Review TRENDS in Neurosciences Vol.29 No.8

Blueprints for behavior: genetic specification of neural circuitry for innate behaviors

Devanand S. Manoli, Geoffrey W. Meissner and Bruce S. Baker

Medical Scientist Training Program, Neurosciences Program and Department of Biological Sciences, Stanford University, Stanford, CA 94305-5020, USA

Innate behaviors offer a unique opportunity to use genetic analysis to dissect and characterize the neural substrates of complex behavioral programs. Courtship in *Drosophila* involves a complex series of stereotyped behaviors that include numerous exchanges of multimodal sensory information over time. As we will discuss in this review, recent work has demonstrated that male-specific expression of Fruitless transcription factors (Fru^M proteins) is necessary and sufficient to confer the potential for male courtship behaviors. Fru^M factors program neurons of the male central and peripheral nervous systems whose function is dedicated to sexual behaviors. This circuitry seems to integrate sensory information to define behavioral states and regulate conserved neural elements for sex-specific behavioral output. The principles that govern the circuitry specified by Fru^M expression might also operate in subcortical networks that govern innate behaviors in mammals.

Introduction

> 'When, as by a miracle, the lovely butterfly bursts from the chrysalis full-winged and perfect,... it has, for the most part, nothing to learn, because its little life flows from its organization like melody from a music box.' – Douglas A. Spalding (1873)

A major goal of neuroscience is to understand in molecular detail how neural circuits are built and subsequently function to permit individuals to perceive the world and carry out specific behaviors based on those perceptions. To gain insights into these issues, neuroscientists have generally either attempted to understand nervous system structure and function from studies of its elementary molecular and cellular components, or examined neural functions and behaviors in intact animals and attempted to relate these to large systems of neurons. Although both approaches have had many notable successes, it has not been clear how the knowledge collected at these two levels can be unified. Here, we focus on recent findings suggesting that developmental genetic and neurogenetic approaches to identifying the neural circuits underlying specific innate behaviors can bridge this gap.

The innate nature of many basic fixed action patterns and fairly invariant species-specific animal behaviors suggests that the underlying neuronal substrates necessary for their execution are genetically determined and developmentally programmed [1]. This makes innate behaviors particularly attractive systems for study, because genetic approaches can be exploited to address questions such as: What does it mean to 'genetically program' a behavior? And what are the elemental computations that behavioral circuits must execute? For example, how are innate behaviors elicited by specific environmental cues? How are sequential motor programs coordinated?

We begin with a brief summary of sex-developmental pathways in the regulation of *Drosophila* courtship, as background for recent work implicating transcription factors encoded by the *fruitless* (*fru*) gene as the crucial components that specify the neural substrates of *Drosophila* sexual behavior. We then discuss the implications of the findings that Fruitless-expressing neurons function at all levels of processing underlying this behavioral program. Based on these organizing principles, we briefly proceed to recent studies in vertebrates that implicate distinct genetically specified circuits – many of which operate through hypothalamic axes – in distinct programs for innate behaviors. Taking these findings together, we suggest that common principles govern the specification and organization of circuitry that underlies complex innate behavioral programs.

Male courtship behavior: the biological system

Sexual reproduction in many species is preceded by elaborate stereotyped courtship behaviors. In *Drosophila* courtship, the male engages in a series of actions including orienting towards and following the female, tapping her with his forelegs, singing a species-specific courtship song by vibrating one of his wings, licking the genitalia of the female, and curling his abdomen to attempt copulation [2,3]. Female behavior consists largely of avoidance and rejection. However, if unmated and sufficiently stimulated by male courtship, the female will slow down, open her vaginal plates and allow copulation.

Courtship involves the integration of stimuli from multiple sensory modalities over time into meaningful, multistage, behavioral output during the courtship ritual. Thus, a male recognizes a female via both visual and olfactory cues. Tapping supplies gustatory information, and auditory information is central to song; during licking the male

Corresponding author: Baker, B.S. (bbaker@pmgm2.stanford.edu).
Available online 27 June 2006

 0166-2236/$ – see front matter doi:10.1016/j.tins.2006.06.006

probably receives gustatory and/or olfactory information, and tactile information directs copulation itself.

Male courtship behavior is largely innate: males know how to court without exposure to another animal and the steps comprising courtship nearly always occur in the aforementioned order. One aspect of courtship – discrimination between suitable mates (i.e. virgin females or females who have not recently mated) and unsuitable mates (i.e. recently mated females or young males) – is modifiable by experience. The robustness and complexity of male courtship behavior, coupled with the fact that it contains both stereotyped and modifiable behavioral components, make it amenable to analysis of sensory processing, sensory integration, coordination of motor programs, and motor output, and also to studies of learning, memory and other experience-dependent behavioral modifications.

Courtship and fruitless*: developmental studies*

The initial characterization of *fru* mutants revealed that they had defective male courtship behavior, but otherwise appeared normal [4–6]. The recognition that *fru* had a central role in courtship behavior stemmed from the finding that the known genes in the sex-determination hierarchy were insufficient to account for how this hierarchy controlled male sexual behavior, thus implicating an additional gene. Ryner *et al.* used molecular knowledge of the sex hierarchy to identify this missing gene and showed it was *fru* [7,8]. Concurrently, Ito *et al.* isolated *fru* as a gene necessary for male courtship based on neurogenetic approaches [9].

fru is a large complex gene (~140 kb) with four promoters (P1–P4) and alternative splicing near both ends of the transcripts [8,10]. Substantial genetic, molecular and phenotypic analyses showed that only the P1 *fru* transcripts are sex-specifically spliced as part of the sex-determination hierarchy, and strongly suggested that only the P1 *fru* products are essential for male courtship behavior [1]. Male-specific P1 *fru* transcripts encode Fru^M proteins, which have a BTB dimerization domain and one of three alternative pairs of zinc fingers, and are thus probably transcription factors. In females, alternative splicing together with translational repression result in the absence of P1-derived proteins [10,11]. Here, we will focus on the P1 *fru* products.

Analysis of various *fru* mutants showed that flies lacking P1-encoded proteins are viable, and male courtship behavior is abolished while other behaviors are normal [9,12–15]. These analyses also established that Fru^M proteins are required for the proper execution of all steps of courtship, from the initial recognition of a potential mate to the transfer of seminal fluids and sperm.

Initial expression studies suggested that Fru^M proteins are expressed exclusively in the CNS, where they are first detectable in a limited number of cells at the end of the third larval instar [8,11,13]. Expression is maximal about two days into the pupal period, when ~2000 cells (~2% of the CNS) express Fru^M. Most Fru^M-expressing cells are found in ~20 clusters of neurons scattered throughout the CNS, including the regions previously implicated in male courtship. Maximal Fru^M expression coincides with the period of major morphogenetic events that shape the adult fly CNS. Taken together, these findings led to the provocative hypothesis that the Fru^M proteins are both necessary and sufficient to build the potential for male courtship behavior into the nervous system [1].

Courtship and fruitless*: neurobiological studies*

The hypothesis that Fru^M functions to build the potential for male courtship behavior into the nervous system was derived from molecular genetic and behavioral studies. As such it raised, but left unaddressed, several key neurobiological questions that can be defined conceptually or operationally. First, and most centrally, is expression of Fru^M alone sufficient to confer the potential for male courtship? If this hypothesis with respect to *fru* is correct, additional questions arise. These include:

(i) How is the circuitry for an innate behavior built? (Or, in operational terms: at both the neuroanatomical and molecular levels, what aspects of neuronal development and differentiation are regulated by Fru^M expression in the many types of neurons that comprise this circuit?)
(ii) What is the precise circuitry responsible for an innate behavior? (Or, operationally: do the Fru^M-expressing neurons comprise a neuroanatomical circuit and, if so, what is the structure of that circuit?)
(iii) How does this circuitry function? (Or, operationally: what are the behavioral roles of individual groups of *fru*-expressing neurons?)

To address these fundamental neurobiological questions with respect to courtship behavior, several groups developed *fru*-based genetic tools that permit the manipulation of Fru^M-expressing neurons, without affecting other neurons. These tools include:

(i) Insertion, via homologous recombination, of the yeast *GAL4* gene into regions of *fru* unique to P1 transcripts in males and females [16,17]. These *fru-GAL4* genes enable the manipulation of only the cells that normally express P1 *fru*, by using GAL4 to drive expression of various UAS constructs in these neurons. Such constructs have been used to visualize the nuclei of Fru^M-expressing neurons (via *UAS-GFPnls*), to visualize the projections of these neurons (*UAS-cd8GFP, UAS-lacZ*), to silence these neurons (*UAS-shi^{ts}*), and to 'change of the sex' of these cells from male to female (*UAS-traF*) or from female to male (*UAS-tra2IR*).
(ii) A gene construct that silences the expression of Fru^M in any cell that expresses both P1 *fru* transcripts and this construct. This construct, *UAS-Fru^{M}IR*, encodes an RNA molecule that is an inverted repeat of sequences encoding the N terminus of Fru^M, and it acts specifically to destroy these transcripts by RNA interference [18].
(iii) A *fru* gene modified by site-directed homologous recombination such that it produces the Fru^M proteins in females as well as males [19].

A circuit sufficient: from stimuli to behavior

One of the most significant findings from recent studies was that expression of male-specific Fru^M isoforms is

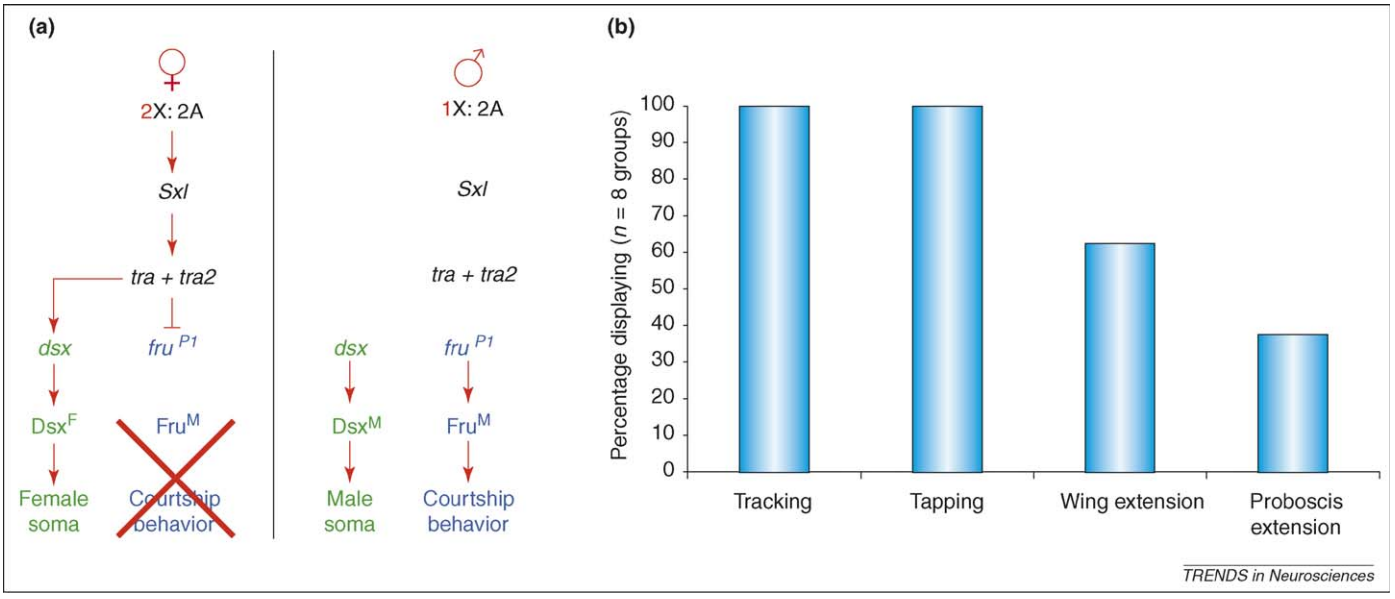

Figure 1. Sex determination and courtship. **(a)** The *Drosophila* sex-differentiation hierarchy regulates the potential for male courtship through its control of *fruitless* splicing and translation. In male flies, the ratio of one X chromosome (X) to two autosomes (A) results in the absence of *Sex lethal* (*Sxl*) and thereby *transformer* (*tra*) activity. The lack of *tra* activity enables the default splicing of P1 *fru* (*fru*^{P1})-derived and *doublesex* (*dsx*) transcripts. Thus male flies produce the male-specific Fruitless isoforms (Fru^{M}) required for courtship behavior, and the male form of Doublesex (Dsx^{M}) required for male somatic differentiation outside the nervous system. In females, the ratio of two X chromosomes to two autosomes activates *Sxl* and thus *tra*, which acts along with *transformer-2* (*tra2*) to splice *dsx* and *fru* into their female forms. This female-specific splicing results in female Dsx isoforms (Dsx^{F}), but the absence of any sex-specific isoforms of Fru. **(b)** Fru^{M} expression confers the potential for male courtship behaviors. Masculinization of neurons that express P1 *fru* transcripts is sufficient to enable male-specific courtship behavior by females. Shown are percentages of groups of females expressing *fru*^{P1}*-Gal4/3x UAS-tra2IR* (to inhibit *tra2* activity in neurons that normally express Fru^{M} in males but not females) that display individual male courtship behaviors (D.S. Manoli and B.S. Baker, unpublished).

sufficient to confer the potential for male courtship behavior in females [16,19] (Figure 1). These experiments made use of the fact that P1 *fru* is transcribed in homologous cells in males and females but, because of post-transcriptional regulation, it normally produces Fru^{M} proteins only in males. Thus, it was possible to manipulate regulation of *fru* to produce Fru^{M} in females in cells homologous to those in which it is normally expressed in males. Such manipulated females showed male courtship behavior, confirming the proposal that *fru* is both sufficient and necessary for establishing the potential for male courtship. This finding strengthens the possibility that other complex innate behaviors are specified by dedicated genetic machinery.

Direct labeling of Fru^{M}-positive neurons by the *fru-GAL4*-driven expression of green fluorescent protein (GFP) provided insights into where Fru^{M} is expressed, and how this expression is temporally regulated [16,17] (Figure 2). These studies also began to delineate how Fru^{M} modifies the nervous system for male courtship behavior. In addition to being expressed in the CNS as previously described, Fru^{M} is also expressed in subsets of primary sensory neurons in all of the sensory systems (i.e. visual, olfactory, gustatory, auditory and tactile) known to be used during male courtship [2,11,16,17]. Identification of Fru^{M}-expressing subpopulations of sensory neurons in the optic lobes, forelegs, antennal segments, wings, mouth-parts and male genitalia suggests that these particular sensory neurons are involved in the reception and processing of sensory cues that mediate courtship initiation, increase courtship drive, and facilitate progression to attempted (and successful) copulation. These observations suggest that, despite there being obvious morphological differences between the sense organs in the two sexes only in the gustatory system, there might be fundamental sexual dimorphism at the level of the detection of sensory input and/or the initial processing of this information in most

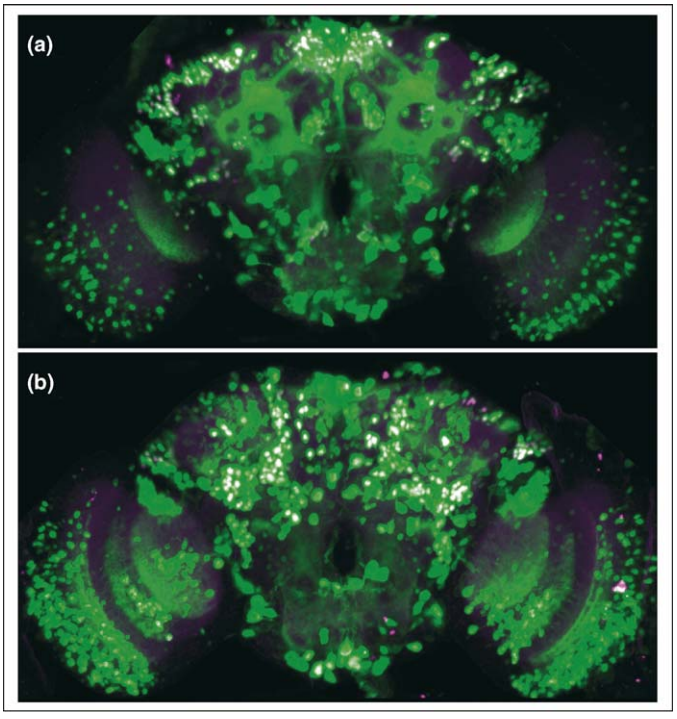

Figure 2. Fru^{M} expression in the *Drosophila* brain. *fru*^{P1}*-GAL4* driving a *UAS-m-CD8-GFP* reporter (green) is present in clusters of neurons on both the anterior **(a)** and posterior **(b)** halves of the *Drosophila* brain. These clusters project along many major tracts in the CNS and components of multiple sensory systems, including the visual and olfactory systems. White indicates the coincidence of reporter expression with anti-Fru^{M} antibody staining (magenta), which labels the nuclei of these neurons [16].

sensory systems. In flies (at least), males and females might perceive the world differently.

Besides being expressed in primary sensory neurons, Fru^M is present in second-order and third-order neurons in the visual and olfactory systems, supporting its role at sequential levels in the relays for multiple sensory modalities [16,17]. Thus, the circuitry identified by Fru^M expression not only functions centrally, as had been previously suggested, to integrate sensory cues to generate specific behavioral states and to initiate or execute sex-specific behavioral programs, but also functions to detect, transmit or integrate the sensory cues that define distinct ethological contexts.

Interestingly, Fru^M expression is notably absent from the central complex, several structures in the central brain that are thought to compute internal comparisons and regulate patterned motor behaviors [20]. This suggests that Fru^M-specified circuits interact with conserved circuits to regulate many aspects of behavior, rather than mediate behaviors directly.

These initial characterizations of Fru^M-expressing neurons also revealed a surprising lack of differences in the gross neuroanatomical features of the *P1-fru*-expressing circuitry in males and females [16,17]. Certain clusters of *P1-fru*-expressing neurons showed some sexual dimorphism in cell number, but no gross differences in neural projections were apparent when membrane-bound GFP was expressed in these cells. These observations suggest that Fru^M functions largely to regulate fine neural connectivity or to alter neural physiology. The most apparent exception to this proposal is the innervation of sex-specific somatic structures, whose direct innervation by Fru^M-expressing neurons was established, suggesting the potential for coevolution in sex-specific anatomy and the neural mechanisms that regulate its function [15,21,22].

Recently however, Kimura *et al.* have identified sexual dimorphism in neuronal survival and projection patterns in a cluster of Fru^M-expressing CNS neurons [23]. Most interestingly, this study showed that the male-specific neurons examined survive as a consequence of Fru^M expression (and in females these cells are removed by apoptosis), suggesting that in certain cases Fru^M might specify a set of male-specific neural elements.

Functional analysis of Fru^M-expressing neurons

Recent studies have provided insights at two levels into the functional roles of Fru^M-expressing neurons. First, the findings that Fru^M-expressing neurons are generally present in small groups throughout the CNS and peripheral sensory system leads to the obvious prediction that groups of these neurons have distinct, specific roles in the reception, processing and transmission of information relevant to courtship or directing behaviors based on that information. Second, the observation that Fru^M-expressing neurons are ~2% of all CNS neurons and higher proportions of the primary sensory neurons in various sensory systems raises the question of whether these neurons are dedicated to sexual behavior or have functions in other behaviors.

With respect to the first of these topics, several experiments have investigated the Fru^M-dependent functions of specific subsets of these neurons in courtship. Additionally, Fru^M expression in several levels of the olfactory system has been shown to mediate key aspects of courtship initiation and modification. Stockinger *et al.* demonstrated that Fru^M-expressing olfactory neurons contribute significantly to courtship initiation (in the absence of visual cues), consistent with the observation by Demir and Dickson that transformation of the pheromone profile of a male is sufficient to elicit courtship by males and Fru^M-masculinized females [17,19].

Using the targeted inhibition of Fru^M expression, Manoli and Baker demonstrated the role of a group of Fru^M-expressing neurons in the sub-esophageal ganglion in coordinating the behaviors that comprise courtship, potentially via the sequential processing or integration of multiple sensory cues to increase courtship drive [18]. This proposal is consistent with earlier studies demonstrating that no single sensory modality is required for courtship behavior in *D. melanogaster*. It is readily apparent how such an approach can be extended to functionally probe many other parts of the Fru^M-specified circuitry [16].

Another proposal arose from the interesting observation that the aberrant courtship behaviors in flies in which Fru^M expression was suppressed in sub-esophageal neurons resembles normal courtship behaviors in other Dipterans. Manoli and Baker suggested that Fru^M might modify circuitry that mediates courtship in many insect species, with subtle changes in that circuitry able to generate species-specific courtship rituals. Indeed, recent work has identified sex-specific regulation and functional conservation of *fru* homologs in Dipterans as far from *D. melanogaster* as *Anopheles gambiae*, and homologies with striking sequence conservation exist in more distant species such as the honeybee *Apis mellifera* and the beetle *Tribolium castaneum* [24]. It will be interesting to determine whether *fru* also functions in the regulation of innate behavioral programs in these distant species, given their complex social organization and mechanisms of sexual differentiation [25,26].

A role for P1 *fru* function in behavioral plasticity

In addition to beginning to characterize the contributions of specific populations of Fru^M-expressing neurons to distinct aspects of male sexual behaviors, Manoli *et al.* recently demonstrated that Fru^M expression in various components of the olfactory system is necessary for different experience-dependent modifications to male courtship [16]. Previous work had shown that, although naïve *Drosophila* males will initially court either male or female targets at first encounter, wild-type males rapidly habituate to other males based on male-specific olfactory cues, resulting in a permanent suppression of male–male courtship [27]. By contrast, inhibition of Fru^M expression in primary or secondary olfactory neurons reduces male–male habituation [16]. The absence of gross sexual dimorphism in these neural populations suggests that Fru^M functions in these neurons to specify the molecular mechanisms necessary for either the detection of or proper adaptation to male-specific cues that mediate such modifications of sexual behavior.

In addition to such habituation, *Drosophila* males also learn to decrease courtship directed towards recently mated females [28]. This associative form of learning

depends both on olfactory cues specific to mated females and on repeated encounters with a recently mated animal [29]. Furthermore, numerous studies have demonstrated that such conditioning requires function of the mushroom body (MB), in addition to molecular mechanisms implicated in associative learning in these neurons [30,31]. Based on the significant levels of P1 *fru* expression identified in the MB, Manoli, *et al.* demonstrated that Fru^M function is required in subsets of MB neurons for the proper conditioning of males to mated females [16]. Again, given the lack of gross sexual dimorphism in MB neurons, it is likely that Fru^M functions in these neurons either to alter fine connectivity necessary for proper associations or to regulate the molecular mechanisms mediating such learning. That a single gene seems not only to specify the circuitry necessary for an innate behavioral program, but also to regulate the means by which such behaviors are altered by experience, suggests the remarkable extent to which genetic elements might influence animal behaviors.

A dedicated circuit for sexual behaviors

Beyond the analysis of specific populations of Fru^M-expressing neurons and their contributions to sexual behaviors, investigation of whether Fru^M-expressing neurons are in fact dedicated to these programs has provided one of the most surprising findings regarding this circuitry: these neurons appear to be specifically dedicated to sexual behaviors. Transient inhibition of neurotransmission in only Fru^M-expressing cells demonstrated that these neurons function largely, if not exclusively, for sexual behaviors, because males in which these neurons are silenced show a complete lack of courtship yet have normal locomotor, flight, geotactic, phototactic and chemotactic behaviors [16,17]. Although the dedicated nature of the circuitry specified by Fru^M is perhaps unsurprising in cases where the neurons are present only in males, such as those identified by Kimura *et al.* [23], the observation that the vast majority of Fru^M-expressing neurons have homologs in females, and that these have largely similar gross morphology, renders the apparent functional autonomy of these neurons in males remarkable. Undoubtedly, characterizing the function of these neurons in females will help to determine whether they represent circuitry for mating that is in both males and females and is specified independently of sex, with sexually dimorphic function for courtship resulting from the presence or absence of Fru^M expression.

At a more general level, the fact that the circuitry identified by Fru^M expression seems to be specifically dedicated to sexual behavior not only suggests that distinct genetic elements might specify the neural substrates of different complex innate behaviors, but also raises the intriguing proposal that such circuits might be largely autonomous within the nervous system. Furthermore, given the observation that inhibition of Fru^M expression in a subset of neurons produces courtship behaviors reminiscent of other species, we suggest that the circuitry underlying complex innate behavior might arise first from the novel association of sensory information with the initiation of specific behavioral modules, and second, from the coordination of these modules within a complex behavioral program. In addition to functioning at all stages of courtship, Fru^M therefore also functions at several different levels within the nervous system, further emphasizing its role as a master regulator of the neural substrates underlying courtship behavior.

General principles for behavioral circuits

Based on the implications of these observations, we propose that for the circuitry specified for particular behavioral programs, distinct neuronal components must: (i) detect and integrate general and context-specific sensory information to identify distinct ethological contexts; (ii) relay such information to central components to determine specific behavioral states; (iii) coordinate and execute programs for behavioral sequences; and (iv) regulate basal elements of motor programs to generate appropriate behavioral output.

Characterization of the mechanisms underlying these levels of processing will undoubtedly shed light on the fundamental principles underlying basic neural computations and mechanisms of behavior. At a primary level however, these findings suggest intriguingly that genetically specified circuits underlying distinct programs for innate behaviors might function largely independently of each other and other components of the nervous system, and interact in specific ethological contexts to give rise to distinct behavioral output. Recent work has suggested that similar principles might also govern the specification of circuitry underlying innate behaviors in other species.

Similarities to vertebrate circuitry

It worth briefly considering three cases that demonstrate genetic regulation of behavioral circuitry in vertebrates. As discussed, if complex behavioral programs are maintained during evolution, it follows that the elements that regulate them are subject to tight genetic control and are likely to be linked to pathways that regulate the highest levels of development. In vertebrates, two developmental programs that regulate conserved and more basic developmental mechanisms throughout the organism are those of axial asymmetry and sex determination. Not surprisingly, both pathways seem to regulate nervous system development and distinct behavioral programs.

Developmental programs and innate behaviors in vertebrates

In humans, asymmetry in cortical function is associated with language and mathematical processing, and with facial and spatial recognition [32]. Abnormalities in cortical asymmetry have been associated with various psychiatric disorders, most significantly schizophrenia and autism [33,34]. Thus, identifying and understanding the initial programs that regulate asymmetry in nervous system development and their target genes underlying specific cortical circuits are a powerful means by which the genetic basis for cognition might be approached. Although many of the pathways that mediate somatic axial asymmetry have been successfully characterized, until recently it had been determined only that most of these mechanisms did not seem to regulate asymmetry in the nervous system [35]. The lack of known phenotype associated with CNS asymmetry in model systems also hindered genetic analysis of this question.

Recently however, Barth and colleagues demonstrated that mutations in the zebrafish *frequent situs inversus* (*fsi*) produced not only visceral but also neuroanatomical and behavioral phenotypes [36]. Thus, based on asymmetries in neuroanatomy as well as gene expression in *fsi* animals, it is now possible to correlate some lateralized behaviors with neural asymmetry.

Similar to studies of *fruitless*, studies in mammals have used the components of sex-determination pathways as molecular entrée into the circuitry underlying sexually dimorphic behaviors. Recent molecular genetic approaches have been combined with earlier use of pharmacological and anatomical manipulations to identify sexual dimorphism both in previously implicated and in uncharacterized regions of the CNS, and to correlate these regions with aspects of sexual behaviors. Thus, expression of estrogen receptors in the ventromedial hypothalamus correlates with the estrogen responsiveness of these regions necessary for female lordosis behavior, and circuitry identified by the expression of the mouse androgen receptor includes the medial preoptic nucleus and afferent projections from the bed nucleus of the stria terminalis, which have both been implicated in male sexual behaviors [37,38].

That the regulators of sexual differentiation in mammals might specify multiple, if not all, parts of the circuitry that underlies specific sexual behaviors, including their volitional components, is supported by the expression of estrogen and androgen receptors in cortical and subcortical regions projecting to hypothalamic nuclei that mediate distinct behavioral programs [39]. It will be interesting to dissect these circuits to identify the contributions of specific neural populations to distinct aspects of behavior. Equally compelling will be to determine how hormone receptors mediating sex determination in these systems interact at cellular and molecular levels with factors that specify local circuits for isolated behavioral modules – such as pattern generators for coordinated motor output – or perhaps even cortical circuitry for higher-order functions [40,41].

Hypothalamic axes and innate behaviors: pathways of processing for behavioral states

Finally, the analysis of innate behaviors in vertebrates has repeatedly suggested a central role of hypothalamic axes in regulating various behavioral programs, through the coordination of diverse sets of neurons via neuroendocrine control [42–44]. Indeed, distinct hypothalamic pathways have been implicated in the regulation of arousal via the orexins [45,46], feeding via leptins [47], sleep [45,48], fear [49], mating [42,50], social competition [51,52] and other behaviors [53,54]. Despite the lesioning or ablation of cortical structures, rodents can be stimulated to display avolitional feeding and defensive behaviors, whereas hypothalamic lesions abolish such behavior. Furthermore, the hypothalamus makes dense interconnections with cortical and subcortical regions to mediate volitional aspects of behavioral control [44,50,55].

The central role of neuroendocrine specification and modulation of behavioral circuits is supported by numerous studies in various systems. First, the observation that expression of the vasopressin receptor in the ventral forebrain of non-pair-bonding voles is sufficient to induce monogamous pairing supports the capacity of these pathways to couple neural activity in specific populations to extant behavioral circuitry [56,57]. More interestingly, recent work has begun to uncover the genetically specified circuitry that, both through and within the hypothalamus, regulates diverse behavioral programs, receives sensory information to determine specific ethological contexts, and mediates interactions between pathways for specific behaviors to define behavioral states.

Early studies indicated that injection of gonadotropin-releasing hormone (GnRH) into the brain can induce sexual behaviors, suggesting that GnRH regulates sexually dimorphic motor output, and perhaps earlier stages of sensory processing [58]. Inspired by such work, Boehm *et al*. and Yoon *et al.* recently used molecular markers to label GnRH-expressing neurons (~800 neurons within the mouse anterior hypothalamus and nearby structures) and to trace connectivity of these neurons throughout the CNS [59,60]. GnRH-expressing neurons were found to make direct, excitatory connections with the hypothalamic nuclei implicated in sexual behaviors, suggesting the neuroendocrine regulation of these circuits. More significantly however, the amygdalar nuclei that synapse with GnRH-expressing neurons seem to receive direct sensory input and to influence sensory perception via significant feedback interconnections. By demonstrating that GnRH-expressing neurons form (i) feedback connections with multiple sensory systems, (ii) interconnections with hypothalamic nuclei implicated in sexual behaviors, and (iii) significant interconnections with cortical and subcortical structures, these studies provide additional evidence for neuroendocrine (GnRH-mediated) regulation of the behavioral (amygdalar and hypothalamic) circuits and volitional (cortical) circuits that regulate innate behaviors.

Specialized neuroendocrine circuits for innate behaviors thus seem to process sensory information relevant to ethological contexts and influence sensory perception and processing; integration by these circuits of multiple pathways of information relevant to different behaviors determines the behavioral state of the animal. Given the sufficiency of neuroendocrine stimulation to elicit sexual behaviors, it is likely that similar regulation and interconnectivity will underlie other components and levels of circuitry necessary to generate behavioral output.

Further evidence suggesting a crucial role of hypothalamic circuits in the determination of behavioral states came from characterization of the connectivity between amygdalar and hypothalamic nuclei. Choi *et al.* used specific transcription factors to identify neurons within amygdalar nuclei that project to distinct hypothalamic nuclei implicated in defensive or reproductive behaviors [61]. This connectivity suggests that the balance of inhibitory and excitatory inputs from amygdalar neurons, which respond to reproductive or defensive stimuli respectively, most likely mediates the determination of behavioral states given multiple olfactory cues. Given the extensive connectivity of limbic structures and hypothalamic circuits with each other, multiple sensory systems and cortical structures, hypothalamic neuroendocrine function can be

modulated to define behavioral states in response to ethologically relevant environmental cues.

Neurogenetic approaches to behavioral circuits: blueprints for behaviors

Based on these principles governing genetically specified neural circuits, it is worth considering how further studies might best capitalize on the molecular and technical facility offered by genetically tractable model systems. The system afforded by *fruitless* represents a remarkable opportunity to explore the molecular, cellular and physiological basis for nervous system development and function, and more significantly how these functions transform information into action. As described, opportunity now exists for directed molecular characterization of Fru^M function. Identifying the targets of regulation by Fru^M, in addition to the factors with which it interacts, is necessary for understanding how expression of Fru^M affects neural function and connectivity. Determination of how Fru^M itself is deployed in the nervous system is crucial to understanding the principles by which dedicated circuitry is built, and perhaps how such networks adapt to produce different behavior between species.

In addition, it now possible to characterize neural mechanisms at various levels of processing in behavioral circuits, to understand the architecture of such innate circuitry and thus to determine how information is both encoded and integrated to drive behavior in distinct contexts. Although rigorous characterizations of Fru^M-dependent behaviors, neuroanatomy and gene expression are necessary for understanding how such circuitry gives rise to sexual behaviors, this alone is not sufficient. It is thus essential to analyze the physiological properties and responses of distinct populations of neurons implicated in courtship. Targeted inhibition and activation of specific neural populations will identify more of the neurons that mediate perception and integration of stimuli to generate behavioral responses appropriate to distinct behavioral states. Taken together, studies that characterize the physiological responses of neural populations to ethologically relevant stimuli, and the behavioral consequences of manipulating the activity of these and other neurons, will begin to address how sensory information is encoded, integrated and transformed to drive behavior.

The studies described here illustrate varied systems in which specific genetic elements define the neural substrates of innate behaviors. At the extreme end of this spectrum *fruitless* – and perhaps sex-determination mechanisms in other species – can transform the entire behavioral identity of an animal. Thus, such elements must specify or regulate all components of the neural circuitry necessary for complete behavioral programs, from sensory input and integration, through the determination of distinct behavioral states, to the regulation and coordination of motor output. Moreover, recent studies have begun to demonstrate that the neural substrates underlying behavioral circuitry are specified also by specific genetic elements in vertebrates. Identification of genes regulating the development of cortical circuitry, combined with studies in other model systems, will begin to reveal the principles governing circuits for complex behavior and cognition and how these networks evolve. Growing evidence supports the alluring idea that hypothalamic neuroendocrine axes have a central role in integrating higher-order behavioral circuits in the vertebrate nervous system. Analysis and characterization of the circuitry underlying innate complex behaviors in these systems will be facilitated by new targeted molecular approaches that enable unbiased neurogenetic study in vertebrate systems, for example by inactivating defined neural populations based their molecular characteristics [62,63]. Just beyond lies the dissection of the pathways by which these circuits interact with cortical processing to mediate volitional and eventually conscious control of the most elemental, but essential, aspects of animal behaviors.

Acknowledgements

We thank the members of the Baker laboratory for helpful comments and discussions. D.S.M. is supported by the MSTP Training Grant. G.W.M. and B.S.B. are supported by grants from the NIH.

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