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In the mood for sex: neural circuits for reproduction

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Sex is pervasive in nature. Yet, despite its importance for species maintenance and evolution, sex is unnecessary for the survival of the individual, it can have a negative impact on fitness and is performed by most species (except our own) without awareness of its consequences: fertilization. A myriad of mechanisms has evolved to promote its fruitful execution, such that sex it promoted when fertilization is most likely to occur and inhibited otherwise. In this review we present recent advances in our knowledge of the neuronal circuits underlying sexual behaviour. We discuss flies, rats and mice to underline the breadth of existing neuronal strategies used to accomplish the appropriate execution of this behaviour, while still highlighting shared principles across such distinct taxa.

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Introduction

Life without sex would not only be less interesting, it would be impossible for most species. Not surprisingly, nature has evolved powerful tools to guarantee that animals have sex, such as turning it into something that is highly rewarding or reinforcing in many species [1,2,3•]. However, sexual behavior should also be tightly controlled to match the behavioral ecology of each individual and species [4]. Therefore, a series of mechanisms that ensure the fruitful execution of sexual behavior have been reinforced, coordinating the reproductive capacity of each individual with their attractiveness, motivation to mate and environmental conditions [4].

This article reviews the recent findings on the neuronal processes underlying the timely execution of sexual behavior. We begin by describing the behavior itself, focusing on flies, mice and rats and the relevant circuitry. Despite the astonishing diversity of sexual behavior, it can be generally divided into three phases that are shared by most species: an appetitive phase, where the suitability of potential partners is assessed, culminating with the choice of a mate; a consummatory phase, where animals copulate and sperm is transferred; a third, inhibitory phase, where the drive to re-start the behavior is highly diminished. We emphasize recent literature offering mechanistic insights into how mating is promoted when fertilization is most likely to occur and inhibited otherwise. Despite the differences, common solutions are emerging across taxa, reminding us that insights gained from different model organisms may guide our pursuit in understanding this fascinating behavior [5].

Basic sequence of sexual behavior

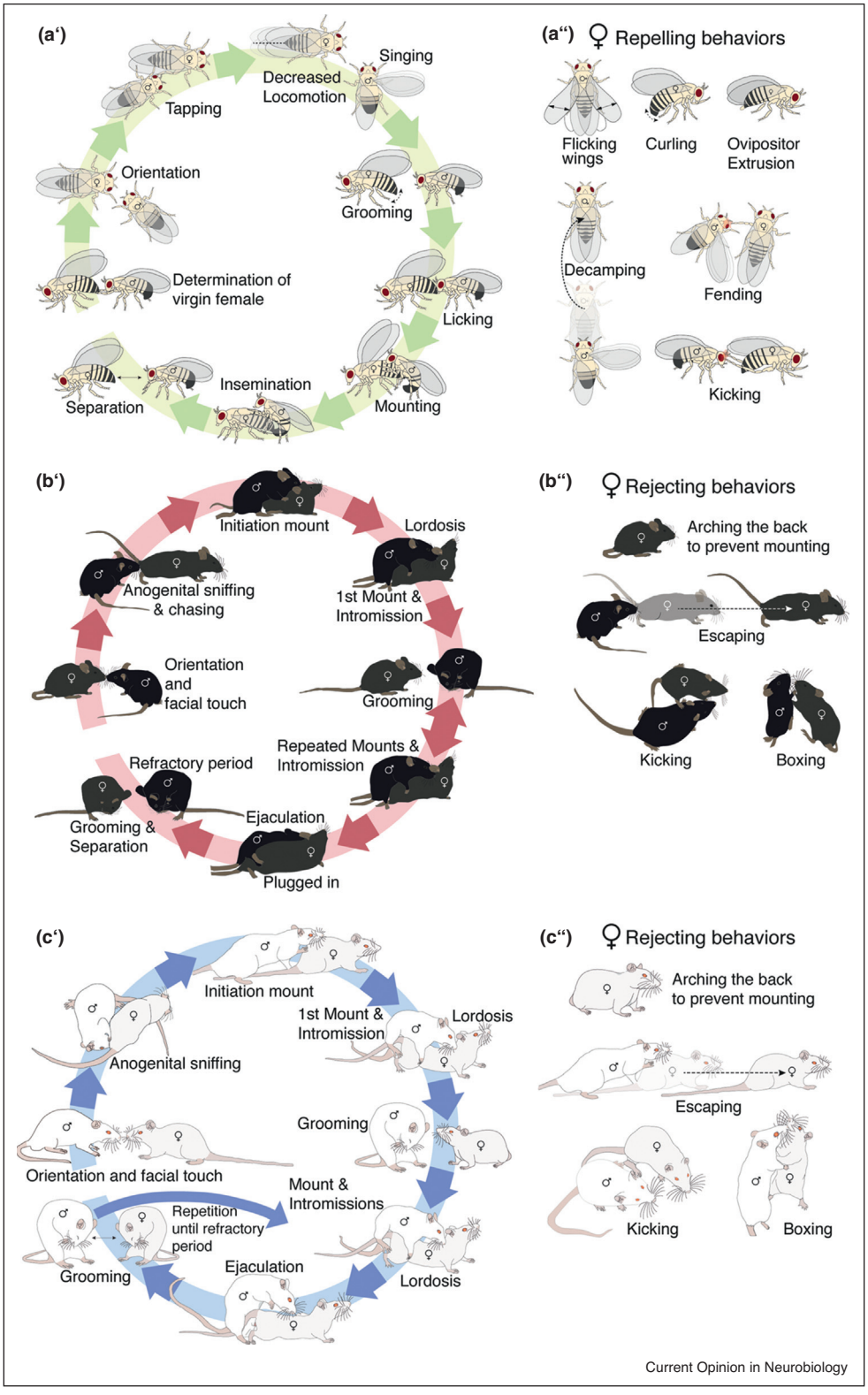
Flies

When a male fly encounters another fly, he will chase it, extend his foreleg and touch it to determine its suitability (Figure 1a') [6,7]. If the other fly is a mature virgin female, courtship is initiated and maintained by the integration of multisensory information [7,8••] and eventually reciprocated by the female [9–11]. Copulation takes place once the female stops and similarly to other species, ejaculation is rewarding for the male [3•]. Inhibition after copulation is primarily imposed by the male, that converts the female into a less desirable and less receptive individual towards other males and aggressive towards other females, mainly by the transfer of sex peptide (SP) (Figure 1a'') [12–14]. The SP-mediated effect is terminated once sperm is depleted, at which point she may mate with another male [15]. During copulation, males also deposit anti-aphrodisiac pheromones on the female, inhibiting courtship attempts by other males [16]. Females can counteract the copulatory aftermath by ejecting sperm [17] and by removing pheromones [16]. Genital sensory stimulation received by the female during copulation also decreases receptivity [9,18•]. After few copulations, males decrease their motivation to re-initiate courtship [19] (For detailed description of *Drosophila* sexual behavior, see Refs. [20,21]).

Mice

Volatile olfactory cues are essential for communication amongst mice, but when in close contact, individuals engage in mutual tactile exploration and are exposed to contact-dependent pheromones (Figure 1b') [4]. Less obvious to the experimenters' eye are ultrasonic vocalizations (For reviews see Refs. [22,23]). Despite initial controversial role [24], male calls enhance female approach behavior [25] and females also emit a courtship song [26]. While the initial social interaction is

Figure 1



Basic sequence of fly **(a)**, mouse **(b)** and rat **(c)** sexual behaviour.
(a') The fly's appetitive or courtship behavior is initiated once the male tastes the pheromones of a virgin female. Male courtship sequence includes: chasing and tapping the female, singing and licking. The sequence is highly variable and occurs until the female either actively accepts or rejects the male [21]. The female shows her willingness to mate by modulating her walking speed and pausing behavior [10,11], and by the

independent of the female's reproductive cycle in some mouse strains [27], its course quickly changes if she is receptive (Figure 1b' versus b'' for non-receptive): the female initiates a series of behaviors including lordosis in response to male's copulation attempts. Consummatory behaviour begins with the first successful mount and the execution of intromissions. A variable number of mounts is executed until reaching ejaculation. The pacing of the sexual stimulation is fundamental for the establishment of pregnancy and the rewarding nature of the interaction (at least for the female), and intromissions and ejaculation are rewarding for the male. Males enter a variable refractory-period after ejaculation that can last up to several days depending on the strain, where they are not attracted by a receptive female [28]. Male mice deposit copulatory plugs that act as a physical barrier, reducing or delaying female re-mating [29]. Vaginal stimulation during copulation can induce a pseudo-pregnant state, and similarly to a pregnant female, she will reject mounting attempts (Figure 1b'') (For detailed description of mouse sexual behavior, see Refs. [4,30]).

Rats

Rat appetitive sexual behavior is comparable to mice (Figure 1c'), with olfaction playing a critical role in female approach behavior [31]. In contrast to mice, ultrasonic vocalizations have no incentive value for both sexes [32,33] and copulation is similar in vocalizing or silent rats [34]. Rats will mount once and perform several intromissions until they ejaculate, but multiple rounds of mounts followed by ejaculation can be executed until the male reaches sexual exhaustion and enters a refractory period that can last for several days and differs across strains. Females can accept multiple ejaculations from the same or different males and if possible, they will pace the rate of the sexual interaction (For detailed description of rat sexual behavior, see Refs. [35,36]).

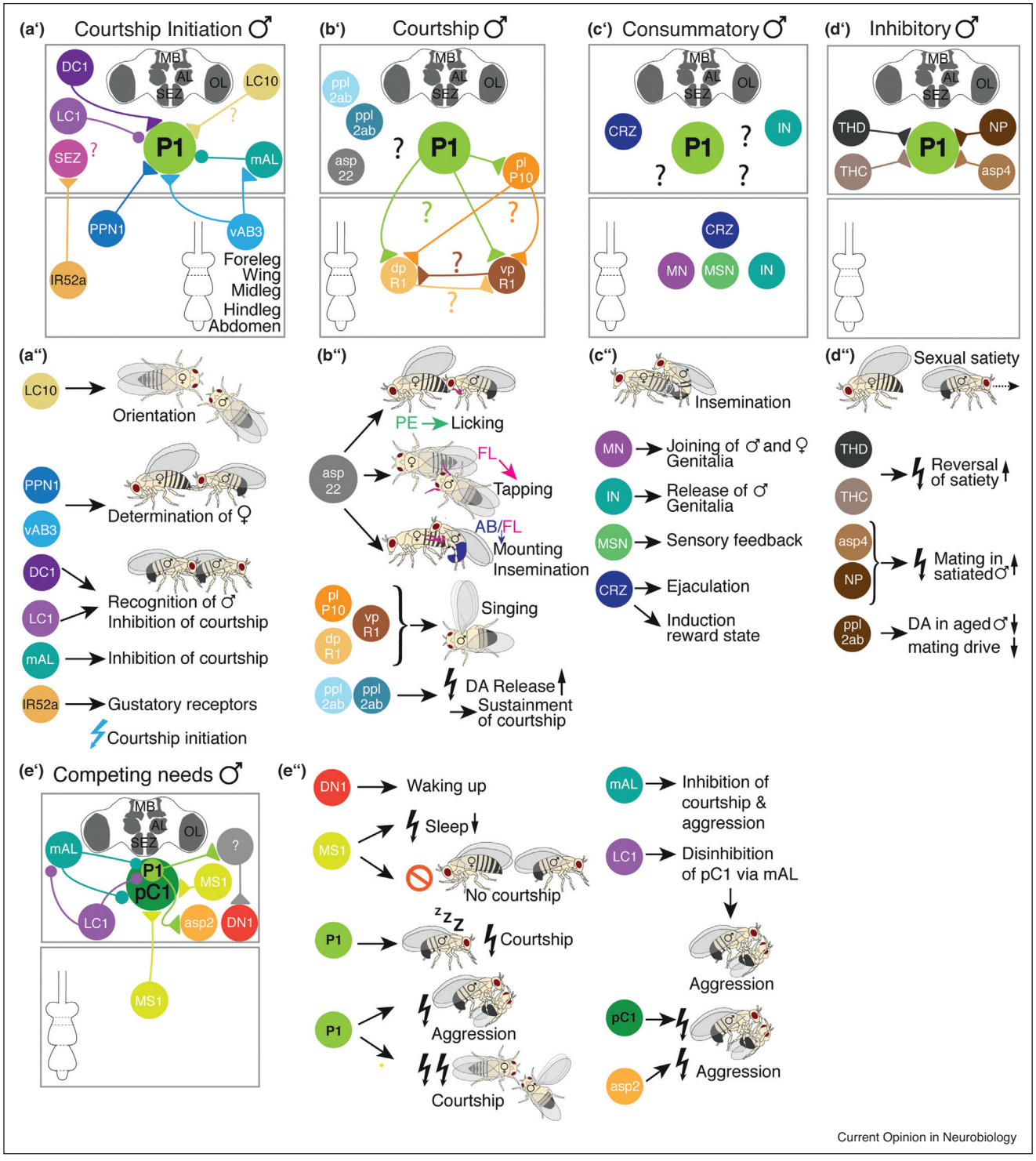
Neuronal processing of fly sexual behavior

A central node for male courtship initiation is the P1 cluster (Figure 2a'), onto which sensory input converge [8••,37–39,40•]. Flies produce a variety of pheromones that modulate courtship behavior in males, as for example the female pheromone 7,11-HD [41] (for a recent review on *Drosophila* chemical signals see [20]; but the logic of pheromone communication is still being uncovered [42]). Pheromonal input reaches P1 neurons through parallel pathways of feedforward excitation and inhibition, such that the net effect of female derived stimuli is excitation [8••,39]. Stimuli like 7,11-HD also gate the impact of other stimuli onto P1 neurons, so that once the behavior is initiated it can be maintained by cues that do not elicit the behavior alone, sustaining persistent courtship to appropriate targets [8••,38]. For example, activation of visual projection neurons (LC10; Figure 2a' and a'') that respond to fly-resembling moving targets, elicits robust courtship in males whose P1 neurons were previously activated [40•]. The wing also senses sexual cues, containing gustatory receptor-expressing neurons (IR52a; Figure 2a' and a'') whose activity modulates male courtship [43]. If and how wing-derived information reaches P1 is unknown.

How P1 interneurons activate the appropriate sequence of descending neurons in charge of each component of male courtship is unresolved, but some of the elements controlling individual actions have been identified (Figure 2b' and b'') [44]. The characterization of half of the fly's descending neurons [45] will hopefully assist in this effort. A single pair of descending neurons (asp22; Figure 2b' and b'') capable of triggering distinct courtship actions in the same sequence as found during courtship was recently identified, a surprising circuit organization that contrasts a parallel arrangement for different actions [46••].

(Figure 1 Legend Continued) opening of the vaginal plates [21]. The male employs its external genitals to hold the female and achieve genital coupling [48••]. During copulation a mixture of sperm and seminal fluid is transferred, upon which the male detaches from the female. Female receptivity is highly diminished after ejaculation and after multiple ejaculations the male's mating drive is also decreased. (Fly drawings were adapted with permission from Ref. [131]).
(a'') Mated and immature females will express multiple rejecting behaviors in the presence of a courting male. (Fly drawings were adapted with permission from Ref. [21]).
(b') In close contact, male and female mice will start tactile exploration of each other's bodies. The male will then actively chase the female, approaching her from behind and positioning his forelimbs on her flanks to perform mount attempts; if willing to mate, she will stop and the somatosensory stimulation of the flanks elicits a dorsiflexion of the female back, the lordosis posture, allowing copulation [4]. The male performs pelvic thrusts with intromissions. In between mounts, animals can groom themselves. Male mice perform a variable number of mounts with intromissions until they ejaculate. During ejaculation, the male stays clasped onto the female. He then collapses to the side, while still holding the female with his forelimbs (Plugged in); after 15–20 s, the couple splits up, usually because the female walks away, and the separation is followed by genital grooming on both sides. Male mice enter a refractory period after having reached ejaculation.
(b'') Similar to flies, immature and non-receptive female mice will perform multiple rejection behaviours.
(c') Rat appetitive sexual behaviour is comparable to mice (facial and anogenital sniffing). Highly receptive females will express spontaneous lordosis posture in the absence of flank stimulation, ear wiggling, hopping and darting. Rats will mount once and perform several intromissions until they ejaculate. Multiple mounts, each one leading to an ejaculation, can be executed within a short post-ejaculatory interval (4−7 min), until the male rat reaches sexual exhaustion which can last for several days. Like mice, male rats leave a plug on the female.
(c'') Unreceptive female rats, similar to flies and mice, express a series of rejecting behaviours.

Figure 2



Neural circuits of male fly sexual behaviour.
(a') Sensory pathways involved in courtship initiation. To initiate courtship towards a female, visual, olfactory and gustatory stimuli converge on the P1 population (made up of ~ 40 neurons). PPN1 and vAB3 neurons (and indirectly inhibitory mAL [39]) relay excitatory female information to P1 [8••]. The information of the male pheromone cVA reaches P1 via a group of excitatory (DC1) and inhibitory visual neurons (LC1). Both branches impinge onto P1, but the net balance is inhibition [8••]. The wing sensilla brings pheromonal input via specialized neurons (IR52a), which project onto second order projection neurons in the suboesophagal zone (SEZ), but how the information reaches P1 is unknown [43].
(a'') Function of sensorial pathways during courtship initiation. LC10 neurons may be involved in the correct orientation of the male towards the female [40•]. PPN1 and vAB3 process female pheromonal cues and promotes courtship, whereas DC1, LC1 and mAL are crucial for the

Much less is known about the circuitry underlying female appetitive behavior, but the tide is changing with the identification of two central clusters, pC1 and pCd (Figure 3a'), which are fundamental for female receptivity and respond to courtship relevant stimuli, such as the volatile male pheromone cVA and the courtship song [47]. Other nodes include *abdominal-B* expressing neurons in the ventral nerve cord (AbdB in VNC; Figure 3a') that control pausing behavior (Figure 3a'') [10], and the *apterous* population in the brain (Ap; Figure 3a'), necessary for reducing walking speed (Figure 3a'') [11]. It remains to be determined if these two populations interact with the pC1 and pCd neurons (Figure 3a').

The control of copulation is less well understood, but recently the circuitry commanding the fly's genitalia was characterized and is composed of VNC glutamatergic motor neurons, GABAergic interneurons that modulate the timing of motor neuron activity and hair-like bristles that provide sensory feedback (MN, IN and MSN; Figure 2c' and c'') [48••]. Interestingly, ejaculation alone, which can be triggered by male-specific neurons present in the VNC-abdominal ganglia, is rewarding (CRZ; Figure 2c' and c'') [3•].

Inhibition after copulation afflicts both males and females. After repeated copulations, males will exhibit decreased sexual drive, dependent on brain dopaminergic neurons that sensitize P1 neurons to inhibition, such that the net effect of female appetitive cues becomes inhibitory (THC/D, asp4 and NP; Figure 2d' and d'') [19]. The inhibition of female re-mating comes in many flavors, but the best studied mechanism relies on SP [13], whose action depends on local (SPS; Figure 3c' and c'') [49] and ascending (SAG; Figure 3c' and c'') [50] VNC-abdominal ganglion neurons that relay the information of uterine SP receptor-expressing sensory neurons to the brain. Inhibition of female re-mating is further mediated by neurons innervating the sperm storage organs (Oct; Figure 3c' and c'') [51]. Recently, two fast-acting SP-independent mechanisms inhibiting female receptivity were described (Figure 3d' and d''). The first involves cVA, which during mating activates additional sensory neurons that inhibit the initial attractive response towards cVA [52]. A second depends on VNC-abdominal ganglion sensory neurons whose terminals in the vagina (piezo-pkk; Figure 3d' and d'') sense the mechanical stimulation received during mating and relay it to ascending neurons in the VNC and the brain (LSAN; Figure 3d' and d'') [18•]. It is unknown how these neurons lead to decreased female receptivity, but anatomical evidence and the fact that decreasing activity in the pC1 and pCd populations leads to decreased female receptivity, suggests that pC1 and pCd neurons might be involved [47]. Active sperm

(Figure 2 Legend Continued) recognition of another male and lead to net inhibition of P1 and courtship/aggression. Activating IR52a leads to robust courtship initiation (Blue lightning bolt: optogenetic artificial activation).

(b') Neuronal clusters involved in courtship maintenance. Courtship patterns include tapping, licking and mounting and the pair of descending asp22 neurons are able to drive these motor actions [46••]. The projection pathway of asp22 to or/and from P1 is unknown. The decision to sing is mainly achieved by P1 and pIP10 in the brain (P1 sends excitatory axons to pIP10) whereas thoracic neurons (dPR1, vPR6) are the motor components that produce the appropriate song pattern [20]. Two clusters of dopaminergic ppl2ab neurons control courtship maintenance once initiated [57].

(b'') Function of neuronal clusters during courtship maintenance. Stimulating asp22 neurons at different intensities elicits courtship actions in the appropriate sequence, from proboscis extension which is needed for licking behaviour, to foreleg lifting, which is part of tapping and needed for successful mounting, up to abdomen bending, which is observed during mounting and insemination [46••]. pIP10, dPR1 and vPR6 are part of the song central pattern generator [20]. (Black lightning bolt: artificial activation).

(c') Neuronal clusters involved in consummatory behaviour. Insemination demands a tight sensory control of the genitalia. VNC glutamatergic motor neurons (MN), GABAergic interneurons (IN) that connect onto the latter and hair-like bristles containing mechanosensory neurons (MSN), make up the genital controlling circuitry [48••]. Corazonine peptide expressing neurons (CRZ) are able to drive ejaculation [3•]. If these groups of neurons are interconnected and how they communicate with the P1 cluster is unknown.

(c'') Function of neuronal clusters involved in consummatory behavior. GABAergic IN control the release of the genitalia, while MNs provide the joining of male and female genitals. MSN give sensory feedback [48••]. Artificially activating CRZ cells leads to ejaculation and induces a rewarding state [3•].

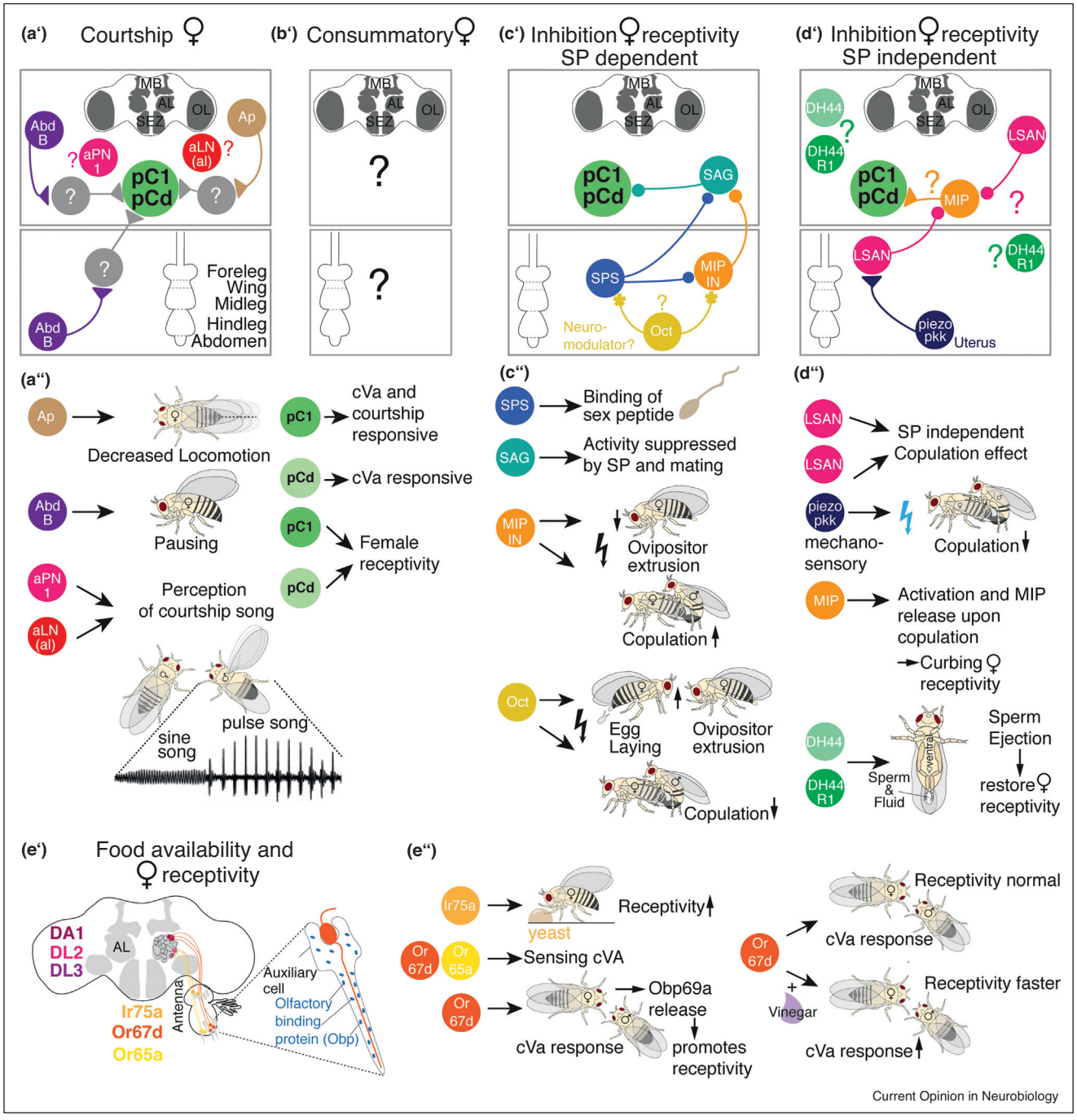
(d') Neuronal clusters involved in post-mating inhibition. Neurites of the dopaminergic neurons TH-C, TH-D and asp4 as well as the line NP5945 (NP) are in close proximity to P1, suggesting direct connection [19].

(d'') Dopaminergic clusters for sexual motivation. Activating TH-C and TH-D in a sexually satiated male, reinitiates courtship with a female. Co-activating of asp4 and all the DAergic neurons in the NP-line increases mating in satiated males [19]. The age-related decrease in DA levels and thus diminished courting behaviour, can be overcome by activating a specific subset of the ppl2ab population [57]. (Black lightning bolt: artificial activation).

(e') Neuronal clusters integrating competing needs. The pC1 aggression center contains the courtship driving P1 cluster. mAL inhibits P1 and pC1. LC1 serves as a neuronal switch in the circuitry, which is able to disinhibit pC1 via mAL, thereby promoting aggression [59]. Further LC1 inhibits P1 and thus courtship [59]. A small octopaminergic population (asp2) involved in aggression was recently identified which receives input from P1 [132]. P1 is further connected with sleep controlling centers (DN1), although the connection is not direct [60] and receives input from sleep-related octopaminergic neurons (MS1) [61].

(e'') Function of clusters integrating competing needs. DN1 neurons are active during wakefulness. During courtship DN1 activity is kept high via excitatory input arriving from P1 [60]. Stimulating P1 neurons in a sleepy fly can promote courtship. Activation of MS1 neurons decreases sleep, while blockade of MS1 disturbs courtship [61]. Even though pC1 was supposed to be exclusively responsible for aggression, lower activity in P1 can lead to aggression too, while higher activity exclusively drives courtship [132]. (Black lightning bolt: artificial activation; forbidden signal: artificial inactivation).

Figure 3



Neural circuits of female fly sexual behaviour.

(a') Neuronal clusters important for the receptive state. The pC1 and pCd clusters are the female homologue of the male P1 [47]. The *apterous* neurons do not have direct connection to pC1 and pCd [11]. The *abdominal-B* neurons are found in the abdominal ganglion and reproductive tract and impinge onto the pC1 and pCd clusters also via an unknown relay [10]. Two interneurons (aPN, aLN) are involved in courtship song processing. How they connect with brain receptivity centers is unknown [133].

(a'') Function of neuronal clusters involved in receptivity/courtship. pC1 and pCd receive courtship relevant stimuli. pCd is exclusively cVa responsive, while pC1 also responds to the males' courtship song. Activating these clusters promotes female receptivity, blocking them renders females unreceptive [47]. *Apterous* neurons promote a decrease in walking speed [11] and *Abdominal-B* neurons control pausing behavior [10]. aPN1 and aLN(al) are required for processing the courtship song; specifically, the pulse song module is integrated in aPN1 [133].

(b) Neuronal clusters involved in female consummatory behaviour. The female neuronal pathways underlying female copulation behavior are unknown.

(c') Neuronal clusters involved in sex peptide mediated post-mating inhibition. Male sex peptide enters the spermatheca and is detected by

ejection by the female is achieved by a brain signaling pathway dependent on the diuretic hormone (DH44R1; Figure 3d' and d'') [17].

Integration of past experience, competing needs and environmental cues

The response of P1 neurons to pheromones is positively correlated with courting vigor, suggesting that these neurons compute the desirability of available targets, promoting or inhibiting courtship [8••,39]. For example, the male pheromones 7-T and cVA suppress inter-male courtship by net inhibition of the P1 cluster [8••,39]. In mated females (which become cVA-coated), cVA also overrides the effect of the female pheromone 7,11-HD [8••]. Courtship inhibition by cVA-coated females is experience-dependent [53]. Remarkably, reproductive barriers between closely related *Drosophila* species can emerge from the evolution of peripheral chemosensory pathways detecting pheromones [54] as well as in diversification of central processing [55]. The coupling of male reproductive maturity and courtship activity depends on a juvenile hormone-dependent sensitization of sensory neurons responding to female appetitive pheromones [56•], one of the first hormone-dependent mechanisms to be described in the fly. In contrast, older flies have a decline in sex drive, dependent on dopaminergic transmission (ppl 2ab; Figure 2d'') [57].

The P1 cluster is also involved when flies must decide between different needs. For example, cVA and 7-T do more than just suppress courtship towards other males, they can elicit inter-male aggression, via the pC1 cluster (which contains the P1 population, Figure 2e' and e'') [58,59]. Also, sleep and sex are two mutually exclusive behaviors, and P1 neurons and sleep-controlling centers communicate to each other (Figure 2e') such that sleep deprivation inhibits P1 activity and decreases courtship, while P1 activity leads to sleep suppression (Figure 2e''), ensuring that courtship is maintained once initiated [60,61]. Homeostatic regulation of sleep is also affected by sex, as sexual arousal can suppress the need to sleep after sleep deprivation [62].

Food availability affect female's behavior (Figure 3e' and e''). In the presence of vinegar, an attractive odor available in fermenting fruits, virgin females become more receptive through enhancement of the cVA response [63•]. The presence of yeast, indicative of a rich nutritional environment, also affects female receptivity by an unknown mechanism [64]. Finally, the social context modulates the response of virgin females to cVa, as male presence upregulates the expression of odorant binding proteins (Figure 3e', zoom in) involved in the perception of cVA [65].

Neuronal processing of mouse/rat sexual behavior

Volatile and contact-dependent pheromones are fundamental for rodent appetitive behavior [66,67]. Despite some controversy, volatile cues sensed by neurons of the main olfactory epithelium (MOE) in the main olfactory system might be crucial for the initial approach behavior (Figure 4a), while contact-dependent cues present in several body fluids are primarily sensed by neurons of the vomeronasal organ (VNO) and the accessory olfactory system when animals are in close contact (Figure 4b).

(Figure 3 Legend Continued) sex-peptide positive sensing neurons (SPSN). SPSNs project to the abdominal ganglion onto SP abdominal ganglion (SAG) neurons which relay the SP information through ascending fibres to the brain [50]. SPSNs make also inhibitory contact onto local inhibitory myoinhibitory peptide (MIP) interneurons. MIP neurons in turn branch onto ascending SAG cells which transmit the SP information to pC1 and pCd clusters [49]. A fourth cluster of octopaminergic neurons is found in the abdominal ganglion, but how they interact with the rest of the circuit is unknown [51].

(c'') Function of neuronal clusters mediating the SP post-mating response. The sex-peptide sensory neurons (SPSNs) sense the SP in the females' reproductive organs and transmit the signal to two other groups of neurons (SAG, MIP). The SAG neuronal activity is suppressed in the presence of SP and mating [50]. Activation of MIP neurons leads to a decrease in ovipositor extrusion and to an increase in copulation [49]. Activating octopaminergic cells drives egg laying and ovipositor extrusion and thus decreases copulation [51].

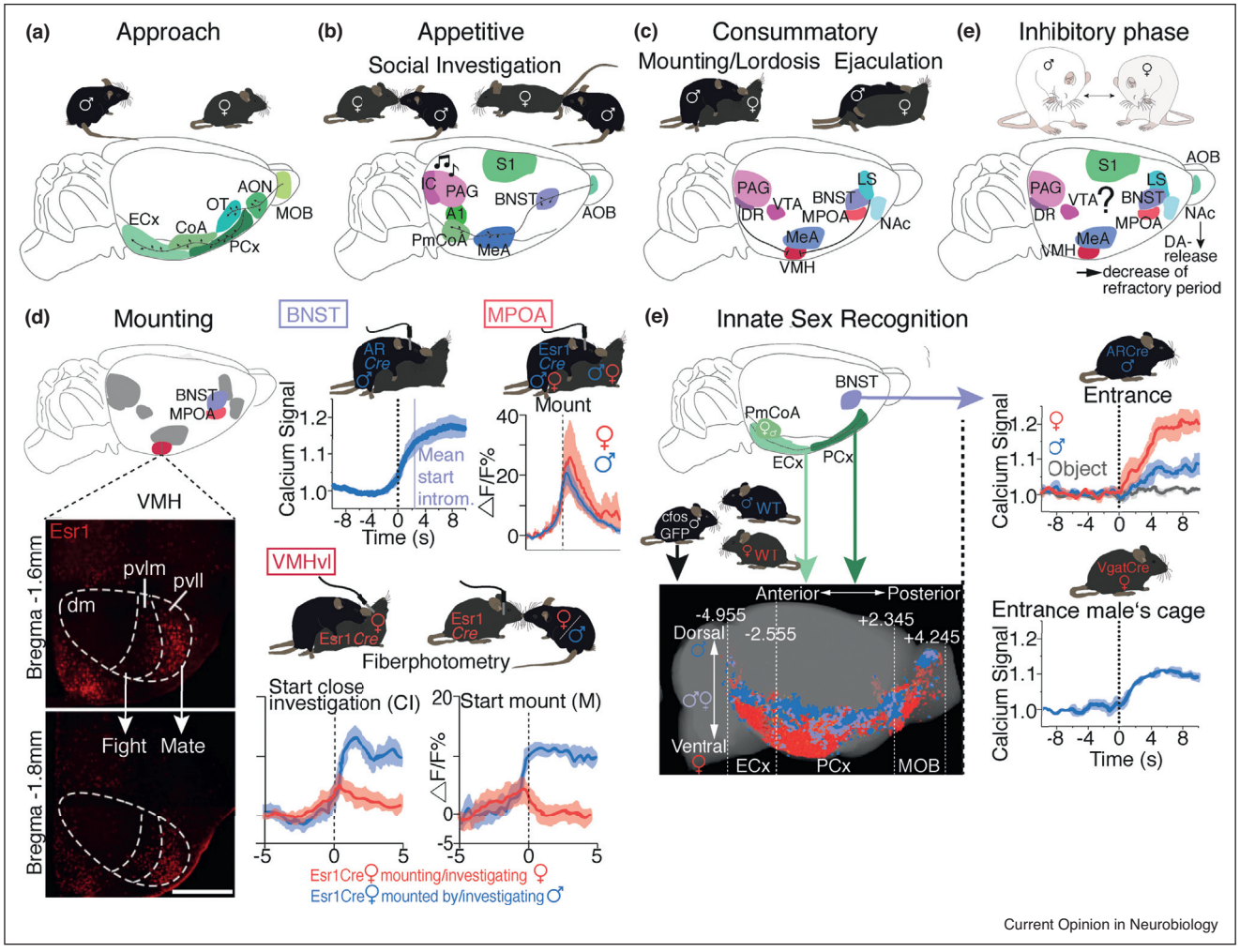
(d') Neuronal clusters involved in sex-peptide independent post-mating inhibition. A group of abdominal ganglion neurons, expressing the mechanosensory channel piezo (piezo-pkk), contains terminals in the vagina where they sense mating related sensory information. This information is relayed to ascending neurons (LSAN), and finally to MIP neurons in the brain (MIP) [18•]. Female flies have the capacity of actively rejecting sperm and become receptive again. DH44 receptor expressing neurons (DH44R1) in the abdomen and DH44 producing and DH44R1 neurons in the brain are crucial for sperm ejection, but their inputs and outputs are currently unknown [17].

(d'') Function of neuronal clusters mediating the SP independent post mating response. Activation of piezo-pkk neurons leads to a decrease in copulation. LSAN neurons mediate the SP-independent copulation effect (females become unreceptive by the mere mechanical stimulation upon mating), relaying information to MIP releasing neurons in the brain that curb female receptivity through an unknown pathway [18•]. Silencing DH44 producing neurons or neurons expressing its receptor (DH44R) accelerates sperm injection, while their activation delays the process [17].

(e') Olfactory sensing neurons integrating food availability information. The cVa sensing neurons (Or67d and 65a) are located on the ventral part of the flies' antennae and project to DA1 (Or67d) and DL3 (Or65a) glomeruli in the antennal lobe (AL) [65]. Activation of Or67d-expressing sensory neurons causes the release of olfactory binding proteins (Obp) in auxiliary cells in the antennae. Yeast sensing Ir75a olfactory neurons are located dorsally and project onto the DL2 glomerulus [64].

(e'') Olfactory sensing neurons and their impact on female receptivity. Female flies' receptivity is increased in the presence of yeast which is sensed by Ir75a [64]. Or67d neurons are responsible for the normal cVa response in the presence of the male. In the presence of vinegar, the cVa response is increased [63•]. Another mechanism through which the female fly becomes more sensitive to cVa, is by the release of odorant binding proteins (Obp69a) which are triggered by the male presence [65].

Figure 4



Neural circuits of rodent sexual behavior.

(a) Brain areas involved in approach behavior. The main olfactory system is a key player during approach behavior. The most prominent output of the main olfactory bulb (MOB) is the olfactory cortex, which is composed by the anterior olfactory nucleus (AON), olfactory tubercle (OT), piriform cortex (PCx), cortical amygdala (CoA) and entorhinal cortex (ECx) [71•].

(b) Brain areas involved in appetitive behavior. The accessory olfactory bulb (AOB) receives strong input from the vomeronasal organ (VNO) which is a specialized structure involved in the detection of pheromones present in sweat, urine, tears or saliva [75]. The AOB output comprises the medial amygdala (MeA), posteromedial cortical amygdala (pmCoA) and the bed nucleus of the stria terminalis (BNST) (all activated during appetitive sexual behaviour). Primary cortical areas (primary somatosensory (S1) and auditory (A1) cortex) are responsive to social stimuli [81,82,86]. Ultrasonic vocalizations emitted by male and female mice and rats were shown to be processed in the inferior colliculus (IC) and periaqueductal grey (PAG).

(c) Brain areas involved in mounting/lordosis and ejaculation.

The medial preoptic area (MPOA) and ventromedial hypothalamus (VMH) are crucial structures for mouse and rat consummatory behaviour. The VMH receives strong inhibition from the dorsal raphe (DR) and lateral septum (LS), which is supposed to be released for consummatory behaviour to be executed. The ventral tegmental area (VTA) and the nucleus accumbens (NAc) are involved in sexual consummation and motivation. The PAG is thought to be the key relay structure for transmitting hypothalamic activity to the spinal cord.

(d) Neuronal activity during mounting in:

BNST: Calcium bulk activity of aromatase receptor-expressing neurons (AR) is elevated before intromissions are performed. Genetic ablation of these cells leads to a marked decrease in the percentage of males performing mounts, intromissions and ejaculation. Graphs adapted with permission from Ref. [77•].

MPOA: Similar recordings in the MPOA reveal that calcium transients in estrogen expressing (Esr1+) neurons ramp up before male and female mice initiated mounting, indicating that the MPOA is active during appetitive and consummatory behavior. Graphs adapted with permission from Ref. [92•].

VMH: Left, the ventrolateral part of the VMH (see larger insets lower panel) shows a topographic organization with lateral parts activated upon mounting, and medial parts involved in aggression. Recording calcium transients in Esr1+ neurons, revealed differential sex responses, with high activity when a female is mounted by a male and suppressed if mounted by a female. Close investigation by a male elicited higher calcium transients when compared to being investigated by another female. Graphs adapted with permissions from Ref. [100••].

Nevertheless, it is well accepted that both systems contribute to sexual behavior in both sexes [66–70]. Information originating from the MOE arrives at distinct regions of the olfactory cortex (Figure 4a) and, together with VNO-derived information (Figure 4b), may contribute for innate sex discrimination, since distinct cortical populations are activated when naïve male mice interact with males or females (Figure 4e, left panel) [71•]. Interestingly, the VNO seems to harbor sensory neurons that drive labeled line-like circuits capable of inducing female receptivity [72,73••], but can also mediate the synergism of cues representing sex and reproductive state, promoting male mounting towards receptive females [74]. The accessory olfactory bulb (AOB) projects to limbic regions, including the medial amygdala (MeA), cortical amygdala (CoA) and the bed nucleus of the stria terminalis (BNST), all activated during appetitive sexual behavior (Figure 4b) [75,76••,77•]. While male and female AOB neurons respond in a similar manner to cues of both sexes, the output regions of these neurons show different response profiles: whereas the posterior dorsal part of the MeA (pdMeA) is dimorphically activated (male and female are more activated by cues of the opposite sex, [75]), the CoA response is monomorphic (both sexes respond stronger to female cues) [71•,78]. Despite its importance in innate conspecific preference [79], the response of pdMeA neurons develops with sexual experience [76••], a process that is oxytocin-dependent, at least in males [80]. The BNST may be involved in sex recognition as well, with neurons of naïve mice responding differentially during encounters with males and females (Figure 4e, right top panel) [77•]. Further, activity of BNST neurons in the male seems to be important for consummatory behavior (but not in females, Figure 4d, upper middle panel) [77•].

Rodents also rely on touch during appetitive behavior, which is initially processed by the primary somatosensory cortex (S1; Figure 4b). While social facial touch drives sex-specific activity in rat S1 neurons [81,82], genital stimulation is represented in the genital portion of the rat [83] and mouse S1 [84], an area that undergoes pubertal expansion [84,85]. The receptive fields of male and female genital cortex seem to reflect the copulation posture, with neurons exhibiting multi-body responses [83]. Despite the non-essential role of USV for rat sexual behavior, conspecifics' vocalizations activate primary auditory cortex neurons [86] and female behavior modulates serotonin levels in auditory areas that perform multisensory integration in the male mouse (Figure 4b) [87].

The medial hypothalamus is one of the main receivers of chemosensory information, in particular the medial preoptic area (MPOA) and the ventromedial hypothalamus (VMH), which are fundamental for the progression from appetitive to consummatory behavior in both sexes [88,89]. Most input arrives from the pdMeA (Figure 4c), which is primarily GABAergic and thus most likely disinhibit the aforementioned areas [90].

The sexually dimorphic MPOA is comprised of an heterogenous population of cells [91], with estrogen receptor expressing neurons (Esr1+) capable of driving mounting behavior in both sexes (Figure 4d, upper right panel) [92•]. However, the MPOA also seems to be important for appetitive behavior and reinforcing aspects of sex [93,94], probably through the disinhibition of dopaminergic neurons of the mesolimbic reward pathway [95,96]. While dopaminergic neurons of the ventral tegmental area (VTA) can modulate social interest [97], optogenetic stimulation of MPOA-VTA projections promotes the development of place preference in males and receptive females [98•]. Furthermore, the VNO-dependent innate preference of male mice to female-derived stimuli seems to be dependent on VTA to nucleus accumbens (NAc) dopaminergic transmission [70]. In contrast, dopamine release in the NAc of male rats exposed to olfactory cues derived from receptive females only occurs after sexual experience [99]. Future studies are needed to understand if separate or overlapping cell populations in the MPOA (and VTA) encode sexual consummation and/or sexual motivation, but recent studies clearly refute the old hypothesis of localized control of appetitive behavior in the BNST and consummatory in the MPOA [92•].

The VMH, in particular its ventrolateral part (VMHvl), is involved in socio-sexual behaviors [67]. The VMHvl comprises primarily excitatory cells with different populations expressing steroid receptors, such as Esr1+ and progesterone receptor (PR+) [18•,100••]. Ablation of PR + neurons reduces female receptivity and male mounting [101]. Female PR + neurons project to the rostral periventricular region of the third ventricle [101], which contains kisseptin producing neurons involved in ovulation [102] and mating [103]. Detailed

(e) Brain areas involved in the inhibitory phase.
The current knowledge regarding brain regions involved in the refractory period in mice and rats is very scarce. Dopamine release in NAc neurons in sexually satiated male rats, led to a shortening of the refractory period [114].

(f) Encoding of innate sex recognition.
Left: Distinct cfos activation patterns in PCx and ECx elicited by male (more dorsal) or female (more ventral) in naïve male mice. Graph adapted with permission from [71•]. The posterior medial cortical amygdala (pmCoA) shows higher activation towards the female sex in both male and female mice [71•,78].
Right: Calcium transients of AR + neurons in the BNST of naïve males are higher when encountering a female compared to a male. GABAergic BNST neurons of naïve females show a strong activation with encountering the opposite sex. Graphs adapted with permission from Ref. [77•].

investigation of the VMHvl Esr1+ cells in females uncovered anatomical distinguishable subdivisions of the posterior VMHvl, with the more lateral part (VMHpvll) regulating mating and the more medial part (VMHpvlm) aggression (Figure 4d, lower left panel) [100••]). Optogenetic stimulation of female [100••] and male [104] Esr1 + VMHvl neurons induces mounting towards both males and females arguing, similarly to the MPOA, for a shared layout of the brain. Besides being active during mounting, Esr1+ VMHvl (Figure 4d, right bottom panel) cells are also active during appetitive behavior (Figure 4d, middle bottom panel) [100••,104] and moderate optogenetic modulation of these neurons affects appetitive interactions [104]. Supporting these results, single-unit electrophysiological recordings of female VMHvl neurons showed strong activation during appetitive behavior [27]. Together with the MeA, the neural representation of conspecifics in the VMHvl evolves with experience [105].

The periaqueductal grey (PAG) is a key structure relaying hypothalamic information to spinal circuits controlling muscle activity (Figure 4b, c, e). Early lesion studies in rats [106] and more recent studies in mice [73••] point to a lordosis modulating role of the PAG, and tracing studies [107–109] reveal multiple PAG-brainstem projections that are activated by mating [100••,107]. Still, this midbrain structure is quite heterogeneous and it remains to be determined how distinct cell populations drive different motor patterns. One nose-to-PAG circuit has been described that transforms the presence of a pheromone present in male's tears to the execution of lordosis in female mice [72,73••,74]. Surprisingly, rather than activating the circuit classically involved in female receptivity (MeApd/VMHvl), ESP1 is processed by a circuit otherwise associated with processing of predator related information [110,111].

Inhibitory mechanisms in rodents are also understudied. While males exhibit a refractory period after ejaculation whose duration is affected by many variables [30,35,112], female receptivity can be modified by vaginal stimulation [113]. As previously discussed, dopaminergic transmission is involved in sexual behavior and activation of dopamine receptors in the NAc of sexually exhausted rats decreases the length of the refractory period (Figure 4e) [114].

Integration of pheromonal/hormonal cues, past experience and competing needs

The probability of copulation in rodents is remarkably well-coordinated with the reproductive capacity of each individual. Sex with non-sexually mature juveniles is inhibited in two ways by ESP22, a pheromone present in the tears of both sexes, which directly inhibits male mounting behavior [115] and counteracts the ESP1-promoting effect on female receptivity [116]. Individual differences in the levels of ESP1 may underlie the interruption of pregnancy observed when a female is exposed to a male different from the one that she had sex with, the Bruce effect [117]. Once sexual maturity is reached, female behavior is dependent on cycling levels of ovarian sex hormones, primarily estrogen and progesterone, which match receptivity with fertility [4,27,101,118]. Female perception is altered at early stages across the reproductive cycle, with progesterone modulating VNO signaling and female attraction to male odors [119•], and whisker-derived information [81,120]. Further in the brain, the activity of single VMHvl neurons also changes across the cycle [27]. In fact, estrogen increases the output of PR+-VMHvl neurons to the anteroventral periventricular nucleus and this change is necessary for mating to occur during the fertile phase [121]. Disruption of estrogen signaling in the dorsal raphe nucleus (DRN; Figure 2c, left panel) abolishes the decrease in female receptivity at the end of the receptive phase [122]. Sexual behavior in pregnant or lactating females is prevented in many ways. For example, the social preference of lactating female rats is altered, such that they prefer pups to males [123]. Even though the VMHvl is involved in sexual behavior, VMHvl-Esr1+ neurons in lactating females can drive aggression towards intruders [124].

Even though sexual behavior is instinctively expressed by rodents, experience modulates early components of the olfactory system [125–127] as well as output regions [76••,94,105].

The nutritional state of mice modulates sexual behavior and hypothalamic agouti-related peptide (AgRP) neurons, which are critical regulators of appetite, can suppress competing motivational drives, such as social interactions [128] and mating [129]. However, if the food source was unavailable animals would resume mating [129]. Neuropeptide Y, which is released during low energy conditions, also decreases sexual behavior via inhibition of serotonergic cells in the DRN [130]. These studies clearly show the existence of a need-processing hierarchy, with hunger being the most prominent to fulfil.

Concluding remarks

From flies to mice and rats, sex behavior is controlled to match the reproductive capacity of each individual and the availability of resources. Despite the differences, recent studies reveal conserved neuronal strategies in the solutions opted by nature to tightly control the execution of sex. For example, hormonal-dependent mechanisms and the experience of copulation itself were recently shown to modulate sexual receptivity in the fly, mechanisms that are well established in rodents. Shared circuits have been shown to control mutually exclusive behaviors, such as sex and aggression (via the P1 cluster and the VMH). Interestingly, the inhibition of sexual behavior towards non-suitable targets is also in part

mediated by conserved strategies, with inhibiting and promoting cues being processed by independent/parallel pathways that ultimately impinge on the same nodes, counteracting each other's effect (for example, cVA/7,11-HD to P1 and ESP22/ESP1 to VMH). This modular organization can allow the rapid evolution of reproductive barriers, for example. Despite all efforts, several components of circuitry are markedly unexplored, such as the pathways controlling copulation in flies and rodents or the refractory period in the male. A comparative approach maybe be helpful in the quest to unravel understudied mechanisms, in particular because each model organism enjoys a different set of tools that might enable progress in different aspects of the behavior at a different pace.

Conflict of interest statement

None declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Martinez I, Paredes RG: **Only self-paced mating is rewarding in rats of both sexes**. *Horm Behav* 2001, **40**:510-517.

2. Matthews TJ, Abdelbaky P, Pfaff DW: **Social and sexual motivation in the mouse**. *Behav Neurosci* 2005, **119**:1628-1639.

3. • Zer-Krispil S *et al.*: **Ejaculation induced by the activation of Crz neurons is rewarding to drosophila males**. *Curr Biol* 2018, **28**:1445-1452.e3
Ejaculation induced by specific activation of male-specific Crz-neurons is sufficient to emulate all the rewarding features attained from a full copulation with a female.

4. Plant TM, Zeleznik A, Knobil E: *Knobil and Neill's Physiology of Reproduction*. edn 4. 2015. 1 online resource (l, 2550, 32, 24 pages).

5. Anderson DJ: **Circuit modules linking internal states and social behaviour in flies and mice**. *Nat Rev Neurosci* 2016, **17**:692-704.

6. Bastock M, Manning A: **The courtship of Drosophila melanogaster**. *Behaviour* 1955, **8**:85-110.

7. Agrawal S, Safarik S, Dickinson M: **The relative roles of vision and chemosensation in mate recognition of Drosophila melanogaster**. *J Exp Biol* 2014, **217**:2796-2805.

8. •• Clowney EJ *et al.*: **Multimodal chemosensory circuits controlling male courtship in Drosophila**. *Neuron* 2015, **87**:1036-1049
Using circuit mapping and imaging techniques, the authors revealed that gustatory and olfactory pheromonal inputs reach P1 neurons through parallel pathways of excitation and inhibition, allowing the integration of stimuli and the tight control of courtship initiation towards appropriate partners.

9. Manning A: **The control of sexual receptivity in female Drosophila**. *Anim Behav* 1967, **15**:239-250.

10. Bussell JJ *et al.*: **Abdominal-B neurons control Drosophila virgin female receptivity**. *Curr Biol* 2014, **24**:1584-1595.

11. Aranha MM *et al.*: **Apterous brain neurons control receptivity to male courtship in Drosophila melanogaster females**. *Sci Rep* 2017, **7**:46242.

12. Connolly KC, Cook R: **Rejection responses by female Drosophila melanogaster: their ontogeny, causality and effect upon the behavior of the courting male**. *Behavior* 1973, **44**:142-166.

13. Liu H, Kubli E: **Sex-peptide is the molecular basis of the sperm effect in Drosophila melanogaster**. *Proc Natl Acad Sci U S A* 2003, **100**:9929-9933.

14. Bath E *et al.*: **Sperm and sex peptide stimulate aggression in female Drosophila**. *Nat Ecol Evol* 2017, **1**:0154.

15. Lupold S *et al.*: **Female mediation of competitive fertilization success in Drosophila melanogaster**. *Proc Natl Acad Sci U S A* 2013, **110**:10693-10698.

16. Laturney M, Billeter JC: **Drosophila melanogaster females restore their attractiveness after mating by removing male anti-aphrodisiac pheromones**. *Nat Commun* 2016, **7**:12322.

17. Lee KM *et al.*: **A neuronal pathway that controls sperm ejection and storage in female Drosophila**. *Curr Biol* 2015, **25**:790-797.

18. • Shao L *et al.*: **A neural circuit encoding the experience of copulation in female Drosophila**. *Neuron* 2019, **102**:1025-1036.e6
This study revealed that vaginal sensory input received during copulation elicits a reduction in female receptivity, which is initiated immediately after copulation and is independent of sex peptide. Sensory input is sensed by abdominal mechanosensory neurons that convey the mating stimulation to the brain via ascending neurons.

19. Zhang SX, Rogulja D, Crickmore MA: **Dopaminergic circuitry underlying mating drive**. *Neuron* 2016, **91**:168-181.

20. Auer TO, Benton R: **Sexual circuitry in Drosophila**. *Curr Opin Neurobiol* 2016, **38**:18-26.

21. Aranha MM, Vasconcelos ML: **Deciphering Drosophila female innate behaviors**. *Curr Opin Neurobiol* 2018, **52**:139-148.

22. Egnor SR, Seagraves KM: **The contribution of ultrasonic vocalizations to mouse courtship**. *Curr Opin Neurobiol* 2016, **38**:1-5.

23. Jouda J, Wohr M, Del Rey A: **Immunity and ultrasonic vocalization in rodents**. *Ann N Y Acad Sci U S A* 2019, **1437**:68-82.

24. Nyby J: **Ultrasonic vocalizations during sex behavior of male house mice (Mus musculus): a description**. *Behav Neural Biol* 1983, **39**:128-134.

25. Asaba A *et al.*: **Male mice ultrasonic vocalizations enhance female sexual approach and hypothalamic kisspeptin neuron activity**. *Horm Behav* 2017, **94**:53-60.

26. Neunuebel JP *et al.*: **Female mice ultrasonically interact with males during courtship displays**. *eLife* 2015, **4**.

27. Nomoto K, Lima SQ: **Enhanced male-evoked responses in the ventromedial hypothalamus of sexually receptive female mice**. *Curr Biol* 2015, **25**:589-594.

28. McGill TE: **Sexual behavior of the mouse after long-term and short-term postejaculatory recovery periods**. *J Genet Psychol* 1963, **103**:53-57.

29. Sutter A, Lindholm AK: **The copulatory plug delays ejaculation by rival males and affects sperm competition outcome in house mice**. *J Evol Biol* 2016, **29**:1617-1630.

30. McGill TE: **Sexual behavior in three inbred strains of mice**. *Behavior* 1962:341-350.

31. Agmo A, Snoeren EM: **A cooperative function for multisensory stimuli in the induction of approach behavior of a potential mate**. *PLoS One* 2017, **12**:e0174339.

32. Snoeren EM, Agmo A: **The role of odors and ultrasonic vocalizations in female rat (*Rattus norvegicus*) partner choice**. *J Comp Psychol* 2014, **128**:367-377.

33. Snoeren EM, Agmo A: **The incentive value of males' 50-kHz ultrasonic vocalizations for female rats (*Rattus norvegicus*)**. *J Comp Psychol* 2014, **128**:40-55.

34. Agmo A, Snoeren EM: **Silent or vocalizing rats copulate in a similar manner**. *PLoS One* 2015, **10**:e0144164.

35. Hull EM, Dominguez JM: **Sexual behavior in male rodents**. *Horm Behav* 2007, **52**:45-55.

36. Dewsbury DA: **A quantitative description of the behavior of rats during copulation**. *Behaviour* 1967, **29**:154-178.

37. Kimura K *et al.*: **Fruitless and doublesex coordinate to generate male-specific neurons that can initiate courtship**. *Neuron* 2008, **59**:759-769.

38. Kohatsu S, Yamamoto D: **Visually induced initiation of Drosophila innate courtship-like following pursuit is mediated by central excitatory state**. *Nat Commun* 2015, **6**:6457.

39. Kallman BR, Kim H, Scott K: **Excitation and inhibition onto central courtship neurons biases Drosophila mate choice**. *eLife* 2015, **4**:e11188.

40. • Ribeiro IMA *et al.*: **Visual projection neurons mediating directed courtship in Drosophila**. *Cell* 2018, **174**:607-621.e18
The authors identified visual projection neurons, the lobula columnar LC10, that respond to moving targets with female-like properties and whose activity is sufficient and necessary for visually guided courtship behavior.

41. Billeter JC *et al.*: **Specialized cells tag sexual and species identity in Drosophila melanogaster**. *Nature* 2009, **461**:987-991.

42. Dweck HK *et al.*: **Pheromones mediating copulation and attraction in Drosophila**. *Proc Natl Acad Sci U S A* 2015, **112**:E2829-35.

43. He Z *et al.*: **Chemosensory sensilla of the Drosophila wing express a candidate ionotropic pheromone receptor**. *PLoS Biol* 2019, **17**:e2006619.

44. Clyne JD, Miesenbock G: **Sex-specific control and tuning of the pattern generator for courtship song in Drosophila**. *Cell* 2008, **133**:354-363.

45. Namiki S *et al.*: **The functional organization of descending sensory-motor pathways in Drosophila**. *eLife* 2018, **7**.

46. •• McKellar CE *et al.*: **Threshold-based ordering of sequential actions during Drosophila courtship**. *Curr Biol* 2019, **29**:426-434.e6
This study revealed the existence of a single pair of descending neurons, aSP22, that controls several male courtship actions. Artificial activation of aSP22 triggers these actions in the same sequence as found during courtship with a virgin female.

47. Zhou C *et al.*: **Central brain neurons expressing doublesex regulate female receptivity in Drosophila**. *Neuron* 2014, **83**:149-163.

48. •• Pavlou HJ *et al.*: **Neural circuitry coordinating male copulation**. *eLife* 2016, **5**
This study identifies a neuronal motor circuit composed of three neuronal classes that mediates male copulation: motor neurons control genital attachment and intromission; GABAergic inhibitory neurons mediate genital uncoupling; mechanosensory neurons activate the two other classes and most likely control fine motor coordination.

49. Jang YH, Chae HS, Kim YJ: **Female-specific myoinhibitory peptide neurons regulate mating receptivity in Drosophila melanogaster**. *Nat Commun* 2017, **8**:1630.

50. Feng K *et al.*: **Ascending SAG neurons control sexual receptivity of Drosophila females**. *Neuron* 2014, **83**:135-148.

51. Rezaval C *et al.*: **Sexually dimorphic octopaminergic neurons modulate female postmating behaviors in Drosophila**. *Curr Biol* 2014, **24**:725-730.

52. Lebreton S *et al.*: **A Drosophila female pheromone elicits species-specific long-range attraction via an olfactory channel with dual specificity for sex and food**. *BMC Biol* 2017, **15**:88.

53. Zhao X *et al.*: **Persistent activity in a recurrent circuit underlies courtship memory in Drosophila**. *eLife* 2018, **7**.

54. Ahmed OM *et al.*: **Evolution of mechanisms that control mating in Drosophila males**. *Cell Rep* 2019, **27**:2527-2536.e4.

55. Seeholzer LF *et al.*: **Evolution of a central neural circuit underlies Drosophila mate preferences**. *Nature* 2018, **559**:564-569.

56. • Lin HH *et al.*: **Hormonal modulation of pheromone detection enhances male courtship success**. *Neuron* 2016, **90**:1272-1285
This study revealed that the copulation advantage of older males is due to a higher sensitivity of olfactory receptor neurons to female-borne attracting pheromones and this sensitization is dependent on juvenile hormone. The hormonal control of mating behavior to match fertility is pervasive in mammals, but mostly unexplored in flies.

57. Kuo SY *et al.*: **PPL2ab neurons restore sexual responses in aged Drosophila males through dopamine**. *Nat Commun* 2015, **6**:7490.

58. Hoopfer ED *et al.*: **P1 interneurons promote a persistent internal state that enhances inter-male aggression in Drosophila**. *eLife* 2015, **4**.

59. Koganezawa M, Kimura K, Yamamoto D: **The neural circuitry that functions as a switch for courtship versus aggression in Drosophila males**. *Curr Biol* 2016, **26**:1395-1403.

60. Chen D *et al.*: **Genetic and neuronal mechanisms governing the sex-specific interaction between sleep and sexual behaviors in Drosophila**. *Nat Commun* 2017, **8**:154.

61. Machado DR *et al.*: **Identification of octopaminergic neurons that modulate sleep suppression by male sex drive**. *eLife* 2017, **6**.

62. Beckwith EJ *et al.*: **Regulation of sleep homeostasis by sexual arousal**. *eLife* 2017, **6**.

63. • Das S *et al.*: **Electrical synapses mediate synergism between pheromone and food odors in Drosophila melanogaster**. *Proc Natl Acad Sci U S A* 2017, **114**:E9962-E9971
This study identified the mechanism by which vinegar enhances the female response to the male pheromone cVA. The synergism is mediated by electrical synapses between excitatory local interneurons receiving input from vinegar-responsive glomeruli and a cVA-responsive glomerulus, such that the response to cVA is enhanced when vinegar is present.

64. Gorter JA *et al.*: **The nutritional and hedonic value of food modulate sexual receptivity in Drosophila melanogaster females**. *Sci Rep* 2016, **6**:19441.

65. Bentzur A *et al.*: **Odorant binding protein 69a connects social interaction to modulation of social responsiveness in Drosophila**. *PLoS Genet* 2018, **14**:e1007328.

66. Dulac C, Wagner S: **Genetic analysis of brain circuits underlying pheromone signaling**. *Annu Rev Genet* 2006, **40**:449-467.

67. Hashikawa K *et al.*: **The neural circuits of mating and fighting in male mice**. *Curr Opin Neurobiol* 2016, **38**:27-37.

68. Fraser EJ, Shah NM: **Complex chemosensory control of female reproductive behaviors**. *PLoS One* 2014, **9**:e90368.

69. Matsuo T *et al.*: **Genetic dissection of pheromone processing reveals main olfactory system-mediated social behaviors in mice**. *Proc Natl Acad Sci U S A* 2015, **112**:E311-20.

70. Beny Y, Kimchi T: **Conditioned odor aversion induces social anxiety towards females in wild-type and TrpC2 knockout male mice**. *Genes Brain Behav* 2016, **15**:722-732.

71. • Kim Y *et al.*: **Mapping social behavior-induced brain activation at cellular resolution in the mouse**. *Cell Rep* 2015, **10**:292-305
Serial two-photon tomography allowed whole-brain visualization and detection of neurons (in the male mouse) activated after interactions with conspeficis of both sexes. Interestingly distinct cortical areas were activated by either male or female stimuli.

72. Haga S *et al.*: **The male mouse pheromone ESP1 enhances female sexual receptive behaviour through a specific vomeronasal receptor**. *Nature* 2010, **466**:118-122.

73. •• Ishii KK *et al.*: **A labeled-line neural circuit for pheromone-mediated sexual behaviors in mice**. *Neuron* 2017, **95**:123-137.e8
The authors characterized a neuronal circuit underlying the enhancement of female receptivity by the male pheromone ESP1, from the periphery to the midbrain, via amygdala/hypothalamus. Interestingly, the regions of the amygdala/hypothalamus involved in the response to ESP1 have been associated with defensive behavior, rather than female receptivity.

74. Haga-Yamanaka S *et al.*: **Integrated action of pheromone signals in promoting courtship behavior in male mice**. *eLife* 2014, **3**:e03025.

75. Bergan JF, Ben-Shaul Y, Dulac C: **Sex-specific processing of social cues in the medial amygdala**. *eLife* 2014, **3**:e02743.

76. •• Li Y *et al.*: **Neuronal representation of social information in the medial amygdala of awake behaving mice**. *Cell* 2017, **171**:1176-1190.e17
The activity of MeA neurons was monitored via calcium dynamics over multiple months using a miniature microscope. Calcium transients reveal sex-specific differences in the encoding of different social stimuli (male, female and pup). Interestingly, the responses to social stimuli where shaped by sexual experience, triggering long-lasting and sex-specific changes.

77. • Bayless DW *et al.*: **Limbic neurons shape sex recognition and social behavior in sexually naive males**. *Cell* 2019, **176**:1190-1205.e20
Fiberphotometry and genetic ablation tools of aromatase expressing neurons in the male mouse BNST reveal that these cells encode an innate female preference and reflect mounting behavior in their calcium transients.

78. Pardo-Bellver C *et al.*: **Synchronized activity in the main and accessory olfactory bulbs and vomeronasal amygdala elicited by chemical signals in freely behaving mice**. *Sci Rep* 2017, **7**:9924.

79. McCarthy EA *et al.*: **DREADD-induced silencing of the medial amygdala reduces the preference for male pheromones and the expression of lordosis in estrous female mice**. *Eur J Neurosci* 2017, **46**:2035-2046.

80. Yao S *et al.*: **Oxytocin signaling in the medial amygdala is required for sex discrimination of social cues**. *eLife* 2017, **6**.

81. Bobrov E *et al.*: **The representation of social facial touch in rat barrel cortex**. *Curr Biol* 2014, **24**:109-115.

82. Lenschow C, Brecht M: **Barrel cortex membrane potential dynamics in social touch**. *Neuron* 2015, **85**:718-725.

83. Lenschow C *et al.*: **Sexually monomorphic maps and dimorphic responses in rat genital cortex**. *Curr Biol* 2016, **26**:106-113.

84. Sigl-Glockner J *et al.*: **Effects of sexual experience and puberty on mouse genital cortex revealed by chronic imaging**. *Curr Biol* 2019, **29**:3588-3599.e4.

85. Lenschow C, Sigl-Glockner J, Brecht M: **Development of rat female genital cortex and control of female puberty by sexual touch**. *PLoS Biol* 2017, **15**:e2001283.

86. Rao RP *et al.*: **Vocalization-whisking coordination and multisensory integration of social signals in rat auditory cortex**. *eLife* 2014, **3**.

87. Keesom SM, Hurley LM: **Socially induced serotonergic fluctuations in the male auditory midbrain correlate with female behavior during courtship**. *J Neurophysiol* 2016, **115**:1786-1796.

88. Paredes RG: **Opioids and sexual reward**. *Pharmacol Biochem Behav* 2014, **121**:124-131.

89. Paxinos G: *The Rat Nervous System*. edn 4. London, UK: Academic Press; 2015. xvi, 1035 pages.

90. Dalpian F, Rasia-Filho AA, Calcagnotto ME: **Sexual dimorphism, estrous cycle and laterality determine the intrinsic and synaptic properties of medial amygdala neurons in rat**. *J Cell Sci* 2019, **132**.

91. Kohl J *et al.*: **Functional circuit architecture underlying parental behaviour**. *Nature* 2018, **556**:326-331.

92. • Wei YC *et al.*: **Medial preoptic area in mice is capable of mediating sexually dimorphic behaviors regardless of gender**. *Nat Commun* 2018, **9**:279
Wei *et al.* showed that the activity of MPOA neurons of male and female mice strongly correlates with male mounting and female pup retrieval. Optogenetic activation, on the contrary, drives these sexual dimorphic behaviors in both sexes arguing for a shared layout of the brain.

93. Meerts SH *et al.*: **Previous sexual experience alters the display of paced mating behavior in female rats**. *Horm Behav* 2014, **65**:497-504.

94. Jean A *et al.*: **Revisiting medial preoptic area plasticity induced in male mice by sexual experience**. *Sci Rep* 2017, **7**:17846.

95. Fang YY *et al.*: **A hypothalamic midbrain pathway essential for driving maternal behaviors**. *Neuron* 2018, **98**:192-207.e10.

96. Ishii KK, Touhara K: **Neural circuits regulating sexual behaviors via the olfactory system in mice**. *Neurosci Res* 2019, **140**:59-76.

97. Gunaydin LA *et al.*: **Natural neural projection dynamics underlying social behavior**. *Cell* 2014, **157**:1535-1551.

98. • McHenry JA *et al.*: **Hormonal gain control of a medial preoptic area social reward circuit**. *Nat Neurosci* 2017, **20**:449-458
Using intersectional genetic tools and two photon imaging this study describes a specific MPOA cell population projecting to the VTA, which preferential encodes attractive male cues and whose activity is modulated by sex hormones. Activation of this pathway promotes social approach and dopamine release in the striatum.

99. Fujiwara M, Chiba A: **Sexual odor preference and dopamine release in the nucleus accumbens by estrous olfactory cues in sexually naive and experienced male rats**. *Physiol Behav* 2018, **185**:95-102.

100. •• Hashikawa K *et al.*: **Esr1(+) cells in the ventromedial hypothalamus control female aggression**. *Nat Neurosci* 2017, **20**:1580-1590
This study revealed that the female VMHvl, similarly to the male, contains estrogen receptor expressing neurons whose activity is fundamental for female aggression. Furthermore, the authors describe two anatomically distinct subdivisions in the posterior VMHvl, with differential gene expression, projection and activation patterns after mating versus fighting.

101. Yang CF *et al.*: **Sexually dimorphic neurons in the ventromedial hypothalamus govern mating in both sexes and aggression in males**. *Cell* 2013, **153**:896-909.

102. Hu MH *et al.*: **Relative importance of the arcuate and anteroventral periventricular kisspeptin neurons in control of puberty and reproductive function in female rats**. *Endocrinology* 2015, **156**:2619-2631.

103. Hellier V *et al.*: **Female sexual behavior in mice is controlled by kisspeptin neurons**. *Nat Commun* 2018, **9**:400.

104. Lee H *et al.*: **Scalable control of mounting and attack by Esr1+ neurons in the ventromedial hypothalamus**. *Nature* 2014, **509**:627-632.

105. Remedios R *et al.*: **Social behaviour shapes hypothalamic neural ensemble representations of conspecific sex**. *Nature* 2017, **550**:388-392.

106. Sakuma Y, Pfaff DW: **Mesencephalic mechanisms for integration of female reproductive behavior in the rat**. *Am J Physiol* 1979, **237**:R285-90.

107. Yamada S, Kawata M: **Identification of neural cells activated by mating stimulus in the periaqueductal gray in female rats**. *Front Neurosci* 2014, **8**:421.

108. Subramanian HH *et al.*: **The physiological motor patterns produced by neurons in the nucleus retroambiguus in the rat and their modulation by vagal, peripheral chemosensory, and nociceptive stimulation**. *J Comp Neurol* 2018, **526**:229-242.

109. Lo L *et al.*: **Connectional architecture of a mouse hypothalamic circuit node controlling social behavior**. *Proc Natl Acad Sci U S A* 2019, **116**:7503-7512.

110. Kunwar PS *et al.*: **Ventromedial hypothalamic neurons control a defensive emotion state**. *eLife* 2015, **4**.

111. Silva BA *et al.*: **Independent hypothalamic circuits for social and predator fear**. *Nat Neurosci* 2013, **16**:1731-1733.

112. Pfaus JG: **Pathways of sexual desire**. *J Sex Med* 2009, **6**:1506-1533.

113. Reuquen P *et al.*: **Prolactin gene expression in the pituitary of rats subjected to vaginocervical stimulation requires Erk-1/2 signaling**. *Reprod Biol* 2017, **17**:357-362.

114. Guadarrama-Bazante IL, Rodriguez-Manzo G: **Nucleus accumbens dopamine increases sexual motivation in sexually satiated male rats**. *Psychopharmacology (Berl)* 2019, **236**:1303-1312.

115. Ferrero DM *et al.*: **A juvenile mouse pheromone inhibits sexual behaviour through the vomeronasal system**. *Nature* 2013, **502**:368-371.

116. Osakada T *et al.*: **Sexual rejection via a vomeronasal receptor-triggered limbic circuit**. *Nat Commun* 2018, **9**:4463.

117. Hattori T *et al.*: **Exocrine gland-secreting peptide 1 is a key chemosensory signal responsible for the Bruce effect in mice**. *Curr Biol* 2017, **27**:3197-3201.e3.

118. Ford CS, Beach FA: *Patterns of Sexual Behavior*. New York: Harper; 1952. viii, 307 p..

119. • Dey S *et al.*: **Cyclic regulation of sensory perception by a female hormone alters behavior**. *Cell* 2015, **161**:1334-1344
Dey *et al.* show that vomeronasal sensory neurons are rendered blind to male urinary proteins during the non-receptive phase of the mouse female reproductive cycle, whereas they are fully functional and responsive to the same ligands when sexually receptive. The silencing of vomeronasal sensory neurons is mediated by progesterone.

120. Clemens AM *et al.*: **Estrus-cycle regulation of cortical inhibition**. *Curr Biol* 2019, **29**:605-615.e6.

121. Inoue S *et al.*: **Periodic remodeling in a neural circuit governs timing of female sexual behavior**. *Cell* 2019, **179**:1393-1408.

122. Sano K *et al.*: **The role of estrogen receptor beta in the dorsal raphe nucleus on the expression of female sexual behavior in C57BL/6J mice**. *Front Endocrinol (Lausanne)* 2018, **9**:243.

123. Ferreno M *et al.*: **Dopaminergic activity mediates pups' over male preference of postpartum estrous rats**. *Physiol Behav* 2018, **188**:134-139.

124. Hashikawa Y *et al.*: **Ventromedial hypothalamus and the generation of aggression**. *Front Syst Neurosci* 2017, **11**:94.

125. Corona R *et al.*: **Sexual behavior increases cell proliferation in the rostral migratory stream and promotes the differentiation of the new cells into neurons in the accessory olfactory bulb of female rats**. *Front Neurosci* 2016, **10**:48.

126. Xu PS, Lee D, Holy TE: **Experience-dependent plasticity drives individual differences in pheromone-sensing neurons**. *Neuron* 2016, **91**:878-892.

127. Santoyo-Zedillo M, Portillo W, Paredes RG: **Neurogenesis in the olfactory bulb induced by paced mating in the female rat is opioid dependent**. *PLoS One* 2017, **12**:e0186335.

128. Burnett CJ *et al.*: **Hunger-driven motivational state competition**. *Neuron* 2016, **92**:187-201.

129. Burnett CJ *et al.*: **Need-based prioritization of behavior**. *eLife* 2019, **8**.

130. Inaba A *et al.*: **Neuropeptide Y signaling in the dorsal raphe nucleus inhibits male sexual behavior in mice**. *Neuroscience* 2016, **320**:140-148.

131. Sokolowski MB: **Drosophila: genetics meets behaviour**. *Nat Rev Genet* 2001, **2**:879-890.

132. Watanabe K *et al.*: **A circuit node that integrates convergent input from neuromodulatory and social behavior-promoting neurons to control aggression in Drosophila**. *Neuron* 2017, **95**:1112-1128.e7.

133. Vaughan AG *et al.*: **Neural pathways for the detection and discrimination of conspecific song in *D. melanogaster***. *Curr Biol* 2014, **24**:1039-1049.