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DO PAINFUL SENSATIONS AND FEAR EXIST IN FISH?

Lynne U. Sneddon*

Abstract

The detection of pain and fear in fish has been subject to much debate and, since fish are a popular experimental model and commercially important in both angling and aquaculture, many procedures that fish are subjected to cause injury, fear and stress. These injuries would give rise to the sensation of pain in humans but whether fish have the capacity for pain is relatively under explored. Recent evidence has shown that fish have the same neural apparatus to detect pain that mammals and humans do, that their brain is active during a potentially painful experience, that fish show negative changes in behaviour and physiology and that this is reduced by administering a pain killer. Experiments demonstrating the significance of pain to fish have been conducted and have shown that fish do not show appropriate fear and anti-predator responses during a painful stimulation. This suggests that they are dominated by the pain state confirming its importance to the fish. However, social context affects the aggressive behaviour of fish when noxiously stimulated. In a familiar group, dominant trout perform much less chasing of conspecifics yet this suspension in aggression is not seen when placed in an unfamiliar group of fish. Therefore, responses to pain are more complex and not simple reflexes. Together, these results demonstrate that pain is an important stimulus for a trout and we should seek to minimise and alleviate pain where possible. Studies have demonstrated that fish are capable of exhibiting signs of fear including avoidance behaviour and they may also anticipate fearful events. Recent evidence shall be discussed with future directions suggested.

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The capacity to experience negative states such as pain and fear are integral to the doctrine surrounding animal welfare. To promote positive welfare animals must be free from affective states that are detrimental physically and mentally. Therefore, proving an animal perceives pain and experiences fear provides convincing evidence that their wellbeing can be compromised. From a moral perspective, humans as intelligent ethical beings must ensure that the use of animals should be conducted in the most humane manner endeavouring to maintain their health and welfare. The question of whether fish possess the capacity for experiencing pain and fear is particularly highly debated and dichotomised into those who whose opinions are entrenched in a semantic argument over human brain anatomy\(^1\) versus scientists who conduct research producing data that demonstrates fish exhibit adverse responses to pain and fear\(^2\). We have a complicated relationship with fish, using them as a foodstuff, catching them for sport, employing them as experimental research models and keeping them as pets and exhibits in public aquaria. Therefore, understanding how the practices we subject fish to affects them is of paramount importance if we are to meet minimum welfare standards. Here, the central tenets of pain and fear that must be fulfilled for an animal to be considered capable of both negative affective states shall be discussed, with evidence from scientific studies exploring these in fish. The possibility of adverse welfare states in fish shall be discussed in relation to current practices such as large scale fisheries, angling, experimentation and the ornamental pet trade.

**PAIN: KEY TENETS**

**Tenet One: Neural apparatus**

To detect a stimulus an organism must have the sensory system attuned to that type of sensation whether it is olfactory (smell), gustatory (taste), visual, auditory or painful. The receptors for each type of sensory stimulation specifically detect these particular cues. Therefore, receptors for perceiving potentially painful stimuli (termed nociceptors) preferentially detect tissue damaging agents such as high mechanical pressure, extremes of temperature and chemicals such as acids. Nociceptors have been well studied in mammals and birds,

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but less so in non-mammalian vertebrates. Sneddon was the first to identify nociceptors in the rainbow trout in 2002 using neuroanatomy (Fig. 1) and by recording electrical activity from nociceptors on the head of the trout (Fig. 2). Further research on the properties of these fish nociceptors have demonstrated they are comparable with mammalian nociceptors and respond to noxious heat and pressure in a similar manner.

Topical application of many noxious chemicals such as acetic acid, agents with low pH and carbon dioxide infused water stimulate trout nociceptors. Any chemicals with such properties which fish encounter in their aquatic environment are, therefore, likely to excite their nociceptors. In humans this would give rise to the sensation of pain.

The possession of nociceptors must be accompanied by relevant pathways from periphery and internal tissues to the central nervous system so that the information is conveyed to the brain for processing. If the nociceptive inputs do not ascend higher than the reflex centres (dorsal root ganglion) of the spinal cord or the trigeminal ganglion in the hindbrain then the perception of the painful stimulus is often accompanied by a reflex withdrawal but this instantaneous perception and response are collectively termed nociception. This does not necessarily lead to pain but may do if the information is conducted to higher brain areas in the forebrain and midbrain and leads to a negative affective experience associated with any damage. Therefore, demonstrating that fish have pathways to the brain and that the higher brain is active in response to potentially painful stimuli is particularly important. The trigeminal (head) and spinothalamic (body) tract that are involved in conveying pain information in mammals have also been identified in fish. Studies have shown that stim-

ulation of the body with noxious mechanical and thermal cues does ascend up through the spinal cord to forebrain areas\(^8\). Many critics of fish pain have stated that fish are only capable of nociception and that noxious stimuli simply evoke a reflex response restricted to hindbrain and spinal cord. Therefore, studies have set out to measure activity in the brain of fish using a variety of techniques including electrical recordings with electrodes placed in key brain areas\(^7\) of goldfish, Atlantic salmon and rainbow trout; global gene expression determining molecular changes in trout and carp brain\(^6\); and using imaging technology such as functional magnetic resonance imaging (MRI) in common carp\(^5,10\). The results are convincing that painful stimulation elicits activity in the forebrain and midbrain as well as hindbrain and spinal cord, and that this change in activity differs from that of innocuous, non-painful stimulation. Therefore, higher brain areas are active during pain in fish. All of this evidence demonstrates that fish do indeed possess the neural machinery to detect and respond to pain.

**Tenet Two: Behavioural and physiological responses**

Pain often motivates us to alter our behaviour to promote healing and recovery as well as preventing further pain by behavioural alterations such as guarding behaviour where one does not use a limb or painful area. It is crucial to demonstrate that an animal’s behaviour is detrimentally affected by a painful stimulation and that this is not an instantaneous reflex response. Prolonged adverse changes in behaviour after a painful event suggest suffering or discomfort. Behavioural responses to pain are also accompanied by measureable physiological reactions such as an increase in respiration rate. Therefore, determining whether alterations in behaviour and physiology occur after potentially painful stimulation provides insight into the subjective experience especially if these responses

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are aversive and normal behaviour is suspended\textsuperscript{11}. In rainbow trout, individuals injected subcutaneously by acetic acid, a noxious chemical, exhibit a dramatic increase in opercular (gill cover) beat rate that remains elevated for 3 to 6 hours compared with sham handled individuals and those injected with non-noxious saline\textsuperscript{12}. Many animals reduce activity after a painful event, and rainbow trout and zebrafish do indeed show a substantial reduction in active behaviours\textsuperscript{11}. Trout and carp also perform anomalous behaviours in response to subcutaneous injection of acetic acid into the frontal lips. These behaviours are not seen in control animals and are reduced by the use of an analgesic or painkiller, morphine. Therefore, these behaviours are specific to pain responses in these fish. Reduction of pain-related responses by the use of analgesics in mammals is accepted as firm evidence that whatever alterations occurred were indicative of pain. Analgesic drugs are now being tested in fish with studies exploring side effects using robust behavioural and physiological indicators that can easily be measured by the animal carer. In the case of reduction in activity and elevation of opercular beat rate when stimulated noxiously, lidocaine administered at the same site significantly reduced these adverse changes after 30 minutes but other drugs injected intramuscularly did not\textsuperscript{13} (Fig. 3). Carprofen did eventually have some action reducing some of these responses, however, buprenorphine was not effective in trout. These studies demonstrate that it is indeed nociceptive pathways that are responsible for the changes in behaviour and that normal feeding, swimming and physiological functions are detrimentally affected. These alterations would be acceptable as indicators of pain in mammals and since they are prolonged and are not immediate, short-lasting reflex responses, these suggest that there is a negative experience associated with painful stimulation in fish.


Tenet Three: Consciousness

To be able to suffer, one must be consciously aware of this negative mental state, such that when experiencing pain we must know we are in pain and that we hurt. This conscious awareness is impossible to measure but as humans who are capable of communicating complex concepts through language we can convey whether we are in pain to one another and the degree of such pain as well as intensity, duration and so on. However, humans can exaggerate and if a person has no means of communication we cannot know how much pain they are in. This is very much the context that we operate in when assessing animal pain. How can we get into the animal mind? Unless one has been an animal it is impossible to know what they experience and unscientific to dismiss the capacity for pain or fear since this is impossible to measure directly. However, clever experimentation with consideration for the behaviour, ecology, life history and evolution of an animal can open up routes into obtaining meaningful information on the subjective experience of animals. Fish live in a very different world to humans and have evolved to meet the demands of an aquatic life. Critics of fish pain anthropomorphise pain stating that animals must have the same brain anatomy as humans to be capable of consciously experiencing pain. Yet many studies have shown that fish are capable of complicated behaviours with a relatively smaller brain and they are one of the most successful animal groups. As discussed previously, the fish forebrain and midbrain are active during painful stimulation and fish exhibit complicated changes in behaviour and physiology that are ameliorated by painkilling drugs. Therefore, fish are likely to experience pain but it may be more primitive than that experienced by mammals, however, it not feasible to measure this so we cannot make a firm conclusion. Welfare assessment should be based upon sound scientific approaches. Certainly, many scientists take the view that as we know very little about the neurobiology of consciousness in humans that it would be foolish to make judgements on animals using this criterion\textsuperscript{14}. Yet other scientists are of the opinion that we know enough regarding consciousness in animals and that the absence of the human neocortex does not prevent an animal having some form of conscious awareness nor experiencing negative affective states associated with suffering\textsuperscript{15}.

\begin{itemize}
\item \textsuperscript{15} http://fcmconference.org/img/CambridgeDeclarationOnConsciousness.pdf.
\end{itemize}
Consciousness studies often employ the mirror self recognition test where animals are able to recognise themselves in a mirror. These have failed for fish and the species tested often react to their own image by attacking it seeing the reflection as an intruder or competitor. However, we must consider the evolution and ecology of fish – when would they come into contact with their own mirror image? Terrestrial animals would come to water bodies to drink and would see their own reflection but this is precluded by living under water. This difference in ecology would influence whether mirror self recognition would work and explains why fish have not evolved to recognise themselves in this way\textsuperscript{16}. However, fish can recognise themselves through smell and considering how fish live in a world where light is filtered out at depth, a reliance on other forms of communication are especially important. Cichlid fish can recognise their own odour distinct from others but also distinct from closely related kin\textsuperscript{17}. Therefore, this is evidence for self recognition and the ability to discriminate one owns smell from others.

In terms of pain, one of the central pieces of evidence is whether animals will self medicate with painkillers when in pain or are willing to pay a cost to access such pain relief. Many studies in birds and mammals have shown animals will eat food dosed with analgesics upon experiencing painful stimuli. However, fish suspend feeding until they have recovered from a painful event. In order to determine whether fish will pay a cost to accessing pain relief, zebrafish were given access to two chambers, one of which was enriched with gravel, plants and a live shoal behind a transparent barrier. The other chamber was made unfavourable by being barren and brightly lit. Fish selected the enriched chamber to spend most time in and when they had selected the chamber six consecutive times they were assigned to a noxiously stimulated group which had acetic acid injected subcutaneously and a control group with innocuous saline injected. Half of each group were then re-tested and continued to spend most time in the enriched chamber. However, when an analgesic was added to the unfavourable chamber only fish experiencing pain spent time in this chamber shifting their preference. This demonstrates that fish sought analgesia and were willing to pay the cost of being in a brightly, lit barren area where their pain was reduced\textsuperscript{18} (Fig. 4).

\textsuperscript{18} Sneddon L.U. Zebrafish shift preferences to access analgesia. MS submitted.
is compelling evidence for a negative affective component when fish experience a painful event.

FEAR: KEY TENETS

Tenet One: Neural apparatus

As with pain, the neural machinery required to detect and react to fear-causing stimuli must be comparable with the mammalian brain circuitry. Fear is generally sensed as an external threat to the whole animal. For example, the predator test is a standard fear paradigm in experiments where an animal is exposed to the sight, odour or some other cue of a predator that elicits a fight or flight response. Thus fear stimuli are psychological threats to the survival of the whole animal and fear motivates the animal to make an appropriate defensive response such as freezing, hiding or fleeing. Fear can either be innate or unlearned whereby the stimulus elicits a fear response without the animal previously being exposed to the stimulus (e.g. the predator test) or fear can be learned and in many experimental studies animals are provided with a non-threatening cue or conditioned stimulus (CS) such as an innocuous light or sound paired with the presentation of a fear causing stimulus such as chasing or confinement (unconditioned stimulus; US) a few seconds later. After repeated trials of the CS-US the animal learns to respond to the CS or innocuous cue by showing a fear response in the absence of the actual fear stimulus. Rodent models have been employed in such paradigms investigating the neuronal circuitry and the mammalian amygdala and hippocampal regions are particularly important in mediating emotions especially fear learning and memory. Experiments in fish have shown comparable behaviours, cognitive mechanisms and brain areas that are homologous to the fear circuitry in mammals. For example, the dorsomedial telencephalon in the forebrain area of goldfish has identical functions in fear conditioning as the amygdala of mammals mediating fear responses and learning whereas the goldfish dorsolateral telencephalon is homologous to the mammalian hippocampus involved in spatial learning and retrieval of memories.

The mammalian habenula is an evolutionarily highly conserved diencephalic brain structure subdivided into medial and lateral regions (MHb and LHb, respectively). The LHb sends efferent neurons to monoaminergic neurons and has been implicated in the control of aversive learning and emotional behaviours. The MHb projects to the interpeduncular nucleus (IPN), and regulates fear responses. The zebrafish dorsal habenula (dHb) also connects with with the interpeduncular nucleus (IPN) and is equivalent to the mammalian medial habenula. Anatomically the habenula system in zebrafish is similar (Fig. 5) and studies have sought to address its function by silencing this system during fear responses. Genetic inactivation of the dHb resulted in zebrafish that froze rather than the normal flight response to a conditioned fear stimulus (Fig. 6), suggesting that the dHb-IPN pathway is important for controlling fear responses\textsuperscript{21}.

**Tenet Two: Consistent behavioural response**

Fear responses should generate a coherent set of behavioural and physiological reactions. Measurements of startle, freezing and other defensive behaviours can be coupled with physiological parameters such as heart rate and release of stress hormones, for example, cortisol. Studies in fish have demonstrated a consistent response to threatening stimuli such as avoidance of novel objects, freezing to reduce conspicuousness; escape or fleeing behaviours; thigmotaxis where the fish swims next to tank walls avoiding open, central areas; sinking to depth; fast start swimming and diving responses; and anti-predator behaviours\textsuperscript{22}. Many rodent tests of fear and anxiety are now routinely applied to fish species such as open field, novel object, classical conditioning; avoidance learning, predator cues and scototaxis (preference for darker areas). Combined with studies on pain, fear responses can be evaluated as to whether pain or fear is more important. In rainbow trout, fish show a classic anti-predator response to alarm substance by performing increased escape responses and also hiding under cover. When trout were given a pain stimulus they did not perform correct fear responses and did not increase their use of


cover nor perform escape reactions demonstrating in this context pain was the imperative\textsuperscript{23} (Fig. 7).

**Tenet Three: Anti-anxiety drugs**

The final key criterion that animals must fulfil is demonstrating that anti-anxiety drugs reduce any fear responses such as those described above. Many agents are used to decrease fear and anxiety including benzodiazepines, opioids, cholinergic and serotonergic agents. Benzodiazepines are a major class of drugs used to treat human anxiety disorders and have been shown to reduce fear in mammalian models. Benzodiazepines act by enhancing the action of a neurotransmitter, GABA (gamma-aminobutyric acid) which has an inhibitory influence thus exerts a sedative effect. Binding sites for these drugs are found in comparable brain areas of fish and several experiments have shown they reduce fear responses in zebrafish\textsuperscript{24}. Piracetam, a derivative of GABA, is prescribed to reduce clinical anxiety in humans. Chronic administration of piracetam also reduces fear behaviour in zebrafish where fish spend more time in a white area in a scototaxic (light versus dark chamber) test\textsuperscript{25} (Fig. 8). The opioidergic system has a key role in the modulation of human and animal fear. Fish possess a functional opioidergic system, including both opioid peptides and their receptors akin to the mammalian system. Opioid administration in zebrafish in a fear test reduced the amount of erratic, flight swimming\textsuperscript{23}. Serotonergic mechanisms are not only implicated in depression but also animal anxiety. Selective serotonin reuptake inhibitors (SSRIs) are potent modulators of brain serotonin and many of these drugs have been employed in mammalian studies seeking to reduce fear. Zebrafish have a well-developed serotonergic system but this is not anatomically nor genetically identical, however, many fish serotonin receptors have similar expression patterns.


binding, and physiological properties compared with mammals. As with rodent and human clinical studies on the use of SSRIs, clear anxiolytic action or diminished fear responses of chronic fluoxetine has been recorded in zebrafish\textsuperscript{23}. The cholinergic system relates to the sympathetic and parasympathetic nerve fibres or neurons in which acetylcholine (ACh) is the neurotransmitter liberated at a synapse. Cholinergic receptors are of two types: nicotinic receptors, which are situated in striated muscles and muscarinic receptors, which are situated in parasympathetically innervated structures. Low choline levels have been related to high anxiety in humans, therefore, attention is now turning to the cholinergic system as a new target for reducing fear. Zebrafish administered with nicotine (nicotinic-cholinergic agonist) were more active and spent less time at the bottom compared with untreated fish who displayed a classic fear response of freezing and remaining on the bottom of the tank in a novel tank test. Thus, the neurobiological mechanisms of fear and the impact of selective drugs to reduce fear in humans and mammals are also apparent in fish.

**IMPLICATIONS FOR THE USE OF FISH**

Fish do fulfil the criteria for both animal pain and fear and if we are to accept that many of the procedures we apply during our use of fish are likely to cause tissue damage giving rise to the sensation of pain and may also be considered life threatening then these are likely to evoke fear. The experimental data for both pain and fear in fish are accepted for mammals yet why do some authors reject these when the same indicators are presented? Perhaps this is due to the varied functions that fish serve as a foodstuff, sport, pet and experimental model. If one enjoys catch and release angling as a means of leisure this does involve hooking the fish causing injury as well as suffocating the fish in air to retrieve the hook. One could argue that if the fish is killed quickly and humanely and used for food then there is a benefit to the human that outweighs the cost to the fish. However, catch and release does involve of course the return of live fish to the water body for the pleasure of the fisher at the expense of any impact upon the wellbeing of the fish. Fish are also farmed in high densities to provide protein for our growing populations and many of the practices such as vaccination, size grading, handling, and slaughter will often result in damage or situations which may result in fear. Studies are exploring ways of improving fish welfare in aquaculture which would improve economic return on healthy, well grown fish. The amount of
fish caught at sea outnumber the number of terrestrial animals used for food and not only are the target species of fish caught but many unwanted species are captured and discarded in the process. Fish are also a popular experimental model and as described above much of the experimental data collected is very similar to mammals and small species such as zebrafish are much easier and economically cheaper to maintain than traditional rodent models. Finally, the ornamental pet trade in both freshwater and marine fish is an important industry with fish now being the third most popular pet behind cats and dogs. The purchase of an aquarium set-up and addition of fish does not require any licensing or training.

The impact upon fish welfare does ignite contentious debate, however, how can we reduce the impact we humans have? Is it feasible for humans to stop eating fish? If you accept that fish suffer pain and fear when caught by current fishing practices, it may be a decision that is made by the individual. Alternatively, more welfare friendly solutions could be proposed by improving fishing gear, capture methods, refining the procedure of capture and slaughter so it less invasive, causes less damage and enhances welfare. Can the time between capture of fish at sea and discarding be reduced such that the chances of survival are better? The public drive improvements in animal welfare and it is public opinion that would provide the strongest motivation for enhancing fish welfare during large scale fisheries. The public are willing to pay more for animals farmed under better welfare or from sustainable stocks, therefore, this could and has been applied to the source and method of catching wild fish. Clearly, there are improvements that may be made to current fishing methods, specifically, reduce the time spent fishing so that fish are landed more quickly, reduce the injuries sustained to the fish by improving equipment; use of quick, efficient humane killing techniques on board; and reduce bycatch. These may be

conveniently considered under the following categories but these are not restrictive:

– Reducing the initial numbers of non-target species (bycatch) captured.
– Increasing the survival chances of discarded bycatch.
– Bycatch should be included in fishing quotas.
– Reducing the duration of the capture experience.
– Mitigating the stressful experience of slaughter for target species.
– Adjusting fishing practices to exclude the use of live bait fish.

Many public and government bodies now consider fish to be capable of perceiving pain and as a consequence suffer when injured. Regulations are strict when considering the use of fish in scientific experimentation (e.g. Scientific Procedures Act in the UK\(^ {31} \); guidelines on scientific research in USA\(^ {32} \)). Farmed fish are also subject to scrutiny and the European Food Safety Authority (EFSA) also consider fish to be capable of suffering when subject to poor welfare\(^ {33} \). The Norwegian Scientific Committee for Food Safety have proposed enhancements to recreational catch and release angling to minimise pain and poor welfare during the practice of catching fish for sport or food by individuals\(^ {34} \). Therefore, we should apply these principles of diminishing the impact of large scale fisheries on fish welfare by demanding better methods of fishing. Some authors misguidedly suggest that it is acceptable to treat wild fish in any way and have little or no regard for their wellbeing as we should consider ourselves as predators\(^ {35} \). However, natural predators only kill to satiate their hunger and stop once satisfied. They do not kill many other non-target animals in the process of killing the fish that they consume and they do not massively disrupt and destroy the environment when doing so. To deliberately cause injury and suffering is unethical and as moral beings we have a duty of care to animals that we place in the com-

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pletely unnatural environment of fishing equipment. The scientific evidence that fish are capable of pain perception and of experiencing fear cannot be ignored and we must factor this into our treatment of fish regardless of the context.

ACKNOWLEDGEMENTS

I am grateful to the organisers Martine Lachance and Thierry Auffret Van Der Kemp for inviting me to participate in the LFDA and GRIDA symposium Animal suffering: from science to law. I wish to thank David C.C. Wolfenden for his useful comments on this text and NC3Rs for Research Grant funding.

FIGURE LEGENDS

Figure 1. Section of the maxillary branch of the trigeminal nerve of the rainbow trout showing the presence of A-delta and C fibres that may act as nociceptors (×1000, scale bar=2 µm. Adapted from Sneddon, L. U. 2002. Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, *Oncorhynchus mykiss*. Neurosci. Letts., 319, 167-171 by kind permission from Elsevier).

Figure 2. Electrophysiological recordings from a nociceptive receptive field on the trout face showing responses of nociceptors to heat stimulation. The instantaneous firing frequency (IFF) is displayed in the centre as scatter graphs. This illustrates sensitization of a mechanothermal receptor to heat following noxious chemical stimulation. The firing response to ramp and hold heat stimulation is shown (A) before and (B) 9 min after subcutaneous injection of 1% formalin < 1 mm from the receptive field. Upper trace shows heat stimulus, middle trace plots instantaneous firing frequency (IFF) and lower trace shows extracellular single unit recording from the trigeminal ganglion. Thermal threshold remains the same but firing frequency is greatly increased following formalin injection. (Adapted from Ashley P.J., Sneddon L.U. & McCrohan C.R. 2007 Nociception in fish: stimulus–response properties of receptors on the head of trout *Oncorhynchus mykiss*. Brain Res. 1166, 47-54 by kind permission from Elsevier).
**Figure 3.** The percentage change in (A) activity and (B) opercular beat rate (OBR) performed by rainbow trout 30 minutes after they were injected subcutaneously with saline or a noxious substance, 0.1% acetic acid (Acid) or acid combined with intramuscular injection of 0.1mg/kg buprenophine (0.1 Bup) or 5mg/kg carprofen (5mg/kg Car) or injected at the same site as the acid with 1mg lidocaine (1.0 Lid). The grey line represents the impact of saline (control) treatment whereas the black line represents the impact of pain (acid injection; adapted from Mettam J.J., Oulton L.J., McCrohan C.R. & Sneddon L.U. 2011 The efficacy of three types of analgesic drugs in reducing pain in the rainbow trout, *Oncorhynchus mykiss*. Appl. Anim. Behav. Sci. 133, 265-274 by kind permission from Elsevier).

**Figure 4.** Time spent in either a favourable or unfavourable chamber by zebrafish that were injected subcutaneously with saline (Control) or injected with 1% acetic acid (Acid) when analgesia was present (+ Analgesia) or absent (-analgesia) in the unfavourable chamber. When analgesia was present zebrafish spent more time in the unfavourable chamber (*P<0.001; Sneddon, MS submitted).

**Figure 5.** Diagrammatic representation of the zebrafish habenula (Hb) system. Asymmetric pathways from dorsal Hb (dHb) to the interpeduncular nucleus (IPN) and parallel pathway from ventral Hb (vHb) to the median raphe (MR). Dorsal oblique view on the left and sagittal view on right. Red, dHbL (lateral)-d/iIPN pathway; green, dHbM (medial)-v/iIPN pathway; blue, vHb-MR pathway (Adapted from Agetsuma M., Aizawa H., Aoki T., Nakayama R., Takahoko M., Goto M., Sassa T., Amo R., Shiraki T., Kawakami K., Hosoya T., Higashijima S. & Okamoto H. 2010. The habenula is crucial for experience-dependent modification of fear responses in zebrafish. Nat. Neurosci. 13, 1354-1356. Suppl. Info.).

**Figure 6.** Examples of the control (a) and dHbL-silenced (b) zebrafish locomotion trajectories during retrieval sessions, before (20 s, red dotted lines), during (8.5 s, red solid lines) and after (20 s, blue lines) the conditioned stimulus (CS) exposure. Silenced fish did not show the classic fear conditioned response (Adapted from Agetsuma M., Aizawa H., Aoki T., Nakayama R., Takahoko M., Goto M., Sassa T., Amo R., Shiraki T., Kawakami K., Hosoya T., Higashijima S. & Okamoto H. 2010. The habenula is crucial for experience-dependent

**Figure 7.** (a) The median (interquartiles) change in percentage time spent active in bold and shy fish injected with either saline (control) or acid (acid) from before to after the addition of alarm substance (predator cue). (b) The median change in duration of time spent under cover by bold and shy fish in the control and acid groups from before to after the addition of alarm substance. The arrows indicate the impact of pain upon these behaviours (\(^*P < 0.01. N = 24;\) Adapted from Ashley, P. J., Ringrose, S., Edwards, K. L., Wallington, E., Mccrohan, C. R. & Sneddon, L. U. 2009. Effect of noxious stimulation upon antipredator responses and dominance status in rainbow trout. *Animal Behaviour*, 77, 403-410 by kind permission from Elsevier).

**Figure 8.** Behavioural effects of chronic piracetam (200 mg/L for 7 days; n = 20–23 per group) on adult zebrafish tested in a light–dark box (day 8) showing time spent in white chamber (#P<0.05; Adapted from Grossman L., Stewart A., Gaikwad S., Utterback E., Wu N., DiLeo J., Frank K., Hart P., Howard H., Kalueff A.V. 2011. Effects of piracetam on behavior and memory in adult zebrafish. *Brain Res. Bull.* 85, 58-63 by kind permission from Elsevier).
Figure 2

Figure 3
Figure 6

Figure 7

Impact of pain
Figure 8

![Graph showing total time in white (s) for Control and Chronic Piracetam conditions. The graph displays a bar chart with error bars, indicating statistical significance.](image)