

Neuropsychology of Learning and Memory in Teleost Fish

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ABSTRACT

Traditionally, brain and behavior evolution was viewed as an anagenetic process that occurred in successive stages of increasing complexity and advancement. Fishes, considered the most primitive vertebrates, were supposed to have a scarcely differentiated telencephalon, and limited learning capabilities. However, recent developmental, neuroanatomical, and functional data indicate that the evolution of brain and behavior may have been more conservative than previously thought. Experimental data suggest that the properties and neural basis of learning and memory are notably similar among teleost fish and land vertebrates. For example, lesion studies show that the teleost cerebellum is essential in classical conditioning of discrete motor responses. The lateral telencephalic pallium of the teleost fish, proposed as homologous to the hippocampus, is selectively involved in spatial learning and memory, and in trace classical conditioning. In contrast, the medial pallium, considered homologous to the amygdala, is involved in emotional conditioning in teleost fish. The data reviewed here show a remarkable parallelism between mammals and teleost fish concerning the role of different brain centers in learning and memory and cognitive processes. These evidences suggest that these separate memory systems could have appeared early during the evolution of vertebrates, having been conserved through phylogenesis.

A NEW UNDERSTANDING OF VERTEBRATE BRAIN EVOLUTION

THE CLASSICAL THEORIES about vertebrate brain and behavior that pervasively dominated neuroscience during most of the twentieth century, sustain that brain evolution occurred in successive stages of increasing complexity and advancement,¹⁻⁴ leading necessarily to the cognition capabilities that characterize mammals. According to this view, the telencephalon of fishes, the 'most primitive' and 'less evolved' vertebrate group, would consist mainly in a subpallium ('paleostriatum') and a very reduced and primitive pallium ('paleocortex'), both entirely dominated by olfactory inputs and relatively simple neural circuits. As

the 'archistriatum' (caudate), 'neostriatum' (putamen), 'archicortex' (hippocampus), and of course the 'neocortex' (i.e., the 'neencephalon') were thought to have evolved later in "more recent and complex" vertebrate groups, the behavior of fishes was considered essentially 'reflex' or 'instinctive.'

The results of the initial studies on the neural basis of behavior in teleost fish were consistent with the anagenetic ideas on brain and behavior evolution that prevailed at the time, as fish telencephalon ablation appeared to produce virtually no effects on sensory, motor, and motivational processes.⁵⁻¹⁰ However, a few decades later, pioneering studies using more sensitive techniques and sophisticated experimental procedures revealed that the forebrain

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of teleost fish, like that of mammals, is involved in emotional, social, and reproductive behavior, as well as in learning and memory¹¹⁻¹⁴ (for reviews, see Savage,¹⁵ Overmier and Hollis^{16,17}).

Furthermore, during the last years of the twentieth century a wealth of new comparative developmental, neuroanatomical, and functional evidence led to an entirely different understanding of vertebrate brain evolution. Vertebrates have not evolved linearly; instead, different parallel radiations evolved independently from a remote common ancestor, from which have inherited some basic features of brain and behavior organization, and increases in brain size and complexity occur in members of all of them, including fish. The brains of extant vertebrates, although showing conspicuous morphological and cytoarchitectural differences, are indeed a mosaic of both primitive and derived characteristics, and can be conceived as variations of a common vertebrate plan.¹⁸⁻²¹ Moreover, we know now that the olfactory areas represent only a limited portion of the fish telencephalon, and the phylogenetic analysis indicates that the main telencephalic pallium subdivisions in the actinopterygian fish are likely homologous to the hippocampus, the amygdala and the isocortex of tetrapods.^{19,20,22,23} In the present review we summarize recent neurophysiological and behavioral evidence that challenges some of those traditional notions on brain and cognition in vertebrates, indicating that the evolution of learning and memory systems and their neural basis could have been more conservative than previously recognized.

NEURAL BASIS OF SPATIAL COGNITION IN TELEOST FISH

A considerable amount of experimental evidence shows that mammals possess multiple and parallel learning and memory systems, which have distinctive properties and depend on separated neural substrata. Relational memory processes, such as spatial cognition, are based on the function of the hippocampal formation, whereas some forms of implicit learning processes, for instance the classical conditioning of simple motor reflexes and emotional

responses, depend on the cerebellum and the amygdala, respectively. Recent evidence shows that at least some learning and memory capabilities of teleost fish are as complex as those of mammals and birds, and that they are likely based on homologous neural mechanisms

Spatial cognition comprises a variety of perceptive and cognitive mechanisms subserved by distinct brain systems, which contribute in different ways to spatial orientation and navigation. These mechanisms process and integrate sensory and motor information, encoding it into multiple reference frameworks, and transforming it into a series of spatial coordinate systems, from receptive surface and other body-centered coordinates, to some more abstract, world-centered, coordinate systems.²⁴⁻²⁶ For example, in mammals, brain structures as the superior colliculus, the cerebellum or the parietal cortex are involved in perception and action based in body-centered or 'egocentric' frames of reference, whereas other brain centers and neural circuits, such as the hippocampal formation, support navigation based on world-centered frameworks or 'allocentric' spatial representations (i.e., cognitive maps). A cognitive map is defined as a map-like 'allocentric' memory representation of the environment, based on encoding the spatial relationships between multiple cues and sensory features, which enables the subject to locate the goal regardless of its own actual position and local view.^{24,27} These map-like representations, which are true relational memories, are considered the clearest animal model of human declarative or episodic memory.^{28,29} The traditional view in comparative psychology and neuroscience is that relational and allocentric spatial memories are attributes that distinguish humans and other mammals from any other vertebrate group, as these high-order cognitive capabilities are supposed to require complex associational structures, in particular a hippocampus and the six-layered neocortex. However, significant naturalistic and laboratory studies reveal that teleost fish, as well as land vertebrates, rely not only on egocentrically referenced mechanisms for orienting, based on stimulus-response simple associations, but also can use allocentric, map-like representations of the environment.^{24,30-33} In fact, the perfor-

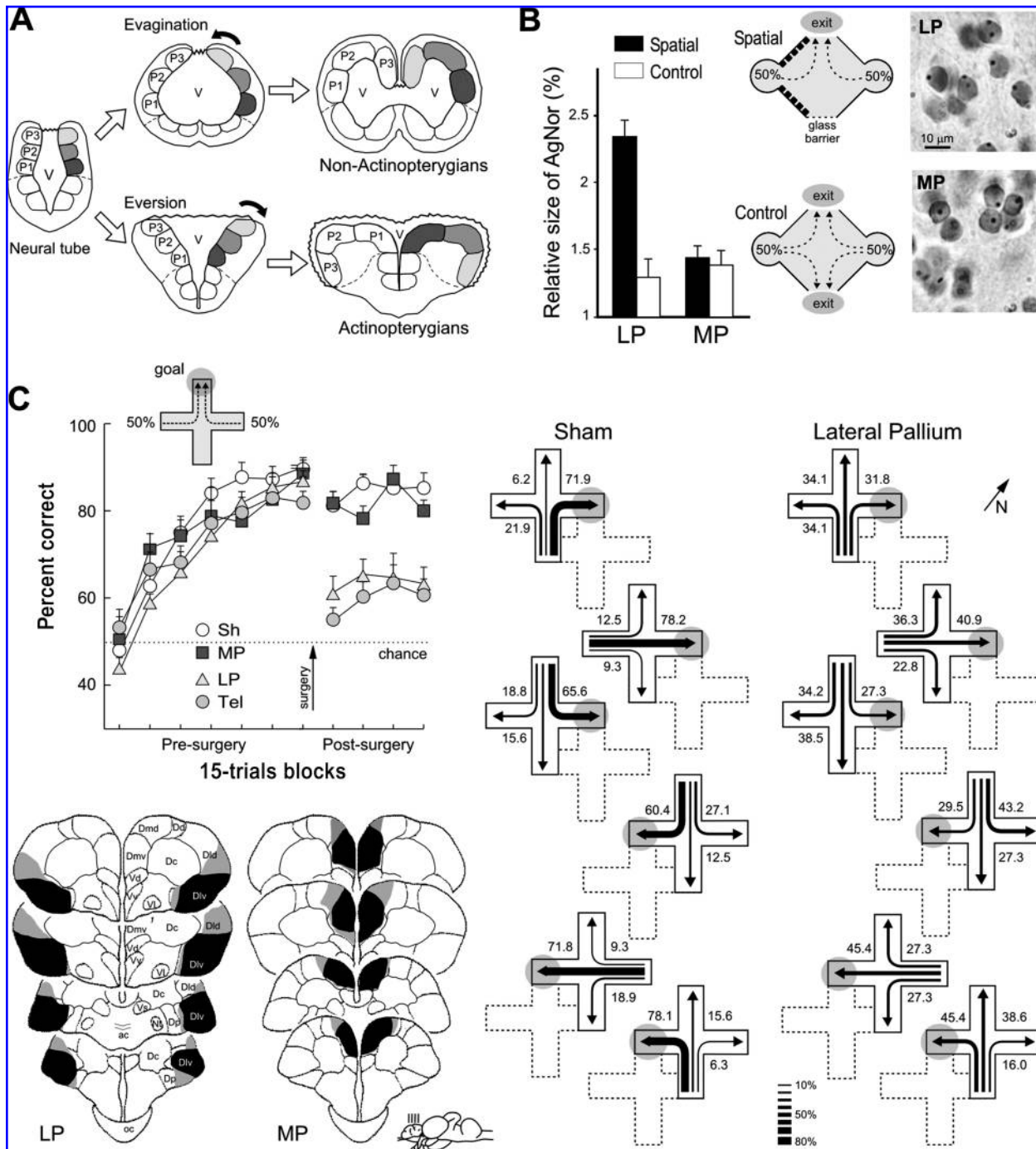
mance of teleost fish trained in standard spatial tasks, such as plus-mazes and other apparatuses, is strikingly similar to that observed in amniotes (reptiles, birds, and mammals). For example, goldfish trained to locate a goal in a four-arm maze surrounded by a wide arrangement of distal visual cues can reach the goal from previously unvisited start locations, approaching from different or even opposite directions, and can readily navigate to the goal using the most direct trajectories adopting spontaneously novel routes, shortcuts or detours, or in the absence of any particular visual cue.³⁴ Moreover, fish, as well as humans and other mammals, can implement simultaneously both, allocentric and egocentric (body-centered) strategies (e.g., using a fix body turn or approximating to a particular visual cue), and use one or the other according to experimental conditions.^{34,35} Interestingly, goldfish are able to use not only the information provided by conspicuous objects in the environment, but also the geometrical attributes of the surroundings for spatial navigation, and conjoin geometric and nongeometric information to reorient themselves.³⁵⁻³⁸ In summary, these findings reveal that teleost fish can reach the goal location by learning its position relative to the landmarks and other features of the environment, by using any available spatial information and by encoding the reciprocal metric and geometric relationships relative to the goal, thus indicating distinctly they have the capacity to use relational memory mechanisms (Fig. 1).

In mammals, birds and reptiles cognitive mapping and other relational memory processes depend on the hippocampus and associated structures.^{24,26,39-44} Substantial evidence show that cognitive mapping abilities also depend on the forebrain in teleost fish,⁴⁵⁻⁴⁸ and particularly, from the hippocampal pallium.^{33,49} The identification of the homologies among the pallial subdivisions of land vertebrates and ray-finned fishes has been hindered because the telencephalon of this fish group presents unique morphological features, consisting mainly of solid telencephalic hemispheres separated by a single ventricular cavity. This particular morphological pattern is consequence of a major variation during the early stages of the embryonic development,

that is, the eversion (bending outward) of the prosencephalic alar plate in the ray-finned fishes, instead of the evagination (bending inward) in every other vertebrate group, that reverses the pallial medial-to-lateral topography observed in land vertebrates (Fig. 1A).

Accordingly, the lateral (LP) and the medial (MP) telencephalic pallium of actinopterygian fish are the most likely homologues of the hippocampus and amygdala of mammals, respectively.^{19,22,50-53} In this regard, functional studies show that the lateral pallium of teleost fish, like the hippocampus of amniotes, is essential for spatial cognition. For example, training goldfish in a spatial task induces a significant and selective spatial learning-related increase in the transcription activity (protein synthesis) of the neurons in the LP, evaluated by means of a silver stain with high affinity for the argyrophilic proteins of the nucleolar organizing region⁵⁴ (Fig. 1B). The selective implication of the teleost LP in spatial cognition is further confirmed by selective lesion studies. LP lesions produce a dramatic impairment in place learning and memory in goldfish trained in a plus-maze surrounded by widely distributed distal visual cues⁴² (Fig. 1C). Following surgery, LP-lesioned fish show a severe and permanent impairment in locating a familiar place (goal location), whenever reaching the goal implies implementing novel routes (transfer trials), but also when the well-trained trajectories are available (training trials). The spatial learning and memory deficits following LP lesions in goldfish are as severe as those produced by the ablation of the whole telencephalon.^{41,42,46,47,55} In addition, the involvement of the LP in spatial cognition seems to be highly selective, as damage to this area does not disrupt cue learning or other egocentrically referenced strategies.^{42,45-47} In contrast, medial or dorsal pallium lesions do not produce any observable impairment in spatial memory.⁴² Convergent evidence is provided by the performance of LP-lesioned goldfish trained to locate one baited feeder (goal) within a 25-feeder matrix surrounded by an array of intra-maze visual cues, in a procedure analogous to the hole-board task used with rats.⁵⁵

Although LP goldfish, as well as MP and control animals learn to solve the task with ac-



curacy, the LP animals, in contrast to MP and control fish, fail to reach the goal when the particular subset of visual cues situated in the proximity of the goal is excluded (test trials), indicating that these animals lack the capacity to encode the goal location relative to multiple environmental features in a unique, map-like representation (place learning), and rely on a guidance strategy to solve the task. These data

clearly indicate that the lateral pallium of teleost fish, proposed as the homologue of the hippocampus, sustains the ability of fish to use allocentric, relational representations of the environment.

In mammals, spatial cognition and behavior involve the interaction of multiple neural mechanisms, and depend on memory systems based on telencephalic and nontelencephalic

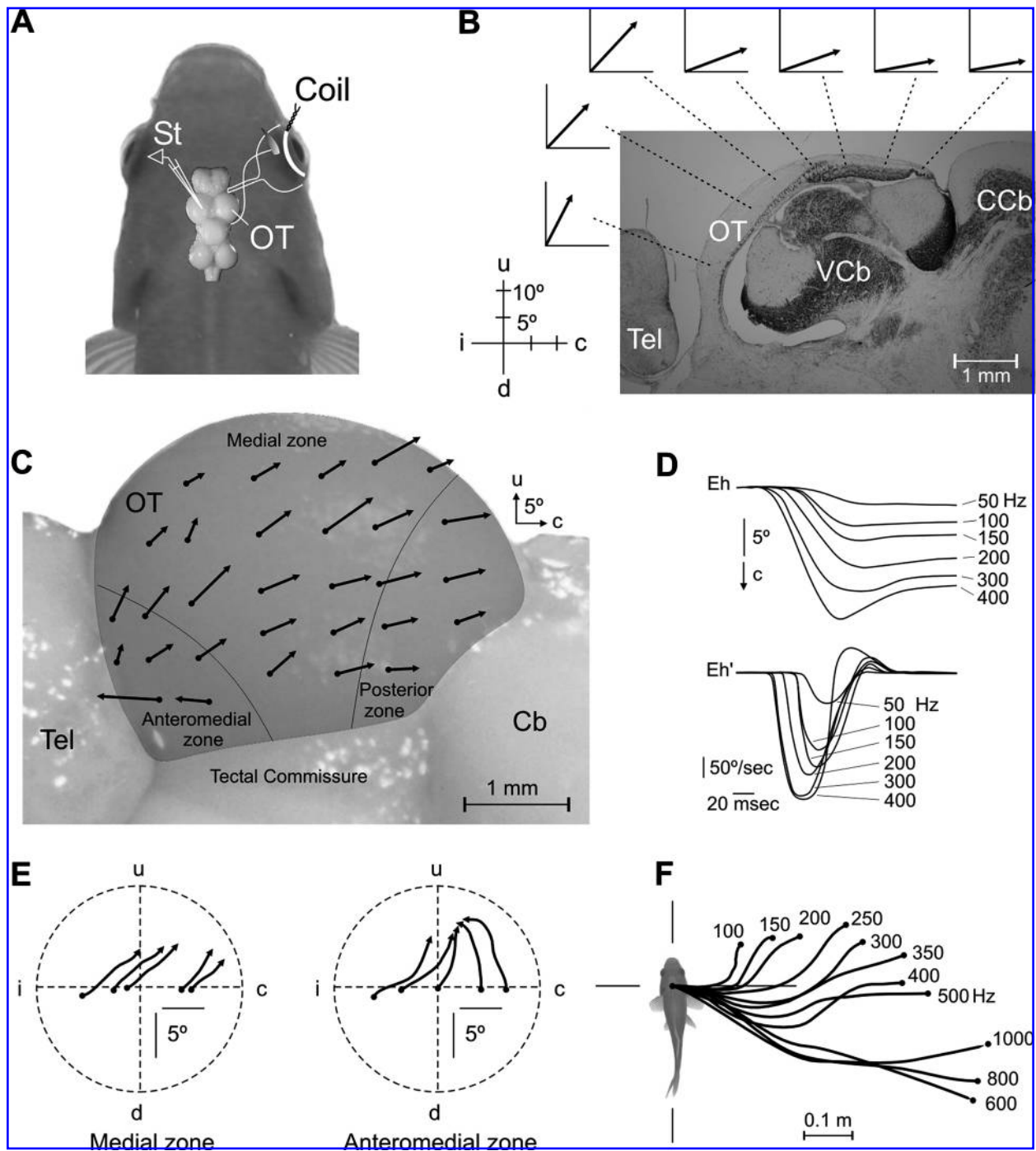
FIG. 1. The lateral telencephalic pallium of goldfish is selectively involved in spatial learning and memory. (A) Schematic representation of the process of evagination and inversion that occurs in the telencephalon of non-actinopterygian vertebrates during embryonic development compared with the eversion or bending outward that occurs in actinopterygians. These different developmental processes produce notable morphological divergence, mainly, paired telencephalic hemispheres with internal ventricles in nonactinopterygians, which contrast with the massive telencephalic hemispheres flanking a single ventricular cavity in the actinopterygian radiation. However, despite these conspicuous differences, the telencephalon of actinopterygian and nonactinopterygian vertebrates present equivalent pallial and subpallial zones, and the pallium of the actinopterygian telencephalon probably contains subdivisions homologous to the hippocampus, amygdala and isocortex of land vertebrates.^{20-22,54} P1, P2, and P3 correspond to the three main subdivisions of the pallium and v to the ventricle. (B) Spatial learning induced a significant increase protein synthesis in the neurons of the lateral pallium evaluated by AgNOR histochemistry in the lateral (LP) but not medial (MP) pallium neurons of goldfish trained in a spatial or a control task. The diagrams show a schematic representation of the spatial and the control procedures. The fish were trained to exit from a box maze with two start compartments and two doors. On the spatial task, two stripped panels signaled indirectly the exit (goal); a transparent glass barrier blocked the second door. The control procedure was identical except that the cues and the glass barrier were excluded, so the two doors remained open. The numbers indicate the percentage of trials initiated from each start compartment. The *arrows* show the routes to the exit. Note that the size of the AgNORs region in the goldfish LP increased significantly after learning the spatial task, relative to controls and to MP neurons. The *photographs on the right* show AgNORs in neurons of the LP and MP. Modified from Vargas *et al.*⁵⁴ (C) Effects of selective pallial lesions on spatial memory in a place-learning task. The *insert* shows a schematic representation of the training procedure. Two different start positions were used and animals were trained to find the goal, situated always in the same place of the room. Note that the animals were required to make a left- or a right-turn, depending on the start arm (i.e., no fixed turn strategy was useful to solve the task). So, animals could only use the extramaze cues to identify goal location. The presurgery performance and the transfer trials results indicate that goldfish are able to build complex spatial representations of their environment and to solve spatial tasks on the basis of allocentric frames of reference. Following surgery, LP-lesioned animals were severely impaired in the solution of the task, whereas MP-lesioned animals did not decrease accuracy relative to sham (Sh) animals. Note that the LP lesion produced an impairment as severe as that observed in the animals with complete ablation of the telencephalon (Tel). The diagrams on the *left* show the trajectories chosen by Sh and LP-lesioned goldfish during the transfer trials conducted after surgery, when the maze was displaced in the room. Note that always the end of one arm was located in the place where the fish was rewarded during training trials, but the start positions and the trajectories were different. The numbers and the relative thickness of the *arrows* denote the percentage of times that a particular choice was made. The position of the maze during training trials is shown by *dotted lines*. The *gray circles* show the goal location during training. Note that during these trials Sh goldfish consistently chose the route leading to the place where they were rewarded during training trials, indicating their ability to use new pathways towards a goal from unfamiliar start points, and to reorganize their spatial strategies in response to an environmental change. In contrast, the random distribution of choices by the LP-lesioned animals indicates a severe spatial deficit. The drawings in the *bottom left* show a schematic representation of the largest (*gray shading*) and smallest (*black shading*) extent of the LP and MP lesions in goldfish, reconstructed in coronal sections at the levels indicated in the lateral view of the brain. Modified from Rodríguez *et al.*⁴²

brain structures and circuits. As mentioned above, LP lesions and telencephalon ablation in teleost fish do not impair, or even facilitate, the use of egocentric strategies for spatial orientation, for example body-turns, simple spatial discriminations, or approaching or avoiding a single cue,^{42,45-47} suggesting that nontelencephalic cerebral centers, such as the optic tectum and the cerebellum, are likely implicated in these processes.

One of the brainstem centers closely associated to spatial cognition and action is the optic tectum (superior colliculus in mammals). The neuroanatomical and functional organization of the optic tectum is notably conserved in vertebrates. For example, in teleost fish the basic pattern of specialized cytoarchitecture and

microcircuitry, the profuse connectivity with other motor and sensory centres,⁵⁶ and the mechanisms for generating coordinated eye, head, and body movements, and for coding the metric and kinetic features of these movements⁵⁷⁻⁵⁹ are remarkably similar to land vertebrates. As in other vertebrates, focal electrical stimulation of the optic tectum of teleost fish elicits coordinated eye and body movements, postural adjustments, and other motor patterns⁵⁶⁻⁶¹ (Fig. 2).

Moreover, electrical stimulation in the tectum of free-moving goldfish evokes different kinds of motor responses (orienting or escape, or a shift from one to the other), according to the location, the intensity, and frequency of the stimulus.⁵⁹ Furthermore, the deep tectal layers



of teleosts are organized in a topographically ordered motor map in correspondence with the retinotopic visual map in the superficial layers, as revealed by the strict dependence of the characteristics of the orienting movements on the active tectal site^{58,59,62} (Fig. 2). These data indicate that the optic tectum of teleost fish, like the superior colliculus of mammals, provides a common, body-centered framework for multi-

sensory integration and sensory-motor transformations,^{25,62} participating in the translation of the sensory inputs coded in spatial coordinates into a temporal signal in the brainstem motor generators,⁶³⁻⁶⁵ and is, thus, crucial for generating actions within an egocentric frame of spatial reference.

An increasing amount of evidence show that the mammalian cerebellum, in addition to be-

FIG. 2. Focal electrical stimulation in the optic tectum of goldfish (**A**) elicits coordinated eye and body movements, postural adjustments, and other motor patterns, revealing that the optic tectum of teleost fish is a crucial center for the generation of egocentrically referenced actions in space. (**B, C**) The amplitude and direction of eye movement vectors depend on the stimulation site within the tectum. The variation of the stimulation site in the rostro-caudal axis produce a systematic change in the amplitude of the horizontal component of the saccade (**B**), whereas the variation of the stimulation site in the medial—lateral axis produce an increase in the vertical component (not shown). (**C**) Characteristic vectors of evoked saccades from different stimulation sites in the right tectum of the goldfish. As in other vertebrates, goldfish orienting-eye movement characteristics depend on the active tectal site, thus revealing a topographically ordered motor map within the optic tectum in alignment with the retinotopic visual map. (**D**) The direction and amplitude of the orienting responses depend not only on the tectal stimulation site, but also on the stimulus parameters. The variation of the stimulation parameters (v.g. frequency) produces systematic changes in the metric and kinetic of the evoked orientation responses. (**E**) The stimulation of anatomically separated tectal areas evokes different types of eye movements. *Medial zone*: Fixed vector movements, independent of the initial eye position, indicating that eye movements are coded retinotopically. *Anteromedial zone*: Goal directed movements, the direction depends on the initial eye position, suggesting a craneotopic codification of the eye movement direction. (**F**) The electrical microstimulation of the optic tectum in free-swimming fish also elicits body movements. Evoked movements consist of complete orientation responses including coordinated movements of the axial musculature, fins, and eyes, which closely resemble the natural responses. The direction and amplitude of the orienting responses depend on the tectal stimulation site and also on the stimulus parameters. Abbreviations: Cb, cerebellum; CCb, corpus cerebellum; Eh, horizontal component of eye position; Eh', eye velocity trace; OT, optic tectum; St, electrode for microstimulation; Tel, telencephalon; VCb, valvula cerebellum; d, u, i, c, downward, upward, ipsiversive, and contraversive direction of evoked eye saccade, respectively. Modified from Salas *et al.*⁵⁸ and Herrero *et al.*⁵⁹

ing a important center for motor coordination, is also involved in spatial learning and memory and other cognitive processes.^{66–68} The cerebellum, as well as the telencephalon, characterizes by a remarkable morphological variability across vertebrate species, but other features, as the pattern of cytoarchitectural organization, the basic intrinsic circuitry, the connectivity with associated brain structures, and the neurophysiological mechanisms, are notably conserved.^{20,69–71} Interestingly, recent lesion studies using a number of standard spatial tasks show that the teleost cerebellum, similar to that of mammals, participates in spatial cognition. For example, cerebellum lesions produce a profound spatial cognition deficit in goldfish trained to locate the only baited feeder within a 25-feeder matrix, surrounded by a stable array of visual cues.^{72,73} Although the performance of the cerebellum-lesioned animals slightly improves along training, the search pattern is stereotyped and inefficient. Moreover, these goldfish never reach the level of accuracy of the control and sham operated animals. At least in part, the poor performance of the cerebellum-lesioned goldfish is related to an inability to generate or use a map-like representation of the environment, as indicated by their performance during test trials. Thus, whereas the animals in the sham operated group navigate readily to the goal regardless

of the removal of any particular visual cue, the lesioned goldfish are further impaired whenever one particular subset of visual cues is removed. Similar results have been observed in mammals trained to locate a goal in a variety of spatial tasks, such as the Morris water maze or the T-maze.^{68,74,75} Moreover, when cerebellum, telencephalon, and sham operated goldfish are trained in a spatial- or a cue-maze learning task,⁷² cerebellum, but not telencephalon lesions, are equally disruptive independently of the task. Typically, in teleost fish, telencephalon lesions and in particular those damaging the hippocampal pallium, impair the performance in the allocentric spatial tasks, but spare cue learning.^{33,42,45,48,49} In contrast, the postsurgery performance of cerebellum-lesioned fish decays to random levels in both, the spatial, and the cue task, thus indicating that the teleost cerebellum is involved also in the association of oriented motor responses with single landmarks and in other egocentric mechanisms. Remarkably, whereas the effects of cerebellum lesions in goldfish are profound and widespread in spatial cognition, impairing the use of both allocentric and egocentric strategies, they do not produce observable sensory-motor impairments or deficits in posture, swimming ability, or obstacle avoidance, which are equally efficient in the control and the cerebellum lesioned animals.^{72,73}

NEURAL BASIS OF CLASSICAL CONDITIONING IN TELEOST FISH

Fish show reliable Pavlovian conditioning in different reflexes and response systems, and in a wide range of conditions. They show sensitivity to the predictive relationship between the conditioned and the unconditioned stimulus, and exhibit overshadowing, blocking, auto-shaping, and higher-order conditioning.^{17,76} In addition, recent evidence suggests that at least some of the neural mechanisms underlying these learning phenomena in teleost fish are strikingly similar to those of mammals. For example, as in mammals,^{67,77} the teleost fish cerebellum is essential for the classical conditioning of motor responses. Cerebellum lesions in goldfish produce a permanent impairment in the classical conditioning of a simple eye-retraction reflex analogous to the eye-blink conditioning procedure commonly used in mammals^{73,78} (Fig. 3). In a typical eye-blink classical conditioning, animals learn to express a conditioned response (CR; an eye-blink or eye retraction movement), to a predictive or conditioned stimulus (CS; light or sound) that is paired with a significant unconditioned stimu-

lus (US; eye air-puff, or mild electric shock). The control fish show a progressive and significant increase in the percentage of CRs to the CS presentation. As in mammals, the percentage of CRs increase with paired CS–US presentations, becoming progressively more accurately timed to the onset of the US, but decrease with CS alone (extinction) or unpaired CS–US presentations. The sensitivity of the performance of goldfish to the variations in the CS–US relationships enables us to disregard pseudoconditioning biases or other nonassociative mechanisms, indicating that also in teleost fish the conditioning of simple motor responses depends on associative rules. In contrast, cerebellum-lesioned goldfish show a severe learning impairment; cerebellum lesions abolish the eye-retraction conditioning; the percentage of CRs does not change significantly independently of training conditions. In fact, the number of CRs does not increase after 300-paired CS–US training trials. It is interesting to note that the deficit observed in the cerebellum lesioned fish is selective to the CRs; cerebellum lesions do not affect the percentage of URs and spontaneous eye movements.

Additional evidence on the involvement of

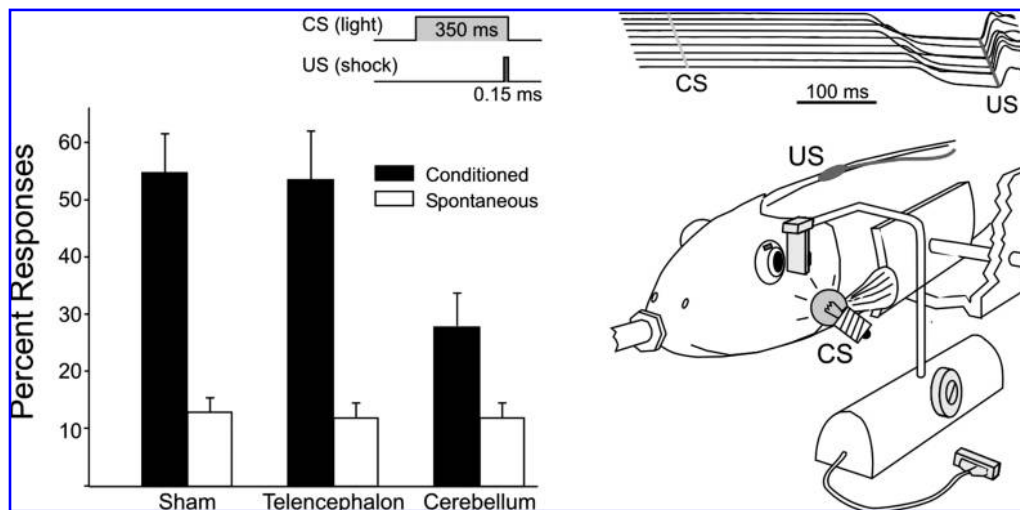


FIG. 3. Cerebellum ablation in goldfish produces a severe impairment in the classical conditioning of a simple eye-retraction response relative to sham and telencephalon-operated animals. Fish were trained in a delay classical conditioning procedure equivalent to the eye-blink conditioning used with mammals. In this procedure, the conditioned stimulus (CS) is a light (350 ms in duration), and the unconditioned stimulus (US) consists of a mild shock (0.15 ms in duration) that elicits an unconditioned eye-retraction reflex or “eye-blink.” In this paradigm, the CS onset precedes the US, but both stimuli overlap in time and co-terminate. The traces on the right corner show some illustrative examples of conditioned responses (CRs: eye movements before the onset of the US) during paired presentations of the conditioned and the unconditioned stimuli in the control animals. Modified from Rodríguez *et al.*^{73,79}

the teleost fish cerebellum in the classical conditioning of simple motor responses was provided by a recent experiment addressed to identify possible learning-related changes in the metabolic activity of the cerebellum of goldfish by means of cytochrome oxidase (COX) histochemistry.^{73,80} Optical densitometry analysis shows an increase in the level of COX activity in the molecular and granular layers of the cerebellum of goldfish trained in a CS-US paired conditioning procedure. In contrast, COX activity does not increase in the cerebellum of goldfish subjected to unpaired presentations of the CS and the US, or in untrained animals. These data permit to exclude the possibility that the increase observed in the COX activity of the goldfish in the paired CS-US condition could be due to uncontrolled factors, such as sensory stimulation or emotional activation, and provide strong evidence concerning specific learning-related neural plasticity in the cerebellum of goldfish.

In teleost fish, as in mammals, the cerebellum and related brainstem circuits provide the essential neural substratum for eye-blink classical conditioning, as forebrain structures are not required for conditioning this simple motor reflex. Telencephalon ablation does not impair eye-retraction conditioning in goldfish^{73,81} (Fig. 3). However, these findings are valid for delay conditioning, but not for trace conditioning. In trace conditioning the end of the CS is separated from the onset of the US by a stimulus-free time gap (trace interval), imposing the contribution of additional neural mechanisms. In mammals, learning under these conditions involves the engagement of some telencephalic structures and circuits, mainly the hippocampus.^{70,82} Interestingly, although the cerebellum is the critical centre for eye-retraction classical conditioning in goldfish, both in the delay and the trace paradigms,^{73,78,81,83} the lateral telencephalic pallium, likely the homologue of the hippocampus (see above), appears to be selectively involved in trace classical conditioning. Lesions to the LP disrupt eye-retraction conditioning, but only when learning implies associating two stimuli separated in time.^{73,81} In contrast, MP lesions do not produce observable deficits in the conditioning of this reflex in either procedure. These findings

indicate that the neural circuits underlying classical conditioning in goldfish are similar to those in mammals, both in the role of the cerebellum and in the more selective involvement of the hippocampal pallium.

BRAIN SUBSTRATES FOR EMOTIONAL MEMORIES

The neuropsychological and experimental research concerning the neural bases of emotion shows that in mammals the amygdala is critical for emotional learning and memory.^{84,85} In teleost fish, the medial pallium is considered homologous to the pallial amygdala.^{19,22,52,53,86} Like the amygdala, the MP seems to play an important role in behaviors that involve significant emotional components. Lesions selective to the MP disrupt or disorganize aggressive, reproductive and parental behavior,^{87,88} and focal electrical stimulation in the MP of free-swimming fish elicits arousal, and defensive and escape responses.^{89,90} Moreover, the MP of teleost fish is involved in emotional learning and memory. For example, MP lesions⁹¹ (Fig. 4A), like damage to the amygdala,^{92,93} impair the retention of a conditioned active avoidance response in goldfish. In this paradigm, the animals learn to avoid an unpleasant stimulus (a mild electric shock) by producing a particular action (such as jumping to a safe area), in response to the presentation of the CS (a light) that signals the oncoming US. It is important to note that the avoidance conditioning in goldfish, in addition to being similar to that of mammals in the behavioral profile, also appears to be based on the acquisition of a mediational state of fear.^{17,94} During post-surgical training, goldfish with MP lesions are able to improve escape, but not avoidance responses.⁹¹ The MP lesion impairment on avoidance memory is as severe as that produced by the ablation of the whole telencephalon, indicating that the MP is a critical area for this function in teleost fish. The selective involvement of the MP in avoidance learning is further confirmed by the failure of MP-lesioned goldfish trained in spaced-trial conditions (one trial per day) to express avoidance responses,^{94,95} procedure that allows the assessment of whether

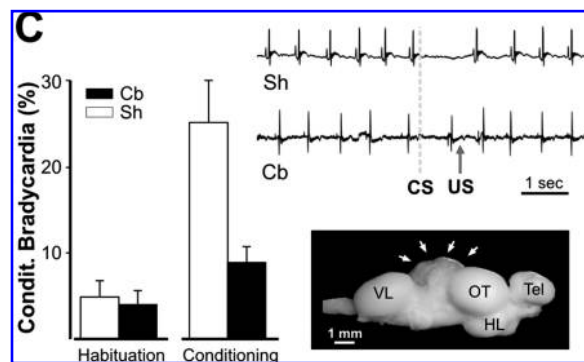
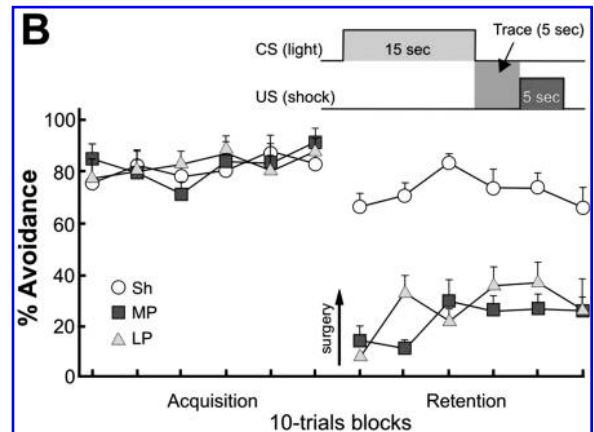
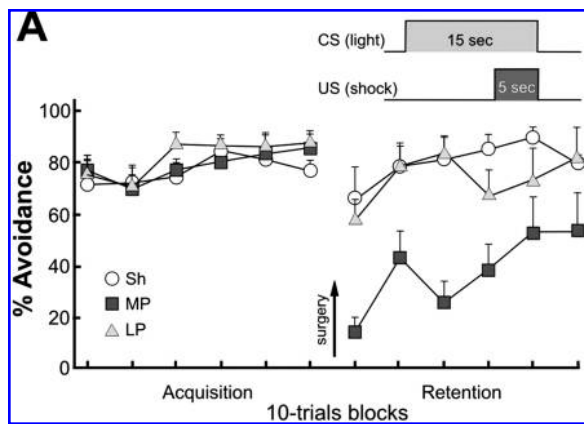


FIG. 4. Neural substrates for emotional learning in goldfish. (**A**, **B**) The medial (MP) and lateral (LP) telencephalic pallium of teleost fish are involved in emotional and temporal learning, respectively. A two-way active avoidance paradigm was used in a shuttle box adapted for goldfish conditioning. This experiment analyzed the effects of MP and LP lesions on the retention of an avoidance response previously acquired, in two different conditioning situations, one with stimuli overlapping [(**A**) nontrace delay procedure] and the other with an interstimuli gap [(**B**) trace procedure]. Results show that damage to the goldfish MP produces a severe deficit in the retention of conditioned avoidance in both procedures. However LP lesions impair performance only in the trace conditioning procedure. These data support the presence of two dif-

ferent systems of memory in fish, based on discrete telencephalic areas: the MP, involved in an emotional memory system; and the LP, involved in a spatial, relational, or temporal memory system. Modified from Portavella *et al.*⁹¹ (**C**) The teleost fish cerebellum (Cb) participates in emotional learning, as indicated by the effects of Cb lesions on fear heart rate conditioning in goldfish. In control fish, the paired presentations of the CS (light) and the US (shock) consistently produce a conditioned bradycardia. However, goldfish with Cb lesions fail to acquire the conditioned bradycardia response. Like in mammals, cerebellum lesions in goldfish impair the acquisition of the conditioned bradycardia without altering the heart rate baseline (habituation trials). The *insert on the right* shows the electrocardiograms of a representative Cb-lesioned and a Sh animal. Note that the Cb-lesioned goldfish does not exhibit the normal conditioned heart rate deceleration response to the CS. The photograph shows a lateral view of a goldfish brain with cerebellum ablation. *Arrows* denote the border of cerebellar tissue removal. Abbreviations: Cb, cerebellum; HL, hypothalamic lobe; OT, optic tectum; Tel, telencephalon; VL, vagal lobe. Modified from Rodríguez *et al.*⁷³

avoidance learning can be accomplished in absence of stimulus carry-over effects from previous trials. These data indicate that the MP, like the amygdala of mammals, is an essential component of an emotional memory system in teleost fish.

Interestingly, the LP of goldfish,⁹¹ like the hippocampus of mammals,^{70,93,97} is involved in trace- but not in delay-avoidance conditioning. In goldfish, LP lesions severely impair conditioned avoidance memory, but only when an interstimulus temporal gap (trace avoidance conditioning) is introduced in the two-way active avoidance procedure⁹¹ (Fig. 4B), providing additional evidence on the role of the LP of teleost fish as a relational memory device and

on its involvement in the processing and encoding of stimuli separated in space (cognitive mapping) and in time (trace memories). In summary, these data show a remarkable functional similarity between the MP and LP of teleost fish and the amygdala and hippocampus of land vertebrates, respectively.

In mammals, intra-amygdaloid infusions of N-methyl-D-aspartic acid (NMDA) antagonists, such as aminophosphonopentanoic acid (AP5) and dizocilpine maleate (MK-801) prevent acquisition of Pavlovian fear conditioning and avoidance conditioning.^{98–101} In goldfish, NMDA receptors are densely concentrated in the telencephalon¹⁰² and intracranial administration of NMDA receptor antagonists impairs

acquisition of avoidance and fear conditioning in a dose-dependent manner.^{103–106} Furthermore, microinjections of D-AP5 to the goldfish telencephalon immediately following training does not impair memory consolidation of avoidance conditioning,¹⁰⁶ indicating that NMDA receptor antagonists impair learning by disrupting the neural mechanisms of acquisition and not by blocking memory storage or retrieval processes. In mammals, NMDA receptors play an important role in long-term potentiation (LTP), a physiological phenomenon of synaptic plasticity universally considered a putative correlate of learning.¹⁰⁷ In teleost fish, NMDA receptors and protein kinases also play important roles in LTP formation in the telencephalon¹⁰⁸ as well as in the optic tectum^{109,110} and brainstem.¹¹¹ In addition, also as in mammals, cell adhesion molecules¹¹² and protein synthesis^{113,114} are involved in consolidation of avoidance and fear conditioning in teleost fish.

A growing number of studies suggest that the cerebellum of mammals participates also in emotional learning.^{115–120} Recent data show that the cerebellum of goldfish is also involved in emotional learning. Cerebellum-lesioned goldfish, as mammals, are impaired in fear heart rate conditioning^{73,78,83,121} (Fig. 4C). In the control goldfish, paired CS–US presentations produce a rapid increase in the percentage of conditioned bradycardia responses (a deceleration of the heart rate during CS–US interval relative to pre-CS baseline), which decreases quickly during extinction training. In contrast, goldfish with cerebellum lesions fail to acquire this conditioned emotional response. It is important to note that no impairment is observed in the autonomic orientation response to the CS, the reflex response to the US, or the heart rate baseline in cerebellum-ablated animals, indicating that the sensorial and motor neural circuits underlying the expression of the unconditioned cardiac responses are spared in cerebellum-ablated goldfish. Thus, the effects of the cerebellum lesions on the cardiac activity of goldfish seem to be selective to the conditioned bradycardia response. Similarly, cerebellum lesions impair the acquisition of the conditioned bradycardia response in rats and rabbits, without altering the heart rate baseline or the orientation response to the CS.^{117,118}

CONCLUSION

The historical trend in comparative cognition and neuroscience, sustained by a predarwinian notion of *Scala naturae* of evolution and intelligence, has regarded fishes as the ‘most primitive’ or ‘less evolved’ vertebrate group, situated at the bottom of the so called ‘phylogenetic scale.’ According to it, fishes have been long perceived as lacking most of the brain centers and neural circuits that support cognitive capabilities, attributed exclusively to the “superior” vertebrate groups (birds, and especially mammals). We have reviewed here recent evidence that challenges this traditional view. The results summarized here indicate that a variety of learning and memory systems, involving the optic tectum, the cerebellum, and the hippocampal and the amygdalar pallium are strikingly similar among teleost fish and land vertebrates. Such notable parallelisms in vertebrate groups that diverged millions of years ago suggest that the emergence of the main features of these memory systems could have occurred early in the phylogenetic history of vertebrates. Moreover, these data suggest the possibility that extant fish and tetrapods, all evolved from an ancestral fish group that lived some 400 million years ago, probably inherited some behavioral and cognitive traits from their common ancestor, that would have been retained during phylogenesis.

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