



Can insects feel pain? A review of the neural and behavioural evidence

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Abstract

The entomology literature has historically suggested insects cannot feel pain, leading to their exclusion from ethical debates and animal welfare legislation. However, there may be more neural and cognitive/behavioural evidence for pain in insects than previously considered. We use Birch et al. 's (2021) eight criteria for sentience to critically evaluate the evidence for pain in insects. We assess six orders (Blattodea, Coleoptera, Diptera,

Hymenoptera, Lepidoptera, and Orthoptera) in at least two life stages (adult and first instar juveniles, as well as other instars where relevant data are found). Other insect orders have not received enough research effort to be evaluated. According to the Birch et al. framework, adult Diptera (flies and mosquitoes) and Blattodea (cockroaches and termites) satisfy six criteria, constituting strong evidence for pain. Adults of the remaining orders (except Coleoptera, beetles) and some juveniles (Blattodea and Diptera, as well as last instar Lepidoptera [butterflies and moths]) satisfy 3–4 criteria, or “substantial evidence for pain”. We found no good evidence that any insects failed a criterion. However, there were significant evidence gaps, particularly for juveniles, highlighting the importance of more research on insect pain. We conclude by considering the ethical implications of our findings where insects are managed in wild, farmed, and research contexts.



1. Introduction

Sentience is the capacity to have feelings—mental states that are consciously experienced as good or bad. Examples include love and hate, joy and anger, excitement and exhaustion, happiness and depression, hunger and thirst. A particularly salient feeling is pain, such as the “sharp pain” of an injection or the “dull throb” of a headache. These feelings have an important evolutionary function: motivating and teaching us to avoid harm, such as sharp objects or bumps on the head (Kolodny et al., 2021). Yet, due to its intrinsic aversiveness, extreme or unnecessary pain leads to major ethical concerns. Many argue that animal welfare only matters if the animal is sentient and can experience pain (Duncan, 1996; Fraser et al., 1997). Feeling pain is therefore central to whether a living being deserves moral consideration.

How can we tell whether an animal feels pain? First, we must distinguish pain from nociception. Nociception is the detection of noxious stimuli (Tracey, 2005, 2017), or stimuli that may cause tissue damage (Cervero and Merskey, 1996). Nociception does not require pain: hand withdrawal from a hot stove is a nociceptive reflex controlled by neurons relaying signals from the nociceptors in the hand, to the spinal cord, and back again (Defrin et al., 2007). All this happens before nerve impulses reach the brain (where pain is experienced). Thus, when animals display nociception, this does not necessarily demonstrate that they can feel pain (Adamo, 2016; Magee and Elwood, 2013; Sneddon et al., 2014). However, when nociceptive signals are transmitted to the brain, this may lead to the aversive, subjective experience of pain (Auvray et al., 2010; Birch et al., 2020).

While it is essential to distinguish between indicators of nociception and pain, the fundamental challenge of pain is that scientists cannot directly

measure this subjective and inherently private experience (Frischenschlager and Pucher, 2002). Even in humans, who can self-report their pain and describe its severity (Heft et al., 1980; Wideman et al., 2019), we can never be certain we are accurately measuring pain (Frischenschlager and Pucher, 2002). This issue is exacerbated in non-human animals who cannot verbally self-report. Therefore, researchers rely mainly on two lines of indirect evidence: (1) whether the animal has a nervous system that might support pain, and (2) whether they exhibit behaviours potentially caused by pain (Briffa, 2022; Crump and Birch, 2022). Given functionally similar neuroanatomy and analogous behavioural responses to harm, few dispute that mammals and birds feel pain (Gentle, 1992). There is also growing expert consensus on pain in other animals, including the invertebrate cephalopod molluscs and decapod crustaceans (Crump et al., 2022; Elwood, 2012).

Some early entomologists and naturalists asserted a belief in insect sentience, such as Charles Darwin (1872) and Charles Henry Turner (Galpayage Dona and Chittka, 2020; Turner, 1912). However, by the mid-20th century, the notion that insects are purely instinctual/reflexive had gained popularity (for a historical overview in myrmecology, see Sleight, 2007). Anecdotal accounts of insects appearing to behave normally after extreme injury were taken as evidence against pain (Eisemann et al., 1984; Wigglesworth, 1980). Despite lacking empirical support, these accounts have received hundreds of citations in entomology, comparative cognition, and welfare/ethics (e.g., Adamo, 2016; Ng, 1995; Sneddon et al., 2014). Another popular argument against insect pain is that insect brains are too small, or lack the appropriate neural connections, to support sentience (Adamo, 2016; Allen-Hermanson, 2008, 2016; Hill, 2016; Key et al., 2016, 2021). For example, Adamo (2019) argued that the lack of direct connections between integrative brain regions that process noxious stimuli, which are essential for pain in vertebrates (Garcia-Larrea and Bastuji, 2018), likely precludes the experience of pain. However, such direct connections have now been found in adult *Drosophila melanogaster* fruit flies (Diptera: Drosophilidae; Li et al., 2020a). This highlights how lack of evidence may serve as a poor guide for drawing accurate conclusions about insect nervous systems and their psychological correlates.

New psychological evidence is consistent with some form of sentience in insects, such as “emotion-like” cognitive biases (Bateson et al., 2011; Solvi et al., 2016). Further, insects display nocifensive behaviour (defensive or protective behaviours in response to noxious stimuli) that different stimuli and contexts can modulate (Gibbons et al., 2022a, b). Although small, insect

nervous systems are exquisitely complex (Chittka and Niven, 2009; Giurfa, 2013) and may perform many of the same functions as mammalian nervous systems, even without homologous brain structures (e.g., Varga and Ritzmann, 2016). Insects do not have a visual cortex, for example, but there is no doubt that they can see. It is thus possible that insects may also experience pain, but underpinned by different neural circuits than mammals (e.g., multiple realizability and related theses: Chittka et al., 2012; Mallatt and Feinberg, 2021).

From an ethical standpoint, whether insects feel pain is an urgent question. Trillions of insects are farmed, managed in the wild, and used for research or other purposes every year. There are currently no guidelines for considering their welfare in these settings, and they are almost universally excluded from animal welfare legislation. This is based, at least in part, on the assumption that insects do not feel pain.

In this article, we assess the evidence for pain in insects. First, we outline the assessment framework (Section 2). We then review the neural and cognitive/behavioural evidence for insect pain across six orders, at different developmental stages (Section 3). We use this framework to judge the current likelihood of pain in insects, and consider the review's limitations (Section 4). Finally, we briefly discuss the contexts in which humans use insects and the potential welfare concerns of such usage (Section 5).



2. How we evaluate evidence for pain

In a report commissioned by the UK government, Birch et al. (2021) developed a new framework for evaluating evidence of animal sentience, with a focus on pain (later published as Crump et al., 2022). Birch et al. (2021) write that “pain is one example within a broader category of negatively-valenced affective states, a category which also includes states of anxiety, fear, hunger, thirst, coldness, discomfort and boredom” (Birch et al., 2021, p. 12). Building on previous work (e.g., Bateson, 1991; Smith and Boyd, 1991; Sneddon et al., 2014), the authors listed eight criteria that, if satisfied, add to the case for pain experiences:

1. **Nociceptors:** The animal possesses receptors sensitive to noxious stimuli (nociceptors).
2. **Integrative brain regions:** The animal possesses integrative brain regions capable of integrating information from different sensory sources.

3. **Integrated nociception:** The animal possesses neural pathways connecting the nociceptors to the integrative brain regions.
4. **Analgesia:** The animal's behavioural response to a noxious stimulus is modulated by chemical compounds affecting the nervous system in either or both of the following ways:
 - a. **Endogenous:** The animal possesses an endogenous neurotransmitter system that modulates (in a way consistent with the experience of pain, distress or harm) their responses to threatened or actual noxious stimuli.
 - b. **Exogenous:** Putative local anaesthetics, analgesics (such as opioids), anxiolytics or anti-depressants modify an animal's responses to threatened or actually noxious stimuli in a way consistent with the hypothesis that these compounds attenuate the experience of pain, distress or harm.
5. **Motivational trade-offs:** The animal shows motivational trade-offs, in which the disvalue of a noxious or threatening stimulus is weighed (traded-off) against the value of an opportunity for reward, leading to flexible decision-making. Enough flexibility must be shown to indicate centralised, integrative processing of information involving an evaluative common currency.
6. **Flexible self-protection:** The animal shows flexible self-protective behaviour (e.g., wound tending, guarding, grooming, rubbing) of a type likely to involve representing the bodily location of a noxious stimulus.
7. **Associative learning:** The animal shows associative learning in which noxious stimuli become associated with neutral stimuli, and/or in which novel ways of avoiding noxious stimuli are learned through reinforcement.
8. **Analgesia preference:** The animal shows that they value a putative analgesic or anaesthetic when injured in one or more of the following ways:
 - a. **Self-administration:** The animal learns to self-administer putative analgesics or anaesthetics when injured.
 - b. **Conditioned place preference:** The animal learns to prefer, when injured, a location at which analgesics or anaesthetics can be accessed.
 - c. **Prioritisation:** The animal prioritises obtaining these compounds over other needs (such as food) when injured.

For our purpose, [Birch et al.'s \(2021\)](#) criteria have several advantages. They are clear, empirically testable, and designed to assess animals that have attracted

little specific pain or sentience research. The criteria were also developed with invertebrates in mind. For example, [Smith and Boyd \(1991\)](#) specified opioids (in Criterion 4), as in humans, while [Birch et al. \(2021\)](#) generalised this to any endogenous neurotransmitters. They also introduced several cognitive and behavioural criteria (Criteria 5–8). Simple nociceptive reflexes cannot satisfy these criteria, unlike [Smith and Boyd's \(1991\)](#) “[Responses] functionally similar to the human response” or [Sneddon et al.'s \(2014\)](#) “Behavioural changes from norm”. Finally, the equal weighting of neural and cognitive/behavioural criteria balances both major lines of evidence for pain. Notably, each of these criteria is likely to be necessary, but not sufficient, for sentience in insects; therefore, we stress that these criteria are only meant to *add to the case* for sentience. [Birch et al.'s \(2021\)](#) framework uses confidence levels to communicate the likelihood that an animal satisfies each criterion. The five possible confidence levels are:

- (1) “Very high confidence”, when the weight of scientific evidence leaves no scope for reasonable doubt;
- (2) “High confidence”, when we are convinced that the animal satisfies or fails the criterion, although scope for reasonable doubt remains;
- (3) “Medium confidence”, when concerns about the evidence’s reliability and quality prevent us from having high confidence;
- (4) “Low confidence”, when there is little or flawed evidence;
- (5) “Very low confidence”, when the evidence is seriously inadequate or when there is no evidence whatsoever to make a determination (e.g., no confidence).

The lower confidence levels do not imply that an animal has failed the criterion—the evidence may simply be low-quality or unavailable. It is important to distinguish between absence of evidence and evidence of absence, as both are “very low confidence” ([Mallatt and Feinberg, 2022](#)). Thus, in this review, we clarify when the absence of evidence is driving the ‘very low confidence’ rating by adding “no research found”.

After summing the information from all the criteria, [Birch et al. \(2021\)](#) suggested a grading scheme for determining our confidence that an animal is sentient (detailed in [section 4](#)). The criteria are intended to cast a wide net for relevant evidence, leaving room for debate about which types of evidence are most salient and why. In other words, the aim is to capture all evidence that shifts the probability of sentience, even if the effect is small. For example, the mere presence of nociceptors (Criterion 1) is clearly weak evidence for sentience by itself, but is included because it is a relevant (if small) part of

the overall picture. Further, there is continuing debate about which specific forms of associative learning are most relevant to questions of sentience and why (Ginsburg and Jablonka, 2019; Birch et al., 2020, 2021). Criterion 7 takes all evidence of associative learning involving noxious stimuli as part of the overall picture, leaving room for this ongoing debate, which may eventually allow us to pin down a subset of this evidence as especially relevant.

As there are over one million described insect species (Zhang, 2011) and many more undescribed (Stork, 2018), we choose to focus our review on six orders of insects: Coleoptera (beetles), Blattodea (cockroaches), Diptera (flies and mosquitoes), Hymenoptera (bees, wasps, sawflies, and ants), Lepidoptera (butterflies and moths) and Orthoptera (crickets, katydids, and grasshoppers), which contain several model species studied for their neuroanatomy and behaviour. These orders cover virtually all commercially important species and include a broad swath of insect diversity. Since only a few species have ever attracted in-depth study within each order, we will tentatively generalise the findings from these insects (listed in Table 1) to the rest of their orders, while acknowledging that more detailed phylogenetic analysis is a goal for the future (Birch, 2017).

In addition to species differences, insects' anatomy and behaviour may change substantially across development. Some insects are hemimetabolous: juveniles are mostly smaller, non-reproductive versions of adults, often occupying similar ecological niches (e.g., crickets and cockroaches: Mito et al., 2010). Other insects are holometabolous: larvae must undergo a complete metamorphosis (during pupation) to obtain their adult form (e.g., moths, flies, bees, beetles). In this case, larvae and adults may use very different ecological niches, with resulting anatomical and behavioural consequences (Rolff et al., 2019). Pupation may involve complete reorganisation of multiple brain regions for some holometabolous taxa, while hemimetabolous taxa see much less drastic change post-embryogenesis (Fahrbach, 2006; Farris and Strausfeld, 2001; Malaterre et al., 2002).

If an animal is capable of pain, this capacity must arise at some point during their development. We have thus assessed our confidence that each taxon meets the criteria separately for both juveniles and adults. In a few cases, data conflict between first and later instar juveniles, or are only available for last instar juveniles. In these cases, we provide two separate ratings in the table, to represent our uncertainty about when during juvenile development this criterion is fulfilled.

Table 1 Guide to the species names and common names of included insects, listed alphabetically by abbreviated name.

Abbreviated name	Species full name (homotypic synonyms)	Order: Family	Genbank common names, and others (if applicable)
<i>A. compressa</i>	<i>Ampulex compressa</i>	Hymenoptera: Ampulicidae	no Genbank name ; Emerald cockroach wasp, jewel wasp
<i>A. diaperinus</i>	<i>Alphitobius diaperinus</i>	Coleoptera: Tenebrionidae	lesser mealworm (beetle)
<i>A. domesticus</i>	<i>Acheta domesticus</i>	Orthoptera: Gryllidae	house cricket
<i>A. gambiae</i> *	<i>Anopheles gambiae</i>	Diptera: Culicidae	African malaria mosquito
<i>A. ipsilon</i>	<i>Agrotis ipsilon</i> (<i>Phalaena ipsilon</i>)	Lepidoptera: Noctuidae	black cutworm moth
<i>A. mellifera</i>	<i>Apis mellifera</i>	Hymenoptera: Apidae	Western honey bee
<i>B. germanica</i>	<i>Blattella germanica</i>	Blattodea: Ectobiidae	German cockroach
<i>B. impatiens</i>	<i>Bombus impatiens</i>	Hymenoptera: Apidae	common eastern bumble bee
<i>B. mori</i>	<i>Bombyx mori</i>	Lepidoptera: Bombycidae	domestic silkworm (moth)
<i>B. terrestris</i>	<i>Bombus terrestris</i>	Hymenoptera: Apidae	buff-tailed bumble bee
<i>C. aethiops</i>	<i>Camponotus aethiops</i>	Hymenoptera: Formicidae	no Genbank name ; carpenter ant
<i>C. pomonella</i>	<i>Cydia pomonella</i>	Lepidoptera: Tortricidae	codling moth
<i>D. coccus</i>	<i>Dactylopius coccus</i>	Hemiptera: Dactylopiidae	no Genbank name ; cochineal (scale bug)
<i>D. melanogaster</i>	<i>Drosophila melanogaster</i>	Diptera: Drosophilidae	fruit fly , vinegar fly (Green 2002)**
<i>D. plexippus</i>	<i>Danaus plexippus</i>	Lepidoptera: Nymphalidae	monarch butterfly
<i>F. rufa</i>	<i>Formica rufa</i>	Hymenoptera: Formicidae	no Genbank name ; wood ants
<i>G. assimilis</i>	<i>Gryllus assimilis</i>	Orthoptera: Gryllidae	no Genbank name (cricket)
<i>G. bimaculatus</i>	<i>Gryllus bimaculatus</i>	Orthoptera: Gryllidae	two-spotted field cricket ; Mediterranean field cricket
<i>G. molesta</i>	<i>Grapholita molesta</i> (<i>Cydia molesta</i>)	Lepidoptera: Tortricidae	oriental fruit moth
<i>G. portentosa</i>	<i>Gromphadorhina portentosa</i>	Blattodea: Blaberidae	giant Madagascar hissing cockroach
<i>G. sigillatus</i>	<i>Gryllobates sigillatus</i>	Orthoptera: Gryllidae	no Genbank name (cricket)
<i>H. armigera</i>	<i>Helicoverpa armigera</i>	Lepidoptera: Noctuidae	cotton bollworm (moth)

<i>H. illucens</i>	<i>Hermetia illucens</i>	Diptera: Stratiomyidae	no Genbank name ; black soldier fly
<i>J. fuscaria</i>	<i>Jankowskia fuscaria</i>	Lepidoptera: Geometridae	no Genbank name ; bark-like moths
<i>K. lacca</i>	<i>Kerria lacca</i>	Hemiptera: Kerriidae	common lac scale (bug)
<i>L. decemlineata</i>	<i>Leptinotarsa decemlineata</i> (<i>Stilodes decemlineata</i>)	Coleoptera: Chrysomelidae	Colorado potato beetle
<i>L. kohalensis</i>	<i>Laupala kohalensis</i>	Orthoptera: Trigonidiidae	no Genbank name ; Hawaiian cricket
<i>L. migratoria</i>	<i>Locusta migratoria</i>	Orthoptera: Acridoidea	migratory locust
<i>M. analis</i>	<i>Megaponera analis</i> (<i>Pachycondyla analis</i>)	Hymenoptera: Formicidae	no Genbank name (ants)
<i>M. domestica</i>	<i>Musca domestica</i>	Diptera: Stratiomyidae	house fly
<i>M. sexta</i>	<i>Manduca sexta</i>	Lepidoptera: Sphingidae	tobacco hornworm (moth)
<i>N. cinerea</i>	<i>Nauphoeta cinerea</i>	Blattodea: Blaberidae	grey cockroach ; speckled cockroach
<i>P. americana</i>	<i>Periplaneta americana</i> (<i>Blatta americana</i>)	Blattodea: Blattidae	American cockroach
<i>P. rapae</i>	<i>Pieris rapae</i>	Lepidoptera: Pieridae	cabbage white (butterfly)
<i>P. terraenovae</i>	<i>Protophormia terraenovae</i> (<i>Phormia terraenovae</i>)	Diptera: Calliphoridae	northern blowfly ; blue-bottle fly
<i>P. xuthus</i>	<i>Papilio xuthus</i>	Lepidoptera: Papilionidae	Asian swallowtail
<i>Pteronemobius†</i>	<i>Pteronemobius sp.</i>	Orthoptera: Gryllidae	no Genbank name ; (cricket)
<i>S. americana</i>	<i>Schistocerca americana</i>	Orthoptera: Acrididae	American grasshopper
<i>S. gregaria</i>	<i>Schistocerca gregaria</i>	Orthoptera: Acrididae	no Genbank name ; desert locust (grasshopper)
<i>S. bullata</i>	<i>Sarcophaga bullata</i> (<i>Neobellieria bullata</i>)	Diptera: Sarcophagidae	grey fleshfly
<i>S. invicta</i>	<i>Solenopsis invicta</i>	Hymenoptera: Formicidae	red fire ant , red imported fire ant
<i>S. littoralis</i>	<i>Spodoptera littoralis</i>	Lepidoptera: Noctuidae	African cotton leafworm (moth)
<i>T. castaneum</i>	<i>Tribolium castaneum</i>	Coleoptera: Tenebrionidae	red flour beetle
<i>T. molitor</i>	<i>Tenebrio molitor</i>	Coleoptera: Tenebrionidae	yellow mealworm (beetle)
<i>Z. nevadensis</i>	<i>Zootermopsis nevadensis</i>	Blattodea: Termitoidae	No Genbank name (termite)

Light purple = holometabolous; light red = hemimetabolous.

* Please note that the *A. gambiae* is a species complex.

** Despite the fact that vinegar fly is likely the correct name for this species (Green 2002), we use fruit fly to avoid confusion for a broader audience and to match the Genbank common name.

† Species not listed.



3. Pain in insects: A review of the evidence

3.1 Criterion 1: Nociception

The animal possesses receptors sensitive to noxious (i.e., potentially or actually harmful, damaging) stimuli (nociceptors)

This criterion specifies the most basic prerequisite for experiencing pain. If fulfilled, the animal has the neurobiological capacity for nociception. Vertebrates detect noxious stimuli through specialised peripheral sensory neurons: nociceptive neurons (Dubin and Patapoutian, 2010), characterised by free nerve endings under the epidermis. Fruit fly larvae have an anatomically-similar type of neuron with complex dendritic branches throughout the epidermal layer (Grueber et al., 2001). Class IV multidendritic neurons (see Fig. 1), present from the first instar, are multimodal but respond only to noxious stimuli (noxious heat, mechanical, and chemical stimuli) and not gentle touch (Gerhard et al., 2017; Hwang et al., 2007; Lopez-Bellido et al., 2019; Tracey et al., 2003; Zhong et al., 2010). The activation of class IV multidendritic neurons facilitates a corkscrew-like rolling behaviour in third instar fruit fly larvae. Blocking the neurons' function via genetic manipulation prevents nocifensive rolling in response to noxious heat and mechanical stimuli, while optogenetic activation produces the rolling response (Hwang et al., 2007; Tracey et al., 2003). Based on their essential role in nociception, these neurons are considered nociceptors.

Fruit fly larvae also have separate multimodal class III neurons that respond to non-noxious stimuli such as gentle touch and noxious cold (Yan et al., 2013). These neurons mediate an activation-level-dependent suite of behaviours that are distinct from those induced by noxious heat (Turner et al., 2016). Therefore, fruit fly larvae have multiple classes of neurons for different types of noxious stimuli.

There is also evidence for nociceptive multidendritic neurons in the larvae of the *Manduca sexta* moth (otherwise known as the tobacco hornworm [Lepidoptera: Sphingidae]). These γ multidendritic neurons have similar anatomical characteristics to the class IV multidendritic neurons in fruit fly larvae (Grueber et al., 2001; Grueber and Truman, 1999). These neurons fire specifically in response to thermal and mechanical noxious stimuli (Caron et al., 2020).

Fruit fly adults also exhibit nocifensive responses specific to noxious stimuli (e.g., jumping on a noxiously-heated plate: Xu et al., 2006). To our knowledge, dedicated sensory neurons that only respond to noxious

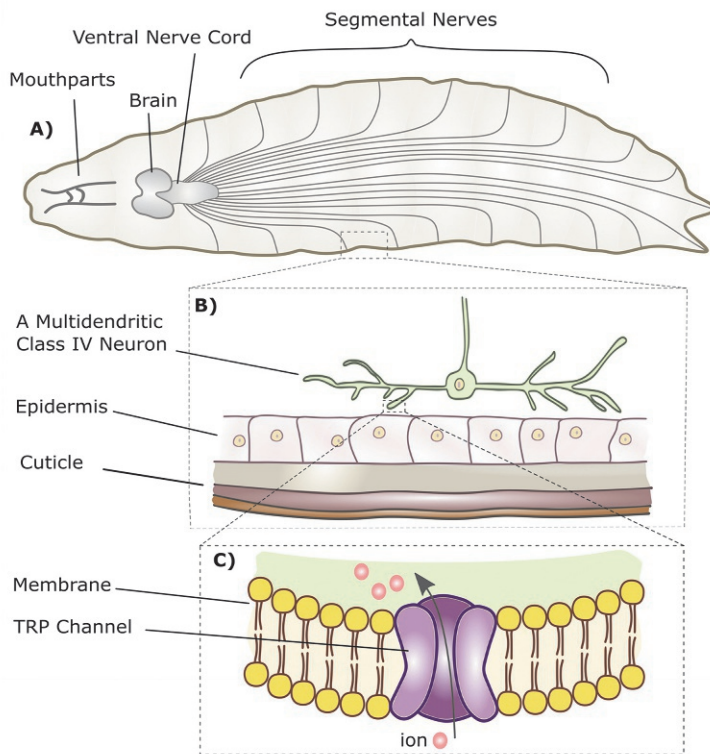


Fig. 1 A *Drosophila melanogaster* fruit fly larva and nociceptor. (A) The mouthparts, brain, ventral nerve cord and segmental nerves. (B) A nociceptive multidendritic class IV neuron, which is seen below the epidermis. (C) The membrane of the multidendritic neuron, which expresses a TRP channel. An ion passes through, to demonstrate the activation of the multidendritic neuron during the detection of a noxious stimulus.

stimuli in adults have not yet been found. Larval class IV multidendritic neurons are pruned and regrow during pupation, developing into complex sensory neurons that cover the body wall of the adult fly (Shimono et al., 2009), but whether these directly respond to noxious stimuli has not been tested. There are adult sensory neurons that regulate nocifensive responses: blocking the sensory neurons expressing the ion channel *pickpocket* prevented flies from displaying a sensitised jump response to noxious heat (Khuong et al., 2019). Therefore, there is evidence of nociceptors in fruit fly adults, but the specific neuron subtypes have not yet been identified.

In the American cockroach (*Periplaneta americana*; Blattodea: Blattidae), activity in the sensory afferent nerve differs in response to noxious stimuli vs. non-noxious tactile ones, demonstrating the presence of nociceptive sensory

neurons (Emanuel and Libersat, 2019). The authors posited that these neurons likely have a similar architecture to the multidendritic neurons found in fruit fly and *M. sexta* moth larvae. Further, application of the noxious chemical allyl-isothiocyanate, which activates nociceptive neurons in mammals (Jordt et al., 2004), causes *P. americana* cockroaches to avoid a noxiously-heated arena more than controls (Maliszewska, 2018b). Topical application of allyl-isothiocyanate also causes a concentration-dependent decrease in the cockroaches' preferred temperature (Maliszewska et al., 2018a). These data indicate *P. americana* cockroaches have nociceptive neurons.

Key features of vertebrate nociceptive neurons are their nociceptive ion channels which are responsible for detecting noxious stimuli. These include transient receptor potential (TRP) ion channels (such as TRPA1: Julius, 2013) and degenerin/epithelial sodium channels (DEG/ENaC: Ben-Shahar, 2011). Both ion channel types occur in the fruit fly class IV multidendritic neurons (see Fig. 1) (Adams et al., 1998; Ainsley et al., 2003; Kim et al., 2012; Tracey et al., 2003). Removing the TRP-channel-coding gene *painless* in mutant larvae suppresses their rolling response to noxious stimuli (Tracey et al., 2003), and calcium imaging of *painless*-expressing human embryonic kidney cells confirms that this channel directly senses noxious heat (Sokabe et al., 2008). Mutant larvae without the gene encoding for *pickpocket* (a DEG/ENaC subunit; Zhong et al., 2010), or the gene for the ion channel *Dmpiezo* (Kim et al., 2012), also have a suppressed nocifensive rolling response, but still respond to gentle touch. These ion channels are thus required for nocifensive responses to noxious heat (*painless*) or noxious mechanical stimuli (*painless*, *pickpocket*, and *Dmpiezo*).

Genes that code for nociceptive ion channels are, in many cases, evolutionarily ancient. Sponges, which diverged from all other metazoans >600 million years ago (Srivastava et al., 2010), have TRPA1 genes (Peng et al., 2015). Besides Hymenoptera, all orders covered in this review have TRPA1, which has been found in the genomes of *M. sexta* moths, *Helicoverpa armigera* (Lepidoptera: Noctuidae) cotton bollworm moths, *Bombyx mori* (Lepidoptera: Bombycidae) domestic silkworm moths, *Pieris rapae* (Lepidoptera: Pieridae) cabbage white butterflies, *Papilio xuthus* (Lepidoptera: Papilionidae) Asian swallowtail butterflies, *Danaus plexippus* (Lepidoptera: Nymphalidae) monarch butterflies, *Blattella germanica* (Blattodea: Ectobiidae) German cockroaches, *Zootermopsis nevadensis* (Blattodea: Termitoidea) termites, *Anopheles gambiae* (Diptera: Culicidae) African malaria mosquitoes, *Tribolium castaneum* (Coleoptera: Tenebrionidae) red flour beetles, and the transcriptome of *Locusta migratoria*

(Orthoptera: Acridoidea) migratory locust eggs (Matsuura et al., 2009; Peng et al., 2015; Tu et al., 2015; Wei et al., 2015).

The nociceptive function of these TRPA1 channels has been tested in the adults of only a few species. RNAi inhibition of TRPA1 function reduces the avoidance of noxious heat (42°C) in *T. castaneum* beetles. Unexpectedly, inhibiting *painless* does not reduce heat avoidance; the authors suggest that *painless* may respond to temperatures above 42°C (Kim et al., 2015). Expressing the *H. armigera* moth TRPA1 channel in *Xenopus* frog oocytes increases electrophysiological activity in response to both noxious temperature and the noxious chemical, allyl-isothiocyanate (Wei et al., 2015). Mutant TRPA1 fruit flies also fail to avoid noxious chemicals (Kang et al., 2010).

Hymenoptera have a TRPA channel specific to their order, HsTRPA, which may have evolved to replace the function of TRPA1 (Mao et al., 2020; Matsuura et al., 2009; Peng et al., 2015; Wang et al., 2018). HsTRPA-expressing human embryonic kidney cells (a technique used for recording stimulus-induced cellular calcium influx) respond to both high temperatures and the application of noxious chemicals, supporting the role of HsTRPA as a replacement for TRPA1 (Kohno et al., 2010). Chemical inhibition of HsTRPA via injection blocks the nocifensive sting extension reflex in Western honey bees (*Apis mellifera*; Hymenoptera: Apidae). However, applying HsTRPA-activating chemicals directly to the cuticle is insufficient to induce the reflex. Junca and Sandoz (2015) posit that the chemicals are unable to diffuse through the bees' cuticle to reach the channel. Expression of red fire ant (*Solenopsis invicta*; Hymenoptera: Formicidae) HsTRPA in TRPA1-mutant adult fruit flies, and in the class IV multidendritic neurons of mutant fly larvae, rescues their nocifensive response to noxious heat. Additionally, four SiHsTRPA-activating compounds repel *S. invicta* ant workers (Wang et al., 2018).

The genes for *painless*, TRPm, and two TRPV channels have also been found in all tested insects, including multiple species of Lepidoptera, Hymenoptera, and Diptera, as well as *T. castaneum* beetles and *B. germanica* cockroaches (Peng et al., 2015; Mao et al., 2020). *Painless* mutant flies display longer nocifensive jump latencies in two noxious thermal assays (Xu et al., 2006), and selectively avoid ingesting foods containing noxious (but not non-noxious) chemicals (Al-Anzi et al., 2006; but see Kang et al., 2010). TRPm is required for fruit fly larval cold nociception by class III neurons (Turner et al., 2016). Capsaicin (a noxious TRPV1 channel agonist in humans; Pingle et al., 2007) decreases the preferred temperature of

T. molitor beetle larvae, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) Colorado potato beetle adults, and *P. americana* cockroaches. *L. decemlineata* beetle temperature preference shifts are life stage-dependent, as the temperature preferred by larvae increases in response to capsaicin; however, these differences may also be due to other changes in capsaicin delivery method between studies (Adamkiewicz et al., 2012; Maliszewska and Tęgowska, 2012; Olszewska and Tęgowska, 2011; Tęgowska et al., 2004).

TRPs are not the only possible nociceptive ion channels present in insects. The two-spotted field cricket (*Gryllus bimaculatus*; Orthoptera: Gryllidae) and Hawaiian cricket (*Laupala kohalensis*; Orthoptera: Trigonidiidae) have the DEG/ENaC channel *pickpocket* class V gene family (Ylla et al., 2021). However, the function of this channel in crickets has not been clarified, and *pickpocket* has many different roles in fruit flies. Finally, the gene *straightjacket* codes for a member of the $\alpha 2\delta$ family of voltage-gated Ca^{2+} channels, whose ortholog is involved in mammalian nociception. *Straightjacket* knockout fruit flies avoid noxious heat less than wild-type flies (Neely et al., 2010), suggesting a possible role in nociception.

In summary, there is both functional and genetic evidence for nociceptive ion channels, and/or evidence of specific sensory neuron populations that respond to noxious stimuli, in **adult Coleoptera**, **Diptera**, **Hymenoptera**, and **Lepidoptera**, as well as juvenile **Diptera** and **Lepidoptera**. These orders fulfil the criterion with very high confidence at these life stages (Table 2). In **adult** and **juvenile Blattodea** and **Orthoptera**, and **juvenile Coleoptera** and **Hymenoptera**, there is

Table 2 Confidence levels for criterion 1 (nociception). Information in the right column is for first instar juveniles.

Insect order	Adult	Juvenile
Blattodea	H	H
Coleoptera	VH	H
Diptera	VH	VH
Hymenoptera	VH	H
Lepidoptera	VH	VH
Orthoptera	H	H

VH = Very high; H = High.

genomic and/or transcriptomic evidence of nociceptive ion channels. However, the expression of these genes in neurons, and their subsequent function, have not been directly tested. Therefore, we only have high confidence for these orders at these life stages. Further data demonstrating the expression of nociceptive ion channels *specifically* in sensory neurons in all orders would be beneficial, uniting the behavioural data on nociceptive responses across orders (Criterion 5–8) and the mostly genetic data on nociceptive ion channels reviewed in this criterion.

3.2 Criterion 2: Sensory integration

The animal possesses brain regions capable of integrating information from different sensory sources

Pain is different from nociception in that the former, but not the latter, is a conscious experience. Consciousness involves integrating multiple sensory inputs to generate a unified stream of experience, with this integration happening in dedicated brain regions (Birch et al., 2020; Feinberg and Mallatt, 2020; Mudrik et al., 2014). It is a point of wide agreement that the presence of integrative brain regions raises the probability of sentience (Smith and Boyd, 1991), despite continuing disagreement about what kinds of integration matter and why (Mudrik et al., 2014; Barron and Klein, 2016; Mashour et al., 2020). Stronger evidence for conscious awareness comes from psychological experiments demonstrating multisensory cognitive operations on mental representations, as found in bees (Giurfa et al., 2001; Lawson et al., 2018; Solvi et al., 2020). However, the Birch et al. (2021) framework aims to cast a wide net in search of relevant evidence for insect sentience, so any evidence of multisensory integration within the brain is considered relevant.

Multisensory integration *outside* the brain is not relevant. Some low-level multisensory integration can occur outside the brain in the spinal cord (in vertebrates: Cervero and Tattersall, 1986) or in the ventral nerve cord (in insects: Ohyama et al., 2015; Wessnitzer and Webb, 2006). However, the brain must be involved to produce subjective experience. This criterion is concerned with the presence or absence of brain regions dedicated to integrative functions.

All our focal orders use multisensory information, which is integrated for learning, memory, and the motor responses necessary for mating, navigation, foraging/feeding, communication, escape, flight control, and more (reviewed in Buehlmann et al., 2020; Kinoshita et al., 2017; Mongeau et al., 2021; Taylor and Krapp, 2007; Thiagarajan and Sachse, 2022).

To fulfil this criterion, we must have evidence in the nervous system (not just behavioural evidence) that integrative brain regions either receive multisensory inputs or have multisensory responses.

The structural organisation of the adult insect brain is broadly conserved across insects (e.g., insect brain database: [Heinze et al., 2021](#)). The brain regions most important to our review are found in the supraesophageal ganglion, which consists of three large divisions: the proto-, deuto-, and tritocerebrum. In the protocerebrum, a few ‘higher order’ brain regions are responsible for most sensory integration across all insect orders: the *mushroom bodies*, *central complex*, and *lateral horn* (see [Fig. 2](#)) ([Thiagarajan and Sachse, 2022](#)). These are the brain regions that we will consider as the ‘integrative brain regions’ for this criterion (and subsequently, Criterion 3).

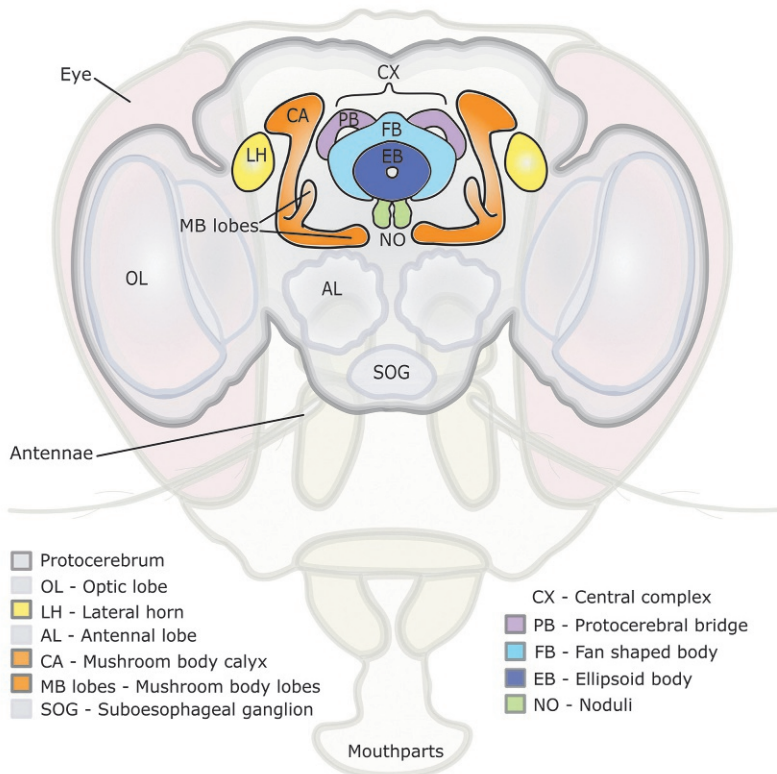


Fig. 2 A frontal view of the *Drosophila melanogaster* fruit fly brain. In colour are the integrative brain regions: the mushroom bodies, central complex and lateral horn. In grey are other sensory regions in the brain. These regions are seen in other insect species, although they differ in multiple ways, such as size or structure.

However, it is important to note that some multisensory processing can occur in other brain regions. As one example, multisensory integration may occur in the primary sensory centres (e.g., some neurons may input bimodal information in the adult *A. mellifera* honey bee and fruit fly larval antennal lobes: [Tiraboschi et al., 2021](#); [Berck et al., 2016](#)).

The mushroom bodies are higher order centres for learning, memory, and sensory integration, and have historically been considered the seat of insect “intelligence” ([Dujardin, 1850](#)). They are present in all adult insects except the Archaeognatha ([Strausfeld et al., 2009](#)), with variation across orders in their volume and structure, as well as the types, numbers, and connections of their cells ([Chittka and Niven, 2009](#); [Farris, 2013](#); [Farris and Strausfeld, 2003](#); [Oya et al., 2017](#)). As one example, the mushroom bodies of adult *A. mellifera* have nearly ~340,000 total intrinsic neurons (40% of total brain cells) while fruit flies have only ~5000 (3–5% of total brain cells: [Witthöft, 1967](#); [Schürmann, 1987](#)). Structural variation may impact the role of the mushroom bodies in specific multisensory integration tasks across taxa ([Farris and Van Dyke, 2015](#); [Strausfeld et al., 1998](#)).

In all focal orders, the mushroom bodies have two broad regions: the calyces and the lobes. The calyces contain the dendrites of the mushroom body intrinsic neurons (known as Kenyon cells). Projection neurons carrying sensory signals from the primary sensory centres (directly, or indirectly via the protocerebral mass) each diverge to meet multiple Kenyon cells in the calyces ([Ehmer and Gronenberg, 2002](#); [Strausfeld et al., 1998](#); [Sun et al., 2021](#); [Thiagarajan and Sachse, 2022](#)). Projection neurons and Kenyon cells may be generally associated, as in locusts, or isolated into parallel subsystems, as in fruit flies ([Martin et al., 2011](#)). Single Kenyon cells play a role in integrating multiple sensory inputs to the mushroom body calyces in some orders ([Farris 2008a](#); [Martin et al., 2011](#)). For example, in *P. americana* cockroaches, Kenyon cell dendrites may span multiple calyx zones that receive different sensory afferents ([Strausfeld and Li, 1999a, 1999b](#)). Multisensory inputs to the mushroom body calyces have been reported in cockroaches, flies, butterflies, crickets, bees, beetles, and ants ([Farris 2008b, 2008c](#); [Gronenberg, 1999, 2001](#); [Kinoshita et al., 2017](#); [Nishino et al., 2012](#); [Strausfeld and Li, 1999a](#); [Yagi et al., 2016](#)).

The second region is formed by the mushroom body lobes, containing the axons of the Kenyon cells. The axons generally bifurcate to produce one or more mushroom body lobes ([Strausfeld, 2002](#)). The lobes may also be supplied directly by afferents from the protocerebrum ([Ito et al., 1998](#); [Li and Strausfeld, 1997](#); [Schildberger, 1984](#); [Strausfeld, 1998](#)).

Connections in the mushroom body lobes between Kenyon cell axons, modulatory neurons and output neurons facilitate learning and coordinate behaviour (Li et al., 2020a). Improved memory of unimodal cues via cross-modal training occurs due to plastic changes in cellular connectivity in the lobes (Okray et al., 2022). Output neurons may thus receive input from multiple sensory modalities to coordinate multisensory responses (Li et al., 2020a). Multimodal responses by neurons in the mushroom body lobes have been recorded in crickets, bees, flies and cockroaches (Li and Strausfeld, 1997; Li and Strausfeld, 1999; Okada et al., 1999; Okray et al., 2022; Rybak and Menzel, 1998; Strube-Bloss and Rössler, 2018).

The mushroom body calyces and lobes are functionally divided by modality in different ways across taxa; this structural variation affects where sensory integration occurs (Farris 2008a; Martin et al., 2011; Strausfeld and Li, 1999b; Thiagarajan and Sachse, 2022). For example, the lip, collar, and basal ring zones of the *A. mellifera* honey bee calyces receive information from olfactory afferents, visual afferents, or a combination of olfactory, visual, and gustatory or mechanosensory afferents (Gronenberg, 2001; Strausfeld, 2002). However, class II Kenyon cells representing all calyx zones supply the same mushroom body lobe for further integration (λ lobe; Strausfeld, 2002). Overall, this organisation suggests that, in multiple orders, each lobe functions to integrate different combinations of stimuli (Li et al., 2020a; Yagi et al., 2016). It is thus well-documented that the mushroom bodies are pivotal in sensory integration in insects.

Another site for multisensory integration is the central complex, a structurally conserved region of interconnected neuropils at the midline of the protocerebrum (Homberg, 1987; Wolff et al., 2015). It has species-dependent roles in spatial navigation, descending control of locomotion, nociceptive perception, memory, and more (Barron and Klein, 2016; Klein and Barron, 2016; Pfeiffer and Homberg, 2014; Plath and Barron, 2015; Thiagarajan and Sachse, 2022). Depending on the order, central complex neurons may respond to visual, olfactory, proprioceptive, mechanosensory, or nociceptive information (Hu et al., 2017; Klein and Barron, 2016; Pfeiffer and Homberg, 2014). Many individual neurons are sensitive to multiple stimuli. For example, specific neurons in the central complex of the grey fleshfly (*Sarcophaga bullata*, Diptera: Sarcophagidae) respond to mechanical (air puffs) as well as visual information (Phillips-Portillo, 2012). Multisensory sensitivity of central complex neurons has been recorded in bees, cockroaches, flies and locusts (Homberg, 1994; Homberg, 1985; Matheson et al., 2021; Milde, 1988; Ritzmann et al., 2008; Rosner and Homberg, 2013; Vitzthum et al., 2002).

Additionally, fruit fly mushroom body output neurons connect to the central complex to coordinate experienced-based motor behaviours (Collett and Collett, 2018; Li et al., 2020a; Plath et al., 2017; Thiagarajan and Sachse, 2022); direct connections between these two brain regions have previously been identified as a necessary prerequisite for pain experience in insects (Adamo, 2019). The central complex clearly plays a role in multisensory integration across taxa.

The third integrative brain region, the lateral horn, has primarily been studied for its role in regulating innate olfactory-driven behaviours (Das Chakraborty and Sachse, 2021; Thiagarajan and Sachse, 2022). The fruit fly lateral horn receives input of visual, gustatory, mechanical, thermal, and auditory information to the ventral zone (Dolan et al., 2019), as well as information from mushroom body output neurons (Bates et al., 2020; Frechter et al., 2019; Li et al., 2020a). Multimodal responses of lateral horn neurons to visual and olfactory stimuli have been demonstrated in the American grasshopper, *Schistocerca americana* (Orthoptera: Acrididae [Gupta and Stopfer, 2012]). Projection neurons from the antennal lobe, which convey thermo-, hydro-, and chemosensory information, co-terminate in one region of the *P. americana* cockroach lateral horn (Nishino et al., 2003). Together, these data suggest that the multisensory integration in the lateral horn may be conserved in the adult insects of many orders.

The mushroom bodies, central complex, and lateral horn may not always be present during juvenile development; without these brain regions, juvenile insects may not fulfil this criterion. In hemimetabolous insects, the structure of these integrative brain regions is apparent following embryogenesis (Farris and Strausfeld, 2001; Farris and Sinakevitch, 2003; Malaterre et al., 2002; Pfeiffer and Homberg, 2014), though development of these regions will continue, sometimes even into adulthood (Cayre et al., 1994). Thus, juvenile hemimetabolous insect orders (Blattodea and Orthoptera in this review) are considered to have the same integrative brain regions as adults, although functional research that confirms these regions' involvement in multisensory integration of early instars would still be valuable.

In holometabolous insects, however, the brain is not fully developed following embryogenesis, and undergoes significant reorganisation during pupation (Fahrbach, 2006). Additionally, different orders of holometabolous insects may differ in the developmental timing of these brain regions, and their cell types (Fahrbach, 2006; Farris and Sinakevitch, 2003; Pfeiffer and Homberg, 2014). Taxonomic variation in the development of integrative brain regions may be the result of the lower adaptive value of

multisensory integration for larvae of species with fewer action selection opportunities (Farris and Rio, 2012; Søvik et al., 2015). For example, the cognitive demands of a caterpillar that must find food and avoid predation may necessitate more sensory integration earlier in development, when compared to a honey bee larva being cared for by adults inside a small wax cell in the hive. For this reason, we suspect, the timing of integrative brain region development in holometabolous insects may vary across (or even within) orders.

First instar fruit fly larval mushroom bodies already have a small subset of the adult fly's Kenyon cells, and these receive multiple sensory inputs (Eichler et al., 2017; Eschbach and Zlatic, 2020; Li et al., 2020a; Li et al., 2020b); at the third instar, larvae can integrate information about tastes and odours (Saumweber et al., 2018). Other first instar larval dipterans also have developed mushroom bodies (Gundersen and Larsen, 1978), as do first instar larval *T. castaneum* beetles (Farnworth et al., 2022). By contrast, in first and second instar *A. mellifera* bee brains, only mushroom body neuroblasts are apparent, and indicators of Kenyon cells and mushroom body lobes do not appear until the third and fourth instar (Farris et al., 1999). First instar *D. plexippus* butterflies only have a few Kenyon cells and poorly developed mushroom bodies (Nordlander and Edwards, 1970). Additionally, calyx formation lags after Kenyon cell and lobe appearance in both larval bees and butterflies, suggesting their Kenyon cell axons are produced before dendrites (Farris and Sinakevitch, 2003). This, alongside late maturation of Kenyon cell dendritic morphology (Farris et al., 2001), could imply delayed development of multisensory integration until late larval, or even pupal, stages in these orders (although this has not been tested).

Central complex development also differs across holometabolous orders. This is likely to correspond with development of larval "legs" in some orders (e.g., moths and beetles vs. bees and flies). Larval legs increase mobility, which may require the central complex's spatial navigation or motor control functions (Pfeiffer and Homberg, 2014). In the legless *A. mellifera* bee and fruit fly larvae, the central complex is absent in the first instar; central complex subunits first develop in the third instar in fruit fly larvae (Young and Armstrong, 2010). By contrast, some central complex subunits are present in first instar *B. mori* and *M. sexta* moths, and *T. molitor* and *T. castaneum* beetles (Farnworth et al., 2022, but see Young and Armstrong, 2010), which have larval legs. Other regions of the central complex may develop before or after pupation, dependent on taxon (Granger et al., 1989; Homberg and Hildebrand, 1994; Wegerhoff et al., 1996; Wegerhoff and Breidbach, 1992;

Young and Armstrong, 2010). Additionally, while parts of the central complex may be developed by a particular larval stage, it is not well-established whether these parts perform multisensory integration. Finally, larval subunits may undergo consistent remodelling during development (*T. molitor*; Wegerhoff and Breidbach, 1992) with unknown effects on sensory integration functions.

Lateral horn temporal development is relatively poorly studied in larval insects. The larval fruit fly lateral horn receives convergent visual, thermosensory, and chemosensory inputs and, like in adults, is expected to play a role in innate valence (reviewed in Eschbach and Zlatić, 2020). Studies of third instar fruit fly larvae suggest connections between the lateral horn and mushroom body output neurons converge on neurons responsible for sensory integration and coordinating behaviour (Cardona and Lungu, 2021; Eschbach et al., 2021). This indicates that, in third instar larval fruit flies, the lateral horn is likely to be involved in sensory integration.

In summary, to fulfil this criterion (sensory integration) with very high confidence, the order must (1) have at least one of the above integrative brain regions, and (2) these brain regions must either receive multisensory inputs or have multisensory responses. All adults in the focal insect orders (**adult Blattodea**, **Coleoptera**, **Diptera**, **Hymenoptera**, **Lepidoptera**, and **Orthoptera**) meet both conditions, alongside **juvenile Dipterans**. In hemimetabolous juvenile insects (**juvenile Blattodea** and **Orthoptera**), multisensory integration has not been directly shown, however they have similar integrative brain regions to the adults. Therefore, we have high confidence of multisensory integration.

In **juvenile Coleoptera**, and first instar **juvenile Lepidoptera**, an incomplete but actively signalling central complex exists, but the central complex's role in multisensory integration is unknown. The mushroom bodies are either partially developed (Coleoptera) or underdeveloped (Lepidoptera); in neither case has multisensory integration been shown. We therefore have low confidence in the sensory integration abilities of these orders at this life stage. For first instar **juvenile Hymenoptera**, the central complex and mushroom bodies are both undeveloped, so we have very low confidence in their sensory integration. Mushroom body development increases with instar in both **juvenile Lepidoptera** and **Hymenoptera**. However, multisensory integration has not been demonstrated at these stages, thus we have medium confidence for last instar juveniles in these orders to meet Criterion 2 (Table 3).

Table 3 Confidence levels for criterion 2 (sensory integration). By default, information in the right column is for first instar juveniles. Where published information on later instars exists, cells are split; left: first instar; right: last instar.

Insect order	Adult	Juvenile	
Blattodea	VH	H	
Coleoptera	VH	L	
Diptera	VH	VH	
Hymenoptera	VH	VL	M
Lepidoptera	VH	L	M
Orthoptera	VH	H	

VH = Very high; H = High; M = Medium; L = Low; VL = Very low. When cells are split for juveniles, left cell indicates the first instar and right cell indicates the last instar.

3.3 Criterion 3: Integrated nociception

The animal possesses neural pathways connecting the nociceptors to the integrative brain regions

A requirement for pain experience is that nociceptor activation (Criterion 1) must lead to the nociceptive signal being integrated in the brain (Criterion 2). This requires connections between the nociceptors and integrative brain regions. Thus, an order cannot have a higher confidence level for Criterion 3 than for either 1 or 2, at a particular life stage. Moreover, some nocifensive responses can occur without the brain in insects (Booker and Quinn, 1981; Horridge, 1962; Ohyama et al., 2015; Yoshino et al., 2017) and vertebrates (Jindrich et al., 2009). Elucidating neural connections between the nociceptors and the brain can help differentiate nociceptive reflexes from possible pain experiences.

Nociception is one of many types of peripheral sensory information integrated in the brain. The ascending neural pathways are broadly similar across all types of sensory information, even if the cells involved may differ. Channels used for sensing stimuli are found in the dendrites of the sensory neurons (Criterion 1). The axons of these neurons project to modality-specific layers within the ventral nerve cord (in a few cases, they may also project directly to the brain). In the ventral nerve cord, these axons connect

with interneuron dendrites. Some of these interneurons project their axons to the brain, eventually converging on the integrative regions (see Criterion 2; Tsubouchi et al., 2017). In this manner, peripheral sensory information reaches integrative brain regions. While nociceptive pathways have not been precisely mapped in most orders/contexts, the same general architecture is likely to underpin the ascending nociceptive pathways of adult insects that meet criteria 1 and 2. Thus, we will focus on specific connections between the nociceptors and the integrative brain regions outlined in Criterion 2 (the mushroom bodies, central complex and lateral horn) as evidence for this criterion.

An ascending nociceptive pathway has been described for adult *P. americana* cockroaches (Emanuel and Libersat, 2019). Extracellular recordings from the axons of sensory neurons projecting to the abdominal ganglia reveal very different response profiles to non-noxious tactile stimuli vs. noxious heat. Recordings of ascending activity between the thoracic and suboesophageal ganglia (i.e., “neck connective”) show a weak response to a continuous tactile stimulus, and a strong and persistent response to noxious heat. Additionally, headless cockroaches, or those with the neck connective severed, lack a full nocifensive response (Emanuel and Libersat, 2019; Rana et al., 2022). The local nerve cord pathway in the body ganglia could only coordinate a startle reflex, not an escape run, suggesting a role for the central complex (Emanuel and Libersat, 2019). In a follow-up study, central complex neurons were deactivated via chemical injection. Suppressing the central complex’s neuronal response significantly reduces nocifensive responses, despite nerve cord interneurons still conveying nociceptive signals to the brain. These data suggest that ascending pathways integrate nociceptive information in the central complex to coordinate full, nocifensive motor responses (Rana et al., 2022).

In the adult fruit fly, numerous distinct cell populations convey context-dependent nociceptive signals to the protocerebrum, using direct and indirect pathways. For example, multidendritic neurons in the abdomen project to the ventral nerve cord (and indirectly to the brain; Tsubouchi et al., 2017), while heat-responsive *ppk* + neurons in the legs project both to the ventral nerve cord and directly to the brain. Similar to the *P. americana* cockroaches, in headless fruit flies, a full escape response to nociceptive thermal stimulation requires the head ganglia (Khuong et al., 2019).

Integrative brain regions are required for both innate and conditioned responses of adult fruit flies to noxious stimuli, indicating that there are connections between the nociceptors and these particular regions. For example, neurons in the fan-shaped body of the central complex respond to electric

shock, and are required for innate avoidance of electric shock and noxious heat (Hu et al., 2018). Innate responses to shock or heat may also involve the lateral horn, suboesophageal ganglion, and/or the protocerebral bridge in the central complex (Ohashi and Sakai, 2018; Xu et al., 2006). Therefore, the central complex, and possibly the lateral horn, are neurally connected to the nociceptors.

Conditioned responses provide more evidence for connections between the nociceptors and the integrative brain regions in adult fruit flies. Whenever another sensory stimulus (e.g., an odour) is paired with a noxious stimulus for learning, sensory integration must occur—indicating that an integrative brain region must be connected to the nociceptors. Multiple studies have supported a role for the mushroom bodies in memory formation in nociception-odour association contexts (Aso et al., 2012; Galili et al., 2014; Oswald et al., 2015). Unique neural pathways regulating conditioned responses to both shock and heat were found to converge on a subset of dopaminergic neurons in the mushroom bodies (Galili et al., 2014, Aso et al., 2012), that connect directly to anterior cells in the superior protocerebrum. The anterior cells may be part of the neural pathway connecting the nociceptors to the mushroom bodies.

Both the mushroom body lobes and central complex may be responsible for integrating nociceptive signals in several different orders, as dopaminergic neurons are known to innervate these regions in both adult flies and cockroaches (Riemensperger et al., 2005; Hamanaka et al., 2016). Neurons in the fan-shaped body of the fruit fly central complex are activated in odour-shock learning (possibly as direct downstream targets of mushroom body output neurons; Li et al., 2020a; Hu et al., 2018). Although the ascending pathways to these regions have not been elucidated, conditioned responses to noxious stimuli demonstrate that the mushroom bodies and central complex are indirectly connected to the nociceptors.

In third instar fruit fly larvae, one nociceptive circuit leading to the brain has been particularly well described (Ohyama et al., 2015). In response to thermogenetic activation of TRPA1 in class IV multidendritic neurons, these neurons activate Basin interneurons. Basins then connect with A00c neurons, which project to the brain and synapse onto third-order interneurons, ipsiphone and contraphone. These third-order brain interneurons receive multisensory input from (minimally) mechanical and nociceptive sensory streams. Ipsiphone and contraphone connect with other brain neurons that send descending axons to Goro, exerting descending control over the rolling behaviour. Silencing the A00c interneurons

(thus inactivating the brain pathway synapsing onto Goro, but leaving the local ventral nerve cord pathway to Goro intact) reduced rolling in response to bimodal stimuli. Thus, while rolling can be modulated by the ventral nerve cord pathway alone, the ascending brain pathway is essential for multisensory integration and the effect of multiple streams of sensory information on larval fruit fly rolling behaviour (Ohyama et al., 2015). This pathway shows that multisensory information is integrated in the brain (though specific roles for the brain regions described in Criterion 2 are not mentioned).

Other connections between nociceptors and the brain have also been reported in third instar fruit fly larvae (e.g., as mediated by A08 neurons: Hu et al., 2017; Vogelstein et al., 2014). These connections can be specific to multisensory contexts. The axons of Class IV multidendritic neurons (and other sensory neurons) connect with Dorsal pair insulin-like-peptide 7-producing (DP-ilp7) neurons in the ventral nerve cord. The axons of these modulatory neurons then project to the brain. Multiple sensory streams were shown to converge onto DP-ilp7 neurons, which facilitate mechanonociceptive (but not thermonociceptive) behaviours (Hu et al., 2017). Exploring nocifensive responses in new contexts will yield better information about the brain regions that may be involved in controlling nocifensive behaviours (Eschbach and Zlatic, 2020). Further, as third instar fruit fly larvae are capable of shock-odour associative learning, they must have ascending nociceptive pathways to an integrative brain region (most likely, the mushroom bodies) (Pauls et al., 2010). While it is clear that third instar larvae have connections between the nociceptors and the brain, it has not been directly tested which integrative brain regions outlined in Criterion 2 are involved in each nociceptive context and exactly how the nociceptive sensory information reaches the brain.

Most studies on the pathways from the nociceptors to the fruit fly larval brain have focused on third instar nocifensive rolling behaviour. However, first and second instar larvae do not perform this behaviour, although general avoidance of noxious stimuli still occurs (Sulkowski et al., 2011). This difference in behaviour, and the continuous development of the brain during the juvenile period (reviewed in Criterion 2), suggests that information about ascending nociceptive pathways in the third instar should not be applied to the first instar in fruit flies without further studies elucidating the neural mechanism underlying these differences in nocifensive motor response. We are not aware of any direct evidence of connections between the nociceptors and brain regions in first instar fruit fly larvae.

In fifth instar *M. sexta* moth larvae, nociceptive sensory neurons project their axons somatotopically to the body ganglia. This axonal organisation allows for site-specific nociceptive striking responses (Caron et al., 2020; Mukherjee and Trimmer, 2020; Tabuena et al., 2017; Walters et al., 2001). Unfortunately, no studies have determined the pathways from these axonal projections to integrative brain centres. However, long pre-sensitization response latencies to noxious stimuli suggest the head ganglia are involved. This is because local interneuron (e.g., ventral nerve cord only) responses would occur with much shorter latencies, such as those seen in the quick motor neuron reflex response (Mukherjee and Trimmer, 2020).

Shock-odour training of third instar *M. sexta* moth larvae resulted in associative learning that lasted through larval development, suggesting there are neural pathways connecting nociceptive neurons and the mushroom bodies (which develops throughout juvenile development in juvenile Lepidoptera; Criterion 2). While both third and fifth instar larvae learned the association, only training at the fifth instar resulted in memory through metamorphosis (Blackiston et al., 2008). This suggests that the earlier development of certain mushroom body lobes may allow for third instar associative learning, while the presumably later development of other mushroom body lobes (e.g., α'/β' , which develop late in fruit fly larval development and persist through metamorphosis: Lee et al., 1999) may be responsible for memory retention after pupation. Specific pathways connecting the mushroom bodies and the nociceptive sensory neurons have not yet been explored and more data on the developmental timing of the different mushroom body lobes in Lepidoptera is needed to confirm this hypothesis.

In summary, we have **very high confidence** of integrated nociception in **adult Diptera** and **Blattodea**. Due to similarities in the nervous system of hemimetabolous insects across development, we also have high confidence in **juvenile Blattodea**. In last instar **juvenile Diptera**, there is evidence of multisensory input to the brain. However, connections to the integrative brain regions outlined in Criterion 2 have not been described. Therefore, we have medium confidence that they fulfil this criterion. In last instar **juvenile Lepidoptera**, there is evidence that the cerebral ganglia are involved in nociceptive responses, and they can form memories of nociceptive shock-odour pairs. Neural pathways probably connect nociceptors to higher order brain regions in the later instars, but no direct evidence of these pathways exists. Thus, we have low confidence that last instars meet this criterion, as the circuits to the brain have not yet been elucidated.

Table 4 Confidence levels for criterion 3 (integrated nociception). By default, information in the right column is for first instars. Where published information on later instars exists, cells are split: left: first instar; right: last instar.

Insect order	Adult	Juvenile	
Blattodea	VH	H	
Coleoptera	VL (no research found)	VL (no research found)	
Diptera	VH	VL (no research found)	M
Hymenoptera	VL (no research found)	VL (no research found)	
Lepidoptera	VL (no research found)	VL (no research found)	L
Orthoptera	VL (no research found)	VL (no research found)	

VH= Very high; H= High; M= Medium; L= Low; VL (no research found)= Very low (no research found). When cells are split for juveniles, left cell indicates the first instar and right cell indicates the last instar.

For **adult** and first instar **juvenile Lepidoptera**, and **adult** and **juvenile Coleoptera**, **Hymenoptera**, and **Orthoptera**, and first instar **juvenile Diptera**, we have very low confidence due to a total absence of evidence (Table 4).

3.4 Criterion 4: Analgesia

The animal’s behavioural response to a noxious stimulus is modulated by chemical compounds affecting the nervous system in either or both of the following ways:

- (a) *The animal possesses an endogenous neurotransmitter system that modulates (in a way consistent with the experience of pain, distress or harm) their responses to threatened or actual noxious stimuli.*

An endogenous neurotransmitter system that modulates responses to noxious stimuli suggests the animal may have mechanisms to reduce pain (not simply nociception). Neural, behavioral or pharmacological evidence may indicate such a system (Gibbons et al., 2022a). In fruit flies, multiple neurotransmitters appear to regulate nociceptive responses. The inhibitory neurotransmitter GABA (important in the mammalian endogenous pain

system: Jasmin et al., 2003) modulates noxious heat responses in fruit flies. When given a GABA receptor agonist, fruit flies are more likely to cross a noxious heat barrier to reach an appetitive light (Manev and Dimitrijevic, 2004). A nerve injury study demonstrated that GABAergic neurons in the ventral nerve cord modulate the nociceptive heat responses of *ppk* + nociceptive neurons (Khuong et al., 2019). Peripheral injury results in GABAergic neuron death and thus a loss of GABAergic inhibition, causing persistent thermal allodynia and nociceptive hypervigilance. Experiments suppressing GABAergic cell death and knocking down GABA receptors confirm that the loss of GABAergic inhibition was responsible for reducing the heat avoidance threshold (Khuong et al., 2019).

Ohashi and Sakai (2018) found that food deprivation reduced jump reflex frequency in response to noxious heat and that this modulation was likely mediated by leucokinin signalling. Leucokinins are a family of neuropeptides that, among other roles, mediate feeding behaviour in flies (Nässel, 2021). Silencing the leucokinin neurons rescues the response to noxious heat in food-deprived flies. Further, this modulation of the nociceptive reflex does not occur in decapitated flies, suggesting the head ganglia are involved. Leucokinin neurons exist in both the lateral horn and the sub-oesophageal ganglion; increased lateral horn leucokinin secretion likely mediates the effect of food deprivation on nocifensive response. Finally, allatostatin-C is the insect orthologue of the neuropeptide somatostatin (involved in the endogenous pain system in mammals: Kecskés et al., 2020). Knockout of allatostatin-C receptors causes insects to escape noxious heat more quickly (Bachtel et al., 2018), suggesting that allatostatin-C may suppress nociceptive responses. These findings suggest that adult fruit flies have an endogenous neurotransmitter system that may regulate responses to noxious stimuli.

Pharmacological studies also indicate that such endogenous modulatory systems may exist in insects. In adult *P. americana* cockroaches, nocifensive escape responses are inhibited following a sting to the brain by the parasitic Jewel wasp (*Ampulex compressa*; Hymenoptera: Ampulicidae; see Fig. 3). The venom also increases the startle threshold in response to electric shocks (Gavra and Libersat, 2011; Jasmin et al., 2003). A neurotrimin/opioid-binding protein/cell adhesion molecule is one component in the wasp venom (Kaiser et al., 2019). Injecting opioid antagonists before a sting rescues the cockroaches' startle threshold, while an opioid agonist mimics the sting's effect on startle threshold (Emanuel and Libersat, 2019), suggesting that an endogenous neurotransmitter system exists in cockroaches. Similarly, in *A. mellifera* bees, injection of isopentyl acetate

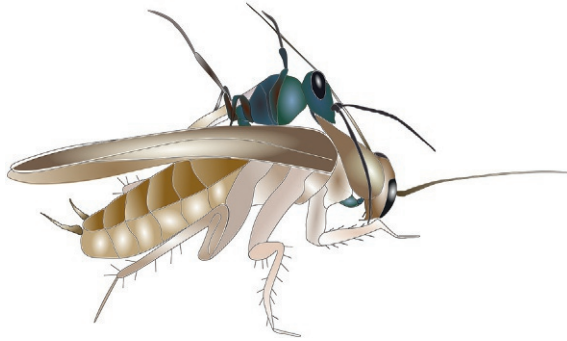


Fig. 3 A jewel wasp (*Ampulex compressa*) attacks a *Periplaneta americana* cockroach by stinging the thorax and the head. The cockroach is subsequently consumed alive by the wasp's larva inside a dedicated borrow. Unlike other parasitoids, these wasps do not fully paralyse their prey – the cockroach can still walk (albeit only under the guidance of the wasp), but loses all self-initiated behaviour. In addition, the venom increases the cockroach's threshold to noxious stimuli, thus potentially activating an endogenous neurotransmitter system for dampening nociception.

(from the bee alarm pheromone) increases their stinging response threshold to an electric shock. Injecting an opioid antagonist returned the threshold to normal (Núñez et al., 1997). This suggests that isopentyl acetate activated an endogenous neurotransmitter system, which opioid antagonists can reduce.

However, the above pharmacological data are difficult to explain in light of recent genetic studies revealing that insects do not have genes for opioid receptors or proneuropeptides (Elphick et al., 2018; Jékely, 2013; Kreienkamp et al., 2002). A genomic analysis confirmed that the opioid system likely emerged and diversified in early vertebrates (Mirabeau and Joly, 2013). When the fruit fly genome was screened for mammalian opioid receptor genes, the sequences with the highest similarity were allatostatin-C receptors (Kreienkamp et al., 2002). Many historic studies appeared to have demonstrated the presence of opioid peptides and binding sites (e.g., Davenport and Evans, 1986; Duve and Thorpe, 1983; Santoro et al., 1990), based on immunocytochemistry, high-pressure liquid chromatography, and opioid receptor binding assays. However, in light of the new genomic evidence, the accuracy or interpretation of these studies is now unclear. It is thus unknown how the above-mentioned opioid agonist and antagonists work in insects. It is possible that opioids bind to a receptor for another peptide (with no sequence homology to opioids) in insects, and thus function as an agonist. Further research will be needed to elucidate the mechanisms underlying the nociceptive modulation induced by these neuropeptides.

Finally, in fruit fly larvae, there also appears to be an endogenous neurotransmitter system modulating nociception. [Oikawa et al. \(2022\)](#) found that Drosulfakinin (DSK)-knockout third instar larvae had reduced rolling latencies in response to noxious heat; the same response occurred with DSK-receptor knockouts. DSK is a homologue of mammalian cholecystokinin, which plays a role in the descending control of nociception. Similarly, the axons of larval DSK neurons descend from the brain to the ventral nerve cord, where DSK receptor activation in ventral nerve cord interneurons mediates nocifensive behaviour. In another study, ectopic expression of neuropeptide F (another mammalian neuropeptide: [Diaz-delCastillo et al., 2018](#)) receptors in *painless*-expressing neurons suppressed third instar larval nocifensive rolling, potentially implicating neuropeptide F in the inhibitory modulation of nociception.

(b) *Putative local anaesthetics, analgesics (such as opioids), anxiolytics or anti-depressants modify an animal's responses to threatened or actual noxious stimuli in a way consistent with the hypothesis that these compounds attenuate the experience of pain, distress or harm.*

A putative local anaesthetic/analgesic should reduce nocifensive behaviour. In *A. mellifera* bees, consumption of ethanol (which can act as an analgesic in humans: [Perrino Jr. et al., 2008](#)) dose-dependently increases the electric shock threshold of the sting extension response ([Giannoni-Guzmán et al., 2014](#)). Ethanol also increases the time spent in an area where bees received electric shocks, and delays the bees' learning to avoid the area (also dose-dependent: [Black et al., 2021](#); [Giannoni-Guzmán et al., 2014](#)). However, ethanol impairs bee learning of sucrose-odour pairs ([Abramson et al., 2000](#)), so delays in avoidance learning may not be due to ethanol's putative analgesic effect.

As described in the previous section, insects do not have opioid receptors. Strangely, however, many opioids have seemingly analgesic effects (see [Table 5](#)), possibly by binding to a different receptor. For example, morphine reduces stinging response to electric shocks in *A. mellifera* bees ([Núñez et al., 1983](#)) and increases noxious-heat escape latency in *P. americana* cockroaches ([Gritsai et al., 2004](#)) and *Pteronemobius sp.* (Orthoptera: Gryllidae) crickets ([Zabala and Gómez, 1991](#)). Various opioid peptides (which can occur naturally in the body or be absorbed from food) also seem to have analgesic effects. β -casomorphins and rubiscoin (both antinociceptive in mammals: [Liu and Udenigwe, 2019](#)) increase the duration that

Table 5 The effects of opioid agonists on nocifensive behaviour in the focal insect orders.

Insect order	Drug/ peptide used	Species	Method of application	Location of application	Volume used (μl)	~ μg/g used	Effect	References
Blattodea	Morphine (?)	<i>Periplaneta americana</i>	Injection	Anterior chest wall between the bases of forelimbs.	2, 5 and 10	50	No effect	Gritsaï et al. (2004)
						200	Antinociceptive	
						300	Antinociceptive	
	Morphine-HCl	<i>Periplaneta americana</i>	Injection	Thorax	15	0.56	No effect	Weiss and Penzlin (1987)
						56	Antinociceptive	
	Heptapeptide β-casomorphine-7	<i>Periplaneta americana</i>	Injection	Anterior chest wall between the bases of forelimbs.	2, 5 and 10	200	Antinociceptive	Gritsaï et al. (2004)
						300	Antinociceptive	
						500	Antinociceptive	
	Pentapeptide β-casomorphine-5 shortened from the C end	<i>Periplaneta americana</i>	Injection	Anterior chest wall between the bases of forelimbs.	2, 5 and 10	200	Antinociceptive	Gritsaï et al. (2004)
						300	Antinociceptive	
						500	Antinociceptive	
	Hexapeptide β-casomorphine-6 shortened from the N end hexapeptide	<i>Periplaneta americana</i>	Injection	Anterior chest wall between the bases of forelimbs.	2, 5 and 10	500	No effect	Gritsaï et al. (2004)

Continued

Table 5 The effects of opioid agonists on nocifensive behaviour in the focal insect orders.—cont'd

Insect order	Drug/ peptide used	Species	Method of application	Location of application	Volume used (μl)	~ μg/g used	Effect	References
	β-casomorphine-7	<i>Periplaneta americana</i>	Injection	Between the bases of the forelegs	1-5	50 100 200	Antinociceptive Antinociceptive Antinociceptive	Gritsaï et al. (2000)
	Rubiscolin-5	<i>Periplaneta americana</i>	Injection	Front segment of the chest between the front leg bases	5	300 500	Antinociceptive Antinociceptive	Gritsaï et al. (2008)
	Met-enkephalin	<i>Periplaneta americana</i>	Injection	Anterior thorax segment between the bases of the insect forelegs.	5 or 10	300 500	No effect Antinociceptive	Gritsaï et al. (2009)
	Dinorphin A	<i>Periplaneta americana</i>	Injection	Anterior thorax segment between the bases of the insect forelegs.	5 or 10	250 500	Antinociceptive Antinociceptive	Gritsaï et al. (2009)
	Exorphine C	<i>Periplaneta americana</i>	Injection	Anterior thorax segment between the bases of the insect forelegs.	5 or 10	300 500	No effect Antinociceptive	Gritsaï et al. (2009)
	Cytochrophin- 4	<i>Periplaneta americana</i>	Injection	Anterior thorax segment between the bases of the insect forelegs.	5 or 10	300 500	No effect No effect	Gritsaï et al. (2009)
	B-neoendorphin	<i>Periplaneta americana</i>	Injection	Anterior thorax segment between the bases of the insect forelegs.	5 or 10	250 500	No effect No effect	Gritsaï et al. (2009)

Hymeoptera	Met-enkephalin	<i>Apis mellifera</i>	Injection	Membrane lying between sternal area of the mesothorax and the coxa of the left middle leg	2	1912.16	No effect	Núñez et al. (1983)
	Leu-enkephalin	<i>Apis mellifera</i>	Injection	Membrane lying between sternal area of the mesothorax and the coxa of the left middle leg	2	1852	No effect	Núñez et al. (1983)
	[D-Ala2]-Met-enkephalinamide	<i>Apis mellifera</i>	Injection	Membrane lying between sternal area of the mesothorax and the coxa of the left middle leg	2	1954.16	No effect	Núñez et al. (1983)
	Morphine-HCl	<i>Apis mellifera</i>	Injection	Membrane lying between sternal area of the mesothorax and the coxa of the left middle leg	2	187.9 927 375.8 751.6	Antinociceptive Antinociceptive Antinociceptive Antinociceptive	Núñez et al. (1983)
Orthoptera	Morphine-HCl	<i>Pteronemobius sp.</i>	Injection	Abdominal haemolymph	10	320	Antinociceptive	Zabala and Gómez (1991)
						500	Antinociceptive	
						520	Antinociceptive	
						690	Antinociceptive	
						1005	Antinociceptive	

P. americana cockroaches stay in a noxiously-heated box (Gritsaï et al., 2000, 2004, 2008). Other opioid peptides also increase noxious heat tolerance in the *P. americana* cockroach (Gritsaï et al., 2008; Gritsaï et al., 2009, Table 5). Oddly, injecting *A. mellifera* bees with some of the same opioid peptides does not affect their response to electric shock (Núñez et al., 1983). It is unclear why these peptides differ in their effects across these orders or contexts (Table 5).

A common experimental flaw in most of the analgesic studies reviewed here is the failure to control for reduction of locomotion and general responsiveness, which may explain a specific reduction in nocifensive behaviour. Another potential confound occurred in studies that required memory in order to measure nocifensive response, as analgesics often reduce learning and memory performance (Izquierdo et al., 1980). Future studies should use paradigms that do not rely on learning or reduced movement.

Besides analgesics and anaesthetics, many drugs have antidepressant and anxiolytic effects in insects (this is not evidence of pain, but of aversive subjective states more broadly). One method used to measure “depression-like” behaviour in fruit flies is the forced-swim test. Flies are placed in a well of harmless liquid (sodium dodecyl sulphate) and the time taken for them to stop moving is recorded (Neckameyer and Nieto-Romero, 2015). This immobility is considered depression-like, because it may indicate resignation to ‘hopeless’ conditions (Porsolt et al., 1977). Under this paradigm, vertebrate antidepressants (psilocybin: Carhart-Harris et al., 2017; citalopram: Jiao et al., 2011) increases the time-to-immobility in a fly strain prone to rapid immobility (Hibicke and Nichols, 2022). Flies can also be subjected to multiple stressors (cold stress, starvation, heat stress and sleep deprivation) to induce immobility in the forced swim test (Araujo et al., 2018). Using this model, Araujo et al. (2021) discovered that γ -oryzanol, a putative antidepressant in vertebrates (Mehdi et al., 2015), increases time-to-immobility and decreases aggressive behaviour. This, and other findings, point to antidepressants reducing depression-like behaviours in fruit flies.

Fruit flies also display an “anxiety-like” state under a variety of stressful conditions, resulting in a preference for dark areas or arena edges, which provide more perceived protection (Neckameyer and Nieto-Romero, 2015). γ -oryzanol, a vertebrate anxiolytic, reduced light avoidance behaviour in flies (Araujo et al., 2021). However, other anxiolytics, diazepam and fluoxetine, did not affect this behaviour (Ramos-Hryb et al., 2021). Thus, more research is needed into anxiolytics and anxiety-like states in fruit flies.

In summary, we consider the highest confidence level for either a) or b) in an insect order as the overall confidence level for the criterion of analgesia. Multiple studies on **adult** and **juvenile Diptera** indicate an endogenous nociceptive neurotransmitter system. Some studies have identified the neurotransmitters involved, and revealed which analgesic drugs work on them. Further, there is some evidence for antidepressants and anxiolytics affecting adult fruit flies. Thus, we have very high confidence they fulfil this criterion. In **adult Blattodea**, there is good evidence from multiple studies for a neurotransmitter system and/or reduced nociceptive responses after analgesic administration; we have high confidence that they fulfil the criterion.

In adult Hymenoptera, one study indicates a neurotransmitter system for nociception, and there is some evidence for possible analgesics, but these studies have locomotion and learning confounds. Thus, we have medium confidence that they fulfil this criterion. In **adult Orthoptera**, morphine agonists affect nociceptive behaviours, but confounds again weaken this evidence, only giving us low confidence. There is no research into endogenous neurotransmitter systems, local anaesthetics or analgesic drugs in **adult Coleoptera** and **Lepidoptera**, or **juvenile Blattodea**, **Coleoptera**, **Hymenoptera**, **Lepidoptera** and **Orthoptera**. Therefore, due to an absence of evidence, we have very low confidence for these developmental stages (Table 6).

Table 6 Confidence levels for criterion 4 (analgesia). Information in right column is for first instars.

Insect order	Adult	Juvenile
Blattodea	H	VL (no research found)
Coleoptera	VL (no research found)	VL (no research found)
Diptera	VH	VH
Hymenoptera	M	VL (no research found)
Lepidoptera	VL (no research found)	VL (no research found)
Orthoptera	L	VL (no research found)

VH=Very high; H=High; M=Medium; L=Low; VL (no research found)=Very low (no research found).

3.5 Criterion 5: Motivational trade-offs

The animal shows motivational trade-offs, in which the negative value of a noxious or threatening stimulus is weighed (traded-off) against the positive value of an opportunity for reward, leading to flexible decision-making. Enough flexibility must be shown to indicate centralised, integrative processing of information involving a common measure of value

Nociceptive motivational trade-offs occur when a competing motivation (e.g., acquiring food) modulates a nociceptive response (Fields, 2006; Navratilova and Porreca, 2014). Such trade-offs may indicate pain, because they show that the nociceptive processing is flexible, context-dependent, and occurs in the brain (Appel and Elwood, 2009; Elwood and Appel, 2009; Millsopp and Laming, 2008). A common argument against pain in invertebrates is that they only respond to injury reflexively (Eisemann et al., 1984). However, if the animal trades off a nociceptive response against competing motivational requirements, the behaviour cannot be a simple reflex.

D. melanogaster fruit flies display some evidence for nociceptive motivational trade-offs. Only flies with nociceptive genes knocked out would cross a 60 °C noxious-heat barrier to reach an attractive light source (Aldrich et al., 2010; Benzer, 1967). Using the same paradigm but with a 42 °C barrier, Manev and Dimitrijevic (2004) found that only 27.5% of wild-type flies would cross at 42 °C (see also Aldrich et al., 2010). While these results may indicate a motivational trade-off, the authors did not confirm that the 42 °C barrier was noxious for the entire population outside the motivational-light context. Interindividual variation in the activation temperature of noxious heat receptors could thus also be responsible for a subset of wild-type flies crossing the barrier at 42 °C. If these individuals were not perceiving the stimuli as nociceptive, then there was no motivational trade-off. Another problem with this fruit fly study is that it does not provide evidence that satisfies this criterion's requirement for "centralised, integrative processing of information involving a common measure of value". When both competing stimuli are experienced simultaneously, the two sensory inputs may interact via simple neural mechanisms without involving centralised integration (as demonstrated in the nematode *Caenorhabditis elegans* [Rhabditida: Rhabditidae]: Ghosh et al., 2016, 2017; Irvine, 2020; Shinkai et al., 2011).

In a more complex study, adult fruit flies were conditioned to associate an odour with an appetitive stimulus (ethanol or sucrose). They were then presented with the odour, which they could only reach by crossing a novel,

electrified barrier. Untrained flies would not cross the barrier at 100 V, demonstrating the shocks were noxious (other studies have shown that flies avoid 30 V shocks: [Hu et al., 2018](#)). Conditioned flies would cross the 100 V barrier to reach odours associated with ethanol or sucrose, but only ethanol-conditioned flies would cross a 120 V barrier ([Kaun et al., 2011](#)). To better determine how flexible this trade-off is, future studies could vary the sucrose or ethanol concentrations. Nonetheless, this study demonstrates a nociceptive motivational trade-off in fruit flies that varies in a context-dependent manner.

More flexible trade-offs provide better evidence for pain. In a study on large earth bumblebees (*Bombus terrestris*; Hymenoptera: Apidae), [Gibbons et al. \(2022b\)](#) demonstrated a nociceptive motivational trade-off that did not require direct sensory input from the competing stimuli, relying instead on memory. Bees could choose to feed from either noxiously-heated (55 °C) feeders with 40% sucrose solution, or alternative unheated feeders with 10%, 20%, 30%, or 40% (equal) sucrose concentrations (see [Fig. 4](#)). These feeders were marked with different colours and spatial locations, and bees learned the feeder contents and temperature based on these cues. When every feeder contained 40% sucrose solution, bees avoided the heated feeders and preferred the unheated feeders. However, when the unheated feeders contained lower sucrose concentrations, bees showed a significant reduction in avoidance of the heated feeders. This occurred despite the bees not directly perceiving either the heat or sucrose when making the trade-off—they relied on conditioned stimuli. Additionally, bees persisted in these behaviours when the feeders did not contain sucrose and were not heated ([Gibbons et al., 2022b](#), unpublished data: [Fig. 4](#) [Memory test data]). Thus, bees trade off nociceptive heat avoidance against their preference for sucrose, based solely on memory.

Future research should seek to identify the neural mechanisms that underpin such trade-offs. It is unclear if simple mechanisms (e.g., in the ventral nerve cord) mediate trade-offs involving the simultaneous presentation of competing stimuli (as in *C. elegans*), while more complex trade-offs may require integrative brain regions. In summary, **adult Diptera** and **Hymenoptera** display complex motivational trade-offs, using conditioned stimuli as the motivational stimuli, thereby meeting the criterion with high confidence. For **adult Blattodea**, **Coleoptera**, **Lepidoptera**, and **Orthoptera**, and **all insect orders at the juvenile stage**, there is no research into trade-off behaviours, and we have very low confidence that they fulfil this criterion ([Table 7](#)).

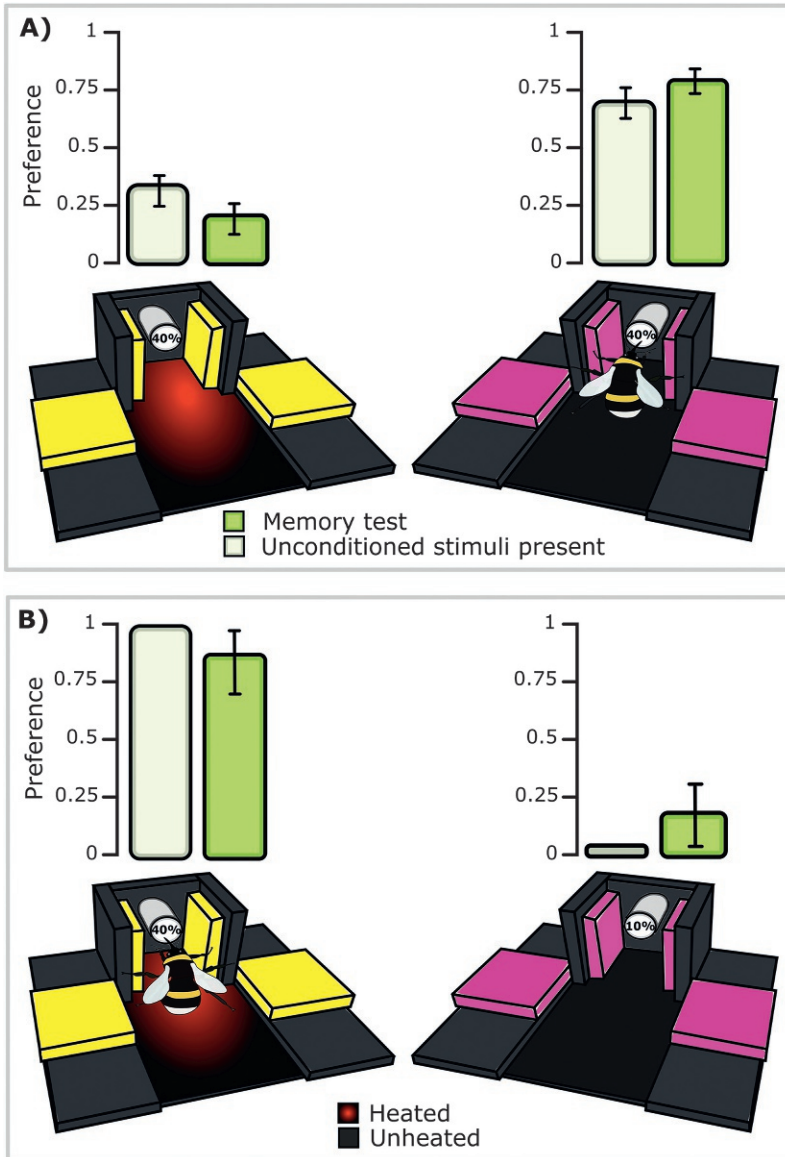


Fig. 4 A nociceptive motivational trade-off based on memory in *Bombus terrestris* bees, where bees have to weigh the benefits of a rewarding food source against the experience of noxious heat. The bars show both the proportion of feedings events at each feeder type in two different conditions: “Unconditioned stimuli present” (data from [Gibbons et al., 2022b](#)), and a subsequent “Memory test” with neither heat or rewards present. (A) When there are two feeders both with 40% sucrose solution, bees feed more at the *unheated* feeder (and subsequently remember the feeders’ nutritious and noxious properties). (B) When there is one feeder with 10% and one with 40% sucrose solution, there are higher proportions of feeding events at the *heated* feeder associated with 40% sucrose solution (and this preference persists into a subsequent memory test without heat or reward present). This demonstrates a motivational trade-off between sucrose solution concentration and noxious heat that is based on a memory of the colour and spatial cues.

Table 7 Confidence levels for criterion 5 (motivational trade-offs). Information in the right column is for first instars.

Insect order	Adult	Juvenile
Blattodea	VL (no research found)	VL (no research found)
Coleoptera	VL (no research found)	VL (no research found)
Diptera	H	VL (no research found)
Hymenoptera	H	VL (no research found)
Lepidoptera	VL (no research found)	VL (no research found)
Orthoptera	VL (no research found)	VL (no research found)

H=High; VL (no research found)=Very low (no research found).

3.6 Criterion 6: Flexible self-protection

The animal shows flexible self-protective behaviour (e.g., wound tending, guarding, grooming, rubbing) of a type likely to involve representing the bodily location of a noxious stimulus

Good evidence for this criterion would be self-protective behaviour targeted at the bodily location of noxious stimulation. Such protective behaviours are used to identify pain in mammals (see Fig. 5A) and non-verbal human patients. For example, the Checklist of Nonverbal Pain

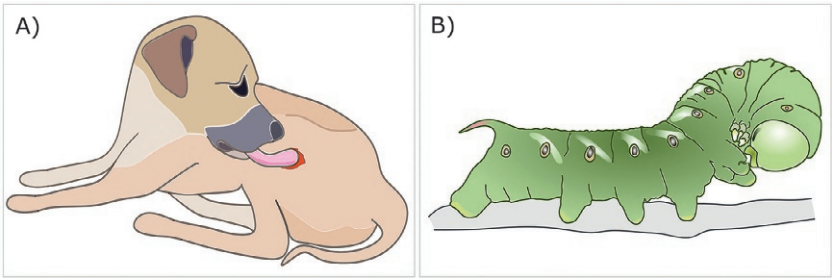


Fig. 5 A self-protective behaviour (wound tending) in a mammal and an insect. Both use their mouth to tend to their wound directly, demonstrating awareness of the site of injury. (A) A dog licking their wound. (B) A *Manduca sexta* larva tending to their wound using their mouthparts.

Indicators includes massaging or clutching the affected area (in humans: Feldt, 2000). Self-protective behaviours suggest animals are aware of the injury location and motivated to protect themselves from further harm (Elwood, 2011). While self-grooming is a common behaviour in insects (e.g., in fruit flies, *B. germanica* cockroaches, and *A. mellifera* bees: El-Awami and Dent, 1995; Ringo, 2020; Russo et al., 2020), very few studies have investigated whether insects groom, rub, or guard a specific site of noxious stimulation.

Eisemann et al. (1984) and Wigglesworth (1980) listed several anecdotal accounts of injured insects not obviously performing protective behaviour. For example, an insect with a crushed tarsus appeared to continue walking on it with the same force (Eisemann et al., 1984). However, the paper provided no experimental evidence for this behavioural observation (e.g., measuring the force applied by the leg compared to control insects), so this cannot count as evidence against self-protective behaviour. In any case, absence of evidence in specific contexts, and where pain behaviours may be suppressed, is not evidence of absence in other contexts (Gibbons and Sarlak, 2020).

Walters et al. (2001) reported anecdotal evidence of wound tending behaviour in last instar *M. sexta* moth larvae. When pinching the abdominal prolegs produced a visible wound, the larvae positioned their heads close to the wound and repeatedly touched the area with their open mouthparts (see Fig. 5B; Walters et al., 2001). This behaviour was aimed specifically at the pinched proleg. The larvae also increased their defensive response (rapidly bending the head towards the noxiously-stimulated site), even in response to non-noxious touch. This behavioural response may suggest they were guarding the site against further injury (Walters et al., 2001). In addition, *P. americana* cockroaches that receive an abdominal puncture wound will groom more, and non-quantified observations of grooming the site of the wound have been reported (Hentschel and Penzlin, 1982).

Although not a self-directed behaviour, healthy ants (*Megaponera analis*; Hymenoptera: Formicidae) tend to the wounds of other colony members after raids on termite colonies (see Fig. 6) (Frank et al., 2017). Injured workers release a 'help' pheromone that attracts healthy colony members, who investigate their wounds (typically lost limbs) and carry them back to the nest. In the nest, ants may work together to remove the termites clinging to the injured worker. Helpers then intensely groom the injury site, sometimes for several minutes. This allogrooming behaviour increased survival of injured ants in an unsterile environment by 70%. However, to our knowledge, the ants have not been observed tending to their own wounds.



Fig. 6 Wound tending behaviour from conspecifics in *Megaponera analis* ants. (I) Firstly, the ant is injured by a termite. This ant signals to the colony mates by changing the way she walks to indicate injury. (II) The colony mate will then pick up the injured ant. (III) The colony mate will groom the injury site directly.

In summary, there is evidence for wound-specific grooming in last instar **juvenile Lepidoptera** and **adult Blattodea**, but only one study in each case. Thus, we have high confidence that these orders fulfil the criterion. There is no research on site-directed, self-protective behaviour in **juvenile Blattodea**, **adult** or first instar **juvenile Lepidoptera**, and **adults** or **juvenile Coleoptera**, **Diptera**, **Hymenoptera**, or **Orthoptera**, so we have very low confidence they fulfil this criterion (Table 8).

Table 8 Confidence levels for criterion 6 (self-protective behaviour). By default, information in the right column is for first instars. Where published information about later instars exists, cells are split; left: first instar; right: last instar.

Insect order	Adult	Juvenile	
Blattodea	H	VL (no research found)	
Coleoptera	VL (no research found)	VL (no research found)	
Diptera	VL (no research found)	VL (no research found)	
Hymenoptera	VL (no research found)	VL (no research found)	
Lepidoptera	VL (no research found)	VL (no research found)	H
Orthoptera	VL (no research found)	VL (no research found)	

H=High; VL (no research found)=Very low (no research found). When cells are split for juveniles, left cell indicates the first instar and right cell indicates the last instar.

3.7 Criterion 7: Associative learning

The animal shows associative learning in which noxious stimuli become associated with neutral stimuli, or in which novel ways of avoiding noxious stimuli are learned through reinforcement

Learning from aversive experiences allows an animal to avoid these experiences in the future. Although not a compelling demonstration of sentience on its own, such learning goes over and above reflex-like responses to noxious stimuli. There is ongoing debate about which forms of associative learning are linked to sentience and conscious awareness (Ginsburg and Jablonka, 2019) with particularly intense recent interest in certain forms of instrumental conditioning (Skora et al., 2021), reversal learning (Travers et al., 2018), and trace conditioning (Droege et al., 2021). To leave room for this debate, without taking a stance, we review both classical conditioning and more complex forms of learning, which may provide stronger evidence for sentience. There is a robust literature (reviewed in Giurfa, 2015) on insect associative learning using appetitive (e.g., sucrose) or non-nociceptive aversive stimuli (e.g., quinine). However, only learning that involves noxious stimuli, such as shocks or very high temperatures, can fulfil this criterion, given its intended link to pain.

Classical conditioning in response to noxious stimuli has been demonstrated in several insect orders. Adult fruit flies sequentially exposed to two odours, and receiving twelve shocks during the first odour, avoided that odour in a subsequent T-maze test 95% of the time. Flies learned associations after just one trial and retained the association for at least 24 h (Tully and Quinn, 1985; Quinn et al., 1974). There is also evidence for classical conditioning in response to noxious shock or heat stimuli in adult Hymenoptera (e.g., *A. mellifera* bees: Abramson, 1986; Nouvian and Galizia, 2019; Roussel et al., 2012; Vergoz et al., 2007; Junca and Sandoz, 2015; and *Camponotus aethiops* (Hymenoptera: Formicidae) carpenter ants: Desmedt et al., 2017) and adult Lepidoptera (e.g., *Agrotis ipsilon* [Lepidoptera: Noctuidae] black cutworm moths: Murmu et al., 2020). Further evidence for classical conditioning is found in juvenile Diptera (e.g., third instar fruit fly larvae: Aceves-Piña and Quinn, 1979; Khurana et al., 2012) and juvenile Lepidoptera (e.g., *M. sexta* moth and *Grapholita molesta* [Lepidoptera: Tortricidae] oriental fruit moth larvae). In *M. sexta* and *G. molesta* moth larvae, the noxious associative memories formed during the last larval instar may persist into adulthood (Blackiston et al., 2008; Sant'Ana et al., 2021).

Operant conditioning is also widespread in insects. Historically, authors have reported that adult fruit flies readily learn operant responses in noxious

heat paradigms (Brembs, 2003; Putz and Heisenberg, 2002; Wustmann et al., 1996). However, flies learn predictive cues (i.e., classical conditioning) preferentially over operant conditioning (Brembs, 2009; Brembs and Plendl, 2008). Operant paradigms must, therefore, avoid any predictive cues (Wiggin et al., 2021). In studies that avoid predictive cues, fruit flies still learn to avoid visual landmarks associated with noxious heat (Brembs, 2011; Brembs and Heisenberg, 2000; Heisenberg et al., 2001). *P. americana* cockroaches and *A. mellifera* bees also readily learn operant responses, for example avoiding a location where they receive noxious electric shocks (Barraco et al., 1981; Abramson et al., 2004). *S. americana* grasshoppers learn to remove their leg from a salt solution where they receive an electric shock (Punzo, 1980), and to move their leg to avoid noxious heat stimulation to their head (Forman, 1984).

Some older studies have been taken as evidence that operant responses to noxious stimuli may not require a brain in insects. Horridge (1962) found that headless *Periplaneta* sp. cockroaches and *L. migratoria* locusts could ‘learn’ a leg position to avoid receiving an electric shock (later replicated in fruit flies; Booker and Quinn, 1981). The headless insects retracted their legs more frequently and for longer durations than “yoked” control insects (with heads attached), which were shocked at the same time as the headless subjects regardless of their leg position. The isolated prothoracic ganglion, which controls limb movement, was sufficient for the headless insects to learn the leg retraction response (Aranda and Luco, 1969; Eisenstein and Carlson, 1994; Eisenstein and Cohen, 1965). However, an experimental design flaw, outlined by Church and Lerner (1976), suggests that differences in yoked and headless insect performance do not necessarily demonstrate learning in the headless insects. The headless insects’ legs are retracted for much more of the training phase than the yoked controls’ legs (as they do not immediately receive a shock after extending their legs again). Computer simulations further demonstrated that this difference in retraction time between groups could be the result of reflex, without learning, on the part of headless insects. Therefore, studies using the Horridge paradigm should not be taken as good evidence for operant conditioning in headless insects.

More complex forms of Pavlovian learning may offer better evidence for sentience (Birch et al., 2020, 2021; Ginsburg and Jablonka, 2019). One example is trace conditioning, a form of classical conditioning with a time interval between the conditioned stimulus (CS) and unconditioned stimulus (US) (Droege et al., 2021). Unlike delay conditioning (where the CS and US overlap), this requires a neural representation of the CS after

it has ceased (Dylla et al., 2013). It has been suggested that when such trace conditioning requires the subject to keep track of the delay between the CS and the US, this requires conscious awareness of the stimuli and interval (Bellebaum and Daum, 2004; Knuttninen et al., 2001). According to Dylla et al. (2013), insects can be faster than vertebrates at learning trace conditioning tasks, making them ideal for studying this behaviour.

Several studies have demonstrated nociceptive trace conditioning in adult fruit flies, pairing odours (CS) with shocks (US; Dylla et al., 2017; Galili et al., 2014; Shuai et al., 2011). However, without proper controls, odours can linger in the apparatus during the time interval (Galili et al., 2011), raising uncertainty about whether experiments are testing trace or delay conditioning (Dylla et al., 2013). To avoid these issues, Grover et al. (2022) developed a novel Pavlovian visual conditioning paradigm (adapted from Brembs and Heisenberg, 2000). Tethered flies were shown one of two shapes (CS), which was paired with aversive heat (US) in either a trace or delay context. Flies were subsequently shown both shapes simultaneously and allowed to orient towards one or the other. If flies oriented away from the shape paired with the noxious heat, this indicated they had learned the association. Flies could learn this task for trace intervals of around 20s (although there was no evidence of them learning the duration of the interval, an important part of trace conditioning tasks in the mammal literature on the involvement of consciousness with such tasks). Further, distracting stimuli, such as air puffs, impaired trace conditioning but not delay conditioning (Grover et al., 2022). This mirrors the responses of mammals to distractions while engaged in conscious (trace) vs. unconscious (delay) learning (Clark and Squire, 1998, 1999; Han et al., 2003).

Another consciousness-linked type of learning has been discussed in the context of reversal learning: classical conditioning after the reinforcement contingencies have reversed. Travers et al. (2018) reported that, in humans, rapid reversal learning was only possible if participants consciously perceived the stimuli. The critical question is whether an animal improves performance with multiple reversals, so that it can be inferred that the animal has understood the principle of the reversal (rather than having to learn *de novo* after each reversal; Shettleworth, 1998). Basic reversal learning has been found in a range of insects. In as little as one trial, fruit flies learned to reverse odour-shock (McCurdy et al., 2021; Shuai et al., 2011; Tully and Quinn, 1985; Wu et al., 2012), odour-heat (Guo and Guo, 2005) and pattern-heat associations (Guo and Guo, 2005; Ren et al., 2012).

Additionally, [Longo \(1964\)](#) reported reversal learning in the grey cockroach (*Nauphoeta cinerea*; Blattodea: Blaberidae) when electric shock reinforcement was swapped between different arms of a T-maze (see also, *P. americana* cockroaches: [Balderrama, 1980](#)).

Numerous studies of non-nociceptive reversal learning have been conducted in fruit fly larvae ([Mancini et al., 2019](#)), and adult honey bees and bumblebees ([Boitard et al., 2015](#); [Chittka 1998](#); [Dyer et al., 2014](#); [Raine and Chittka, 2012](#)). In one study on serial reversal learning, honeybees failed the criterion of “learning to reverse learn” ([Mota and Giurfa, 2010](#)); conversely [Chittka \(1998\)](#) trained a single common eastern bumblebee (*Bombus impatiens* [Hymenoptera: Apidae]) through several reversals over multiple days; this individual became highly efficient at the end of training and switched preference after only two errors following each reversal. However, nociceptive reversal learning that could be used as evidence to fulfil this criterion has not been tested in these taxa; for the others, the key criterion of learning to reverse-learn remains to be tested.

More experiments are needed to confirm the link between abilities like trace conditioning, or rapid reversal learning, and consciousness in insects. Future researchers could demonstrate a conscious/unconscious distinction by “switching off” the abilities in a putative unconscious condition, perhaps by masking the stimuli ([Birch, 2020](#)) or distracting subjects with a competing task ([Droege et al., 2021](#)). The strongest evidence would come from consciousness-linked forms of learning that have unconscious analogues. For example, while trace conditioning seems to require conscious awareness in humans ([Clark and Squire, 1998](#); [Clark et al., 2001](#); [Clark and Squire, 1999](#)), this appears not to be the case for delay conditioning ([Clark and Squire, 1999](#); [Han et al., 2003](#); [Knight et al., 2006](#)). A similar pattern of results for multiple consciousness-linked learning abilities would bolster the case for insect consciousness ([Birch, 2020](#); [Crump and Birch, 2022](#); [Shea and Bayne, 2010](#)). Overall, there are multiple studies demonstrating nociceptive associative learning in **adult Blattodea, Diptera, Hymenoptera, Lepidoptera, and Orthoptera**, as well as last instar **juvenile Diptera and Lepidoptera**, so we have very high confidence that these orders fulfil this criterion at these life stages. To our knowledge, there is no direct evidence of *nociceptive* associative learning in **adults** or **juveniles** of any **adult Coleoptera, juvenile Blattodea, Coleoptera, Hymenoptera, Orthoptera** or first instar **juvenile Diptera and Lepidoptera**; thus, we have very low confidence (no research found) ([Table 9](#)).

Table 9 Confidence levels for criterion 7 (associative learning). By default, information in the right column is for first instars. Where published information on later instars exists, cells are split; left: first instar; right: last instar.

Insect order	Adult	Juvenile	
Blattodea	VH	VL (no research found)	
Coleoptera	VL (no research found)	VL (no research found)	
Diptera	VH	VL (no research found)	VH
Hymenoptera	VH	VL (no research found)	
Lepidoptera	VH	VL (no research found)	VH
Orthoptera	VH	VL (no research found)	

VH = Very high; H = High; VL (no research found) = Very low (no research found). When cells are split for juveniles, left cell indicates the first instar and right cell indicates the last instar.

3.8 Criterion 8: Analgesia preference

Animals can show that they value a putative analgesic or anaesthetic when injured in one or more of the following ways

(a) *The animal learns to self-administer putative analgesics or anaesthetics when injured*

When an injured animal self-administers an analgesic drug, they demonstrate a motivation to cease their perception of the injury. A local anaesthetic works peripherally, suggesting (minimally) that the animal is motivated to end nociceptive processing of the injury. A drug that works in the brain, possibly on areas involved in pain experience, suggests the animal is motivated to end the feeling of pain.

To self-administer analgesics, an injured animal must know: (1) the behavioural response necessary to administer the drug, and (2) the drug’s analgesic effect. In vertebrates, condition 1 is usually trained by associating a conditioned stimulus (e.g., colours, spatial locations) with the unconditioned stimulus (e.g., analgesic drug or control). For condition 2, the analgesic and control are presented successively, with a time gap when the drug’s effects can be experienced. This ensures that the animal only associates the analgesic effect with the drug conditioned stimulus, and not the control stimulus.

The vertebrate literature includes self-administration experiments that rigorously satisfy both conditions. As an example, Colpaert et al. (2001) trained arthritic and healthy rats (*Rattus norvegicus domestica*; Rodentia: Muridae) to drink from a feeder containing fentanyl, an analgesic drug, for one day. The day after, they offered both the fentanyl feeder (in the same location) and a control feeder (in a different location). Arthritic rats drank from the fentanyl feeder more than healthy rats.

Unfortunately, the only insect study on analgesic self-administration does not satisfy condition 2. Groening et al. (2017) offered feeders containing either morphine sulphate (as the analgesic) or no morphine (as the control) to injured and healthy *A. mellifera* bees. Injured bees did not use the morphine feeders more than healthy bees, so the authors concluded that injured honey bees do not self-administer morphine. However, both feeders were presented simultaneously. If morphine's analgesic effects are not immediate, and the bees were not using the drug feeder when the effect kicked in, then the bees would not have the chance to associate the drug's effect with the morphine feeder.

Additionally, Groening et al. (2017) chose morphine sulphate as the analgesic, but insects do not have opioid receptors (Elphick et al., 2018; Jékely, 2013; Kreienkamp et al., 2002; Mirabeau and Joly, 2013). Older morphine studies that reported "analgesic" effects in insects (Núñez et al., 1983; Zabala et al., 1984) possibly reported artefacts based on binding of opioids to non-opioid receptors, or documented effects on general locomotion and responsiveness, rather than nociception (see Criterion 4). Therefore, future studies should use analgesics validated for insects, such as GABA agonists (Bowery, 2006).

Groening et al. (2017) also highlighted that uninjured and injured bees *all* consumed less morphine-sucrose solution than control solution, indicating a bias against the morphine-sucrose solution. This is probably because morphine has a bitter taste (Chen et al., 2014), which bees quickly learn to avoid (Chittka et al., 2003). Thus, even if bees could associate the feeder with the drug's effects, they would also have to overcome a taste aversion to use the morphine feeders. Future studies using validated analgesics should mask aversive tastes with appetitive flavours. If this cannot be achieved, topical application or injection are alternate delivery methods (Barron et al., 2007). Given these complications—the simultaneous presentation of treatment and control, the choice of an analgesic that might not work in bees, and the undisguised aversive taste of morphine—Groening et al.'s study provides poor evidence against analgesic self-administration in bees.

There are no other analgesic/anaesthetic self-administration studies on insects. However, one study found that parasitised *B. terrestris* bumblebees will self-medicate with nicotine, which has antimicrobial properties (Baracchi et al., 2015). Multiple studies also demonstrate self-administration of ethanol and psychotropic drugs in fruit flies (Devineni and Heberlein, 2009; Rigo et al., 2021). These studies used healthy, uninjured flies, and therefore do not fulfil the criterion. However, their protocols should be considered for future self-administration studies on insects.

(b) *The animal learns to prefer, when injured, a location at which analgesics or anaesthetics can be accessed.*

To our knowledge, no insect studies have investigated whether analgesics or anaesthetics affect conditioned place preference.

(c) *The animal prioritises obtaining these compounds over other needs (such as food) when injured.*

To our knowledge, there are no insect studies investigating whether injured insects prioritise obtaining analgesics/anaesthetics over other needs.

Due to a lack of evidence (apart from very weak negative evidence in *A. mellifera* bees) we have very low confidence one way or another for **all focal orders at all life stages** regarding analgesic self-administration in injured insects. This represents a major knowledge gap (Table 10).

Table 10 Confidence levels for criterion 8 (analgesia preference). Information in the right column is for first instars.

Insect order	Adult	Juvenile
Blattodea	VL (no research found)	VL (no research found)
Coleoptera	VL (no research found)	VL (no research found)
Diptera	VL (no research found)	VL (no research found)
Hymenoptera	VL	VL (no research found)
Lepidoptera	VL (no research found)	VL (no research found)
Orthoptera	VL (no research found)	VL (no research found)

VL=Very low; VL (no research found)=Very low (no research found).



4. Summary of evidence for insect pain

In [Section 3](#), we assessed the evidence for each criterion in adults and juveniles of six insect orders. [Table 11](#) summarises our confidence levels for adults, and [Table 12](#) summarises our ratings for first (and last) instar juveniles.

Birch et al. (2021) suggested an approximate grading scheme for communicating the strength of evidence for sentience (specifically for pain). The five grades were:

- 1. Very strong evidence:** High or very high confidence that 7–8 criteria are satisfied. Welfare protection clearly merited. No urgent need for further research into pain experience in this taxon.
- 2. Strong evidence:** High or very high confidence that 5–6 criteria are satisfied. If remaining indicators are uncertain rather than shown absent, further research is advisable. However, these animals should be regarded as sentient (or capable of pain) in the context of animal welfare legislation.
- 3. Substantial evidence:** High or very high confidence that 3–4 criteria are satisfied. If remaining indicators are uncertain rather than shown absent, further research is strongly recommended. Despite the scientific

Table 11 Confidence level for each criterion for adults of each focal insect order.

Adults	Criterion 1: Nociception	Criterion 2: Sensory integration	Criterion 3: Integrated nociception	Criterion 4: Analgesia	Criterion 5: Motivational trade-offs	Criterion 6: Flexible self- protection	Criterion 7: Associative learning	Criterion 8: Analgesia preference
Blattodea	H	VH	VH	H	VL (no research found)	H	VH	VL (no research found)
Coleoptera	VH	VH	VL (no research found)	VL (no research found)	VL (no research found)	VL (no research found)	VL (no research found)	VL (no research found)
Diptera	VH	VH	VH	VH	H	VL (no research found)	VH	VL (no research found)
Hymenoptera	VH	VH	VL (no research found)	M	H	VL (no research found)	VH	VL
Lepidoptera	VH	VH	VL (no research found)	VL (no research found)	VL (no research found)	VL (no research found)	VH	VL (no research found)
Orthoptera	H	VH	VL (no research found)	L	VL (no research found)	VL (no research found)	VH	VL (no research found)

VH = Very high; H = High; M = Medium; L = Low; VL = Very low; VL (no research found) = Very low (no research found).

Table 12 Confidence level for each criterion for juveniles of each focal insect order.

Juvenile	Criterion 1: Nociception	Criterion 2: Sensory integration	Criterion 3: Integrated nociception		Criterion 4: Analgesia	Criterion 5: Motivational trade-offs	Criterion 6: Flexible self- protection	Criterion 7: Associative learning	Criterion 8: Analgesia preference		
Blattodea	H	H	H		VL (no research found)	VL (no research found)	VL (no research found)	VL (no research found)			
Coleoptera	H	L	VL (no research found)		VL (no research found)	VL (no research found)	VL (no research found)	VL (no research found)			
Diptera	VH	VH	VL (no research found)	M	VH	VL (no research found)	VL (no research found)	VL (no research found)	VH	VL (no research found)	
Hymenoptera	H	VL	M	VL (no research found)		VL (no research found)	VL (no research found)	VL (no research found)		VL (no research found)	
Lepidoptera	VH	L	M	VL (no research found)	L	VL (no research found)	VL (no research found)	H	VL (no research found)	VH	VL (no research found)
Orthoptera	H	H	VL (no research found)		VL (no research found)	VL (no research found)	VL (no research found)	VL (no research found)		VL (no research found)	

When cells are split for a criterion, left cell indicates the first instar and right cell indicates the last instar juvenile. VH = Very high; H = High; M = Medium; L = Low; VL = Very low; VL (no research found) = Very low (no research found).

uncertainty regarding these animals, it might still be reasonable to include them within the scope of animal welfare legislation, e.g., if they are closely related to animals that have been more extensively studied and for which the evidence is stronger.

- 4. **Some evidence:** High or very high confidence that 2 criteria are satisfied. Sentience (or pain) should not be ruled out. If remaining indicators are uncertain rather than shown absent, further research might provide insight.
- 5. **Capacity for pain unknown or unlikely:** High or very high confidence that 0–1 criteria are satisfied. If remaining indicators are uncertain rather than shown absent, sentience (or pain) is simply unknown. However, if high-quality scientific work shows the other indicators to be absent, pain is unlikely.

Using these ratings, we found that **adult Blattodea** and **Diptera** satisfy 6 criteria with high or very high confidence. According to the Birch et al. framework, this constitutes strong evidence of the capacity for pain. **Last instar juvenile Diptera** and **Adult Hymenoptera** satisfy 4 criteria with high or very high confidence. **Adult Lepidoptera** and **Orthoptera**, **juvenile Blattodea**, first instar **juvenile Diptera**, and last instar

juvenile Lepidoptera satisfy 3 criteria with high or very high confidence. This amounts to substantial evidence of pain experiences. **Adult Coleoptera** and **juvenile Orthoptera** satisfy 2 criteria—some evidence of pain. **Juvenile Coleoptera** and **Hymenoptera**, and first instar **juvenile Lepidoptera** satisfy one criterion, which by our criteria means that the capacity for pain experiences is unknown. We found no robust evidence that any order definitively failed any criteria.

Our assessment here is limited by the state of the scientific literature. Even just for the six focal orders in this review, there was no evidence at all for many criteria (especially for juveniles). For insect orders we did not include in this review, there is even less evidence; we cannot reliably generalise from our focal orders to all insects. Further, most research within our focal orders was conducted on a few “model” species. To determine our confidence levels for each order, we were forced to generalise from just a few species. These species may not be representative of the vast majority of their order.

For juvenile insects, which are especially understudied, these generalisations may be even more problematic. For example, much of our neural data on juvenile Hymenoptera came from honey bee larvae, which live inside protecting wax cells and are cared for by adults; they thus have fewer demands during development, including for self-protection or cognition. Sawfly larvae (also hymenopterans) are solitary and free-living, and may have many more cognitive demands during development as a result. These key behavioural differences may necessarily change the temporal development of the neural substrates required for sentience or pain, even for species within the same order. Future work should look for evidence across development in understudied orders, as well as in non-model species within our focal orders.

Additionally, our review stuck closely to the original Birch et al. framework. As noted by Irvine (2022), there are interdependencies and redundancies between criteria (e.g., Criteria 2 and 3), and the eight criteria do not provide equivalent weight in assessing the likelihood of pain vs. nociception – yet they are given equal consideration in drawing a final conclusion about pain in this section. Caution is also warranted when interpreting behavioural data in insects as evidence for emotion-like states (Baracchi et al., 2017). Future work may continue to improve this framework as our scientific understanding of pain continues to advance through collaborations between biologists and philosophers.



5. Ethical considerations for the use or management of insects

Insects are managed in a variety of contexts that may raise welfare concerns, including the food and feed industry, silk/shellac/dye production, waste management, pest/invasive species management, wildlife conservation, beekeeping, zoos and insectariums, research/education settings, the entertainment industry, in medicine, and as pets. By far, the largest number of insects with welfare impacted by human management will be in wild/agricultural settings, followed by the growing insects as food and feed industry (Rowe, 2020a, 2020b).

Wild insect populations are managed using pesticides, parasitoids, infectious agents, reproductive control, sticky traps, and more. Few estimates are available to determine how many insects such practices affect. However, some estimates posit that the number could be as large as 10 quadrillion annually (Rowe, 2020a), and many management practices are likely to be low welfare. For example, pesticides have negative physiological impacts on insects, including deleterious effects on the nervous system, myocardial cell dysfunction, developmental perturbations resulting in malformation, reproductive system abnormalities, reduced longevity, decreased immune function, and at high levels of exposure, immediate death (Desneux et al., 2007).

The UN Food and Agriculture Organisation has promoted insect farming as a way to help feed a global population of 10 billion people by 2050 (Baillie and Zhang, 2018). Compared to traditional livestock, insects have a higher nutritional value (van Huis and Tomberlin, 2017), are cheaper to farm (Lambert et al., 2021), and can result in lower greenhouse gas emissions (Oonincx et al., 2010). The insects as food and feed industry slaughters over a trillion individuals annually (Rowe, 2020b) and is projected to grow rapidly (de Jong and Nikolik, 2021). For context, about 79 billion terrestrial mammal and bird livestock are slaughtered for meat annually, worldwide (Šimčikas, 2020). The insects as food and feed industry will, thus, become the largest livestock rearing project in human history.

The current lack of guidance on insect pain and welfare is particularly concerning, since the insect farming industry might involve serious welfare issues in both rearing and slaughter. For example, starvation of adults used for breeding, inhumane slaughter, unmet needs for mating behaviour, nutritional inadequacies, disease, and larval crowding/overheating are all welfare issues for farmed black soldier flies (Barrett et al., 2022). However, there is

interest by producers, academics, and consumers in species-specific insect welfare investigations (e.g., Barrett et al., 2022, Kortsmit et al., 2022) that can inform industry best practices, particularly given the large number of insects involved (Bear, 2019, 2021; de Goede et al., 2013; Delvendahl et al., 2022; Erens et al., 2012; Gjerris et al., 2016; IPIFF, 2019; van Huis and Tomberlin, 2017; van Huis, 2021).

Seven farmed species are currently cultivated in truly massive numbers for food and feed, with another four species being managed intensively for other products (see Table 13; Rowe, 2020a; Rowe, 2021). Given the large numbers of individuals impacted, the practical importance of studying

Table 13 Most farmed insect species.

Species name	Order: Family	Common name	Main Use
<i>Acheta domesticus</i>	Orthoptera: Gryllidae	house cricket	Food and feed industry
<i>Alphitobius diaperinus</i>	Coleoptera: Tenebrionidae	lesser mealworm beetle	Food and feed industry
<i>Apis mellifera</i>	Hymenoptera: Apidae	western honey bee	Pollination services, honey production
<i>Bombyx mori</i>	Lepidoptera: Bombycidae	domestic silkworm (moth)	Silk production
<i>Dactylopius coccus</i>	Hemiptera: Dactylopiidae	cochineal bug	Dye production
<i>Gryllus assimilis</i>	Orthoptera: Gryllidae	Jamaican field cricket	Food and feed industry
<i>Gryllodes sigillatus</i>	Orthoptera: Gryllidae	banded cricket	Food and feed industry
<i>Hermetia illucens</i>	Diptera: Stratiomyidae	black soldier fly	Food and feed industry
<i>Kerria lacca</i> *	Hemiptera: Kerridae	common lac scale	Lac production
<i>Musca domestica</i>	Diptera: Muscidae	house fly	Food and feed industry
<i>Tenebrio molitor</i>	Coleoptera: Tenebrionidae	yellow mealworm beetle	Food and feed industry

* Most commonly farmed species, but other species such as *Kerria yunnanensis* are also farmed in large numbers. In addition, strain-specific differences in ecology may be especially important when considering the welfare concerns of this species.

a limited number of species, and the precedent for regulating the welfare of livestock, research should begin with these eleven insects, using or expanding upon the model established in [Barrett et al. \(2022\)](#).

Considering insect welfare in these settings presents major practical and ethical challenges ([Barrett & Fischer, 2022](#)). For example, assessment tools for livestock are designed for use with vertebrates, such as Brambell's Five Freedoms ([Brambell, 1965](#)). These tools do not easily translate to insects, a limitation admitted by many of the entomologists adopting them for early explorations of farmed insect welfare ([Barrett et al., 2022](#); [de Goede et al., 2013](#); [Erens et al., 2012](#); [van Huis, 2021](#)). Additionally, the vast majority of farmed holometabolous insects are slaughtered as larvae. As our review demonstrates, there has been little research on larval pain. Given this uncertainty, should the welfare of the larger number of larval insects be treated in the same way as adults? How we address these challenges (among others) could have tremendous impacts on the welfare of the insects, wild and farmed vertebrates, and humans relying on insect farming or management for food or economic security – as well as the cost, feasibility, and design of production facilities and wildlife management tools.

Finally, insects are widely used in research, although probably significantly fewer individuals than in the aforementioned contexts (minimally 100 million: [Rowe, 2020b](#)). While vertebrate research is increasingly legislated, insects may be subjected to invasive procedures, kept in poor conditions, and killed *en masse* with no consideration for welfare. Some scientists have argued for the consideration of insect welfare independent of any formalised processes ([Drinkwater et al., 2019](#); [Sandall and Fischer, 2019](#); [Smith, 1991](#)). Taking insect welfare into consideration is valuable for the sake of the insects, and also necessary in practice, given changing public perception of insect pain. A survey of nearly 5000 American adults found that 52%, 56%, and 65% of the population believed that termites, ants, and bees are capable of pain, respectively. Only 8–13% disagreed in each case, and the rest were unsure ([Dullaghan et al., 2021](#)). [Drinkwater et al. \(2019\)](#) suggest that, given shifting opinions on invertebrate sentience, the scientific community may lose public support if the field does not revisit the ethics of invertebrate research. This position is corroborated by recent data from Canada showing that public trust in scientists decreases when oversight of invertebrate research is absent ([Brunt et al., 2022](#)).

Many of the challenges discussed above also apply to insect welfare in research settings. Additionally, there are interspecific welfare trade-offs to be considered, as many experimenters reduce their use of vertebrates by

replacing them with insects. In most insect species, there are almost no data on humane treatment in the lab or field. For example, while general anaesthetics are known to reduce overall sensory processing, or possibly conscious perception in a wide variety of insects at both the juvenile and adult stage (Abe et al., 2014; Campbell and Nash, 1994; Gooley and Gooley, 2021; Lizé et al., 2010; Lum and Flaherty, 1972; Ma et al., 2022; MacMillan et al., 2017; McCallion et al., 2021; Ribbands, 1950; Sandstrom, 2004; Takami, 2003; Yang et al., 2016), there are no standardised protocols on the humane use of these anaesthetics for experimental manipulation or sacrifice. The lack of guidelines is a practical challenge for experimenters looking to adopt higher-welfare practices (although see Zemanova, 2020; database in Zemanova, 2022; for many non-lethal/non-invasive techniques).

The new challenges that considering insect welfare will bring in these contexts require interdisciplinary collaboration between ethicists, entomologists, comparative cognition scientists, animal welfare researchers, policy-makers, economists, industry and wildlife management professionals, in order to make decisions that protect the interests of all animals.



6. Conclusion

Using the Birch et al. (2021) framework, we reviewed the evidence for sentience (and specifically pain) in six insect orders across their development. We found “strong evidence” for pain experiences in adults of two orders, Diptera (flies and mosquitoes) and Blattodea (cockroaches and termites). There was also “substantial evidence” in adult Hymenoptera (bees, wasps, ants, and sawflies), Orthoptera (crickets and grasshoppers), and Lepidoptera (butterflies and moths), and “some evidence” in adult Coleoptera (beetles). Evidence was generally weaker for juvenile stages, but we nonetheless found “substantial evidence” in juvenile Blattodea and Diptera, as well as last instar Lepidoptera, and “some evidence” in juvenile Orthoptera. Juvenile Hymenoptera and Coleoptera, as well as first instar Lepidoptera, were categorised as “pain experiences unknown”. We found no good evidence against sentience, or specifically pain perception, in any insect orders at any life stage—lower ratings invariably reflected absence of evidence, rather than negative evidence. More insect research is crucial to address these knowledge gaps, as well as to investigate and minimise potential welfare issues. Overall, we hope this review convinces even sceptical readers that insect pain is plausible and deserves further study.

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Conflict of interest

Meghan Barrett reports a relationship with Rethink Priorities that includes: consulting.

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