The anxiogenic-like effects of dehydration in a semi-desert rodent *Meriones shawi* indicating the possible involvement of the serotoninergic system

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ABSTRACT

Dehydration is a powerful stimulus causing disequilibrium in homeostasis of water and electrolytes resulting from depletion in total body water. Most studies have focused on domestic and laboratory animals; however, the study of desert animals allows improved understanding about water balance and resistance to dehydration and associated behavioral changes, including those related to mood disorders. *Meriones shawi* (Shaw's Jird) is a desert rodent characterized by its resistance to long periods of thirst that can extend for several months. In the present study, *M. shawi* were subjected to water deprivation for 1 and 3 months. We used 5-HT immunohistochemistry to evaluate the effects of prolonged dehydration on the serotoninergic system in both dorsal and median raphe nuclei (DRN, MRN), which are the main sources of 5-HT input to several brain areas. In addition, a dark/light box was used to evaluate the anxiolytic-like or anxiogenic-like effects of dehydration on *M. shawi*. The results showed a reduction in the 5-HT immunolabelling in both DRN and MRN following 1 and 3 months of dehydration. This diminution of serotonin immunoreactivity was accompanied by noticeable changes in anxiety behavior of *Meriones*, with animals spending more time in the light box, suggesting anxiogenic-like effects caused by dehydration. Overall, the results indicate that dehydration is able to reduce serotoninergic neurotransmission, which might be involved in generating anxiety behavior in this desert animal.

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Introduction

The study of desert animals allows us to improve our understanding of water balance and resistance to dehydration and associated behavior patterns, including mood disorders. Unlike the rat, *Meriones shawi* (Shaw's Jird) is a semi-desert rodent that has a particular ability to survive long periods of dehydration extending over several months (Laalaoui et al., 2001; Elgot et al., 2009). This rodent presents many characteristics and behavioral adaptations that allow survival in very dry and hot seasons. In our laboratory, unlike in the rat, it has been shown that the supraoptic nucleus of this rodent is very developed and spread throughout the optic chiasm (Gamrani et al., 2011). Nephrons of the *Meriones* kidney are very well developed, allowing excretion of a highly concentrated urine and effective water retention (Rabhi et al., 1996).

Dehydration is associated with osmotic and nutritional problems and involves depletion in total body water content, which may be due to several conditions such as pathologic fluid losses, decreased fluid intake, or both (Gross et al., 1992). Water deprivation is a potent stimulus, which modifies water and electrolyte balance which results in dehydration of the two major body fluid compartments (extracellular and intracellular) (Ramsay et al., 1977). This disturbance in water balance affects many neuronal systems, including the serotoninergic one (Elgot et al., 2009), and commands the behavior of water intake (Epstein, 1990; Berridge, 2009), however, information on the effects of dehydration on anxiety behavior disorders remain obscure.

There is considerable evidence supporting the involvement of serotonin (5-hydroxytryptamine; 5-HT) in the physiopathology of anxiety disorders (Blier and de Montigny, 1999; Millan, 2003; Lowry et al., 2005; Maron and Shlik, 2006) and many currently available anxiolytic drugs are known to act either by interfering with 5-HT reuptake or directly on 5-HT receptors (Argyropoulos et al., 2000; Hoyer et al., 2002; Millan, 2006). Despite intensive research, there remain many controversies regarding the specific role of 5-HT in the physiopathology of anxiety (Glennon, 1990; Handley, 1995). The dorsal raphe nuclei (DRN) and median raphe nuclei (MRN) provide almost all the 5-HT innervation to the forebrain (Dahlstrom and Fuxe, 1964; Moore et al., 1978). Their projections are organized in such a way that some forebrain areas receive mixed DRN/MRN projections, while others have selective DRN or MRN inputs (Azmitia and Segal, 1978; Charara and Parent, 1994). Several studies have shown that raphe neurons innervating

the forebrain play a critical and diverse role in regulating autonomic functions, including mood (Smits et al., 1978; Piper and Goadsby, 1985). Deakin and Graeff (1991) have proposed that the 5-HT system, originating from the dorsal raphe nucleus (DRN), is implicated in anxiety behavior. Moreover, it has been shown that 5-HT pathways departing from MRN play an important role in anxiety processing, with implications in pathologies such as generalized anxiety disorder (Dos Santos et al., 2005).

The objective of this study was to assess using an experimental dark/light box test and 5-HT immunohistochemical localization, whether the serotoninergic system originating from raphe nucleus (from DRN and MRN) is implicated in anxiety disorder behavior under the effects of prolonged dehydration following water deprivation for one and three months.

Materials and methods

Animals

Experiments were carried out in *M. shawi*, captured around the semi-desert regions of Marrakech and kept in captivity in our breeding farm for several generations. Animals 1 month old of both sexes were used for both behavioral and immunohistochemical studies including 5 controls (drinking water *ad libitum*) and 10 dehydrated animals for a period of either 1 or 3 months ($n = 5$ per group). The rodents were housed at a constant room temperature ($25\,°C$), with a 12 h dark/light cycle and free access to food for all study groups. Water bottles were provided freely to controls, but not to water-deprived animals. All animals were treated in compliance with guidelines of the Cadi Ayyad University, Marrakech (Morocco), and all efforts were made to minimize animal suffering and to reduce the number of animals used.

Dark/light box test

The exterior size of the dark/light box is 50 cm length, 20.5 cm width, and 19 cm height. It consists of two unequal compartments, one dark (one third) and one white (two-thirds) illuminated by a 60 W lamp. The opening between the two adjacent compartments is no more than 7 cm. Each *Meriones* was tested by placing it in the center of the white area, facing away from the dark region, and it was allowed to explore the novel environment for 5 min. The periods spent in the illuminated side were measured. The model is based on the observation that although nocturnal rodents such as *Meriones* will naturally tend to explore a novel environment, they show a general fear of bright light.

Immunohistochemistry

After anesthesia with sodium pentobarbital (40 mg/kg i.p.), all animals were perfused transcardially with chilled physiological saline and paraformaldehyde (4%) in phosphate buffer (PBS, 0.1 M, pH 7.4). Brains were post-fixed in the same fixative for 12 h at $4\,°C$, dehydrated in graded ethanol solutions (70–100%), passed through serial polyethylene glycol solutions (PEG) and embedded in pure PEG. Frontal sections (20 μm) were cut with a microtome, collected and rinsed in PBS to wash out the fixative. Sections were performed throughout both DRN and MRN; the immunolabelling was performed on six selected sections in the median part of each nucleus.

Free floating sections were incubated in 5-HT (Miles, Elkhart, IN) diluted 1/2000 in PBS containing 0.1% Triton X-100 and 1% bovine serum albumin. After three washes in the same buffer, sections were incubated for 2 h at room temperature in goat anti-rabbit biotinylated immunoglobulins (1/2000; Dako, Glostrup, Denmark) and then, after washing, incubated in streptavidin peroxidase (1/2000; Dako). Peroxidase activity was revealed by incubating sections in 0.03% DAB (3,3-diaminobenzidine (DAB), Sigma–Aldrich, Oakville, Canada) in 0.05 M Tris buffer, pH 7.5, containing 0.01% H_2O_2. The sections were then collected, dehydrated and mounted in Eukitt® (Sigma–Aldrich, St. Louis, MO, USA) for optical microscopy observation. The specificity (negative controls) of the immunoreactivity was tested by subjecting the slides to the same immunohistochemical protocol described above by either using the preimmune serum or omission of the primary antibodies. These tests showed that the primary antibodies used against 5-HT display specific labelling.

Immunolabelling quantification and statistical analysis

The quantification of the immunorectivity was performed according the protocol published by Vilaplana and Lavialle (1999). Briefly, the digitization and storage of images were performed using a Zeiss-Axioskop 40 microscope fitted with a Canon digital camera. Images were digitized into 512×512 pixels with eight bits of grey resolution and were stored in TIFF format. Image processing and quantification were performed using Adobe Photoshop® v.6.0 (Adobe Systems, San Jose, CA, USA). After transformation of each image to the binary mode, the percentages of black pixels were obtained using the image histogram option of Adobe Photoshop®. This percentage corresponds to the 5-HT immunopositive areas of each nucleus (DRN and MRN). For controls and water-deprived animals, five sections were randomly chosen for the quantification of each group. Data are reported as mean ± SEM, and were subjected to a one-way analysis of variance (ANOVA). Post hoc differences between group means were tested with the Tukey test. Values of *p* lower than 0.05 were considered significant. Statistical analyses were performed using the computer software SPSS 10.0 for Windows® (IBM, Chicago, IL, USA).

Results

Effects of dehydration on 5-HT system in both DRN and MRN

All immunohistochemical results that we collected were conducted on the two main raphe nuclei: the dorsal raphe nucleus (DRN) and median raphe nuclei (MRN) (Fig. 1A–C).

The DRN of the adult *Meriones* showed five distinct delimited regions: namely the dorsal, the ventral, the medial and two dorsolateral areas (Fig. 1: a1). After application of the anti-5-HT antibody in control *Meriones*, our data show significant 5-HT immunoreactive labelling in the DRN, with many 5-HT neurons appearing strongly immunoreactive throughout all regions of this nucleus (Fig. 1: a1). Similarly at the DRN, the MRN of control *Meriones* (Fig. 1: a2) shows an intense 5-HT immunoreactivity, therefore, many 5-HT neurons are classified longitudinally forming a neuronal network.

In the 1-month water-deprived group (Fig. 1B), a major reduction in the 5-HT immunolabelling was observed, this decrease is very marked in both dorso-lateral regions of DRN (Fig. 1: b1). This diminution also applies to the MRN where a few neurons are grouped at the front of this nucleus (Fig. 1: b2).

Compared to both control group and 1-month dehydrated groups, the 3-month water-deprived group (Fig. 1C), shows a net and significant decrease in 5-HT immunoreactivity. This decrease involves all raphe nucleus. Moreover, only a few scattered neurons in both DRN and MRN are discernible (Fig. 1: c1 and c2).

Quantification of the immunoreactive 5-HT neurons in DRN and MRN showed a statistically significant reduction in both water-deprived groups, and also between treated groups (Fig. 2) [**$p = 0.009$ vs. control; *$p = 0.014$ vs. control; $\mu,\mu$$p = 0.008$ vs. dehydrated groups; $\mu$$p = 0.014$ vs. dehydrated groups; $p < 0.05$].