Sentience in Cephalopod Molluscs: An Updated Assessment

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Abstract. This article evaluates the evidence for sentience - the capacity to have feelings - in cephalopod molluscs: octopus, cuttlefish, squid, and nautilus. Our framework includes eight criteria, covering both whether the animal's nervous system might support sentience and whether their behaviour indicates sentience. There is very strong evidence of sentience in octopuses, which satisfy seven of the eight criteria. There is less evidence for cuttlefish and squid (4/8 criteria), although this is still substantial evidence. By contrast, whether nautiluses are sentient remains unknown (1/8 criteria), since this group of cephalopods have attracted little research. This reflects a general pattern: when criteria were not satisfied, this invariably reflected a lack of positive evidence. In no cases were we confident that a taxon failed a criterion. In our review, we explore the nuances of evidence for sentience, examining both neural and behavioural markers. This paper draws from and updates our review (Birch et al., 2021), shedding light on the implications for ethical treatment and welfare within this class of animals while also revealing areas where further research is needed.

Keywords: Invertebrate, emotions, welfare, pain, nociception, octopus, cuttlefish, squid, nautilus, consciousness

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I | INTRODUCTION

Sentience, as defined for the purposes of this article, is the capacity to experience feelings that are pleasant (e.g., warmth, comfort, pleasure, excitement, joy) and unpleasant (e.g., hunger, thirst, boredom, distress, anxiety, pain). When defined in this way, sentience does not require sophisticated forms of cognitive awareness: a sentient being might not have the capacity to reflect on its own affective states or understand those of others. However, sentience does imply the brain monitors bodily states, experiences these states as pleasant or unpleasant, and then uses that assessment to guide action.

Are some invertebrates sentient? The case of cephalopod molluscs (octopus, cuttlefish, squid, and nautilus) has long been a source of both scientific interest and public concern. Cephalopod molluscs (or "cephalopods", for short) are drawing increasing interest from the scientific community as a research model and from the aquaculture industry as a food resource (O'Brien *et al.,* 2018; Vidal *et al.,* 2014). Since 2010, the European Union (EU) and United Kingdom (UK) have included all cephalopods within the scope of animal experimentation laws, implicitly recognizing them as sentient beings capable of feeling pain (and the UK took this step for *Octopus vulgaris* much earlier, in 1993). Cephalopods have also gained recognition in several national codes: Canada (1991), New Zealand (1999), Australia (2004), Switzerland (2011), and Norway (2011) (see Smith *et al.,* 2013 for details). In 2021, the report by Birch and colleagues (2021) provided critical evidence of sentience in cephalopods and decapods, which informed the UK government's decision to extend the Animal Welfare (Sentience) Bill to include these invertebrate groups. Most recently, recognition of sentience has extended to legislative efforts in the U.S., where the bipartisan Octopus Act (2024) seeks to ban octopus farming and the import of farmed octopuses due to ethical considerations. The state of Washington has already enacted such a ban, with California and Hawaii considering similar measures. Despite these regulations, cephalopods remain wholly unprotected in most jurisdictions. This background has made the cephalopods a highly important case-study for examining the question: what counts as evidence for or against sentience in an invertebrate animal, and why?

Past reviews have addressed these questions, reaching careful evaluations of evidence for sentience in cephalopods (Andrews *et al.,* 2013; Fiorito *et al.,* 2015). However, an updated review is warranted for two reasons. First, since 2015, a significant amount of new evidence has emerged. Second, earlier reviews were necessarily constrained by the available evidence at the time and could not provide a detailed comparative assessment across different cephalopod groups. This updated review presents an up-to-date evaluation of cephalopod sentience, considering differences across species and highlighting key evidence gaps. An initial version of the present review was included by the authors in a policy report (Birch *et al.,* 2021), but the present version, which aims to include all relevant evidence up to 2024, is intended as the version of record.

There are over 800 extant cephalopod species, and all are descended from hard-shelled ancestors (Jereb *et al.,* 2005; 2010; 2016). The nautiloids (subclass Nautiloidea, consisting between 7–11 species; Saunders, 2010; Ward *et al.,* 2015) retain this shell. By contrast, in the coleoids (subclass Coleoidea), informally known as the soft-bodied cephalopods, the shell has either been reduced and internalised or else eliminated altogether. The coleoids, and especially the octopus, have been the main topic of discussion in debates about sentience. As our review will show, the nautiloids have been comparatively neglected.

Although phylogeny is not the focus of this review, the most recent molecular phylogeny of cephalopods will guide our generalisation across species (Tanner *et al.,* 2017; Sanchez *et al.,* 2018). There is clear phylogenetic distinction between the octopus (order Octopoda consisting of some 300 species), in which no internal shell is present, and the other coleoids, in which there are internal shells of various kinds. Much of the relevant evidence concerning these other coleoids comes from cuttlefish (order Sepiida, consisting of some 100 species), with some evidence from various squid species (superorder Decapodiformes, consisting of some 350 species, including order Teuthoidea, order Sepiolida, and order Spirulida).

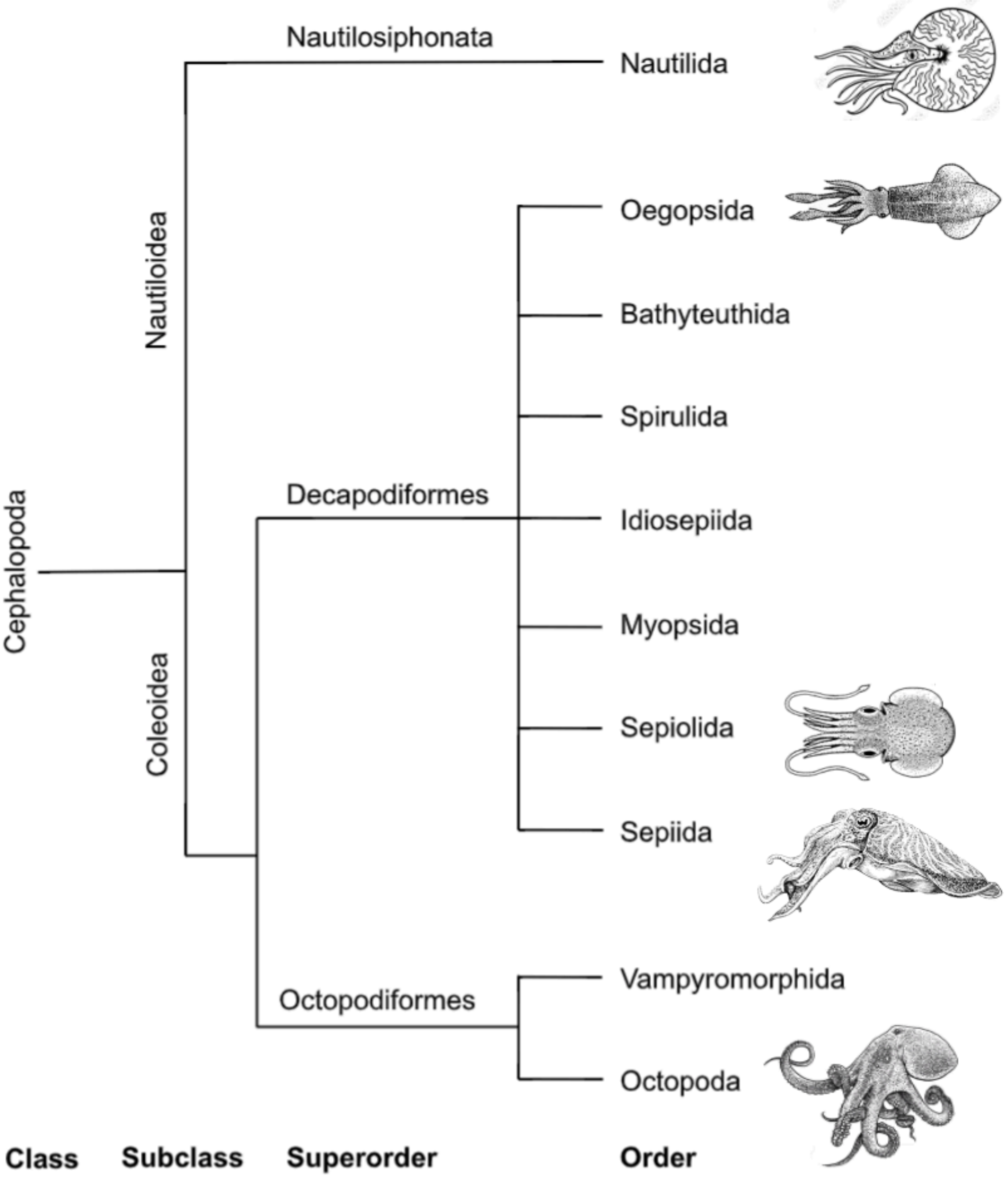

Figure 1. An overview of the main phylogenetic relationships among cephalopods, according to the molecular phylogeny of Tanner et al. (2017). There are over 750 extant cephalopods species in total.

II | A FRAMEWORK FOR ASSESSING EVIDENCE OF SENTIENCE

There are major challenges in conclusively proving the presence of sentience in any animal. However, in the case of mammals such as mice and rats there is a long tradition in science taking a pragmatic approach that considers a multitude of behavioural and neural markers.

These markers do not deliver conclusive proof (and are not intended to do so) but they do raise the probability of sentience to a level where it becomes irresponsible to ignore that possibility (NRC 2009). There is no justification for taking this approach *only* with mammals. We need a consistent attitude, whereby indicators that are accepted as relevant to questions of sentience in mammals are also accepted as relevant when found in other animals.

Past reviews have, of course, grappled with this question too, and they have proposed lists of relevant criteria. Our own framework adapts and improves upon an influential set of criteria first proposed in 1991 (Smith & Boyd, 1991). In this article, we use this framework to evaluate the evidence from cephalopods. However, the framework has been designed to allow fair evaluations of the evidence from a wide range of taxa. Separate articles apply our framework to the cases of decapod crustaceans (Crump *et al.,* 2022) and insects (Gibbons *et al.,* 2022). Here, we reviewed over 120 scientific studies to investigate whether cephalopods meet our criteria for sentience.

Our framework consists of eight criteria, intended to address the questions of whether the animal's nervous system has the right features to support sentience (criteria 1–4) and whether the animal's behaviour is consistent with a role for sentience (criteria 5–8). Even though sentience includes a wider range of affective states than just pain, the criteria are pain-focussed, because their purpose is to aid in the evaluation of the existing evidence, most of which has looked for markers of pain (we will return to this issue later, when discussing future directions). Our criteria are:

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. **Sophisticated associative learning:** The animal shows forms of associative learning in which noxious stimuli become associated with neutral stimuli, or in which novel ways of avoiding noxious stimuli are learned through reinforcement. These forms of associative learning go beyond classical conditioning in which a single conditioned stimulus overlaps temporally with an unconditioned stimulus. Note: forms of associative learning that are linked, at least tentatively, to sentience in humans (such as instrumental, reversal learning, and trace conditioning) provide stronger evidence than other forms.
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.

We have designed these criteria to provide a more inclusive and balanced approach to assessing sentience across diverse taxa, addressing and refining the influential framework first proposed by Smith and Boyd (1991). While we acknowledge that behavioural and cognitive criteria (criteria 5–8) hold particular importance for invertebrates, our framework also integrates neurobiological criteria (criteria 1–4) to ensure a balanced consideration of both cognitive/behavioural and neurobiological evidence (Crump & Birch, 2022; Edelman *et* *al.,* 2005; Edelman & Seth, 2009; Paul *et al.,* 2020). This approach allows us to build upon and improve criteria such as those developed by Sneddon et al. (2014) for animal pain, which, although valuable, contain redundancies and place heavier emphasis on behavioural over neurobiological criteria.

It is important to clarify that none of these criteria, when considered in isolation, conclusively demonstrates sentience. For instance, criterion 1, which could be met by a non-sentient animal, does not stand as a definitive marker. However, as part of a broader mechanism for detecting noxious stimuli – an underlying factor in the experience of pain in humans – the presence of nociceptors slightly raises the probability of an organism's capacity for pain. While criterion 1 cannot alone distinguish between nociception and experienced pain, criteria 2 and 3 help address this distinction. Collectively, every criterion contributes to the strength of the overall case for sentience.

Our criteria 1–3 retain elements from Smith and Boyd's (1991) criteria but shift the focus from "higher" and "lower" brain regions to the role of integrative brain regions. There is broad consensus in consciousness science that, in humans and other mammals, sentience depends on brain regions that integrate information from various sources (Firth, 2021; Seth, 2022). Although there is debate over which specific brain regions are crucial, the consensus suggests that sentience is more likely in species with such integrative regions. Further, the adaptive function of sentience plausibly lies in its ability to integrate different information sources, enabling animals to coordinate appropriate behavioural responses.

In revising Smith & Boyd's behavioural criteria (criteria 6 and 7), we have developed more detailed and rigorous cognitive and behavioural criteria (our criteria 5–8). These criteria are designed to align with many plausible views about the adaptive value of sentience, without committing to any specific theoretical perspective. They identify four primary types of cognitive and behavioural abilities that likely involve negatively valenced affective states: motivational trade-offs, flexible self-protective behaviour, sophisticated associative learning, and behaviours indicating the value of analgesics or anaesthetics when injured (e.g., self-administration, conditioned place preference, or prioritisation).

While our behavioural criteria are more concrete than those of Smith and Boyd (1991), they are intentionally less extensive than those proposed by Sneddon and colleagues (2014), to avoid treating similar behaviours as independent lines of evidence. For example, Sneddon and colleagues list “self-administration of analgesia” and “pay cost to access analgesia” separately, whereas our criterion 8 treats these as different ways of meeting the same criterion. Our criteria also make a clearer distinction between nociceptive and pain-related behaviours. For instance, a nociceptive reflex could satisfy Sneddon et al.’s “movement away from noxious stimuli” (Campbell *et al.,* 1991), but the behavioural part of our framework demands evidence of capacities going beyond reflexes.

Our criteria also allow for the interpretation of empirical evidence on a case-by-case basis. Flexibility in behaviour, for example, does not necessarily imply a capacity for planning ahead or reflection; rather, it may indicate an ability to respond adaptively in a variety of ways to the same noxious stimulus depending on the situation. This kind of flexibility, which can vary between individuals and change if a response proves ineffective, contrasts with fixed, reflexive behaviour that is species- and context-specific. However, even simple organisms like sea anemones show some flexibility, such as reflexes that can be inhibited by another stimulus (Haag & Dyson, 2014). Therefore, criteria 5 and 6 emphasize specific *types* of flexibility likely to involve centralized, integrative information processing.

Criterion 7 focuses on sophisticated associative learning, which may be facilitated by sentience through the ‘labelling’ of previously neutral stimuli or behaviours with positive or negative value. This capability allows animals to respond more beneficially in future similar situations. However, simple forms of associative learning may occur unconsciously, even in humans (Greenwald & De Houwer, 2017), leading to ongoing debate about the link between associative learning and sentience (Birch, 2020; Birch *et al.,* 2020*a*). More complex forms of learning, such as instrumental learning (Skora *et al.,* 2021) and trace conditioning (Clarke *et al.,* 2002), are more strongly associated with sentience, and so our framework gives special weight to these abilities. But rather than expressing a fixed view on which learning abilities are most relevant, the framework calls for close attention to the latest findings from consciousness science, where new results are appearing regularly.

While physiological stress responses to noxious stimuli have been proposed as evidence of animal sentience (Sneddon *et al.,* 2014), we have chosen not to include stress-based criteria in our framework. This decision is based on the understanding that physiological stress is neither a necessary condition for pain nor always associated with pain (Stevens *et al.,* 2016). Although stress responses can sometimes help explore other criteria, such as in studies using anxiolytic drugs (Fossat *et al.,* 2014), our criteria focus more on behavioural and cognitive evidence. Our framework prioritizes more direct and consistent evidence of cognitive and behavioural responses that are more clearly associated with the animal's subjective experience of pain. We note the distinction between physiological and psychological stress, and that psychological stress can be part of the pain experience.

Finally, these criteria, while more precisely worded than previous attempts, still leave room for further scientific debate (*e.g.,* what kinds of self-protective behaviour are likely to involve representing the bodily location of a noxious stimulus?). The aim is not to conduct an algorithmic evaluation of the evidence that shuts down debate and leaves no role for expert judgement. On the contrary, many subtle scientific judgements are involved in applying these criteria, and our framework aims to facilitate (not circumvent) debate about these judgements.

III | Review of the Evidence

Questions of sentience are subject to substantial uncertainty. We aim to convey that uncertainty, not suppress it. The language of confidence levels is a helpful shorthand for this purpose. It provides a way of communicating our overall qualitative assessment of the evidence regarding each criterion. To be precise, we will use the following terminology:

> Very high confidence: The weight of scientific evidence leaves no scope for reasonable doubt that the animal satisfies/fails the criterion.
> High confidence: There is high-quality evidence that the animal satisfies/fails the criterion, and either no countervailing evidence or else the countervailing evidence is clearly outweighed.
> Medium confidence: There are concerns about the reliability, quality or quantity of the evidence, or there is countervailing evidence that is not clearly outweighed, preventing us from having high confidence that the animal satisfies/fails the criterion.
> Low confidence: There is some relevant evidence, but not enough high-quality evidence to allow even medium confidence that the animal satisfies/fails the criterion, or else the evidence is too finely balanced to allow even medium confidence.
> No research found: There is no relevant evidence that can be used to evaluate this question.

3.1 Criterion 1: Nociceptors

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

Nociceptors are “high-threshold sensory receptor[s] of the peripheral somatosensory nervous system that [are] capable of transducing and encoding noxious stimuli” (International Association for the Study of Pain, 2017). Unlike other sensory receptors, nociceptors have relatively high thresholds before they fire. This means that they are only activated by extreme (e.g., intense, prolonged, or repeated) stimuli, thus representing an actual or potential threat of tissue damage. Some nociceptors cannot be activated unless they are sensitised by inflammatory molecules, which are released when tissue is damaged (Smith & Lewin, 2009). As specialist detectors of noxious stimuli, nociceptors are necessary – although not sufficient – for pain.

There are different types of nociceptors. Some respond to extreme mechanical, heat, cold, chemical, or light stimulation, whilst others are polymodal, meaning they respond to two or more classes of stimuli (Sneddon *et al.,* 2014; Walters, 2018). Nociceptors can also vary in how quickly they respond to stimuli, with some only responding to prolonged stimulation. Several earlier reviews have concluded that the presence of nociceptors in cephalopods is “likely, but not proven” (Andrews *et al.,* 2013; della Rocca *et al.,* 2015; Fiorito *et al.,* 2015), but these predate the more recent experimental work described below.

Hague and colleagues (2013) found that severed arms of *Octopus vulgaris* show rapid reflex withdrawal responses to noxious stimuli (forcep pinches, fresh water, and acetic acid) but not innocuous stimuli (gentle touch and seawater). These results complement early findings by Rowell (1963), who noted that severed arms showed immediate reflexive full withdrawal when encountering noxious stimuli, as compared to merely skin flinching and orientation of the suckers in response to lighter pricking. Altman (1971) also observed that amputated and denervated octopus arms would withdraw from food pieces treated with quinine hydrochloride. An early study in octopus (*O. vulgaris*) arms found some neurons that only fired in response to forcefully applied mechanical stimuli such as blows or pinches (Rowell, 1966).

Several more recent studies have looked directly at neural firing in response to tissue damage or noxious stimuli, in both octopus and squid. Crook and colleagues (2013) demonstrated mechanosensitive nociceptors in the fin of squid (*Doryteuthis pealeii*, also known as *Loligo pealeii*). These nociceptors only activated in response to filaments that produced tissue damage and were sensitised by both these stimuli and by crush injuries to the fin, an effect that injection of local anaesthetic suppressed. Sensitisation occurred across the whole body, rather than just as a localised response, which may suggest induction of a general cautious state rather than specific wound-tending (see criterion 6).

These tests were performed on both attached and excised fins. When the fin was attached, squid showed behavioural sensitisation (increased escape response) after an injury. Spontaneous neural activity continued for at least 24 hours following injury, but only in attached fins, suggesting necessary engagement with other parts of the body or nervous system. These findings were supported by Howard et al.'s (2019) recent study on bobtail squid *Euprymna scolopes* (order Sepiolida). Sensitisation of the peripheral nerves occurred after crush injury, as well as lasting lifetime neural excitability in animals injured in their early life.

Alupay et al. (2014) and Perez et al. (2017) found similar results in octopuses (*Abdopus aculeatus* and *Octopus bocki*, respectively). Alupay and colleagues applied a crush injury to the arms and observed an immediate behavioural response, as well as a decreased sensory threshold for response to subsequent stimuli on both these arms (as well as nearby arms) and in whole-body responses for the 24 hours after injury. The arms were then removed to test neural firing. The authors identified neurons that only fired in response to noxious stimuli, as well as increased sensitisation on injured arms and those nearby (increased neural firing in response to the 'damaging' but not the 'light' filaments). Measurements were taken at the axial nerve cord, implying that information from arm mechanosensors was being passed through to at least this part of the central nervous system.

Similarly, Perez et al. (2017) again found that octopus have neurons that show short-term sensitisation and spontaneous firing after crush injury in the mantle. Their measurements were taken at the pallial nerve, the primary nerve connecting the mantle to the brain. In a study of the Hawaiian bobtail squid, *Euprymna scolopes* (order Sepiolida), Bazarini and Crook (2020) found increased firing rates in the pallial nerve in response to noxious stimuli.

Recently, Crook (2021) took electrophysiological measures of the brachial connectives (which connect arm nerve cords to the brain) in Bock's pygmy octopus (*O. bocki*). This revealed ongoing activity after applying a noxious stimulus (injected acetic acid), which an anaesthetic (lidocaine) silenced – strong evidence that these signals are relayed from the arms to the central nervous system. A new study by Crook (2022) on bobtail squid (*Euprymna*) has demonstrated the first direct visualisation of mechanoreceptive and mechano-nociceptive neurons firing in response to firm touch in a localized area on the body surface. The results suggest that the stellate ganglion (collection of nerves) in the squid contains key neurons involved in mechano-afferent signalling to the central nervous system. Early research laid the foundation for understanding cephalopod neural pathways, with studies identifying excitatory and inhibitory pathways in the octopus arm (Rowell 1963; 1966) and investigating motor projections from the stellate ganglion (Bühler *et al.,* 1975). However, Crook (2022) provides the first evidence of somatotopic organization in a cephalopod - where neurons in the stellate ganglion of the squid are approximately grouped to support efficient neuronal processing, decrease axon tangling, and permit neighbour-type computations such as contrast enhancement (i.e., neighbouring neurons can connect in way that perceiving stimuli intensity can be heightened in the region it occurs and suppressed in surrounding areas). This may support the efficient processing of sensory inputs, potentially including those related to nociception. Further, this arrangement of neurons might be designed to enhance the processing of sensory information, which could be crucial for responding to noxious stimuli.

There is also molecular evidence of nociceptors in octopus arms. In a detailed study of *O. vulgaris*, di Cristina (2017) found markers associated with detecting noxious stimuli in the arm tips. Di Cristina observed "putative nociceptive fibres" running along the axial nerve of the arm. These results suggest the presence of peripheral nociceptors and their connection to the central nervous system. We note, however, that these results are reported in a PhD thesis, rather than a peer-reviewed journal.

The presence of nociceptors in other related species can also serve as evidence of nociception, via evolutionary/phylogenetic reasoning (Andrews *et al.,* 2013), since nociceptive processes appear highly conserved across taxa. Crook and Walters (2011) and Walters (2018) describe evidence for nociception in a range of molluscs, primarily gastropods. For example, the gastropod mollusc *Aplysia* has nociceptors. The presence of nociceptors in other molluscs makes their presence in cephalopods more likely. Ecological considerations also speak in favour of the presence of nociceptors in cephalopods. As soft-bodied, mobile animals, cephalopods are at great risk of damage and predation, but they also have the capacity to avoid or escape, so nociception would be highly beneficial to these animals.

Finally, there is indirect behavioural evidence of nociceptors. Octopus learn to avoid noxious stimuli, suggesting they can differentially detect and process these inputs (see criterion 7).

For example, Ross (1971) observed that octopus (*O. vulgaris*) would learn to avoid hermit crabs with sea anemones on their shells. Contact with the stinging anemones would trigger retreat behaviour and the octopus would not eat these crabs. However, behavioural evidence will be considered later, under other headings, so here we focus on neurophysiological evidence.

In summary, there is high-quality evidence that octopods (particularly *O. vulgaris*) and squid have afferent sensory neurons that respond differentially to noxious stimuli, and which undergo sensitisation and show spontaneous activation following exposure to noxious stimuli. Octopods also have molecular markers of nociceptors in their arms. We, therefore, have very high confidence that octopods (order Octopoda), myopsid squid (Myopsida) and bobtail squid (Sepiolida) satisfy criterion 1. Based on evolutionary considerations and evidence from non-cephalopod molluscs with much simpler nervous systems, we have high confidence that other cephalopods also satisfy criterion 1, including other squid, cuttlefish (order Sepiida) and nautiloids (Nautilida).

3.2 Criterion 2: Integrative Brain Regions

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

Sensory integration is a core function of sentience (Dehaene & Changeux, 2011; Palmer & Ramsey, 2012; Tononi, 2004). The human brain unifies information from different sensory modalities to generate a single subjective point-of-view (Birch *et al.,* 2020*b*). In non-human animals, too, integrative brain regions might plausibly underpin sentience. Such brain regions can further illustrate that stimulus responses are more complex than simple reflexes.

Coleoid cephalopods have a brain-to-body ratio higher than most fish and reptiles (Packard, 1972). Early studies on *Octopus vulgaris* (reviewed in Wells, 1978; Young, 1963*a*), *Loligo* squid (Messenger, 1979; Young, 1974; 1976; 1977; 1979) and *Sepia* cuttlefish (Boycott, 1961; Sanders & Young, 1940) outlined the structure of the cephalopod nervous system and central brain, which most subsequent research rests on. The structure and organisation of the coleoid cephalopod brain is now well documented (reviewed in Budelmann, 1995; Hochner, 2012; Hochner *et al.,* 2006; Shigeno *et al.,* 2018; Zullo *et al.,* 2009; Zullo & Hochner, 2011).

In octopuses, the brain consists of ~170 million nerve cells, with 130 million in the optic lobes and 40 million in the central brain. It has a complex structure, made up primarily of the sub- and supra-oesophageal masses (both containing numerous lobes, around 30 in total; Nixon & Young, 2003), as well as the optic lobes. There is clear hierarchical organisation and high connectivity between centres. The sub-oesophageal mass (~40 million nerve cells) is primarily a lower motor control centre and plays a crucial role in coordinating arm movements. The supra-oesophageal mass contains intermediate and higher motor control centres, as well as memory and learning centres. The supra-oesophageal mass probably plays a role in resolving potential conflicts between input and action patterns on each side of the body. The higher motor centres connect to the lower for input and output.

Shigeno et al. (2018) draws structural and functional analogies between regions of the cephalopod brain and the vertebrate brain. The sub-oesophageal mass is roughly equivalent to the vertebrate spinal cord, and other regions of the supra-oesophageal mass to the hypothalamus, thalamus, basal ganglia, and cerebellum. Of greatest interest is the frontal-vertical lobe as an analogue of the cerebral cortex, hippocampus, and amygdaloid complex. This lobe is important in learning and memory, and probably in evaluation and decision-making (Young, 1963*b*; 1991).

The vertical lobe is often described as the ‘highest’ cephalopod brain centre, analogous to the mammalian hippocampus (Maldonado 1965; Hochner *et al.,* 2006; Shomrat *et al.,* 2015; 2018; Shigeno et al. 2018). It contains ~25 million of the brain’s 40 million cells (Shomrat *et al.,* 2015). It also appears to have a distinct cell type: Small cells hypothesised to have an inhibitory function (Young, 1963*a*). Brown and Piscopo (2013) found that there is distinct synaptic plasticity within the vertical lobe of cephalopods, a feature associated with the learning and memory centres of vertebrates.

The vertical lobe system receives various inputs from the entire body, including eyes, arms, mouth, and mantle (Young, 1979). There is evidence for integration across senses, since *O. vulgaris* can combine peripheral arm information with visual information to guide movement in a maze task (Gutnick *et al.,* 2001). New research on the vertical lobe in *O. vulgaris* has characterised and localized a rich array of neuropeptides and neuromodulators, which are likely involved in transmitting punishment (i.e., pain) and reward signals (Stern-Mentch *et al.,* 2022). The study reveals that the network in the vertical lobe is more complex than previously thought (previously described in Shomrat *et al.,* 2011; Ponte & Fiorito 2015; Shigeno & Ragsdale 2015). Specifically, the interneurons (amarcine cells) appear to be functionally and chemically heterogeneous. Moreover, the vertical lobe’s capacity to control behaviour appears to be intricately governed by both excitatory and inhibitory pathways, which provides elaborated pathways (i.e., a process where material is extended or added to make information more memorable) for memory acquisition (Stern-Mentch *et al.,* 2022). Most of this evidence regards the octopus, but similar findings have been seen across coeloid taxa. The main differences are that octopus brains are more centralised, while cuttlefish and squid have larger optic lobes (Budelmann, 1995; Boycott, 1961; Packard, 1972). Squid and cuttlefish also have a reduced inferior frontal lobe system, inferior tactile discrimination and learning (Young, 1991), and a structurally different vertical lobe complex (Young, 1979). Nautiloids appear to have simpler brains with quite complex structures containing multiple lobes, but lack the 'higher' brain structures associated with learning and memory (Budelmann, 1995). Even so, Nixon and Young (2003) suggest that the cerebral cord may function as a 'higher' integrative centre.

An unusual feature of cephalopod neuroanatomy is the peripheral distribution of processing. The peripheral nervous system makes up almost two-thirds of the total number of neurons, with ~300 million cells in the arm cords (Young, 1963*a*). There is relatively low connectivity between the brain and the periphery, indicating a lot of peripheral processing, while the central brain is mainly responsible for coordinating information and decision-making (Hochner, 2012). The arm cords appear to act as reflex centres for the individual arms, in some sense elaborating on orders received from the brain (Wells, 1978). However, the central brain is still highly sophisticated.

Overall, there is extremely strong evidence that coleoid cephalopods have complex, centralised brains that integrate different types of information, including nociceptive. Although no structure has been identified as a direct analogue to the mammalian cerebral cortex, the vertical lobe is the coleoid brain centre responsible for learning and memory. We, thus, have very high confidence that coleoid cephalopods (octopods, squid, cuttlefish) satisfy criterion 2. The vertical lobe is absent in nautiloids, so we have low confidence that they fulfil this criterion.

3.3 Criterion 3: Integrated Nociception

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

This criterion links criterion 1 (Nociceptors) and criterion 2 (Integrative Brain Regions). Here, we are looking for evidence of neural pathways between the two, which would demonstrate centralised, integrative processing of nociceptive information.

In multiple studies reviewed under criterion 1, electrophysiological measurements were taken at the nerve cords linking peripheral nerves to the central brain (Alupay *et al.,* 2014; Bazarini & Crook, 2020; Crook, 2021; Crook *et al.,* 2013; Howard *et al.,* 2019; Perez *et al.,* 2017). These found increased activity in response to noxious stimuli demonstrating that nociceptive information reaches the central nervous system in both squid and octopus. Despite compellingly showing that signals from nociceptors reach the brain, this evidence does not show that signals reach the vertical lobe system. Past research has documented many connections between the peripheral nervous system and the vertical lobe, but it has tended to assume (rather than explicitly demonstrate) that these connections transmit nociceptive information.

The severed arms of *O. vulgaris* quickly withdraw in response to noxious stimuli (forcep pinches, fresh water, and acetic acid) (Hague *et al.,* 2013). These were severed arms and thus not connected to the central nervous system. Clearly, the presence of nociceptors in a severed arm, while not irrelevant to questions of sentience, could only ever be a small part of the picture. However, Hague and colleagues also found that severing the axial nerve cord in the arm eliminated the response, suggesting a connection to more central pathways.

Patterns of connectivity have also been suggested in squid, *Loligo pealeii* (Crook *et al.,* 2013). After individuals sustained fin crush injuries, spontaneous neural activity persisted for at least 24 hours, but was observed only in the attached fins, not in the severed ones. This suggests that ongoing neural activity requires the integration of signals from other parts of the body or nervous system. Measurements taken at the fin nerve, which connects the fin nerve branches to the brain, suggest the presence of pathways connecting peripheral nociceptors to the central nervous system.

When discussing the functions of the brain lobes, Young (1963*a*) refers to an input to the brain which is "presumed to be of nocifensor (pain) fibres". However, this is hypothesised based on functional rather than structural considerations. Young (1979) describes several afferent pathways to the vertical lobe system as possibly conveying nociceptive signals, and

Nixon and Young (2003) similarly assume that the vertical lobe system processes pain signals from the body. Young (1991) describes the connectivity of the nervous system, including afferent fibres connecting the arms to the lateral inferior frontal lobe, which then progress to the superior frontal and vertical lobe system. Although this is not directly related to nociceptors, he presumes that pain/trauma signals are part of this pathway. Budelmann and Young (1985) found that afferent fibres from the arms pass through to the frontal and subvertical lobes (though not the vertical lobe; information is taken to be passed there from these lobes) and speculate that they could relate to nociception. There is high connectivity between brain regions, particularly between the 'lower' control regions of the sub-oesophageal mass and the 'higher' supra-oesophageal mass (e.g., Shigeno *et al.,* 2018), but this is not direct evidence of nociceptive signals being transferred.

The distributed nature of the cephalopod nervous system further complicates this picture. Many peripheral afferent nerves (particularly in the arms) do not connect directly to the central nervous system, but instead to the central ganglia in the arms, which then pass reduced information to the brain (di Cristina, 2017). Around 140,000 afferent neurons transmit sensory information from the arms to the central brain (Hochner, 2012; Levy & Hochner, 2017; Zullo *et al.,* 2019), though not all necessarily project directly. Some of these neurons contribute to pathways associated with the frontal lobe system (Budelmann & Young 1985; Nixon & Young, 2003), however, what type of information is lost in this 'compiling' and what is transmitted needs further investigation.

Studies on anaesthesia offer one potential source of information (see also criterion 4). Local and general anaesthetics shut down both afferent and efferent neural signals to/from the brain (Butler-Struben *et al.,* 2018). As the stimuli involved were potentially-noxious forcep pinches, this suggests cessation of nociceptive transmission. The lack of response to other surgical procedures under anaesthetic is also suggestive, though care must be taken to separate immobility effects from true anaesthesia and loss of sensation.

A recent study by Crook (2021) on *Octopus bocki* recorded electrophysiological activity in the brachial connectives, which connect the arm nerve cords to the brain. These revealed a prolonged period of activity, which an injection of anaesthetic (lidocaine) silenced (this study is primarily discussed under criterion 8).

Behavioural evidence also suggests that information about noxious stimuli is processed in central brain regions. For example, because of sophisticated behavioural responses to noxious stimuli in their tests, Alupay et al. (2014) infer that perception of noxious stimuli in the arms and mantle was conveyed to "higher processing centres". However, this evidence is discussed under other headings, and (as in Section 2.1) our focus is neurophysiological evidence in this section.

Past reviews of the evidence for connections between nociceptors and the vertical lobe conclude it is "uncertain" (Andrews *et al.,* 2013) or "likely, but not proven" (Fiorito *et al.,* 2015; Zarrella *et al.,* 2015). We agree with these assessments. In our framework, we have high confidence that there are such connections, but not very high confidence.

A recent study by Crook (2022) on bobtail squid (*Euprymna*) has provided functional evidence to demonstrate that the stellate ganglion is organized somatotopically (i.e., topographic sensory and motor representation of different body parts). This finding suggests that somatotopy might be found in higher circuits because organizational consistency is likely maintained throughout lower to higher sensory centres (i.e., PNS and CNS). However, this study does not imply central processing or direct connectivity to integrative brain regions. Whilst ascending pathways of nociceptive circuits are somatotopic in vertebrates, we cannot assume that the existence of somatotopy in the stellate ganglion of squid suggests that there are also affective circuits processing that information in the central brain. Further research is required to determine the circuit pathways in squid.

In summary, there is indirect evidence for connections between cephalopod nociceptors and integrative brain regions. The peripheral nervous system is highly connected to the central brain, and the different lobes of the brain are also very well connected. These pathways could relay nociceptive signals to integrative brain regions, but this has not yet been demonstrated beyond doubt. As such, we have high confidence that coleoid cephalopods (octopods, cuttlefish, and squid) satisfy criterion 3. More neurophysiological evidence would be required for very high confidence. Since there is no direct evidence of integrated nociception in nautiloids, we have low confidence that they satisfy criterion 3.

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. *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 actual noxious stimuli in a way consistent with the hypothesis that these compounds attenuate the experience of pain, distress or harm*

Neurotransmitters are signalling molecules that cross the synapses between neurons. Endogenous neurotransmitters are produced in the body, whereas exogenous compounds are introduced from outside. This criterion may indicate sentience because, in humans, endogenous modulation of nociception can affect pain (Morélot-Panzini *et al.,* 2014; Rhudy *et al.,* 2006; 2018). Therefore, mammal researchers quantify pain by measuring behaviours susceptible to endogenous modulation (Sadler *et al.,* 2022). Rodent examples include reduced feeding (Negus *et al.,* 2010), locomotion (Negus *et al.,* 2010), and burrowing (Jirkof *et al.,* 2010). Exogenous compounds can also reveal a shared neural basis for felt experiences. We are especially interested in whether anaesthetics, analgesics, anxiolytics, or anti-depressants produce behaviours consistent with (respectively) unconsciousness, pain, anxiety, and depression.

Past cephalopod reviews have concluded that an endogenous neurotransmitter system managing responses to injury is likely (Andrews *et al.,* 2013; Fiorito *et al.,* 2015; Zarrella *et al.,* 2015) but cephalopod brains have many identified neurotransmitters (Ponte & Fiorito 2015), and none have yet been implicated in responses to noxious stimuli.

There is some evidence for opioids and similar compounds (enkephalin-like peptides) in octopus. For instance, immunohistochemical studies have identified Leu-, met-, and delta-enkephalin in *Octopus ocellatus* (Sha *et al.,* 2012), which bind to opioid receptors in the brain and delta opioid receptors in the peripheral nervous system, suggesting their role in nociceptive pathways. Martin et al. (1979) used antibodies to identify Met-enkephalin-like proteins in the octopus vena cava, but the opioid antagonist naloxone did not affect their action (Voight *et al.,* 1981). Stefano et al. (1981) found that opioids (morphine and met-enkephalin) suppressed dopamine release in octopus (*Octopus bimaculatus*) brain tissue. Naloxone reversed the effect, implying mediation by opioid receptors. However, Frazier et al.

(1973) found that opioids and antagonists both played the same inhibitory role on the squid (*Loligo pealei*) axon, suggesting that the opioids were not acting as analgesics.

In a PhD thesis, Di Cristina (2017) found transcripts designated as opioid receptors and opioid-like peptides in the suboesophageal mass and optic lobe in the *Octopus vulgaris* brain, implying a pain-modulating system. However, as these molecules can play multiple roles apart from modulating responses to noxious stimuli, further work is needed on their effects, including the effects of opioid-antagonists such as naloxone.

Through phylogenetic reasoning, the fact that opioid receptors are widespread and highly conserved across vertebrate and invertebrate taxa is reason to think they are present in cephalopods (Andrews *et al.,* 2013, though cf. Crook & Walters, 2011). However, even if this were the case, we would still need further evidence that the system modulates nociceptive pathways.

Notwithstanding the typical focus on opioids, other compounds such as cannabinoids or steroids may function as endogenous modulators for nociceptive processing (Andrews *et al.,* 2013). Studies on other molluscs suggest that, although enkephalins were not promising, FMRFamide may be a good candidate for nociceptive signalling (Crook & Walters, 2011). FRMFamide is primarily known as a neuropeptide, but its role in nociception remains unclear and warrants further exploration. Loi & Tublitz (1997) identified FRMFamide-like proteins in the brains of cuttlefish (*Sepia officinalis*), but only in the role of chromatophore regulation. Wollensen et al. (2008) found FMRFamide-like immunoreactivity throughout the brain of pygmy squid (*Idiosepius notoides*). di Cristina (2017) found transcripts of genes for FMRFamide receptors in *O. vulgaris* brain and body tissues. These findings collectively suggest that FMRFamide may play a broader role in cephalopod physiology, yet more research is needed to clarify its precise function in nociception.

Endogenous oestrogens modulate nociceptive processing in mammals, and some evidence hints at a similar phenomenon in cephalopods. Bazarini & Crook (2020) examined the role of oestrogens in processing and responding to noxious stimuli in Hawaiian bobtail squid (*E. scolopes*). Environmental oestrogen exposure altered behavioural responses to noxious (fin crush) and potentially threatening (vibration) stimuli by lowering responsiveness to the former and creating hypersensitivity to the latter. Oestrogen exposure also impaired sensitisation of neural firing in response to injury. These results suggest that oestrogens modulate nociceptive responses in this species. However, this result alone is not enough to conclude that squid satisfy criterion 4.

Serotonin is involved in nociceptive sensitisation following noxious stimulation in molluscs, and modulating nociceptive signals in vertebrates (Perez *et al.,* 2017). Octopus (*Octopus bimaculoides*) have serotonin transporter binding sites orthologous to those in humans (Edsinger & Dölen, 2018). Perez et al. (2017) tested the effect of fluoxetine (a serotonin reuptake inhibitor that increases the serotonin concentration) on neural nociceptive responses in Bock's pygmy octopus (*O. bocki*). Fluoxetine treatment increased rates of spontaneous firing after injury, though neural sensitisation was not affected. Elevated serotonin levels may, therefore, enhance neural and behavioural responses to tissue injury and spontaneous firing may play a role in injury guarding and escape behaviours. However, as these tests involved prepared tissue samples from euthanised animals, they only show changes in afferent firing, rather than changes in the brain. This cannot tell us that fluoxetine attenuates an experience of pain, distress, or harm in a live animal.

Serotonin also appears to modulate learning in octopuses, because it is active in the vertical lobe (Shomrat *et al.,* 2010). This may occur through modulating signals for reward/punishment (Shomrat *et al.,* 2015), which could signal involvement in nociceptive pathways and decision-making, but we cannot yet be confident of this.

Zarrella et al. (2015) describe a range of genes that increase or decrease expression in response to fear conditioning (e.g. genes for stathmin, tyrosine hydroxylase, dopamine transporter, octopressin, cephalotocin). In particular, an increase in stathmin under innate and learned fear responses suggests it plays a similar role in cephalopods as in the vertebrate amygdala during fear memory formation and expression of fear responses.

The efficacy of different anaesthetic agents has been investigated in various cephalopod species (also discussed in Lopes *et al.,* 2017). Both ethanol and magnesium chloride induced general anaesthesia in juvenile octopus (*Octopus maya*) but this was only measured through monitoring respiratory rates (Roumbedakis *et al.,* 2020). A different study investigated local and general anaesthesia in cephalopods (including *Sepia bandensis, Octopus aculeatus, Octopus bocki*) (Butler-Struben *et al.,* 2018). Of particular relevance to criterion 4, lidocaine and magnesium chloride were effective local anaesthetics, suppressing activity in the peripheral nervous system (pallial nerve) as measured by electrodes. Another study on juvenile pharaoh cuttlefish (*Sepia pharaonis*) demonstrated that magnesium chloride reduced the content of excitatory neurotransmitter Dopamine by increasing the content of tyrosine and thereby achieving anaesthetization. Magnesium chloride also indirectly increased the content of inhibitory neurotransmitter glycine in the juvenile cuttlefish (Yang *et al.,* 2020). Other anaesthetics tested on marine animals proved ineffective; clove oil did not meet the criteria for general anaesthesia, and MS-222 was unable to anesthetize cuttlefish and also induced a strong stress response. However, this does not necessarily constitute negative evidence for the criterion, as the failure of specific anaesthetics does not equate to a general lack of response to anaesthesia. Note that the use of MS-222 in cephalopods could interfere with the pH of the solution, as MS-222 is acidic, which may cause discomfort or stress and affect the results.

More recently, a study on two temperate species (*Octopus bimaculoides* and *Sepia officinalis*) showed that both ethanol and magnesium chloride can successfully anaesthetize adult and senescent animals. Indeed, both agents were effective at depressing activity in the pallial nerve, which provides neural network control for breathing and skin patterning on the side of the mantle (Abbo *et al.,* 2021). Finally, a study on the embryos of common squid (*Loligo vulgaris*) has shown that both ethanol and magnesium chloride had depressive effects on both heart and respiratory rates after immersion (Sprecher *et al.,* 2022; see also Young 1971). However, these studies did not link the local anaesthetic to behavioural responses to injury.

Recent research provides this missing piece of the puzzle. One study demonstrates that lidocaine abolished injury-directed grooming behaviour in Bock’s pygmy octopus (*O. bocki*), discussed in greater detail under criterion 8 (Section 2.8). Similarly, another study shows that pharaoh cuttlefish (*S. pharaonis*) reduced injury-directed grooming behaviour when administered lidocaine (Kuo *et al.,* 2022). Collectively, these studies convincingly demonstrate the effectiveness of lidocaine in modulating responses to noxious stimuli in octopods and cuttlefish, satisfying criterion 4b. A recent study shows, for the first time, evidence for systemic analgesia in a cephalopod (Deustche *et al.,* 2023). Specifically, analgesics from several different drug classes (ketorolac, dexmedetomidine, and buprenorphone) appear to be effective in the Hummingbird bobtail squid (*Euprymna berryi*). The three drug classes were administered, at similar doses to those used in fish, and revealed promising effects on baseline nociceptive thresholds, excitability of peripheral sensory nerves and behavioural responses to noxious stimuli. The same study demonstrated that bobtail squid can be anaesthetised using a mix of ethanol and magnesium chloride, akin to octopods and cuttlefish.

We have found no work exploring the effects of anxiolytics in cephalopods. However, some studies have examined the effects of antidepressants. In *Sepia officinalis,* venlafaxine exposure has been linked to neurochemical changes, altered brain development, and impaired camouflage ability (Bidel *et al.,* 2016). Additionally, fluoxetine and venlafaxine exposure during early development have been found to delay predatory behaviour maturation, reduce food intake, and impact growth (Chabenat *et al.,* 2021). Separately, Edsinger & Dölen (2018) found that MDMA increased social behaviour in octopuses (*Octopus bimaculoides*), but no work was done on whether MDMA affects decision-making or responses to noxious stimuli.

In conclusion, there is evidence for the relevant endogenous neurotransmitters and receptors (including enkephalins, oestrogen and serotonin) in cephalopods, but these have not been directly linked to nociceptive pathways. Research also points to magnesium chloride and ethanol acting as a local anaesthetic in octopods, cuttlefish, and squid. The recent notable studies in octopus and cuttlefish demonstrates a local anaesthetic (lidocaine) modifies responses to noxious stimuli. The study in bobtail squid showed evidence for systemic analgesia with three distinct classes of drugs. Studies on fluoxetine and venlafaxine in common cuttlefish indicate that serotonergic modulation can influence cognitive and behavioural processes, highlighting the need for further research into how the serotonergic system affects decision-making and pain-related responses. We, therefore, have high confidence that all coleoid cephalopods satisfy criterion 4. However, due to lack of direct research on analgesia in nautiloids, we have low confidence that they satisfy the criterion. Further research is required to refine analgesic dosing in sepiolid squids and to test whether octopods and cuttlefish respond similarly to the effects of analgesics.

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

If an animal responds to noxious stimuli using simple nociceptive reflexes, the same stimulus should always elicit the same behaviour. However, if competing motivations affect the animal's response, it cannot be a simple reflex (Appel & Elwood, 2009; Elwood & Appel, 2009). This suggests that the response may also involve pain. Moreover, a proposed function of feelings, such as pleasure and pain, is to serve as a "common currency", which enables animals to weigh disparate motivations and make flexible, context-dependent decisions (Cabanac, 1992). For this criterion, then, we are looking for robust evidence that an animal is motivated to avoid a noxious stimulus, and that this motivation is *weighed* (*traded off*) against other motivations in a flexible decision-making system. This requires more than a simple change in one behaviour in the presence of a competing motivation, but a more flexible response that can change contextually as the strength of the different motivations change.

Although various studies have investigated cephalopod behaviour in response to environmental stimuli, there is no clear-cut evidence demonstrating motivational trade-offs. The following sections summarise research findings that explore potential competing motivations, as well as those that may be better explained by sensitisation responses.

Potential Competing Motivations

Wilson et al. (2018) examined the behaviour of common cuttlefish (*S. officinalis*) when exposed to infrasonic pulses mimicking predatory attacks. The study found that food-deprived cuttlefish exhibited significantly higher escape thresholds at 3 Hz but not 9 Hz, suggesting a potential motivational trade-off where hunger may influence the decision to prioritise feeding over threat avoidance. However, this effect could also be explained by hunger inhibiting threat detection, a phenomenon seen in other species, such as the nematode worm (*Caenorhabditis elegans*) (Ghosh *et al.,* 2016). Further data are required to distinguish between these explanations, ideally by controlling for hunger level, threat level, and signal strength while varying the quality of the food reward.

Ross (1971) provided another example of potential motivational trade-offs, demonstrating that octopuses (*O. vulgaris*) initially attacked hermit crabs with anemones on their shells but eventually ceased the attacks after repeated stings. This suggests a sensitivity to pain and a trade-off between the risk of being sting and the reward of the prey. However, this study did not directly test whether octopuses would tolerate higher risks for higher quality rewards.

Zarrella's doctoral thesis (2011) examines how *O. vulgaris* negotiates conflicting motivations when acquiring and responding to fear. In a fear conditioning protocol, octopuses encountered a coloured ball, that, once paired with a mild electric shock, shifted from a neutral or potentially attractive objects to one they learned to avoid. Over successive trials, animals showed progressively longer latencies to contact the ball – an outcome that reflects a trade-off between their initial curiosity or past positive associations and the new negative value imposed by shock. In a separate experiment, the presence of conspecifics – an inherently stressful cue for this solitary species –suppressed predatory attacks on live prey, suggesting that the aversive social stimulus could outweigh the motivation to feed. At the molecular level, changes in expression of learning- and fear-related genes (*e.g.,* CREB, dopamine-linked markers) and increased protein activation were detected across multiple brain regions (optic lobes, supra-oesophageal mass, sub-oesophageal mass) following both learned and innate fear tasks. Although the thesis does not explicitly invoke a "common measure of value", the fact that overlapping neural substrates and pathways are engaged during both shock avoidance and social fear implies centralised integration of positive and negative inputs.

Further support for motivational trade-offs comes from a behavioural battery in *O. vulgaris,* combining neophobia and individual learning tests (Borrelli *et al.,* 2020). In the neophobia task, octopuses were offered a tethered crab (familiar prey) alongside a novel object. Although they continued to attack, latencies were significantly higher in the presence of the object – particularly on a second exposure – suggesting flexible decision-making based on competing motivations. In the individual learning task, animals first formed a preference for a coloured ball through positive reinforcement. In subsequent phase, interacting with the same stimulus triggered a mild electric shock. Over trials, octopuses showed increasing hesitation and ultimately avoided the object, reversing their initial preference. This shift from approach to avoidance reflects the integration of positive and negative values in guiding behaviour. While the underlying mechanisms remain unclear, these results are compatible with centralised processing and could suggest that octopuses may use a common value system to flexibly weight costs and benefits, but simpler explanations would need ruling out for this to be conclusive.

Further evidence comes from a recent study where *O. vulgaris* were presented with puzzle-box task developed to assess problem-solving, which inherently elicited motivational conflict (Dissegna *et al.,* 2023). To access a visible crab, octopuses had to manipulate an unfamiliar transparent box–requiring engagement with a novel, potentially threatening object to obtain a reward. Individuals varied in performance: while some consistently solved the task, other disengaged or never attempted to open the box. To disentangle individual differences in problem-solving from underlying motivational traits, a separate neophobia test was conducted, where a crab was presented either alone or adjacent to a novel object. Animals that exhibited neophilia (*i.e.,* reduced latency to attack prey in the presence of novelty) were significantly more likely to solve the puzzle-box task. This suggests that tolerance for novelty modulated willingness to engage, consistent with the weighing of positive (food) and negative (neophobia) valences. While the task does not include a noxious stimulus per se, it reflects a flexible behavioural response to competing motivations. A more robust test of motivational trade-offs would involve systematically varying the reward's value – such as by increasing food quality or quantity–to assess whether animals tolerate higher costs (*e.g.,* greater novelty, threat, or effort) in proportion to anticipated benefit.

Sensitisation Responses

Other studies suggest that certain cephalopod behaviours, particularly those observed after injury, may be explained by sensitisation rather than central decision-making. Crook et al. (2011) found that injured squid (*D. pealeii*) showed increased responsiveness to visual threats, escaping earlier and for longer periods. This heightened response may indicate sensitisation of visual and tactile receptors rather than calculated motivational trade-off. Crook et al. (2014) further supported this interpretation, showing that injured squid had longer alert and flight distances, with no evidence of altered behaviour due to central decision-making processes.

Similarly, Oshima et al. (2016) demonstrated that injured squid made different schooling decisions based on the nature of the threat, potentially due to increased sensitivity to stimuli rather than awareness of injury. While injured squid positioned themselves centrally within a school in response to visual predator cues, they moved to the periphery when exposed to olfactory cues, perhaps to engage in predator inspection behaviour.

Finally, Howard et al. (2019) reported that early-life injuries in Hawaiian bobtail squid (*E. scolopes*) led to long-term changes in defensive behaviour and short-term memory, with increased caution around predators but an inability to learn to inhibit behaviour when prey was present. Although this study did not test for motivational trade-offs, it demonstrates that injury can produce long-term effects that may influence behaviour in complex ways.

In this section, we found no study that tests for motivational trade-offs in cephalopods using appropriate controls. Various studies have shown sustained behavioural change after injury – findings compatible with cephalopods being aware of their injuries and changing their priorities when injured. However, they are also compatible with the hypothesis that injury directly increases sensitivity to threat. As a result, there is insufficient evidence for us to have high confidence that any cephalopod mollusc satisfies criterion 5. Indirect evidence from coleoid cephalopods nonetheless hints at motivational trade-offs, especially when these studies are taken together, allowing medium confidence.

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

Here, we are looking for self-protective behaviours that go beyond reflexes. To meet this criterion, the animal should vary its response in a targeted way, according to where on the body the noxious stimulus was administered. This indicates that the animal can internally represent the injury location.

Alupay et al. (2014) provide strong evidence to support criterion 6 in octopods, demonstrating flexible self-protective behaviours in algae octopus (*Abdopus aculeatus*). All injured octopuses (*i.e.,* arm crush) immediately held the arm stump or wound site in their beak for at least 10 mins. At 6 h, octopuses did not exhibit ongoing grooming, and mechanical stimulation did not re-induce it. Rather, octopuses contracted the injured area, keeping it close to the body. Three of the five injured subjects used adjacent arms to guard their injury, wrapping their uninjured arms around the injured site. After 24 h, the injured site was no longer contracted, but light touch induced contraction. Control subjects did not exhibit grooming or guarding behaviour.

A separate study on a different species, the lesser octopus (*Eledone cirrhosa*), also reports protective responses to injury (Polglase *et al.,* 1983). Injured octopuses attended to puncture wounds between the mantle apex and the siphon by stroking the tip of an arm across the injury. Note that this study does not report whether a subset of the subjects acted as control individuals. Nevertheless, similar wound-tending behaviour has been observed in octopuses following surgery to the optic capsule or cranium, although this observation is anecdotal (I. Gleadall, personal observation cited in Andrews *et al.,* 2013). G. Fiorito also reports that octopuses guard the mantle or cranium post-surgery (unpublished data and cited in Fiorito *et al.,* 2015).

In a study discussed primarily under criterion 8, Crook (2021) found that octopods (*O. bocki*) injected with dilute acetic acid would groom the site with their beak, including stripping away some skin. As grooming is seen for other types of injury (arm crush, skin pinch, skin slice), but the skin-stripping behaviour is not, it could work for noxious stings (to release the poison). If correct, this suggests that the octopus can represent the type of pain (mechanical or chemical), as well as its location.

Several studies on different species of octopod have shown that they withdraw, in a way that seems self-protective, from hermit crabs with stinging anemones on their shell (Boycott, 1954; Brooks, 1988; Hand, 1975; McClean, 1983; Polimanti, 1910; Ross, 1971). This is not, by itself, compelling evidence in relation to criterion 6, because it is difficult to be sure whether such behaviours involve centralised representation of the bodily location of an injury or noxious stimulus. Common octopuses (*O. vulgaris*) are capable of reflex withdrawal in response to a noxious stimulus without reference to the brain (Hague *et al.,* 2013).

Research on the regenerative abilities of octopuses offers additional insight into their self-protective behaviours. Following injury, such as the transection of the pallial nerve, octopuses can rapidly restore crucial function, including skin patterning, which plays a vital role in communication and concealment. This ability to recover functional capacities allows them to maintain behaviours essential for self-protection, even after injury (Imperadore *et al.,* 2019). The rapid regeneration of neural connections, including those involved in behaviour control, enables octopuses to continue performing targeted responses such as guarding and grooming, post-injury.

A recent study on juvenile pharaoh cuttlefish (*S. pharaonis*) has demonstrated that individuals perform wound-directed grooming (brushing their arms over the injected site) to areas that have been injected with acetic acid (Kuo *et al.,* 2022), whereas sham controlled individuals showed no grooming behaviour. After being pinched, common cuttlefish (*S. officinalis*) avoid the claws of crab prey, instead learning to attack from behind (Boal *et al.,* 2000). Anecdotal evidence also suggests that cuttlefish can discriminate between different crab species and avoid attacking or hunting more aggressive crab species after being pinched (A.S. Darmaillacq, personal observation communicated in Andrews *et al.,* 2013). Moreover, following surgery to the optic capsule, the cranium, the skin or the arms, common cuttlefish will direct wound attention and grooming, brushing their arms across the surgery site for several days to weeks (A.K. Schnell and C. Jozet-Alves, personal observation communicated to A.K. Schnell). Quantitative data on these observations were not recorded, but they can be regarded as credible anecdotal observations from cephalopod biologists with expertise in neuroethology.

Bazarini and Crook (2020) report defensive behaviours in Hawaiian bobtail squid (*E. scolopes*) following an injury to the left fin. Squid responded to tactile and vibratory sensory tests through defensive arm posture, sometimes accompanied by escape jetting or inking. Although wound grooming or guarding is not reported, the left fin would be difficult to reach with the squid's arms.

Another study by Crook et al. (2011) showed that minor arm injury caused long-lasting enhancement of defensive responses to visual and tactile stimuli in squid (*D. pealeii*). All injured animals responded with escape jetting and ink release. However, squid never displayed wound-directed attention (i.e. grooming or guarding). This absence is probably not due to the inability to reach or manipulate the injured area, because injured subjects were observed manipulating other areas close to the injury.

What explains the lack of site-specific wound-directed behaviour after injury in squid? The absence of pain, or something else? Crook et al.'s (2013) findings are relevant here. Peripheral injury caused pronounced, long-lasting spontaneous activity, as well as sensitisation to mechanical stimuli, in afferent neurons not only near the injury site but also on the other side of the body. The lack of localisation is consistent with the hypothesis that enhanced activity is part of a general behavioural state after injury in squid. This general behavioural state increases reactions to tactile stimulation anywhere on the body surface. By contrast, mammalian nociceptors are assumed to be spatially associated with an injury, prompting self-protective behaviours directed at wound sites. Results from Crook et al.'s study demonstrates that, following injury, nociceptive sensitisation in squid appears to be widespread. This phenomenon might initiate a generalised vigilance state – an explanation consistent with other findings that minor injury in squid increases predation risk (Crook *et al.,* 2014). Therefore, a generalised vigilance state might help injured animals avoid approaching predators.

To summarise, the strongest evidence of wound-grooming and guarding comes from octopods, where injured individuals curl their adjacent arms around the injured site or attempt to scrape away a noxious stimulus. We thus have very high confidence that octopods satisfy criterion 6. A notable study on injury-related grooming in cuttlefish suggests directed yet flexible self-protection in this group, allowing high confidence. In squid, there is evidence of widespread nociceptive sensitisation following injury, but no evidence of protective behaviour directed specifically towards the wound. We therefore have low confidence that squid and nautiloids fulfil this criterion.

3.7 Criterion 7: Sophisticated Associative Learning

The animal shows forms of associative learning in which noxious stimuli become associated with neutral stimuli, or in which novel ways of avoiding noxious stimuli are learned through reinforcement. These forms of associative learning go beyond classical conditioning in which a single conditioned stimulus overlaps temporally with an unconditioned stimulus. Note: Forms of associative learning that are linked, at least tentatively, to sentience in humans (such as instrumental, reversal learning, and trace conditioning) provide stronger evidence than other forms.

For this criterion, we ask whether the animal can form associations between noxious stimuli and neutral stimuli by, for example, learning to associate a particular place, or an otherwise neutral odour, with a noxious stimulus. We are also looking for evidence that an animal can learn a novel behaviour (distinct from any pre-existing reflex responses) that allows it to avoid a noxious stimulus.

Associative learning requires integrative processing, because representations of both stimuli must come together (Birch *et al.,* 2020*a*; Ginsburg & Jablonka, 2019). There is some evidence of unconscious associative learning (Greenwald & De Houwer, 2017), leading to ongoing inquiry about which types of associative learning are most strongly linked to sentience and why. Instrumental learning (Skora *et al.,* 2021), reversal learning (Travers *et al.,* 2017), learning "incongruent" spatial relationships (Ben-Haim *et al.,* 2021), and learning across temporal gaps between stimuli ("trace conditioning"; Clark & Squire, 1998; 1999; Clark *et al.,* 2002) seem to have a particularly strong link to sentience. Here, we prioritise these types of associative learning as evidence for this criterion, without assuming which types require sentience.

There is a clear scientific consensus that octopods and cuttlefish are readily capable of sophisticated forms of associative learning (Hanlon & Messenger, 2018; Hochner *et al.,* 2006; Marini *et al.,* 2017; Mather, 1995; 2008; Schnell *et al.,* 2021*a*). However, the evidence is somewhat weaker in squid and nautiloids.

Coleoid cephalopods have a brain functionally specialised to facilitate learning. Based on electrophysiological studies, Hochner et al. (2006, p. 315) suggested that: "a convergent evolutionary process has led to the selection of similar networks and synaptic plasticity" involved in learning and memory in cephalopods and mammals. In particular, the vertical lobe-median superior frontal lobe complex has learning and memory functions analogous to the mammalian hippocampus (Hochner *et al.,* 2006, Shomrat *et al.,* 2015). Lesions inhibit performance in long-term learning tasks, such as visual discriminations, without affecting other survival behaviours (Boycott & Young, 1955; Maldonado, 1965; Young, 1960), and this structure develops concurrently with learning abilities in octopus (Fiorito & Chichery, 1995) and cuttlefish (Dickel *et al.,* 2001).

Octopods. Octopods can learn to associate reward or punishment with various visual and tactile stimuli (reviewed in Fiorito & Scotto, 1992; Kawashima *et al.,* 2020; Mackintosh, 1962; Mackintosh & Mackintosh, 1963; 1964; briefly in Marini *et al.,* 2017; Schnell *et al.,* 2021*a*; Sutherland, 1962; Tokuda *et al.,* 2015). For example, Papini and Bitterman (1991) trained the day octopus (*Octopus cyanea*) to associate a neutral stimulus with a food reward. Subjects that received larger rewards learned faster than subjects that received smaller rewards. Moreover, consistent reinforcement improved subsequent performance.

Rather than explicitly testing whether octopods can learn associatively, recent studies usually train octopods on some association as a first step towards testing another cognitive ability. For instance, octopods can perform spatial learning (Boal *et al.,* 2000), social learning (Amodio & Fiorito, 2013; Tomita & Aoki, 2014), conditional learning (Hvorecny *et al.,* 2007; Tokuda *et al.,* 2015), and reversal learning (Mackintosh, 1962; Mackintosh & Mackintosh, 1963; 1964) (but see Bublitz *et al.,* 2017). Studies of *O. vulgaris* dominate the literature, but some work has focused on *Octopus bimaculoides* (Boal *et al.,* 2000), *Octopus ocellatus* (Tomita & Aoki, 2014), *Octopus aegina* (Kawamura *et al.,* 2001) and *Abdopus aculeatus* (Kawashima *et al.,* 2020).

Cuttlefish. In cuttlefish, learning has been extensively studied using the prawn-in-a-tube test (Agin *et al.,* 1998; 2006*a*; 2006*b*; Boycott, 1961; Cartron *et al.,* 2013; Chichery & Chichery, 1992; Dickel *et al.,* 2000; Messenger 1971; 1973; Sanders & Young, 1940). This paradigm presents a shrimp inside a glass beaker or test tube. Initially, the subject attacks the shrimp, but it quickly learns that the prey item cannot be obtained. There is no doubt that cuttlefish can learn this task, but a major challenge for prawn-in-a-tube researchers is to show that success involves associative learning (specifically, instrumental conditioning) and not just habituation (Agin *et al.,* 2006*b*).

A key characteristic of habituation is dishabituation: The tendency for novel stimulus presentations to reverse the habituation process (Pinsker *et al.,* 1970). Agin et al. (2006*a*) tested dishabituation by giving the cuttlefish an alternative prey item (crab; exp. 1-2) or a novel stimulus (flashing light; exp. 3), before presenting the prawn-in-a-tube again. Despite the novel stimuli, cuttlefish did not resume attacking prawns. Nonetheless, null results in such a small sample (exp. 1: $n = 8$; exp. 2: $n = 13, 9$; exp. 3: $n = 7$) are poor evidence of the absence of dishabituation.

Similar considerations apply to another study that attempted to disentangle associative learning from habituation (Purdy *et al.,* 2006). The study found no evidence of a dishabituation effect in a study involving two groups of seven cuttlefish (*S. officinalis*). This is also a small sample, but the two negative results taken together offer stronger evidence than either in isolation.

In the first cephalopod study on taste aversion learning, Darmaillacq et al. (2004) first established whether cuttlefish (*S. officinalis*) preferred crab or shrimp. They then repeatedly presented the preferred prey with distasteful quinine. Compared to controls, these subjects rapidly learned to avoid unpalatable prey items – an effect that persisted for at least 72 hours.

Cuttlefish research has focused on avoidance learning and mostly used *S. officinalis*, although other species learn the prawn-in-a-tube task (e.g. *Sepia bandensis*: Bowers *et al.,* 2020; *Sepia pharaonis*: Purdy *et al.,* 2006). Cuttlefish also exhibit classical conditioning (Agin *et al.,* 1998; 2003; Cole & Adamo, 2005; Messenger, 1971), spatial learning (Alves *et al.,* 2007, 2008; Scatà *et al.,* 2016), conditional learning (Hvorecny *et al.,* 2007), reversal learning (Schnell *et al.,* 2021*b*), and temporal learning (Jozet-Alves *et al.,* 2013; Schnell *et al.,* 2021*c*).

Squid. We consider it unlikely that associative learning would be present in both octopods and cuttlefish but not squid. However, few squid learning studies have been carried out.

Allen et al. (1985) investigated visual discrimination in Atlantic brief squid (*Lolliguncula brevis*). In the first experiment, subjects were trained to attack a horizontal rectangle for a food reward and avoid a vertical rectangle that resulted in a 20 V electric shock. Squid subsequently attacked the horizontal rectangle in significantly more trials than the vertical rectangle. There was some evidence for task retention after nine days, although no statistical analysis was reported. In the second experiment, the positive stimulus was a white ball, and the negative stimulus was a black ball. The white sphere was attacked in significantly more trials than the black sphere. A limitation of this study was that the stimuli were not counterbalanced, making it difficult to disentangle learning from behaviour driven by stimulus properties (e.g., visibility).

In a recent associative learning study, Zepeda et al. (2017) tested Hawaiian bobtail squid (*E. scolopes*) on the prawn-in-a-tube task. Training sessions were either massed (three 10-minute trials with 10-minute intervals) or spaced (three 10-minute trials with one-day intervals). The squid significantly reduced responses across the first trial. This reduced tendency to respond appeared to be retained between tests for 8 days in the massed treatment and 10 days in the spaced treatment – a form of long-term memory. However, this study compared the number of strikes in the first half of the trial with the number of strikes in the second half. Depleted energy levels, rather than learning, could explain reduced responses. Even if learning were responsible, Zepeda et al. (2017) did not establish whether it was habituation or associative learning.

Nautiloids. Nautiloids have fewer neurons than coleoids and lack clearly differentiated lobes, including the vertical lobe-median superior frontal lobe complex linked to coeloid learning and long-term memory (Young, 1965; 1991). There is nonetheless evidence for classical conditioning and potentially spatial learning in nautiloids.

Crook and Basil (2008) trained chambered nautiluses (*Nautilus pompilius*) on a classical conditioning task. The unconditioned stimulus was food; the conditioned stimulus was a 0.5s blue light; and the responses were tentacle extension and rapid breathing. Although the authors had no criteria to establish that subjects had learned the task, the conditioned stimulus increased tentacle extension and breathing rates in the treatment group compared to the control group, both three minutes and one hour after conditioning (i.e., short-term memory). There was no treatment difference for either measure at one hour, but significant differences reappeared at six and 12 hours (i.e. long-term memory). Crook and Basil equated this to the biphasic short- and long-term memory curve observed in coleoids (Agin *et al.,* 2003; 2006*b*). This functional analogy is surprising, given the structural differences between nautiloids and coleoids.

However, further research would be needed to allow high confidence that nautiloids satisfy criterion 7. The p-values for several time intervals, especially at three and 30 minutes, were only borderline significant (between 0.02 and 0.05). Moreover, it would have been appropriate to correct for multiple comparisons, such as by applying a Bonferroni correction. Had a correction been applied, the borderline significant findings may have been non-significant.

In another nautiloid study, Crook et al. (2009, exp. 1) found tentative evidence for spatial learning (learning the spatial configuration of a maze) in chambered nautilus (*N. pompilius*). Subjects were placed in an open-field maze with aversive bright light and shallow water. To escape these unconditioned stimuli, nautiluses needed to use an exit hole signalled by bubble wrap, a visual and tactile conditioned stimulus. Exit latency significantly decreased across the five training trials and remained significantly below the naïve latency for up to 21 days. This retention time was much longer than the 12 hours observed in Crook and Basil's (2008) classical conditioning task.

However, it is unclear what the nautiloids were learning in this study. One interpretation is that they learned to associate the bubble wrap with the exit hole. Alternatively, they began every trial opposite (180°) the exit hole, so may have learned the orientation to escape, rather than the conditioned stimulus. We also cannot rule out a general reduction in exploratory behaviour on repeated exposure to the same arena – a form of habituation.

Taken together, the evidence for associative learning in octopods (Octopoda) and cuttlefish (Sepiida) is overwhelming, particularly evidence for complex forms of associative learning that have been linked to sentience. We thus have very high confidence that both taxa fulfil criterion 7. Few squid studies have investigated associative learning, but the overall evidential picture points towards associative learning in all coleoid cephalopods. There are also few nautiloid studies, but the existing evidence hints at a capacity for associative learning. As a result, we have high confidence that squid satisfy this criterion and medium confidence for nautiloids.

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. 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*

If an animal selectively self-administers an analgesic or anaesthetic when injured, it demonstrates a motivation to cease perception of the injury. Local anaesthetics work peripherally, suggesting (minimally) that the animal wants to end nociceptive processing. However, a drug that works in the brain, possibly on areas involved in pain, suggests the animal is motivated to end the feeling of pain. This criterion also builds on criterion 4b by introducing clear hypotheses. Rather than simply observing behavioural responses consistent with the drugs alleviating pain, we can predict that animals in pain will exhibit drug-induced self-administration, conditioned place preference, or prioritisation over competing motivations.

A recent study bears on criterion 8. Crook (2021) asked: Will an octopus (*O. bocki*), after being injected with acetic acid and placed in their preferred chamber, learn to avoid that chamber in the future? Moreover, will they learn to *prefer* a chamber where they receive a local anaesthetic (lidocaine) when injured? Lastly, does this preference depend on injury, so the lidocaine-associated chamber is not preferred without the acid injection? This type of study can provide quality evidence for criterion 8 (via 8b) by showing that the animal values an anaesthetic when injured.

Crook's (2021) results answered "yes" to all three questions. Octopuses subsequently avoided their initially preferred chamber, where they were confined after acid injection. When given tonic pain relief (i.e., topical injection of lidocaine), subjects changed their preference to the chamber where they received pain relief. By contrast, control animals did not change their chamber preference after saline injection, and lidocaine injection did not influence their chamber preference. The overall structure of Crook's experiment is shown in **Figure 2** (from Crook, 2021).

The study's design includes a notable limitation: to show a conditioned place preference for the lidocaine-paired chamber, it would be necessary to have a control group that has the *exact same sequence of experiences* (i.e. acetic acid, then a second injection, then 20 minutes in the initially non-preferred chamber) but with saline instead of lidocaine in the second injection. There was no such control group: the experiment only included a control with saline in place of acetic acid, then a lidocaine injection for all subjects. The absence of this control means that, while the study convincingly demonstrates conditioned place avoidance of the acid-paired chamber, plus an effect of lidocaine on behaviour and on nervous system response, , it leaves some uncertainty about whether the octopuses valued pain relief when injured, which is our criterion 8. Further investigation with additional controls may help clarify this aspect.

To conclude, a recent study found that, when exposed to a noxious stimulus, octopuses learn to avoid the chamber paired with that stimulus, preferring a chamber paired with a local anaesthetic. While this study provides evidence of conditioned place avoidance, some uncertainties remain due to limitations in the control conditions. Despite these limitations, the study's design offers valuable insights, warranting medium confidence. Criterion 8 has not been tested in other cephalopods.

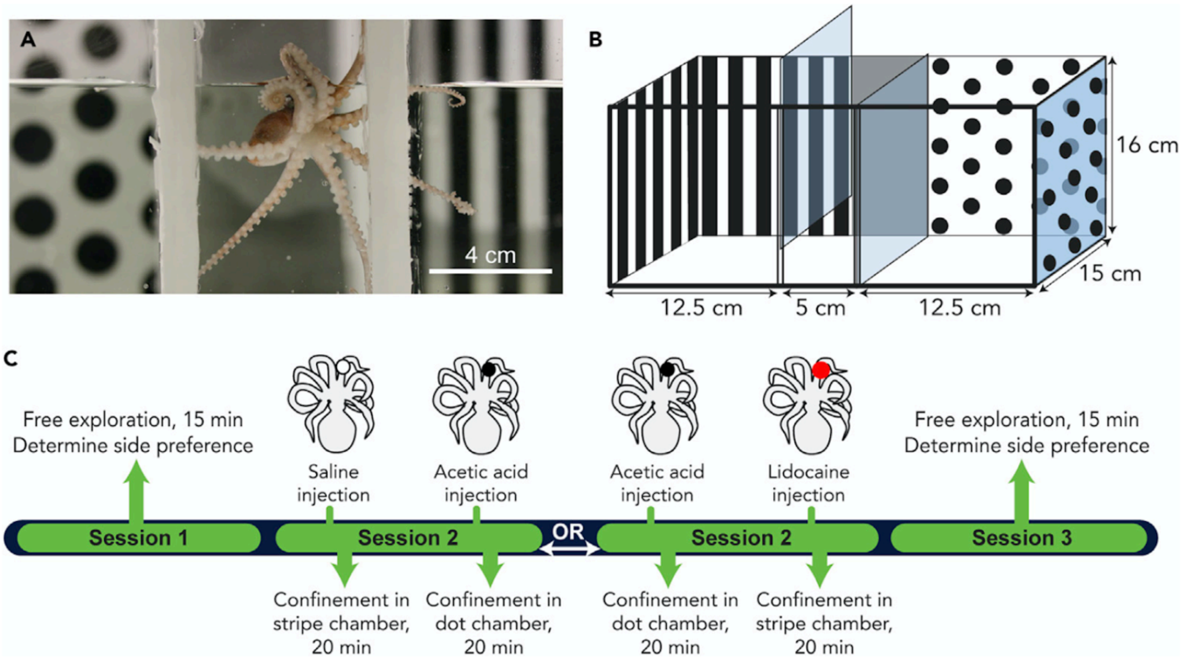

Figure 2a. A key figure from Crook (2021). The experiment (which is relevant to our criteria 4, 5 and 8) involved four groups of animals (with either 7 or 8 in each group): (A) shows an octopus in the apparatus; (B) is a representation of the apparatus; and (C) is a timeline of the experiment. In this representation the octopus exhibits an initial preference in session 1 for dot chamber and thus was subsequently given an injection of acetic acid prior to confinement in the dot chamber and/or lidocaine prior to confinement in the striped chamber, to see whether its preferences would reverse. The figure is © Robyn Crook 2021, CC-BY-NC-ND 4.0 licensed.

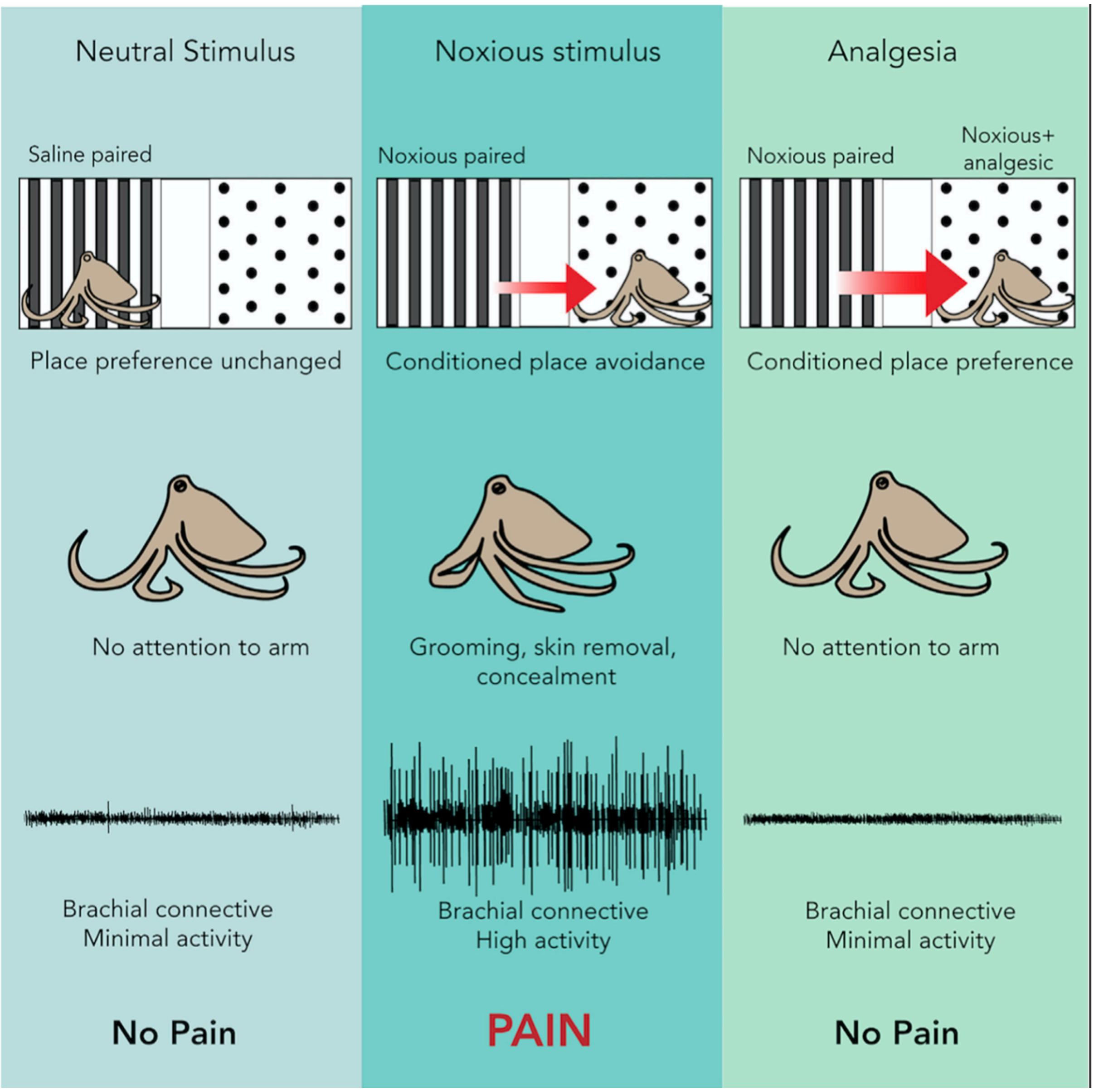

Figure 2b. A graphical representation from Crook's (2021) conditioned place preference study. The experiment involved four groups of animals (with either 7 or 8 in each group): a group injected with only saline solution; a second group injected with acetic acid; a third group injected with acetic acid and, later, lidocaine; and a fourth group (not shown) injected with saline and then lidocaine. After receiving acetic acid, the affected animals showed directed self-protective behaviour, increased neural activity, and avoidance of the chamber where they had received it. Lidocaine silenced the heightened neural activity, stopped the self-protective behaviour, and led to a conditioned preference for the chamber where the effects of the lidocaine were experienced. The figure is © Robyn Crook 2021, CC-BY-NC-ND 4.0 licensed. See the original source for further methodological details.

IV | DISCUSSION

The strength of the evidence of sentience is not equal across cephalopod groups. Octopods exhibit compelling evidence (high or very high confidence in 6/8 criteria). Cuttlefish and squid, belonging to the broader coleoid cephalopod group, present substantial evidence (4/8 criteria). In contrast, nautilus shows a dearth of evidence (1/8 criteria): a significant gap in our understanding (Table 1 & 2). These findings build on previous reviews (Andrews *et al.,* 2013; Fiorito *et al.,* 2015), which broadly suggested potential for sentience across cephalopods. Here we provide a more detailed understanding, highlighting the strong evidence in octopods and significant gaps in nautilus.

Our review emphasizes a shift towards more specific ethical considerations in cephalopod research. While earlier reviews laid the groundwork for ethical consideration, our findings highlight the need to address welfare issues with greater precision. We avoid making definitive statements about the “levels” of sentience across different cephalopod groups due to existing research gaps. It is not clear, in any case, that sentience comes in “levels” (*Birch et al.,* 2020*b*). Still, these nuances between cephalopod groups hint at differences in their forms of sentience.

Understanding the implications of sentience in cephalopods is crucial, particularly in light of various welfare risks associated with their treatment (see Browning *et al.* 2025 for a detailed review). Commercial fisheries pose several welfare risks, including physical trauma for capture techniques, rough handling, and rapid ascent, which can lead to buoyancy issues and injuries (A.K. Schnell, personal observation; Forsythe *et al.,* 1991; McDonald 2011; Sherrill *et al.,* 2000). Netting methods often result in exhaustion, suffocation, or crushing under the weight of other animals, with subsequent risks of disease and predation (Boyle 2010; Gestal *et al.,* 20191; Hanlon *et al.,* 1984). For instance, skin and fin injuries can increase predation risk and reduce the ability to respond to environmental changes (Crook *et al.,* 2014; Hanlon *et al.,* 1983). Prolonged confinement in traps or pots further exacerbates stress, aggression, and cannibalism (Aguado-Gimémenz & Garcia Garcia, 2002; Budelmann, 2010; Florini *et al.,* 2011; Ibáñez & Keyl, 2010; Jacquet *et al.,* 2019), while sensitivity to environmental changes such as salinity and temperature can cause additional stress (Moltschaniwskyj *et al.,* 2007).

Slaughter methods present further welfare concerns, with inhumane practices such as asphyxiation and clubbing reported in some regions (Pereira & Lourenco, 2014). While efforts are underway to improve euthanasia techniques for captive cephalopods in research settings (Abbo *et al.,* 2021; Andrews *et al.,* 2013; Butler-Struben *et al.,* 2018), these methods are not suitable for commercial use. There is a need for further research to develop humane, practical slaughter methods for commercial fisheries.

Cephalopod aquaculture, while still small-scale, raises additional welfare concerns. Hatchling mortality is high due to temperature, water quality, and nutrition issues (Boyle, 2010; Iglesias *et al.,* 2007; Vaz-Pires *et al.,* 2004). Prolonged transport of wild cephalopods for aquaculture can lead to asphyxiation from ink contamination (Hayter, 2005; McDonald, 2011). Inadequate nutrition and unmet cognitive needs in capacity also contribute to stress and behavioural problems, including self-cannibalism (Cooke & Tonkins, 2015; Jacquet *et al.,* 2019). The lack of appropriate shelter (Cooke *et al.,* 2019; Tonkin *et al.,* 2015) and high-speed collisions in tanks further exacerbate these issues. Maintaining optimal water quality and addressing factors such as lighting and temperature are crucial to ensuring cephalopod welfare in aquaculture.

Our objective in this review is not to prescribe specific legislative measures or to say what constitutes a proportionate response to the identified risks. Instead, we aim to emphasise that the body of evidence we have reviewed should be regarded as affirming the existence and legitimacy of these risks. While the determination of appropriate responses remains a multifaceted and context-dependent task, the empirical foundation we have presented highlights the significance of addressing and mitigating these risks in a manner consistent with evolving ethical and scientific understandings. Historically, invertebrate welfare and ethical considerations have been marginalised. Our findings provide reasons to take the issues more seriously. This recognition is essential for shaping responsible policies and practices that acknowledge the well-being of *all* sentient beings, regardless of whether they have a backbone.

Moreover, against a background of apparently growing interest in octopus farming, careful consideration of the welfare needs of cephalopods can help humanity avoid repeating the mistakes made in other kinds of animal farming, such as the systemic welfare problems associated with the intensive farming of pigs and chickens (Duncan, 2001; Faure & Jones, 2002; Mason & Mendl, 1993; 2004). For all the evidence gaps that remain, current knowledge can provide a foundation for establishing ethical guidelines in both research and industry. It can help stakeholders develop practices that prioritise animal welfare and promote responsible and humane treatment. While empirical research has advanced our understanding of cephalopod neurobiology and behaviour, key questions – such as the evolutionary origins of complex nervous systems – remain challenging to address using non-invasive methods. This leads to an ethical quandary: is it justifiable to conduct further invasive research to address these questions, knowing the potential for causing pain? At minimum, all such research must adhere to the 3Rs (Replacement, Reduction, and Refinement), employ appropriate anaesthesia and analgesia, refine experimental protocols to mitigate stress, and make full use of non-invasive alternatives. But it is also crucial to assess whether the likely benefits of any given study, including benefits to the animals themselves through greater understanding of their welfare needs, are large enough to justify the harms.

Table 1. Neural evidence relevant to cephalopod sentience

Neural criteria	Octopods	Cuttlefish	Squid	Nautilus
Nociceptors	Possess nociceptors that respond to noxious stimuli	Direct evidence is lacking, but evolutionary consideration suggest they possess nociceptors	Possess nociceptors that respond to noxious stimuli	Direct evidence is lacking, but evolutionary considerations suggest they possess nociceptors, supported by insights from non-cephalopod molluscs
Integrative Brain Regions	Nociceptors are indirectly suggested to connect ton higher-order brain regions, specifically the vertical lobe	N /A	Nociceptors are indirectly suggested to connect ton higher-order brain regions, specifically the vertical lobe	Lack a vertical lobe
Integrated Nociception	Nociceptors and other sensory information are integrated, facilitated by connections to higher-order brain regions	N/A	Nociceptors and other sensory information are integrated, facilitated by connections to higher-order brain regions	N/A
Analgesia (Endogenous & Exogenous)	Local anaesthetics (magnesium chloride, ethanol), affect the nervous system; lidocaine modifies responses to noxious stimuli	Local anaesthetics affect the nervous system; lidocaine modifies responses to noxious stimuli	Local anaesthetics (magnesium chloride, ethanol) affect the nervous system; evidence suggests analgesic effects in bobtail squid	Local anaesthetic effects are uncharted, indicating a need for research

Table 2. Behavioural evidence relevant to cephalopod sentience

Behavioural criteria	Octopods	Cuttlefish	Squid	Nautilus
Motivational Trade-Offs	Insufficient evidence to determine motivational trade-offs directly; sustained behavioural changes after injury suggest awareness and adjustment of priorities	Insufficient evidence to determine motivational trade-offs directly	Insufficient evidence to determine motivational trade-offs directly	Insufficient evidence to determine motivational trade-offs directly
Flexible Self-Protection	Exhibit injury-related grooming, indicating flexible self-protection mechanisms	Exhibit injury-related grooming, indicating flexible self-protection mechanisms	Exhibit nociception sensitisation but does not show protective behaviour toward the wound	Limited evidence; further research needed to establish self-protective behaviours
Sophisticated Associative Learning	Extensive evidence supports associative learning, particularly in avoiding noxious stimuli	Extensive evidence supports associative learning, particularly in avoiding noxious stimuli	Evidence suggests associative learning, though fewer studies are available	Limited evidence; hints at associative learning, but further research is needed
Analgesia Preference	Evidence of preference for local anaesthetic when injured	Limited research; no clear evidence of analgesia preference	Limited research; no clear evidence of analgesia preference	Gap in research' further investigations needed to understand pain responses and preferences

V | CONCLUSION

1. Evidence variability: The evidence of sentience varies across cephalopod groups, with octopods showing strong support, cuttlefish and squid presenting moderate evidence, and nautilus displaying minimal evidence. This variability might not only be due to inherent differences between these groups but also reflects the unequal weight of research efforts, with octopods being more extensively studied than other cephalopod groups. This disparity highlights gaps in our understanding, particularly for nautilus, where more research is needed to draw stronger conclusions about their sentience.

2. Ethical implications: The review highlights the need for refined ethical considerations in cephalopod research, advocating for more precise welfare guidelines to address the unique challenges of different cephalopod species.
3. Welfare risks: Commercial fisheries and aquaculture pose substantial welfare risks to cephalopods, urgently requiring the development of humane capture, handling, and slaughter methods, as well as improvement in captive conditions, if indeed captive conditions can ever sufficiently meet the needs of cephalopods to be publicly acceptable.
4. Legitimacy of welfare concerns: The reviewed evidence highlights the legitimacy of welfare concerns in cephalopods, emphasizing the need for these issues to be taken seriously in both research and industry practices.
5. Avoiding past mistakes: The review advocates for a protective approach toward cephalopod welfare, particularly in emerging industries like octopus farming, where there is still the opportunity to avoid the welfare issues historically associated with intensive farming of other animals and prevent the need for later reform.

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