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Neuroscience: Plasticity Matters for Mating

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It remains unclear how hormonally mediated internal states affect specific brain circuits to modify behaviour. A new study reveals that a hypothalamic projection pathway critical for female sexual receptivity is extensively remodelled during the estrous cycle.

Animals are equipped with sets of instinctive behaviours, such as feeding, parenting and aggression, which can be performed without (or with very little) prior experience. The neural circuits controlling such behaviours are thought to be genetically specified during brain development. Critical neuronal populations within these circuits have been mapped to the hypothalamus, an evolutionarily highly conserved brain region which is often assumed to be largely hard-wired [1–3]. In contrast, functional and structural neural plasticity are more commonly studied in neocortex and hippocampus, brain structures associated with learning, memory and flexible cognitive processing [4,5]. In a new study in *Cell*, Inoue *et al.* [6] now demonstrate that hormonal changes during the rodent estrous cycle extensively remodel a hypothalamic projection crucial for female sexual behaviour.

Instinctive behaviours are profoundly shaped by physiological states [7]. For instance, hypothalamic neurons embedded within feeding circuits mediate taste preference depending on hunger state — resulting in diametrically opposed behavioural responses to highly-caloric food [8]. Such observations suggest that even developmentally sculpted hypothalamic circuits can undergo considerable plasticity in the adult brain, but the degree to which this occurs is not well understood. In principle, two broad categories of neural plasticity can be distinguished: *functional plasticity*, in which the strength of existing connections is modified, for example by synaptic plasticity and/or neuromodulation; and *structural plasticity*, in which circuits are remodelled by adding, moving or removing (sub) cellular elements.

While functional plasticity has been rather well studied, comparatively little is known about the extent and nature of large-scale structural plasticity in the adult brain, especially in subcortical areas

such as the hypothalamus. Several observations indicate that hypothalamic neurons and circuits are surprisingly malleable. The morphological reorganization of oxytocinergic neurons during lactation is a classic example [9], and structural plasticity is also well-documented in hypothalamic feeding circuits [10]. Another recent study [11] found a substantial increase in the number of tyrosine hydroxylase-expressing neurons in a hypothalamic nucleus in mothers as compared to virgin females. While these observations testify to the ability of hypothalamic areas for extensive remodelling, it remains largely unclear how such plasticity alters circuit-level information processing and, eventually, behaviour.

Inoue *et al.* [6] focussed their investigations on progesterone receptor (PR)-expressing neurons in the ventrolateral subdivision of the ventromedial hypothalamus (VMHvl^{PR}). This neuronal population co-expresses estrogen receptor 1 (Esr1) and was previously found to be crucial for female sexual receptivity [12]. Estrogen levels peak during the ovulatory phase of the estrous cycle (estrus; Figure 1), a temporal window during which females are sexually receptive. The authors therefore hypothesized that VMHvl^{PR} neurons are a possible link between behavioural (female receptivity) and physiological estrus.

In order to test whether these neurons are active during female sexual behaviour, Inoue *et al.* [6] performed population-level calcium imaging from VMHvl^{PR} neurons in ovariectomised females during interactions with a male conspecific. VMHvl^{PR} neurons were active during these encounters in hormonally primed (receptive) and non-primed (unreceptive) females (Figure 1). VMHvl^{PR} neurons are thus implicated in female sexual behaviour regardless of physiological status. Subsequent chemogenetic and optogenetic manipulations corroborated the necessity of VMHvl^{PR} neuronal function for female sexual behaviour [6]. Surprisingly, however, artificial activation of these neurons in non-primed females was *insufficient* to elicit sexual behaviour, suggesting that VMHvl^{PR} neuronal activity cannot be relayed during a non-receptive state equivalent to diestrus.

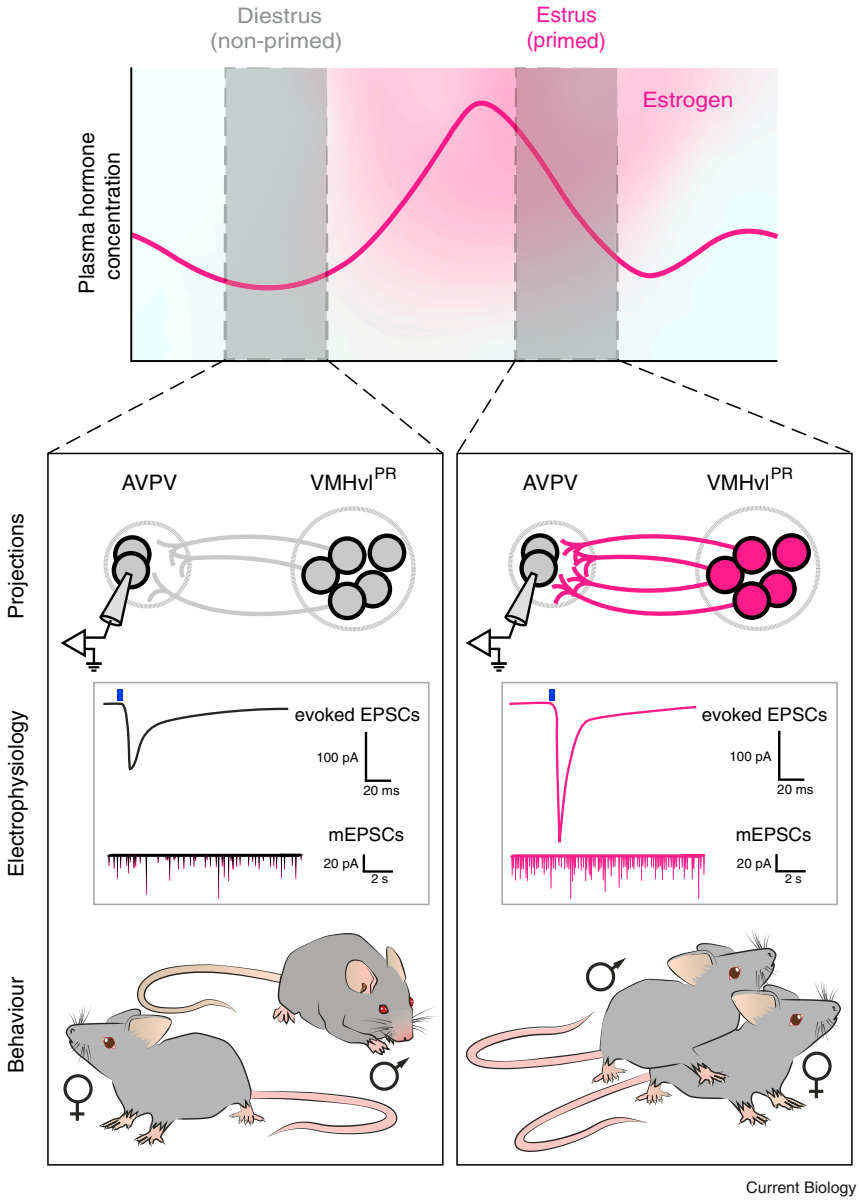

Figure 1. Estrogen induces morphological remodelling of a hypothalamic projection crucial for female sexual behaviour.
Cyclic increase in estrogen levels during the estrous cycle (4–5 days in mice) leads to increased density of projections between progesterone receptor (PR)-expressing neurons in the ventromedial hypothalamus (VMH) and the anteroventral periventricular nucleus (AVPV) [6]. Electrophysiological recordings confirm that this increased anatomical connectivity results in increased functional connectivity, as demonstrated by larger optogenetically evoked excitatory post-synaptic currents (EPSCs) and more frequent miniature excitatory post-synaptic currents (mEPSCs) in estrus. Females in estrus (high-estrogen state) display higher sexual receptivity, and the VMH^{PR}-to-AVPV projection is critical for this behaviour.

Inoue *et al.* [6] therefore next explored whether VMHvl^{PR} projections are subject to estrous cycle-driven changes. They virally expressed a synaptically targeted fluorophore in VMHvl^{PR} neurons and quantified the abundance of presynaptic punctae in the anteroventral periventricular nucleus (AVPV), an important hub for sex-specific behaviours which is more densely innervated by VMHvl^{PR} neurons in females [11–14].

Intriguingly, a three-fold increase in labelled presynaptic termini was observed in primed *versus* non-primed females (Figure 1). This difference was seen neither in two other VMHvl^{PR} projection sites, the preoptic hypothalamus (POA) and the periaqueductal gray (PAG), nor in males subjected to an estrus-mimicking hormonal regime. These data suggest that VMHvl^{PR}-to-AVPV projections are substantially and selectively remodelled during the estrous cycle.

Does this striking anatomical change have functional consequences? Chemogenetic stimulation of (glutamatergic) VMHvl^{PR} neurons *in vivo* resulted in higher numbers of activated AVPV neurons in primed females, and *ex vivo* whole-cell patch clamp recordings confirmed that the observed increase in synapse number is accompanied by increased functional connectivity: AVPV neurons of primed females receive higher frequencies (but not amplitudes) of miniature excitatory postsynaptic currents (mEPSCs), as well as larger peak amplitudes of optogenetically evoked EPSCs (Figure 1). These physiological data corroborate the finding that ovarian sex hormones enhance VMHvl^{PR}-to-AVPV excitatory projections by increasing synapse number, but not the strength of individual synapses [6].

In a final set of experiments, Inoue *et al.* [6] addressed which mechanisms are responsible for this form of neuronal plasticity. Estrus is typically induced by sequential stimulation with estrogen and progesterone. However, PR expression is induced by estrogen, and estrogen alone is sufficient to maximally stimulate female receptive behaviour [15,16]. Indeed, priming with estrogen alone recapitulated the increase in VMHvl^{PR}-to-AVPV projections, while VMHvl-specific deletion of Esr1 abolished this priming-induced effect. Estrogen signalling via Esr1 is therefore critical for estrous cycle-dependent morphological remodelling of this pathway [6].

Overall, Inoue *et al.* [6] provide compelling evidence for hormonally induced large-scale rewiring of a hypothalamic circuit element. Naturally, these new findings raise several important questions. First, the extent and subcellular localization of this remodelling remain to be addressed. Are entire VMHvl^{PR} axons remodelled (or even generated?) during periods of merely 2–3 days? Or is the observed increase in presynaptic termini the result of smaller-scale outgrowth of axonal boutons? A simple way to address this would be to investigate whether coronal brain slices between VMHvl and AVPV contain a larger number of axonal cross-sections during estrus. Second, an intriguing mechanistic problem is posed by the fact that the majority of VMHvl neurons seem to send out highly branched projections (fan-in, fan-out model) [17]. Because VMHvl^{PR} projections to the MPOA and PAG seem to be structurally unaffected by estrus, how does estrogen selectively remodel some axons but not others?

Finally, while it is clear that the VMHvl^{PR}-to-AVPV projection pathway is critical for female sexual behaviour, and that its remodelling is hormonally controlled, it would be exciting to directly address the behavioural role of this type of plasticity, for example by examining the consequences of selectively rendering this projection hormone-insensitive. In conclusion, this fascinating study by Inoue and colleagues highlights an astonishing degree of adult structural brain plasticity, occurring over a surprisingly short time course [6]. This study therefore reveals a soft, malleable side to hard-wired hypothalamic circuits.

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