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Analgesia for non-mammalian vertebrates

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Analgesia encompasses drugs used to reduce perceived pain. The attribution of pain to non-mammalian vertebrates has widened markedly over the last 20 years, based on common nociceptive pathways, the presumption of evolutionary advantage in the experience of pain, and particularly, behavioral findings. Central processing of pain in non-mammalians is not well understood which has hindered both the attribution of pain and the emphasis placed on analgesia in non-mammalian vertebrates. Nociception (the processing of harmful stimuli, which does not require the emotive aspect of pain) triggers a wide-ranging physiological cascade that, irrespective of the attribution of pain, is sufficient to warrant the use of analgesic drugs. Consolidated research between physiologists and veterinarians is required into the dosages and efficacies of analgesics in the many and varied taxa of physiological interest.

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Introduction

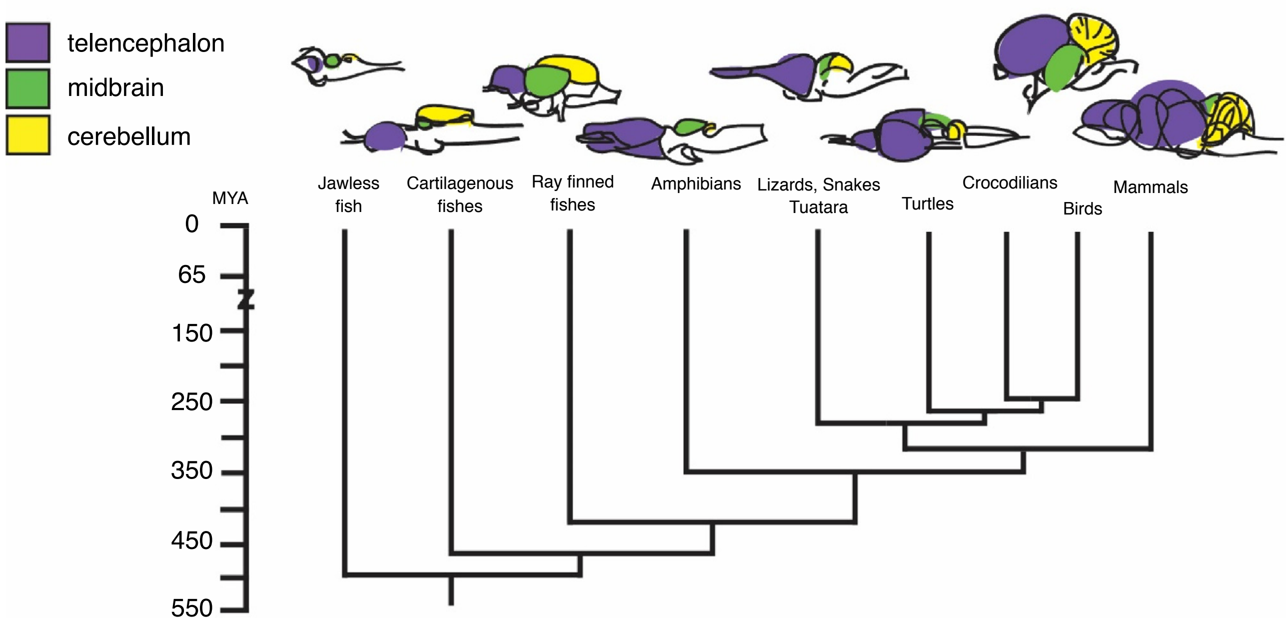
Nociception — the neural process of encoding noxious stimuli — is of obvious importance across animals, as it allows immediate responses to detrimental conditions that could compromise survival or reduce fitness. There is broad consensus that nociception evolved early amongst multicellular animals and certainly before the division of extant vertebrates and invertebrates over 500 million years ago [1•,2,3]. Pain has distinct significance because it is believed to enable longer-term learning and avoidance behavior and by definition includes an 'affective or emotional component in response to actual or potential tissue damage or described in terms of this damage' [4]. The sensation of pain is therefore perceived to possess evolutionary advantages that outweigh its immediate negative effect on welfare. Antinociception and analgesia describe the reduction or abolition of nociception and pain, respectively. Therefore, if defined strictly, the need to provide analgesia rests on the attribution of pain to organisms. Drugs which act to provide analgesia in those animals where pain is attributed can act either centrally or peripherally, and often therefore are described as anti-nociceptive if their action is peripheral, or in animals where pain is not experienced, for example, those undergoing concurrent anaesthesia.

The distinction between pain and nociception is relatively straight-forward in communicating humans, but more challenging in non-verbal humans, such as infants. It is even more problematic and contentious in other animals, particularly non-mammalian vertebrates; probably explaining why renaissance scientists reasoned that animals live senseless lives without the ability to feel pain. This Cartesian view was paraphrased by French priest and philosopher Nicolas Malebranche (1638–1715) proclaiming "*animals eat without pleasure, cry without pain, grow without knowing it; they desire nothing, fear nothing, know nothing*". Into the 1980s there were controversies as to whether human babies benefit from analgesia [5]. Our views have changed profoundly in recent decades and there is now general academic consensus that all mammals are endowed with the ability to feel pain [6]. However, although all vertebrates (as well as cephalopods) are now covered by many legislations on animal experiments [7], there continues to be intractable controversy regarding the emotional component in the definition of pain in non-mammalian vertebrates. The debate is most prevalent in fish [8,9,10••,11•,12••] leading to geographical differences in legislation and policies in scientific journals as to how analgesia is viewed and applied. Parts of the dispute reside with the perceived link of pain to consciousness.

Here we discuss the use of analgesia for non-mammalian taxa. We begin with the anatomy of pain, demonstrating that virtually all vertebrates possess the neural circuitry to carry nociceptive information to the central nervous system (CNS) (Figure 1), but that there has been considerable controversy as to whether the structures in the CNS enable the emotional state of pain. We then discuss whether long-term behavioral changes in response to noxious stimuli may provide a better indication of pain in non-verbal animals. Finally, we argue, separate from the unresolved discussion

Figure 1

	Jawless fish sea lamprey	Cartilagenous fishes stingray	Bony fishes trout	Amphibians grass frog	Lizards, Snakes Tuatara crotaline snake	Turtles	Crocodilians	Birds chicken	Mammals mouse
Nociception									
chemical	NT	-	+	+	+	+	+	+	+
thermal									
heat	+	-	+	+	+	+	+	+	+
cold	-	-	-	+	NT	NT	NT	+	+
mechanical	+	+	+	+	+	+	+	+	+
Nociceptors									
(C) unmyelinated	+	(very low)/-	+ (low)	+	+	NT	NT	+	+
(Aδ) myelinated	-	+	+	+	+	NT	NT	+	+
Pathway to CNS			+	+	+	+	+	+	+
Central processing			+/?	?	?	?	?	+	+
Endogenous opiates and Substance P in nociceptive tracts		+	+	+	+	+	+	+	+
Response to exogenous opiate in nociceptive test		-	+	+	+/-	+	+	+	+



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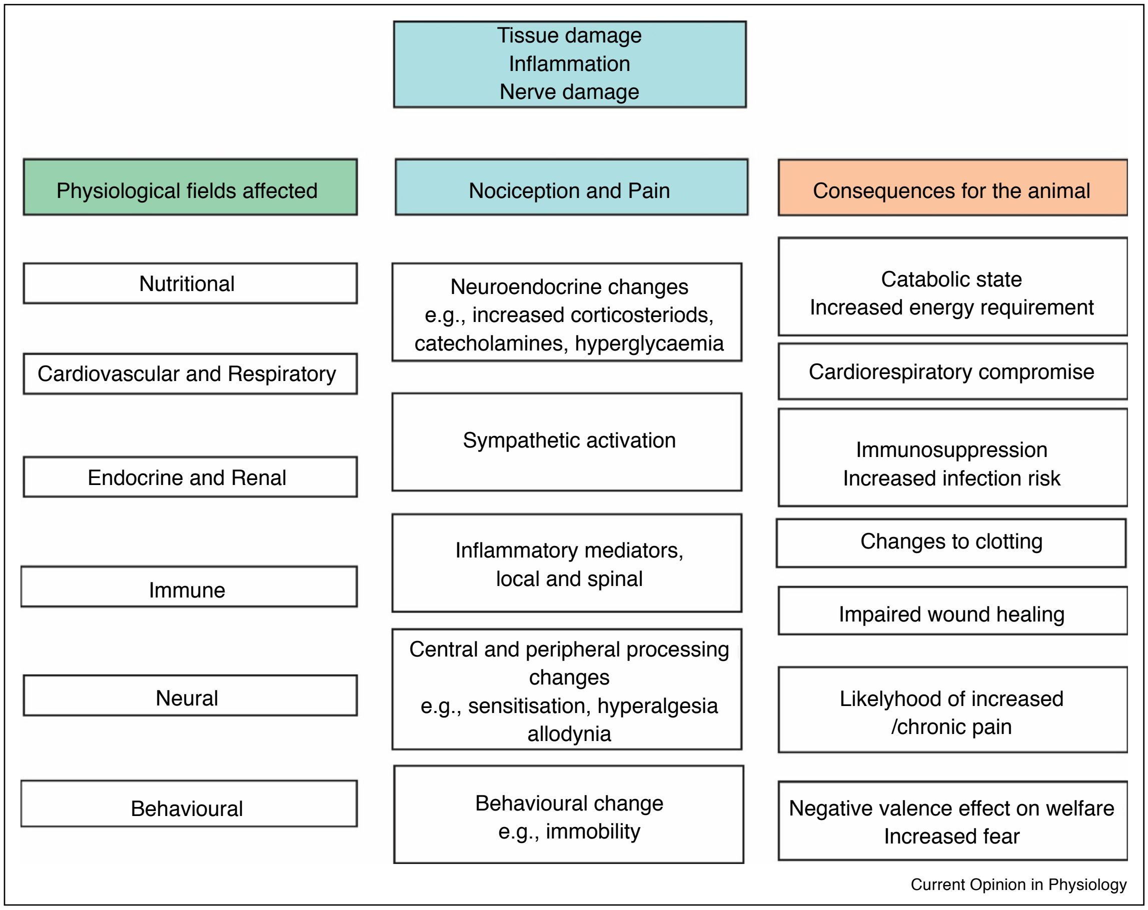
A phylogeny of vertebrates with diagrams of gross brain regions, and description of nociceptive pathways, modified from Refs. [12••,137] with additional data from literature referenced in the text. NT = not tested,? = disputed in the literature. Substance P = a neurotransmitter and neuromodulator which is released from specific sensory nerve terminals often associated with inflammation and pain.

of whether non-mammalian vertebrates experience pain, that nociception, irrespective of its emotional correlates, triggers parallel cascades of physiological responses that can be mitigated or alleviated by proper anti-nociception (Figure 2). Hence, the use of appropriate drugs is central to acquisition of good quality physiological data from animals that have been subject to interventions with tissue damage, regardless of their capacity to experience pain (Figure 3). This is as part of a wider goal of reducing stress in experimental animals, refining experimental planning, for the

Figure 2



The potential effects of nociception and additionally of pain (central column), with consequences of for the animal (right hand column), and for the physiologist (left hand column) Modified from Ref. [138].

provision of good quality science as well as animal welfare, within the framework of recommended or mandated 3Rs provision and legislation [7,13,14].

How to study pain in non-mammalian species

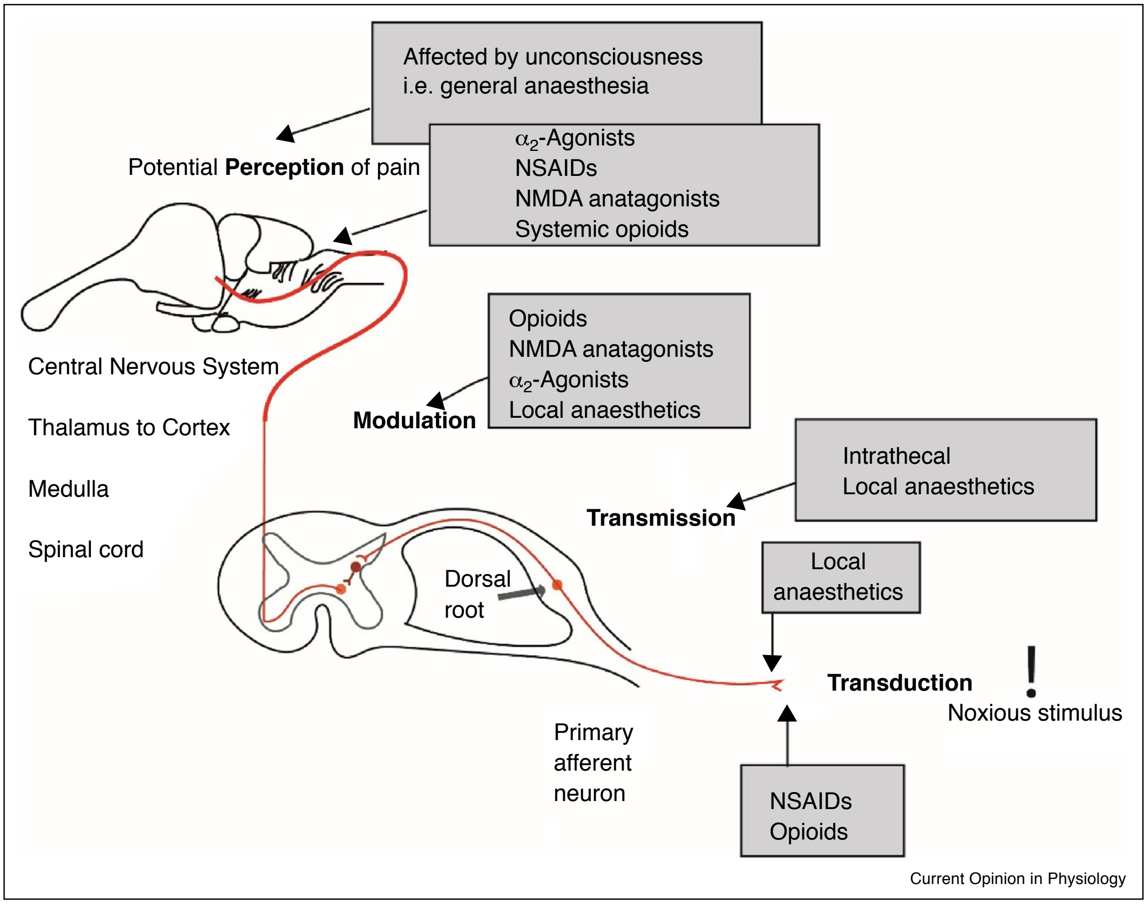
Given the inability of animals to declare whether or not they feel pain, we must resort to indirect approaches to distinguish pain from nociception [12••]. An immediate withdrawal in response to a harmful stimulus does not suffice because this type of reflex does not require emotional input. Two different approaches have been advanced; one focusing on the presence or absence of peripheral and central anatomy required for nociception and pain, [10••,15] and another interrogating behavioral changes in response to harmful stimuli [16,17•]. As reviewed below, we believe these approaches complement each other.

The anatomy of pain

Nociceptors, primary neurons and the spinal cord

Nociceptors were defined by Sherrington as a distinct class of nerve fibers conveying noxious information more than a century ago [18]. Nociceptors in mammals are classically polymodal, responding to a variety of noxious or potentially noxious signals, such as intense mechanical pressure, extreme temperature, low pH and certain chemical stimuli. Their receptors include families of acid sensing receptors (ASICs) and transient receptor potential receptors (TRPs), which are widely present amongst sensory systems in vertebrates and invertebrates [1•,19–21]. Nociceptors are characterized by free nerve endings connected to a small diameter myelinated fiber (Aδ) or un-myelinated C fiber. These fiber types provide the sharp early sensation of pain and later dull/burning sensation, respectively, due to their different conduction velocities. These nerves can be sensitized by a milieu of inflammatory mediators leading to increased firing, or recruited to fire when sensitized, thus giving rise to increased pain from the same stimulus (hyperalgesia). Nociceptive fibers synapse within the dorsal horn of the spinal cord, where further sensitization can take place in response to injury, recruiting non-nociceptors, such as Aβ mechanoreceptors, producing sensations of pain from

Figure 3



A nociceptive pathway illustrated for the snake, with sites of action of the major classes of analgesic drugs. Cell bodies illustrated for known sites, thicker red line for unknown pathways in reptiles. Modified from Refs. [101,139–141].

non-noxious stimuli (allodynia). Sensation from the head, including nociception, proceeds to the central nervous system in the trigeminal cranial nerve. Nociceptive information from the body mainly crosses the spinal cord, moves in the spinothalamic tract to the thalamus and thence to central processing areas in the mammal brain.

Nociceptors have been characterized in most major groups of vertebrates (Figure 1). Broadly, slower conduction compared to non-noxious stimuli, and responses to multiple classes of noxious stimuli are conserved. There are however taxonomic differences in threshold and response of the putative nociceptive receptors, for example, of the TRP-V1 channel to capsaicin reactivity between mammals and other phyla. Differing responses of TRP-A to heat, noxious heat or cold potentially align with adaptations, such as the apparent emergence of cold nociception with the transition to terrestrial living or endothermy [1•,22–27]. Differences in transmission of nociceptive stimuli are not only found in non-mammalian systems; specialized mammals have different nociceptive thresholds, such as naked mole rats that are insensitive to acid [28], and grasshopper mice that are insensitive to bark scorpion toxin [29].

Within agnathans, modern representatives of the earliest vertebrate group, sea lampreys have small diameter un-myelinated fibers running to the spinal cord and in the trigeminal nerve with a polymodal profile responding to extreme heat and intense mechanical pressure but not cold [30,31], although enkephalin opiates appear to be absent from the spinal cord [32]. The presence of nociception in elasmobranchs is questioned [33] due to very low numbers of unmyelinated fibers in somatic nerves of fully grown specimens (0.7–1.2%), along with a lack of some dorsal horn nociceptive areas, and an absence of classic polymodal nociceptors (despite detection of some myelinated mechanical nociceptors in sting rays [34]). It will be interesting to unravel whether this represents a loss of function in transmitting information on chemical and thermal damage. However, more work is required to rule out elasmobranch nociception, especially given high concentrations of enkephalin and neuromodulators, such as Substance P, in the spinal cord [35], as well as the presence of acid sensing receptors in the central nervous system [34,36]. There is speculation that localized chemical and thermal stimuli may simply not require responses in a marine environment (Leonard, 1985, however *c.f.* their sensation in teleost fish) or that integration of other sensory modalities, for example, electroception, may give earlier warning of potential damage, rendering local nociception unnecessary [33]. Polymodal and bi-modal nociceptors, with Aδ and C fiber projections to the spinal cord dorsal horn or in trigeminal nerve have been found in teleost fish [22,37•,38], amphibians [39,40,41•], reptiles [42–44], and birds [45–47]. The proportion of C fibers, for example, in the trigeminal nerve varies widely from 65% in amphibians and 40–50% in mammals, to 28–30 % in birds and 4% in the rainbow trout [47,48], but the functional significance of these differences is largely unknown and we must also account for effect of body temperature on nerve conduction.

Central processing

Central processing of pain in humans and other mammals is a rapidly evolving research area, where neuroimaging has recently allowed the emergence of the 'pain matrix'. This is a network of areas activated in pain after relay of information through the thalamus, including the primary and secondary somatosensory cortex, posterior and anterior insula and anterior cingulate cortices, pre frontal cortex and amygdala [49–53]. Of these areas, the first three have been linked to somatosensory pain, with the latter three permissive of emotional pain experience [54,55]. Whether these areas and their connections have homologues in the non-human and non-mammalian brains has understandably attracted attention. The presence of a layered cortex with interconnected association areas has for some researchers been non-negotiable in terms of attributing pain [10••,15,41•,56]. However, despite a lack of columnar cortical structure, and a much less developed research field than that active in mammals, central projections of nociceptive pathways in fish have begun to be identified *i)* to the telencephalon showing amplitude variable responses to stimulus intensity [57•,58–60] *ii*) projecting to areas considered homologous to the amygdala (medial telecephalonic pallium) and *iii)* implicated in avoidance learning, leading to changes to behavior upon lesioning [61]. These projections lead to species-specific findings of nociceptive localization, learning and post nociceptive behavior [58,62,63], and are subject to central modulation [64,65,66•,67]. Hence, mounting evidence supports substantive central nociceptive modulation in fish, which together with increasing evidence of complex cognitive ability, is supportive of pain attribution [11•,68,69]. In birds, reptiles and amphibians, primary spinal neurons and dorsal horn characteristics are similar to those in mammals, but central projections to the thalamus and telencephalon are less well understood. There are reports of projections to and from the medullary reticular formation [70,71] and primordial hippocampus [72] reviewed for amphibians [41•] and birds [47].

Some CNS measures such as electroencephalogram (EEG) or functional magnetic resonance imaging (fMRI) blood-oxygen-level-dependent imaging (BOLD-contrast imaging) pattern activity changes following nociceptive stimulation may be highly instructive in non-mammalian vertebrates, but could suffer methodologically from the difference in architecture of the pallium, such as a deeper non-columnar structure in birds and smaller CNS of amphibians, reptiles and fish [73••]. A deeper understanding of nociceptive neuroanatomy in non-mammalian species is required to ground future studies.

In mammals, descending modulation of spinal nociceptive signals is coordinated by the periaqueductal gray (PAG), and rostroventral medial medulla (RVM), leading to facilitation or depression of ascending spinal pathways. These are classically a site of high opiate receptor concentration, along with the dorsal horn of the spinal cord. Non-opiate modulation, for example by oxytocin in the PAG, is common in rodents, but less repeatable in human studies [74]. Endogenous opiates and their receptors have been found in the CNS of reptiles, amphibians, fish and birds [75–78], allowing modulation by endogenous opiates of nociceptive responses in response to conspecific alarm and stress in fish [64,79]. Behavioral evidence of the efficacy of exogenous opioids as analgesics in non-mammalian animals has been another bulwark of attribution of pain in these species [12••]. Effective doses of drugs such as morphine are higher in ectotherms, than those necessary for antinociception in mammals, [39,80–82] and while physiological side effects such as respiratory depression, sedation or hyperexcitability are noted, they also occur at doses much higher than the therapeutic index in mammals.

Central processing and experience of any sensory input are not going to be the same in humans and other vertebrates with markedly different natural histories, evolutionary trajectories and CNSs. However, the shared features of nociceptive pathways, central modulation and descending influence of opiate system argue for a common framework for the processing of noxious stimuli and their integration into a sensory milieu that informs CNS function. High level cortical functions, such as learning and memory, have different CNS anatomical substrates in birds to that in mammals but similar networks and abilities [83], and the same may be true for the complex production of pain. Hence, neuroanatomy cannot provide the only way to understand this important question.

Behavior as a measure of pain in animals?

Measuring the behavioral output of an organism is the other arm of research investigating nociception and pain in non-mammalian animals, and may be sensitive to different neurological systems' potential to produce an organismal effect in response to noxious stimuli. Behavioral tests can be divided into nociceptive tests and those testing more complex outcomes. The former test reflex outcome to standardized noxious stimuli such as thermal and electrical stimuli, used in rodents and adapted for birds [84,85••], amphibians [86,87] reptiles [81,88,89,90•,91•,92] and fish [59,60,93–95]. Thermal nociceptive tests have been productive, but could be prone to misinterpretation as exposure to different temperature can have complex effects in ectotherms where temperature conditions may vary in terrestrial versus aquatic habitats [86,87,90•]; temperature changes may be locally noxious but if experienced systemically also cause obvious changes to all physiological functions and therefore render interpretation difficult. Well-established tests in mammalian research use exposure to noxious chemicals such as acetic acid, formalin and capsaicin to produce a potentially longer-lasting nociceptive stimulus, causing immediate physiological or behavioral changes, for example, in amphibians [39,86], reptiles [96–98] and longer term behavioral change such as change to locomotion, abnormal rocking and rubbing behaviors when injected in fish [37•,63,82,99], and delayed return to normal feeding, mimicking that of a surgical stimulation under anesthesia in pythons [17•]. These can also be the substrate for testing the efficacy of analgesics [99,100] (Figure 3).

These nociceptive tests validate stimuli then used to measure more complex outcomes, such as clinical examination of the whole organism [101] and the change in behavior from a normal ethogram [102•], and in mammalian literature the recent development of the pain face, which can be dissociated from nociceptive reflexes by lesion of the anterior insula [49]. The 'pain face' explosion in mammalian literature [103,104] is limited in its application to non-mammals by the profound differences in facial skeleton and musculature. Long-term feeding behavior and associative learning, such as place preference and other changes in long-term behavioral patterns, may be more amenable to transfer and give more information about affective state, for example, cognitive bias. Nociceptive tests while useful for drug screening cannot fully replicate the tonic spontaneous pain associated with surgery, or injury, and may not reveal the full efficacy of analgesics in these cases, so surgical or spontaneous models of pain are used within both mammalian and non-mammalian systems. These have included behavioral and physiological sparing effects of opiate or nonsteroidal analgesia following surgery in fish and reptiles, [82,105•,106,107•,108,109], surgery or spontaneous orthopedic injury in chickens [110,111]. One difficulty of using more complex behaviors to assess analgesic activity is the need to fully validate the measures of species-specific behavior before testing and rule out effects of the analgesics themselves on behavior [13,102•]. Improved knowledge of opiate systems is required given inconsistencies across taxa in efficacy in nociceptive tests [91•].

The classes of drugs that are available for use in vertebrates act at the nociceptive pathway to block transduction (local anesthetics), the inflammatory milieu surrounding nociceptors in inflamed areas (non-steroidal anti-inflammatories), or on central and descending pathways (opiates, NMDA antagonists, alpha 2 agonists, oxytocin like molecules and neuraxial use of local anesthetics) Figure 3. The possibilities mirror those currently used in mammalian systems, but more research is required in non-mammalian organisms into which groups, drugs dosages and means of application are effective for which taxa. Options are reviewed in laboratory animal and veterinary literature for fish, reptiles, amphibians and birds [101,112••,113–116].

Since analgesic drugs interact with widespread receptors if given systemically, they may also have their own physiological effects, especially undesired within experimental situations [96,107•,117] requiring greater study of the appropriate drugs, dosages and routes across taxa. Some of the criticisms that have been made of nociceptive research in non-mammalian animals focus on the potential for different pharmacokinetics and dynamics of the dosages used and the need for consideration of non-analgesic effects [107•,118,119•].

Conclusions, perspectives, and recommendations

For the authors, the evidence for central modulation of nociceptive inputs and the balance of behavioral evidence reinforces the attribution of pain and therefore the necessity for effective analgesia in non-mammalian vertebrates. Even if pain is not ascribed, the effect of tissue damage without antinociception has causative and parallel cascades of effects on stress, behavior and immunofunction, profoundly affecting most physiological data. Effective pharmacological anti-nociceptives which act as analgesics therefore form part of a holistic stress-reducing set-up to improve the animal's experimental environment and the physiological data collected whether in non-verbal humans [120] or non-mammalian animals. Analgesia can run parallel with good handling and surgical practice, for example, the Keepemwet campaign and the effect of reducing surgical invasiveness and improving surgical practice on physiological variables [121,122]. Analgesia is a mainstay of veterinary medicine; using opioid analgesia in surgery can reduce the anaesthetic agent concentration required (i.e. Minimum Anaesthetic Concentration (MAC) sparing effect) therefore reducing the anaesthetic's off-target physiological effects [123,124]. Pre-emptive analgesia, for example, premedication before anaesthesia for surgery, or use of local anaesthesia before incision, prevents sensitization at the level of the spinal cord, production of PGE2 in the brain and activation of the pituitary adrenal stress axis in mammals [125,126]. Multimodal and balanced analgesia, a concept brought from human to veterinary medicine [127,128] allows multiple pathways to nociception and pain to be blocked or modulated, effecting post-operative analgesia with lower drug doses, thus reducing their unwanted physiological effects. These techniques are now routine and advocated in non-mammalian veterinary care [101,129] and can be employed by comparative physiologists in non-mammalian species following verification of efficacy. Physiologists working on terminal preparations gain from the reduction in off-target effects after multimodal analgesia as well as the avoidance of complex immune/neural networks of effects following nociception via pre-emptive and peri-surgical anti-nociception (Figures 2 and 3). Those working on animals after recovery from surgery additionally benefit from the effects of post-operative analgesia.

The argument for effective analgesia mirrors the progression of knowledge within our species; while human infants were not believed to 'feel' pain the physiological consequences of providing them with antinociceptives under surgery were stark [5] and evidence of a discrimination of pain from other sensory modalities has later reinforced this view [130]. Imaging and electrical mapping of pain and efficacy of analgesics in infants [131,132] are a useful mirror for experimental design in non-mammalian vertebrates, but differences in pallial architecture must be taken into account [73••]. Non-mammalian vertebrates, especially zebrafish, are increasingly used as model systems, including in pain and nociception [133–136]. It is key that comparative physiology is central to these experiments given physiologists' knowledge of the biology of their study species. Collaborations between physiologists and veterinary professionals can aid drug selection in specific experiments, but also enable the experimental planning required to illuminate non-mammalian nociception and pain [13].

Conflict of interest statement

Nothing declared.

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References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. • Smith ESJ, Lewin GR: **Nociceptors: a phylogenetic view**. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2009, **195**:1089-1106.
Thorough review of nociception including vertebrate and invertebrate taxa.

2. Sneddon LU: **Comparative physiology of nociception and pain**. *Physiology* 2018, **33**:63-73.

3. Kumar S, Hedges SB: **A molecular timescale for vertebrate evolution**. *Nature* 1998, **392**:917-920.

4. IASP: **IASP taxonomy**. *Update from Pain Terms, A Current List with Definitions and Notes on Usage. Classification of Chronic Pain*. edn 2. 2012:209-214:. IASP Task Force on Taxonomy.

5. Anand K, Sippell W, Green A: **Randomised trial of fentanyl anaesthesia in preterm babies undergoing surgery: effects on the stress response**. *Lancet* 1987, **1**:243-248.

6. Flecknell P: **Analgesia from a veterinary perspective**. *Br J Anaesth* 2008, **101**:121-124.

7. European Council: **Directive 2010/63/EU of the European parliament and of the council on the protection of animals used for scientific purposes**. *Off J Eur Union* 2010, **1**:33-79.

8. Braithwaite VA, Huntingford FA: **Fish and welfare: do fish have the capacity for pain perception and suffering?** *Anim Welf* 2004, **13**:87-94.

9. Weary DM, Droege P, Braithwaite VA: **Behavioral evidence of felt emotions: approaches, inferences, and refinements**. *Adv Stud Behav* 2017, **49**:28-47.

10. •• Key B: **Why fish do not feel pain**. *Anim Sentience* 2016, **3**:1-33.
Starting point for robust discussion of fish pain in the responses to this article.

11. • Brown C: **Comparative evolutionary approach to pain perception in fishes**. *Anim Sentience* 2016:0-11. Commentary on Key on Fish Pain.
Key response article.

12. •• Sneddon LU, Elwood RW, Adamo SA, Leach MC: **Defining and assessing animal pain**. *Anim Behav* 2014, **97**:201-212.
Broad ranging pertinent review of animal pain.

13. Chatigny F: **The controversy on fish pain: a veterinarian's perspective**. *J Appl Anim Welf Sci* 2018:1-11.

14. *Guide for the Care and Use of Laboratory Animals*. National Academies Press; 2011.

15. Rose JD: **Pain in fish: weighing the evidence**. *Anim Sentience* 2016, **032**:1-3.

16. Mettam JJ, Oulton LJ, McCrohan CR, Sneddon LU: **The efficacy of three types of analgesic drugs in reducing pain in the rainbow trout, *Oncorhynchus mykiss***. *Appl Anim Behav Sci* 2011, **133**:265-274.

17. • James LE, Williams CJA, Bertelsen MF, Wang T: **Evaluation of feeding behavior as an indicator of pain in snakes**. *J Zoo Wildl Med* 2017, **48**:196-199.
Study using different nociceptive stimuli, surgery, and chemical, and assessing a long term behavioral response in reptiles.

18. Sherrington C: **Qualitative difference of spinal reflex corresponding with qualitative difference of cutaneous stimulus**. *J Physiol* 1903, **30**:39-46.

19. Julius D: **TRP channels and pain**. *Annu Rev Cell Dev Biol* 2013, **29**:355-384.

20. Lynagh T, Mikhaleva Y, Colding JM, Glover JC, Pless SA: **Acid-sensing ion channels emerged over 600 Mya and are conserved throughout the deuterostomes**. *Proc Natl Acad Sci U S A* 2018, **115**:8430-8435 http://dx.doi.org/10.1073/pnas.1806614115.

21. Deval E, Lingueglia E: **Acid-sensing ion channels and nociception in the peripheral and central nervous systems**. *Neuropharmacology* 2015, **94**:49-57.

22. Ashley PJ, Sneddon LU, McCrohan CR: **Nociception in fish: stimulus-response properties of receptors on the head of trout *Oncorhynchus mykiss***. *Brain Res* 2007, **1166**:47-54.

23. Gracheva EO, Ingolia NT, Kelly YM, Cordero-Morales JF, Hollopeter G, Chesler AT, Sánchez EE, Perez JC, Weissman JS, Julius D: **Molecular basis of infrared detection by snakes**. *Nature* 2010, **464**:1006-1011.

24. Saito S, Fukuta N, Shingai R, Tominaga M: **Evolution of vertebrate transient receptor potential vanilloid 3 channels: opposite temperature sensitivity between mammals and western clawed frogs**. *PLoS Genet* 2011, **7**:e1002041.

25. Saito S, Banzawa N, Fukuta N, Saito CT, Takahashi K, Imagawa T, Ohta T, Tominaga M: **Heat and noxious chemical sensor, chicken TRPA1, as a target of bird repellents and identification

of its structural determinants by multispecies functional comparison. *Mol Biol Evol* 2014, **31**:708-722.

26. Saito S, Nakatsuka K, Takahashi K, Fukuta N, Imagawa T, Ohta T, Tominaga M: **Analysis of transient receptor potential ankyrin 1 (TRPA1) in frogs and lizards illuminates both nociceptive heat and chemical sensitivities and coexpression with TRP vanilloid 1 (TRPV1) in ancestral vertebrates**. *J Biol Chem* 2012, **287**:30743-30754.

27. Hamamoto DT, Simone DA: **Characterization of cutaneous primary afferent fibers excited by acetic acid in a model of nociception in frogs**. *J Neurophysiol* 2003, **90**:566-577.

28. Smith ESJ, Omerbasic D, Lechner SG, Anirudhan G, Lapatsina L, Lewin GR: **The molecular basis of acid insensitivity in the African naked mole-rat**. *Science (80-)* 2011, **334**:1557-1560.

29. Rowe AH, Xiao Y, Rowe MP, Cummins TR, Zakon HH: **Voltage-gated sodium channel in grasshopper mice defends against bark scorpion toxin**. *Science* 2013, **342**:441-447.

30. Martin BAR, Wickelgren W: **Sensory cells in the spinal cord of the sea lamprey**. *J Physiol* 1971, **212**:65-83.

31. Matthews G, Wickelgren WO: **Trigeminal sensory neurons of the sea lamprey**. *J Comp Physiol* 1978, **123**:329-333.

32. Buchanan JT, Brodin L, Hökfelt T, Van Dongen PAM, Grillner S: **Survey of neuropeptide-like immunoreactivity in the lamprey spinal cord**. *Brain Res* 1987, **408**:299-302.

33. Snow PJ, Plenderleith MB, Wright LL: **Quantitative study of primary sensory neurone populations of three species of elasmobranch fish**. *J Comp Neurol* 1993, **334**:97-103.

34. Leonard RB: **Primary afferent receptive field properties and neurotransmitter candidates in a vertebrate lacking unmyelinated fibers**. *Prog Clin Biol Res* 1985, **176**:135-145.

35. Snow PJ, Renshaw GMC, Hamlin KE: **Localization of enkephalin immunoreactivity in the spinal cord of the long-tailed ray *Himantura fai***. *J Comp Neurol* 1996, **367**:264-273.

36. Springauf A, Gründer S: **An acid-sensing ion channel from shark (*Squalus acanthias*) mediates transient and sustained responses to protons**. *J Physiol* 2010, **588**:809-820.

37. • Sneddon LU: **The evidence for pain in fish: the use of morphine as an analgesic**. *Appl Anim Behav Sci* 2003, **83**:153-162.
Early evidence for pain in fish.

38. Roques JAC, Abbink W, Geurds F, van de Vis H, Flik G: **Tailfin clipping, a painful procedure: studies on *Nile tilapia* and common carp**. *Physiol Behav* 2010, **101**:533-540.

39. Stevens C: **Analgesia in amphibians: preclinical studies and clinical applications**. *Vet Clin North Am Exot Anim Pract* 2011, **14**:33-44.

40. Adrian ED, Cattell M, Hoagland H: **Sensory discharges in single cutaneous nerve fibres**. *J Physiol* 1931, **72**:377-391.

41. • Stevens CW: **Opioid research in amphibians: an alternative pain model yielding insights on the evolution of opioid receptors**. *Brain Res Brain Res Rev* 2004, **46**:204-215.
A review of amphibian nociception and opioid evolution.

42. Liang YF, Terashima SI: **Physiological properties and morphological characteristics of cutaneous and mucosal mechanical nociceptive neurons with A-delta peripheral axons in the trigeminal ganglia of crotaline snakes**. *J Comp Neurol* 1993, **328**:88-102.

43. Bryant BP, Kraus F: **Neural basis of trigeminal chemo- and thermonociception in brown treesnakes, *Boiga irregularis* (Squamata: Colubridae)**. *J Comp Physiol A* 2018, **0**:0.

44. Liang Y-F, Terashima S-I, Zhu A-Q: **Distinct morphological characteristics of touch, temperature, and mechanical nociceptive neurons in the crotaline trigeminal ganglia**. *J Comp Neurol* 1995, **360**:621-633.

45. Gentle MJ: **Pain in birds**. *Anim Welf* 1992, **1**:13.

46. Zhai XY, Atsumi S: **Large dorsal horn neurons which receive inputs from numerous substance P-like immunoreactive axon terminals in the laminae I and II of the chicken spinal cord**. *Neurosci Res* 1997, **28**:147-154.

47. Dubbeldam JL: **The trigeminal system in birds and nociception**. *Cent Nerv Syst Agents Med Chem* 2009, **9**:150-158.

48. Sneddon LU: **Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, *Oncorhynchus mykiss***. *Neurosci Lett* 2002, **319**:167-171.

49. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, Glick S, Ingrao J, Klassen-Ross T, Lacroix-Fralish ML *et al.*: **Coding of facial expressions of pain in the laboratory mouse**. *Nat Methods* 2010, **7**:447-449.

50. Segerdahl AR, Mezue M, Okell TW, Farrar JT, Tracey I: **The dorsal posterior insula subserves a fundamental role in human pain**. *Nat Neurosci* 2015, **18**:499-500.

51. Baumgärtner U, Buchholz H-G, Bellosevich A, Magerl W, Siessmeier T, Rolke R, Höhnemann S, Piel M, Rösch F, Wester H-J *et al.*: **High opiate receptor binding potential in the human lateral pain system**. *Neuroimage* 2006, **30**:692-699.

52. Brooks J, Tracey I: **From nociception to pain perception: imaging the spinal and supraspinal pathways**. *J Anat* 2005, **207**:19-33.

53. Tracey I: **Imaging pain**. *Br J Anaesth* 2008, **101**:32-39.

54. Barthas F, Sellmeijer J, Hugel S, Waltisperger E, Barrot M, Yalcin I: **The anterior cingulate cortex is a critical hub for pain-induced depression**. *Biol Psychiatry* 2015, **77**:236-245.

55. Xiao X, Zhang YQ: **A new perspective on the anterior cingulate cortex and affective pain**. *Neurosci Biobehav Rev* 2018, **90**:200-211.

56. Rose JD, Arlinghaus R, Cooke SJ, Diggles BK, Sawynok W, Stevens ED, Wynne CDL: **Can fish really feel pain?** *Fish Fish* 2014, **15**:97-133.

57. • Dunlop R, Laming P: **Mechanoreceptive and nociceptive responses in the central nervous system of goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*)**. *J Pain* 2005, **6**:561-568.
Early evidence of central nervous system involvement in nociceptive processing in fish.

58. Dunlop R, Millsopp S, Laming P: **Avoidance learning in goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*) and implications for pain perception**. *Appl Anim Behav Sci* 2006, **97**:255-271.

59. Nordgreen J, Horsberg TE, Ranheim B, Chen ACN: **Somatosensory evoked potentials in the telencephalon of Atlantic salmon (*Salmo salar*) following galvanic stimulation of the tail**. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* 2007, **193**:1235-1242.

60. Ludvigsen S, Stenklev NC, Johnsen HK, Laukli E, Matre D, Aas-Hansen Ø: **Evoked potentials in the Atlantic cod following putatively innocuous and putatively noxious electrical stimulation: a minimally invasive approach**. *Fish Physiol Biochem* 2014, **40**:173-181.

61. Portavella M, Torres B, Salas C: **Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium**. *J Neurosci* 2004, **24**:2335-2342.

62. Reilly SC, Quinn JP, Cossins AR, Sneddon LU: **Behavioural analysis of a nociceptive event in fish: comparisons between three species demonstrate specific responses**. *Appl Anim Behav Sci* 2008, **114**:248-259.

63. Deakin AG, Spencer JW, Cossins AR, Young IS, Sneddon LU: **Welfare challenges influence the complexity of movement: fractal analysis of behaviour in zebrafish**. *Fishes* 2019, **4**:8.

64. Wolkers CPB, Barbosa Junior A, Menescal-de-Oliveira L, Hoffmann A: **Stress-induced antinociception in fish reversed by naloxone**. *PLoS One* 2013, **8**:e71175.

65. Ehrensing RH, Michell GF, Kastin AJ: **Similar antagonism of morphine analgesia by MIF-1 and naloxone in *Carassius auratus***. *Pharmacol Biochem Behav* 1982, **17**:757-761.

66. • Wolkers CPB, Barbosa Junior A, Menescal-de-Oliveira L, Hoffmann A: **GABAA-benzodiazepine receptors in the dorsomedial (Dm) telencephalon modulate restraint-induced antinociception in the fish *Leporinus macrocephalus***. *Physiol Behav* 2015, **147**:175-182.

The cross over between central nervous system modulation, stress and nociception in a fish.

67. Wolkers CPB, Menescal-de-Oliveira L, Hoffmann A: **Cannabinoid system of dorsomedial telencephalon modulates behavioral responses to noxious stimulation in the fish *Leporinus macrocephalus***. *Physiol Behav* 2017, **179**:504-509.

68. Brown C: **Fish intelligence, sentience and ethics**. *Anim Cogn* 2015, **18**:1-17.

69. Braithwaite V: *Do Fish Feel Pain?* OUP Oxford; 2010.

70. Donkelaar HJT, De Boer-Van Huizen R: **Ascending projections of the brain stem reticular formation in a nonmammalian vertebrate (the lizard *Varanus exanthematicus*), with notes on the afferent connections of the forebrain**. *J Comp Neurol* 1981, **200**:501-528.

71. Ebbesson SOE: **Brain stem afferents from the spinal cord in a sample of reptilian and amphibian species**. *Ann N Y Acad Sci* 1968, **167**:80-101.

72. Vesselkin NP, Agayan AL, Nomokonova LM: **A study of thalamo-telencephalic afferent systems in frogs**. *Brain Behav Evol* 1971, **4**:295-306.

73. •• McIlhone AE, Beausoleil NJ, Kells NJ, Mellor DJ, Johnson CB: **Effects of noxious stimuli on the electroencephalogram of anaesthetised chickens (*Gallus gallus* domesticus)**. *PLoS One* 2018, **13**:1-12.

A well discussed attempt to bring mammalian central nervous techniques into an avian model.

74. Boll S, Almeida de Minas AC, Raftogianni A, Herpertz SC, Grinevich V: **Oxytocin and pain perception: from animal models to human research**. *Neuroscience* 2018, **387**:149-161.

75. Reiner A: **The distribution of proenkephalin-derived peptides in the central nervous system of turtles**. *J Comp Neurol* 1987, **259**:65-91.

76. Vecino E, Pinuela C, Arevalo R, Lara J, Alonso JR: **Distribution of enkephalin-like immunoreactivity in the central nervous system of the rainbow trout: an immunocytochemical study**. *J Anat* 1992, **180**:435-453.

77. Larhammar D, Bergqvist C, Sundström G: **Ancestral vertebrate complexity of the opioid system**. *Vitam Horm* 2015, **97**:95-122.

78. Xia Y, Haddad GG: **Major difference in the expression of δ- and μ-opioid receptors between turtle and rat brain**. *J Comp Neurol* 2001, **436**:202-210.

79. Alves FL, Júnior AB, Hoffmann A: **Antinociception in piauçu fish induced by exposure to the conspecific alarm substance**. *Physiol Behav* 2013, **110–111**:58-62.

80. Jones S, Kamunde C, Lemke K, Stevens ED: **The dose – response relation for the antinociceptive effect of morphine in a fish, rainbow trout**. *Vet Pharmacol Ther* 2011, **35**:563-570.

81. Sladky KK, Kinney ME, Johnson SM: **Effects of opioid receptor activation on thermal antinociception in red-eared slider turtles (*Trachemys scripta*)**. *Am J Vet Res* 2009, **70**:1072-1078.

82. Deakin AG, Buckley J, AlZu'bi HS, Cossins AR, Spencer JW, Al'Nuaimy W, Young IS, Thomson JS, Sneddon LU: **Automated monitoring of behaviour in zebrafish after invasive procedures**. *Sci Rep* 2019, **9**:9042.

83. Shanahan M, Bingman VP, Shimizu T, Wild M, Güntürkün O: **Large-scale network organization in the avian forebrain: a connectivity matrix and theoretical analysis**. *Front Comput Neurosci* 2013, **7**:1-17.

84. Hughes RA: **Strain-dependent morphine-induced analgesic and hyperalgesic effects on thermal nociception in domestic fowl (*Gallus gallus*)**. *Behav Neurobiol* 1990, **104**:619-624.

85. •• Caplen G, Baker L, Hothersall B, McKeegan DEF, Sandilands V, Sparks NHC, Waterman-Pearson AE, Murrell JC: **Thermal nociception as a measure of non-steroidal anti-inflammatory drug effectiveness in broiler chickens with articular pain**. *Vet J* 2013, **198**:616-619.

A well substantiated study investigating the use of nonsteroidals in decreasing hyperalgesia in poultry.

86. Coble DJ, Taylor DK, Mook DM: **Analgesic effects of meloxicam, morphine sulfate, flunixin meglumine, and xylazine hydrochloride in African-clawed frogs (*Xenopus laevis*)**. *J Am Assoc Lab Anim Sci* 2011, **50**:355-360.

87. Vachon P: **Hargreaves does not evaluate nociception following a surgical laparotomy in *Xenopus leavis* frogs**. *Res Vet Sci* 2014, **97**:470-473.

88. Leal WP, Carregaro AB, Bressan TF, Bisetto SP, Melo CF, Sladky KK: **Antinociceptive efficacy of intramuscular administration of morphine sulfate and butorphanol tartrate in tegus**. *Am J Vet Res* 2017, **78**:1019-1024.

89. Mans C, Lahner LL, Baker BB, Johnson SM, Sladky KK: **Antinociceptive efficacy of buprenorphine and hydromorphone in red-eared slider turtles (*Trachemys scripta elegans*)**. *J Zoo Wildl Med* 2012, **43**:662-665.

90. • Sladky KK, Kinney ME, Johnson SM: **Effects of opioid receptor activation on thermal antinociception in red-eared slider turtles (*Trachemys scripta*)**. *Am J Vet Res* 2009, **70**:1072-1078.

Example of a thermal nociceptive test in reptiles with opioid antinociception successful in reptiles.

91. • Kharbush R, Gutwilling A, Hartzler K, Gardner A, Abbott A, Cox S, Watters J, Sladky KK, Johnson SM: **Antinociceptive and respiratory effects following application of transdermal fentanyl patches and assessment of brain μ-opioid receptor mRNA expression in ball pythons**. *Am J Vet Res* 2017, **78**:785-795.

Example of the diversity of reptile responses to opioids, with conserved effects on respirator physiology but not in anti-nociception.

92. Couture ÉL, Monteiro BP, Aymen J, Troncy E, Steagall PV: **Validation of a thermal threshold nociceptive model in bearded dragons (*Pogona vitticeps*)**. *Vet Anaesth Analg* 2017, **44**:676-683.

93. Curtright A, Rosser M, Goh S, Keown B, Wagner E, Sharifi J, Raible DW, Dhaka A: **Modeling nociception in zebrafish: a way forward for unbiased analgesic discovery**. *PLoS One* 2015, **10**:1-18.

94. Chervova LS, Lapshin DN: **Opioid modulation of pain threshold in fish**. *Dokl Biol Sci* 2000, **375**:590-591.

95. Nordgreen J, Garner JP, Janczak AM, Ranheim B, Muir WM, Horsberg TE: **Thermonociception in fish: effects of two different doses of morphine on thermal threshold and post-test behaviour in goldfish (*Carassius auratus*)**. *Appl Anim Behav Sci* 2009, **119**:101-107.

96. Williams CJA, James LE, Bertelsen MF, Wang T: **Tachycardia in response to remote capsaicin injection as a model for nociception in the ball python (*Python regius*)**. *Vet Anaesth Analg* 2016, **43**.

97. Kanui TI, Hole K, Miaron J: **Nociception in crocodiles: capsaicin instillation, formalin and hot plate tests**. *Zool Sci* 1990, **7**:537-540.

98. Wambugu SN, Towett PK, Kiama SG, Abelson KSP, Kanui TI, Effects TI, Box PO: **Effects of opioids in the formalin test in the Speke's hinged tortoise (*Kinixys spekii*)**. *J Vet Pharmacol Ther* 2009, **33**:347-351.

99. Correia AD, Cunha SR, Scholze M, Stevens ED: **A novel behavioral fish model of nociception for testing analgesics**. *Pharmaceuticals* 2011, **4**:665-680.

100. Lopez-Luna J, Al-Jubouri Q, Al-Nuaimy W, Sneddon LU: **Reduction in activity by noxious chemical stimulation is ameliorated by immersion in analgesic drugs in zebrafish**. *J Exp Biol* 2017, **220**:1451-1458.

101. Sladky KK, Mans C: **Analgesia**. In *Mader's Reptile and Amphibian Medicine and Surgery.* Edited by Divers SJ, Stahl SJ. Elsevier Health Sciences; 2019:465-474.

102. • Kinney ME, Johnson SM, Sladky KK: **Behavioral evaluation of red-eared slider turtles (*Trachemys scripta elegans*)**

administered either morphine or butorphanol following unilateral gonadectomy. *J Herpetol Med Surg* 2011, **21**:54-62.
Surgery as a nociceptive stimulus, with opioid analgesia assessed via behavioral outcomes: ethogram and feeding.

103. Gleerup KB, Forkman B, Lindegaard C, Andersen PH: **An equine pain face**. *Vet Anaesth Analg* 2015, **42**:103-114.

104. Keating SCJ, Thomas AA, Flecknell PA, Leach MC: **Evaluation of EMLA cream for preventing pain during tattooing of rabbits: changes in physiological, behavioural and facial expression responses**. *PLoS One* 2012, **7**:e44437.

105. Harms CA, Lewbart GA, Swanson CR, Kishimori JM, Boylan SM:
• **Behavioral and clinical pathology changes in koi carp (*Cyprinus carpio*) subjected to anesthesia and surgery without intra-operative analgesics**. *Comp Med* 2005, **55**:221-226.
The effects of analgesia over surgery in fish, showing mild anti-inflammatory effects of carprofen, and behavioral sparing of butorphanol, with a main effect of anaesthesia and surgery on behavior and biochemistry.

106. Newby NC, Wilkie MP, Stevens ED: **Morphine uptake, disposition, and analgesic efficacy in the common goldfish (*Carassius auratus*)**. *Can J Zool* 2009, **87**:388-399.

107. Newby N, Gamperl A, Stevens D: **Cardiorespiratory effects and**
• **efficacy of morphine sulfate in winter flounder (*Pseudopleuronectes americanus*)**. *Am J Vet Res* 2007, **68**:592-597.
Physiology paper assessing the advantages and disadvantages for comparative physiology measurement of the use of morphine.

108. Baker T, Baker B: **Comparative analgesic efficacy of morphine sulfate and butorphanol tartrate in koi (*Cyprinus carpio*) undergoing unilateral gonadectomy**. *J Am Vet Med Assoc* 2013, **243**:882-890.

109. Olesen M, Bertelsen M, Perry S, Wang T: **Effects of preoperative administration of butorphanol or meloxicam on physiologic responses to surgery in ball pythons**. *J Am Vet Med Assoc* 2008, **232**:1183-1188.

110. Nasr MAF, Nicol CJ, Murrell JC: **Do laying hens with keel bone fractures experience pain?** *PLoS One* 2012, **7**.

111. Desmarchelier M, Troncy E, Fitzgerald G, Lair S: **Analgesic effects of meloxicam administration on postoperative orthopedic pain in domestic pigeons (*Columba livia*)**. *Am J Vet Res* 2012, **73**:361-367.

112. Chatigny F, Creighton CM, Stevens ED: **Updated review of fish**
•• **analgesia**. *J Am Assoc Lab Anim Sci* 2018, **57**:5-12.
Balanced contemporary review of analgesia options in fish.

113. Chatigny F, Kamunde C, Creighton CM, Stevens ED: **Uses and doses of local anesthetics in fish, amphibians, and reptiles**. *J Am Assoc Lab Anim Sci* 2017, **56**:244-253.

114. Sladky KK, Mans C: **Clinical analgesia in reptiles**. *J Exot Pet Med* 2012, **21**:158-167.

115. Hawkins MG: **The use of analgesics in birds, reptiles, and small exotic mammals**. *J Exot Pet Med* 2006, **15**:177-192.

116. Sneddon LU: **Clinical anesthesia and analgesia in fish**. *J Exot Pet Med* 2012, **21**:32-43.

117. Williams CJA, Alstrup AKO, Bertelsen MF, Jensen HM, Leite CAC, Wang T: **When local anesthesia becomes universal: pronounced systemic effects of subcutaneous lidocaine in bullfrogs (*Lithobates catesbeianus*)**. *Comp Biochem Physiol Part A Mol Integr Physiol* 2017, **209**:41-46.

118. Stevens ED, Balahura RJ: **Aspects of morphine chemistry important to persons working with cold-blooded animals, especially fish**. *Comp Med* 2007, **57**:161-166.

119. Grans A, Sandblom E, Kiessling A, Axelsson M: **Post-surgical**
• **analgesia in rainbow trout: is reduced cardioventilatory activity a sign of improved animal welfare or the adverse effects of an opioid drug?** *PLoS One* 2014, **9**.
Discussion of the effects of opioids on cardiorespiratory activity.

120. Lago P, Garetti E, Bellieni CV, Merazzi D, Savant Levet P, Ancora G, Pirelli A: **Systematic review of nonpharmacological analgesic interventions for common needle-related procedure in newborn infants and development of evidence-based clinical guidelines**. *Acta Paediatr Int J Paediatr* 2017, **106**:864-870.

121. Sandblom E, Axelsson M: **Autonomic control of circulation in fish: a comparative view**. *Auton Neurosci Basic Clin* 2011, **165**:127-139.

122. Danylchuk AJ, Danylchuk SC, Kosiarski A, Cooke SJ, Huskey B: **Keepemwet fishing—an emerging social brand for disseminating best practices for catch-and-release in recreational fisheries**. *Fish Res* 2018, **205**:52-56.

123. Lang E, Kapila A, Shlugman D, Hoke J, Sebel P, Glass PS: **Reduction in isoflurane minimum alveolar concentration by remifentanyl**. *Anesthesiology* 1996, **85**:721-728.

124. Brosnan RJ, Pypendop BH, Siao KT, Stanley SD: **Effects of remifentanil on measures of anesthetic immobility and analgesia in cats**. *Am J Vet Res* 2009, **70**:1065-1071.

125. Shavit Y, Weidenfeld J, DeKeyser FG, Fish G, Wolf G, Mayburd E, Meerson Y, Beilin B: **Effects of surgical stress on brain prostaglandin E2 production and on the pituitary–adrenal axis: attenuation by preemptive analgesia and by central amygdala lesion**. *Brain Res* 2005, **1047**:10-17.

126. Goldkuhl R, Klockars A, Carlsson H-E, Hau J, Abelson KSP: **Impact of surgical severity and analgesic treatment on plasma corticosterone in rats during surgery**. *Eur Surg Res* 2010, **44**:117-123.

127. Corletto F: **Multimodal and balanced analgesia**. *Vet Res Commun* 2007, **31**:59-63.

128. Slingsby L: **Multimodal analgesia for postoperative pain management**. *In Pract* 2008, **30**:208-212.

129. West G, Heard D, Caulkett N: *Zoo Animal and Wildlife Immobilization and Anesthesia*. end 2. 2014.

130. Fabrizi L, Slater R, Worley A, Meek J, Boyd S, Olhede S, Fitzgerald M: **A shift in sensory processing that enables the developing human brain to discriminate touch from pain**. *Curr Biol* 2011, **21**:1552-1558.

131. Hartley C, Duff EP, Green G, Mellado GS, Worley A, Rogers R, Slater R: **Nociceptive brain activity as a measure of analgesic efficacy in infants**. *Sci Transl Med* 2017, **9**.

132. Goksan S, Baxter L, Moultrie F, Duff E, Hathway G, Hartley C, Tracey I, Slater R: **The influence of the descending pain modulatory system on infant pain-related brain activity**. *eLife* 2018, **7**:1-16.

133. Demin KA, Meshalkina DA, Kysil EV, Antonova KA, Volgin AD, Yakovlev OA, Alekseeva PA, Firuleva MM, Lakstygal AM, de Abreu MS *et al.*: **Zebrafish models relevant to studying central opioid and endocannabinoid systems**. *Prog Neuro Psychopharmacol Biol Psychiatry* 2018, **86**:301-312.

134. Taylor JC, Dewberry LS, Totsch SK, Yessick LR, DeBerry JJ, Watts SA, Sorge RE: **A novel zebrafish-based model of nociception**. *Physiol Behav* 2017, **174**:83-88.

135. Costa FV, Rosa LV, Quadros VA, Santos ARS: **Understanding nociception-related phenotypes in adult zebra fish: behavioral and pharmacological characterization using a new acetic acid model**. *Behav Brain Res* 2019, **359**:570-578.

136. Ellis LD, Berrue F, Morash M, Achenbach JC, Hill J, Mcdougall JJ: **Comparison of cannabinoids with known analgesics using a novel high throughput zebra fish larval model of nociception**. *Behav Brain Res* 2018, **337**:151-159.

137. Naumann RK, Ondracek JM, Reiter S, Shein-Idelson M, Tosches MA, Yamawaki TM, Laurent G: **The reptilian brain**. *Curr Biol* 2015, **25**:R317-R321.

138. Cooley K: **Physiology of pain**. *Pain Management for Veterinary Technicians and Nurses*. John Wiley & Sons, Ltd.; 2017:30-41.

139. Pyati S, Gan TJ: **Perioperative pain management**. *CNS Drugs* 2007, **21**:185-211.

140. Stein C, Lang LJ: **Peripheral mechanisms of opioid analgesia**. *Curr Opin Pharmacol* 2009, **9**:3-8.

141. Kardong KV: *Vertebrates: Comparative Anatomy, Function, Evolution*. McGraw-Hill Education; 2014.