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KEYWORDS

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ABSTRACT

Recent evidence has shown that fish display aversive behavioral and physiological reactions and a suspension of normal behavior in response to noxious stimuli that cause pain in other animals and humans. In addition to these behavioral responses, scientists have identified a peripheral nociceptive system and recorded specific changes in the brain activity of fish during noxious stimulation. As a result of these observations teleost fish are now considered capable of nociception and, in some opinions, pain perception. From both an experimental and an ethical perspective, it is important that scientists be able to assess possible pain and minimize discomfort that may result from invasive or other noxious procedures. If scientists accept that the definition of pain in animals cannot include direct measurement of subjective experience (the standard for humans), then fish fulfill the criteria for animal pain. In this review, recent evidence for pain is discussed in terms of the physiological properties of nociceptors, central responses to noxious stimulation, and changes in behavior and physiology that are indicative of nociception and are responsive to analgesia. To enable the assessment of potential pain, there are descriptions of newly identified robust indicators and species-specific responses that are easily measurable. The article concludes with a discussion of humane endpoints and of the need for alleviation of pain through the use of analgesia and anesthesia.

Nociception and Pain in Animals

The definition and recognition of pain in humans depend on the human ability to communicate “feelings” or subjective state (Chandroo et al. 2004). In the absence of such communication, the assessment of pain in animals is challenging and frequently relies on human definitions (Rose 2002). But because animal neuroanatomy can be very different from that of humans and animals it is essential to develop clear, easily identifiable, species-specific indicators.

The accepted definition of human pain is “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (IASP 1979). According to this definition, pain comprises both sensory and negative affective aspects and is a conscious experience (i.e., the individual knows it is in pain). Perception in animals of stimuli that cause pain in humans—such as significant mechanical pressure, extremes of temperature, and nociceptive chemicals (e.g., acids, venoms)—is easily demonstrable. But measuring the negative affective component, which can be described as discomfort and suffering or consciousness of pain, is problematic—it is simply not possible

to know how an animal “feels.” However, the IASP (1979) adds that “The inability to communicate verbally does not negate the possibility that an individual is experiencing pain”; although this text refers to neonates and infants, it can be applied to animals.

Scientists can assess the likelihood of pain in animals through indirect measures (Molony 1997; Zimmerman 1986), such as adverse changes in behavior and physiology in response to a potentially painful event and the reduction or elimination of these behaviors by the administration of analgesia. Definitions of animal pain thus rely on what can be observed and measured rather than on subjective states.

In general, pain in animals can be thought of as the perception and aversive sensory experience of a noxious stimulus associated with potential or actual injury. The animal should move away almost immediately from the stimulus as a reflex response and the injury should be associated with vegetative responses including inflammation and cardiovascular changes. The animal should also quickly learn to avoid the noxious stimulus and demonstrate sustained changes in behavior that have a protective function to reduce further injury and pain, prevent the injury from recurring, and promote healing and recovery.

In addition to these observable measures, criteria based on both neurobiology and behavior have been suggested to determine whether an animal is capable of pain perception (Bateson 1991; Sneddon 2004). These criteria include the presence of a nociceptive system similar to that of mammals, relevant brain areas to process pain or nociceptive information, pathways from the periphery to these brain areas, and the existence of opioid receptors and endogenous opioids. A thorough assessment of neurobiological and behavioral indicators yields a robust indication of whether an animal is likely to experience pain.

Notwithstanding these criteria and study findings, there is still much debate as to whether animals are capable of pain, and it is particularly vehement regarding fish. Critics acknowledge that fish are capable of nociception (simple detection and reflexive withdrawal in response to noxious stimuli), but there is disagreement as to whether fish can consciously experience the negative affective component of pain (Rose 2002; Sneddon 2004, 2006). The argument centers on brain anatomy—specifically, comparison of the fish and human forebrain (Rose 2002)—and posits that only humans and other primates with a highly developed neocortex are able to suffer from pain and that fish cannot because their cortex is smaller and has fewer neurons. Not only does such an argument defy the laws of evolution, by suggesting that a function suddenly arises with no primitive ancestor (Bekoff and Sherman 2004), it also means that cats, dogs, birds, and other vertebrates are unable to experience pain.

Rather than suggesting that animal pain has to be identical to human pain and so dismissing the possibility of other species experiences of pain (Rose 2002), it seems reasonable to view animal pain as a more rudimentary experience and perhaps consider it on a phylogenetic sliding scale (Bekoff and Sherman 2004). Thus humans have the most advanced, complicated experience of pain and fish have a more primitive form of pain perception. Whereas previous reviews have focussed on the question of whether fish have the capacity or not to experience pain (Chandroo et al. 2004; Rose 2002), this review presents more recent evidence to identify indicators of potential pain in fi sh and humane endpoints in experimentation. Regardless of characterization, as morally and ethically advanced individuals humans should consider pain a significantly negative experience for animals and should seek to avoid, minimize, and alleviate it whenever possible.

Evidence of Pain Perception in Fish

Nociceptors and Brain Involvement

Nociceptors were first characterized in bony or teleost fish in 2002 (Sneddon 2002), and subsequent work has shown that they are physiologically identical to those of mammals, responding to extreme heat, mechanical pressure, and noxious chemicals (acetic acid and bee venom; Ashley et al. 2006, 2007; Sneddon 2003a, 2004; Sneddon et al. 2003a). In the rainbow trout (*Oncorhynchus mykiss*) they are present in the skin all over the head and in the cornea of the eye, and may have been characterized in the ancestor of modern fishes, the lamprey (*Petromyzon marinus*), in which sensory neurons responded to burning and piercing of the skin (Matthews and Wickelgren 1978). However, nociceptors have yet to be found in elasmobranch fish (sharks, skates, and rays). The major tracts that convey pain information from the periphery to the brain are the spino-thalamic tract (body) and the trigeminal tract (head). Both have been studied in agnathans, teleost, and elasmobranch fish (review in Sneddon 2004; e.g., trigeminal in the common carp, *Cyprinus carpio*, Luiten 1975; spinothalamic tract in the sea robin, *Prionotus carolinus*, Finger 2000). Researchers have suggested that responses to nociception in fish are simply reflexive and do not ascend further than the spinal cord or hindbrain to involve higher brain areas (Rose 2002). Recent studies refute this idea since electrical activity during noxious stimulation has been recorded in the forebrain and midbrain of rainbow trout, goldfish (*Carassius auratus*), and Atlantic salmon (*Salmo salar*) (Dunlop and Laming 2005; Nordgreen et al. 2007), and this electrical activity differed according to stimulus type (e.g., simple touch vs. noxious, potentially painful stimuli).

Molecular techniques have also demonstrated that the brains of rainbow trout and common carp exhibit global changes at the level of gene expression (Reilly et al. 2008a), according to separate measurements in the forebrain, midbrain, and hindbrain both during a noxious stimulus and for up to 6 hours afterward. Most of the changes occurred in the forebrain, suggesting that this is the most important site in the physiological processing of noxious information and mirrors the importance of the forebrain in mammalian pain processing. Together these studies demonstrate that higher brain areas are implicated in the fish response to potentially painful events and that their response is not a simple reflex.

Furthermore, researchers have identified opiate and opioid receptors in the fish brain (Alvarez et al. 2006; Buatti and Pasternak 1981; Li et al. 1996; Porteros et al. 1999) as well as enkephalin-like (endogenous) substances in goldfish (Finger 1981; Schulman et al. 1981) and rainbow trout (Vecino et al. 1991). Opioid receptors and endogenous substances are present in the neural regions involved in the processing of nociceptive and pain information in mammals—the spinal cord, the raphe nucleus, the reticular formation, the periaqueductal gray, and the thalamus (Simantov et al. 1977). Enkephalins in the fish brain show a similar distribution pattern to that of higher vertebrates (Vecino et al. 1992). In the fish spinal cord, enkephalin-like immunoreactivity is most dense in the superficial portion of lamina A, which is thought to be similar to the mammalian substantia gelatinosa that is important for nociception (Snow et al. 1996). Thus opioid receptors and endogenous substances are present in the fish nervous system (Gonzalez-Nunez and Rodríguez 2009, in this issue).

Behavioral and Physiological Changes

Behavioral changes that indicate a protective function in response to a potentially painful event are important indicators of a negative affective component associated with the sensory experience. Thus the ability to learn to avoid a noxious stimulus is a very useful indicator of whether an animal experiences a stimulus as aversive, and studies using classical conditioning with negative reinforcement have shown that teleost fish are capable of learning to avoid a noxious stimulus (Sneddon 2004). For example, Ehrensing and colleagues (1982) showed that goldfish learned to avoid an electric shock but that learning did not occur if they received the analgesic morphine. The aversive nature of the stimulus was important for learning and memory consolidation. Similarly, rainbow trout, common carp, and zebrafish (*Danio rerio*) that experienced a noxious stimulation exhibited rapid changes in physiology and behavior that persisted for up to 6 hours (Reilly et al. 2008b; Sneddon et al. 2003a,b) and thus were not simple reflexes. In other

studies, rainbow trout that received an injection of dilute acetic acid to the upper and lower frontal lips rubbed the affected area against the available substrates (Ashley et al. 2009; Sneddon 2003a); in contrast, saline-injected fish did not demonstrate this anomalous behavior, which may have had the function of reducing the intensity of the noxious sensation, as has been described in humans and mammals that rub an affected area to reduce pain (Roveroni et al. 2001).

A reduction in swimming and other activity in rainbow trout and zebrafish may also have a protective role in terms of energy expenditure (permitting the diversion of energy to recovery) and the prevention of further damage and pain (Ashley et al. 2009; Reilly et al. 2008b; Sneddon et al. 2003a). Trout suspended normal feeding behavior after the injection of acid in their lips and resumed feeding only when the adverse changes in physiology subsided (Sneddon 2003b; Sneddon et al. 2003a). Such a behavioral change may have a protective function comparable to guarding behavior in an animal that reduces the use of a painful area or limb to protect it from further pain or injury; birds, mammals, and humans all display guarding behavior (Banik et al. 2005; Shega et al. 2008; Wylie and Gentle 1998).

Fish that experience a noxious stimulus also display a dramatic rise in gill ventilation rate (number of gill beats per minute); in trout and zebrafi sh, for example, it almost doubles from normal rates (Ashley et al. 2009; Reilly et al. 2008; Sneddon 2003b; Sneddon et al. 2003a). Trout normally exhibit these rates only at their maximum swimming speed (Altimiras and Larson 2000), but the fi sh responding to noxious stimuli showed a reduction in activity.

The administration of an analgesic (morphine) significantly reduced all of the behavioral and physiological changes described above, further demonstrating that they were specifically due to pain (Sneddon 2003b).

More recent studies have attempted to determine the significance of these noxious stimulations to fish. Using competing stimuli, investigators demonstrated that responses to pain took precedence in trout that had experienced a noxious stimulus, whereas the fish did not show an appropriate fear response by either avoiding novel objects or exhibiting antipredator behavior (Ashley et al. 2009; Sneddon et al. 2003b; for a review of fear in fish, Ashley and Sneddon 2007). Studies in goldfish and trout showed that they can learn to avoid a noxious electric shock, as they avoided entering the area where the shock was given even when food was present (Millsopp and Laming 2008); however, avoidance behavior depended on hunger levels, as previously shocked fish would enter to obtain food if they had been starved. Together, these findings demonstrated that a potentially painful event was significant to the fish, altering both their behavior and physiology and deleteriously affecting their normal behavioral responses.

Observable Indicators of Pain

Most behavioral changes in fish after noxious stimulation occur in the period immediately after the treatment for up to 120 minutes, peaking between 60 and 90 minutes. Such changes include anomalous behaviors such as rubbing the affected area, rocking on the substrate to and fro on both pectoral fins, and swimming less. These behaviors are conspicuous and easily observable if fish are in glass tanks; observation is more difficult in opaque tanks and in large numbers of fi sh. A reduction in activity is easily measurable, but requires an existing measure of normal pretreatment behavior; most published studies record baseline observations for 15 to 30 minutes, so this is not usually too costly in terms of time, although an assessment of high numbers of fish would require more time.

Another robust indicator that can easily be measured is ventilation rate. In rainbow trout, a normal average rate of 54 beats/min increased to over 90 beats/min after a potentially painful event (Sneddon et al. 2003a). Zebrafish showed a similar change in ventilation rate, whereas common carp showed no such change (Reilly et al. 2008b). These results illustrate species-specific responses and the necessity of

meaningful and reliable indicators of pain assessment for any animal to enable ready detection and intervention. But current behavioral studies of pain in fish are restricted to a very small number of species (rainbow trout, common carp, and zebrafish) and to one model of pain (subcutaneous injection of acetic acid) (Reilly et al. 2008b; Sneddon 2003b; Sneddon et al. 2003a). There is a significant need for more research to include a wider range of species, different pain measures, and different models of pain (e.g., noxious stimulation of fins and body, fin clipping or other tissue damage, disease, and parasite infestation).

Humane Endpoints for Experimental Studies

Moral and ethical questions, including the determination of humane endpoints, must be addressed in the use of animals especially when a procedure results in tissue damage that would give rise to pain in humans. It is clear from the research evidence that fish are capable of nociception and that their experience meets the criteria for animal pain and is biologically important to the individual. Scientists should therefore administer analgesia if it does not interfere with the study results, and ideally should apply humane endpoints before an animal experiences pain and suffering, although if pain is the subject of the study it may be unavoidable.

The definition of specific endpoint criteria is difficult due to the lack of research on fish pain and suffering. Possible humane endpoints include early termination of a study or changes that avoid, reduce, minimize, or alleviate pain. One might end an experiment at the peak response to pain (60-90 mins), rather than allowing a longer experimental period, if enough relevant data have been collected. Alternatively, analgesia might be provided at the peak point or, if pain is not the objective of the study, before, during, and after any invasive treatment. In toxicological, disease, and neurobiological studies, tissue damage may occur as a result of exposure to a noxious chemical, pathogen, parasite, or surgery; if the resulting pain is not the focus of these studies, the animals should receive analgesia.

It is well known in the veterinary field that drug types, administration methods, and doses differ between mammalian species; there may similarly be species differences in fish, but there has been very little research on the effects of analgesia in fish. Morphine has been validated as an effective analgesic for trout as it did not affect their normal behavior, feeding, and physiology (Sneddon 2003b). Validation of other opioids, nonsteroidal anti-inflammatory drugs (NSAIDs), and local anesthetics in fish is vital to show that they are safe to use and will not confound research results as well as to determine species-specific efficacy and dosage.[1]

Any invasive procedures involving the penetration or removal of tissue (e.g., surgery or tagging) should take place under anesthesia to ensure that the sedated fish does not experience pain. This is standard practice in mammalian research and has been shown to effectively reduce post-procedural pain (Richardson and Flecknell 2005). Research on fish anesthetics (e.g., Kiessling et al. 2009) is covered elsewhere in this issue (Neiffer and Stamper 2009).

Finally, trained personnel with experience in fish behavior are necessary to effectively characterize changes in behavior and/or ventilation rate. A pilot study may also be necessary to identify humane endpoints before the execution of a larger study and to ensure that the use of anesthesia and analgesia does not compromise achievement of the research objectives. In addition to these currently applicable measures, research is needed to reliably identify humane endpoints in fish and incorporate them as common practice in experimental studies.

[1] For example, mammalian studies have demonstrated that the use of analgesics to alleviate pain from ocular toxicology tests does not interfere with the test objectives (Patrone et al. 1999; Peyman et al. 1994; Stiles et al. 2003).

Conclusions

Pain in fish is a controversial issue, but the research evidence confirms that fish are capable of nociception and appear to experience a negative affective state as well. Researchers should therefore consider fish to be capable of some form of pain and aim to minimize or alleviate it. Much research is still necessary, however, to identify species-specific responses to different types of pain, analgesics, and doses. Such research could identify and validate robust indicators for use in assessing pain in fish. Researchers should also consider alternatives and humane endpoints where fish may be subject to tissue damage or noxious treatment.

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References

Altimiras J, Larsen EJ. 2000. Non-invasive recording of heart rate and ventilation rate in rainbow trout during rest and swimming: Fish go wireless! J Fish Biol 57:197-209.

Alvarez FA, Rodriguez-Martin I, Gonzalez-Nunez V, Fernández de Velasco EM, Sarmiento RG, Rodríguez RE. 2006. New kappa opioid receptor from zebrafish *Danio rerio*. Neurosci Lett 405:94-99.

Ashley PJ, Sneddon LU. 2007. Pain and fear in fi sh. In: Branson E, ed. Fish Welfare. Oxford: Blackwell Publishing. Ashley PJ, Sneddon LU, McCrohan CR. 2006. Properties of corneal receptors in a teleost fish. Neurosci Lett 410:165-168.

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

Ashley PJ, Ringrose S, Edwards KL, Wallington E, McCrohan CR, Sneddon LU. 2009. Which is more important in fish: Pain, anti-predator responses or dominance status? Anim Behav 77:403-410.

Banik RK, Subieta AR, Wu CR, Brennan TJ. 2005. Increased nerve growth factor after rat plantar incision contributes to guarding behavior and heat hyperalgesia. Pain 117:68-76.

Bateson P. 1991. Assessment of pain in animals. Anim Behav 42:827-839.

Bekoff M, Sherman PW. 2004. Reflections on animal selves. Trends Ecol Evol 19:176-180.

Buatti MC, Pasternak GW. 1981. Multiple opiate receptors: Phylogenetic differences. Brain Res 218:400-405.

Chandroo KP, Yue S, Moccia RD. 2004. An evaluation of current perspectives on consciousness and pain in fishes. Fish Fish 5:281-295.

Dunlop R, Laming P. 2005. Mechanoreceptive and nociceptive responses in the central nervous system of goldfi sh (*Carassius auratus*) and trout (*Oncorhynchus mykiss*). J Pain 6:561-568.

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

Finger TE. 1981. Fish that taste with their feet: Spinal sensory pathways in the sea robin, *Prionotus carolinus*. Biol Bull 161:154-161.

Finger TE. 2000. Ascending spinal systems in the fish, *Prionotus carolinus*. J Comp Neurol 422:106-122.

Gonzalez-Nunez V, Rodríguez RE. The zebrafish: A model to study the endogenous mechanisms of pain. ILAR J 50:373-386.

IASP [International Association for the Study of Pain]. 1979. Pain terms: A list with defi nitions and notes on usage. Pain 6:249-252.

Kiessling A, Johansson, Zahl IH, Samuelsen OB. 2009. Pharmacokinetics, plasma cortisol and effectiveness of benzocaine, MS-222 and isoeugenol measured in individual dorsal aorta-cannulated Atlantic salmon (*Salmo salar*) following bath administration. Aquacult 286:301-308.

Li X, Keith DE Jr, Evans CJ. 1996. Mu opioid receptor-like sequences are present throughout vertebrate evolution. J Molecular Evol 43:179-184.

Luiten PGM. 1975. The central projections of the trigeminal, facial, and anterior lateral line nerves in the carp (*Cyprinus carpio* L.). J Comp Neurol 160:399-418.

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

Millsopp S, Laming P. 2008. Trade-offs between feeding and shock avoidance in goldfish (*Carassius auratus*). Appl Anim Behav Sci 113:247-254.

Molony V. 1997. Comments on Anand and Craig (Letters to the Editor). Pain 70:293.

Neiffer DL, Stamper MA. 2009. Fish sedation, anesthesia, analgesia, and euthanasia: Considerations, methods, and types of drugs. ILAR J 50:343-360.

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

Patrone G, Sacca SC, Macri A, Rolando M. 1999. Evaluation of the analgesic effect of 0.1% indomethacin solution on corneal abrasions. Ophthalmologica 213:350-354.

Peyman GA, Rahimy MH, Fernandes ML. 1994. Effects of morphine on corneal sensitivity and epithelial wound healing: Implications for topical ophthalmic analgesia. Br J Ophthalmol 78:138-141.

Porteros A, García-Isidoro M, Barrallo A, González-Sarmiento R, Rodríguez RE. 1999. Expression of ZFOR1, a δ-opioid receptor, in the central nervous system of the zebrafish (Danio rerio). J Comp Neurol 412:429-438.

Reilly SC, Quinn JP, Cossins AR, Sneddon LU. 2008a. Novel candidate genes identified in the brain during nociception in common carp. Neuro Sci Lett 437:135-138.

Reilly SC, Quinn JP, Cossins AR, Sneddon LU. 2008b. Behavioral analysis of a nociceptive event in fish: Comparisons between three species demonstrate specific responses. Appl Anim Behav Sci 114:248-249.

Richardson CA, Flecknell PA. 2005. Anesthesia and post-operative analgesia following experimental surgery in laboratory rodents: Are we making progress? ATLA 33:119-127.

Rose JD. 2002. The neurobehavioral nature of fishes and the question of awareness and pain. Rev Fish Sci 10:1-38.

Roveroni RC, Parada CA, Cecilia M, Veiga FA, Tambeli CH. 2001. Development of a behavioral model of TMJ pain in rats: The TMJ formalin test. Pain 94:185-191.

Schulman JA, Finger TE, Brecha NC, Karten HJ. 1981. Enkephalin immunoreactivity in Golgi cells and mossy fibres of mammalian, avian and teleost cerebellum. Neurosci 6:2407-2416.

Shega JW, Rudy T, Keefe FJ, Perri LC, Mengin OT, Weiner DK. 2008. Validity of pain behaviors in persons with mild to moderate cognitive impairment. J Am Geriatrics Soc 56:1631-1637.

Simantov R, Kuhar MJ, Uhl GR, Snyder SH. 1977. Opioid peptide enkephalin: Immunohistochemical mapping in rat central nervous system. Proc Natl Acad Sci U S A 74:2167-2171.

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

Sneddon LU. 2003a. Trigeminal somatosensory innervation of the head of a teleost fish with particular reference to nociception. Brain Res 972:44-52.

Sneddon LU. 2003b. The evidence for pain in fish: The use of morphine as an analgesic. Appl Anim Behav Sci 8:153-162.

Sneddon LU. 2004. Evolution of nociception in vertebrates: Comparative analysis of lower vertebrates. Brain Res Rev 46:123-130.

Sneddon LU. 2006. Ethics and welfare: Pain perception in fish. Bull Eur Assoc Fish Path 26:6-10.

Sneddon LU, Braithwaite VA, Gentle MJ. 2003a. Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. Proc R Soc Lond B Biol Sci 270:1115-1121.

Sneddon LU, Braithwaite VA, Gentle MJ. 2003b. Novel object test: Examining nociception and fear in the rainbow trout. J Pain 4:431-440.

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

Stiles J, Honda CN, Krohne SG, Kazacos EA. 2003. Effect of topical administration of 1% morphine sulfate solution on signs of pain and corneal wound healing in dogs. Am J Vet Res 64:813-818.

Vecino E, Ekstrom P, Sharma SC. 1991. Enkephalin-like immunoreactive cells in the mesencephalic tegmentum project to the optic tectum in *Salmo gairdneri* and *Salmo salar*. Cell Tissue Res 264:133-137.

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

Wylie LM, Gentle MJ. 1998. Feeding-induced tonic pain suppression in the chicken: Reversal by naloxone. Physiol Behav 64:27-30.

Zimmerman M. 1986. Physiological mechanisms of pain and its treatment. Klinische Anaesthesiol Intensivtherap 32:1-19.