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Neural representation of sexual satiety in mice

Mating behaviors are controlled by genetically hardwired circuits (Figure 1A), which endow sexually mature animals with the ability to respond appropriately upon encounters with conspecifics of the opposite sex without needing prior training, thereby facilitating propagation of the species (Chen & Hong, 2018; Wei et al., 2021; Xiao et al., 2022). When a male mouse meets a female mouse in estrus and wants to initiate mating, he usually shows appetitive investigation by approaching the female and sniffing her genital area; afterward, the male places his forepaws on the back of the female, a behavior called mounting (Figure 1B). If the female accepts the male, she arches her back, a behavior known as lordosis, to permit rhythmic genital penetration by the male, known as intromission. Ejaculation typically occurs after several rounds of intromission (Figure 1B). Although instinctual, the display of mating behaviors is not a reflex, where a specific sensory stimulus triggers the same motor program. In other words, the presence of appropriate mating partners does not guarantee the display of mating behaviors. Instead, animals of both sexes only perform mating routines under conditions of appropriate internal states.

Successful mating triggers a period of sexual quintessence that lasts for several days. During this time, animals rarely resume consummatory mating actions despite the presence of mating partners and appetitive social interactions between them. This process, sometimes called sexual satiety, is evident in many species, including humans (Phillips-Farfán & Fernández-Guasti, 2009). Biologically speaking, repeated ejaculations cause a marked decline in both the total number of sperm per ejaculate and number of sperm transported to the uterus in rats, leading to slightly diminished fertility (Austin & Dewsbury, 1986). Thus, imposing a brake on subsequent mating after successful ejaculation may be evolutionarily adaptive, especially considering the energy cost of mating and possible danger of predation during the pursuit of mates. Despite the well-documented phenomenon of sexual satiety in scientific and popular literature, neural encoding of the sexual satiety state in the central nervous system remains poorly understood.

In a recent paper published in *Science*, Zhou and coworkers identified persistent increases in the neural activity of estrogen receptor 2 (*Esr2*)-expressing neurons in the bed nucleus of the stria terminalis ($BNST^{Esr2}$) as a central mechanism that encodes the sexual satiety state and suppresses mating in mice (Zhou et al., 2023) (Figure 1C).

First, to characterize the process of sexual satiety based on behavior, the authors found that after successful ejaculation, animals of both sexes showed suppressed mating, as evidenced by a diminished transition from appetitive investigation to consummatory mating actions, indicating a changed behavioral state after ejaculation. In male mice, normal sexual behaviors gradually reappeared within about 7 days of ejaculation. In female mice mated with vasectomized males, normal sexual behaviors typically reappeared at 14 days after ejaculation. If the initial mating process was interrupted before ejaculation, both males and females continued to mate until ejaculation finally occurred to trigger the state of sexual satiety and subsequent suppression of mating.

The authors next investigated preferentially activated brain areas in post-ejaculated mice based on regions showing the highest changes in immediate early gene c-Fos expression post-ejaculation. Interestingly, the principal nucleus of the BNST (BNSTpr) showed the highest fold-change in number of c-Fos+ cells in post-ejaculated male mice compared to control males that mated but did not ejaculate. This finding is consistent with other rodent species showing activation of BNSTpr neurons after ejaculation (Coolen et al., 1996). The BNSTpr is a node in the male mating circuit (Figure 1A) thought to relay information about sex-specific cues from the olfactory pathways to hypothalamic nuclei to control male mating and other reproductive behaviors (Yang et al., 2022). Bayless et al. (2019) showed that BNSTpr aromatase-expressing cell activity is required for male preference of female cues and male mating behavior. Thus, the BNSTpr is well-positioned to encode the sexual satiety state and modulate the display of mating behaviors.

Moreover, ejaculation-triggered c-Fos expression in the BNSTpr appears to be cell-type specific. Based on analysis of published single-cell RNA sequencing data, the authors identified two largely non-overlapping neuronal types in the BNSTpr that express the estrogen receptor 2 gene (*Esr2*) and suppression of tumorigenicity 18 gene (*St18*), respectively. Curiously, ejaculation-induced c-Fos signals were restricted to BNSTpr neurons expressing *Esr2* ($BNST^{Esr2}$), indicating these two neuronal populations exhibit distinct functions in mating regulation. By implanting a head-mounted microendoscope to monitor the activity of these two groups of neurons at single-cell resolution during mating, the authors found that largely distinct populations of $BNST^{Esr2}$ were activated by social sniffing and ejaculation in both sexes. In contrast, although different subsets of BNSTpr *St18*-expressing neurons ($BNST^{St18}$) were activated during social sniffing and intromission, few were activated during ejaculation. Taken together, these results indicate that ejaculation-responsive BNSTpr neurons are a subset of $BNST^{Esr2}$ neurons.

Strikingly, in addition to an acute increase in neural activity triggered by ejaculation, spontaneous activity in $BNST^{Esr2}$

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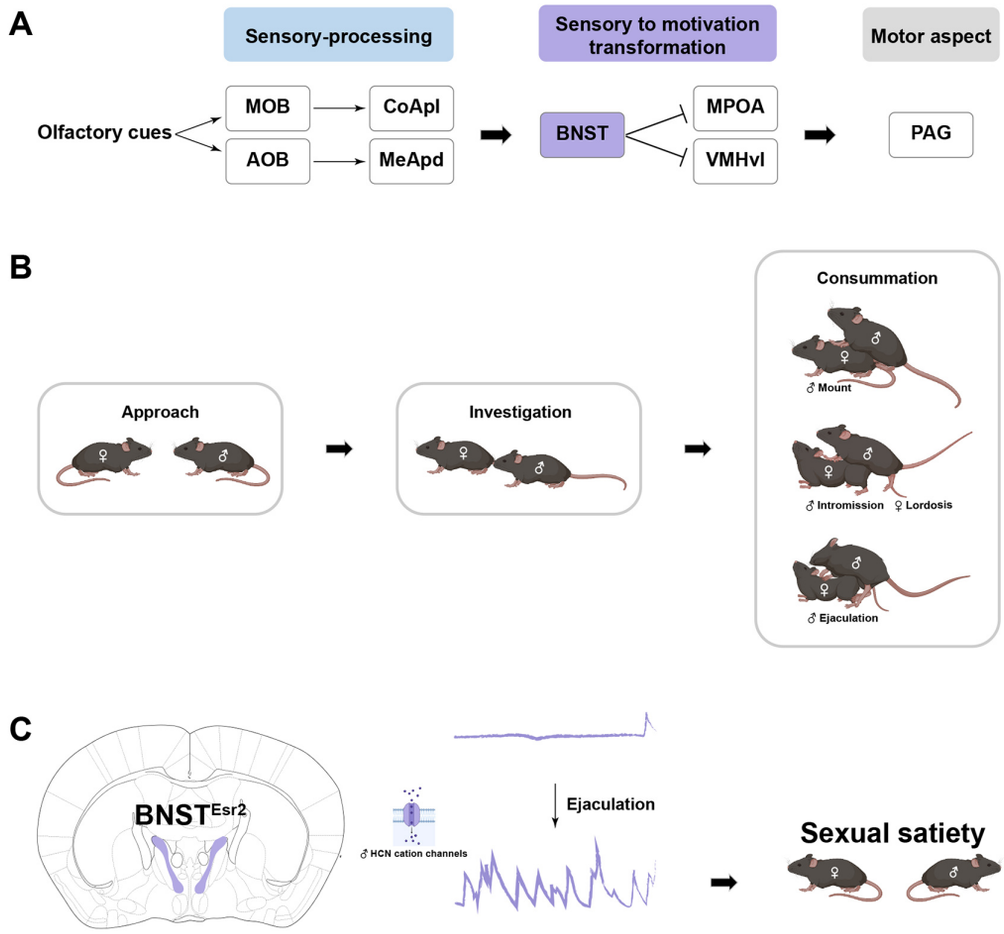

Figure 1 Hardwired neural circuits and typical behavioral rituals of mating in mice

A: Mating behaviors are controlled by genetically hardwired circuits. B: Typical mating behaviors in mice. C: Persistent and increased BNST^{Esr2} neuronal activity encodes sexual satiety, and HCN channels contribute to hyperexcitability of BNST^{Esr2} in males. MOB, main olfactory bulb; AOB, accessory olfactory bulb; CoApl, posterolateral cortical amygdala; MeApd, posterodorsal medial amygdala; BNST, bed nucleus of the stria terminalis; MPOA, medial preoptic area; VMHvl, ventrolateral division of the ventromedial hypothalamus; PAG, periaqueductal gray; HCN, hyperpolarization-activated cyclic nucleotide-gated.

neurons also showed long-lasting increases after ejaculation, as evidenced by the higher firing frequency and larger amplitude of spontaneous Ca^{2+} transients in BNST^{Esr2} neurons in satiated mice of both sexes compared to the unsatiated group. Moreover, these changes lasted for days before gradually returning to pre-ejaculation levels, coinciding with the gradual recovery of mating in satiated mice after ejaculation. Together, these results established a firm and specific correlation between ejaculation-driven increases in BNST^{Esr2} neuronal activity and sexual satiety.

To further explore the causal relationship between elevated BNST^{Esr2} activity after ejaculation and sexual satiety, the authors artificially activated or inhibited these neurons and tested the effects on mating behaviors in satiated animals. Their results showed that chemogenetic inhibition of BNST^{Esr2} hastened the recovery of mating behaviors in sexually satiated mice of both sexes. Notably, the same manipulation also increased mating efficiency in virgin males, as evidenced by the increased probability of sniff-to-mount transition in regular male-female mating tests, even eliciting mounting toward male conspecifics. Similarly, ablation of BNST^{Esr2} led to deficits in achieving sexual satiety in both sexes, albeit without influencing normal mating behaviors. Conversely, chemogenetic activation of BNST^{Esr2} neurons almost completely abolished male mating without influencing female mating behaviors. In comparison, chemogenetic activation of BNST^{St18} neurons promoted rather than inhibited male mating. These findings established a negative correlation between BNST^{Esr2} activity and mating, especially in males, suggesting that ejaculation-driven increases in BNST^{Esr2} activity encode the sexual satiety state to inhibit mating after ejaculation.

To understand how increased BNST^{Esr2} activity suppresses mating, the authors optogenetically activated these neurons in a temporally resolved manner during different stages of the mating routine. They found that activation of BNST^{Esr2} during early sniffing selectively blocked the transition from the appetitive phase to consummatory stage and reduced the time males spent investigating the female, producing similar behavioral changes as seen in sexually satiated males. In comparison, optogenetic activation or inhibition of BNST^{Esr2} during the consummatory mating stage in male mice did not affect ongoing sexual behavior. These results suggest that BNST^{Esr2} neuronal activity controls the probability of behavioral transition from appetitive to consummatory actions in male mating, with higher neuronal activity blocking the behavioral switch.

Finally, to dissect the molecular and electrophysiological changes underlying the persistent activity changes in BNST^{Esr2} neurons after ejaculation, the authors performed whole-cell patch-clamp recording in brain slices. Consistent with the *in*

vivo Ca^{2+} recording results, the $BNST^{Esr2}$ neurons displayed more spontaneous firing in brain slices obtained from sexually satiated mice of both sexes compared to naïve or behaviorally recovered animals. Notably, $BNST^{Esr2}$ neurons in satiated animals showed higher resting membrane potentials (RMPs) and lower rheobases, indicating they are capable of firing action potentials with less excitation or are more excitable. Furthermore, the authors reported a larger depolarizing voltage "sag" mediated by the hyperpolarization-activated cyclic nucleotide-gated (HCN) cation channels in $BNST^{Esr2}$ neurons of sexually satiated males than unsatiated males or females. Consistently, the authors found higher expression levels of the *Hcn1* subunit in $BNST^{Esr2}$ neurons in satiated males than in virgin or recovered males. Importantly, pharmacological blockade of the BNSTpr HCN channel or genetic deletion of the *Hcn1* gene in $BNST^{Esr2}$ neurons via CRISPR-Cas9 blocked sexual satiety and ejaculation-triggered mating suppression without changing normal mating behaviors in male mice. However, interference with HCN currents did not affect sexual satiety in females.

Taken together, the authors showed that elevated spontaneous activity in $BNST^{Esr2}$ neurons encoded a brain state that prevented mating behaviors from transitioning from the appetitive stage to consummatory stage after ejaculation. They further showed that post-ejaculation increases in *Hcn1* expression and HCN currents contributed to hyperexcitability of $BNST^{Esr2}$ neurons in sexually satiated male mice. These results open up exciting new avenues to explore questions regarding neural modulation in mating, sexual dysfunction, and libido imbalance. For instance, the $BNST^{Esr2}$ neurons showing persistent, long-lasting changes in activity after ejaculation appear to represent only a fraction of the entire population. Thus, one immediate follow-up question is pinpointing this subset of $BNST^{Esr2}$ neurons. Establishing a more refined molecular marker for this ejaculation-responsive and sexual satiety encoding population will help to reveal the underlying molecular, neurochemical, and neural circuit mechanisms by which transient ejaculation events lead to long-lasting changes in *Hcn1* expression and neuronal firing patterns. In addition, by specifically targeting this population, we could determine whether the same neuronal population also plays a role in other behavioral functions assigned to the BNSTpr, such as preference for female cues, encoding of sex-specific conspecific information, regulation of aggression, and parental care, or whether it is dedicated to the regulation of mating behavior and sexual satiety. Could the activity of this population be modulated by other life events, such as stress and hormonal changes, to affect mating? Furthermore, what are the downstream targets of these neurons that mediate their effects on mating?

Previous research showed that dopamine neurons in the medial preoptic area (mPOA, a central brain site that drives male mating behavior) play crucial roles in the mating drive of male mice. Specifically, mPOA dopamine transients are abolished after successful mating, contributing to a lack of mating after ejaculation (Zhang et al., 2021). Thus, it would be interesting to investigate whether ejaculation-responsive $BNST^{Esr2}$ neurons directly project to mPOA dopamine neurons to mediate mating suppression during the sexual satiety period in males. Relatedly, sex differences in encoding sexual satiety and $BNST^{Esr2}$ neurons deserve more in-depth study. The authors recorded similar increases in $BNST^{Esr2}$ neuronal activity in male and female mice after ejaculation. However, chemogenetic inhibition of $BNST^{Esr2}$ neurons promoted mating in male but not female mice. Furthermore, the voltage sag in $BNST^{Esr2}$ neurons was much smaller in females than in males, and disruption of *Hcn1* in $BNST^{Esr2}$ neurons did not restore sexual receptiveness in satiated females. Indeed, female sexual behavior and mating motivation are influenced by cycling hormones and mediated by a different neural circuit than that in males. Thus, other neuronal populations and molecular mechanisms may exist in $BNST^{Esr2}$ neurons to encode sexual satiety in females, which awaits further analysis.

The work by Zhou et al. (2023) significantly advances our understanding of the neural encoding of the sexual satiety state. More importantly, their results highlight how the display of innate behaviors, though genetically hardwired, is also highly plastic and state-dependent (Wei et al., 2021). Experience-dependent changes in the excitability and synaptic connections of neurons in the circuit node of distinct neural networks that govern innate behaviors can initiate rapid transitions in the expression of a single behavior or even an entire behavioral pattern. This adaptive mechanism provides both robustness and malleability and represents an interesting new research direction for future study of innate behaviors.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

M.J.L. and X.H.X. wrote the draft manuscript. All authors read and approved the final version of the manuscript.

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