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

Raja Bhattacharya & Michael M Francis

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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.

**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 searching).
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, 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 (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 dopaminergic regulation of DVA activity might provide a mechanism for controlling release of NLP-12 during local searching.

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. What are the respective contributions of these sensory modalities to local food searching? Elegant Ca٢⁺ 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 (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 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.

**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) involves 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.
sensory neurons can have profound effects demonstrating the activity state of some pre-
esting possibility is that olfactory and
influenced by descending olfactory informa-
trons, the activity pattern of these motor
innervation from the AIA, AIB and AIY
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 neu-
rons that play direct roles in controlling
locomotory output. Due to strong synaptic
innervation from the ASK chemosensory
neurons, the activity pattern of these motor
circuit neurons is likely to also be heavily
influenced by descending olfactory informa-
tion about food availability. Thus, one inter-
esting possibility is that olfactory and me-
chanosensory information about food
availability converge at the level of premotor
and motor neurons. Notably, recent work
demonstrates the activity state of some pre-
motor interneurons downstream of chemo-
sensory neurons can have profound effects
on neural responses to odors, providing an
additional avenue by which peptide modu-
lation 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 cir-
cuit 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 follow-
ing removal from food. In contrast,
dopaminergic neurons seem to be acti-
vated directly by the presence of food,
making it difficult to understand how sig-
naling through this pathway would modu-
late behavioral responses to removal from
food. One possible explanation is that
dopamine-mediated signaling events eli-
cited 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 pro-
duce 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. 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. Fur-
ther, manual bending of the worm was
sufficient to produce trp-4-dependent
Ca++ increases in DVA. These results sug-
gested 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 modu-
lation of body bend amplitude may
occur through NLP-12 potentiation of
neuromuscular transmission.

In our work we used a genetic strategy
to increase body bend depth, and discov-
ered 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 acetyl-
choline 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 con-
trast, DVA ablation in wild type worms
caused no obvious effect on movement,
suggesting that DVA is recruited to modu-
late locomotion only under certain con-
ditions, for example during the execution
of deep body bends—a result consistent
with previous evidence indicating a pro-
 proprioceptive function for DVA. In support
of this idea, the enhanced bending that
was produced from NLP-12 overexpres-
sion was suppressed by a deletion muta-
tion that eliminates L-AChR function in
the muscles.

Our findings using the L-AChR(gf)
receptor provide support for a proprioce-
tive function for DVA, while our analysis
of area-restricted search suggests context-
dependent regulation of DVA activity
based on food availability. Do these repre-
sent distinct functions of DVA? How
might DVA distinguish between these
sensory cues to appropriately shape behav-
ior? 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 informa-
tion 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 dimor-
phic manner. Males deficient in the
neuropeptide nematocin perform poorly in
several steps of mating, including diffi-
culty with turning. Either DVA ablation
or DVA-specific disruption of the ntc-
1 gene in males similarly disrupted mating
behavior. Interestingly, other sensorimo-
tor functions of ntc-1 mutant males were
normal, consistent with a specific function
of this peptide signaling system in the con-
text of mating. Likewise, ntc-1 mutant
ermaphrodites 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 path-
ways to alter neuronal properties over
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 ekr-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 ekr-2 were each required for short-term plasticity in cholinergic synaptic release at the NMJ.14 ekr-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 ekr-2 gene 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; 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.

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.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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