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Chemosensory Recognition of Olfactory Individuality

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Introduction

Body odors regulate social, sexual and endocrine responses of many species. Specialized structures have evolved to produce and detect odorous signals. Individual identity, often communicated through body odor, can be critical in mate choice, incest avoidance, parental care and other inter-individual interactions. Because of the importance of individual identity, particularly among social species, we have focused our research program on understanding how odors code for individuality and in what contexts individual signals modulate social behavior. In particular, we have been investigating how one set of genes, those of the MHC (see below), are involved in producing an animal's self odor.

The major histocompatibility complex (MHC)

MHC gene-encoded proteins play a critical role during immune recognition by serving as antigen receptors that bind peptide fragments for cell-surface presentation to T-lymphocytes (Germain, 1994). This family of ~50 genes is characterized in many species by their extreme diversity. In fact, the number of potential MHC types, comprising two MHC sets in each diploid individual, could exceed the population of a given species. The great diversity of the MHC implies an evolutionary investment in mechanisms that promote it. One such mechanism is mating preference that favors outbreeding and MHC disparity (Yamazaki *et al.*, 1976; Potts *et al.*, 1991). Inbreeding is particularly likely in animals like mice that in one season may generate large populations comprising many generations from a single pair.

Lewis Thomas (1975) originally suggested that MHC genes would be excellent candidates for marking each individual of a species with a unique odortype (genetically programmed body odors that distinguish one individual from another) because of their diversity (see also Boyse *et al.*, 1987). Since Thomas's suggestion, our laboratory and, more recently, other laboratories, have verified Thomas's remarkable predictions in mice (Boyse *et al.*, 1991; Brown and Eklund, 1994), rats (Brown *et al.*, 1987) and perhaps humans (Wedekind *et al.*, 1995; Jacob *et al.*, 2002).

In particular, our past studies have revealed that: (i) mice differing only at the MHC or even in one gene of the MHC have a unique odor; (ii) these unique odors apparently modulate mating preferences, mother infant interactions and physiological responses to other mice; and (iii) The odorants involved apparently are made up of patterns of volatiles found prominently in urine but also in serum. Here we describe three recent studies that contribute to our understanding of MHC odor communication in mice. Prior to describing these studies, we describe the training methods used in both studies.

Y-maze

We have used the Y-maze extensively to study odortypes. Briefly for the Y-maze, adult mice are trained, using water reward, to detect differences in urine samples based on MHC-determined odortypes (Yamaguchi *et al.*, 1981; Beauchamp and Yamazaki, 2003). After successful training (>80% concordance), unrewarded trials are interspersed, at an average frequency of one in four, with rewarded trials to accustom the mice to occasional absence of reward after a correct response. The mice typically perform with comparable accuracy during these trials. Generalization trials are instituted so as to test novel urine samples without reward and thus insure that the trained mice learn to distinguish the class distinction rather than learn to distinguish the individual samples used during training. The generalization procedure lends itself to blind testing of coded samples, because the operator of the maze is not required to supply reward for concordant choices.

MHC odor against a different genetic background

We investigated (Yamazaki and Beauchamp, unpublished) whether the signal determined by an animal's MHC genes is detected against a varied odor background. If MHC-determined odor signals of individual identity are to play a significant role in regulating social behavior (e.g. mate preferences; parent–infant interactions) and reproductive physiology in natural populations, then the signals must be salient against a changing background of odor signals arising from genetic and environmental variation. To evaluate the prominence of MHC-determined odor, we investigated whether mice trained to discriminate MHC types on one genetic background (C57BL/6 mice) generalized this without further training to another (BALB/c mice). That is, we trained mice to discriminate between the congenic strains C57BL/6 versus C57BL/6-H-2k. These mice are identical throughout their genome except for the MHC genes (0.2% of the total genes). Once the animals had learned this problem, we gave them a choice between two other congenic strains that also differed in the same MHC genes but on another (BALB) background. The trained mice were never reinforced for this new pair of odors. Hence, if the respond appropriately then this demonstrates that they recognize the MHC-determined odor even against a novel genetic background. As hypothesized, the trained mice generalized appropriately. MHC-determined odor apparently remains constant regardless of the genetic background. In previous work we have shown that background genes contribute to individual odor. It was possible that these genes could modify the MHC-pattern according to some idiosyncratic rule that would alter the odor depending on the background. Apparently, MHC odors are, as we have hypothesized, constant and relatively uninfluenced by other genetic variation.

Pups and fetal odortypes

Our discovery (Yamazaki *et al.*, 1992) that MHC odortypes are evident in mice as young as 1 day raised the possibility that MHC odortypes could underlie discrimination. Since MHC odortypes are demonstrable as early as one day of age, we tested for the presence of fetal odors in maternal urine. MHC-determined odors are produced by fetuses: Mice can be trained to discriminate between genetically identical pregnant females carrying 9–18-day-old fetuses of differing MHC type (Beauchamp *et al.*, 1994). This demonstrates that fetal odortypes are expressed in maternal odors and that the MHC type of

the fetus may modulate adult male and female behavior. Theoretically, it should be possible for a mouse to determine the MHC type of the sire based on the odortype of the pregnant female.

Recent studies show that there is bi-directional cell traffic between mother and fetus during pregnancy. We investigated whether trained mice can discriminate fetal odor remains in mothers' circulation after postpartum. Preliminary data demonstrate that the fetal odortype signal remains for a substantial time after the fetuses have been born. This result is consistent with chimeric persistence of fetal cells in the mother's circulation.

Removal of the vomeronasal organ (VNO) does not disrupt MHC odortype discrimination

At present, the respective contributions of the olfactory epithelium and the vomeronasal organ (VNO) in the recognition of individual odortypes are not well defined. It has been increasingly clear that VNO plays an important role in reception and processing of chemical signals involved in social and reproductive behavior and physiology. We examined a possible role for the VNO in the recognition of MHC-odortypes in mice by first removing the organ (VNX) (Wysocki and Wysocki, 1995) and then training the mice to distinguish the odors of two congenic strains of mice that differed only in their MHC type using our standard Y-maze training paradigm. B6-H-2^b mice and B6-H-2^k provided urine for sensory testing. Eight VNX and six sham-operated mice were trained to make the discrimination. Neither the number of training trials-to-criterion nor the rate of learning differed significantly for VNX and sham-operated mice.

Given the apparent complexity of the chemical code for MHC odortypes (differences in proportions of volatile chemicals (Singh *et al.*, 1988; Singer *et al.*, 1997)), we have assumed that the main olfactory system is primarily or completely responsible for detecting odortype information. This is consistent with a recent study (Schaefer *et al.*, 2001, 2002) demonstrating different patterns of c-fos activity in the main olfactory bulbs of mice exposed to urine odors of bb versus kk males. Nevertheless, there are indications that the accessory olfactory system does, in some cases, process complex information on individual recognition. Results from the current studies demonstrate that the VNO is not necessary for MHC odortype discrimination when animals are trained to make this discrimination. However, it is possible that it could be involved in mediating natural behavioral responses to odortypes.

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