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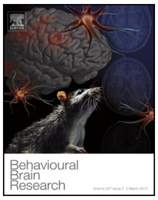

Research report

Spatial learning-related changes in metabolic brain activity contribute to the delimitation of the hippocampal pallium in goldfish

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HIGHLIGHTS

- Teleostean Dl telencephalic area seems not to be a single functional division.
- Spatial learning increases metabolic activity in ventral but not in dorsal Dl.
- Spatial learning does not activate the Dm telencephalic pallial area.
- Dlv region might be functionally comparable to the hippocampus.

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ABSTRACT

Comparative neuroanatomical, developmental and functional evidence suggests that the lateral division of the area dorsalis telencephali (Dl) of the teleost fish is homologous to the hippocampus of tetrapods. Nonetheless, some important aspects of the organization of the hippocampal pallium of teleosts are still under discussion and conflicting hypotheses regarding the extension and demarcation of this region have been proposed. Thus, whereas some authors suggest that the entire Dl region, including its dorsal (Dld) and ventral (Dlv) subdivisions, is homologue to the mammalian hippocampus, others claim that only Dlv should be considered as such. To further elucidate this debate, we investigated the role of Dld and Dlv in one of the most unambiguous functions of the hippocampus, spatial learning. We trained goldfish in a spatial constancy task and mapped the activity of Dld, Dlv, and the medial division of the area dorsalis telencephali (Dm) by means of cytochrome oxidase (CO) histochemistry. The results revealed that training goldfish in the spatial constancy task significantly increased the metabolic activity in Dlv, but not in Dld or Dm, suggesting that only Dlv is critically involved in spatial learning and in this regard comparable to the hippocampus. These data provide additional functional support to the hypotheses that consider Dl as a heterogeneous pallial region and propose that Dlv, but not Dld, might be homologous to the hippocampus.

1. Introduction

Among vertebrates, the actinopterygian fish, e.g., teleosts, represent a separate case because of the particular anatomical organization of their forebrain. The telencephalic hemispheres of this vertebrate group undergo a unique embryonic development (i.e., the eversion or outward bending of the distal walls of the prosencephalic vesicle) that produces solid telencephalic hemispheres separated by a single ventricle, as well as the reversal of the pallial topography observed in non-actinopterygians, e.g., land vertebrates [1–7]. As a consequence, the teleostean area homologous to the hippocampal or medial pallium is hypothesized to occupy a lateral position in the telencephalic pallium, whereas the region comparable to the pallial amygdala would occupy a medial position [2,4,8,9]. In fact, on the basis of topological, hodological, neuroanatomical, histochemical, developmental, and functional comparative evidence, a broad consensus has been obtained in considering that at least part of the lateral division of area dorsalis telencephali (Dl) is homologous to the mammalian hippocampus, and that at least part of the medial division of area dorsalis telencephali (Dm) might be considered homologue to the amygdala [5,10–13]. In agreement with this view, lesions of the ventral part of Dl (Dlv) in goldfish produce severe spatial learning and memory impairments, comparable to those observed after hippocampus damage in land vertebrates [14–18]. Furthermore, higher spatial behavior demands in natural environments have been associated with increases in the relative size of Dlv [19], and morphofunctional studies have shown changes in the transcriptional activity of the neurons in Dlv, related to spatial learning [16,20]. Regarding

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the Dm region, it has been shown that its ventromedial area, similar to the mammalian amygdala, plays a critical role in the emotional conditioning as well as in encoding values and motivational signals [15,21–27].

However, as the precise mechanisms of eversion have not been thoroughly identified and the functional organization of the teleost telencephalon is not completely understood, several contending hypotheses about the homologies of the teleost pallium subdivisions have been proposed [7,28–30]. For example, it is currently assumed that the Dl can be cytoarquitectonically divided into a dorsal (Dld) and a ventral subdivision (Dlv) throughout most of its rostrocaudal extent [5], but is still under discussion whether the entire Dl, i.e., Dld plus Dlv, or by contrast Dlv exclusively, are comparable to the hippocampal pallium. Thus, some hypotheses propose Dlv as the homologue to the mammalian hippocampus and Dld as a part of the dorsal pallium or isocortex [11,28,31]. In particular, it has been proposed that Dld is homologue to the primary, geniculate recipient visual pallial region in the dorsal pallium or isocortex of other vertebrates [32–34]. The topological position of this region, its visual ascending inputs, and its visually related activity argue in favor of this hypothesis [11,13,31]. In contrast, other hypotheses, based on a topological criterion and the presumable similarity in the pattern of connections of the Dld and the Dlv, propose that the whole Dl should be viewed as a single primary pallial division, homologous to the mammalian hippocampus [5,35].

In the present work we investigated further the regions of the teleost fish pallium involved in spatial learning. With this objective, we trained goldfish in a spatial task and measured the learning-related neural activity in Dld, Dlv and Dm regions using quantitative cytochrome oxidase (CO) histochemistry. Since CO is a mitochondrial enzyme involved in the generation of ATP associated with cell metabolic demands, and therefore in neuronal activation, CO histochemistry is a useful tool to index regional functional activity in the brain [36,37] and, in particular, to identify sustained activity changes induced by learning situations [38,39]. Thus, the present experiment could contribute to clarify the identity of Dlv, Dld, and Dm by means of assessing their possible involvement in spatial learning, one of the most unambiguous functions of the hippocampus.

2. Methods

2.1. Subjects

Goldfish (*Carassius auratus*) 11 to 13 cm in length were obtained from a local supplier and were kept in large tanks with aerated and filtered water at 19 ± 1 °C on a 14/10-light/dark cycle for several weeks prior to the experiments. A week before the experiments, animals were distributed into small groups of four subjects and kept in the same conditions. At the end of each experimental session, the fish were returned to their home aquaria and fed with Tetrapond Pondsticks (Ulrich Baensch GmbH, Germany). Fish were identified individually based on phenotypic features. All animal procedures were performed in accordance with Directive 86/609/CEE of the European Community Council and Spanish legislation (R.D. 53/2013).

2.2. Apparatus

The apparatus and training procedure have been described previously [16,40]. Briefly, the maze consisted of a diamond-shaped enclosure (choice compartment) and two identical, removable circular start compartments that were placed diagonally in the middle of an 85 × 85 cm aquarium. The aquarium was filled with aerated and filtered water at 19 ± 1 °C to a depth of 30 cm. The experimental apparatus was made of dark gray PVC. The choice compartment, which was 324 cm^2 and 24 cm high, had four openings that were 6.5 cm wide and 20 cm high. One opening was placed in each corner. Two of the diagonally opposite openings led to the start boxes, and the two remaining openings served as doors (see Fig. 1A). One of the doors remained open and provided the only exit (goal), and the other door was blocked with a transparent glass barrier. In each trial, only one start compartment was used, and the access to the opposite compartment was blocked with a sliding dark gray PVC barrier. The visual cues consisted of two removable gray PVC panels with five 2-cm-wide white vertical stripes on each that entirely covered two walls of the choice compartment. The other two remaining walls were dark gray. To ensure that no other cues were available except those provided by the experimental setting, the walls of the experimental room were homogeneously covered with dark gray curtains. Illumination was provided by an 18-W fluorescent light placed at a height of 25 cm and oriented along the axis between the two start compartments. For each trial, the observer, who was behind the start compartment, recorded the animal's behavior. Sessions were conducted with groups of four fish, but the animals were trained individually. Two glass enclosures placed in the aquarium behind the start compartments served as waiting areas during inter-trial intervals. The subject within the maze could not see the others until it completed the trial by exiting the choice compartment through the exit.

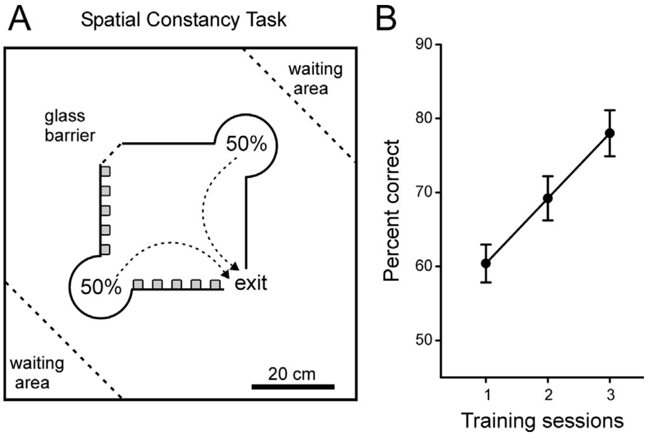

Fig. 1. (A) Schematic representation of the spatial constancy task showing the choice compartment, the location of the cues (gray boxes), the start compartments, the exit door (goal), the glass barrier, and the waiting areas. The numbers represent the percentage of trials on which each start compartment was used. The arrows represent the most appropriate trajectory to the exit during the training sessions. The glass barrier was not used during habituation. (B) The percentages of correct choices during the training sessions. Data are represented as mean ± SEM.

2.3. Behavioral procedure

Fish were randomly distributed into two groups: naive ($n = 8$), and a group trained for 3 sessions in a spatial constancy task ($n = 10$). Fish in the naive group were not exposed to the apparatus. The trained animals were first individually allowed to explore the experimental apparatus by swimming out of each start box five times (i.e., 10 total trials). The order of the beginning start box was random. Because the glass barrier was not used in these trials, the animals made spontaneous exits through either door. After this habituation session, the fish were trained for three 25-trial sessions on consecutive days. During training, the exit door (goal) was located in a constant spatial position relative to the entire experimental setup that was formed by the choice compartment and the cues (striped panels); thus, the spatial position of the goal was independent of the entrance point. The other door was blocked with a transparent glass barrier (see Fig. 1A). To make egocentric (turn) responses irrelevant, the start boxes were