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Molecular mechanisms underlying sexual differentiation of the nervous system

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A long-standing goal in developmental neuroscience is to understand the mechanisms by which steroid sex hormones pattern the mammalian central nervous system along male or female pathways to enable subsequent displays of sexually dimorphic behaviors. In this article, we review recent advances in understanding the epigenetic and transcriptional mechanisms mediating sexual differentiation of the brain in mammals, flies, and worms. These studies suggest a model of sexual differentiation wherein master regulators of sex determination initiate a cascade of sexually dimorphic gene expression that controls development of neural pathways and behavioral displays in a strikingly modular manner. With these advances in molecular genetics, it is now feasible to disassemble different components of sexually dimorphic social behaviors without disrupting other behavioral interactions. Such experimental tractability promises rapid advances in this exciting field.

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Introduction

Sexually reproducing species exhibit sex differences in social interactions, presumably to enhance reproductive success and survival of progeny. Many of these differences are acquired traits that depend on experience. Nevertheless, a core set of sex-typical behavioral displays such as mating, territorial aggression, and parental care, although modifiable by experience, are innate in the sense that they can be displayed without prior training. These sexual dimorphisms in innate behavior reflect the action of a sexually differentiated nervous system [1–3]. While the chromosomal and endocrine mechanisms mediating sexual differentiation can vary across taxa, such mechanisms converge to regulate sexually dimorphic gene expression that in turn guides sex-typical developmental trajectories and adult function of neural pathways [4]. The molecular mechanisms underlying these sexually dimorphic gene expression programs in the central nervous system remain poorly understood. Because sexual differentiation in mammals depends on the long-lasting actions of early life exposure to steroid hormones, it is likely that epigenetic mechanisms play an important role [5,6]. In this review, we highlight recent advances in understanding the transcriptional and epigenetic mechanisms of sexual differentiation of the central nervous system. We illustrate these advances with examples from flies, worms, and mice, and we discuss how modern molecular and genetic approaches should enable unprecedented insights into sexual differentiation of the nervous system.

Development and function of sex-specific circuits in *Caenorhabditis elegans*

The nematode worm *C. elegans* is unique in allowing investigation of sexual differentiation at the level of individual identified neurons, as its nervous system is numerically simpler and mapped in great detail [7,8], with 294 neurons shared between both sexes, 8 neurons that are hermaphrodite-specific, and 91 neurons that are male-specific. These sex-divergent neurons enable molecular dissection of sex-specific neural pathways that mediate specific adaptive behavioral programs in this organism. Hermaphrodites have two X chromosomes whereas males have one (XO), and the number of X chromosomes (or X-to-autosome ratio) determines the sex of the worm [9]. Sexual differentiation occurs in a largely cell-autonomous manner under control of the zinc-finger transcription factor TRA-1 [10–12]. In hermaphroditic cells, TRA-1 represses male-specific genes. In the male, TRA-1 is degraded by proteolysis, allowing development of the male phenotype [13,14]. Importantly, as a result of this system sexually mosaic animals can be generated by regulating TRA-1 expression in specific cells [15]. This allows the generation of 'male' neurons in hermaphrodites, or vice-versa, allowing elegant dissection of sex-specific circuits.

Remarkably, in addition to sex-specific neurons, male and hermaphrodite *C. elegans* also possess shared neurons that nevertheless form sex-specific projections and synapses. Recent studies by Oliver Hobert's group have made

progress in describing the cellular and transcriptional mechanisms that mediate the development of sex-specific connections of shared neurons [16••]. The shared PHB sensory neuron forms synapses on to three downstream interneurons in hermaphrodites, while in males it forms synapses on to a different shared interneuron as well as male-specific motor, sensory and inter-neurons. These sexually dimorphic connections appear to arise by at least two distinct mechanisms. PHB efferent synapses develop in both males and hermaphrodites at early larval stages but connections to different shared interneurons are selectively lost in the two sexes ('selective pruning'). In some other neurons, connections are formed only in larval males and these persist in adult males, providing evidence for a 'pre-patterning' mechanism for circuit wiring. PHB neurons mediate sex-specific functions: disruption of the PHB neuron altered locomotion and the behavioral response to noxious chemicals in hermaphrodites, whereas it reduced the ability of males to mate. Sex-specific synapse pruning of PHB connections is cell-autonomous since masculinization or feminization of this neuron elicited pruning of the connections appropriate to the sex of the PHB neuron and not to the sex of the post-synaptic cells. Remarkably, the authors found that the sex of the post-synaptic neurons was also sufficient to lead to rewiring of PHB connectivity such that it was now appropriate to the sex of the post-synaptic neuron. In this latter instance, the authors showed that such rewiring was dependent on the expression of DMD-5 and DMD-11, members of the DM family of transcription factors that govern sex determination or differentiation across metazoans [4,16••]. It will be interesting to understand the mechanisms whereby such selective sexual differentiation of either a sensory neuron or its post-synaptic partner leads to re-wiring of the pathway.

Work by Hobert's group on the sex-shared PHC neurons provides new molecular insights into repurposing of shared neurons [17••]. PHC neurons appear to be typical sensory neurons in hermaphrodites, with connections to a limited number of shared neurons, that are required for response to harsh touch. By contrast, PHC neurons resemble hub neurons in males, with extensive connectivity with male-specific neurons and to different shared neurons, and they are required for specific aspects of the male mating ritual. This sex-specific connectivity is controlled to a large extent cell-autonomously because altering sexual identity of PHC by manipulating expression of TRA-1, the master regulator of sexual differentiation in worms, rewires connectivity of this neuron accordingly. In keeping with the greater connectivity of male PHC neurons, there is a corresponding transcriptional scaling of many components of the synaptic machinery, including the vesicular glutamate transporter EAT-4. Expression of EAT-4 was dependent on shared transcriptional factors as well as sexually dimorphic utilization of *cis*-regulatory elements that repressed expression in hermaphrodites and boosted it in males. Remarkably, the neuritic arbor of as well as transcriptional scaling in male PHC neurons was regulated cell autonomously by DMD-3, yet another member of the DM family of transcriptional regulators. Whether DMD-3 acts directly by binding to the regulatory elements of the *eat-4* locus is unknown.

Elegant studies in *C. elegans* point the way to a systems-level understanding of how internal states such as hunger and age interact with shared circuitry in a sex-specific manner to enable presumptively adaptive male or hermaphrodite-specific responses [18••]. Adult hermaphrodites show a stronger attraction toward the compound diacetyl (a food cue) than do males. This attraction is mediated by two pairs of sex-shared neurons (AWA and AWC) in hermaphrodites but by AWC in males. This is associated with sex-specific expression of the odorant receptor ODR-10 in hermaphrodite AWA neurons. This sex difference only exists in well-fed adults. Larval AWA neurons of both sexes express this chemoreceptor, and both males and hermaphrodites show equivalent attraction to diacetyl. Food deprivation upregulates *odr-10* in male AWA neurons and increases food-seeking behavior at the expense of seeking mates. Genetically specified circuits can thus be modulated dynamically by external cues to regulate sex differences in behavior; it will be interesting to identify the signaling cascades that modulate chemoreceptor expression in an age and nutritional status dependent manner. Sex-shared sensory neurons can also guide sexually dimorphic responses to pheromones in worms. Males but not hermaphrodites are attracted to ascarosides, components of the worm cuticle that can act as pheromones [19]. This attraction is mediated by a pair of sex-shared sensory neurons (ADF), and feminization of these neurons results in males being repelled by rather than attracted to ascarosides [20]. The ability of ADF to detect and mediate attraction to ascarosides was dependent on the transcriptional regulator MAB-3, a founding member of the DM family of transcription factors. How MAB-3 regulates ADF function is presently unknown.

Together, these pioneering studies in worms show how sexually dimorphic wiring and behaviors can emerge from neurons present in both sexes. Moreover, they reveal a profound sexually dimorphic reconfiguration of morphology and function of shared sensory neurons (PHA, PHB, ADF, AWA). Whether such sexually dimorphic reconfiguration is present in other worm sensory neurons, and more generally, is common to sensory neurons in flies or mammals is an open question.

Sexual differentiation in *Drosophila*: the search for isoform- and sex-specific targets of master transcriptional regulators

In the fruit fly *Drosophila melanogaster*, sexual determination is chromosomal as in the worm, such that the number