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MHC peptides and the sensory evaluation of genotype

Thomas Boehm[1] and Frank Zufall[2]

[1]Department of Developmental Immunology, Max-Planck-Institute of Immunobiology, D-79108 Freiburg, Germany
[2]Department of Anatomy and Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Social interactions, such as finding and identifying a mate, often rely on the ability to sense molecular cues carrying information about genetic relationship and individuality. We summarize recent evidence for an unexpected mechanistic link between the immune and olfactory systems in enabling this identification process. In addition to their established role in the immune response, peptide ligands of major histocompatibility complex (MHC) molecules constitute a previously unknown family of social recognition signals detected by specific subsets of sensory neurons in the mammalian nose. This sensing of MHC peptides can be viewed as a form of functional genome analysis by the nose. Behavioral studies in mice and fish show that MHC peptides are accepted as olfactory cues that influence mate choice decisions and selective pregnancy failure. These findings provide a molecular mechanism by which an individual can sense the composition and compatibility of vital immune system molecules of a conspecific, with direct consequences for social behavior.

Introduction

An important advance of recent years has been the identification of several evolutionarily conserved genes that are required for basic aspects of social behavior [1]. Together, such studies have established that social behavior often has a genetic basis. For instance, neurotransmitters and their receptors are involved in the establishment and maintenance of social hierarchies and dominance interactions [2], certain transcription factors are involved in vocal learning and vocalization in birds and primates [3], particular neuropeptides function in regulation of parental care in various species [4], and the ion channel TRPC2 is essential for the display of aggression in mice [5].

Chemical communication among individuals of the same species is a versatile and widely used means of social interaction. It can be conceptualized as a three-component system (Figure 1) that involves production, transmission and perception of semiochemicals – that is, chemical signals used for communication. Importantly, these signals are probably used in a combinatorial, hierarchical and context-dependent manner to enable animals to adjust their behavioral responses to specific needs. Chemical signals are produced via different metabolic pathways; consequently, these signals have a wide variety of chemical structures, including such diverse molecules as steroid and peptide pheromones [6–9]. Additionally, the production of such signals might be either constitutive or inducible (e.g. depending on gender or maturation stage), which could further enhance the information content of these signals. The chemical nature of these signals is probably constrained for reasons of efficient transmission and specific function: chemosignals can be nonvolatile or ephemeral and might (e.g. [10]) or might not require specific carriers. Therefore, with respect to different signals, transmission can be either indiscriminate or selective. On the receiving end of chemical communication systems, the olfactory system has a crucial role in the evaluation of chemical signals. Olfactory recognition can be broadly or finely tuned, depending on the particular nature of a given chemical and its role in the communication process.

Signals of individuality carry genotypic information

Social interactions often require information about genetic relationship (individuality), and these signals are particularly important for mate choice decisions and post-mating behaviors such as kin recognition. How can we define the functional properties of chemical signals carrying information about individuality? Signals of individuality are probably structurally polymorphic molecules that are superimposed on other structurally invariant species-specific signals. In any case, signals of individuality must be specifically linked to the genetic composition of the individual producing it, enabling the receiver to compare its own genotype with that of a conspecific.

There are two general ways by which individuals can generate unique chemical signatures that reflect certain aspects of their genotype (Figure 1). In the first model, transmission of a common pool of signals is affected by the use of structurally diverse carrier molecules. Although each individual produces the same mixture of signals, its composition is quantitatively (and perhaps also qualitatively) altered during transmission. The highly polymorphic major urinary proteins that have been

Corresponding authors: Boehm, T. (boehm@immunbio.mpg.de), Zufall, F. (fzufa001@umaryland.edu).

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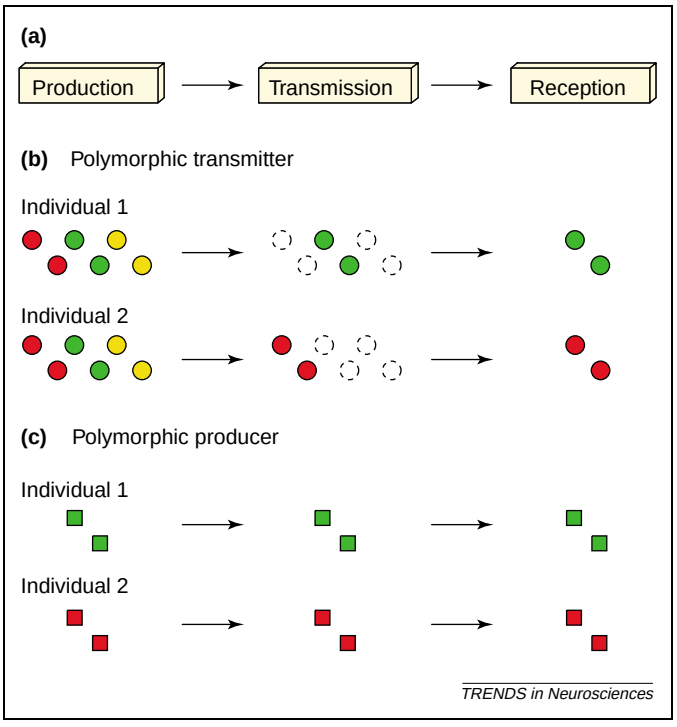

Figure 1. Three-component system of chemical communication. **(a)** Chemical signaling between individuals requires production of signals, their transmission and their sensory reception. Individuality can be imposed on these signals in at least two ways. **(b)** A set of signals, common to all individuals of one species is filtered during the transmission stage such that only a subset of these signals is available for the receiver. Polymorphic lipocalins, such as major urinary proteins, could modify signal content in this way. **(c)** Signals could be produced through biosynthetic pathways utilizing structurally polymorphic molecules. The presence of MHC molecules and/or their peptide ligands might yield such specific signals (see main text for details). In this scenario, transmission is indiscriminate. Reception of signals, for instance via neurons in the peripheral olfactory system, could be broadly or finely tuned, presumably depending on the chemical nature of the signal and constraints imposed during the transmission process.

implicated in scent marking of mice and other rodents [10] might be employed for this purpose (Figure 1a). This is a versatile means to alter the bouquet of volatile semiochemicals, enabling information about individuality to be conveyed indirectly. In an alternative model, the sender could employ a metabolic pathway with structurally polymorphic components, such that the original signals themselves are unique among individuals of the same species (Figure 1b). The polymorphic major histocompatibility (MHC) proteins [11,12] (Box 1) could be employed to achieve this end. Pheromones, as classically defined [13], would not be affected by either mechanism because they function as structurally invariant signals among individuals of the same species; however, this definition is currently in flux and might need to be revised [14]. For the perception of an individuality signal, the sensory apparatus of the receiver must be flexible enough to utilize the information contained in its chemical diversity. Perception of structurally invariant compounds is considerably less demanding. In mechanistic terms, this implies co-evolution between production and reception of signals of individuality. One of the central features in the classical definition of a pheromone is its species-specificity [13]. Because the overall structure of MHC peptides is conserved among higher vertebrates, they would not be considered pheromones according to the classical definition. However, it should be kept in mind that in natural biological situations, signals of individuality are likely to gain species-specificity in an indirect fashion, through their use in a specific signaling context where species-specific signals are evaluated alongside those encoding individuality.

MHC system and signals of individuality

It was shown almost 30 years ago that genes at the MHC locus influence behavioral decisions in the context of social recognition in mice [15,16]. Subsequently, it became clear that MHC genes have similar roles in fish [17,18], birds [19] and humans [20,21]. MHC genes are among the most polymorphic multi-gene families known [11,12]. Historically, they have been investigated for their role in cellular immunity, because they enable intracellular protein

Box 1. Information content of MHC–peptide complexes

MHC–peptide complexes present at the cell surface carry information about the genetic make-up of cells (Figure 2). MHC molecules function as specialized display devices that bind and shuttle to the cell surface intracellular peptides that are generated as intermediates during intracellular proteolytic degradation [11,12]. In the context of immune surveillance, these complexes are assessed by T lymphocytes through their highly diverse antigen receptors. Because the ligand-binding pockets of MHC molecules are diverse, only subsets of peptides are displayed at the cell surface. In other words, the range of peptides displayed by the MHC molecules of an individual mirrors the structural diversity of its MHC alleles. The structural diversity of peptide ligands of each MHC molecule in terms of their primary amino acid sequences might be too large to provide an unambiguous signal. However, different peptide ligands of one particular MHC molecule share common residues (so-called anchors) whose side chains fit into the characteristic binding pockets of the MHC molecule [11,12]. For instance, almost all ligands of the mouse MHC class I molecule K^d share a tyrosine residue at position 2 and an isoleucine residue at the C-terminal end, at position 9 [12]. This suggests that anchor residues could be the defining feature of peptides and olfactory assessment might focus on them. MHC class I and class II molecules bind their peptide cargos according to different rules, which are reflected in peptide motifs [12]. These motifs mirror the overall structure of the ligand-binding pocket of MHC molecules and describe the lengths and the preferential occurrences (usually less pronounced in MHC class II ligands) of certain amino acid residues at certain positions of peptide ligands. However, as discussed previously [26], the core peptide of MHC class II molecules is also nine amino acids long (i.e. identical to typical MHC class I ligands); both might thus be amenable to the same mechanism of structural assessment. Indeed, the C-terminal amino acid residue of the core sequence of MHC class II peptides always functions as an anchor residue, further highlighting the structural similarity of MHC class I and II ligands. This structural similarity explains why nonamer peptides are accepted as odor signals in olfactory assessment of MHC class II genotypes [26]. Hence, based on the known structure of MHC binding pockets and their corresponding ligands, C-terminal residues of peptides could be a common target of sensory discrimination in the process of evaluating MHC diversity [26]. MHC–peptide complexes are shed from the cell surface and their fragments appear in serum, saliva, sweat and urine [24]. These truncated MHC molecules are believed to have a reduced affinity to their peptide ligands [51] and are thus likely to release them into the extracellular space. Peptides could then interact with other types of receptors expressed in sensory neurons of the olfactory system (Figure 2).

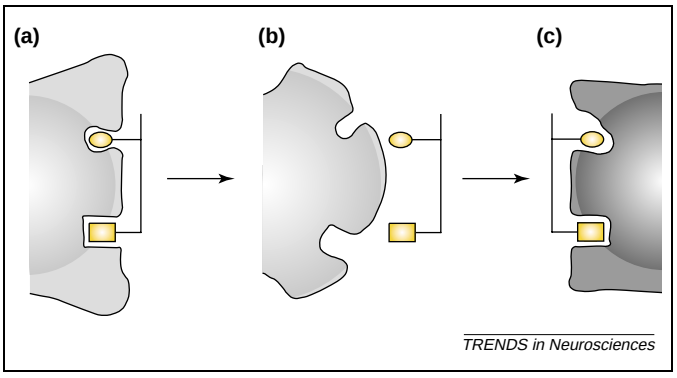

Figure 2. Conversion of MHC genotype into a chemosensory quality. The peptide-binding groove of MHC molecules accommodates peptides of various lengths, depending on the class and type of MHC molecule **(a)**. Irrespective of the exact primary sequences, the most significant contacts between peptides and the MHC molecules (light gray) are mediated through the side chains of so-called anchor residues of the peptide (yellow) fitting into pockets of the MHC molecule. The specificity and precise location of these pockets vary among different MHC molecules; in this way, the structural polymorphism of MHC molecules influences peptide-binding specificity. When the MHC–peptide complex is destroyed, for instance by proteolytic shedding from the cell surface **(b)**, the peptide ligands are liberated and thus become available for interaction with other receptors (dark gray), such as those on olfactory sensory neurons **(c)**.

synthesis to be monitored on a near real-time basis (Box 1). Several hypotheses have been put forward to explain mechanistically how MHC genes can influence social recognition [16]. For instance, MHC molecules, their fragments, degradation products of their peptide ligands and products of MHC-dependent microflora have all been considered as potential odorants. Considerable evidence suggests a volatile nature of olfactory cues influenced by MHC genes [22]. In what has previously been called the 'peptide hypothesis' [16], MHC peptide ligands were suggested to be the precursors of volatile odorants including volatile organic acids such as phenylacetic acid [23]. In a properly folded molecule, most of the structural diversity of MHC molecules contributes to the binding groove for peptides (Figure 2a). Because MHC molecules are transmembrane molecules, they must be proteolytically shed from the cell surface to appear in bodily fluids [24] and to become available for assessment by other individuals. This ectodomain shedding has three important consequences for MHC-dependent social recognition mechanisms. First, the 3D structure of the MHC fragment is probably altered to the effect that the peptide-binding groove is no longer functional (Figure 2b); hence, polymorphic residues originally clustered in a specific region of the molecule become spatially dispersed in the unfolded state and less amenable for structural assessment. This argues against MHC molecules themselves being chemosignals. Second, as a further consequence of unfolding, the binding affinity of the peptide ligand is probably altered such that the peptide can be released; in this way, the anchor residues of liberated peptides become re-accessible for binding by other molecules (Figure 2b,c). Third, the structurally altered peptide-binding groove of the soluble MHC molecules could interact with low-molecular-weight products of endogenous metabolism or of commensal flora. Although attractive, this third scenario suffers from the problem that the type of chemical that would function as signal of individuality is unpredictable and perhaps variable overtime, thus challenging the discriminatory power of the sensory evaluation process.

Modification of behavior by MHC peptide ligands

On the basis of these theoretical considerations of the information content of MHC–peptide complexes (Box 1), MHC peptide ligands themselves can be considered the most likely candidates for individuality signals (Figure 2). This hypothesis has recently been tested in two model systems, mice [25] and fish [26] (Table 1). Whether MHC peptides could function as signals of individuality in the context of pregnancy block was investigated in mice [25]. In this paradigm (Box 2), recently mated female mice return to estrus when they are exposed to the urine of a male genetically different from the stud male [27]. To show that MHC peptides provide a crucial signal among the plethora of other cues present in male urine, peptides characteristic of a disparate MHC molecule were synthesized and added to the urine of a familiar male. Unlike cognate peptides, which had no effect, disparate peptides caused pregnancy block when added to otherwise familiar urine [25]. These results clearly established that MHC peptides are accepted as natural odors in a distinct social behavior.

In natural populations of the three-spined stickleback (*Gasterosteus acculeatus*), individuals with an intermediate number of different MHC alleles represent the most frequent genotype [17,18]. Interestingly, under both field [28] and laboratory conditions [29,30], such animals best resist natural parasites. During mate choice, female sticklebacks use an odor-based selection strategy to achieve this optimal level of MHC diversity for their offspring (Box 2). It has therefore been postulated that male sticklebacks produce and release MHC-related odors that provide the information about their genotypes that is used by the female to choose among males. This hypothesis was tested by exposing female sticklebacks to water from males with sub-optimal, optimal or supra-optimal MHC diversity relative to the particular test female. The water was then spiked with a mixture of synthetic MHC peptide ligands and the change in preference of the female was recorded. Indeed, synthetic MHC peptides made water from sub-optimal males more attractive, whereas they decreased attractiveness of optimal and supra-optimal water sources [26]. These results suggest an evolutionarily conserved function of MHC peptide ligands in social behaviors of different species.

Table 1. Role of MHC peptides in social behavior

Species	Behavior	Signal conveyed by peptides	Mediated by	Refs
Mouse	Pregnancy block	Strain specificity of male urine[a]	VNO	[25]
Stickleback	Mate choice	MHC diversity of mate	?[b]	[26]

[a]Because these experiments were performed using inbred mouse strains in which all members of a given strain are genetically identical, strain specificity could be equated with individual specificity in outbred strains.
[b]Although this recognition is odor-based, it is not yet clear which sensory neuron type mediates this effect. Functionally distinct olfactory subsystems have been identified in the olfactory rosette of fish [62].

Box 2. Pregnancy block (Bruce effect) in mice and mate choice in sticklebacks

In the Bruce effect, exposure to urine from an unfamiliar male prevents embryo implantation, causing a recently mated female mouse to return to estrus [27]. By contrast, if the female mouse is exposed to urine from her mate, implantation is not prevented and pregnancy ensues. This is due to the ability of the female during the period around mating [38] to form a memory to the chemosensory cues of the male with which she has mated. The subsequent recognition by an impregnated female of these familiar chemosignals from the mating male prevents her from aborting his own offspring. The vomeronasal system is essential for the pregnancy-block effect and the accessory olfactory bulb has been identified as the site in the vomeronasal pathway of the neural changes underlying this discrimination [52–56].

Female sticklebacks chose their mates according to visual and olfactory cues [57]. Female sticklebacks prefer bright-red males as mates; it has been shown that red reflects physical condition and the state of parasitization [58] – in essence, red represents a surrogate for the state of the male's immune system. Male odor is another source of information about immunological status, and female sticklebacks use it to achieve an optimal diversity of MHC genes [17,28–30,34]. It has recently been shown that MHC peptide ligands form part of the natural odor cues that signal MHC diversity [26]. During mate choice under natural conditions, females probably use both types of information to extrapolate immune function in prospective offspring.

Neurophysiological basis of peptide recognition

The olfactory assessment of MHC peptides at the level of individual neurons was examined in mice [25] (Figure 3). The mouse olfactory system is divided into at least two anatomically distinct organs, the vomeronasal organ (VNO) and the main olfactory epithelium (MOE) [31]. Although initially viewed as functionally non-overlapping, with the VNO being responsible for detection of non-volatile molecules and the MOE being responsible for detection of volatile chemicals, this strict distinction has become increasingly questionable [32–34]. Hence, peptide recognition was examined in both the VNO [25] and the MOE [34].

Using peptide ligands of two different MHC molecules encoded by two different haplotypes, in combination with confocal Ca^{2+} imaging and patch-clamp recording in VNO tissue slices, it was shown that these ligands activate subsets of VNO sensory neurons in a sequence-specific manner, with minimal spatial overlap [25] (Figure 3b,c). Interestingly, peptide-responsive cells often occurred in clusters [25], suggesting a possible clonal relationship. Additional experiments showed an important contribution

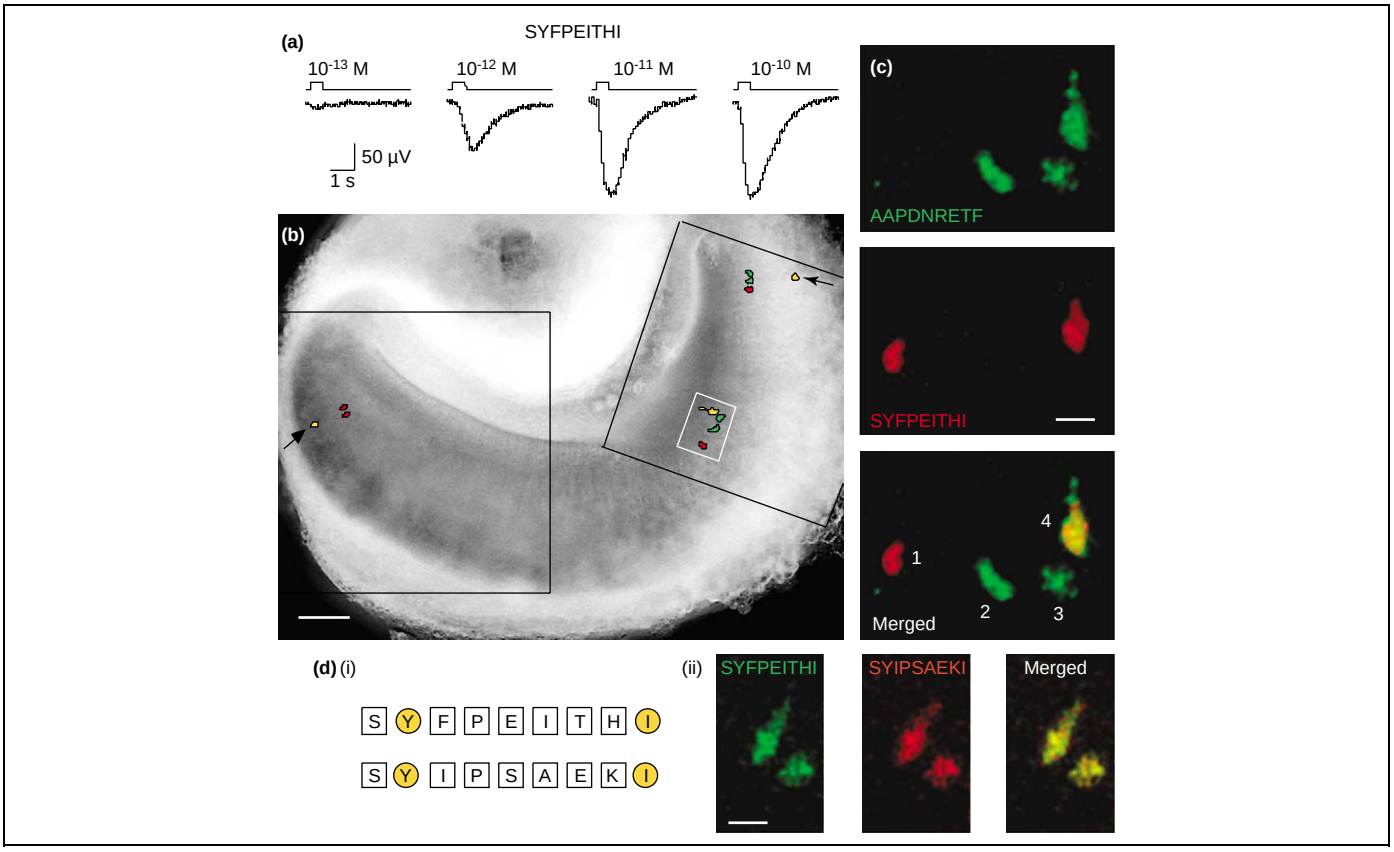

Figure 3. Spatial representation and tuning of peptide-induced neuronal activity in the VNO. **(a)** Ultrasensitive detection of the MHC class I ligand SYFPEITHI by vomeronasal sensory neurons (VSNs). Traces are summed field potentials evoked by brief pulses of increasing concentrations of ligand, as indicated. **(b)** Spatial representation of peptide-induced activity in VNO sensory epithelium using an acute slice preparation. Shown are reconstructed sensory neuron response maps (ΔF/F confocal Ca^{2+} images digitally superimposed onto a transmitted light image of the same slice) for the MHC class I ligands AAPDNRETF (10^{-12} M, green) and SYFPEITHI (10^{-12} M, red). Cells responding to both peptides are shown in yellow. Black arrows indicate peptide-sensitive neurons that are localized at the base of the epithelium. Black boxes indicate regions that were imaged in these experiments; the area within the white box is shown at higher magnification in (c). Scale bar, 100 μm. **(c)** Pseudocolor images of the relative increase in peptide-induced Ca^{2+} fluorescence. In this example, AAPDNRETF (10^{-12} M, green) activated three VSNs (cells 2, 3 and 4) and SYFPEITHI (10^{-12} M, red) activated two VSNs (cells 1 and 4). Cell 4 responded to both ligands. Scale bar, 10 μm. **(d)** Evidence that the anchor positions of MHC peptides are crucial for sensory recognition in the VNO. (i) Two different MHC class I ligands, SYFPEITHI and SYIPSAEKI (each at 10^{-12} M), which share the same anchor residues (Y and I, yellow) but differ substantially in the other positions, activated the same two neurons (ii). Female C57BL/6 mice were used in these experiments. Adapted, with permission, from [23].

of the peptide anchor residues to the specificity of sensory activation [25,26] (Figure 3d). Hence, it appears that the specific anchor residues rather than the exact primary sequences of peptides carry crucial information that is used by sensory neurons for the recognition process. Peptide-specific neuronal activation patterns could provide a sensory basis for the formation and maintenance of social memories induced by signals of individuality. Interestingly, VNO neurons responding to peptides were located in the basal layer of the sensory epithelium, where V2R-type receptors and the G protein $G\alpha_o$ are expressed [25], and activation by peptides was reversibly blocked by 2-aminoethoxydiphenyl borate, a blocker of Ca^{2+}-permeable diacylglycerol-gated cation channels [25]. This suggests that peptide detection by VNO neurons involves, in part, previously characterized signal transduction pathways [35].

A second set of experiments examined whether sensory neurons located in the mouse MOE also respond to MHC peptides. This was indeed found to be the case, suggesting that anatomical segregation of peptide responsiveness is not absolute [34]. MOE peptide recognition involves canonical cAMP signaling and the cAMP-gated CNG cation channel [34]. Interestingly, the specificity of peptide detection in the MOE appears to be different from that in the VNO, suggesting that different receptors might be used for peptide recognition in both systems. Together, these findings support the notion that peptide activation of sensory neurons in the VNO and MOE of mice might be based on different signal transduction pathways and that activation of each system causes differential behavioral outcomes. Indeed, the two routes might have evolved separately to be used for different behavioral decisions, because the neurons connect to distinct regions of the olfactory bulb and higher olfactory centers [31,36,37]. In conclusion, these studies clearly indicate that diverse olfactory mechanisms have evolved to recognize and assess the structural diversity of MHC peptides.

Integration of signals of individuality with other chemical signals

Chemosensory cues recognized by the VNO and MOE, although initially processed separately [31,36], converge at the level of the amygdala [31,37]. Thus, the neuronal hardware exists to regulate behavioral responses mediated by the two systems both separately and coordinately. It has also become clear that innate behavioral responses are not the only ones regulated by the VNO: in the pregnancy-block phenomenon, the behavioral outcome depends on prior olfactory memory formation (i.e. olfactory learning) [31,36,38]. It is also likely that these signals of individuality are assessed alongside other signals that inform about species-identity (e.g. unique peptides [39]), gender (e.g. (methylthio)-methanethiol, present only in male urine [40]), maturation stage (e.g. 2,5-dimethylpyrazine, which interferes with sexual maturation in mice [41]) and fitness (e.g. metabolites, such as ketone bodies as markers of starvation [42]). Indeed, the outcome of the pregnancy-block experiment itself illustrates that sensory activation by MHC peptides is processed in a context-specific manner, because the effect of these peptides depends on the genotype of the previous mating combination [25]. All this indicates that the recognition of and response to signals of individuality is probably regulated at multiple layers in the CNS.

Encoding and decoding signals of individuality

How specific is an individuality signal based on the use of MHC peptide ligands? Addressing this question requires further consideration of the information content of MHC–peptide complexes. Peptides are bound by the MHC molecules through interactions of two to three amino acid side chains with complementary pockets in the binding groove of the MHC molecule [11,12] (Figure 2; Box 1). If all 20 common amino acids were to occupy these two or three anchor residues, ~400–8000 different peptide anchor signatures could be generated. However, not all amino acids are equally likely to be used as anchors [11,12] and MHC molecules show a certain degree of promiscuity in their binding pockets, tolerating for example isoleucine and leucine at one anchor position [11,12]. Although this tends to decrease the sequence complexity of peptide ligands, the preference of MHC molecules for certain residues at sites other than the anchors increases it [11,12]. The structural complexity of MHC peptide ligands found in an individual is also determined by the number of different alleles that are expressed. The large number of different MHC alleles usually present in natural populations ensures that the peptide signatures of two randomly chosen individuals are unlikely to be identical. Thus, at least at the level of MHC peptide diversity, a vast repertoire of chemically distinct combinations can be generated.

What do we know about sensory discrimination of MHC peptides? It is clear that a given sensory neuron in the VNO can be activated by different peptides that share the same anchor residues but differ substantially in the other positions [25] (Figure 3d). However, discrimination at the cellular level must involve more than their anchor residues, because a small fraction of VNO sensory neurons respond to multiple peptides with different anchors [25] (Figure 3c). There has been considerable speculation about the nature of the peptide receptors (Box 3). Neurons activated by peptides are located in the basal layer of the VNO sensory epithelium. These cells express V2R-type G-protein-coupled receptors [31,41,43–45], in addition to non-classical MHC class I genes (those encoding MHC Ib) [46,47]. MHC Ib molecules are unlikely to function as peptide receptors for two reasons. First, they show limited diversity among mice with different classical MHC class I genes [48]; second, a recent analysis of the 3D structure of MHC Ib provided no evidence for bound peptides [49]. Classical MHC class I molecules are almost certainly not involved in peptide recognition because this is independent of MHC haplotype [25]. This leaves members of the V2R family as the most likely candidates for specific peptide receptors. According to a recent computational analysis of mouse genome sequences, mice possess ~60 functional V2R genes that fall into three different families [50]. Hetero-oligomerization of V2R receptors would enable significant discriminatory power (e.g. 3600

Box 3. Outstanding questions

(i) Ecological considerations

One could imagine several systems where MHC peptides have a role as signals of individuality. One interesting area is kin recognition, which could be studied in mammals, birds or fish. It would also be important to determine whether the occurrence of peptides in bodily secretions is constitutive or inducible. If this step is inducible, the regulatory mechanisms would be an interesting area of research.

Discovery of the dual role of MHC peptides for immune defense and social behavior could provide a mechanistic underpinning of the Red Queen hypothesis, which aims to explain the advantage of sexual over asexual reproduction [59]. Its central argument is that only sexual reproduction can achieve optimal combinations of multi-allelic resistance genes quickly enough to fight off rapidly evolving parasites in the environment. If mate selection is based on MHC peptides, immune function can be prospectively evaluated. If olfactory assessment of immune function and genetic individuality were central to the evolution of sexual reproduction, systems functionally equivalent to MHC should exist in invertebrates, because MHC appears first evolutionarily in early vertebrates. In this context, it would also be interesting to determine whether nasal peptide detection coincides with the evolutionary appearance of MHC molecules.

(ii) Molecular identity of peptide receptors

Several functional and structural aspects of peptide recognition require further study. For instance, it would be interesting to see whether peptide receptors are finely or broadly tuned and whether VNO and MOE sensory neurons differ in this respect. It will also be important to examine in detail the signal transduction components in vomeronasal and olfactory sensory neurons. The receptors for peptides have not yet been determined. Classical MHC molecules are unlikely to be involved because peptide recognition is independent of MHC haplotype [25,34]; non-classical MHC class I molecules are also unlikely to function as peptide receptors because peptides do not bind to them [49]. The sensory neurons in the basal layer of the vomeronasal sensory epithelium that are activated by peptides expressing the V2R class of vomeronasal receptors [31,41,43–45]. These receptors are characterized by a large N-terminal extracellular domain reminiscent of metabotropic glutamate receptors and are likely candidates for peptide recognition. Expression of these receptors in heterologous systems or directed mutation by gene targeting will be required to examine their role, if any, in this process. The potential requirement of receptor homo- or hetero-oligomerization or peptide recognition also needs to be addressed. Another interesting issue is whether the same or different receptors are used for peptide recognition in the main and accessory olfactory systems. For these studies, final proof would come from targeted inactivation of such receptors and accompanying specific behavioral phenotypes.

(iii) Combinatorial and hierarchical assessment of semiochemicals

It seems likely that signals of individuality can be interpreted only in the context of other signals, such as those conferring information about species identity, gender, sexual maturation or fitness. It will be particularly interesting to examine what these signals are, where they are detected, and how this information is integrated. In view of the evidence for MHC-related volatile odorants [16,20–23], future work should examine their ability to activate nasal sensory neurons, and whether and how nonvolatile MHC peptide ligands and volatile urinary components interact to convey genotypic information.

(iv) Identification of neural networks that process and integrate individuality cues

Although we now know that peripheral sensory neurons can be activated by MHC peptides, we know virtually nothing about how this information is relayed to higher levels. Sensory neurons of the VNO project to the accessory olfactory bulb; axons from apical and basal zones of the VNO project to anterior and posterior subdivisions of the accessory olfactory bulb, respectively. However, further projections from these areas overlap in the bed nucleus of the accessory olfactory tract, the bed nucleus of the stria terminalis, and the medial and posteromedial areas of the amygdala. From the amygdala, connections exist to the hypothalamus, which is a central regulator of neuroendocrine status. The main olfactory neurons project to the main olfactory bulb, which connects to the piriform cortex, the amygdala and many other brain structures. A combination of functional recordings and genetic tracing studies [60,61] will be required to characterize the higher neuronal networks essential for processing of individuality signals.

heterodimers) that is probably sufficient to assess the structural diversities of MHC peptides. Given that the peptide receptors on sensory neurons probably co-evolve with MHC molecules according to the structural rules imposed by the latter, absolute specificity cannot be expected, and is perhaps not desirable to maintain sufficient flexibility in ligand recognition. However, genetic individuality as measured by peptide structure is but one of the many signals that need to be integrated during social recognition processes such as mate choice (Box 3). Nevertheless, it will be important to examine the exact tuning properties of peptide receptors and the specificity of peptide discrimination. Only then will it be possible to estimate the information content conveyed by structurally distinct peptides and to simulate the impact of MHC-peptide-based distortions of random mating patterns.

Functional genome analysis by the nose

In summary, the assessment of an individuality signal by the nose can be viewed as a sensory mechanism to determine genome composition. The recently discovered role of MHC peptides in this process described here reveals an unexpected mechanistic link between the immune and nervous systems in processing genotypic information. However, it appears that the immune and nervous systems decode the information contained in the structure of MHC peptides in a fundamentally different manner. Because the immune system depends on real-time monitoring of protein synthesis in cells, MHC–peptide complexes must turn over rapidly to be able to respond adequately to potentially devastating infections. To a first approximation, the immune system focuses on the exact primary sequence of the peptides to distinguish between their self or non-self origins. By contrast, although the nervous system also evaluates the structure of the MHC peptides, it focuses on communalities (i.e. anchor residues) rather than differences among the peptides. It therefore ignores possible changes in intracellular protein synthesis, monitoring of which is vital for an effective immune surveillance. Hence, it seems that the olfactory system has evolved a receptor modality that mimics the MHC mode of peptide binding and thereby directs its sensory capabilities towards the genome of conspecifics.

Conclusion, future prospects and questions

As indicated by the evidence summarized here, the role of MHC peptides as signals of individuality appears to be evolutionarily conserved. This will prompt further studies

using various animal models – including humans – to determine whether they employ a similar sensory mechanism (Box 3). With their vast structural repertoire, the MHC peptides represent the first large family of social recognition signals detected by the nose, but it is important to note that they might not be the only chemical cues involved in the sensing of individuality (Box 3). Indeed, as illustrated in Figure 1, several mechanisms could cooperate to achieve versatile and robust individuality signaling in diverse behavioral contexts. A major goal for future research is to establish the molecular identity of nasal peptide receptors and their associated signaling pathways (Box 3). The specificity of neuronal activation by structurally diverse peptides might lend itself to the study of social memories that are formed within the main and accessory olfactory systems. In any case, the discovery of MHC peptide ligands as sensory stimuli in the olfactory system has provided a simple molecular scheme by which genotypic diversity can be converted into a chemosensory quality. This knowledge should enable us to examine the problem of how signals of individuality are encoded and processed by neuronal circuits and how this ultimately leads to behavioral changes.

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