Circuit modules linking internal states and social behaviour in flies and mice

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Abstract | Goal-directed social behaviours such as mating and fighting are associated with scalable and persistent internal states of emotion, motivation, arousal or drive. How those internal states are encoded and coupled to behavioural decision making and action selection is not clear. Recent studies in *Drosophila melanogaster* and mice have identified circuit nodes that have causal roles in the control of innate social behaviours. Remarkably, in both species, these relatively small groups of neurons can influence both aggression and mating, and also play a part in the encoding of internal states that promote these social behaviours. These similarities may be superficial and coincidental, or may reflect conserved or analogous neural circuit modules for the control of social behaviours in flies and mice.

An organism encountering another creature must identify it as friend (conspecific) or foe (predator), male or female, juvenile or adult, and, based on this, decide how to respond to it. These decisions are typically accompanied by internal states, such as motivation, arousal, drive or emotion (BOX 1). These states share characteristic properties such as persistence and scalability[1]. Owing to the lack of consensus operational definitions for such states that apply across species[1–5], for the purposes of this Review, I refer to such internal states generically as 'π states'.

Our subjective experience tells us that the quality of these states, and their intensity, influences our behavioural choices; we are more likely to react violently to an insult or challenge if we are feeling irritable or aggressive than if we are calm. Yet relatively little is known about how such internal states are encoded in animal brains and whether they causally influence behavioural decision making or are epiphenomena. The important role of such states in controlling behaviour has been recognized and considered both by psychologists[2,6] (BOX 1) and by ethologists such as Tinbergen[7] and Lorenz[8] (BOX 2).

Reproductive social behaviours such as mating and fighting provide rich territory in which to investigate this issue in non-human animals. These behaviours are crucial for survival and are typically directed towards females and males of a species, respectively, in a mutually exclusive manner. The decision of whether to mate or fight is therefore closely linked to the identification of an intruder as a conspecific female or male[9,10]. However, encounters between male conspecifics do not invariably lead to fighting, just as encounters between male and female conspecifics do not inevitably lead to mating. Whether these encounters progress from their appetitive phases to their respective consummatory phases is determined by the immediate context, the experience and the internal state of the interacting animals[7,11,12].

Earlier studies of internal states relevant to mating and aggression have focused on the role of neurohormones and neuromodulators such as sex steroids[13] (reviewed in REFS 7,14–18) and biogenic amines (reviewed in REFS 9,19). However, less is known about the neural circuit-level mechanisms that contribute to the encoding of internal states; such states are probably encoded by the interaction between patterns of circuit activity and neuromodulation[20], not just by the modulators themselves.

In this Review, I discuss recent studies of the relationship between courtship and aggression circuitry in flies and mice, focusing on (relatively) small populations of genetically identified neurons that control (directly or indirectly) both of these opponent social behaviours, as well as associated internal states (FIG. 1). The finding of such shared nodes is arguably surprising, as some investigators have proposed that mating and aggression are largely mediated by parallel, non-overlapping circuits[21,22] (FIG. 1a). These cross-species similarities may be superficial, or may reflect a conservation of common circuit modules[23] controlling sexual and aggressive behaviour, as suggested by Tinbergen[7,24] and others[25]. Here, I sequentially summarize studies from each model organism, from the perspective of aggression and its relationship to mating, and then provide a more detailed comparison of the similarities as well as the important differences between the two systems. Although these topics have been separately reviewed elsewhere[26–30], a comparative perspective affords the opportunity to consider whether there may be conserved circuit 'motifs' (REF. 31) for the control of these evolutionarily ancient, innate social behaviours.

π states
A generic term, introduced for the purposes of this Review, denoting persistent and scalable internal brain states.

Appetitive phases
The phases of a goal-directed activity that involve seeking or investigative actions; in social behaviour, these include approach and ano-genital or head-directed sniffing.

Consummatory phases
In aggression, the phase that involves overt attack behaviours such as biting; in mating, the phase that includes intromission (pelvic thrusting with vaginal penetration) and ejaculation.

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Box 1 | **Motivation, arousal, drive and emotion**

Motivation, arousal, drive and emotion are internal, central states that influence our behaviour, physiology and conscious experience. The definition of these states may be theoretical or operational, and varies by field (for example, in psychology versus neuroscience; see REFS 2,6). There is general agreement that motivation, arousal and drive apply to humans and non-human animals, whereas there is continuing disagreement about whether emotions can be ascribed to animals[1,3] or (as argued recently by LeDoux[2] and by Tinbergen[7]) should be considered as purely subjective phenomena that apply only to humans[4].

In general, arousal states typically involve an increase in physiological, autonomic and motor activity, and increased sensitivity to sensory cues[139]. Arousal states can increase the probability of engaging in a behaviour or can influence the choice between behaviours (such as during an escalating social or predator–prey interaction)[9,19]. There is ongoing debate as to whether arousal is exclusively a generic state or whether there are behaviour-specific forms of arousal[14,140]. Increased arousal can occur in the absence of motivation, for example during the transition from sleep to waking.

Drive and motivation are conceptualized as internal states that promote goal-directed behaviours. Although drive and motivation may co-occur with increased arousal states, they can be independent (for example, motivation to go to bed early when sleepy in order to obtain sufficient rest). Drive states are typically homeostatic in nature and triggered by interoceptive cues (known as 'needing' states — for example, hunger and thirst[141]). Motivational states subsume homeostatic drive states but extend more broadly to states triggered by exteroceptive stimuli — for example, an incentive that predicts a positive reinforcer or a threat that predicts a punishment[6] — and include 'wanting' as well as needing states. Wanting states are also distinguished from 'liking' states, which are the hedonic responses to the consumption of a reward[142], as measured by orofacial expressions in humans, non-human primates and rodents[143]. In psychology, motivational states are usually studied using incentive-guided learned instrumental behaviours (operant conditioning)[2,6]. Whether motivational or reward states apply to purely Pavlovian (associative) learning paradigms in organisms such as *Drosophila melanogaster*, for which operant conditioning paradigms are scant[144], is a matter of debate[5,6,145,146].

Emotion states often include a motivational component and an associated goal-directed behaviour, as well as varying levels of arousal; however, emotions are more flexible than motivation states and can have a communicative or expressive function as well[3]. Some emotion theorists have used the term 'emotion' to refer to observable behaviour, which can be studied in both humans and animal models, and the term 'feeling' to refer to the subjective experience of emotion states, which is accessible only by verbal report and therefore can only be studied in humans[147]. More recently, others have rejected this view and suggested that the term 'emotion' should be reserved for subjective feeling states, which by definition are accessible to study only in humans[2,4]. An alternative view is that emotions are central states, of which subjective feelings are only one expression (or 'readout') and which can and should be studied in non-human animal models, for example by focusing on features of these states called 'emotion primitives': properties such as valence, scalability, persistence and generalization[1].

Social behaviour and π states in flies

Aggression and internal states in arthropods. Neuromodulators, including biogenic amines and neuropeptides, are well known to be involved in the control of internal states[20,32]. Much research has implicated biogenic amines in the control of aggression-related internal states in arthropods (reviewed in REF. 33). For example, in crustaceans, serotonin (5-HT) injection restores aggressiveness to subordinates[34]; a similar function has been attributed to octopamine (OA) in crickets[35–37]. Studies of aggression in *Drosophila melanogaster*[38,39] have shown that OA[40,41] and 5-HT[42] promote fighting in that species as well. The availability of genetic tools in flies has allowed the identification of small populations of neurons expressing dopamine, 5-HT or OA that regulate aggressiveness[43–47]. However, whether OA regulates aggression specifically or a state of arousal more generally (analogous to noradrenaline in vertebrates[19]) is not clear, as OA influences many other behaviours in *D. melanogaster* (reviewed in REF. 48). The identification of OA receptor-expressing neurons that control aggression in *D. melanogaster* should help to resolve this issue.

Several neuropeptides promote aggression in flies, including Neuropeptide F[42] and *Drosophila* Tachykinin (Tk); the latter has been suggested to increase aggressive arousal[49]. Interestingly, Tk homologues (including substance P) have been implicated in aggression in several mammalian models[50–52] and in humans[53]. Tachykinin 1 is also expressed in a subset of mouse oestrogen receptor 1-positive (ESR1$^+$) neurons in the ventromedial hypothalamic nucleus (VMH)[54], which have been implicated in aggression by optogenetic[55] and other studies (see below). Thus, evidence suggests that there is some conservation in the neuromodulatory regulation of aggressiveness; however, these neuromodulators are not specific for aggression and regulate many behaviours in different species[20,32,56,57].

***Courtship and internal states in* D. melanogaster.** The circuitry controlling male courtship behaviour in *D. melanogaster* is one of the most intensively studied neural systems in this species and arguably one of the best systems in any genetic model organism for understanding how the brain controls an innate reproductive social behaviour (reviewed REFS 29,30,58). For the purposes of this Review, it is important to highlight the important discovery that many neurons in the male courtship circuitry are marked by the expression of the male-specific form of the sex-determination transcription factor-encoding gene *fruitless* (*fru*^{M})[59,60]. This discovery has been exploited to generate comprehensive anatomical maps of FruM-positive (FruM$^+$) neurons[61,62] and to conduct systematic, unbiased functional screens for neurons that promote courtship when activated[63]. Through these and more recent studies[64–66], a detailed picture is emerging of the circuitry through which the detection of female sensory cues leads to the expression of male-specific courtship behaviours, such as singing (reviewed REFS 26,29).

Until recently, the study of FruM$^+$ circuits has focused primarily on the delineation of pathways that control specific courtship-related actions, rather than on the encoding of internal states that are associated with such behaviour. Below, I review recent data identifying a subpopulation of FruM$^+$ neurons involved in both aggression and courtship, and provide evidence of their role in promoting an internal state that stimulates both social behaviours. Other subpopulations of FruM$^+$ neurons that exclusively control aggression have been identified (reviewed in REF. 67).

P1^a interneurons can promote aggression and courtship. One of the most intensively studied subsets of FruM$^+$ neurons is called the P1 cluster (also known as pMP4/e neurons[61,62], and not to be confused with the *fru* P1 promoter[30]). These neurons integrate female cues of different sensory modalities, such as contact

Scalability
In the context of this Review, the property of a π state to exhibit graded and time-varying changes in its intensity, such as escalation during a social encounter.

and volatile chemosensory signals[64–66,68,69] (reviewed in REF. 26), and are thought to control the decision to engage in courtship[29]. P1 neurons comprise a group of ~20–25 male-specific interneurons per hemibrain that were functionally identified in screens for subsets of FruM-expressing neurons that promote courtship song[63,68–70], as well as by anatomical studies[61,62] (reviewed in REFS 26,30,67) (FIG. 1c). All P1 neurons also express DsxM, the protein product of the male sex-determination gene *doublesex* (*dsx*)[69,70] (although DsxM is also expressed in a larger cluster of ~40 neurons per hemisphere called pC1 (REFS 70,71), which includes FruM$^-$ neurons[22,72]). Importantly, although P1 neurons have been defined by their characteristic anatomy[69] and

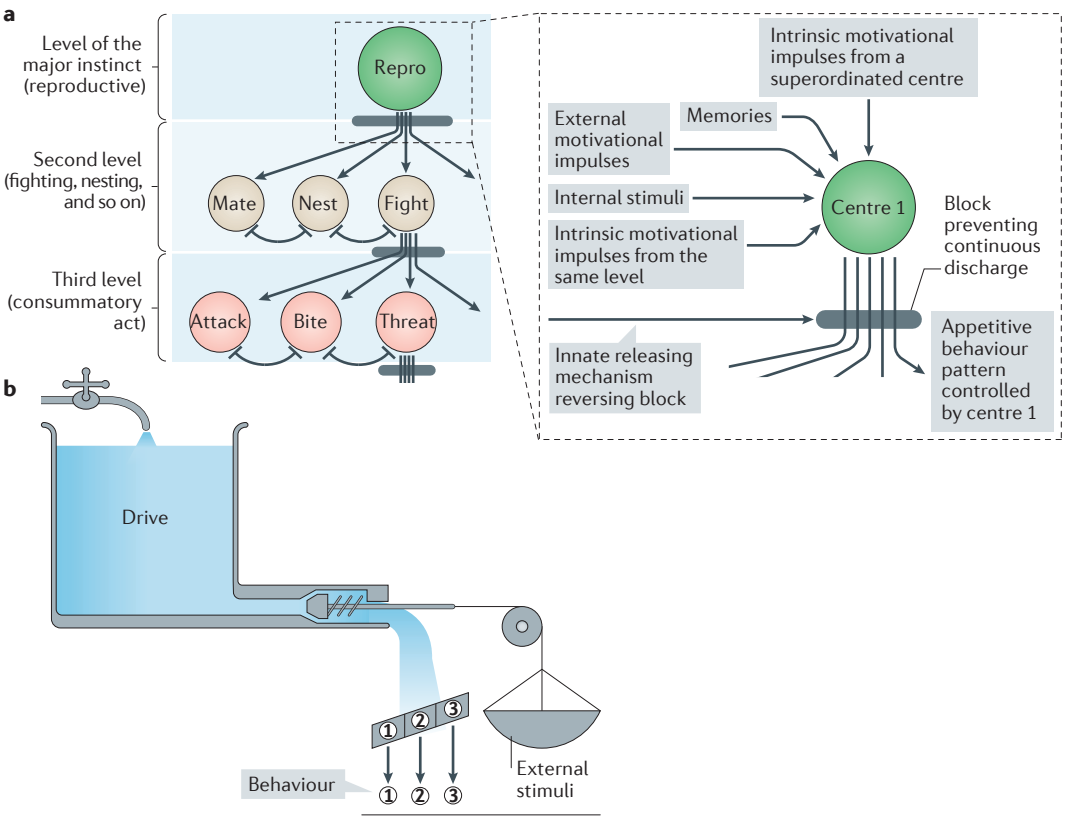
Box 2 | **Tinbergen and Lorenz: hierarchies and hydraulics**

The Nobel prize-winning neuroethologists Niko Tinbergen and Konrad Lorenz were fascinated by the relationship between behavioural decisions and motivation, arousal and drive states (herein referred to as π states), but had different views on how behavioural decisions and such internal states might interact.

Tinbergen[7,24] viewed behavioural decisions as occurring in a hierarchical manner and postulated an underlying hierarchical organization of circuit nodes organized in a feedforward manner (see the figure, part **a**; left panel). Fighting and mating were envisioned as being organized as separate nodes under a common 'reproductive' ('repro') hierarchy (see the figure, part **a**; 'Second level'). Tinbergen envisioned that external and internal inputs that promote motivational states (for example, pheromonal cues or caloric deprivation), as well as hormones, would exert their influence at the apex node of such a hierarchy (centre 1) (see the figure, part **a**; right panel). However, this model does not make explicit reference to the scalability (intensity) of internal states, and the intensity of the state does not influence the choice between different nodes at subordinate levels of the hierarchy.

By contrast, Lorenz[8] proposed that the intensity (level) of internal drive states, in concert with external 'releasing' stimuli, would influence the behavioural choice (see the figure, part **b**). The amount of drive 'released' by an external stimulus and allowed to 'flow' through the nervous system, together with the inherent level of drive that is needed to activate different behaviours, would determine the sequence (in the figure, indicated by '1', '2' and '3') in which certain behaviours were exhibited. Thus, certain behaviours would only be released if the level of drive was sufficiently high and if the external releasing stimulus was sufficiently strong. Therefore, in Lorenz's view, drive states not only causally promote behaviour, but their strength (intensity) influences which behaviours are expressed. Unlike Tinbergen, Lorenz did not attempt to instantiate this 'hydraulic' model at the level of neural circuit organization, preferring to leave it as a metaphor.

One way to reconcile these views is to combine features of both models: the level of drive or motivation is encoded in the level of activity of a higher-order centre, such that the level of output from this centre determines which subordinate centres are activated[95]. Interestingly, neither Lorenz nor Tinbergen explicitly considered how the persistent nature of π states might be encoded or how their levels might decay over time. Part **a** of the figure is adapted from REF. 7 by permission of Oxford University Press. Part **b** of the figure is adapted from REF. 6, Elsevier.



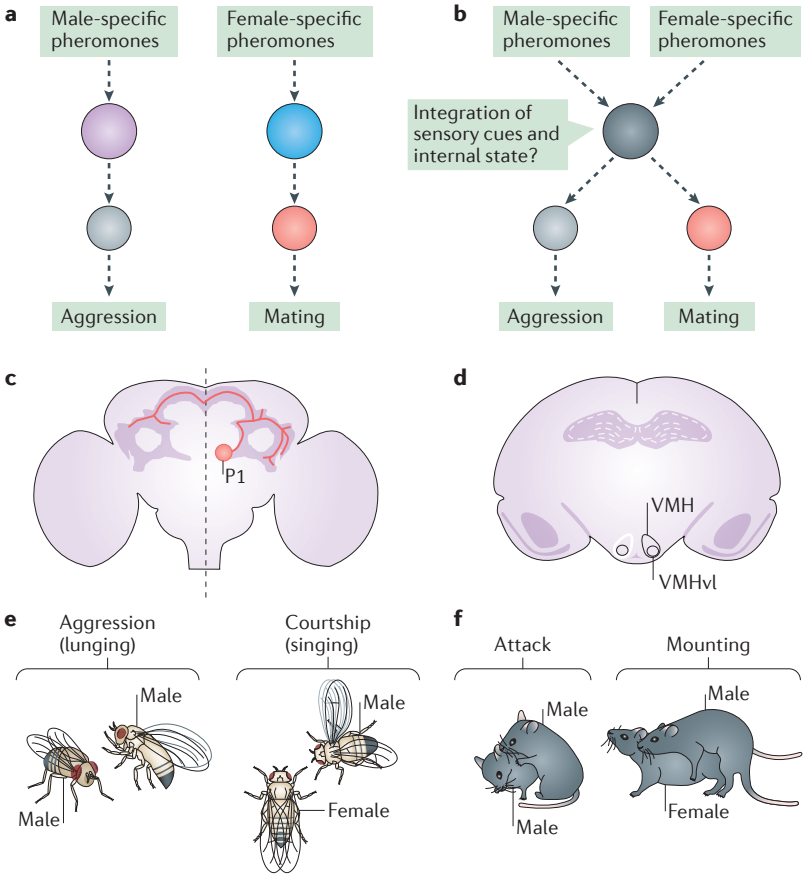


Figure 1 | **P1 and VMHvl neurons control multiple social behaviours. a**,**b** | Simplified schematics illustrating extreme alternative views of the possible relationship between aggression and mating circuits. Spheres indicate circuit nodes containing multiple neurons. Signals (for example, pheromones) from male and female conspecifics may activate parallel pathways to release aggression and mating, respectively (part **a**), or these pathways may converge on a common node that controls both of these social behaviours (part **b**). In part **a**, reciprocal inhibition between pathways is omitted for clarity. **c** | Schematic illustrating a *Drosophila melanogaster* brain showing the location of the P1 cluster and major projections. **d** | Coronal section of a mouse brain indicating the location of the ventrolateral subdivision of the ventromedial hypothalamic nucleus (VMHvl). **e**,**f** | Behavioural phenotypes promoted by optogenetic stimulation of P1 neurons in male flies (part **e**) or VMHvl oestrogen receptor 1-positive neurons in male mice (part **f**). Part **c** is adapted from REF. 30, Nature Publishing Group. Fly images in part **e** adapted from drawings courtesy of H. Chiu, California Institute of Technology, USA.

derivation from FruM-expressing precursors[61–63,73], in adult male flies not all neurons in the P1 cluster express FruM[74]. Thus, different studies may use slightly different criteria when referring to P1 neurons, depending on the genetic reagents used to identify or manipulate these cells[22,63,65,66,74,75].

Recently, an unbiased screen of 3,000 Gal4 lines[76] of *D. melanogaster* for neurons that increase aggression when thermogenetically activated using *Drosophila* Transient receptor potential cation channel A1 (dTrpA1)[77] identified three independent hits that promoted increases in both male–male courtship and aggression[74]. Pairwise intersectional combinations between these hits, using the split Gal4 system[78,79], labelled a common cluster of 8–10 cells per hemibrain with a P1-like morphology[61–63]; of this cluster, ~60–80% of the cells expressed FruM[74]. Thermogenetic activation of one of these intersectional combinations (line 71G01 ∩ line 15A01), called P1^a, caused interspersed bouts of wing extension and aggression (lunging) (FIG. 2a), confirming that these P1^a cells are sufficient to promote both social behaviours. Importantly, intersectional labelling between the parental Gal4 lines and FruM-expressing cells (using *fru*–FLP)[62] labelled an even smaller subset of these P1^a neurons (3–5 cells per hemibrain), which, when thermogenetically activated, could promote aggression without wing extension (FIG. 2b). These data confirmed that the fighting phenotype was indeed due to activation of a subset of P1 neurons (as defined using *fru*–FLP labelling) and is not an indirect social response to increased wing extension[74].

How can P1^a neurons promote two social behaviours that are usually mutually exclusive? Optogenetic activation, using red-shifted opsins such as ReaChR[80] or CsChrimson[81], provided insight into this question because of its higher temporal resolution and broader dynamic range compared with thermogenetic activation[75]. Optogenetic activation of P1^a neurons at a low frequency (10–20 Hz) promoted aggression (FIG. 2c; left; 'ON'; blue rasters) but not wing extension, whereas higher-frequency stimulation (>30 Hz) evoked wing extension (FIG. 2c; right; 'ON'; red rasters) as well as aggression. Therefore, P1^a neurons might differentially control these two behaviours according to the level of activity, number or type of active neurons in the population.

During high-frequency optogenetic stimulation of P1^a neurons, aggression was elevated after, rather than during, photostimulation and was suppressed during the light-ON phase (FIG. 2c; right; 'OFF'; blue rasters)[74]. This observation suggests that P1^a neurons either indirectly promote aggression at the circuit level — for example, by inhibiting aggression neurons, which then rebound from such inhibition following the offset of P1^a photostimulation (FIG. 2d) — or have a direct effect to activate aggression neurons that is supervened by wing extension, locomotor arrest[74] or an inhibitory effect of photostimulation on the flies' visual system (FIG. 2e). At higher frequencies of photostimulation, optogenetically evoked wing extension was rapidly suppressed following light offset (FIG. 2c; right, 'OFF'; red rasters), as a consequence of the resumption of aggression[74]. Thus, P1^a neuron activation promoted wing extension and aggression in a threshold-dependent and inverse manner, perhaps reflecting reciprocal inhibition between these two behaviours[7,24].

P1^a interneurons promote a π state. Activation of P1 neurons in single male flies promoted persistent wing extension[66,75,82] (FIG. 3a). By contrast, activation of P1^a neurons in pairs of male flies triggered aggression that persisted for minutes after photostimulation offset (FIG. 2c; 'OFF'; blue rasters)[74]. This persistent aggression did not simply reflect social perpetuation of fighting by iterative cycles of attack and counterattack: transient activation of P1^a neurons in pairs of solitary males that were initially separated by a partition triggered an

Gal4 lines
Inbred lines of transgenic flies in which the expression of the yeast transcriptional activator Gal4 is restricted to specific neuron subsets by regulatory DNA fragments.

Split Gal4 system
Intersectional labelling of neurons by expressing the DNA-binding and transcriptional activation domains of *GAL4* from two separate transgenes under the control of different but overlapping promoters.

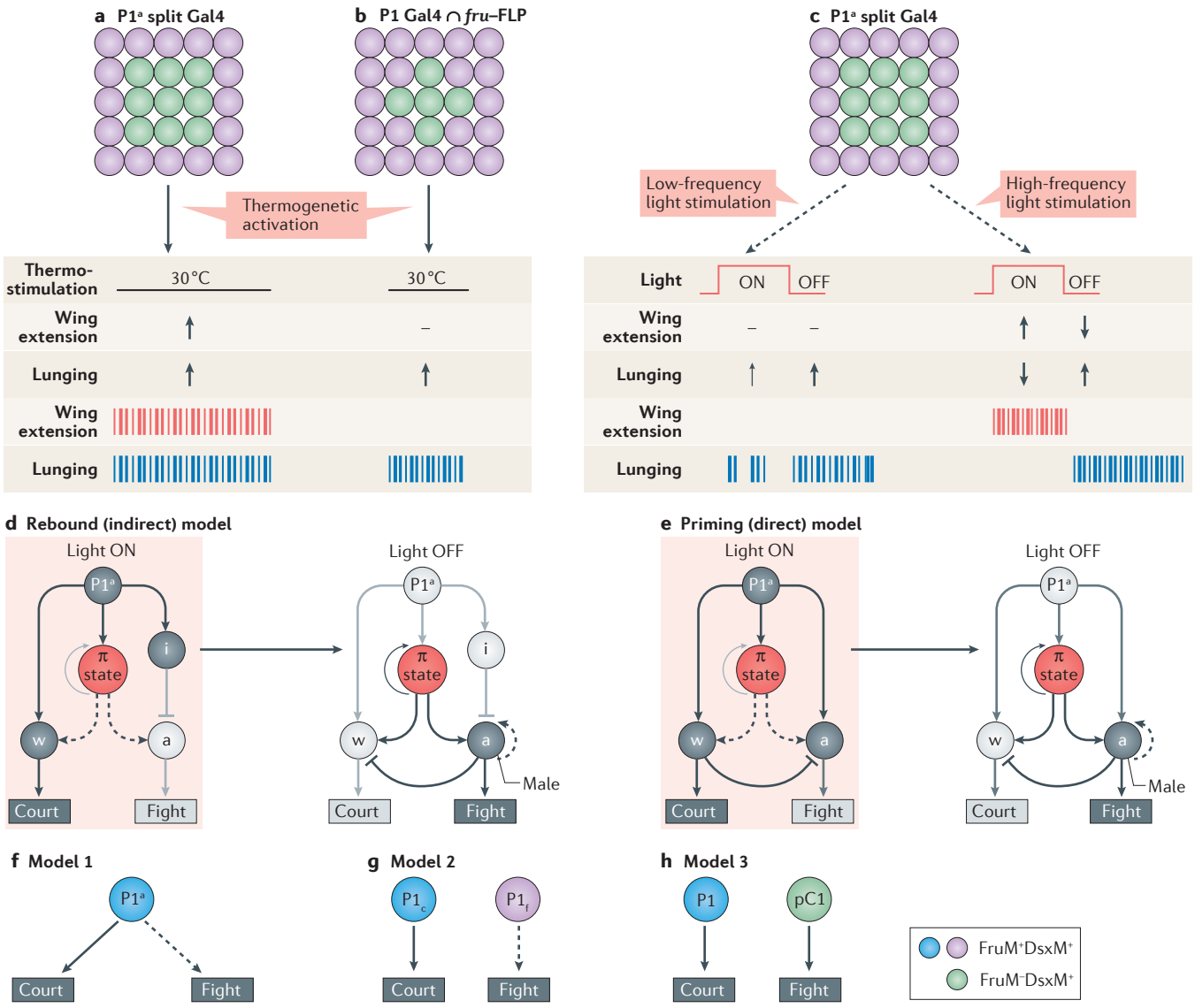


Figure 2 | **Effects of P1 neuron activation on social behaviour in flies.** **a**–**c** | Effects of activating different populations of P1 neurons either thermogenetically[77] using dTrpA1 (parts **a**,**b**) or optogenetically using ReaChR[75,80] or CsChrimson[81] (part **c**). Green spheres indicate the subset of P1 neurons that are genetically labelled in each case; purple spheres indicate unlabelled P1 neurons. 'P1ª split Gal4' (parts **a**,**c**) indicates a subset of 8–10 P1 neurons[63] (green shading) that are labelled by a genetic intersection between lines 71G01 (REFS 69,74) and 15A01, which were recovered in the original screen[74]. 'P1 Gal4 ∩ *fru*–FLP' (part **b**) indicates a subset of ~3–5 P1 neurons that are identified by the intersection of 71G01-Gal4 and *fru*–FLP[62,63]. Behavioural readouts (wing extension and lunging) are denoted using arrows: thick arrows indicate an increase in behaviour in pairs of male flies following P1 subset activation; the thin arrow indicates a weaker behavioural phenotype. '–' indicates that there was no observed change in behaviour. Simulated behavioural rasters (red and blue vertical tick marks) are also shown. **d**,**e** | Two speculative circuit models to explain the inverse control of courtship and aggression by optogenetic activation of P1ª neurons. In the rebound (indirect) model, the influence of P1ª neurons on aggression circuits is indirect and inhibitory (part **d**). During the light-ON phase, P1ª neurons activate a wing-extension circuit[63] (indicated by 'w'), trigger a persistent internal state (π state) and suppress aggression-promoting neurons (indicated by 'a') through a putative inhibitory interneuron (indicated by 'i'). After light offset (light OFF), and in the presence of a conspecific male, aggression circuits, which are no longer suppressed by the putative inhibitory interneuron, show rebound activity, which persists (dashed arrow) and suppresses wing extension[74]. In the priming (direct) model, the influence of P1ª neurons on aggression circuits is direct and excitatory (part **e**). During photostimulation (light ON), P1ª neurons activate wing-extension and prime aggression circuits ('a'; grey shading), but overt aggressive behaviour is inhibited by downstream courtship circuitry, locomotor arrest or an influence of light[74]. After light offset (light OFF), the primed aggression circuit activates its downstream targets and the wing-extension circuit is suppressed. In both models, persistent aggression is driven by the internal state. Thin and thick arrows indicate effects requiring low- and high-frequency photostimulation, respectively. **f**–**h** | Three possible models to explain the relationship of optogenetically induced social behavioural phenotypes to the cellular composition of the P1ª population. In the first model (part **f**), common P1ª neurons promote both courtship and aggression; the dashed arrow indicates that the effect on aggression may be indirect (as in part **d**) or direct (as in part **e**). In the second model (part **g**), separate subpopulations of FruM-positive (FruM⁺) cells within the P1ª population (as in part **a**) may separately promote courtship ($P1_c$) and fighting ($P1_f$). In the third model (part **h**), FruM⁺DsxM⁺ P1 cells (P1) exclusively promote courtship and inhibit aggression, and aggression is promoted by FruM⁻DsxM⁺ cells (pC1)[22]. DsxM, male-specific Doublesex.

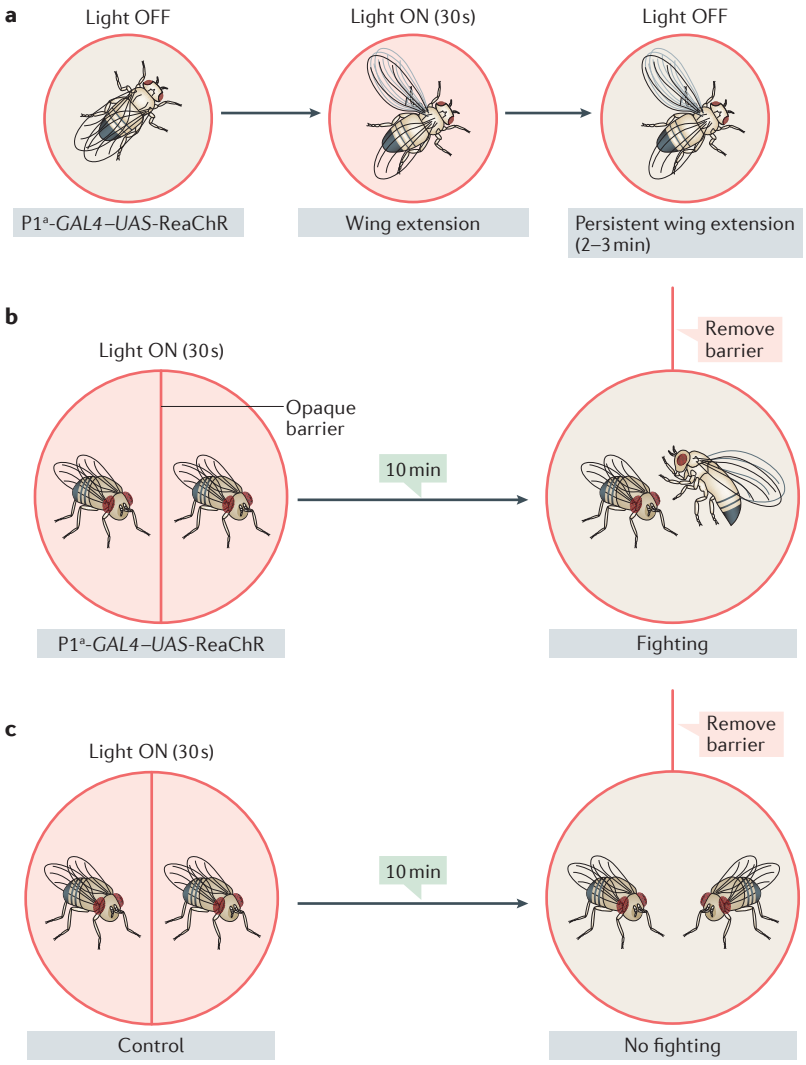


Figure 3 | **P1ᵃ neurons promote a persistent internal state of social arousal or motivation. a** | Transient optogenetic activation[66,75] (middle panel; light ON) or thermogenetic activation[82] (not illustrated) of P1 neurons in solitary male flies triggers persistent wing extension. **b**,**c** | Schematics illustrating an experiment to reveal the persistent internal state promoting aggression in *Drosophila melanogaster*[74]. Circles represent arenas containing two genetically identical male flies that are separated by a removable partition (red vertical lines). Following 30 s of optogenetic stimulation (left panels; pale red shading), the flies are allowed to recover for 10 min; after this, the partition is removed (right panels) to allow the flies to interact for 5 min. Fighting between flies in which P1ᵃ neurons were transiently activated is observed following partition removal (part **b**; right panel), whereas control flies do not fight (part **c**; right panel). Before partition removal, solitary flies exhibit persistent wing extension for 2–3 min (not illustrated), as in part **a**. *UAS*, upstream activating sequence. Fly images in parts **b** and **c** adapted from drawings courtesy of H. Chiu, California Institute of Technology, USA.

enduring internal state that promoted aggression when the partition was subsequently removed, even tens of minutes after photostimulation[74] (FIG. 3b,c).

In summary, these data identify two novel features of P1ᵃ neurons. First, their artificial activation can promote not only courtship[63,69] but also (directly or indirectly) aggression. Second, P1ᵃ activity also promotes a persistent π state[75,82] that can enhance both types of social behaviour[74].

Wing extension
A social behaviour in which a male fly extends one wing towards another fly and vibrates it at specific frequencies to generate a courtship 'song'.

These observations raise several important new questions about P1ᵃ neuron function in social behaviour. First, how is the persistent internal state that is triggered by P1ᵃ neurons instantiated in the brain? Second, do the same or different P1ᵃ neurons promote courtship and aggression (FIG. 2f–h)? Third, do P1ᵃ neurons promote aggression at the circuit level indirectly or directly (FIG. 2d,e)? Fourth, how is the threshold-dependent control of social behaviour by P1ᵃ neurons achieved? Last, do P1ᵃ neurons normally control the decision between courtship and aggression[22], and, if so, what is their role in this process?

Given the role of P1ᵃ neurons in promoting internal states, it is interesting that the excitability of P1ᵃ neurons can itself be modulated by other internal states that influence social behaviour, such as those produced by social isolation[75,83] or by sexual exhaustion/satiety[84,85]. Clearly, P1 neurons are fascinating; they have attracted the attention of many laboratories (reviewed in REFS 26,67), and new insights into their function and circuitry will be forthcoming over the next few years.

Mating and aggression circuits in mice

Although the elucidation of the brain circuits controlling mating and aggression in flies is a compelling research objective in its own right, it is important from an evolutionary perspective to understand whether such circuits exhibit any organizational or functional similarities to those mediating analogous behaviours in vertebrates, including humans. To make such a comparison, it is necessary to investigate how mating and aggression are controlled in the mammalian brain, despite its enormous complexity. Below, I provide some historical background to the study of aggression circuits in mammals.

The seminal discovery that electrical activation of the lateral hypothalamus in cats could evoke an aggressive response known as 'defensive rage' (REF. 86) has since been extended to several other species (reviewed in REFS 87,88). Detailed microstimulation studies in rats delineated a so-called hypothalamic attack area (HAA), into which injection of current could elicit attack[89]. The HAA spans portions of the ventrolateral subdivision of the VMH (VMHvl) (FIG. 1d) and the adjacent intermediate hypothalamic area (IHA)[90]. These studies have raised several outstanding questions, two of which are outlined here.

First, which neurons are responsible for brain stimulation-evoked aggression? Kruk[88] proposed that three criteria should be met to satisfy this identification: activation of the neurons should be sufficient to trigger attack; inhibition of these neurons should impair normal aggression; and these neurons should be active during aggressive encounters. Antibody–toxin conjugate-mediated ablation of neurons expressing neurokinin 1 receptor (NK1R; also known as tachykinin receptor 1) in the rat HAA was shown to reduce naturally occurring aggression[50]. However, owing to the dearth of genetic tools, it was difficult to further define or specifically activate these neurons. Such tools are available in mice[91], but historically most brain-stimulated aggression research in rodents has been performed in rats and hamsters[92,93].

Second, what is the relationship of aggression-promoting neurons to those controlling mating behaviour? Newman[25] has proposed that aggression and mating in rodents are controlled by a distributed network involving multiple limbic structures that participate in both behaviours, and that the relative level of activity across these nodes determines which behaviour will be expressed. Alternatively, different brain structures and neuronal subpopulations may control these two behaviours[21]. A recent quantitative brain-wide survey of neuronal activation during mating and aggression in mice revealed both substantial overlap and differences in the structures that are activated during these social behaviours[94]. Below, I outline advances in delineating aggression-promoting neurons in mice and their relationship to those controlling mating behaviour.

Aggression-promoting neurons in the mouse hypothalamus. Over the past 5 years, considerable progress has been made towards identifying aggression-promoting neurons in mice (reviewed in REFS 23,27,95–97). Briefly, optogenetic gain- and loss-of-function studies[55,98], as well as genetically based cell-ablation[99] and RNAi-mediated knockdown experiments[100], have identified a population of ~2,000 glutamatergic neurons co-expressing ESR1 and the progesterone receptor (PR) in the VMHvl as being necessary and sufficient for normal levels of aggression. Importantly, optogenetic activation of VMHvl ESR1$^+$ neurons had little obvious behavioural effect in solitary animals[55]. By contrast, optogenetic activation of steroidogenic factor 1 (SF1; also known as NR5A1)-expressing neurons in the adjacent dorsomedial-to-central subdivision of the VMH (VMHdm/c) elicited defensive behaviours in solitary animals and inhibited social behaviours such as aggression[101,102]. Finally, electrophysiological and *Fos* expression studies have shown that VMHvl ESR1$^+$ neurons are activated during inter-male social interactions that include aggressive encounters[55,95] (although whether they are specifically activated during attack is not yet clear).

Collectively, these studies suggest that ESR1$^+$PR$^+$ neurons in the VMHvl are candidates for attack-promoting neurons, according to the Kruk criteria[88,95]. Notably, the VMHvl is subsumed within the HAA in rats, but the latter is (proportionally) a much larger region that also includes the IHA[90]. In mice, electrophysiological recordings in the IHA failed to detect units specifically active during social encounters, and optogenetic stimulation in this region failed to evoke attack[98]. It is possible that the attack evoked by electrical stimulation of the IHA in rats reflects the activation of fibres of passage derived from VMHvl neurons or their inputs (see below). Alternatively, 'attack neurons' in rats may be more broadly distributed than in mice.

Importantly, in mice, attack can also be evoked by optogenetic stimulation of GABAergic neurons in the medial amygdala[103], which provides direct and indirect input to the VMHvl[104,105]. Furthermore, the VMHvl projects to many downstream structures, including the dorsal periaqueductal grey[106] and anterior hypothalamic nucleus, which have been implicated in maternal aggression in rats[107] and aggression in hamsters[92], respectively. Therefore, it is not yet clear whether the motor programme for attack is encoded in the VMHvl itself or in one of its direct or indirect targets. Clearly, no single structure or neuronal population encodes aggression in the brain; rather, a distributed circuit controls this behaviour. The aforementioned studies provide points of entry into this circuit; the next challenge will be to understand what input–output transformations are performed at different nodes in this circuit and the underlying cellular implementation of these transformations.

VMHvl neurons in appetitive and sexual behaviours. Based on earlier rat studies, male mating behaviour was attributed to the medial preoptic nucleus[16,23], whereas female mating behaviour was assigned to the VMHvl[108,109]. It was therefore surprising that single-unit multi-electrode recordings in the VMHvl of freely behaving male mice revealed that some neurons were active during male–female as well as male–male social encounters[98]. Compartment analysis of temporal activity by fluorescence *in situ* hybridization (catFISH) experiments examining *Fos* expression indicated an overlap of ~25% between VMHvl neurons activated during a social encounter with another male and those activated 30 minutes later during an encounter with a female[98]. The number of ESR1$^+$ neurons expressing *Fos* in the VMHvl was approximately twofold higher after fighting than after mating[55].

What is the function of VMHvl neurons activated during male–female social encounters — to promote mating, to inhibit attack, or both? Genetically restricted optogenetic manipulation of ESR1$^+$ neurons in the VMHvl (~40% of total VMHvl neurons) revealed that weak activation of these neurons in socially inexperienced animals could promote close investigation and mounting (towards males or females), whereas strong activation promoted attack[55]. Conversely, optogenetic inhibition of these neurons could interrupt either close investigation or attack, depending on whether photostimulation was delivered during the approach to the intruder or after the initiation of fighting[55]. Although optogenetic inhibition of VMHvl ESR1$^+$ neurons failed to interrupt ongoing male–female mounting, genetic ablation of PR-expressing VMHvl neurons[99], or RNAi-mediated knockdown of *Esr1* in the VMHvl[100], caused a statistically significant (albeit incomplete) reduction in male mating behaviour. Together, these loss- and gain-of-function studies reveal a causal role for VMHvl ESR1$^+$PR$^+$ neurons in both the appetitive and consummatory phases[7] of aggressive behaviour in males and also in male sexual behaviour. Whether these behavioural functions are mediated by common or distinct subsets of ESR1$^+$ neurons is an important open question.

VMHvl neurons play a part in promoting a π state. The finding that neuronal activity in the VMHvl is necessary and sufficient for normal levels of male aggressive and mating behaviour and is also elevated during these social behaviours leaves open the precise functional role of this node. Several lines of evidence suggest that the VMHvl does not have a purely sensory or motor function but may contribute to internal states associated with mating and/or

Fos
An immediate early gene, the transcription of which is rapidly induced by elevated intracellular free calcium and therefore serves as a surrogate marker of neuronal activation.

Fibres of passage
Axons that pass through a given brain region *en route* to a distant target without forming local synapses; such axons can nevertheless be electrically stimulated.

Compartment analysis of temporal activity by fluorescence *in situ* hybridization
(catFISH). A method for comparing immediate-early gene activation in the same neuron in response to two sequential stimuli.

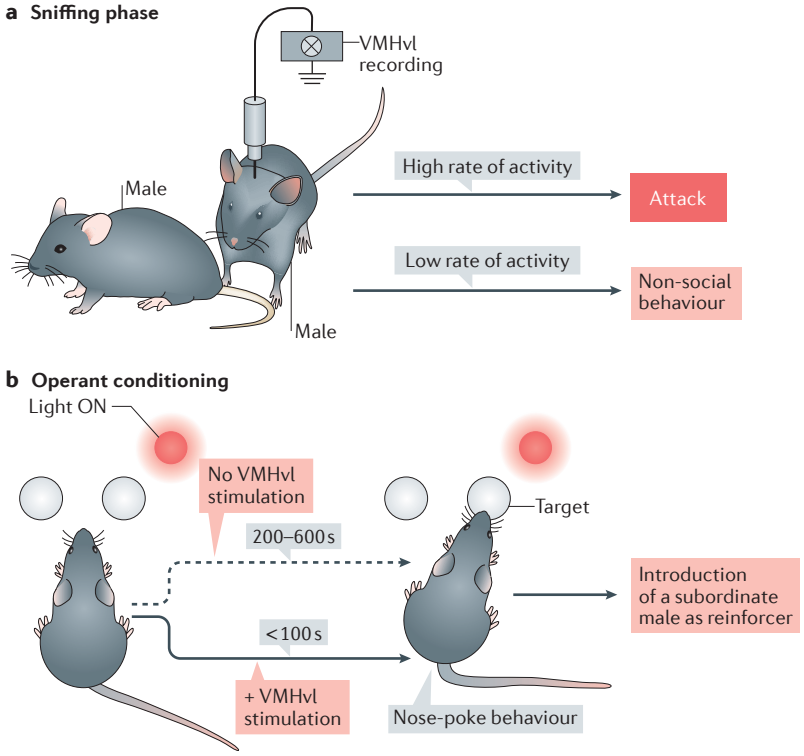


Figure 4 | **VMHvl neurons promote aggressive motivation or arousal.** **a** | Electrophysiological recordings from the ventrolateral subdivision of the ventromedial hypothalamic nucleus (VMHvl) in awake, behaving male mice[98] sniffing an intruder male reveal that the average spiking rate during sniffing predicts the likelihood and duration of ensuing attack[110]. **b** | Mice in an operant conditioning chamber can be trained to nose poke for access to a subordinate male that they can attack and defeat[112,113]. Optogenetic stimulation of non-genetically targeted neurons in the VMHvl reduces the latency to initiate nose-poke behaviour from 200–600 s to less than 100 s. Pharmacogenetic inhibition of the VMHvl reduced nose poking (not shown). Together, these data imply a role for the VMHvl in promoting aggression-seeking behaviour[114].

fighting. For example, analysis of single-unit activity in the VMHvl recorded during male–male social interactions[98] (FIG. 4a) revealed an increase in average spiking rate during the 400 milliseconds of sniffing preceding attack[110]; the peak value reached during sniffing was markedly higher for sniffs followed by attack than for those followed by non-social interactions (FIG. 4a). Moreover, the average spiking rate during the sniff phase predicted the duration of, and (inversely) the latency to, attack[110]. These data suggested that a component of activity in the VMHvl might reflect or encode a scalable π state, and/or a neural integrator[111], that must reach a certain threshold to evoke attack, consistent with the results of optogenetic activation experiments[55].

Motivation (a type of π state) is typically studied using operant conditioning paradigms[6] (BOX 1). Mice can be trained to perform an instrumental task to gain access to a subordinate male that they can attack[112,113], indicating that this social interaction is rewarding or reinforcing (FIG. 4b). A small percentage of units recorded in the VMHvl are active specifically during performance of the operant behaviour, and optogenetic activation of VMHvl neurons can decrease the latency to perform this operant task[114] (FIG. 4b). Thus, VMHvl activity can promote (and is required for) aggression-seeking behaviour, suggesting a potential role for these neurons in encoding an internal state of aggressive motivation or arousal.

Arousal is typically characterized by a reduced threshold for responses to behaviourally relevant stimuli (BOX 1). Electrical pre-stimulation of the HAA in solitary rats or hamsters can decrease the threshold of the electrical stimulation that is necessary to evoke a subsequent attack towards an intruder. This effect, known as 'priming' (REF. 115), is persistent and has been interpreted to reflect an elevation of aggressive arousal[116]. Preliminary data suggest that such priming effects can be elicited by optogenetic stimulation of VMHvl ESR1$^+$ neurons in mice as well (H. Lee, D.-W. Kim and D.J.A., unpublished observations). Together, these data suggest that VMHvl ESR1$^+$ neurons may have a role in promoting π states underlying aggressive behaviour. Whether the ESR1$^+$ population also promotes sexual motivation in males, as it seems to do in females[117], is not yet clear. Finally, it remains to be determined whether the effects of optogenetic manipulation of ESR1$^+$ neurons to trigger overt mounting and attack are mediated indirectly, through the promotion of a π state (or states), or whether these two influences are controlled in parallel (FIG. 5c).

Coincidence or conservation?

It is striking that parallel studies in both flies and mice have identified small populations of neurons that control mating and aggression in males, in a manner that also involves the promotion of internal states. Such an apparent correspondence might not have been predicted, as P1 neurons are not located in the pars intercerebralis, the region of the fly brain that is traditionally considered to be analogous to the vertebrate hypothalamus[118]. Is this similarity superficial and coincidental, or does it reflect an evolutionarily conserved, circuit-level motif for the control of social behaviours — perhaps corresponding to a node at the apex of the 'reproductive hierarchy' envisioned by Tinbergen[7,24,96] (BOX 2)? Below, I consider the similarities and differences between results obtained in the two systems and highlight open questions for further investigation.

Do overlapping P1^a and VMHvl neurons control mating and aggression? As described above, artificial activation of neurons in the P1^a cluster and VMHvl populations can promote either mating behaviour or aggression, depending on the precise conditions of stimulation[55,74]. An obvious question raised by these observations is whether the same or different neurons within these nodes promote each type of social behaviour (FIG. 2f–h). A recent study has argued that FruM$^+$ P1 neurons exclusively promote courtship, and that aggression is instead promoted by a population of ~20 FruM$^-$DsxM$^+$ neurons[22] called pC1 cells[71] (FIG. 2h) — subsets of which may 'contaminate' apparently P1-specific *GAL4* drivers. However, as mentioned above, thermogenetic activation of an intersectional subset of 3–5 P1 neurons per hemibrain labelled using *fru*–FLP[62] can promote aggression without inducing wing extension[74] (FIG. 2b), a result confirmed using optogenetic activation of neurons labelled using a triple intersection

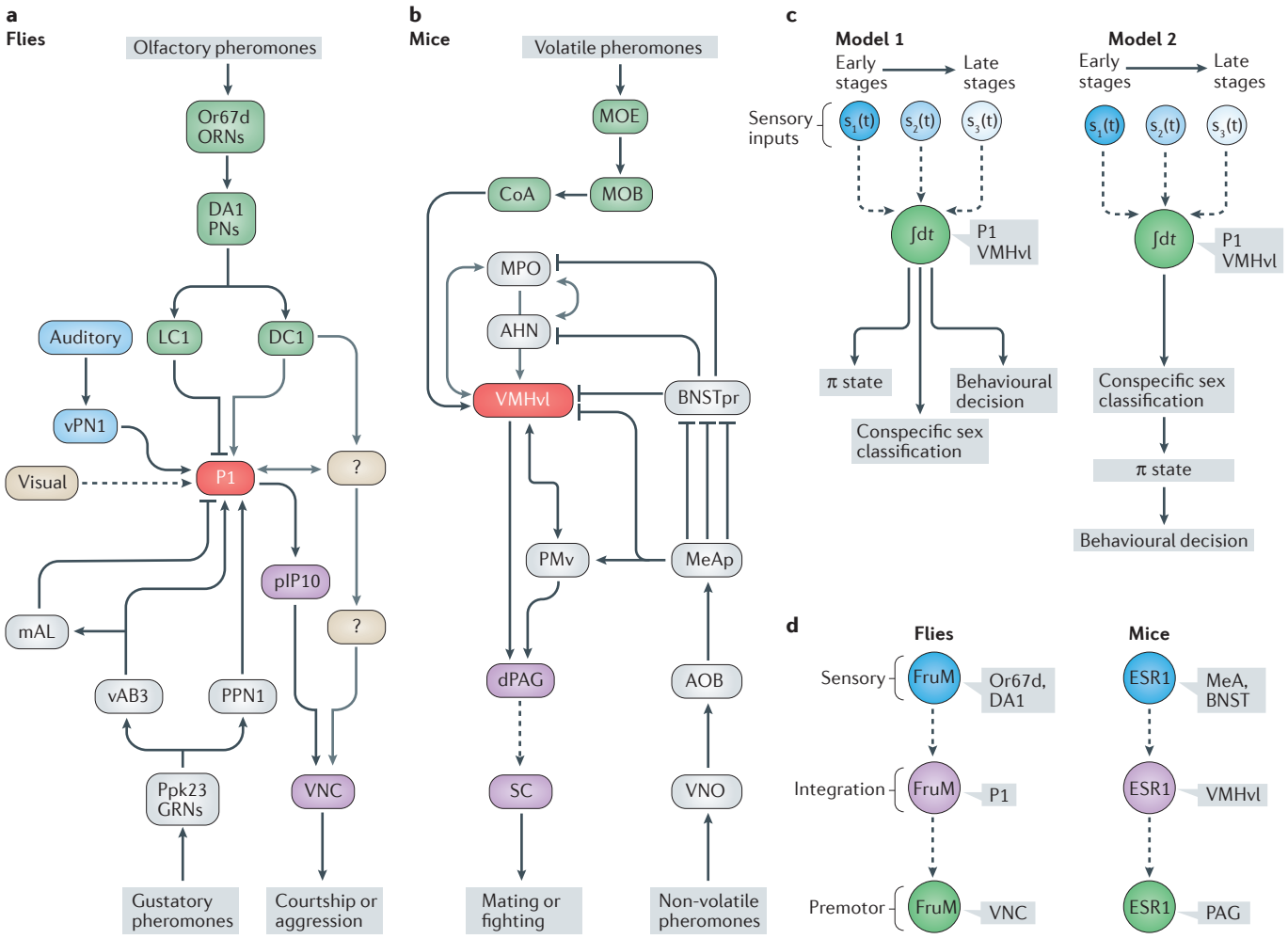


Figure 5 | **P1 and VMHvl neurons receive ascending inputs from multiple chemosensory systems. a** | Schematic illustrating inputs onto P1 neurons from the olfactory system and gustatory system, which detect volatile and non-volatile pheromones, respectively (see also REFS 26,30). **b** | Schematic illustrating inputs onto the mouse ventrolateral subdivision of the ventromedial hypothalamic nucleus (VMHvl) from the main and accessory olfactory systems. Only a subset of connections is illustrated. Data from REF. 125. In parts **a** and **b**, black arrows indicate excitatory connections, flat-headed arrows indicate inhibitory connections, dashed arrows indicate probable indirect connections, and beige boxes and grey lines illustrate hypothetical connections (of unknown sign) based on extrapolations from known connections[26,65,66]. **c** | P1[a] and VMHvl oestrogen receptor 1-positive (ESR1$^+$) neurons may promote multiple functions. These neurons may accumulate (integrate) information about multiple sensory cues (s_1–s_3) at different times over the course of a social encounter (early stages → late stages) and transform this information into an internal state, classification of conspecific sex and/or behavioural decision. These functions may be exerted via the outputs of these structures in parallel (model 1), in series (model 2) or in some combination of the two (not illustrated). **d** | In flies, anatomically defined putative inputs onto and outputs from P1 neurons (dashed lines) express the male sex-determination transcription factor FruM, which is encoded by the male-specific form of *fruitless*[60,62] (left panel). Analogously, in mice, anatomically defined inputs onto and outputs from the VMHvl express ESR1 (REF. 135), which is required for masculinization of social-behaviour circuits[148] (right panel). Electrophysiological confirmation of direct synaptic connectivity between FruM-positive (FruM$^+$) neurons has been demonstrated in only a few cases (for examples, see REFS 66,149,150) and has not yet been shown for ESR1-positive (ESR1$^+$) neurons in this circuit. AHN, anterior hypothalamic nucleus; AOB, accessory olfactory bulb; CoA, cortical amygdala; dPAG, dorsal periaqueductal grey; BNST, bed nucleus of the stria terminalis; BNSTpr, principal division of the BNST; DC1 and LC1, FruM$^+$ interneurons; GRNs, gustatory receptor neurons; MeA, medial amygdala; MeAp, posterior MeA; MOB, main olfactory bulb; MOE, main olfactory epithelium; MPO, medial preoptic nucleus; Or67d, Odorant receptor 67d; ORNs, olfactory receptor neurons; pIP10, pIP10 descending neurons; PMv, ventral pre-mammillary nucleus; PNs, projection neurons; Ppk23, Pickpocket 23; PPN1, pheromone projection neuron class 1; SC, spinal cord; VNC, ventral nerve cord; VNO, vomeronasal organ; vPN1, ventrolateral protocerebrum projection neuron 1.

strategy combining *fru*–FLP and the P1[a] split *GAL4* driver (E. D. Hoopfer and D.J.A., unpublished observations). Therefore, although there may be an aggression-specific subset of neurons within the P1[a] population (FIG. 2g), these neurons express *fru*–FLP. Computational analysis of MARCM (mosaic analysis with a repressible cell marker) labelling experiments has revealed at least ten anatomically distinct neuron subclasses within the P1 cluster[73]. Identifying more specific *GAL4* drivers for each of these subsets should enable future studies to determine whether these cells have distinct roles in courtship, aggression or other aspects of social behaviour.

In male mice, electrophysiological recordings and *Fos* catFISH experiments in the VMHvl have revealed evidence of functional heterogeneity, including neurons that are preferentially activated during male–male versus male–female social encounters[98]. However, it is not yet clear which of these populations is responsible for the behavioural phenotypes that are observed upon functional manipulation of VMHvl ESR1$^+$PR$^+$ neurons[55,99]. *In vivo* microendoscopic imaging[119] has revealed that functionally distinct subpopulations of GABAergic neurons in the lateral hypothalamus are active during appetitive versus consummatory phases of feeding behaviour[120]. Therefore, it will be interesting to see whether this is true for neurons that are active during appetitive versus consummatory aspects of social behaviour in the VMHvl. New methods for single-neuron transcriptional profiling[121,122], together with functional manipulation and imaging of genetically defined VMHvl subpopulations, should help to clarify the behavioural roles of these cells. In summary, whether the P1^a and VMHvl clusters exert their dual effects on mating and aggression through common or distinct subpopulations of neurons remains to be resolved.

Threshold dependence of behavioural control. Optogenetic activation of P1^a neurons or VMHvl ESR1$^+$ neurons promotes different social behaviours in a scalable, threshold-dependent manner: the intensity or frequency of stimulation determines the type of behaviour that is evoked[55,74] (FIG. 2c). A similar scalable control of defensive behaviours has been observed after optogenetic activation of SF1-expressing neurons in the VMHdm/c[101,102]. This threshold dependence of behaviour as a function of stimulation intensity is well documented in the electrical brain-stimulation literature[123] and is reminiscent of Lorenz's 'hydraulic' model for the control of instinctive behaviours according to the level of drive (BOX 2). Interestingly, however, the threshold dependence of mating and fighting in flies and mice is reversed: in flies, P1^a activation evokes aggression at a lower threshold than is required to evoke wing extension[74], whereas aggression in mice requires a higher level of activation of ESR1$^+$ neurons than does close investigation or mounting[55]. Whether these threshold-dependent effects of optogenetic stimulation are relevant to the normal role of these neurons in social behaviour is not yet clear; resolving this will require *in vivo* analysis of neuronal population activity during social behaviour.

Dual behaviour effects: direct or indirect? The fact that optogenetic activation of P1^a and VMHvl neurons promotes both sexual and aggressive behaviour raises the question of whether such dual effects are direct or indirect (FIG. 2d,e). One difference between the results in the two species is that, in mice, activation can promote either close investigation and mounting or attack during the photostimulation period (depending on the light intensity or the number of channelrhodopsin-2-expressing neurons), whereas, in flies, high-frequency activation of P1^a neurons exclusively promotes wing extension during photostimulation, whereas aggression is increased after the offset of photostimulation (FIG. 2c; right; 'OFF'; blue rasters). As discussed above, one interpretation of this observation is that P1^a neurons may indirectly promote aggression via inhibition of attack neurons that rebound after the offset of such inhibition[22] (FIG. 2d). Notably, however, P1^a activation did not inhibit ongoing aggression at low photostimulation frequencies (below the threshold for eliciting wing extension)[74].

Loss-of-function experiments, as well as recording or imaging neuronal activity during behaviour, can help to resolve these issues. In flies, silencing or ablation of P1^a neurons did not measurably reduce levels of aggression[74], although it reduced (but did not eliminate) male–female courtship[63,74]. Whether this negative result reflects technical or biological factors is not clear. Recently, calcium imaging of P1^a neuronal activity in freely behaving flies during courtship was reported using a novel technique called Flyception[124]. The extension of this exciting new approach to aggression should help to elucidate the circuit mechanism through which P1^a neurons exert their effect to promote this behaviour.

In mice, different loss-of-function manipulations indicate that VMHvl ESR1$^+$ neurons are required in males for normal consummatory sexual, as well as aggressive, behaviours[55,99,100]. However, electrophysiological recordings have indicated that overall activity in the VMHvl decreases as mating progresses from mounting to intromission and ejaculation, whereas it remains elevated during attack[98]. Thus, it is conceivable that the dual role of the VMHvl in sex and aggression may be relevant primarily to the appetitive (motivational) phase of a social encounter, whereas its function during the consummatory phase may be more selective for aggression[95]. In summary, P1^a neurons may promote courtship and aggression in flies via circuit-level mechanisms that are different from those by which VMHvl neurons promote mounting and attack in mice.

P1 and VMHvl populations as integrators of multisensory inputs. Another similarity between P1 and VMHvl neurons lies in their input circuitry (FIG. 5a,b). Both structures receive input from pheromonal processing pathways: the P1 cluster receives inputs from ascending interneurons that process volatile or non-volatile chemosensory cues[65,66], whereas the VMHvl receives inputs from the medial amygdala and the bed nucleus of the stria terminalis[104,125] — relays that process pheromones detected by the accessory olfactory system[126] — as well as from the cortical amygdala[127], which processes input from the main olfactory system[128]. The accessory and main olfactory systems are both required for aggression in mice[129–132], and VMHvl neurons respond to chemical cues in urine[98,110]. However, whether the VMHvl integrates volatile and non-volatile chemosensory cues has not been directly tested. The presence of pheromonal inputs onto P1^a and VMHvl ESR1$^+$ neurons is consistent with a role for these nodes in generating π states related to social behaviour (FIG. 5c), as pheromones are well known to evoke, for example, sexual arousal[133,134].

In flies, the available data suggest that P1 neurons receive inputs from non-chemosensory as well as chemosensory modalities, including visual and auditory cues

(reviewed in REF. 26). Whether VMHvl ESR1$^+$ neurons also receive inputs from such modalities has not yet been addressed. Importantly, optogenetic stimulation of VMHvl neurons does not evoke aggression when there is no attackable object present[55,98], suggesting that convergence with visual input may occur downstream. Alternatively, activation of the VMHvl by pheromonal cues might sensitize these neurons to visual input, as was shown recently for P1 neurons[64,69]. Whatever the case, the location of P1 and VMHvl neurons in their respective circuits is well suited to integrate complex, multimodal sensory inputs. Interestingly, FruM and ESR1 are expressed by anatomically defined inputs onto, and outputs from, P1 (REF. 62) and VMHvl neurons[135], respectively (FIG. 5d). Moreover, both of these transcription factors are important for the development of sexually dimorphic circuits that mediate social behaviour[15,30,58], further underscoring the analogy between the two systems.

P1 and VMHvl neurons promote π states. As discussed above, both P1 and VMHvl neurons seem to have a role in promoting π states that are associated with social behaviour. However, the evidence is different in the two systems. In single male flies, transient P1 activation promotes wing extension that persists for several minutes after the offset of stimulation[66,75,82]. In pairs of male flies initially separated by a partition, P1^a activation evokes a behaviourally latent internal state that endures for tens of minutes (after wing extension is no longer elevated) and that is manifested as aggression once contact between the males is permitted[74] (FIG. 3b,c).

By contrast, in mice, there is no report that VMHvl stimulation can trigger a persistent internal state that lasts for minutes. However, the average VMHvl neuron spiking rate during social investigation predicts the duration and (inversely) the latency of impending attack[110] (FIG. 4a), consistent with a role in coding a scalable internal state that reflects the level of aggressiveness. Activation of VMHvl neurons also promotes instrumental behaviour during an operant conditioning paradigm in which the opportunity to attack is the reinforcer[114] (FIG. 4b), arguing for a role in aggressive motivation. In addition, some VMHvl neurons exhibit persistent activity following attack offset *in vivo*[110], and persistent spiking activity lasting minutes can be evoked by transient optogenetic activation of ESR1$^+$ neurons in VMHvl acute slices (D.-W. Kim and D.J.A., unpublished observations).

Thus, although P1 and VMHvl ESR1$^+$ neurons are implicated in π states, whether these states have similar or different functions in the two species is not known. It is also not yet clear whether these π states are generated in parallel, or in series, with other potential functions of P1 and VMHvl ESR1$^+$ neurons, such as object (intruder-sex) classification and behavioural decision making (FIG. 5c). Part of the difficulty in making this comparison lies in the different methods that are used to study internal states in the two systems: for example, there is (thus far) no fly equivalent to the operant conditioning paradigm that is used to assess aggressive motivation in mice[113]. Clearly, an important objective going forward is to elucidate the circuit-level and neurochemical mechanisms underlying these internal states in each of the two systems. Such mechanistic studies should provide further insights into the comparative questions raised here.

Conclusions and outlook

The studies summarized in this Review highlight how independent lines of investigation in flies and mice have converged on an at least superficially similar phenomenology in the neural control of reproductive social behaviour: common circuit nodes for mating and aggression that also control associated π states. These internal states may have a role in promoting arousal, motivating social behaviour, encoding reward[136] and/or controlling the progression from appetitive to consummatory phases[7] of mating or aggression. Improved behavioural assays to measure such internal states, especially in flies, will be important to resolve these issues. Irrespective of the precise nature of these states, however, these findings provide an entry point to determine where and how these internal states are encoded and whether they have a causal role in the control of behavioural decisions.

Given that mating and fighting are closely related, but mutually exclusive, social behaviours, it may not be surprising that circuit-level perturbations of one behaviour affect the other[137]. However, that is not always the case: there are clear examples in which perturbations of neurons that control fighting have no influence on mating[22,49] and vice versa[74,100]. The identification of small populations of neurons in which functional perturbations influence both behaviours (in the same or in opposite[45] directions) suggests that these neurons act at a level of the circuit that controls internal state and/or decision making. Going forward, it will be crucial to investigate the connectional and functional interactions between P1^a or VMHvl ESR1$^+$ neurons and other interconnected populations implicated in social behaviours. The study of these interactions will in turn require the ability to monitor activity simultaneously in multiple, distributed structures in the brains of freely behaving or head-fixed animals[138]. Such studies should take us closer to answering the central questions of how decisions between opponent social behaviours are implemented in brains, and of whether the associated internal states play a causal role to influence these decisions.

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Competing interests statement
The author declares no competing interests.