A tale of two receptors

Alison Philbrook, Belinda Barbagallo & Michael M Francis

To cite this article: Alison Philbrook, Belinda Barbagallo & Michael M Francis (2013) A tale of two receptors, Worm, 2:3, e25765, DOI: 10.4161/worm.25765

To link to this article: http://dx.doi.org/10.4161/worm.25765
A tale of two receptors

Dual roles for ionotropic acetylcholine receptors in regulating motor neuron excitation and inhibition

Alison Philbrook, Belinda Barbagallo, and Michael M Francis*
Department of Neurobiology; UMass Medical School; Worcester, MA USA

Nicotinic or ionotropic acetylcholine receptors (iAChRs) mediate excitatory signaling throughout the nervous system, and the heterogeneity of these receptors contributes to their multifaceted roles. Our recent work has characterized a single iAChR subunit, ACR-12, which contributes to two distinct iAChR subtypes within the *C. elegans* motor circuit. These two receptor subtypes regulate the coordinated activity of excitatory (cholinergic) and inhibitory (GABAergic) motor neurons. We have shown that the iAChR subunit ACR-12 is differentially expressed in both cholinergic and GABAergic motor neurons within the motor circuit. In cholinergic motor neurons, ACR-12 is incorporated into the previously characterized ACR-2 heteromeric receptor, which shows non-synaptic localization patterns and plays a modulatory role in controlling circuit function. In contrast, a second population of ACR-12-containing receptors in GABAergic motor neurons, ACR-12\textsubscript{GABA}, shows synaptic expression and regulates inhibitory signaling. Here, we discuss the two ACR-12-containing receptor subtypes, their distinct expression patterns, and functional roles in the *C. elegans* motor circuit. We anticipate our continuing studies of iAChRs in the *C. elegans* motor circuit will lead to novel insights into iAChR function in the nervous system as well as mechanisms for their regulation.

Introduction

A rich diversity of neurotransmitter receptors regulates the excitability of neurons and drives the activity of neuronal networks, ultimately shaping behavior. Ionotropic (nicotinic) receptors for the neurotransmitter acetylcholine (iAChRs) mediate excitatory signaling in nervous systems ranging from nematodes to mammals. Heterogeneity in the composition of iAChRs gives rise to a wide variety of receptor complexes with distinct functional properties. Mammals possess 17 iAChR subunits, and pentameric iAChRs are expressed in both the central and peripheral nervous systems. The contributions of specific receptor types to neuronal communication are determined by functional characteristics and patterns of subcellular localization that vary with receptor subunit composition. While mammalian iAChRs are primarily postsynaptic at autonomic synapses and at the neuromuscular junction, they are mostly localized presynaptically or extrasynaptically in the central nervous system. Accordingly, fast cholinergic transmission drives synaptic activity in the autonomic ganglia and at the NMJ, while iAChRs in the brain may serve primarily modulatory roles. Activation of brain iAChRs underlies the physiological effects of nicotine and is required for its addictive properties. Decrements in the function of specific iAChR subtypes are also implicated in a variety of neurological disorders, including bipolar disorder and Alzheimer disease.

Compared with mammals, the model organism *Caenorhabditis elegans* possesses an expanded family of 29 iAChR subunits, most of which share obvious sequence similarity with specific vertebrate
iAChR subunits. At least 120 of the 302 neurons in the *C. elegans* nervous system are cholinergic, and iAChR subunits are expressed in both neurons and muscles.12 Our laboratory has been studying cholinergic signaling through iAChRs in the context of neural circuitry required for *C. elegans* movement. While the anatomical connectivity of the motor circuit has been reconstructed through serial electron microscopy studies,13 the nature of functional connectivity and the signaling mechanisms involved remain less well understood. Knowledge of the so-called wiring diagram, coupled with the ease of genetic manipulations in *C. elegans*, allows for detailed investigations into functional roles for specific receptor subtypes in the context of an anatomically defined circuit. Our intention here is to summarize our recent work with a particular emphasis on insights gained into mechanisms driving sinusoidal movement and roles for specific iAChR classes in regulating the activity of motor neurons.

*C. elegans* moves by propagating waves of dorso-ventral muscle flexures along the length of the body, resulting in a sinusoidal motor pattern. Cholinergic motor neurons arrayed on the length of the animal receive electrical and chemical synapses from premotor interneurons. In turn, these excitatory motor neurons make extensive synaptic contacts onto body wall musculature, providing the excitatory drive required for muscle contraction and movement. Additionally, cholinergic motor neurons make synaptic contacts onto inhibitory motor neurons that project to opposing musculature. This pattern of anatomical connectivity has led to the notion that opposing cycles of inhibition and excitation are required for generating sinusoidal body bending during movement. However, GABA-deficient mutants remain capable of performing sinusoidal locomotion, albeit with reduced amplitude and at a slower rate than the wild type,14 suggesting that alternative mechanisms drive production of the sinusoidal wave. Recent studies have provided evidence that patterned activation of specific cholinergic motor neuron classes and proprioceptive coupling between motor neurons are both important for propagation of the sinusoidal wave.15,16 However, several important questions remain:

1. How is the activity pattern of motor neurons established and maintained?
2. Are there conditions or behaviors during which inhibitory motor neuron signaling may be more strongly required?
3. What are the molecular mechanisms underlying communication between cholinergic and GABA motor neurons? Recent studies of iAChRs expressed in motor neurons have begun to address some of these questions.

**ACR-2/12 iAChRs modulate the activity of cholinergic motor neurons.** Several early expression profiling studies revealed enhanced expression of genes encoding iAChR subunits in ventral cord motor neurons.17,18 Among these, the *acr-2* gene showed exclusive expression to cholinergic motor neurons.19 The restricted expression pattern of *acr-2* suggested that receptors incorporating this subunit may mediate specialized functions, such as synaptic activation of cholinergic motor neurons by premotor interneurons. To evaluate this idea, we examined whether deletion of *acr-2* altered motor behavior, both under normal conditions and in pharmacological assays that targeted cholinergic signaling.1 We found that deletion of *acr-2* reduced movement velocity and caused resistance to the cholinesterase inhibitor aldicarb. Surprisingly, however, the effects were not as dramatic as would be expected if ACR-2 heteromeric receptors were required for cholinergic motor neuron activation during movement. Taken together with results from another study that reported similar findings,20 our work pointed toward a complexity for iAChR function that had been previously unappreciated in *C. elegans*. We focused our subsequent efforts on distinguishing between alternative models for ACR-2 receptor function. We hypothesized ACR-2 receptors may act similarly to iAChRs in the mammalian brain and play a primarily modulatory role in regulating motor neuron activity. Alternatively, ACR-2 receptors may act redundantly with other receptor types present on cholinergic motor neurons to mediate synaptic activation of cholinergic motor neurons by premotor interneurons.

To further investigate potential functional roles for ACR-2 receptors, we examined the subcellular localization of an ACR-2-GFP fusion protein in cholinergic motor neurons. We observed diffuse fluorescence throughout motor neuron processes without obvious local increases in intensity. As receptors clustered at postsynaptic sites would be expected to display regions of locally increased punctate fluorescence, we interpreted this result to indicate ACR-2 receptors were not primarily localized to synapses. A later study examining the subcellular localization of ACR-2-GFP specifically expressed in the DA9 motor neuron obtained similar results.21 Additionally, we determined that a cholinergic marker (*unc-17::GFP*) was not strongly expressed by premotor interneurons, indicating these neurons were not primarily cholinergic. Thus, communication between premotor interneurons and motor neurons must occur via some other mechanism. Recent work suggests a major component of this signaling occurs at electrical rather than chemical synapses.22,23 Our findings indicate ACR-2 iAChRs primarily act extrasynaptically in cholinergic motor neurons to modulate excitatory tone rather than directly mediating synaptic drive from premotor interneurons. Interestingly, iAChRs in the mammalian brain often regulate neurotransmitter release or neuronal activity from presynaptic or extrasynaptic sites. Extrasynaptic ACR-2 iAChRs may similarly potentiate ACh release onto the muscles and inhibitory motor neurons that are the primary post-synaptic targets of cholinergic motor neurons.

Synaptic outputs from cholinergic motor neurons occur at dyadic synapses. At these specializations, cholinergic motor neurons form en passant contacts onto motor neuron processes and onto membrane extensions from the muscles, called muscle arms.13 While synaptic connectivity between cholinergic and GABAergic motor neurons has been clearly established by electron microscopy, the receptors responsible have not yet been identified. Intriguingly, additional studies of ACR-2 iAChRs supplied a potential candidate. Our work showed expression of a mutated ACR-2 subunit, in which we had engineered an amino acid substitution in the pore-lining transmembrane region of ACR-2, initiated necrotic-like death of cholinergic motor neurons.1
Interestingly, loss-of-function mutations in genes encoding other iAChR subunits expressed in motor neurons (unc-38, unc-63, and acr-12) suppressed this toxicity, suggesting these were essential partnering subunits with ACR-2. Work from Jin and colleagues had identified these same genes in a genetic screen for suppressors of the effects of a previously isolated gain-of-function allele of acr-2. Further, they showed expression of these subunits in Xenopus oocytes, together with ACR-2 and ACR-3, constituted a functional pentameric receptor. We examined the expression of each of these subunits in the nervous system and found that only acr-12 was highly expressed in both cholinergic and GABAergic motor neurons. This observation led us to hypothesize that ACR-12 may contribute to distinct iAChR subtypes in excitatory and inhibitory motor neurons.

ACR-12 contributes to two receptor populations with distinct patterns of localization and functional roles. To explore whether ACR-12 may contribute to iAChRs with functional roles that were distinct from those of ACR-2 iAChRs, we again turned to experiments using aldicarb. Interestingly, we found acr-12 mutants were hypersensitive to the paralyzing effects of aldicarb. In contrast, our previous work had shown acr-2 mutants were slightly resistant to aldicarb. We hypothesized the differing effects of aldicarb across acr-2 and acr-12 mutants may reflect a requirement for ACR-12 iAChR function in GABA neurons where acr-2 is not expressed. Thus, loss of ACR-12 iAChRs may lead to decreased levels of GABA motor neuron activity and reduced inhibitory signaling onto muscles. Consistent with this idea, specific expression of ACR-12 in GABA neurons rescued the aldicarb phenotype while specific expression in cholinergic motor neurons was not sufficient for rescue. To directly examine the requirement for ACR-12 in regulating motor neuron activity, we measured the frequency of synaptic events at the NMJ. Interestingly, we found that deletion of acr-12 significantly reduced the rate of both endogenous excitatory and inhibitory post-synaptic currents (PSCs), indicating loss of ACR-12 iAChRs causes reduced motor neuron excitability and decreased neurotransmitter release at the NMJ. Specific expression of a rescuing acr-12 construct in GABAergic motor neurons was sufficient to normalize the decreased frequency of inhibitory PSCs, providing further evidence that ACR-12 iAChRs act cell autonomously in GABA motor neurons to regulate their activity. But do these ACR-12 iAChRs act in a modulatory fashion similar to what we had previously observed for ACR-2/12 iAChRs in cholinergic motor neurons, or might they instead mediate synaptic connectivity between cholinergic and GABA motor neurons? To address this question, we examined the subcellular localization of ACR-12-GFP in cholinergic and GABA motor neurons. ACR-12-GFP was diffusely distributed in cholinergic motor neurons. This result was similar to what we had previously observed for ACR-2-GFP and consistent with the model that ACR-12 forms non-synaptic heteromeric complexes with ACR-2 in cholinergic motor neurons. Specific expression of ACR-12-GFP in GABAergic motor neurons, however, produced punctate fluorescence, as would be expected if ACR-12 iAChRs were clustering at post-synaptic sites on GABA motor neurons (Fig. 1). Additional support for this idea came from our finding that ACR-12 puncta in GABAergic motor neurons localized opposite synaptic vesicle clusters visualized in cholinergic motor neurons by expression of mCherry-RAB-3.

Our data provided strong evidence that post-synaptic ACR-12-GABA receptor complexes regulate levels of GABA motor neuron activity and inhibitory signaling, but what is their contribution to overall motor circuit function and movement? Deletion of acr-12 caused a more dramatic reduction in movement velocity than we had previously observed for acr-2 deletion. In addition, we noticed variability in the sinusoidal motor pattern of acr-12 mutants, and a small reduction in the amplitude of body bends, phenotypes that were not present in acr-2 mutants. The movement deficits associated with acr-12 deletion demonstrated clear involvement of ACR-12-GABA iAChRs in motor circuit function, but these effects were relatively modest during normal exploratory movement. By comparison, the effects of acr-12...
deletion were more prominent when synaptic acetylcholine levels were increased with aldicarb treatment. These findings led us to propose that specific synapses and/or receptor classes may be differentially activated with changing activity levels. For example, increases in cholinergic motor neuron activity may lead to recruitment of inhibitory motor neurons through activation of ACR-12\textsubscript{GABA} and other iAChRs. This recruitment of inhibitory motor neurons may reinforce the sinusoidal motor program and counterbalance hyperactivation of muscles under particular conditions or during specific behavioral states when cholinergic motor neuron activity is elevated. In contrast, synaptic activation of GABA motor neurons may be less stringently required during normal movement.

Future Studies and Concluding Remarks

Our work to date has uncovered important and previously unrecognized roles for iAChR-mediated signaling in the motor circuit. However, a number of key questions remain unanswered. Most importantly, we would like to gain a more complete understanding of the mechanisms by which cholinergic neurons drive the activity of GABA neurons. A crucial next step toward this will be identifying the full complement of iAChR classes present on GABA motor neurons, including subunits that co-assemble with ACR-12. Two essential subunits of the ACR-2/12\textsubscript{ACh} receptor (ACR-2 and ACR-3) are not expressed in GABAergic motor neurons,\textsuperscript{1,20} suggesting that ACR-12\textsubscript{GABA} receptors have a distinct subunit composition from that of ACR-2/12\textsubscript{ACh} receptors. A complete description of the iAChR types responsible for regulating GABA motor neuron activity will likely require direct measurements of ACh-gated currents from GABA motor neurons. Recently developed methods for culturing cells from post-embryonic animals may facilitate these kinds of detailed patch clamp studies of larval stage motor neurons.\textsuperscript{24} Additionally, calcium imaging or other optical methods for measuring motor neuron activity in intact, freely moving animals may allow further dissection of motor neuron activity patterns during movement and the requirements for specific receptor types.\textsuperscript{15,21,22,27}

Finally, development of additional gain-of-function approaches similar to those used in studies of ACR-2 may prove useful. Ultimately, mutant combinations that completely lack iAChR subunit expression in motor neurons could prove very powerful for teasing apart motor circuit function.

Signaling through mammalian iAChRs has been shown to regulate the activity of inhibitory neurons in various brain regions. For example, iAChR subunits are strongly expressed by GABAergic interneurons in the hippocampus. Cholinergic stimulation can profoundly alter hippocampal interneuron activity and synaptic plasticity, and these effects are strongly dependent on the subunit composition of the iAChRs involved.\textsuperscript{28-30} Our studies to date have revealed similar roles for C. elegans iAChRs in regulating GABA neuron activity, and ongoing work in C. elegans may elucidate general principles by which subunit composition regulates receptor localization and function, as well as define new mechanisms for iAChR regulation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

4. Lendvai B, Vizi ES. Nonsynaptic chemical transmission through nicotinic acetylcholine receptors. Physiol Rev 2008; 88:333-49; PMID:18399166; http://dx.doi.org/10.1152/physrev.00004.2006
8. Leslie FM, Mojica CY, Reynaga DD. Nicotinic receptors in addiction. Alcohol Mol Pharmacol 2013; 83:753-8; PMID:23247824; http://dx.doi.org/10.1124/mol.112.083659
28. Alkondon M, Albuquerque EX. Nicotinic acetylcholine receptor alpha7 and alpha4beta2 subtypes differentially control GABAergic input to CA1 neurons in rat hippocampus. J Neurophysiol 2001; 86:3043-55; PMID:11731559