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SYMPOSIUM

Chemical Communication in Lizards and a Potential Role for Vasotocin in Modulating Social Interactions

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Synopsis Lizards use chemical communication to mediate many reproductive, competitive, and social behaviors, but the neuroendocrine mechanisms underlying chemical communication in lizards are not well understood and understudied. By implementing a neuroendocrine approach to the study of chemical communication in reptiles, we can address a major gap in our knowledge of the evolutionary mechanisms shaping chemical communication in vertebrates. The neuropeptide arginine vasotocin (AVT) and its mammalian homolog vasopressin are responsible for a broad spectrum of diversity in competitive and reproductive strategies in many vertebrates, mediating social behavior through the chemosensory modality. In this review, we posit that, though limited, the available data on AVT-mediated chemical communication in lizards reveal intriguing patterns that suggest AVT plays a more prominent role in lizard chemosensory behavior than previously appreciated. We argue that these results warrant more research into the mechanisms used by AVT to modify the performance of chemosensory behavior and responses to conspecific chemical signals. We first provide a broad overview of the known social functions of chemical signals in lizards, the glandular sources of chemical signal production in lizards (e.g., epidermal secretory glands), and the chemosensory detection methods and mechanisms used by lizards. Then, we review the locations of vasotocinergic populations and neuronal projections in lizard brains, as well as sites of peripheral receptors for AVT in lizards. Finally, we end with a case study in green anoles (*Anolis carolinensis*), discussing findings from recently published work on the impact of AVT in adult males on chemosensory communication during social interactions, adding new data from a similar study in which we tested the impact of AVT on chemosensory behavior of adult females. We offer concluding remarks on addressing several fundamental questions regarding the role of AVT in chemosensory communication and social behavior in lizards.

Introduction

Chemical communication is the most ancient and taxonomically widespread form of communication in animals (reviewed in Steiger et al. 2011; Wyatt 2014). Reptiles, like many vertebrates (Wyatt 2014), use chemical signals to mediate competitive and reproductive social behavior, including territoriality and courtship (Madison 1977; Halpern 1992; Mason 1992; Houck 2009; Mason and Parker 2010; Martín and López 2011). As in other vertebrates, neuroendocrine systems regulate many aspects of these behaviors in reptiles. However, our understanding of the neuroendocrine mechanisms underlying the production of, and behavioral responses to, social signals in reptiles is limited and relies heavily on studies in other sensory systems (i.e., visual) or chemical communication in non-reptilian animal models (i.e., mammals and fish; Stoka 1999).

This lack of research on neuroendocrine modulation of chemosensory behavior in reptiles represents a major gap in our knowledge of the evolutionary mechanisms shaping chemical communication. By implementing a neuroendocrine approach to the

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study of chemical communication in reptiles, we can address this gap. Sinervo and Miles (2011) argued that the neurohormone arginine vasotocin (AVT) and its receptors (along with prolactin) are the most likely evolutionary mechanisms responsible for the broad spectrum of diversity in strategies for reproductive, competitive, and social behavior. Here, we focus on AVT in reptiles (Wilczynski et al. 2017), reviewing the available literature on the relationship between AVT and chemical communication in lizards. Integrating these two bodies of research will offer evolutionary insights into the mechanisms used by AVT to regulate social behavior.

Neuroendocrine signals likely influence chemosensory communication by modifying the motivation for chemical detection, the sensitivity of chemosensory receptors, the neural pathways activated by chemosensory stimuli, or the behavioral output of sensory processing (Goodson and Bass 2001). The present review aims to build upon previous work by synthesizing the available evidence for the role of AVT in modulating intraspecific chemical communication among squamates, with a particular emphasis on lizards. The historic and ongoing male bias in behavioral neuroscience research, particularly in lizards, reinforces the need for more studies in females (Beery and Zucker 2011). In an effort to balance out this sex bias in AVT-modulated social behavior research in lizards, we present data from a case study of the behavioral effects of exogenous AVT in female green anoles (*Anolis carolinensis*), framing new data in the context of previously published data.

The specific site or sites that AVT acts to modulate sensorimotor responses to olfactory or vomerolfactory stimuli are still unknown in lizards (Meylan et al. 2017). In many mammals, arginine vasopressin (AVP) modulates social odor processing within the olfactory bulb and anterior olfactory nucleus (Wacker et al. 2011; Wacker and Ludwig 2019). AVT is also involved in social (including chemical) communication modulation of non-mammalian taxa (Rose and Moore 2002; Wacker et al. 2011; Albers 2012; Wacker and Ludwig 2019). Dense AVT projections from forebrain to motor and other hindbrain regions may influence social approach behaviors via evolutionarily conserved autonomic regulatory mechanisms, as has been suggested for goldfish and other vertebrates (Thompson and Walton 2009). In cichlids, AVT concentrations are at their highest within the olfactory bulbs relative to all other brain regions (Almeida et al. 2012), suggesting a critical role for AVT in olfaction across many vertebrates. AVT also plays an important role in reptilian social communication (Woolley et al. 2004; Wilczynski et al. 2017). We posit that AVT also plays a modulatory role in chemosensory communication among reptiles.

Chemical signaling in lizards

Chemical communication is widely used among squamates, such that pheromones are important for snake reproduction and many lizard taxa have specialized chemical producing scent glands (Mason 1992; Houck 2009). Many in-depth reviews detail chemical communication in reptiles (Madison 1977; Halpern 1992; Mason 1992; Houck 2009; Mason and Parker 2010; Martín and López 2011; Sinervo and Miles 2011) and, more broadly, the evolution of vertebrate chemosensory systems (Bertmar 1981; Eisthen 1997; Baxi et al. 2006; Dehara et al. 2012; Vandewege et al. 2016; García-Roa et al. 2017; Baeckens et al. 2017b; Baldwin and Ko 2020). Therefore, we will instead briefly review the possible social functions of chemical signaling, followed by methods of chemical signal production and detection.

Chemical sources

Chemical information may also be transmitted from saliva, skin, and the cloaca. Lizards have multiple types of follicular epidermal glands, including femoral (Cole 1966; García-Roa et al. 2017), precloacal (Escobar et al. 2001; Pincheira-Donoso et al. 2008), and cloacal glands, similar to some snake species (Trauth et al. 1987; Siegel et al. 2014). Some lizard (Mason and Gutzke 1990; Whiting et al. 2009) and snake species (Shine et al. 2002) also use skin-derived chemical signals. For example, female snakes actively eject epidermal lipids to solicit male courtship using a suite of behaviors including scale movements, skin stretching, and hyperventilation (Garstka and Crews 1981). Fecal and uric acid deposits from the cloaca also function as sources of chemical information, similar to chemical signaling via urine in fish (Laberge and Hara 2001; Almeida et al. 2012), arthropods, and some mammals (Wyatt 2014). Although this review primarily focuses on lizard taxa, it should be noted that other types of scent glands used in communication are present in reptilian taxa, such as the mandibular glands in alligators (reviewed in Mason and Parker 2010), mental glands in tortoises (Kelley et al. 2021), and inguinal, axial, and Rathke's glands in turtles (Bezerra et al. 2020).

Many environmental and behavioral factors have been linked to species' investment in chemical signal production and composition. *Sceloporus* lizard

species evolving in an arboreal context have significantly fewer femoral pores relative to terrestrial species (Ossip-Klein et al. 2013), suggesting habitat is an important factor that shapes species' reliance on chemical signal production. In addition, the transition to sociality (social groups comprised adults and juveniles) in lizard taxa was facilitated by the presence of epidermal scent glands according to a phylogenetic comparative study of 911 lizard species (Baeckens and Whiting 2021). Chemical signal composition is shaped by environmental conditions, such as temperature (Campos et al. 2020a) or humidity (Baeckens et al. 2018), and potentially predator presence (Donihue et al. 2020). Also, chemical signal composition can alter receiver responses (Romero-Diaz et al. 2021). Neither the intensity of sexual selection (Baeckens et al. 2017c) nor the breadth of diet was associated with chemical signal composition in at least one lizard family, Lacertidae (Baeckens et al. 2017a), but species that ate only arthropods produced a lower total number of different compounds.

Endocrine signals likely impact chemosensory communication in squamates by influencing the frequency or amount of chemical signal deposits, or related behaviors that increase the scent output, such as locomotion. For example, androgens including testosterone and dihydrotestosterone both activate and increase the production of femoral gland secretions in lizards (Fergusson et al. 1985; Hews and Moore 1995). Similar links between androgen activity in scent glands and chemical signal output have been found in birds (Whittaker et al. 2018). Alternatively, the production level of sex pheromones by female garter snakes (*Thamnophis sirtalis*) is estrogen-dependent, with high-estrogen females having greater pheromone production and also soliciting higher rates of courtship from males relative to low-estrogen females (Crews 1976). The pheromone mixture is released from the dorsal skin of a female when elevated estrogen levels in the bloodstream are detected by estrogen receptors in the liver, boosting pheromone production in the liver (Garstka and Crews 1981). Reptiles also have AVT receptors in the liver (Bradshaw and Rice 1981). Although some lizard studies have described correlated changes in AVT (exogenous administration or vasotocinergic neural activity) and testosterone levels (Hillsman et al. 2007; Kabelik et al. 2008; Houck 2009), no direct link between AVT and chemical signal production has yet been identified.

Chemical detection

Many animals, including squamates (lizards and snakes), have a secondary chemosensory processing organ called the vomeronasal organ (VNO), in addition to volatile chemical detection with olfactory receptors in olfactory epithelium. For example, the vomeronasal system is required for male garter snakes to detect and perform courtship in response to female sex pheromones (Noble 1937; Kubie et al. 1978). Squamates engage in tongue flicking, using the tongue to collect volatile compounds from the air. Lizards may lick, or touch the tongue to, some substrate or conspecific to collect compounds from a surface, or may open the mouth to draw in air and presumably odorants (lip smacks; Campos et al. 2020b). Finally, a gular pump occurs when the mouth is closed and the buccal floor is inflated (Owerkowicz et al. 1999) and is correlated with interest in conspecific glandular secretions in alligators (Johnsen and Wellington 1982). The tongue paired with the VNO operates as a chemical delivery system in squamates (Filoramo and Schwenk 2009), with the tongue collecting molecules from the surrounding environment, delivering those chemicals to a pair of ducts within the dorsal oral palette, which lead to the VNO. One proposed mechanism for chemical delivery to the VNO fenestrae is via a hydraulic mechanism by which the tongue tip acts as a piston, forcefully pumping a mixture of chemicals with sublingual gland secretions into the dorsal ducts toward VNO receptors (Filoramo and Schwenk 2009). Squamates, with and without bifurcated tongues, sample chemicals in this way (Filoramo and Schwenk 2009; Baeckens et al. 2017b). Attempts to label the vomerolfactory and olfactory sensory systems as specialized for the detection of pheromonal versus generalized odorants, or water-borne versus airborne odorants, largely ignore data that directly contradict these hypotheses (Eisthen 1997; Baxi et al. 2006). While the two systems may indeed serve distinct functions, the precise differences in compound type specialization are still debated.

Comparative work in Lacertidae lizard species suggests that species' degree of investment in chemical signaling, rather than foraging behavior, could drive diversity in vomeronasal-lingual morphology (Baeckens et al. 2017b). Similar to the aforementioned link between arboreality and reduced chemical communication investment in lizards, extant arboreal lizards also have smaller and less well developed VNOs than do reptiles that are ground dwelling (Bertmar 1981). For the VNO to be functional in terrestrial squamates, aqueous secretions must be produced in and near the VNO since its receptors only work in the aqueous phase, a vestigial property from when VNOs evolved in fish ancestors (Bertmar 1981). With the exception of our recent findings on the impacts of vasotocin on chemosensory behavior

in *A. carolinensis* males (discussed below in Concluding remarks and remaining questions section), there have been no other findings, of which we are aware, to establish a role for neuroendocrine regulation of chemical detection behavior in lizards.

Chemical signal function

Despite the diversity of lizard social systems, this diversity remains largely underutilized in addressing questions underlying the neuroendocrine mechanisms of social behavior. This is in part due to the misleading perception that lizards are non-social (Doody et al. 2013), but there is abundant evidence supporting lizard social communication via chemical signals (Houck 2009; Mason and Parker 2010). For example, odor cues are necessary for recognizing familiar individuals in Iberian wall lizards (*Podarcis hispanica*; López and Martín 2002). The gregarious lizard species *Egernia stokesii* can differentiate between members of its own social group and non-group individuals, responding with increased chemosensory interest and time spent near the odors of lizards from a different group (Bull et al. 2000). Iberian rock lizards (*Lacerta monticola*) can differentiate between familiar and unfamiliar conspecifics using chemical cues (Aragón et al. 2001). Likewise, common wall lizards (*Podarcis muralis*) and Iberian rock lizards (*Iberolacerta cyreni*) behaviorally distinguish between their own secretions and other individual's secretions (Mangiacotti et al. 2019, 2020). Even juvenile common lizards (*Lacerta vivipara*) recognize the odors of familiar siblings and their mothers, despite this species having no maternal care, suggesting a chemosensory role in lizard kin recognition (Léna et al. 1998). Chemical signals that convey female reproductive status have been described in lizards (Cooper and Pèrez-Mellado 2002) and, in multiple species of sea snakes (*Laticauda*), males perform chin presses in response to female skin lipids (Shine et al. 2002), suggesting some squamates rely on chemical communication for reproduction.

Lizards can also determine health (Martín et al. 2007a), age (López et al. 2003; Martín and López 2006; Nisa Ramiro et al. 2019), competitive ability (Martín and López 2007), and social status (Martín et al. 2007b) based on conspecific chemical signals. Territorial space use patterns are modified in response to male femoral gland secretions (Campos et al. 2017), and this territorial function for scent gland signals is also broadly found in insects, fish, birds, and mammals (Wyatt 2014). At least one lizard species has been shown to preferentially deposit fecal pellets on the largest available rock in its territory (Baeckens et al. 2019), presumably advertising ownership of the surrounding space.

Social information in chemical signals is likely conveyed by the presence and proportions of different compounds (Wyatt 2014), given that receiver behavior depends on signal composition (Romero-Diaz et al. 2020). For example, in male *Acanthodactylus boskianus* lizards, cholesterol and alcohol compounds derived from femoral gland secretions elicited increased receiver avoidance and aggressive behavior (Khannoon et al. 2011). In addition, signal composition is species-specific (Gabirot et al. 2010a, 2010b; Campos et al. 2020a), and is associated with phenotypic qualities of signalers (Martín and López 2007; Martín et al. 2007a, 2007b; Campos 2018; Campos et al. 2020a). For example, male rock lizards (*L. monticola*) that are larger-bodied, which is associated with a better fighting ability, also produce femoral gland secretions with higher cholesterol levels (Martín and López 2007). Minor modifications in chemical abundances can impact chemosensory interest of receivers and single compounds can elicit tongue flick rates equivalent to those elicited by the whole chemical signal (Romero-Diaz et al. 2021). Thus, lizards routinely use chemical signaling as a regular part of their social behavior repertoire.

Vasotocin physiology: central and peripheral functions

Vertebrate social behaviors are modulated by AVT with regional specificity, acting within a number of different brain regions, on sensory and motor pathways with temporal variation and at multiple levels in a behavioral sequence. AVT modulates the responsiveness of neurons to behaviorally relevant sensory stimuli and also modifies the behavioral output (Rose and Moore 2002). Social behavior has been evolving alongside the vasotocin-like family of peptides (Moore 1992; Acher and Chauvet 1995; Moore and Lowry 1998; Hoyle 2011; Baran 2017; Wilczynski et al. 2017). Integrative and comparative research on the distribution of AVT/AVP neurons suggest that the neuroanatomy of the AVT/AVP system, along with the mesotocin (MT)/oxytocin (OT)/isotocin (IT) system (Song and Albers 2018), is conserved among vertebrates (Moore and Lowry 1998; Goodson and Bass 2001; Goodson and Kabelik 2009; Albers 2015). For example, AVT/AVP somas have been found in the preoptic area (POA), bed nucleus of the stria terminalis (BNST), and suprachiasmatic nucleus, all brain regions for which there is abundant evidence for vertebrate homology.

Therefore, it is likely that the AVT/AVP cells within these regions originated from common ancestral AVT cell groups and diverged as the functional needs of each species shifted over evolutionary time (Moore and Lowry 1998) along with shifts in brain shape and volume in squamates (Macrì et al. 2019). Here, we discuss the functional behavioral significance of central AVT cell populations and peripheral targets in lizards with an evolutionarily comparative lens.

Functional significance of central AVT

AVT is produced in the hypothalamus (HYP), similar to MT, being synthesized in neurons within the POA, supraoptic nuclei (SON), paraventricular nuclei (PVN), and ventromedial nuclei for Lacertidae lizards and at least one Gekkonidae species (Bons 1983). The most conspicuous brain regions with AVT neurons in lizards include the PVN, SON (Barka-Dahane et al. 2010), and POA (Bons 1983; Wilczynski et al. 2017). MT-producing neurons are also located in the PVN and SON in lizards (Thepen et al. 1987; Kabelik and Magruder 2014). AVT neurons have been identified in periventricular nuclei and in scattered neurosecretory cells of the HYP in the lizard *Uromastix acanthinurus* (Barka-Dahane et al. 2010). In snakes, both magnocellular and parvocellular AVT cell bodies have been identified in the PVN, whereas only magnocellular AVT cell bodies have been found in the SON (Silveira et al. 1992). Some lizards also have AVT cell bodies in the BNST (Stoll and Voorn 1985; Thepen et al. 1987; Hillsman et al. 2007; Kabelik et al. 2013), the hindbrain (Stoll and Voorn 1985; Thepen et al. 1987), and the medial septum (Propper et al. 1992b).

AVT fibers have been identified throughout the brain (Wilczynski et al. 2017), with especially prominent fibers passing through the olfactory bulb (Stoll and Voorn 1985; Thepen et al. 1987; Propper et al. 1992b; Kabelik et al. 2008; Wilczynski et al. 2017). AVT fibers extend rostrally through the olfactory bulb in three species of desert lizards, but not in a temperate species (Bons 1983). Other lizard brain regions with prominent AVT fibers include the lateral septum (LS), dorsal cortex, nucleus accumbens (NAcc), medial amygdala (AMY; MA), periaquaductal gray (PAG), and locus coeruleus (Stoll and Voorn 1985; Thepen et al. 1987; Propper et al. 1992b; Kabelik et al. 2008; Wilczynski et al. 2017). In snakes, AVT fibers have been found in the NAcc and lamina terminalis, with a dense AVT fiber network in the external zone of the median eminence (ME) near the hypophysial portal system (Silveira et al. 1992).

The AMY is one region of interest that needs more targeted studies on AVT and chemical communication in lizards (see Fig. 1 for summary of possible impacts of central AVT on lizard chemical communication). The AMY is involved in both reproductive and aggressive behavior of lizards (Greenberg et al. 1984b), and AVT fibers have been identified within centrally-projecting AMY fibers in *Urosaurus ornatus* tree lizards (Kabelik et al. 2008). The MA integrates olfactory or vomerolfactory sensory information (or both) with the endocrine and autonomic systems, receiving input along with the olfactory cortex from both the main and accessory olfactory bulbs (AOBs; Abellán et al. 2013). Olfactory information is then relayed from the medial extended AMY, where vasotocinergic cell groups are sexually dimorphic, to the medial HYP (Martínez-García et al. 2008). This suggests the MA may be an important processing center for chemical signals in lizards.

In mammal and avian taxa, OXT/MT has prosocial effects centrally and is involved in modulation of stress and fear with mostly anxiolytic effects (Neumann 2008). While a broad body of literature can be found on the role of OT in mammalian social behavior, far less research has been conducted on the role of MT in reptilian social behavior, centrally. One exception is the Kabelik and Magruder (2014) study, which reported a positive correlation between MT cell activity (based on MT colocalization with the immediate early gene Fos, a marker for neural activation) and male courtship displays, but not aggressive displays, in brown anoles, implicating MT in courtship behavior of lizards. No studies have examined the social effects of MT in female lizards to date. However, in one species of live-bearing snakes that display maternal care (Pygmy rattlesnakes, *Sistrurus miliarius*), blocking the VT1a receptors, which bind AVT and MT, in mothers eliminated their spatial aggregation behavior with their neonates (Lind et al. 2017).

Sex differences in vasotocinergic projections may contribute to observed sex differences in chemosensory behavior during social encounters in squamates. Sexual dimorphism in the AVT/AVP system is well documented (De Vries and Panzica 2006) and taxonomically widespread (Moore and Lowry 1998), such that the evolutionary maintenance of sexual differentiation in the AVT/AVP system is likely fundamental to functional differences in social and reproductive behavior (De Vries and Panzica 2006). These differences often manifest as males having more cells and

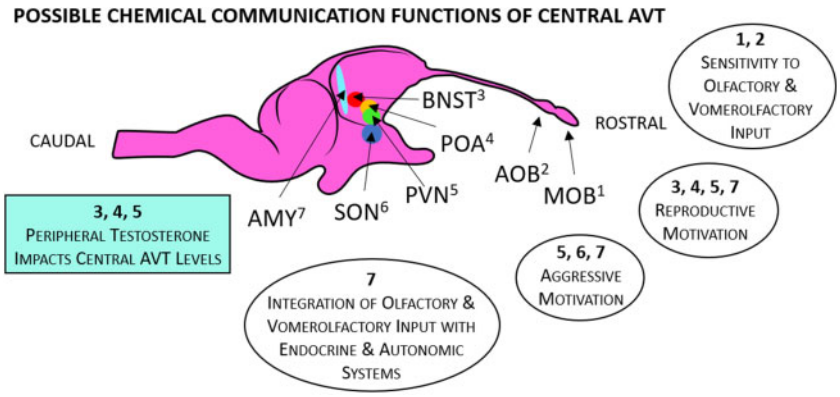

Fig. 1. Illustration of the possible impacts of central AVT on chemical communication in lizards. Arrows point to lizard brain regions that are each labeled with a superscript number. These numbers correspond to the physiological or behavioral effects listed inside ovals or influenced by peripheral physiology in the shaded rectangle. While the lizard brain is modeled after an Anolis brain, the brain regions and effects of AVT are more broadly representative of all lizards and were identified using the Hoops et al. (2018) lizard brain atlas. MOB, main olfactory bulb (encompasses several species-specific nuclei).

denser neural projections relative to females (De Vries and Panzica 2006), although females may have more AVT cells within specific brain regions (Wilczynski et al. 2017). For example, male geckos (*Gekko gecko*) have a greater density of vasotocinergic innervation in the LS, ventrocaudal telencephalon (nucleus sphericus), and the PAG relative to females (Stoll and Voorn 1985). Male tree lizards have greater densities of centrally projecting AVT fibers within the limbic system relative to females (Kabelik et al. 2008) and male whiptails have higher AVT fiber densities in the POA than do females (Hillsman et al. 2007).

Central AVT mediates social behavior in both sex-dependent (Albers 2015) and context-dependent ways. For example, red-sided garter snakes exhibit seasonal variation in AVT cell numbers, where females have significantly higher number of AVT cells in the HYP and males have more AVT cells in the BNST during the spring breeding season compared to fall (Lucas et al. 2017). Both sexes have a higher number of AVT cells in the SON during the fall than in the spring. in brown anoles, activation of AVT neurons (based on colocalization with Fos) within the SON occurred nonspecifically with participation in either aggressive or sexual encounters, whereas activation of AVT neurons in the POA and BNST was associated with engagement in sexual behaviors (Kabelik et al. 2013). The density of AVT fibers in some brain regions also differs between breeding and nonbreeding conditions in anoles (Kabelik et al. 2008), whereas the number of region-specific AVT cells can depend on circulating levels of other sex hormones, such as testosterone (Hillsman et al. 2007).

Both circulating levels and neuronal activity of AVT in the brain change with levels of other hormones, such as testosterone. For example, exogenous AVT led to a significant reduction in circulating testosterone in male *Zootoca vivipara* lizards (Meylan et al. 2017) and an increase in AVT expression in the brain in both unisexual and sexual whiptails, with male whiptails expressing higher AVT immunoreactivity (AVT-ir) in regions that influence rates of courtship and copulation (Hillsman et al. 2007). Testosterone also regulates AVT-ir in brain regions of the limbic system. For example, testosterone influences AVT-ir within the BNST and in peripherally projecting clusters of cells, as well as the size of AVT somas in the PVN (Kabelik et al. 2008).

Despite testosterone being linked to both aggression and AVT expression, evidence linking AVT directly to aggression is conflicting in lizards. Vasotocinergic neurons in the PVN and SON of the brown anole (*Anolis sagrei*) are activated during aggressive encounters (Kabelik et al. 2013), but at least one study found no correlation between AVT expression in the brain and aggression levels in *U. ornatus* lizards (Kabelik et al. 2008). In addition, peripheral injections of AVT in green anoles reduced aggressive push up displays performed by adult males only when males were presented with a mirror stimulus (perceived competitor) and not when given a live stimulus (Dunham and Wilczynski 2014). In male contests, green anoles match the aggression level and tactics of their opponents (Jenssen et al. 2005), which may help explain why males, that presumably matched levels of aggression, displayed similar rates of aggression when interacting with live stimuli. There is considerable debate surrounding whether peripheral AVT crosses the blood–brain barrier in squamates, thus whether these effects were due to central actions of AVT is unclear. However, there is evidence that peripheral actions of AVT may

indirectly affect behavior, which we will review below.

Functional significance of peripheral vasotocin

AVT serves numerous physiological functions in the periphery of squamates that may indirectly influence social behavior (see Fig. 2 for summary of possible impacts of peripheral AVT on lizard chemical communication). Hypernatremia triggers an AVT release, increasing circulating levels of AVT in the blood in lizards and snakes (Ford 2005; Ladyman et al. 2006). AVT is elevated in the bloodstream when lizards are dehydrated, causing oliguria (Ford 2005), and when plasma osmolality increases (Rice 1982). AVT is released by the pars nervosa into the blood stream, reaches AVT receptors in the liver and kidneys of reptiles, and plays a major role as an antidiuretic (Bradshaw and Rice 1981). AVT's antidiuretic function has important implications for chemical communication since it impacts urine storage in vertebrates and urine is used in scent marking. Other peripheral regions with AVT receptors include the cloaca, colon, and cephalic salt-secreting glands, where transmural fluxes of water and electrolytes could be influenced by AVT (Bradshaw and Bradshaw 2002). Whether AVT receptors are present in the skin or exocrine glands of reptiles is not known, although such receptors have been identified in the skin of some amphibians (Kohno et al. 2003; Boyd 2006). In female lizards, AVT is also involved in stimulating oviposition, parturition, and uterine contractions of some species (Guillette 1979; Guillette and Jones 1982; Atkins et al. 2006), but not others (Propper et al. 1992a). In the periphery, OXT/MT are also involved in smooth muscle contraction and oviposition across vertebrates, although AVT is reportedly a more potent stimulator of oviposition in lizards (Guillette and Jones 1982). By influencing these basic physiological processes in lizards, AVT is involved in shifting the motivational state of individuals between essential and social activities (Meylan et al. 2017).

AVT has been linked to both basking behavior and preferred body temperature in lizards and snakes (Ladyman et al. 2006). The impact of AVT on preferred body temperature in lizards is species-specific, such that AVT decreased preferred body temperature in agamids (*Ctenophorus ornatus*; Bradshaw et al. 2007), but increased it in male common lizards (*Z. vivipara*; Meylan et al. 2017). Exposure of *Lacerta muralis* to cold temperatures (4°C) for only 75 min led to AVT accumulation in the ME, suggesting AVT is primed for release into the bloodstream (Bons 1983). The same cold exposure also led to an accumulation of MT in the internal zone of the ME and in adenohypophyseal cells. Through behavioral thermoregulation, by modifying basking and microhabitat use, lizards can compensate for temperature variation (Adolph 1990). Lizards must balance their time during the active period between essential and social activities and AVT may be involved in mediating this trade-off (Huey and Slatkin 1976; Kearney et al. 2009). For example, peripheral administration of AVT increased time spent basking and inhibited social interactions of male common lizards (*Z. vivipara*; Meylan et al. 2017). Conversely, in least one lizard species (*Pseudemoia entrecasteauxii*), males with higher preferred body temperatures are also more socially active, having higher rates of aggression and courtship (Stapley 2006).

AVT and lizard chemosensory behavior

Aside from our data in green anoles, which we will expand upon below, direct evidence for a role of AVT in chemosensory communication in lizards comes from one study in *Z. vivipara* (Meylan et al. 2017). Following a single intraperitoneal (I.P.) injection of AVT (AVT-males) orPBS (control males) in adult males, small AVT-males avoided areas scent marked with conspecific odors (3 days after injection) more than did small control males, whereas larger males did not differ in time spent near conspecific odors. These results suggest that the impact of AVT on chemosensory behavior is context dependent. AVT-males also had significantly lower plasma testosterone levels on Day 6 post-injection, spent more time basking on Day 2 post-injection, and had higher endurance compared to control males, suggesting the impact of AVT on chemosensory social behavior may be partially mediated by correlated changes in androgens or shifts in thermoregulatory behavior.

The impact of AVT on chemosensory behavior in green anoles

Green anoles (*A. carolinensis*) have been a prominent animal model of behavioral neuroendocrinology for over a century (Monks 1881) due to their neuroendocrine regulation of vibrant visual displays (Greenberg 1977; Crews 1980; Greenberg et al. 1984a; Wade 2011). This long history of using green anoles to study behavioral neuroendocrinology is evident in the wide range of topics investigated, including the neuroendocrine correlates of behaviors involved in aggression, reproduction, locomotion, thermoregulation, and oviposition. However,

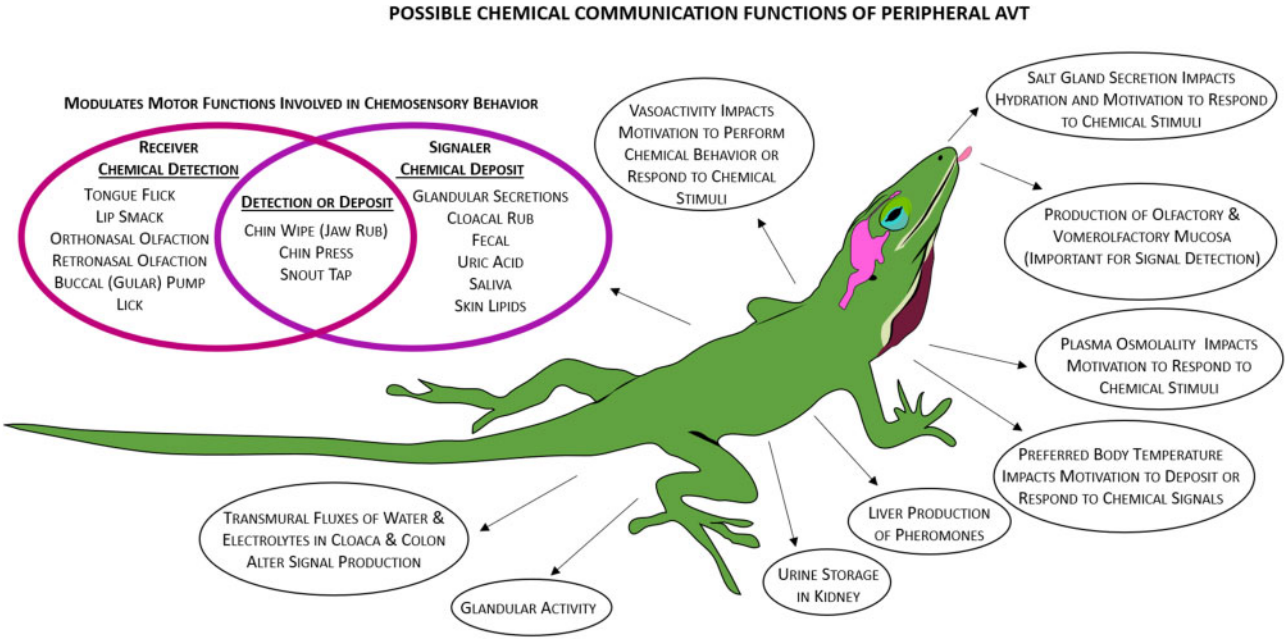

Fig. 2. Possible impacts of peripheral AVT on chemical communication in lizards. Many of the peripheral effects of AVT on behavior may occur indirectly and in connection with the central nervous system by influencing lizard motivation to respond to chemical stimuli or to deposit chemical signals. Black ovals identify the known peripheral functions of AVT in lizards and may also give the possible impact on chemical communication. The colored ovals in the upper left list behaviors that are or may be involved in chemical detection (pink), behavior or chemical sources used in depositing chemical signals (purple), or both (overlap). See text for details.

chemical communication in anoles has been largely ignored and overshadowed by visual communication research on due to the perception of anoles as a microsmatic lizard (Armstrong et al. 1953).

The microsmatic reputation of anoles is not completely unwarranted since anoles have highly reduced olfactory structures, including the main and AOBs (Armstrong et al. 1953; Greenberg 1982). Anoles also perform low rates of spontaneous tongue flick behavior during undisturbed behavioral trials (Gravelle and Simon 1980; Greenberg 1993). Unlike actively foraging lizards, which rely heavily on chemosensory detection behavior to locate prey (Cooper 1995), green anoles are sit-and-wait foragers and do not increase tongue flick rates in response to prey odors (Cooper 1989; Cooper and DePerno 1994).

Since green anoles do not rely heavily on chemosensory behavior for foraging or to detect predators, tongue flicking could instead function primarily to detect social information (see Cooper 1994). While evidence for anole use of the chemosensory modality to process social odors is scarce, the few studies that are available do indicate that chemical social information is detectable and alters receiver behavior. Results from this handful of studies warrant further investigation into the role of social chemicals in anoles. For example, in at least one species (*A. sagrei*, brown anoles), males perform more chemical displays and move around their environment more in response to female odor cues (Baeckens et al. 2016). This evidence suggests males can detect and change their activity patterns in response to female odor cues. Furthermore, female green anoles can differentiate males with experimentally elevated levels of peripheral AVT from males treated with saline (Dunham and Wilczynski 2014), despite similar visual display rates by males in both treatments. Thus, it is possible that females are able to detect a chemical difference in AVT-males, or in some other unmeasured subtle behavioral cue.

Anolis carolinensis males housed in dyads for 10 days form stable dominant–subordinate relationships (Greenberg and Crews 1990). This experimental paradigm is a forced social hierarchy characterized by highly agonistic male–male interactions, and behavioral data on free-range green anoles do not support the establishment of dominant–subordinate hierarchies in natural populations (Jenssen et al. 1995). Nonetheless, these intrasexual relationships offer insight into the neuroendocrine mechanisms that underly social behavior by generating exaggerated social phenotypes that can then be applied to more ethologically relevant study designs. Dominant males have a greater number of vasotocinergic cells in the POA relative to subordinate males, which also have fewer cells than isolated males or

males housed with a female (Hattori and Wilczynski 2009). This suggests that males subjected to prolonged social stress undergo a reduction in vasotocinergic cells within the POA, which processes visual information. It also suggests that the dominant phenotype is the default for male green anoles since the number of vasotocinergic cells in dominant males was similar to males housed singly or with a female. A similar regulation of dominant–subordinate phenotypes via levels of neuropeptides within the vasopressin family has been demonstrated in cichlids, with high levels of AVT in the pituitary gland of subordinate fish relative to dominants and higher IT levels in the hindbrain of dominants relative to subordinates (Almeida et al. 2012). Along with our data, these data suggest that AVT serves a role in regulating social stress in subordinate phenotypes, but that the regulation of central and peripheral AVT levels is locally specific across tissues. All vertebrates examined to date share the anatomical and physiological structures of the hypothalamic–pituitary–adrenal (HPA) axis that help regulate stress responses, but there is large temporal, seasonal, individual, and species variation in how this pathway is regulated, contributing to large variation in the activation and functional output of the HPA axis (reviewed in Romero and Gormally 2019).

AVT in male green anoles

Our recent study aimed to determine the direct impact of AVT on chemosensory and related behavior in male–male and male–female interactions of green anoles (Campos et al. 2020b). We kept lizards under temperature, humidity, and light conditions that simulated breeding season months (although the study was technically conducted after peak-breeding season). We gave adult males an I.P. injection of AVT (AVT ~3 ug/body mass g) in a vehicle of saline (reptile ringer's solution, CON), a dose based on previous AVT studies in green anoles (Dunham and Wilczynski 2014) and amphibians (Burmeister et al. 2001; Coddington and Moore 2003). We found that AVT-males were more than three times faster than CON-males to perform a tongue flick toward untreated conspecifics (Fig. 3A) in 30 min trials (Campos et al. 2020b). These data suggest that AVT increased a male's initial interest in the chemical information available during social encounters. We could not determine whether a live stimulus is necessary for this increased interest in chemical information. Thus, whether males respond similarly to isolated social odor cues remains to be addressed.

Interestingly, AVT in the male signaler also impacts the behavioral responses of social partners toward AVT-males, such that social partners increase rates of some chemical sampling behaviors and decrease locomotion rates (Campos et al. 2020b). Here, we use the term "social partner" to refer to any conspecific that interacts with the focal individual, but in the Campos et al. (2020b) study, these untreated social partners were referred to as "Intruders." Male social partners increased tongue flick (Fig. 3B) and lip smack behavior when interacting with AVT-males compared to CON-males. Social partners of both sexes that interacted with an AVT-male were less active, performing fewer movements or locomotive behaviors than social partners of CON-males (Fig. 3C). Despite no measurable differences in the visual or chemosensory display rates of signaler males, untreated social partners still responded differently to AVT-males relative to CON-males. Thus, AVT could impact signaler behavior in more subtle ways that we did not measure in this study.

Contrary to our prediction, we found no significant differences in the rates of tongue flick behavior performed by female social partners of AVT-males versus CON-males (Fig. 3D) that could have explained the increase in push up displays toward AVT-males in the previously published study by Dunham and Wilczynski (2014). In contrast to this study, we did not find any differences in visual displays performed by untreated females toward AVT- versus CON-males. Although females perform many of the same social displays as males, it is generally assumed that push up displays are a form of solicitation when performed by a female toward a male, courtship when performed by a male toward a female, but agonistic when performed by a male toward a male (e.g., Dunham and Wilczynski 2014). Furthermore, our study was not conducted within peak breeding season, which may have led to motivational differences in untreated females despite housing animals in environmental conditions that simulated conditions during peak breeding months.

AVT in female green anoles

Similar to males, female green anoles will form dominate–subordinate relationships in captivity, such that dominant females perform higher frequencies of assertion displays, challenge displays, attacks, and bites relative to subordinates (Summers and Andrews 1996). Female social status does not affect perch site selection, body color, or prey capture success and latency, as has been shown for males. Dominant females did respond to male courtship

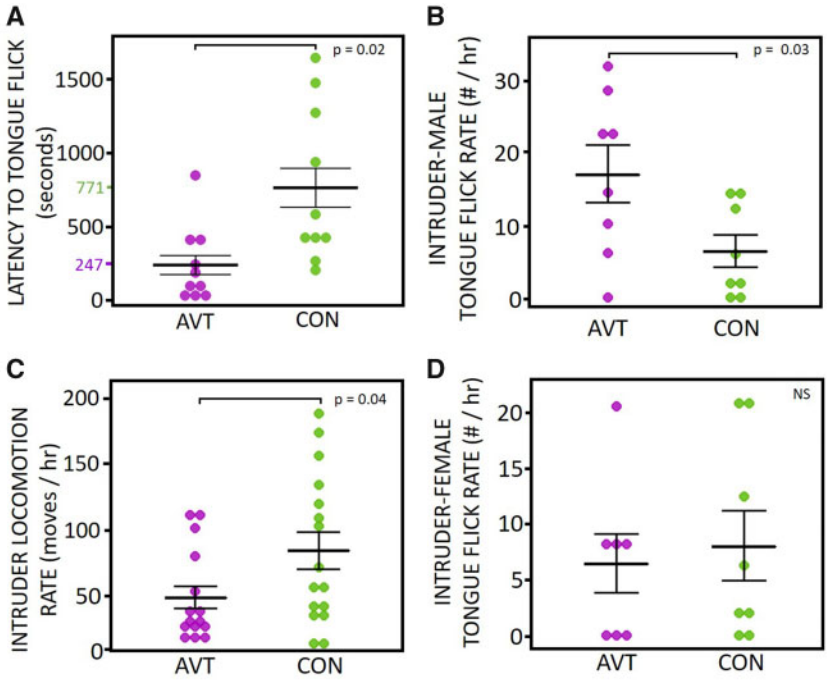

Fig. 3. Impacts of AVT on behavior of *A. carolinensis* males and on the behavior of untreated social partners ("Intruders") from Campos et al. (2020b). Figure modified and reproduced with permission. (**A**) Latency to perform an initial tongue flick in treatment males. Of males that performed at least one tongue flick in response to a social partner, males that received a peripheral AVT injection were over three times faster to do so than control injected males (CON) (mean of AVT in purple and CON in green). (**B–D**) Behavior of untreated social partners in response to AVT-males versus CON-males. (**B**) Untreated social partners moved less when paired with AVT-males than did those paired with CON-males. (**C**) Untreated males also performed higher rates of tongue flick behavior in response to AVT-males than to CON-males (**D**) but untreated females did not differ in tongue flick rates.

displays by performing their own displays significantly more often than subordinate females. When female dominance is coerced by placing females in social groups that compete for a single male mate, reproductively subordinate females have elevated serotonergic activity in the brainstem and some females exhibit recrudescing ovaries. Reproductively dominant females have elevated serotonergic and dopaminergic activation in the telencephalon (Summers et al. 1997). Collectively, these data suggest that female social encounters are impacted by neuroendocrine activities and social contexts.

AVT is involved in stimulating physiological functions such as parturition, oviposition, and uterine contractions in female lizards. AVT concentrations within the SON are higher in green anole females with large pre-ovulatory follicles than those with small pre-ovulatory follicles despite similar plasma levels of AVT across different stages of oviposition and estrous (Propper et al. 1992a). Reported mean levels of plasma AVT ranged from AVT 0.35 to 0.50 ng/plasma milliliter in females across different oviposition stages and from AVT 2.5 to 3.3 ng/plasma milliliter in females across different stages of estrous. Thus, AVT clearly plays a role in female reproductive physiology, but its impact on social behavior in green anoles is unknown, and much less known is the impact of either peripheral or central AVT on female chemosensory behavior.

To test whether exogenous AVT altered the chemosensory behavior of female green anoles, we performed a second study using the same methods as in the previously published Campos et al. (2020b) study. Briefly, we gave adult females an I.P. injection of AVT (AVT 15 μg in 100 μL of saline) or saline (100 μL, CON). Each female received both injections with a 7-day period of rest between injections, and we randomized the injection order. We presented 12 resident females with an untreated male stimulus 10 min after the injection and recorded behavior of 30 min interactions. We measured chemical display behavior, which encompassed any behavior that may be involved in the detection or deposit of chemical information including tongue flicks, lip smacks, licks (substrate or conspecific), chin wipes (also called jaw rubs in the literature), fecal deposits, cloacal rubs, gular pumps, and nose taps to substrate. We also separately analyzed rates of behavior and latencies to perform an initial behavior for tongue flicks, lip smacks, locomotion, and visual displays (i.e., push

up bouts). We found no significant differences in the chemosensory display rates of females across AVT and CON treatments in response to untreated males (Fig. 4A) based on paired *t*-tests. Similarly, we found no significant differences between treatments in female rates of or latencies to perform tongue flicks (Fig. 4B), lip smacks, locomotion, or visual displays. This lack of a difference in female social behavior contrasts with our earlier findings in males and may indicate that peripheral AVT is not important for chemosensory behavior in *A. carolinensis* females, that the influence on chemosensory behavior is context or dose-dependent, or AVT impacts other subtle behavior not measured. In addition, we administered AVT peripherally, but it is still possible that AVT administered centrally will result in different behavioral outcomes.

Concluding remarks and remaining questions

The impacts of AVT on chemosensory communication in reptiles are a relatively unexplored frontier in behavioral neuroendocrinology, despite an abundance of evidence supporting distinct social functions for chemical communication and for AVT in lizards. Thus, research spanning across all four of Tinbergen's categories of questions are needed to address this deficit (Tinbergen 1963), including research on the ontogenetic, mechanistic, adaptive, and evolutionary roles of AVT in regulating chemosensory behavior in lizards and, more broadly, in reptiles. Future studies are needed to address several fundamental questions regarding the role of AVT in chemosensory communication and social behavior in lizards.

Foremost among these questions is whether peripheral or central AVT alter the production, deposition, or composition of an individual's chemical signal. In addition, whether peripherally administered AVT can cross into the central nervous system via the blood–brain barrier (Banks et al. 1987; Zlokovic et al. 1990). Peripheral mechanisms may modify the likelihood of a social partner to use chemosensory detection behavior and need to be addressed with pharmacological studies, using agonists and antagonists to assess both peripheral and central impacts of AVT on chemically mediated social behavior. In cichlids, dominant and subordinate males differ in their levels of AVT and IT (the teleost homolog to OT/MT) across different brain regions, and the olfactory bulbs express the highest levels of AVT relative to all other brain regions (Almeida et al. 2012). Whether the outcome of competitive challenges depends on a lizard's level of AVT/MT in the olfactory bulb or in other brain regions still needs to be investigated.

Transgenic manipulations and more targeted transcriptomic studies will help fill in major gaps in our current understanding of the role AVT plays in chemical communication across reptiles. Only within the last 3 years has the revolutionary gene editing technique CRISPR-Cas9 been successfully applied to an *Anolis* species. By directly injecting immature oocytes of *A. sagrei* with Cas9 ribonucleoprotein complex while unfertilized eggs were still inside the female's ovaries, targeted mutations can be transmitted through the germline (Rasys et al. 2019). AVT-knockout and ME-knockout lizards are within reach, and more targeted studies on the role of AVT in chemical communication of reptiles are necessary. Further, future transcriptomic studies on changes

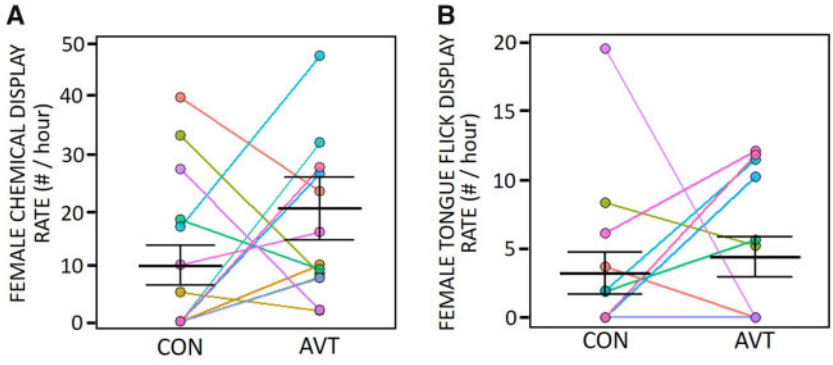

Fig. 4. The impact of exogenous AVT on *A. carolinensis* female rates of (**A**) chemical display behavior and (**B**) tongue flicks in response to untreated male social partners. (**A**) The change in an individual female's (each represented by a different color and line) chemical display rate between AVT and CON treatments is not significantly different from 0, based on a paired *t*-test ($N = 12$, $t = 1.0$, $P = 0.4$). (**B**) Similarly, we found no significant change in female tongue flick rates between AVT and CON treatments ($N = 12$, $t = 0.5$, $P = 0.6$). While this study was conducted with lizards housed under temperature, humidity, and light regimes that simulate peak breeding season, this study took place in months after peak breeding season.

in gene expression within neural tissue, including main and AOBs, in response to the presentation of conspecific chemical signals can provide insight into the potential mechanisms used by AVT to modulate production of and responses to chemical signals. In addition, whether lizards or other reptiles have AVT receptors in the skin or chemical secreting glands would be a worthy area of investigation.

Whether the sex differences in AVT-modulated chemosensory interactions have reproductive or competitive consequences needs further investigation. While our studies in green anoles were conducted in lizards that we housed under conditions that simulated the environmental conditions of peak breeding season, these studies took place in the months after peak breeding season has concluded in wild populations. Thus, studies during peak breeding season when reproductive motivation is high may produce different, more ethologically relevant results for competitive and sexual social encounters. Whether AVT-modulated chemosensory behavior of females is present during breeding season, or varies with reproductive condition, will offer important insight into potential adaptive functions of AVT stimulated chemosensory behavior. Few studies have measured plasma concentrations of AVT in lizards (but see above) and establishing these baseline AVT levels will also help inform the dose-dependent effects of AVT on chemosensory behavior. Similarly, future work must integrate neuroendocrine studies on the effects of plasma AVT and vasotocinergic neuronal activation on competitive and reproductive social interactions with behavioral studies of chemical communication in lizards.

Finally, the role of AVT and related peptides (e.g., MT) in chemically mediated social interactions between lizards is likely species-specific. Future work must investigate neuroendocrine regulation of chemical communication across species that span diverse habitats, environmental conditions, and social structures, or vary widely in their reliance on chemical communication for survival or social reasons. We have only brushed the surface of work to be done on the neuroendocrine regulation of chemical communication in reptiles and the chemical secretions of lizards have much to reveal.

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Conflicts of interest

The authors have no conflicts of interest.

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