variants and then find the tissue in which the same set of genes is expressed. Campbell *et al.*[2] take this strategy one step further by using their scRNA-seq data to identify the specific subtype of neuron that is most relevant for bodyweight, a trait that has previously been associated with the Arc-ME. In this study, a method developed for bulk RNA-seq was used for the gene set enrichment[9]. As it is becoming increasingly evident that computational strategies developed specifically for scRNA-seq tend to outperform those developed for bulk RNA-seq[11], a method specifically developed for gene set enrichment analysis in scRNA-seq data is likely to provide more accurate results. Considering the widespread use of both GWAS and scRNA-seq, there is considerable scope for developing better computational methods for integrating the two types of data.

The identification of a specific neuronal cell type is important for follow-up studies of the mechanisms involved in bodyweight regulation. Not only is it helpful to have a better understanding of what subset of neurons are involved, knowing which genes are expressed in these neurons also makes it possible to further investigate the relevant gene regulatory network. Notably, a list of target genes opens up new possibilities for developing pharmacological treatments. Moreover, the marker gene makes it possible to carry out follow-up studies involving creating a cell line or mouse line that can be used to further characterize this neuronal population.

Overall, this study illustrates how the catalog generated by a scRNA-seq experiment can be used to enhance GWAS (**Fig. 1**). It is a demonstration of how one can combine a genetic catalog and a transcriptomic catalog to identify the cellular basis of a complex trait, thereby providing important biological and clinical insights. Projects such as the NIH BRAIN Initiative (https://grants.nih.gov/grants/guide/rfa-files/RFA-MH-17-230.html) and the Human Cell Atlas (https://www.humancellatlas.org/) are aiming to generate a detailed catalog of the entire brain and human body. The next major challenge to the field will be to integrate large data sets of different resolution and modalities to attain biological and clinical insights. The work by Campbell *et al.*[2] is an important contribution toward the goal of understanding how the individual parts of a tissue sum up to a whole.

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Setting the mood for love

Gül Dölen

McHenry and colleagues delineate a neural circuit controlling female sexual behavior. These experiments shed light on how the brain optimizes reproductive behavior to coincide with phases of peak fertility.

Most female mammals become sexually receptive only when they are fertile. Even in humans, who are sexually active throughout the reproductive cycle, there is evidence that sexual encounters and interest in sexual cues peaks during the ovulatory phase, when pregnancy is achievable[1,2]. This behavioral adaptation is thought to balance reproductive drives (for example, mating) with the demands imposed by other evolutionary selection pressures (for example, food acquisition and territorial defense)[3]. In this issue of *Nature Neuroscience*, McHenry *et al.* report a series of elegant experiments delineating a brain circuit mechanism that sheds light on how the switch between these behavioral states is controlled[4].

While men may wine and dine, light candles, and play romantic music, it has long been known that estrogen is what puts a woman in the mood for love. Specifically, experimentally removing the ovary (ovariectomy) eliminates sexual activity in most mammals, while administrating exogenous estradiol, an estrogen hormone whose circulating level peaks around the time of ovulation, restores sexual activity[5]. Moreover, previous studies have shown that estrogen exerts its motivational effects in the medial preoptic area (mPOA), a subdivision of the hypothalamus of the brain[6]. Microdialysis studies in rats have shown that dopamine, the neurotransmitter most often linked to rewarding and appetitive behaviors, is released into the nucleus accumbens (NAc) when a female interacts with a sexually active male[7]. Furthermore, as in humans, this activity is rewarding in rodents, so long as the female controls the mating interval[8]. Still, the mPOA sends widespread projections throughout the brain and has also been implicated in other types of behavior, so the explicit neural circuit controlling the appetitive and rewarding aspects of female sexual preference behavior was heretofore unknown.

McHenry *et al.*[4] focused on a subpopulation of neurotensin peptide-positive (Nts^+) neurons in the mPOA that project to the ventral tegmental area (VTA) and express estrogen receptor α (Esr1) (**Fig. 1**). While the Nts^+ neurons that project to the VTA have been described[9] and Nts^+ neurons in the mPOA are known to be estrogen responsive[10], the innovation of the studies carried out by McHenry *et al.*[4] is to use modern technologies to gain experimental control over this subpopulation and examine the functional consequences of activation of this circuit in the context of female sexual preference behavior. Using Nts-IRES-Cre mice[11] to express calcium sensors (GCaMP6) in Nts^+ neurons, they directly viewed the neural activity of mPOA neurons, using gradient refractive index (GRIN) lenses and two-photon imaging in awake, behaving animals. Notably, a subset of the Nts^+ neurons in the mPOA was preferentially excited by male odor cues, which were delivered directly to the female mouse via an air puff aimed at her nose.

To determine whether this neural activation in response to male odor cues is gated by estrogen, McHenry *et al.*[4] next repeated this experiment in ovariectomized female mice, following either estrogen replacement or vehicle control. These studies revealed that both

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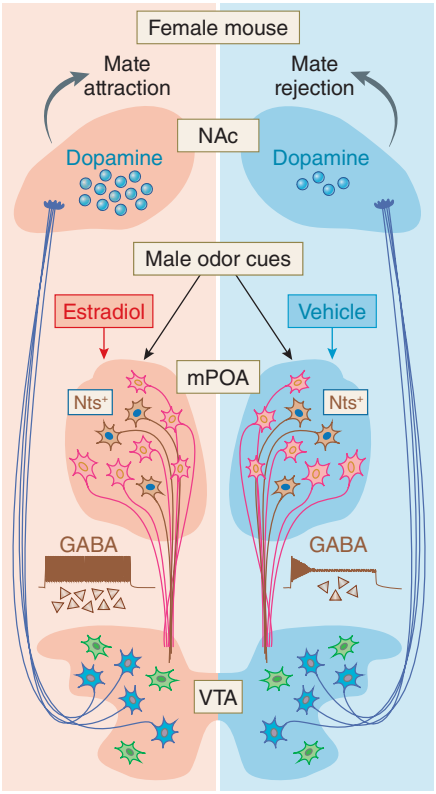

Debbie Maizels/Springer Nature

Figure 1 A neural circuit for mate attraction and rejection. Circulating estrogens (for example, estradiol) peak during the proestrus phase of the female reproductive cycle. Estrogen binding in Nts$^+$ neurons of the mPOA, which express Esr1, enhances the intrinsic excitability of these GABAergic neurons. Via intermediate synapses within the VTA, this estradiol-induced change in basal firing properties of Nts$^+$mPOA neurons, combined with male odor cues, causes an increase in dopamine released in the NAc and is correlated with increased appetitive female sexual behavior.

the amplitude of response and proportion of Nts$^+$ cells in the mPOA excited by male odor cues, but not by other rewarding odors (such as peanut oil), increased following estrogen replacement compared to vehicle. Moreover, in nonovariectomized females, the response to male odor cues varied across the estrus cycle and was significantly greater during proestrus, when estradiol levels peak, compared to estrus. These findings indicate that Nts$^+$mPOA neurons adjust their male odor cue encoding properties in response to changes in circulating estrogen.

To determine how estrogen might alter the firing properties of Nts$^+$mPOA neurons, McHenry *et al.*[4] next used whole-cell patch-clamp electrophysiology to record from these cells in acute (freshly cut) brain slices of the hypothalamus. These experiments revealed that estradiol priming changes the basal firing properties of Nts$^+$mPOA neurons, rendering them more excitable, in part by increasing A-type K$^+$ channel conductance (**Fig. 1**). Since these channels are delayed-rectifier K$^+$ channels, they allow the neurons to rapidly reset their membrane potential following a spike, thus enabling high-frequency firing. Future studies aimed at uncovering how estrogen is able to increase A-type K$^+$ channel conductance in these cells will be informative.

To determine the functional consequences of activation of the subpopulation of Nts$^+$mPOA neurons, McHenry *et al.*[4] next expressed channelrhodopsin-2 (ChR2) in these cells and implanted optical fibers above the mPOA to stimulate the neurons in question with light. In addition, they combined this approach with projection-target specification to express ChR2 in the subset of VTA-projecting Nts$^+$mPOA neurons. In both experiments, stimulation of Nts$^+$mPOA neurons was behaviorally reinforcing, with the highest amount of reinforcement occurring during proestrus (in intact females) or following estradiol replacement (in ovariectomized females). While previous findings had correlated changes in estrogen and Nts$^+$mPOA neuron activation with receptive behaviors, these experiments harness the power of optogenetics to demonstrate their sufficiency for reinforcement behaviors.

Drawing on previous studies in female rats demonstrating that interaction with a male induces increased dopamine release in the NAc[7] and is rewarding[8], McHenry *et al.*[4] next showed that optical stimulation of the Nts$^+$mPOA projections to the VTA in female mice similarly led to increased dopamine, as measured by fast-scan cyclic voltammetry, and increased time spent interacting with a male in a choice assay. In addition, following expression of the inhibitory light-gated chloride pump halorhodopsin (NpHR) in Nts$^+$mPOA neurons, the experimenters used photoinhibition to demonstrate that this molecularly specified circuit is necessary for male-odor preference and preferential investigation of males.

The study by McHenry *et al.*[4] makes a compelling argument that Nts$^+$mPOA neurons that project to the VTA are necessary and sufficient for appetitive sexual behavior in females. These Nts$^+$mPOA neurons are GABAergic, so their increase in excitability in response to estrogen would be expected to increase suppression of downstream target neurons in the VTA, and yet activation of this pathway leads to the release of dopamine in the NAc. Thus, it will be critical to understand which neurons in the VTA receive Nts$^+$ GABAergic inputs from the mPOA. The VTA is a heterogeneous brain structure that is composed of anatomically and functionally distinct dopaminergic subpopulations that are intermingled and connected with GABAergic and glutamatergic neurons[12]. Since estradiol increases the excitability of the inhibitory Nts$^+$ inputs from the mPOA and their activation leads to dopamine release, it seems reasonable to suppose that these projections form synapses onto GABAergic neurons. Alternatively, Nts could be released directly into the VTA and directly affect the activity of local neurons. At the same time, oxytocin, another hypothalamic peptide implicated in a variety of social behaviors[13], has also been shown to act in concert with estrogens in the mPOA to enable sexual receptivity and lordosis, and the promoter region of the oxytocin gene contains estrogen-response elements[14]. At this time, it is unclear whether oxytocin acts on the same Nts$^+$ cell population described by McHenry *et al.*[4] or on a distinct subset of cells within the mPOA and how the activity of these neuropeptides is coordinated to regulate sexual behaviors in females. Although a number of mechanistic questions remain, the current work is a necessary first step toward identifying neural circuit and modulatory players, and it lays out a framework for future investigations.

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