Viewpoints

Excreted Steroids in Vertebrate Social Communication

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Steroids play vital roles in animal physiology across species, and the production of specific steroids is associated with particular internal biological functions. The internal functions of steroids are, in most cases, quite clear. However, an important feature of many steroids (their chemical stability) allows these molecules to play secondary, external roles as chemical messengers after their excretion via urine, feces, or other shed substances. The presence of steroids in animal excretions has long been appreciated, but their capacity to serve as chemosignals has not received as much attention. In theory, the blend of steroids excreted by an animal contains a readout of its own biological state. Initial mechanistic evidence for external steroid chemosensation arose from studies of many species of fish. In sea lampreys and ray-finned fishes, bile salts were identified as potent olfactory cues and later found to serve as pheromones. Recently, we and others have discovered that neurons in amphibian and mammalian olfactory systems are also highly sensitive to excreted glucocorticoids, sex steroids, and bile acids, and some of these molecules have been confirmed as mammalian pheromones. Steroid chemosensation in olfactory systems, unlike steroid detection in most tissues, is performed by plasma membrane receptors, but the details remain largely unclear. In this review, we present a broad view of steroid detection by vertebrate olfactory systems, focusing on recent research in fishes, amphibians, and mammals. We review confirmed and hypothesized mechanisms of steroid chemosensation in each group and discuss potential impacts on vertebrate social communication.

Key words: vertebrate; olfaction; chemosensation; steroid; bile acid; pheromone

Introduction

Olfactory pathways have evolved to interpret environmental cues that support survival and reproduction. Most vertebrates possess multiple olfactory systems that allow them to detect and interpret social chemosignals. These parallel olfactory systems support a wide range of important behaviors, including mating, aggression, and predator avoidance (Belanger and Corkum, 2009; Houck, 2009; Bazáes et al., 2013; Stowers and Kuo, 2015). Throughout vertebrate evolution, the ability to detect certain informative chemical classes (e.g., biogenic amines) has been conserved (Liberles and Buck, 2006; Liberles, 2009; Ferrero et al., 2011; Zhang et al., 2013). Steroids (sex steroids, glucocorticoids, neurosteroids, and bile acids) are increasingly being recognized for their capacity to serve as activating ligands for vertebrate olfactory neurons (W. Li et al., 1995; Friedrich and Korsching, 1998; Nodari et al., 2008; Isogai et al., 2011; Haga-Yamanaka et al., 2014; Doyle et al., 2016; Greer et al., 2016). Nuclear receptors (for review, see Evans and Mangelsdorf, 2014) and several steroid-sensitive plasma membrane receptors (Kawamata et al., 2003 for review, see Wang et al., 2014) are known to play important roles in mammalian physiology. However, evidence suggests that it is not these receptors, but

chemosensory GPCRs and 4-transmembrane domain receptors that sense environmental steroids (Isogai et al., 2011; Haga-Yamanaka et al., 2014; Greer et al., 2016). Steroids have the potential to convey information about reproductive status and health, and accordingly some, but not all, steroid ligands have been linked to social and reproductive behaviors (Hurk and Lambert, 1983; Stacey and Sorensen, 1986; Sorensen et al., 1995; Poling et al., 2001; W. Li et al., 2002; Haga-Yamanaka et al., 2014; Fu et al., 2015). Steroids are not the only hormones that also serve as external chemosignals (Yabuki et al., 2016); but in the interest of clarity, we focus exclusively on steroid chemosignals in this manuscript. The topics of olfactory receptor evolution (Fleischer et al., 2009; Korsching, 2009; Niimura, 2009), olfactory system organization and function (Houck, 2009; Bazáes et al., 2013; Buchinger et al., 2015; Stowers and Kuo, 2015), and steroid synthesis and metabolism (Miller and Auchus, 2011; Evans and Mangelsdorf, 2014; Copple and Li, 2016) have all been recently covered, and we refer readers interested in each of these topics to the relevant reviews. Reptiles also possess chemosensory systems capable of detecting steroids (Houck, 2009) but are not discussed here. In this Viewpoints article, we begin by briefly reviewing chemical features of steroids and then discuss steroid chemosensory research in fishes, amphibians, and mammals. We conclude by mentioning areas we believe are ripe for discovery, and discussing potential implications of olfactory steroid sensing for vertebrate behavior.

Steroids are a structurally diverse, information-rich family of molecules

Steroids perform diverse functions in the body; and as derivatives of cholesterol, they share common structural features. Steroids contain a core of four fused rings that can take on a flat (*cis*- or α)

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Figure 1. Steroid olfactory cues. *a*, Steroid nucleus structure. Carbons are indicated by numbers and rings by letters. *b*, Steroid classes. The number in parentheses indicates the number of carbon atoms present in each class. *c*, Simplified diagram of steroid biosynthesis. Steroids that have been identified as vertebrate olfactory cues have solid backgrounds. Some intermediate steroids are omitted for clarity. Arrows indicate the proposed directions of synthesis. Reversals and alternative methods are possible. Colors are the same as in *b*.

or bent (trans or β) conformation based on the orientation of a hydrogen at the fifth carbon (Fig. 1*a*) (Hagey et al., 2010b; Kasal, 2010). An important location of structural diversity among steroids is at the 17th carbon, where side chains of varying lengths are attached (Fig. 1*b*) (Henley et al., 2005; Kasal, 2010). In general, the number of carbon atoms is used to classify steroids. Cholestanes, like cholesterol, contain 27 carbons. Cholanes, such as the bile acid cholic acid, have 24 carbons. Pregnanes, such as hydrocortisone and progesterone, have 21 carbons. Androstanes, including the predominant male sex steroid testosterone, have 19 carbons but lack a side chain at carbon 17. Androstanes are further metabolized to 18-carbon estrane, for example, the female sex steroid estrogen (Fig. 1*b*) (Henley et al., 2005; Kasal, 2010; Miller and Auchus, 2011).

Steroids have well-known functional roles in the body, including sexual development (sex steroids), food digestion (bile acids), stress responses (glucocorticoids), and brain function (neurosteroids) (Henley et al., 2005; Kasal, 2010; Miller and Auchus, 2011). Steroids are typically detected in the body through binding to nuclear receptors (for review, see Evans and Mangelsdorf, 2014) or plasma membrane receptors (for review, see Wang et al., 2014). The different functional roles of steroids in the body tend to be associated with the number of carbons and stereochemistry

of the molecule. For example, estranes are enriched for estrogens that regulate the ovarian cycle (Henley et al., 2005; Do Rego et al., 2009; Kasal, 2010; Finco et al., 2015).

Because of their potent biological activity, there is tight regulation of steroid synthesis, modification, and excretion. The addition of conjugate groups, such as sulfate, glucuronidate, or taurine, supports steroid mobilization and excretion (Russell, 2003; Fraser et al., 2010; Hofmann et al., 2010; Holder et al., 2010; Miller and Auchus, 2011). Steroid conjugation is most common at free hydroxyl groups, typically those on the third or 17th carbon of the steroid nucleus (Holder et al., 2010; Sjövall et al., 2010). For example, in cholane bile acids, taurine and glycine conjugation is most common at the carboxyl group at the end of the side chain (Hagey et al., 2010a; Hofmann et al., 2010; Sjövall et al., 2010). Conjugation increases water solubility and, in many cases, allows the molecules to be excreted via urine and/or feces. Conjugation can also modify steroid biological activity. For example, neurosteroids, including pregnanes and certain androstanes, are potent modulators of GABA receptors in the brain and have greater biological activity after the addition of a sulfate moiety (Paul and Purdy, 1992; Do Rego et al., 2009; Miller and Auchus, 2011). Excreted steroids are remarkably stable (Stroud et al., 2007). This environmental stability allows excreted steroids to be detected by

Figure 2. Olfactory steroid detection in bony fish. *a*, Early olfactory system anatomy of bony fish (teleosts). Steroids are detected by sensory neurons in the olfactory epithelia (OE) and are initially processed in the olfactory bulb (OB). *b*, Anatomy of the teleost olfactory epithelium. Teleost sensory neurons include neurons with cilia or microvilli. Crypt cells consist of ciliated and microvillar subpopulations. The steroid-sensing neuronal populations in lampreys (which have only ciliated sensory neurons) and elasmobranchs (which lack ciliated sensory neurons) differ considerably. *c*, Selected fish steroid olfactory cues. Petromyzostrosterol is a lamprey-specific estrane. $17\alpha,20\beta$ -Dihydroxyprogesterone sulfate (also known as $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one 20-sulfate) is a pregnane that guides mating in goldfish. Androstenedione is an androstane that regulates aggression in goldfish. Petromyzonol sulfate is a bile alcohol that acts as a migratory pheromone in lamprey. Taurocholic acid is a bile acid that is a common vertebrate olfactory cue.

transmembrane receptors expressed in olfactory tissues and be used as chemosignals that reveal information about the status of the emitter to the outside world.

Fishes use excreted steroids as pheromones

Fish are the earliest evolving vertebrates and include jawless, cartilaginous, and bony fishes (Brazeau and Friedman, 2015). Fish live in water and in low-visibility environments and are especially reliant on water-soluble chemical cues to guide reproductive behavior. Jawless fish (e.g., lampreys and hagfishes), evolved before jawed cartilaginous fish (elasmobranchs; e.g., sharks and rays), which evolved before bony fish (teleosts; e.g., goldfish and salmon) (Huertas et al., 2010; Bazáes et al., 2013; Brazeau and Friedman, 2015). Work in fish olfactory systems has confirmed sensitivity to environmental steroids, leading to the belief that most, if not all, fishes use steroids for chemical communication (Bazáes et al., 2013). The majority of this research has focused on lampreys and teleosts.

In lampreys, excreted steroids have been directly linked to migration and reproduction (W. Li et al., 1995, 2002; Robinson et al., 2009). In their larval phase, lampreys feed on detritus in streambeds, then as adults migrate downstream to feed on other fish. Near the end of their adulthood, lampreys migrate upstream to mate (Buchinger et al., 2015). Unlike migratory species, such as salmon, which learn the chemical composition of their birthplace and return to it in adulthood, lampreys migrate to wherever fed larvae are present (Bett and Hinch, 2016). Fed larvae excrete large amounts of sulfated bile alcohols that attract adults (W. Li et al., 1995; Vrieze and Sorensen, 2001; Fine et al., 2004; Fine and Sorensen, 2008; Yun et al., 2011; Buchinger et al., 2013, 2015).

Several bile alcohols, many of which contain the prefix “petromyzon” after the lamprey taxonomic order “Petromyzontiformes,” act in a blend to promote adult migration. The active lamprey steroids include petromyzonol sulfate, petromyzonamine disulfate, petromyzestrosterol, petromyzosterol disulfate, petromyzones, petromyzenes, and allocholic acid (W. Li et al., 1995; W. Li and Sorensen, 1997; Sorensen et al., 2005b; K. Li et al., 2012, 2013a, 2017a,b; Johnson et al., 2014). These steroid ligands are active at nanomolar to subnanomolar concentrations, which is important because these pheromones must diffuse through large volumes of turbid water before reaching their targets (W. Li et al., 1995; K. Li et al., 2012).

Excreted steroids also guide lamprey mating behaviors. Males release a blend of bile alcohols (along with additional unidentified compounds), which potently attract females. Males secrete oxidized forms of the same bile alcohols released by larvae, including 3-ketopetromyzonol sulfate and 3-ketoallocholic acid (Johnson et al., 2009; Buchinger et al., 2013, 2015). Other components of the male pheromone blend include diketo petromyzonene sulfate and an unconjugated estrane petromyzestrosterol (K. Li et al., 2012, 2013b; Buchinger et al., 2015; Brant

et al., 2016). The observation that male lampreys release oxidized versions of larval migratory pheromones seems to indicate that they have coopted larval signals for the purpose of attracting mates (Buchinger et al., 2013). The pheromone activity of petromyzestrosterol and other nonbile alcohol steroids further demonstrates that the lamprey olfactory system can discriminate between members of multiple steroid classes.

The molecular and cellular mechanisms underlying steroid detection by lampreys are not fully understood. Lampreys possess both a main olfactory epithelium and an accessory olfactory organ (not to be confused with the vomeronasal organ found in amphibians and mammals; Fig. 2) (Chang et al., 2013; Buchinger et al., 2015; Daghfous et al., 2016). Neuroepithelial cells in both sensory tissues have ciliated dendrites and express receptors that are related to the olfactory receptor (OR), trace amine-associated receptor (TAAR), and vomeronasal Type I receptor (V1R) families (Grus and Zhang, 2009; Buchinger et al., 2015). Although it is currently unknown which lamprey receptors respond to steroids, there are indications that a specific subpopulation of receptors is involved. The lamprey medial olfactory bulb, which receives dense projections from the accessory olfactory organ, responds most strongly to steroids (Green et al., 2017). However, the medial olfactory bulb does receive a minority of its innervation from the main olfactory epithelium, and steroids also activate the lateral olfactory bulb; so the steroid sensing receptors remain unclear. However, these data do demonstrate that neurons in the accessory olfactory organ are steroid detectors (Chang et al., 2013; Buchinger et al., 2015; Green et al., 2017).

Steroid detection has been confirmed in elasmobranchs, which can detect common cholestane bile acids found in all ver-

tebrates. Confirmed ligands in sharks and rays include cholic, conjugated chenodeoxycholic, and conjugated lithocholic acid (Meredith et al., 2012). Cholic acid and chenodeoxycholic acid are produced in the liver (so-called primary bile acids), whereas lithocholic acid is a secondary bile acid produced by anaerobic bacteria in the gut (Russell, 2003). Sensitivity to bile acids was observed in the micromolar to nanomolar range, a concentration range similar to other species, indicating that a population of bile acid-sensitive receptors is maintained and/or refined in elasmobranch evolution (W. Li et al., 1995; Michel and Lubomudrov, 1995; Meredith et al., 2012; Doyle et al., 2016). It may be the case that bile acid-responsive receptors in sharks are most sensitive to other naturally secreted steroids other than those currently tested. For example, shark bile is enriched for a sulfated bile alcohol named scymnol sulfate (Hagey et al., 2010b), but more work is needed to determine whether this molecule is a natural ligand with behavioral significance. As in lampreys, the steroid-sensitive neurons and receptors in elasmobranchs remain unclear.

In both lampreys and elasmobranchs, the majority of identified steroid ligands are bile acids and their conjugate salts (W. Li et al., 1995; Siefkes and Li, 2004, 2005; Meredith et al., 2012; Buchinger et al., 2014). Bile salt detection is maintained in teleosts, with responses observed in multiple species (Døving et al., 1980; Hellström and Døving, 1986; Quinn and Hara, 1986; Morin and Døving, 1992; Sola and Tosi, 1993; Michel and Derbidge, 1997; Friedrich and Korsching, 1998; Hara and Zhang, 1998; Zhang et al., 2001; Frade, 2002; Baker et al., 2006; Giaquinto and Hara, 2008; Velez et al., 2009; Zhang and Hara, 2009; Huertas et al., 2010). In addition to bile salt responses, many have noted olfactory sensitivity to steroid hormones in teleosts, such as salmon, trout, and goby (Moore and Scott, 1991, 1992; Essington and Sorensen, 1996; Lastein et al., 2006; Colombo et al., 2009). This has been most deeply studied in *Carassius auratus*, the common goldfish. In goldfish, increased levels of a pregnane, 17,20 α , 20 β -dihydroxy-4-pregnen-3-one (also called 17,20 β -dihydroxyprogesterone), promote oocyte maturation (Van der Kraak et al., 1989). Both conjugated and unconjugated forms of this steroid are released by ovulating females and are detected by male goldfish at nanomolar concentrations (Sorensen et al., 1987, 1995; Poling et al., 2001). These molecules induce courtship behaviors in males, such as chasing and nudging, confirming its action as a pheromone (Poling et al., 2001). An unconjugated androstane, androstenedione, is both produced and detected by mature males and has been shown to promote aggression (Poling et al., 2001; Sorensen et al., 2005a). Zebrafish (*Danio rerio*) also show strong olfactory responses to conjugated and unconjugated sex hormones. Zebrafish olfactory systems respond to pregnanes, including 17 α ,20 β -dihydroxy-4-pregnen-3-one 20-sulfate, and glucuronidated androstanes and estranes (Hurk and Lambert, 1983; Michel and Lubomudrov, 1995; Friedrich and Korsching, 1998). The round goby (*Neogobius melanostomus*) and African cichlid (*Haplochromis burtoni*) also respond to steroid hormones (Robison et al., 1998; Murphy et al., 2001; Cole and Stacey, 2006). The diverse steroid sensitivities observed in multiple teleost species indicate that these fish evolved highly specialized sets of steroid chemoreceptors.

The teleost olfactory epithelium possesses three well-studied populations of sensory neurons: ciliated, microvillar, and crypt cells (Bazáes et al., 2013; Kermen et al., 2013). Rarer cell populations have recently been identified but have not been linked to steroid detection (Ahuja et al., 2014; Wakisaka et al., 2017). Ciliated sensory neurons are located basally and their dendrites are long, whereas microvillar cells are located apically and have

shorter dendrites. Crypt cells are located superficially and have a short sensory dendrite (Bazáes et al., 2013; Kermen et al., 2013). As is the case in lamprey, teleost steroid responses are enriched in the medial olfactory bulb, which is innervated by both ciliated sensory neurons and crypt cells (Friedrich and Korsching, 1998; Hansen et al., 2003; Laberge and Hara, 2004; Rolén and Caprio, 2007; Yaksi et al., 2009; Ahuja et al., 2013; Bazáes et al., 2013). It is hypothesized that ciliated sensory neurons respond to bile acids, but the neuronal population responsible for sex steroid detection remains unclear (Thommesen, 1983; Bazáes et al., 2013). Sex steroid responses have been reported in crypt cells, but some studies found only amino acid responses (Vielma et al., 2008; Hamdani et al., 2008; Bazáes and Schmachtenberg, 2012). Intriguingly, in some species, the number of crypt cells fluctuates throughout the year, with increases seen during the mating season (Hamdani et al., 2008; Bazáes et al., 2013). In other species, crypt cells expand in number and/or change their preferred ligands following the transition to adulthood, suggesting that the repertoire of putative steroid-detecting neurons may be plastic throughout the life cycle (Bazáes and Schmachtenberg, 2012). Although the receptors responsible for detecting sex steroids in teleosts remain unknown, ciliated sensory neurons are known to express ORs and crypt cells express a V1R-like receptor, indicating that at least one of these receptor families may detect environmental steroid pheromones (Oka et al., 2012; Bazáes et al., 2013; Kermen et al., 2013).

Sulfated steroids are potent olfactory cues in larval amphibians

Amphibians transition from a purely aquatic larval phase to a mixed aquatic and terrestrial adulthood (Hansen et al., 1998; Dittrich et al., 2016). The olfactory systems of *Xenopus* frogs (*X. tropicalis* and *X. laevis*) have been particularly well studied, particularly during the aquatic larval phase (tadpoles; Fig. 3). *Xenopus* tadpoles contain two olfactory cavities, the principal cavity and vomeronasal organ, both of which are exposed to water-borne odorants (Hansen et al., 1998; Belanger and Corkum, 2009; Dittrich et al., 2016). The tadpole principal cavity contains both ciliated and microvillar sensory neurons, whereas the vomeronasal organ contains only microvillar neurons (Belanger and Corkum, 2009; Dittrich et al., 2016). Bile salts activate the tadpole principal cavity, potentially activating both ciliated and microvillar sensory neurons (Gliem et al., 2013). Although the bile salt sensitivity of the tadpole principal cavity is clear, these molecules were delivered as blends, leaving in question the specific activities of each component molecule and its receptor(s) (Gliem et al., 2013). Both the principal cavity and the vomeronasal organ respond to sulfated pregnanes and estranes, including 17 β -estradiol sulfate (Sansone et al., 2015). 17 β -Estradiol sulfate is also a potent olfactory cue in the teleost *H. burtoni*, suggesting a conserved capacity for detecting this important sex steroid (Robison et al., 1998; Cole and Stacey, 2006). The 17 β -estradiol sulfate receptor(s) in both species remain unclear. V2Rs are the only class of receptors known to be expressed in both the principal cavity and the vomeronasal organ in amphibians (Syed et al., 2013, 2017; Sansone et al., 2015). V2Rs in both sensory organs may be the 17 β -estradiol sulfate receptors, but it is also possible that receptors from multiple receptor classes are sensitive to this ligand.

During amphibian metamorphosis, a third cavity develops, called the middle cavity, while the principal cavity is remodeled (Hansen et al., 1998; Dittrich et al., 2016). In adulthood, the newly restructured principal cavity acts as a detector for airborne

Figure 3. Olfactory steroid detection in amphibians. **a**, Early olfactory system anatomy of *Xenopus* tadpoles. Premetamorphic larva detect odorants through the main olfactory epithelium (MOE) in the principal cavity and in the vomeronasal organ (VNO). Sensory neurons in the MOE project to the main olfactory bulb (MOB), and in the VNO to the AOB. During metamorphosis, the middle cavity housing a third olfactory epithelium develops. **b**, Anatomy of the tadpole olfactory epithelial neurons. V2Rs are expressed in microvillar and ORs in ciliated cells. In tadpoles, it is unknown which cells express V1Rs, although in adults it is in ciliated sensory neurons. **c**, Example sulfated estranes and pregnanes that activate the tadpole olfactory system. 17 β -dihydroequilin and 17 β -estradiol are sulfated estranes, and allopregnanolone is a sulfated pregnane. These cues also activate the vomeronasal organ of the mouse.

odorants, whereas the middle cavity and vomeronasal organ continue to detect aqueous-phase cues (Hansen et al., 1998). The adult frog vomeronasal organ contains microvillar sensory neurons, whereas the middle cavity and premetamorphic principal cavity contain both ciliated and microvillar sensory neurons (Hansen et al., 1998). The adult principal cavity only contains ciliated sensory neurons (Dittrich et al., 2016). In adult frogs, the anatomical segregation in the nose is maintained in the olfactory bulb. The adult main olfactory bulb is innervated by the principal and middle cavities and the accessory olfactory bulb (AOB) is innervated by the vomeronasal organ (Belanger and Corkum, 2009). Even though receptor composition within the sensory organs is different from amphibians, anatomical segregation of parallel chemosensory streams is conserved in some terrestrial mammals and is thought to reflect specialization of neural pathways that are used to guide innate and learned behaviors (Stowers and Kuo, 2015). The steroid sensitivity of the adult frog olfactory systems has not been studied in depth, but recent work in mammals has revealed a conserved olfactory capacity to detect steroid ligands (Nodari et al., 2008; Isogai et al., 2011; Haga-Yamanaka et al., 2014; Doyle et al., 2016).

Steroids are potent regulators of mating in mammals

Most terrestrial mammals have two major olfactory subsystems: the main olfactory system and accessory olfactory system (AOS; Fig. 4) (Stowers and Kuo, 2015). In mice, the main olfactory system detects airborne volatile odorants and consists of a main olfactory epithelium with ciliated sensory neurons that innervates the main olfactory bulb (Dulac and Wagner, 2006). The AOS detects aqueous-phase odorants in the vomeronasal organ via microvillar sensory neurons and projects to the AOB (Dulac and Wagner, 2006). This anatomical segregation in the olfactory bulb is reminiscent of the adult frog and indicates a conserved aspect of parallelized olfactory processing in the brain (Belanger and Corkum, 2009).

Recent studies in mice have revealed that several classes of steroids activate neurons of the AOS. Initial studies identified sulfated pregnanes, androstanes, estranes, and glucocorticoids as mouse vomeronasal sensory neuron ligands (Nodari et al., 2008;

can evaluate a nearby animal's stress from urinary cues (Nodari et al., 2008). The behavioral relevance of the capacity to detect glucocorticoids is not yet fully understood, but it is noteworthy that sulfated estranes (17 β -estradiol sulfate) and glucocorticoid acids (cortigynic and corticosteronic acids) have been implicated in guiding male mating behaviors (Haga-Yamanaka et al., 2014; Fu et al., 2015).

Recent work from our laboratory has shown that unconjugated bile acids, well-known steroid ligands in fishes and amphibians, are present in mouse feces and are potent activators of the mouse AOS. The primary bile acids cholic and chenodeoxycholic acid and their secondary gut derivatives deoxycholic and lithocholic acid produce robust activity in the AOB (Doyle et al., 2016). AOB neuronal activity patterns were discriminable for each of the four bile acids and also distinguishable from those elicited by sulfated steroid ligands (Doyle et al., 2016). A bile acid that has high levels in rodent bile, ω -muricholic acid, activated a small number of mouse neurons, indicating that the AOS detects bile acids that are relatively unique to a given taxon (muricholic acid) and those that are present across the animal kingdom (cholic and chenodeoxycholic acid) (Doyle et al., 2016). Similar to fish, these results support the hypothesis that bile acids may convey general information about other animals in the environment. Many active bile acids are present in multiple species, whereas others convey taxon-specific information (Hagey et al., 2010a, b; Hofmann et al., 2010). Our initial experiments focused on unconjugated bile acids, but future experiments will determine the breadth of bile acid tuning (e.g., including common conjugates) in the rodent AOS and their roles in guiding animal behavior.

The majority of identified steroid responses in mice have been in the AOS, which begins in the vomeronasal organ. Vomeronasal sensory neurons are microvillar and express V1Rs, V2Rs, formyl peptide receptors, and nonclassical major histocompatibility complex proteins (Dulac and Axel, 1995; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997; Pantages and Dulac, 2000; Loconto et al., 2003; Dulac and Wagner, 2006; Liberles et al., 2009; Rivière et al., 2009). Several V1R-expressing neurons have been shown to be sensitive to sulfated steroids (Isogai et al., 2011; Haga-Yamanaka et al., 2014), and both sulfated steroids

Meeks et al., 2010; Isogai et al., 2011; Celsi et al., 2012; Turaga and Holy, 2012; Hammen et al., 2014). These ligands are quite potent, and the neuronal responses demonstrate highly specific steroid tuning profiles (Nodari et al., 2008; Meeks et al., 2010; Turaga and Holy, 2012; Fu et al., 2015; Haga-Yamanaka et al., 2015), but it remains unknown how many of these active steroids are naturally occurring ligands. However, several steroid cues have been identified in relevant biological excretions. The first such molecules were two sulfated glucocorticoids, corticosterone sulfate and cortisol sulfate, which were identified in mouse urine (Nodari et al., 2008). Subsequent research has shown that mice can also detect glucocorticoids with a modified carboxylic acid side chain (Fu et al., 2015), making them structurally similar to bile acids. Sulfated glucocorticoids are upregulated in urine in response to stress, and their detection suggests a neural mechanism by which mammals

and bile acids activate the anterior sub-region of the AOB (Meeks et al., 2010; Hammen et al., 2014; Doyle et al., 2016), which is selectively innervated by V1R-expressing sensory neurons (Belluscio et al., 1999; Rodriguez et al., 1999). These results indicate that V1Rs are likely to be the predominant detector of steroids in the mouse. However, responses to sulfated pregnanes have been observed in the posterior AOB (Hammen et al., 2014), which is innervated by V2R-expressing neurons (Del Punta et al., 2002). This indicates that members of both the V1R and V2R families may serve as steroid detectors in mice. Additional recent work has revealed that neurons expressing members of the MS4A protein family in the “cul-de-sac” regions of the main olfactory epithelium are sensitive to steroids, indicating that subsets of main olfactory sensory neurons are capable of interpreting airborne environmental steroid cues (Greer et al., 2016).

Other mammals, including pigs and humans, have been shown to respond to unconjugated steroids. The best-known steroid ligands in nonrodents are unconjugated volatile androstanes, which are active in both pigs and humans (Melrose et al., 1971; Dorries et al., 1995; Keller et al., 2007). In pigs, androstenone is present in male pig saliva and induces attraction and mating behaviors in estrous females (Melrose et al., 1971; Dorries et al., 1995). Humans are also able to detect the volatile androstanes androstenone and androstadienone (Keller et al., 2007). For humans, these steroids do not appear to have a set valence. Some individuals report them as having pleasant odor qualities, whereas others find them aversive, which is thought to reflect genetic variations affecting receptor sensitivity in the human population (Wysocki and Beauchamp, 1984; Keller et al., 2007). In both pigs and humans, volatile androstanes are detected by the main olfactory epithelium, demonstrating that responses to steroids do not necessarily require a pathway specialized for nonvolatile ligands (Dorries et al., 1997; Keller et al., 2007). It is unknown what receptors detect the odorants in pigs, but in humans, volatile androstanes are detected by ORs (Keller et al., 2007). MS4a receptors may play a role, but their expression and ligand sensitivities in humans remain to be studied (Greer et al., 2016). The ability of humans to detect environmental steroids shows that vertebrates spanning hundreds of millions of years in evolution have maintained the ability to detect external steroids.

What are the steroid chemosensory receptors?

Despite a wealth of information about steroids that are detected by chemosensory systems, we still know strikingly little about the relationship between these ligands and their chemosensory receptors. The four major classes of transmembrane chemosensory receptors in fish, amphibians, and mammals are ORs, V1Rs, V2Rs, and TAARs (Buck and Axel, 1991; Dulac and Axel, 1995; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997; Pantages and Dulac, 2000; Liberles and Buck, 2006). Chemosensory receptor genes have undergone rapid evolution and the exact comple-

Figure 4. Olfactory steroid detection in mammals. *a*, Early olfactory system anatomy of the mouse. The two major olfactory systems are as follows: (1) the main olfactory system, consisting of the main olfactory epithelium (MOE) and main olfactory bulb (MOB); and (2) the accessory olfactory system, consisting of the vomeronasal organ (VNO) and AOB. The apical layer of the VNO projects to the anterior AOB, whereas sensory neurons in the basal layer of the VNO project to the posterior AOB. Omitted for space are two additional olfactory tissues, the septal organ and Grueneberg ganglion. *b*, Anatomy of mouse olfactory tissues. Ciliated neurons in the MOE express ORs, whereas microvillar cells in the VNO express V1Rs and V2Rs. V1Rs are expressed apically and V2Rs basally. *c*, Example mammalian social cues. 17 β -Estradiol is a sulfated estrane that regulates male mouse mating behaviors. Testosterone sulfate is an androstane detected by mice. Androstenone is an androstane detected by pigs and humans. Corticosterone sulfate is a pregnane detected by mice. Cortigynic is a glucocorticoid acid that guides male mouse mating. Deoxycholic acid is a bile acid detected by multiple vertebrates.

ment of expressed receptors changes not only among orders but also among different species (Emes et al., 2004; Grus and Zhang, 2004; Lane et al., 2004; Grus et al., 2005; Kurzweil et al., 2009; Young et al., 2010). This rapid evolution allows for changes in the binding pockets of receptors, allowing them to bind selectively to molecules that differ by only minor changes in structure (Isogai et al., 2011; Haga-Yamanaka et al., 2014, 2015). The lamprey genome contains multiple ORs and TAAR-like genes, a few V1Rs, and no V2Rs (Grus and Zhang, 2009; Libants et al., 2009). V2R-like genes appear in cartilaginous fish alongside OR-like, TAAR-like, and V1R-like receptors (Grus and Zhang, 2009). In most teleosts, the major receptors are ORs, TAARs, and V2R-like receptors (termed olfc receptors), with only a few V1R-like (ora) receptors (Hussain et al., 2009; Behrens et al., 2014; Saraiva et al., 2015). *Xenopus* express all of the major chemosensory receptor classes and an expanded V2R repertoire (Mezler et al., 1999; Hagino-Yamagishi et al., 2004; Date-Ito et al., 2008; Syed et al., 2013, 2017). Mice also express all of the major classes of chemosensory receptors with an expansion of V1Rs (Young et al., 2005; Zhang et al., 2007, 2010).

In some cases, anatomy provides clues about the most likely steroid-sensitive receptor classes. In teleosts, sex steroids may activate crypt cells, which express V1R-like receptors, and bile salts are active in regions of the olfactory bulb that are innervated by OR-expressing neurons (Thommesen, 1983; Hansen et al., 2003; Sato et al., 2005; Oka et al., 2012; Biechl et al., 2017). Teleosts may also detect steroids through noncanonical olfactory receptors; for example, goldfish and zebrafish olfactory epithelia express a group of transmembrane progesterone receptors (Kolmakov et al., 2008). In frogs, steroids activate both the principal cavity and the vomeronasal organ. Both organs express V2Rs, but

Table 1. Example steroid cues with known effects on social behaviors^a

Common name	Chemical name	Structural class	Animals	Behavior
Petromyzonamine disulfate	5 α -cholestane-7 α ,24-diol-N-(3-aminopropyl)2-pyrrolidinone 7,24-disulfate	Cholestane	Fish	Migration
Petromyzosterol disulfate ^b	(3 β ,12 α ,22E,24S)-ergost-22-ene-3,12,24-triol, 12,24-disulfate	Ergostane	Fish	Migration
Petromyzonol sulfate	5 α -cholan-3 α ,7 α ,12 α ,24-tetrol 24-sulfate	Cholane	Fish	Migration
3-keto petromyzonol sulfate	5 α -cholan-7 α ,12 α ,24-triol-3-one 24-sulfate	Cholane	Fish	Mating
3,12-diketo-4,6-petromyzonene-24-sulfate	4,6-cholestadien-24-ol-3,12-one 24-sulfate	Cholane	Fish	Mating
17 α ,20 β -dihydroxyprogesterone	4-pregnen-17 α ,20 β -diol-3-one	Pregnane	Mammal	Mating
17 α ,20 β -dihydroxyprogesterone sulfate	4-pregnen-17 α ,20 β -diol-3-one 20-sulfate	Pregnane	Mammal	Mating
Corticosteronic acid	4-pregnen-21-oic acid-11,20-diol-3-one	Pregnane	Mammal	Mating
Cortigynic acid	4-pregnen-21-oic acid-11,16,20-triol-3-one	Pregnane	Mammal	Mating
Androstene	5 α -androst-16-en-3-one	Androstane	Mammal	Mating
Androstenedione	4-androsten-3,17-dione	Androstane	Fish	Aggression
17 β -estradiol disulfate	1,3,5(10)-estratrien-3,17 β -diol 3,17-disulfate	Estrane	Mammal	Mating
17 β -estradiol sulfate	1,3,5(10)-estratrien-3,17 β -diol 17-sulfate	Estrane	Mammal	Mating

^aA comprehensive list of all known steroid ligands and citations for these ligands are provided in Tables 1–1 (available at <https://doi.org/10.1523/JNEUROSCI.2488-17.2018.t1-1>) and 1–2 (available at <https://doi.org/10.1523/JNEUROSCI.2488-17.2018.t1-2>).

^bPetromyzosterol disulfate is an ergostane, structurally similar to a cholestane.

the steroid-sensitive receptors remain unknown (Syed et al., 2013, 2017; Sansone et al., 2015). In mice, sulfated steroids and bile acids potently activate the anterior AOB, consistent with detection by V1R receptors, but sulfated pregnanes strongly activate the V2R-targeted posterior AOB (Meeks et al., 2010; Hammen et al., 2014; Doyle et al., 2016), suggesting that both families may contain steroid receptors (Hammen et al., 2014). Candidate gene approaches to identifying steroid ligand-receptor pairs are starting to bear fruit. For example, recent studies have confirmed the steroid sensitivities of individual receptors, marking the beginning of an important phase of discovery in olfaction. In mice, sulfated estranes are detected by *Vmn1r85*, *Vmn1r89*, and *Vmn1r237*, whereas *Vmn1r226* and *Vmn1r227* detect sulfated corticosteroids (Isogai et al., 2011; Haga-Yamanaka et al., 2014). Humans detect androstanes through the OR *ORD74* (Keller et al., 2007). Overall, candidate receptor approaches have been slow. Recent technical advances in activity-mediated transcriptional profiling will enable major gaps in our knowledge of steroid chemosensation to be filled (Jiang et al., 2015; von der Weid et al., 2015). Such unbiased approaches may help to discover other steroid-sensitive receptors, whether within or outside the known complement of olfactory receptors. Overall, the evidence obtained across vertebrate species indicates that a single receptor class is unlikely to serve as a steroid specialist. Instead, it seems apparent that, throughout evolution, receptors in multiple families have acquired steroid sensitivity.

How does steroid chemosensation influence vertebrate behavior?

Structurally similar steroids typically serve similar internal functions across vertebrate species (e.g., for estranes, this includes modulating sexual maturation and the ovarian cycle) (Henley et al., 2005). At face value, the detection of excreted derivatives of some steroid classes could provide interpretable signals for recipient animals that guide behavior (Table 1; Tables 1–1, available at <https://doi.org/10.1523/JNEUROSCI.2488-17.2018.t1-1>; and 1–2, available at <https://doi.org/10.1523/JNEUROSCI.2488-17.2018.t1-2>). For example, in mice, estranes are released by females and drive courtship by males (Haga-Yamanaka et al., 2014). Similarly, in goldfish, females emit pregnanes that drive male mating behavior (Poling et al., 2001). In these cases, the connection between the physiological role of the steroid for the emitter and the behavioral response it generates in the detector are congruent. However, such a relationship is not always clear. For example, the

carboxylated glucocorticoid cortigynic acid, which has unknown internal functions in female mice, is a major driver of male mouse attraction (Fu et al., 2015). Internally, bile acids are used in the digestion of lipids, but these molecules also act as lamprey pheromones, driving female sexual attraction (Buchinger et al., 2013).

In addition to the identification of internal physiological states, vertebrates may use olfactory steroid receptors to determine an animal's taxon. Bile salts are potent olfactory cues across vertebrates that may, in part, support this function. This is seen most clearly with lamprey mating and migration, both of which rely on the detection of lamprey-specific bile alcohols, such as petromyzonol sulfate and 3-ketopetromyzonol sulfate (W. Li et al., 2002; Johnson et al., 2009). In other vertebrates, the bile acid complement is more complex and overlapping (Hagey et al., 2010a, b), but the specific combination of bile acids has been hypothesized to be a “molecular fingerprint” useful for identifying taxon (Hofmann et al., 2010). The biological significance of taxon-identifying steroid cues remains to be fully explored, but it seems likely that the behavioral response to such cues will depend on the identity of the detector. For example, detection of a taxon-specific cue may elicit an attractive response (e.g., migration, mating) in conspecifics, but in other animals could produce different behavioral effects. For example, prey could use steroids released by predators, such as the shark-enriched scymnol sulfate, as avoidance cues. In support of the hypothesis that steroids may provide taxon-specific information, it has been reported that a bile acid enriched in lampreys can be detected by teleosts, although the behavioral relevance of this cue is unknown (Baker et al., 2006).

In addition to roles in identifying mates and other species, steroids may also provide other information about the health of other animals or their diet. The first urinary steroid ligands discovered in mice, for example, were sulfated glucocorticoids that increased in mouse urine after acute stress (Nodari et al., 2008). Excreted bile acids vary with diet and gut flora, potentially providing information about the emitters health and vigor (Chiang, 2004). Determining the biological relevance of these and other verified natural steroid ligands has proven difficult, perhaps because the information in these cues is used for subtle distinctions that are difficult to quantify in laboratory behavioral assays.

The full complement of steroids expressed by prominent model organisms and during fundamental physiological states (age, sex, etc.) is far from complete, but it is becoming apparent that the blend of steroids excreted by an individual provides the

outside world with a readout of its internal biological state (e.g., a potential “honest” signal). Decoding state and/or identity from complex ligand blends is an intrinsic challenge for all chemosensory sensory pathways. At this time, the computational strategies used by steroid-detecting chemosensory pathways remain largely unexplored. At a fundamental level, however, the complexity of steroid chemosensation suggests that there is no universal rule governing the relationship between a particular steroid cue and (1) the receptor class involved in its detection, (2) the pathway into the brain it activates, or (3) the behavioral response. Unraveling the logic of steroid chemosensation represents a major challenge in neuroscience, and answering the many outstanding questions will fill major gaps in our understanding of vertebrate biology.

Outlook

Steroid detection by olfactory systems is an important aspect of vertebrate biology and supports the survival and reproduction of species from early diverged fishes to mammals. Decades of research have begun to unravel the roles that steroids play in olfactory-mediated behavior, but more research is needed to understand the molecular mechanisms underlying steroid detection, the logic of steroid-decoding neural circuits, and the complement of steroid-mediated behaviors. The growing evidence that mammalian genomes contain multiple chemosensory steroid receptors raises interesting questions about their potential for modulating mammalian physiology. Understanding the “how” and the “why” of olfactory steroid detection will add new insights into brain evolution, and doing so will improve our capacity to understand how the brain uses environmental cues to guide behavior.

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