A primitive social circuit: vasotocin–substance P interactions modulate social behavior through a peripheral feedback mechanism in goldfish

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Abstract
At its core, the polyvagal theory proposes that peptides affect simple social behaviors through influences on hindbrain autonomic processes. To test this mechanism, we compared the effects of fore- and hindbrain infusions of vasotocin (VT) on social approach behavior in goldfish. VT infusions into the 4th ventricle, which ink infusions verified did not move rostrally to the forebrain, inhibited social approach at a lower dose than did infusions into the 3rd ventricle, which did diffuse to the hindbrain. Thus, VT actions in the hindbrain appear to modulate this simple social behavior. We then identified a population of substance P (SP)-immunoreactive cells in the hindbrain that are encapsulated by putative VT terminals, and determined that those cells project to the periphery. Injecting SP peripherally, as with infusing VT centrally, inhibited social approach, and peripheral injections of an SP antagonist, but not central infusions, abolished the behavioral effects of central VT infusions. We therefore propose that VT inhibits social approach by activating SP cells in the hindbrain, which then induce changes in body state that feed back to the brain. Central VT infusions did not inhibit feeding, suggesting that this VT mechanism selectively affects appetitive social responses. Because VT projections to the hindbrain are highly conserved in vertebrates, influences on peripheral feedback processes like the one we have described in goldfish may reflect how VT affected simple social behaviors in ancestral vertebrates and thus preadapted members of this peptide family to play increasingly complex roles in social and emotional regulation in modern animals.

Introduction
Comparative studies suggest that functions of vasotocin (VT) and vasopressin (VP) related to the control of social behavior have, in general, been highly conserved during vertebrate evolution (Goodson & Bass, 2001). However, we still know little about how ancestral peptide circuits may have influenced simple social behaviors and, perhaps as a consequence, provided a foundation for the subsequent evolution of increasingly complex social regulatory mechanisms in modern vertebrates. We therefore do not yet know whether any behavioral mechanisms associated with such primitive circuits, if conserved, continue to influence social behavior in extant species, perhaps through interactions with more derived peptide circuits that are important for the regulation of stereotypical, species-specific forms of social behavior.

The most ancestral anatomical characteristics of these peptide systems in vertebrates include VT preoptic/hypothalamic cell groups and fiber projections to the pituitary and to the tegmentum and hindbrain. These characteristics are present in non-mammalian jawed and jawless vertebrates (Hoheisel et al., 1978; Goodson & Bass, 2001), whereas extrahypothalamic VT and VP cell populations in areas such as the bed nucleus of the stria terminalis and the amygdala, as well as the associated forebrain projections that modulate some forms of social behavior, are only found in tetrapods. The ability to influence peripheral processes, either through release from the neurohypophysis into circulation, by stimulating pituitary hormone secretion or by affecting autonomic output, is thus one of the most conserved functions of these peptides. Influences on hindbrain autonomic processes, in particular, have been suggested as a primitive mechanism through which these peptides may affect basic tendencies to approach or withdraw from social interactions (Porges, 2001), perhaps by inducing peripheral physiological changes that feed back to the brain to promote ‘fight or flight’ tendencies. However, a central peptide mechanism that depends on feedback from the periphery to induce its behavioral effects has not yet been demonstrated in any species.

In goldfish, central VT inhibits approach responses towards other goldfish in the absence of olfactory stimuli that would otherwise elicit stereotypical courtship and aggressive responses (Thompson & Walton, 2004). This suggests that VT indeed affects just such a simple approach/withdrawal process in this highly social teleost. To determine whether VT actions in the hindbrain inhibit social approach, we compared the behavioral effectiveness of hindbrain and forebrain infusions of VT. To determine if this inhibition depends on a peripheral feedback mechanism, we immunocytochemically identified a population of cells that VT terminals make contact with in the hindbrain, verified that they project to the periphery, and tested if blocking the peripheral actions of those cells would block the behavioral effects of centrally infused VT.

Experimental procedures

Subjects
All subject animals were adult comet goldfish (*Carassius auratus*) 5–6 inches (12.7–15.2 cm) in length and typically weighing between 30
and 50 g. Fish were housed in groups in tanks of circulating, dechlorinated water. Photoperiod and water temperature varied, depending on the time of year, from 14:10 h light/dark and 20 °C for fish in spring/summer breeding condition to 10:14 h light/dark and 14 °C for fish in fall/winter non-breeding condition. All surgical methods, behavioral protocols and methods used to kill the animals were approved by and in accordance with guidelines for the use of vertebrate animals established by the Research Oversight Committee (IACUC) at Bowdoin College.

VT ventricular dose responsiveness

Our dose–response studies were conducted in February, a time when fish are not in breeding condition and so cannot be reliably sexed. We therefore used both male and female goldfish from mixed-sex housing. We have found that central infusions of VT similarly inhibit social approach in both sexes and at the same doses, and that VT similarly inhibits approach responses towards the visual cues of same- or opposite-sexed fish at that time of year (unpublished data). We used the same surgical method previously described to place a permanent cannula into the 3rd ventricle or the 4th ventricle in different groups of fish (Thompson & Walton, 2004). Briefly, fish were anesthetized in 0.1% MS-222 (Sigma), and a hole was drilled above the junction of the telencephalon and optic tectum for 3rd ventricle injections and above the vagal lobes for 4th ventricle injections. The cannula was lowered approximately 1 mm and fixed in place with dental cement anchored to bone screws in the skull.

Behavioral tests were conducted 3 and 5 days after surgery. Each fish was tested once after an infusion of 1 μL saline and once after an infusion of a single dose of VT dissolved in saline (200 ng, 3rd ventricle n = 14, 4th ventricle n = 11; 40 ng, 3rd ventricle n = 7, 4th ventricle n = 8; 5 ng, 3rd ventricle n = 8, 4th ventricle n = 10), in counterbalanced order. For each test, fish were placed into the central compartment (70 L) of a rectangular tank with two stimulus compartments (5 L) on each end, separated by sealed Plexiglas to prevent chemical communication. Time spent within 2.5 cm of each partition during a 15-min baseline was recorded with a video tracking system (Limelight; Coulbourne Instruments, Whitehall, PA, USA). Fish were then captured and infused, then placed back into the central test tank. Five minutes later, a stimulus fish was placed in the side compartment behind the partition where the fish spent the least amount of time during the baseline period, and time within 2.5 cm of that partition was again recorded for 15 min. Corrected proximity scores were calculated by subtracting the baseline time in proximity to that partition from the time in proximity during the 15 min while the stimulus fish was present. After the last day of testing, 1 μL India ink was infused through the cannula. Fish were killed 10 min later by an overdose of MS-222 and the brains removed to evaluate the spread of ink through the ventricle system. Any fish with no ink in the ventricles was excluded from the analysis.

Neuroanatomy

For immunocytochemical (ICC) studies, male and female goldfish in various reproductive states were transcardially perfused with heparinized cold teleost ringer followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Brains were then removed, postfixed overnight in 4% paraformaldehyde, rinsed in 0.1 M phosphate buffer (PB) and then cryoprotected by sinking them in 30% sucrose in PB. Brains were then cut on a cryostat in 30-μm serial sections, thaw-mounted on positively charged slides (Fisher) and stored at −80 °C until processed.

Slides were warmed to room temperature, rinsed for 20 min in PBS and blocked for 20 min in PBS containing 2.5% bovine serum albumin (BSA). Primary antibodies, diluted in PBS + 2.5% BSA + 0.1% Triton X-100, were then added, and sections were incubated overnight at 4 °C in a humid chamber. The following day slides were rinsed twice for 20 min in PBS, rinsed twice for 20 min in PBS + 2.5% BSA, and then incubated for 2 h at room temperature with the appropriate fluorescently conjugated secondary antibody diluted 1 : 500 in PBS + BSA. Slides were then rinsed for 40 min in PBS before mounting and coverslipping.

For VT ICC, a rabbit polyclonal anti-VT (courtesy of F. Van Leeuwen, Netherlands Institute for Brain Research) was used at a 1 : 4000 dilution and visualized with an Alexa 594-conjugated anti-rabbit secondary antibody (Molecular Probes, Eugene, OR, USA), and sections were mounted with 80% glycerol or with a media containing a fluorescent nissl stain (NeuroTrace 500/525; Molecular Probes, n = 6). For VT combined with substance P (SP) ICC (n = 6), a polyclonal guinea-pig anti-VP (1 : 1000; Bachem, San Carlos, CA, USA) and a polyclonal rabbit anti-SP (1 : 500; Immunostar, Hudson, WI, USA) were used and visualized with Alexa 594- and CY2- (Jackson ImmunoResearch, West Grove, PA, USA) conjugated secondary antibodies. Specificity of all antibodies in areas where we report labeling was verified by blocking the signal via preincubation of the primary antibody in 1–100 μg solutions of the respective peptides and by ensuring a lack of signal in the same areas on slides without primary antibodies added.

For tract tracing, male and female goldfish in various reproductive states were injected i.p. with 40 μL of 2% Fluorogold (Fluorochrome, Denver, CO, USA) dissolved in teleost ringer saline and, 3 days later, perfused transcardially. Brains were removed and processed for VT ICC, as described above, using the rabbit VT antibody (n = 4). A separate group of mixed-sex fish (n = 4) was anesthetized in 0.1% MS-222, and the skull over the hindbrain was removed to expose the vagal lobes. The vagal lobe on one side was gently moved aside, and the vagus nerve on that side was severed. The opening was then sealed with dental cement, and the fish were held for 4 days to give the severed nerve time to degenerate. Fish were then injected i.p. with 40 μL of 2% Fluorogold in ringer solution. After 3 days fish were perfused transcardially and alternate sections of the brains were processed for VT and SP ICC, as described above.

Substance P behavior tests

These tests were conducted between March and July, when fish display secondary sexual characteristics and thus could be sexed. Separate tests were done in males and females using same-sex stimuli. We first wanted to test if peripheral injections of SP, as with central infusions of VT, inhibit social approach behavior. To do this, fish were placed in the central compartment of the same test tank described above, and baseline side preferences were recorded for 15 min. Fish were then injected i.p. with 25 μL saline or SP (25 μg) dissolved in saline, in counterbalanced order, on 2 days of testing 48 h apart (males, n = 10; females, n = 10). A stimulus fish was added to the side compartment on the least preferred side of the tank 5 min later, and social proximity (time within 2.5 cm of the partition) was measured for 30 min. Time spent in proximity to the partition during the 15-min baseline was then subtracted from the time spent in proximity to the same partition during the first 15 min after the stimulus fish was added and during the second 15 min after the stimulus fish was added so we could determine the time course of any effects of SP on social approach.
In a separate test of SP effects on activity, fish from a mixed-sex group were individually placed in the same testing tank and given 15 min to habituate. The number of times they crossed the midline was then counted for 15 min. Fish were then captured and injected i.p. with saline on one test day and SP (25 µg) dissolved in saline on the other, in counterbalanced order (n = 10). The number of times the fish crossed the midline during the next 30 min were counted. Activity change in response to the injections was calculated by subtracting the number of midline crossings during the initial 15-min baseline period from the number of crossings during the first 15 min after the injection and from the number of crossings during the second 15 min after the injection.

Additionally, we tested if IP injections of the neurokinin 1 receptor antagonist Sendide (Bachem, King of Prussia, PA, USA) could block the effects of peripherally injected SP on social approach behavior in male goldfish. Although neurokinin receptors have not yet been sequenced in teleosts, neurokinin 1 receptor antagonists have been used in jawless fish to block physiological responses to SP (Parker & Grillner, 1996; Perez et al., 2007), which suggests that related receptors have likely been conserved during vertebrate evolution. These experiments were conducted between September and December, and because we have observed that 25 µg of SP does not inhibit social approach behavior at this time of year (data not shown), we first tested if a higher dose of SP would inhibit social approach. To do this, all fish received an initial i.p. injection of saline on both test days. Thirty minutes later side preferences were recorded for 15 min, as described above. Then, fish were recaptured and injected i.p. with saline on one test day and SP (50 µg) on the other, in counterbalanced order, and social approach behavior was measured for 30 min (n = 8). To determine if Sendide affects social approach, a second group of fish was initially injected i.p. with saline on one test day and with Sendide (50 µg) on the other, in counterbalanced order, and then with saline on the second injection, 45 min later, on both test days (n = 8). Finally, to see if Sendide blocks the effects of SP, fish in a third group received an initial i.p. injection of saline on one test day and of Sendide (50 µg) on the other, in counterbalanced order, and then all fish received a second i.p. injection of SP (50 µg) on both test days (n = 8). There are no published reports using Sendide in fish; the dose we used, which is approximately 1.25 mg/kg, depending on the exact size of the fish, was chosen because it is within the range of doses used to measure the effects of peripherally administered Sendide on SP-mediated physiological responses in ferrets (0.1 mg/kg when given intravenously, 3.0 mg/kg when given subcutaneously; Minami et al., 1998).

VT/SP behavioral interactions
These tests were conducted during the late winter/early spring (late February/March), a time of transition into the breeding season. To ensure that fish did not undergo gonadal recrudescence during the experiments, we increased water temperature and photoperiod to those used in jawless fish to block physiological responses to SP (Parker & Grillner, 1996; Perez et al., 2007), which suggests that related receptors have likely been conserved during vertebrate evolution. To see if central VT specifically affects social approach behavior, we also tested the effects of central VT infusions on approach responses towards a non-social appetitive stimulus, goldfish food pellets (Wardley). Cannula were surgically inserted into the 3rd ventricle of male goldfish, as described above. We then tested effects of VT on latency to feed in two ways. In the first, we wanted to test fish in a novel environment, which meant we could not do repeated tests with the same fish. We therefore put fish in a novel test tank for 15 min of habituation, then captured them and infused one group with saline (n = 8) and one group with 200 ng VT dissolved in saline (n = 8). They were then returned to the tank and, 5 min later, 3–5 food pellets were dropped in a floating ring in the tank, and the latency to initiate feeding was recorded. In the second experiment, we tested the effects of 1 µg VT – a dose that we have previously shown has similar effects on social approach behavior as the 200-ng dose (Thompson & Walton, 2004) – on latency to initiate feeding in the same tank, but after the fish had multiple experiences feeding in that environment. In this test, all fish (n = 19) were given three pretest feeding trials in the test tank. After a 15-min habituation period, 3–5 food pellets were dropped into a floating ring in the center of the test tank, and the latency to initiate feeding was recorded. Preliminary studies indicated that latency to feed decreases across the first three feeding trials, but not on subsequent days; therefore, only fish that fed on all three pretest trials were included in the final analysis (n = 8). On the 4th and 5th days, each fish was infused with 1 µL saline and 1 µL VT (1 µg) dissolved in saline, in counterbalanced order, after the 15-min habituation period. Food pellets were added 5 min later and latency to feed was again recorded during a 15-min test. For all feeding tests, fish were fed ad libitum in their home tanks 20–24 h before testing.

Statistics
For all of our within-groups behavioral tests each fish was tested in two different drug conditions, so paired t-tests were used to compare behavioral responses in each. For the between-groups test of latency to feed, a Mann–Whitney test was used because several fish did not feed at all, and the data were therefore not normally distributed.

Results

VT ventricular dose responsiveness

When 200 ng of VT was infused into the 3rd or 4th ventricle, social approach behavior was similarly inhibited 5–20 min after the infusion (3rd ventricle, \( t_{13} = 3.29, P = 0.006; \) 4th ventricle, \( t_{10} = 2.81, P = 0.017; \) Fig. 1). By contrast, social approach was not inhibited by infusions of 40 ng VT into the 3rd ventricle (\( t_8 = 1.23, P = 0.27 \)), but was inhibited when the same dose was infused into the 4th ventricle (\( t_7 = 4.86, P = 0.002 \)). Infusions of 5 ng into the 3rd or 4th ventricle did not inhibit social approach behavior (3rd, \( t_7 = 0.07, P = 0.94; \) 4th, \( t_9 = 1.57, P = 0.15 \)). Ink infusions after testing showed that infusions into the 3rd ventricle resulted in the spread of ink caudally throughout the ventricle system, including the 4th ventricle. However, because ventricular circulation moves rostrocaudally, ink infusions into the 4th ventricle did not diffuse rostrally from the 4th ventricle.

SP behavioral tests

Peripheral injections of 25 \( \mu \)g SP, as with central infusions of VT, inhibited social approach behavior in male and female goldfish tested in spring/summer breeding conditions, although the effects of these peripheral injections appeared strongest 15–30 min after the injections, particularly in males (males; 1st 15 min, \( t_9 = 1, P = 0.34; \) 2nd 15 min, \( t_9 = 4.39, P = 0.002; \) females: 1st 15 min, \( t_9 = 2.42, P = 0.039; \) 2nd 15 min, \( t_9 = 2.68, P = 0.025; \) Fig. 3A and B). The ability of SP to inhibit social approach did not appear to be a secondary consequence of an influence on general activity, as peripheral injections of the same dose of SP did not significantly affect the number of midline crossing in a mixed-sex group of fish tested at the same time of year (1st 15 min, \( t_9 = 0.3, P = 0.77; \) 2nd 15 min, \( t_9 = 1.76, P = 0.11 \); see Fig. 3C).

When male fish were injected with saline on both test days and then with saline or SP 45 min later on different test days in an experiment conducted during the fall, a higher dose (50 \( \mu \)g) of SP again inhibited social approach behavior 15–30 min after the injection (\( t_7 = 3.44, P = 0.011; \) Fig. 3D). Sendide or saline did not significantly affect social approach during the same time interval (15–30 min after an injection of saline and thus 60 min after the Sendide injection; \( t_9 = 0.17, P = 0.87; \) Fig. 3D). However, when fish were injected with SP 45 min after an initial injection of Sendide or saline, they spent significantly more time in proximity to the stimulus fish 15–30 min after the SP injection than they did when the SP injection followed an initial saline injection (\( t_9 = 2.79, P = 0.027; \) Fig. 3D). Thus, Sendide blocks the behavioral effects of peripheral SP.

VT/SP behavioral interactions

Intraperitoneal injections of Sendide (50 \( \mu \)g) completely blocked the ability of centrally administered VT to inhibit social approach in males and females; 200 ng VT infused centrally significantly inhibited social approach in fish injected i.p. with saline 45 min before the central infusions (males; \( t_{11} = 3.98, P = 0.002; \) females; \( t_{11} = 2.6, P = 0.025 \)), but not in fish injected i.p. with Sendide 45 min before the central infusions (males; \( t_{10} = 0.09, P = 0.93; \) females, \( t_9 = 1.23, P = 0.25; \) Fig. 4A and B). Because Sendide may cross the blood–brain barrier, we also tested if central infusions of a smaller dose of Sendide could block the behavioral effects of centrally administered VT. When 500 ng of Sendide was infused into the 3rd ventricle 45 min before central saline and VT infusions, VT still significantly inhibited social approach behavior (\( t_7 = 3.23, P = 0.014; \) Fig. 4C).

VT feeding test

When two groups of fish were tested in a novel environment, VT tended to decrease the latency to feed (mean ± SEM: saline, 947 ± 165 s; VT, 574 ± 159 s), though the effect was not significant (\( U = 46, P = 0.12 \)). Likewise, there was not a significant effect of VT on the latency to initiate feeding in a group of fish that was infused with saline on one day and VT on the other after multiple experiences.
feeding in the test environment (saline, 566.8 ± 201 s; VT, 705.7 ± 231 s; \(t_7 = 0.13, P = 0.9\)).

Discussion

We have demonstrated a novel mechanism through which the neuropeptide VT can influence social behavior in vertebrates, one that is initiated within the hindbrain and that depends on peripheral processes mediated by neurokinin receptors. We propose that VT stimulates SP cells in the DMV, which then influence peripheral processes that feed back to the brain to affect the animal's behavior during the ongoing social engagement. We cannot yet say what the specific peripheral changes associated with elevations of central VT and peripheral SP are in goldfish, but they may include changes in blood pressure, as central VT and peripheral SP increase blood pressure in another teleost, rainbow trout (Le Mevel et al., 1993; Kagstrom et al., 1996). Additionally, cells in the DMV project to the heart in goldfish (Morita & Finger, 1987), and SP terminals are present in teleost cardiac ganglia (Davies et al., 1994). It is therefore also possible that the VT-wrapped SP cells innervate the cardiac ganglia and directly influence heart rate in goldfish. SP can inhibit post-ganglionic acetylcholine cell activity in vertebrates (Cuevas & Adams, 2000; Zhang et al., 2001), which means the social effects of central VT in goldfish could ultimately depend on SP-mediated parasympathetic

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Fig. 2. Immunocytochemical characterization of a VT terminal field in the goldfish hindbrain. VT fibers sweep dorsomedially into the dorsal motor vagus (DMV) and innervate a population of large cells (A; VT fibers are red; nissl-stained cells are green; arrow indicates region shown in the box in the inset). Those cells are backfilled by i.p. injections of fluorogold, indicating they project into the periphery (B; VT fibers are red, backfilled cells are blue; midline is on the left). The VT fibers encapsulate SP-immunoreactive cells (C, midline on the right; D, midline on the left; SP is red, VT is green). The inset in (D) is a confocal zoom of the area indicated by the arrow. Scale bars in the lower left corner are all 50 \(\mu\)m; the scale bar in the insert in D is 5 \(\mu\)m.
modulation. Consistent with this hypothesis, we have observed that blocking the parasympathetic system, as with infusing VT into the brain, inhibits social approach behavior (unpublished data).

However, the neurokinin 1 receptor antagonist did not stimulate social approach behavior, which indicates that the endogenous VT–SP pathway was not engaged during those behavioral tests. Although we have found that central infusions of a V1 receptor antagonist stimulate social approach behavior in male and female goldfish (Thompson & Walton, 2004; unpublished data), thus indicating that endogenous VT does influence levels of sociality in both sexes in this species, they only do so if the fish are tested at the height of the breeding season, which is typically in May and early June. It is therefore possible that our experiments with Sendide, which were all completed between October and March, were done before the endogenous VT–SP pathway was fully functional. It is also possible that the stress associated with the extra handling and injections used in these experiments masked any influences of that pathway on social approach behavior. Alternatively, it may be that the peripheral feedback associated with neurokinin receptor activation is necessary for central VT to exert its behavioral effects, but not part of a serial circuit through which endogenous VT regulates sociality. Future studies testing the effects of a V1 antagonist delivered to the hindbrain and of peripheral Sendide on social approach in differing environmental and social contexts will therefore be necessary to determine if and how the endogenous VT–SP pathway contributes to the regulation of sociality, as will tests of how selectively knocking out hindbrain SP cells affects social approach behavior and responsiveness to exogenous VT.

Central VT inhibited approach responses to other goldfish, but not approach responses to food. This suggests that if the behavioral effects of VT do depend on the serial activation of SP cells in the hindbrain and subsequent changes in body state, then that pattern of peripheral changes selectively influences social processes. At the very least, it indicates that central VT does not inhibit social responses to other goldfish through a general anxiogenic mechanism which, if activated,

![Figure 3](https://example.com/figure3.png)

**Fig. 3.** The effects of peripheral substance P (25 µg) on social approach behavior, measured as the time spent in proximity to the visual stimulus of a conspecific (mean ± SEM) in males and females tested during the breeding season (A, B), and on activity, measured as the change in the number of midline crossings before and after injections (mean ± SEM), in a mixed-sex group of fish (C). Intraperitoneal injections of 25 µg SP significantly inhibited social approach in males, but did not significantly affect general activity. In tests done during the non-breeding season, subjects spent significantly less time in proximity to a stimulus fish 15–30 min after injections of 50 µg SP (which was given 45 min after an initial i.p. injection of saline) than they did after receiving a second saline injection (D). Sendide did not significantly affect social approach responses during the same period, but fish injected i.p. with SP 45 min after a previous injection of Sendide spent significantly more time near a stimulus fish than when injected with SP after a previous injection of saline. **P < 0.01, *P < 0.05.**
should have also increased latency to feed, a behavioral index of anxiety in teleost fish as well as in mammals (Rehnberg et al., 1989; Merali et al., 2003). Although some studies have suggested that increasing levels of VP may reduce anxiety (Appenrodt et al., 1998; Everts & Koolhaas, 1999), the bulk of evidence indicates that VP is anxiogenic (Landgraf et al., 1995; Liebsch et al., 1996; Griebel et al., 2002; Bielsky et al., 2004, 2005), and it is possible that some of the influences of VP on social behavior, particularly non-aggressive social investigation, are a secondary consequence of heightened anxiety (Beiderbeck et al., 2007). By contrast, VP effects on social recognition and affiliation processes do not appear to be related to the peptide’s influence on anxiety (Bielsky et al., 2005; Hammock & Young, 2005; Hammock et al., 2005). In birds, VT neuropeptidation within the septum affects neuronal responsiveness to non-social stressors as well as to social challenge, and so could influence states related to general anxiety and/or stress-coping strategies (Goodson & Evans, 2004), although some VT neurons in the bed nucleus of the stria terminalis appear to respond selectively to positive social stimuli and thus likely modulate specific social behaviors (Goodson & Wang, 2006). Together, these studies suggest that VT/VP modulate general stress circuits that may provide an internal signal related to the context of the social interaction as well as dedicated social circuits, and that the particular behavior exhibited by an individual during any given social interaction likely depends upon an interaction between the two. Although our current data suggest that VT effects on social approach in goldfish are not a secondary consequence of peptide influences on anxiety, additional tests of central, particularly hindbrain, VT influences on other indexes of anxiety should be done to determine more conclusively if this particular circuit is truly dedicated to the regulation of social processes.

VT and VP projections to the hindbrain, which originate, at least in rats, from cells in the paraventricular nucleus of the hypothalamus (Sawchenko & Swanson, 1982; De Vries & Buijs, 1983), are among the most conserved in vertebrates. Peptide actions in the hindbrain that affect simple social approach/withdrawal responses could therefore be widespread across species. If so, it would suggest that VT actions within this circuit are part of a primitive mechanism through which it influenced social behavior in vertebrates. Because approach/withdrawal processes underlie most if not all social interactions, this circuit could have preadapted VT and related peptides to play increasingly complex roles in the regulation of species-specific forms of social behavior. In most vertebrates, however, hindbrain VT/VP terminals are predominately found in visceral sensory areas, not motor areas. However, VP receptor (V1b) gene expression does occur in the DMV in rats (Vaccari et al., 1998), and VP can influence the physiology of cells in this area (Mo et al., 1992). VT/VP influences on parasympathetic motor output could therefore be more widespread than previously thought and, if so, be part of a conserved vertebrate mechanism through which these peptides influence peripheral processes and, potentially, social approach behaviors. It is also possible that the ability of VT/VP to affect social approach by influencing hindbrain autonomic regulatory mechanisms is generally conserved, but that the specific cellular mechanisms through which they induce changes in peripheral body states and thus behavior differ across species. For example, VT/VP release within sensory hindbrain areas, which lowers baroreflex set points and thus increases sympathetic outflow, could also be tied to social regulation (Porges, 2001). In fact, the specific pathway through which we propose VT hindbrain actions inhibit social approach behavior in goldfish may be unique, as we are unaware of any other species in which VT/VP terminals in the hindbrain have been shown to interact with SP cells. It will obviously be important to look specifically for such interactions in other species to test that hypothesis.

Most of the research generated by William James’ proposal that emotions depend on peripheral body states has focused on the nature of that relationship in humans, the only species that can verbally report subjective emotional experiences (James, 1884). Although studies in patients with reduced or non-existent autonomic feedback capabilities have shown that peripheral feedback is not necessary to feel emotions (Heims et al., 2004), it does appear to be related to the intensity of emotional experience (Pollatos et al., 2007), and there may even be unique peripheral response patterns that influence specific emotional experiences.
states (Rainville et al., 2006). It is possible that the ability of body state to influence emotional experience evolved from basic mechanisms through which peripheral responses to emotional stimuli, including conspecifics, particularly in reproductive and/or aggressive contexts, influenced behavior. Our data suggest that central VT can selectively influence social behavior through a mechanism involving a peripheral feedback loop initiated in the hindbrain like the one James proposed affects subjective emotional experience. If this mechanism has been conserved during vertebrate evolution, it might therefore not only participate in the regulation of social behavior in other extant species, perhaps modulating the intensity of stereotypical behaviors affected by peptide actions in more derived forebrain circuits, but also contribute to subjective emotional experiences in species that have the neuronal capacity to interpret the peripheral changes induced by hindbrain peptide actions. Although we have not yet identified the stimuli that drive VT release in goldfish brains, the VT/VP system is responsive to social stimuli in other vertebrates (Delville et al., 2000; Ebner et al., 2005; Goodson & Wang, 2006; Gobrogge et al., 2007), so it is possible that social stimuli induce unique patterns of peripheral change that influence specific social emotions through actions in such primitive VT/VP circuits.

Because VT/VP do consistently affect social interactions in emotional contexts in vertebrate animals, it has been suggested that disruptions of the VP system in humans may underlie the social/emotional abnormalities associated with autism (Insel et al., 1999; Lim et al., 2005). Indeed, recent studies have shown that, as in other animals, central VP can affect social communication in humans (Thompson et al., 2004, 2006) and that there is linkage disequilibrium between allelic variation in the V1a receptor gene and autism (Kim et al., 2002; Wassink et al., 2004; Yirmiya et al., 2006). However, it is still unclear how that variation is related to difficulties in social/emotional regulation. In parallel, studies have shown that dysfunctions in parasympathetic regulatory mechanisms are associated with autism (Jensen et al., 2006) and that vagus nerve stimulation can alleviate some of the social symptoms associated with the disorder (Warwick et al., 2007). Together, these studies suggest that disrupted autonomic regulatory mechanisms may contribute to those symptoms. In light of the linkages between autism, the VP system and peripheral autonomic feedback, our current finding that the ancestral homolog of VP can influence social behavior through a peripheral feedback mechanism initiated in the hindbrain suggests that disrupted VP functions within this part of the brain, perhaps associated with abnormal V1a expression patterns in visceroaotor motor areas such as the dorsal motor vagus and/or the nucleus ambiguus, could contribute to some of the social/emotional disturbances observed in autistic individuals.

In summary, we have demonstrated that VT can influence social behavior through a novel hindbrain mechanism that involves peripheral feedback mediated by tachykinins. We propose that hindbrain influences on simple social approach responses may be, as originally proposed by Porges (2001), a primitive mechanism through which these peptides influence social behaviors and which could even be part of the neurobiological foundation upon which the capacity for social emotions evolved.

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Abbreviations

DMV, dorsal motor vagus; ICC, immunocytochemistry; SP, substance P; VT, vasotocin; VP, vasopressin.

References


