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In the proper context: Neuropeptide regulation of behavioral transitions during food searching

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Neuromodulation enables transient restructuring of anatomically fixed neural circuits, generating alternate outputs and distinct states that allow for flexible organismal responses to changing conditions. We recently identified a requirement for the neuropeptide-like protein NLP-12, a *Caenorhabditis elegans* homolog of mammalian Cholecystokinin (CCK), in the control of behavioral responses to altered food availability. We showed that deletion of *nlp-12* impairs turning during local food searching while *nlp-12* overexpression is sufficient to induce deep body bends and enhance turning. *nlp-12* is solely expressed in the DVA interneuron that is located postsynaptic to the dopaminergic PDE neurons and presynaptic to premotor and motor neurons, well-positioned for modulating sensorimotor tasks. Interestingly, DVA was previously implicated in a NLP-12 mediated proprioceptive feedback loop during *C. elegans* locomotion. Here, we discuss the modulatory effects of NLP-12 with an emphasis on the potential for circuit level integration with olfactory information about food availability. In addition, we propose potential mechanisms by which DVA may integrate distinct forms of sensory information to regulate NLP-12 signaling and mediate context-dependent modulation of the motor circuit.

Keywords: foraging, motor control, neuropeptide, neuromodulation, synapse

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Introduction

Survival in a changing environment requires the ability to adapt and respond appropriately. Consequently, animals display a remarkable capacity for flexibility in their behavioral responses to changes in either their external environment or their internal physiological state. In many cases, such behavioral changes are mediated through the actions of neuromodulators, such as neuropeptides. Neuromodulators have the capacity to generate alternate behavioral states by reconfiguring hard-wired neural circuits in order to allow alternative paths of information flow through the nervous system.[1-4] We have a growing knowledge of the actions of specific neuromodulators at the level of cellular physiology. In contrast, we have a more limited understanding of how neuromodulatory systems act *in vivo* to alter the activity of intact neural circuits and generate specific behavioral outcomes. Several recent studies utilizing invertebrate model organisms have started to address these questions.[5-8] Here we will focus on recent work from our laboratory describing a requirement for the cholecystokinin-like neuropeptide NLP-12 in a context-dependent *C. elegans* foraging behavior.[9]

Changes in food availability are among the most variable and challenging environmental factors that animals face. Thus, mechanisms that regulate food-seeking behaviors are particularly important for survival. Recently, we reported that the *C. elegans* neuropeptide NLP-12, a homolog of mammalian Cholecystokinin (CCK), coordinates behavioral responses to changes in food availability.[9] Specifically, we discovered that NLP-12 signaling is required for behavioral transitions into a local searching motor program that is triggered by removal from food. Immediately following removal from food wild type *C. elegans* alter their locomotory pattern by increasing high angle turning and restricting movement to their local environment (local or area-restricted

search).[10-13] With more prolonged food deprivation (20–30 minutes), animals transition to a dispersal-like behavior, characterized by long forward runs of movement that carry the animal into previously unexplored territory. We found that *nlp-12* was required for wild type performance of local searching. *nlp-12* deletion produced a significant reduction in high-angle turning during the local search phase of food-searching. Importantly, *nlp-12* deletion did not have significant effects on movement when food was present, raising the interesting possibility that NLP-12 signaling mediates context-dependent modulation of motor circuit activity during local searching.

nlp-12 is solely expressed in the neuron DVA,[9,14,15] an interneuron that is well-positioned to integrate sensory information and shape locomotory behavior. DVA is postsynaptic to PDE dopaminergic neurons that are involved in food-sensing, and presynaptic to both motor neurons and interneurons previously demonstrated to be involved in the control of movement[16] (**Fig. 1A**). We hypothesized that dopaminergic regulation of DVA activity might provide a mechanism for controlling release of NLP-12 during local searching. Consistent with this idea, we found that acute exposure to exogenous dopamine enhances turning in an *nlp-12* dependent manner. Dopamine exposure also decreased NLP-12::Venus fluorescence in the DVA process, suggesting that dopamine exposure elicits NLP-12 secretion from DVA. The NLP-12::Venus fluorescence changes were reversed by mutation of *dop-1*, a D1-like dopamine receptor that we found is also expressed in DVA. Conversely, overexpression of *nlp-12* heightens turning events, inducing a chronic local search-like state (**Fig. 1B, C**). Our findings suggest a model where dopamine-mediated sensory information about food availability shapes context-dependent foraging behavior by regulating NLP-12 release from DVA, thereby modulating motor circuit activity. In the following we discuss important questions raised by our work and related studies in the field.

The interneuron DVA and local search behavior

Prior work has demonstrated that specific classes of olfactory and gustatory neurons – in particular the olfactory neuron AWC – play central roles in monitoring the availability of food in the environment.[17,18] As noted above, sensory information about food availability is also transmitted through mechanosensory activation of dopaminergic neurons (CEP, ADE and PDE).[19] What are the respective contributions of these sensory modalities to local food searching? Elegant Ca^{2+} imaging and behavioral studies have demonstrated that the AWC neuron responds robustly to odor removal, and promotes local searching behavior through synaptic contacts onto interneurons involved in the control of movement (AIA, AIB, AIY).[10,18,20] DVA makes synaptic contacts both onto interneurons that are primary postsynaptic partners of AIA, AIB and AIY, and onto downstream motor neurons (**Fig. 1A**). As noted above, DVA is strongly innervated by the dopaminergic neuron PDE. Mindful of this connectivity pattern, we proposed that changes in food

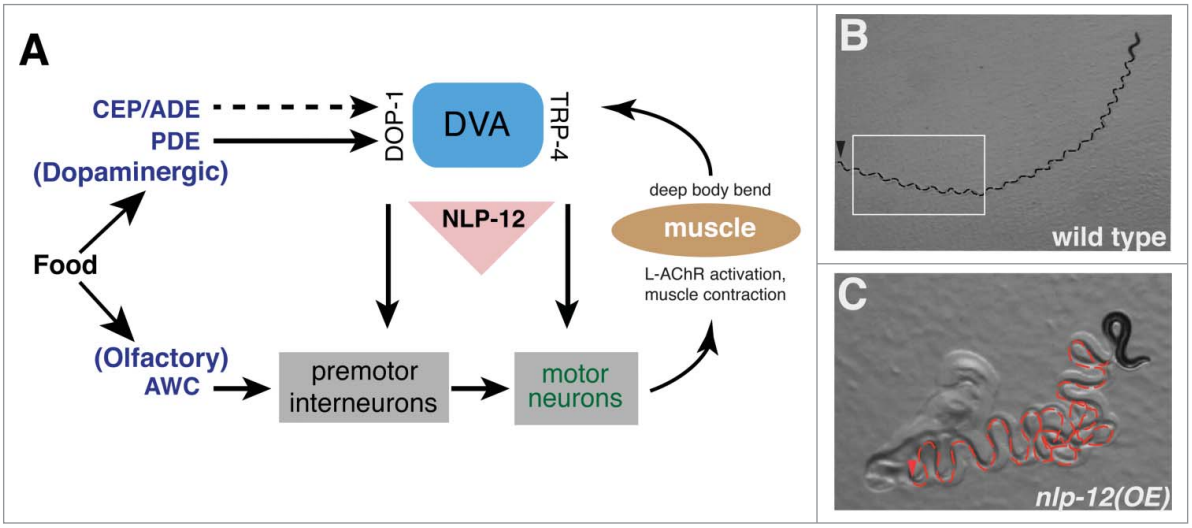

Figure 1. NLP-12 release from DVA modulates locomotion during local search. (**A**) Schematic of neural circuit underlying DVA activation and NLP-12 modulation of local search behavior. See text for details. Synaptic inputs onto DVA from PDE are denoted by a solid black arrow. Potential extrasynaptic actions of dopamine on DVA are represented by a dashed arrow. Solid arrows from DVA denote direct synaptic connections onto premotor (AVA, AVE, RIM, AVB, PVC) and motor neurons (DA, VA, DB, VB). Pink triangle represents potential hormonal actions of NLP-12. Proprioceptive feedback resulting from muscle stretch (L-AChR activation, muscle contraction) involving the mechanosensory channel TRP-4 is represented by the curved arrow. Synaptic connections are as described by White et al. (1986) and wormwiring.org. (**B, C**) Still images of wild type (**B**) and *nlp-12(OE)* (**C**) animals following 30 s movement on NGM agar plates seeded with bacteria. *nlp-12(OE)* refers to a transgenic strain stably expressing high levels of the wild type *nlp-12* genomic sequence. Note the convoluted track of the *nlp-12(OE)* animal (red dashed line) compared to the uniform track (black dashed line) of the wild type animal. The *nlp-12(OE)* image is at 3X higher magnification than the wild type in order to show the movement pattern more clearly. The starting point of each movie is indicated by an arrowhead (wildtype: black; *nlp-12(OE)*: red). The white rectangle in (**B**) shows the approximate size of the still image in (**C**) for comparison.

availability may regulate NLP-12 release through dopaminergic inputs onto DVA from PDE. While direct dopaminergic regulation of DVA activity through synaptic release onto DVA is an appealing idea, it is also possible that other dopaminergic neurons influence DVA activity through an extrasynaptic mechanism. For example, the dopaminergic CEP neurons receive synaptic innervation from the ASK chemosensory neurons, and this could provide a parallel route for dopaminergic control. Control of NLP-12 release via dopaminergic regulation would provide a mechanism for conditional modulation of interneurons and motor neurons that play direct roles in controlling locomotory output. Due to strong synaptic innervation from the AIA, AIB and AIY neurons, the activity pattern of these motor circuit neurons is likely to also be heavily influenced by descending olfactory information about food availability. Thus, one interesting possibility is that olfactory and mechanosensory information about food availability converge at the level of premotor and motor neurons. Notably, recent work demonstrates the activity state of some premotor interneurons downstream of chemosensory neurons can have profound effects on neural responses to odors,[21] providing an additional avenue by which peptide modulation of premotor and motor activity may shape local search responses.

If this is the case, it will be interesting to determine the temporal relationship between these sensory stimuli, motor circuit activity, and local search behavior. The AWC neuron is activated by odor removal, consistent with the notion that AWC activation may act as a trigger for initiation of local search behavior following removal from food.[18] In contrast, dopaminergic neurons seem to be activated directly by the presence of food, making it difficult to understand how signaling through this pathway would modulate behavioral responses to removal from food. One possible explanation is that dopamine-mediated signaling events elicited by food exposure may not terminate immediately upon removal from food. In this case, the activation of dopaminergic neurons in the presence of food may produce a persistent elevation in DVA activity and NLP-12 release that extends beyond the duration of food exposure. Thus, mechanosensory information signaling recent food exposure may act in concert with olfactory information signaling food removal in order to shape local searching behavior.

DVA integration of sensory information

Previous work has suggested that DVA performs a proprioceptive function during locomotion.[22] The transient receptor potential channel TRP-4 (homolog of mechanosensory TRPN channels) is expressed in DVA and deletion of *trp-4* enhances body bend amplitude during forward locomotion. This effect is reversed by either DVA ablation or DVA-specific rescue of *trp-4* expression. Further, manual bending of the worm was sufficient to produce *trp-4*-dependent Ca^{2+} increases in DVA. These results suggested that DVA is activated by stretch in order to modulate body bend depth. A subsequent study provided evidence that cholinergic synaptic release at the NMJ was potentiated by NLP-12 and also required *trp-4*, suggesting that DVA modulation of body bend amplitude may occur through NLP-12 potentiation of neuromuscular transmission.[14]

In our work we used a genetic strategy to increase body bend depth, and discovered a similar requirement for NLP-12 signaling. We engineered a mutation into muscle acetylcholine receptor (L-AChR, where L denotes levamisole-sensitive class) subunits that prolongs receptor activation and heightens synaptic activation of muscles in response to endogenous acetylcholine release. Expression of this gain of function receptor [L-AChR(*gf*)] produces a significant increase in body bend depth during movement (**Fig. 2**). We found that *nlp-12* was required for these behavioral effects. Laser ablation of DVA reversed the exaggerated body bend phenotype of L-AChR(*gf*) expressing animals. In contrast, DVA ablation in wild type worms caused no obvious effect on movement, suggesting that DVA is recruited to modulate locomotion only under certain conditions, for example during the execution of deep body bends–a result consistent with previous evidence indicating a proprioceptive function for DVA. In support of this idea, the enhanced bending that was produced from NLP-12 overexpression was suppressed by a deletion mutation that eliminates L-AChR function in the muscles.

Our findings using the L-AChR(*gf*) receptor provide support for a proprioceptive function for DVA, while our analysis of area-restricted search suggests context-dependent regulation of DVA activity based on food availability. Do these represent distinct functions of DVA? How might DVA distinguish between these sensory cues to appropriately shape behavior? We observed that wild type animals transiently increase their body bend depth following removal from food, perhaps suggesting that the proprioceptive and context-dependent functions of NLP-12 signaling are related. We propose that DVA integrates mechanosensory information encoded by dopaminergic neurons with cell-intrinsic information about stretch (via TRP-4 signaling) in order to shape locomotory output. In this case, the levels and timing of NLP-12 release would be regulated by the activity pattern of DVA and thereby alter behavioral responses accordingly.

A recent study reported an additional neuropeptide signaling system that resides in DVA and functions in sexually dimorphic manner.[23] Males deficient in the neuropeptide nematocin perform poorly in several steps of mating, including difficulty with turning. Either DVA ablation or DVA-specific disruption of the *ntc-1 g*ene in males similarly disrupted mating behavior. Interestingly, other sensorimotor functions of *ntc-1* mutant males were normal, consistent with a specific function of this peptide signaling system in the context of mating. Likewise, *ntc-1* mutant hermaphrodites exhibited no discernable defects in locomotion. Thus, DVA may perform differing functions in the context of the specific behavioral state that is active, perhaps through selective release of peptide neuromodulators such as NLP-12 or NTC-1.

Mechanism of NLP-12 action

Most neuropeptides mediate their actions by activating specific G-protein coupled receptors (GPCR) that act through second messenger signaling pathways to alter neuronal properties over

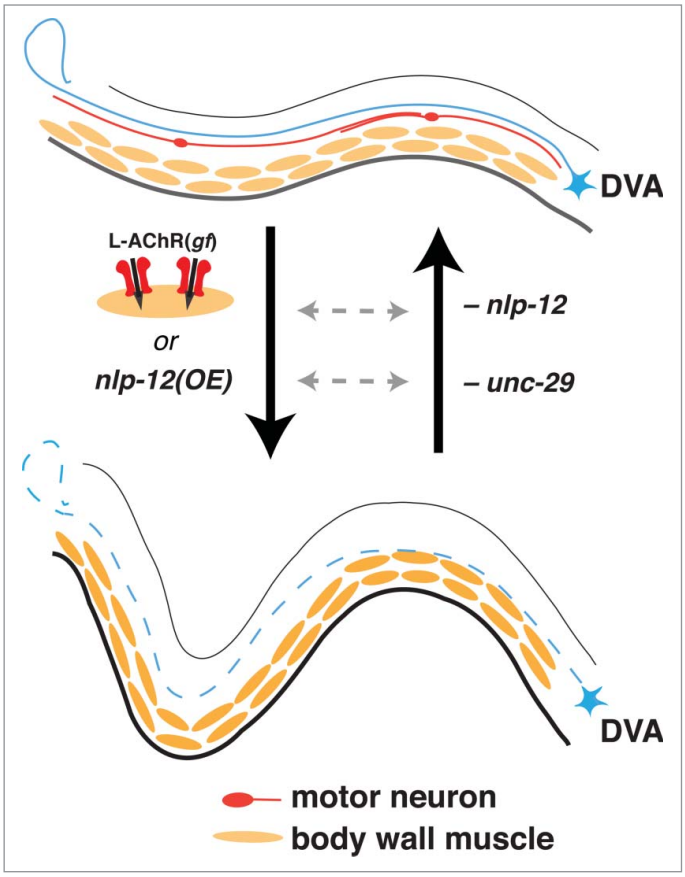

Figure 2. Molecular signals in the control of body bend depth. Cartoon representing genetic manipulations that alter body bend depth. Transgenic expression of L-AChR(*gf*) receptors in muscles or DVA-specific overexpression of *nlp-12* (*nlp-12(OE)*) increase body bend depth. Deletion of *nlp-12* suppresses the locomotory effects of L-AChR(*gf*) expression. Conversely, mutation of the *unc-29* AChR subunit (required for L-AChR function in muscles) suppresses the locomotory effects *of nlp-12* overexpression. See text for additional details.

prolonged timescales compared with fast synaptic transmission. NLP-12 shares sequence similarity with mammalian gastrin/CCK peptides that have important peripheral roles in signaling satiety.[24] CCK is also abundant in the mammalian brain, though precise roles for CCK in modulation of brain circuits are only now beginning to emerge.[25] The effects of CCK in mammals are mediated through 2 GPCRs, CCK1 and CCK2, with distinct though partially overlapping distributions. CCK1 is abundant in peripheral tissues and is present in discrete brain areas, while CCK2 is predominant in the CNS.[26-28] In *C. elegans*, the Cholecystokinin-like Receptor 2 (CKR-2) GPCR was previously shown to bind synthetic NLP-12 peptides with high affinity *in vitro*.[15] Moreover, *nlp-12* and *ckr-2* were similarly implicated in the regulation of fat storage, suggesting that this transmitter system plays conserved roles between nematodes and mammals.[15,29] Likewise, *nlp-12* and *ckr-2* were each required for short-term plasticity in cholinergic synaptic release at the NMJ.[14] *ckr-2* expression in cholinergic motor neurons was required for this effect, supporting the idea that NLP-12 acts via CKR-2 in cholinergic motor neurons to regulate their activity.

In contrast, we found that deletion of the *ckr-2 g*ene did not impair food searching to the same degree as deletion of *nlp-12*, raising the interesting possibility that additional NLP-12 signaling pathways may function either independently or in parallel with CKR-2. Major postsynaptic targets of DVA include several command interneurons (AVE, AVA, AVB, PVC) as well as motor neurons (SMB, VA, VB and DB) that are directly involved in locomotion;[16] thus, GPCRs expressed in these neurons might be prime candidates. In addition, NLP-12 might act in a hormonal manner through volume transmission, affecting GPCRs expressed on neurons that are not direct synaptic partners of DVA. Identifying the precise expression pattern of CKR-2 and any other GPCRs that bind NLP-12 will be essential for distinguishing between these modes of action, and for gaining a complete understanding of how NLP-12 regulates the local food search circuitry. For example, preferential activation of specific GPCR subtypes might underlie context-specific modulatory effects of NLP-12. One interesting possibility is that GPCRs which bind NLP-12 ligand with differing affinities might be differentially active depending upon levels of NLP-12 release.

In conclusion, our recent work in *C. elegans* has revealed a novel role for the NLP-12/CCK transmitter system in regulating behavioral responses to changes in food availability. Further, we demonstrated that NLP-12/CCK release is regulated by dopamine, and provided evidence that dopaminergic regulation of NLP-12 release plays a central role in shaping a context-dependent behavior, local food searching. Interestingly, similar interactions between dopamine and CCK have been described in mammalian neurons. Given the conserved nature of these signaling systems, we anticipate that ongoing investigation of this pathway will provide new insights into fundamental mechanisms that regulate feeding/foraging behaviors, and further, improve our understanding of general principles by which neuromodulators shape circuit activity and promote behavioral flexibility.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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