Histomorphogenesis of cranial nerves in *Huso huso* larvae

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**Abstract**

In this study the cranial nerves development of *H. huso* are explained from 1 to 54-days-old (1, 3, 6, 15, 21 and 54 days). Despite all the researches on fish brain, there are no study on nerves evolution on *H. huso* during their larvae life. For this research 40 samples of larvae *H. huso* were obtained (from each age, about six samples were selected). The specimens were maintained in fiberglass tank, then histological samples were taken from tissues and stained with hematoxylin and eosin for general histological studies using light microscope. According to the results, on 1 and 3-days-old, no nerve was observed. The terminal nerve and their dendrites were observed around the nasal cavity and the axons projected to different areas in forebrain especially around olfactory bulb diffusely, on 6-day-old fish. Also, olfactory, optic, oculomotor, trochlear, trigeminal, lateral line and vagus nerves were detected on 6-day-old fish, however two parts of lateral line nerve were separated on 54-day-old. Three nerves, profundus, facial and octaval were observed on 54-day-old, however, up to this age, epiphysial nerve was not observed.

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**Key words:** Brain nerves, development, histology, *Huso huso*, larvae.

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**Histоморфогенез аусєбов магзи в дарою філ махі**

*He*ıstemorfofоzus ауєсбуов магзи ды *лароу* філ махі

چکیده

در این مطالعه تکامل اعصاب مغزی در دوران لاروی فیل ماهی از 1 تا 45 روزه (سنین 1, 3, 6, 15, 21 و 45 روزه) شنا داده شده است. با وجود تحقیقات زیادی که بر روی ماهیان انجام شده است، تاکنون مطالعه ای درخصوص تکامل اعصاب مغزی فیل ماهی در دوران لاروی انجام نشده است. بدین منظور تعداد 40 نمونه فیل ماهی (از هر سن حدوداً شش نمونه انتخاب شده) به همراه دیگر عناصر آماده بودند. در این مطالعه ماهیان که در فیل‌های اولاری مورد مطالعه قرار گرفتند، از آن‌ها میکروسکوپی نوری پیاده‌سازی و فرآیند مطالعه قرار گرفتند. بر اساس نتایج که در ماهیان شناسایی شدند، ماهیانی که در هر سن حاصل شدند، بافت شناسی همراه با رنگ آمیزی با هماتوکسیلین و اوزین، بر روی سلول‌های مختلف مغزی مورد تحقیق قرار گرفتند. در نتیجه، اعصاب از جمله: اعصاب بویایی، بینایی، اکولوموتور، تروکلئار، ترگیال، خط جانبی، و اعصاب جانبی و ماهیان واقع در هر سن حاصل شدند. با توجه به نتایج، در این مطالعه تکامل اعصاب مغزی فیل ماهی به فرموله زیر در جدول ۱ حاصل شد:

| سال | اعصاب موجود | گروه | دستگاه | ماهیان |
|-----|--------------|------|--------|--------|
| 1   | نا رخ داده شد | نا رخ داده شد | نا رخ داده شد | نا رخ داده شد |
| 3   | نا رخ داده شد | نا رخ داده شد | نا رخ داده شد | نا رخ داده شد |
| 6   | آرایش و ساخت | آرایش و ساخت | آرایش و ساخت | آرایش و ساخت |
| 15  | آرایش و ساخت | آرایش و ساخت | آرایش و ساخت | آرایش و ساخت |
| 21  | آرایش و ساخت | آرایش و ساخت | آرایش و ساخت | آرایش و ساخت |
| 45  | آرایش و ساخت | آرایش و ساخت | آرایش و ساخت | آرایش و ساخت |

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Introduction

The sturgeons are most valued fishes in Caspian Sea and H. huso is one of the most long life sturgeon species. They are pelagic fishes which the adults usually live in middle depths sea and these fishes do not live in sea bottom. However, they require rather low light environments and use of special senses for life activities like the ability of their perception of earth magnetic circuit to navigate their migration path without using other environmental factors. Other sturgeon species are normally benthic and swimming bottom sea.

Variation of ray-finned fish's brain structure happens independently during development and it is reflective of key points in relation to evolution of nerves.

Despite all the researches on fish brain, there are few studies on nerve development. The primary projection of the trigeminal nerve was studied in 1984 on Acipenser oxyrhynchus and Acipenser scaphirhynchus. However, there is no study on nerves evolution on H. huso during their larvae life.

Nerve fibers in actinopetrygians are responsible for different behaviors and senses such as vision, gustatory, smell, balance and eye movements. The nerves may be sensory and carry impulses to the different areas of brain, or may be motor and carry information from brain to other structures of body, and finally they may be mixed (motor and sensory).

Sensory system is classified into chemical senses (i.e. taste and smell), mechanical senses (i.e. hearing and touch) and electromagnetic senses (i.e. visual and electroreceptive).

Motor system is classified into: dorsal, ventral and parasympathetic parts, similar to sensory system, that innervate various areas. In ray-finned fishes like catfishes motor systems especially V nerve, distinguish edible stuff in the near water and produce the necessary suction for ingestion of food. The sensory and motor partitions of nerves are fragmentally formed and the positions which they enter or exit the brain reflect their developmental period. In most of fishes, all the brain nerves are identifiable and contain 22 nerves. The first four nerves are in the forebrain and entirely are sensory including: terminal (Te), olfactory (I), optic (II) and epiphysis (Ep) nerves. Midbrain includes two motor nerves of: oculomotor (III) and trochlear (IV). The remaining cranial nerves originate from hindbrain. All fishes lacked XI and XII nerves. Moreover, Huge fishes do not have the optic and epiphysis nerves. The nerves in all vertebrates have the same names and functions. Oculomotor nerve is the somatic motor nerve of eye muscles and innervates the extrinsic extra orbital eye muscles.

The trigeminal nerve (V) arises from the anterior end of the medulla. It is a mixed motor and sensory nerve which has four branches that innervate the face, eyes, mouth and jaws. The superficial ophthalmic nerve is of the four branches. It has a general sensory function for the skin of the rostrum. Maxillary branch innervates superior jaw and mandibular branch innervates the structures of mandible and tongue. The most of fishes profundus nerve (Pro) innervates to the mucosal layer of lateral line.

The vagus nerve (X) is the longest of the cranial nerves. It is a mixed motor and sensory nerve that arises at the posterior end of the medulla. It innervates the gills, throat, esophagus, stomach, intestines and body wall.

Fishes and other aquatic animals bear the mechanosensory lateral line. This structure is located on the surface of the head and the body. The sturgeons similar to other fishes have six detached nerves including: anterio-dorsal, anterio-ventral, otic, middle, supra-temporal and posterior lateral line nerves. Amphibian ambystoma has five lateral line nerve and lacks otic nerve.

The highly economic importance of H. huso is for the caviar and also it is valuable meat. This study illustrated cranial nerves morphogenesis in H. huso larvae from 1 to 54-days of age.

Materials and Methods

For this research we obtained 40 samples of H. huso larvae with total average weight of 0.81 g and length of 2.03 cm. The ages of 1, 3, 6, 15, 21 and 54 days posthatching (dph) were obtained from Shahid Marjani’s Aghghala propagation station, Gorgan, Iran. The specimens were maintained in fiberglass tank. At first morphometric parameters like total length and weight were measured caliper and digital balance device (Model 47257 SKU; Pittsburgh®, Camarillo, USA), then Six larvae of each age were fixed in 10% buffered formalin (Merck, Darmstadt, Germany) in 48 hr, dehydrated with ethanol (Merck) series 50% to 100%, cleared in xylene (Merck) and embedded in paraffin (Merck) by tissue processing device (Pouyan Teb Khadem, Mashhad, Iran). Because the brain was very small in size, total head was fixed and embedded in paraffin blocks. We used lockhart’s molds for making paraffin blocks. Blocks were cut frontally and sagittally in serial sections and ribbon shaped with regular intervals into 6 µm thickness from the primary specimens to ends by using an auto cut rotary microtome (Leica, Wetzlar, Germany). Tissue sections were deparaffinized and stained with hematoxylin and eosin (H & E) and about 10 sections from each specimens were selected and observed by a camera attached to the light microscope (Model CX22; Olympus, Tokyo, Japan), with 200× and 400× magnifications for general histological studies.

Results

According to the results of the current research, on 1 and 3-day-old fishes, the cranial nerves were not observed...
in _H. huso_ larvae. Histomorphogenesis of cranial nerve in _H. huso_ larvae began on 6-day-old fish.

On 6-day-old fish, the Te nerves and their dendrites were observed around the nasal cavity and the axons were projected to different areas in forebrain especially around olfactory bulb diffusely (Fig. 1). The olfactory nerve (I) was branched from the olfactory placode and was pairwise. The nerve carried olfaction formations to olfactory bulb at the rostral part of telencephalon and was distinguished on 6-day-old fish and with the growth of larvae became thicker. The nerve was formed from appendices of efferent bipolar olfactory cells (Fig. 1).

The optic nerve (II) was large and originated from the neurons in retina and was pairwise. This nerve was located caudo-medial from the surface of orbit eye in _H. huso_ and detected on 6-day-old fish. With growth of larvae this nerve was larger and in chiasma optic coursed each other. Epiphysial nerve (Ep) was not distinguished up to 54-days in _H. huso_. Oculomotor nerve (III) exited from the middle tectum nearby ventro-medial midbrain and was observed on 6-day-old fish.

Trochlear nerve (IV) was the only nerve exited from the caudo-dorsal area of midbrain and innervated upper oblique of the eye muscle of the opposite side and was detected on 6-days in _H. huso_ larvae (Fig. 2).

The Pro nerve was very small. It is a sensory component. The Pro nerve exited from rostral part of the hindbrain and was located toward snout. The Pro nerve was distinguished on 54-day-old fish (Fig. 3).

Trigeminal nerve (V), originated bilaterally from caudo-ventral of hindbrain and lied on toward rostrally area of brain on 6-day-old (Fig. 2). Two nuclei were observed in this area: trigeminal sensory and descending trigeminal stripe nuclei. These nuclei were located in rostro-caudal area and formed long cell sensory columns on 54-day-old fish (Fig. 3).

Lateral line nerve (LLn) contained anterior lateral line (ALLn) and posterior lateral line (PLLn). The LLn was observed without any separation bilaterally in rostro-dorsal, rostro-ventral and inner otic area on 6-day-old fish. The ALLn was located in the ventro-medial part of the inner ear (Fig. 4). The PLLn was located in the dorso-caudal part of the vagal lobe and both two parts were separately detected on 54-day in _H. huso_ larvae (Fig. 3).

Facial nerve (VII) was located in rostro-ventral part of the medulla oblongata and observed on 54-day-old fish (Fig. 4). It formed cranial part of the facial-octaval ganglion complex. The central root of the facial nerve entered the hindbrain in a similar manner to position of their motor root.

![Fig. 1. Frontal section of the brain from 6-day-old, OL: olfactory bulb, I: olfactory nerve, Te: terminal nerve (H & E).](image1)

![Fig. 2. Sagittal section of the brain from 6-day-old, OT: optic tectum, F: forebrain, H: hindbrain, II: optic nerve, III: oculomotor nerve, IV: trochlear nerve, V: trigeminal nerve, ey: eye (H & E).](image2)

![Fig. 3. Frontal section of the brain from 54-day-old, V: trigeminal nerve, H: hindbrain, M: midbrain, Vn: trigeminal nucleus, Pro: profundus nerve, X: vagus nerve, PLLn: posterior lateral line nerve (H & E).](image3)
Octaval nerve (VIII) formed the dorso-caudal part of the facial-octaval ganglion complex and originated from hindbrain on 54-day-old fish (Fig. 4) and entered to the lateral surface of the brain stem and was related with inner ear.

Vagus nerve (X) was a complex nerve with some branches and located in the caudal part of hindbrain. It was observed on 6-day-old fish and with aging up 54-day became thicker in *H. huso* larvae (Fig. 3).

The Te nerve was first described in cartilaginous fishes in 1905. This nerve is a bipolar free nerve end and located in the nasal epithelium and olfactory bulb diffusely. Its modality is not detected as yet. The Te nerve derives from the olfactory placode and projects to the areas in ventral forebrain and only in the ray-finned fishes projects to the retinal region. Many of its fibers, cell bodies contain a hormone, named luteinizing-hormone releasing hormone (LHRH) or gonadotropin-releasing hormone (GnRH), that controls the production of the sex hormones and affect reproductive behaviors.

The olfactory nerve (I) is created from the efferent fibers of the olfactory bipolar cells. This nerve is observed in this fishes with the sessile olfactory bulb and its function is received by smell senses. Nerve I derives from the olfactory placode and translates olfactory information to the olfactory bulb. This nerve also has an important role in detection of chemical factors.

The optic nerve (II) arises from retinal neurons and is a sensory nerve. This nerve in the pelagic fishes is large and thick. The retinal bipolar neuron cells derive from neural tube rather than the neural crest and placode. Nerve II courses caudally and medially from the surface of orbit in all fishes. Optic nerves cross together in the optic chiasm and in this area the axons are decussated to the adverse side.

Epiphysial nerve (Ep) is a sensory nerve is a second vision nerve in diencephalon jawed fishes. Ep nerve originates from epiphysial nerve cell bodies which obtain visual informations from photoreceptor cells in the pineal gland. This nerve regulates the dark-light circadian cycle, which were not distinguished up to 54-days in *H. huso* in this study.

Oculomotor nerve (III) originates the midbrain in nearly the ventral surface of midline and is motor for extraocular muscles and parasympathetic for intraocular muscles. The nerve III only contains efferent fibers. The oculomotor complex is usually compared to some relation nuclei as oculomotor nucleus. In the fishes similar to stargazer *Astroscopus*, in part of the extraocular muscles (the roof of skull muscle) it has an important structure such as electrical organ, thus the oculomotor nucleus contains oculomotor and electromotor parts.

The oculomotor nerve fibers end in the extraocular muscles for regulation of eye movements. The electromotor nerve fibers end in the electrical organ and control related structures to this organs. This process is observed in adult sturgeons. The intraocular muscles of function control the eye structures and innervate parasympathetic nerve fibers and rotates eye to the upward and outward. According to the previous reports the optic and oculomotor nerves were not observed in the sea lamprey up this age. They appear later in sea lamprey than in other vertebrates. The late development of the eye-related nerves in lamprey appears to be related to the late development of the eye.
Trochlear nerve (IV) originates from the dorsal surface of midbrain, however in the initial stages of growth have ventro-rostral position. This nerve innervates superior oblique muscles of the eye on the opposite side, and is detected on 6-day-old H. huso larvae. Nerve IV innervates the contralateral superior oblique muscle in fishes. This nerve also can rotate eye to the downward and outward.

The Pro nerve was a very small somatosensory component for upper face region. It exited from rostral part of the hindbrain and was located toward snout and is detected on 54-day-old fish. This nerve may correspond to deep ophthalmic part of the trigeminal nerve in the most vertebrates. Profundus and trigeminal ganglion separate in cartilaginous and some ray-finned fishes, however, in agnathans, some teleosts and sarcopterygian radiations are fused. In Crossopterygii, Pro innervates the skin of snout and rostral part of tubular organs mucosal walls in which ateral line receptors are located.

Trigeminal nerve (V) appears in 6-day-old fish and course bilaterally from caudo-ventral hindbrain and is located rostrally. It contains somatosensory component for face region and motor components for visceral arch muscles. Somatosensory components contains afferent and efferent nerve fibers in fishes. The afferent fibers are sensitive to the magnetic pulses specially in same teleosts. Trigeminal afferent fibers terminate to some sensory nuclei in the rostro-caudal part of the brain. Efferent fibers innervate the respiratory system and support breathing action. The motor component innervates the visceral arch and muscles of mandible in teleost fishes. In lampreys, it innervates the mandibular muscles for feeding in the sucking mouth and rasping organ. In fighting fish as Betta splendens, the trigeminal nerve innervates the dilator operculi muscle used for aggressive opercular demonstration behavior.

Trigeminal nerve has two branches in lampreys and sturgeon, however, it has a single origin in sharks and teleosts.

The LLn contains ALLn and PLLn. The LLn is observed bilaterally in rostro-dorsal, rostro-ventral and inner otic area on 6-day-old fish. The PLLn is located in the dorso-caudal part of vagal lobe and detected on 54-day-old fish. This nerve includes about six nerves in fishes: Antero-dorsal, antero-ventral, otic, middle, supratemporal and posterior. Lateral line nerves originate from the hindbrain and in this area form the lobes in some teleosts. These nerves are machano-sensory system of all fishes and are used in the recognition of water movements and also innervate the electro-sensory receptors.

Facial nerve (VII) is located in rostro-ventral part of the medulla oblongata and observed in 54-day-old fish. Its function is gustatory and mixed. Motor component innervates visceral arch muscles in dorsal part and sensory component innervates the anterior oral cavity, lips and body surface and is responsible for gustatory sense in ventro-lateral part.

Octaval nerve (VIII) is formed the dorso-caudal part of the facial-octaval ganglion complex and originates from hindbrain on 54-day-old fish. This nerve is mixed and is classified as specially somatic afferent which project to the octaval nuclei in hindbrain. It innervates the inner ear structures which contains semicircular canals that recognize angular velocity, the utriculus that distinguishes displacement caused by gravity and sacculus and lagena that recognize displacement caused by sound waves.

Vagus nerve (X) is a complex nerve with some branches and situated in caudal part of hindbrain and observed on 6-day-old fish. Vagus nerve is mixed and contains gustatory (sensory) and motor components. Gustatory nerve fibers innervate the branchial arch region, specially epibranchial organ in osteoglossid fish, also support tactile and proprioceptive innervation to the similar respective pharyngeal areas. The motor component nerve is located in dorsal area and innervate visceral arch muscles and appear from the lateral part of hindbrain. It is related to the visceral cavities as heart, gastrointestinal tract, swim bladder and pneumatic duct. In cyprinidae vagal motor nerve innervate the muscles of palatal structure, muscular structure in the roof of mouth, and gill rakers. The cranial nerves in vertebrates are responsible for different behaviors, including olfaction, vision, taste, balance and eye movements. The vagus nerve has two peripheral branches in larval lampreys.

With respect to above studies of the embryological development of the nerves, it seems that in H. huso larvae, the activities are mostly depended on the nerves development that provide key information on the interrelationship of the different structures of the body as ear, lateral line organ on H. huso larvae. We suggest to study cranial nerves development on the other species and compare the point in the time of growth of the nerves.

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