

## Hippocampal activation of immediate early genes Zenk and c-Fos in zebra finches (*Taeniopygia guttata*) during learning and recall of a spatial memory task

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### ARTICLE INFO

#### Article history:

Received 22 September 2009

Revised 18 November 2009

Accepted 23 November 2009

Available online 26 November 2009

#### Keywords:

Birds

Avian brain

Hippocampus

Immunohistochemistry

Spatial orientation

### ABSTRACT

Zebra finches (*Taeniopygia guttata*) are able to learn the position of food by orienting on spatial cues in a 'dry water maze'. In the course of spatial learning, the hippocampus shows high expression of the immediate early genes (IEGs) Zenk and c-Fos, indicating high activation of this area during learning. In contrast, the IEG activity is nearly absent if the birds do not have to rely on spatial cues. In the present experiment it was investigated whether hippocampal activation can also be observed if the learned spatial task is recalled. For this purpose, the hippocampal Zenk and c-Fos activation of birds in an early learning stage was compared with that of others having well reached their maximal performance. The results show that the avian hippocampus is also active during recall of a learned spatial task, but the activation is significantly lower than in animals learning actually. As in previous experiments, hippocampal IEG expression showed strong variation not only in the position of the active patches of neurons, but also in size and cell density. The observed difference contributes to the view that immediate early genes may not be indicators of activation alone, but may be due to a combination of activation and plastic changes.

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### 1. Introduction

Spatial orientation and memory is an essential skill for coping with daily demands in most animals. Studies initially performed with rats indicate a crucial role of hippocampus in spatial memory processing (O'Keefe & Dostrovsky, 1971). It was presumed that hippocampus may contain a 'cognitive map' which provides a representation of an animal's environment (O'Keefe & Nadel, 1978). Such a representation allows the animal to find its way irrespective of its starting point in a known area. Hippocampal damage disrupts spatial orientation on distant cues in rats, but does not affect the orientation on visible proximal cues in a water maze (Morris, Garrod, Rawlins, & O'Keefe, 1982). This suggests that hippocampus plays a specific role in spatial memory.

The avian hippocampus is believed to be homologous to the mammalian hippocampus and is also involved in spatial navigation (Atoji & Wild, 2006; Clayton, 1995; Clayton & Lee, 1998; Colombo & Broadbent, 2000; Watanabe, 2006). It is larger in food hoarding birds compared with non-hoarding species (Krebs, Healy, & Shettleworth, 1990; Sherry, Vaccarino, Buckenham, & Herz, 1989) and lesioning this structure impairs the use of spatial memory (Bischof, Lieshoff, & Watanabe, 2006; Hampton & Shettleworth, 1996; Sherry & Vaccarino, 1989; Shiflett, Smulders,

Benedict, & DeVoogd, 2003; Watanabe, 2001; Watanabe & Bischof, 2004). The hippocampus of black-capped chickadees (*Parus atricapillus*) shows seasonal changes in size and is larger during the peak of food hoarding behaviour in the fall (Smulders, Sasson, & DeVoogd, 1995). This may at least partly be due to the net addition of newly generated neurons (Barnea & Nottebohm, 1994; Barnea & Nottebohm, 1996), resulting in an increased cell number (Smulders & DeVoogd, 2000). Although most of the investigations were performed with food hoarding birds, more recently it was shown that non-storing birds are also able to solve spatial memory tasks, e.g., great tits (Hodgson & Healy, 2005), pigeons, and zebra finches (Patel, Clayton, & Krebs, 1997; Sanford & Clayton, 2008; Watanabe & Bischof, 2001). Lesioning studies revealed that also in these species hippocampus is crucial for spatial memory (Bischof et al., 2006; Fremouw, JacksonSmith, & Kesner, 1997).

Zebra finches are able to find the position of the accessible food feeder in a 'dry version of the Morris water maze' task (Watanabe & Bischof, 2001). During their search, the birds rely on a combination of extra-maze cues and cage wall cues, i.e., they are using a spatial map. By damaging hippocampus before and after learning, Bischof et al. (2006) demonstrated that the zebra finch hippocampus is involved in learning and recall of the spatial task. In another experiment, immediate early genes (IEGs) were used as markers for active neurons (Bischof et al., 2006). IEGs are rapidly activated after neuronal activity increases and are supposed to play a crucial role in the transformation of short term enhancement of synaptic

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<sup>1</sup> Grant sponsor: Deutsche Forschungsgemeinschaft, Grant No. BI 245/19-1.

efficiency into long-term efficiency changes or, in other words, in learning situations (Guzowski, 2002; Jones et al., 2001; Kubik, Miyashita, & Guzowski, 2007; Lanahan & Worley, 1998). In zebra finches, the activation of immediate early genes were high during learning of the spatial task, but almost absent if the birds did not have to rely on spatial cues to find food (Bischof et al., 2006). The IEG activation pattern in the learner group showed big variation not only in the position of the patches of active neurons, but also in size and cell density. The cause for this variation is not known as yet. A plausible hypothesis would be to presume that the birds were in different stages of learning when they were sacrificed for IEG immunohistochemistry.

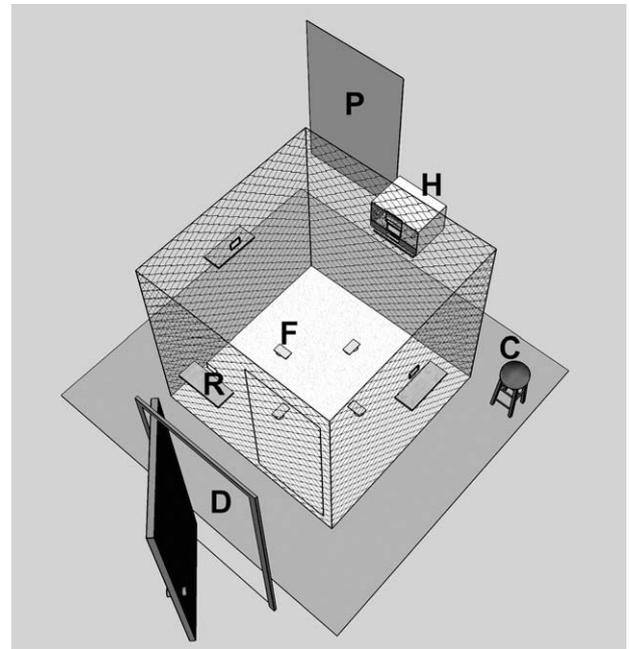
To examine this idea, the present study focuses on the hippocampal activation of the immediate early genes *Zen* and *c-Fos* at two different stages. We examined the density and distribution of the IEGs in birds which were just at the beginning of learning the spatial task, and compared the results with those of birds which had completely learned it. Birds which have to perform a task which they have learned previously have to recall this previously learned task. One therefore can also express the goal of our experiments as to examine differences of hippocampal activation, as indicated by the expression of the immediate early genes *c-Fos* and *Zen*, between learning and recall of a spatial task. To separate the effects of learning or recall, respectively, from activation due to the pure motor behaviour performed by the birds to reach a food tray in the experimental situation, we also added an experimental group where the birds got food in trays without having to learn or recall a spatial position.

## 2. Materials and methods

Twenty-one zebra finches (*Taeniopygia guttata*) of either sex, between 14 and 21 months old, from the institute's stock were used for this study. They were housed in individual cages with water freely available. The light cycle was 12L:12D. Food was removed at 8 p.m., 30 min before the light was switched off, and was ad libitum provided after the end of the daily experiments which started at 9 a.m.

The experimental setup was a 'dry' version of the Morris water maze (Fremouw et al., 1997; Watanabe & Bischof, 2001), consisting of a cubic aviary positioned in the middle of a chamber (3 m × 3 m × 2.50 m) with extra-aviary visual cues (Fig. 1). The aviary (180 cm × 180 cm × 180 cm) was made of wire mesh. Each wall of the aviary included a flap door (10 cm × 7.5 cm) with a wooden platform attached to the outer side. The individual home cages (42 cm × 22 cm × 24 cm) could be placed on these platforms to enable the release of an animal into the experimental arena. Each individual home cage was equipped with a lamp at the ceiling (DC 12 V). There was one long perch (27 cm) below each flap door and seven short perches (9 cm) attached to the walls inside the aviary. The floor was covered with white paper. Four food feeders were placed on the floor in a distance of 40 cm to the walls. The feeders were made from the base part of commercially available pet bird feeders in the shape of cylindrical white cups (3.5 cm in diameter, 3.5 cm high), each with a hollow arm (1.0 cm wide, 3.5 cm long) allowing the bird to reach the mixed grain. The cups were covered with yellow carton sheets (6 cm × 9 cm), so that the birds could not see the inside of the arm until they came closer to the feeder. As extra-aviary cues the experimental chamber contained a coloured poster on one wall, a punched board on another, and the room entrance door with a chair aside.

For the first two days of the experiments, the birds were released into the aviary in groups of four or five to habituate to the setup. The trial continued for a maximum of two hours, so that all birds had enough time to explore the new environment and



**Fig. 1.** Sketch of the experimental setup (view from above). The experimental aviary (180 cm × 180 cm × 180 cm) was placed in the middle of a chamber (3 m × 3 m × 2.50 m). R = releasing site (150 cm above the floor, one at each side of the arena); F = food feeders on the floor (only one was accessible for food); H = home cage (placed at one of the four releasing sites); P, D, C = extra-maze cues (poster, door, chair).

to find the food feeders on the floor. After two days of such group adaptation, each bird was released alone. During the habituation training the birds had to learn to enter the aviary when the experimental chamber illumination was switched on. They were trained to eat food from the cups on the floor and to fly back to their home cage when the illumination of the chamber was switched off and the ceiling lamp of their home cage was switched on. For this purpose, the individual home cage was placed on the platform. The birds were released by opening the guillotine door with a string attached to it. After reaching one of the feeders they were allowed to eat food for two minutes. The aviary illumination was then switched off whereas the illumination of the home cage was switched on. In this situation, most of the birds returned spontaneously back to their illuminated home cage. If not, they were gently chased to the correct direction. After each trial, the home cage was removed and the floor of the aviary was cleaned in order to avoid possible local landmarks. The releases occurred from four different positions (releasing sites of the aviary) in random order. The training trials of each bird were separated by at least 90 min. The habituation training consisted of two training trials per day and continued for five days, until the birds had learned to eat food from the feeders and to return to their home cage. More details of the experimental setup are given in Watanabe and Bischof (2001).

During discrimination training following habituation training, the birds had to learn that food was accessible in only one of the four feeders. The other three also contained food, but it was covered with a transparent adhesive tape in order to enable the birds to see the food when they approached the feeder, but to prevent them from reaching it. As in the habituation training, the birds were released from four different positions randomly chosen for each trial (two trials per day), whereas the position of the accessible feeder was fixed. A circular area with a radius of 12 cm around the cup was defined as the "choice area". The time each bird needed to reach the correct feeder after entering the aviary and the number of incorrect visits of other feeders were measured. A

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