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METABOLIC BRAIN ACTIVITY UNDERLYING BEHAVIORAL PERFORMANCE AND SPATIAL STRATEGY CHOICE IN SEDENTARY AND EXERCISED WISTAR RATS

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Abstract—We have studied the performance of a spatial reference memory task, the navigation strategy and the changes in the cytochrome c oxidase activity (COx) in different brain regions in exercised (forced exercise, 10 consecutive days, 15 min/day) and non-exercised adult Wistar rats. The spatial learning task was carried out in the radial-arm water maze (RAWM) for four days with six daily trials, and on the fifth day, a probe session was run, in which we rotated the position of the distal cues 90° in a clockwise direction. During the four days of training, the exercised group showed shorter latency and distance traveled to find the platform, as well as fewer memory errors and reduced use of non-appropriate navigation strategies according to the protocol of the task (egocentric). Interestingly, the rotation of the cues did not affect the performance of the exercised group, in contrast to the non-exercised group, which spent more time in the center of the maze and traveled longer distance to find the platform. Finally, higher COx activity in the cingulate and the retrosplenial cortices, as well as in the dorsal CA1 and CA3 was found in the exercised group. All in all, it seems that the exercise favored the configuration of an efficient and accurate cognitive map of the environment, which was supported by our finding that the rotation of the cues, without altering their overall configuration, did not affect performance. The brain regions with higher COx activity in the exercised group seem to be involved in this function.

Key words: forced exercise, spatial memory, navigation strategy, cytochrome c oxidase, Wistar rat.

INTRODUCTION

Effective spatial navigation is crucial to the survival of most animal species. For example, rats and other mammals can orient themselves in their habitat and locate several goals using different spatial navigation strategies that involve qualitatively different stimulus types, psychological processes and neural substrates (Bird and Burgess, 2008; Mosser et al., 2008; Rubio et al., 2012). In general, it has been shown that targets can be encoded in memory with respect to egocentric frames of reference, which are based on vestibular information about turns and self-motion (Lemay and Stelmach, 2005), whereas other studies have indicated that target locations can be represented relative to surrounding objects within the environment, defined as allocentric frame of reference (Olson, 2006). The findings infer that the choice of strategy depends on several factors, such as the organism's perceived orientation in space (Gibson et al., 2001), the shape or design of the spatial environment (Ramos, 2000), the availability of distal visual cues (Rodrigo et al., 1997) and the amount of training given in the environment (Hicks, 1964; Hamilton et al., 2008).

Regarding the allocentric strategy, it has been shown that, on the one hand, the animals can associate the location of a target, for example a platform in the radial-arm water maze (RAWM), with a particular cue, as the *list hypothesis* suggests (Suzuki et al., 1980), or, on the other hand, use information about the relationships among several extramaze cues, as the map *hypothesis* describes (Tolman, 1948; O'Keefe and Nadel, 1978). These map-like representations of the environment are more flexible and allow a more accurate performance, as neither rotating nor removing some of the cues affects the performance (Cohen and Bussey, 2003). However, if we vary the spatial configuration of the distal cues by transposition, this would be expected to adversely affect behavioral performance (Fenton et al., 1994). O'Keefe and Nadel hypothesized that the location of this cognitive

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Abbreviations: Acb, accumbens nucleus; BlA, basolateral amygdala; BNST, bed nucleus stria terminalis; CeA, central amygdala; Cg, cingulate cortex; COx, cytochrome c oxidase activity; dCA, dorsal hippocampal cornu ammonis; dDG, dorsal dentate gyrus; EX, exercise; LS, lateral septal nucleus; MANOVA, multivariate analysis of variance; MC, motor cortex; MM, medial mammillary nucleus; MO, medial orbital cortex; MWM, Morris water maze, OD, optical density; RAWM, radial-arm water maze; RSC, retrosplenial cortex; SED, sedentary group; SPL, Spatial learning group; SuM, supramammillary nucleus; vCA, ventral hippocampal cornu ammonis; vDG, ventral dentate gyrus; VTA, ventral tegmental area.

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map was in the hippocampus by the discovery of the place cells (O’Keefe and Nadel, 1978). However, new research indicates that the cognitive map is represented in several brain systems including not only the hippocampus, but also the entorhinal cortex (Mosser et al., 2008), the pre- and parasubiculum (Epstein, 2008), the retrosplenial cortex (RSC) (Vann et al., 2009), the frontal cortex (Silver and Kastner, 2009) and the parietal cortex (Whitlock et al., 2008), among other areas.

Interestingly, a growing body of scientific evidence has demonstrated that aerobic exercise improves task acquisition and/or retention of several spatial memory tasks (Hoveida et al., 2011) such as, the Morris water maze (MWM) (Ang et al., 2006) the radial-arm maze (RAM) (Schweitzer et al., 2006) and the RAWM (Pietrelli et al., 2012). Since these are hippocampal-dependent tasks, it has been postulated that the hippocampus is involved in the spatial memory improvements following exercise (Griffin et al., 2009). Hence, due to the relationship of the hippocampus with the configuration of a cognitive map, it would be possible to hypothesize that aerobic exercise may facilitate the fast acquisition of an accurate spatial configuration of the environment.

Therefore, our aim was to assess the effect of a protocol of forced exercise on the performance of a spatial reference memory task with distal cues available and, at the same time, to check if the exercised rats had established a cognitive map of the environment. For that purpose, during a probe session, we rotated the position of the cues 90° in a clockwise direction, which if a previous cognitive map of the environment had been formed, would not affect performance. In this regard, our study is innovative as it is the first to study in more detail the behavioral data, showing the effects of exercise on the navigation strategy, as to date most studies have focused on analyzing only the typical behavioral variables, such as latency and distance traveled. Besides, the RAWM, in contrast to the MWM, allows us to obtain reliable data regarding performance (errors and spatial strategy) without video cameras or computers (Morgan, 2008). On the other hand, and as a first approach to the effects of a spatial memory task on the brain activity in exercised and non-exercised rats, we consider that the cytochrome histochemical measurements are the most appropriate, because biochemical assays or spectroscopy provide accurate quantification of COx activity, but they lack the precise anatomical localization achieved by histochemical staining. Moreover, for learning and memory studies aimed at mapping changes in brain activity at regional and system levels, the light microscopic resolution of COx histochemistry is required (Poremba et al., 1997) and it represents an index of the energy demands after prolonged stimulation of neurons (Villarreal et al., 2002). Mapping of COx activity with quantitative histochemistry appears to be the most adequate method to detect learning effects produced by training paradigms, because other techniques may fail to detect small activity changes (Bruchey and Gonzalez-Lima, 2008; Begega et al., 2012; Mendez-Lopez et al., 2013). Also, a previous study with Western Blot analysis did not find significant differences in the content of COx subunit I after a spatial memory task. The subunit I is part of the catalytic center of protein, so it might play a more important role in perceiving energy requirements than other nuclear encoded subunits (Fidalgo et al., 2012). COx activity has also been studied after exercise protocols (McCloskey et al., 2001; Sampedro-Piquero et al., 2013).

EXPERIMENTAL PROCEDURES

Animals

A total of 24 3-month-old male Wistar rats from the vivarium of the University of Oviedo were used. The subjects were housed in groups. All the animals had access *ad libitum* to food and tap water and were maintained at constant room temperature (20–21 °C), with a relative humidity of 65–70% and artificial light–dark cycle of 12 h (8:00–20:00 h light/20:00–8:00 h dark). The procedures and manipulation of the animals were carried out according to the Directive 86/609/EEC (The Council Directive of the European Community) concerning the protection of animals used for experimental and other scientific purposes. The National legislation, in agreement with this Directive, is defined in Royal Decree N° 53/2013.

The rats were randomly assigned to three groups: sedentary group (SED: 274.4 g; $n = 8$), Spatial learning group (SPL: 256.4 g; $n = 8$), and exercise + Spatial learning group (EX + SPL: 251.8 g; $n = 8$). The animals were housed in standard cages (55 × 20 × 34 cm) in groups of four rats per cage. Rats of different experimental groups were not housed together. The SED group consisted of animals without learning or exercise experience. The SPL group was handled, habituated and trained in the RAWM, as was the EX + SPL group, which, in addition, performed the forced exercise protocol. The animals were handled daily for seven days for a period of 10 min (even the SED group) in order to avoid stress reactions to subsequent human contact.

Forced exercise protocol

The Rat-Rotarod (47700, Ugo Basile) was used with individual compartments for each rat. The EX + SPL group was subjected to running exercise for 10 consecutive days. Forced exercise was performed for 15 min/day with 5-min rest periods for every 5 min of exercise to avoid stress and fatigue. The Rotarod had a 6-cm diameter and the rod was elevated 40 cm above the floor. The apparatus rotated at 10 rpm and the rate was held constant during all the sessions. The body of the rat was placed perpendicular to the rotating axis with its head in the opposite direction of the rotation; therefore, the animal had to move forward in order to stay on the rod. During the 15 min of exercise, we supervised the behavior of the rats to ensure that they had a correct performance, and when a rat fell off, it was immediately placed on the rod again. The animals did not receive stimulation (appetitive or aversive) to motivate them to run. After the exercise program, the