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Sex differences in learning and memory following short-term dietary restriction in the rat

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ABSTRACT

Introduction: Acute or prolonged dietary restriction has been shown to have significant effects on learning and memory, and also on the food seeking behaviour in animals.
Aim: This study investigated whether or not there are sex differences in spatial learning and memory following short-term dietary restriction in the rat.
Methods: Two month-old male (n = 24) and female (n = 24) Sprague-Dawley rats were randomly assigned to either (1) male or female control (i.e. normal diet regimen); (2) male or female 40% dietary restriction for either 2 hours (2 h-DR) or 2 weeks (2 wks-DR) duration. Following the restriction paradigm (i.e. either 2 h or 2 weeks), animals were weighed, and learning and memory was assessed daily for a total of 5 days by Morris Water Maze.
Results: Dietary restriction for 2 h provoked high speed swimming in female rats compared to controls. However the females performance in water maze was inferior to the control animals. Two weeks after 40% DR resulted in reduction of male's body weight by 20% compared to their control group. However, both males and females showed difficulties in water maze learning and memory test after the 2 weeks 40% DR. The animals swam longer distance and took longer time to reach the platform when compared to their age-matched controls.
Conclusion: In fully developed brain, adult animal's cognitive performance is shown to be affected by acute and prolonged stress in the form of food restriction. Interestingly, the impact of this stress was different according to the sex of the animals. In female rats, dietary restriction has a negative effect on learning and memory after 2 h and 2 weeks intervals. In male rats, 2 h of DR has a positive effect on learning and memory; however this effect is not maintained and by 2 weeks there is a negative effect similar to that seen in female animals.

1. Introduction

Ageing is known to affect learning and memory in animals (Mizumori and Kalyani, 1997; Barnes, 1998; Hansalik et al., 2006; Dal-Pan et al., 2011) and humans (Craik, 1990; Albert, 1993; Maki and Resnick, 2000; Witte et al., 2009). In rodents, these age-related affects can be demonstrated for example, in the Morris water maze, radial arm maze and tunnel maze task (Goodrick, 1984; Beatty et al., 1987; Van der Staay, 1997; Hansalik et al., 2006; Carter et al., 2009; Khabour et al., 2010).

Long-term dietary restriction (DR), or more specifically calorie restriction of 20–40%, is a non-genetic manipulation that has been shown to increase longevity by ~40% and to improve metabolic markers such as glucose homeostasis, insulin sensitivity, and resistance to several acute oxidative stressors (Speakman and Mitchell, 2011; Trapenowski et al., 2011). There is also evidence that long-term DR can ameliorate age-related impairments in learning and memory across a wide variety of species including rodents, dogs, non-human primates and humans (Goodrick, 1984; Pitsikas et al., 1990; Means et al., 1993; Markowska and Savonenko, 2002; Hashimoto and Watanabe, 2005; Fontàn-Lozano, 2007; Witte et al., 2009; Dal-Pan et al., 2011). The mechanisms underlying these benefits to learning and memory at least partly involve increased

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expression of NMDA receptor subunit NR2B (Fontàn-Lozano, 2007). Conversely, some studies have reported that long-term DR is either of no benefit (Beatty et al., 1987; Bond et al., 1989; Markowska, 1999; Khabour et al., 2010), or may even have negative effects on cognitive performance (Yanai et al., 2004). There are a number of possible explanations for these inconsistent findings. For example, greater caloric restriction increases longevity, until the level of caloric restriction hits the threshold that would produce starvation (Merry, 2002; Hashimoto and Watanabe, 2005). Studies involving reduction/manipulation of nutrient intake (i.e. dietary restriction) suggest that neither carbohydrate nor lipid restriction are factors with respect to longevity (Isawaki et al., 1988; Shimokawa et al., 1996). However, restriction of protein – and in particular the amino acid methionine – appears to increase longevity by ∼20%, approximately half the effect of calorie restriction (Pamplona and Barja, 2006; Caro et al., 2009). Finally, evidence from rodent studies suggests that food-motivated tasks (e.g. radial arm maze; Bond et al., 1989) are inappropriate for examining the effects of long-term DR on cognitive function (Markowska, 1999). In such food motivated tests, a greater incentive value of food reinforcement for diet restricted animals can play a significant role. The animal in the water maze of this experiment should use its memory to locate the (rescue) platform to escape swimming, and not to be reinforced by finding food as in the radial maze.

Short-term DR can broadly defined as a period of restriction ranging from 1 day to several months (Robertson and Mitchell, 2013). Studies have shown beneficial effects of short-term dietary restriction in animals across a range of conditions including for example brain ischaemia (Marie et al., 1990) and stroke (Chiba and Ezaki, 2010), hepatic and renal ischaemia-reperfusion injury (Mitchell et al., 2010), and immune responses to endotoxic shock (MacDonald et al., 2011). However, there are few studies concerning learning and memory. A 3-month study of 30% DR in elderly humans has shown increases of 20% in verbal memory scores which is correlated with decreases in fasting plasma insulin (Witte et al., 2009). In addition, 20 days of 20% DR results in significant improvement in long-term memory, and up-regulation of synapsin I, in young mice (Deng et al., 2009). In contrast, a rodent study of DR between 2 and 6 weeks duration failed to show any benefits to learning and memory when assessed using passive avoidance and Y-maze tests (Mahdavi et al., 2009).

Feeding is regulated by the hypothalamus, which controls and regulates the endocrine functions of the pituitary gland and the responses to stressful situations through the Hypothalamo-pituitary-adrenal cortical axis. Big fundamental differences exist between males and females in these interconnected functional systems. Food scarcity triggers a stressful situation affecting food seeking behaviour (like increased activity) and cognitive responses (as learning and memory). Both of these parameters were not extensively studied or compared in males and females animals.

The aim of this study was to investigate whether or not there are sex differences in spatial learning and memory following short-term dietary restriction in the rat.

2. Methods

2.1. Animals

Animals were housed in cages on sawdust and maintained on a 12 h–12 h light–dark cycle, light out at 1300 h. Behavioural testing was performed after 1400 h. Two month-old (i.e. adult) male ($n = 24$) and female ($n = 24$) Sprague-Dawley rats were equally and randomly assigned to either (1) male or female control (i.e. normal diet regimen) groups; (2) male or female dietary restriction for 2 h (2 h-DR) and (3) 2 weeks of 40% dietary restriction for 2 weeks duration (2 wks-DR). The latter was determined after measurement of the daily food intake in a metabolic cage, then 40% of daily food intake was given for 2 weeks. Food restriction by 40% of ad libitum was used to study effects of calorie restrictions in animals on various parameters (Trapenowski et al., 2011). Despite having their caloric intake reduced, the animals are provided sufficient amounts of nutrients and vitamins so as to avoid malnutrition. In the first experiment (2 h-DR), the animals were tested for learning and memory for five consecutive days immediately after the daily 2 h of DR. In the second experiment (40% DR for 2 weeks), the animals were weighed, and learning and memory was assessed daily for a total of 5 days by the Morris Water Maze.

2.2. Water maze

Animals were assessed daily for a total of 5 days in the Morris water maze, as previously described (Biessels et al., 1996; Kamal et al., 2006). The maze consisted of a circular swimming pool (140 cm diameter and 50 cm height, filled to a depth of 30 cm); the water was maintained at room temperature. The maze was housed in a darkened room, rich in extra-maze visual cues, and illuminated by sparse red light. It was divided into 4 equal quadrants by two imaginary diagonal lines.

Each rat was given five acquisition trials/day for five consecutive days to learn the position of a hidden 'escape' platform, submerged 2 cm below the water surface, at a fixed location inside the pool. On each trial, the rats were released from one of four predetermined positions on the perimeter of the pool. The starting position was varied on each trial in a quasi-random sequence. Animals were given a maximum of 2 min to find the platform, and were allowed to remain on the platform for 30 s. Rats that failed to locate the disc were put onto it by the experimenter.

The position and movement of the animals, in the pool, was captured and analysed every 0.2 s, using a video-camera computer system, and ANY-maze video-tracking system (Stoelting Co, U.S.A). Outcome measures were latency time and distance swum to reach the platform. These measures were considered most relevant for spatial learning and memory (Gallagher et al., 1993). Performance in the five daily trials was averaged to yield one data point per rat per day. Speed of swimming (which is a measure of motor function (Lindner, 1997)) was measured as control between the groups. To determine any bias, a trial was conducted following the last acquisition training on day-5. The platform was removed and each animal was allowed to swim for 60 s. In this probe trial, the selective search strategy was indicated if animals performed significantly above chance (25%).

2.3. Data analysis

All statistical analyses were performed with Microsoft EXCEL (version 2010) and a Addinsoft™ XLSTAT (Version 2012.6.06). For the Morris maze data, daily averages for all the acquisition trials for each animal were calculated. Comparisons within- and between-treatment groups were conducted using repeated measures ANOVA and post hoc Tukey test. Data was expressed as mean ± SEM. Statistical significance was set at a P value of less than 0.05.

2.4. Ethical approval

The experimental procedures were approved by the Research and Research Ethics Committee, Arabian Gulf University, Bahrain.

3. Results

3.1. Effects of dietary restriction on body weight

No body weight differences were recorded between the control animals (free access to food) and 2 h DR group. However, the male animals in experiment two that were subjected to 40% DR for 2 weeks showed 20% reduction of body weight ($P < 0.01$) compared to their control group (Fig. 1A). The female body weight after 40% DR was not different (Fig. 1B).

3.2. Effects of 2 h of dietary restriction on performance in the water maze

Acute food restriction in female rats resulted in increased swimming speed but also into deterioration in the performance in the water maze test for learning and memory. The swimming speed in the maze was higher when compared to controls during days 1–2 ($P < 0.01$) and 3 ($P < 0.05$) of the water maze trials (Fig. 2B); however the DR females also swam significantly longer distance on days 2 and 3 ($P < 0.01$) (Fig. 3B) and took longer (so called 'escape latency') to reach the platform on days 1–3 ($P < 0.01$) (Fig. 4B) compared to controls.

However, while the 2 h food restriction did not affect the swimming speed of the male rats in the maze, it enhanced their performance in the learning and memory tests.