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# Trigeminal Somatosensory Innervation of the Head of a Teleost Fish with Particular Reference to Nociception

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KEYWORDS

Nociception, mechanoreception, Trigeminal nerve, Cutaneous receptor, Teleost fish

ABSTRACT

Trigeminal somatosensory receptors have not been characterised in teleost fish and studies in elasmobranchs have failed to identify nociceptors. The present study examined the trigeminal nerve of a teleost fish, the rainbow trout (*Oncorhynchus mykiss*) to determine what types of somatosensory receptors were present on the head of the trout specifically searching for nociceptors. Single unit recordings were made from receptive fields on the head of the fish innervated by the trigeminal nerve. Each receptive field was tested for sensitivity to mechanical, thermal and chemical stimulation. Five different receptor types were found: fast adapting receptors responding to mechanical stimulation; slowly adapting receptors responding to mechanical stimuli; polymodal nociceptors responding to mechanical, noxious thermal and chemical stimulation; mechanothermal nociceptors responding to mechanical stimulation and noxious heat; and mechanochemical receptors responsive to mechanical and chemical stimulation. Mechanical thresholds, receptive field diameter, conduction velocities and thermal thresholds of the receptors were determined and there was no significant difference between the receptor types in terms of these properties. Three shapes of action potential (AP) were recorded from these receptors: type 1 with no inflexion; type 2 with an inflexion on depolarisation; and type 3 with an inflexion on repolarisation. Conduction velocity, amplitude and duration of the APs, after hypolarisation amplitude and duration, as well as the maximum rate of depolarisation were measured for each action potential type. No major differences were found when making comparisons within receptor type and between receptor types. The fish nociceptors had similar physiological properties to nociceptors found in higher vertebrates.

## 1. Introduction

In vertebrates, the trigeminal nerve, the fifth cranial nerve, conveys somatosensory information from the head and mouth to the brain [7] and [40]. As well as mechanical and chemosensory information, the trigeminal plays an important role in nociception, the detection of noxious, tissue damaging stimuli [5], [12], [25], [43] and [46]. Trigeminal nociception has been extensively studied in higher vertebrate groups (e.g., birds [8] and [12], mammals [5] and [33]; and humans [24]) and nociceptive or painful experiences from oral and facial areas are a common clinical occurrence in humans [10] and [36]. However, relatively little is known about trigeminal innervation of the head in fish or indeed nociception.

Various types of receptors have been identified in higher vertebrates that are innervated by the trigeminal nerve [7]. Cutaneous touch receptors that convey mechanical information regarding pressure, stretch and vibration have been described as well as chemoreceptors in oral mucosa [7] and [43] and the cornea [25]. Trigeminal nociceptors have been characterised in birds and mammals and respond preferentially to noxious, damaging stimuli [5], [12], [13] and [33]. These tend to have higher mechanical thresholds than touch receptors and also respond to noxious heat above 40 °C classifying them as mechanothermal nociceptors [27]. If these nociceptors also respond to noxious chemical stimulation, they are classified as polymodal nociceptors [27]. Although chemoreceptors and mechanoreceptors have been characterised from other cranial nerves in fish (e.g., olfactory (I) [21]; facial (VII) [20] and [28]; vestibulocochlear (VIII) [45]; glossopharyngeal (IX) [3] and [4]), trigeminal receptors have not been fully characterised. However, the anatomical attributes of the trigeminal nerve and tract in fish and primitive vertebrates, such as the lamprey and hagfish, have received much attention [26], [30], [31] and [37].

Nociceptors have not been identified in fish and studies in elasmobranchs failed to find mechanothermal nociceptors [23]. However, receptors that responded to physical damage of the receptive field were identified in the lamprey and it was suggested that these may have a nociceptive function [29]. It has been hypothesised that the ability to recognise and react to aversive stimuli is a fundamental property of animals [6]. Nociceptors have also been characterised in invertebrate animals (e.g., the leech, Hirudo medicinalis; and the mollusc, Aplysia californica; review in Ref. [17]). Teleost fish can learn to avoid a noxious stimulation such as electric shock [9] and, furthermore, learning is hindered by the administration of opiate analgesia [32]. This would suggest that teleost fish have nociceptors, yet no study has confirmed their existence [38].

Nociceptors in higher vertebrates are of two fibre types; A-delta and C fibres [27]. Cutaneous A-delta fibres are thought to be involved in immediate pain or pricking pain whereas C fibres are believed to be involved in second pain or dull pain and are crucial in chronic pain [44]. Nociceptors can be classified into various types according to their physiological properties [34], [35] and [46] Amongst cutaneous nociceptors, there are two main classes: A-delta high threshold mechanonociceptors and C polymodal nociceptors. Types and frequency distribution appear to vary amongst different body sites and animal species [1], [22], [34], [35] and [46]. An anatomical study on the trigeminal nerve in the rainbow trout demonstrated the presence of A-delta and C fibres [41] and in vivo behavioural experiments showed that administration of algesic chemicals adversely affected behaviour and physiology of this fish [42]. These A-delta and C fibres could potentially act as nociceptors on the head of the trout. Therefore, the aim of the present study was to investigate what receptor types are located on the head of the rainbow trout, a teleost fish, and specifically explore the head for nociceptors. Electrophysiological recordings from the trigeminal nerve were made to assess the properties of the somatosensory units.

## 2. Materials and methods

These experiments were conducted in an ethical manner in accordance with Home Office (UK) guidelines.

### *2.1. Maintenance of animals*

Rainbow trout (750±100 g, *n*=10) were supplied by a commercial fish supplier. They were maintained in two black circular tanks (130 gallons; *n*=5 fish maximum per tank) with a constant flow of filtered freshwater at 15 °C. They fish were fed ad libitum daily and kept on a 12:12 light–dark regime. The top of the each tank was half covered by an opaque lid to provide an area for sheltering.

### *2.2. Surgical procedure*

Trout were caught individually by netting and initially anaesthetized by immersion in buffered MS 222 (50 mg/l; Sigma–Aldrich, UK; level of anaesthesia was medium plane indicated by loss of muscle tone) to facilitate weighing and injection of Saffan (0.3 ml/100 g; Schering-Plough Animal Health, Welwyn Garden City, UK). Once deep anaesthesia was achieved as indicated by loss of gill movements, the fish was placed into a stainless steel cradle cushioned with wet paper towel and held in position with Velcro straps. The fish had to be ventilated by flushing fresh water over the gills by means of a tube held in place by a specially constructed mouth piece. The heart rate was monitored continuously and the animal was closely observed to ensure that deep plane anaesthesia was maintained until decerebration. Skin and bone were removed above the brain and then the olfactory and optic lobes and cerebellum were removed via a suction tube connected to a vacuum pump. After decerebration a neuromuscular blocker, Pavulon (pancurorium bromide 2 mg/ml; Sigma–Aldrich) was injected intramuscularly (0.08 ml/100 g fish weight) to prevent muscular twitching. Bone was removed to expose the left trigeminal ganglion and the ganglion was desheathed and covered in paraffin to prevent moisture loss.

### *2.3. Electrophysiological recordings*

Glass insulated tungsten microelectrodes (tip diameter 10 μm) were used to record from afferent cell bodies. The extracellular action potentials were amplified using an NL100 head stage connected to a NL104 preamplifier (Neurolog System, Digitimer, UK). The signal was displayed on a storage oscilloscope (5113, Tektronix) and stored on a personal computer using a Micro 1401 interface and Spike 2 software (CED, UK).

Following the application of stimuli to the head of the fish, evoked neural activity was recorded from single cells in the trigeminal ganglion. The skin was kept moist throughout the recording period so that responses would be fairly similar to a submerged fish. A glass mechanical probe (0.1 mm diameter) was lightly applied to the facial skin in order to locate a receptive field. Once located, the mechanical threshold of the receptor was determined by applying von Frey hairs (0.1–15.0 g at 0.1 g intervals) to the receptive field. The diameter of the receptive field was measured to 0.1 mm using Vernier calipers. The receptor was then tested for thermal and chemical sensitivity. A thermal stimulator was placed 1 mm above the area of the receptor field so that it did not burn the skin and the stimulator raised the temperature to 58 °C. Thermal sensitivity was determined by heating the skin at a rate of 1 °C/s up to 58 °C using a prefocussed quartz glass light bulb with built in reflector (A1231, 12v, 100w Wotan) orientated vertical to the skin. If the receptor responded to the increase in temperature, the threshold was determined and the response had to be repeatable. Temperature was measured using a type K thermocouple placed in the centre of the bulb focus and was controlled by a feedback circuit. The skin temperature was held at 58 °C for 10 s after which it rapidly returned to normal. The temperature increase of 1 °C/s allowed the threshold to be determined. To ascertain chemosensitivity, a single drop of 1% acetic acid (pH 2.8) was placed onto

the skin at the centre of the receptive field. Since these units responded to mechanical stimulation, any responses within the first 5 ms after drop application were disregarded as possible mechanical artefacts. Acetic acid was chosen since it has deleterious behavioural and physiological effects when administered subcutaneously to the lips of the trout [42]. This stimulation was repeated to ensure the reaction to acid was a genuine response. None of the units responded to a central drop of water placed on the receptive field. Conduction velocities were obtained by placing silver wire stimulation electrodes onto the skin at the centre of the receptive field and stimulating the receptor directly by a square-wave electrical pulse using an isolated stimulator (D52; Digitimer). This stimulated an action potential from the afferent unit and the conduction velocity was estimated using the time that the action potential was recorded after the stimulus and the approximate distance travelled from the receptive field to the recording electrode in the trigeminal ganglion.

### *2.4. Statistics*

The Spike 2 software allowed the following parameters of the action potentials to be measured: spike shape, amplitude and duration; afterhyperpolarisation (AHP) amplitude and duration and maximum rate of depolarisation ($dV/dt_{max}$). All of these parameters were compared along with conduction velocities using analysis of variance (ANOVA). Differences between units types were analysed as well as differences between different shapes of action potentials.

## 3. Results

A number of different receptor types were located on the head of the rainbow trout (Fig. 1). The number of units recorded from individual fish ranged between five and 13 units. Only the mechanochemical and mechanothermal units described below were not found in all fish. The areas indicated in Fig. 1 were chosen due to anatomical information regarding the branches of the trigeminal nerve, i.e., area 1 is innervated by both the ophthlamic and maxillary branches; area 2 is innervated by the mandibular branch; area 3 is innervated by the ophthalmic and area 4 is innervated by both the mandibular and maxillary branches [30]. These units responded to mechanical stimulation by either a fast adapting or slowly adapting response (Fig. 2). Recordings were made from the left trigeminal ganglion and so all 58 units described in this study were found on the left side of the head. The fish body temperature was kept at 20 °C and so all conduction velocities are approximately four-times slower than those of warm-blooded vertebrates. The calculated conduction velocities shown in the following descriptions are those recorded at 20 °C but these were multiplied by 4 to determine whether they were A or C fibres to make them comparable to mammalian units and to identify the unit type (see Refs. [3], [4], [23], [28] and [29]). This is justified by an anatomical study that showed the diameter of C and A fibres are identical to mammalian fibres [41]. Occasionally a unit was lost before all measurements could be completed and this is reflected in the graphs and statistics.

### *3.1. Fast adapting mechanoreceptors*

Out of 62 units, 16 were fast adapting mechanoreceptors (FAST) that did not respond to thermal or chemical stimulation (Fig. 2A). Three of these units were located on the dorsal area of the head, above the eye (Fig. 1, area 1). Two were located directly below the eye (Fig. 1, area 3). Six units were located on the lower jaw ventral to the mouth (Fig. 1, area 2) and five were found posterior to the mouth in area 4 (Fig. 1). The diameter of the receptive fields ranged from 1.2 to 4.6 mm×1 mm with a mean of 2.54 (±0.1) mm (Fig. 3A). The mean mechanical thresholds of these units was 1.13 (±0.5) g and these ranged from <0.1 to 7.1 g (Fig. 3B). Conduction velocities of these units had a mean of 3.56 (±0.3) m/s ranged from 1.62 to 6.32 m/s, which means that all these units were A-delta fibres (Fig. 3 and Fig. 4). The action potentials from these units were found to have three different shapes: type 1 that had no inflexion ($n$=9);

type 2 that had a depolarisation inflexion (n=1); and type 3 that had an inflexion on repolarisation (*n*=8; Fig. 5). The properties of the action potentials are shown in Table 1 separately for each action potential shape. There were no significant differences between the three action potential types in terms of conduction velocity ($F_{2,12}$=0.20; $P$=0.821; Fig. 4B), amplitude ($F_{2,12}$=0.35; $P$=0.707) and duration ($F_{2,12}$=0.90; $P$=0.426) of the action potential, AHP amplitude ($F_{2,12}$=1.43; $P$=0.270) and duration ($F_{2,12}$=2.19; $P$=0.146), and maximum rate of depolarisation [$dV/dt_{max}$ (V/s); $F_{2,12}$=0.40; $P$=0.680].

**Fig. 1.** Position of the five different receptor types on the head of the rainbow trout showing the four different areas [fast adapting mechanoreceptors (∗); slowly adapting mechanoreceptors (+); polymodal nociceptors (●); mechanothermal nociceptors (○); mechanochemical receptors (×)].

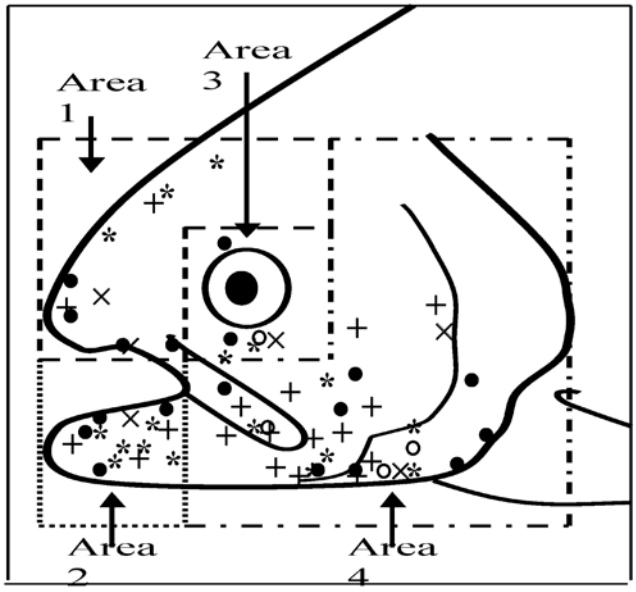
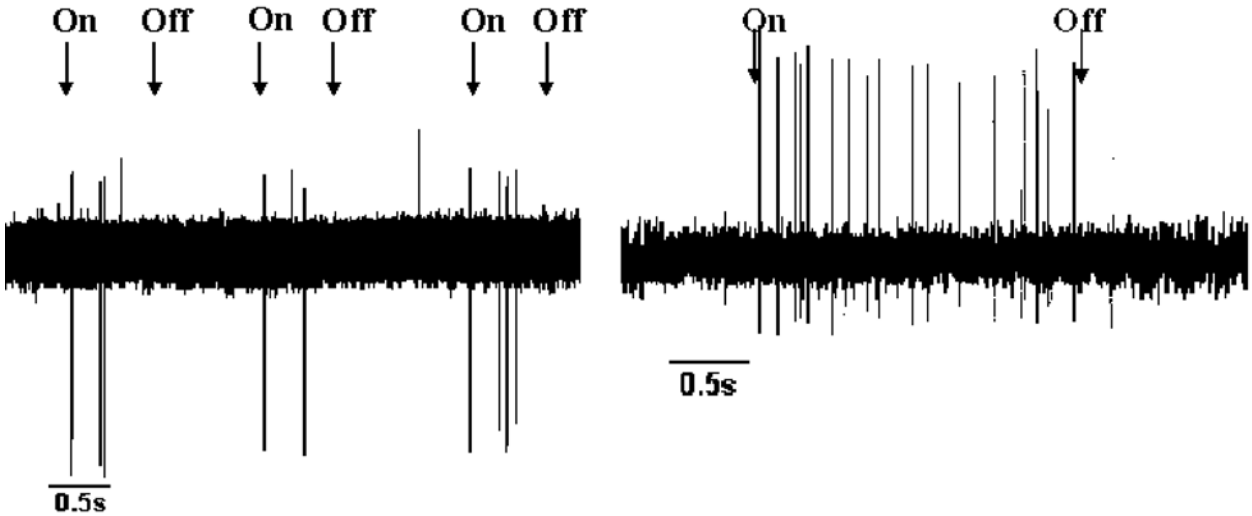



**Fig. 2.** Two responses to mechanical stimulation. (A) Fast adapting response whereby the unit fires a few times and then quickly stops. (B) Slowly adapting response whereby the unit fires continuously during the mechanical stimulation but the firing rate decreases with time.



*3.2. Slowly adapting mechanoreceptors*

There were 18 slowly adapting mechanoreceptors (SLOW) identified on the trout head that did not respond to thermal or chemical stimulation (Fig. 2B). One receptor was located on the dorsal area of the head above the eye and another was found anterior to the eye on the lip of the upper jaw (Fig. 1, area 1). Four units were located on the lower jaw ventral to the mouth (Fig. 1, area 2) and 12 were found posterior to the mouth on the opercular area (Fig. 1, area 4). The diameter of the receptive fields ranged from 0.8 to 7.2 mm×1 mm with a mean of 3.49 (±0.1) mm (Fig. 3A). Mechanical thresholds ranged from <0.1 to 3.1 g [mean 0.744 (±0.3) g; Fig. 3B] and conduction velocities were measured between 1.4 and 6 m/s with an average of 3.56 (±0.2) m/s, which is the range for A-delta fibres (Fig. 3 and Fig. 4). There were three shapes of action potentials from these units: type 1 ($n$=5); type 2 ($n$=4); and type 3 ($n$=7; Fig. 5). The properties of the action potentials are shown separately for each action potential shape in Table 1. There were no significant differences between the three action potential types in terms of conduction velocity ($F_{2,14}$=3.08; $P$=0.080; Fig. 4B), amplitude ($F_{2,14}$=1.27; $P$=0.312) and duration ($F_{2,14}$=3.09; $P$=0.080) of the action potential, AHP amplitude ($F_{2,14}$=0.81; $P$=0.466) and duration ($F_{2,14}$=1.14; $P$=0.350), and maximum rate of depolarisation [d$V$/d$t_{max}$ (V/s); $F_{2,14}$=0.07; $P$=0.935].

*3.3. Polymodal nociceptors*

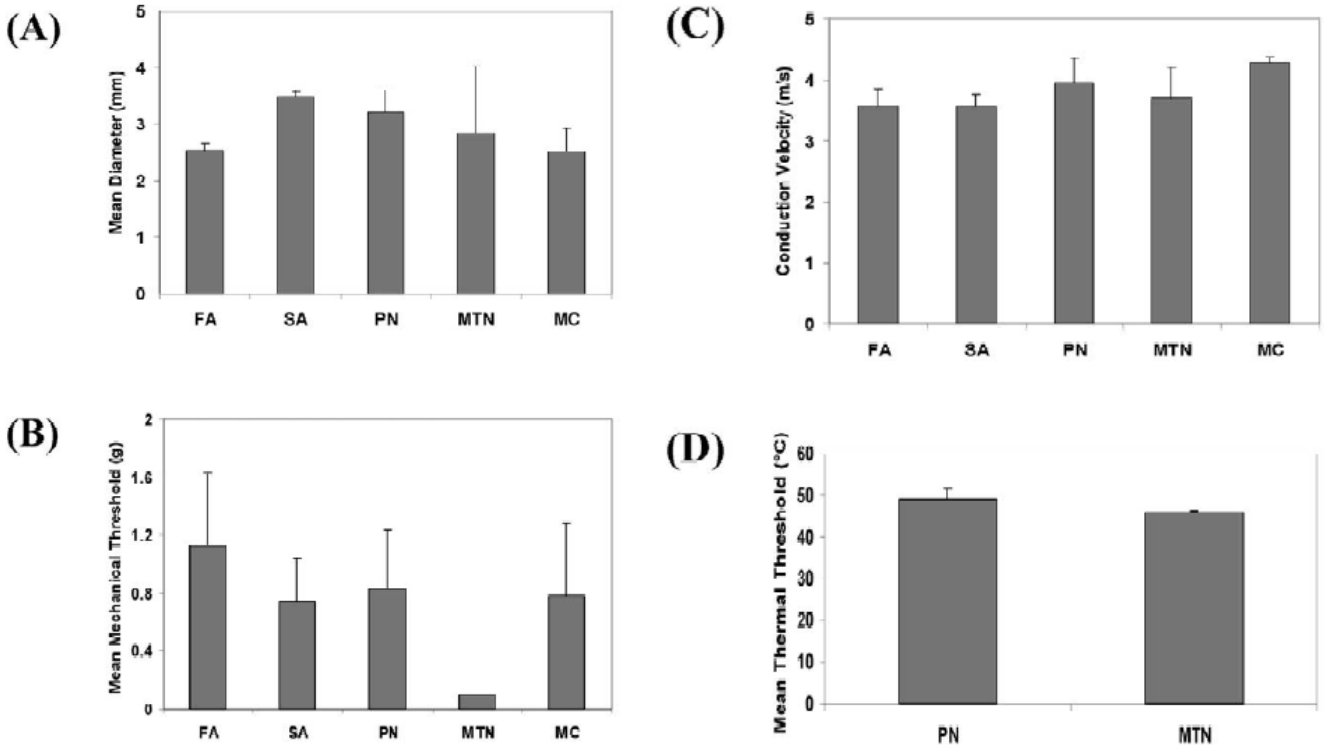
Out of 62 units, 18 were polymodal nociceptors (PNs). These units showed a slowly adapting response to mechanical stimulation and responded to noxious heat and noxious chemical stimulation. One of the units was located above the eye and three units were found anterior to the eye on the upper jaw (Fig. 1, area 1). Two units was found directly below the eye (Fig. 1, area 3) and four were found on the lower jaw (Fig. 1, area 2). The eight remaining units were located posterior to the mouth on area 4 (Fig. 1). The diameter of the receptive fields had a mean of 3.20 (±0.4) mm and ranged from 1.6 to 9 mm×1 mm (Fig. 3A). The mean mechanical threshold was 0.83 (±0.4) g and these were measured between <0.1 and 7.1 g (Fig. 3B). Conduction velocities ranged from 0.67 to 8.5 m/s with a mean of 3.96 (±0.4) m/s (Fig. 3 and Fig. 4). Only one of the units recorded from was a C fibre and the remaining units were A-delta fibres. Thermal responses only occurred above 40 °C with a mean threshold of 49.3 (±2.4) °C and ranged between 40 and 58 °C (Fig. 3D). There were three shapes of action potentials from these units: type 1 ($n$=5); type 2 ($n$=4); and type 3 ($n$=7; Fig. 5). The properties of the action potentials are shown separately for each action potential shape in Table 1. There were no significant differences between the three action potential types in terms of conduction velocity ($F_{2,16}$=0.56; $P$=0.585; Fig. 3B), amplitude ($F_{2,16}$=1.93; $P$=0.182) and duration ($F_{2,16}$=2.80; $P$=0.090) of the action potential, AHP amplitude ($F_{2,16}$=1.19; $P$=0.335) and duration ($F_{2,16}$=0.05; $P$=0.948), and maximum rate of depolarisation [d$V$/d$t_{max}$ (V/s); $F_{2,16}$=2.10; $P$=0.160].

*3.4. Mechanothermal nociceptors*

Four units responded to noxious heat but not to chemical stimulation (MTN). One of these units was located directly below the eye (Fig. 1, area 3) and the rest were found posterior to the mouth on the opercular area (Fig. 1, area 4). The diameter of the receptive fields ranged between 1.3 and 3.9 mm×1 mm with a mean of 2.83 (±1.2) mm (Fig. 3A). Thermal responses were only elicited above 40 °C with a mean threshold of 46.2 (±0.4) °C and ranged between 40 and 45 °C (Fig. 3D). All mechanical thresholds were below 0.1 g (Fig. 3B). Conduction velocities ranged between 4 and 4.7 m/s with a mean of 3.71 (±0.5) m/s and as such, these were A-delta fibres (Fig. 3 and Fig. 4). There were three shapes of action potentials from these units: type 1 ($n$=1); type 2 ($n$=2); and type 3 ($n$=1; Fig. 5). The properties of the action potentials are shown separately for each action potential shape in Table 1. There were no significant differences between the three action potential types in terms of conduction velocity ($F_{2,1}$=4.09; $P$=0.330; Fig. 4B), and maximum rate of depolarisation [d$V$/d$t$max (V/s); $F_{2,1}$=0.35; $P$=0.662]. There was a significant difference in amplitude ($F_{2,1}$=269.5; $P$=0.043) but not in duration ($F_{2,1}$=2.80; $P$=0.090) of the

action potential types. There was also a significant difference in AHP amplitude ($F_{2,1}$=326.7; P=0.039) but not in duration ($F_{2,1}$=0.21; $P$=0.837).

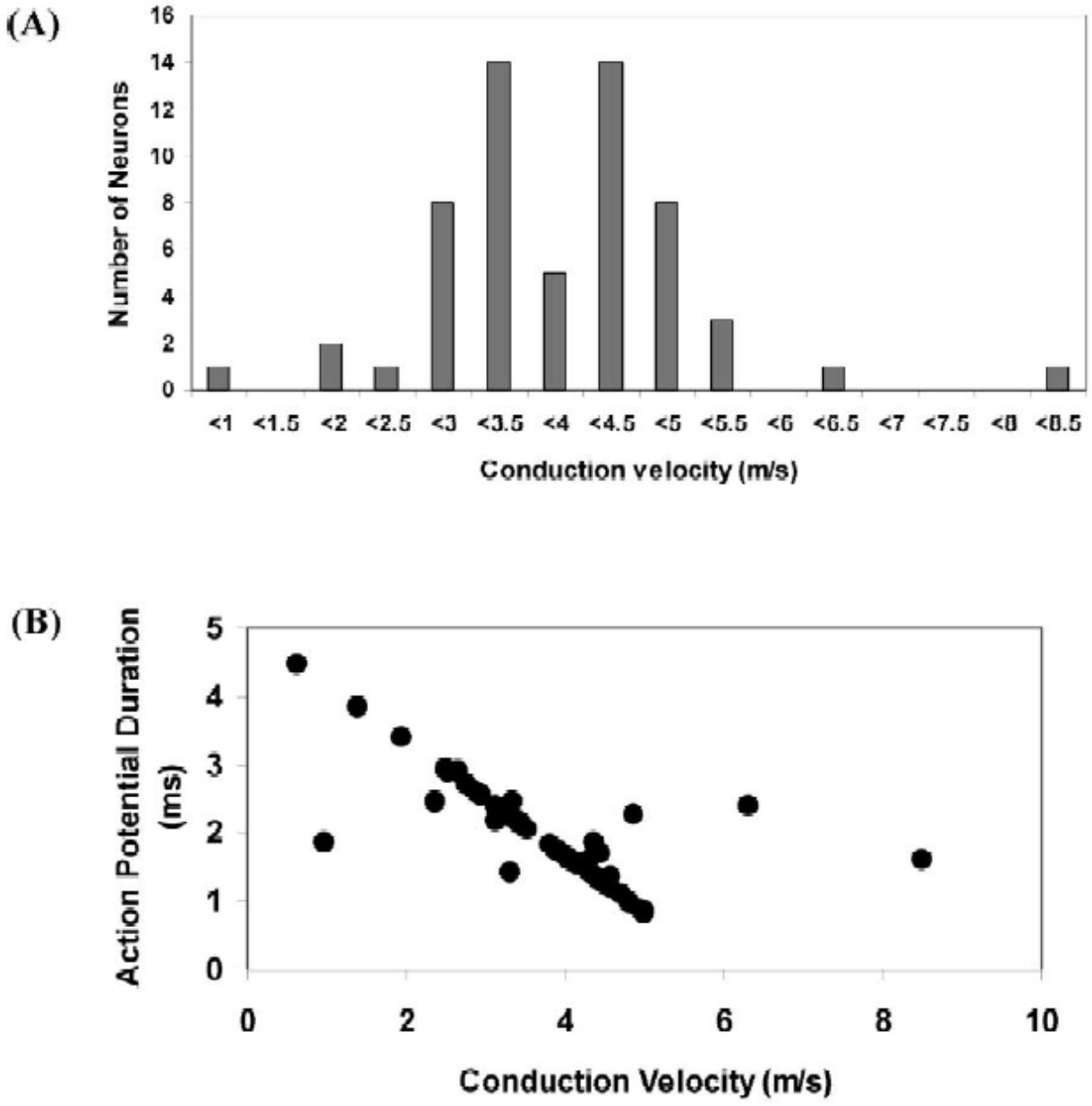
**Fig. 3.** (A) The mean (+S.E.M.) diameter of the receptive fields of the five different receptors types (FA=fast adapting mechanoreceptors; SA=slowly adapting mechanoreceptors; PN=polymodal nociceptors; MTN=mechanothermal nociceptors; MC=mechanochemical receptors). (B) The mean (+S.E.M.) mechanical threshold for each receptor type. (C) The mean (+S.E.M.) conduction velocity for each receptor type. (D) The mean (+S.E.M.) thermal threshold for the nociceptive receptors.




*3.5. Mechanochemical receptors*

Six of the 62 units responded to chemical stimulation but not to thermal stimulation. Two of these units were found on the upper jaw (Fig. 1, area 1) and one on the lower jaw (Fig. 1, area 2). One was also found directly beneath the eye (Fig. 1, area 3) and the remaining three were found posterior to the mouth (Fig. 1, area 4). The diameter of the receptive fields ranged from 1.2 to 5.8 mm×1 mm with a mean diameter of 2.52 (±0.4) mm (Fig. 3A). The mechanical thresholds were measured between <0.1 and 3.4 g with a mean threshold of 0.78 (±0.5). Conduction velocities had a mean of 4.28 (±0.1) m/s were measured in the range of 2.58 and 5 m/s, which means these units were A-delta fibres (Fig. 3 and Fig. 4). There were only two shapes of action potentials from these units: type 1 ($n$=2); and type 3 ($n$=4; Fig. 5). The properties of the action potentials are shown separately for each action potential shape in Table 1. There were no significant differences between the two action potential types in terms of conduction velocity ($F_{1,5}$=1.71; $P$=0.282; Fig. 3B), amplitude ($F_{1,5}$=8.23; $P$=0.064) and duration ($F_{1,5}$=1.77; $P$=0.275) of the action potential, AHP amplitude ($F_{1,5}$=0.36; $P$=0.591) and duration ($F_{1,5}$=9.44; $P$=0.054), and maximum rate of depolarisation [d$V$/d$t$max (V/s); $F_{1,5}$=4.16; $P$=0.134].

**Fig. 4.** (A) The number of neurons for successive conduction velocities at intervals of 0.5 m/s ranging from 0 to 9 m/s. (B) The relationship between action potential duration and conduction velocity.

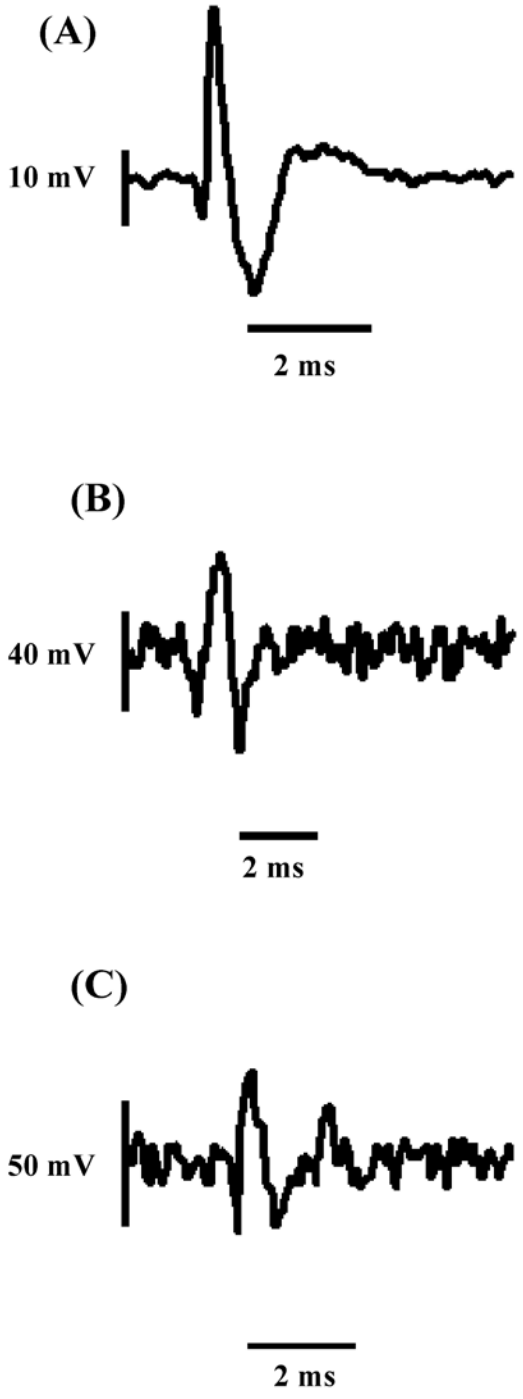



*3.6. Comparison of the receptor types*

The receptor types had similar properties. There was no significant difference between the five different receptor types in terms of the diameter of the receptive field ($F_{4,53}$=0.88, $P$=0.481); the mechanical thresholds ($F_{4,53}$=0.39, $P$=0.818); or in the speed of conduction ($F_{4,53}$=0.62, $P$=0.650). There was no difference between the thermal thresholds of the polymodal nociceptors and the mechanothermal nociceptors ($F_{1,20}$=0.95, $P$=0.341). There was also no difference between the five receptor types in action potential amplitude ($F_{4,54}$=0.72, $P$=0.581); duration ($F_{4,54}$=1.35, $P$=0.262); AHP amplitude ($F_{4,54}$=0.59,

$P$=0.669) or duration ($F_{4,54}$=0.89, $P$=0.475) and maximum rate of depolarisation ($F_{4,54}$=1.17, $P$=0.333). The conduction velocity of the majority of the units are shown in Fig. 4A indicating that most of the units have a velocity between 2.6 and 5.5 m/s. As expected, action potential duration was negatively correlated with conduction velocity ( Fig. 4B).

**Fig. 5.** The three different types of action potential from polymodal nociceptive receptors. (A) Type 1—an action potential without an inflexion; (B) type 2—an action potential with an inflexion on depolarisation; (C) type 3—an action potential with an inflexion on repolarisation.



## 4. Discussion

Five different receptor types that were innervated by the trigeminal nerve were found on the head of the rainbow trout. Over half of these units were monomodal and only responded to mechanical stimulation. The fast adapting mechanoreceptors are possibly touch units and these have been described in mammalian skin [39]. These units also had low mechanical thresholds, which is characteristic of mammalian touch units. The slowly adapting mechanoreceptors are possibly pressure units and these also have low mechanical thresholds similar to mammalian skin pressure receptors [39]. Two of the other receptor types were bimodal with both of these types having mechanical sensitivity and were responsive to either thermal or chemical stimulation. The mechanothermal (MT) units only responded to temperature in the noxious range (>40 °C [27]) and as such can be classified as mechanothermal nociceptors [27] and [43]. The mechanochemical (MC) units could potentially be nociceptive but without further testing this cannot be concluded from the present study. The predominating receptor type was polymodal, responding to both noxious heat and chemical stimuli, and as such can be classified as nociceptive (PN) [27].

The diameter of the receptive fields of each receptor type was similar to those found in higher vertebrates (e.g., birds [14], mice [43]). The mechanical thresholds were lower and so the nociceptors were much more sensitive than those found in bird and mammalian skin [14] and [15]. Mammalian skin nociceptors need a pressure of above 0.6 g before they are mechanically stimulated [27]. This may be due to the porous, more easily damaged nature of the fish skin. However, the fish nociceptors have mechanical thresholds that are similar to those found in corneal nociceptors which have lower mechanical thresholds than nociceptors found in mammalian skin or oral mucosa [2] and [25].

From the peripheral conduction velocities, all but one of the units recorded from were A-delta fibres. Only one C fibre was found in this study and it was a polymodal nociceptor. It may be difficult to locate a C fibre since only 4% of total trigeminal fibres are C fibres whereas A-delta fibres comprise 25% of fibre type in the rainbow trout [41]. This is in contrast to the situation in amphibia, birds and mammals where C fibres compose 50–65% of total fibre type [47]. This may represent an evolutionary difference between teleost fish and higher vertebrates. In higher vertebrates, touch and pressure units are generally A-beta fibres with much higher conduction velocities than those recorded in the trout [39]. This suggests that A-beta fibres may have a different role in teleosts and future studies should investigate their function.

Since nearly all of the units were A-delta fibres, this may explain why there were no major differences between their electrophysiological properties. Three different shapes of action potentials were found and only one receptor type, the MC units, did not have all three shapes. This could be due to the low number of MC units found in this study ($n$=6). Apart from the MTN units there were no differences in the physiological characteristics of the different shaped action potentials for the other four receptor types. The dissimilarity shown by the statistical analyses for the MTN units cannot be conclusive since the sample sizes were too small ($n$=4).

The conduction velocities and action potential durations of the PN and MTN units are similar to the properties of mammalian corneal nociceptors [25]. The duration of the fish nociceptors action potentials are longer than mammalian nociceptors but this could be due to the difference in body temperature between a fish (~20 °C) and a mammal (37 °C). Therefore, the fish nociceptive units fire at a much slower rate due to this temperature difference. This could also explain why the maximum rate of depolarisation is also slower in the fish nociceptors [25]. The AHP amplitudes of the fish nociceptors are comparable to those in the mammal but they are of shorter duration [25]. When examining the relationship between action potential duration and conduction velocity, a typical mammalian A-delta polymodal nociceptor relationship is found [25]. Mammalian polymodal nociceptors have been observed

to have large, broad action potentials and slow depolarisation as well as an inflexion on repolarisation but a third of the A-delta nociceptors have no inflexion [11] and [25]. Here in the fish many of the nociceptors had broad, slow action potentials with either an inflexion on repolarisation or no inflexion. However many of these nociceptors had an inflexion on depolarisation and this may be an attribute that is not found on the action potentials of mammalian nociceptors and is, therefore, peculiar to the rainbow trout. It would be interesting to examine other fish species from a comparative point of view.

**Table 1.** Characteristics (mean±S.D.) of the action potentials of the five different receptor types

| **Receptor** | **Type** | **CV (m/s)** | **$AP_{amp}$ (mV)** | **$AP_{dur}$ (ms)** | **$AHP_{amp}$ (mV)** | **$AHP_{dur}$ (ms)** | **$dV/dt_{max}$ (V/s)** |
|---|---|---|---|---|---|---|---|
| FAST | 1 | 3.4±1.6 | 32.4±45 | 2.4±1.0 | 4.1±3.6 | 0.7±0.5 | 127±132 |
| | 2 | 4.3±0.0 | 26.0±0.0 | 1.6±0.0 | 10.0±0.0 | 1.0±0.0 | 162±0.0 |
| | 3 | 3.5±0.7 | 18.0±15 | 2.0±0.6 | 3.1±4.2 | 0.3±0.3 | 89.8±58 |
| SLOW | 1 | 2.9±1.0 | 22.0±22 | 2.6±0.9 | 5.0±4.0 | 2.5±3.8 | 74.9±47 |
| | 2 | 4.2±0.7 | 10.0±4.0 | 1.5±0.6 | 3.0±3.0 | 0.7±0.7 | 65.2±19 |
| | 3 | 3.7±0.7 | 12.0±6.0 | 1.9±0.6 | 3.0±3.0 | 0.7±0.6 | 72.0±42 |
| PN | 1 | 4.1±0.6 | 26.0±15 | 1.6±0.5 | 5.5±6.2 | 0.6±0.6 | 160±52 |
| | 2 | 3.2±0.8 | 33.0±44 | 2.4±0.4 | 3.5±5.7 | 0.7±0.9 | 128±167 |
| | 3 | 3.7±1.4 | 10.0±3.0 | 1.7±0.4 | 1.8±1.3 | 0.6±0.4 | 63.3±29 |
| MTN | 1 | 2.6±0.0 | 90.0±0.0 | 0.9±0.0 | 4.5±0.0 | 1.0±0.0 | 80.5±0.0 |
| | 2 | 3.6±0.6 | 10.0±3.0 | 2.0±0.5 | 0.1±0.1 | 0.5±0.7 | 226±214 |
| | 3 | 5.0±0.0 | 6.0±0.0 | 0.8±0.0 | 1.1±0.0 | 0.6±0.0 | 71.4±0.0 |
| MC | 1 | 4.1±0.1 | 6.1±0.2 | 1.6±0.1 | 1.4±1.6 | 1.3±0.1 | 38.1±3 |
| | 2 | None | | | | | |
| | 3 | 4.4±0.4 | 13.7±3.5 | 1.3±0.3 | 2.8±3.0 | 0.4±0.4 | 112±48 |

Action potentials had three different shapes: type 1 which had no inflexion; type 2 which had a depolarisation inflexion; and type 3 which had a repolarisation inflexion. Properties shown are conduction velocity (CV); action potential amplitude ($AP_{amp}$) and duration ($AP_{dur}$); afterpolarisation amplitude ($AHP_{amp}$) and duration ($AHP_{dur}$); and maximum rate of depolarisation ($dV/dt_{max}$)

This study has shown that the head of the rainbow trout has a fine innervation of somatosensory information from the trigeminal nerve. Many units, including some of the nociceptors, had mechanical thresholds of less than 0.1 g. Polymodal A-delta fibres predominated in the recordings from the trigeminal ganglion of the trout. Polymodal A-delta nociceptors are rarely found in higher vertebrate skin [22] but are commonly found in oral mucosa [43]; skeletal muscle [18]; and visceral organs [16] and [19]. It was suggested that the nociceptors of these areas are mainly polymodal since they come into contact with various aqueous and hard substances that give a mixture of mechanical, thermal and chemical stimulation. The rainbow trout inhabits an aqueous environment and thus it is intuitive that the majority of nociceptors are polymodal. The fish nociceptors share common elements with mammalian nociceptors and, therefore, these properties are possibly fundamental aspects of structure and function of nociceptive neurons.

It is interesting that a fish species, which inhabits environments between 0 and 25 °C usually of a relatively neutral pH in freshwater, possesses nociceptors that respond to temperatures above 40 °C and are stimulated by acid at pH 2.8. It may be possible that this species evolved in a hot, acidic environment in the distant past and this explains the presence of these receptors. Although it would be expected that these receptors would be lost in more recent times if they are not used. An evolutionary approach assessing climate and water quality is necessary to explore this hypothesis. Industrial and agricultural effluents are discharged into waterways and these are possibly of a high temperature and/or acidic so it is crucial for the fish's survival to be able to detect these noxious stimuli and avoid them and so nociceptive capability is a necessity.

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**References**

[1] P.W. Beck, H.O. Handwerker, M. Zimmerman Nervous outflow from the cat's foot during noxious radiant heat stimulation Brain Res., 67 (1974), pp. 373–386

[2] C. Belmonte, J. Gallar Corneal nociceptors C. Belmonte, F. Cervero (Eds.), Neurobiology of Nociceptors, Oxford University Press, Oxford (1996), pp. 146–183

[3] M.L. Burleson, W.K. Milsom Sensory receptors in the first gill arch of the rainbow trout Resp. Physiol., 93 (1993), pp. 97–110

[4] M.L. Burleson, J.D. Soard, L.P. Elikan Branchial mechanoreceptor activity during spontaneous ventilation in the channel catfish Comp. Biochem. Physiol. A, 128 (2001), pp. 129–136

[5] P.M.B. Cahusac, R. Morris, R.G. Hill A pharmacological study of the modulation of neuronal and behavioural nociceptive responses in the rat trigeminal region Brain Res., 700 (1995), pp. 70–82

[6] L.S. Chervova Pain sensitivity and behavior of fishes J. Ichthyol., 37 (1997), pp. 98–102

[7] W.K. Dong, E.H. Chudler, K. Sugiyama, V.J. Roberts, T. Hayashi Somatosensory. multisensory, and task-related neurons in cortical area 7B (PF) of unanaesthetised monkeys J. Neurophysiol., 72 (1994), pp. 542–564

[8] J.L. Dubbeldam The sensory trigeminal system in birds: input, organisation and effects of peripheral damage. A review Arch. Physiol. Biochem, 106 (1998), pp. 338–345

[9] R.H. Ehrensing, G.F. Michell, A.J. Kastin Similar antagonism of morphine analgesia by MIF-1 and

[10] P.K. Eide, T. Rabben Trigeminal neuropathic pain: pathophysiological mechanisms examined by quantitative assessment of abnormal pain and sensory perception Neurosurgery, 43 (1998), pp. 1103–1109

[11] R. Gallego The ionic basis of action potentials in petrosal ganglion cells of the cat J. Physiol., 342 (1983), pp. 591–602

[12] M.J. Gentle Cutaneous sensory afferents recorded from the nervus intramandibularis of Gallus gallus var. domesticus J. Comp. Physiol. A, 164 (1989), pp. 763–774

[13] M.J. Gentle, F.L. Hill Oral lesions in the chicken: behavioral responses following nociceptive stimulation Physiol. Behav., 40 (1987), pp. 781–783

[14] M.J. Gentle, V.L. Tilston Nociceptors in the legs of poultry: implications for potential pain in pre-slaughter shackling Anim. Welfare, 9 (2000), pp. 227–236

[15] H.O. Handwerker, F. Anton, P.W. Reeh Discharge patterns of different cutaneous nerve fibres from the rat's tail during prolonged noxious mechanical stimulation Exp. Brain Res., 65 (1987), pp. 493–504

[16] P. Haupt, W. Janig, W. Kohler Response pattern of visceral afferent fibers, supplying the colon, upon chemical and mechanical stimuli Eur. J. Physiol., 398 (1983), pp. 41–47

[17] M. Kavaliers Evolutionary and comparative aspects of nociception Brain Res. Bull., 21 (1988), pp. 923–931

[18] T. Kumazawa, K. Mizumura Thin-fibre receptors responding to mechanical, chemical and thermal stimulation in the skeletal muscle of the dog J. Physiol., 273 (1977), pp. 179–194

[19] T. Kumazawa, K. Mizumura Chemical responses of polymodal receptors of the scrotal contents in dogs J. Physiol., 299 (1980), pp. 219–230

[20] S. Kiyohara, I. Hidaka, J. Kitoh, S. Yamashita Mechanical sensitivity of the facial nerve fibers innervating the anterior palate of the puffer, Fugu pardalis, and their central projection to the primary taste center J. Comp. Physiol. A, 157 (1985), pp. 705–716

[21] K. Kotrschal Taste(s) and olfaction(s) in fish: a review of specialized sub-systems and central integration Eur. J. Physiol., 439 (Suppl.) (2000), pp. R178–R180

[22] E. Lang, A. Novak, P.W. Reeh, H.O. Handwerker Chemosensitivity of fine afferents from the rat skin in vitro J. Neurophysiol., 63 (1990), pp. 887–901

[23] R.B. Leonard Primary afferent receptive field properties and neurotransmitter candidates in a vertebrate lacking unmyelinmated fibres Prog. Clin. Res., 176 (1985), pp. 135–145

[24] F. Lobbezoo, M. Trulsson, R. Jacobs, P. Svensson, S.W. Cadden, D. van Steenberghe Topical review: modulation of trigeminal sensory input in humans: mechanisms and clinical implications J. Oralfacial Pain, 16 (2002), pp. 9–21

[25] M. López deArmentia, C. Cabanes, C. Belmonte Electrophysiological properties of identified trigeminal ganglion neurons innervating the cornea of the mouse Neuroscience, 101 (2000), pp. 1109–1115

[26] P.G.M. Luiten Proprioceptive reflex connections of head musculature and mesencephalic nucleus in carp J. Comp. Neurol., 183 (1979), pp. 903–912

[27] B. Lynn The fibre composition of cutaneous nerves and the classification and response properties of cutaneous afferents, with particular reference to nociception Pain Rev., 1 (1994), pp. 172–183

[28] T. Marui, J. Caprio Electrophysiological evidence for the topographical arrangement of taste and tactile neurons in the facial lobe of the channel catfish Brain Res., 231 (1982), pp. 185–190

[29] G. Matthews, W.O. Wickelgren Trigeminal sensory neurons of the sea lamprey J. Comp. Physiol. A, 123 (1978), pp. 329–333

[30] J.G. New, R.G. Northcutt Primary projections of the trigeminal nerve in two species of sturgeon: Acipenser oxyrynchus and Scaphirhynchus platorynchus J. Morphol., 182 (1984), pp. 125–136

[31] R.G. Northcutt Experimental determination of the primary trigeminal projections in lampreys Brain Res., 163 (1979), pp. 323–327

[32] R.D. Olson, A.J. Kastin, G.F. Mitchell, G.A. Olson, D.H. Coy, D.M. Montalbano Effects of endorphin and enkephalin analogs on fear habitutation in goldfish Pharmacol. Biochem. Behav., 9 (1978), pp. 111–116

[33] J. Pajot, T. Pelissier, F. Sierralta, P. Raboisson, R. Dallel Differential effects of trigeminal tractotomy on Aδ and C-fiber-mediated nociceptive responses Brain Res., 863 (2000), pp. 289–292

[34] E.R. Perl Pain and nociception J.M. Brookhart, V.B. Mountcastle (Eds.), Handbook of Physiology, Section I, The Nervous System, Sensory Process, American Physiological Society, Bethesda, MD (1984), pp. 915–975

[35] P.W. Reeh Sensory receptors in mammalian skin in an in vitro preparation Neurosci. Lett., 66 (1986), pp. 141–146

[36] A. Romaniello, G. Cruccu, A.S. McMillan, L. Arendt-Nielsen, P. Svensson Effect of experimental pain from the trigeminal muscle and skin on motor cortex excitability in humans Brain Res., 882 (2000), pp. 120–127

[37] M. Ronan The sensory trigeminal tract of Pacific hagfish. Primary afferent projections and neurons of the tract nucleus Brain Behav. Evol., 32 (1988), pp. 169–180

[38] J.D. Rose The neurobehavioral nature of fishes and the question of awareness and pain Rev. Fish. Sci., 10 (2002), pp. 1–38

[39] M.R. Rosenzweig, A.L. Leiman, S.M. Breedlove Biological Psychology. An Introduction To Behavioral, Cognitive and Clinical Neuroscience (2nd Edition), Sinauer Associates, Sunderland, MA (1999), pp. 189–218

[40] W.L. Silver, T.E. Finger The trigeminal system T.V. Getchell (Ed.), Smell and Taste in Health and Disease, Raven Press, New York (1991), pp. 97–107

[41] L.U. Sneddon Anatomical and electrophysiological analysis of the trigeminal nerve in a teleost fish, Oncorhynchus mykiss Neurosci. Lett., 319 (2002), pp. 167–171

[42] L.U. Sneddon, V.A. Braithwaite, M.J. Gentle, Do fish have nociceptors: evidence for the evolution of a vertebrate sensory system. Proc. Roy. Soc. Lond. B, in press.

[43] K. Toda, N. Ishii, Y. Nakamura Characteristics of mucosal nocieptors in the rat oral cavity: an in vitro study Neurosci. Lett., 228 (1997), pp. 95–98

[44] H.E. Torebjörk, J.L. Ochoa New method to identify nociceptor units innervating glabrous skin of the human hand Exp. Brain Res., 81 (1990), pp. 509–514

[45] T.C. Tricas, S.M. Highstein Action of the octavolateralis efferent system upon the lateral line of free swimming toadfish, Opsanus tau J. Comp. Physiol. A, 169 (1991), pp. 25–37

[46] W.D. Willis The Pain System: The Neural Basis of Nociceptive Transmission in the Mammalian Nervous SystemKarger, Basel (1985)

[47] R.F. Young Fiber spectrum of the trigeminal sensory root of frog, cat and man determined by electron microscopy D.L. Anderson, B. Matthews (Eds.), Pain in the Trigeminal Region, Elsevier, Amsterdam (1977), pp. 137–160