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COMMENTARY

The stalk-eyed fly as a model for aggression – is there a conserved role for 5-HT between vertebrates and invertebrates?

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ABSTRACT

Serotonin (5-HT) has largely been accepted to be inhibitory to vertebrate aggression, whereas an opposing stimulatory role has been proposed for invertebrates. Herein, we argue that critical gaps in our understanding of the nuanced role of 5-HT in invertebrate systems drove this conclusion prematurely, and that emerging data suggest a previously unrecognized level of phylogenetic conservation with respect to neurochemical mechanisms regulating the expression of aggressive behaviors. This is especially apparent when considering the interplay among factors governing 5-HT activity, many of which share functional homology across taxa. We discuss recent findings using insect models, with an emphasis on the stalk-eyed fly, to demonstrate how particular 5-HT receptor subtypes mediate the intensity of aggression with respect to discrete stages of the interaction (initiation, escalation and termination), which mirrors the complex behavioral regulation currently recognized in vertebrates. Further similarities emerge when considering the contribution of neuropeptides, which interact with 5-HT to ultimately determine contest progression and outcome. Relative to knowledge in vertebrates, much less is known about the function of 5-HT receptors and neuropeptides in invertebrate aggression, particularly with respect to sex, species and context, prompting the need for further studies. Our Commentary highlights the need to consider multiple factors when determining potential taxonomic differences, and raises the possibility of more similarities than differences between vertebrates and invertebrates with regard to the modulatory effect of 5-HT on aggression.

KEY WORDS: Serotonin, 5-HT receptors, Monoamines

Introduction

Aggressive behavior is ubiquitous for gaining access to desirable resources such as territory, food and mates (Edwards and Herberholz, 2005; Summers et al., 2005a,b), and hence aggression is critical for determining individual fitness. However, fighting is energetically costly and potentially injurious. As a consequence, diverse species have evolved signaling strategies during aggressive encounters with conspecifics to minimize physical engagement, often comprising elaborate displays incorporating various morphological ornaments and armaments. Across the majority of animal taxa, the ability to modulate aggressive responses appears to be governed by monoaminergic activity (Alekseyenko et al., 2013; Hoopfer, 2016; Rillich and Stevenson, 2014; Zhou et al., 2008), with serotonin (5-hydroxytryptamine, 5-HT) playing a key role (Bubak et al., 2015; Takahashi et al., 2012). In stalk-eyed flies, 5-HT appears to mediate appropriate behavioral responses upon perception of aggressive signals (Bubak et al., 2014a).

5-HT, 5-HT receptor structure and function, and the 5-HT transporter (SERT), which removes 5-HT from the synaptic cleft to terminate 5-HT signaling (Fig. 1), are phylogenetically conserved (Blenau and Baumann, 2001; Martin and Krantz, 2014). Despite this, 5-HT appears to play generally opposing roles in the generation of the complex behaviors associated with aggression in invertebrates and vertebrates (see Table S1). However, we propose that this seemingly contrasting role of 5-HT may be an overly simplistic generalization. In this Commentary, we will briefly outline the known functions of serotonergic signaling in aggression across invertebrates and vertebrates (for more comprehensive reviews, see Alekseyenko and Kravitz, 2015; de Boer et al., 2016; Takahashi et al., 2012), combined with findings from our stalk-eyed fly model (Box 1), to demonstrate that 5-HT plays a much more nuanced role when factors such as receptor subtype, other neuromodulators and specific phases within aggressive interactions are taken into consideration. The emerging picture suggests that the serotonergic mechanisms governing invertebrate aggression may be more reminiscent of those of vertebrates than previously thought.

The role of 5-HT in vertebrate and invertebrate aggression

In most vertebrates, 5-HT is largely viewed as an inhibitory neuromodulator of aggression (Carrillo et al., 2009; de Almeida et al., 2015; Nelson and Chiavegatto, 2001; Summers et al., 2005a; but see de Boer et al., 2015, 2016). This interpretation is principally based upon studies showing that reductions in 5-HT in vertebrates typically increase aggression (Table S1; Audero et al., 2013; Caramaschi et al., 2008; Cervantes and Delville, 2007; Mosienko et al., 2012; Perez-Rodriguez et al., 2010). Conversely, augmenting 5-HT availability, through either dietary supplementation or reducing SERT-mediated 5-HT clearance, suppresses aggression (Höglund et al., 2005; Holmes et al., 2002).

In contrast to the effect in vertebrates, most studies suggest 5-HT increases aggression in invertebrates (Table S1). Acute 5-HT injection into the hemolymph of crustaceans induces subordinate males to re-engage in confrontations with dominant opponents while decreasing their willingness to retreat (Antonsen and Paul, 1997; Huber et al., 1997; Livingstone et al., 1980; Panksepp et al., 2003), and, in some species, increases the probability of winning a fight (Momohara et al., 2013). Retention of dominant status in crayfish is also enhanced by increasing synaptic 5-HT through SERT blockade (Huber et al., 1997; Momohara et al., 2013; Panksepp and Huber, 2002). However, the role of 5-HT is likely to be more complex than a simple enhancement of aggressive behavior. In paired fights with a size discrepancy between opponents, injection of 5-HT increases aggression in smaller crayfish while decreasing aggression in larger animals, suggesting that, as in vertebrates (Blanchard and Meyza, 2019), 5-HT may alter risk assessment (Bacqué-Cazenave et al., 2018). Manipulations that

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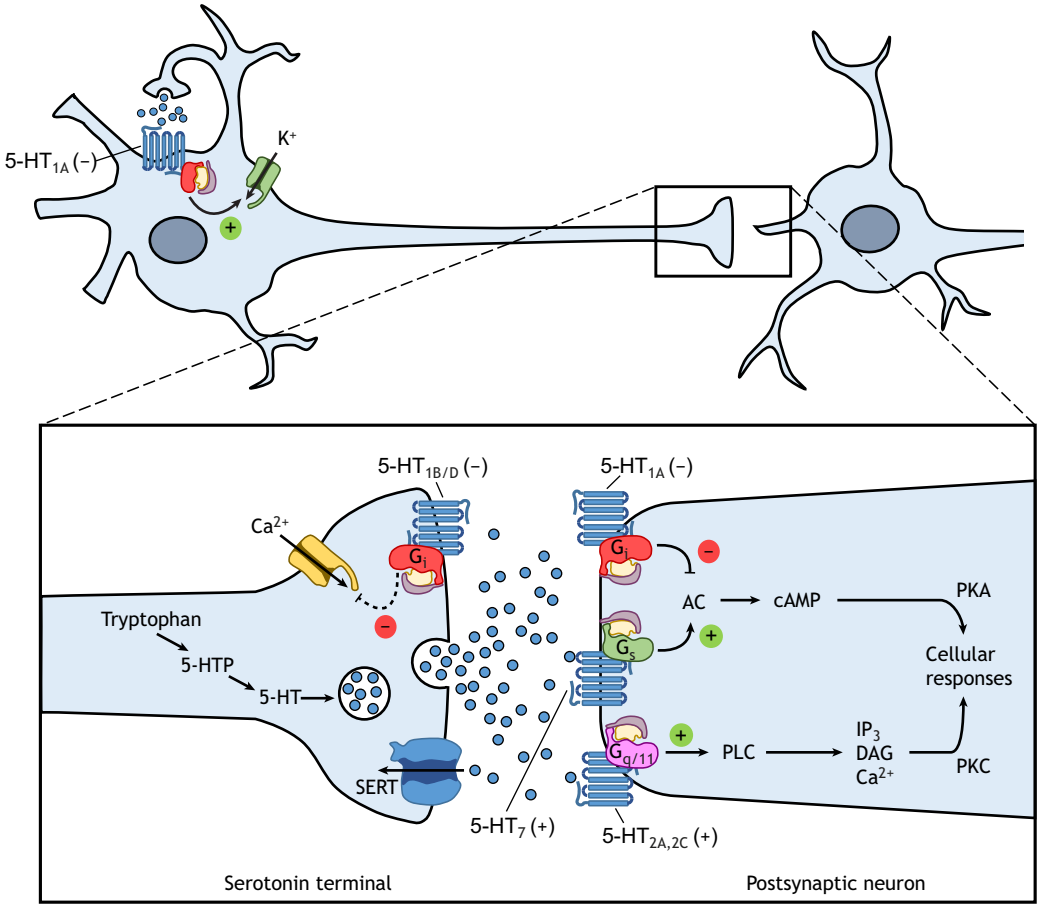


Fig. 1. A representative serotonin (5-HT) neuron and synapse. The figure shows predominant cellular locations of 5-HT receptors discussed in the text (5-HT$_1$, 5-HT$_2$ and 5-HT$_7$), with their net effect on cellular activity denoted as excitatory (+) or inhibitory (–). The amino acid tryptophan is hydroxylated into 5-hydroxytryptophan (5-HTP), which then undergoes decarboxylation to produce serotonin (5-hydroxytryptamine, 5-HT). Once released, 5-HT can negatively modulate postsynaptic neurotransmission by binding to G$_i$-coupled 5-HT$_{1A}$ receptors, which inhibit adenylyl cyclase (AC) to restrict the production of cyclic AMP (cAMP) and dampen protein kinase A (PKA) activity. Excitatory postsynaptic effects can be mediated either by G$_s$-coupled 5-HT$_7$ receptors (activate AC) or G$_{q/11}$-coupled 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors that activate the phospholipase C (PLC)/inositol 1,4,5-trisphosphate (IP$_3$)/diacylglycerol (DAG) pathway to increase cytosolic calcium (Ca^{2+}) and stimulate protein kinase C (PKC). Presynaptic 5-HT$_{1B}$ (5-HT$_{1D}$ in humans) receptors located on 5-HT terminals are also G$_i$ coupled, and serve as autoreceptors to limit further 5-HT release by closing Ca^{2+} channels and preventing docking of vesicles at the synaptic membrane. Note that 5-HT$_{1B}$ receptors can also be located on non-5-HT terminals, where they can act as heteroreceptors to similarly inhibit release of other neurotransmitters. The 5-HT transporter (SERT) takes 5-HT back up into the terminal, where it can be repackaged into vesicles for future use, and so plays a key role in regulating the duration of presynaptic or postsynaptic receptor activation by controlling extracellular 5-HT availability. Activation of somatodendritic 5-HT$_{1A}$ autoreceptors by 5-HT release at the level of the cell body inhibits neuronal firing by opening inwardly rectifying potassium (K^+) channels, providing another mechanism to determine the amount of 5-HT released in terminal fields. For further reading, see Aggarwal and Mortensen (2017), Masson et al. (2012) and Sari (2004).

elevate 5-HT tend to increase aggression in several insect species (Alekseyenko et al., 2010; Bubak et al., 2014b; Dierick and Greenspan, 2007; Dyakonova and Krushinsky, 2013; Kostowski and Tarchalska, 1972; Szczuka et al., 2013). These studies, in which 5-HT is experimentally increased just prior to a conflict, may be consistent with the rapid and transient increase in endogenous 5-HT observed in highly aggressive individuals during vertebrate interactions (de Boer et al., 2015; Matter et al., 1998; Summers et al., 2005a; Takahashi et al., 2012). In contrast to findings in vertebrates, decreasing 5-HT function prior to interaction does not appear to affect subsequent expression of aggressive behavior in either male *Drosophila* or male crickets (Dierick and Greenspan, 2007; Rillich and Stevenson, 2018; Stevenson et al., 2000), suggesting 5-HT is permissive but not essential for invertebrate aggression.

Caveats in understanding invertebrate 5-HT and aggression

There are a number of caveats that are problematic with respect to our understanding of the role of 5-HT in invertebrate aggression. First, although the 5-HT–aggression relationship has been well studied in a range of vertebrate models (de Boer et al., 2015, 2016; Table S1), relatively little attention has been paid to the role of 5-HT in aggression in invertebrates other than arthropods (but see Edsinger and Dölen, 2018). A second issue involves assessing behavioral changes following systemic injection of 5-HT. Insects and crustaceans have a hemolymph–blood barrier (HBB) that is functionally analogous to the vertebrate blood–brain barrier (Otopalik et al., 2012; Schirmeier and Klämbt, 2015), which should prevent diffusion of 5-HT from the hemolymph to the brain. However, systemic administration of 5-HT clearly influences aggression in lobsters (Antonsen and Paul, 1997; Bacqué-Cazenave et al., 2018; Huber et al., 1997; Momohara et al., 2013; Peeke et al., 2000) and ants (Kostowski and Tarchalska, 1972; Szczuka et al., 2013), suggesting that effects are modulated by mechanisms outside the brain, or that the invertebrate HBB is permeable to monoamines. The latter possibility is suggested by data from our laboratory showing that, in mantis shrimp

Journal of Experimental Biology (2020) 223, jeb132159. doi:10.1242/jeb.132159

Box 1. The stalk-eyed fly as a model for aggression

Stalk-eyed flies (Diptera; Diopsidae) provide an ideal model to study aggression, from both a neurophysiological and an evolutionary perspective. All species have eye bulbs displaced on the ends of eye stalks that serve as ornamental signals in both intrasexual and intersexual interactions (Wilkinson and Dodson, 1997; Wilkinson and Johns, 2005). In sexually dimorphic species, such as *Teleopsis dalmanni*, females prefer males with longer eye spans (Wilkinson et al., 1998; Burkhardt and de la Motte, 1988): (A) male and female *T. dalmanni* copulating (photo credit: Amy Worthington). Furthermore, males with larger eyespans typically win contests for food and mates (Lorch et al., 1993; Panhuis and Wilkinson, 1999; Egge et al., 2011): (B) males of the sexually dimorphic *Teleopsis pallifacies* fighting (photo credit: Jerry Wilkinson).

Like many species in the family, male *T. dalmanni* use eye stalks to both convey and assess aggressive intent in interactions with rivals. A contest typically comprises three distinct sequential stages: (1) initiation – one individual approaches the other, initiating the fight (de la Motte and Burkhardt, 1983; Panhuis and Wilkinson, 1999); (2) escalation – opponents line up their eyestalks, which appears to be mutual assessment (Bubak et al., 2016a; but see Brandt and Swallow, 2009), followed by low-intensity posturing behaviors that can escalate to higher-intensity physical contact exchanges; and (3) termination – one rival capitulates and retreats (Egge et al., 2011). Female *T. dalmanni* also engage in intrasexual contests, but at lower intensity, rarely escalating to high-intensity behaviors (Bath et al., 2015).

The easily characterized and quantifiable aggressive interactions in stalk-eyed flies provide a useful model to uncover proximate neurobiological mechanisms governing individual and sex differences in behavioral expression. By combining behavioral measurements with pharmacological treatments, measurements of brain neurochemistry and manipulations of endogenous 5-HT receptor subtypes in the stalk-eyed fly, we can test hypotheses relating to the role of 5-HT in modulating aggression in insects, and compare these results with findings from other taxa (Bubak et al., 2013; Bubak et al., 2019).

(*Neogonodactylus oerstedii*), systemic dopamine and 5-HT both cross the HBB (K.J.R., unpublished results), and from studies indicating that systemically administered dopamine can directly alter nervous system development and locomotion in *Drosophila* larvae (Budnik et al., 1989; Wakabayashi-Ito et al., 2011). Thus, results obtained by systemically injecting 5-HT do not rule out potential confounds from neurohormonal or negative feedback effects rather than direct effects on the brain. Third, some vertebrate studies suggest that the degree to which 5-HT affects aggression may depend on individual social status established after repeated interactions. For example, aggression-reducing effects of elevating 5-HT in male lizards are only seen in dominant males (Summers et al., 2005b), and in hamsters and some teleost fish, 5-HT is associated with the acquisition and maintenance of subordinate status (Harvey et al., 2012; Backström and Winberg, 2017). Although differential actions of 5-HT following repeated fights and social status have been noted in crayfish (Huber et al., 1997) and male crickets (Rillich and Stevenson, 2018), the majority of invertebrate studies only utilize single interactions between unfamiliar opponents. Finally, most work on vertebrate aggression has focused on males, although there is some evidence suggesting 5-HT may increase or have minimal effects on aggression in female rodents (see Table S1; de Boer and Newman-Tancredi, 2016; Joppa et al., 1997; Terranova et al., 2016; Villalba et al., 1997; but see Heiming et al., 2013; Kästner et al., 2019). Even less is known about the role of 5-HT in female invertebrate aggression. Thus, there is a clear need for further studies using multiple species before conclusions about the activational or sex-specific role of 5-HT in aggression across invertebrates can be drawn. Such knowledge is crucial for understanding not only how individual or sex-specific aggression can be discretely modulated by 5-HT activity but also why functional homologies or differences in such a conserved neurotransmitter system would have evolved across vertebrates and invertebrates.

5-HT receptor subtypes and aggression

In vertebrates, progress has been made in understanding how 5-HT modulates aggression through differential binding of specific 5-HT

receptors, with 5-HT_{1A}, 5-HT_{1B}, 5-HT_2 and 5-HT_3 subtypes being involved (Box 2; Juárez et al., 2013; Morrison et al., 2015; Popova et al., 2010; Takahashi et al., 2012). In general, systemic activation of each subtype dampens vertebrate aggression, but the opposite effect can be induced in mammals when these receptors are activated either in specific brain regions or during certain contexts such as maternal aggression or self-defense (de Almeida and Lucion, 1997; Takahashi et al., 2012). In contrast, the contribution of 5-HT receptor subtypes to invertebrate aggression is not as well understood. Of the seven known 5-HT receptor families in mammals, three (5-HT_1, 5-HT_2 and 5-HT_7) have been described with notable sequence and functional homology in insects (Box 3; Tierney, 2018; Vleugels et al., 2013). As in vertebrates, adenylate cyclase activity and cAMP production are decreased by G_i-coupled 5-HT_1-like receptors but increased by G_s-coupled 5-HT_7-like receptors to exert inhibitory and excitatory effects, respectively, whereas excitatory 5-HT_2-like receptors function through Gq proteins to stimulate phospholipase C and subsequently increase Ca^{2+} (Tierney, 2018; Fig. 1).

Similar to findings in vertebrates, 5-HT_1-like and 5-HT_2-like receptor subtypes are implicated in insect aggression (see Table 1). In male *Drosophila*, aggression is reduced by 5-HT_2 receptors but enhanced by activation of 5-HT_{1A} receptors (Johnson et al., 2009). Further, the role of each subtype is specific to the type of aggressive behavior, with 5-HT_{1A} receptors predominantly affecting low-intensity aggression seen at contest initiation, such as threat displays, whereas 5-HT_2 receptors mediate high-intensity aggressive behaviors, such as lunging (Johnson et al., 2009). Our recent studies using the stalk-eyed fly (*Teleopsis dalmanni*) indicate a similar role for 5-HT_{1A} and 5-HT_2 receptors, respectively, in enhancing and reducing aggression (Bubak et al., 2019), suggesting receptor subtype activation as one mechanism to explain the generally opposing role of 5-HT in vertebrate versus invertebrate aggression. However, the story is not as simple as this; consideration of other factors points to more similarities than differences between invertebrates and vertebrates in how 5-HT can modulate aggression. In the following sections, we provide a summary of our work on aggression using the stalk-eyed fly. With this model, we hope to expand our knowledge of the role of 5-HT in altering aggressive behaviors with respect to discrete components of an aggressive interaction, such as contest initiation, intensity and termination, and we hope to determine how the actions of 5-HT may differ between the sexes (Box 1).

The stalk-eyed fly (*T. dalmanni*) as a case study

Sex differences in 5-HT receptors and aggression

Pharmacologically increasing neural 5-HT using the precursor 5-hydroxytryptophan (5-HTP) increases high-intensity (defined by contact) behaviors in male stalk-eyed flies (Bubak et al., 2014b). This is consistent with studies demonstrating a positive relationship between increased 5-HT and aggression in other invertebrates (Table S1; Antonsen and Paul, 1997; Bubak et al., 2016b; Huber et al., 1997; Livingstone et al., 1980; Panksepp et al., 2003; Momohara et al., 2013; Dierick and Greenspan, 2007). However, females pretreated with 5-HTP exhibit no difference in either behavioral output or fight outcome (Bubak et al., 2019). Thus, as in some vertebrates (Joppa et al., 1997; Terranova et al., 2016; Villalba et al., 1997), there appears to be a sex difference in how 5-HT modulates aggression in stalk-eyed flies. This is supported by sex differences in components of 5-HT signaling, with males having higher 5-HT_{1A} but lower 5-HT_2 receptor expression, whereas 5-HT_7 receptor expression is equivalent between the sexes (Bubak et al., 2019). In addition, males show much lower expression of SERT, which would presumably result in reduced 5-HT clearance. Combined, this suggests the higher levels of aggression displayed by males are a result of elevated levels of 5-HT acting at 5-HT_{1A} receptors. Further, these findings indicate that 5-HT_2 activation may inhibit aggression in male *T. dalmanni*. The fact that administration of selective 5-HT_{1A} and 5-HT_2 agonists increases or decreases, respectively, inter-male aggression in *Drosophila* supports this hypothesis (Johnson et al., 2009). Therefore, the lower expression of 5-HT_{1A} and higher expression of 5-HT_2 in female stalk-eyed flies may account for the difference in aggressive behavior seen between sexes of this species.

Results available from insect systems suggest some similarities in the role(s) of 5-HT receptors in modulating aggression when compared with vertebrates. In both vertebrates and arthropods, 5-HT_2 receptors appear to dampen aggression (Bubak et al., 2019; Johnson et al., 2009; Muehlenkamp et al., 1995; Takahashi et al., 2011; Ten Eyck, 2008), but in contrast to the majority of rodent studies, 5-HT_{1A} activation appears to increase insect aggression (Bubak et al., 2019; Johnson et al., 2009). However, this discrepancy in 5-HT_{1A} modulation of aggression may owe more to whether the selected agonist is acting on somatodendritic 5-HT_{1A} autoreceptors located presynaptically or on postsynaptic 5-HT_{1A} heteroreceptors in terminal fields. For example, rodent aggression is reduced by 5-HT_{1A} agonists when they are either injected directly into the dorsal raphe or given systemically (de Boer et al., 2016; Van der Vegt et al., 2003a,b; Calcagnoli et al., 2015), which seems contradictory, as activating autoreceptors in the raphe causes a reduction in 5-HT neuron firing and thus a decrease in 5-HT availability (Fig. 1), whereas activating postsynaptic 5-HT_{1A} heteroreceptors mimics increased 5-HT signaling. Although the latter fits with an inhibitory role of 5-HT in vertebrate aggression, the anti-aggressive effects of autoreceptor activation suggest that 5-HT stimulates aggression. In support of this, social challenge in rodents and lizards is accompanied by rapid phasic increases in 5-HT in various brain regions (Watt et al., 2007; Nakazato, 2013; Takahashi et al., 2015). Combined, the overlap in functional effects of receptor subtypes again points to more similarities than differences between invertebrates and vertebrates in 5-HT-mediated modulation of aggression.

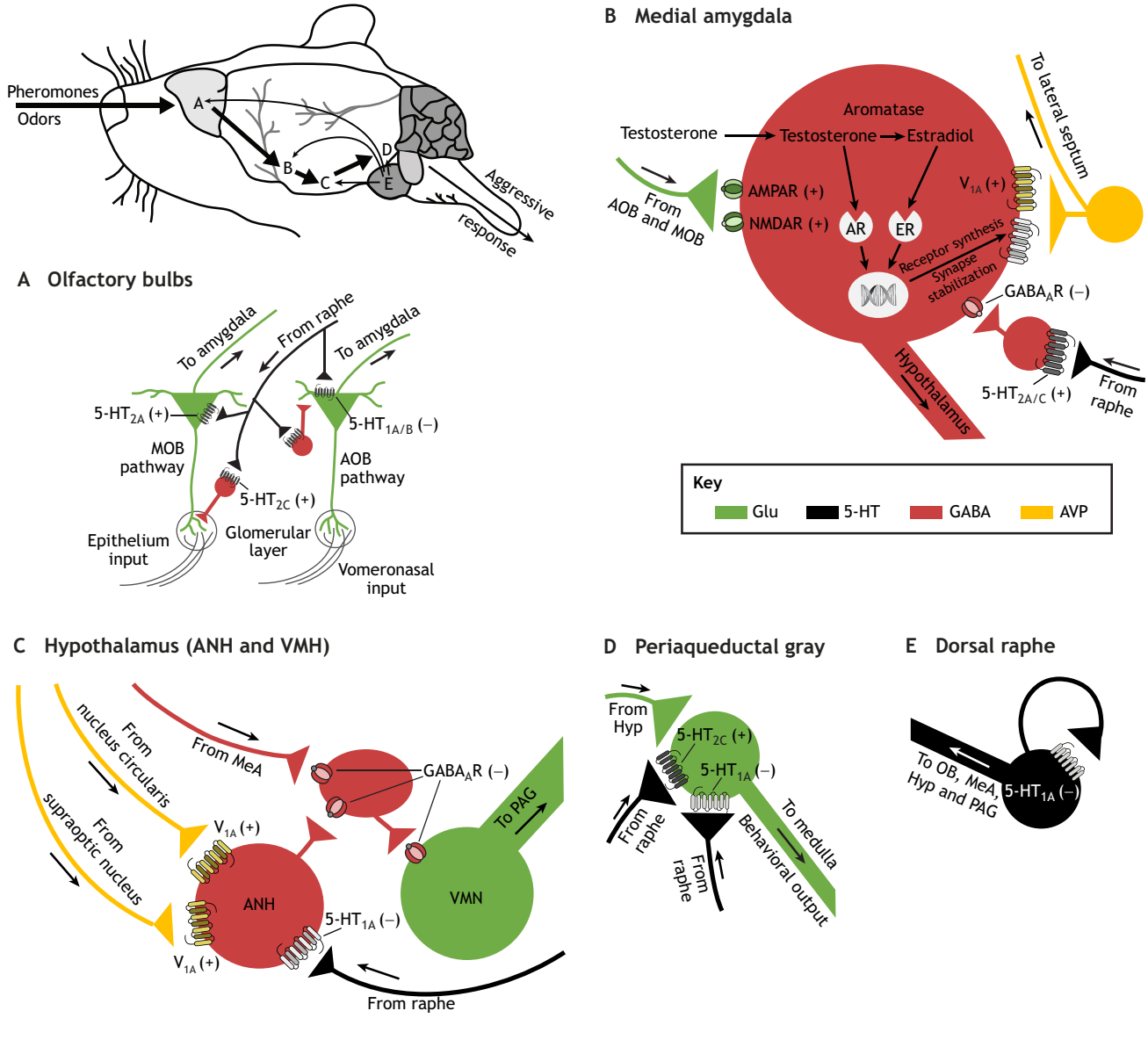
Social isolation, 5-HT and insect aggression

Social isolation increases aggressive behavior in both vertebrates and invertebrates (Twenge et al., 2001; Wongwitdecha and Marsden, 1996; Alexander, 1961; Johnson et al., 2009). Male *Drosophila* reared in isolation also show changes in 5-HT receptor expression compared with group-housed males (Johnson et al., 2009). Similarly, socially isolated male stalk-eyed flies are more aggressive, performing more high-intensity behaviors and contest initiations than their socially reared opponent (Bubak et al., 2019). Socially isolated males also have lower 5-HT_2 expression levels than their socially housed opponents, while expression of 5-HT_{1A} and 5-HT_7 receptors appears to be independent of rearing condition. This pattern of receptor expression changes is opposite to that seen in *Drosophila*, where isolation reduces expression of 5-HT_{1A} while 5-HT_2 expression increases (Johnson et al., 2009). In contrast, social isolation has no effect on the expression or intensity of aggressive behavior or the expression of any of the 5-HT receptor subtypes measured in female stalk-eyed flies (Bubak et al., 2019).

Social isolation has similar augmenting effects on aggression in rodents. For example, male rats reared in isolation exhibit abnormally high levels of unprovoked and contextually inappropriate violent

Journal of Experimental Biology (2020) 223, jeb132159. doi:10.1242/jeb.132159

Box 2. Regulation of vertebrate aggression circuitry through serotonergic signaling



(A) In rodents, aggression requires sensory activation of glutamatergic mitral cells of the main (MOB) and accessory olfactory bulbs (AOB) (Mandiyan et al., 2005; Stowers et al., 2002). This is enhanced by 5-HT_{2A} binding in the MOB but dampened by 5-$HT_{1A/B}$ signaling in the AOB, whereas 5-HT_{2C} receptor excitation of GABAergic interneurons inhibits both pathways (Huang et al., 2017). Net effects on cellular activity are denoted as excitatory (+) or inhibitory (–). (B) Excitatory olfactory bulb (OB) output is received by AMPA and NMDA receptors located on aggression-promoting GABAergic neurons in the posterior dorsal (pd) medial amygdala (MeA) that project to the hypothalamus (Hyp) (Hong et al., 2014). Anti-aggressive effects of 5-HT in the MeA (Puciłowski et al., 1985) may be mediated via 5-$HT_{2A/C}$ receptors on GABAergic interneurons (Asan et al., 2013) to inhibit MeA output. MeApd cells also contain aromatase (which converts testosterone to estrogen), and via androgen (AR) and estrogen receptors (ER) these steroids may have an organizational effect during puberty to dampen 5-HT signaling in the adult MeA and promote aggression (Grimes and Melloni, 2002; Puciłowski et al., 1985). Similarly, these steroids regulate activity of excitatory V1 vasopressin (AVP) receptors that enhance aggression (Koolhaas et al., 1990; Murakami et al., 2011). (C) In the anterior hypothalamus (ANH), V_1 receptors promote aggression, which is countered by 5-HT_{1A} binding (Ferris et al., 1997, 1999). The ventromedial hypothalamus (VMH) receives inhibitory projections from the ANH (Lo et al., 2019) and MeA (Canteras et al., 1995), which target GABAergic interneurons to disinhibit VMH output to the periaqueductal gray (PAG) and increase aggression (Lin et al., 2011). (D) In the cat PAG, 5-HT promotes or suppresses reactive aggression via 5-HT_{2C} and 5-HT_{1A} receptors, respectively (Shaikh et al., 1997). In contrast, both of these receptors in the PAG suppress maternal aggression in rats (de Almeida and Lucion, 1997; de Almeida et al., 2005). (E) Aggression decreases upon activation of inhibitory somatodendritic 5-HT_{1A} autoreceptors (de Boer and Newman-Tancredi, 2016), suggesting the transient 5-HT increase seen at the initiation of aggression in many vertebrates is mediated by negative feedback at the dorsal raphe.

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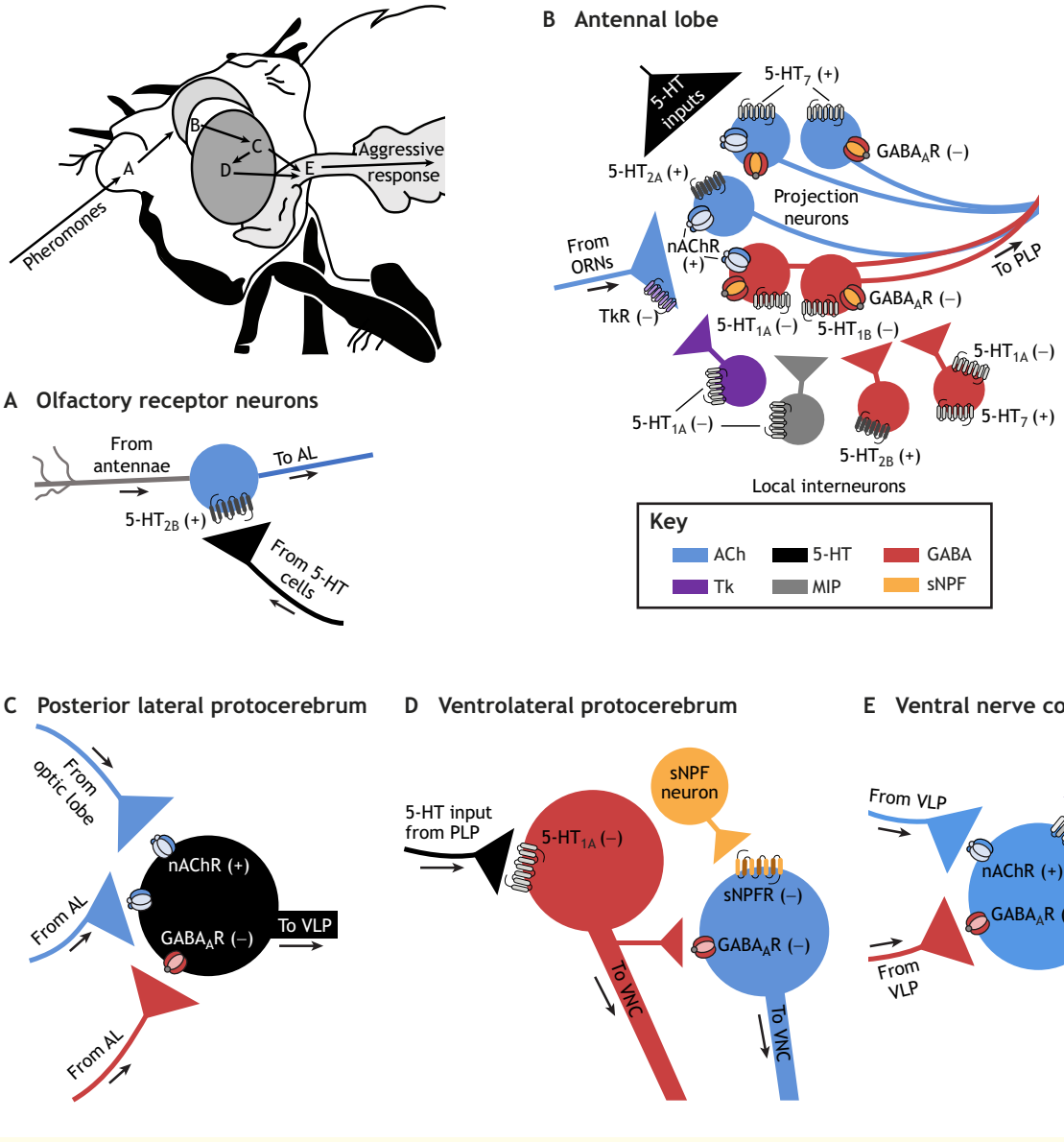
Box 3. Regulation of invertebrate aggression circuitry through serotonergic signaling



(A) In *Drosophila*, male aggression is prompted by male pheromone activation of cholinergic olfactory receptor neurons (ORNs; Wang and Anderson, 2010), which is enhanced by 5-HT_{2B} receptor binding (Sizemore and Dacks, 2016). Net effects on cellular activity are denoted as excitatory (+) or inhibitory (–). (B) Olfactory signals are processed by the antennal lobes (ALs), which comprise interconnected projection neurons (PNs) and local interneurons, most of which express different 5-HT receptor subtypes specific to neuronal type. Activation of excitatory cholinergic PNs by ORN afferents (Barbara et al., 2005) is enhanced by 5-HT_{2A} and 5-HT_7 receptors, whereas stimulation of inhibitory GABAergic PNs is dampened by 5-HT_{1A} and 5-HT_{1B} receptors (Sizemore and Dacks, 2016). Local inhibition of PNs and ORN terminals is provided by GABAergic and peptidergic [tachykinin (Tk) and myoinhibitory peptide (MIP)] interneurons (Bicker, 1999; Ignell et al., 2009). Peptidergic interneurons only express 5-HT_1-type receptors (Sizemore and Dacks, 2016), and so are suppressed by 5-HT. In contrast, GABAergic interneurons express a combination of 5-HT_1, 5-HT_2 and 5-HT_7 receptors (Sizemore and Dacks, 2016). In this manner, 5-HT can fine-tune AL output through both direct (stimulation/inhibition of PNs) and indirect (feedforward inhibition and disinhibition of PNs by interneurons) actions. (C) The AL targets the posterior lateral protocerebrum (PLP) (Tanaka et al., 2012), which contains densely arborized 5-HT neurons specifically implicated in male aggression (Alekseyenko et al., 2014) that may be modulated by incoming olfactory and visual information (Otsuna and Ito, 2006; Tanaka et al., 2012). (D) PLP 5-HT afferents regulate activity in the neighboring ventrolateral protocerebrum (VLP) to promote aggression, which may result from 5-HT_{1A} receptor-mediated suppression of inhibitory GABAergic output neurons and concurrent disinhibition of excitatory cholinergic output (Alekseyenko et al., 2019). These cholinergic neurons also possess inhibitory short neuropeptide F (sNPF) receptors (Alekseyenko et al., 2019), which are functionally distinct from aggression-dampening NPF receptors (Dierick and Greenspan, 2007; Bubak et al., 2019) but possibly receive input from locomotion circuits (Nässel and Wegener, 2011) activated during aggression. (E) The PLP and VLP send descending projections, including aggression-promoting Tk neurons (Asahina et al., 2014), to the ventral nerve cord to control behavioral expression (Namiki et al., 2018).

Table 1. Roles of serotonin receptor subtypes in insect aggression

Receptor subtype	Study species	Manipulation/measure	Outcome	Net effect of subtype on aggression	Reference
5-HT_{1A}-like	*Drosophila*	Oral administration (in food) of agonists or antagonists	Activation promotes low-intensity aggression	Increase	Johnson et al., 2009
		Thermal activation of dTrpA1 channel in *5-HT_{1A}-Gal4* driver flies	Releasing 5-HT_{1A}-mediated neuronal inhibition decreases aggression	Increase	Alekseyenko et al., 2014
	Stalk-eyed flies	Sex-specific expression of 5-HT_{1A} receptor	Males are more aggressive than females and show higher 5-HT_{1A} expression	Increase	Bubak et al., 2019
5-HT_2-like	*Drosophila*	Oral administration (in food) of agonists or antagonists	Activation decreases high-intensity aggression	Decrease	Johnson et al., 2009
	Stalk-eyed flies	Sex-specific expression of 5-HT_2 receptor	Males are more aggressive than females and show lower 5-HT_2 expression	Decrease (males)	Bubak et al., 2019
		Social isolation	Reduced 5-HT_2 expression plus increased high-intensity aggression (males only)	Decrease (males)	Bubak et al., 2019
		Selective knockdown of brain 5-HT_2 using siRNA injection	Increases motivation to engage in fight in males, no effect on female aggression	Decrease (males)	Bubak et al., 2019
	Crickets	Injection of antagonist before fight	Facilitates post-fight recovery of aggression in losing males and protects against subordination effects of chronic social defeat	Decrease (only after initial contest)	Rillich and Stevenson, 2018
	Honey bees	Topical application of preferential antagonist	Decreases likelihood of stinging attacks during colony defense	Increase (colony defense)	Nouvian et al., 2018

aggression (Toth et al., 2011). Similarly, socially isolated arthropods express high levels of aggression (Johnson et al., 2009; Sibbald and Plowwright, 2014; Stevenson and Rillich, 2013) and abnormal high-intensity attacks (Bubak et al., 2019) relative to socially raised controls. Further, serotonergic modulation of aggressive responses through changes in the expression of 5-HT_{1A} and/or 5-HT_2 has been implicated for both socially isolated vertebrates (reviewed in Veneema, 2009) and invertebrates (Bubak et al., 2019; Johnson et al., 2009; Yeh et al., 1996). The role(s) of the 5-HT receptors appears to be species specific in insects (Johnson et al., 2009; Bubak et al., 2019). Similarly, in vertebrates, there are species differences in both 5-HT receptor subtype expression following social isolation as well as the brain region affected (Bibancos et al., 2007; Preece et al., 2004; Ross et al., 2019; Schiller et al., 2003).

5-HT_2 receptors and aggression

Use of small interfering RNA (siRNA) to selectively knock down 5-HT_2 receptors in stalk-eyed flies decreases the receptor expression by approximately 30% in males, similar to that observed following social isolation (Bubak et al., 2019). Behaviorally, siRNA-treated males initiate more fights but perform the same amount of high-intensity aggression compared with their vehicle-treated opponents. Female aggression, as with both isolation and 5-HTP pretreatment, does not change following reduction of 5-HT_2 receptors (Bubak et al., 2019). These findings also suggest 5-HT_2 may modulate the willingness of males to engage in a fight, whereas escalations to potentially injurious levels are mediated by a separate mechanism. This differs from *Drosophila*, where stimulation of 5-HT_2 receptors in isolated males reduces high-intensity aggression but not fight initiation (Johnson et al., 2009). However, social isolation increases expression of 5-HT_2 receptors in male *Drosophila* (Johnson et al., 2009), whereas the opposite effect is seen in stalk-eyed flies (Bubak et al., 2019). In male crickets, activation of 5-HT_2 receptors inhibits aggression in subordinates, but only after they have fought (Rillich and Stevenson, 2018). In contrast, aggressiveness during honey bee colony defense appears to be increased by 5-HT_2 activation (Nouvian et al., 2018). These differences among insects in how 5-HT_2 receptors mediate the type of aggressive behavior expressed, along with social isolation effects on receptor expression, highlight the need for studying multiple species before making general conclusions about the role of 5-HT in invertebrate aggression.

The results discussed above suggest some similarity in the role of 5-HT_2 receptors in vertebrates and invertebrates. In crickets, stalk-eyed flies and *Drosophila*, 5-HT_2-like receptors appear to inhibit components of aggressive behavior such as fight initiation, intensity and reduction of aggression after defeat (Bubak et al., 2019; Johnson et al., 2009; Rillich et al., 2019). Similarly, selective 5-HT_2 agonists are effective in decreasing aggression in several vertebrate species (Muehlenkamp et al., 1995; Ten Eyck, 2008; Takahashi et al., 2012; but see Juárez et al., 2013).

Interactions between 5-HT and neuropeptides in stalk-eyed fly aggression

Serotonin modulates a variety of other neurochemical systems, including the neuropeptides tachykinin (Tk; invertebrate equivalent to substance P) and neuropeptide F (NPF; invertebrate equivalent of neuropeptide Y), each of which has been linked to aggressive behavior in vertebrates and invertebrates (Katsouni et al., 2009; Takahashi et al., 2012). Other neuropeptides, such as oxytocin and vasopressin, have been shown to be important mediators of mammalian aggression (Caldwell, 2017); however, the role played by their functional orthologs (inotocin in insects; oxytocin/vasopressin-like peptide in crustaceans) in invertebrate aggression is largely unknown (Gruber, 2014; Liutkeviciute et al., 2016), although a recent study showed no relationship between the expression of inotocin receptors and aggression in mated ant queens (Chérasse and Aron, 2017). In contrast, Tk/substance P increases aggression across both taxa (Asahina et al., 2014; Halasz et al., 2009; Katsouni et al., 2009), whereas NPY/NPF suppresses aggression (Dierick and Greenspan, 2007; Karl et al., 2004). Both neuropeptides are influenced by 5-HT activity (Guiard et al., 2007; Hennessy et al., 2017; Karl et al., 2004; Sergeyev et al., 1999). In stalk-eyed flies, manipulation of serotonergic function alters the expression of Tk and NPF to modulate specific components of aggressive behavior, and effects differ as a function of sex and receptor subtype (Bubak et al., 2019).

Social isolation increases both contest initiation and escalation exclusively in male stalk-eyed flies. In addition, there is an increase in Tk expression in isolated males that is not evident in females

(Bubak et al., 2019). Recent work in male mice also shows that social isolation increases the expression of a closely related neuropeptide, tachykinin 2, in portions of the limbic stress circuit, and increased fear and aggressive behaviors can be blocked in these animals by tachykinin 2 receptor antagonists (Zelikowsky et al., 2018). Pretreating socially raised male stalk-eyed flies with 5-HTP to increase 5-HT also increases Tk and high-intensity aggression, but does not affect contest initiation. Comparing the effects of isolation and 5-HTP treatment suggests that Tk may primarily control behaviors associated with fight escalation, but not necessarily affect other less-intense aggressive behaviors (Bubak et al., 2019). Similarly, reducing Tk signaling reduces high-intensity attacks but leaves milder aggressive behaviors unaffected in rats (Halasz et al., 2009). In *Drosophila*, sexually dimorphic Tk neurons also regulate male aggression, but this extends to both low- and high-intensity aggressive behaviors (Asahina et al., 2014), initially suggesting that Tk regulation of discrete types of aggression may represent an evolutionarily derived state possessed by vertebrates. However, the finding that Tk is most closely associated with high-intensity aggression in male *T. dalmanni* argues against this, and instead points to convergence in Tk function at the level of the species rather than phylum.

The association between Tk and 5-HT in mediating aggression in vertebrates versus invertebrates is less clear. Tachykinin receptors on 5-HT neurons in the mammalian hindbrain can directly modulate neuronal firing and release of 5-HT in terminal regions (Maejima et al., 2013), and there is evidence for 5-HT and Tk co-release from neurons in mammals (Chan-Palay et al., 1978). In contrast, Tk and 5-HT do not appear to be co-localized in neurons in the majority of invertebrates (Boyan et al., 2010; Boyer et al., 2007; Chamberlain et al., 1986; Langworthy et al., 1997). However, the finding that 5-HTP treatment elevates both Tk expression and high-intensity behaviors in male stalk-eyed flies (Bubak et al., 2019) implies an interaction between 5-HT, Tk and aggression. Whether this represents a direct functional interaction as opposed to an additive effect produced by independent actions of 5-HT and Tk is unknown. Serotonin neurons do appear to synapse on to Tk-immunoreactive terminals in desert locust brain (Ignell, 2001), suggesting a direct relationship via synaptic contact that may also be present in *T. dalmanni*.

Willingness to engage in a fight increases following administration of 5-HT_2 siRNA in male but not female stalk-eyed flies, despite similar reductions in 5-HT_2 expression levels in the two sexes. This may result, in part, from a sex-dependent interactive role between the 5-HT_2 receptor and the NPF system, as knockdown of the 5-HT_2 receptor only reduces NPF receptor expression in males (Bubak et al., 2019). Although this is consistent with an inhibitory role reported for NPF in modulating aggression in *Drosophila* and mice, decreases in NPF/NPY in these species specifically suppress high-intensity behaviors (Dierick and Greenspan, 2007; Karl et al., 2004). In contrast, reductions in NPF receptor expression following 5-HT_2 siRNA treatment have no effect on expression of high-intensity aggression in male stalk-eyed flies (Bubak et al., 2019). Further, 5-HT and NPF pathways appear to act independently in regulating aggression in male *Drosophila* (Dierick and Greenspan, 2007) and mice (Karl et al., 2004), whereas a direct positive relationship between 5-HT_2 receptors and NPF is indicated for male *T. dalmanni*.

Combined, the findings from the stalk-eyed fly system generate a complex picture of interplay among serotonergic and peptidergic pathways that may fine tune the expression of aggressive behavior as appropriate for that particular context. These studies suggest that although 5-HT has a critical role in male aggression, precisely how the confrontation proceeds is governed by selective activation of 5-HT receptor subtypes along with changes in activity of NPF and Tk. Reductions in 5-HT_2 activation seem to promote the motivation to engage with an opponent, which may be potentiated by reductions in NPF signaling. Once committed, the two opponents typically express equivalent amounts of low-intensity aggressive behaviors, but a sharp increase in expression of high-intensity behaviors in the last stages of the confrontation is shown by those that eventually win the fight (Bubak et al., 2016a). Thus, while a balance of signaling in favor of 5-HT_{1A} versus 5-HT_2 receptor activation may be sufficient to initiate a confrontation and maintain expression of low-intensity aggression, the shift to high-intensity aggressive behaviors required for winning depends upon an additional mechanism, such as increased Tk signaling. In contrast, the lower levels of aggression in female stalk-eyed flies appear to be maintained by heightened 5-HT_2 receptor activity, with NPF and Tk having no apparent function in this behavior. These findings suggest that 5-HT modulation of aggression in this species is permissive or inhibitory depending on receptor subtype, intensity of aggression, neuropeptide involvement and the sex of the individual.

Comparing the role of 5-HT in aggression in vertebrates and invertebrates – where do we go from here?

Several studies show that, as in invertebrates, increases in 5-HT in vertebrates are associated with enhanced aggression. For example, administration of 5-HTP to mice increases 5-HT turnover and aggression intensity (Kulikov et al., 2012). Similar results are obtained in insects following 5-HTP pretreatment (Dierick and Greenspan, 2007; Bubak et al., 2013). Other studies have shown that 5-HT increases in specific brain regions in several vertebrate species just before or during aggression (Summers et al., 2003; van der Vegt, et al., 2003a,b; Watt et al., 2007). Because 5-HT is an evolutionarily ancient neurotransmitter present in all animal lineages (Moutkine et al., 2019), it is perhaps not surprising that a broadly shared role in aggression has been retained in both phyla, as seen for modulation of other behaviors critical for survival such as feeding, motor control and reproduction (Weiger, 1997).

The 5-HT receptors that modulate aggression appear to be similar in structure and function in invertebrates and vertebrates (Tierney, 2018; Vleugels et al., 2013, 2015), although experiments studying the effects of 5-HT receptor function in invertebrate aggression are limited. In crickets, stalk-eyed flies and *Drosophila*, 5-HT_2-like receptors appear to inhibit aggression, whereas 5-HT_{1A}-like receptor activation increases aggression (Table 1), and these effects may be exerted postsynaptically (Alekseyenko and Kravitz, 2015). In vertebrates, these two receptor subtypes also appear to strongly influence the expression of aggression, but to have a dampening effect. As discussed above, the anti-aggressive effects of 5-HT_{1A} agonists in rodents may result from a depression of serotonergic activity/release through actions at presynaptic somatodendritic autoreceptors (de Boer and Newman-Tracredi, 2016), implying that increased levels of 5-HT actually have a facilitatory role in vertebrate aggression similar to that demonstrated for arthropods. Further, it could be argued that what were presumed to be opposing effects of 5-HT_{1A} receptors on aggression between invertebrates and vertebrates are largely dependent on the balance of presynaptic versus postsynaptic activation, and that activation of postsynaptic 5-HT_{1A} receptors mimicking elevated 5-HT release should promote aggression in both groups. This premise is supported, in part, by the finding that infusion of 5-HT_{1A} agonists into some 5-HT terminal regions of the rodent brain can enhance aggression (Takahashi et al., 2012). However, this is only seen with

specific types of aggression, such as maternal defense or alcohol-enhanced aggression (Takahashi et al., 2012).

This raises an important point, in that the degree to which behavioral outputs differ between species may depend on the context in which the aggressive confrontation is taking place (e.g. Ling et al., 2010; Harvey et al., 2012; Backström and Winberg, 2017; Rillich and Stevenson, 2018), making it difficult to parse out specific mechanisms. Therefore, aggression should be investigated under different contexts and at different stages of the interaction (e.g. fight initiation, escalation, termination), particularly those relevant to the life history of the species or sex. For example, pitting female stalk-eyed flies in a forced-fight paradigm with food being the incentive may be a less powerful stimulus to provoke aggressive confrontations than access to egg-laying sites. To obtain a more fundamental understanding of how aggression is differentially modulated between the sexes by receptor subtypes, different combinations of selective knockdown or conditional gene overexpression could be linked with delivery of specific pharmacological agents. Work with *Drosophila* shows that strains can be created in which expression of genes controlling key aspects of neural signaling are restricted to particular brain regions, allowing fine-tuned analysis of how transmitters such as 5-HT mediate specific behaviors (Alekseyenko et al., 2014; Alekseyenko and Kravitz, 2015). Application of similar techniques to other arthropods could provide powerful tools for elucidating how and why aggression regulation by 5-HT has either diverged or remained similar in response to diverse evolutionary pressures.

Conclusions

So, does 5-HT have divergent or similar functions in aggression between invertebrates and vertebrates? Based on our studies using stalk-eyed flies, along with the available literature, the answer seems to depend on exactly how the question is posed. Systemically induced increases in 5-HT in invertebrates largely enhance components of aggressive behaviors (Table S1; Antonsen and Paul, 1997; Bubak et al., 2014a,b; Dierick and Greenspan, 2007; Huber et al., 1997; Livingstone et al., 1980; Panksepp et al., 2003; but see Stevenson and Rillich, 2017). In contrast, 5-HT historically has been viewed as an inhibitory neuromodulator of aggression in vertebrates (Table S1; Nelson and Chiavegatto, 2001; Summers et al., 2005a; Summers and Winberg, 2006). However, several studies suggest that the role of 5-HT in modulating aggression is more complicated, and depends on the subtype of 5-HT receptor activated, effects of 5-HT within specific brain regions, the type of aggression studied and the use of animal models selected for high aggression (de Boer et al., 2015, 2016; Nelson and Trainor, 2007; Takahashi et al., 2012). At least, elements of serotonergic function appear to be conserved phylogenetically.

The major classes of serotonergic receptors (5-HT_1, 5-HT_2 and 5-HT_7) are estimated to have diverged over 800 million years ago (Peroutka and Howell, 1994), with a subsequent differentiation and appearance of new 5-HT receptors (including the 5-HT_{1A} receptor) when ancestral vertebrates appeared some 600–700 million years ago (Peroutka and Howell, 1994; Blair and Hedges, 2005). Thus, there may truly be a broad phylogenetic divergence in how 5-HT_{1A} receptors influence aggression, with the promoting effects in invertebrates representing a more ancestral state that has only been conserved in the vertebrate brain for mediating specific types of aggression. In contrast, the aggression-inhibiting role of the evolutionarily older 5-HT_2 receptors appears to have been conserved phylogenetically. However, these hypotheses will remain speculative until additional studies with different invertebrate species are conducted. Further, there is some debate as to whether pharmacological agents used to manipulate specific 5-HT receptor subtypes in vertebrate studies are equally efficacious in their invertebrate orthologs (Vleugels et al., 2015; Tierney, 2018), and sophisticated genetic manipulations to target specific brain regions have primarily been restricted to *Drosophila* (Alekseyenko et al., 2014; Alekseyenko and Kravitz, 2015). Despite this, considerable evidence is steadily accumulating to suggest that there is indeed a shared facilitatory role for 5-HT in vertebrate and invertebrate aggression.

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Competing interests

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Supplementary information

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