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Impairment of emotional behavior and spatial learning in adult Wistar rats by ferrous sulfate

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ABSTRACT

The aim of this study was to investigate the effects of $FeSO_4$ on the behavior of adult Wistar rats. Rats were treated with moderate doses of iron (1.5 or 3.0 mg/kg) for 5 consecutive days, and the effects of iron supplementation on emotional behavior were studied. One group of rats was tested in elevated plus-maze and in open field, and other group was tested for learning abilities in water maze and for motor skills in rotarod task. Iron level in the brain was measured in the frontal cortex, cerebellum, basal ganglia and hippocampus. The effects of the iron treatment (in particular, a dose of 3.0 mg/kg) on emotional behavior in the elevated plus maze and in the open field were significant. The effects of iron on spatial learning were less pronounced, but significant impairments due to the treatment were observed during the probe test. Motor skills and procedural learning in the rotarod task were not significantly affected by the treatment. These behavioral impairments were associated with significant iron accumulations in the hippocampus and basal ganglia of rats treated with 3.0 mg/kg iron and are discussed in terms of possible neuronal impairments of these structures. Thus, $FeSO_4$ administration at 3.0 mg/kg for 5 consecutive days in adult rats overcomes the mechanisms that shield the brain from iron intoxication and leads to behavioral impairments, in particular with respect to emotional behavior.

1. Introduction

Iron is a metallic element that ranks second in abundance among metals and fourth among elements. Iron is also the most abundant metal found in human body. It is essential for all living organisms as it is involved in fundamental processes such as transport and exchange of oxygen, enzyme action, and DNA, RNA and protein syntheses. Its brain metabolism [e.g. 12,40] depends on its balanced concentrations in various brain regions. Indeed, impairments of iron metabolism have been demonstrated in several pathologies such as Alzheimer's and Parkinson's disease, epilepsy, stroke and amyotrophic lateral sclerosis [9,11,14–16,25,33,34,57,59].

The physiological and psychological effects of iron metabolism impairments have been studied under the conditions of iron deficiency in human adults [5,63] and children [37], and in animal models [3,10]. In animal models, iron deficiency has been reported to affect sensorimotor development [62] or emotional processes such as startle response [8,61], which were found to be associated with modifications of homeostatic mechanisms [56]. Thus, iron supplementation studies are useful in understanding pathologies such as anemia or developmental deficiency.

However, caution should be exercised while conducting these studies and the effects of iron supplementation studied more extensively since iron overload has some adverse effects in human and animal models [27,64]. Iron transport to the brain is controlled by transferrin receptors present at the blood–brain barrier [30], and iron homeostasis is regulated by cerebrospinal fluid bulk flow [7]. Rate of brain iron uptake is high during postnatal period or aging, but is low in adult brain [19,51,58]. Thus, iron overload during these periods causes physiological and behavioral impairments (*see infra*). The possibility of free-radical formation and oxidative stress induced by iron accumulation in the brain is higher not only in undeveloped and aged individuals but also in adults with iron supplementation. From a neurobiological point of view, accumulation of iron in the brain may be cytotoxic, leading to behavioral disruptions [56]. Iron infusions into the substantia nigra can induce monoamine variations (particularly in the dopaminergic system) and behavioral impairments symptomatic of Parkinson's disease [6,54,55]. In addition to these motor impairments, learning abilities were found to be disrupted during postnatal development in mice [23]. However, there are only a few studies that have investigated the effects of iron overload on emotional processes.

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Iron supplementation in anemic women has been reported to affect emotional processes such as anxiety or depression [5], but there are only few studies on animals exposed to iron overload at adult age.

In the present study, to examine the effects of iron on emotional behavior of adult rats, Wistar rats were injected with ferrous sulfate (0, 1.5 or 3 mg/kg) for five consecutive days and then tested in the elevated plus maze and in an open fields. Learning abilities and motor performances were measured in separate groups of animals. Behavioral effects and neurobiological effects were studied by measuring the brain iron levels in the frontal cortex, basal ganglia and hippocampus.

2. Materials and methods

2.1. Animals

Male Wistar rats (SIPHAT, Tunisia), weighing 150–170 g at the beginning of the experiment were randomly assigned to a control or an iron-treated condition. Three groups of rats (control, iron dose 1 and iron dose 2) were used as to evaluate anxiety behaviours. Three other groups of rats similarly treated were used as to evaluate learning abilities and motor skills ($n=10$ in each group). Animals were housed in groups of 5/6 in cages at 25 °C, under a 12:12 light/dark cycle (lights on at 07:00), with free access to food and water. Animals were cared for in compliance with the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes. The experimental protocols were approved by the Faculty Ethics Committee (Faculté des Sciences de Bizerte, Tunisia).

2.2. Treatment

Animals received on five consecutive days a single daily IP injection with either a dose of 9‰ sodium chloride (control group), a 1.5 mg/kg (Iron-1.5 group) or a 3 mg/kg (Iron-3.0 group) of ferrous sulfate ($FeSO_4$) that was dissolved in sodium chloride (9‰). The doses were chosen on the basis of previous works that demonstrated effects of iron overload in rodent neonatal pups or adults [13,20,21,23]. As one study demonstrated some effects with a dose as low as 3.7 mg/kg for 3 days in neonatal mice, we choose the 3.0 mg/kg dose for 5 days along with an even more moderate dose of 1.5 mg/kg [22].

2.3. Behavioral testing

2.3.1. Emotional behavior

Behavioral experiments began the day following the five days' treatment and were performed in a sound-proofed chamber between 08:00 am and 01:00 pm. Animals were first tested in the elevated plus maze and thereafter were submitted to a daily session of open field on the three consecutive days. All tests were video recorded for a latter analysis with Etholog 2.25 [43]. The elevated plus maze and the open field testing took place in rooms that were lit from above with two 36-W fluorescent lamps. The rats were tested with a 30 lx ambient light intensity.

2.3.1.1. Elevated plus maze. The elevated plus maze test was used accordingly to previously published methodologies [24,45,52]. The maze was made of clear painted wood. The arms were 50 cm long and 10 cm width and the apparatus was elevated at a height of 60 cm. The closed arms were surrounded by a 50 cm wall while open arms had 0.5 cm edges in order to maximize open arms entries [60]. The test was 5 min long and began with the placement of a rat in the centre of the maze, with its head facing an open arm. The time spent in the different parts of the maze (i.e. open arms, closed arms and central part) was recorded along with the numbers of entries into closed and open arms. [46,49]. In addition, total activity into the maze was evaluated via the total number of arm and central part entries. Since total activity was found to be different among groups, a ratio for open arm entries and open arm time was also calculated [36,45]. Finally, number of rears, SAPs (stretched attend postures) and head scans (the animal is exploring an open arm from the central part with its head) were measured.

The maze was cleaned with a 10% alcohol solution between each animal.

2.3.1.2. Open field. Control and iron-treated rats were tested in the open field on three daily sessions of 5 min. The apparatus consisted of yellow plastic circular area (100 cm in diameter) surrounded by a 50 cm wall. The apparatus was divided by black lines into one central and six peripheral parts of equal surface and three identical objects (white glass incubation tray) were placed in the field. The experiment took place in the same room as the plus maze experiment and, therefore, under very similar conditions (intensity of light). At the beginning of the test, the rat was placed in a peripheral part of the open field. Number of entries into the central part of the field were measured and considered as an approach–avoidance measure and a reliable index of anxiety since it responds to anxiolytic agents [48] and it is sensitive to stress-induced anxiety states [17,29,44]. The following other behavioral components were measured: locomotion in the peripheral part (i.e. number of peripheral quadrants crossings), rearing (standing upright on the hind legs), frequency of contact with an object and total number of faecal boli (defecation). Total locomotion was calculated from central and peripheral locomotion. The open-field apparatus was also wiped out using a 10% alcohol solution before the next animal was introduced, as to preclude the possible cuing effects of odors left by previous subjects.

2.3.2. Learning abilities and motor skills

Rats from this protocol were tested during the course of 15 days after the termination of the 5 days of iron treatment. Performances in the Morris water maze were evaluated throughout different procedures in order to evaluate: initial learning (days 1–4) and probe test (day 4), retention (day 11) and motivational/visuo-spatial abilities via a visible platform test (day 11). Acquisition of the rotorod task was evaluated on days 12–15 and motor skills (dynamic equilibrium and muscular strength) were assessed by the stationary beam and suspended string tests on day 15.

2.3.2.1. Morris water maze. The water maze protocol was based on the work by Morris et al. [41] and on previous publication from our team [31,32]. The maze was made of opaque Plexiglas, measuring 90 cm in diameter, with walls 30 cm in height and water level (22 °C) maintained at 15 cm. The escape platform (diameter: 8 cm) was covered with a grid in order to provide firm gripping. Small black and white plastic beads covered the water surface in order to hide the escape platform from the animal's view. The maze was placed in a room with various extra maze visual cues such as light fixtures and black and white geometric figures fixed on the walls outside the maze. On days 1–4 of this task, acquisition of spatial learning in the Morris water maze was assessed. The rats were placed next to and facing the wall successively in the north (N), east (E), south (S), and west (W) positions in a semi-random order during four trials per day with an intertrial interval of 10–15 min. The escape platform was hidden 1 cm beneath water level in the middle of the NW quadrant. The pool was virtually divided into four quadrants. The number of quadrant entries and the escape latencies were measured. Whenever a rat failed to find the platform within the maximally allowed time of 60 s, it was manually placed on it for 5 s. A probe test was conducted on day 4, 20 min after the final trial of acquisition. The rats were placed in the centre of the pool and the time spent in the training quadrant was measured (entire body criterion) in a single trial of 60 s. Seven days after the final day of acquisition, a four trials retention test was given under the same experimental conditions as on days 1–4. A visible platform test was conducted 2 h after the retention test. The escape