1-1-2003

The Neural Connectivity of the Torus Semicircularis in the Cave Dwelling Form of Astyanax Fasciatus

Timothy B. Angel

THE NEURAL CONNECTIVITY OF THE TORUS SEMICIRCULARIS

IN THE CAVE DWELLING FORM OF

ASTYANAX FASCIATUS

by

Timothy B. Angel

Dissertation submitted to
The Graduate College
of
Marshall University
In partial fulfillment of the requirements
For the degree of

Doctor of Philosophy
in
Biomedical Science

Approved by

Stephen E. Fish, Ph.D., Committee Chairperson
Mitchell L. Berk, Ph.D.
Todd L. Green, Ph.D.
Lawrence M. Grover, Ph.D.
William B. Rhoten, Ph.D.

Department of Anatomy, Cell, and Neurobiology

APPROVAL OF EXAMINING COMMITTEE

_____
Mitchell L. Berk, Ph.D.

_____
Todd L. Green, Ph.D.

_____
Lawrence M. Grover, Ph.D.

_____
William B. Rhoten, Ph.D.

_____
Date

_____
Stephen E. Fish, Ph.D., Chair

Accepted by Graduate College

_____
Date

_____
Leonard J. Deutsch, Ph.D., Dean

ABSTRACT

THE NEURAL CONNECTIVITY OF THE TORUS SEMICIRCULARIS IN THE CAVE DWELLING FORM OF *ASTYANAX FASCIATUS*

by Timothy B. Angel

Afferent projections of the torus semicircularis were traced in the blind cave form of *Astyanax fasciatus* using HRP tract-tracing techniques, and the results were compared to those found in previous studies of closely related sighted teleosts.

Following unilateral injections of tracer to the nucleus centralis of the torus semicircularis, retrogradely labeled cells were found in two octaval nuclei of the medulla. The descending octavolateralis nucleus was labeled bilaterally and the anterior octaval nucleus was labeled primarily on the ipsilateral side. Both the descending and anterior octavolateralis nuclei are known to receive auditory input from CN VIII in sighted fish. In addition, labeled commissural fibers could be traced across the midline to labeled cells in the nucleus centralis of the contralateral torus semicircularis.

Large Purkinje-like neurons of the contralateral medial octavolateralis nucleus of the medulla were labeled with HRP injections to the nucleus lateralis. The medial octavolateralis nucleus receives input from the anterior and posterior lateral line nerves known to carry mechanosensory information to the CNS. In addition, labeled cells in the contralateral descendens nucleus of V in the caudal medulla and in the ipsilateral ventromedial nucleus of the thalamus were found. Both of these nuclei process somatosensory information. Labeled cells were also located in the anterior tuberal nucleus of the hypothalamus and minor nuclei of the mesencephalon.

These results suggest that the neural input to the torus semicircularis in cave *A. fasciatus* differ little, if any, from those previously found in sighted teleosts studied. Further, these findings also suggest that the torus semicircularis functions as a multimodal processing center with auditory input from the descending and anterior octavolateralis nuclei of the medulla, mechanosensory information relayed by the medial octavolateralis nucleus, and somatosensory input from the descendens nucleus of V and the ventromedial nucleus of the thalamus.

In Loving Memory of My Mother

Opal Lorraine "Connie" Angel
1935-2001

ACKNOWLEDGMENTS

Many people are instrumental to one's success in reaching goals in life, however a select few truly stand out. I would first like to acknowledge the one person in my life that was always there for encouragement and guidance, no matter how difficult the situation. That person was my mother, who I lost during the course of my graduate career. She was my best friend and I miss her dearly.

Those who have been so understanding and supportive through some really tough times during graduate school will remain forever in my memory. Dr. William Rhoten was the one who encouraged my continuance in the doctoral program after a trying first semester. If it had not been for him, I would have walked away. Dr. Rhoten will always be special to me.

To other members of the Department, past and present, Sharon France, Dr. Tracy Soltez, Dr. Sasha Zill, Dr. Rudy Wang, Dr. Laura Richardson, Karen Lucas and Mr. Aslam Chaudhry, thank you.

To my committee members Dr. Todd Green, Dr. Larry Grover, Dr. William Rhoten, and Dr. Mitchell Berk who have been wonderful. I appreciate your support, suggestions, and understanding and I am glad you have been such an important part of my life. A special thanks goes to Dr. Patrick Brown who, by retirement, did not continue on the committee. Thanks Dr. Brown for your guidance and input.

I will never be able to thank enough the most important person in my graduate education, Dr. Stephen Fish, my teacher, mentor, and friend enough. He has been a blessing to my graduate education from the beginning to the end and without him completion of this degree would have never been possible. Steve has always been there through the highs and lows of graduate school and even in my personal life when tragedy struck my family. Without his support and encouragement, I would never have returned to finish the degree. I owe it all to him and will never forget him. He has been so much more than a teacher. He is a true friend.

Finally, special thanks goes to my many personal friends and family who have offered their encouragement and support over the course of this work and especially to Stan who has been there through the best and the worst of times.

TABLE OF CONTENTS

ABSTRACT……..……iii

DEDICATION…….……...……v

ACKNOWLEDGMENTS……...……...…..vi

TABLE OF CONTENTS…….…….……...……..viii

LIST OF FIGURES…….…….ix

LIST OF SYMBOLS ……...……...…...……xi

INTRODUCTION……..…1

REVIEW OF LITERATURE……...……4

MATERIALS AND METHODS……26

RESULTS…….……34

DISCUSSION……...……72

CONCLUSIONS……...……77

BIBLIOGRAPHY……...……..80

LIST OF FIGURES

Figure 1 Line drawing of the lateral view of brain of cave *A. fasciatus*, atlas section of planes, principal regions discussed……..……...……38

Figure 2A Atlas section of HRP injection site to the NC of the TS…….…...……39

Figure 2B Photos of HRP injection site to the NC of the TS……...…….……..….40

Figure 3A Atlas section of the AON and DON of the medulla…...…..…….…….41

Figure 3B Photos of AON and DON of the medulla……..……42

Figure 4A Atlas section of the DON bilaterally……..……43

Figure 4B Photo of the DON bilaterally……...……..44

Figure 5A Atlas section of the AON……...……45

Figure 5B Photo of the AON……..46

Figure 6 Photos of combination of the AON and DON…….…..47

Figure 7A Atlas section of RF in the medulla ……..……..48

Figure 7B Photo of the RF with bilateral labeling…….……49

Figure 8 Photo of fibers in the dorsomedial LL……...……50

Figure 9A Atlas section of labeled periventricular cells in the medulla……..…51

Figure 9B Photo of labeled periventicular cells and apparent terminals surrounding the medulla …….….52

Figure 9C Photos of labeled periventricular cells and apparent terminals seen in Figure 9B……..…….53

Figure 10A Atlas section of HRP injection site to the NC of the TS……...….54

Figure 10 B Photos of HRP injection site to the NC of the TS, labeled cells in the contralateral torus, commissural fibers connecting the two tori……55

Figure 11A Atlas section of HRP injection site to the NL of the TS……....56

Figure 11B Photo of HRP injection site to the NL of the TS……...57

Figure 12A Atlas section of labeled cells in the DNV in the caudal medulla……..…..58

Figure 12B Photo of labeled cells in the DNV in the caudal medulla…….…….59

Figure 12C Photo of labeled cells in the DNV in the rostral medulla……...…60

Figure 12D Photo of fiber bundles leaving the DNV……...….61

Figure 12E Photo of ascending fibers from the DNV decussating across the midline through the MLF……..……..62

Figure 12F Photos of fibers from the DNV decussating through the MLF and ascending in the TBT…….….63

Figure 13A Atlas section of labeled cells and dendrites of the MON of the medulla……..64

Figure 13B Photo of the labeled cells and dendrites of the contralateral MON……...…65

Figure 14 Photo of labeled fibers of the contalateral MON decussating across the midline to ascend in the ipsilateral LL……...……..…..66

Figure 15A Atlas section of the labeled cells of the NR……..…….67

Figure 15B Photo of labeled cells in the NR……68

Figure 16 Photos of topographically arranged fibers ascending from the MON in the dorsolateral LL…….……..69

Figure 17A Atlas section of the Vm of the thalamus and the AT of the hypothalamus……...70

Figure 17B Photos of labeled cells in the Vm and in the AT of the hypothalamus……..…71

LIST OF SYMBOLS

AON	anterior octaval nucleus
AT	anterior tuberal nucleus of the hypothalamus
CB	cerebullum
CrCB	cristi cerebelli
DNV	descendens nucleus of V
DON	descending octaval nucleus
HYP	hypothalamus
LL	lateral lemniscus
M	medulla
MLF	medial longitudinal fasciculus
MON	medial octavolateralis nucleus
MRF	medial reticular formation
NC	nucleus centralis
NL	nucleus lateralis
NR	nucleus reticularis
OT	optic tectum
OpT	optic tract
PG	lateral preglomerular nucleus
RF	reticular formation
TBT	tectobulbar tract
TEL	telencephalon
TL	torus longitudinalis
TS	torus semicircularis
Vm	ventral medial nucleus of the thalamus

INTRODUCTION

Cave animals are inherently interesting due to their ability to survive in an unusual habitat. Once thought to exemplify degeneration, cave animals are now known to represent a constantly changing form of life. Many regressive characteristics exist in cave animals, but there are many examples of constructive evolution as well. The animal model used in this study was the cave-dwelling, or hypogean, form of the fish *Astyanax fasciatus* which also exists as a river or epigean form.

Regressive evolutionary change is the loss of complexity of a body structure. Constructive or progressive evolutionary change is the movement toward increased levels of complexity of a body structure. In addition to body structures, behavioral traits can undergo regressive or progressive evolutionary changes.

Examples of regressive evolutionary change in cave dwelling animals in general are a decreased number of thoracic vertebrae, loss of skin pigmentation, and reduction or degeneration of eyes (Jeffery, 2001). Some examples of constructive changes in cave-dwelling animals are longer life spans, specialized appendages, hypersensitive olfactory, gustatory, and mechanosensory system organs (McCormick, 1983; Nieuwenhys, 1983). In fish, the mechanosensory system refers to the lateral line which includes lateral line canals containing neuromasts and free neuromast organs scattered over the body surface (McCormick, 1981; Nieuwenhys, 1983).

Jeffery (2001) reviewed the literature on *A. fasciatus* and classified specific traits as examples of regressive or progressive change. The anatomy of the eye has undergone many regressive changes including the absence of a cornea, iris, and anterior and posterior chambers. The lens and optic nerve, although present, show considerable degeneration. The optic tectum, pineal gland, vertebrae, scales and pigmentation have all shown regression. In addition to regressive changes in structure, there is notable regression in metabolism, circadian activity, schooling and aggressive behavior.

In contrast to regressive changes, constructive specializations have been documented in the feeding apparatus; the jaws, taste buds and teeth have all been refined or increased in number. The infraorbital bones, egg size, fat content and metabolism have all shown progressive evolutionary changes in the cave dwelling form of *A. fasciatus*.

It should be noted that the auditory (Popper and Fay, 1980) and olfactory systems (Riedel and Krug, 1997) in cave *A. fasciatus* are not enhanced constructively to compensate for lack of sight. On the contrary, these fish exhibit hypersensitivity of the mechanosensory system (Jeffery, 2001) to compensate for the absence of vision.

Because cave dwelling animals are an evolutionary descendant of their surface ancestors, comparisons can be made between them. However, many of the surface ancestors are now extinct making such studies more difficult. In the case of cave *A. fasciatus*, that is not so. Although there are several different isolated colonies of these fish in Mexico, their common ancestor is a sighted river fish or epigean form (river *A. fasciatus*) that is found in the same area.

Further, cave and river *A. fasciatus* are completely interfertile (Sadoglu, 1956; Wilkens, 1987) and can be considered to be divergent forms of the same species that live in very different environments. This provides a unique opportunity to make direct comparisons between the ancestral and descendant forms of a species, and makes these fish excellent specimens for evolutionary study. Although beyond the scope of this discussion, *A. fasciatus* has been used as an excellent model system for genetic research (see Wilkens, 1971; Wilkens, 1987; Yokoyama et al., 1995; Behrens et al., 1998; Jeffery et al., 2001; Yamamoto and Jeffery, 2000 for examples).

Cave *A. fasciatus* were readily obtainable from breeding stock, while importation of river *A. fasciatus* was difficult and made their use in this study impossible. In the present study, data obtained from the cave form of *A. fasciatus* will be compared to those from studies of related sighted species. Future research could explore information obtained from the river form and compare it to the present information from cave *A. fasciatus*.

The focus of this dissertation is a neuroanatomical tract tracing study of the torus semicircularis (TS) in the brain of cave *A. fasciatus*, a region that has not been previously studied. The TS is located in the midbrain of fish and is believed to be analogous to the inferior colliculus in mammals. In sighted teleosts this region is thought to be a processing center for auditory, visual, and mechanosensory functions, and the neuronal connectivity is relatively constant (Knudsen, 1977; Northcutt, 1980; Schellart, 1983). In *A. fasciatus*, which has undergone evolutionary regression of the visual system and progression of the mechanosensory system, a significant change in TS connectivity is predicted. This hypothesis forms the basis for this dissertation.

LITERATURE REVIEW

Introduction

Many advances in the biological sciences have occurred through the observation of species that thrive under unusual circumstances. One example, cave *Astyanax fasciatus,* is the focus of this dissertation. Cuvier first conducted observational field studies of the sighted ancestor of these fish in 1819 and coined the scientific name *Astyanax fasciatus*. Since the early scientific studies of Hubbs and Innes (1936), much has been learned, not only about this particular species, but also about the ecology of their habitat. Sighted river *A. fasciatus* are found in a region extending from the northern part of South America to southern Texas. Blind cave *A. fasciatus* dwells in caves only in Mexico, with the largest concentration confined to the Sierra de El Abra region of San Luis Potosi and Tamaulipas, Mexico (Mitchell et al., 1977; Miller et al., 1985).

Astyanax fasciatus was originally thought to consist of five species and subspecies (Wilkens, 1985, 1988); however, later studies revealed that the *Astyanax* occurring in Mexico is a variable form. Further, genetic analysis of the two does not allow separation into different species (Avise and Selander, 1972; Mitchell et al., 1977; Jeffery, 2001).

Many laboratory studies and field observations have shown that river and cave *A. fasicatus* can hybridize (Sadoglu, 1956: Wilkens, 1988; Jeffery, 2001). Therefore, the hypogean fish could be characterized as cave dwelling populations of *A. fasciatus* (Wilkens, 1985, 1988) and could be considered to be the descendent of their extant surface ancestors. Although the same species (meaning completely interfertile), certain features suggest that these two populations exist as different forms. River *A. fasciatus* is characterized by an elongated body with silver to black

pigmentation and prominent eyes. Cave *A. fasciatus* are characterized by a whitish to pink body color (due to reduced skin pigmentation) and the reduction or absence of identifiable eyes.

Twenty-nine Mexican caves are known to be inhabited by cave-adapted *Astyanax.* The caves are grouped by location: the southern El Abra caves, Los Sabinos caves, Yerbaniz caves, northern El Abra caves, Micos caves, Nicholas Perez caves, Chamal caves and Gomez Faria caves (Mitchell et al, 1977). These fish are plentiful in the Sierra del Abra region, a ridge of highly cavernous limestone 125 km long (Mitchell et al., 1977).

Cave Fish (*A. fasciatus*) Habitat

Formation of Mexican limestone caves inhabited by cave *A. fasciatus* has been reviewed by several investigators (Breder, 1942; Barr, 1968; Poulson and White, 1969; Mitchell et al., 1977; Howarth, 1980; Wilkens, 1988). Limestone accumulated in warm shallow seas and was later elevated due to continental uplift. Cracks and crevices gradually developed in the limestone allowing water to seep through. Limestone was solublized by organic acids from the soil including nitric, sulfurous, sulfuric, and carbonic acid to enlarge the cracks. Enlargement of these solutional channels by abrasion took place when cobbles, pebbles, and sand derived from overlying strata were swept through the caves by flood waters.

Surface water drainage is an important factor for cave *A. fasciatus* inhabitation of limestone caves. Water from overflow of small streams in the surrounding region further erode the limestone and allow small pools to form (Mitchell et al., 1977). Fish may be washed into these pools with the floodwater. Over time, populations of fish trapped in the pools from flooding

form isolated colonies. During the rainy season, flooded streams also supply a source of food to these fish that normally survive in an energy poor environment (Mitchell et al., 1977).

The cave environment is separated into a twilight zone near the entrance, a middle zone of complete darkness and variable temperature, and a deep zone where the temperature remains constant and it is completely dark (Poulson and White, 1969; Howarth, 1980). The unique deep zone of the cave environment is where its obligate (troglobitic) fauna appear (Poulson and White, 1969).

The climate of the cave interior is much less variable than surface environments. The temperature is approximately the same as the mean annual temperature of the region. The cave atmosphere is humid, commonly between 95 and 100 percent (Poulson and White, 1969). Evaporation rates are usually low, but cave air is not necessarily still. Air currents and even strong winds activated by chimney effects and by changes in barometric pressure occur at great distances from entrances. A phenomenon known as “cave breathing” occurs in which air currents flow back and forth through constricted passages; these can resonate for periods of a few seconds to a few minutes (Poulson and White, 1969).

Colonization of caves by different species is influenced by several factors. Important factors are energy sources and the physical constraints of the caves. The low food input into a cave system makes colonization difficult, however the constant temperature and relative humidity is conducive to survival. Over time, the arrangement of passageways in cave systems changes due to erosion and substrate deposition via floodwaters, and interior collapse. This may contribute to

the isolation of animals within deep sections of a cave system. All isolated animals that have colonized the deep interior of caves exhibit a common set of physical characteristics over time, including reduced skin pigmentation and metabolism, reduced eyes, longer limbs and sensory alterations.

Breder's (1942) observational study of La Cueva Chica cave in Mexico noted few food sources for cave *A. fasciatus*. Bats and insects were the predominant terrestrial inhabitants. When specimens of cave *A. fasciatus* were dissected their stomach contents consisted of bat guano droppings, smaller fish and their eggs. Breder suggested that the only regular input of energy was from the bats. Barr (1968) suggested that flooding streams bring in an annual supply of leaves, twigs and other organic debris that would help support the insect population such as spiders, beetles, flies. These insects, along with fleas, mites and streblid flies, all parasites of bats, also contribute to the overall food chain of cave *A. fasciatus*.

In summary, the cave environment is relatively constant. In *A. fasciatus* caves, the influx of energy sources is limited throughout most of the year and all inhabitants ultimately depend on bats for food. Infrequent seasonal flooding contributes to the energy source.

***Astyanax fasciatus* Cave Populations**

Four major populations of cave fish have been described. The first population of cave *A. fasciatus* was described by Hubbs and Innes (1936) as "*Anoptichthys jordani*" or "fish without eyes". These fish were named for Mr. C. B. Jordan who supplied them to Hubbs and Innes (Breder, 1942). These fish were discovered in the southern El Abra cave, La Cueva Chica.

Histological and genetic studies by Avise and Selander (1972) and Wilkens (1985, 1987) showed that the Chica fish is a hybrid of the surface form (Wilkens, 1988). Chica fish are raised commercially and are commonly found in pet stores.

Other populations of cave *A. fasciatus* have been discovered including the Sabinos fish from La Cueva de los Sabinos. These fish were originally described as "*Anoptichthys hubbsi*" by Alvarez (1947). In addition to Sabinos, other populations have also been located in the Yerbaniz caves, and most recently in the La Cueva del Rio Subterraneo (Micos fish) (Wilkens, 1988). Alvarez (1946) described the population from La Cueva de El Pachon, found in the northern El Abra region, as "*Anoptichthys antrobius*". Morphological studies showed that the Pachon population is the most distinct population in the Sierra de El Abra. This is probably due to the cave's considerable vertical isolation from the base level waters (Avise and Selander, 1972). Cave *A. fasciatus* from the Pachon caves were used in this research because they are least likely to be hybridized with the surface form (Wilkens, 1988).

Despite variations in the nomenclature, the different cave populations and the river form are genetically similar and are classed together as one interfertile species: *Astyanax fasciatus* (Sadoglu, 1956; Avise and Selander, 1972; Wilkens, 1988).

Special Traits of River and Cave *A. fasciatus*

Comparative studies of the river and cave forms of *A. fasciatus* have been conducted. These studies have examined both behavior and sensory characteristics (Breder, 1943; John, 1957; Fricke, 1988; Romero, 1984; Schemmel, 1980; Parzefall, 1986; Wilkens, 1988; Teyke, 1990; Langecker et al., 1995). Comparisons with other sighted species will help to define the specialized adaptation of *A. fasciatus* to cave life.

General Behavior

Schooling

In a surface habitat, river *A. fasciatus* normally schools (Parzefall, 1986). However, in darkness or when blinded, river *A. fasciatus* do not school and swim in a random spatial distribution (Wilkens, 1988). Unlike river *A. fasciatus*, cave *A. fasciatus* do not school either in light or darkness (Wilkens, 1988), but do have a tendency to congregate based on olfactory cues (Parzefall, 1986).

Aggression

Breder (1943) described the aggressive behavior characteristics of river *A. fasciatus* as "erratic viciousness". Several factors influence the aggressiveness of these fish. When left in their natural habitat, these fish tend to school and are aggressive only during feeding. However, under laboratory conditions when these fish are maintained in smaller aquariums, their aggressive tendencies increase dramatically. River *A. fasciatus* will attack their mirror image as well as specimens separated by a plane of glass. Aggressive behavior also is directed against the phenotypically different cave *A. fasciatus*. This aggressive behavior is released visually

(Wilkens, 1988). In darkness or in enucleated river *A. fasciatus*, aggressive behavior is practically nonexistent. Cave *A. fasicatus* exemplify a reduced aggressive behavior and diminished territoriality.

Fright Reaction

In river *A. fasciatus* the fright reaction response was documented (Pfeiffer, 1963 as translated and reviewed by Wilkens, 1988; Fricke, 1988). Experiments by Fricke (1988) concluded that an alarm substance, secreted into the surrounding water when damage occurs to the body of another fish, causes river *A. fasciatus* to react violently with characteristic zig zag movements and erratic behavior. This fright reaction can last for several hours. Cave *A. fasciatus* do not react in this manner. Fricke (1988) demonstrated that only during feeding do the cave populations avoid an area where the alarm substances have been released. He believes this avoidance reaction is the only part of the alarm behavior still present in cave *A. fasciatus* (Fricke, 1988; Parzefall, 1986).

Special Senses

Olfactory

There are minor differences between the olfactory systems of cave and river *A. fasciatus*. While their noses are similar, cave *A. fasciatus* have shallower olfactory pits with a larger naris thus exposing their olfactory epithelial folds to a greater extent (Wilkens, 1988). These findings might suggest differences in this system may impact the olfactory neural connections of the brain, however more research would have to be done to make these determinations.

Gustatory Behaviors

River *A. fasciatus* school in rivers and clear fresh water ponds. They catch living and dead prey by active visually guided movement. When river *A. fasciatus* feed on the bottom they do so at a steep angle of 80° (Parzefall, 1986; Wilkens, 1987). In contrast cave *A. fasciatus* search the floor of their environment at a 55° angle. At this angle they expose their taste buds that can be found on the ventral side of their head (Schemmel, 1967 [German language] reviewed in Wilkens, 1988). The average angle maintained between the body and ground does not differ between most cave populations (Schemmel, 1980). The Micos fish, which have a functional but reduced eye, show significant variability (Wilkens, 1988). Schemmel (1980) bred the cave and river forms and found the offspring showed a variable expression of the feeding trait. This suggests the angle at which these fish feed on the bottom has a genetic component.

Auditory Ability

Fish detect sounds similarly to other vertebrates (Popper and Fay, 1980, 1993) using an inner ear and peripheral structures that carry sound to the inner ear (Popper and Fay, 1980). The inner ear in most fish, including teleosts, has three semicircular canals and three otolith organs, the saccule, utricle, and lagena (Popper and Fay, 1993). In teleosts, these otolith organs are separate structures rather than a gelatinous mass as in primitive bony and cartilaginous fishes. The sensory epithelium or macula associated with each of these otoliths contains sensory hair and supporting cells (Popper and Fay, 1980). The sensory hair cells have an apical ciliary bundle consisiting of a kinocilium and a large number of stereocilia. The kinocilium is always positioned to one side of the bundle and the lengths of the stereocilia decrease further away from

the kinocilium. The sensory hair cells are physiologically polarized when the otolith organs respond to motion. Afferents from these otoliths target several medullary areas such as the anterior, tangential, magnocellular, medial, and descending octaval nuclei via the eighth cranial nerve (CN VIII). Information is then relayed to the auditory midbrain regions including the torus semicircularis from the auditory medulla (Popper and Fay, 1980).

Breder's (1943) observational studies concluded no significant difference in auditory ability for the different populations of cave *A. fasciatus*. Popper and Fay (1980) studied the hearing ability in eight species of fish including cave *A. fasciatus* using behavior sound pressure audiograms and concluded both river and cave *A. fasicatus* had comparable hearing capacities over a wide range of sounds.

Lateral Line

Many aquatic and marine vertebrates possess a lateral line, a sensory system composed of lateral line canal organs and free neuromasts that detect low frequency vibrations and water movements around the animal (Webb, 1989; Northcutt et al., 2000). A neuromast is the sensory organ of the lateral line system. Each neuromast consists of hair cells and supporting cells covered by a gelatinous cupulae similar to those in the semicircular canals of all vertebrates (Popper and Fay, 1980; Katsuki and Yanagisawa, 1982) and are arranged in a canal alongside of the animal just caudal to the operculum to the caudal peduncle, or just prior to the tail. The head portion of the lateral line canal system divides into the supra and infraorbital region (Wilkens, 1988).

The lack of eyes in cave *A. fasciatus* leads to considerable variability and fragmentation in the lateral line around the orbit. However, there are no other differences between the head portion of the lateral canal system of river or cave *A. fasciatus* (Wilkens, 1988).

In river *A. fasciatus*, free neuromasts (found outside the lateral line canal) are distributed over the head, body, and caudal fin, as is typical among other teleosteans (Schemmel, 1967 [German language] reviewed in Wilkens, 1988). With respect to total number and distribution, no significant differences in free neuromasts are found between river or cave *A. fasciatus* (Wilkens, 1988).

Teyke (1990) found micro-anatomical differences in neuromasts of river and cave *A. fasciatus.* In cave *A. fasciatus* the cupulae of the neuromasts are larger than those of river *A. fasciatus* (300 vs. 50 µm). The length of the cupulae is an important determinant of the sensitivity of the neuromasts, as longer cupulae protrude further out of the boundary layer that surrounds the fish during swimming and, are therefore exposed to faster water currents (i.e., they receive a stronger hydrodynamic stimulus). Teyke suggested that the longer cupulae of neuromasts are a functional improvement of the lateral line system that would compensate for the loss of vision. He reported a further difference from the river form, with the cave *A. fasciatus* having holes at the distal ends of the two lateral flanges of the neuromast cupulae. No hypothesis for the functional significance of this modification was given.

John (1957) conducted a behavioral study of the lateral line ability of cave and river *A. fasciatus.* He put the fish in a tank with a variety of solid objects they had to learn to swim around without

colliding with them. John concluded that both naturally blind cave fish and river fish blinded by the investigator depended upon their mechanosensory (lateral line) system instead of spatial learning ability when detecting solid objects in their environment.

Abdel-Latif et al. (1990) disrupted both cave and river *A. fasciatus* lateral line organs by placing hairs directly in the canals. Care was taken not to damage the free neuromasts. They recorded spontaneous swimming behavior. All fish with disrupted lateral line systems swam at slower velocities than those in the control group with normal lateral line canals. These results suggested that normal swimming requires a functioning lateral line system.

Problems with Fish Brain Nomenclature

Multiple naming schemes exist for different nuclei and regions of fish brains. This has created confusion in the nomenclature (see Braford and Northcutt, 1983; McCormick, 1983; Nieuwenhuys and Pouwels, 1983; Northcutt, 1983; Northcutt and Davis; 1983 chapters for comparisons). While the diencephalon is one of the most well documented regions of the brain, it is also a good example of confused nomenclature. Nonhomologous cell groups were given the same name by different investigators, and homolgous structures were given different names (Striedter, 1990a, 1990b).

To alleviate confusion, Northcutt and Braford (1984) compared the diencephalon of the teleost species *Carassius auratus*, the common goldfish, to non-teleost bony fishes, including the longnose gar, *Lerisosteus osseus* and bichir, *Polypterus*. Northcutt and Braford established that some cell groups could be recognized in all three taxa, while others differed from species to

species. With nearly 30,000 living species of teleosts, and additional variations among them, Northcutt and Braford proposed that the description of the goldfish brain be used as a standard for comparison with other teleosts in the future. While many investigators have followed Northcutt and Braford nomenclature, others have not and confusion still exists. A recent trend in the literature has been to apply Northcutt and Braford's terminology to the brain of other teleosts, even when it is not clear that the cell groups being named are indeed homologous to those of the same name in goldfish (Striedter, 1990). To eliminate as much confusion as possible, the nomenclature of Northcutt and Braford will be used in this dissertation.

Brain of the Cave *Astyanax fasciatus*

There have been few investigations involving the brain of river or cave *A. fasciatus*. Voneida's (1973) comparative study of retinotectal projections in cave and river forms of *A. fasciatus* revealed fibers terminating in the most rostral portion of the tectum. Sligar and Voneida (1976a, 1976b) investigated tectal efferents in cave *A. fasciatus*. They examined ascending, descending, and intertectal projections of the tectum using lesions and stains for degenerating fibers. The primary fiber bundle that exits the tectum is a major pathway that bifurcates into two fascicles. The large fascicle becomes a contralateral projection as part of the tectobulbar tract that descends along the lateral edge of the TS. Some fibers leave this bundle to synapse in the torus. A comparative study of retinal projections of cave and river *A. fasciatus* was also published the same year by these authors.

Reidel (1997a, 1997b) published a study mapping the forebrain of cave *A. fasciatus*. He used degeneration, DiI, and HRP axonal tracing techniques with similar results. The first portion (1997a) covered general anatomy of the telencephalon including gross morphology and a histological description of the olfactory bulbs and telencephalon proper. He plotted the connections of the forebrain with other brain areas by the medial and lateral forebrain bundles. Riedel examined the projections of the olfactory bulb in the forebrain of cave *A. fasciatus* and traced the connections of olfactory tracts to a variety of telencephalic areas.

Fish and Ghosh (1992) did a comparative study of the six occulomotor muscles in cave and river *A. fasciatus*. While the extraocular muscles contained a slightly reduced number of fibers, extraocular motor nuclei located in the brainstem were comparable in number and overall morphology. Further, studies have been made on cave *A. fasciatus* involving changes in the

central nervous system and the reduction of visual input (Voneida and Fish, 1984). In addition, retinotectal and tectal synaptic organization has been studied. These studies showed the optic tectum responded to mechanosensory information. In addition, central projections of the trigeminal and lateral line nerves in cave *A. fasciatus* were examined by Voneida et al. (1983). Further, Fish and Voneida (1979) studied the neurons in the optic tectum of both cave and river *A. fasciatus*.

Torus Semicircularis

Little is known about the torus semicircularis (TS) in cave *A. fasciatus*, however by comparing them with closely related fish, inferences may be drawn about the connectivity of the TS in the cave fish. The TS is found in the brain of most fish and other aquatic vertebrates such as frogs and crocodiles and is thought to be a multimodal-sensory processing center (Pritz and Stritzel, 1990; Feng and Lin, 1991). In teleost fish, the TS is situated dorsolaterally in the mesencephalic tegmentum and is thought to integrate information coming from the auditory and lateral line systems in fish (Cuadrado, 1989). Acoustic information and lateral line information are processed separately in two nuclei of the torus. Nucleus centralis (NC) receives auditory input and lies medial, while nucleus lateralis (NL) lies lateral and processes lateral line input (for examples see Page and Sutterlin, 1970; Echteler, 1984; Cuadrado, 1989 and see review by Braford and Northcutt, 1983).

While cytoarchitectural studies of the TS reveal many details of the cellular arrangement of the TS, they contribute limited information to the understanding of its connectivity. There have been several indirect studies of the connections of the TS while examining other brain areas such as

the tectum in sighted teleosts. Information obtained from these studies was used when identifying like brain areas connected to the TS in the brain of cave *A. fasciatus*. These studies will be discussed in detail.

Cytoarchitecture of the Torus

Knudsen (1977) conducted an extensive study of the nuclear subdivisions of the TS using two species of catfish, *Ictalurus nebulosus* and *Ictalurus punctatius*. He used both degeneration experiments and Golgi-Cox staining techniques to identify the TS. The TS is bounded by the nucleus isthmi ventrally and laterally by the lateral lemniscus and NL while the mesencephalic ventricle forms the dorsal and medial boundaries.

Both the the NC and NL are found at caudal levels, but only NL is found at the rostral pole of the TS. NC is described as a cylindrical group of cells with an overlying covering or rind of fibers beginning at the caudal pole of the TS and stopping prior to the posterior commissure. The NC begins from its caudal perspective as an enlarged circular cell group that flattens against the ventricular surface and decreases in size as it projects rostrally.

NL is shaped like an "L" with its base at the rostral end of the TS. This portion of the cell group is tipped in a medioventral orientation and is bounded by the mesencephalic ventricle dorsally and NC medially. The tectobulbar tract (TBT) contacts the NL ventrally. At rostral regions of the TS, the tectobulbar tract transects the NL (Knudsen, 1977).

Knudsen's axonal degeneration tract tracing experiments demonstrated that fibers projecting to the NC travel in the medial half of the lateral lemniscus. He saw a major ascending output exiting the rostral end of NC and projecting to the ventromedial thalamus. The NL receives bilateral input from the lateral line lobes traveling in the lateral bundle of the lateral lemniscus. Incoming lemniscal fibers enter the nucleus lateralis ventrally and terminate throughout most of its dorsoventral extent.

Two regions have been identified within the NL, one medial (pars medialis) and the other lateral (pars lateralis). They are separated by a fibrillar sulcus and both nuclei have similar if not the same structure (Knudsen, 1977; Cuadrado, 1989). In addition to the acoustic and lateral line inputs to the TS, some teleosts receive electroreceptive impulses, for example, catfish (Knudsen, 1977). In electroreceptive fish, a region of the nucleus lateralis receives electrical impulses. In addition to medial-lateral segregation of sensory inputs, the TS is somatotopically organized. Lateral line inputs from the head project to rostral areas and inputs from the tail to caudal areas (Knudsen, 1977). Knudsen examined, in the same study, the TS from three other families of catfish including a South American catfish, *Pimelodus clarias,* and two East Asian species, *Plotosus anguillaris* (marine) and *Parasilurus asotus* (fresh water). He concluded that the structural and functional subdivision of the TS into an auditory NC and a lateral line NL is likely to apply to teleost fish in general.

Cuadrado (1989) found four cell layers in the NL of the *Barbus meridionalis,* alternating between cell-poor and cell-rich layers: (1) subependymal, (2) small cell, (3) fibrillar and (4) disperse layers. Cuadrado compared his data to other teleosts including carp and found the

lamina are basically the same. TS neurons of *B. meridionalis* are classified in three categories, small (7-12µm), medium (12-17µm), and large (17-25µm). Cuadrado et al., (1992) found the layers of the TS to be consistent among the species studied. Ito (1974) published photographs of the torus semicircularis in non-electroreceptive teleosts including trout, carp, loach, and eel. Tori of these fish showed a clearly laminated NL and a poorly differentiated NC.

In the next three sections, the regions of the brain that support the multimodal sensory input of the torus are reviewed. Other known connections are also discussed.

Auditory Connections to the Torus

Echteler (1984) studied the connectivity of the TS to the medulla in carp by injecting HRP into the NC. Retrograde labeling was observed in six nuclei of the medulla. Cells in the octavus nucleus and descending octavus nucleus were labeled along with the ipsilateral superior olive, fibers of the lateral lemnisci and the medullary reticular formation (MRF) bilaterally. Cells near the IVth ventricle and cells in medial octavolateralis nucleus (MON) were also found. Retrograde labeling of the contralateral TS and ipsilateral optic tectum was also seen. Efferent projections were found in the ipsilateral superior olive, ipsilateral medullary reticular formation, the deep layers of the optic tectum and contralateral TS. Fibers and terminals were observed in the anterior tuberal nucleus of the hypothalamus and central posterior thalamic nucleus. Echteler separated auditory and lateral line regions of the TS using electrophysiological recordings and focused his anatomical study primarily on auditory connections.

Lateral Line Connections to the Torus

Nieuwenhuys and Pouwels (1983) studied the octavolateralis system in fish. This system consists of auditory, vestibular and lateral line input. In teleosts, they divided the octavolateral area into a dorsal lateral line zone and a ventral auditory-vestibular zone. The dorsal zone contains the MON that is the primary target for the anterior and posterior lateral line nerves. These nerves carry input from neuromasts of the lateral line canal and free neuromasts (Northcutt, 1983). The ventral auditory-vestibular zone contains several cell masses. Different names have been used throughout the literature when describing these nuclei (McCormick, 1983).

Nieuwenhuys and Pouwels (1983) demonstrated a large number of efferents leaving the octavolateral area as decussating fibers and running rostral toward the tegmentum of the midbrain in the lateral lemniscus where most terminate in the TS. Efferents of the MON decussate in the medial medulla and pass rostrally in the lateral portion of the lateral lemniscus to terminate in the NL (Northcutt, 1983). Nieuwenhuys and Pouwels (1983) also described a cell mass associated with the lateral lemniscus as analogous to the superior olivary nucleus of McCormick and Braford (1993).

Tectal Connections to the Torus

Luiten (1981) studied afferent and efferent connections of the optic tectum in carp. Injection of several regions of the tectum with HRP revealed afferent projections from the TS. A large number of fibers entered the TS after leaving the tectum. The cell bodies of these afferents were found in the NL. Reciprocal connections were also noted from the tectum to the TS. Tectal efferents ran medially and projected to the central core of the TS. A contralateral projection to

the TS consisted of many fine efferent fibers that decussated in the tectal commissure. Luiten concluded the tectum provides visual input to the TS. Fiebig et al. (1983) studied the afferent connections of the optic tectum in piranha and noted afferents from the NC of the TS on the ipsilateral side and the NL bilaterally.

While the fish used in the next two studies reviewed are not teleost, it is interesting to note the similarities between their brains and those of teleosts. Ariens Kappers et al. (1960) studied the TS of plagiostomes (sharks) and compared them to bony fishes. The plagiostome TS sends efferent fibers to the nucleus isthmi and to the tectum. However, differences exist in the termination of the lateral lemniscus between sharks and bony fishes. In teleosts, the lateral lemniscus terminates in the TS and the nucleus isthmi but not in the tectum.

Reciprocal connections between the torus and the optic tectum were demonstrated in the longnose gar (*Lerisosteus osseus)* by Northcutt and Butler (1980) and Northcutt (1982). The long nose gar (*L. osseus*) is a bony fish, however it is not a teleost. Anterograde transport of HRP from tectal injections demonstrated tectal efferents to the contralateral TS with commissural fibers and ipsilateral projections specifically to the NC of the TS.

Other Neuronal Connections with the Torus Semcircularis

Striedter (1992) studied the connections of the lateral preglomerular nucleus (PG) in several teleosts including the common goldfish, *Carassius auratus*, red-belly pacu, *Colossoma bidens*, chocolate ghost knife fish, *Apteronotus leptorhynchus*, and channel catfish, *Ictalurus punctatus*. The PG receives primary input from the NC with minor input from the NL. It functions as a relay

nucleus to the thalamus. He applied DiI to the lateral preglomerular nucleus and to the TS. Labeled cells were found in NC and in a restricted portion of NL. The TS of the pacu can be divided into the same general regions as in the goldfish. After DiI application to the lateral preglomerular nucleus, which is larger in the pacu than in the goldfish, labeled cells were found in the NC and in the rostral portion of the NL. In catfish labeled cells were seen in both the NC and NL. In the chocolate ghost, the NL is larger, highly laminated and receives electrosensory input in addition to lateral line information. DiI labeled cells were found in both the NC and NL.

Wullimann and Northcutt (1988) traced the connections between the TS and the cerebellum (CB) in the green sunfish and the common goldfish. They found ascending efferent fibers running dorsorostrally from the cerebellum and terminating in the NC of the TS. This was consistent in both species examined.

Electrophysiological Studies

Page and Sutterlin (1970) used electophysiological recordings to study the visual and auditory unit responses in the goldfish tegmentum. Recordings were made in several areas of the brain including the TS and optic tectum. Most mesencephalic units that responded to sound were located in the dorsal region of NC. No auditory units were found in the tectum or valvula cerebelli although numerous penetrations were made through these areas in order to reach the TS. The TS was found to respond only to sound and no response was elicited to visual stimulation. Lu and Fay (1993) made single unit recordings of cells in the NC of goldfish using auditory tuning curves. Schellart (1983) studied both acousticolateral and visual processing in the TS of trout. The recordings from the cells in the NC demonstrated a strong response to

auditory stimuli. Two cells (Schellart, 1983) showed a response that could be classified in the range of the lateral line frequencies. He suggested that some cells have bimodal characteristics. Schellart found that only sudden changes in the visual field seemed to be of importance.

Functional regions of the catfish TS were demonstrated in the electrophysiological studies of Knudsen (1977). Recordings showed a distinct separation of stimulus processing in the TS. Auditory responses were found in NC while cells responding to lateral line stimuli were always located in NL. Knudsen noted that while it appeared that a response could be elicited by multiple stimuli in the same area, there was always a clear domination of the functional region by a single modality.

In addition to HRP-tract-tracing, Echteler (1984) used electrophysiological recordings to study the connections to the TS. He found a distinct segregation of auditory responsive cells in the TS confined to the medial portion of the nucleus. A larger concentration of cells responding to auditory stimulation was located at more caudal levels of the TS with a decreasing number found at more rostral regions. Cells responding to lateral line stimuli were always located lateral to the auditory cells at all rostrocaudal levels. Echteler's study seemed to support Knudsen's earlier findings of distinct functional regions of the TS.

In summary, the TS is the known processing center for mechanosensory processing of lateral line system information and therefore could possibly show progressive evolution in its gross connectivity of associated brain areas in cave *A. fasciatus.* This fish is already known to have demonstrated minor progressive modifications in its lateral line sensory organs or neuromasts. In

addition, the literature reviewed of the torus semicircularis and related brain regions provided valuable information when identifying similar regions in the brains of cave *A. fasciatus*. These studies gave a solid background of information about the anatomical brain structures and connections associated with them and therefore a basis to compare the brains of cave *A. fasciatus*.

MATERIALS AND METHODS

Subjects

Experiments were performed on 62 Pachon cave *A. fasciatus*. Subjects ranged between 5.5 to 9.6 centimeters long and 1.8 to 13.6 grams in weight.

Preparation for Surgery

Each animal was prepared for surgery by immersion in an anesthetic solution of 0.1% tricane methanesulfonate (MS-222, Finquel, Ayerst). An appropriate level of anesthesia was obtained by observing respiratory gill movement. Once gill motion ceased, the animals were immediately placed in a special head holder that clamps to the top of the head with a tube placed in the mouth through which a solution of 0.05% MS-222 was passed to perfuse the gills. This special head holder was mounted in a Kopf stereotaxic frame. The body of the fish was kept moist by covering it with absorbent tissue (Kimwipes) saturated with fish Ringer's solution. An animal could be successfully maintained in this state for procedures lasting in excess of four hours.

Surgical Procedures

The skin covering the cranium was incised at the midline with a scalpel and a flap was carefully laid back. A small section of the skull plate covering the right half of the optic tectum was removed using a dental drill and forceps. The piece of bone was stored in sterile fish Ringer's and used to close the opening after the injection. Fatty deposits were gently removed by flushing the cranial cavity with a stream of fish Ringers solution delivered by a pipet tipped Nalgene squeeze bottle.

Stereotaxic procedures

A stereotaxic atlas was prepared from microscope slides of cave *A. fasciatus* brains on sections that contained the TS. In a surgically exposed brain the visible region overlying the TS is the optic tectum. Therefore, landmarks used for measurement on the atlas sections were anterior and posterior poles of the tectum for anterior-posterior position, and midline and lateral tectum for medial-lateral measurements. Dorsal-ventral measurements were from the tectal surface at a specific anterior-posterior/medial-lateral position. During an experiment, the visible landmarks and measurement from the atlas slides, applied to the three dimensional coordinate system of the stereotaxic apparatus, were used to position an HRP injection pipette tip in the TS. Care was taken to try to keep the body length and weight of fish reasonably similar in order to minimize the differences in brain size, but the unavoidable variability, and the small size of the brain, resulted in a large amount of inaccuracy of injections sites. Measurements were modified with each subsequent experiment to improve accuracy and insure that all regions of the TS and adjacent brain areas were injected with tracer.

Pressure Injections of HRP

The injection technique for placing nl range HRP pressure injections in the brain is described in detail in Fish and Rhoades (1981). Briefly, 20 µl Drummond Microtrol Glass micropipettes, which come with a fitted plunger, were converted into micro syringes. The pipettes were heated and pulled to a closed point with a Kopf vertical pipette puller, and then the tips were broken back to a tip diameter of 10-25 microns. The micropipette was clamped in a modified carrier on a Kopf stereotaxic apparatus and the plunger was attached to the slave cylinder of a hydraulic microdrive. Retracting the microdrive with the tip in a 10% solution of HRP (Sigma) in 0.1 M

Tris buffer (pH 7.4) filled the pipette. Advancing the microdrive by 5 µm steps ejected 1 µl of the HRP solution.

Iontophoretic Injections of HRP

Radnoti Starbore glass capillary tubing (1.2mm) were prepared, as above for the micropipette glass, and filled with the HRP solution by capillary action. A tungsten wire inserted in the HRP solution and connected to a Midgard Constant Current Source was placed in the pipettes. Pipettes were mounted in a Kopf microelectrode carrier and tracer was delivered by passing a +2µA current (1 to 3 minutes, 2 seconds on 2 seconds off, 50% duty cycle). During insertion and removal from the brain, a negative backing current was applied to the electrode to minimize leakage of HRP in the electrode track.

Animal Recovery

When the deposition of HRP was complete, the pipette was withdrawn and the surface of the brain was rinsed with fish Ringer's solution and a small pledget of Gelfoam, soaked in fish Ringer's, was inserted into the cranial cavity. The piece of skull that was removed was then repositioned and the skin was laid back into place. The surgical site was sealed with Vetbond Tissue Adhesive. The gill perfusion anesthetic solution was then replaced with fresh water and the gills were perfused until evidence of respiration (gill and muscle movement) was observed, typically within 5 to 15 minutes. The animal was then moved to a recovery tank and allowed to survive for 6 to 8 days in order for axonal transport of HRP to occur.

Fixation Perfusion Technique

After the survival period, the fish were reanesthetized using the previous method. They were placed in the surgical apparatus with the ventral side of the animal exposed. The cardiac cavity was surgically exposed by gentle dissection and the fish were transcardially perfused through the conus arteriosus with cold 0.1 M phosphate buffer (pH 7.4). In order to flush the circulatory system, the atrium was incised and the gills were observed until a change in color was detected. Once the gills were pale pink and no blood was observed exiting the atrial incision, indicating a flushed vascular system, the phosphate buffer was replaced with a solution of 1% paraformaldehyde and 2% gluteraldehyde. Fixation was allowed to continue for 45 minutes after which the brain was carefully dissected from the animal's cranium and placed in a rinse of fish Ringer's for 20 minutes. The brain was rinsed in distilled water and blocked from the caudal telencephalon to the caudal brainstem. The brain was then positioned for sectioning in the coronal plane before being embedded in an agar block.

Tissue Processing

The brain was sectioned on an Electron Microscopy Sciences vibratome in 100 micron sections. The sections were processed using diaminobenzidine (DAB, Sigma) (technique of Adams, 1977; refer to Mesulam, 1982) as a substrate for the identification of the HRP enzyme, and sections were serially mounted on gelatin-coated slides and allowed to air-dry overnight. Sections were counterstained with thionin and cover slipped. The tissue was analyzed using light microscopy to determine the location of the reaction product in nerve fibers, cells, and terminals.

Identification of Brain Anatomy in Cave *A. fasciatus*

The findings of previous researchers were critical when identifying pertinent brain nuclei and regions in the brain of cave *A. fasciatus*. Several regions were analyzed including brain areas associated with auditory input (Knudsen, 1977; Popper and Fay, 1980; McCormick, 1983; Nieuwenhuys and Pouwels, 1983; Meredith et. al, 1983; Schellart, 1983; Murakami et al., 1986; Striedter and Northcutt, 1989; Striedter, 1990a, 1990b; Bleckmann et al., 1991; Striedter, 1991; McCormick and Braford, 1993; Northcutt and Butler, 1993a, 1993b; Bass et al., 2001), lateral line and general sensory inputs (Luiten, 1975; Luiten and Van der Pers, 1977; Northcutt and Braford, 1984; Fernald and Shelton, 1985; Gomez-Segade and Anadon, 1988; Wullimann and Northcutt, 1988; Diaz and Anadon, 1989; Webb, 1989; Diaz-Regueira and Anadon, 1990; Schlussman et al., 1990; Teyke, 1990; Bensouilah et al., 1991; Bleckmann et al., 1991; Butler et al., 1991; Cuadrado et al., 1992; Pinuela et al., 1992; Northcutt and Butler, 1993a, 1993b; New et al., 1996; Pombal et al., 1997; Saidel and Butler, 1997a, 1997b; New et al., 1998; Rupp and Northcutt, 1998; Kiyohara et al., 1999; Northcutt et al., 2000; Boudriot and Reutter, 2001)

Maintenance of the Breeding Colony

The establishment and maintenance of a breeding colony of cave *A. fasciatus* has been an important component of this research. Two colonies of cave *A. fasciatus* are maintained in our laboratory, one originating from the Pachon cave stock and another of Chica cave origin. The original colony of 9 (Pachon) cave *A. fasciatus* was obtained through the courtesy of Dr. Horst Wilkens while the Chica stock was acquired commercially. In order to have sufficient numbers of fish to conduct this research, it was necessary to breed the original colony. Many techniques were attempted to increase their spawning activity. These included increasing the food supply,

altering the water temperature, changing the ambient lighting, and use of human gonadotropin hormone (Zeitlin, 1973). Best results were obtained by dropping the water temperature by 7º to 10º F by adjusting the aquarium heater and decreasing the food supply for approximately two weeks, replicating the conditions these fish might experience during the winter season (Sadoglu, 1979). After this period, the fish were provided a variety of foods including frozen brine shrimp and krill until the females were heavy with eggs. Frequent water changes of approximately 20% 3x per week were required to maintain the appropriate water chemistry (6.0-7.0 pH with low ammonium and nitrate levels) (Andrews, 1986). This was necessary due to the increased food supply and resulting waste excreted into the water. The water temperature was then increased to 80º to 82ºF to duplicate spring-like temperatures. Disruption of the gravel at the bottom of the aquarium seemed to cause increased breeding activity and promote spawning. This could have been due to increased supply of food from debris in the gravel or to some chemical change that would require further investigation. Spawning usually occurred within 12 to 18 hours after completion of these manipulations.

Newly laid eggs were caught in a double-layered screen placed in the bottom of the aquarium to prevent the adult fish from eating them. After they were trapped in the screen, the screen was very carefully removed from the aquarium and gently washed in the water of a nearby five-gallon glass hatch tank. The fertile eggs were extremely fragile and sticky and hatched within 24 hours when maintained at 85º to 88º F. New hatchlings remained stuck to the sides and bottom of the aquarium by a sticky patch on their dorsal side. They lived on nutrients in an abdominal egg sack for approximately five days after which the fry became free swimming. Most hatchlings

were lost during this time due to parasitic (gill worms of the *Dactilogyridea* family, diagnosed in our laboratory) and fungal infections (Untergasser, 1989) in the hatching aquarium.

To combat the parasitic and fungal infections that destroyed several hatches, several remedies were experimented with including commercially available products intended to protect eggs and hatchlings. The most effective treatment was the use of methylene blue (Fisher), a common histological stain, and a commercially available product, fish Egg Guard (Jungle Laboratories). These two chemicals were used along with gentle infusion of air from an airstone. The powdered methylene blue was mixed in a stock solution of 1 gram to 1 liter of water. For treatment, 1 ml of stock solution was added for each liter of water in the hatch tank. Egg Guard was applied according to the manufacturers instructions. Care was taken to remove all charcoal filtration during the treatment period.

Once the fry were free swimming, the fish egg guard treatment was replaced with a treatment of Small Fish Saver (Jungle Laboratories). Again, the manufacturers directions were followed. Approximately one cup of water was removed from the hatch tank daily and replaced with fresh water. Treatment chemicals were monitored and replaced weekly.

Feeding of the free swimming fry began once the egg sack was depleted. Their diet consisted of newly hatched brine shrimp and micro-food (Ocean Star International, Inc) fed 3x daily. Care was taken to adjust the amount of food to insure no debris remained in the tank between feedings. Fry were maintained on this diet until they were large enough (about 2 cm long) to eat frozen brine.

Adult fish were maintained on a diet of commercially produced food supplemented periodically with fresh and frozen brine shrimp. Constant monitoring of the water chemistry was necessary in order to keep the animals in good health. Currently, there are several generations of these fish maintained in the laboratory including individuals from the original stocks.

RESULTS

The neural connections examined in this study were the afferents to two regions of the TS in cave *A. fasciatus*. The first region discussed is the NC, known to receive auditory input from the ocatavolateralis region of the medulla. The second region that will be discussed is the NL, known to receive physiological input derived from the anterior and posterior lateral line nerves. The NL serves as a mechanosensory processing-center in fish.

These two nuclei were injected with HRP and the connections to both traced independently. The results are separated into medial and lateral injections to the TS and the details of the results are given. Of the 62 experiments performed, 21 cases yielded data with 5 being NC injections and 7 NL injections. The remaining 9 cases were mixed injections involving both the NC and NL or other regions of the brain. In all figures, regions where cell bodies were found are indicated by solid circles.

Medial Injections

The major input to the NC of the TS was from the octavolateralis region of the medulla. When the NC was injected unilaterally with HRP (Figures 2A and 2B), labeled cells were found in two octaval nuclei of the medulla, the DON and the AON (Figures 3A and 3B). Large distinct cells were located in the DON while the cells in the AON were considerably smaller. Cells were found bilaterally (Figures 4A and 4B) in the DON, however the AON was labeled primarily on the ipsilateral side (Figures 5A and 5B). On the ipsilateral side, large fibers from the AON could be seen extending to the region of the DON (Figure 6). Fibers from these two nuclei pass

together to ascend in the dorsomedial portion of the LL (Figure 8). Labeled fibers were found bilaterally in the LL.

Small isolated cells and fine fibers were also seen in the reticular formation (RF) (Figure 7A and 7B). In more rostral sections, fibers were seen in the RF and in the DON. There were no apparent terminals in the AON, however an area of probable terminals could be found in a region adjacent to the ventricle on the ipsilateral side (Figure 9A-9C). Further analysis would have to be done to confirm this information.

Labeled cells and fibers were observed in the contralateral TS (Figure 10A-10B). Commissural fibers were seen crossing to the contralateral torus through the commissura ansulata (Figure 10A-10B) at more rostral levels of the midbrain caudal to the optic chiasm. Cells in the contralateral TS are labeled when either the NC or NL are injected with HRP. The two areas appear to be topographically related. A few labeled fibers were also seen in the deep layers of the OT.

Lateral Injections

Injections of HRP were placed in the NL of the TS. These were rather large injections that covered the majority of the nucleus (Figures 11A and 11B) resulting in labeling of cells and fibers in the contralateral descendens nucleus of V (Figure 12A) that consists of a column of cell bodies in the dorsolateral medulla along most of its rostral-caudal extent. Cells were found in a diffuse group with intermingled fibers (Figure 12B). These cells and fibers were isolated to the dorsolateral side throughout the length of the medulla contralateral to the injection site (Figure

12C). Fibers leave this nucleus and run ventral splitting into two fascicles before decussating across the midline between the MLF to ascend in the ipsilateral tectobulbar tract (TBT) and LL (Figure 12D-12F). Together, these two pathways are referred to as the bulbar lemniscal system in the literature (Nieuwenhuys and Pouwels, 1983).

The MON of the medulla is heavily labeled after injections to the NL. Large Purkinje cell-like neurons of the MON have large horizontally directed dendrites that curve dorsally into the overlying cerebellar crest (Figure 13A and 13B). Neurons in the MON were also labeled on the side contralateral to the injection with a distinct fiber pathway traveling medially toward the ventricle, where it decussates across the midline to ascend in the contralateral LL (Figure 14) to the NL.

The fibers of the LL are topographically arranged. Fibers ascending from the MON to the NL pass in the ventral lateral portion of the LL (Figure 16) and fibers ascending from the AON and DON to the NC pass in the dorsomedial portion of the LL.

Several fine fibers were traced to the ipsilateral OT, however only a few cells were located within the deep layers. A cluster of labeled cells was located in the nucleus reticularis (NR) (Figure 15A and 15B). In caudal regions of the midbrain, several scattered cells were seen in the tegmentum dorsolateral to the LL (Figure16).

Cells of the ipsilateral ventromedial nucleus of the thalamus (Vm) were labeled (Figure 17A and 17B) with fibers running caudally then dorsolaterally to enter the TS. Labeled cells were also found in the ventral anterior tuberal nucleus of the hypothalamus (AT) (Figure 17A and 17B). These cells were greater in number on the ipsilateral side, however no fibers were present.

In cases where the injection site was mixed and included portions of both the NC and the NL, a combination of the results were found. These cases were used to classify pure injections to each region of the TS and will not be discussed further.

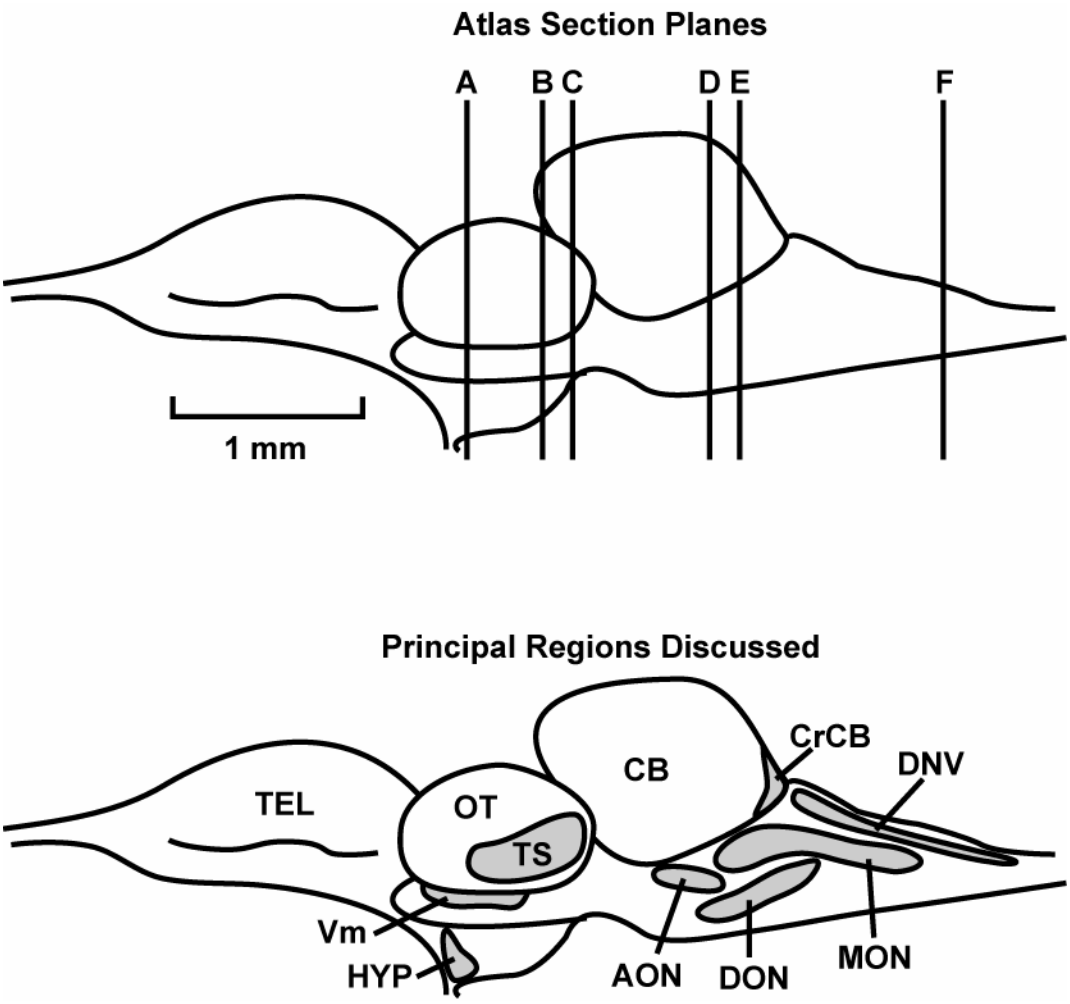


Figure 1. This line drawing shows a lateral view of the brain of cave *A. fasciatus*. In the top illustration the vertical lines indicate planes of section designated with capital letters. These sections are used in subsequent figures to map data. The lower illustration shows the approximate locations of principal regions discussed and their relationship to each other.

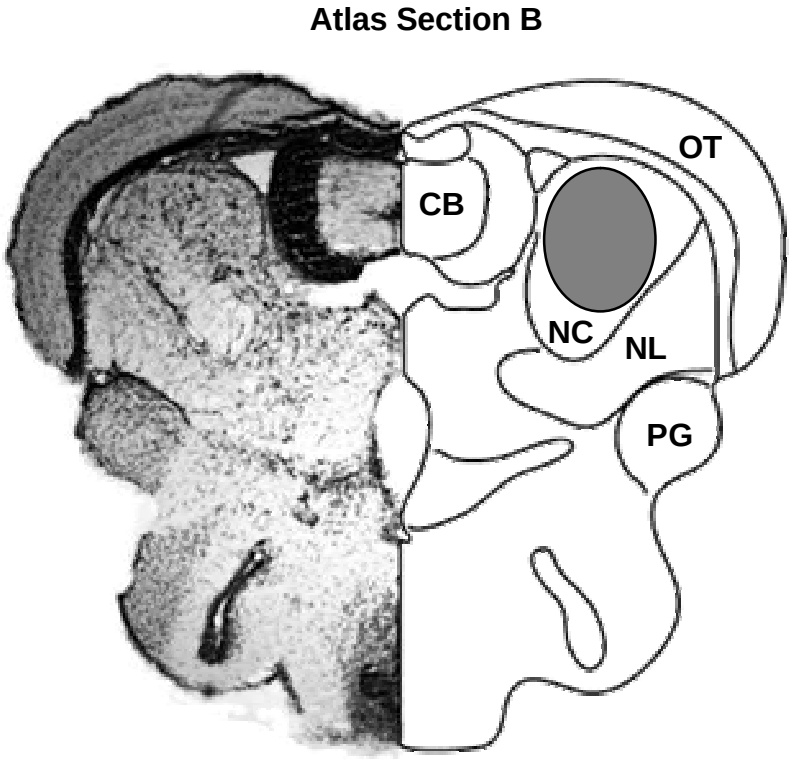


Figure 2A. Drawing of injection site of HRP to the NC. A large portion of the NC is covered indicated by the gray oval. The entire TS includes both the NC and the nucleus lateralis (NL). In all atlas drawings, such as Figure 2A, normal brain sections stained for cell bodies are presented on the left with a comparable line drawing on the right. These are for reference and orientation purposes for the reader.

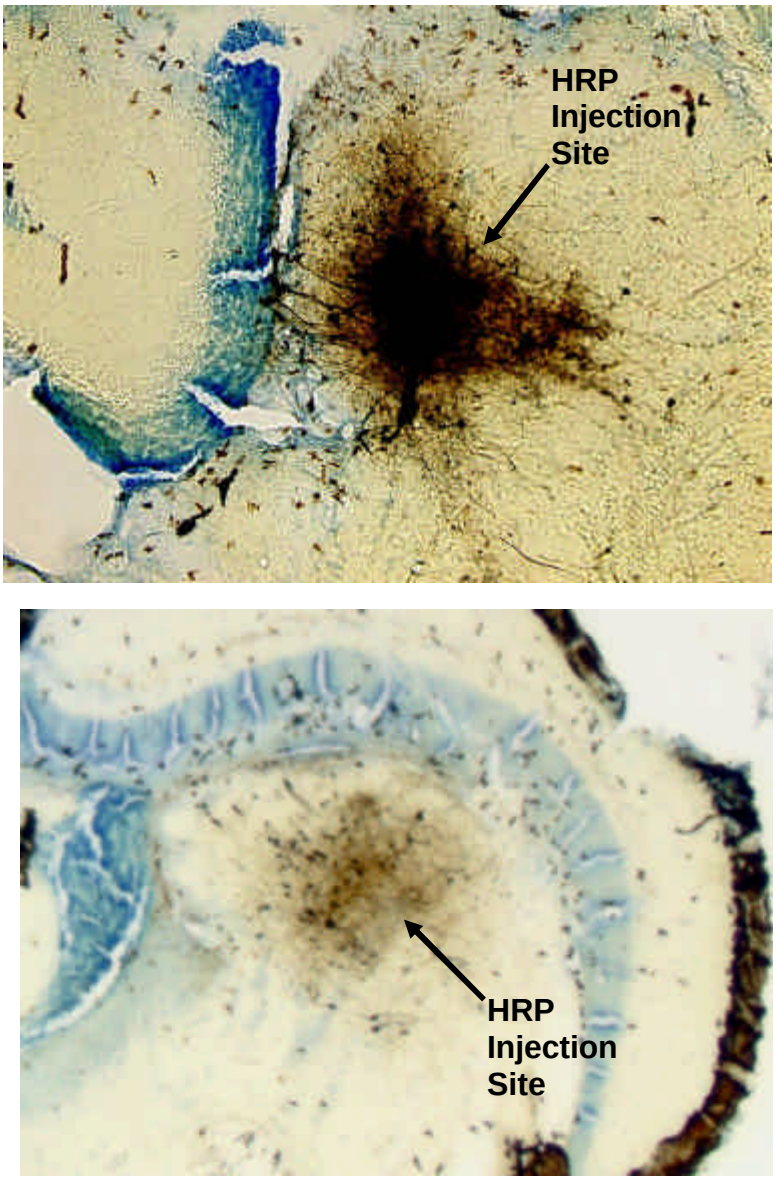


Figure 2B. Photos shown above are examples of HRP injection sites to the NC of the TS

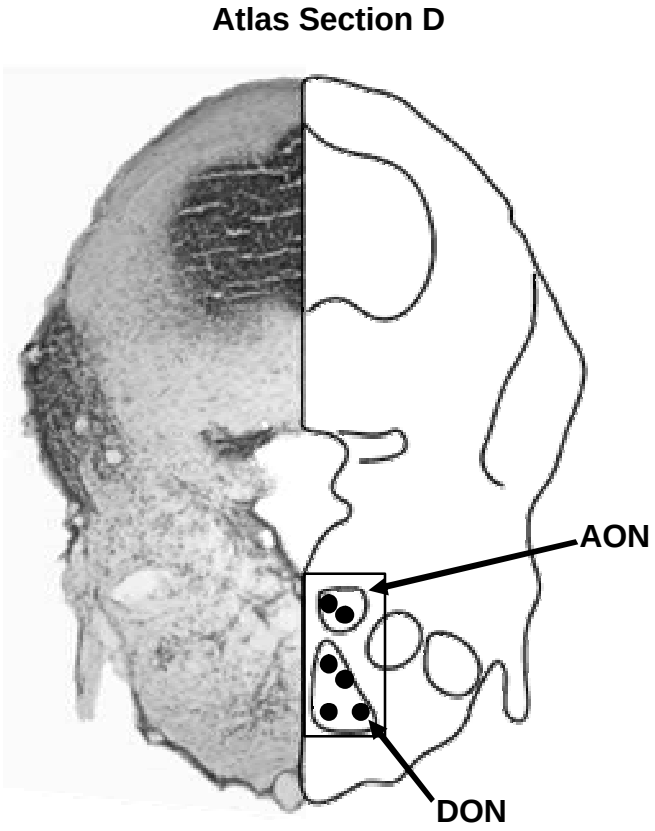
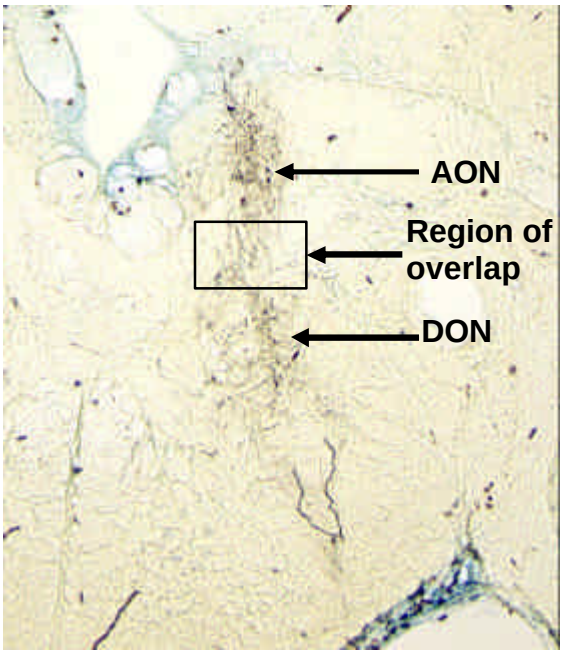
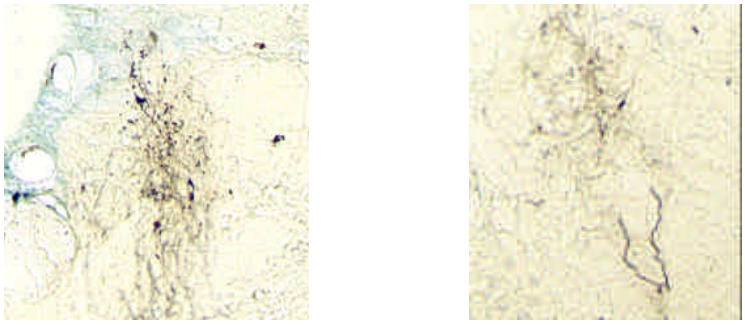


Figure 3A. Two octavolateralis nuclei are labeled with medial injections of the TS. The anterior octavolateralis nucleus (AON) and the descending octavolateralis nucleus (DON) relay information to the NC from CN VIII. The area indicated by the box can be seen in more detail in Figure 3B.





A B

Figure 3B. In injections of the NC, two octavolateral nuclei are labeled. The AON is shown in inset "A" while the DON is seen in inset "B". Some overlap of cell processes seem to occur between these two nuclei (top photo).

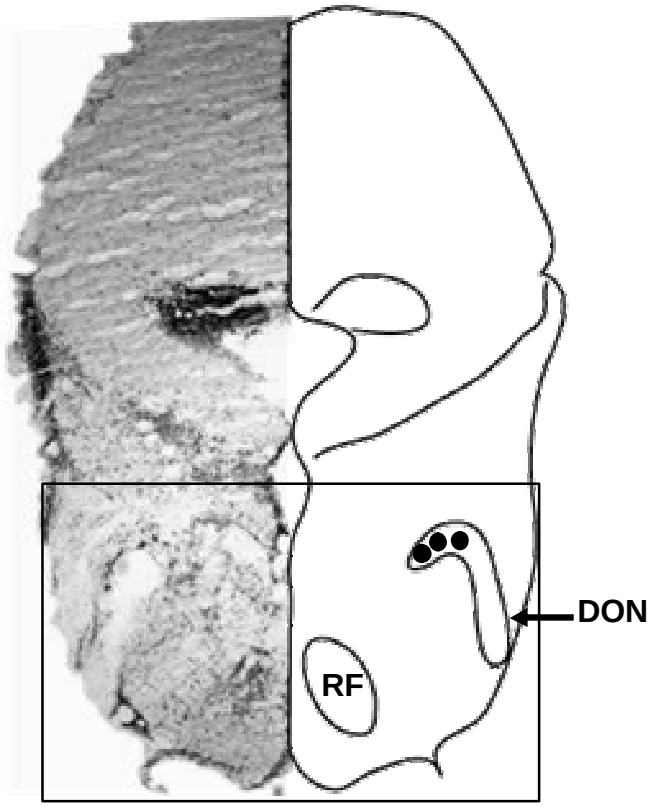


Figure 4A. The descending octaval nuclei are located bilaterally in the medulla and are labeled when the NC is injected with HRP. The DON receives input from CN VIII and relays this information to the NC. The area outline by the box can be seen in Figure 4B.

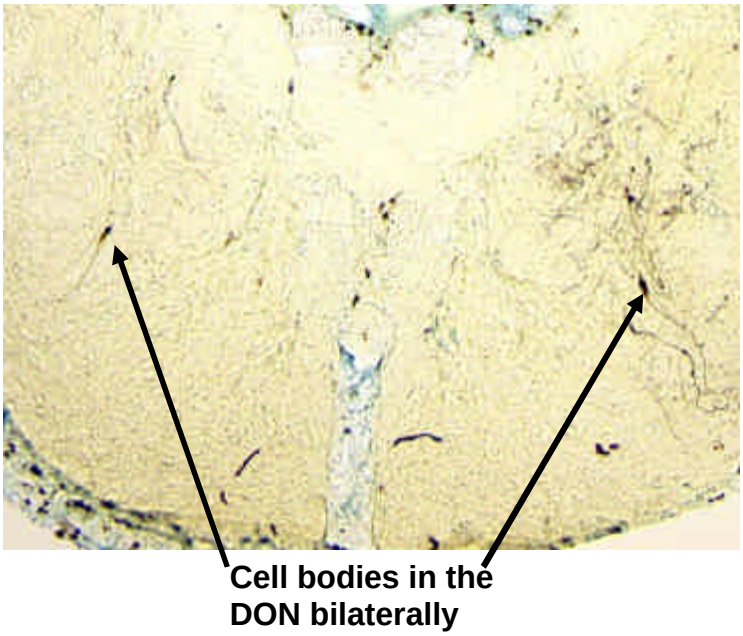


Figure 4B. With unilateral injections to the NC, spindle shaped cells of the descending octaval nuclei are labeled bilaterally. More labeling of the DON occurs on the ipsilateral side.

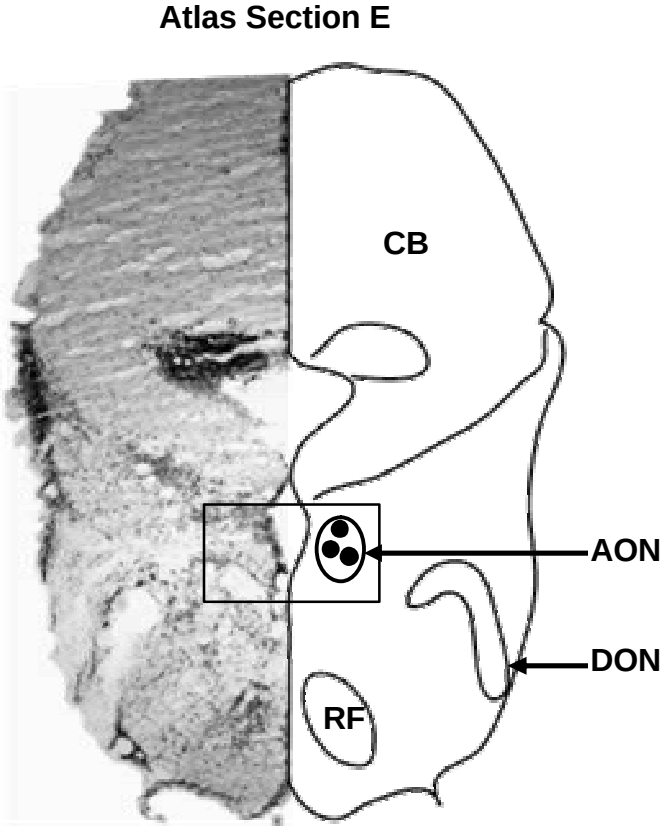


Figure 5A. The AON is labeled when the NC is injected with HRP. However, the labeling is more predominant on the ipsilateral side of the injection site. The box indicates the area shown in Figure 5B.

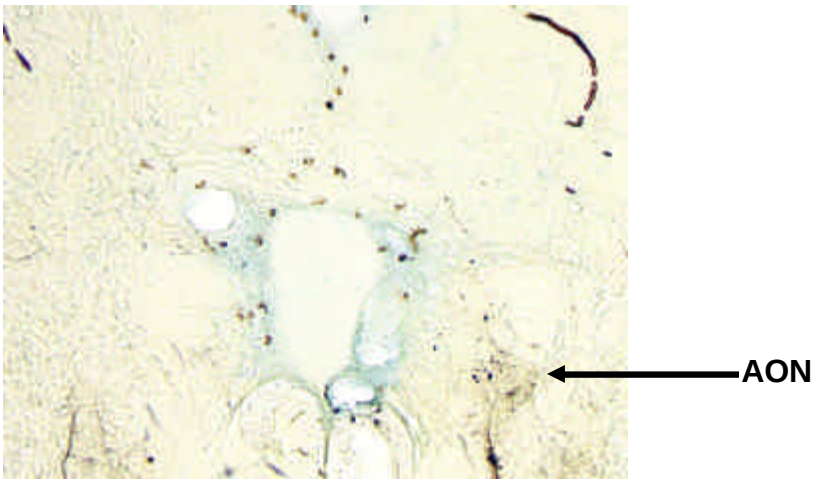


Figure 5B. The AON is labeled primarily on the ipsilateral side with HRP injections to the NC.

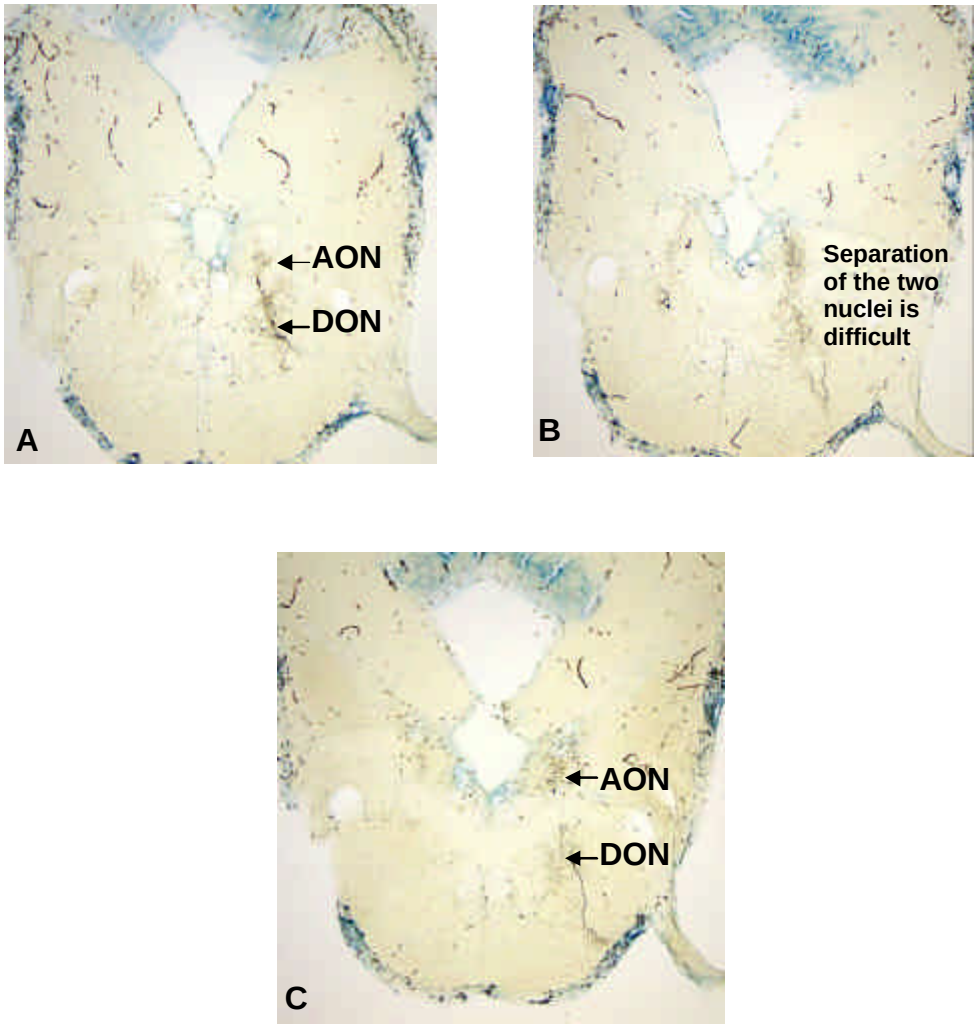


Figure 6. The above series of photographs of the medulla were taken from a brain with an HRP injection to the NC. While all photos show both the AON and the DON, the mixing of fibers from both is apparent. Photo (A) is caudal to (B). In a more rostral section (C), the two nuclei are distinguishable once again.

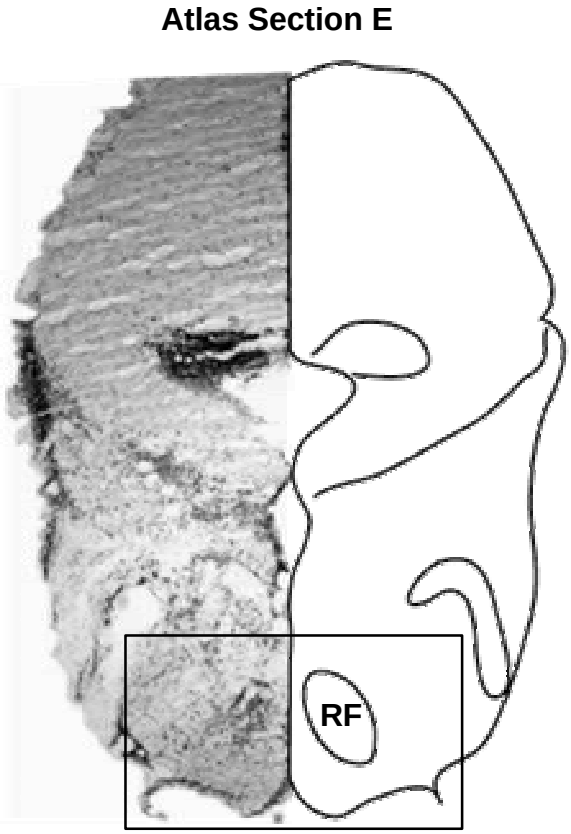


Figure 7A. Scattered fibers were found throughout the extent of the reticular formation (RF) from medulla to midbrain. The box indicates the area shown in Figure 7B.

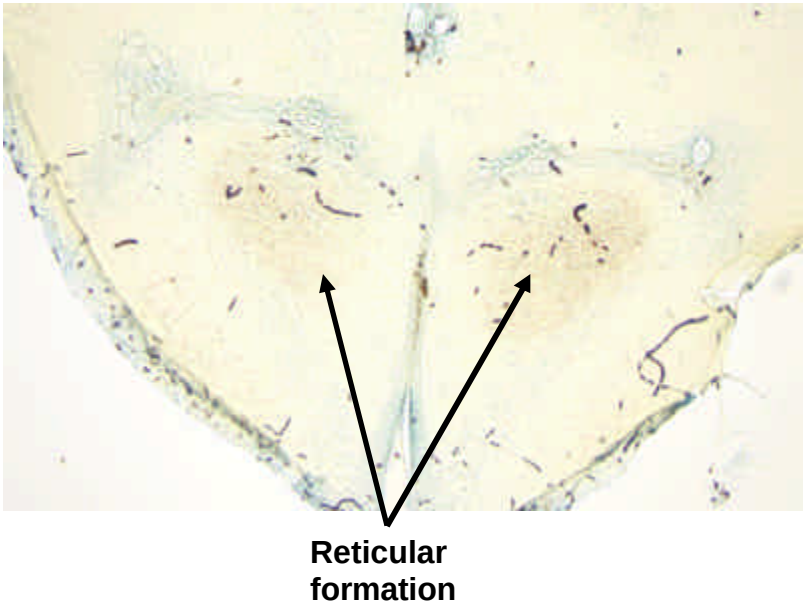


Figure 7B. Small isolated cells were found in the RF bilaterally with injections to the NC throughout the medulla and midbrain

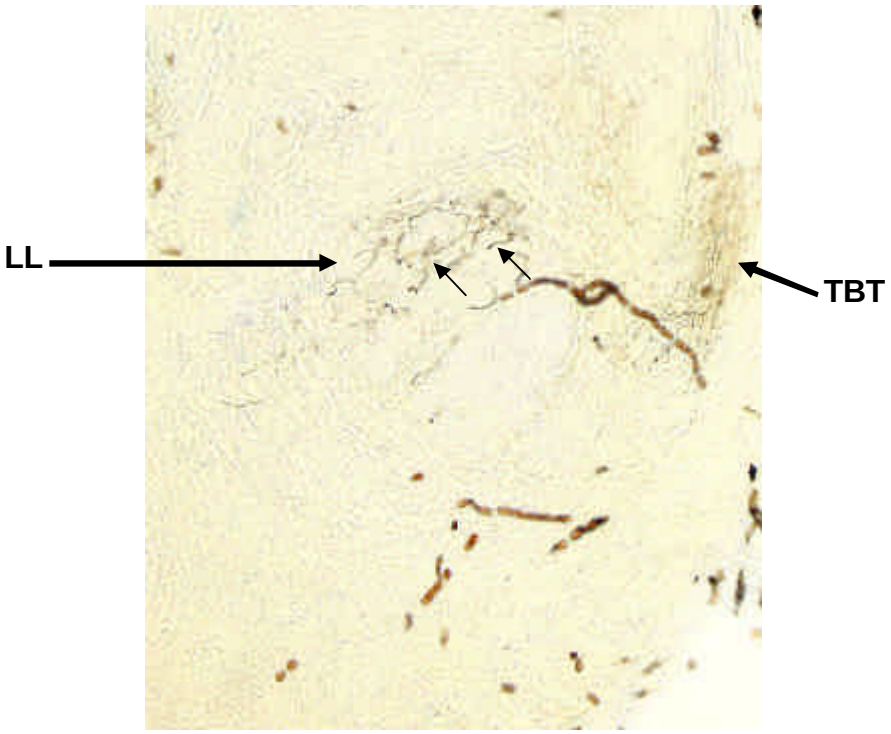


Figure 8. With injections of HRP to the NC, labeled fibers are found in the dorsomedial lateral lemniscus (LL). Small arrows indicate individual fibers in the LL. The tectobulbar tract (TBT) can be seen to the right of the LL.

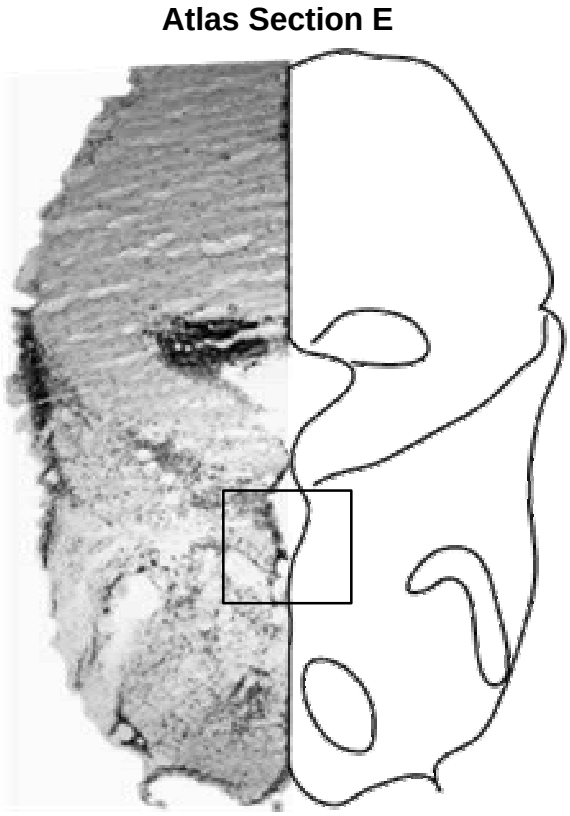


Figure 9A. Labeled cells and fibers were seen in close proximity to the ventricle in the medulla. The identity of these cells is unknown. The area indicated by the box is shown in Figure 9B.

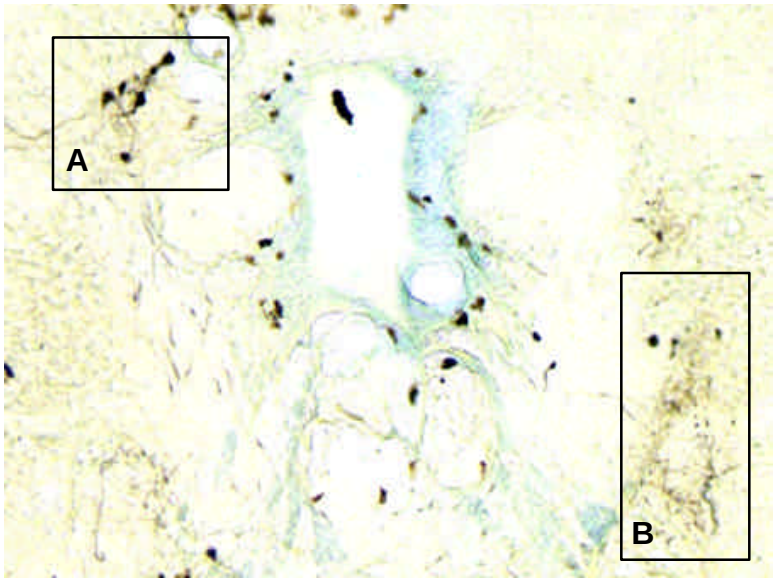


Figure 9B. Periventricular cells were found after injections to the NC. The identity of these areas remains unknown at this time. The labeled boxes are shown in more detail in Figure 9C.

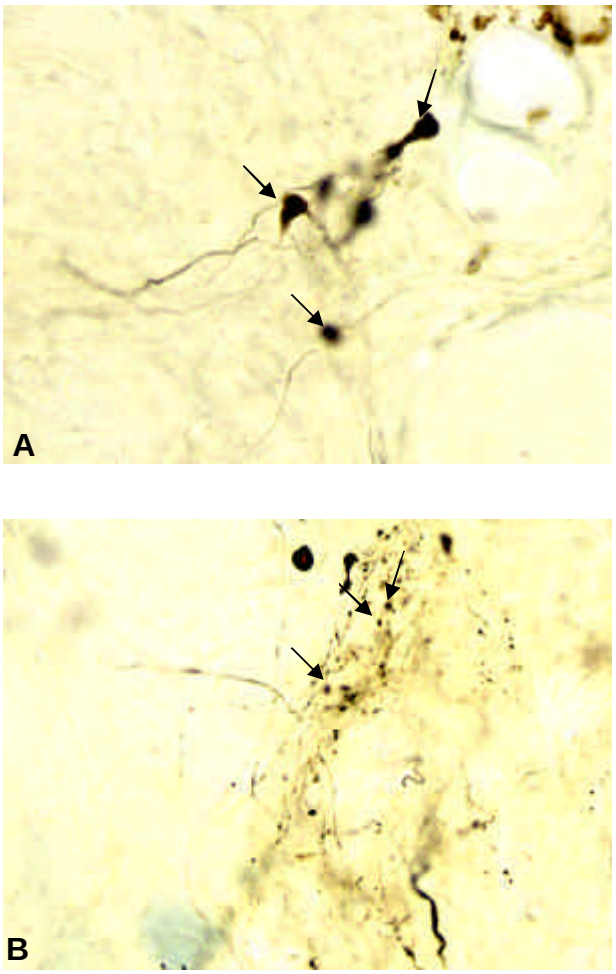


Figure 9C. Labeled cells and fibers could be seen in close proximity to the ventricle. Neurons (indicated by small arrows in A) with laterally extending processes and possible terminals (indicated by small arrows in B)

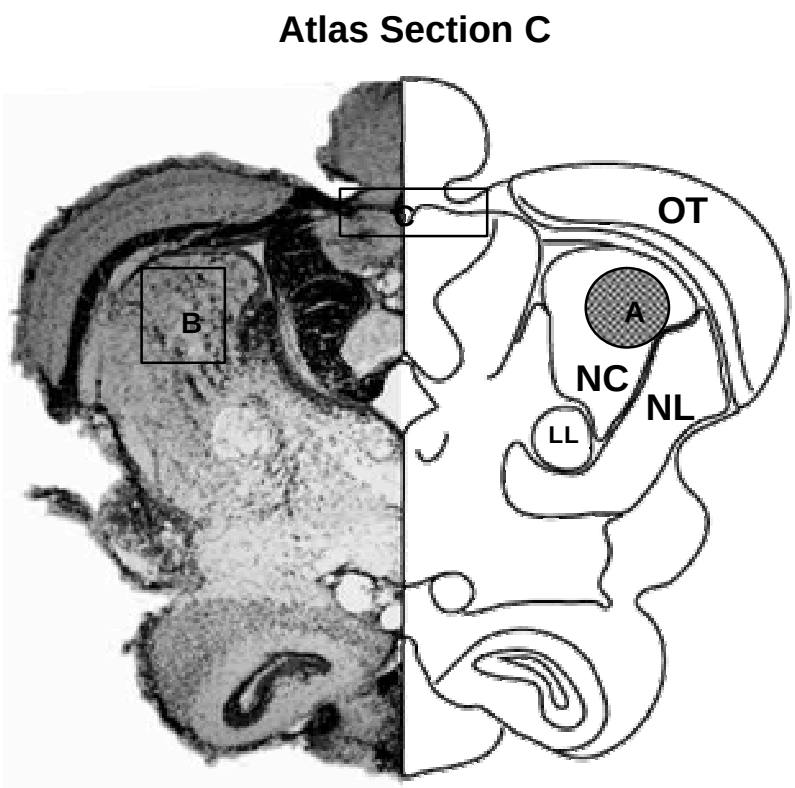


Figure 10A. A HRP injection to the NC indicated by the large gray circle “A”, results in labeled cells in the contralateral TS indicated by “B”. Commissural fibers passing from the ipsilateral to the contralateral TS are indicated by “C”. The letters marked in the boxes and injection site correspond to the lettered photos in Figure 10B.

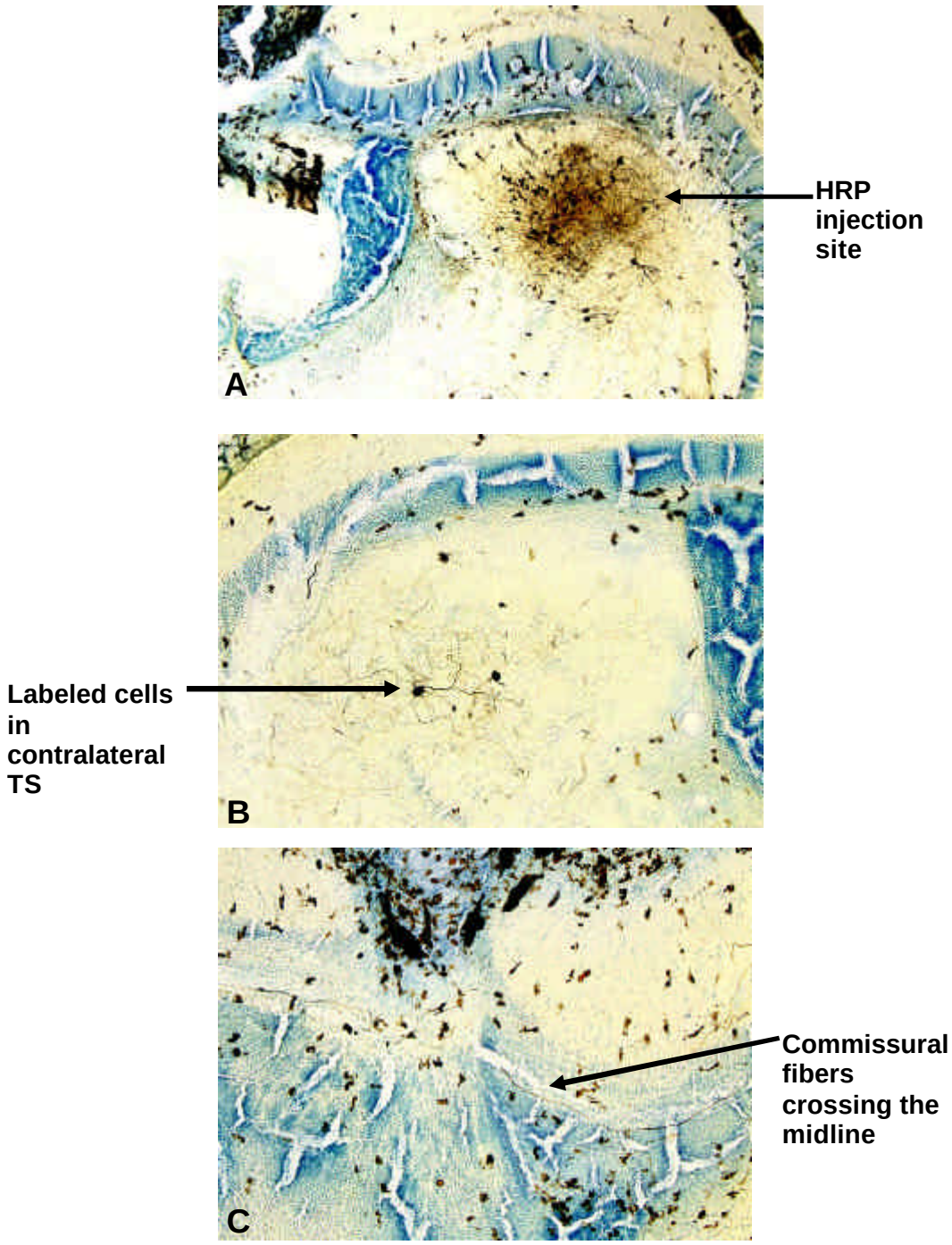


Figure 10B. When the TS is injected with HRP (A), cells in the contralateral torus (B) are also labeled. Commissural fibers (C) connecting the two tori can be identified.

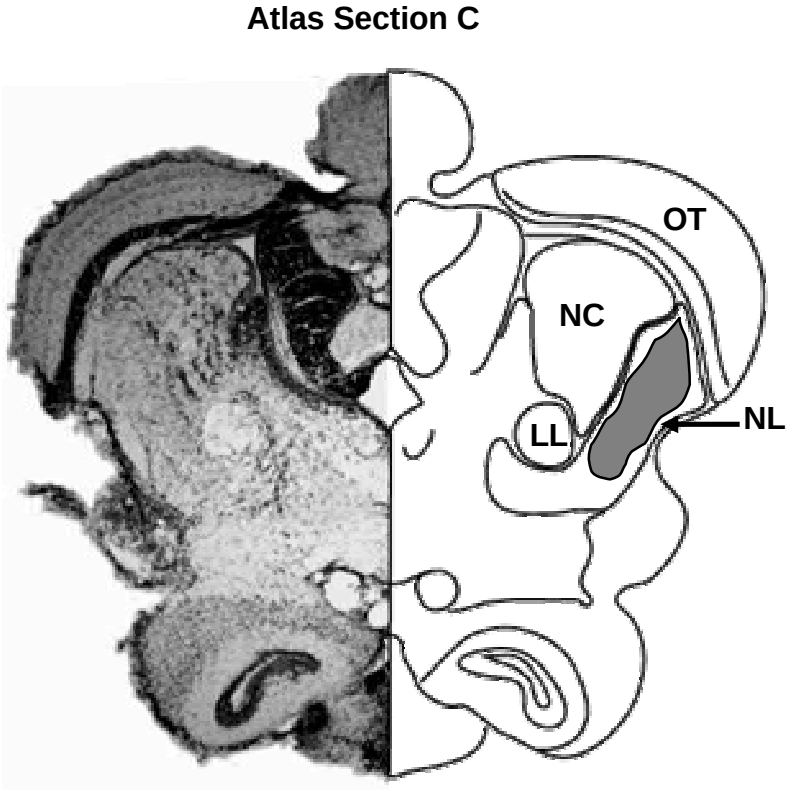


Figure 11A. The gray area indicates a HRP injection site in the nucleus lateralis (NL) in the TS.

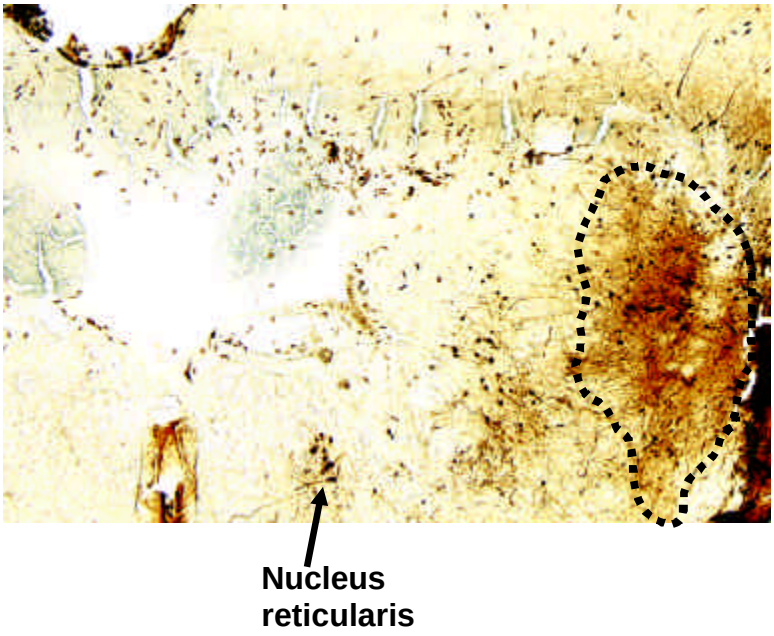


Figure 11B. Lateral injection site in the NL indicated by the broken line. Note the labeling of the nucleus reticularis in the lower center of the photo.

Atlas Section F

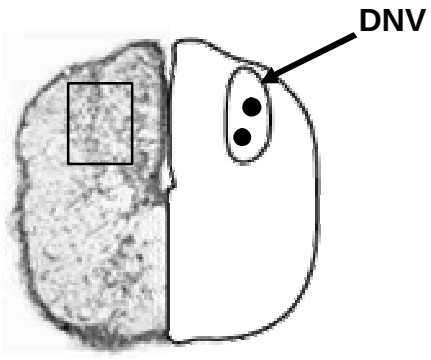


Figure 12A. Labeled cells, indicated by solid circles, are found in the descendens nucleus of V (DNV) when HRP injections are placed in the NL. The DNV is located in the dorsolateral medulla (see Figure 1). These cells show extensive branching processes as shown in Figure 12B in the area indicated by the box.

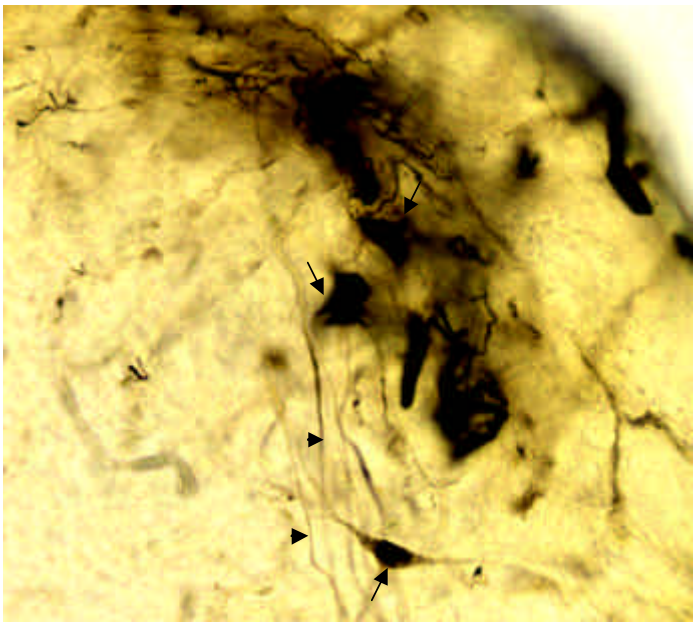


Figure 12B. Labeled cells, indicated with small arrows, and fibers, indicated by arrowheads, are found at caudal levels of the medulla contralateral to the injection site in the DNV when HRP is injected to the NL.

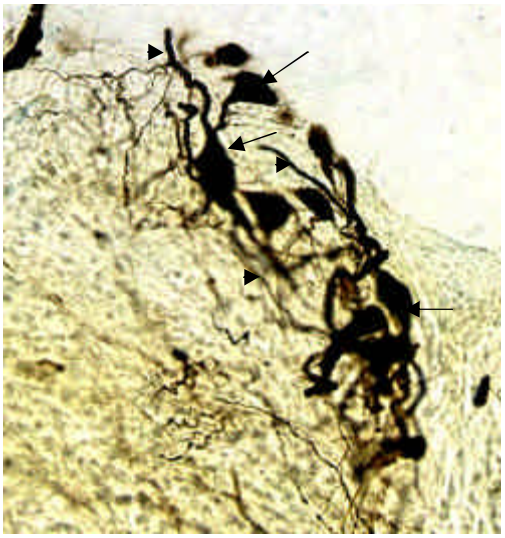


Figure 12C. Labeling in the DNV in the rostral medulla. Labeled cell bodies are indicated by small arrows while labeled fibers are indicated by arrowheads.

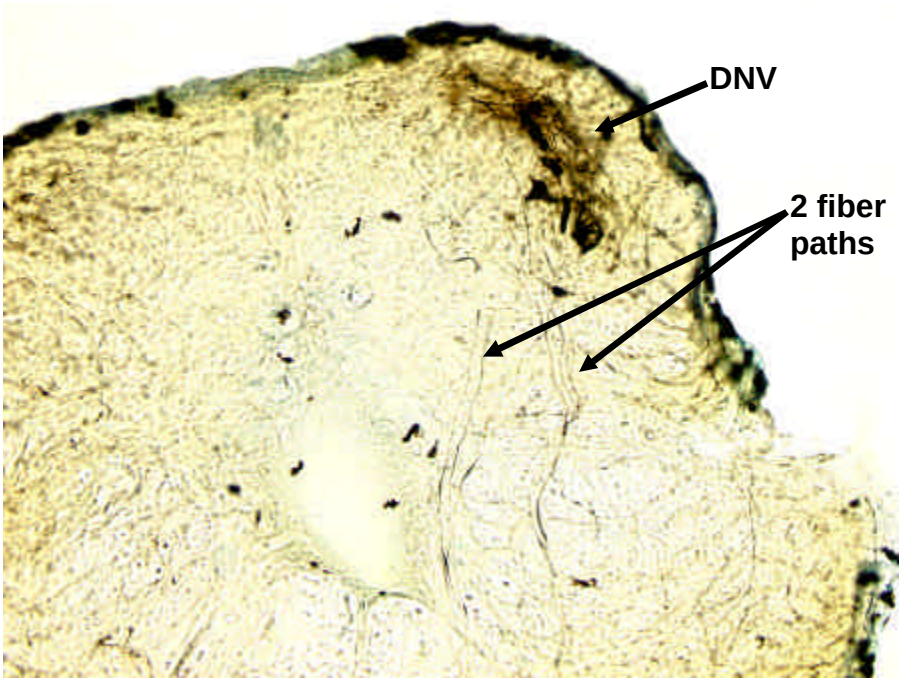


Figure 12D. Fibers split into 2 separate bundles as they leave the DNV. The fibers ascend in the TBT and the LL.

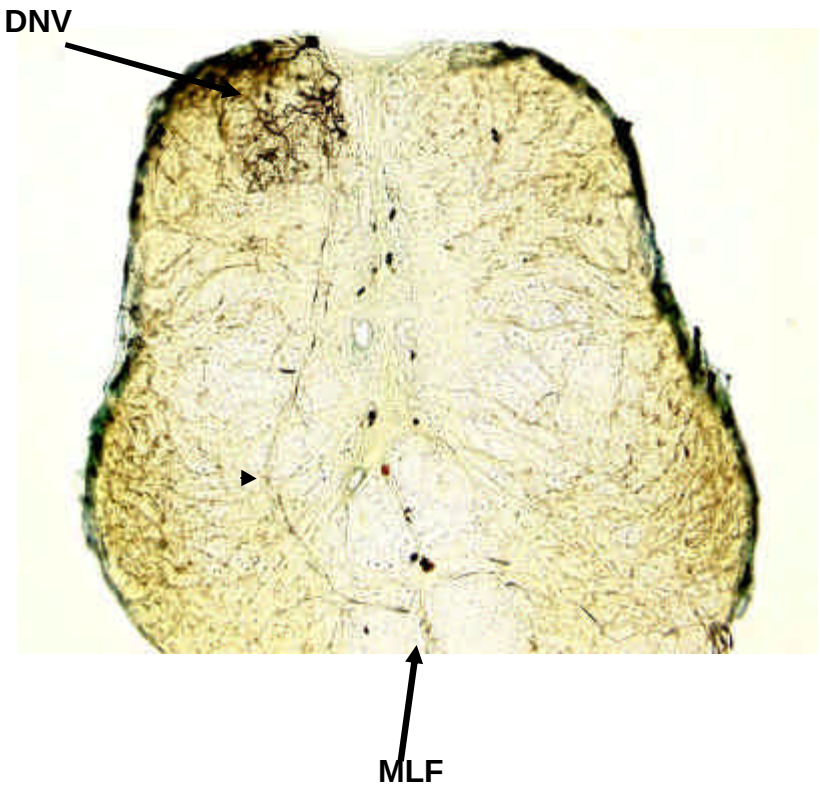


Figure 12E. The photograph shows the DNV with labeled fibers, indicated by arrowheads, leaving the nucleus in a ventral direction to decussate across the midline at the medial longitudinal fasciculus (MLF).

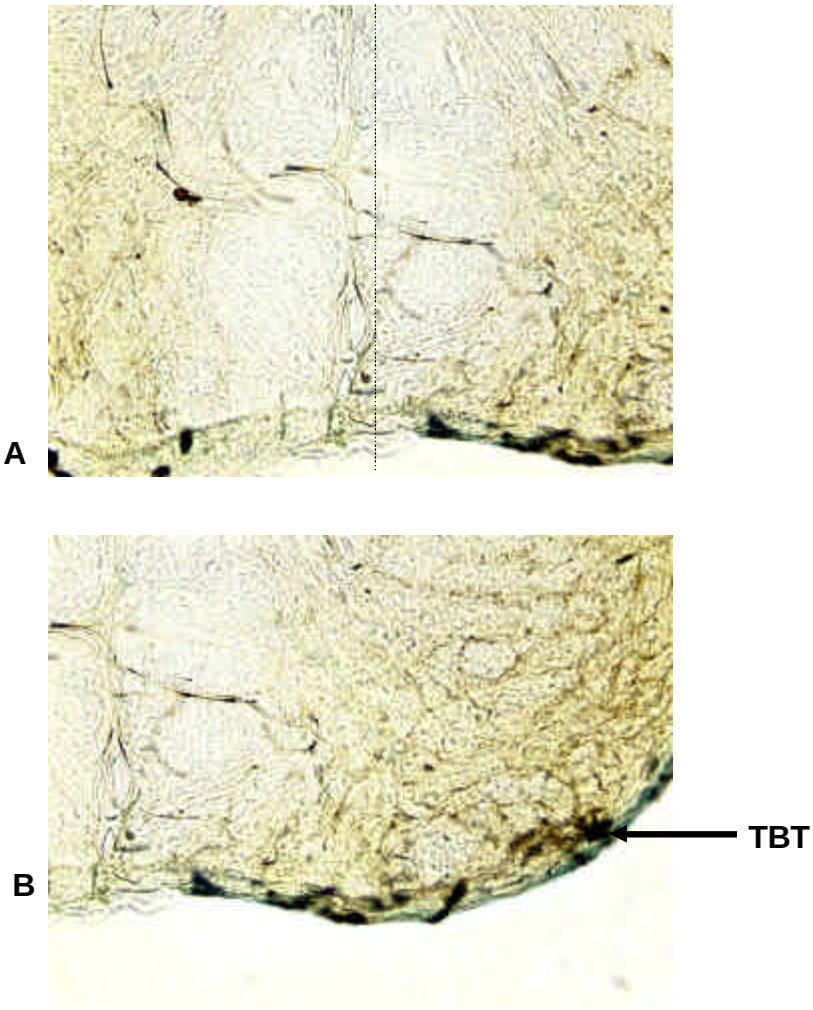


Figure 12F. The decussation of fibers across the midline through the MLF is shown in photograph A. These fibers cross the midline, indicated by the broken line in photograph A, to the ventral lateral side of the medulla to pass in the TBT as shown in photograph B.

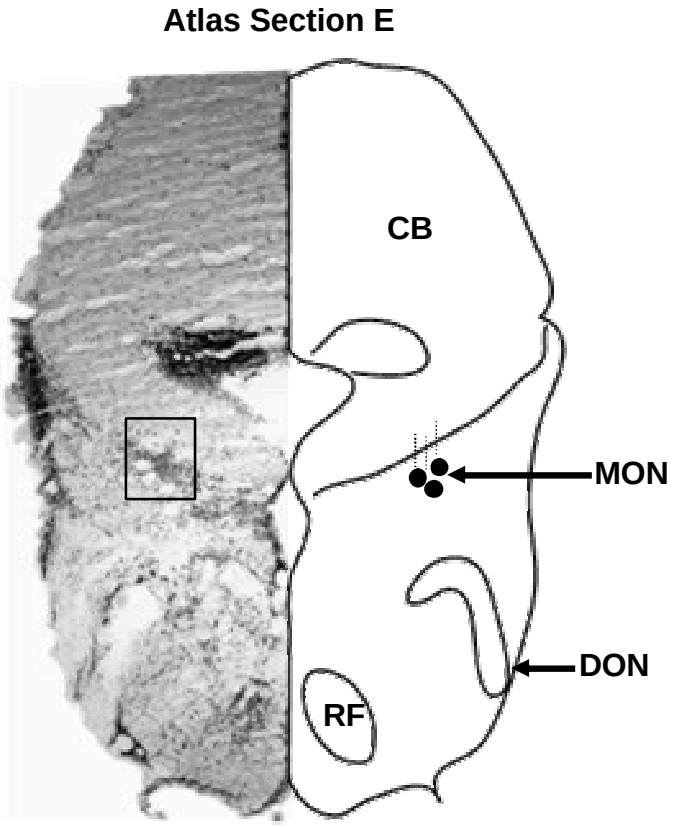


Figure 13A. Purkinje-like cells are labeled in the medial octavolateralis nucleus with lateral injections of the TS. These cells send dendritic branches into the overlying cerebellum indicated by the dotted lines. The area in the box is shown in Figure 13B and 13C.

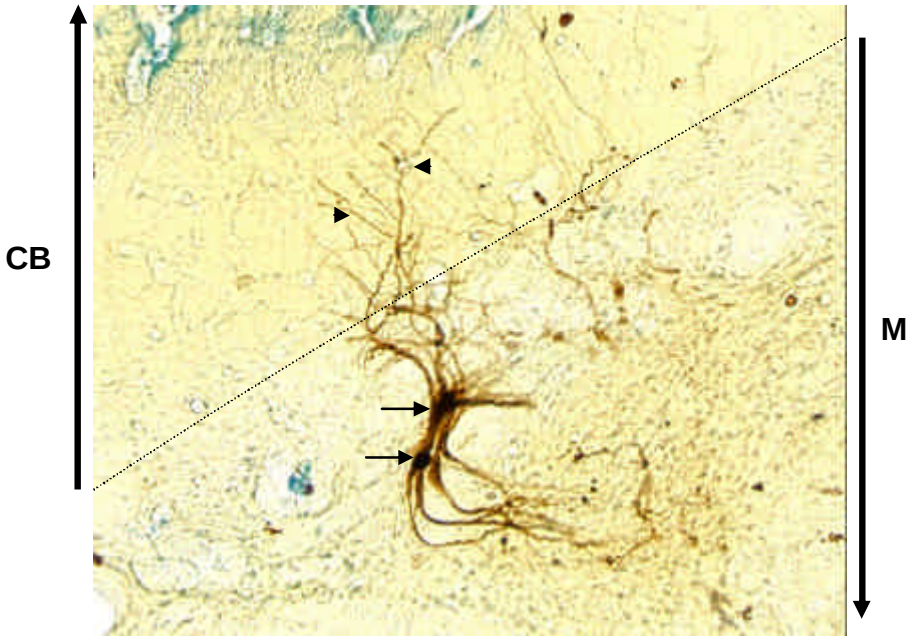


Figure 13B. Cells and processes of the MON labeled from an injection in the NL. Note the dorsal projection of dendrites into the crest of the cerebellum. The cerebellum is indicated by the region labeled CB and the medullary region is labeled M. Labeled cell bodies are indicated by the small arrows and labeled dendrites are marked with arrowheads.

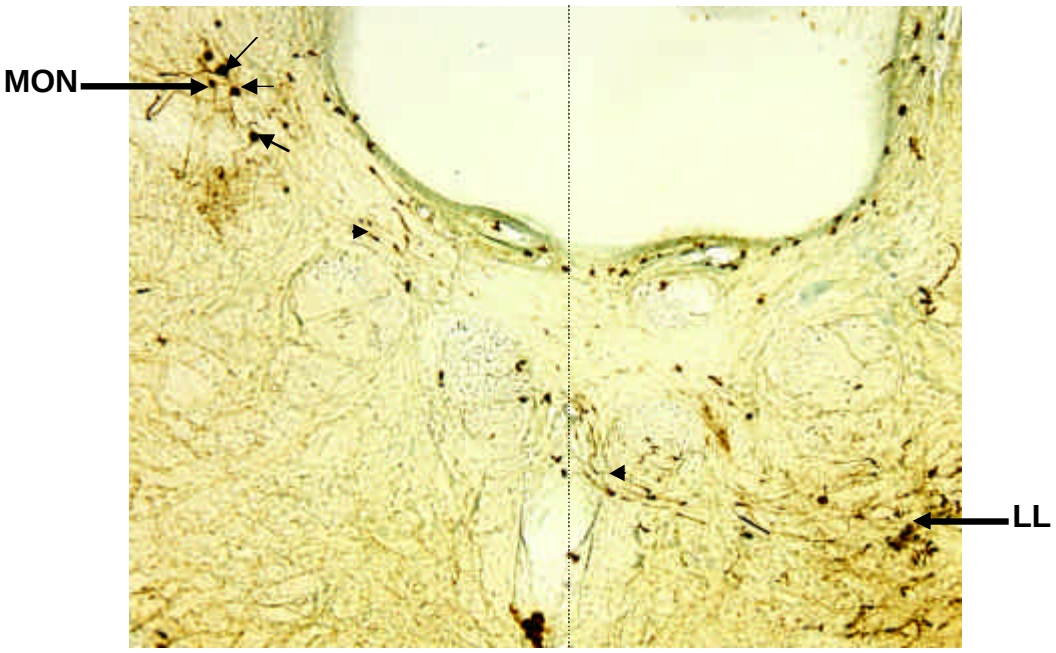


Figure 14. Labeled fibers (indicated by arrowheads) from cells located in the MON decussate across the midline, indicated by the broken line, to pass in the lateral lemniscus (LL) to the NL. Labeled cell bodies in the MON are indicated by small arrows.

Atlas Section A

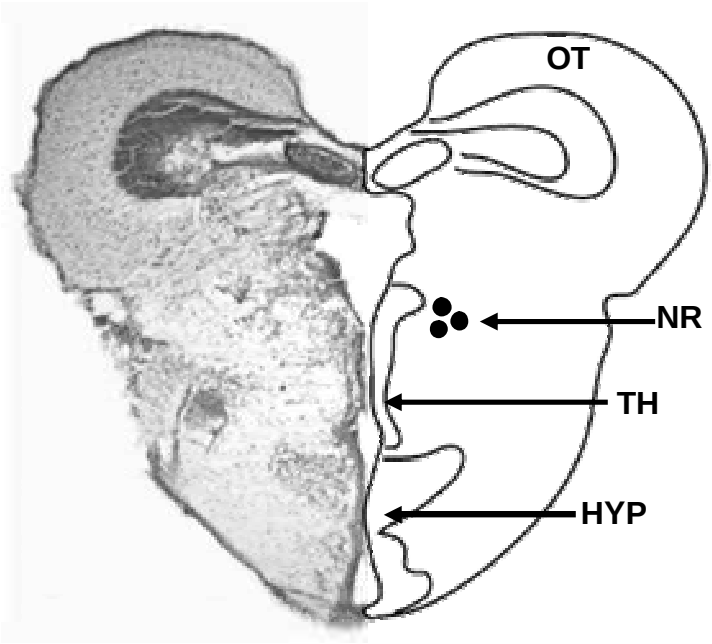


Figure 15A. Labeled cells are found in the nucleus reticularis (NR) with injections of HRP to the NL. These cells are indicated by the solid black circles and can be seen in Figure 15B.

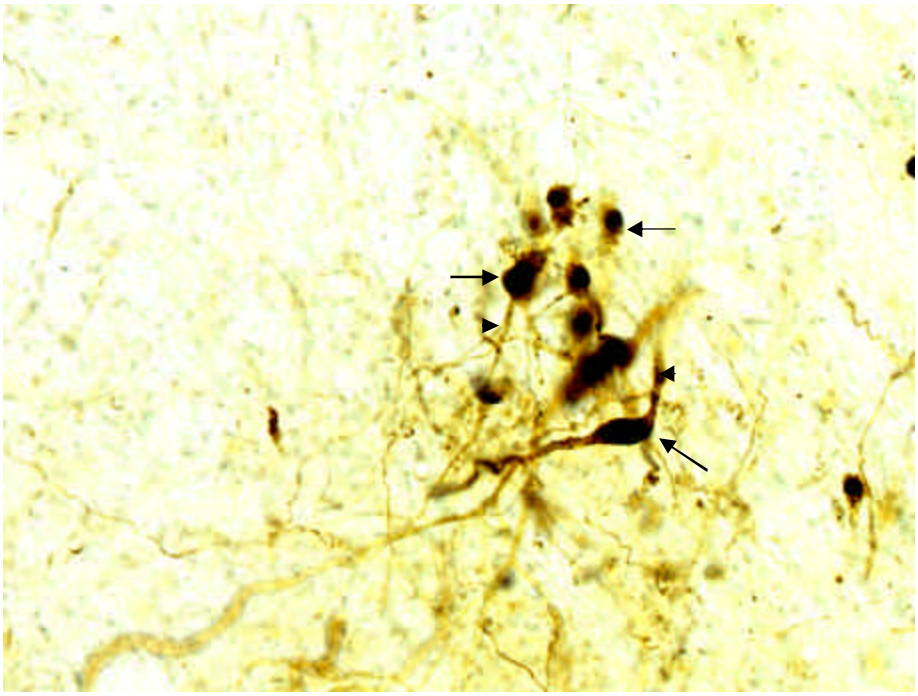


Figure 15B. Labeling in the nucleus reticularis consisted of a tight cluster of cell bodies and fibers. Labeled cell bodies are indicated by small arrows while labeled fibers are marked with arrowheads.

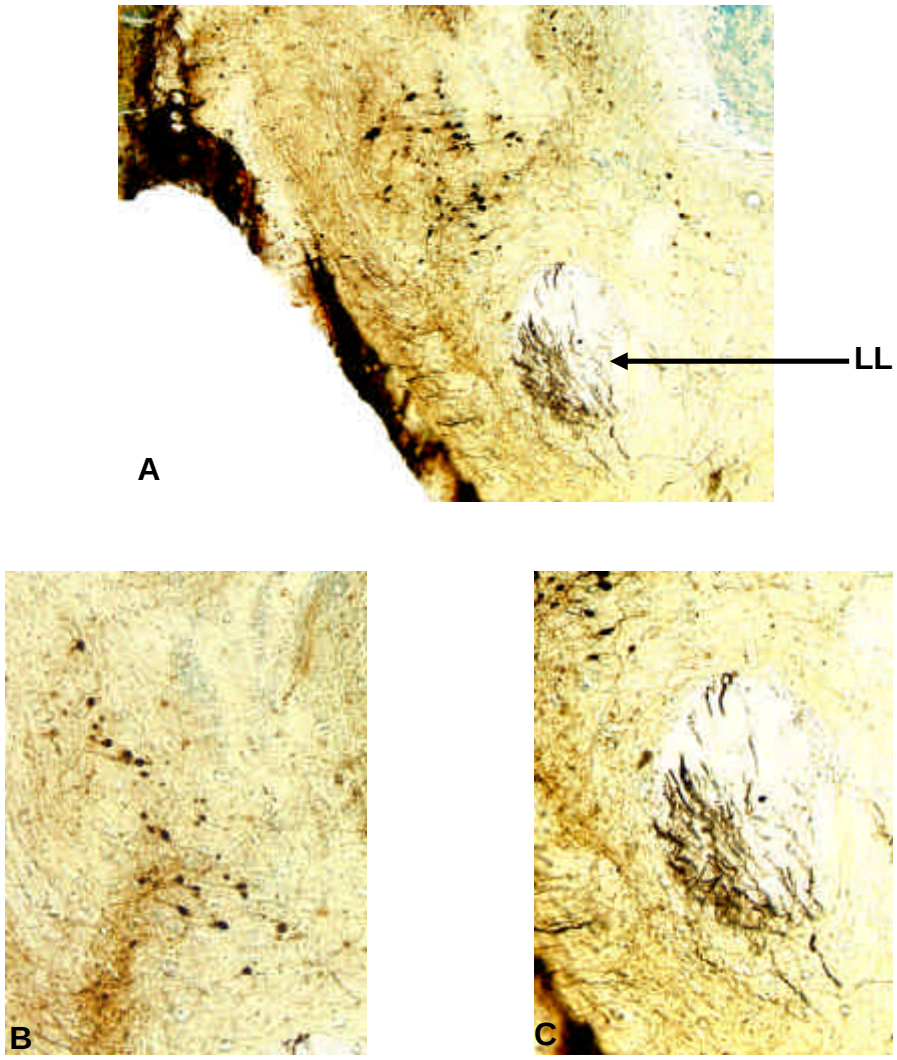


Figure 16. The above photographs are of the caudal midbrain of a fish with a lateral injection to the NL. Several scattered cells were seen in the tegmentum dorsolateral to the lateral lemniscus as seen in A. Fibers passing in the LL are topographically arranged with injections to the NL labeling fibers in the ventral lateral portion of the tract (see photo C). These fibers orginate in the octavolateralis region of the medulla with the majority of fibers ascending from the MON.

Atlas Section A

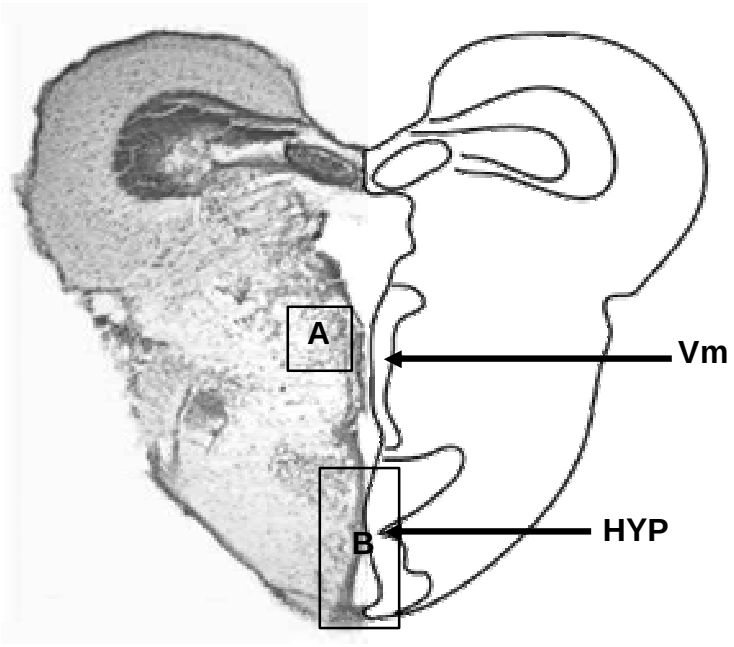


Figure 17A. In more rostral sections of the brain, labeled cells and fibers can be found in the ventralmedial nucleus of the thalamus (Vm) and scattered cells are seen in the anterior tuberal nucleus of the hypothalamus (HYP). Areas outlined by boxes A and B are shown in Figure 17B.

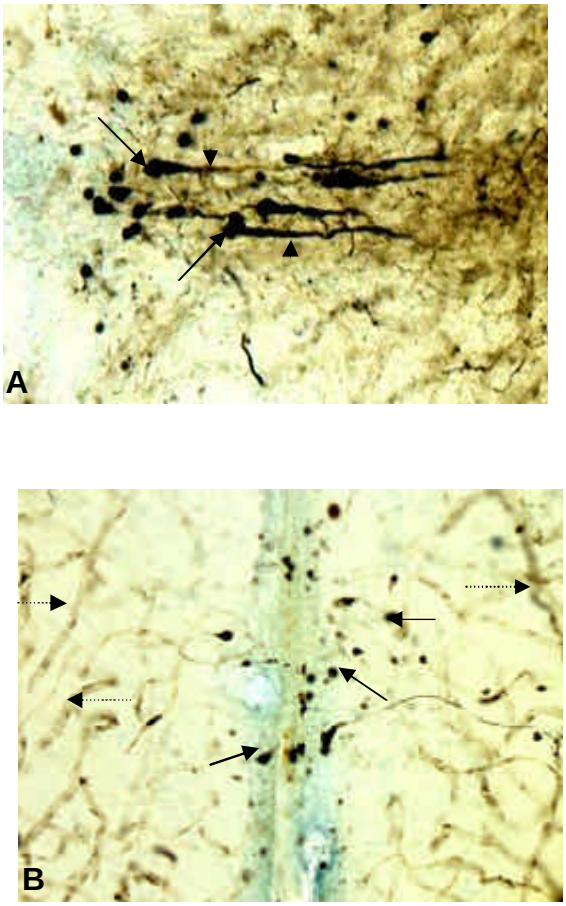


Figure 17B. Labeled cells (indicated by small arrows) with laterally directed processes (indicated by arrowheads) were located in the ventromedial nucleus of the thalamus (A). These cells are found only at rostral levels with HRP injections to the NL. Scattered cells (indicated by small arrows) can be seen in the hypothalamus (B). The enzymatic reaction that occurs at times when using HRP in the endothelial cells of blood vessels (indicated by broken arrows) results in the artifact seen in the field of view in B.

DISCUSSION

The main finding of this study of torus semicircularis afferents in cave *A. fasciatus* are consistent with previous research on sighted fish. The NC receives a substantial input from the AON and DON of the medulla (Echteler 1984; Murakami et al., 1986) while the NL receives primary afferents from the MON, a source of lateral line information (Schellart, 1983; Murakami et al., 1986).

NC Afferents

The majority of NC afferents arise from the medulla and Echteler (1984) focused his research on these connections. The results found in cave *A. fasciatus* paralleled Echteler's results for the AON and DON to the TS in *Cyprinus carpio*. Likewise, Murakami et al. (1986) found similar connections with HRP injections to the NC in *S. marmoratus*. The AON and DON, both receive input from CN VIII projections (Echteler 1984; Northcutt 1980). Consistent with this finding, Echteler documented electrophysiological recordings from these two nuclei in carp indicating auditory responses. Since labeled neurons were seen in both of these nuclei in cave *A. fasciatus*, like *C. carpio* and *S. marmoratus*, cave *A. fasciatus* has a dual input auditory pathway to the NC of the TS.

Echteler identified a medial and lateral olivary nucleus, however these two nuclei were not identified by HRP labeling in cave *A. fasciatus*. In addition, previous investigators have not seen evidence of the superior olivary nucleus in fishes, however Echteler reported cells in the medullary region of *C. carpio* that he described as comparable to the superior olivary nucleus. Data in this study suggest this same cluster of cells, however with the close proximity of the cells

of the DON it remains unclear if true boundaries exist between these two nuclei and therefore the existence of the superior olivary nucleus in cave *A. fasciatus* is inconclusive.

Echteler concluded auditory information is relayed to higher brain areas, including the TS, within the lateral lemniscus. This pathway for ascending auditory information in the lateral lemniscus was also found in the brain of cave *A. fasciatus*. Fibers from the AON and DON of the medulla passed to the NC in the dorsomedial region of the LL, while the fibers from the MON passed in the ventrolateral portion of the LL to the NL.

Cell labeling between the telencephalon and the TS was not evident in this study. Echteler (1984) reported minor projections between these two brain regions. However, Murakami et al. (1986) did not find telencephalic projections to the TS. A possible explanation for the lack of evidence of this connection in cave *A. fasciatus* might be the area of the TS injected. Possibly only extreme rostral injections may show the telencephalic connection based on the topography of the TS. While injections used in this research were large enough to cover the majority of the TS, invariably some regions may have been missed. Further studies would have to be made to ascertain any connections the telencephalon may have with the TS in cave *A. fasciatus*.

Labeled cells were also found in the contralateral TS and deep layers of the ipsilateral OT with HRP injections to the NC. In addition, labeled commissural fibers passed along the lower border of the deep layers of the optic tectum to the labeled cell bodies in the contralateral NC. Both of these ipsilateral OT and contralateral NC afferent connections have been reported by previous investigators (Knudsen, 1977; Grover and Sharma, 1981).

Murakami et al. (1986) reported labeled neurons in caudal regions of the contralateral cerebellum of *S. marmoratus* with injections to the NC, however these cells were not found in cave *A.*

fasciatus. Cave *A. fasciatus* may differ from *S. marmoratus* in this projection, or this discrepancy may be explained by the position of the injection site. A neural projection from the CB to the TL has been previously been reported (Ito, 1978; Northmore, 1984). The torus longitudinalis (TL) is dorsomedial to the TS and in close proximity. HRP from a large or extremely dorsomedial injection in the NC could spread to terminals of CB neurons in the TL, thus labeling cells in the CB.

Sparse bilateral fibers and cells were found in the medial reticular formation (MRF) by Echteler (1984), Northcutt (1980) and McCormick (1981) with injections in the NC. Northcutt and McCormick identified these same cells and fibers in the MRF of the longnose gar and carp respectively. While several fibers were found in the MRF with injections to the NC, cells within the MRF were not found in cave *A. fasciatus*. None of these authors offered an explanation for the function of this projection.

NL Afferents

When the NL was injected with HRP (it is a rather difficult target to hit due to it's diagonal positioning in the midbrain), the majority of labeled cells were found in the contralateral MON. These consisted of large Purkinje-like cells with dorsally directed dendrites that invaded the overlying cerebellar crest. The MON consumes a large portion of the lateral region of the brainstem and is a primary, if not an exclusive target of the lateral line nerves (Schellart, 1983). This nucleus extends from the spinomedullary junction to the molecular layer of the cerebellum (McCormick, 1983). These findings are consistent with previous observations (Schellart, 1983; Murakami et al., 1986; New et al., 1996) and demonstrates that lateral line information is a primary input to the NL.

A minor afferent projection of the anterior tuberal nucleus (AT) of the hypothalamus was reported by Striedter using DiI. Labeled cells from these injections were observed bilaterally in the NL. The same afferent neurons were found in cave *A. fasciatus* with injections of HRP to the NL.

Another source of toral afferents to the NL were from a distinct cell group corresponding to the nucleus decendens of V (DNV) (Nieuwenhuys and Pouwels, 1983; Murakami et al., 1986). According to Woodburne (1936) and Nieuwenhuys and Pouwels (1983) the DNV is composed of small cells with interspersed fibers present throughout the length of the nucleus. This nucleus extends from the caudal pole of the trigeminal nucleus caudally to the dorsal horn of the spinal cord. Labeled afferent neurons were found throughout the entire rostrocaudal extent of the contralateral DNV in cave *A. fasciatus*. Ariens Kappers (1936) thought the ascending fibers from this group carry general tactile and pain information and Woodburne (1936) suggested the fibers from this nucleus decussated to form a trigeminomesencephalic and tectal tract, a sensory component of the tectobulbar tract. The finding of a contralateral projection from DNV in cave *A. fasciatus* is consistent with Woodburne's proposal.

Labeled cells were found in the Vm in cave *A. fasciatus* with HRP injections to the NL. When Murakami et al. (1986) injected HRP in the Vm of the teleost, *Sebastiscus marmoratus,* they found descending fibers running caudally and then turning dorsolaterally to terminate in the TS. Murakami et al. (1986) also noted fibers crossing from the Vm through the posterior commissure to descend to the contralateral TS. Striedter (1991) showed similar results in the channel catfish, *Ictalurus punctatus* with DiI injections to the NL. These injections revealed labeled cells ipsilaterally in the caudal Vm paralleling the same results in cave *A. fasciatus*.

The TS of cave *A. fasciatus* could be considered multimodal as it receives input from the AON and DON, both auditory nuclei, and lateral line input from the MON. In addition to auditory and mechanosensory information, the TS also receives somatosensory (tactile and pain) from the DNV and the Vm (first suggested for fish by Arien Kappers, 1936). Both the DNV and the Vm carry somatosensory information from head regions to the NL. The significance of this input to the NL and its impact on lateral line processing remains an interesting question.

CONCLUSIONS

Horseradish peroxidase is a classic neuroanatomical tract tracing technique. This research utilized this neural tracer to demonstrate the primary afferents of the torus semicircularis in cave *A. fasciatus*. The results of this study suggest the connections of the TS in cave *A. fasciatus* are very similar, if not identical, to the connections of the TS in sighted teleosts studied previously with HRP and DiI.

The original hypothesis of this research was that the brain of naturally blind cave *A. fasciatus* would exhibit differences in the afferent connections of the TS, relative to sighted teleosts, due to evolutionary enhancement of the lateral line sense to compensate for the loss of vision. However, the hypothesized differences in connectivity were not found. For the most part, the major identifiable connections of the TS with the nuclei of the medulla are the same as those found in other teleosts with functional visual systems.

There are several possible explanations for the lack of change in connectivity:

(1) ***Lack of evolutionary time.*** Due to the relatively short time (50,000-100,000 years) these fish have evolved from the sighted form (Wilkens, 1985, 1988), it is possible the neural connections have not had enough time to show the changes that might occur and are, therefore still in the process of change. In many other species of cave fish the lateral line organs have undergone extensive hypertrophy (Poulson and White, 1969). Cave *A. fasciatus* does show minor modifications in structures of the lateral line. Therefore, the changes in the sensitivity of the lateral line system in cave *A. fasciatus* may be in progress.

(2) ***Environmental conditions.*** Due to the fact that typical cave environments are characterized as energy poor with low influx of food (Poulson and White, 1969), cave inhabitants usually must adapt to survive. Most species of cavefish have greatly hypertrophied lateral line systems for efficient localization of food (Poulson and White, 1969). However, the limestone caves of Mexico have a considerable seasonal influx of food and are not considered as energy poor as other cave environments (Breder, 1942; Mitchell et al., 1977). Therefore, further changes in brain connectivity of cave *A. fasciatus* may not be required to compensate for extreme energy deprivation like that experienced by inhabitants of other caves. Further, the minor changes that have occurred in the neuromast of cave *A. fasciatus* (Wilkens, 1985, 1988) that have provided a slightly more sensitive mechanosensory input to the TS may be all that is required for survival in their environment.

(3) ***Interneuron connectivity.*** This study uses HRP to examine gross connections that carry sensory information from one brain region to another. Perhaps no change or additional gross lateral line input connections are required for good TS function. However, possible increases in their ability to find food and improve navigation may be due to improved processing of lateral line information by the TS by changes in interneuron connectivity. This would not be detectable with HRP tract tracing techniques.

(4) ***Changes in other brain areas.*** Perhaps changes in the connections of the TS are not required at all to improve the functioning of the lateral line system. There may be increased sensitivity of the lateral line sense due to evolutionary modifications in areas of the brain not studied. For example, mechanosensory responses have been

recorded in the OT in cave *A. fasciatus* (Fish and Voneida, 1983; Voneida and Fish, 1984), but not in the sighted form of *A. fasciatus* (Fish, unpublished) or reported in the literature for any teleost species. Since the OT (superior colliculus in mammals) subserves behaviors for orienting to environmental cues in all vertebrates studied (Kandel et al., 1991) it is possible that the OT in cave *A. fasciatus* is evolutionarily modified for improved lateral line orienting behavior. These speculations would require additional research.

References

Abdel-Latif H, Hassan ES, von Campenhausen C (1990) Sensory performance of blind Mexican cave fish after destruction of the canal neuromasts. *Naturwissenschaften* 77:237-239.

Andrews C (1986) A Fishkeeper's Guide to Fish Breeding. Salamander Books Ltd., London, UK

Ariens Kappers CU, Huber GC, Crosby EC (1936) The Comparative Anatomy of the Nervous System of Vertebrates Including Man. Reprinted 1960. Harper Publishing Company, NY

Avise JC, Selander RK (1972) Evolutionary genetics of cave-dwelling fishes of the Genus *Astyanax. Evolution* 26:1-19

Barr, Jr. TC (1968) Cave ecology and the evolution of troglobites. Evolutionary Biology. Plenum Press, New York, NY

Bass AH, Bodnar DA, Marchaterre MA (2001) Acoustic nuclei in the medulla and midbrain of the vocalizing gulf toadfish (*Opsanus beta*). *Brain, Behavior and Evolution* 57:63-79

Bensouilah M, Denizot JP (1991) Taste buds and neuromasts of *Astyanax jordani*: Distribution and immunochemical demonstration of co-localized substance P and enkephalins. *European Journal of Neuroscience* 3:407-414

Bleckmann H, Niemann U, Fritzsch B (1991) Peripheral and central aspects of the acoustic and lateral line system of a bottom dwelling catfish, *Ancistrus* sp.. *Journal of Comparative Neurology* 314:452-466

Boudriot F, Reutter K (2001) Ultrastructure of the taste buds in the blind cave fish *Astyanax jordani* ("*Anopticthys*") and the sighted river fish *Astyanax mexicanus* (Teleostei, Characidae). *Journal of Comparative Neurology* 434:428-444

Braford, Jr. MR, Northcutt RG (1983) Organization of the diencephalon and pretectum of the ray-finned fishes. Fish Neurobiology 1:117-164 (Northcutt RG, Davis RE eds.) The University of Michigan Press, Ann Arbor, MI

Breder, Jr. CM (1942) Descriptive ecology of La Cueva Chica, with special reference to the blind fish, *Anoptichthys*. *Zoologica* 27:7-15

Breder, Jr. CM (1943) Problems in the behavior and evolution of a species of blind cave fish. *Transactions of the New York Academy of Sciences* 5:168-176

Butler AB, Wullimann MF, Northcutt RG (1991) Comparative cytoarchitectonic analysis of some visual pretectal nuclei in teleosts. *Brain, Behavior and Evolution* 38:92-114

Cuadrado MI (1989) A quantitative study of the torus semicircularis of *Barbus meridionalis* Risso. *Journal fur Hirnforschung* 30(3):371-374

Cuadrado MI, Covenas R, Tramu G (1992) Neuropeptides in the torus semicircularis of the carp (*Cyprinus carpio*). *Brain Research Bulletin* 28:593-598

Cuadrado MI, Covenas R, Tramu G (1992) Neuropeptides and monoamines in the torus semicircularis of the carp (*Cyprinus carpio*). *Brain Research Bulletin* 29:529-539

Diaz SM, Anadon R (1989) Central projections of the lateral line nerves of *Chelon labrosus* (Teleosts, Order Perciformes). *Journal fur Hirnforschung* 30(3):339-347

Diaz-Regueira S, Anadon R (1990) Primary nerve projections and primary nuclei of the octaval nerve in the teleost *Chelon labrosus*. An HRP study. *Journal fur Hirnforschung* 31(6):705-714

Echteler SM (1984) Connections of the auditory midbrain in a teleost fish, *Cyprinus carpio. Journal of Comparative Neurology* 230:536-551

Feng AS, Lin W (1991) Differential innervation patterns of three divisions of frog auditory midbrain (Torus semicirularis). *Journal of Comparative Neurology* 306:613-630

Fernald RD, Shelton LC (1985) The organization of the diencephalon and the pretectum in the cichlid fish, *Haplochromis burtoni*. *Journal of Comparative Neurology* 238:202-217

Fricke, D (1988) Reaction to alarm substances in cave populations of *Astyanax mexicanus* (Characidae, Pisces). *Ethology* 76:305-308

Fiebig E, Ebbesson SOE, Meyer DL (1983) Afferent connections of the optic tectum in the piranha (*Serrasalmus nattereri*). *Cell and Tissue Research* 231:55-72

Fish SE, Ghosh P (1992) Occulomotor system of a blind cave fish. *Society of Neuroscience Abstracts* 18:327

Fish SE, Rhoades RW (1981) A method for making very small, quantifiable micropipette injections of axonal tracer substances. *Journal of Neuroscience Methods* 4:291-297

Fish SE, Voneida TJ (1979) Extravisual neurons in the optic tectum of a sighted and unsighted fish. *Society for Neuroscience Abstracts* 5:784

Gomez-Segade, P, Anadon, R (1988) Specialization in the diencephalon of advanced telelosts. *Journal of Morpholgy* 197:71-103

Grover BG, Sharma SC (1981) Organization of extrinsic tectal connections in goldfish (*Carassius auratus*). *Journal of Comparative Neurology* 196:471-488

Howarth FG (1980) The zoogeography of specialized cave animals: A bioclimatic model. *Evolution* 34(2):394-406

Ito H, Kishida R (1978) Afferent and efferent fiber connections of the carp torus longintudinalis. *Journal of Comparative Neurology* 181:465-476

Jeffery WR (2001) Cavefish as a model system in evoulutionary developmental biology. *Developmental Biology* 231:1-12

John KR (1957) Observations on the behavior of blind and blinded fishes. *Copeia* 2:123-132

Kandel ER, Schwartz JH, Jessell TM (1991) Principles of Neural Science. Elsevier Science Publishing Company, NY

Katsuki Y, Yanagisawa K (1982) Chemoreception in the lateral line organ. Chemoreception in Fishes (TJ Hara, ed.). Elsevier Science Publishing Company, NY

Kiyohara S, Yamashita S, Lamb CF, Finger TE (1999) Distribution of trigeminal fibers in the primary facial gustatory center of channel catfish, *Ictalurus punctatus*. *Brain Research* 841:93-100

Knudsen EI (1977) Distinct auditory and lateral line nuclei in the midbrain of catfishes. *Journal of Comparative Neurology* 173:417-432

Langecker TG, Neumann B, Hausberg C, Parzefall J (1995) Evolution of the optical releasers for aggressive behavior in cave-dwelling *Astyanax fasciatus* (Teleostei, Characidae). *Behavioural Processes* 34:161-168

Lu Z, Fay RR (1993) Acoustic reponse properties of single units in the torus semicircularis of the goldfish, *Carassius auratus*. *Journal of Comparative Physiology* 173:33-48

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

Luiten PGM, Van der Pers JNC (1977) The connections of the trigeminal and facial motor nuclei in the brain of the carp (*Cyprinus carpio* L.) as revealed by anterograde and retrograde transport of horseradish peroxidase. *Journal of Comparative Neurology* 174:575-590

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

McCormick CA (1983) Organization and evolution of the octavolateralis area of fishes. Fish Neurobiology 1:179-213 (Northcutt RG, Davis RE eds.) The University of Michigan Press, Ann Arbor, MI

McCormick CA, Braford, Jr. MR (1993) The primary octaval nuclei and inner ear afferent projections in the ostophysan *Ictalurus punctatus*. *Brain, Behavior and Evolution* 42:48-68

Meredith GE, Butler AB (1983) Organization of eighth nerve afferent projections from individual endorgans of the inner ear in the teleost, *Astronotus ocellatus*. *Journal of Comparative Neurology* 220:44-62

Miller RR, Smith ML (1985) Origin and geography of the fishes of Central Mexico. The Zoogeography of North American Freshwater Fishes (Hocutt CH, Wiley EO eds.). John Wiley and Sons, New York, NY

Mitchell RW, Russell WH, Elliott WR (1977) Mexican eyeless characin fishes, Genus *Astyanax*: Environment, distribution, and evolution. Special Publications The Museum. Texas Tech Press, Lubbock, Texas

Murakami T, Fukuoka T, Ito H (1986) Telencephalic ascending acousticolateral system in a teleost (*Sebastiscus marmoratus*), with special reference to the fiber connections of the nucleus preglomerulosus. *Journal of Comparative Neurology* 247:383-397

New JG, Coombs S, McCormick CA, Oshel PE (1996) Cytoarchitecture of the medial octavolateralis nucleus in the goldfish, *Carassius auratus*. *Journal of Comparative Neurology* 366:534-546

New JG, Snyder BD, Woodson KL (1998) Descending neural projections to the spinal cord in the channel catfish, *Ictalurus punctatus*. *The Anatomical Record* 252:235-253

Nieuwenhuys R, Pouwels E (1983) The brain stem of actinopterygian fishes. Fish Neurobiology 1:25-87 (Northcutt RG, Davis RE eds.) The University of Michigan Press, Ann Arbor, MI

Northcutt RG (1980) Retinal projections in the Australian lungfish. *Brain Research* 185:85-90

Northcutt RG (1982) Localization of neurons afferent to the optic tectum in longnose gar. *Journal of Comparative Neurology* 204:325-335

Northcutt RG (1983) Evolution of the optic tectum in ray-finned fishes. Fish Neurobiology 1:1-42 (Northcutt RG, Davis RE eds.) The University of Michigan Press, Ann Arbor, MI

Northcutt RG, Butler AB (1980) Projections of the optic tectum in the longnose gar, *Lepisosteus osseus*. *Brain Research* 190:333-346

Northcutt RG, Butler AB (1993) The diencephalon and optic tectum of the longnose gar, *Lepisosteus osseus*: Cytoarchitectonics and distribution of acetylchoinesterase. *Brain, Behavior and Evolution* 41:57-81

Northcutt RG, Butler AB (1993) The diencephalon of the pacific herring, *Clupea harengus*: Retinofugal projections to the diencephalon and optic tectum. *Journal of Comparative Neurology* 328:547-561

Northcutt RG, Braford, Jr. MR (1984) Some efferent connections of the superficial pretectum in the goldfish. *Brain Research* 296:181-184

Northcutt RG, Davis RE (1983) Telencephalic organization in ray-finned fishes. Fish Neurobiology 1:203-236 (Northcutt RG, Davis RE eds.) The University of Michigan Press, Ann Arbor, MI

Northcutt RG, Holmes PH, Albert JS (2000) Distribution and innervation of lateral line organs in the channel catfish. *Journal of Comparative Neurology* 421:570-592

Northmore DPM (1984) Visual and saccadic activity in the goldfish torus lontiudinalis. *Journal of Comparative Physiology A* 155:333-340

Page CH, Sutterlin AM (1970) Visual-auditory unit responses in the goldfish tegmentum. *Journal of Neurophysiology* 33:129-136

Parzefall J (1986) On the heredity of behavior patterns in cave animals and their epigean relatives. *NSS Bulletin* 47(2):128-135

Pinuela C, Baatrup E, Geneser FA (1992) Histochemical distribution of zinc in the brain of the rainbow trout, *Oncorhynchos myciss*. *Anatomy and Embryology* 186:275-284

Pombal MA, Alvarez-Otero R, Rodicio MC, Anadon R (1997) A tract-tracing study of the central projections of the mesencephalic nucleus of the trigeminus in the guppy (*Lebistes reticulates, Teleostei*), with some observations on the descending trigeminal tract. *Brain Research Bulletin* 42(2):111-118

Popper AN, Fay RR (1980) Structure and function in teleost auditory systems. Comparative Studies of Hearing in Vertebrates. Springer-Verlag, New York, NY

Popper AN, Fay RR (1993) Sound detection and processing by fish: Critical review and major research questions. *Brain, Behavior, and Evolution* 41:14-38

Poulson TL, White WB (1969) The cave environment. *Science* 165:971-981

Pritz MB, Stritzel ME (1990) Thalamic projections from a midbrain somatosensory area in a reptile, *Caiman crocodilus*. *Brain, Behavior and Evolution* 36:1-13

Untergasser D (1989) Handbook of Fish Diseases. T.F.H. Publications, Inc., Neptune City, NJ

Reidel G (1997) The forebrain of the blind cave fish *Astyanax hubbsi* (Characidae). *Brain, Behavior and Evolution* 49:20-38

Reidel G, Krug L (1997) The forebrain of the blind cave fish *Astyanax hubbsi* (Characidae). *Brain, Behavior and Evolution* 49:39-52

Romero A (1984) Behavior in an "intermediate" population of the subterranean-dwelling characid *Astyanax fasciatus*. *Environmental Biology of Fishes* (4)10:203-207

Rupp B, Northcutt RG (1998) The diencephalon and pretectum of the white sturgeon (*Acipenser transmontanus*): A cytoarchitectonic study. *Brain, Behavior and Evolution* 51:239-262

Sadoglu P (1956) A preliminary report on the genetics of the Mexican cave characins. *Copeia* 2:113-114

Sadoglu P (1979) A breeding method for blind *Astyanax mexicanus* based on annual spawning patterns. *Copeia* 2:369-371

Saidel WM, Butler AB (1997) An atypical diencephalic nucleus in actinopterygian fishes: Visusal connections and sporadic phylogenetic distribution. *Neuroscience Letters* 229:13-16

Saidel WM, Butler AB (1997) Visual connections of the atypical diencephalic nucleus rostrolateralis in *Pantodon buchholzi* (Teleostei, Ostelglossomorpha). *Cell and Tissue Research* 287:91-99

Schellart NAM (1983) Acousticolateral and visual processing and their interaction in the torus semicircularis of the trout, *Salmo gairdneri*. *Neuroscience Letters* 42:39-44

Schlussman SD, Kobylack MA, Dunn-Meynell AA, Sharma SC (1990) Afferent connections of the optic tectum in channel catfish *Ictalurus punctatus*. *Cell and Tissue Research* 262:531-541

Sligar CM, Voneida TJ (1976) Tectal Efferents in the blind cave fish *Astyanax hubbsi*. *Journal of Comparative Neurology* 165:107-124

Striedter GF (1990) The diencephalon of the channel catfish, *Ictalurus punctatus*. *Brain, Behavior and Evolution* 36:329-354

Striedter GF (1990) The diencephalon of the channel catfish, *Ictalurus punctatus*. *Brain, Behavior and Evolution* 36:355-377

Striedter GF (1991) Auditory, electrosensory, and mechanosensory lateral line pathways through the forebrain in channel catfishes. *Journal of Comparative Neurology* 312:311-331

Striedter GF (1992) Phylogenetic changes in the connections of the lateral preglomerular nucleus in ostariophysan teleosts: A pluralistic view of brain evolution. *Brain, Behavior and Evolution* 39:329-357

Striedter GF, Northcutt RG (1989) Two distinct visual pathways through the superficial pretectum in a percomorph telelost. *Journal of Comparative Neurology* 283:342-354

Teyke T (1990) Morphological differences in neuromasts of the blind cave fish *Astyanax hubbsi* and the sighted river fish *Astyanax mexicanus*. *Brain, Behavior and Evolution* 35:23-30

Voneida TJ (1973) A comparative sudy of retinotectal projections in the blind cave characin, *Astyanax hubbsi*, and its sighted ancestor, *Astyanax mexicanus*. *Anatatomical Record* 175:462-463

Voneida TJ, Fish SE (1984) Central nervous system changes related to the reduction of visual input in a naturally blind fish (*Astyanax hubbsi*). *American Zoologist* 24:775-782

Voneida, TJ, Fish SE, Cauller, TC (1983) Central projections of trigeminal and lateral line nerves in the blind cave fish, *Astyanax hubbsi*. *Society of Neuroscience Abstracts* 9:1167

Voneida TJ, Sliger CM (1976) A comparative neuroanatomic study of retinal projections in two fishes: *Astyanax hubbsi* (the blind cave fish) and *Astyanax mexicanus*. *Journal of Comparative Neurology* 165:89-106.

Webb JF (1989) Gross morphology and evolution of the mechanoreceptive lateral line system in teleosts fishes. *Brain, Behavior and Evolution* 33:34-53

Wilkens H (1971) Genetic interpretation of regressive evolutionary processes: Studies on hybrid eyes of two *Astyanax* cave populations (Characidae, Pisces). *Evolution* 25:530-544

Wilkens H (1985) The evolution of polygenic systems, studied on epigean and cave populations of *Astyanax fasciatus* (Characidae, Pisces). *NSS Bulletin* 47(2):101-108

Wilkens H (1987) Genetic analysis of evolutionary processes. *International Journal of Speleology* 16:33-58

Wilkens H (1988) Evolution and genetics of epigean and cave *Astyanax fasciatus* (Characidae, Pisces). *Evolutionary Biology* 23:271-367

Wullimann MF, Northcutt RG (1988) Connections of the corpus cerebelli in the green sunfish and the common goldfish: A comparison of perciform and cypriniform teleosts. *Brain, Behavior and Evolution* 32:293-316

Yamamoto Y, Jeffery WR (2000) Central role for the lens in cave fish eye degeneration. *Science* 289:631-633.

Zeitlin SM (1973) Hormonal induction of ovulation and spawning in the blind cave fish, *Anoptichthys jordani* with the use of human chorionic gonadotropin. *Experientia* 20:461-462