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Emotional responses and memory performance of middle-aged CD1 mice in a 3D maze: Effects of low infrared light

S. Michalikova [a], A. Ennaceur [a,*], R. van Rensburg [b], P.L. Chazot [b]

[a] University of Sunderland, Sunderland Pharmacy School, Wharncliffe Street, Sunderland SR1 3SD, UK
[b] University of Durham, School of Biological and Biomedical Sciences, Durham, UK

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Abstract

Non-thermal near infra-red (IR) has been shown to have many beneficial photobiological effects on a range of cell types, including neurons. In the present study, a pretreatment with a daily 6 min exposure to IR1072 for 10 days yielded a number of significant behavioral effects on middle-aged female CD-1 mice (12-months) tested in a 3D-maze. Middle-aged mice show significant deficits in a working memory test and IR treatment reversed this deficit. Interestingly, the IR treated middle-aged group despite making less memory errors than sham middle-aged group spent longer time in different parts of the maze than both the young group (3-months) and sham-middle-aged group (12-months). Young mice appeared more anxious than middle-aged mice in the first sessions of the test. Exposure to IR appeared to have no significant effects upon exploratory activity or anxiety responses. However, it elicited significant effects on working memory, with the IR middle-aged mice being more considerate in their decision making, which results in an overall improved cognitive performance which is comparable to that of young CD-1 mice. The present study describes a novel method for assessing emotional responses and memory performance in a 3D spatial navigation task and demonstrates the validity of our new all-in-one test and its sensitivity to ageing and non-invasive beneficial IR treatment.

Keywords: Aging; Ageing; Amnesia; Amyloid; APP; Alzheimer; Hippocampus; Rats

1. Introduction

Sunlight is the most important and universal source of non-ionising radiation essential for life on Earth. Non-thermal near infra-red (IR) (700–2000 nm) light has high penetrability of biological tissues, with low degrees of light scatter, and has been shown to have a wide range of therapeutic benefits, for example, in the treatment of musculoskeletal disorders (Ceylan, Hizmetli, & Silig, 2003), healing indolent wounds (Karu, 2003; Vinck, Cagnie, Cambier, & Cornelissen, 2001; Whelan et al., 2003) and reducing pain (Honmura, Ishii, Yanase, Obata, & Haruki, 1993). IR improved recovery from ischemic injury of the heart (Kawasuji et al., 2000), prevents the development of oral mucositis in pediatric bone marrow transplant patients (Whelan et al., 2002), and attenuates the developmental toxicity of dioxin in chicken embryos (Yeager et al., 2005). It can also generate significant biological effects including cellular proliferation, collagen synthesis, and the release of growth factors from cells (Conlan, Rapley, & Cobb, 1996; Karu, 1999; Leung, Lo, Siu, & So, 2002).

A growing number of reports have shown effects of IR on the nervous system. Low power laser irradiation has been shown also to improve retinal function and attenuate degeneration of injured optic nerves induced by methanol-derived formic acid in rat models (Eells et al., 2003) and has also been shown to alter gene expression in retinal neurons (Eells et al., 2004) and olfactory ensheathing cells *in vitro* (Byrnes, Wu, Waynant, Ilev, & Anders, 2005). Furthermore, effects upon neurotransmission has been described, modulating cerebral blood flow and changes in energy metabolism, including selective rises in ATP levels (Moc-

* Corresponding author.
E-mail address: abdel_ennaceur@yahoo.com (A. Ennaceur).

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hizuki-Oda et al., 2002; Schaffer et al., 2000), and activation of mitochondrial respiratory chain components resulting in initiation of a signaling cascade that promotes cellular proliferation and cytoprotection (Grossman, Schneid, Reuveni, Halevy, & Lubart, 1998; Karu, 1999; Wong-Riley, Bai, Buchmann, & Whelan, 2001).

CD1 mice have been shown to display tumors, skin lesions, and abscesses (Chandra & Frith, 1992; Homburger et al., 1975; Maita et al., 1988; Son, 2003; Son & Gopinath, 2004). Notably, a high incidence of systemic amyloidosis in CD-1 mice has been reported (Chandra & Frith, 1994; Engelhardt, Gries, & Long, 1993; Frith & Chandra, 1991; Gruys, Tooten, & Kuijpers, 1996; Homburger et al., 1975; Lavie & Weinreb, 1996; Maita et al., 1988; Rao, Birnbaum, Collins, Tennant, & Skow, 1988). It was observed in 50% of CD-1 mice of both sexes, beginning at 8 months in males and 12 months in females (Frith & Chandra, 1991; Homburger et al., 1975). It was found to be higher than in other strains of mice and as one of the major factors contributing to death in aging CD-1 mice (Lavie & Weinreb, 1996; Majeed, 1993). Numerous studies suggest that more than 50% of CD1 mice from both sex die within 2 years. The survival rate was higher in females than in males (Homburger et al., 1975; Maita et al., 1988; Navarro, Sanchez-del-Pino, Gomez, Peralta, & Boveris, 2002; Son, 2003). This strain of mice may also develop cognitive deficits earlier in life, which may have an immunological basis. A recent study from our laboratory demonstrates that middle-aged CD1 male and female mice (12 months) made significantly more errors than young CD1 female mice (4 months) in a 3D maze (Ennaceur, Michalikova, Van Rensburg, & Chazot, 2007). CD1 mice might be useful as a model of age-related disorders, and for screening drug and therapeutic manipulations.

In the present study, we examined the effects of IR1072 on the behavior of CD1 middle-aged mice on emotional responses to novelty and open-space and on the acquisition of a working memory test in a 3D maze. IR 1072 nm light was chosen for this study as it represents a peak in the transmission spectrum of the water molecule (Bradford, Barlow, & Chazot, 2005). Two recent clinical studies have shown wavelength-specific beneficial effects of IR upon cold sores (Dougal & Kelly, 2001; Hargate, 2006). Our *in vitro* evidence confirmed the wavelength specificity, with IR1072 showing most benefit for protection against human lymphocyte cytotoxicity (Bradford et al., 2005).

The 3D maze is a modification of an eight-arm radial maze. In this maze, animals need to cross a bridge to reach an arm. Animals are introduced to the maze without prior habituation. They can be assessed for their emotional responses to novelty and open spaces in a single or a few sessions without food deprivation (Ennaceur, Michalikova, van Rensburg, & Chazot, 2006). They can also be food-deprived and tested in a working memory paradigm in 16 sessions and the first sessions are assessed for emotional responses. In our view, the first sessions of exposure to the test are likely to induce anxiety in animals that would subside over time after repeated exposures while learning takes place. Therefore, it is possible to assess emotional responses and working memory performance within the same experimental settings and testing conditions. The 3D maze was developed to assess spatial navigation from different view perspectives (Mostafa, Michalikova, & Ennaceur, 2002). In the present report we describe the behavior of mice in the raised arm configuration only (Ennaceur et al., 2006). A detailed analysis of the behavior is provided by examining a variety of spatio-temporal measures of animals' responses induced by exposure to the maze such as frequencies, latencies and duration of visits to bridges and arms (Ennaceur et al., 2006).

2. Methods

2.1. Animals

Thirty female mice from CD1 mice strains were obtained from our in-house colony. They were constituted of a group of 3-month old mice ($n = 10$) and a group of 12 months old mice ($n = 20$). The colony room was held under a 12 h light/12 h dark cycle (light 0700–1900 h at 180 Lux) and at 23 ± 1 °C. In order to avoid unequal light exposure, the upper shelf was occupied with empty plastic cages filled with sawdust. Mice were housed in a group of five mice per cage. Individual mice could be identified by their cage number and their ear tags. All mice had *ad libitum* access to food and water. During their stay in respective housing conditions, they were removed twice a week from their cages for cleaning the cages and renewing their food and water supply. Animal treatment and husbandry were in accordance with approved use of animals in scientific procedures regulated by the Animals (Scientific Procedures) Act 1986, UK.

2.2. Treatment

CD-1 middle-aged female mice (12 months old, $n = 10$) were exposed to IR 1072 in a purpose built box (full body exposure) for 6 min session a day over a 10 day period. A parallel set of CD-1 female mice of the same age ($n = 10$) were exposed to the same box without IR 1072 exposure. Young CD1 female mice (3-months old, $n = 10$) that were not exposed to IR treatment are included in this experiment to examine whether IR treatment would align the performance of middle-aged CD1 mice to that of untreated CD1 young control. All mice were introduced to the 3D maze in which they were trained to retrieve one food pellet from the end of the arms. Only 1 food pellet was available on each arm. One middle-aged died before the start of the experiment and two young mice died a few sessions after the start of the experiment.

2.3. Apparatus and recording

The maze is made from grey PVC (5 mm thick). It consists of eight arms radiating from a central platform (Fig. 1). Each arm (51 × 11.2 cm) is made from two segments, extended from an octagonal shaped central hub (30 cm in diameter) and can be manipulated independently. The first segment of an arm (15.2 × 11.2 cm) directly attached to the central platform can be tilted (max. 90°) and constitutes a bridge that allow access to the second segment (35 × 11.2 cm) of the arm which is presented horizontally either at the same, below or above the level of a central platform. The bridge forms a slope which is inclined by about 60°. In order to change the configurations of the maze, the central platform is raised or lowered by about 15 cm. The floor of the bridges is covered with wire mesh. Each entry to a bridge is narrowed either on the left or on the right side by short wire mesh wall (width 5 cm × height 5 cm). The narrowing of the entries to and exits from the bridges are designed to prevent mice

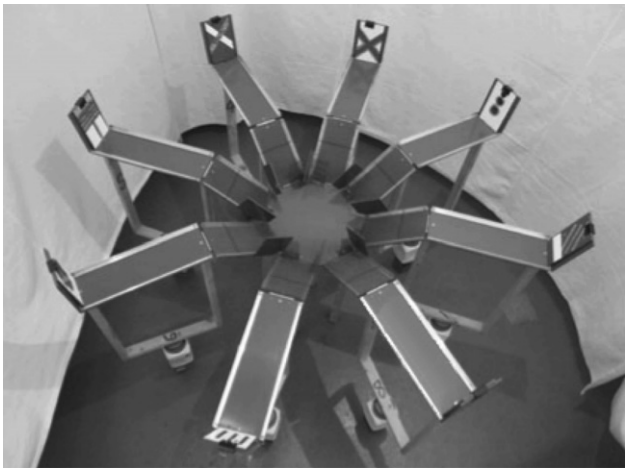

Fig. 1. 3D maze with a raised arm configuration. Note the narrowing of the entries to and exits from the bridges to prevent mice relying entirely on sequential arm choices.

relying entirely on sequential arm choices. The end of each arm is extended with panels of identical size (20.2 × 11.2 cm). These panels are used for holding intra-maze cues made of distinctive pattern drawings designed on plastic adhesive material and attached to a PVC board (18 × 11.2 cm). Sidewalls, about 1 cm high, extended the length of each arm. The maze is totally surrounded with a heavy beige-light colored curtain and it is placed in a sound attenuated room with a masking noise. The ambient light at the surface of the central platform is 180 Lux.

During the test, mice were observed on a screen monitor connected to a video camera suspended 160 cm above the test arena. Using an in-house computer program we are able to record in sequential order the start and end of each entry to the different parts of the maze. This record provides us with a variety of measures such as frequency, latency, duration, and the sequence order of each visit to the central platform, bridges and arms of the maze (see measurements below).

2.4. Measurements and statistical analysis

A session lasts until 8 choices are made or 10 min elapsed. An entry to an arm or a bridge is recorded whenever a mouse crosses with all four paws the line that delimits these areas. Several measurements were considered: (1) Number of bridges and arm entries: An entry to a bridge is recorded only once if a mouse enters a bridge and continues to an arm. A return from an arm to a bridge within the same visit is not recorded; (2) Number of bridge entries before first arm visit: The total number of bridge entries is recorded for those animals that did not visit any arm; (3) Number of arm entries before first repeated arm visit: An arm that is visited only once during a session is considered as a unique entry to that arm. A mouse that did not visit any arm will be recorded as having made 0 unique visits before first repeat. A mouse that have made less than 8 choices with no repeat will be recorded as having made his first repeated choice after his last arm visit. (4) Number of repeated visits to arms: This measure refers to incorrect choices or errors. Each entry to an arm is considered as one choice. An animal can make up to 8 visits to the same arm. The first visit is considered a unique visit or a correct choice and subsequent visits are considered repeated choice or incorrect choices. In the first few sessions a mouse may visit a fewer arms or may not visit any arm. In this case, a mouse that did not visit any arm will be receiving a penalty of 8 repeated visits. For a mouse that have made less than 8 choices, the number of the remaining arm choices is added to their actual number of repeated arm visits. For example, a mouse that has made 5 choices and 2 repeat visits to arms will be recorded having made 3 unique visits and 5 repeated visited (the actual 2 repeats + the remaining arm choices 3). A mouse that has made 5 choices and 0 repeat visit to arms will be recorded having made 5 unique visits and 3 repeated visited. (5) Number of sessions and unique arm visits to criterion: a criterion is set to a total of five repeated choices maximum obtained in 5 consecutive sessions. (6) Latency of first entry to a bridge: The time spent by a mouse in the central platform before it enters for the first time in one of the bridges with all four paws. A mouse that did not enter a bridge receives the highest score which is 10 min. (7) Latency of first entry to an arm: The time spent by a mouse in the central platform and bridges before it enters for the first time in an arm. A mouse that did not enter any arm receives the highest score which is 10 min. (8) Latency of first repeated visit to an arm: The time spent by a mouse in a session before it makes a first repeat visit to an arm. A mouse that did not enter any arm receives the lowest score which is 0 min. (9) Total latencies between exits from and entries to arms: The time is recorded when a mouse exits from an arm with all four paws until its next re-entry to an arm (the same or another arm). A mouse that did not enter an arm receives the highest score which is 10 min. (10) Total duration of centre re-entries: The time is recorded from the entry of a mouse with all four paws in the central platform until it exits to a bridge. (11) Total duration of bridge entries: The time is recorded from the entry of a mouse with all four paws in a bridge until it exits to the central platform or to an arm. If a mouse enters a bridge and continues to an arm, then the time on an arm is subtracted from the time spent on a bridge. (12) Total duration of arm entries: The time is recorded from the entry of a mouse with all four paws in an arm until it exits to the bridge. (13) Total duration of unique visits to arms: The time spent by a mouse on each first visit to an arm in a session. (14) Session duration: the time required to perform 8 choices.

Differences among group means for each of the above measurements were tested for significance with one way ANOVA repeated measures. This was followed up with Newman–Keuls post-hoc comparisons. Statistics were calculated using the statistical package Statistica for Windows (version 5.5). Results were considered significant when $p \leqslant .05$. When $p \leqslant .10$, the value was reported and rounded up to the nearest value.

3. Results

3.1. Anxiety responses to novelty and open space

As our mice were introduced to the maze without prior habituation, we examined some parameters of the test that would reflect the emotional responses of mice to novelty and open-spaces. Emotional animals are unlikely to adventure beyond the bridges and cross to an arm of the maze; they would take longer time and make numerous entries to bridges before any visit to an arm. Examination of the first sessions revealed that the latency of first entry to an arm (Fig. 2b) was significantly high in the first sessions block in young mice compared to sham middle-aged ($p < .02$) and significantly low in the second sessions block in young mice compared to IR-treated mice ($p < .01$). The number of arm visits (Fig. 2c) was significantly low in the first session compared to the subsequent 3 sessions ($p < .0001$) and in the second session compared to the third ($p < .01$) and fourth ($p < .02$) sessions. In the first session, young mice entered significantly less number of arms than sham-middle-aged ($p < .004$) and IR-treated mice ($p < .02$). In the third session young mice entered more arms than IR treated mice but this was not significant ($p < .07$). Young mice entered more bridges before first arm visit than sham-middle-aged ($p = .01$) and IR-treated ($p = .03$) mice, and the number of entries to bridges was significantly high in the first block of 4 sessions than in the subsequent 3 blocks ($p < .0001$) (Fig. 2d).

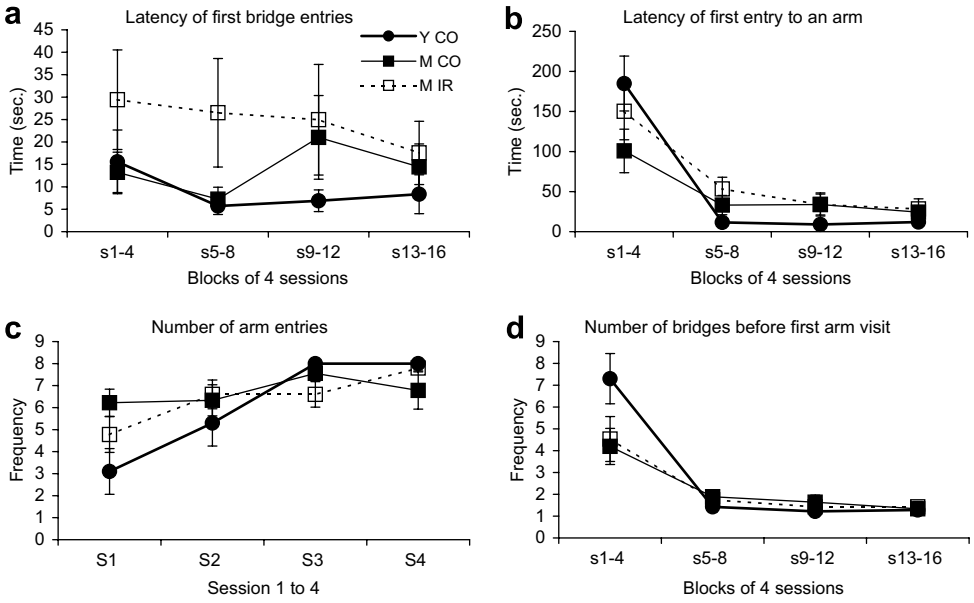

Fig. 2. Each block represents an average of four sessions except for (c). (a) IR-treated mice compared to sham-middle-aged and young mice in the first session block ($p < .09$ and $p < .08$, respectively), and in the second session block ($p < .02$ and $p < .04$, respectively). (b) Young mice compared to sham middle-aged ($p < .02$) in the first sessions' block and compared to IR-treated mice ($p < .01$) in the second sessions' block. (c) Young mice compared to sham-middle-aged ($p < .004$) and IR-treated mice ($p < .02$) in session 1. Young compared to IR-treated mice ($p < .07$) in session 3. (d) Young mice compared to sham and IR-treated middle-aged mice ($p < .0004$) in session block 1.

3.2. Learning and memory performance

Young mice committed their first repeated arm visit after fewer arm entries than sham-middle-aged ($p < .02$) and IR treated middle-aged mice ($p < .02$) in the first session block (Fig. 3a). This is reversed in the second and final session blocks with both young and IR-treated mice making significantly more arm entries before a first arm repeat than sham-middle-aged mice ($p < .02$ and $p < .05$, respectively). Young and IR treated middle-aged mice made also less arm repeats than sham middle-aged mice in the second ($p < .04$) and the final ($p < .05$) block of 4 sessions (Fig. 3b). The number of repeated visits to arms and the number of unique arm visits before first repeated entry to an arm measures suggest that middle-aged mice are impaired in the 3D maze compared to young mice, and this deficit is reversed by IR treatment. In addition, IR-treated mice took significantly longer time to commit a repeated visit compared to young in the second ($p < .002$) and third ($p < .008$) sessions' blocks (Fig. 3c). They took also longer time compared to sham-middle-aged in session blocks 2 ($p = .06$) and 3 ($p = .10$), although this did not reach significance. However, young mice took less time to commit a repeated visit compared to sham-middle-aged in the second session block but this again was not significant ($p < .07$).

IR-treated mice spent significantly longer time on the central platform than young ($p = .009$) and sham-middle-aged ($p < .02$) mice. This difference was evident in the second session block between young and IR treated middle-aged ($p < .03$) mice but not between young and sham-middle-aged ($p < .07$) mice (Fig. 4d). The time spent between re-entries to arms was significantly high in IR-treated mice compared to young mice ($p < .04$) in the first 3 session blocks and it was high but not significant in IR treated mice compared to sham-middle-aged ($p < .06$) in the first session block. It was also significantly high in sham middle-aged treated mice compared to young mice in the second ($p < .05$), but not in the third ($p = .07$) session block (Fig. 4a).

Young CD-1 mice spent less time on the arms compared to IR-treated mice ($p < .02$) but not compared to sham-middle-aged mice ($p = .09$). This difference was evident in session blocks 3 ($p = .02$) and 4 ($p < .05$); it was not significant in session block 2 ($p < .08$) (Fig. 4b). They also spent less time on unique arm visits compared to IR-treated mice ($p = .02$). This difference was evident in session blocks 3 ($p = .02$) and 4 ($p < .05$); it was not significant in session block 2 ($p < .09$) (Fig. 4c). In addition, young mice took less time to perform the test than IR middle-aged treated mice ($p < .004$) in the first 3 session blocks. They also took less time than sham-middle-aged mice in the second ($p < .03$) and third ($p < .04$) session blocks. IR middle-aged-treated mice took longer time to perform the test than sham-middle-aged in the first session block ($p < .09$) and than young mice in the last session block ($p < .08$) but this was not significant (Fig. 4d).

There were no differences between animals on the number of entries on the bridges and time spent on the bridges. However, IR treated mice took longer time to enter a bridge (Fig. 2a), this was significant compared to young mice ($p < .05$) but not compared to sham-middle-aged mice ($p = .10$).

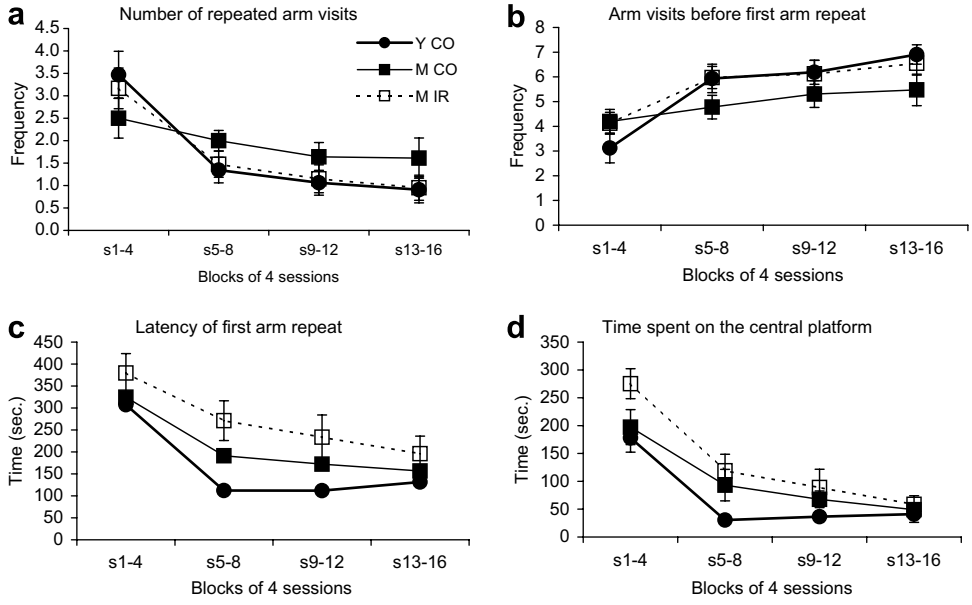

Fig. 3. Each block represents an average of four sessions. (a) Young and IR-treated middle-aged mice compared to sham-middle-aged mice in the second ($p < .04$) and the last ($p < .05$) session blocks. (b) Young mice compared to sham-middle-aged and IR-treated middle-aged mice ($p < .02$) in the first session block. Young and IR-treated compared to sham-middle-aged mice ($p < .02$ and $p < .05$, respectively) in the second and fourth session blocks. (c) IR-treated compared to young in the second ($p < .002$) and third ($p < .008$) sessions' blocks. IR-treated compared to sham-middle-aged in session block 2 ($p = .06$) and 3 ($p = .10$). Young compared to sham-middle-aged in the second session block ($p < .07$). (d) IR-treated mice compared to young ($p = .009$) and sham-middle-aged ($p < .02$) mice in the first session block. Young compared to IR-treated middle-aged ($p < .03$) and sham-middle-aged ($p < .07$) in the second session block.

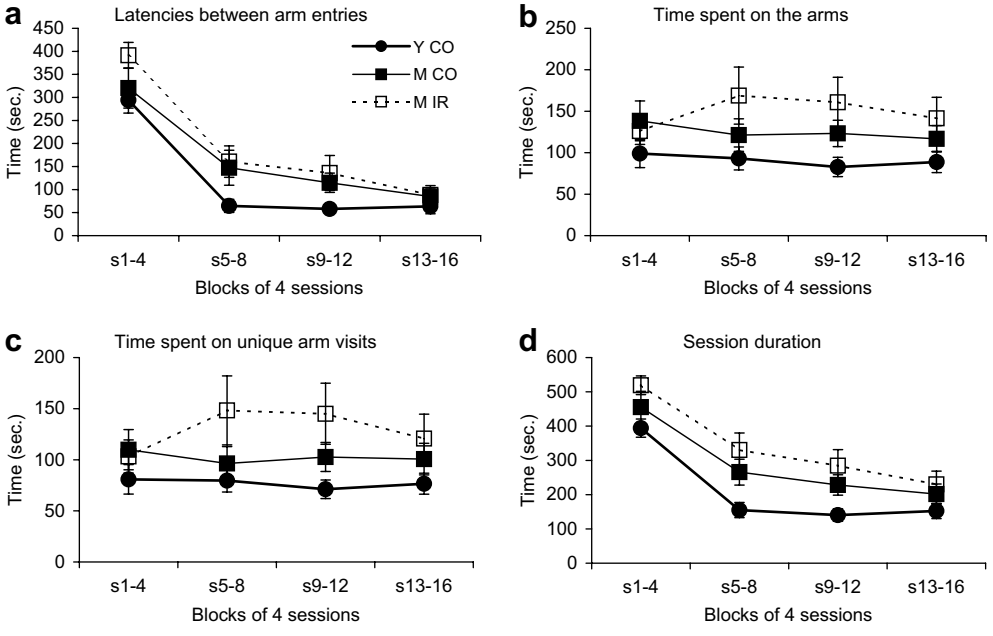

Fig. 4. Each block represents an average of four sessions. (a) IR treated mice compared to young mice ($p < .04$) in the first 3 session blocks and IR-treated mice compared to sham-middle-aged ($p < .06$) in the first session block. Sham middle-aged treated mice compared to young mice in the second ($p < .05$) and in the third ($p = .07$) session blocks. (b) Young mice compared to IR-treated mice in session block 2 ($p < .08$), session blocks 3 ($p = .02$) and session blocks 4 ($p < .05$). (c) Young control compared to IR-treated mice in session block 2 ($p < .09$), 3 ($p = .02$) and 4 ($p < .05$). (d) Young mice compared to IR-treated mice ($p < .004$) in the first 3 session blocks. Young compared to sham-middle-aged mice in the second ($p < .03$) and third ($p < .04$) session blocks. IR middle-aged-treated compared to sham-middle-aged in the first session block ($p < .09$) and compared to young mice in the last session block ($p < .08$).

4. Discussion

Non-thermal near infra-red (IR) (700–2000 nm) has been shown to have many beneficial photobiological effects on a range of cell types, including neurons. In the present study, a pretreatment with a daily 6 min exposure to IR1072 for 10 days yielded a number of significant behavioral effects on middle-aged female CD-1 mice. Young mice

committed their first repeated arm visit after fewer arm entries than both groups of CD-1 middle aged mice in the first sessions of the test. This is reversed in the subsequent sessions with young mice and middle-aged IR-treated mice making significantly more arm entries before a first arm repeat than sham-middle-aged mice. Furthermore, young and middle-aged IR treated middle-aged mice made less arm repeats than sham-middle-aged mice in the second ($p < .04$) and the final ($p < .05$) block of 4 sessions. Interestingly, the middle-aged IR treated group, despite making less errors than sham middle-aged group, spent longer time in different parts of the maze than both the young group and sham middle-age group. Exposure to IR1072 appeared to have no significant effects upon motor activity or emotional responses in the first sessions of the test. However, it elicited significant effects on the acquisition phase, with the middle-aged mice being more considerate in their decision-making which resulted in an overall improved cognitive performance which was comparable to that of young CD-1 mice. These results suggest a beneficial effect of IR1072 nm wavelength on the acquisition of working memory spatial navigation task in the 3 D maze. The mechanism by which this effect is induced is unknown, but may involve a number of possible mechanisms including improved energy metabolism, neurogenesis and/or neuroprotection (Grossman et al., 1998; Karu, 1999; Mochizuki-Oda et al., 2002; Wong-Riley et al., 2001).

CD-1 mice demonstrate a high incidence of spontaneous neoplastic and non-neoplastic lesions (Chandra & Frith, 1994; Engelhardt et al., 1993; Homburger et al., 1975; Maita et al., 1988) and record a high mortality rate at about 24 month of age (32.6% and 28.6% at 83 weeks of age and 66.4% and 63.3% at 109 weeks of age for male and females, respectively (Maita et al., 1988). Amyloidosis is considered one of the major factors that contribute to death in aging CD-1 mice (Lavie & Weinreb, 1996; Majeed, 1993). Amyloid deposits represent frequent histological findings in this strain of mice (Gruys et al., 1996) but their observation was limited to peripheral organs; there are no reports on histological brain tissues. It is possible that the premature deficit in the acquisition of a working memory task observed in this and a previous experiment in the 3D maze can be due to a high level of circulating amyloid or possibly to a natural occurrence of amyloidosis in the brain of CD-1 mice. This is currently investigated in our laboratory. In the present study, middle aged CD-1 mice are impaired in the acquisition of the memory test; they made a first arm repeat after fewer arm choices and made more repeated visits to arms than young CD-1 mice. Middle-aged CD-1 mice were as active as young CD-1 mice although taking longer time to perform the test. Middle-aged mice also made as many entries to bridges as young mice in the first sessions' block (average for Young $= 10.29 + 0.60$, Middle-aged $11.15 + 0.86$) and throughout the 16 sessions (average for Young $= 14.78 + 1.13$, Middle-aged $15.72 + 1.47$). However, they visited more arms than young mice on the first four sessions of the test (Fig. 2), indicating that young mice were more anxious than middle-aged mice.

There are only a few studies devoted to age-related memory performance in CD-1 mice. In one study (Navarro et al., 2002) 13 weeks old male and female CD-1 mice were tested every two weeks over 65 weeks in a T-maze spontaneous exploration task. Performance decreased with age in both male and female mice but females performed better and showed a greater exploratory activity than males. Exploration time was longer in young and old males than in young and adult females. In another recent study, adult (5 months) vs. middle-aged (13 months) male mice were examined in a water-maze (Francia et al., 2006). The authors report that middle-aged mice show a deficit in the acquisition of the test as they displayed longer latencies to find the platform location than adults, showed longer cumulative distance from both the acquisition quadrant and platform location area than adults, and tended to spend more time in the outer area. They also report no age differences in swimming path or swimming speed. Similar deficits were reported in C57BL/6Nia mice at age 10 months (Magnusson et al., 2003), 15 months (Verbitsky et al., 2004), 22 months (Forster et al., 1996) and 24–26 months (Frick, Burlingame, Arters, & Berger-Sweeney, 2000; Magnusson et al., 2003). A deficit in the water-maze is also observed in aged C57BL/6J mice (18–20 months old) (Benice, Rizk, Kohama, Pfankuch, & Raber, 2006; Vicens, Bernal, Carrasco, & Redolat, 1999; Vicens, Redolt, & Carrasco, 2002) but middle-aged (10–12 months old) mice were comparable to 3–4 months old mice (Benice et al., 2006). These results from the water maze are difficult to compare with the 3D maze or the standard radial maze which involve choices between various alternatives and are based on food reward and not an aversive reinforcer. In addition, in the standard radial-maze and the 3D maze memory performance is inferred from the type of choices made by animals during a session whereas in the water maze they are inferred from latencies, duration and distance which can be confounded with alterations in motor activity (see Ennaceur et al., in press).

Several studies have reported an increase of anxiety with age, as assessed by performance of mice in different behavioral anxiety tasks such as the elevated plus-maze, the open field test or the light/dark box (Acevedo, Ohtsu, Benice, Rizk-Jackson, & Raber, 2006; Benice et al., 2006; Boguszewski & Zagrodzka, 2002; Darwish, Koranyi, Nyakas, & Almeida, 2001; File, 1990; Francia et al., 2006; Frick et al., 2000; Frussa-Filho, Otoboni, Uema, & Sa-Rocha, 1991; Imhof, Coelho, Schmitt, Morato, & Carobrez, 1993; Lamberty & Gower, 1991; Miyagawa et al., 1998). In a recent study (Francia et al., 2006) middle-aged CD-1 mice were reported to be more anxious than adult mice. But this conclusion is not compatible with the data and the statement made by the authors of this study. A careful examination of this report, suggests that middle-aged CD-1 mice are not different from young mice in the plus-maze. In fact, middle-aged mice are less anxious than young mice in

the open-field as they approached the object in the centre of the field and spent longer time at the object. In our present study, middle aged CD-1 mice appear less (emotionally) affected by exposure to novelty and open spaces compared to young mice. They record a high number of entries to arms, a low number of entries to bridges before first arm visit and a short latency for first entry to an arm in the first sessions of the test. Young CD-1 mice appear more anxious than middle aged CD-1 mice, based on a behavioral task which does not provide animals with any protective space to escape to, analogous to human anxiety where people feel unable to escape or avoid the source of threat.

In human studies, cognitive functions have been reported to decline with age, these include episodic memory (Nilsson et al., 1997), working memory (Czaja, 1996), spatial ability and spatial memory (Light & Zelinski, 1983; Salthouse, 1982, 1991). Older subjects were found to require more time to solve a 3D spatial navigation task (Sjölinder, Höök, Nilsson, & Andersson, 2005) and to acquire spatial information in a novel environment compared to younger adults (Kirasic, 1991). The effect of IR may have been beneficial on the acquisition of a working memory task by increasing the duration of encoding the particularities of visited arms and/or increasing the time to make a choice decision. Indeed, aging has been reported to affect information processing speed in humans (Lachenmayr et al., 1994; McDowd & Shaw, 2000) and animals (Ison, Bowen, & del Cerro, 1998) which may account for the behavior of middle-aged CD-1 mice in our experiment. IR-treated middle aged mice seem to benefit from spending longer time on the central platform and on the arms before making the correct choice. The present effect of IR on middle aged mice raises the issue that an improvement of cognition in ageing does not need to match all aspects of behavior with that of young mice. Performing a cognitive task at a slower more considered pace can benefit performance in the aged subject.

Disclosure statement

A preliminary account of some of the present findings has been submitted in abstract to the Society for Neuroscience 2007.

None of the authors has any actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within 3 years of beginning the work submitted that could inappropriately influence (bias) our work. We also do not have contracts relating to this research through which ours or any other organization may stand to gain financially now or in the future. Our Institutions do not have a financial interest in this work.

Animals used in the study were handled in accordance with approved use of animals in scientific procedures regulated by the Animals (Scientific Procedures) Act 1986, UK.

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