed to novel male urine (H-2^k) (Fig. 2*A–D*). We averaged the four arrays from different individuals to better illustrate central tendencies (Fig. 2*F*). Exposure to urine odor from H-2^k males resulted in consistent activation in the

Figure 1. Mapping *c-fos* glomerular activation patterns in the MOB on exposure to urine odor. *A*, Diagram illustrating the sagittal aspect of the MOB. *1st*, The rostral limit is defined as the section 432 μ m posterior to the first section that contains complete mitral cell and external plexiform layers. The label *B* depicts the location of the section shown in part *B* at 864 μ m posterior to the rostral limit. *36th*, The caudal limit is defined as the location just posterior to the AOB at 2592 μ m posterior to the rostral limit. *B*, Representative section demonstrating *c-fos* DIG-cRNA labeling in an H-2^d female mouse exposed to H-2^k male urine odor. Radial measurements are shown marking “positive” glomeruli surrounded by *c-fos* DIG-cRNA labeling of juxtaglomerular cells. *C*, High-power magnification of boxed portion in *B*. *G*, Glomerulus; *Epl*, external plexiform layer; *PG*, periglomerular cell; *D*, dorsal; *L*, lateral. Dashed line is marking the 0° to 180° axis parallel to the subependymal layer.

ventral, lateral, caudomedial, and to some extent dorsomedial regions of the bulb (Fig. 2*A–F*). Thus, the odor representation of excreted male urine is similar between animals, but no two representations are exactly alike.

A small number of functional studies in rats have indicated that the total number of glomeruli activated by complex odors is of the same order of magnitude as the number stimulated by simple (monomolecular) odorants (Teicher et al., 1980; Astic and Cattarelli, 1982; Astic and Saucier, 1982). In contrast, with the advent of more sophisticated mapping procedures, it has recently been suggested that odors produced by mixtures of dissimilar chemical odorants may elicit more complex spatial representations as well as activate much of the olfactory bulb surface (Johnson et al., 1999). Here, we investigated the extent of glomerular activation elicited by H-2^k urine odor. Only ~10% (190 ± 30) of the estimated 1800 glomeruli were activated in mice exposed to 20% v/v of urine (1:5 dilution) (Fig. 2*A–D, F*). In control mice exposed to fresh air, significantly fewer glomeruli were activated ($30 \pm 12 \approx 2\%$ of total bulb glomeruli) (Fig. 2*G*). Thus, urine odor activates only a small portion of the glomerular surface.

A quantitative analysis of the variability between the urine-exposed animals shows that, in general, the regions showing activity above that of the fresh air control (>16% of maximal glomerular activation for any bin) have the least fractional variability and are therefore the most meaningful (Fig. 2*H, blue areas*). In all mice, exposed to urine, the ventral region of the bulb displays the greatest density of active glomeruli and also corresponds to the largest region of low variability, perhaps reflecting its importance in the overall representation of urine odor (Fig. 2*A–D, F, H*).

Figure 2. *c-fos* glomerular activation patterns for H-2^k male urine odor in the main olfactory bulbs of H-2^d female mice. *A–D, F–G*, Color contour maps of evoked glomerular activity. *A–D*, Individual H-2^k urine odor maps. *F*, Average H-2^k urine odor map constructed from *A–D*. *E*, Diagram illustrating regions of activity in the main olfactory bulb as shown in *F*. *L*, Lateral; *V*, ventral; *CM*, caudomedial; *DM*, dorsomedial. *G*, Control showing average map for mice exposed to fresh air ($n = 3$). *H*, Normalized SD (SD/average) map for *A–D*. Color bar on the top of *A* shows the density of active glomeruli (number of positive glomeruli per bin) for *A–D* and *F–G*. Color bar on the top of *H* shows the range of the variability (two is most variable) in *H*.

The spatial representation of urine odor is maintained over a wide range of concentrations

To assess concentration dependence of the odor representation for H-2^k male urine in the MOB of H-2^d female mice, we compared the glomerular activation maps of mice exposed to six different urine dilutions. Comparison of the average patterns yields the qualitative impression that the spatial pattern is maintained from 0.8 to 20% v/v of urine (Fig. 3*A–E*). The activated regions: ventral, lateral, caudomedial, and to some extent dorso-

Figure 3. Concentration dependence of *c-fos* glomerular activity for H-2^k male urine odor in the main olfactory bulbs of H-2^d female mice. *A–F*, Color contour maps of the average evoked glomerular activity at different urine concentrations. *A*, 0.8% v/v (% volume of urine/volume of 20 mM HEPES, pH 7) ($n = 2$). *B*, 2% v/v ($n = 4$). *C*, 4% v/v ($n = 4$). *D*, 10% v/v ($n = 4$). *E*, 20% v/v ($n = 4$). *F*, 40% v/v ($n = 3$). Color bar shows the color code for the density of active glomeruli. *G*, *H*, Analysis of the mean number of positive glomeruli at each urine concentration. *G*, Total number of positive glomeruli per olfactory bulb. * $p < 0.05$ versus fresh air. *H*, Quadrant analysis of the number of positive glomeruli. RD, Rostrodorsal (375–1125 μm , -45 to 45°); RL, rostrorolateral (375–1125 μm , 45 – 135°); RV, rostrorodorsal (375–1125 μm , 135 – 225°); RM, rostromedial (375–1125 μm , 225 – 315°); CD, caudodorsal (1125–1875 μm , -45 to 45°); CL, caudolateral (1125–1875 μm , 45 – 135°); CV, caudoventral (1125–1875 μm , 135 – 225°); CM, caudomedial (1125–1875 μm , 225 – 315°). Error bars plotted in one direction to avoid overlap represent SEM.

medial remain similar with respect to overall position, but an increase in the density of active glomeruli is apparent (Fig. 3*A–E*). The total number of positive glomeruli per bulb increases with urine concentration (Fig. 3*G*). The result of the 40% v/v

urine exposure was the exception. The regions of activation appear more diffuse, and the total number of positive glomeruli is not significantly different from the 20% v/v urine exposure (Fig. 3*E–G*). Specifically, the ventral region that dominated the pattern at lower concentrations is now more diffuse and contributes less to the overall pattern.

Because changes in spatial activity patterns could underlie altered odor perception across odorant concentrations (Johnson and Leon, 2000b), we sought to gain a better understanding of how domains of activation change with respect to each other across different urine concentrations. Therefore, we compared the density of active glomeruli in individual quadrants (Fig. 3*H*). The quadrant analysis is consistent with the relative invariance of the pattern in the middle concentration range. That is, the number of positive glomeruli in each quadrant increases from 0.8 to 20% v/v, albeit with different concentration dependencies. This presumably reflects the recruitment of neighboring glomeruli of related specificity (Yokoi et al., 1995; Johnson et al., 1999). The ventral quadrants reach half-maximal activity at a urine concentration of 10% v/v, whereas other quadrants, for example the rostrorodorsal, medial, and dorsal, do not appear to have reached maximal activity even at the highest concentration of urine tested. Also, some quadrants (e.g., rostromedial and dorsal) become activated only at higher concentrations. This difference in the rate of glomerular recruitment within each quadrant is expected from the fact that olfactory neurons, expressing receptors with different affinities, project their axons to one or a few glomeruli in a particular quadrant or quadrants. In addition, some quadrants (e.g., rostrorodorsal) appear saturated at 40% v/v, under the given stimulus duration. Thus, the differences in the rate of glomerular recruitment and saturation (and possibly adaptation and fatigue) give rise to changes in the overall spatial pattern, but are relatively invariant within the range of 0.8–20% v/v.

The spatial representations of congenic urine odors differ

To determine whether spatial maps of neuronal activity within the MOB contain sufficient information to allow discrimination of H-2 determined body odors, we tested whether urine odors from congenic mice (H-2^k and H-2^b haplotypes) evoke distinct maps of neuronal activation. The rationale was such that if two perceptually different urine odors elicit distinct odor maps then recognition of individuals by their unique body scent may be mediated at least in part by spatially encoded information in the MOB. Maps showing the average glomerular activity from four mice exposed to either H-2^k or H-2^b urine were constructed (Fig. 4*A,B*). The mean number of activated glomeruli in mice exposed to 20% v/v of urine was 190 ± 30 for H-2^k and 160 ± 75 for H-2^b (~10% of total bulb glomeruli). Both urine odors activated similar regions of the bulb—ventral, lateral, and medial aspects. The ventral band of activation is conspicuous in both urine maps and spans nearly the entire length of the bulb rostrocaudally. Within this region, H-2^k urine odor activated glomeruli located in a more lateral and rostral aspect with peak activation at 175° and 1200 μm . H-2^b urine odor activation, however, is located more medial and caudal with peak activation at 225° and 1750 μm .

To test whether the differences in glomerular activation patterns between these congenic odors were statistically significant we performed a Mann–Whitney *U* Test (Johnson and Leon, 1996) (Fig. 4*C*). The largest region showing a statistical difference

Figure 4. Comparison of *c-fos* glomerular activity elicited by H-2^b and H-2^k male urine in the main olfactory bulbs of H-2^d female mice. *A, B*, Color contour maps of the average evoked glomerular activity by either H-2^b (*A*) or H-2^k (*B*) urine. *C*, Map of the difference between H-2^b and H-2^k urine odor representations. *p* values for Mann–Whitney *U* tests are shown at each bin within the map. Color bar on the top of *A* shows the density of active glomeruli. Color bar on the top of *C* shows *p* values. The black border was drawn to indicate the limits of the Bonferroni Correction ($p < 6 \times 10^{-5}$) in *C* and used to overlay maps in *A* and *B*. *D*, Prediction of H-2 haplotype using H-2^b and H-2^k average odor maps in *A* and *B*, respectively, as templates. All individual maps used to construct averages in Figures 2 and 3 were tested. The graph shows the mean SSQ ratio at each urine odor concentration. A value <1 means the H-2 haplotype was correctly predicted from the individual map. Error bars plotted in one direction to avoid overlap represent SEM.

($p = 0.001$ to 10^{-6}) is located in the ventral to ventrolateral aspect of the MOB and spans 1330 μm rostrocaudally. This region was still significant after applying the Bonferroni Correction ($p < 6 \times 10^{-5}$), an ultraconservative correction for multiple comparisons (Miller, 1981) (Fig. 4*C*, black border). Two smaller regions located dorsomedially and caudoventrally span 310 and 260 μm , respectively. These areas are dominated by H-2^k. An additional small region located caudomedially spans ~ 330 μm . This area is dominated by H-2^b. Thus, even using a measurement technique limited in temporal resolution (*c-fos* is essentially a leaky integrator of neuronal activation), we find that these two maps are significantly different over a large area that is dominated by the H-2^k urine.

The spatial representations of congenic urine odors are sufficient for H-2 discrimination

We next developed a method to test whether the average patterns were consistent enough to predict the H-2 haplotype of urine donors blindly. First, we performed a least squares fit of the data in an unknown urine sample to each of the H-2^k and H-2^b average datasets measured at 20% v/v multiplied by a scaling variable. The smallest sum of squares (SSQ) was used as the criterion to classify the unknown as H-2^k or H-2^b. Both initial unknown donors were correctly identified as H-2^k. To illustrate how predictive these values and their respective donors were, we calculated a sum of squares ratio (SSQ for known correct answer/SSQ for known incorrect answer) for the maps of all the urines used in this publication (23 H-2^k and 4 H-2^b) (Fig. 4*D*). We were able to correctly predict (SSQ ratio <1.0) the H-2 haplotype of all the urine donors within the range of 2–20% v/v (18 H-2^k and 4 H-2^b). The maps lost predictability at the lowest and highest concentrations, 0.8 and 40% v/v, respectively (Fig. 4*D*; see also Fig. 3*A,F*). These results show that over a wide range of concentrations the spatial pattern of urine odor is maintained and sufficient to identify the H-2 haplotype of urine donors.

DISCUSSION

A quarter century ago Lewis Thomas hypothesized that the ability of the immune system to recognize self from nonself as exemplified by antigens encoded by the MHC evolved from the

necessity of primitive organisms to detect differences between self and nonself via exteroceptive chemoreception (Boyse et al., 1987). He further postulated that such an evolutionary origin for MHC might still be reflected in chemical sensory signaling of individuality among members of advanced taxa such as mammals. Coincidentally, a technician at Sloan Kettering noticed that male mice appeared to consort with female mice of MHC dissimilar type to the relative exclusion of females with MHC type identical to the males. These observations were followed by a multitude of behavioral studies that appear to show that mice detect individuality by smell and that MHC-determined body odors have profound effects on social interactions (Boyse et al., 1987).

The role for MHC in mammalian social interactions through smell has tantalized immunologists and neurobiologists alike for the last quarter century. However, the lack of an understanding of the mechanisms that mediate the biological effects of MHC-determined body odors has hampered further progress in this area. Efforts to approach the problem in an analytical manner by trying to determine which volatile compounds in urine mediate the effect have not yet lead to identification of relevant compounds because of the complexity of the stimulus (urine) and the difficulty of the behavioral bioassays (Singh et al., 1987; Eggert et al., 1996; Singer et al., 1997). Our manuscript represents a paradigm shift in the study of MHC-determined body odors. Rather than taking the analytical approach, we asked what patterns of neural activity the MHC-determined odors elicit. Our results are surprising: urine, a complex stimulus, elicits relatively simple spatial patterns of activation in the mouse olfactory bulb, and urine from MHC dissimilar types of mouse elicit distinct patterns of activity.

We examined urine odor-evoked spatial activity patterns in the MOB by mapping odor-induced *c-fos* mRNA expression. *c-fos* is an ideal reporter for the study of biologically meaningful stimuli as it is known to be influenced by primary olfactory receptor neurons and centrifugal afferents (e.g., noradrenergic afferents from locus ceruleus) (Guthrie and Gall, 1995a). Furthermore, *c-fos* mRNA expression levels are differentially influenced by learning and experience in a nonhomogenous and odor-specific manner in the MOB (Johnson et al., 1995). Because early odor

learning and experience are known to significantly alter behavior and perhaps perception, we felt this was a critical component to our analysis. For example, male mice typically prefer to mate with females of a different, nonself H-2 haplotype (Beauchamp et al., 1985; Yamazaki et al., 1988). However, when a mother differing at the H-2 locus fosters them, they reverse their mating preference to favor the same H-2 type. Thus, exposure history altered behavior.

To understand how mice recognize one another by their unique body scents we chose to functionally map urine (a potent source for H-2 body odors)-evoked response in the MOB, an organ known to be modified postnatally during the critical period of familial imprinting. First, we systematically analyzed evoked response of a novel urine odor (H-2^k) in naive H-2^d female mice. Next, we compared H-2^k urine odor with a perceptually different urine odor (H-2^b) to determine if their identity could be spatially represented in the MOB. We found distinct spatial patterns of glomerular activation within the MOB of awake and behaving untrained mice exposed to urine odor. These complex, biologically meaningful odors (urine) activated only a small portion of the glomerular surface, much as was the case for nest and fox odor in rats (Astic and Saucier, 1982). Thus, complex odors (those with many components) seem to resolve as relatively simple bulb activation patterns, much as they seem to resolve perceptually as a single gestalt. Analysis of the spatial patterns showed they were consistent between individuals and over a wide range of concentrations, similar to what has been shown for monomolecular odors in rats (Skeen, 1977; Guthrie et al., 1993; Guthrie and Gall, 1995a; Rubin and Katz, 1999; Johnson and Leon, 2000b). We identified two types of concentration-dependent changes. As we increased urine concentration from 0.8 to 20% v/v, we saw an increase in the number of positive glomeruli. This presumably reflects the recruitment of neighboring glomeruli of related specificity (Yokoi et al., 1995; Johnson et al., 1999). In contrast, at the highest concentration tested, 40% v/v, we saw no significant total increase but rather a shift in the active regions dominating the pattern. These data suggest that the change in spatial pattern that arises between the 20 and 40% v/v urine may result in altered odor perception (Johnson and Leon, 2000a).

H-2^k urine odor representation was distinctly different from a perceptually different urine odor (H-2^b). The odor maps were overlapping but distinct. Comparison of neural patterns at different urine concentrations with the neural patterns evoked by the two urines at 20% v/v was sufficient to identify the urine donor within a wide range of concentrations. These results are consistent with the hypothesis that complex, biologically relevant odors are represented by spatial activity maps (Astic and Cattarelli, 1982; Astic and Saucier, 1982; Vickers et al., 1998; Galizia et al., 1999), but do not rule out a role for temporal cues (Laurent, 1999). Nonparametric analysis showed the ventrolateral aspect of the MOB was significantly different between the two urines. The H-2^k urine dominates this pattern. This result cannot be explained as a simple difference in urine concentration because the pattern is maintained over a wide range of concentrations. The unique regions of activity could be explained by differences in the composition of congenic urine odors. These results support gas chromatographic evidence that the difference between these two individual body scents may be the result of a significant difference in either the ratio of some volatile component or components (Singer et al., 1997) or in a unique volatile component or components (Eggert et al., 1996).

In conclusion, these results show that over a wide range of concentrations the spatial pattern of urine odor is maintained and consistent enough to predict the H-2 haplotype of urine donors. In fact, we show these spatial patterns can be used much like an “olfactory fingerprint” to predict the H-2 haplotype of the donor. These data demonstrate distinct physiological responses within the MOB on exposure to two genetically different urine odors that influence social interactions such as mother–offspring relationships and mate choice. These experiments represent a radical shift in the approach to the study of MHC-dissimilar body odors because we can now bring to bear powerful molecular and biophysical methods to study MHC-determined body odors. Based on this work, we are now identifying the olfactory receptors responsible for generating these unique H-2-specific neural activity patterns in the MOB.

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