ARTICLE

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Neurexin controls plasticity of a mature, sexually dimorphic neuron

Michael P. Hart[1] & Oliver Hobert[1]

During development and adulthood, brain plasticity is evident at several levels, from synaptic structure and function to the outgrowth of dendrites and axons. Whether and how sex impinges on neuronal plasticity is poorly understood. Here we show that the sex-shared GABA (γ-aminobutyric acid)-releasing DVB neuron in *Caenorhabditis elegans* displays experience-dependent and sexually dimorphic morphological plasticity, characterized by the stochastic and dynamic addition of multiple neurites in adult males. These added neurites enable synaptic rewiring of the DVB neuron and instruct a functional switch of the neuron that directly modifies a step of male mating behaviour. Both DVB neuron function and male mating behaviour can be altered by experience and by manipulation of postsynaptic activity. The outgrowth of DVB neurites is promoted by presynaptic neurexin and antagonized by postsynaptic neuroligin, revealing a non-conventional activity and mode of interaction of these conserved, human-disease-relevant factors.

Experience modifies the structure and function of neurons and circuits in the brain through multiple mechanisms of neuronal plasticity[1,2]. Plasticity in adult brains refines circuits in response to experience in order to mediate adaptation and homeostasis, and as a cellular correlate of learning and memory[1,3,4]; this type of plasticity includes extension and retraction of dendrites and axons[5–7]. The molecular mechanisms that underlie morphological plasticity in adult neurons are not well understood. Similarly, though the sexual identity of an organism influences the function and plasticity of its nervous system, the molecular and cellular bases of such sexual dimorphism are also not fully understood.

Morphological plasticity in adult male DVB neuron

The GABAergic motor neuron/interneuron DVB is located in the tail of *C. elegans* and projects anteriorly in the ventral nerve cord in both sexes (Fig. 1a). We used fluorescent reporter gene technology to visualize DVB and found that it displays extensive post-developmental morphologic plasticity exclusively in males, characterized by the progressive extension of new neurites posteriorly into the tail (Fig. 1b; Extended Data Fig. 1). The total neurite length and the number of neurite junctions increase significantly ($P < 0.001$) from day 1 to day 5 of adult life (Fig. 1c, d). The branching pattern of male DVB neurites lacks any overt stereotypy (Extended Data Fig. 2a, b). The generation of new DVB neurites in males is accompanied by the addition of presynaptic boutons containing the synaptic marker RAB-3, suggesting that these neurites are axon-like (Fig. 1b, Extended Data Fig. 1); electron microscopy analysis supports this conclusion[8,9]. We have not identified other neurons that undergo comparable neurite outgrowth in adulthood (Fig. 1b, Extended Data Fig. 2c–h).

Dimorphic DVB connectivity influences behaviour

In hermaphrodite worms, DVB controls defecation behaviour[10]; in males it also contributes to protraction of the male-specific spicule structures, which are inserted into the hermaphrodite vulva during copulation[11] (Fig. 1e–g). Consistent with a sexually dimorphic function, the synaptic wiring pattern of DVB is also notably sexually dimorphic[8,9] (Fig. 1g). To test for functional roles of DVB neurite outgrowth, we examined DVB function over the period of DVB neurite outgrowth. Day 1 males have been shown to protract their spicules briefly following the expulsion step of defecation, owing to connections between defecation and spicule circuits[11]. This seemingly pointless protraction can result in chronic protraction of spicules, which is detrimental to male mating ability. We found that day 1 males, but not day 3 males, frequently protracted spicules during expulsion[12] (Extended Data Fig. 3b). To determine whether DVB was involved in this change, we silenced DVB using expression of a histamine-gated chloride channel (*lim-6^{int4}::HisCl1* with histamine), which resulted in increased protraction of spicules with expulsion at day 3 (Extended Data Fig. 3b). The time between consecutive expulsions was unchanged between day 1 and day 3 in controls, but slightly increased in DVB-silenced day 3 males (Extended Data Fig. 3c). These results suggest that DVB plays a role in reducing expulsion-associated spicule protraction during the period of neurite outgrowth, probably through inhibition of spicule circuit components that connect with the defecation circuit. Moreover, laser ablation of DVB in day 1 males (Extended Data Fig. 3d) resulted in a reduction in the number of males with chronically protracted spicules compared to controls, whereas ablation of DVB on each day after day 2 resulted in a progressive increase in worms with chronically protracted spicules (Fig. 2a). Thus, DVB contributes to spicule protraction at day 1 and inhibits spicule protraction after day 2, with a functional consequence of suppressing spicule protraction during expulsion.

We validated these findings using expression of channelrhodopsin in DVB (Extended Data Fig. 3e). Light-induced activation of DVB in day 1 adult males resulted in observable movement of spicules, whereas activation of DVB at day 5 resulted in only rare movement of spicules (Fig. 2b, Supplementary Video 1). Expression and activation of channelrhodopsin in the spicule protraction neurons and muscles always resulted in spicule protraction at days 1 and 5 (Fig. 2b, Supplementary Video 2, Extended Data Fig. 3f). The fraction of male worms exhibiting spicule movement after channelrhodopsin-mediated DVB activation at day 1 was unchanged in males lacking GABA signalling components (*unc-25*/GAD or *unc-49*/$GABA_A$ receptor mutants; Fig. 2c), indicating that DVB may signal through electrical connections and/or neuropeptides[11]. Although DVB neurite outgrowth was not affected in *unc-49* mutants, these worms did show a reduction in

[1]Department of Biological Sciences, Howard Hughes Medical Institute, Columbia University, New York, New York, USA.