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Pheromones enhance somatosensory processing in newt brains through a vasotocin-dependent mechanism

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We tested whether the sex pheromones that stimulate courtship clasping in male roughskin newts do so, at least in part, by amplifying the somatosensory signals that directly trigger the motor pattern associated with clasping and, if so, whether that amplification is dependent on endogenous vasotocin (VT). Female olfactory stimuli increased the number of action potentials recorded in the medulla of males in response to tactile stimulation of the cloaca, which triggers the clasp motor reflex, as well as to tactile stimulation of the snout and hindlimb. That enhancement was blocked by exposing the medulla to a V1a receptor antagonist before pheromone exposure. However, the antagonist did not affect medullary responses to tactile stimuli in the absence of pheromone exposure, suggesting that pheromones amplify somatosensory signals by inducing endogenous VT release. The ability of VT to couple sensory systems together in response to social stimulation could allow this peptide to induce variable behavioural outcomes, depending on the immediate context of the social interaction and thus on the nature of the associated stimuli that are amplified. If widespread in vertebrates, this mechanism could account for some of the behavioural variability associated with this and related peptides both within and across species.

Keywords: sensorimotor; pheromone; vasopressin; medulla; sex; multisensory

1. INTRODUCTION

Social stimuli often evoke stereotypical behavioural responses in conspecifics, potentially by activating endogenous neuropeptide systems, notably those related to vasotocin (VT) and its mammalian derivative, vasopressin (VP). These peptides influence a variety of species-specific social behaviours across vertebrates (reviewed in Goodson & Bass 2001), and social stimuli can activate VT/VP cells and drive release of the peptides in the brains of at least some species. For example, social challenge activates VP cells in the hypothalamus in hamsters and prairie voles (Delville *et al.* 2000; Gobrogge *et al.* 2007) and evokes dendritic VP release within the hypothalamus of some rats (Ebner *et al.* 2005). In hamsters, at least, it may be the odours of conspecifics that are critical for stimulating VP neurons; a V1 antagonist blocks odour-induced flank marking (Ferris *et al.* 1985), suggesting that such cues typically stimulate flank marking by driving endogenous VP release. On the other hand, positive social stimuli, including potential mates and, in gregarious species, same-sex conspecifics, activate VT cells in the medial bed nucleus of the stria terminalis in several avian species (Goodson & Wang 2006). Similarly, some cue received through contact with females probably triggers VP release in male prairie vole brains, as suggested by the ability of a V1a antagonist to block mating-induced pair bond formation (Winslow *et al.* 1993). However, studies in species from additional taxa are necessary to determine whether VT/VP responsiveness to social stimuli is widespread across vertebrates and is thus a conserved feature of these otherwise evolutionarily malleable systems.

There are numerous VT/VP circuits within the brain, so once released, potentially in response to social stimuli, these peptides are capable of influencing behaviour through a variety of mechanisms. They can affect how social stimuli are processed in brain areas that generate stereotypical motor responses (Rose & Moore 2002), affect tendencies to approach or withdraw from social stimuli (Thompson & Walton 2004), influence specific motivational states (Chu *et al.* 1998; Kime *et al.* 2007) and/or modulate reward circuits once social contact is made (Lim & Young 2004). However, it is not yet clear if any or all of these VT/VP behavioural mechanisms are species-specific adaptations to unique social pressures, or if some may be conserved mechanisms that influence processes common to the social repertoires of many species but which could, especially if activated in different social contexts, lead to different behavioural outcomes across species or even among individuals within a species. Clarifying when and how these mechanisms work in the species in which they have already been identified will therefore not only help to predict when the same mechanisms might be operative in other species and thus guide our attempts to determine how widespread each is across vertebrates, but also help us to understand more fully how the diversity of behavioural effects associated with these peptides is generated.

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In roughskin newts, one of the species in which detailed mechanistic studies have been done, VT affects how sensory information is translated into motor output. In this species, VT stimulates amplectic clasping, a stereotypical courtship response in which a male approaches a female, positions himself on her dorsum and then wraps his fore- and hindlimbs around her (Moore & Miller 1983). Thompson & Moore (2000) showed that VT enhances approach responses not only towards the visual cues associated with a potential mate but also towards those associated with food (live worms), although it does not stimulate feeding. This suggests that VT enhances a visuomotor response towards a stimulus feature common to the food source and females, potentially movement. Additionally, VT stimulates medullary neuronal responses to cloacal pressure (Rose *et al.* 1995), a tactile stimulus that elicits the clasp motor reflex in the male's hindlimbs when he presses down on the female. Together, these behavioural and neurophysiological results show that VT can influence processes associated with the direct coupling of sensory input to motor output.

There are clearly seasonal and steroidal influences on the VT system in roughskin newts, indicating that its ability to influence sensorimotor processes and thus behaviour depends on reproductive context (Zoeller & Moore 1982, 1986; Moore *et al.* 1992, 2000). Most notably, VT levels in the optic tectum, a sensory integration area involved in the generation of orientation responses to visual stimuli, are highest during the breeding season (Zoeller & Moore 1986). Thus, environmental cues that trigger breeding probably prime the visual system with VT so that males will rapidly approach females when they enter the ponds to mate. By contrast, increased cloacal responsiveness is probably only advantageous once a female is clasped. The contexts in which endogenous VT enhances somatosensory responsiveness may therefore depend on the presence of appropriate social releasing stimuli associated with amplexus. Among such stimuli are the female sex pheromones that elicit amplexus in male newts (Thompson *et al.* 1999; Thompson & Moore 2000). We therefore hypothesized that pheromone exposure might induce VT release onto medullary neurons that regulate amplectic clasping, enhancing their responsiveness to the cloacal stimulation provided by the female and, as a result, strengthening and/or prolonging the motor pattern elicited by that stimulation. To test this hypothesis, we used extracellular recording techniques to measure the effects of pheromone exposure on medullary neuronal responses to somatosensory stimuli. We predicted that pheromone exposure would, like exogenous VT, enhance the responsiveness of medullary neurons to cloacal pressure, but that exposing the medulla to a VT receptor antagonist would prevent that enhancement.

2. MATERIAL AND METHODS

(a) *Subjects*

Adult male *Taricha granulosa* were captured from ponds in Benton County, Oregon and shipped to Bowdoin College between November and March. They were maintained in tanks of aerated, dechlorinated water with neutral pH between 13 and 15°C on a 8 L : 16 D cycle. All newts used in the experiment were sexually mature adults that weighed more than 12.0 g, and all had toepads and an enlarged cloaca gland, both of which are secondary sexual characteristics typical of male newts in reproductive condition. Newts were fed raw beef liver or heart once a day, 5 days a week. All methods were in accordance with guidelines for the use of vertebrate animals established by the Research Oversight Committee (IACUC) at Bowdoin College.

(b) *Surgery*

Thirty minutes before surgery, newts were anaesthetized by placing them into a 0.1% solution of ethyl 3-aminobenzoate methanesulfonate salt (MS-222; Sigma-Aldrich, St Louis, MO) in dechlorinated tap water and then chilled on ice. After newts were clearly anaesthetized (15–20 min), they were weighed and the hindbrain was exposed surgically.

To expose the hindbrain, a $2 \times 1.5\ mm^2$ rectangular piece of skin covering the dorsal surface of the skull was removed. A 1.2 mm flat-head steel screw was then placed into the anterior skull. A 5.0 in. V-shaped stabilization bar was attached to the head of the implanted screw using dental cement. The ends of the bar were pinned into the base of a Sylgard-based surgical dish to ensure complete immobilization of the newt throughout the surgery and recording. Neck muscles, subcutaneous fat and tissue layers were cauterized to expose the skull overlying the rostral medulla, which was removed with rongeurs. Absorbent dental points were used to reposition the underlying plexus such that the midline of the rostral medulla was fully exposed. Subsequent bleeding was controlled through the use of absorbent dental points. Once the midline of the rostral medulla was exposed, the newt was taken out of anaesthesia and rinsed with 150 ml chilled dechlorinated water. Time spent under anaesthesia was recorded and used to determine expected time of recovery from the anaesthesia before electrophysiological recordings could begin. Recovery typically took approximately the same amount of time that the newt spent under anaesthesia.

(c) *Electrophysiological recordings*

Recordings were made under the same conditions described in previous studies in roughskin newts (Rose *et al.* 1993, 1995; Coddington *et al.* 2007). Twenty minutes before the estimated time of recovery, newts immobilized on the surgical recording platform were placed within a Faraday cage and given a 0.2 ml intraperitoneal injection of a 2% solution of the muscle relaxant Flaxedil (98% gallamine triethiodide; Sigma, Milwaukee, WI). Neuronal activity is reduced or absent in amphibian brains during anaesthesia because tricaine, an active ingredient in MS-222, affects Na^+ channels (Frazier & Narahashi 1975; Arnolds *et al.* 2002), so we waited until newts had recovered from the MS-222 anaesthesia, as evidenced by the presence of electrical activity, before initiating experimental recordings. Subsequent injections of 0.2 ml of Flaxedil were given approximately every half hour to ensure complete immobilization of the newt. Previous investigations (Rose *et al.* 1993) had demonstrated that newts prepared in this way are not stressed. All newts were continuously superfused with chilled dechlorinated water (14–15°C when it entered the bath, 16–17°C at the point of outflow).

Extracellular single neuron recordings were obtained by lowering a two-stranded microwire electrode, fabricated from Teflon-insulated 50 μm diameter nichrome wire (impedance less than 0.9 MΩ), onto the surface of the rostral medulla with a micromanipulator (figure 1*a*). Although conventional sharp-tipped microelectrodes can be used for recording from

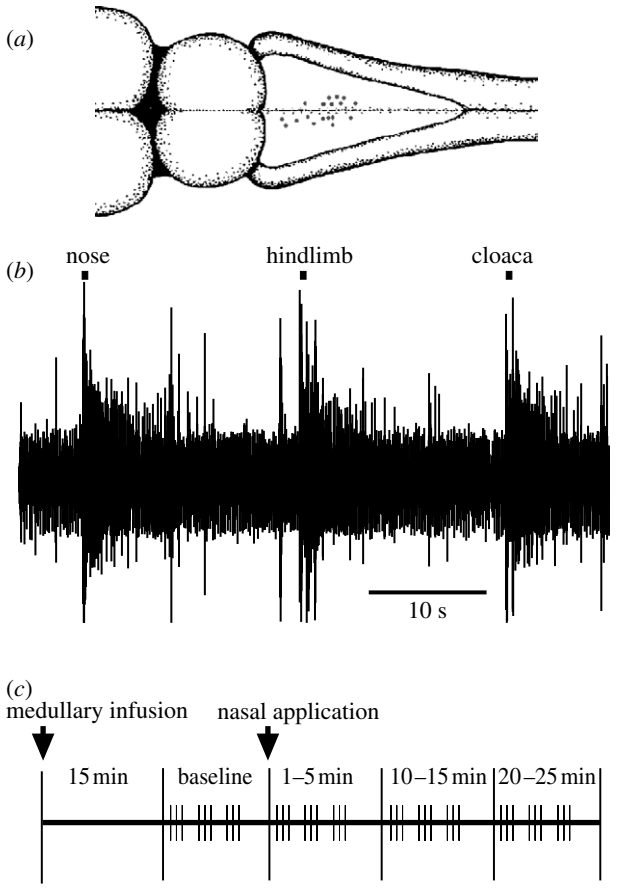

Figure 1. (*a*) Diagram of the area where recordings were made (dots indicate representative recording sites), (*b*) a representative trace of neuronal activity in response to the three different types of somatosensory stimulation as well as (*c*) a timeline of events during a recording session.

the newt medulla (Rose *et al.* 1993), microwire electrodes have proved to be preferable because they can be more readily positioned and maintained with stability on the dorsal surface of the medulla where neuronal somata are concentrated (see figure 1*b* for a sample recording). Neural activity was amplified using an AC amplifier (A–M systems, Model 1700, filter settings at 3 kHz–300 Hz), visualized using a two-channel digital storage oscilloscope (Tektronix, TDS 2002), and recorded through the use of a 1401 K data acquisition unit and the computer program Spike2 (v. 5.0, CED, England). Sites over the medial reticular formation in the rostral medulla were chosen because this is where neuronal firing patterns related to the regulation of clasping can be recorded (Rose *et al.* 1995).

Female-scented water (FSW) used for olfactory stimuli was collected from water housing reproductively active females captured in Oregon as they migrated to ponds to mate in late winter. Females were housed in Tupperware containers (10 ml of water per female) overnight. The next day, the water was collected in 2 ml aliquots, then snap-frozen on dry ice and stored at −80°C until use. A new aliquot was used for each subject. We have previously shown that male newts will clasp rubber newt models as if they were females only if the models are scented through direct contact with reproductively active females or by soaking them in the FSW collected from such females (Thompson *et al.* 1999; Thompson & Moore 2000). Although we do not yet know what the biologically active molecule(s) is/are, it can be extracted from FSW with organic solvents such as methylene chloride and ethyl acetate, and it is not disrupted by boiling or protease treatment. Together, these findings suggest that while the biologically active molecule(s) can be collected in FSW, it is probably not a diffusible protein signal used for long-distance communication. Rather, it may be a non-polar molecule used by males to determine a female's reproductive state once contact is made.

Somatosensory stimuli were delivered by pressing the newt for 3 s with a plastic pipette tip attached to a piano wire. This method of stimulation allowed us to apply a constant pressure of 20 g for the duration of each stimulus regardless of individual variation in how hard the experimenter pressed the wire. Before each surgery, we used a scale to confirm that presses with the piano wire consistently resulted in 20 g of pressure. The primary site of interest was the cloaca, which was made accessible to touch by holding the males' tails up with a clamp during the entire recording session. To assess specificity of pheromone effects, we also measured somatosensory responses in two control areas, the top of the snout and the hindlimb. All sites were tested initially for somatosensory responsiveness (and to ensure the newts had recovered from the anaesthesia) by recording the response to pressure on the recording sites with a wet cotton probe. If no responses were observed, the electrode was moved to a new site and/or more time was given for the newt to recover from anaesthesia.

Once a recording site was established, 2 µl of newt saline or the V1a receptor antagonist [3-mercapto-3,3-cyclopentamethylene-propionyl^{1}, *O*-Me-Tyr^{2}, Arg^{8}]-vasopressin (Manning compound; 2.5 µg µl^{-1}) were perfused manually onto the newt's medulla through a 30 g needle attached by PE10 tubing to a 25 µl Hamilton syringe. Preliminary data indicated that perfusing saline onto the medulla after a recording site was established did not affect subsequent recordings. Manning compound blocks VT-induced receptor internalization in the newt medulla (Lewis *et al.* 2004) and, at the dose used, courtship clasping (Moore & Miller 1983). After 15 min, baseline responses to stimulation of the cloaca, snout and hindlimb were recorded (see figure 1*b* for sample responses to each type of stimulation). FSW or dechlorinated tap water was then applied continuously to the nares of the newt over the next 30 min of recording by draping a Kim wipe saturated with the FSW or tap water onto the newt's nares and placing the ends of the Kim wipe in a mini-perfusion dish holding 2 ml of FSW or tap water. Capillary action onto the nose allowed continuous olfactory exposure to FSW or tap water. Thus, we tested four groups of male newts: one that was perfused with saline onto the medulla and then had the nares exposed to tap water; one that was perfused with saline onto the medulla and then had the nares exposed to FSW; one that was perfused with Manning compound onto the medulla and then had the nares exposed to tap water; and one that was perfused with Manning compound onto the medulla and then had the nares exposed to FSW. For all groups, tactile stimuli were applied 1–5, 10–15 and 20–25 min after the onset of olfactory stimulation (see figure 1*c* for a schematic timeline). Three rounds of recordings, approximately 1 min apart, were taken during each recording interval. Each of the three stimulus locations was touched once, 15–30 s apart, during each round. Responses for the three rounds of stimulation to each site during each interval were averaged. Experimenters were blind as to whether saline or Manning was perfused onto the

medulla during the recordings. At the end of the recordings, newts were again deeply anaesthetized in MS-222 and then euthanized.

(d) *Data analysis and statistics*

To quantify the neuronal responses to pressure, we used the analysis functions of SPIKE2 to discriminate extracellularly recorded action potentials from background noise. To discriminate action potentials associated with somatosensory stimulation from spontaneous activity, we subtracted the number of action potentials 1 s before stimulation from the number during the first second of stimulation. We did not attempt to discriminate the responses of individual neurons, but rather focused our analysis on the activity of the entire population of neurons being recorded by our extracellular electrodes.

One-way repeated measures ANOVA were run on responses to tactile stimulation across recording intervals. If an overall main effect was found, Bonferonni corrected, planned comparisons were made between control responses prior to olfactory stimulation and responses during each subsequent time interval. A 2×3 repeated measures ANOVA with medullary perfusion (saline or Manning) as a between groups factor and stimulus location (cloaca, nose and hindlimb) as a within subjects factor was run on baseline responses prior to olfactory stimulation to see if Manning compound affected somatosensory responsiveness independently of FSW exposure. We also ran separate 2×4 repeated measures ANOVA for each stimulus location in the two groups of animals exposed to tap water, with medullary perfusion (saline or Manning) as the between subjects factor and recording interval as the within subjects factor, to see if Manning compound affected responsiveness during later recording intervals independently of FSW exposure.

3. RESULTS

All sites recorded in the medulla showed spontaneous activity, although overall spike frequency varied from site to site. Spike frequency increased, and remained elevated for up to several seconds after the application of pressure to the cloaca, the snout or the hindlimb (figure 1*b*). Perfusions of Manning compound onto the medulla did not affect somatosensory responsiveness independently of olfactory stimulation; there was not a main effect of perfusion ($F=0.42$, $p=0.71$; 23 animals infused with saline, 14 infused with Manning) or a significant interaction between infusion and stimulus location ($F=0.12$, $p=0.89$) for baseline responses before olfactory stimulation. There was a trend for Manning compound to decrease responses to snout pressure in animals exposed to tap water when we looked across all recording intervals (main effect of perfusion: $F=4.3$, $p=0.06$; eight animals infused with saline and eight animals infused with Manning). However, there were no significant main effects of perfusion or any significant interactions between perfusion and recording interval in any of the separate ANOVA's run for each recording site in the groups of animals exposed to tap water after medullary perfusions of saline or Manning (all other p-values >0.1), indicating that the V1 antagonist does not affect responsiveness independently of FSW exposure at later time points after drug administration, either.

Of the four groups of newts, only those exposed to FSW in the absence of the V1a antagonist showed any change in responsiveness. In animals with saline perfused onto the medulla and continuously exposed to FSW, neuronal responses to cloacal pressure changed significantly across recording intervals ($n=15$, $F=6.51$, $p=0.001$); there were significantly more action potentials both 10–15 min ($t=3.16$, $p<0.01$) and 20–25 min ($t=3.89$, $p<0.01$) after FSW application than during baseline recordings (figure 2*a*). By contrast, there were no significant changes in the response to cloacal pressure across recording intervals in animals perfused with saline and exposed to tap water ($n=8$, $F=0.34$, $p=0.78$; figure 2*b*) or in animals perfused with Manning and then exposed to either FSW ($n=6$, $F=0.20$, $p=0.89$; figure 2*c*) or tap water ($n=8$, $F=0.27$, $p=0.85$; figure 2*d*).

As was the case with cloacal pressure, there was a significant change in the neuronal response to snout pressure across the recording intervals in animals with saline perfused onto the medulla and continuously exposed to FSW ($n=15$, $F=4.39$, $p=0.009$); there were again significantly more action potentials generated in response to snout pressure 10–15 min ($t=2.98$, $p<0.05$) and 20–25 min ($t=3.04$, $p<0.05$) after FSW application than during baseline recordings (figure 3*a*). However, there were no significant changes in the response to snout pressure across recording intervals in animals perfused with saline and exposed to tap water ($n=8$, $F=2.31$, $p=0.11$; figure 3*b*), in animals perfused with Manning and exposed to FSW ($n=6$, $F=0.14$, $p=0.94$; figure 3*c*), or in animals perfused with Manning and exposed to tap water ($n=8$, $F=0.19$, $p=0.90$; figure 3*d*).

There was also a significant change in the neuronal response to hindlimb stimulation across recording intervals that was a function of FSW exposure. Again, we recorded an increase in the number of action potentials recorded in animals in which saline was perfused onto the medulla and the nares were subsequently exposed to FSW ($n=15$, $F=3.94$, $p=0.01$). However, it took longer for FSW to enhance hindlimb somatosensory responses than cloaca or snout responses; there were significantly more action potentials recorded in response to hindlimb stimulation 20–25 min after FSW application than in the baseline condition ($t=3.43$, $p<0.01$), but not 10–15 min after FSW application (figure 4*a*). As with the other somatosensory stimuli, there were no significant changes in the neuronal response to hindlimb stimulation across recording intervals in animals perfused with saline and exposed to tap water ($n=8$, $F=0.34$, $p=0.80$; figure 4*b*) or in animals perfused with Manning and exposed to either FSW ($n=6$, $F=0.48$, $p=0.70$; figure 4*c*) or tap water ($n=8$, $F=0.40$, $p=0.76$; figure 4*d*).

4. DISCUSSION

We have demonstrated that female olfactory cues enhance somatosensory responsiveness in the medulla of male newts, indicating that olfactory social stimuli have cross-modal sensory influences in this species. We propose that sex pheromones present in FSW, which elicits male-typical courtship clasping (Thompson *et al.* 1999; Thompson & Moore 2000), were responsible for those effects. Consistent with this hypothesis, preliminary studies indicate that food odours do not enhance somatosensory responsiveness,

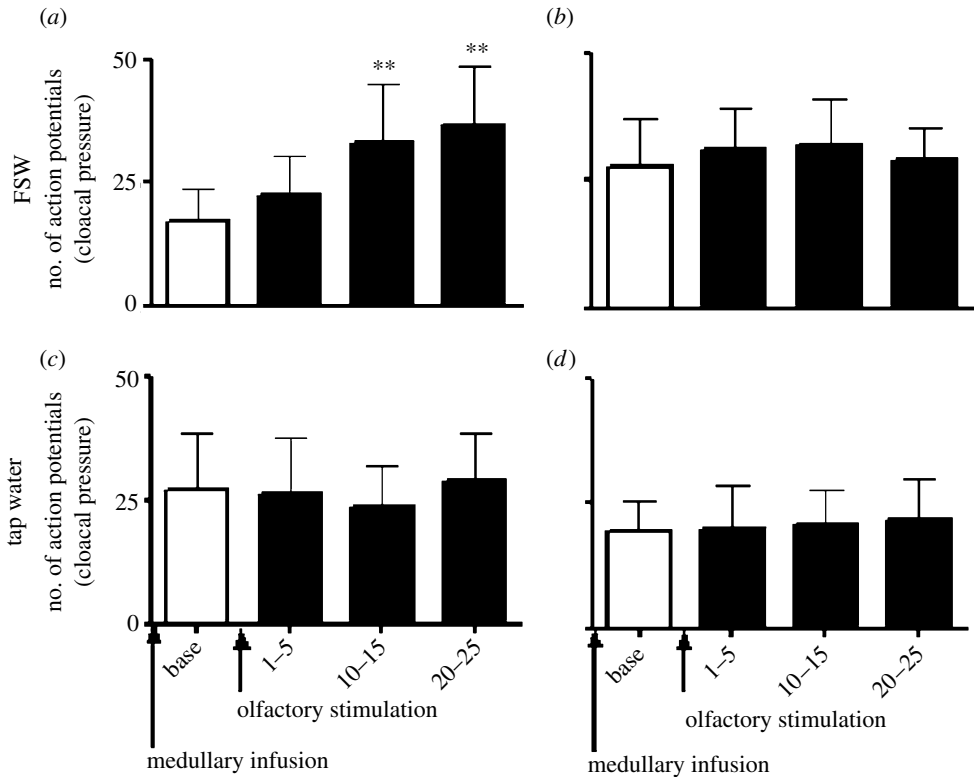

Figure 2. When FSW was applied continuously to the nares for 30 min, the medullary response to cloacal pressure increased across recording intervals during exposure. However, no increase in responsiveness was seen in the animals whose medulla was perfused with Manning compound or in those whose nares were exposed to tap water, regardless of medullary treatment. Mean ± s.e.m. of responses to cloacal pressure across recording intervals (*a*) in animals with saline perfused onto the medulla and FSW applied to the nares, (*b*) in animals with Manning perfused onto the medulla and FSW applied to the nares, (*c*) in animals with saline perfused onto the medulla and tap water applied to the nares and (*d*) in animals with Manning perfused onto the medulla and tap water applied to the nares. **Indicates significantly more action potentials than were recorded during baseline conditions prior to olfactory stimulation, $p < 0.01$.

although further tests with male and non-social odours would be necessary to make that determination conclusively. The influences of FSW were blocked by a V1a receptor antagonist, which demonstrates that they are dependent on endogenous VT acting on V1a-related receptors. Because the antagonist did not affect somatosensory responses independently of pheromone exposure, endogenous VT's ability to influence medullary sensorimotor processes in this species appears to depend on social context, specifically on the presence of female olfactory stimuli. Furthermore, we have elucidated a novel mechanism by which VT can influence social behaviour in vertebrates: it effectively couples sensory systems together so that social stimuli processed within one system can influence how an animal responds to stimuli that are associated with the same social interaction but are processed in another sensory system.

It seems probable that the ability of the V1a antagonist to block the olfactory-induced enhancement of somatosensory responsiveness was mediated near the perfusion site within the medulla, where ascending sensory projections are integrated into motor output associated with clasping, particularly the activity of descending reticulospinal neurons that influence the onset, offset and duration of the clasp motor reflex (Naujoks-Manteuffel & Manteuffel 1988; Rose *et al.* 1995). There are VT terminals (Lowry *et al.* 1997) and receptors (Boyd & Moore 1991; Lewis *et al.* 2005) in the newt medulla, although the source of those projections and the circuits through which olfactory stimuli activate them have not yet been identified. Additionally, although we did not characterize individual neuronal responses as did Rose *et al.* (1995), and thus cannot say whether individual neurons are firing more or previously unresponsive neurons are being recruited in response to FSW, our present results are consistent with what they observed when exogenous VT was applied to the medulla, which was that neuronal responses to somatosensory stimuli typically increased. It should be noted, however, that the volumes perfused onto the medulla were high, so it is also possible that diffusion to other brain areas mediated the effects of the antagonist. It also remains possible that the pheromone effects could depend on interactions with the mesotocin system, as this oxytocin homologue can also bind and activate VT receptors in amphibians, though less potently than VT (Kohno *et al.* 2003; Acharjee *et al.* 2004). However, there is not yet any evidence that a V1 antagonist blocks endogenous mesotocin effects in any species or that mesotocin has any behavioural effects in this species.

The ability of the V1a antagonist to block the olfactory-induced enhancement of somatosensory responses without changing baseline responsiveness indicates either that tonic VT release only affects medullary responses to tactile stimulation when female olfactory stimuli are present, or that such cues actually induce VT release within the brain. That exogenous VT can increase somatosensory responsiveness in medullary neurons in the absence of female

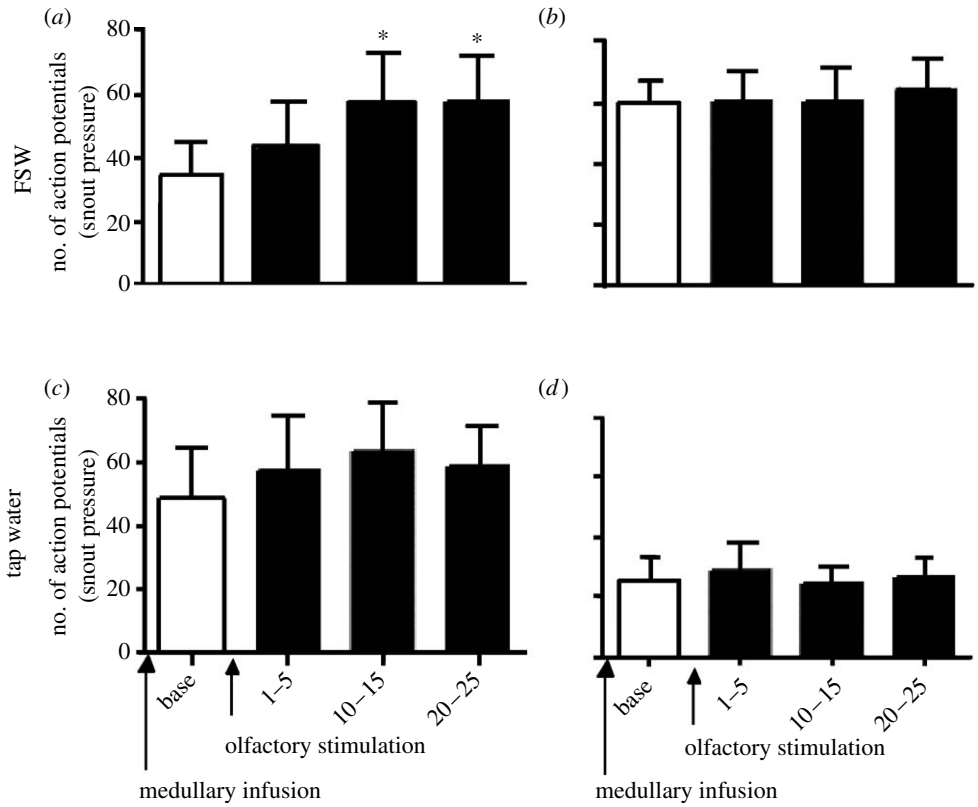

Figure 3. When FSW was applied continuously to the nares for 30 min, the medullary response to snout pressure increased across recording intervals during exposure. However, no increase in responsiveness was seen in the animals whose medulla was perfused with Manning compound or in those whose nares were exposed to tap water, regardless of medullary treatment. Mean ± s.e.m. of responses to snout pressure across recording intervals (*a*) in animals with saline perfused onto the medulla and FSW applied to the nares, (*b*) in animals with Manning perfused onto the medulla and FSW applied to the nares, (*c*) in animals with saline perfused onto the medulla and tap water applied to the nares and (*d*) in animals with Manning perfused onto the medulla and tap water applied to the nares. *Indicates significantly more action potentials than were recorded during baseline conditions prior to olfactory stimulation, $p < 0.05$.

olfactory stimuli (Rose *et al.* 1995) argues against the former possibility. On the other hand, our results are consistent with numerous studies showing that social stimuli can drive endogenous VT/VP cell activation and peptide release in vertebrates (see §1). We therefore propose that sex pheromones present in FSW stimulate VT neurons that project to the medulla, where they release the peptide, a hypothesis that could be tested in the future with cFOS and microdialysis experiments. Whatever the mechanism through which female olfactory stimuli and the VT system interact, our present results clearly show that VT can act as a link between sensory systems, allowing social stimuli processed within one modality to amplify responses to stimuli processed in a different modality.

We had anticipated that sex pheromones present in FSW would primarily affect neuronal responsiveness to cloacal pressure, a somatosensory input that helps male newts maintain amplexus by continuously triggering a clasp motor reflex after they have mounted a female. Males typically maintain amplexus for hours to induce female receptivity (Propper & Moore 1991), and they constantly rub their snouts into the female during amplexus. Although we do not yet know the identity of the biologically active molecule(s) in FSW, our previous studies suggest that it is probably a contact pheromone secreted from the female's skin and that males are in contact with it throughout amplexus (Thompson *et al.* 1999). The delayed enhancement of cloacal responsiveness that occurred after exposure to female odours may therefore be more important for prolonging the duration of the clasp than for modulating initial phases of clasping, though it is also possible that exposure to more concentrated amounts of the biologically active molecule(s) through natural exposure would induce faster effects.

However, the effects of FSW do not exclusively affect the processing of somatosensory stimuli directly related to clasping, i.e. cloacal pressure; FSW also enhanced the somatosensory responsiveness of other parts of the body, including the snout and, though more slowly, the hindlimb. Similarly, exogenous VT applied to the medulla also generally increased somatosensory responsiveness in the study by Rose *et al.* (1995). As mentioned, male newts do continually press their snouts into the dorsum of the female during amplexus, potentially to increase pheromone exposure and/or to deliver their own pheromones to the female to induce receptivity (Propper & Moore 1991), so enhanced responsiveness to snout pressure may enhance this courtship-related behaviour. On the other hand, we were touching an area of the hindlimb that is usually not in contact with the female during amplexus and is thus not obviously related to courtship. However, other males often try to dislodge a male engaged in amplexus, sometimes forming large mating balls around the courting pair, and males in amplexus will show vigorous swim responses with the female in tow when touched (R. R. Thompson 1998, personal observations).

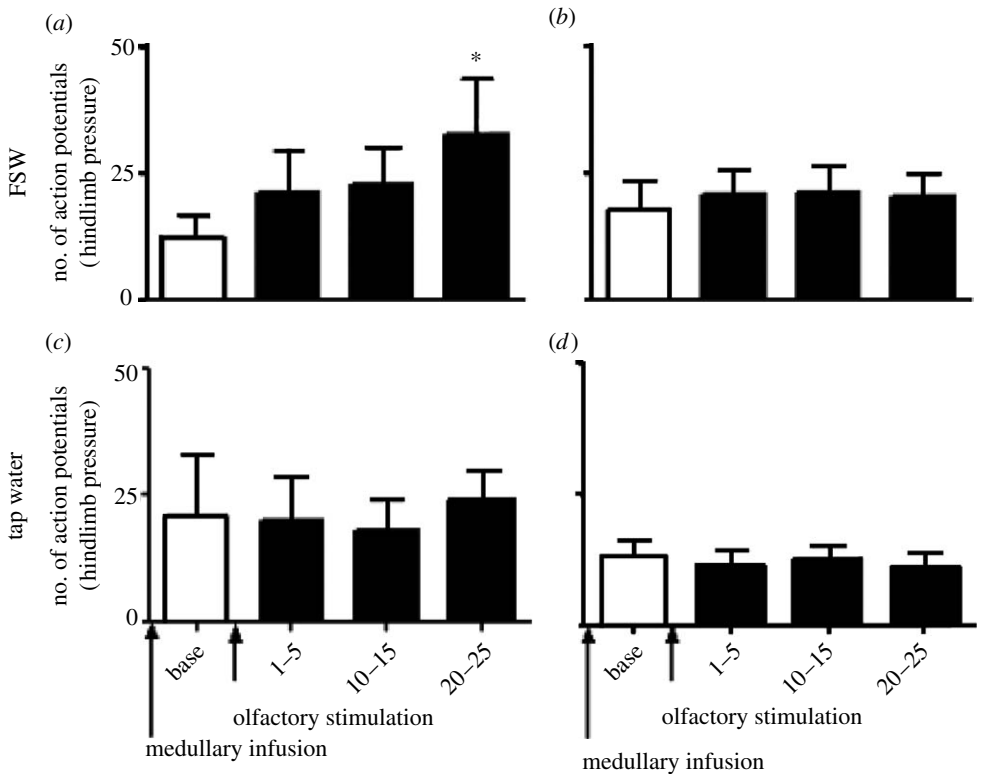

Figure 4. When FSW was applied continuously to the nares for 30 min, the medullary response to hindlimb pressure increased across recording intervals during exposure, but more slowly than did cloacal and snout responsiveness. However, no increase in response was seen in the animals whose medulla was perfused with Manning compound or in those whose nares were exposed to tap water, regardless of medullary treatment. Mean $\pm$ s.e.m. of responses to hindlimb pressure across recording intervals (*a*) in animals with saline perfused onto the medulla and FSW applied to the nares, (*b*) in animals with Manning perfused onto the medulla and FSW applied to the nares, (*c*) in animals with saline perfused onto the medulla and tap water applied to the nares and (*d*) in animals with Manning perfused onto the medulla and tap water applied to the nares ($n=8$). *Indicates significantly more action potentials than were recorded during baseline conditions prior to olfactory stimulation, $p<0.05$.

Generally, enhanced somatosensory responsiveness could make males more sensitive to the attempts of other males to disrupt amplexus and, as a result, help them avoid that competition and thus increase their reproductive success.

Nonetheless, it did take longer for FSW to enhance hindlimb somatosensory responsiveness than cloaca or snout responsiveness, which suggests that there are different cellular mechanisms through which FSW enhanced responsiveness for the different sites. Supporting that hypothesis, we have observed that a single application of FSW, in contrast to continuous exposure, does not significantly affect hindlimb responsiveness, although it does increase cloacal and snout responsiveness (G. M. Civiello 2003, unpublished data). It is possible that female olfactory stimuli initially activate cells that release VT at synapses directly related to the regulation of amplexus, that is, those that affect incoming somatosensory information from the cloaca and snout, but that prolonged exposure activates more and more cells, which then release VT at additional synapses where somatosensory responses to different parts of the body are processed. Alternatively, prolonged exposure may not activate additional neurons, but rather induce more VT release from a limited neuronal population, creating a diffusion gradient that is high in areas directly involved in the regulation of amplexus and lower in areas that process incoming sensory input from other parts of the body, although it is unclear at this time that there is a demarcated somatotopic organization of the newt medulla. Similarly, it has been proposed that high concentrations of peptide release from the dendrites of magnocellular hypothalamic cells in rats may diffuse throughout the brain as a paracrine signal, with the behavioural effects dependent on the size of the diffusion gradient and thus the amount of spread throughout the brain (Landgraf & Neumann 2004).

In summary, we have shown that social stimuli processed within one sensory modality can affect how sensory information coming in through other sensory modalities is processed in male roughskin newt brains, most likely by inducing endogenous VT release. VT may therefore promote focused behavioural responses towards females in this species by amplifying cues coming from the female through multiple sensory modalities once contact is made. However, because that amplification does not appear limited to sensory input from the female, VT may also increase the likelihood the animal will detect and respond to competing social or environmental challenges that arise during the interaction and thus also promote behavioural adjustments to those challenges. In cases where those challenges are strong and feedback from the female is weak, such a mechanism could even increase the likelihood that the male will terminate amplexus altogether to focus on those challenges. Such a sensory coupling mechanism could therefore lead to alternative behavioural outcomes within an individual animal, depending on the immediate social context in which the behaviour takes place and thus on the nature of the associated stimuli that are amplified.

It will therefore be interesting to see if a sensory coupling mechanism like the one we have described in roughskin newts is present in other vertebrates and to see if it does result in context-dependent behavioural effects in newts or any other species in which it is operative. In fact, if the same mechanism is present in different species, but the contexts in which individuals typically interact differ, then this mechanism could help explain some of the different behavioural effects that VT and related peptides have across species on responses to the same types of social stimuli.

Our findings are relevant not only to questions regarding olfactory social communication and the mechanisms by which VT and related peptides influence diverse social behaviours in vertebrates, but also to more general questions regarding how animals focus neural resources on relevant social stimuli. Emotional stimuli, including those related to social encounters, are known to enhance early stages of cortical processing within the modality in which the stimuli are processed in humans, most likely via back projections to those areas (Lang *et al.* 1998; Keil *et al.* 2003), and species-specific social cues processed within one modality can amplify or suppress cortical responses to social cues processed in another modality in rhesus monkeys (Ghazanfar *et al.* 2005; Sugihara *et al.* 2006). The present results extend our knowledge of sensory amplification and multisensory processing related to social communication by showing that species-specific social stimuli can amplify the processing of stimuli coming in through other sensory modalities in non-cortical brain regions that directly couple sensory input to motor output, and that they can do so in non-primate, even non-mammalian, species. Furthermore, we have identified a putative neurochemical mechanism, in the form of local VT release, associated with that socially induced sensory amplification.

All methods were in accordance with guidelines for the use of vertebrate animals established by the Research Oversight Committee (IACUC) at Bowdoin College.

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