ABSTRACT

Title of Document: THE MECHANOSENSORY LATERAL LINE SYSTEM: MORPHOLOGICAL, PHYSIOLOGICAL, AND BEHAVIORAL STUDY IN PRE- AND POST-METAMORPHIC LAMPREYS

Semen Gelman, Doctor of Philosophy, 2007

Directed By: Professor Avis H. Cohen, Department of Biology

Lampreys are extant jawless vertebrates. The oldest lamprey-like fossil, which dates from approximately 360 million years ago, exhibits many external morphological similarities with modern lampreys. It is thought that lampreys have undergone very conservative evolutionary changes and therefore retain many ancestral characters. Studying lamprey sensory physiology may shed light on the phylogenetic development of various sensory systems in vertebrates.

Electrophysiological and microscopic methods were used to investigate the morphology and physiology of the peripheral lateral line system of lampreys with special emphasis on the metamorphic changes. It was established that larval lampreys possess a functional mechanosensory lateral line system. Morphology of larval neuromasts was found to be similar to that of adults.

Metamorphic transformations in the lateral line included functional and morphological changes. A general re-patterning of the system of neuromasts on the

head and trunk was observed. It appears that three processes are involved in the re-patterning: an increase in neuromasts number, their re-distribution within the pit lines, and shifts of the pit lines relative to external features.

Response of the trunk lateral line nerve (TLLN) fibers to vibrational stimulation was qualitatively similar in adults and larvae. Both showed two populations of neurons responding to opposite directions of water flow, with the response magnitude monotonically increasing with stimulus amplitude. At low frequencies, the phase lag of the response with respect to the stimulus maximum was approximately 220°, and the gain depended approximately linearly on frequency, confirming the notion that superficial neuromasts are velocity detectors. The changes in phase lag with increasing stimulus frequency were steeper in larva suggesting slower conductance. The response of adults to different frequencies indicated a narrower range of frequency discrimination.

The observed changes were hypothesized to be of preparatory nature, as the metamorphosis is in general, making this sensory system more suitable for the active life style of adult lampreys. In light of this hypothesis, the behavioral involvement of the lateral line in locomotion was investigated. It was found that the locomotion of lamprey in still water was not affected by blocking the lateral line. This may indicate that the intraspinal system of mechanoreceptors, so called 'edge' cells, is dominating locomotor feedback during such experimental conditions and is sufficient for normal locomotion. However, the question remains unanswered whether the movement-related feedback from the 'edge' cells is sufficient in complex flow conditions. It may very well be that the information provided by the lateral line in such demanding

conditions is necessary for swimming. Thus the behavioral function of the lamprey lateral line remains unknown.

THE MECHANOSENSORY LATERAL LINE SYSTEM: MORPHOLOGICAL, PHYSIOLOGICAL, AND BEHAVIORAL STUDY IN PRE- AND POST-METAMORPHIC LAMPREYS

By

Semen Gelman

Dissertation submitted to the Faculty of the Graduate School of the
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Advisory Committee:
Professor Avis H. Cohen, Chair
Professor Richard Payne
Professor Catherine E. Carr
Professor Elizabeth Quinlan
Professor David Yager

Preface

Lateral line of aquatic vertebrates is a remarkable sensory system that endows these animals with the ability to sense hydrodynamic environment at some distance away. Sven Dijkgraaf (1908-1995), a prominent zoologist, who studied the lateral line, called it ***Ferntastsinn***, which can be translated from Dutch, as 'touch at a distance'. During the course of evolution terrestrial organisms have lost this sensory system. This fact adds an extra level of difficulty in studying the lateral line, because it is hard to imagine something so foreign to us. Quite often, when I would think about the lateral line in conjunction with behavioral experiments, I would erroneously draw analogies with the visual system. For some reason, a temptation to draw analogy with the sense that we possess is strong. At some point, I came up with an analogy that seemed better than the visual one, but probably equally useless. By blowing air at some object or a wall with the eyes closed, one can feel the reflected flow of air with the touch receptors on the face. It is even possible to distinguish small and large objects by performing such an exercise. This is essentially what fish do with their lateral lines, but much more precise of course. ***Ferntastsinn*** is possible only through a medium with certain physical properties. The sense of distance-touch is not possible in air. Water, where the organisms have evolved the lateral line, is such medium. Therefore full understanding of the lateral line system requires understanding of the properties of fluids.

The lateral line research is an integrated field, posing questions and using approaches from evolutionary biology, genetics, sensory physiology, development, physics, signal processing, and mathematical modeling. The list is long.

My graduate work is a small contribution to our understanding of the lateral line in an ancient vertebrate - lamprey. Carl Rovainen, another prominent zoologist, who studied all aspects of the biology of lampreys, noted that 'the lateral line system of lampreys has to be studied systematically'. This dissertation is an attempt in that direction.

In the introductory chapter of this dissertation I tried to review the knowledge about the lateral line system of various organisms. Due to the extensive literature on this subject, and my inability to read all of it, I cannot give an exhaustive overview here, but only information that I came across during my graduate work and that seemed educational and helpful to me in studying the lateral line system. The importance and necessity of reading the literature on the lateral line system in its entirety cannot be overestimated, due to the great diversity of this sensory system in aquatic animals. I regret that my knowledge of a large portion of research in this field is still lacking. For complete reviews on the subject the reader is referred throughout the thesis to the experts in this field.

Dedication

To my parents, Alik and Nadejda Gelman

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I would like to express my gratitude to a number of people who helped me throughout my research and work on this dissertation.

My deepest gratitude goes to my thesis advisor, Professor Avis Cohen. Avis was my first science mentor. She introduced me to the challenging, difficult, but immeasurably rewarding scientific process. She taught me not only to explore and understand my experiments in great detail, but also to keep in mind the reality of science. Her insistence on clear and rigorous explanations nurtured in me a need to critically look at every aspect of my research. Our numerous discussions helped me to see how my research fits into a "big picture". Her enthusiasm was always inspiring. Her words of encouragement kept me going on many occasions.

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subject stimulated me to critically rethink everything that I was doing. I greatly enjoyed working with Amir.

I would like to thank Dr. Tim Kiemel. The many insightful discussions on the mathematical aspects of my work greatly improved it. His mathematical model, which formed a part of the chapter III of this thesis, gave the previously made conclusions, a more rigorous mathematical basis.

I am also thankful to Dr. Elena Sanovich and Mr. Timothy Moguel for help with the electron microscopy.

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Finally, I want to thank my parents and my brother. Without their love and support, it would have been impossible to reach this point.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ALLN:	anterior lateral line nerve
PLLN:	posterior lateral line nerve
TLLN:	trunk lateral line nerve
LLS:	lateral line system
LLN:	lateral line nerve
TS:	torus semicircularis
CN:	canal neuromast
SN:	superficial neuromast
SO:	supra-orbital canal
IO:	infra-orbital canal
PM:	pre-operculomandibular canal
OT:	otic canal
PO:	post-otic canal
ST:	supra-temporal commissural canal
T:	trunk canal
SB:	supra-branchial pit line
V:	ventral pit line
D:	dorsal pit line
M:	main trunk pit line

CHAPTER ONE: GENERAL INTRODUCTION

The mechanosensory lateral line

Synopsis

The mechanosensory lateral line system of cyclostomes, fishes and amphibians detects and processes hydrodynamic stimuli. Peripherally, the lateral line consists of aggregations of neuromast organs innervated by the lateral line nerves. Neuromasts are composed of sensory epithelia (rows of hair cells) and supporting cells. In general, neuromasts comprise two populations: canal neuromasts, which are enclosed in intraepidermal bony or cartilaginous canals, and superficial neuromasts, located in the epidermis of the skin or scales of fish.

Functionally and morphologically, the peripheral lateral line is subdivided into canal and superficial neuromasts, which detect acceleration and velocity of water motion, respectively. Because of this functional division, it is believed that the two classes of neuromasts probably mediate different types of behaviors.

In most species, neuromasts organs are innervated by two cranial nerves: the anterior and posterior lateral line nerves. Fibers of these nerves terminate in the dorsal area octavolateralis (lateralis area) of the brainstem. The ventral part of the area octavolateralis (octaval area) is the site of the VIIIth nerve projections. In most modern actinopterygian fishes, lateralis area consists of medial and caudal nuclei. In agnathans, chondrichthyans, and some other fishes, lateralis column consists of dorsal

and medial nuclei. The dorsal nucleus is the site of terminations of electrosensory fibers and the medial nucleus is the site of terminations of mechanosensory fibers. In lampreys, unlike other fishes, posterior lateral line nerve fibers also project to the contralateral medial nucleus. The area octavolateralis sends projections through lateral lamniscus to the contralateral torus semicircularis in the midbrain tegmentum. The torus semicircularis projects to the contra- and ipsilateral dorsal thalamus and glomerulosus complex of the diencephalons, which in turn project to the telencephalon.

Neuromast morphology

The lateral line receptor organs are classified as ordinary or specialized. The ordinary organs are neuromasts and the specialized organs are ampullary, tuberous, and end bud electroreceptors. The neuromast is the basic functional unit of the mechanosensitive lateral line system. It consists of a patch of sensory hair cells, surrounded by supporting cells and mantle cells (Schellart and Wubbels 1998). Supporting and mantle cells are not sensory cells, as their names imply. The former are dispersed between hair cells, while the latter are found on the periphery of neuromasts (Coombs et al. 1988). The apical surface of the neuromast is covered by a gelatinous cupula, which is dome-shaped structure, which has two layers The inner core is presumably secreted by support cells and the outer core by mantle cells (Munz 1979; Coombs et al. 1988).

The apical specializations of the hair cells consist of ciliary bundles projecting into cupula. The bundles are made of several stereocilia of graded length. The tallest streocilium is adjacent to a single kinocelium, which has a “9 + 2” microtubule

structure. Bending the stereocilia towards the kinocelium results in depolarization of the hair cell, and bending in the opposite direction in hyperpolarization (Flock 1967). Thus the morphological polarization of the hair cells leads the functional polarization.

Canal versus superficial neuromasts

Generally, neuromasts are subdivided into two categories based on their relationship to the epidermal structures.

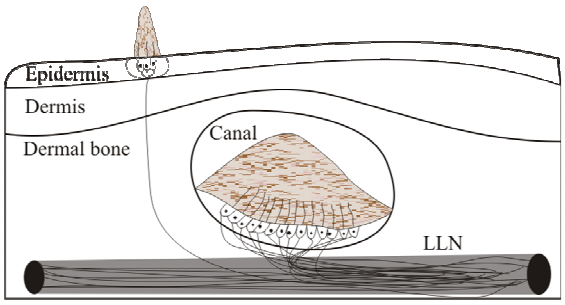

Figure 1-1.
Canal and superficial neuromast.
Modified from (Flock 1967).

The canals, either formed by bony grooves covered with skin as in the head region or by specialized scales in the trunk region, are filled with endolymphatic fluid (Schellart and Wubbels 1998). These canals are in contact with the outside water through pores. Differential pressure at two adjacent pores will cause the fluid in the canal between the pores to move, bending or sliding the cupula and exciting the hair cells. This essentially makes the canal neuromasts detectors of pressure gradients at the surface of the fish. The pressure gradient can be created by an accelerating body of fluid; thus canal neuromasts can also be thought of as detectors of fluid acceleration (Kroese and Schellart 1987, 1992). A flow with constant velocity will not produce a pressure gradient at the surface of the animal and the fluid in the canal organ will remain at rest (Kalmijn 1988).

Bony fishes have two patterns of the neuromasts distribution in the canals (Webb and Northcutt 1997). In one there are multiple neuromasts between pores and in the other there is only one neuromast between adjacent pores. Most actinopterygian

(ray-finned) fishes exhibit the latter configuration (Webb and Northcutt 1997). Some dipneustean fishes, such as African lungfish *Protopterus*, possess multiple neuromasts between adjacent pores. Chondrichthyan fishes also have multiple neuromasts between canal pores (Hama and Yamada 1977; Webb and Northcutt 1997). Petromyzontiformes (i.e. lampreys) and eptatretidian hagfishes lack canals altogether and possess only superficial neuromasts (Yamada 1973; Braun 1996). Finally hagfishes of the family Myxinidae secondarily lost the lateral line system (Braun 1996).

Superficial or free neuromasts are located in the epidermal layer of the skin or scales of fish, either in specialized pits or on top of stalks that protrude into the surrounding space (Flock 1967; Coombs et al. 1988). The cupulae of superficial neuromasts directly contact the surrounding water. The cupulae are thus bent or displaced even by uniform, constant velocity flow. The displacement is proportional to the velocity of the fluid motion (Kalmijn 1988). Hence superficial neuromasts are considered to be velocity detectors (Kroese and Schellart 1987, 1992).

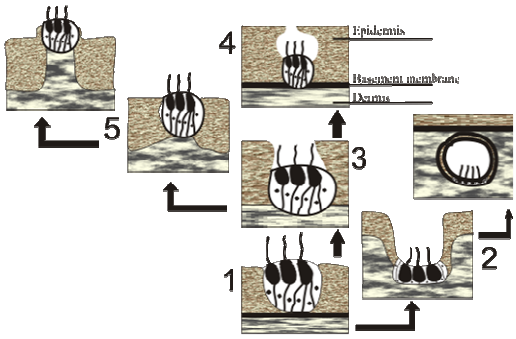

Figure 1-2.
Classification of superficial neuromasts based on the relationship to the epidermis and dermis. Arrows indicate possible ontogenetic sequence. Modified from (Coombs et al. 1988).

Several types of superficial neuromasts can be distinguished based on their relation to the epidermis, dermis, and the basal membrane of the skin (Coombs et al. 1988). *Flush neuromasts* (1) sit on top of a relatively straight basement membrane with apical surfaces at the level of the surrounding epidermal layer.

Dermal pit organs (2) are located in dermal depressions: a groove or a pit formed by the invagination of the basement membrane into the dermis. *Epidermal pit organs* (3) are located in pits formed by a gap in the epidermis. *Ampullary pit organs* (4) are superficially similar to electroreceptive organs. They are located on the relatively straight basement membrane at the bottom of an ampulla within the epidermis. *Papillate neuromasts* (5) are positioned on a dermal protuberance formed by the evagination of the basement membrane (Coombs et al. 1988).

Distribution of neuromasts

A generally common pattern of the distribution of canal and free neuromasts is observed among almost all fishes. Within that pattern, the diversity of the distribution is great (Coombs et al. 1988; Northcutt 1989; Puzdrowski 1989; Webb and Northcutt 1997). The canal system comprises the following canals: the supra-orbital canal (SO), the infra-orbital canal (IO), the preoperculomandibular canal (PM), the otic canal (OT), the post-otic canal (PO), the supra-temporal commissural canal (ST), and the trunk canal (T). Infraorbital and supraorbital canals are located below and above the orbit, respectively. The preopercular portion of the preoperculomandibular canal is located rostral to the opercle and runs dorso-ventrally. The mandibular portion is located in the lower jaw region. The otic and post-otic canals are usually located caudal to the eye, behind the fusion point of the supra- and infraorbital canals.

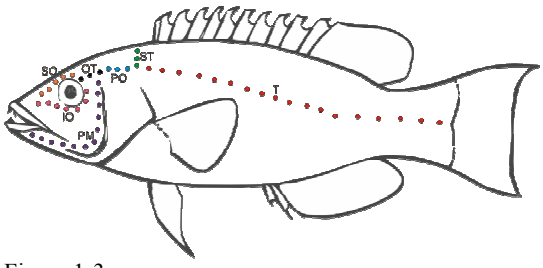

Figure 1-3.
The distribution pattern of canal neuromasts generally observed in actinopterigian fishes. See text for details.

Supra-temporal canal is caudal to the post-otic canal and connects the left and right side of the head. The trunk canal is usually located in the middle of the trunk, though variations can be observed. There is a considerable variability in the number of neuromasts per canal and the exact arrangement of these canals. Also some groups of fishes may lack all or some of the canals. In those groups the canals are substituted by the pit lines of free neuromasts or absent entirely.

Lateral line nerves and the central organization of the lateral line system.

Cole, in the nineteenth century, was the first to realize that lateralis nerves were a separate set of cranial nerves, distinct from facial and vagal nerves. Lateralis nerves possess a separate set of ganglia and have distinct central projections, separate from facial and vagus nerves (McCormick 1983). In addition, the primary projections are distinct from those of the anterior and posterior rami of the octaval (VIII) nerve, which carry vestibular and acoustic inputs from the inner ear (Maler et al. 1973b; Maler et al. 1973a; McCormick 1981a).

In general, canal neuromasts and superficial neuromasts of the head region are bilaterally innervated by anterior lateral line nerves. Some species have more than one anterior lateral line nerve on each side of the head. Cyprinids, like *Carrassius auratus,* have dorsal and ventral anterior lateral line nerves, which have separate ganglia (Puzdrowski 1989). Amiiforms, like *Amia calva,* have only one anterior lateral line nerve (McCormick 1981a). Usually there is only one posterior lateral line nerve, which innervates canal and superficial neuromasts of the trunk region. In some species, a third, middle lateral line nerve innervates sensory organs of post-otic canal

and associated free neuromasts (Puzdrowski 1989). In any case, each nerve will have its own ganglion.

All lateral line nerves enter the brain at the level of rhombencephalic alar plate. The rhombencephalon of actinopterygian fishes, in general, is divided into basal (motor) and alar (sensory) plates. The boundary between these areas, on the ventricular side, is marked by the sulcus limitans of His (Nieuwenhuys 1982). The basal and alar plates are functionally divided into somatic and visceral zones. Thus in the dorsoventral order there are four zones: somatosensory, viscerosensory, visceromotor, and somatomotor. These zones are arranged longitudinally throughout entire rombencephalon (Nieuwenhuys 1982). The somatosensory dorsal part of the alar plate is largely the site of terminations of the lateral line nerves and the VIIIth nerve. In fishes, this area is called area octavolateralis. The dorsal portion of the area octavolateralis, called lateralis column, contains nuclei associated with the lateral line nerves' terminations. The ventral area, called the octavus column, contains nuclei associated with the VIIIth nerve terminations. Generally, the lateralis column consists of two nuclei: the nucleus medialis and the nucleus caudalis. In agnathans and cartilaginous fishes, the lateralis column consists of a nucleus dorsalis (anterior lateral line lobe of cartilaginous fishes) and nucleus medialis (posterior lateral line lobe of cartilaginous fishes) (Boord and Campbell 1977). Ventrally, both lateralis nuclei border with octaval nuclei. Dorsally, the medial nucleus borders with the cerebellar crest, which is a caudal continuation of the molecular layer of the cerebellum (McCormick 1981b).

When entering the rhombencephalon, anterior and posterior lateral line nerves form ipsilateral ascending and descending projections, which terminate throughout nucleus medialis and nucleus caudalis (or dorsalis and medialis). In *Carrassius auratus*, there appears to be some, though imprecise topographical organization of the area lateralis (Puzdrowski 1989). The posterior lateral line nerve projects to the dorsal part of nucleus medialis and nucleus caudalis; the anterior lateral line nerves project to the ventral parts of these nuclei (Puzdrowski 1989). Another set of ipsilateral terminations could be found in eminantia granularis of the cerebellum.

Cerebellum of actinopterygian fishes consists of three structures: the corpus cerebelli, the valvula cerebelli, and the lobus vestibulolateralis. The lobus vestibulolateralis caudally joins the area octavolateralis of rhombencephalon. In chondrostean fishes (includes family Polyodontidae – paddlefishes and family Acipenseridae – sturgeons), brachiopterygian fishes (includes family Polypteridae – bichirs), and halecomorphs (includes family Amiidae – bowfin *Amia*) the vestibulolateral lobe in its rostrolateral parts forms a pair of auriculae. In teleosts these structures are called eminentiae granulares (granular eminences) (Nieuwenhuys 1982). Huesa et. al. (2003) report that in a sturgeon *Acipenser baeri* the cerebella auricles receive ipsilateral and some contralateral afferent connections from the medial nucleus of the octavolateralis area. In addition, they observed that some fibers of the posterior lateral line and ventral anterior lateral line nerves terminate in the ipsilateral cerebellar auricles as well (Huesa et al. 2003). The auricular efferents included ascending fibers to mostly contralateral thalamus and descending fibers to the area octavolateralis (Huesa et al. 2003). This pattern of cerebellar connections

related to the lateral line system is similar to that of teleosts (Nieuwenhuys 1982). Puzdrowski (1989) reports that in a teleost, *Carrassius auratus,* rami of all lateral line nerves project to the nucleus medialis and nucleus caudalis of the lateralis column, to the eminentia granularis, and to the magnocellular nucleus of the octaval area. In *Carrassius,* the eminantia granularis also exhibits a topographical arrangement of inputs. The anterolateral part receives inputs from anterior lateral line nerves and the caudal part receives inputs from posterior lateral line nerve (Puzdrowski 1989). Approximately the same arrangement is seen in a halecomorph, *Amia* (McCormick 1981a). In this species, the anterior lateral line nerves terminate in the ventral to ventromedial parts of nucleus medialis and caudalis. The posterior lateral line nerve terminates in the dorsal to dorsolateral portion of the lateralis nuclei. The lateral part of the eminantia granulares receives fibers of the posterior lateral line nerves and the medial part from the anterior lateral line nerves (McCormick, 1981, 1983). In a cichlid *Astronotus osellatus,* similar findings have been reported (Meredith 1984).

From the rhombencephalon, the lateral line and octaval system form secondary projections to the contralateral torus semicircularis (TS), a mesencephalic area. The mesencephalon of fishes consists of the ventral tegmentum and dorsal tectum. The dorsal tectum is also called optic tectum because it receives direct projections of the optic nerve. The medial part of the tegmentum is also designated as tegmentum motoricum. It contains motor nuclei belonging to IIIrd and IVth cranial nerves and part of the reticular formation. The lateral tegmentum contains the terminations of the secondary lateral line and acoustic projections, coming from the rhombencephalic octavolateralis area. The torus semicircularis (TS) is one of the two

ventricular protrusions of the mesencephalon. The other one is torus longitudinalis (TL). TS is a ventricular protrusion of the lateral tegmentum and TL is a bilateral structure formed by the medial tectal halves (Nieuwenhuys 1982). Actinipterygian fishes have a third torus, torus lateralis, which is an external protrusion of the tegmentum (Nieuwenhuys 1982). Just like in the octavolateralis area of the rombencephalon, the lateral line and octaval system nuclei of the TS do not overlap with each other. It was shown (Knudsen 1977) that in the catfish *Ictalurus* the electrosensory, mechanosensory, and auditory projections from area octavolateralis terminate in distinct areas of TS. These fibers project via the lateral lamniscus from area octavolateralis to the contralateral torus semicircularis. In TS, the auditory nucleus is located in the medial area (nucleus centralis), the electrosensory inputs go to a lateral area (nucleus lateralis, pars lateralis), and the mechanosensory projections terminate in an intermediate area (nucleus lateralis, pars medialis) (Knudsen 1977; Nieuwenhuys 1982). Efferents of the TS include projections to the central posterior nucleus of the dorsal thalamus and the glomerulosus complex, both of which are parts of the diencephalon (Nieuwenhuys 1982). In the catfish *Ictalurus* the mechanoreceptive part of TS also projects to the ventral thalamus of the diencephalon (Finger 1980). The ventral thalamus of *Ictalurus*, projects to the central field of the telencephalon – area dorsalis centralis (Dc). Some, very sparse retrograde labeling of torus semicircularis also occurs when HRP (horseradish peroxidase) is injected into the area dorsalis medialis (Dm) of telencephalon. The two findings indicate that there are limited ascending projections from TS to Dc and Dm areas of telencephalon (Finger 1980).

Peripheral physiology.

Most bony and cartilaginous fishes possess both superficial (SN) and canal (CN) neuromasts. Evidences suggest that the two groups are functionally and neuronally separated (Munz 1985; Kroese and Schellart 1992; Bleckmann 1994). In terms of innervations, it has been shown that a single CN is innervated by a single afferent fiber. On the contrary, a single afferent fiber with its collaterals can innervate an entire field/row of SNs (Munz 1985). No common afferent fiber innervates both CNs and SNs (Munz 1985). In the cichlid *Sarotherodon niloticus,* fibers with a diameter greater than 7μm are exclusively afferent (Munz 1985). Efferent fibers innervate both CNs and SNs. Based on the innervation pattern, a single CN forms a functional unit and a field or a row of SNs form a functional unit.

Almost all afferent lateral line fibers show resting activity (Munz 1985; Kroese and Schellart 1992; Bleckmann 1994). Based on the shape of the inter-spike interval histograms, Munz (1985) classified the resting activity of the fibers into three categories. Type I fibers, which formed a majority of the recorded units, possessed a Poisson-like distribution of the inter-spike intervals. Type I units innervated either CNs or SNs. Units innervating CNs showed higher mean resting activity than units innervating SNs (Munz 1985). This could probably stem from the larger number of hair cells in CNs than in SNs, which could mean that CN fibers innervate more hair cells than SN fibers (Munz 1985). Fibers of type I units have a diameter in the range from 8 to 15 μm. Type II fibers have more regular and symmetrical inter-spike interval distribution (Munz 1985). Most of the units with type II resting activity innervated canal neuromasts. Type III units exhibit a bimodal or "burst like"

distribution of the inter-spike intervals of the resting activity (Munz 1985). These units mostly innervated canal neuromasts and were shown to be the most sensitive units of all. However, there are evidence suggesting that the type III resting activity is not spontaneous, but is a result of unintentional stimulation by the background noise (Wubbels et al. 1990). The spectral components of type III spontaneous activity are very similar to the spectral components of the acceleration of the experimental table (Wubbels et al. 1990). This artifact could be explained either by the uncontrolled vibrations of the recording electrode which would depolarize the neuronal membrane or it is also possible that the hair cells were mechanically stimulated during an experiment. This mechanical stimulation produced action potentials in the afferent fibers in a normal way (Wubbels et al. 1990). Thus, Wubbels et al. (1990) conclude that the bimodal, "bursting" spontaneous activity in lateral line fibers must be considered an artifact.

The center frequency (i.e. the frequency of the stimulus that elicited the strongest response) of SNs lies between 10 and 70 Hz. The center frequency for CN fibers lies in the range from 100 Hz to 200 Hz. Thus center frequencies of CN fibers are higher than those of SN. Adaptation of the response has not been observed either for SN or CN units (Munz 1985).

A positive correlation between the rate of spontaneous activity and the sensitivity and conduction velocity in afferents of lateral line system has been found. Fibers that exhibit higher resting activity are usually more sensitive to stimuli and have larger diameters. This general positive correlation could be a common characteristic of afferent fibers of the octavolateralis system (Munz 1985).

Kroese and Schellart (1992) have shown that the afferent fibers of lateral line system of the trout could be divided into two populations based on the differences in the frequency of the maximal sensitivity, the low-frequency response slope, and the low-frequency phase lag. By comparing their results with other data on the frequency responses of the lateral line afferents from other species, they concluded that the two populations of afferents innervate either canal or superficial neuromasts. They found that afferents presumably innervating CNs have a higher frequency for maximal sensitivity than the SNs. CNs and SNs afferents were maximally active at ~90Hz and ~40Hz, respectively. The low-frequency slope of the frequency response curves of CN and SN afferents was ~35 dB/decade and ~20 dB/decade, respectively. The low-frequency phase lead of CN fibers was ~180° and that of SN fibers ~120°. The frequency of maximal sensitivity, the low-frequency slope of the frequency response, and the low-frequency phase angles indicate that canal neuromasts detect water acceleration and superficial neuromasts detect water velocity (Kroese and Schellart 1992; Bleckmann 1994). These data are in accord with the theoretical studies of Kalmijn, which stipulate that the velocity detector should have the low frequency gain of about 20 dB/decade and the phase lag or lead of about 90° (270°) relative to the stimulus displacement maximum, and the acceleration detector's gain and phase lag should be about 40dB/decade and about 180° (0°), respectively (Kalmijn 1988; Bleckmann 1994).

Dijkgraaf (1963) hypothesized that canal and superficial neuromasts are functionally different hydrodynamic receptors. SNs are permanently stimulated by the water flow around the fish, produced either by currents or by swimming motions of

the fish. Thus they are not ideal or may even be useless in detecting specific biologically relevant stimuli, generated by prey or predator. On the other hand, canal neuromasts, due to the high-pass mechanical filtering provided by the canals, should be much less sensitive to uniform currents of water motion and thus can function as detectors of specific biological disturbances. This functional dichotomy was shown in the New Zealand eel anterior lateral line fibers. One type of fibers was shown to be flow sensitive and the other was not (Voigt et al. 2000). The flow sensitive fibers possess frequency response characteristics of velocity detectors (Engelmann et al. 2000; Engelmann et al. 2002). These fibers innervated superficial neuromasts. In addition, the responses of these fibers to sinusoidal water motions were masked by the running water. Contrary to that, flow insensitive fibers had frequency characteristics of acceleration detectors and their responses to sinusoidal stimuli were not masked by the noise from the unidirectional water flow. Thus the flow insensitive fibers probably innervate canal neuromasts.

Behavioral significance of the mechanosensory lateral line.

Physiological and anatomical data suggest that the two populations of neuromasts (e.g. SN and CN) may mediate different kinds of behaviors (Munz 1989; Voigt et al. 2000). Biologically relevant hydrodynamic stimuli are very complex in nature. Swimming movements of fish and motion of body appendages of invertebrate organisms produce high-frequency oscillations. These oscillations are usually superimposed on low-frequency transients or unidirectional currents. Uniderectional water motions may be produced by mass currents in rivers, lakes or oceans (Bleckmann 1994). As mentioned above, the lateral line system of fish is functionally

subdivided to separately detect unidirectinal and oscillating components of hydrodynamic stimuli. Because of this the lateral line system mediates a large repertoire of behaviors.

Rheotaxis.

To the contrary of established ideas (Dijkgraaf 1963), the lateral line system plays a major role in rheotaxic behavior of fish – orientation and swimming against the current (Northcutt 1997). Superficial and not canal neuromasts are involved in rheotaxic behavior (Montgomery et al. 1997). The threshold (the minimum velocity of the unidirectional current that elicits rheotaxis) for a rheotaxic response is significantly elevated after pharmacological treatment of fish with cobalt and/or streptomycin. Cobalt and streptomycin treatment blocks all receptors of the lateral line system (Kroese and van der Bercken 1982; Karlsen and Sand 1987). On the other hand, application of gentamicin, which inhibits only canal neuromasts (Song 1995), has no effect on the rheotaxic behavior. Physical ablation of superficial neuromasts elevates the rheotaxic threshold too (Montgomery et al. 1997). Thus it seems that superficial neuromasts and not canal neuromasts are involved in the mediation of rheotaxis.

Feeding.

The unconditional bite response of mottled sculpin is elicited by a vibrational (AC) signal, and thus probably is mediated by an acceleration sensitive component of lateral line system, i.e. canal neuromasts (Janssen et al. 1990). Experiments also indicate that fish can be trained to produce a conditioned turn response to uniform (DC) signals (Janssen et al. 1990). Results show that fish sense both DC and AC

signals, but that AC signals are easier to localize than DC signals, thus not requiring training to elicit strike response.

Schooling.

The lateral line system has also been implicated in schooling behavior of fish. Particularly, it is important for monitoring the speed and direction of travel of neighbors (Pitcher et al. 1976; Partridge and Pitcher 1980). Relative roles of visual and lateralis system were determined by blindfolding and/or cutting the lateral line nerves of the schooling aggregates of seith, *Pollachius virens.* Both systems are important for normal maintenance of the school. Results indicate that position and angle between fish is monitored by vision and that lateralis system is used to monitor the swimming speed and direction of travel (Pitcher et al. 1976; Partridge and Pitcher 1980).

Detection of prey.

It has been shown (Montgomery and Macdonald 1987) that vibratory oscillations of crustaceans due to swimming movements are a potent natural stimulus for a zooplankton feeding antarctic fish *Pagothenia borchgrevinki.* The frequency response of the anterior lateral line afferents of this fish corresponds strongly with the power spectra of swimming movements of their prey. By placing the crustacean near the experimental animal, the authors were able to record bursts of action potentials with each power stroke and recovery of the crustacean locomotor cycle. This indicates that the lateral line system is very much suitable for the detection of prey in these ant fish (Montgomery and Macdonald 1987).

The general biology of lampreys.

Lamprey life cycle

Lampreys and hagfishes are the two extant representatives of agnathan fishes (Hardisty 1979). Both groups, collectively called cyclostomes, are considered phylogenetically to be the sister groups to gnathostomes (Hubbs and Potter 1971). Modern lampreys inhabit non-tropical regions of both hemispheres (Hubbs and Potter 1971; Hardisty 1979). Lampreys can generally be divided into parasitic and non-parasitic forms, depending on whether they feed after the completion of metamorphosis. The two forms are very similar morphologically and have been termed the "paired species". It is thought that the non-parasitic species are derived from the parasitic ones (Hardisty and Potter 1971c). The non-parasitic forms, after metamorphosing, reproduce and die, while the parasitic ones become predators and live for a few more years, reproducing yearly (Hardisty and Potter 1971c). Larval lampreys, called ammocoetes, are freshwater, filter-feeding organisms that spend most of their lives burrowed in the mud of river banks, occasionally swimming out when disturbed and changing the cite of the burrow (Hardisty and Potter 1971a). After metamorphosis, the parasitic species, can either stay in their native streams (freshwater species) or migrate down the rivers to the sea (anadromous species) and lead quite active predatory life styles (Hardisty 1979). The predatory adults attach themselves with the suctorial disc (mouth or oral disk), which has sharp teeth-like structures, to other fishes and feed by sucking blood and small pieces of muscle tissue through a hole produced by rasping motions of the teeth (Hardisty 1979).

Reproductive behavior.

Two particularly interesting behavioral adaptations of adult lampreys deserve a little more attention in this short account of the ecology of these organisms. Both behaviors are directly related to reproduction and can be thought of as courtship.

The first is the pre-spawning behavior, involving a quite elaborate and energy-costly nest building. In this act, a male lamprey constructs an approximately circular nest, by moving the stones with its suctorial disk and by vigorously vibrating its body to create a depression up to 10 cm in depth (Hardisty and Potter 1971b). The dimensions of the nest depend on the species and size of the male and can be as large as 1.5 m in diameter for *Petromyzon marinus*. However, most experts think that nest-construction is not a true building activity, but a consequence of the pre-spawning vibratory activity. Nevertheless, everybody agrees that it is a necessary pre-spawning courtship behavior (Hardisty and Potter 1971b). While the male lamprey is building a nest, the female is engaged in her own courtship behavior in which she repeatedly glides over the rostral part of the male (Hardisty and Potter 1971b).

The second behavior is the spawning act itself. During pairing, the female attaches with the suctorial apparatus to a large stone in the nest, while the male approaches her from the downstream direction. When the male reaches the level of the female's branchial region, it attaches with the oral disc to the dorsal side of the female's head. The mail then proceeds to curl its tail around the female's trunk, rostral to the cloaca, forming a loop and moving caudally until both cloacal regions are aligned. The caudal movement of the loop squeezes the eggs out of the female at which point they are fertilized by the male's sperm (Hardisty and Potter 1971b).

In regard to such a peculiar mode of reproduction in lower vertebrates, it is worthwhile to note that the lateral line system of some teleost fish have been shown to play a role in reproductive courtship behavior (Dijkgraaf 1967; Satou et al. 1994). Due to the complex nature of lampreys' reproductive courtship and the spawning act itself, it would be interesting to know whether the lateral line system also takes the courtship behavior. Unfortunately, lamprey spawning in laboratory conditions has rarely been achieved, making it almost impossible to test the involvement of the lateral line.

The lateral line system of lampreys.

The lateral line nerves of lampreys, which are distinct cranial nerves, innervate three types of peripheral epidermal sensory receptors (Nieuwenhuys and Nicholson 1998). These receptors include photoreceptors, electroreceptors, and mechanoreceptors.

Overall anatomical organization of the lateral line system.

Photoreceptors and mechanoreceptors of the trunk are innervated by the posterior lateral line nerve (PLLN). Mechanoreceptors of the head and electroreceptors of the head and trunk are innervated by braches of the anterior lateral line nerve (ALLN) (Nieuwenhuys and Nicholson 1998). The PLLN has one ganglion located caudally to the otic capsule. The ALLN also has one ganglion located caudal to the maxilomandibular ganglion of the trigeminal nerve, medial to the rostral half of the otic capsule. Distal to the ganglion the ALLN branches into four rami. The recurrent ramus extends dorso-caudally around the otic capsule and fuses with the

PLLN to form the trunk lateral line nerve (TLLN). The hyomandibular ramus courses ventrally down to the underside of the head. The buccal ramus runs dorso-ventrally below the orbit. The superficial ophthalmic ramus extends dorso-rostrally joining the nervus profundus (Ronan and Northcutt 1987; Koyama et al. 1990; Gonzalez and Anadon 1992). The primary projections of the lateral line nerves terminate in the octavolateralis area of the rhombencephalon, which consists of three sensory nuclei in the alar plate, arranged longitudinally (Nieuwenhuys 1972). The ventral nucleus receives afferents from the VIIIth nerve. Medial and dorsal nuclei receive afferents from the lateral line nerves. The cell bodies of the medial and dorsal nuclei are located in a thin periventricular plate with the dendrites extending to the lateral neuropil. The medial nucleus is somewhat larger then the dorsal nucleus. It terminates more rostrally then the dorsal nucleus. Degenerating fiber studies show that the PLLN distributes its fibers rostral and caudal to its entry into the medulla throughout the medial nucleus. Ipsilateral projections terminate in the medial neuropil of the medial nucleus. These fibers continue rostrally into the cerebellum where they cross the mid-line in the dorsal part of the fiber layer above the cerebellar periventricular gray. On the contralateral side they terminate in the central neuropil throughout the medial nucleus. Degeneration of the ALLN fibers shows that ALLN terminations fill the dorsal nucleus and occupy the lateral part of the medial nucleus on the ipsilateral side. ALLN fibers continue rostrally into the cerebellum, where they reach the mid-line ventrally to the crossing PLLN fibers. Ipsilateral ALLN projections do not reach the contralateral side. The ALLN of lampreys projects to medial and dorsal nuclei via a separate set of fibers. The dorsal roots of ALLN project to the dorsal nucleus and the

ventral roots of ALLN project to the medial nucleus. The PLLN enters the brainstem as a single root (Nieuwenhuys 1972; Ronan and Northcutt 1987; Koyama et al. 1990; Gonzalez and Anadon 1992).

Photoreception.

Parker in 1905 first showed that larval lampreys are sensitive to light but that this sense is not mediated by the paired eyes. He found that illumination of the tail of ammocoetes elicited a negative photokinesis in a form of delayed locomotion (Young 1935a). Young in 1935 showed that the most photosensitive region of larval as well as adult lampreys was located along the base line of the caudal fin. By his behavioral experiments he determined that the minimum stimulation time to elicit a photokinetic response was 0.4 seconds and that the shortest reaction time was 1.5 seconds. Both of these parameters depended on the intensity of light and the tail area stimulated. The responses to illumination of either side of the tail appeared to lack directionality. Consequently, Young in this regard concluded that the effect of shining light on the tail is only of general excitatory nature and produces a non-orientating motor response, hence 'photokinetic' as opposed to 'phototactic' response. His most relevant finding was that the sensitivity of light is transmitted to the central nervous system by the lateral line nerves and not by the dorsal roots of the spinal cord. Sectioning the trunk lateral line nerves on both sides of adult and larval lampreys completely abolished the photokinesis. Young also showed that the pineal eye is not involved in the photokinesis (Young 1935b). Further investigations of the larval lampreys' photoresponse revealed that the wavelengths of light of highest sensitivity lie in the range between 400 nm and 550 nm. In addition, a strong positive thigmotaxis

(tendency to contact the substrate) can overcome the photokinesis in ammocoetes (Francis and Horton 1936). Investigations directed to determine the nature of the photosensitive element in the skin of ammocoetes yielded information on the dynamic properties of the photoresponse. Steven (1950) showed that the response adaptation to darkness (the decrease in the threshold intensity of light that elicits photokinesis) is slow, reaching the steady-state after 20-30 minutes in dark. The increase in sensitivity at the completion of dark adaptation was relatively small and the intensity discrimination at different levels of light was limited (Steven 1950). Steven also hypothesized that porphyropsin may be the photopigment in the receptor cells, based on the finding that the peak sensitivity was at 530 nm. A roughly estimated number of photoreceptive cells in the tail of ammocoetes is approximately 50, based on silver stained transverse sections of the lateral line nerves at the level beyond the last neuromast (Steven 1951). More recently, Ronan and Bodznick (1991) showed that the tail photoreceptors are innervated by the PLLN and not the recurrent branch of the ALLN. The photoresponse was not affected by the sectioning of the recurrent ramus of the anterior lateral line nerve, which runs together with the PLLN in the trunk lateral line nerve (TLLN). However, cutting the PLLN completely abolished the response. The photosensory afferents in the nerves and in the medial nucleus of the octavo-lateralis area exhibit irregular spontaneous activity, respond to light by bursts of action potentials after latencies of 1-4 seconds, and have response thresholds of 0.1-0.9 mW/cm^2 (Ronan and Bodznick 1991).

The epidermal photoreceptors have not been positively identified yet. A number of cells were proposed to be possible candidates, including clusters of cells

containing yellow pigment and innervated by axonal ramifications (Steven 1951) and multivillous cells with numerous microvilli at the apical surface (Whitear and Lane 1983).

Electroreception.

Electroreception in lampreys had been presumed before it was shown physiologically based on neuroanatomical evidence: in particular, the presence of the dorsal nucleus in the octavolateralis area and the associated dorsal root of ALLN (McCormick 1981b; Fritzsch et al. 1984; Ronan 1986). Such morphology is also observed in elasmobranchs (Boord and Campbell 1977), chondrosteans (New and Northcutt 1984), and lepidosirenid lungfishes (Northcutt 1983). In sharks, it is believed that the electrosensory organs – ampullae of Lorenzini – are innervated exclusively by the dorsal root of the ALLN and project to the anterior lateral line lobe (dorsal nucleus) of the medulla (Boord and Campbell 1977). Subsequently, larval and adult lampreys were shown to be receptive to weak electric fields by electrophysiological recordings. (Bodznick and Northcutt 1981; Akoev and Muraveiko 1984; Ronan 1988). The threshold sensitivity to uniform electric fields was determined to be 0.1 - 20 μV/cm, which is as low as thresholds of the electroreceptive freshwater fish. Evoked potentials with peak latencies of 65 msec, 75-85 msec, and 140 msec were recorded in dorsal medulla, torus semicircularis and the optic tectum, respectively. Bodznick and Northcutt (1981) also recorded visually evoked potentials in the same areas of mesencephalon, suggesting a convergence of visual and electrosensory information in torus semircularis and the optic tectum.

Somatotopy has been observed in the electro-receptive posterior lateral line lobe of gymnotiform fishes (Carr et al. 1982) as well as in elasmobrachs (Bodznick and Schmidt 1984) and lepidosirenid lungfishes (Northcutt 1983). In lampreys, on the other hand, the primary electro-sensory afferents extensive overlap in the dorsal nucleus (Ronan and Northcutt 1987). The nucleus medialis, on the contrary, exhibits a clear somatotopic organization. The cephalic mechanoreceptive neuromasts are represented laterally and the trunk and tail neuromasts, medially (Ronan and Northcutt 1987). A number of gnathostome groups also exhibit a somatotopic organization in the nucleus medialis.

Recordings of afferent fibers in the ALLN of larval and adult lampreys indicate that the electroreceptors are excited by weak cathodal and inhibited by weak anodal electric fields. The response polarity reverses with strong stimuli (Bodznick and Preston 1983; Ronan 1986, 1988).

Ampullary electroreceptive organs are not found in lampreys (Ronan 1986). Instead, the goblet-shaped structures, originally thought to be taste buds and then photoreceptors (Whitear and Lane 1981) in the skin are considered to be electroreceptive (Ronan and Bodznick 1986). They have been termed the end buds. These epidermal structures consist of about 3-25 narrow sensory cells, with 80-90 microvilli at the apical surface, and numerous supporting cells (Whitear and Lane 1981; Ronan 1986). Groups of end buds are innervated by a single afferent fiber forming bar synapses with the sensory cells (Whitear and Lane 1981). The electroreceptive end buds are distributed on the head and trunk regions of the body, though the highest density is observed on the head (Bodznick and Preston 1983).

On the basis of anatomical and physiological data, lamprey's electro-sensory system together with that of chondrichthyans and primitive bony fishes (Acipenseriformes and Lepidosireniformes) are homologous and represent an ancestral condition (Bullock et al. 1983; Ronan and Northcutt 1987). Teleosts have lost the electro-receptive capabilities in the course of evolution, but several groups independently re-evolved electroreception. In these groups (mormyrids, gymnotids, and suluriforms) the structure that receives the electrosensory fibers – the electrosensory posterior lateral line lobe - is not homologous to the dorsal nucleus (Ronan and Northcutt 1987). The electrosensory posterior lateral line lobes of those groups of teleosts are believed to have evolved independently from parts of the mechanoreceptive nucleus medialis (Carr et al. 1982; Bullock et al. 1983).

The functional significance of electroreception in lamprey behavior is unknown. They obviously can passively detect electric fields at some distance generated by other animals. This ability may be utilized for prey or predator detection. In addition, there are evidence suggesting that electroreception can be used actively. Voltage spikes, synchronous with the respiratory movements, can be recorded in the space surrounding the animal. It was shown that these spikes, in case of adult *Petromyzon marinus*, range from 200-300 μV at a distance of 15-20 mm above the eye (Kleerekoper and Sibakin 1956). Such potentials can produce electric fields extending several centimeters away from an animal, in fresh water. The field was found to be symmetrical relative to the longitudinal midline of the animal and diminished sharply caudal to the last gill slit (Kleerekoper and Sibakin 1956). It is possible that lampreys can detect changes in the electric field with their

electroreceptors. The finding that the head region has the highest density of electroreceptors (Bodznick and Preston 1983) may supports the notion of active electro-location in lampreys. However, up to the present this possibility has not been investigated.

Mechanoreception.

Much less attention has been given to the mechanoreceptive component of lamprey lateral line system than to the other lateral line modalities. Most work concerned the anatomical description, including determination of the primary projections and the fine structure of the neuromasts. The primary projections and the medial nucleus of the octavolateralis area were described above (see the section on the *Overall anatomical organization of the lateral line system*).

The neuromast structure appears to be generally similar to that described in fishes and amphibians. It is composed of the sensory hair cells, supporting cells, and an innervating nerve fiber. The neuromast is about 200 μm long, 60-80 μm wide, and 70-90 μm tall. The organ is located in the epidermis with its basal part situated on the dermal evagenation or eminence and basal lamina. The apical surface of the organ does not reach the surface of the epidermis, which situates the hair cells in a groove. Hair cells exhibit the usual morphological polarity, with half of the kinocelia situated at one side of the stereocilia complex pointing to the head and half situated on the opposite side pointing to the tail of the animal (Yamada 1973; Katori et al. 1994).

However, based on certain ultrastructural differences, the lamprey neuromast may represent an ancestral form (Yamada 1973; Katori et al. 1994). The major differences are the absence of the cupula at the apical surface of the hair cell bundles,

the absence of efferent synapses at the base of the hair cells, and the shortness of the stereocilia (Yamada 1973; Lane and Whitear 1982; Katori et al. 1994). Cupulae are very delicate structures and are easily destroyed during the preparations of the specimen (Yamada 1973). Indeed, Yamada reports that the free apical surface of the neuromast is covered by a dense filamentous coating, which could be the remnant of the cupula. Despite its uniqueness, the absence of the efferent innervation of the hair cells has to be taken as a fact, because the evidence from the transmission electron microscopy is unambiguous. The shortness of the stereocialia compared to the microvilli is an unusual finding, which is not observed in teleost fishes. Similar observations were made in shark neuromasts, *Mustelus antarcticus*, (Peach and Rouse 2000), and in lungfish (Webb and Northcutt 1997). The shortness of the stereocilia relative to the surrounding microvilli may also represent an ancestral character. Yamada (1973) also speculated on the functional significance of such morphology. His idea was that in lampreys' hair cells, the stereocilia may not play a role in the mechanical transduction.

Despite the peculiarity and an evolutionary standing of lampreys, the physiological and functional aspects of the mechanosensory lateral line have barely been investigated. Recordings in the PLLN of adult lampreys indicate the presence of mechanoreceptive fibers responding to water movements relative to the skin (Rovainen 1971; Akoev and Muraveiko 1984). Detailed knowledge of lamprey mechanosensation, its physiological properties, behavioral significance, and developmental changes is lacking. The subsequent chapters of this dissertation will describe experiments and observations designed to study some of the aspects of the

mechanosensory lateral line in larval and adult lampreys. In the second chapter, data is presented establishing that larval lampreys possess a functional lateral line system. The third chapter deals with physiological and morphological changes in the lateral line associated with the metamorphosis of lampreys. The final, fourth chapter of this thesis is devoted to the role of the lateral line system in lamprey locomotion.

Further important aspects of the biology of larval and adult lampreys pertinent to this thesis will be given in the introductions to subsequent chapters.

CHAPTER TWO: LARVAL LAMPREYS POSSESS A FUNCTIONAL LATERAL LINE SYSTEM

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Abstract

Morphology of larval lampreys' neuromasts was found to be very similar to that of adults. Activity in the lateral line nerve, elicited by a vibrating ball, indicated a functional lateralis system. Analysis revealed at least two populations of afferents, responding to opposite directions of water flow, with adapting responses. The response magnitude increased monotonically with stimulus amplitude. Larval lampreys' neuromasts were less sensitive than those of teleosts. At low frequencies the response showed a phase lead of 200° - 220° with respect to the maximum of the ball displacement and a gain that was approximately linearly proportional to frequency.

Introduction

Most fish and many aquatic amphibians possess sensory lateral line organs, which are part of the acoustico-lateralis system (Dijkgraaf 1963; Lannoo 1987; Coombs et al. 1988; Bleckmann 1994). The mechanoreceptive component of this system is sensitive to water motions in the vicinity of the animal (Dijkgraaf 1963; van Netten 2005). Lateral line systems (LLS) play an active role in various behaviors including prey detection and capture (Montgomery and Macdonald 1987; Coombs et al. 2001; Pohlmann et al. 2004), surface feeding (Bleckmann 1988; Bleckmann et al.

1989), underwater feeding (Janssen et al. 1990), detection of stationary objects (Weissert and Campenhausen 1981), schooling (Pitcher et al. 1976), rheotaxis (Cahn and Shaw 1963; Montgomery et al. 1997; Kanter and Coombs 2003), and social display (Dijkgraaf 1963; Satou et al. 1994).

The receptor organ of the LLS is the neuromast. Teleost fishes possess two types of neuromasts: canal neuromasts located in intradermal canals, and intraepidermal superficial neuromasts. Canal and superficial neuromasts are considered to be detectors of water flow acceleration and velocity, respectively (Kroese and Schellart 1987; Kalmijn 1988; van Netten and Kroese 1989; Kroese and Schellart 1992) .

The lamprey is a basal vertebrate (Nelson 1984) extensively used in the study of locomotion (Cohen 1988; Grillner et al. 1998b). Its LLS mediates photoreception (Young 1935a; Deliagina et al. 1995), electroreception (Bodznick and Northcutt 1981), and mechanoreception (Rovainen 1982; Akoev and Muraveiko 1984). Lampreys lack canal neuromasts (Yamada 1973; Lane and Whitear 1982; Katori et al. 1994), but their superficial neuromasts have a similar morphology and distribution to those in gnathostome fishes (Northcutt 1989; Braun 1996). The ultrastructure of the neuromasts is generally comparable to that of jawed fishes, except that lamprey neuromasts lack gelatinous cupulae, structures that cover the apical surface of the supporting cells and hair cells and couple the sensory hair cells to the motion of water (Yamada 1973; Lane and Whitear 1982; Katori et al. 1994; Braun 1996). In addition, Katori et. al. (1994) also report that the length and diameter of the hair cells stereocilia is almost half that of hair cells stereocilia in other animals. The absence of

cupulae may affect the dynamic properties and sensitivity of hair cells. However the detailed physiology of the mechanoreceptive component and its involvement in locomotion or any other behavior is practically unknown in adult or larval lampreys. Qualitatively, it has been shown that mechanical stimuli elicit activity in lamprey's posterior lateral line nerve (PLLN) (Rovainen 1982; Akoev and Muraveiko 1984).

During its life span, the lamprey undergoes metamorphosis from a larval stage, called an ammocoete, to an adult stage. The two developmental forms differ remarkably in life style. While adult lampreys are free swimmers, larval lampreys burrow into the mud of freshwater streams and swim only to change their burrowing site (Hardisty and Potter 1971a). Nevertheless, the primary lateral line projections (Ronan and Northcutt 1987; Koyama et al. 1990; Gonzalez and Anadon 1992) and distribution of neuromasts in ammocoetes and adults is very similar (Johnston 1905; Rovainen 1982). It is unknown whether ammocoetes' lateral line differs physiologically from that of adults.

Here we report a preliminary study on the surface ultrastructure and physiology of ammocoetes' LLS. We show that larval lampreys possess a functional mechanoreceptive LLS with neuromasts that do not differ qualitatively from those of adults. Our results will serve as a basis for future comparative and developmental work and will help to examine the role of the LLS in swimming behavior of lampreys.

Materials and Methods

Animals.

Ammocoetes, *Petromyzon marinus*, (length: 11.5 – 13.0 cm) were obtained from fishermen along Lake Michigan, US and kept in aerated aquaria with sand bottoms at 12°C.

Scanning electron microscopy.

Three animals were used for scanning electron microscopy (SEM). In each animal we examined neuromasts from infra-orbital and infra-branchial lines. The number of neuromasts in the infra-orbital line ranged from 7 to 13. The number of neuromasts in the infra-branchial line ranged from 6 to 8.The following procedure was used for scanning electron microscopy. Special care was taken not to damage the neuromasts during preparation: no mucolitic treatments were applied in the preparation. Before fixation, animals were euthanized by an overdose of tricaine methane sulfonate (MS222; ARGENT Chemical Laboratories, Fisheries Chemical Division, Redmond, Washington). Animals were fixed in 2% glutaraldehyde diluted in aquarium water (pH = 7.5) for 60 min at room temperature and kept overnight at 4°C. Specimens were dissected in two lateral halves along the dorsal midline; each half was cut transversely into pieces 1 cm in length. After 12 hours the pieces were washed (3x10 min) in distilled water (pH = 7.5) to remove excess glutaraldehyde and post-fixed in 1-2% OsO_4 in distilled water (pH = 7.5) for 60 min. Excess OsO_4 was washed away with double distilled water (3x10 min). Pieces of the tissue were dehydrated (10 min) in a graded series of ethanol solutions (75%, 95%, 100%, 100%,

100%), and critical-point dried in liquid CO_2 using a Denton DCP-1 critical point dryer (Denton Vacuum, Moorestown, NJ, US), mounted on aluminum stubs, and coated with gold-palladium alloy in a Denton DV 502 vacuum evaporator. Specimens were observed with an Amray 1820D scanning electron microscope (Amray, Inc., Bedford, Mass.).

Physiological preparation.

For physiological study we used 3 animals. Prior to surgery, animals were anesthetized in 0.18g/L buffered MS222. After reflex and breathing ceased, animals were transferred to a chilled surgical dish. The PLLN was exposed by removing the skin and muscles from a 3x6 mm region just posterior to the last gill slit on the animal's left side. Finally, the membrane covering the PLLN was removed. Prior to the cessation of anesthesia, animals were injected with 0.3 – 0.4 ml of 1 mg/ml solution of a neuromuscular blocker, gallamine triethiodide (Sigma-Aldrich, Inc., St. Louis, MO, US), and transferred into a 30x40 cm experimental tank. The tank was divided into two sub-chambers: the head and the site of surgery with the exposed nerve were located in one sub-chamber, filled with saline (NaCl: 91 mM, KCl: 2.1 mM, $CaCl_2$-$2H_2O$: 222.6 mM, $MgCl_2$-$6H_2O$: 1.8 mM, glucose: 4.0 mM, $NaHCO_3$: 20.0 mM); the trunk was located in the second sub-chamber, filled with aquarium water. Temperature was kept at 8-10 °C.

Stimulus generation.

Water flow was induced with an oscillating ball (9 mm diameter), attached to a speaker diaphragm through a metal shaft. The ball's axis of oscillations was

perpendicular to the fish's longitudinal axis. The center of the ball was placed 10 mm above the fish's trunk. Sinusoidal oscillations of the speaker diaphragm were generated by a custom-written LabVIEW (version 7, National Instruments, Austin, TX, USA) program. The stimulus waveform was passed from computer through an audio amplifier (PZR 600, PYLE PRO Audio, Inc., Brooklyn, NY, US) to the speaker. The stimulus consisted of 10 repetitions of a sinusoidal waveform 1 second in duration, 10 seconds apart. The ball motion was filmed with a high-speed digital camera (NAC HotShot, 1280, NAC Image Technology, Simi Valley, CA, US) at 200 frames/second for 5, 10, and 30 Hz and at 500 frames/second for 40 and 50 Hz. The peak-to-peak amplitude of the ball oscillations was determined by analysis of the video. Water motion amplitude at the surface of the animal's skin was estimated by the following formula:

$$A = U(R/D)^3,$$

where A is the amplitude of water motion at the surface of the animal, U is the displacement amplitude of the ball, R is the radius of the ball, and D is the distance between the center of the ball and the surface of the skin (Kroese and Schellart 1992). Based on this approximation the peak-to-peak amplitudes of water motion near the neuromasts were 33 – 278 μm. This formula does not take into account the presence of the fish and is derived for an incompressible fluid. In addition, its validity lies in the range of displacement amplitudes of the ball (U) so small that D can be considered constant. Therefore, the estimates of the water motion amplitudes obtained with this formula are only useful to give a sense of the order of magnitude.

Physiological data collection and analysis.

Extra-cellular multi-unit recordings on 3 animals were made with glass microelectrodes filled with 3 M KCl solution. The electrodes were lowered and inserted slightly into the PLLN using a manual micromanipulator (KITE-R, WPI, Inc., Sarasota, FL). Recordings began four hours after the animal was transferred to the experimental chamber, due to inhibitory effect of the MS-222 on the activity of the lateral line. Responses were amplified by an AC differential amplifier (DAM 80, WPI, Inc., Sarasota, FL), and digitized at 5 kHz synchronously with the stimulus signal. Both the evoked responses and stimulus waveforms were stored on a computer for off-line analysis.

Parameters of the multi-unit response were determined by setting a threshold above the noise level and isolating all spikes that were above the threshold level. Measured parameters included the firing frequency in action potentials per second (response magnitude) and the relative phase of the action potentials with respect to ball displacement. To eliminate transient activity associated with the start of the stimulus we disregarded 1-10 cycles at the beginning of the stimulus (depending on the stimulus frequency). The response magnitude for each trial was normalized by dividing by the spontaneous activity of the PLLN in each animal. The phase of the response was determined by computing the phase of each spike with respect to the central position of the ball as determined from the video.

Gain of the response is defined as the ratio between the change of response magnitude and the corresponding change in the stimulus amplitude. At each frequency, the gain was estimated as the slope of the line fitted to the low-frequency linear portion of the amplitude response (response magnitude vs. stimulus amplitude).

Theoretically, the change in gain with changing frequency indicates some underlying properties of the neuromast sensor. Given a stimulus displacement of $A \sin(\omega t)$, where A is amplitude, ω is frequency and t is time, then stimulus velocity is $A \omega \cos(\omega t)$ and acceleration is $-A \omega^2 \sin(\omega t)$. If the gain is constant with frequency, then the lateral line is responding to stimulus displacement, but if the gain increases linearly or quadratically with frequency, then the lateral line is responding to stimulus velocity or acceleration, respectively. Expressed in decibels, it is equivalent to say that velocity detectors and the acceleration detectors have gains relative to frequency of 20 dB/decade and 40 dB/decade, respectively. Also, at a fixed frequency, both velocity and acceleration are linearly proportional to the amplitude of the displacement.

Results

Neuromasts on the head were clearly visible (Fig. 2-1a).

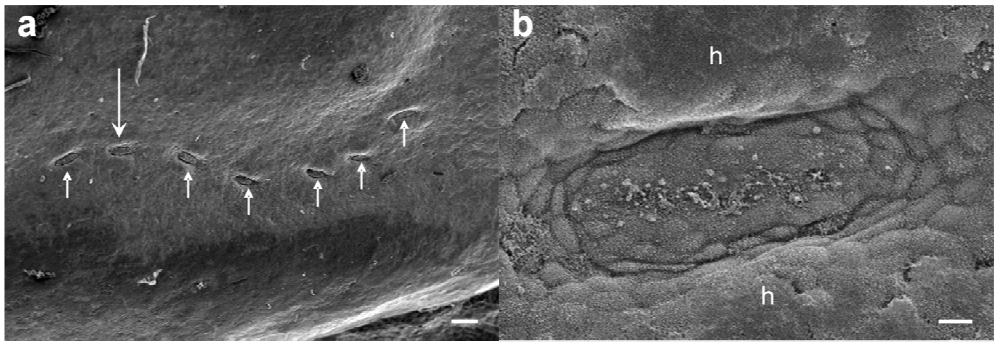

Figure 2-1.
Scanning electron micrographs showing the larval lampreys' skin surface.
(a) Infra-orbital row of 7 superficial neuromasts. Magnification x65.5. Horizontal scale bar is 100 μm long (arrows point to the neuromasts; the large arrow points to the neuromast in part b).
(b) Magnified view of one neuromast in the infra-orbital row. Sensory strip consisting of hair cells can be observed in the middle of the neuromast along the longer dimension of the receptor organ. The neuromast is surrounded by rising hillocks (h). Magnification x830. Horizontal scale bar is 10 μm long.

Observed neuromasts of the infra-orbital and infra-branchial lines had an oval-shaped form with its long axis oriented approximately parallel to the longitudinal axis of the fish. Their length ranged from 85 μm to 120 μm and their width from 20 μm to 25 μm. The neuromasts were situated in epidermal pits, surrounded by a moat partially filled with mucous secretion. The epidermal cells formed hillocks around the neuromast (Fig. 2-1b). The sensory surface, area occupied by the hair cells, of observed neuromasts greatly varied. Some organs appeared to lack the sensory area completely, which could be due to damage during preparation.

As demonstrated in Figure 2-2a, the PLLN recording shows spontaneous

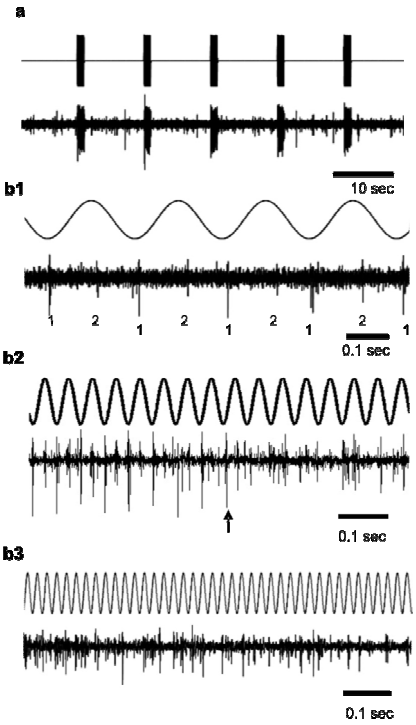

Figure 2-2.
Responses of fibers in the posterior lateral line nerve of larval lampreys to an oscillating ball motion of varying frequencies. Peak-to-peak stimulus amplitude is 230 μm in all cases.**(a)** Spontaneous activity and response to oscillating water motion. Scale bar is 10 sec. **(b1)** Stimulus frequency 5 Hz. Unit 1 is active with a phase lead of ~110° to the displacement of the ball. Unit 2 is active with a phase lead of ~310° to the displacement of the ball. **(b2)** Stimulus frequency 20 Hz. Unit 1 and 2 are active in the first 9 cycles of the stimulus. After the ninth cycle (arrow) unit 1 stops and unit 2 fires less regularly. **(b3)** Stimulus frequency 50 Hz. Response is more random. Scale bars in b1, 2, and 3 are 0.1 sec.

activity and a clear response to vibrating stimuli, indicating that the LLS of ammocoetes is functional. Action potentials from at least two distinct units could be identified in this recording (Fig. 2-2b1, 2). For 5 and 10 Hz stimuli, the neurons showed a non-adapting response (Fig. 2-2b1); units from both populations fired every

cycle for the entire duration of the stimulus. At frequencies of 20 Hz and above, the response was adapting; neurons would often skip some cycles of the stimulus or stop firing after a few cycles (large units in Fig. 2-2b2). The phase locking between the stimulus and the response became more irregular at the highest stimulus frequencies (Fig. 2-2b3).

Figure 2-3a depicts a histogram of spike phases from all three animals at the 5 Hz stimulus with the amplitude of 230 μm. Two distinct peaks of the distributions clearly indicate two populations of neurons that generate action potentials ~180° apart with respect to the stimulus waveform. One population of units was active with a phase lead of ~110° in relation to the displacement of the ball. Another population of units was active with a phase lead of ~310° (Fig. 2-2b1, 2-2b2). At higher frequencies (10, 20 Hz), spike phases shifted due to transduction delays, but the distance between the two peaks remained ~180°. The phase of the two populations of neurons with respect to the displacement of the ball was approximately invariant with the stimulus amplitude (fig. 2-3b and 2-3c).

In all animals and at all tested frequencies, the magnitude of the response monotonically increased with increasing amplitude of the stimulus. While the absolute magnitude of the response varied considerably among animals, this variation was strongly correlated to the spontaneous activity of the nerve (which ranged between 2.0 and 13.2 Hz in different individuals). The evoked activity followed the same pattern of variation, such that the animal with higher spontaneous activity had larger evoked responses. We normalized the multi-unit evoked responses by the level of spontaneous activity in the entire nerve to minimize the differential influence of

any one animal on the averaged responses plotted (Fig. 2-3d). Rate of change of the response magnitude with respect to the stimulus amplitude (gain) depended on frequency. It increased in the 5-20 Hz frequency range and stayed almost the same above 20 Hz, as can be seen from the slopes of the curves (Fig.2- 3b). At low frequencies, the gain increased approximately linearly (and not quadratically) with frequency (Fig. 2-3e).

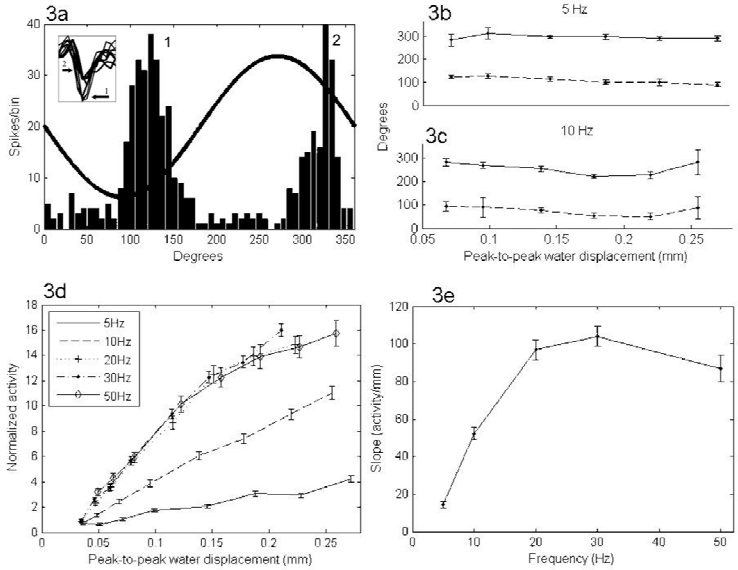

Figure 2-3.
Physiological characteristics of the response of the fibers in the larval lampreys' posterior lateral line nerve. **(a)** Histogram of spike phases with respect to the displacement of the ball at 230 μm peak-to-peak amplitude and 5 Hz frequency. Two peaks ~180° apart indicate the presence of two populations of neurons that respond to opposite directions of water flow. The histogram was constructed by combining the spike phases from 3 animals with 11 recordings from each animal. Inset depicts examples of units that fall in the first and the second peaks, respectively.**(b, c)** The phase of the mean response with respect to the amplitude of the stimulus. Continuous and broken lines represent phases of the two populations of neurons responding to opposite directions of the flow, respectively. Each point is a mean of 3 animals; vertical bars are the standard error of the mean. (b) Stimulus frequency 5 Hz, **(c)** Stimulus frequency 10 Hz.
(d) Amplitude response of fibers in the larval lampreys' posterior lateral line nerve. Normalized activity elicited by stimuli of varying frequency. At all frequencies, the magnitude of the response increases monotonically with increasing stimulus amplitude. Vertical bars indicate standard error of the data combined from 3 animals and 11 stimulus presentations for each animal. When not visible, the standard error is very small.**(e)** The gain of the response with frequency for the posterior lateral line nerve fibers. The gain at each frequency was estimated by the slope of the line fitted to the linear portion of the amplitude response. At low frequencies, the gain showed an approximately linear and not quadratic increase with the stimulus frequency. Vertical bars indicate standard error of the data combined from 3 animals and 11 stimulus presentations for each animal.

Discussion

It appears that the sensory surfaces of neuromasts belonging to *P. marinus* ammocoetes are still in development. Previously reported (Lane and Whitear 1982) SEM images of neuromasts of adult *Lampetra planeri* and *Lampetra fluviatilis* exhibit a more developed sensory area, with a larger portion of the neuromast occupied by the hair cells. Comparing these to our own (Fig. 2-1) indicates that there are no qualitative differences between adults' and ammocoetes' neuromasts. They only differ in the numbers of developed hair cells.

Our physiological results indicate that ammocoetes' LLS is functional. It also appears that larval lampreys' neuromasts are less sensitive then those of other fishes. Results of our experiments revealed that there was no increase in the spike rate below the stimulus amplitude of ~50 μm, whereas Bleckmann et. al. (1989) report minimal peak-to-peak displacements that elicit a neural response to be 0.2 – 0.4 μm in striped panchax, *Aplocheilus lineatus* and Kroese and Schellart (1992) report the range of sensitivities of superficial neuromasts to be 1-57 nm in the rainbow trout, *Sarotherodon niloticus*. This was observed in all three animals, for all recording trials. It is possible that modulation of spontaneous activity took place during stimulation at lower amplitudes.

The extent of LLS utilization in larval behavior is unknown. Mechanoreception is probably rarely used, because larvae spend most of the time burrowed in the silt of riverbeds. Nevertheless, it may be important during the relocation of larvae from one site to another. Information about direction and strength of local currents may be helpful for finding the optimal location for the burrowing

site. Water velocity was found to be the most significant and constant factor determining the location of the ammocoete habitat (Hardisty and Potter 1971a). Low sensitivity of neuromasts and response adaptation indirectly supports this hypothesis. High sensitivity is not necessary for a simple determination of the direction and magnitude of relatively constant and strong water currents in rivers inhabited by larval lampreys. Also, due to relative regularity of river conditions, an adapting response can provide sufficient information about water currents in a short period of time.

Results of this study are insufficient to make a conclusion on the dynamic properties of lamprey neuromasts. In general, superficial neuromasts in teleosts are considered to be water velocity detectors (van Netten and Kroese 1987; Kalmijn 1988; van Netten and Kroese 1989; Kroese and Schellart 1992). Theoretical and experimental studies show that velocity (acceleration) detectors should respond at a phase of 90°-180° (>180°) to the maximum displacement of the source, respectively. The lamprey neuromasts show a peak at 110º with respect to the zero stimulus displacement (central position of the ball) and another peak at 310º (Fig. 2-3a). The second peak appears to be from hair cells that respond to water velocity in the opposite direction as the first peak, because it is 130º (which is 310º minus 180º) after the beginning of motion in the opposite direction. Thus, relative to motion in either direction, the peaks of the response occur at 110º and 130º with respect to the beginning of that motion. For comparison with the results reported by Kroese and Shellart (1992), who put the phase lead for superficial neromasts at ~120°, the phase has to be shifted by 90° to make it relative to the maximum displacement. Thus the

phase lead of 200°- 220° may imply that ammocoetes' superficial neuromasts partially respond as acceleration detectors. On the other hand, our data suggest that the gain of the response with frequency is not quadratically increasing, but may increase linearly (Fig. 2-3e), which is consistent with neuromasts that respond to water velocity. Based on our results, it is not clear whether lampreys' neuromasts are sensitive to velocity or acceleration or to a combination of both. Similar conclusion have been reached by Weeg and Baas (2002), who concluded that midshipman superficial neuromasts cannot be considered as solely velocity detectors, at least at the level of the afferent neurons.

It appears that ammocoetes' peripheral LLS encodes both stimulus amplitude and frequency with spike rate. In addition, up to about 20 Hz, some information about the frequency may also be encoded by the gain of the response.

The LLS of larval lampreys is therefore sufficiently developed to have behavioral significance. Absence of cupulae may endow lampreys' neuromasts with different dynamic properties, though direct evidence for this conjecture is lacking. Further research is necessary to elucidate the mechanisms underlying the function of the LLS and its role in the behavior of lampreys.

CHAPTER THREE: METAMORPHOSIS-RELATED CHANGES IN THE LATERAL LINE SYSTEM OF LAMPREYS, *PETROMYZON MARINUS*

Abstract

Lampreys undergo metamorphosis during which most systems show at least some modification, leading to considerable changes in morphology and overall behavior. Our knowledge of the lamprey mechanosensory lateral line system (LLS) and specifically metamorphic changes in the system is limited. We have recently reported that larval lampreys posses a functional LLS. Here we further investigated pre- vs. post-metamorphosis characteristics of the peripheral LLS using light and electron microscopy. Functional modifications were studied by recording the trunk lateral line nerve (TLLN) of larval and adult lampreys during stimulation of the receptors with sinusoidal water motions. We found a general re-patterning of the system of neuromasts on the head and trunk. It appears that three processes are involved in the re-patterning: an increase in neuromasts number, their re-distribution within the pit lines, and shifts of the pit lines relative to external features. Response of the TLLN to stimulation was qualitatively similar in adults and larvae. Both showed two populations of neurons responding to opposite directions of water flow, with the response magnitude monotonically increasing with stimulus amplitude. At low frequencies, the response phase lag with respect to the stimulus maximum was approximately 220°, and the gain depended approximately linearly on frequency, confirming the notion that superficial neuromasts are velocity detectors. The changes

in phase lag with increasing stimulus frequency were steeper in larva suggesting slower conductance. The response of adults to different frequencies indicated a narrower range of frequency discrimination. We hypothesize that the observed changes make this sensory system more suitable for the active life style of adult lampreys.

Introduction

Lampreys belong to the agnathan class Cephalaspidomorphi (Nelson 1984). All extant species of lampreys undergo metamorphosis – a transformation from a larval stage, called ammocoete, to the adult form (Hardisty 1979). Müller in 1856 was the first to recognize that the members of the genus Ammocoete were larvae of lampreys. Some lampreys are euryhaline anadromous forms as adults; living in both fresh water and marine environment. These migrate upstream to their native brooks to spawn. Others are land-locked and spend both the larval and adult stages in fresh water (Hubbs and Potter 1971). Anadromous forms undergo "true" metamorphosis in a sense that morphological and physiological changes prepare them to enter a drastically different ecological environment (Wald 1958). The land-locked forms, even though not migrating to the sea, nevertheless undergo equivalent somatic changes during metamorphosis (Youson 1980) and change their fresh-water ecological niche, transforming from burrowing, filter-feeding animals into the free swimming parasitic form. Members of the species *Petromyzon marinus* are either land-locked in the Great Lakes or anadromous (Nelson 1984). External morphological changes during the metamorphosis of both land-locked and

anadromous *Petromyzon marinus* were described in detail previously (Manion and Stauffer 1970; Potter et al. 1978). Such descriptions provide precise criteria for the determination of the metamorphic stages. It appears that all systems and organs go through at least some morphological and physiological changes during metamorphosis (Youson 1980). In the current work we focus on metamorphosis-related changes in morphology and physiology of the lampreys' mechanosensory lateral line system (LLS).

Most aquatic anamniotes possess a mechanosensory LLS (Dijkgraaf 1963; Lannoo 1987; Coombs et al. 1988; Bleckmann 1994). This system is sensitive to minute water motions in the vicinity of the animal (Dijkgraaf 1963; van Netten 2005). The lateral line plays an active role in various behaviors including prey detection and capture (Montgomery and Macdonald 1987; Coombs et al. 2001; Pohlmann et al. 2004), surface feeding (Bleckmann 1988; Bleckmann et al. 1989), underwater feeding (Janssen et al. 1990), detection of stationary objects (Weissert and Campenhausen 1981), schooling (Pitcher et al. 1976), rheotaxis (Cahn and Shaw 1963; Montgomery et al. 1997; Kanter and Coombs 2003), and social display (Dijkgraaf 1963; Satou et al. 1994). The periphery of the LLS consists of the collection of sensory hair cells, neurons of the anterior and posterior lateral line cranial nerves that innervate them, and supporting cells. Discrete aggregations of hair cells and supporting cells are called neuromasts. Most fishes possess two types of neuromasts: canal neuromasts located in intradermal canals, and intraepidermal superficial neuromasts. Aquatic stages of anuran amphibians, cyclostomes, and some fishes possess only superficial neuromasts. Canal and superficial neuromasts are considered to be detectors of water

flow acceleration and velocity, respectively (Kroese and Schellart 1987; Kalmijn 1988; van Netten and Kroese 1989; Kroese and Schellart 1992) .

Studies on the metamorphic transformations in the lateral line system of anuran amphibian, *Xenopus laevis*, indicate modifications in the distribution of sense organs and marked alterations in the activity pattern of the efferent, though not afferent component of the LLS (Shelton 1970, 1971). Similar knowledge related to the mechanosensory LLS of lampreys and specifically changes in the characteristics of the system during metamorphosis is missing.

Lampreys' lateral line system was reported to mediate photoreception (Young 1935a; Deliagina et al. 1995), electroreception (Bodznick and Northcutt 1981), and mechanoreception (Rovainen 1982; Akoev and Muraveiko 1984). Recently we showed that larval lampreys possess a functional mechanosensitive LLS (Gelman et al. 2007). We demonstrated that trunk lateral line nerve (TLLN) fibers have a preference for a direction of water flow, which is a consequence of the polarity of the hair cells that these fibers innervate. At low stimulus frequencies the phase lag of the response was 210°-220° with respect to the maximum of the stimulus, and the response gain was approximately linearly dependent on stimulus frequency (Gelman et al. 2007).

We hypothesized that due to considerable differences in behavior of pre- and post-metamorphic lampreys the LLS may acquire a different biological function after metamorphosis. A change in behavioral utilization of the system can be produced by modifications of central pathways, as well as changes in the peripheral part of the system. Here we further investigated the peripheral LLS of *Petromyzon marinus*. We

present general characteristics of this system in larvae and adults, in addition to anatomical and physiological metamorphosis-related modifications.

Materials and Methods

Animals

Fully transformed adult (length in cm: 13.6 ± 0.8 S.D.) and larval (length in cm: 12.4 ± 1.2 S.D.) lampreys, *Petromyzon marinus*, were purchased from suppliers in the area of Lake Michigan, USA. Adult and larval lampreys were kept in separate aerated aquaria (water pH = 7.5 – 8.0) at 5ºC and 12ºC, respectively. Adult lampreys were not fed prior to the experiments, but the larvae were fed once a week with powdered brewer's yeast 500 (General Nutrition Corp., Pittsburgh, PA, USA).

The stages of pre- and post metamorphic animals were determined according to Manion and Stauffer (Manion and Stauffer 1970). Figure 3-1 shows distinguished characteristics of the larva and adult useful for staging. Large ammocoetes were characterized by the following key features: a notch between the lateral (L) and transverse (T) oral hood lips, a ventral depression caudal to the oral hood (VD), eyes appearing like small dark spots (E), furrow connecting the branchiopores (F), dorsal surface being dark brown, ventral surface light brown to slightly grayish color. Young adults were characterized by the following features: yellow, sharp-pointed teeth, prominent lingual lamina, and dark bend surrounding the eyes (E).

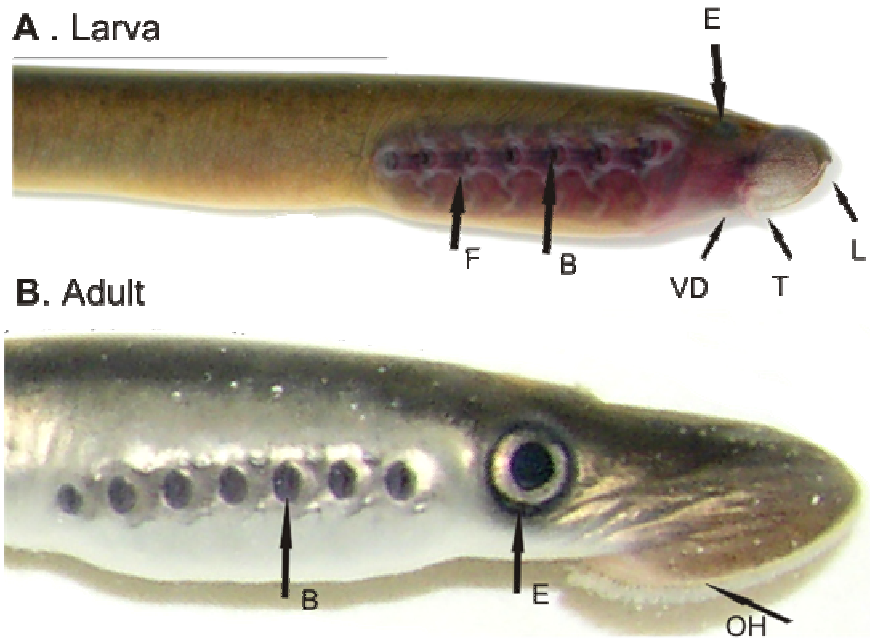

Figure 3-1.
Some unique features of pre- and post-metamorphic lampreys used for staging the experimental animals. Large ammocoete (top, Larvae) are characterized by split lateral (L) and transverse (T) oral hood lips, branchiopores connected by a furrow (F), small, dark eye spot (E), and dark brown and light brown dorsal and ventral surfaces, respectively. Transformed, migrating. Adults (bottom) are characterized by their round, complete oral hood (OH), completely formed, separate branchiopores (B), large, laterally positioned eyes with black surrounding strip (E), yellow, sharp teeth (not shown), black dorsal and silver ventral surfaces.

Morphology and anatomy of the lateral line system

Prior to all microscopy work animals were deeply anesthetized by immersion in buffered (pH = 7.5) MS-222 solution (0.2 g/L) for 30 minutes. The neuromast organs' distribution was examined with a dissecting microscope in 5 adult and 5 larval unstained specimens. The neuromasts counts on the left and right side of the animals were averaged for each animal and then averaged among animals for subsequent comparison.

One adult and one larval specimen were used for the light and electron microscopy. The skin and superficial layer of muscle tissue was removed from a 3 mm x 6 mm region just caudal to the last branchiopore. The exposed area was filled

with 2% gluteraldehyde in Millonig's buffer (0.1 M phosphate buffer, pH = 7.3, osmolarity ~= 280 mOsm) (Millonig 1961; Millonig 1962). A rectangular 3 mm by 5 mm piece of tissue containing a layer of muscles, part of the TLLN and the underlying membrane was dissected out. The tissue was then transferred into a small dish and fixed in 2% gluteraldehyde in Millonig's buffer for 60 minutes at room temperature and then overnight at 4ºC. The tissue was washed in Millonig's buffer (3 x 10 minutes) to remove excess gluteraldehyde and post fixed in a solution of OsO_4 and Millonig's buffer for 60 minutes. After that OsO_4 was washed out with double distilled water and the tissue en bloc fixed in 2% uranyl acetate for 60 minutes. Following fixation, the tissue was dehydrated in a series of graded ethanol solutions (35%, 50%, 75%, 95%) for 10 minutes each, 100% for 10 minutes (x3), and then in propylene oxide three times for 10 minutes. Then the tissue was infiltrated with a series of mixtures of propylene oxide and Spurr's resin (1:1, 1:2, 1:3) for 60 minutes each, Spurr's resin (100%) for 60 minutes, embedded in fresh Spurr's resin for another 60 minutes, and afterwards incubated at 70ºC for 8 hours. The block was then sectioned with a microtome. . The thick sections (0.5 μm) were stained with toluidine blue solution, washed with double distilled water, and examined under the light microscope (Nicon Eclipse E400, Nicon Inc., Japan). Images of the slides were taken with a microscope digital camera (Optronics). Thin sections (50 – 70 nm) were viewed and photographed on JEOL 1010 transmission electron microscope at 80 kV acceleration potential. In order to obtain an entire view of the nerve, an overlapping series of photographs was taken at x1000 magnification. The photographic negatives were digitized using a scanner.

In order to count the number of axons in the nerve and to determine their geometric parameters, digital images (1000x magnification) were pre-processed in Corel PHOTO-PAINT X3 (Corel Corporation, Ottawa, Ontario, Canada). Pre-processing included contrast improvement using standard techniques and a montage of a series of overlapping images to obtain a combined image of an entire area of the nerve. Edges of axons were visually identified; the enclosed areas were cut out using a cut-out utility and converted into black and white image. Subsequent analysis was performed using a custom MATLAB 7.3 (MathWorks, Inc., Natick, MA, USA) program. The black and white image with the axonal areas was analyzed to determine the equivalent circle diameters (ECD) of axons:

$$ECD = 2\sqrt{(A/\pi)} ,$$

where A is the cross sectional area of an axon. The area of each axon was computed by using standard MATLAB functions.

Physiological preparation and data collection

For the main physiological study we used 9 adult and 7 larval animals. An additional one larva was used to determine the sensitivity of the lateral line to vibrations propagated in granular media. Preparations followed our previous work on larval lampreys (Gelman et al. 2007). Prior to surgery, animals were anesthetized in 0.2g/L buffered MS222. After checking for the absence of reflexes and breathing movements of the gills, animals were transferred to a chilled dissecting dish. The TLLN was exposed by removing the skin and muscles from an approximately 3x6 mm region just caudal to the seventh (last) branchiopore on the animal's left side. The membrane covering the TLLN was removed. After the dissection was completed, 0.3

– 0.4 ml of 0.002 M solution of a neuromuscular blocker, gallamine triethiodide (Sigma-Aldrich, Inc., St. Louis, MO, US), was administered intramuscularly and the animal was transferred into a 30x40 cm experimental tank. The tank was divided into two sub-chambers: the head and the site with the exposed nerve were located in one sub-chamber, filled with physiological saline (NaCl: 91 mM, KCl: 2.1 mM, $CaCl_2$-$2H_2O$: 222.6 mM, $MgCl_2$-$6H_2O$: 1.8 mM, glucose: 4.0 mM, $NaHCO_3$: 20.0 mM); the trunk was located in the second sub-chamber, filled with aquarium water. In one experiment, the second chamber was filled with wet aquarium sand to a level that covered the entire trunk of the larval animal and then water was added to fill the chamber. This recreated conditions mimicking a burrowed animal. Temperature was kept at 8-10 °C with a temperature control system (Forma scientific).

Extra-cellular multi-unit recordings were made with glass microelectrodes filled with 3 M KCl solution. The electrodes were lowered and inserted slightly into the TLLN using a manual micromanipulator (KITE-R, WPI, Inc., Sarasota, FL). Recordings began 1-2 hours after the animal was transferred to the experimental chamber. Responses were amplified by an AC differential amplifier (DAM 80, WPI, Inc., Sarasota, FL), and digitized at 10 kHz synchronously with the stimulus signal. Both the evoked responses and stimulus waveforms were stored on a computer for off-line analysis.

Stimulus generation

Water vibrations were induced with an oscillating ball (9 mm diameter), attached to a speaker diaphragm through a metal shaft. The ball's axis of motion was perpendicular to the animals' longitudinal axis. The center of the ball was placed 15

mm above the fish's trunk at the caudal end. Sinusoidal oscillations of the speaker diaphragm were generated by a function generator (LG 1301, Leader Instruments Corporation, Cypress, CA, US). The stimulus waveform was passed from the function generator through an audio amplifier (PZR 600, PYLE PRO Audio, Inc., Brooklyn, NY, US) to the speaker. The stimuli consisted of one repetition of a sinusoidal waveform, 10 seconds in duration, for each amplitude and frequency combination. Inter-stimulus duration ranged between 20 and 30 seconds. The ball motion was filmed with a high-speed digital camera at 500 frames per second (Lihgtning RDT, DRS Data & Imaging Systems, Inc., Oakland, NJ, USA) at the different frequencies used and peak-to-peak amplitude of the ball oscillations was determined by analysis of the video. The amplitudes of the ball motion were 0.32 mm – 5.22 mm. Water motion amplitude at the surface of the animal's skin can be roughly estimated by the following formula:

$$A = U(R/D)^3,$$

where A is the amplitude of water motion at the surface of the animal, U is the displacement amplitude of the ball, R is the radius of the ball, and D is the distance between the center of the ball and the surface of the skin (Kroese and Schellart 1992). Based on this approximation the peak-to-peak amplitudes of water motion near the neuromasts were 19 μm – 312 μm (see chapter II, Methods for details on the accuracy of the formula).

In the experiment where the larva was submerged in sand, an 80 ms voltage step was passed to the speaker, which created a one-time vertical motion of the ball disturbing the sand in the vicinity of the covered animal.

Data analysis

Parameters of the recorded multi-unit response were determined by setting a threshold above the noise level and detecting all spikes that were above the threshold level. Measured parameters included the firing frequency in action potentials per second (response magnitude) and the relative phase of the response. The latter was determined by computing the phase of each spike with respect to the maximum of the voltage trace passed to the speaker. To eliminate transient activity associated with the start of the stimulus we disregarded 1-10 cycles at the beginning of the stimulus (depending on the stimulus frequency). The response magnitude for each trial was adjusted by subtracting the spontaneous activity of the TLLN recorded before each stimulus trial in each animal. The effect of the spontaneous activity on the response was determined to be additive, therefore subtraction of spontaneous activity was appropriate. Gain of the response is defined as the ratio between the adjusted response (spikes/second) and the stimulus amplitude (mm).

In order to acquire insights into the dynamic properties of the peripheral lateral line system, we used the gain and phase data up to stimulus frequency of 20 Hz and tested the best linear model to fit the data parameters. The model used was

$$b_2\ddot{x}(t) + b_1\dot{x}(t) + x(t) = c_1\dot{u}(t-\tau), \qquad (1)$$

where $u(t)$ is the position of the ball and $x(t)$ is the lateral line response at time t. The model parameters are the velocity coupling strength c_1, the time delay τ, and the coefficients b_1 and b_2. The model was fit for each animal by minimizing

$$\sum_{k=1}^{m} \left| H_{\text{data}}(f_k) - H_{\text{model}}(f_k) \right|^2,$$

where the f_k are the stimulus frequencies and H_{data} and H_{model} are the frequency response functions of the data and model, respectively. A frequency response function is a complex-valued function of frequency whose absolute value is gain and argument is phase. Other linear models with position and acceleration coupling were used, but the best fit in a sense of least squares was found for the model with the velocity coupled forcing term.

Results

Distribution of neuromasts.

The distribution of superficial neuromasts of adult and larval lampreys is depicted in figure 3-2. It was observed that both adult and larva possess 8 distinguishable pit lines on the head and trunk regions. The pit lines are generally symmetrical with respect to the midline of the animal though the numbers of neuromasts may slightly differ between the left and right sides. The oral line (Or) runs caudally from the most rostral point of the dorsal aspect of the oral hood to the level of the nasohypophysial opening in ammocoetes. The Or line of adults does not run as far as in ammocoetes, instead it terminates some distance rostral to the hypophysial opening. The infraorbital (I) line begins ventral to the developing eye in ammocoets and slightly more dorsal in the adults and runs to about the level of termination of the Or line. Due to the more lateral positions of the eyes in adults, the infraorbital line is located more laterally in adults. The otic/post-otic (Otic) line begins caudal and slightly dorsal to the eye and runs obliquely to about the level of the first branchiopore in both adult and larvae. The pineal (P) line is located at the

level of the pineal organ in adults, but in ammocoetes it occupies a more caudal position. In adults the ventral (V) line begins at the level of the termination of the

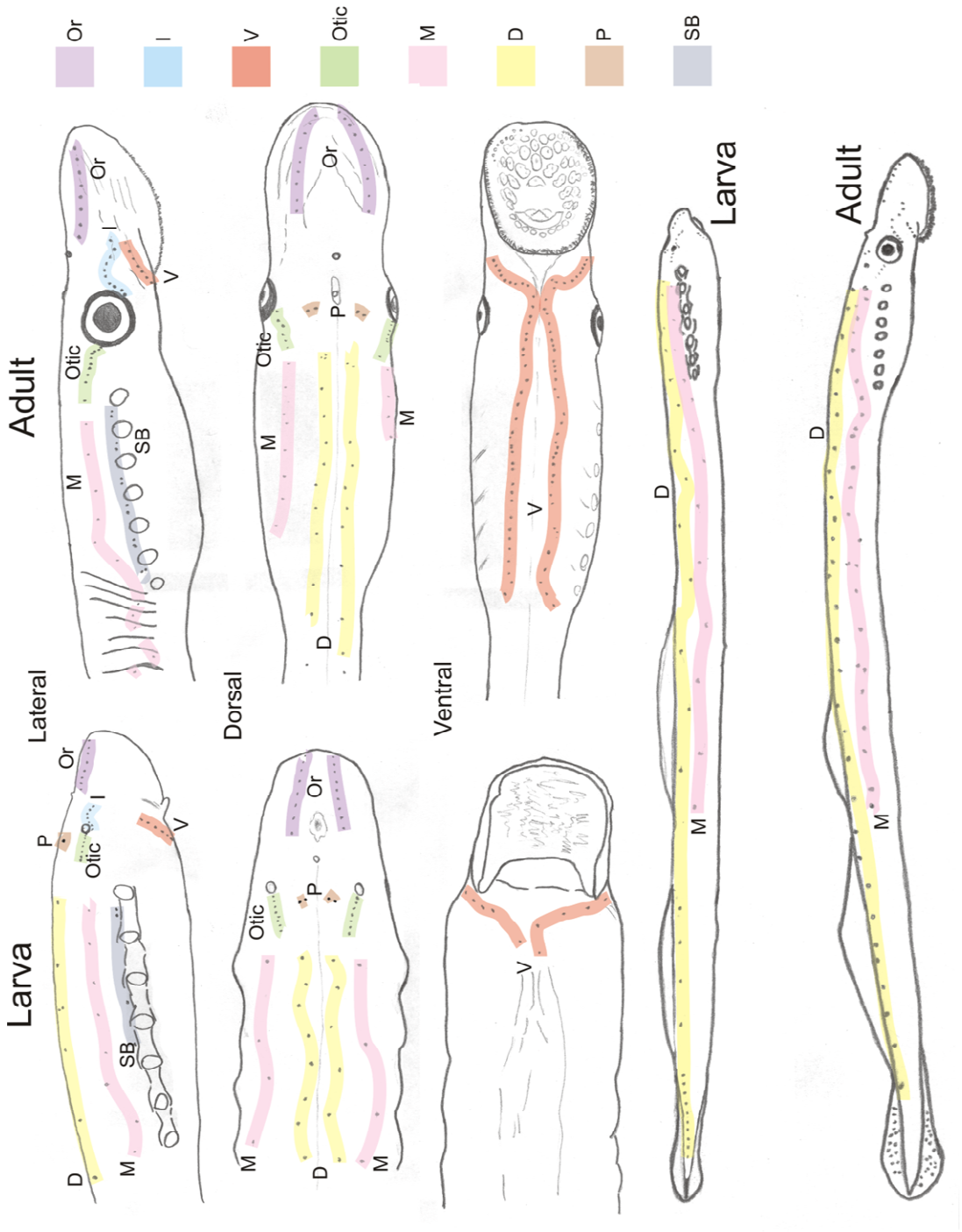

Figure 3-2.
Neuromast distribution on the head and trunk of larval and adult lampreys. Eight pit lines are present in both Larval and adult. The different lines are color coded (right) and marked on the different views of the head and trunk region as follows. Abbreviations: Or – oral, I – infraorbital, V – ventral, Otic, M – main trunk, D – dorsal, P – pineal, SB – suprabrancial.

infraorbital line and runs ventro-laterally to the ventral midline, where it makes a sharp turn and then closely follows the midline until the seventh branchipore. In ammocoetes this line appears to be less developed; it terminates right after the midline turn. Adults have a well developed suprabranchial (SB) line, with paired (sometimes tripled) neuromasts located slightly dorsal and between the branchiopores. In ammocoetes this line is not as well developed, numbering fewer neuromasts. In both adult and larval lampreys, the dorsal (D) and the main (M) trunk lines begin at the level of the first branchiopore and extend rostrocaudally in the dorsal and dorso-lateral aspect of the trunk, respectively. Initially, after the last branchiopore, the neuromasts of dorsal and main trunk lines appear in every myotome, in both adults and larva. The myotomal distance between the neuromasts increases in the rostrocaudal direction in pre- and post-metamorphic animals. The dorsal line appears to continue with some gaps all the way to the tail fin. The main trunk line, on the other hand, terminates at the caudal end of the first dorsal fin in both adults and ammocoetes.

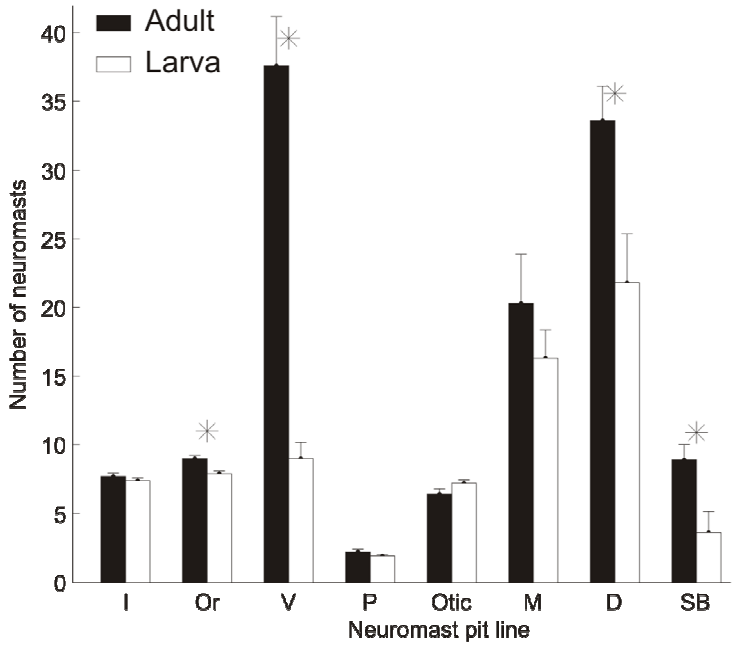

Figure 3-3.
The number of neuromasts in the different pit lines of larval and adult lampreys. Data presented as the mean and standard deviation calculated for the left and right sides of 5 larval and 5 adult animals. Black bars – adults, white bars – ammocoetes. Asterisks indicate significant differences (two-tailed *t*-test $p = 0.0054$ (Or), 0.0001 (V), 0.0268 (D), 0.0241 (SB).

Larval lampreys possess fewer neuromasts in their oral, ventral, suprabranchial, and dorsal lines compared to the corresponding lines in adults (Fig. 3-3). The difference was statistically significant (two-tailed *t*-test $p = 0.0054$ (Or), 0.0001 (V), 0.0268 (D), 0.0241 (SB)). In addition the distribution pattern of the more caudal part of the dorsal line is altered during metamorphosis. The most caudal stretch of the dorsal line in ammocoetes is constituted of a straight row of neuromasts, located approximately equidistant from each other. This arrangement is not seen in transformed adults (whole body panel, Fig. 3-2). In adults the dorsal line terminates slightly more caudal to the second dorsal fin and does not reach the very end of the trunk.

Size and composition of the TLLN

In lampreys, the trunk lateral line nerves run along the lateral aspect of the cartilaginous case enclosing the spinal cord on both sides (fig. 3-4A1 and 3-4B1). For adequate comparisons, cross sections of adult and larval TLLNs were taken 1 mm caudal to the last branchiopore. The longest and shortest dimensions of the adult TLLN at this level were 160.0 μm and 95.0 μm, and those of larva were 107.0 μm and 80.0 μm, respectively. The number of axons was 325 and 397 for larva and adult respectively.

Figures 4 show detailed views of cross sections of an adult and larval TLLN, from which the equivalent circle diameters (ECD) of axons were computed (fig. 3-5; measurements were made from the corresponding TEM images). The ECD of axons ranged from 0.7 μm to 10.3 μm and 0.7 μm to 10.5 μm in adult and larva, respectively. Yet, the distribution of axonal diameters within these ranges was clearly different for adult and larva (fig. 3-5). The mean ECDs were 4.2±2.5 μm compared to 3.6±2.1 μm, in adult and larva, respectively. In both cases the histograms of the ECD showed an approximate tri-modal distribution, putatively indicating three populations of axons.

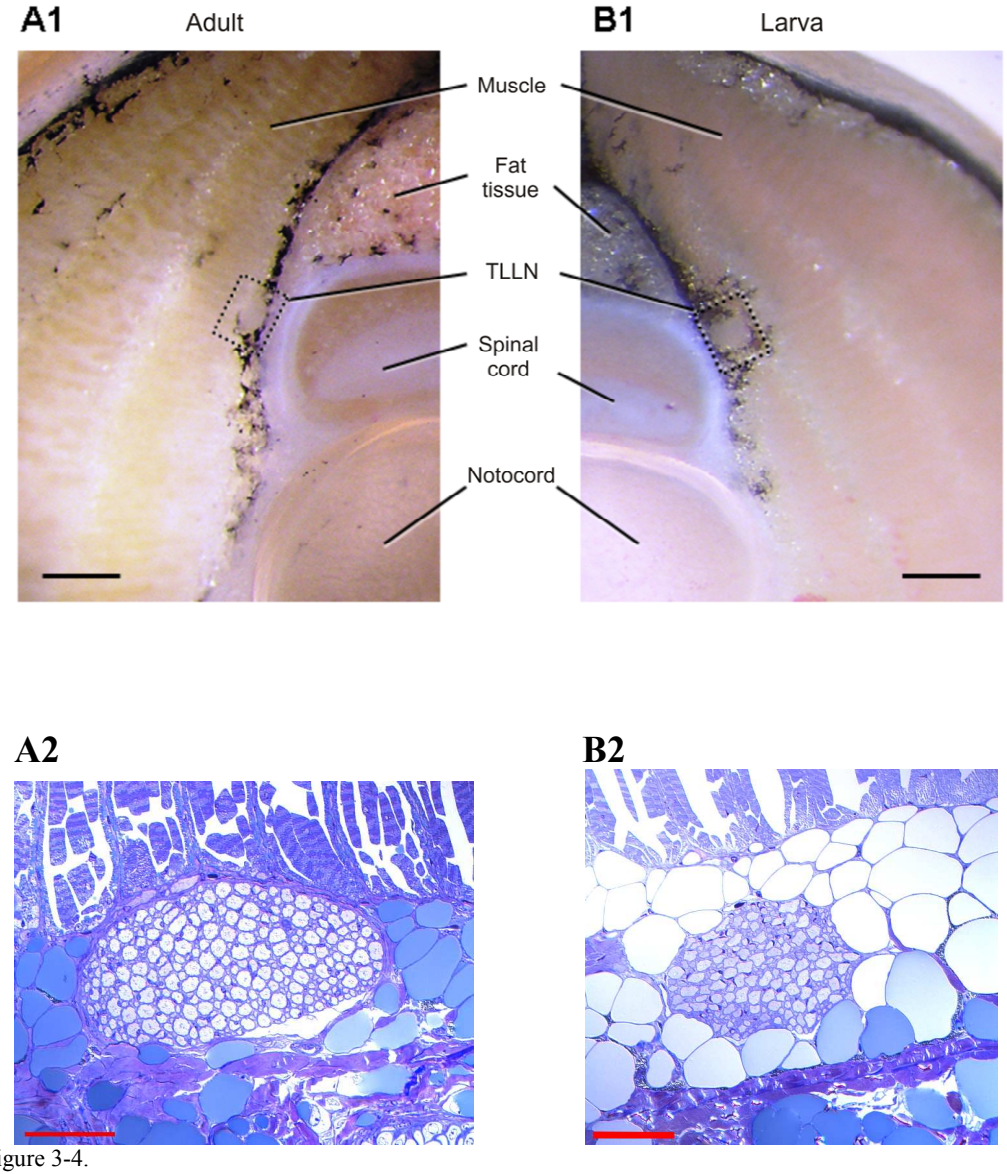

Figure 3-4.
Gross and fine anatomy of the lateral line nerve of adult (A) and larval (B) lampreys. In both, panel 1 shows a transverse section (unfixed tissue, only the dorsal left or right quadrants are shown) showing the relative position of the trunk lateral line nerve (TLLN) and major related tissue. The outlines of the nerve and the spinal chord are traced by dashed lines for clarity. The lower panels (2) are light microscope images from a transverse section of the TLLN. Note that the nerve of the ammocoete is surrounded by fat tissue while there is only little fat at the central aspect (to the right and left of the nerve) in the adult. The overall diameter of the adult nerve is larger and there seem to be larger diameter axons in it. Scale bars are 500 µm (A1, B1), 50 µm (A2, B2,).

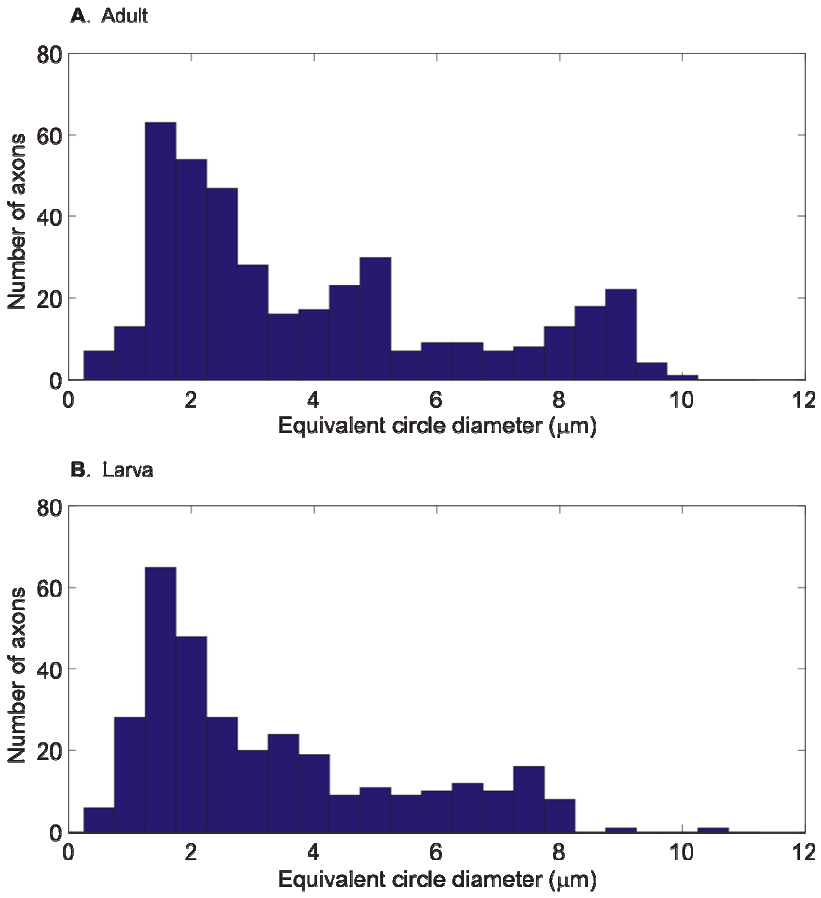

Figure 3-5.
The diameters of the trunk lateral line nerve axons in adult (A) and larval (B) lampreys. Data were measured from transverse electron microscope images (x 1000) of the nerve, and demonstrated as distribution of the diameters of equivalent circles (based on axonal cross section area), in 0.5 μm bins. Data for the adult axons show a clear inclination toward the larger diameters.

Peripheral physiology.

Fibers of the TLLN of both larval and adult lampreys were spontaneously active. The frequency of the multiunit spontaneous activities was not significantly different: 18.99±9.75 spikes/second and 24.45±7.48 spikes/second for adults and ammocoetes respectively (mean± standard deviation; *t*-test p=0.2404). As previously reported (Gelman et al. 2007), the TLLN of larval lampreys is constituted of mechanosensitive fibers that innervate hair cells of opposite polarity. Hence these

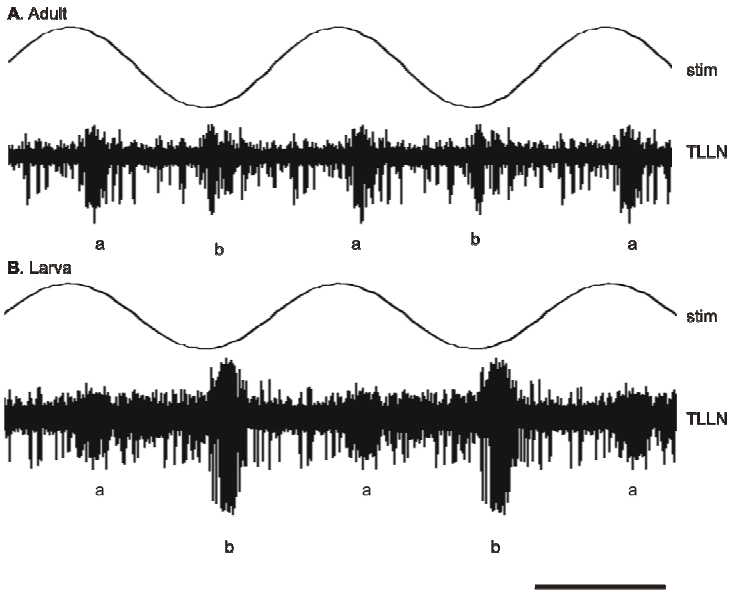

Figure 3-6.
The response of the trunk lateral line nerve to sinusoidal stimulus generated by 5 Hz sinusoidal ball motion (top traces). Extracellular nerve recordings (10 overlaid traces) from both adult (A) and larva (B) show clear two populations of nerve fibers (a and b) responding by firing action potentials during opposite directions of the water flow. Scale bar is 0.1 sec.

fibers respond to the opposite directions of water flow. The same directional sensitivity was observed in adult lampreys (Fig 3-6). Figure 3-6 shows 10 overlaid TLLN responses to a 5 Hz stimulus, 0.2 mm peak-to-peak amplitude of larvae and adults. Two populations of spikes (a and b) are seen, which respond at different times in the same stimulus cycle, corresponding to opposite directions of the stimulus. We have previously shown and re-confirm here that the larval mechanosensitive fibers respond at a phase of approximately 220° with respect to the maximum displacement of the ball at the stimulus frequency of 5 Hz. This phase relationship is approximately invariant and independent of the stimulus amplitude, which is an indication of the linearity of the system. The independence of phase on amplitude was also observed in adult lampreys (Fig. 3-7, next page). Here again a phase lag of approximately 220º was observed at the stimulus frequency of 5 Hz (Fig. 3-7). In general, the phase lag changes with frequency (Fig. 3-8). This may be due to several factors, including constant time delays and dynamic time constants. The constant time delay is presumably a direct result of synaptic transmission and the

fiber's finite transduction velocity (Kroese and Schellart 1992), which do not depend on frequency of the stimulus and do not effect the gain of the response. As can be seen in figure 3-8, the increase in the phase lag with frequency is faster for ammocoetes then for adults. This difference may indicate that the lateral line system of larval lampreys is slower then that of adults.

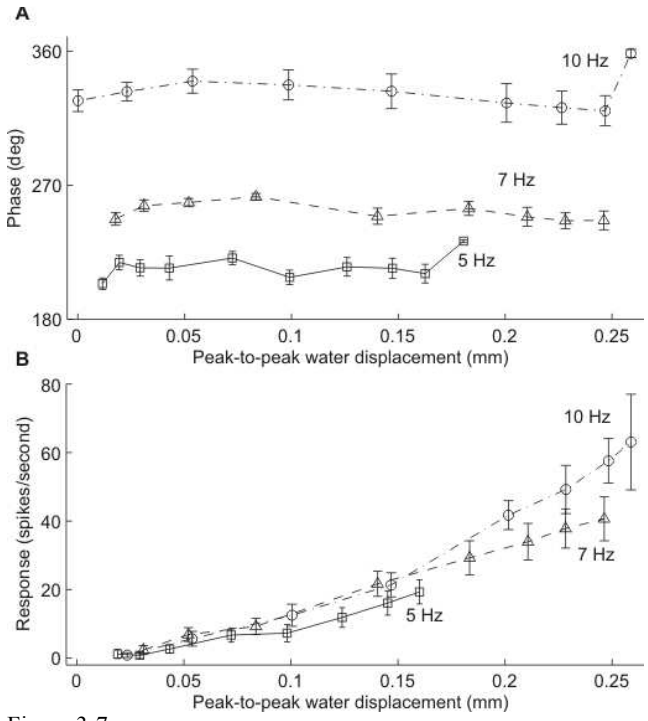

Figure 3-7.
The linear nature of the response of the lateral line fibers of adults. **A**. The phase of the response is approximately constant with changing stimulus amplitude. Data show the mean of 7 preparations with standard error for three different stimulus frequencies. **B**. Magnitude of the response is approximately linearly dependent on the stimulus amplitude (mean and standard error, n=9).

The magnitude of the response of adult and larval lateral line systems increased mostly monotonically with amplitude without apparent saturation (Fig. 3-9). As can be seen in figure 3-10A, for a given stimulus amplitude the larval response showed larger amplification with frequency. Expressed in terms of frequency discrimination, larval lateral line appears to better differentiate between frequencies.

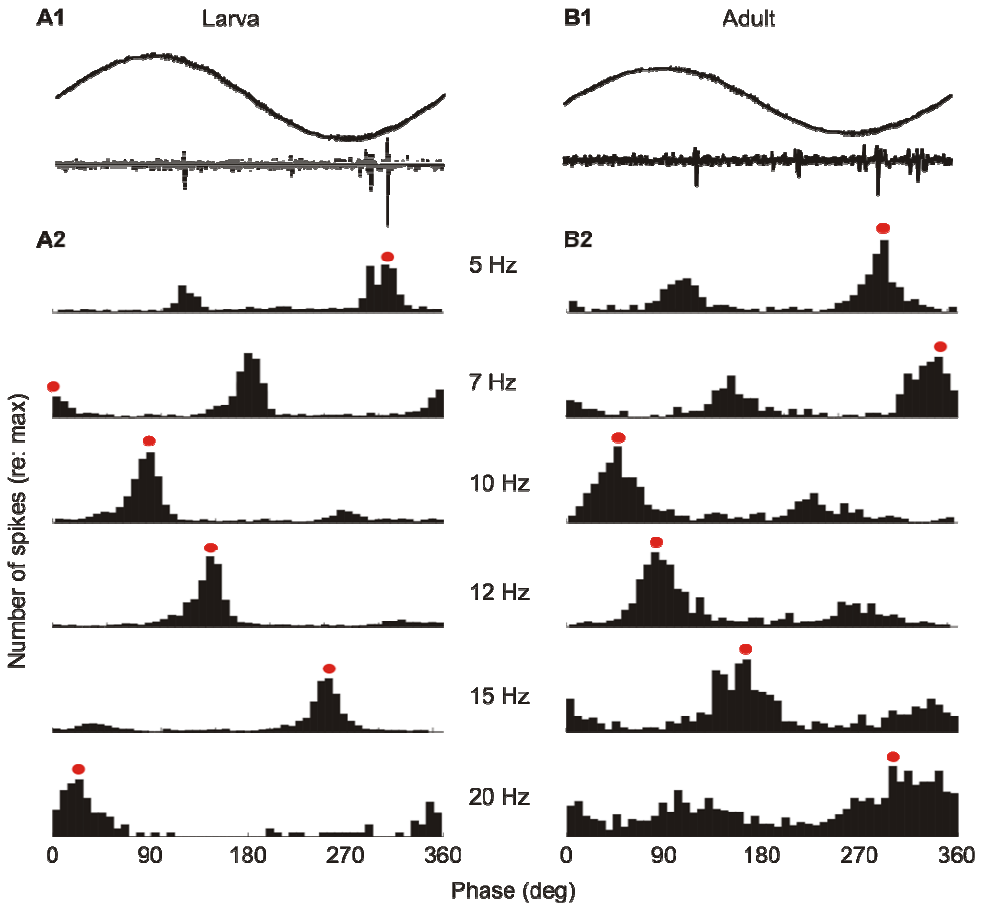

Figure 3-8.

The phase lags between the stimulus and the response of the lateral line nerve fibers is dependent on the stimulus frequency. The top panels (**A1** and **B1**) show raw data of a 5 Hz sinusoidal stimulus and the extracellular recorded response. Note different spike types at different phases of the stimulus. The different phase-response histograms were constructed from examples of data recorded from one larva and one adult preparation. Note how the phase of the peak response (red dot) changes with the stimulus frequency (**A2** and **B2**). The shift in the phase of the adult response for every frequency step is smaller and accordingly the overall change throughout the entire frequency range is smaller in adults.

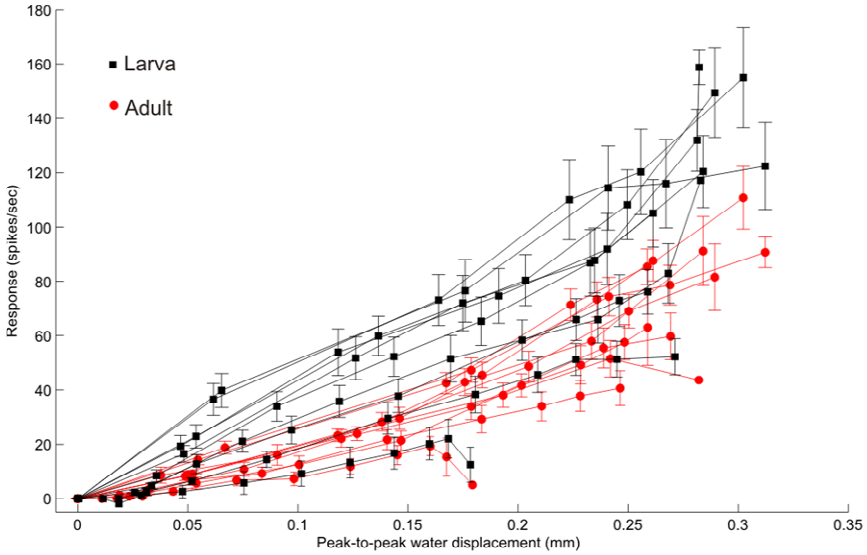

Figure 3-9.

Amplitude response curves of fibers in the larval (black) and adult (red) lampreys' trunk lateral line nerve. Evoked activity elicited by the stimuli of varying frequency (5, 7, 10, 12, 15, 20, 25, 30, 40, 50 Hz). Data points show the mean and standard error for 9 animals. At all frequencies, the magnitude of the response increases monotonically with increasing stimulus amplitude. The amplitude response in larvae is more frequency dependent that in adults as indicated by the larger range of responses per given amplitude.

Figure 3-10 (page 66) shows the dependency of gain and phase on stimulus frequency. The gain of the response for both adult and larvae appear to saturate above the 20 Hz stimulus frequency. Significant differences in gain between larvae and adults were found at 20, 25, 30, 40, 50 Hz stimulus frequency (t-test p = 0.0007, 0.0126, 0.0006, 0.0036, 0.0121 respectively). The phase linearly depended on frequency for adult and larval preparations. Linear regression lines fitted into adult and larval phase data were significantly different with slopes of -22.2°/Hz and -29.4°/Hz (ANOCOVA p = 0.000002) and y-intercepts of 265.4° and 294.1° (ANOCOVA p = 0.00000009) for adult and larval preparations respectively.

For stimulus frequencies up to 20 Hz, gain and phase were reasonably well fit by a linear second-order model with a time delay and only velocity coupling (Eq. 1 in Materials and methods). The time delay was significantly shorter for the adults (45.4±2.1 ms) than for the larvae (63.3±1.8 ms) (unpaired *t*-test, p=0.0001). The other model parameters did not show significant differences between the groups.

As mentioned, the larval lamprey spends most of its time submerged in sand. We thus also determined that the larval lateral line system is sensitive to mechanical disturbances propagated in granular media (sand). Figure 3-11 shows an example of a response elicited by stimuli applied to the surface of the sand covering the larval preparation. A clear response can be seen elicited by stimulation.

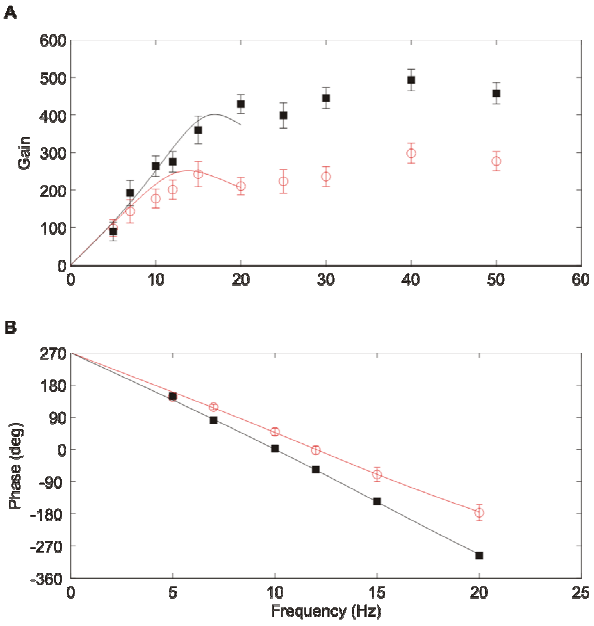

Figure 3-10.
A. The gain of the response with frequency for the trunk lateral line nerve fibers of larvae (black) and adults (red). The gain at each frequency and amplitude was defined as the ratio of the response (spikes/second) and the stimulus amplitude (mm). B. The phase of the response with frequency. By convention, zero phase is when spikes coincided with the stimulus maximum (lowest position of the ball); positive and negative phase is phase lead and lag, respectively. Solid lines represent gain and phase of the model frequency response fitted into the adult and larval data (up to 20 Hz). As can be seen the model generated a reasonable fit to the experimental data.

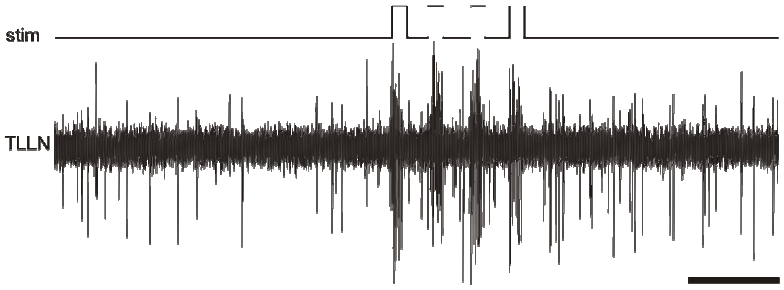

Figure 3-11.
Responses of fibers in the trunk lateral line nerve of a larval lamprey fully submerged in sand to vibrations of the substrate. Stimuli (top trace) were generated by single voltage steps passed to a speaker moving a metal rod and ball placed in the sand. Scale bar is 0.5 sec.

Discussion

Neuromast distribution.

In a recent report we have shown that larval lampreys posses a functional lateral line system (Gelman et al., 2007). In accordance with this report we show here that pre-metamorphic lampreys appear to possess all the lateralis pit lines present in adults. The morphological metamorphic transformations of the system of superficial neuromasts include however an increase in the number of neuromasts, they become distributed differently, and the pit lines change their positions relative to the external features.

While the overall number of neuromasts increases during the metamorphosis, statistically significant increase occurs only in the oral, ventral, suprabranchial, and dorsal lines. Two types of post-embryonic mechanisms for the formation of new neuromasts are known from studies on an amphibian, *Xenopus laevis*, and a teleost fish, *Danio rerio*: splitting or budding of existing neuromasts (Pichon and Ghysen 2004) and addition of new neuromasts by the secondary primordia, i.e. a repetition of the process responsible for the first deposition of neuromasts (Ledent 2002; Sapede et al. 2002). At this stage, neither mechanisms could be eliminated in lamprey, as the metamorphosis could be thought of as a re-initialization of dormant embryonic developmental processes (Balinsky 1965).

Distributive alterations were found to occur predominantly in the dorsal pit line. The observed shifts in position of neuromast lines may be due to general morphological transformations of the head. Metamorphic changes in the arrangement of neuromasts at the tail may be a developmental adaptation to the burrowing life

style of ammocoetes. Occasionally, during burrowing, larval lampreys stick their tails out of the silt or mud of riverbanks. This behavior is also observed in the holding aquaria in the laboratory. Increased number of neuromasts in the tail region may allow the animal to image the hydrodynamic surroundings while in the burrowing site. Higher density of terminal neuromasts in larval lampreys is reminiscent of the early post-embryonic posterior lateral line pattern formation in ostariophysians (*Danio rerio* and *Astyanax fasciatus*), atherinomorphs (*Oryzias latipes*), and percomorphs (*Psetta maxima*) (Pichon and Ghysen 2004). In adults, the neuromasts of the main trunk and the dorsal lines are distributed more uniformly and the increased density at the tail region is not observed. This relatively more regular distribution may be more suitable for sampling the water flow along the entire trunk of the animal and may accompany the changes in behavior toward a free-swimming life style.

At this point however it is still unclear to what extent do the changes in number, distribution, and position of the lateral line neuromasts during the metamorphosis of lampreys bear functional significance and are related to changes in behavior.

Our data also indicates that there are interspecific differences in the neuromast distribution pattern. Katori et al. (1994) have described the neuromast distribution on the head of adult *Lampetra japonica*, it appears that this species of lampreys has an extra group of neuromasts located ventral and slightly rostral to the first branchiopore (Katori et al. 1994). In addition, there is another line of neuromasts connecting two

post-otic lines, running slightly caudal to the pineal line across the dorsal aspect of the head. We did not observe these lines in adult or larval *Petromyzon marinus*.

Composition of the TLLN

Parallel to the changes in the number of neuromasts and probably the number of hair cells, the number and size of axons running in the TLLN was observed to increase during metamorphosis. The increase in the numbers of axons is an indication that at least some new neuromasts, whether formed by budding or by the secondary primordium, must be innervated by new axons. This observation raises an interesting and general developmental question of how these new axons find their targets. One might also ask another related question concerning the coupling of the processes of formation of new hair cells/neuromasts and their innervating axons. Do they possess a common molecular/genetic trigger or does one initiate the other? In zebrafish and some other fish it was shown that if new neuromasts are generated by budding of an existing one, the axon that innervated the hair cells of the old neuromast organ sends a collateral to the newly formed hair cells (Ledent, 2002). On the other hand if new neuromasts are formed by the secondary primordium, the new axons closely follow the migrating promordial cells (Ledent 2002). The developmental mechanisms responsible for the described metamorphic transformations in lampreys are unknown yet.

Peripheral physiology

Our physiological data again indicate that the peripheral lateral line systems of adult and larval lampreys differ only quantitatively. Qualitatively the responses to the vibrating ball were similar. The frequency response of adult as well as larval lamprey

(at least up to stimulus frequency of 20 Hz) can be described by a second order dynamics with velocity coupling, supporting the general notion that superficial neuromasts are velocity detectors (Kroese and Schellart 1987; Kalmijn 1988; Kroese and Schellart 1992). Nevertheless, the time delay constant was larger in ammocoetes then in adults, indicating that the response of the former is slower. The time delay is mostly due to the delay associated with the finite conduction velocity. Delays associated with the mechanotransdunction in the hair cells and the synaptic delay between the hair cells and the afferent neurons also contribute to some extent (fig 3-12).

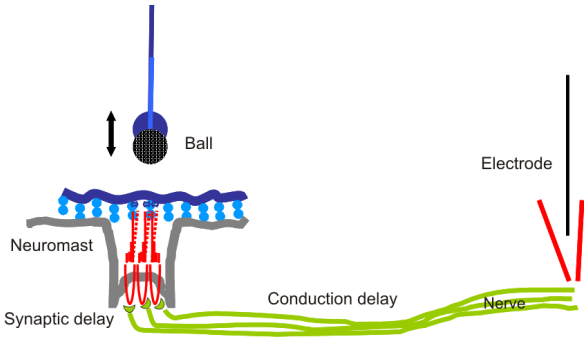

Figure 3-12.
The time delay between the stimulus and recorded response. Hair cell mechanotransduction, synaptic delays and the delays associated with the finite conduction velocity of the nerve fibers contribute to the observed delay.

Comparing the magnitude and the gain of the response with changing frequencies in the larval and adult lampreys may indicate that the larval lateral line system is more frequency discriminating. On the one hand, such changes could be merely a result of general maturation processes that only parallel the metamorphosis process. On the other hand, these changes may indeed be of a preparatory nature; transforming the lateral line system into a sensory system more suitable for the drastically different life style of adult lampreys.

As mentioned, the behavioral significance of the lateral line system of lampreys is not yet fully established. Hence we can only speculate on the biological

function of this system in larval vs. adult lampreys and on the behavioral relevance of the reported metamorphosis-related physiological differences. The evidence for the response of the larval lateral line system to vibrations propagated in the sand in addition to the already mentioned wider frequency discrimination range may indicate that this system is used to detect various disturbances in the substrate surrounding the burrowing site of ammocoetes. Such disturbances, among others, may include the digging actions of predators such as crayfish and some amphibians which were reported to prey on the lamprey larva (Nickerson et al. 1983; Cochran and Gripentrog 1992). Anecdotal evidence also exists indicating that juvenile salmons dig the ammocoetes out of the burrowing sites. It is also reported that mechanical disturbances in the vicinity of the ammocoete burrowing site cause them to withdraw deeper in the burrow (Hardisty 1979). The adult lampreys, on the other hand, have very few predators, including seals and sea lions, animals of a much larger size, possibly producing a narrower range of vibration frequencies than small crustaceans and amphibians.

It is very possible that the major stimuli that the adult lateral line system is handling are self-induced water motions during swimming. Thus the major changes accruing during the lamprey metamorphosis are reflecting the more active and free swimming life style of the adult.

CHAPTER FOUR: STUDIES OF THE BEHAVIORAL FUNCTION OF THE LATERAL LINE SYSTEM OF LAMPREYS

Abstract

The mechanosensory lateral line system of fishes and amphibians participates in a variety of behaviors. These behaviors could be caused by hydrodynamic disturbances emanating from biological or non-biological sources. However, the behavioral function of the lamprey mechanoreceptive lateral line is unknown. The study of lateral line function in these animals is complicated by the difficulty of eliciting stereotyped behaviors in laboratory conditions.

Many morphological features of lampreys are considered to be plesiomorphic (ancestral) to other vertebrates. Based on this, I propose that the lamprey lateral line system is functionally unspecialized. This would make the lateral line participate in many different behaviors, depending on the environmental conditions and the state of the animal. For instance, detection of self-induced water motions during swimming may be one of the functions of the lateral line of lampreys. This hypothesis is supported by the absence of the efferent innervation to the hair cells of lamprey neuromasts. Here this hypothesis was tested by analysis of swimming kinematics in normal animals and in animals with pharmacologically blocked lateral line system. Animals were filmed using high speed camera during swimming in still water.

The kinematics of swimming were not found to be different between control and treated animals. This study concludes that the lateral line system is not necessary

for swimming, at least in stationary water. However, the results of these experiments do not rule out the lateral line's role in the control of swimming in uniform flow, such as during upstream migration or highly complex turbulent flows.

Introduction

The lateral line systems of cyclostomes, fishes, and amphibians detect minute water motions generated in the vicinity of an animal (Dijkgraaf 1963; Dijkgraaf 1967; Gorner 1973; Rovainen 1982). The sources of hydrodynamic disturbances are very diverse and can be of biological or non-biological nature. In addition to what could be called passive detection of water flow, animals generate water disturbances by their own motion and measure the changes in the flow resulting from the interaction of the flow with other animals or objects, thereby actively imaging the hydrodynamic environment in the vicinity (Weissert and Campenhausen 1981; Campenhausen et al. 1981; Hassan 1989). The hydrodynamic image of the surroundings is crucial for the animals' survival in dark or murky waters. However, the relevance of information provided by the lateral line system may greatly vary for different animals. In fact, individuals of different species of fishes or amphibians respond in quite dissimilar ways to identical hydrodynamic stimuli. Numerous studies on the behavioral or biological significance of what has been called ‘sense of touch at a distance’ (or *Ferntastsinn*, introduced by Sven Dijkgraaf in his doctoral dissertation in 1933 (Dijkgraaf 1989)), have shown that the lateral line sense is used by some aquatic animals for detection (Campenhausen et al. 1981), others for orientation (Montgomery et al. 1997; Bleckmann 2004), and still others for communication

(Satou et al. 1994), and possibly for stabilization and/or control of locomotion (Hoagland 1933; Liao 2006).

Despite the functional differences listed above, the peripheral physiological characteristics of the lateral line system of various species of fishes and amphibians show extensive similarities. Recordings of the afferent activity from the peripheral nerves innervating neuromasts' hair cells indicate that the receptors possess properties of a mechanical low-pass filter, detecting the water flow velocity (Kroese et al. 1978; Kroese and Schellart 1992; Bleckmann 1994). Thus, even if behaviors are different, the pattern of the peripheral activity is similar among various diverse species, suggesting that the diversity of the lateral-line-associated behaviors arises from a higher level of the system organization. Peripheral physiology of lamprey lateral line system is similar to fish's and amphibians' even in the presence of difference in hair cell morphology and the absence of the efferent component. In the next few paragraphs the lateral lines of various fish and amphibians are compared with the lamprey lateral line.

The superficial neuromasts of fish and amphibians exhibit properties of a linear system for the stimuli with sufficiently small amplitudes (Kroese et al. 1978; Munz 1985; Kroese and Schellart 1987; van Netten and Kroese 1987). Table 1 shows ranges of the stimulus amplitudes (ball displacement amplitudes) and the distance between the ball and the skin at which the superficial neuromasts in some fish and amphibians exhibit linear properties (the references are given in the last column). The amplitude of the ball displacement and the distance between the ball and the skin are important experimental parameters that among other factors determine the amplitudes

of water movement near the neuromast, which is harder to measure experimentally (Kroese et al. 1978).

Animal	Amplitude (ball displacement)	Ball diameter	Distance from the surface of the ball to skin	Reference
Xenopus	2-36 µm	3.1 mm	<3 mm	(Kroese et al. 1978)
Trout	max = 47 µm	3.0 mm	>1 mm and < 4 mm	(Kroese and Schellart 1992)
Stingray (*Dasyatis sabina*)	0.3 µm – 1.3 mm	6-9 mm	2-3 mm	(Maruska and Tricas 2004)
Lamprey	0.32-5.22 mm	9 mm	10 mm	This thesis, chapters II and III

Table 1. Stimulus parameters used by various research groups for the lateral line experiments. The superficial neuromasts excited by the stimuli in these ranges exhibit linear response properties.

The factors in determining the linear nature of the neuromasts include the linear relation between the response magnitude and the stimulus amplitude (or constant response gain with the stimulus amplitude) and the invariance of the response phase with the stimulus amplitude. In case of lampreys, both larval and adult, approximate linearity of the lateral line organs is observed with much larger stimulus amplitudes. This may be a consequence of the unusual structure of lamprey hair cells and different dynamics of the putative cupula covering the neuromasts. Figures 2-3 and 3-7 (chapter II and III) show that for adult and larval responses, both linearity conditions, linear relation between the response magnitude and the stimulus amplitude and the invariance of phase with the stimulus amplitude are approximately satisfied. Thus lamprey neuromasts can be considered linear in a wider stimulus amplitude range.

The frequency response of adult and larval superficial neuromasts confirms that these receptors at least up to the stimulus frequency of 20 Hz can be considered as detectors of water flow velocity (fig. 3-10, chapter III). The low-frequency gain of the best fit linear model (Eq. 1, chapter III) was linearly dependent on the stimulus frequency and the low-frequency phase lag (the y-intercept of the model phase curve, fig. 3-10, chapter III) was 270° (which is equivalent to 90°) with respect to the stimulus maximum. The actual phase at 5 Hz stimulus frequency was ~220°, which adjusted to the convention (360° - 220° = 140°) gives 140° is close to the reported values in fish and amphibians (see table 2). But since at low frequencies the phase did not appear to level off (fig. 3-10, chapter III) it is reasonable to assume that the y-intercept of 270° given by the model (Eq. 1, chapter III) is a true phase at low stimulus frequencies. Such gain and phase of the response is expected from a velocity detector. If the gain of the response is expressed in decibels, it is equivalent to say that velocity detectors have gains with frequency of 20 dB/decade at low-frequencies (see chapter II for more details). The frequency at which the response gain of lampreys (adults and larvae) superficial neuromasts appears to level off or saturate was between 15 and 20 Hz. These values are lower in comparison with the available data for trout and Xenopus superficial neuromasts, but larger than for the stingray (table 2).

	Trout	Xenopus	Stingray (*Dasyatis sabina*)	Lamprey (*Petromyzon marinus*)
Frequency of maximum sensitivity, Hz	36	30	~9	15-20
Low-frequency slope, dB/decade	20	25	~20	Linear (equivalent to 20 dB/decade)
Low-frequency phase	121°	125°	~90°	~140° (model result 270°)
References	(Kroese and Schellart 1992)	(Kroese et al. 1978)	(Maruska and Tricas 2004)	This thesis

Table 2. Some properties of the response of the superficial neuromasts in fish and amphibians. Low-frequency slopes and gains indicate the velocity detector properties.

Therefore, lamprey lateral line system at the peripheral level, particularly the frequency response of the superficial neuromasts, appears to be similar to the lateral line superficial neuromasts in fish and amphibians. At least, it indicates that the lamprey lateral line is sufficiently developed at the peripheral level to detect similar biologically relevant hydrodynamic stimuli as fish and amphibians. However, it does not necessarily mean that the behavioral function of the lamprey lateral line is equivalent to fish and amphibians. The more behaviorally relevant differences, as mentioned above, may lie in the processing and motor circuitry of the lateral line in the central nervous system. Differences in CNS may result in different reactions of lampreys to identical hydrodynamic stimuli or utilization of the lateral line for the purposes of the locomotor feedback. This latter function appears not to be the main function of the lateral line systems of fish and amphibians. In these animals the lateral line system seems more essential for the functions of localization of the sources of the hydrodynamic disturbances. However, even in fish the lateral line may still have

some role in locomotion in turbulent flows as was recently shown for a trout swimming (Liao 2006).

In lampreys, the behavioral function of the lateral line system is unknown. This vertebrate group is thought to have undergone very conservative evolutionary development retaining many plesiomorphic characters (Hardisty 1979). As indicated by the fossil record (fig. 4-1), agnathan forms, very similar to the modern lampreys, appeared as early as 360 million years ago. The earliest known fossil of a jawless animal very similar to modern lampreys, *Priscomyzon riniensis* (Gess et al. 2006) dates back to the Frasnian-Famennian boundary of the Upper Devonian (~360 mya), a period when most of the ostracoderms (armored agnathan fishes) suddenly disappeared. Other Palaeozoic lampreys include *Mayomyzon pieckonsis* (Bardack and Zangerl 1968) and *Hardistiella montanensis* (Janvier and Lund 1983) both found in Carboniferous/Pensilvannian marine deposits (~310 mya). The most recent fresh water lamprey fossil, *Mesomyzon mengae* (Chang et al. 2006) dates from the Lower Cretaceous epoch (~ 125 million years ago) and is morphologically very similar to the modern lampreys. The fossil data indicate that the general morphological traits of lampreys have been remarkably stable for

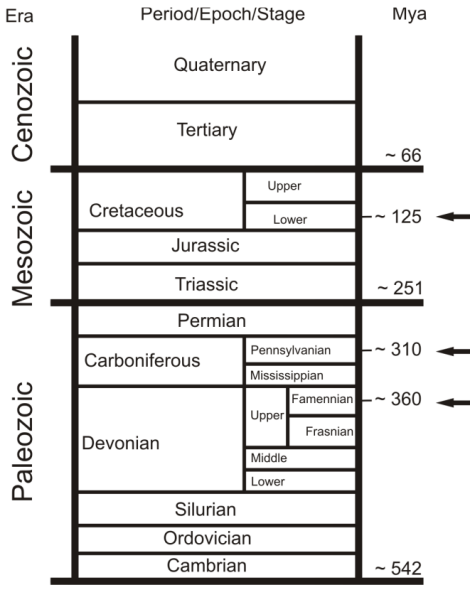

Figure 4-1.
Occurrence of lampreys in the fossil record. The earliest lamprey-like fossil dates about 360 million years back in Fammenian epoch of Upper Devonian. The diagram represents a geologic time scale. Arrows indicate the dates of lamprey fossils (see text for details). Mya – millions of years ago.

approximately 360 million years of evolution (Janvier 2006). It is therefore likely that the lamprey lateral line system represents a plesiomorphic and relatively unspecialized condition. The biological function of this system may encompass a broad range of behaviors, lacking a particular specialization.

Morphological evidence, such as the absence of efferent innervation of the hair cells (Yamada 1973), suggests that the lateral line system of lampreys may function as a locomotor feedback system providing proprioceptive information during swimming. In fishes, feedforward efferent innervation inhibits the hair cells during locomotion, serving to prevent their de-sensitization (Roberts 1972), and thus assuring that the hair cells are dynamically responsive at the times they are required to discern hydrodynamic stimuli (Russell and Roberts 1972; Roberts and Russell 1972). The absence of the efferent component in the lateral line of lampreys suggests that the system either functions only during the times of inactivity or that it is an evolutionary adaptation to detect self-induced water motions for the purposes of locomotor control.

Even though swimming motions of fishes exhibit considerable diversity, it is possible and instructive to group fish swimming into more or less defined categories, based on the extent of body undulations. Breder described three main modes of fish locomotion (Breder 1926). Anguilliform, or eel-like swimming, is defined by relatively large undulations along entire body. In contrast, in the thuniform mode, undulations are performed only by the tail of the fish. An intermediate category, termed carangiform, or jack-like swimming, is characterized by undulations that

become pronounced at the caudal half or third of the animal (Breder 1926; Videler 1993).

The fundamental breakthrough in understanding aquatic locomotion came from the now classic studies of James Gray (Lauder and Tytell 2004). In the first paper of a series of three papers published in 1933, Gray quantitatively analyzed the kinematics of swimming and showed how the motion of the swimming animal can generate forces propelling it forward, laying the framework for further research on fish locomotion (Gray 1933a). In the second paper he related the thrust produced by the motion of the fish to the waves of the muscular activity during swimming (Gray 1933b). In the third paper the ideas developed from the analysis of the anguilliform swimming were extended to the carangiform mode (Gray 1933c). These studies form a basis of our understanding of fish locomotion. They also, as Gray himself did, raise a question whether the lateral line system is involved in locomotion.

If the lateral line is required for swimming behavior, blocking it will produce deficits in the swimming in locomotion. The principle research paradigm in the determination of the biological functions of the lateral line system involves an inhibition of the lateral line sense followed by observations or experimental tests designed to measure animal's performance in a particular stereotypical behavior. Cobalt chloride has been extensively used to inhibit the lateral line in behavioral experiments with teleost fishes (Satou et al. 1994; Montgomery et al. 1997). This mode of pharmacological inhibition is more complete and less invasive then historically preceding method of transecting the lateral line nerves (Dijkgraaf, 1963). Treatment with $CoCl_2$ in conjunction with low Ca^{2+} concentration selectively inhibits

the lateral line hair cell transduction and does not effect the function of the inner ear in teleosts (Karlsen and Sand 1987).

Here I will describe results of the experiments that were designed to shed light on the biological function of the lateral line system of this ancient vertebrate group of animals. The goal of these experiments was to test the hypothesis that the lateral line system of lampreys is involved in a form of proprioception, or, more precisely, detection of self-generated water motions during swimming. However, this hypothesis should not be understood to suggest that proprioception is the exclusive function of the lateral line system of these animals. In fact, proprioception may be just one part of the diverse functional repertoire of what I suggest is an unspecialized lateral line system. To test this hypothesis, the swimming kinematics of normal and cobalt-treated animals was compared.

Materials and Methods

Animals

One larval (length 13.0 cm) and four adult (length 13.2 -15.0 cm) lampreys, *Petromyzon marinus* L., were used in behavioral experiments. One larval animal was used in a physiological experiment to confirm the inhibitory effect of $CoCl_2$ solution on the activity of the lateral line. Live specimens, obtained from a supplier in the area of Lake Michigan, USA, were kept in separate aerated aquaria (water pH = 7.5 – 8.0) at 5ºC and 12ºC, respectively. The larvae were fed once a week with powdered brewer's yeast 500 (General Nutrition Corp., Pittsburgh, PA, USA). Due to slow metabolism at low temperature, adult lampreys did not require feeding.

Swimming protocol

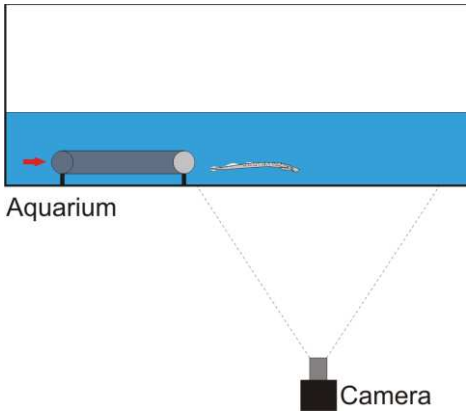

Figure 4-2.
The experimental set-up used for swimming experiments. Lampreys were first induced to swim in the plastic cylinder. This made their trajectories straighter and allowed video to be taken of a few cycles of steady swimming. The camera was mounted below the tank.

Swimming experiments were performed in a room with a temperature of 12ºC. For these experiments, an 120x45x55 cm aquarium filled with water to the level of 15 cm from the bottom was used (fig. 4-2). After an acclimation period of about 30 minutes, animals were induced to swim freely with approximately straight trajectories by introducing them into a cylinder on the bottom of the aquarium. The animals were filmed with a high-speed camera from below (Lightning RDT, DRS Data & Imaging Systems, Inc., Oakland, NJ, USA) at 400 frames per second. Ten to fifteen trials of swimming were recorded for each animal.

Cobalt treatment

Physiological confirmation of the inhibitory effect of $CoCl_2$ on the afferent activity in the TLLN generally followed the procedure equivalent to that of Gelman et. al. (2007). After a normal response to the vibrating ball was recorded, a 2 mM solution of $CoCl_2$ in standard fresh water with low calcium concentration (0.025 mM KCl, 0.05 mM KNO_3, 0.05 mM NaH_2PO_4, 0.2 mM NaCl, 0.1 mM $MgSO_4$ (Sand 1975; Karlsen and Sand 1987)) was added to the experimental chamber without

changing the position of the recording electrode and the stimulating ball. Activity in the TLLN was continuously recorded during the experiment.

For behavioral experiments, normal swimming of each animal was first filmed as described above. After that animals were transferred for 2 hours to a small aquarium filled with 2 mM solution of $CoCl_2$, prepared in the same way as for the physiological experiment, Cobalt treated animals were then filmed following the same protocol as for untreated animals. After 10-15 trials of swimming animals were transferred to a holding aquarium where they were monitored for a few days to determine if the cobalt treatment altered their normal behavior.

Data analysis

Video data was analyzed using a custom Matlab 7.3 (MathWorks, Inc., Natick, MA, USA) program provided by Dr. Eric D. Tytell. In short, this program automatically detected the midlines of the animal by performing a 1-D template matching between a known template of the width of the animal at the position of the transect and the values of the pixels along sequential transects between the head and tail and (Tytell 2004; Tytell and Lauder 2004).

The measured kinematic parameters included: forward velocity (*V*, in body

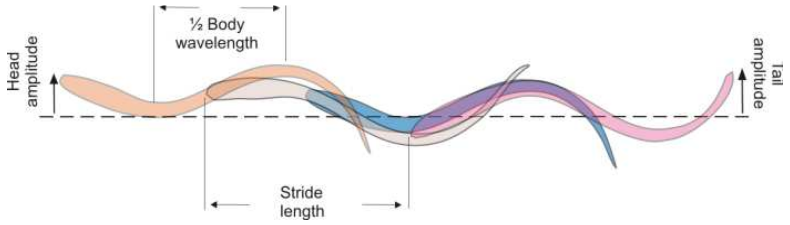

Figure 4-3.
Outlines of a lamprey obtained from four video frames. Some of the analyzed kinematic parameters are illustrated. Head and tail amplitudes are maximal excursion from the axis of swimming (dashed line). Stride length is the distance in body lengths per one tail beat or cycle of swimming. Body wavelength is the distance in body length between the points of maximum curvature.

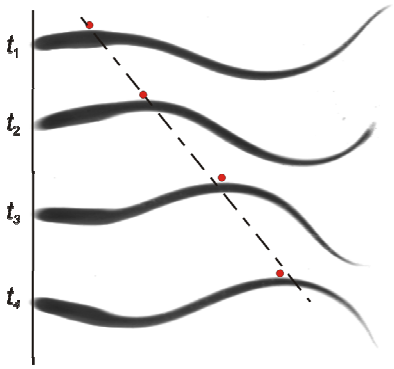

Figure 4-4.
Backward propagating wave of lateral undulations. The snout of a lamprey at different time instances has been aligned to show the backward propagating point of maximum curvature (red dot). During swimming, the lamprey progresses forward with some velocity V, while the wave of lateral undulations moves backward in the fish's frame of reference with speed U, which is larger than V. The ratio V/U is called slip.

length per second L/t), body wave speed (U, the speed with which lateral undulations travel backwards, in body length per second, L/t), slip (the ratio of forward velocity to body wave speed, V/U), tail beat frequency (f, Hz), amplitude of lateral undulations of the head and tail (A_H and A_T, in body length L), wavelength of lateral undulations (λ), stride length (distance in body lengths traveled per one tail beat), and Strouhal number ($2f A_T/U$, a dimensionless number related to the efficiency of swimming (Triantafyllou et al. 2000). Some of these parameters are illustrated in figures 4-3 and 4-4.

Results

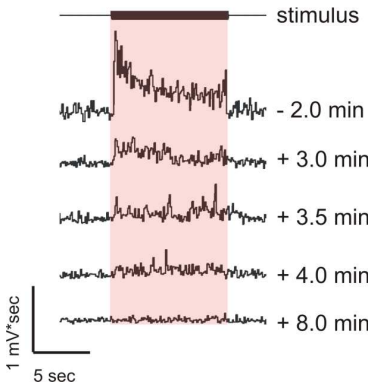

Figure 4-5.
Inhibition of the evoked PLLN activity by Co^{2+}. An example of rectified (full-wave) and integrated (integration bin is 100 ms) traces of the multiunit evoked responses to a 20 Hz stimulus (vibrating ball). Negative and positive times indicate responses before and after application of Co^{2+}.

Physiology experiment and general effects of cobalt on behavior

In light of the distant relation between agnathans and gnathostome fishes, the possibility that cobalt treatment is ineffective in inhibition of the lateral line of lampreys could not be disregarded. Therefore, a physiology experiment was performed to confirm the treatment's effectiveness, even though physiological

confirmation is not usually done in behavioral experiments with various species of gnathostome fishes. As shown in figure 4-5, evoked activity in the TLLN was completely inhibited within 10 minutes after the change to the cobalt-containing solution. In the study conducted by Karlsen et. al. (1987) on the freshwater fish, *Rutilus rutilus*, 1 mM $CoCl_2$ was used and the time course of inhibition indicates a period of 75 minutes for 100 % reduction of the evoked activity. Decrease in evoked activity to 50% was observed in about 30 minutes. Since I used a concentration 2 times larger than that, the fast inhibitory effect is not surprising. It should be noted that Karlsen et. al. (1987) recommend 0.01 mM $CoCl_2$ solution and a treatment time of 24 hours. However, some subsequent behavioral experiments by other groups were conducted using much higher concentrations of cobalt solution, but with shorter exposure times (Montgomery et al. 1997; Baker and Montgomery 1999). Some other workers in the field criticize such high concentrations of Co^{2+}, on the account that it makes fish sick (Janssen 2000). However it is also known that Co^{2+} toxicity varies amongst fish species (Janssen 2000). Therefore, as mentioned in Materials and Methods section, care was taken to observe the general behavior of treated lampreys.

Animals did not exhibit an abnormal behavior either during or after exposure to cobalt solution. In the holding aquarium, adult lampreys swam around for a few minutes and then attached themselves with their suckers to the glass wall of the aquarium, as normal animals do. The larval, post-experimental, cobalt–treated lamprey also swam a few minutes around the tank and then burrowed under the sand. Neither larval nor adult lampreys showed any behaviors or symptoms that are usually

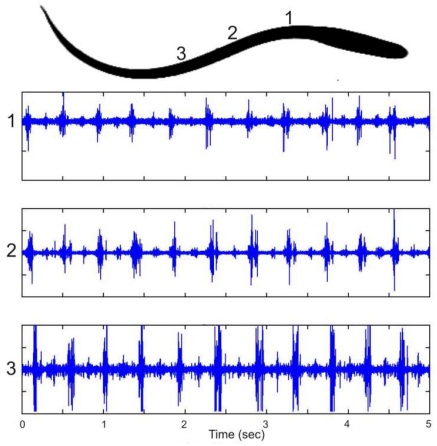

Figure 4-6.
Electromyogram activity (EMG) along the body of the swimming lamprey (top). Three traces are EMG recordings from three points on the body indicated by numbers. Note the lag in the EMG bursts in the caudal direction, which indicates sequential muscle contraction along the body.

indicative of metal poisoning in fish, such as 'coughing' and copious secretion of mucus (Sorensen 1991; Janssen 2000).

Swimming kinematics and the lateral line inhibition effect

In order to quantitatively assess the effect of the lateral line inhibition on swimming of lampreys, it was first necessary to describe the kinematics of movement in untreated animals. Therefore some of the previously reported results on fish and lamprey swimming had to be reproduced. Here I will present the data collected, in addition to giving references of previous work. Lampreys swim by sequentially contracting the myotomal muscle segments in a rostro-caudal direction (fig. 4-6) (Wallen and Williams 1984; Williams et al. 1989), which produces transverse undulations of the body traveling from head to tail (fig. 4-4) with speed U. Complex interaction of the body with water generates a thrust propelling the animal forward with velocity V, which is generally smaller then U (Gray 1933a). When the animal swims in an approximately straight trajectory, all points on the body follow approximately sinusoidal trajectories (fig. 4-7, top panel) with the amplitude of oscillations increasing with the distance along the body from the head to tail (fig. 4-7, middle and lower panel).

A number of kinematic parameters can be obtained from the above illustrated description of the swimming motions of lamprey. Here the comparison of kinematic

parameters is made between control and cobalt-treated animals. Studies on the Mexican blind cave fish show that animals with pharmacologically inhibited lateral line tend to swim faster then the control ones, to compensate the lack of normal lateral line input (Hassan et al. 1992). I did not find this to be the case with lampreys on average. Figure 4-8 shows swimming velocities plotted against time for each trial of normal and cobalt-treated animals. Apart from small number of trials, treated lampreys swam with the same mean velocities (control: 1.09 L/s ± 0.18 SD; treated: 1.12 L/s ± 0.28 SD; two-sample t-test p = 0.5982). Swimming velocities were also variable within a trial for both groups (fig. 4-8).

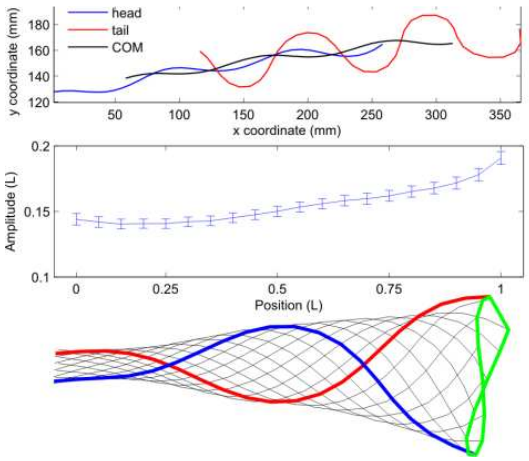

Figure 4-7.
An anguilliform mode of locomotion. (Top panel). Trajectories of the tip of the tail (red curve), center of mass (COM, black curve), and tip of the head (blue curve) are approximately sinusoidal. (Middle panel). Mean amplitudes of lateral undulations (n = 40; midlines from 4 adult lampreys, 10 trials of swimming each) at 20 points on the body. Vertical bars are standard error of the mean. Note how amplitude increases in the caudal direction. (Lower panel).Midlines of the swimming lamprey aligned to the tip of the head. Red and blue lines are midlines at the beginning and mid-point of the swimming cycle. Note a characteristic figure of eight of the moving tail tip.

Figure 4-9 shows a panel of histograms depicting kinematic parameters averaged within a trial for all control animals. By comparing this figure with figure 4-10, which shows the equivalent data for cobalt-treated animals, it can be seen that all averaged kinematic parameters fall in the same range for both groups. Analysis of variance (ANOVA) indicates that the means of the kinematic parameters are not different between groups (table 3, fig. 4-11). The analyzed kinematic parameters generally depend on swimming velocity. Therefore, it was meaningful to

check whether the relationship between these parameters and the swimming velocity was affected by cobalt treatment. Tail beat frequency, stride, wave speed, and slip were positively correlated with swimming velocity as judged by the coefficient of correlation (table 4) and linear regression fits (fig. 4-12). Wave length was not

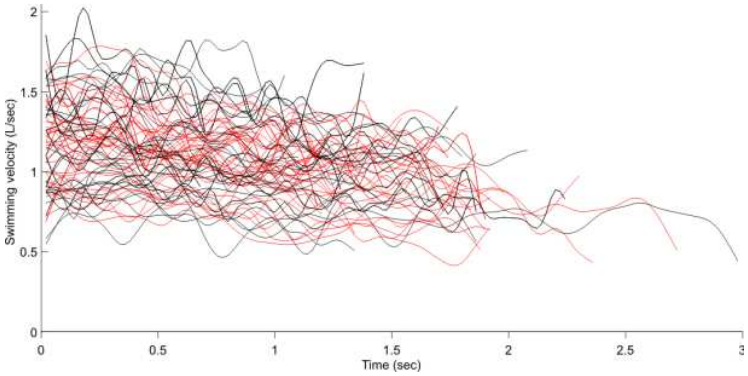

Figure 4-8.
Swimming velocities in each trial of control (45 trials, red lines) and cobalt-treated lampreys (44 trials, black lines). On average control and cobalt-treated lampreys swam with similar velocities.

correlated with the velocity of swimming. The correlation of tail and head amplitude with the swimming velocity was weakly positive. Strouhal number was negatively correlated with swimming velocity (table 4, fig. 4-12). Linear regression lines, fitted into the data for both control and cobalt-treated animals were not different (fig. 4-12). Analysis of covariance (ANOCOVA) confirms the dependences of the kinematic parameters on the swimming velocity concluded from the correlation coefficients (table 5).

Variable\Statistic	*F*	*p*
Swimming velocity	0.2797	0.5982
Tail beat frequency	1.1957	0.2772
Stride	1.0624	0.3055
Wave speed	1.0482	0.3087
Wave length	0.1971	0.6582
Slip	0.5858	0.4461
Strouhal number	1.2527	0.2699
Tail amplitude	0.3428	0.5599
Head amplitude	0.9939	0.322

Table 3.
Summary table of the ANOVA test on the means of kinematics parameters for control and cobalt conditions. *F* statistics and *p* values indicate that the means of the kinematics variables for the two conditions are equal.

Condition	*Control*		*Cobalt*	
Variable\Statistic	*R*	*p*	*R*	*p*
Tail beat frequency	0.6196	0	0.7567	0
Stride	0.31	0.0382	0.6441	0
Wave speed	0.8314	0	0.8292	0
Wave length	-0.0038	0.9803	0.1235	0.419
Slip	0.5462	0.0001	0.6146	0
Strouhal number	-0.6525	0	-0.6319	0
Tail amplitude	0.0642	0.6752	0.3423	0.0214
Head amplitude	0.2461	0.131	0.5824	0.0001

Table 4.
Summary table of the correlation coefficients between kinematics parameters and swimming velocity. Tail beat frequency, stride, wave speed, and slip showed significant positive correlation with the swimming velocity for control and cobalt-treated conditions. Wave length and tail amplitude were not correlated with velocity. Head amplitude was weakly correlated and Strouhal number was significantly negatively correlated with swimming velocity for both conditions.

	Joint Slope (p value)	*Interecept (p value)*	*Slope (p value)*
Tail beat	0	0.3218	0.2561
Stride	0	0.1376	0.3745
Wave speed	0	0.3036	0.7709
Wave length	0.5481	0.637	0.6691
Slip	0	0.1905	0.9994
Strouhal number	0	0.067	0.966
Tail amplitude	0.0511	0.4833	0.4646
Head amplitude	0.0001	0.2417	0.4832

Table 5.
Summary table of the ANOCOVA test on the linear regression coefficients.
Joint Slope p values indicate whether slopes of the linear regression fits in figure 12 are significantly different from zero, i.e. whether the kinematics parameters tested depend on the swimming velocity, for control and cobalt-treated conditions. *Intercept* and *Slope* p values indicate whether the difference in these values was different between control and cobalt conditions. Results indicate that none of the linear regressions were statistically different between control and cobalt conditions.

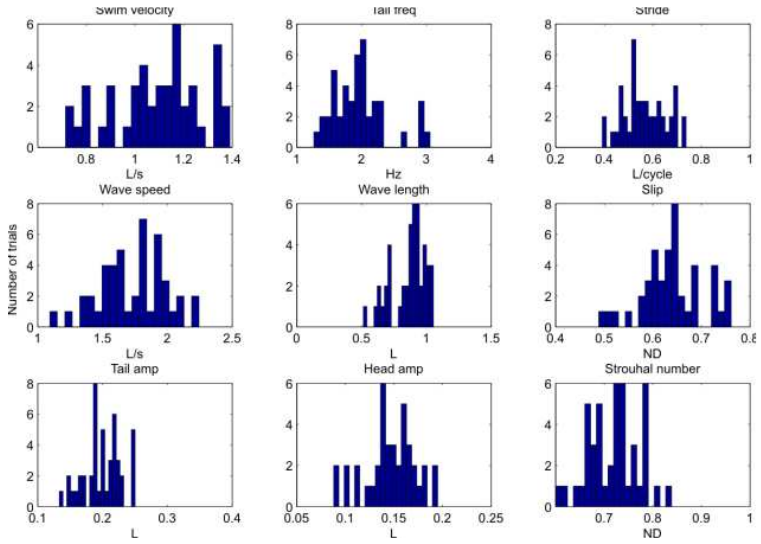

Figure 4-9.
Histograms of the kinematics parameters for control swimming. Titles indicate the variable; x-axis labels indicate the units of measurements. ND stands for non-dimensional.

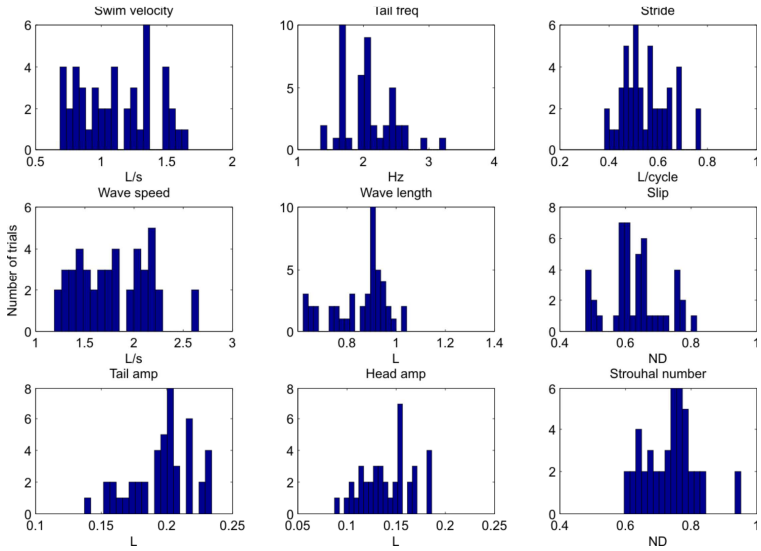

Figure 4-10.
Histograms of the kinematics parameters for cobalt-treated swimming. Titles indicate the variable; x-axis labels indicate the units of measurements. ND stands for non-dimensional. Note that the range of variables is very similar to the control ones (see fig. 4-9).

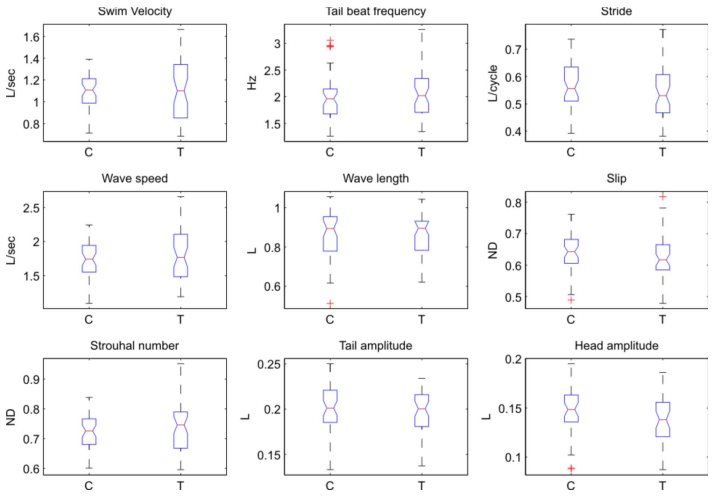

Figure 4-11.
Standard statistical box plots of the kinematics variables for control (C) and cobalt-effected (T) swimming. The boxes extend from the 25th to 75th quartile (a region of 50% of the data); red lines represent the median of the data; red pluses are outliers. The error bars above and below the boxes extend to the maximum and minimum values. The notches in the boxes represent approximate 95% confidence intervals. Overlapping of the notches in two boxes indicates that the data are not statistically different. The kinematic variables between the two groups were not statistically different. Combined data from 45 trials for each condition of 4 lampreys.

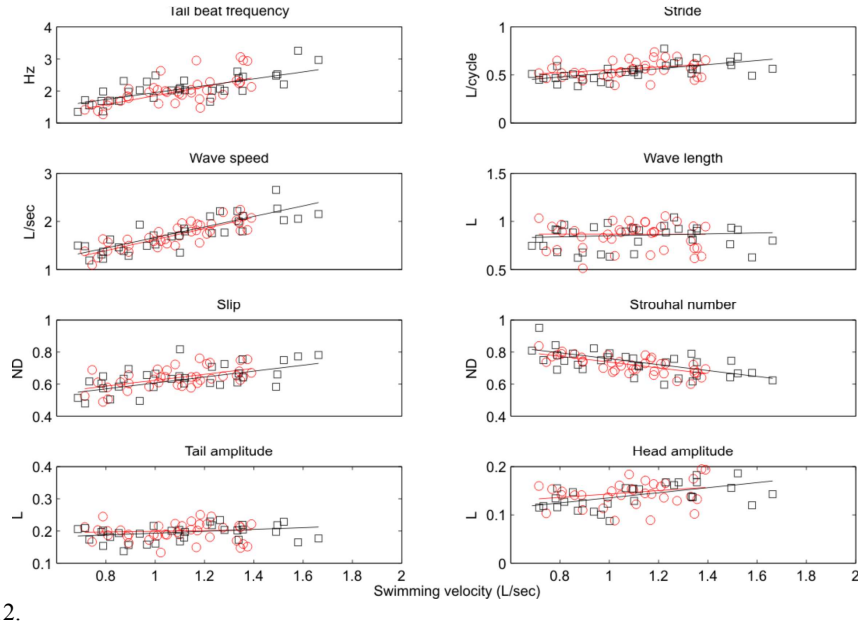

Figure 4-12.
Relationship between the kinematics variables and the swimming velocity for control (red circles) and cobalt-treated (black squares) lampreys. Each data point represents a mean value of the variable from each trial (45 control trials and 44 cobalt trials). Lines are linear regressions to the data points. Note that there is no difference in regression lines between control and cobalt-treated groups.

Discussion

Results of the cobalt experiments indicate that the inhibition of the lateral line system does not produce a noticeable deficiency in the lampreys' swimming, at least at the level of the performed analysis. All examined kinematics parameters were not different from the controls. It is probably safe to conclude on the basis of these experiments that the lateral line system is not essential for the regulation of the normal swimming in conditions of still water, i.e. in such conditions it is not involved in the cycle by cycle control of swimming. It is well established on the isolated spinal cord preparations that the segmental central pattern generators (CPGs) in the lamprey can produce alternating, caudally propagating pattern of motorneuron activity without any sensory feedback (Grillner et al. 1995; Grillner et al. 1998a; Grillner et al. 1998b). Sensory feedback is nevertheless important for *in vivo* locomotion (Grillner et al. 1998b; Cohen and Boothe 1999; Guan et al. 2001). Completely eliminating all sensory feedback during swimming will probably create motor deficiencies. However, due to the presence of mechanosensitive stretch receptors in the spinal cord of lampreys, blocking the lateral line does not eliminate all sensory feedback. The stretch receptors or 'edge cells' are located in the lateral margins of the spinal cord (Rovainen 1982). These cells are excited when the spinal cord is stretched (Grillner et al. 1982; Grillner et al. 1984). Stretch receptors are known to excite certain ipsilateral and inhibit other contralateral interneurons of the pattern generating spinal networks (Viana Di Prisco et al. 1990; Viana di Prisco et al. 2000). Entrainment of the ventral root bursting frequency during fictive swimming in the isolated spinal cord by

rhythmic bending of one end of the spinal cord is mediated by stretch receptors (Andersson et al. 1981; Grillner et al. 1981; McClellan and Sigvardt 1988). Thus the edge cells can modulate the timing of the onset and offset of the activity of the segmental CPGs based on the local curvature of the body (McClellan and Jang 1993). This modulation occurs not only on the cycle-by-cycle basis, but also has a long lasting effect seen over a few cycles (Kiemel and Cohen 2001). Presence of such powerful and fast sensory feedback provides sufficient movements-related feedback to the CPG (Grillner et al. 1981). Consequently the lateral line, even if being a complementary regulatory system in locomotion, may not be necessary for stabilization or other control in simple laboratory conditions. Evidence exists indicating that the swimming performance of blinded minnows is not affected by the surgical transection of the lateral line nerves (Dijkgraaf 1963). On the other hand, importance of the lateral line may increase in uniform flow or in turbulent waters when precise information about the flow is necessary.

Behavioral experiments described here do not shed much light on the involvement of the lateral line in the control of locomotion either in laminar flows or in complex conditions of turbulent flows. Recently it was shown that trout with pharmacologically blocked lateral line exhibited altered kinematics during a swimming pattern called a Kármán gate, a presumably energy-efficient way of station holding utilizing the presence of vortices in the flow (Liao 2006). Reported changes in the kinematics included increase in the body wavelength and the wave speed compared to control animals (Liao 2006). It would be very interesting to conduct similar experiments with lampreys. Effect of blocking the lateral line on performance

and swimming kinematics of lampreys in complex flows should be comprehensively tested.

Even though results of this study support the ingrained and largely unchallenged notion that the lateral line is not involved in a regulatory function in locomotion, which stems from Dijkgraaf's behavioral experiments on blinded minnows (Dijkgraaf 1963), it nevertheless appears plausible that under certain conditions and at least in some species, particularly lampreys, the mechanosensory lateral line system is involved in some aspects of locomotor control.

SUMMARY AND FURTHER DIRECTIONS

The work described in this dissertation is an initial step in studying the mechanosensory lateral line system of lampreys.

Electrophysiological recordings of fibers in the trunk lateral line nerve indicate that larval lampreys are mechanoreceptive. This finding compliments earlier work that established photo- and electro-reception in pre-metamorphic lampreys (Ronan 1988; Ronan and Bodznick 1991). Thus the full functionality, which includes the three sensory modalities, of the lateral line system is already present in larval lampreys. However, as opposed to photo- and electroreception, mechanoreception appears to change during the metamorphosis of lampreys. The morphological changes include an increase in the number and re-distribution of neuromasts and an increase in the number and cross sectional dimensions of the axons in the trunk lateral line nerve that innervate mechanoreceptive hair cells. Physiological metamorphic changes include a narrowing range in the frequency discrimination, a decrease in the gain of the response, which most likely indicate a decrease in the sensitivity to mechanical stimuli, and a decrease in the time constants or delays at the peripheral level, which may be caused by faster afferent conduction or some other so far unknown changes in the transduction mechanisms. More experiments have to be done to establish that these changes are truly metamorphosis-related and not just a consequence of the animal's growth. The behavioral significance of the reported changes is still far from being clear. Investigation of this problem is complicated by the lack of understanding of the lateral line's role in lamprey behavior in general.

Further work on the lateral line system in adult and larval lampreys should focus on the following problems.

First of all, the mechanisms of the central processing of the hydrodynamic information should be investigated. This aspect is not clearly understood even in the model organisms like Xenopus and goldfish. In the case of lampreys, an additional interest lies in the crossing of the primary mechanoreceptive projections, i.e. a bilateral, double representation of the lateral line inputs exists in ipsi- and contra-lateral octavolateralis area. What kind of processing is implemented with such bilateral representation? Histological evidence points to segregation of the anterior and posterior lateral line nerves' projections in the medial nucleus of the octavolateralis area (Ronan and Northcutt 1987; Koyama et al. 1990; Gonzalez and Anadon 1992). How precise is this topography? Is this topography also kept for the anterior and posterior lateral line fibers separately? Another set of similar questions is related to midbrain structures – torus semicircularis and optic tectum.

Second of all, the question related to the metamorphic changes in the central processing of the hydrodynamic stimuli deserves attention. It is not known whether the peripheral changes in the lateral line are accompanied by the changes in the central nervous system.

Third of all, similarities and differences in the processing of electric field and hydrodynamic stimuli present an interesting question. In principle electroreception and mechanoreception are sensory modalities devoted to perception of vector field quantities, albeit of different physical nature. After initial transduction steps, the parameters of the electric and velocity fields are transformed or encoded in the

pattern of neuronal activity. Does the central nervous system processes the spike pattern in the electroreceptive and mechanoreceptive pathways differently? After all, the nature of inputs that the CNS receives is already equivalent and the central neuronal pathways are very similar if not identical for both modalities. Another question is concerned with the integration of the two types of information. It is conceivable that a particular, biologically relevant stimulus will simultaneously produce changes in the electric and hydrodynamic fields around the animal. The CNS has the task of integrating this information and at some level forming a combined representation of the stimulus. Are there neurons in torus semicircularis or optic tectum that respond both to electric and hydrodynamic field stimuli?

Finally, it is very important for all of the above directions, to understand the behavioral roles of both electroreception and mechanoreception in lampreys. Determination of the specific behaviors should be the first step in approaching neuroethology of the lateral line mediated behaviors. In fact, this should go to the beginning of the list of problems. In addition, the issue of metamorphosis arises again. Considering the changes in the life style brought about by the metamorphosis, do the behaviors mediated by the lateral line system change too?

APPENDIX I: DETERMINATION OF AXONAL DIMENSIONS

In chapter three of this thesis the equivalent diameters of axons in the adult and larval TLLN were compared. Here the technical details of the intermediate steps in the image analysis procedure are described in depth.

To count the number of axons in the trunk lateral line nerves of adult and larval specimens and to determine for comparative purposes their geometric parameters, including equivalent circle diameters, elliptic eccentricity, and perimeters, digital images (1000x magnification) were pre-processed in Corel PHOTO-PAINT X3 (Corel Corporation, Ottawa, Ontario, Canada). Pre-processing included contrast improvement using standard techniques and a montage of a series of overlapping images (fig. A1-1) to obtain a combined image of an entire area of the nerve. Due to the large sizes of axons and their relatively small number, axonal membranes were visually identified and manually traced; the enclosed areas were cut out using a cut-out utility and converted into black and white image (fig. A1-2). Subsequent analysis was performed using a custom MATLAB 7.3 (MathWorks, Inc., Natick, MA, USA) program. The objects representing the edges of axons in black and white images were separated using standard MATLAB function *bwlabel*. This function operates on the black and white image and labels or separates the objects based on the pixel connectivity of their boundaries. The output of *bwlabel* is an array of coordinates of all the objects' boundaries in the image. Figure A1-3 depicts the axons boundaries. The array of boundary coordinates was then passed to another

MATLAB function, *regionprops*. This function computes the geometric properties of the objects from the areas enclosed by the boundaries.

Figure A1-4, in addition to already presented (see chapter three) data on the equivalent circle diameters, shows histograms of the equivalent elliptic eccentricities and perimeters of the axons for adult and larval TLLN.

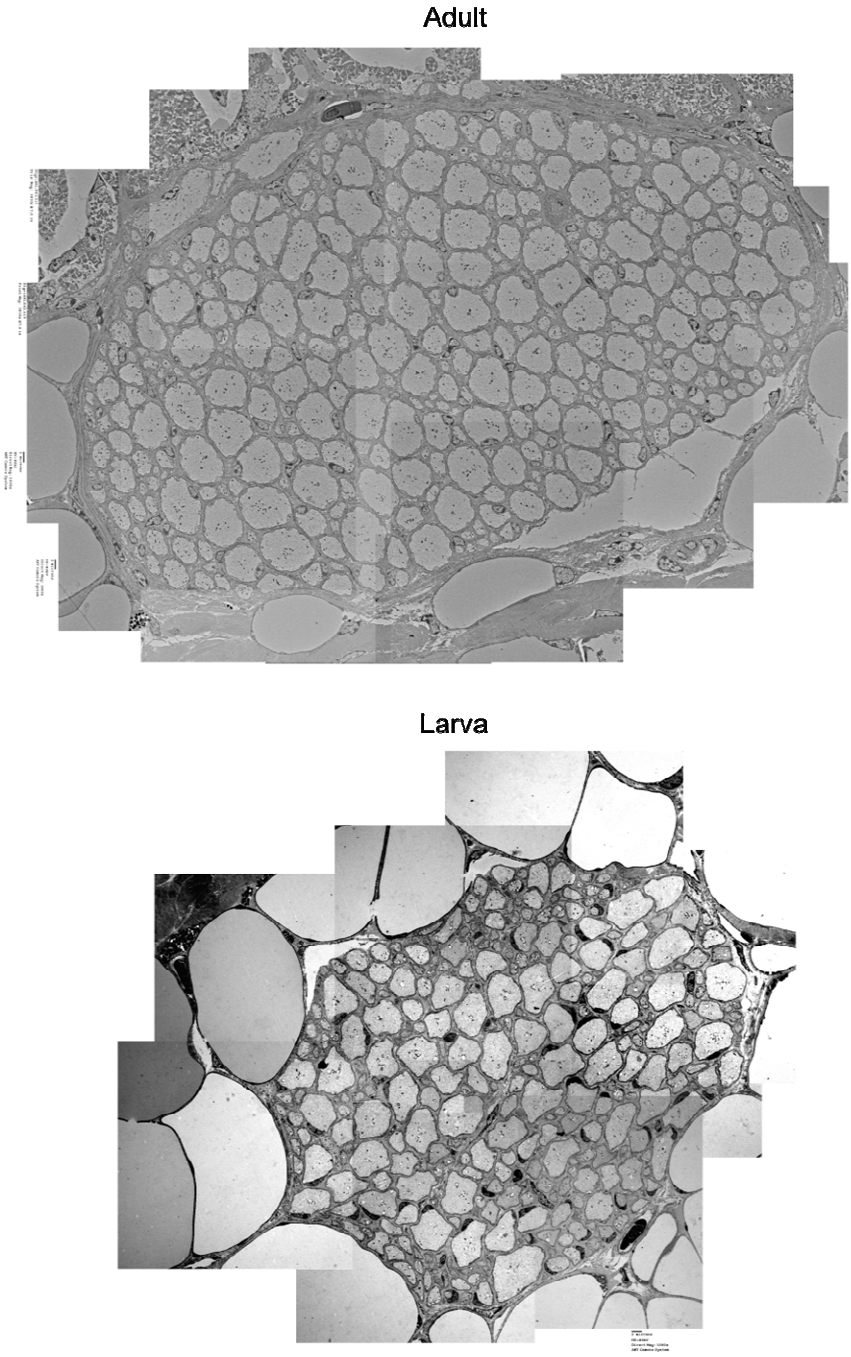

Figure A1-1.

The montage images of the adult and larval TLLN cross sections constructed from x1000 TEM images. These images were used to visually identify the axons and to manually delineate the axonal membranes.

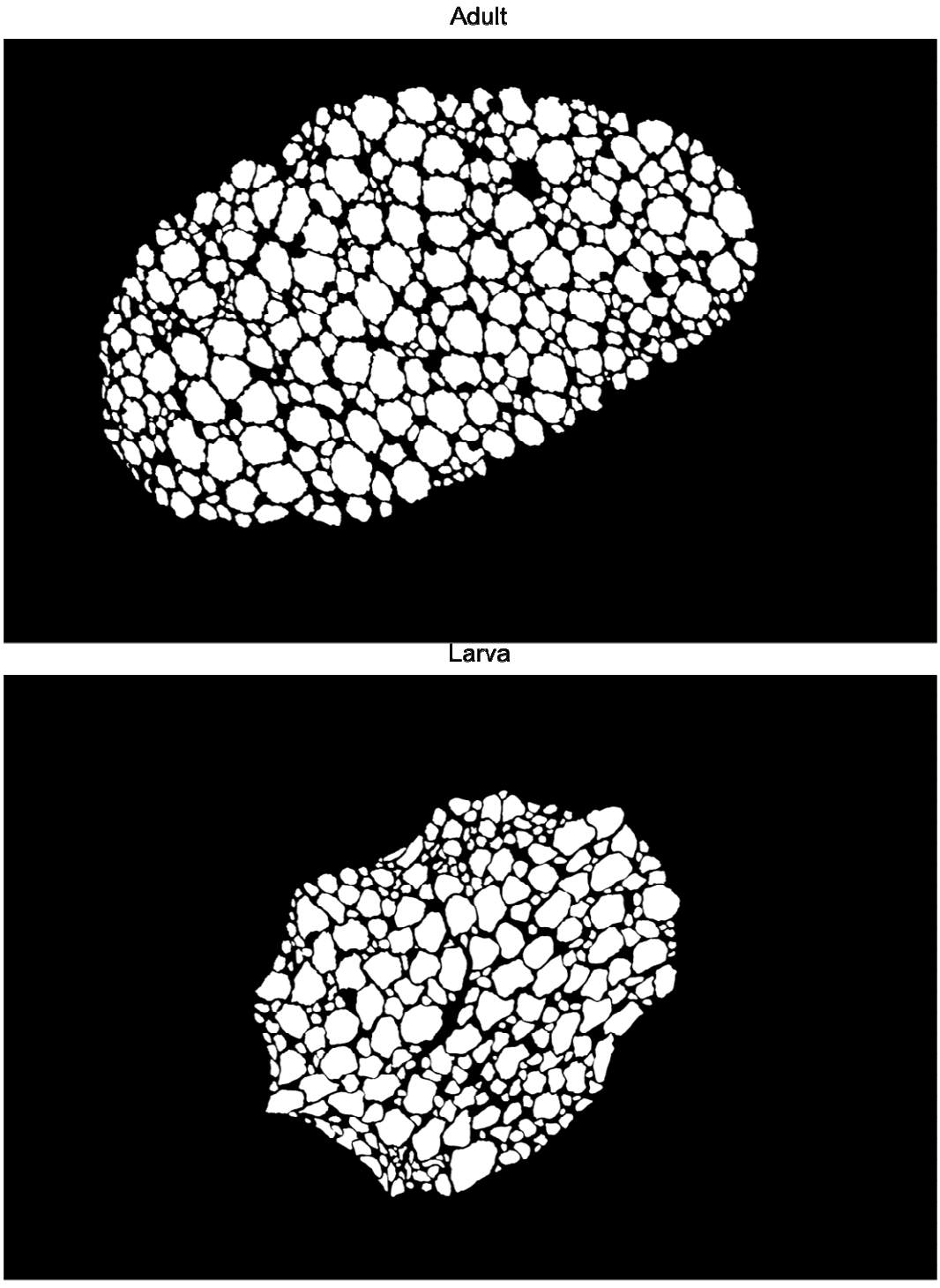

Figure A1-2.

The black and white images of the adult and larval TLLN cross sections.

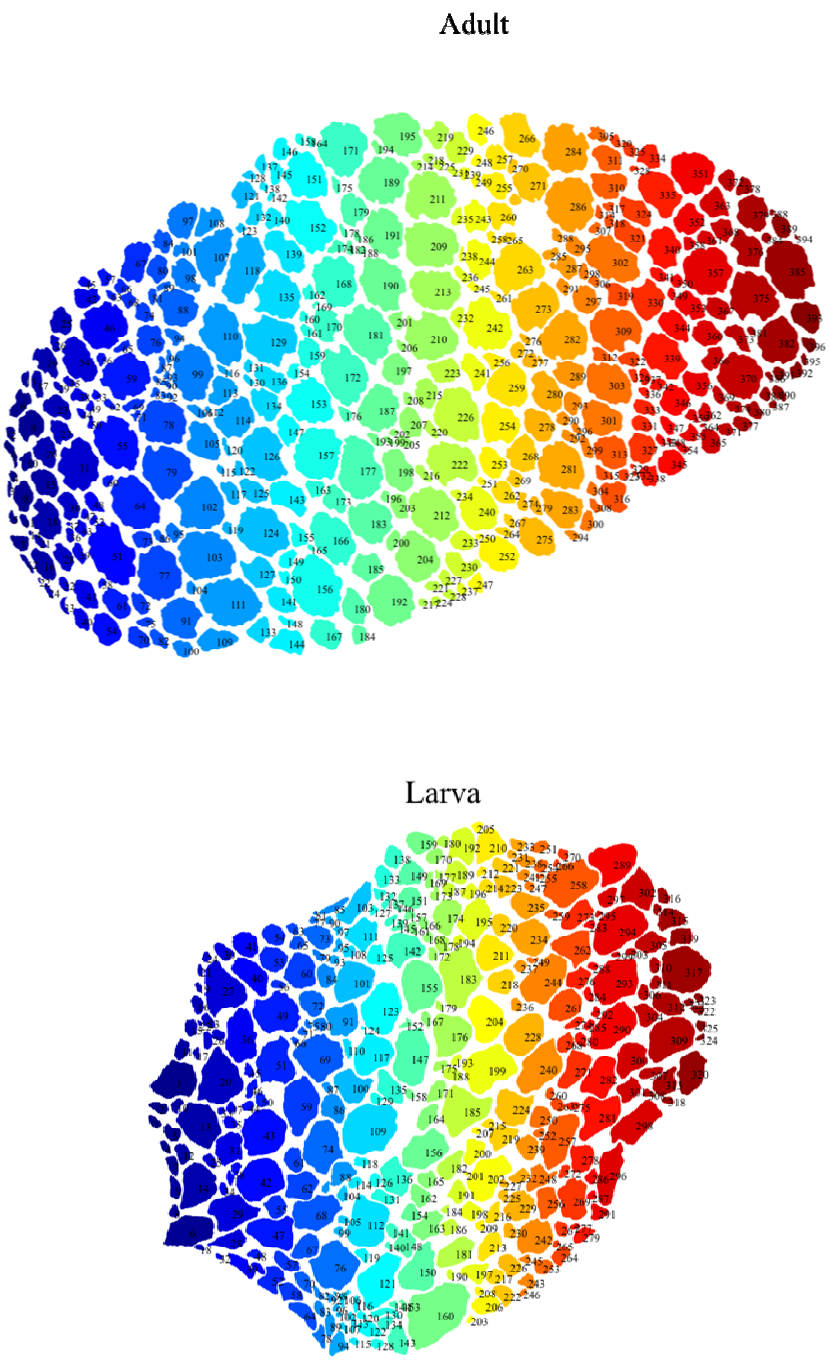

Figure A1-3.

The separated and numbered axons of adult and larval TLLN cross sections.

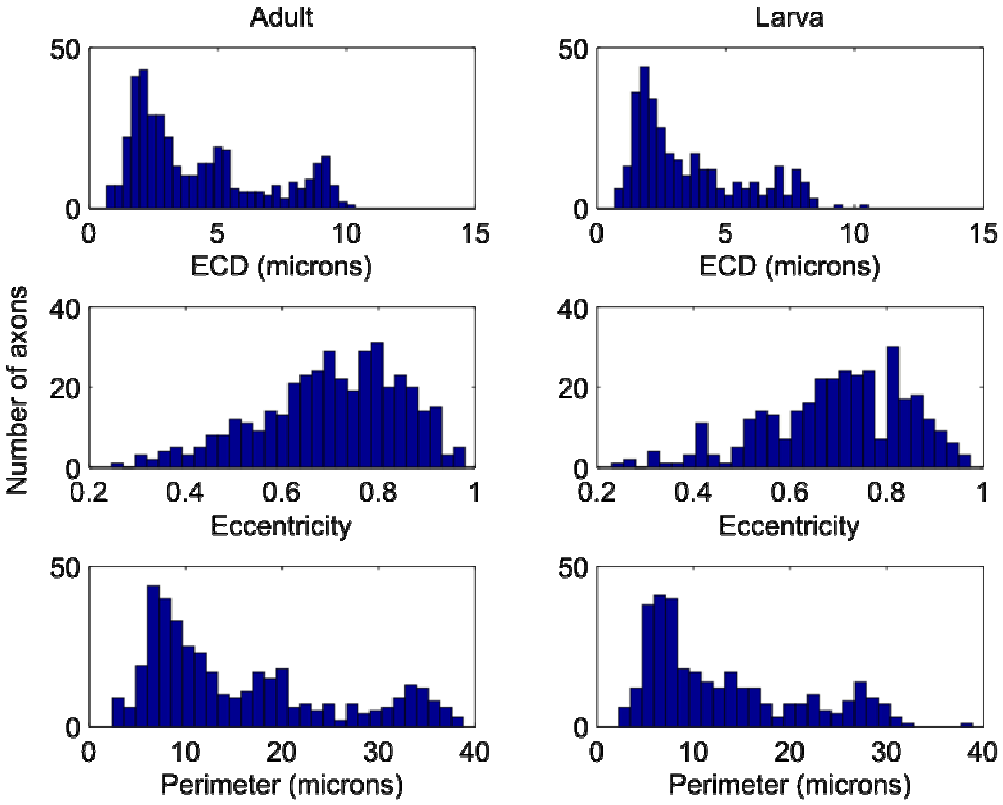

Figure A1-4.

Histograms depicting the distributions of the geometric parameters of adult and larval axons in the TLLN. Equivalent circle diameters, equivalent elliptic eccentricity, and the perimeters were computed using standard MATLAB function, regionprops. Eccentricity of an ellipse is defined as the ratio between its longest axis and the focal length. The circle is a degenerate ellipse in which the radius is equal to its focal length, giving the ratio of 1.

APPENDIX II: ULTRASTRUCTURAL COMPOSITION OF THE TRUNK LATERAL LINE NERVE OF ADULT AND LARVAL LAMPREYS.

Abstract

The ultrastructure of the trunk lateral line nerve of adult and larval lampreys was studied with transmission electron microscope. It was confirmed that the compact myelin ensheathment is absent in lampreys. Nevertheless, all examined axons were wrapped by the Schwann cell processes. In adult nerve, the Schwann cell ensheathment was complete. In larval nerve, on the contrary, the gaps between the Schwann cell processes were observed; the axolemma in those places was covered only by a basal lamina. The perineurium of the adult nerve consisted of at least three layers of fibroblasts separated by collagen fibrils. The larval perineurium was thinner and the fibroblasts contained large amounts of glycogen granules. The larval epineurium contained large fat cells, separated from each other by single fibroblast processes. This large layer of fatty tissue was absent in adult preparation. Both adult and larval trunk lateral line nerves possessed a number of fascicles weakly defined by a thin layer of the perineurial fibroblasts.

Introduction

In general, the vertebrate peripheral nervous system consists of the cranial and spinal nerves. The axons of these nerves are wrapped by Schwann cells and can be myelinated or not myelinated (Peters et al. 1991). Nerves are usually surrounded with the three sheaths of connective tissue (Key and Retzius 1876; Nakao and Ishizawa 1987; Peters et al. 1991). Epineurium (Ep) surrounds the outside of the nerve. It contains the collagen bundles, arranged transversely and longitudinally, and scattered fibroblasts, masts cells and blood vessels. Perineurium (Per), the intermediate sheath, surrounds each nerve fascicle, and also contains collagen fibers and fibroblasts. Cellular components of the perineurium are compactly packed together, forming from 3 to 10 layers separated by the collagen containing connective tissue. The cells of the perineurium, unlike those of epineurium, have a basal lamina (fine connective tissue layer) on each side. In electron micrographs the basal lamina looks like a dark diffuse band. The adjacent perineurial fibroblasts are usually interdigitated, forming sleeves with tight junctions connecting the cells. Endoneurium immediately surrounds the nerve fibers, axons and the Schwann cells. It consists of all the connective tissue within the perineurium. The collagen fibers are smaller than in epineurium and perineurium, but still discernable (Peters et al. 1991).

In the periphery, the Schwann cells surround each axon forming the glial sheath. These glial cells enwrap the axon with the concentric layers of cytoplasmic extensions, which later on in development loose the cytoplasm and become tightly packed layers of the lipid membranes. The enwrapping process begins with the formation of mesaxon, which is an initial interdigitation of the Schwann cell

cytoplasmic protrusions. The lamprey nervous system lacks compact myelin (Schultz et al. 1956; Rovainen 1982; Bullock et al. 1984; Nakao and Ishizawa 1987), but the glial wrapping is present and appears to proceed initially as in other vertebrates, forming a mesaxon (Peters 1960; Fraher and Cheong 1995; Fraher 2002).

In lampreys, the bipolar cells in the posterior lateral line nerve (PLLN) ganglion send their processes to both peripheral photo- and mechano-receptors in the skin (Ronan 1986; Ronan and Northcutt 1987). The ganglionic cells of the anterior lateral line nerve (ALLN) send processes to the periphery that innervate the trunk electroreceptors (Ronan 1986). The TLLN is a mixed nerve because it consists of the afferent fibers innervating all three types of receptors. The ALLN and PLLN join caudal to the otic capsule, forming the TLLN. In chapter three it was suggested that the three types of afferents may be distinguished on the basis of the cross sectional dimensions.

Here some aspects of the ultrastructure of the adult and larval trunk lateral line nerve (TLLN) are presented. The description of the specimen preparation for transmission electron microscopy was given in the Materials and Methods section of the chapter three of this thesis.

Results

Only one blood vessel was observed in adult and larval specimens embedded in the epineurium or perineurium (fig. A2-1,). It was difficult to distinguish between the two outer sheaths surrounding the nerves (fig. A2-2, A2-3). However, it appears that the connective tissues surrounding the outer border of the TLLN are much less developed in larval lamprey. Particularly the presumed perineurium is much thicker

in the adult nerve and appears to have a number of layers of fibroblast cells densely packed together. Longitudinally and transversely running collagen fibers fill the spaces between the processes of fibroblasts. In the larval preparation, fewer fibroblasts were observed and most of the outer neuronal sheath was filled with the

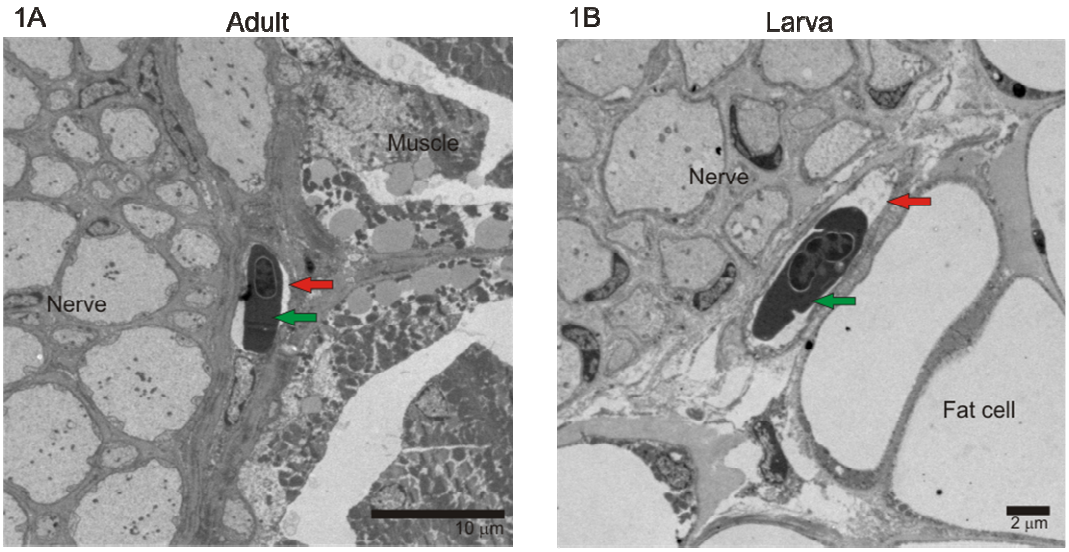

Figure 2A-1.
Cross sections of the adult and larval TLLN at the level of the last branchiopore. In both cases only one blood vessel was observed (red arrows). A nucleated erythrocyte was seen within each blood vessel (green arrows). Blood vessels are usually located in the epineurial sheath. In the adult nerve, a perineurial sheath (Per) is seen between the blood vessel and the nerve. In the larval nerve, on the contrary, the perineurial sheath appears to be very thin. The epineurium, on the contrary, is thickened by the presence of large fat cells.

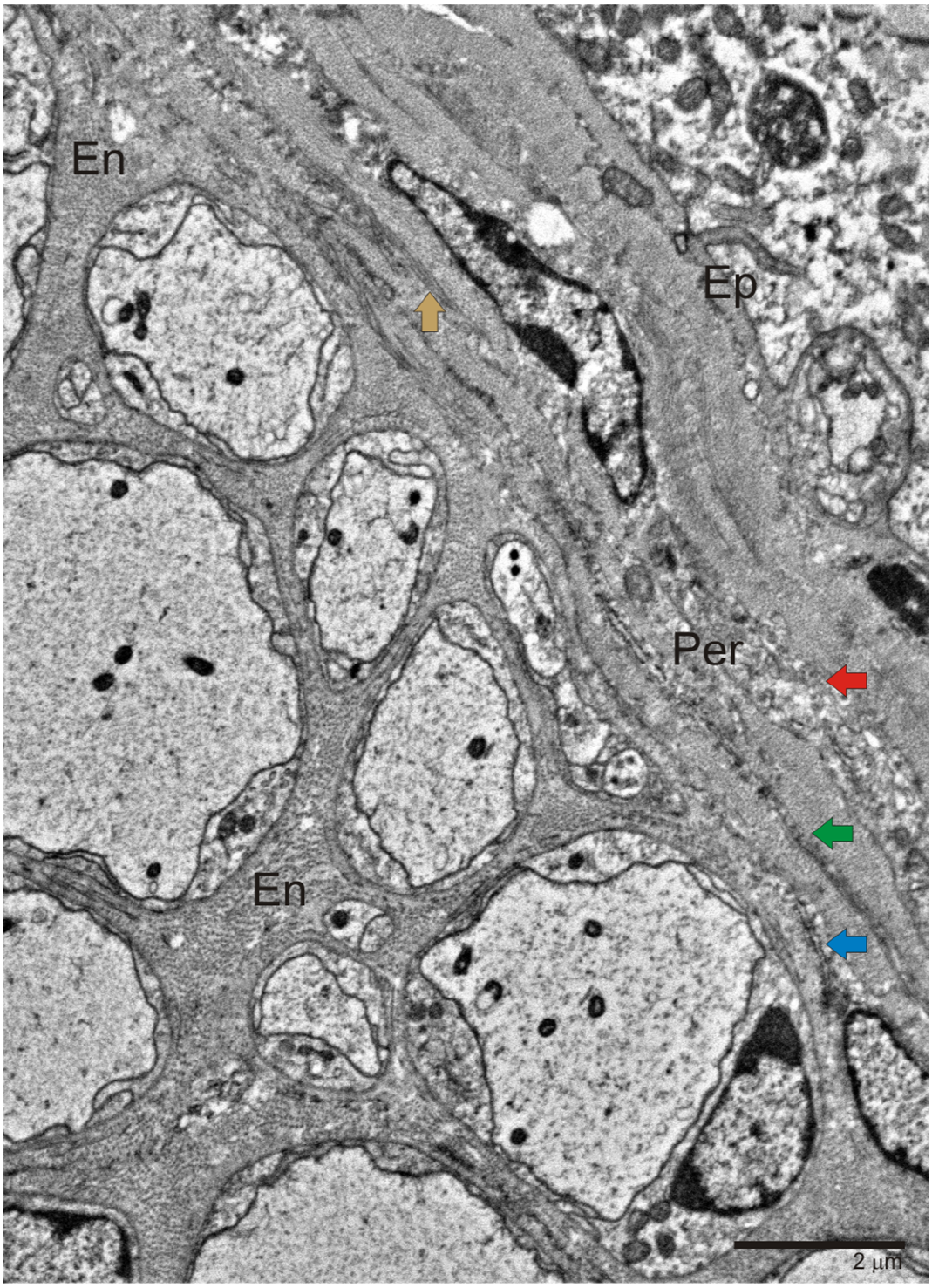

Figure 2A-2.
Connective tissue sheathes of the adult TLLN. In this transverse section the perineurium (Per) with three thin layers of fibroblast processes (red, blue, and green arrows) extends from upper left to lower right. The cellular layers of the perineurium alternate with collagen fibrils, which are seen running transversely and longitudinally (gold arrow). Epineurium (Ep) is to the right. It contains fewer fibroblasts, loosely arranged, and more collagen fibrils. Endoneurium (En) is seen in the space between the Schwann cell wrapped axons. The density of the endoneurial collagen fibrils is smaller and they also appear thicker than the perineurial and epineurial collagen.

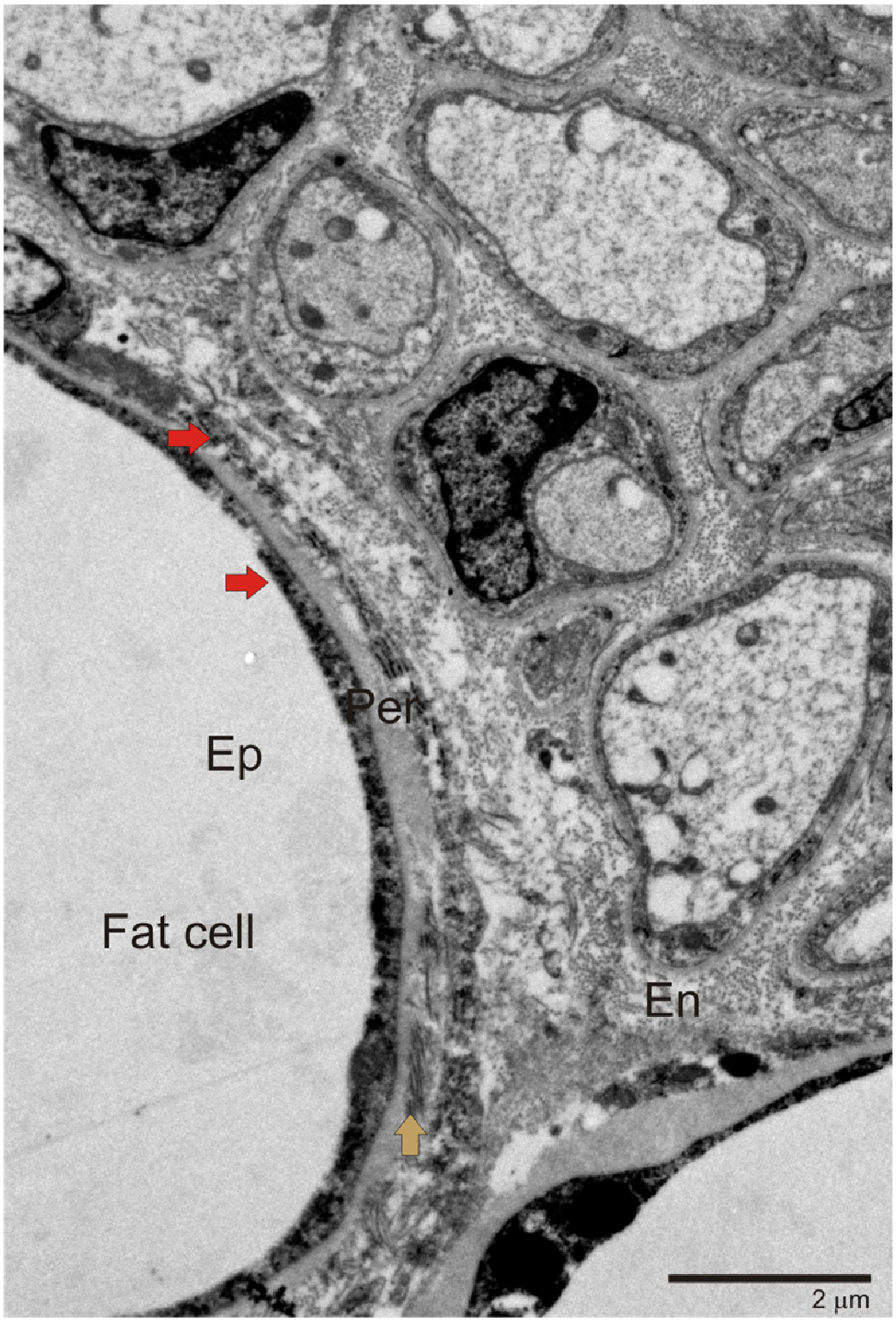

Figure 2A-3.
Connective tissue sheathes of the larval TLLN. The epineurium (Ep) contains large fat cells. The perineurium (Per) has fewer layers of the fibroblasts cells (red arrows). Fibroblast processes are filled with glycogen granules. Collagen fibrils are present between the perineurial fibroblasts, arranged transversely and longitudinally (gold arrow). Collagen fibrils of endoneurium appear to be less dense than in adult nerve.

the nerve fibers, was readily seen in both larval and adult nerves (fig. 2A-4). The cellular components of endoneurium, however, particularly fibroblasts, were difficult to distinguish. Endoneurial fibroblasts are usually identified by the absence of the basal lamina (Peters et al. 1991). Fasciculation within the adult and larval nerve was not clearly observed. If some groups of fibers are enclosed in fascicles, the perineurium surrounding them must be very thin. Figures 2A-5 and 2A-6 show putative segments of the fascicular sheath in adult and larval nerve, respectively.

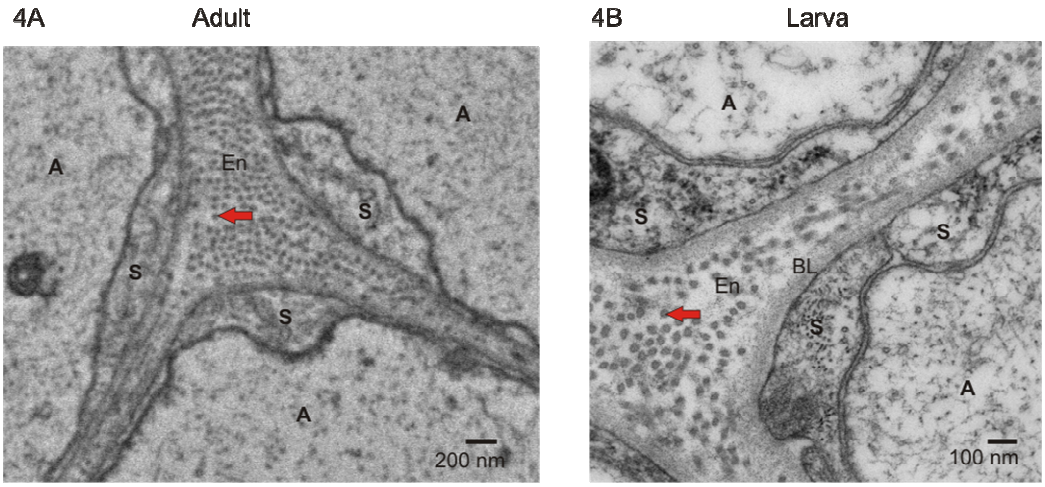

Figure 2A-4.
Enlarged view of the endoneurial tissue (En) in spaces between the axons (A) wrapped by the Schwann cells (S) in adult (4A) and larval (4B) TLLN. Arrows point to the collagen fibrils. Basal lamina (BL) surrounds the nerve fibers, seen as a dark diffuse band between the Schwann cell membrane and the collagen fibers of the endoneurium (4B).

As expected no compact myelin was observed in adult and larval TLLN. It appears that in most cases axons are wrapped by one Schwann cell, but occasionally nuclei of two Schwann cells were visible around the axon (fig. 2A-5). Sometimes no nuclei of the Schwann cell were visible, but in any case the glial membrane was always present around an axon. Putative mesaxons were observed in both adult and larval TLLN. In the larval preparation, in addition to mesaxons, gaps between the Schwann cell cytoplasmic protrusions were observed (fig. 2A-7).

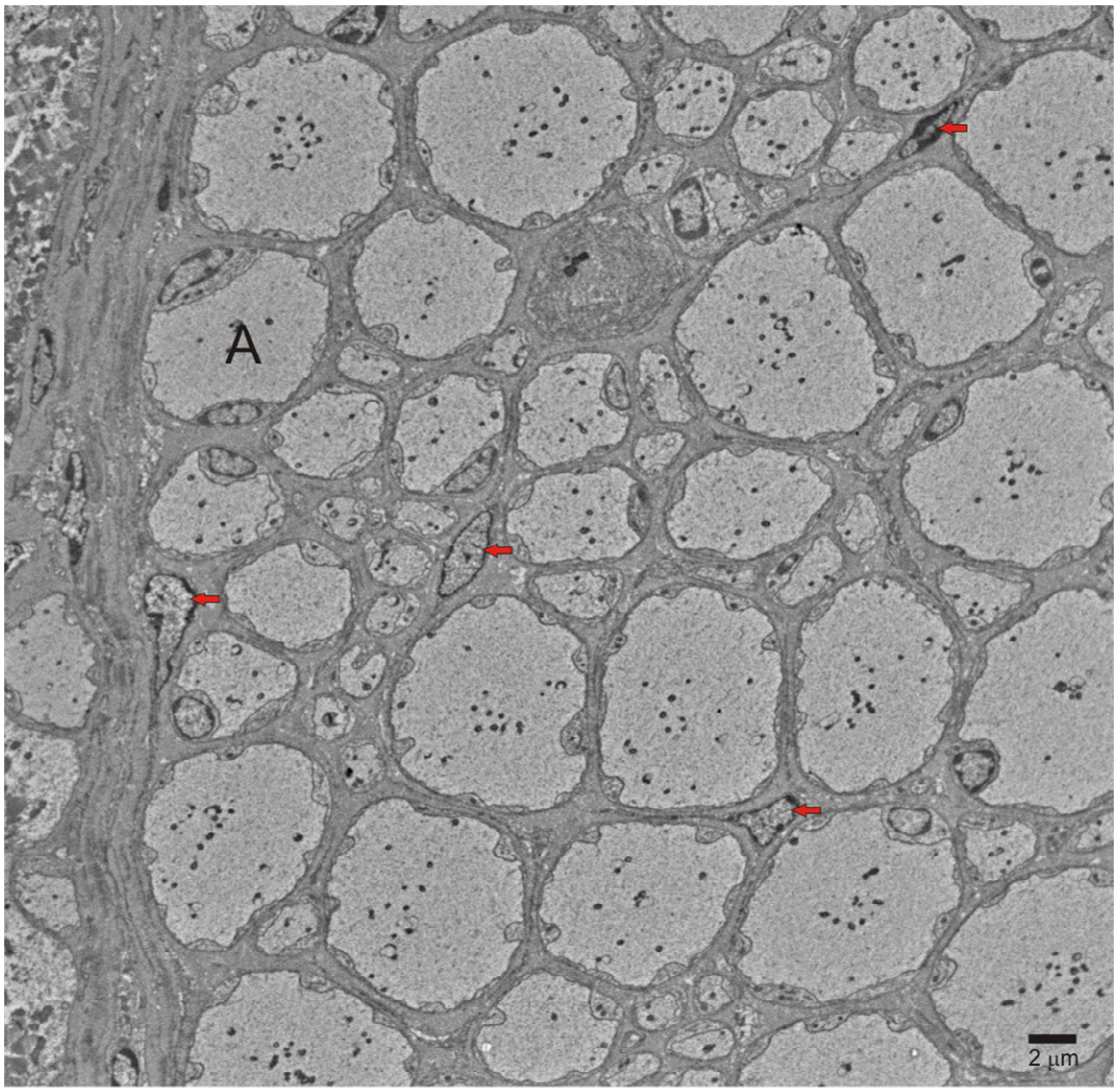

Figure 2A-5.
Fasciculation in the adult TLLN. Within this portion of the TLLN, perineurial fibroblasts are visible. The thin fibroblast processes appear to separate the nerve into fascicles. Arrows point to the elongated or fusiform nuclei of the perineurial fibroblasts. Mitochondria are visible in the cytoplasm of the axons. Outer connective sheaths are seen to the left.

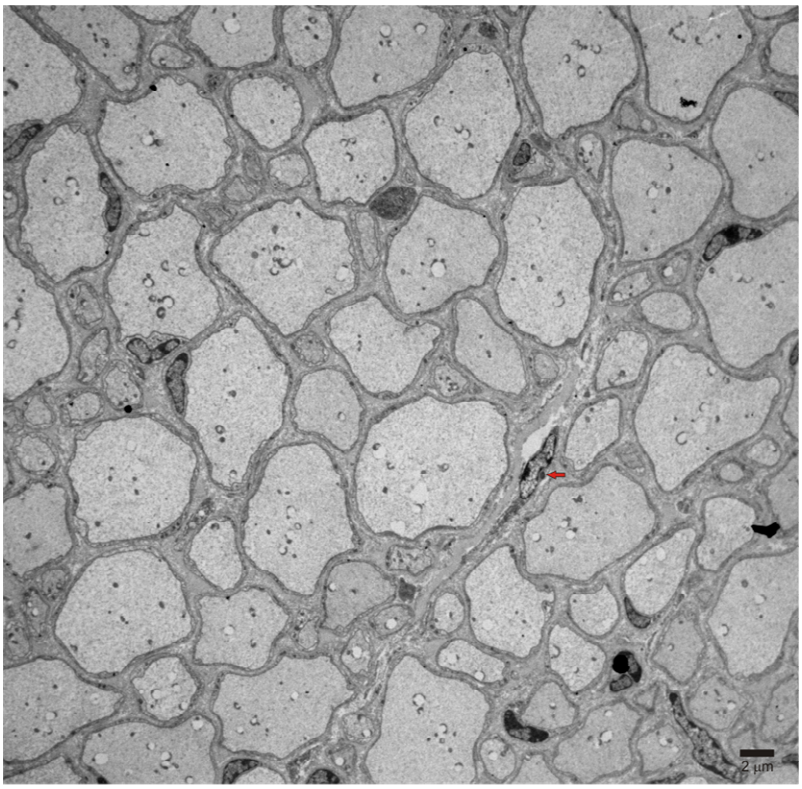

Figure 2A-6.
Fasciculation in the larval TLLN. A perineurial fibroblast with a fusiform nucleus (arrow) is seen extending the thin processes, which form the boundary of a fascicle.

A basal lamina surrounded the membranes of the Schwann cells. The endoneurial space was filled with longitudinally and transversely running collagen fibers. Rough endoplasmic reticulum studded with ribosomes was seen in the cytoplasm of Schwann cells. Occasionally pinocytotic vesicles were observed attached to the Schwann cell membrane (fig. 2A-7D). Mitochondria, microtubules and neurofilaments were present in the cytoplasm of axons (fig. 2A-8).

The majority of axons in the adult preparation were oval shaped. The larval axons were more irregularly shaped. In larval preparation, some axons had a greater density of neurofilaments, the cytoplasm of which appeared darker then others (fig. 2A-8).

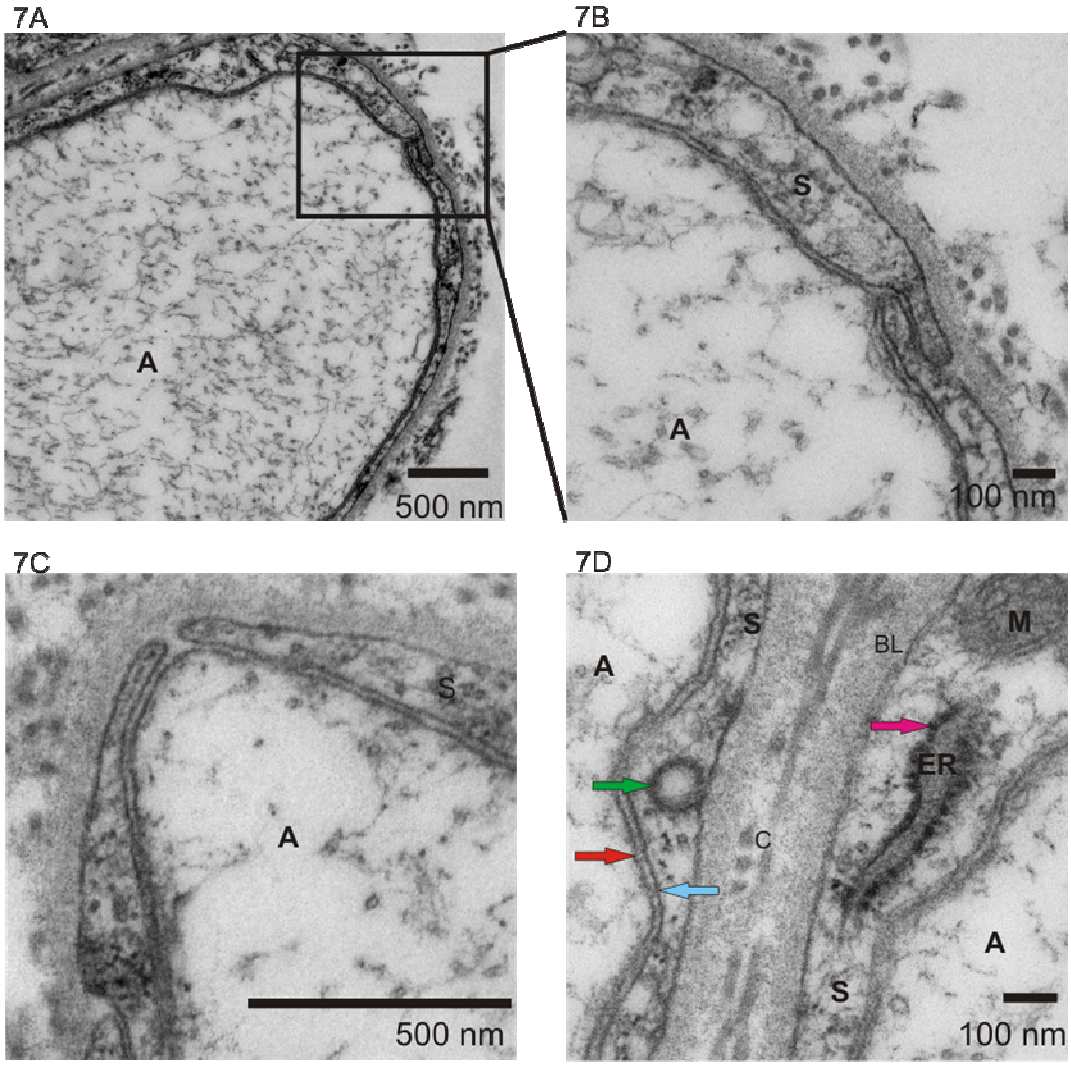

Figure 2A-7.
Schwann cell ensheathment of the axons in the larval TLLN. Each Schwann cell (S) appeared to be associated only with one axon (A).
7A. The enveloping processes of the Schwann cell overlap each other forming a parallel opposition of the cell membranes. The pair of membranes is called the *mesaxon*.
7B. Enlarged view of the mesaxon in 7A.
7C. The axon is only partially enveloped by the Schwann cell. A gap in the ensheathment was formed by an incomplete overlapping of the processes of the Schwann cell. A small portion of the axonal plasma membrane is covered only by the basal lamina (BL).
7D. Two adjacent nerve fibers. A pinocytotic vesicle (green arrow) was visible in apposition to the Schwann cell membrane. The rough endoplasmic reticulum (ER) studded with ribosomes (magenta arrow) was seen in the cytoplasm of another Schwann cell. Opposing membranes of the axon and the Schwann cell are indicated by the red and blue arrows. The basal lamina was clearly visible around each nerve fiber, separated by the layer of collagen fibers (C).

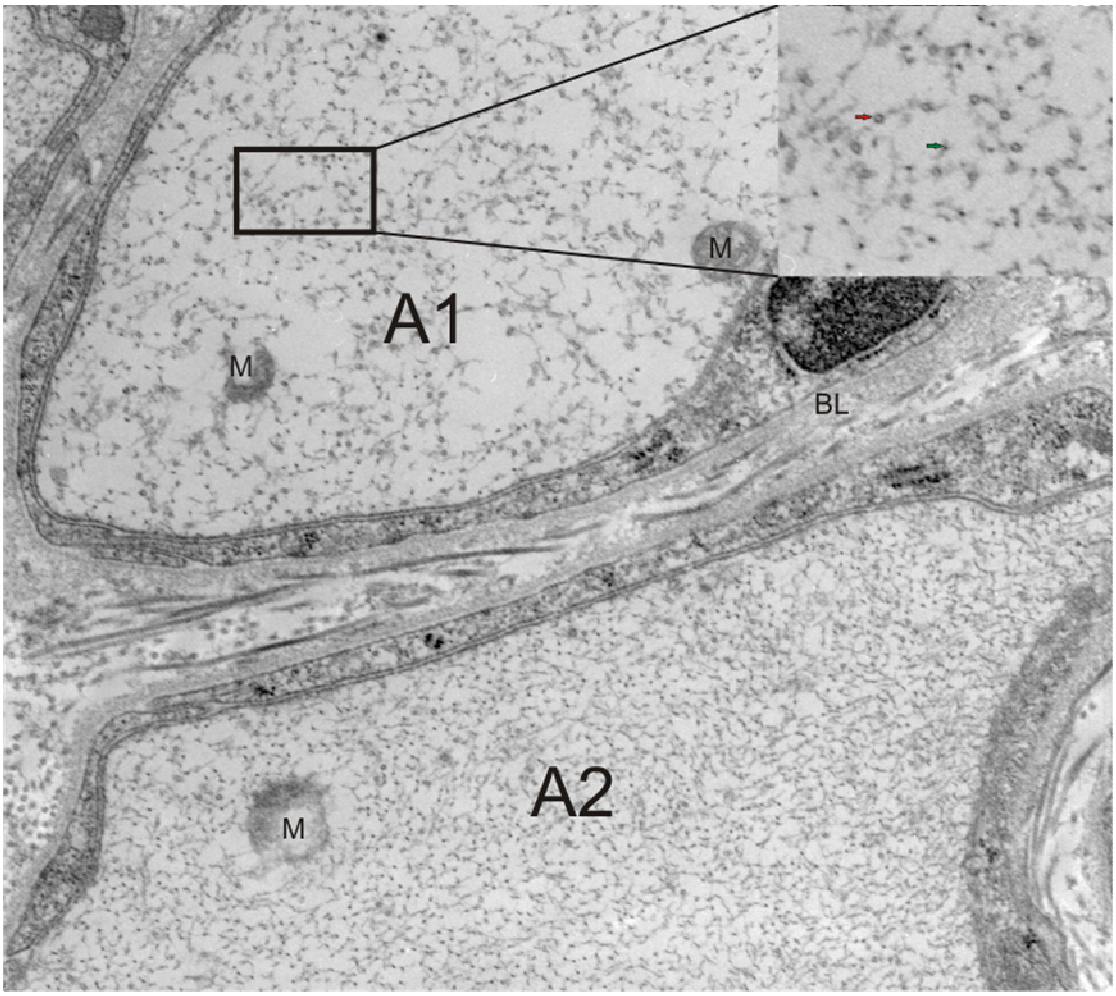

Figure 2A-7.
Axons (A1 and A2) in the larval TLLN with different densities of the neurofilaments. Mitochondria (M) are seen in the cytoplasm of each axon. Basal lamina (BL) surrounds separate nerve fibers. Inset depicts the presence of microtubules (red arrow) and neurofilaments (green arrow). Magnification x20000.

APPENDIX III: HIERARCHY OF HIGHER CATEGORIES OF FISHES

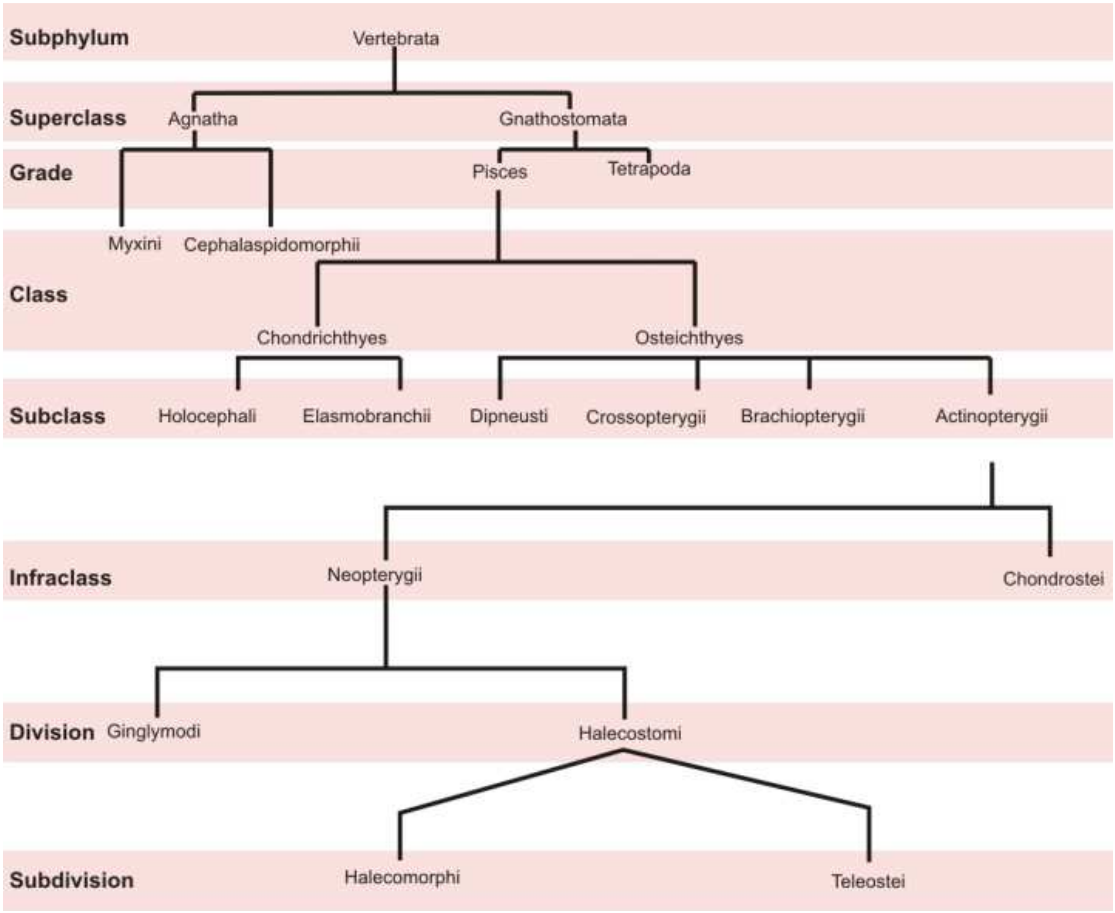

Figure 3A-1.
A simplified hierarchy of higher categories of fishes. Modified from (Nelson 1984).

BIBLIOGRAPHY

Akoev GN, Muraveiko VM (1984) Physiological properties of lateral line receptors of the lamprey. Neurosci Lett 49:171-173

Andersson O, Forssberg H, Grillner S, Wallén P (1981) Peripheral feedback mechanisms acting on the central pattern generators for locomotion in fish and cat. Canadian Journal of Physiology and Pharmacology 59:713-726

Baker CF, Montgomery JC (1999) The sensory basis of rheotaxis in the blind Mexican cave fish, *Astyanax fasciatus*. J Comp Physiol A 184:519-527

Balinsky BI (1965) An Introduction to Embryology. W. B. Saunders Company, Philadelphia and London

Bardack D, Zangerl R (1968) First Fossil Lamprey: A Record from the Pennsylvanian of Illinois. Science 162:1265-1267

Bleckmann H (1988) Prey identification and prey localization in surface-feeding fish and fishing spiders. In: Atema J, Fay RR, Popper AN, Tavolga WN (eds) Sensory biology of aquatic animals. Springer-Verlag, New York, pp 619-641

Bleckmann H (1994) Reception of hydrodynamic stimuli in aquatic and semiaquatic animals. In: Rathmayer W (ed) Progress in Zoology. Gustav Fischer, Stuttgart, Jena, New York pp 1-115

Bleckmann H (2004) 3-D-orientation with the octavolateralis system. J Physiol (Paris) 98:53-65

Bleckmann H, Tittel G, Blubaum-Gronau E (1989) The lateral line system of surface-feeding fish: anatomy, physiology, and behavior. In: Coombs S, Gorner P,

Munz H (eds) The mechanosensory lateral line: neurobiology and evolution. Springer-Verlag, New York, pp 501-526

Bodznick D, Northcutt RG (1981) Electroreception in lampreys: evidence that the earliest vertebrates were electroreceptive. Science 212:465-467

Bodznick D, Preston DG (1983) Physiological characterization of electroreceptors in the lampreys *Ichthyomyzon unicuspis* and *Petromyzon marinus*. J Comp Physiol A 152:209-217

Bodznick D, Schmidt AW (1984) Somatotopy within the medullary electrosensory nucleus of the little skate, *Raja erinacea*. J Comp Neurol 225:581-590

Boord RL, Campbell CBG (1977) Structural and functional organization of the lateral line system of sharks. Am Zool 17:431-441

Braun CB (1996) The sensory biology of the living jawless fishes: a phylogenetic assessment. Brain Behav Evol 48:262-276

Breder CM (1926) The locomotion of fishes. Zoologica 4:159-297

Bullock TH, Bodznick D, Northcutt RG (1983) The phylogenetic distribution of electroreception: evidence for convergent evolution of a primitive vertebrate sense modality. Brain Research Reviews 6:25-46

Bullock TH, Moore JK, Fields RD (1984) Evolution of myelin sheaths: Both lamprey and hagfish lack myelin. Neurosci Lett 48:145-148

Cahn PH, Shaw E (1963) Lateral line activity in rheotactic orientation of schooling fishes. Am Zool 3:168

Campenhausen C, Riess I, Weissert R (1981) Detection of stationary objects by the blind cave fish *Anoptichthys jordani* (Characidae). J Comp Physiol A 143:369-374

Carr CE, Maler L, Sas E (1982) Peripheral organization and central projections of the electrosensory nerves in gymnotiform fish. J Comp Neurol 211:139-153

Chang M-m, Zhang J, Miao D (2006) A lamprey from the Cretaceous Jehol biota of China. Nature 441:972-974

Cochran PA, Gripentrog AP (1992) Aggregation and spawning by lampreys (genus *Ichthyomyzon*) beneath cover. Environmental Biology of Fishes 33:381-387

Cohen AH (1988) Evolution of the vertebrate central pattern generator for locomotion. In: Cohen AH, Rossignol S, Grillner S (eds) Neural control of rhythmic movements in vertebrates. Wiley-Interscience, New York, pp 129-166

Cohen AH, Boothe DL (1999) Sensorimotor interactions during locomotion: Principles derived from biological systems. Autonomous Robots 7:239-245

Coombs S, Braun CB, Donovan B (2001) The orienting response of Lake Michigan mottled sculpin is mediated by canal neuromasts. J Exp Biol 204:337-348

Coombs S, Janssen J, Webb JF (1988) Diversity of lateral line systems: evolutionary and functional considerations. In: Atema J, Fay, R. R., Popper, A. N., Tavolga, W. N. (ed) Sensory biology of aquatic animals. Springer-Verlag New York, pp 553-586

Deliagina TG, Ullen F, Gonzalez MJ, Ehrsson H, Orlovsky GN, Grillner S (1995) Initiation of locomotion by lateral-line photoreceptors in lamprey - behavioral and neurophysiological studies. J Exp Biol 198:2581-2591

Dijkgraaf S (1963) The functioning and significance of the lateral-line organs. Biol Rev 38:51-105

Dijkgraaf S (1967) Biological significance of the lateral line organs. In: Cahn P (ed) Lateral Line Detectors. Indiana University Press, Bloomington, pp 83-95

Dijkgraaf S (1989) A short personal review of the history of lateral line research. In: Coombs S, Gorner P, Munz H (eds) The mechanosensory lateral line: neurobiology and evolution. Springer-Verlag, New York, pp 7-14

Engelmann J, Hanke W, Bleckmann H (2002) Lateral line reception in still- and running water. J Comp Physiol A 188:513-526

Engelmann J, Hanke W, Mogdans J, Bleckmann H (2000) Hydrodynamic stimuli and the fish lateral line. Nature 408

Finger TE (1980) Nonolfactory sensory pathway to the telencephalon in a teleost fish. Science 210:671-673

Flock A (1967) Ultrastructure and function in lateral line organs. In: Cahn PH (ed) Lateral Line Detectors Indiana University Press., Bloomington London, pp 27-44

Fraher J (2002) Axons and glial interfaces: Ultrastructural studies. J Anat 200:415-430

Fraher J, Cheong E (1995) Glial-Schwann cell specialisations at the central-peripheral nervous system transition of a cyclostome: An ultrastructural study. Acta Anat 154:300-314

Francis ETB, Horton FM (1936) Some Reactions of the Ammocoete. J Exp Biol 13:410-415

Fritzsch B, de Caprona MDC, Wachtler K, Kortje KH (1984) Neuroanatomical evidence for electroreception in lampreys. Z Naturforsch 39:856-858

Gelman S, Ayali A, Tytell ED, Cohen AH (2007) Larval lampreys possess a functional lateral line system. J Comp Physiol A 193:271-277

Gess RW, Coates MI, Rubidge BS (2006) A lamprey from the Devonian period of South Africa. Nature 443:981-984

Gonzalez MJ, Anadon R (1992) Primary projections of the lateral line nerves in larval sea lamprey, *Petromyzon marinus* L - an HRP study. J Hirnforsch 33:185-194

Gorner P (1973) The importance of the lateral line system for the perceptiuon of surface waves in the clawed toad, *Xenopus laevis* Daudin. Experientia 29:295-296

Gray J (1933a) Studies in Animal Locomotion: I. The Movement of Fish with Special Reference to the Eel. J Exp Biol 10:88-104

Gray J (1933b) Studies in animal locomotion: II. The relationship between waves of muscular contraction and the propulsive mechanism of the eel. J Exp Biol 10:386-390

Gray J (1933c) Studies in animal locomotion: III. The propulsive mechanism of the whiting (*Gadus merlangus*). J Exp Biol 10:391-400

Grillner S, Deliagina T, Ekeberg Ö, Elmanira A, Hill RH, Lansner A, Orlovsky GN, Wallén P (1995) Neural networks that coordinate locomotion and body orientation in lamprey. Trends in Neurosciences 18:270-279

Grillner S, Ekeberg Ö, El Manira A, Lansner A, Parker D, Tegner J, Wallén P (1998a) Intrinsic function of a neuronal network - a vertebrate central pattern generator. Brain Research Reviews 26:184-197

Grillner S, McClellan A, Perret C (1981) Entrainment of the spinal pattern generators for swimming by mechanosensitive elements in the lamprey spinal cord in vitro. Brain Res 217:380-386

Grillner S, McClellan A, Sigvardt K (1982) Mechanosensitive neurons in the spinal-cord of the lamprey. Brain Res 235:169-173

Grillner S, Parker D, El Manira A (1998b) Vertebrate locomotion - a lamprey perspective. Ann NY Acad Sci 860:1-18

Grillner S, Williams T, Lagerback PA (1984) The edge cell, a possible intraspinal mechanoreceptor. Science 223:500-503

Guan L, Kiemel T, Cohen AH (2001) Impact of movement and movement-related feedback on the lamprey central pattern generator for locomotion. J Exp Biol 204:2361-2370

Hama K, Yamada Y (1977) Fine structure of the ordinary lateral line organ. II. The lateral line canal organ of spotted shark, *Mustelus manazo*. Cell Tissue Res 176:23-36

Hardisty MW (1979) Biology of the Cyclostomes. Chapman and Hall Ltd., London

Hardisty MW, Potter IC (1971a) The behaviour, ecology, and growth of larval lampreys. In: Hardisty MW, Potter IC (eds) The biology of lampreys. Academic Press, New York, pp 85-127

Hardisty MW, Potter IC (1971b) The general biology of adult lampreys. In: Hardisty MW, Potter IC (eds) The Biology of Lampreys. Academic Press, London, New York, pp 127-206

Hardisty MW, Potter IC (1971c) Paired species. In: Hardisty MW, Potter IC (eds) The Biology of Lampreys. Academic Press, London, New York, pp 249-273

Hassan ES (1989) Hydrodynamic imaging of the surroundings by the lateral line of the blind cave fish *Anoptichthys jordani*. In: Coombs S, Gorner P, Munz H (eds) The mechanosensory lateral line: neurobiology and evolution. Springer-Verlag, New York, pp 217-227

Hassan ES, Abdel-Latif H, Biebricher R (1992) Studies on the effects of Ca^{2+} and Co^{2+} on the swimming behavior of the blind Mexican cave fish. J Comp Physiol A 171:413-419

Hoagland H (1933) Electrical responses from the lateral line nerves of catfish. J Gen Physiol 16:695

Hubbs CL, Potter IC (1971) Distribution, phylogeny and taxonomy. In: Hardisty MW, Potter IC (eds) The Biology of Lampreys. Academic Press, London

Huesa G, Anadón R, Yáñez J (2003) Afferent and efferent connections of the cerebellum of the chondrostean *Acipenser baeri*: A carbocyanine dye (DiI) tracing study. J Comp Neurol 460:327-344

Janssen J (2000) Toxicity of Co^{2+}: implication for the lateral line studies. J Comp Physiol A 186:957-960

Janssen J, Coombs S, Pride S (1990) Feeding and orientation of mottled sculpin, *Cottus bairdi*, to water jets. Env Biol Fish 29:43-50

Janvier P (2006) Palaeontology: Modern look for ancient lamprey. Nature 443:921-924

Janvier P, Lund R (1983) *Hardistiella montanensis* n. ge. et sp. (Petromyzontida) from the Lower Carboniferous of Montana, with remarks on the affinities of the lampreys. Journal of Vertebrate Paleontology 2:407-413

Johnston JB (1905) The cranial nerve components of *Petromyzon*. Morph Jb 34:149-203

Kalmijn AJ (1988) Hydrodynamic and acoustic field detection. In: Atema J, Fay, R. R., Popper, A. N., Tavolga, W. N. (ed) Sensory biology of aquatic animals. Springer-Verlag, New York, pp 83-130

Kanter MJ, Coombs S (2003) Rheotaxis and prey detection in uniform currents by Lake Michigan mottled sculpin (*Cottus bairdi*). J Exp Biol 206:59-70

Karlsen HE, Sand O (1987) Selective and reversible blocking of the lateral line in freshwater fish. J Exp Biol 133:249-262

Katori Y, Takasaka T, Ishikawa M, Tonosaki A (1994) Fine structure and lectin histochemistry of the apical surface of the free neuromast of *Lampetra japonica*. Cell Tissue Res 276:245-252

Key AH, Retzius G (1876) Studien in der Anatomie des Nervensystems und des Bindegewebes. Samson & Wallin, Stockholm

Kiemel T, Cohen AH (2001) Bending the lamprey spinal cord causes a slowly-decaying increase in the frequency of fictive swimming. Brain Res 900:57-64

Kleerekoper H, Sibakin K (1956) An investigation of the electric 'spike' potentials produced by the sea lamprey (*Petromyzon marinus*) in the water surrounding the head region. Journal of Fisheries Research Board of Canada 13:375-383

Knudsen EI (1977) Distinct auditory and lateral line nuclei in the midbrain of catfishes. J Comp Neurol 173:417-432

Koyama H, Kishida R, Goris RC, Kusunoki T (1990) Organization of the primary projections of the lateral line nerves in the lamprey *Lampetra japonica*. J Comp Neurol 295:277-289

Kroese ABA, Schellart NAM (1987) Evidence for velocity-sensitive and acceleration-sensitive units in the trunk lateral line of the trout. J Physiol (London) 394:P13-P13

Kroese ABA, Schellart NAM (1992) Velocity- and acceleration-sensitive units in the trunk lateral line of the trout. J Neurophysiol 68:2212-2221

Kroese ABA, van der Bercken J (1982) Effects of ototoxic antibiotics on sensory hair cell functioning. Hearing Res 6:183-197

Kroese ABA, van der Zalm JM, van der Bercken J (1978) Frequency response of the lateral line organ of *Xenopus laevis*. Pflugers Arch 375:167-175

Lane EB, Whitear M (1982) Sensory structure at the surface of fish skin. II. Lateralis System. Zool J Linn Soc-Lond 76:19-28

Lannoo MJ (1987) Neuromast topography in anuran amphibians. J Morphol 191:115-129

Lauder GV, Tytell ED (2004) Three Gray classics on the biomechanics of animal movement. J Exp Biol 207:1597-1599

Ledent V (2002) Postembryonic development of the posterior lateral line in zebrafish. Development 129:597-604

Liao JC (2006) The role of the lateral line and vision on body kinematics and hydrodynamic preference of rainbow trout in turbulent flow. J Exp Biol 209:4077-4090

Maler L, Finger T, Karten HJ (1973a) The central connections of the anterior lateral line nerve of *Gnathonemus petersi*. J Comp Neurol 151:67-85

Maler L, Karten HJ, Bennett MVL (1973b) The central connections of the posterior lateral line nerve of *Gnathonemus petersi*. J Comp Neurol 151:57-66

Manion PJ, Stauffer TM (1970) Metamorphosis of the landlocked sea lamprey, *Petromyzon marinus*. J Fish Res Board Can 27:1735-1746

Maruska KP, Tricas TC (2004) Test of the mechanotactile hypothesis: neuromast morphology and response dynamics of mechanosensory lateral line primary afferents in the stingray. J Exp Biol 207:3463-3476

McClellan A, Sigvardt K (1988) Features of entrainment of spinal patern generators for locomotor activity in the lamprey. J Neurosci 8:133-145

McClellan AD, Jang WC (1993) Mechanosensory Inputs to the Central Pattern Generators for Locomotion in the Lamprey Spinal-Cord - Resetting, Entrainment, and Computer Modeling. Journal of Neurophysiology 70:2442-2454

McCormick CA (1981a) Central projections of the lateral line and eigth nerves in the bowfin, *Amia calva*. Journal of Comparative Neurolology 197:1-15

McCormick CA (1981b) The organization of the octavolateralis area in actinopterygian fishes: A new interpretation. J Morphol 171:159-181

McCormick CA (1983) Organization and evolution of the octavolateralis area in fishes. In: Northcutt GR, Davis, R. E. (ed) Fish Neurobiology. The University of Michigan Press, Ann Arbor, pp 179-213

Meredith GE (1984) Peripheral configuration and central projections of the lateral line system in *Astronotus ocellatus* (Cichlidae): a noneletroreceptive teleost. J Comp Neurol 228:342-358

Millonig G (1961) A modified procedure for lead staining of thin sections. The Journal of Cell Biology 11:736-739

Millonig G (1962) Further observations on a phosphate buffer for osmium solutions in fixation. In: Breese S, S., Jr. (ed) Fifth International Congress For Electron Microscopy. Academic Press New York, p 2

Montgomery JC, Baker CF, Carton AG (1997) The lateral line can mediate rheotaxis in fish. Nature 389:960-963

Montgomery JC, Macdonald JA (1987) Sensory tuning of lateral line receptors in antarctic fish to the movement of planktonic prey. Science 235:195-196

Munz H (1979) Morphology and innervation of the lateral line system in *Sarotherodon niloticus*. Zoomorphologie 93:73-86

Munz H (1985) Single unit activity in the peripheral lateral line system of the cichlid fish *Sarotherodon niloticus* L. J Comp Neurol 157:555-568

Munz H (1989) Functional organization of the lateral line periphery. In: Coombs S, Gorner, P., Münz, H. (ed) The mechanosensory lateral line: neurobiology and evolution. Springer-Verlag, New York, pp 285-298

Nakao T, Ishizawa A (1987) Development of the Spinal Nerves of the Larval Lamprey IV. Spinal Nerve Roots of 21-Mm Larval and Adult Lampreys with Special Reference to the Relation of Meninges with the Root Sheath and the Perineurium. J Comp Neurol 256:386-399

Nelson SJ (1984) Fishes of the world. John Wiley & Sons, New York

New JG, Northcutt RG (1984) Central projections of the lateral line nerves in the shovelnose sturgeon. J Comp Neurol 225:129-140

Nickerson MA, Ashton RE, JR.,, Braswell AL (1983) Lampreys in the diet of the hellbender *Cryptobranchus alleganiensis* (Daudin), and the Neuse River waterdog *Necturus lewisi* (Brimley). Herpetological Review 14:10

Nieuwenhuys R (1972) Topological analysis of the brainstem of the lamprey *Lampetra fluviatilis*. J Comp Neurol 145:165-178

Nieuwenhuys R (1982) An overview of the organization of the brain of actinopterygian fishes. Am Zool 22:287-310

Nieuwenhuys R, Nicholson C (1998) Lampreys, Petromyzontoidea. In: Nieuwenhuys R, ten Donkelaar, H. J., Nicholson, C. (ed) The Central Nervous System of Vertebrates. Springer Verlag, Berlin, Heidelberg

Northcutt GR (1989) The phylogenetic distribution and innervation of craniate mechanoreceptive lateral lines. In: Coombs S, Gorner, P., Munz, H. (ed) The

mechanosensory lateral line: neurobiology and evolution. Springer-Verlag, New York, pp 17-78

Northcutt GR (1997) Swimming against the current. Nature 389:915-916

Northcutt RG (1983) The primary lateral line afferents in lepidosirenid lungfishes. Society for Neuroscience 9:1167

Partridge BL, Pitcher TJ (1980) The sensory basis of fish schools: relative roles of lateral line and vision. J Comp Physiol A 135:315-325

Peach M, B.,, Rouse G, W. (2000) The morphology of the pit organs and lateral line canal neuromasts of *Mustelus antarcticus* (Chondrichthyes: Triakidae). Journal of the Marine Biological Association of UK 80:155-162

Peters A (1960) The structure of the peripheral nerves of the lamprey (*Lampetra fluviatilis*). J Ultrastruct Res 4:349-359

Peters A, Palay SL, Webster H, deF. (1991) The Fine Structure of the Nervous System. Oxford University Press, New York Oxford

Pichon F, Ghysen A (2004) Evolution of posterior lateral line development in fish and amphibians. Evolution and Developement 6:187-193

Pitcher TJ, Partridge BL, Wardle CS (1976) A blind fish can school. Science 194:963-965

Pohlmann K, Atema J, Breithaupt T (2004) The importance of the lateral line in nocturnal predation of piscivorous catfish. J Exp Biol 207:2971-2978

Potter IC, Wright GM, Youson JH (1978) Metamorphosis in the anadromous sea lamprey, *Petromyzon marinus* L. Canadian Journal of Zoology 56:561-570

Puzdrowski RL (1989) Peripheral distribution and central projections of the lateral line nerves in goldfish, *Carassius auratus*. Brain Behav Evol 34:110-131

Roberts BL (1972) Activity of Lateral-Line Sense Organs in Swimming Dogfish. J Exp Biol 56:105-118

Roberts BL, Russell IJ (1972) The Activity of Lateral-Line Efferent Neurones in Stationary and Swimming Dogfish. J Exp Biol 57:435-448

Ronan M (1986) Electroreception in cyclostomes. In: Bullock TH, Heiligenberg W (eds) Electroreception. John Wiley & Sons, Inc., New York, pp 209-224

Ronan M (1988) Anatomical and physiological evidence for electroreception in larval lampreys. Brain Res 448:173-177

Ronan M, Bodznick D (1986) End buds: non-ampullary electroreceptors in adult lampreys. J Comp Physiol A 158:9-15

Ronan M, Bodznick D (1991) Behavioral and Neurophysiological Demonstration of a Lateralis Skin Photosensitivity in Larval Sea Lampreys. J Exp Biol 161:97-117

Ronan M, Northcutt RG (1987) Primary projections of the lateral line nerves in adult lampreys. Brain Behav Evol 30:62-61

Rovainen CM (1971) Neurophysiology. In: Hardisty MW, Potter, I.C. (ed) The Biology of Lampreys. Academic Press, Inc., New York, pp 65-69

Rovainen CM (1982) Neurophysiology. In: Hardisty MW, Potter, I. C. (ed) The biology of lampreys. Academic Press, London, pp 1-136

Russell IJ, Roberts BL (1972) Inhibition of Spontaneous Lateral-Line Activity by Efferent Nerve Stimulation. J Exp Biol 57:77-82

Sand O (1975) Effects of different ionic environments on the mechano-sensitivity of lateral line organs in the mudpuppy. J Comp Physiol A 102:27-42

Sapede D, Gompel N, Dambly-Chaudiere C, Ghysen A (2002) Cell migration in the postembryonic development of the fish lateral line. Development 129:605-615

Satou M, Takeuchi HA, Nishii J, Tanabe M, Kitamura S, Okumoto N, Iwata M (1994) Behavioral and electrophysiological evidences that the lateral line is involved in the inter-sexual vibrational communication of the himε salmon (landlocked red salmon, *Oncorhynchus nerka*). J Comp Physiol A 174:539-549

Schellart NAM, Wubbels RJ (1998) The auditory and mechanosensory lateral line system. In: Evans DH (ed) The physiology of fishes. CRC Press, Boca Raton New York, pp 283-312

Schultz R, Berkowitz EC, Pease DC (1956) The electron microscopy of the lamprey spinal cord. J Morphol 96:251-274

Shelton PMJ (1970) The lateral line system at metamorphosis in *Xenopus laevis* (Daudin). J Embryol exp Morph 24:511-524

Shelton PMJ (1971) The structure and function of the lateral line system in larval *Xenopus laevis*. Journal of Experimental Zoology 178:211-231

Song J, Yan, H.Y., Popper, A.N. (1995) Damage and recovery of hair cells in fish canal (but notsuperficial) neuromasts after gentamicin exposure. . Hearing Res 91:63-71

Sorensen EM (1991) Metal poisoning in fish. CRC Press, Boca Raton

Steven DM (1950) Some Properties of the Photoreceptors of the Brook Lamprey. J Exp Biol 27:350-364

Steven DM (1951) Sensory cells and pigment distribution in the tail of the ammocoete. Quarterly Journal of Microscopical Science 92:233-247

Triantafyllou MS, Triantafyllou GS, Yue DKP (2000) Hydrodynamics of fishlike swimming. Annual Review of Fluid Mechanics 32:33-53

Tytell ED (2004) The hydrodynamics of eel swimming II. Effect of swimming speed. J Exp Biol 207:3265-3279

Tytell ED, Lauder GV (2004) The hydrodynamics of eel swimming: I. Wake structure. J Exp Biol 207:1825-1841

van Netten SM (2005) Hydrodynamic detection by cupulae in a lateral line canal: functional relations between physics and physiology. Biol Cybern:1-19

van Netten SM, Kroese ABA (1987) Laser interferometric measurements on the dynamic behavior of the cupula in the fish lateral line. Hearing Res 29:55-61

van Netten SM, Kroese ABA (1989) Dynamic behavior and micromechanical properties of the cupula. In: Coombs S, Gorner P, Munz H (eds) The mechanosensory lateral line: neurobiology and evolution. Springer-Verlag

Viana di Prisco G, Pearlstein E, Le Ray D, Robitaille R, Dubuc R (2000) A cellular mechanism for the transformation of a sensory input into a motor command. J Neurosci 20:8169-8176

Viana Di Prisco G, Wallen P, Grillner S (1990) Synaptic effects of intraspinal stretch receptor neurons mediating movement-related feedback during locomotion. Brain Res 155:182-186

Videler JJ (1993) Fish Swimming. Chapman & Hall, London

Voigt R, Carton AG, Montgomery JC (2000) Responses of anterior lateral line afferent neurons to water flow. J Exp Biol 203:2495-2502

Wald G (1958) The significance of vertebrate metamorphosis. Science 128:1481-1490

Wallen P, Williams TL (1984) Fictive locomotion in the lamprey spinal cord in vitro compared with swimming in the intact and spinal animal. Journal of Physiology (Lond) 347:225-239

Webb JF, Northcutt RG (1997) Morphology and distribution of pit organs and canal neuromasts in non-teleost bony fishes. Brain Behav Evol 50:139-151

Weissert R, Campenhausen C (1981) Discrimination between stationary objects by the blind cave fish *Anoptichthys jordani* (Characidae). J Comp Physiol A 143:375-381

Whitear M, Lane EB (1981) Bar synapses in the end buds of lamprey skin. Cell Tissue Res 216:445-448

Whitear M, Lane EB (1983) Multivillous cells: Epidermal sensory cells of unknown function in lamprey skin. Journal of Zoology (London) 201:259-272

Williams T, Grillner S, Smoljaninov VV, Wallén P, Kashin S, Rossignol S (1989) Locomotion in lamprey and trout: The relative timing of activation and movement. J Exp Biol 143:559-566

Wubbels RJ, Kroese ABA, Duifhuis H (1990) Afferent bursting activity of ruff lateral line induced by background noise stimulation. J Comp Neurol 166:585-588

Yamada Y (1973) Fine structure of the ordinary lateral line organ. I. The neuromast of lamprey, *Entosphenus japonicus*. J Ultrastruct Res 43:1-17

Young JZ (1935a) The photoreceptors of lampreys I. Light-sensitive fibres in the lateral line nerves. J Exp Biol 12:229-238

Young JZ (1935b) The Photoreceptors of Lampreys: II. The Functions of the Pineal Complex. J Exp Biol 12:254-270

Youson JH (1980) Morphology and physiology of lamprey metamorphosis. Can J Fish Aquat Sci 37:1687-1710