Research report

Cerebellum and spatial cognition in goldfish

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HIGHLIGHTS

- Goldfish cerebellum contributes to spatial cognition.
- Cerebellar lesions impairs the procedural and the declarative component of navigation.
- Cerebelloctomized goldfish are unable to elaborate a cognitive map.
- Cognitive functions of the cerebellum are conserved across vertebrates.

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ABSTRACT

The cerebellum of mammals has recently been linked to spatial navigation, as indicated by the results of a number of studies performed in animal models with cerebellar abnormalities. However, nothing is known about the contribution of this structure to spatial cognition in other vertebrate groups such as teleost fish. To investigate the involvement of the teleostean cerebellum in navigation, sham-operated (Sh) and cerebellum-ablated (Cb) goldfish were trained in a "hole-board" task in which they had to locate the baited feeder within a 5×5 feeder matrix surrounded by visual cues. Cb goldfish were significantly impaired in the acquisition and performance of the task, as revealed by their low spatial accuracy, the number of errors committed, and the stereotyped searching pattern exhibited relative to Sh goldfish. Probe tests, performed during the final training sessions, showed that Cb animals could not integrate experimental cues into an internal representation of the environment (as an allocentric strategy would require) and they resorted to a guiding strategy to locate the goal. The results of this experiment demonstrated that the cerebellum might have a modulatory role in the declarative component of navigation by which an animal develops an internal spatial representation. Our results constitute the first evidence of the involvement of the fish cerebellum in spatial cognition. Our results also suggest that the cognitive functions of the cerebellum may have appeared early in vertebrate evolution and been conserved throughout the phylogenetic history of extant vertebrates.

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1. Introduction

Traditionally, the cerebellum has been considered a neural structure devoted to motor control. However, many studies gathered during the previous two decades indicate that cerebellar functions extend beyond this specific motor domain. Thus, imaging and clinical studies in humans, and those performed in animal models of cerebellar abnormalities, reveal that the cerebellum serves critical roles in different cognitive functions, such as attention, emotion, perception, language, working memory, procedural learning, and spatial navigation [1,2].

Spatial function of the cerebellum has been clearly demonstrated by a variety of experiments performed on rats and mice. Studies involving different strains of cerebellar mutant mice have reported a selective deficit in the spatial orientation of these animals when they are required to locate a hidden platform from random departure locations in the traditional Morris water maze paradigm [3–7]. Similar results were obtained in other studies using the hidden version of the Morris water maze when granular cell depletion was induced in rats by X-irradiation of the cerebellum [8,9] or immunotoxin OX7-saporin was used to selectively destroy cerebellar Purkinje cells [10]. Other authors using hemicerebellectomy combined with a wide range of protocols, notably the Morris water maze paradigm, have demonstrated that hemicerebellectomised rats displayed a severe impairment in coping with spatial information, executing only a characteristic peripheral displacement denoted circling [11–14]. Some of these studies have explicitly proposed that such a noticeable impairment

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is more related to the inability to organise and execute complex and effective exploration behaviours (the procedural component of navigation) than to an inability to develop an internal map of the environment (the declarative component of navigation). However, recent studies suggest that the cerebellum might also contribute to the construction of hippocampal spatial representations [15–18]. Although the exact role of the mammalian cerebellum in spatial cognition remains unclear, these experimental studies indicate that this structure must be added to the neural network involved in navigation.

Fish exhibit sophisticated spatial abilities, comparable to those observed in mammals and birds, based on allocentric and egocentric frames of reference [19]. Several studies analysing the neural substrates of spatial cognition in teleost fish have demonstrated that, like the hippocampal pallium in land vertebrates, the ventrolateral region of the dorsal telencephalic area is selectively involved in the use of map-like or relational spatial memory representations for allocentric navigation [20–22]. Other studies have identified an additional teleostean structure, the optic tectum, as a crucial centre for sensorimotor integration and the generation of egocentrically referenced actions in space [19,23]. In addition to these two structures, no other brain centre in teleost fish has been identified in the neural network involved in spatial cognition. Recent studies have shown that the teleost fish cerebellum is involved in classical conditioning [24–26] and inhibitory avoidance [27] which, together with the fact that the cerebellum in all vertebrates is citoarquitectonically and hodologically similar [28–33], suggests that this teleostean structure may also be involved in a wide range of cognitive functions, including spatial cognition, as is observed in mammals.

To identify the possible role of the teleost fish cerebellum in navigation, this study aimed to analyse the possible spatial deficits produced by cerebellar lesions in goldfish trained in a hole-board spatial task [22]. With this aim, sham (Sh) and cerebellar-ablated (Cb) goldfish were trained to locate the baited feeder within a 5×5 feeder matrix surrounded by several visual cues that maintained stable spatial relationships relative to the goal.

2. Methods

2.1. Subjects

Sixteen goldfish (*Carassius auratus*), 12–14 cm in length and obtained from a local supplier, were maintained for 2 months prior to the experiment in 200 l glass aquaria with aerated and filtered water at $20 \pm 2\,^{\circ}C$ on a 14/10 h light/dark cycle. The animals were fed once a day with dry food for pond fish (Tetra pond, Ulrich Baemsch, GmbH, Melle, Germany). Throughout the experiment, the fish only consumed the food-sticks they obtained in the daily session. Each food-stick weighed ~20 mg. Prior to the experiment, each animal was randomly assigned to two different surgical groups: sham operation ($n = 8$) or cerebellum ablation ($n = 8$). Animal care was performed according to the protocols and guidelines approved by the European Communities Council Directive of November 24, 1986 (86/609/EEC) and the Spanish Real Decreto 1201 of October 10, 2005. All efforts were made to minimise the number of animals used and their suffering.

2.2. Surgery

The cerebellar lesions were performed by aspiration according to previously described methods [24,34]. The animals were anesthetised by immersion in a 1:20000 solution of tricaine methanesulfonate (MS222, Sigma, USA) and then immobilised in a surgical chamber. An adjustable plastic tube inserted in the mouth of the animal provided a constant flow of aerated water through the gills that contained the identical concentration of anaesthesia. Surgery was performed under visual control using a binocular microscope. The cephalic skin and a section of the dorsal skull were carefully removed, and the fatty tissue inside was aspirated to expose the brain. The lesions were performed using a glass micropipette connected to a manual vacuum system. In the Cb group, the most dorsal region of the corpus cerebelli was carefully aspirated, thus interrupting the cerebellar circuit. The valvula cerebelli and vestibulolateral lobe were spared. Following ablation, the piece of skull was replaced in its original position, fixed with cyanoacrylate glue, and covered with dental cement. The operated area was treated with a topical antiseptic. The sham operations were performed identically in the Cb group, except that the brain tissue was not injured. After reanimation, the animals were returned to their home aquarium and allowed to recover for 4 days before behavioural training.

2.3. Apparatus

The experimental setting has been previously described [22]. The apparatus was a circular tank (140 cm in diameter, 50 cm high) composed of white vinyl and filled with aerated and filtered water at $20 \pm 1\,^{\circ}C$ to a depth of 18 cm. A square matrix (80 cm in length) consisting of 25 equidistant feeders was positioned on the floor and in the centre of the tank (Fig. 1A). Each feeder consisted of an opaque glass cup (4.5 cm in diameter, 4.5 cm high), which enclosed a latex tube (1.5 cm long) that enabled a food stick or fake stick to be hidden. The fake sticks were composed of light brown latex to simulate real food. The animals could obtain the food by applying suction to the latex tube but could not remove the false sticks because they were glued to this tube.

Five different objects (cues) placed within the tank around the feeder matrix and maintaining stable spatial relationships relative to the goal, served as landmarks (Fig. 1A). The following cues were used: cue *a*, a plastic grey disc (25 cm in diameter); cue *b*, a blue plastic litter bin (20 cm in diameter × 30 cm high); cue *c*, a plastic red funnel (20 cm in diameter on the base); cue *d*, a grey plastic vessel (20 cm in diameter × 35 cm high); and cue *e*, a yellow plastic box ($20 \times 20 \times 10$ cm). Grey curtains hung from the ceiling to the floor and surrounded the maze to exclude the possible use of uncontrolled cues. A square array of four 60 W fluorescent lights on the ceiling was used to illuminate the maze. A ceiling-mounted video camera allowed us to monitor behaviours and record the trajectory paths of the fish.

2.4. Behavioural procedure

2.4.1. Pre-training

Before training, and for 11 consecutive days, the fish were habituated to the testing environment. In the initial 5 days, the animals were individually trained to obtain food from a single feeder that was re-baited until the fish had consumed 6 sticks. On the following day, the fish were habituated to the maze in groups of four by allowing them to freely explore the experimental tank for 1 h. No feeders or experimental cues were present during this habituation session. In the final 5 days, the fish were individually habituated to retrieve food from the 25-feeder matrix in the experimental tank. No landmarks were present during these days, and only 6 feeders, chosen at random, were baited, whereas the remaining feeders contained fake food. During each one of these days, the goldfish remained in the tank until the 6 food sticks were consumed or 20 min had elapsed. After this habituation period, the animals were deprived of food for 2 days before the experiment proper.