Neurotoxic Effects of Repetitive Inhibition of Oxidative Phosphorylation in Young Adults Surfacing With Deficits of Spatial Learning in Old Age

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Little is known about whether events in youth impact performance in old age. We examined spatial navigation in young (4.5 months) and middle-aged (9 months) CD-1 mice in a complex maze after treatment with 3-nitropropionate (3-np; 20 mg/kg body weight; 9 injections intraperitoneally [i.p.] every other day). Young mice treated with 3-np were examined in a mirror version of this maze in old age (22 months) and with a nonreference memory task of an eight-arm radial maze. The performance of young mice was affected to a small degree by treatment with 3-np. However, the performance of middle-aged mice severely declined on 3-np treatment. Animals treated at a young age with 3-np showed learning deficits in old age for both the complex maze and the radial maze. We conclude that exposure to repetitive inhibition of oxidative phosphorylation in youth leads to impairment of spatial learning surfacing in old age.

Mazes have been used to study learning in rodents since the turn of the last century (1,2). Animal research, as well as the study of normal individuals and patients with brain lesions, have demonstrated the crucial roles of the hippocampus and the hippocampal formation for the cognitive representation of space and navigation (3–11). In addition, the parietal lobes and prefrontal areas have been shown to be active in complex navigational tasks (11–13). Age-related deficits of spatial memory and nonspatial tasks have been demonstrated repeatedly (14–20). Middle-aged rats with hippocampal lesions show impairment when completing spatial tasks but normal performance on nonspatial tasks, whereas both rats with hippocampal lesions and sham-operated old rats show impaired performance on both spatial and nonspatial tasks (21).

In recent years, neurodegenerative diseases have been linked to an impairment of mitochondrial energy metabolism (22,23). However, mild impairment of energy metabolism can actually be protective (24). Depending on the dosage and application interval, 3-nitropropionate (3-np), an inhibitor of mitochondrial complex II, can cause either neuroprotection or neurodegeneration in the hippocampus (25–27).

It has been established that prenatal and neonatal events may have an impact on the development of cognitive abilities and lead to cognitive impairment in adulthood (28). Recently, we showed that inhibition of maternal energy metabolism by treatment with 3-np prior to conception may become obvious in the adult offspring (29). It was the goal of the present study to assess long-term effects of repetitive inhibition of oxidative phosphorylation on spatial learning in young and middle-aged female CD-1 mice and to investigate whether treatment of young adult animals bears long-term effects on spatial learning in old age.

Materials and Methods

Animals

Four groups of female CD-1 mice (Charles River Laboratories, Sulzfeld, Germany) at three different ages (i.e., young [4.5 months], middle aged [9 months], and old [22 months]) were used. Five animals were housed in a cage under controlled conditions of temperature (22 ± 2°C) and humidity (55 ± 5%) and were kept on a 12-hour light/dark cycle, with water available ad libitum. Three days before testing, animals were deprived of food so that their body weight attained 90%–85% of initial weight before fasting. Food deprivation was maintained during the whole experimental period. In part, animals were treated with 3-np at 20 mg/kg body weight (see “Treatment Groups” and Table 1). Previously it was shown that short time intervals between injections of 3-np induce neuronal dysfunction (27,30). In these and other following studies animals received intraperitoneally 3-np at 20 mg/kg body weight every other day for a total of 18 days of treatment. Treating female animals with such a dosage regimen induces neuronal impairment even in their offspring (31). Following up on this study and considering the importance of studies on female rodents given the higher life expectancy in female humans, we used female animals for the present study.

Mazes

We used a complex maze with several crossings, t crossings, blind alleys, a goal zone described elsewhere (29,31,32), and an identical but mirror-inverted complex maze (hereafter referred to as the “m-complex maze”). The mazes were made of rigid gray polyvinyl chloride (PVC) and were surrounded by a gray curtain with geometric cues.
(triangle, circle, rhombus, and square) at four cardinal points (see Figure 1).

Furthermore, we used a nonspatial memory task of an eight-arm radial maze. To control for extraneous spatial cues, the maze was surrounded by a gray curtain.

**Treatment Groups**

An overview of treatment groups is shown in Table 1. Group G1 animals were sham-treated with nine intraperitoneal injections of NaCl and performed the complex maze task at the age of 4.5 months. Group G2 was treated with nine injections of 3-np at the age of 4.5 months. One day after the last injection, the mice performed the complex maze task and were trained for 8 (consecutive) days in the complex maze (G2A). At the age of 22 months, these animals performed the m-complex maze task as well as the radial maze task (G2B). Group G3 mice were sham-treated at the age of 9 months with nine injections of NaCl. One day after the last injection they performed the complex maze task (G3A) and were trained for 8 days in the complex maze. At the age of 22 months, this same group performed both the m-complex maze task and the radial maze task (G3B). Group G4 mice were treated with nine intraperitoneal injections of 3-np at the age of 9 months and performed the complex maze task.

**Behavioral Testing**

In young and middle-aged animals, behavioral testing took place 1 day after the last intraperitoneal injection with 3-np and NaCl, respectively. In the complex and m-complex maze, animals were rewarded with a food pellet in the goal zone. They were trained five times a day for 8 consecutive days and were given a maximum time limit of 300 seconds to find the exit, as previously reported (31).

In the eight-arm radial maze, a food pellet was offered in a bowl at the end of each arm. Animals were tested three times a day until they had visited all arms and consumed the food pellet in each arm, or for a maximum time of 300 seconds per trial. A mouse entering the same arm more than once was coded as an error.

Experiments were recorded by a tracking system (Multitrack; AccuScan Instruments, Inc., Columbus, OH).

**Statistical Analysis**

Data were analyzed by using a Kruskal–Wallis analysis of variance (ANOVA) with a Bonferroni t or Dunn’s test (when normality tests failed). Statistical significance was set at \( p < .05 \).

**RESULTS**

**Treatment of Young and Middle-Aged Animals**

The time for sham-treated 4.5-month-old animals (G1; \( n = 10 \)) to reach the goal zone declined from 108.8 ± 9.2 seconds (mean and standard error) on the first day to 32.0 ± 16.0 seconds on day 8 (\( p < .01 \) to first day to onset) and from 140.4 ± 15.0 seconds to 26.9 ± 4.4 seconds (\( p < .01 \) to first day) in 9-month-old sham-treated mice (G3A; \( n = 10 \)) (Figure 2). A day to day comparison of the groups shows significant differences from day 1 to day 3 (day 1: \( p < .05 \), day 2: \( p < .01 \), day 3: \( p < .02 \)) and on day 7 (\( p < .02 \)) favoring the younger animals.

In 4.5-month-old 3-np-treated animals (G2A; \( n = 10 \)), the time to reach the goal zone declined from 153.1 ± 15.5 seconds on day 1 to 36.8 ± 4.9 seconds on day 8 (\( p < .01 \) to first day). In contrast, 9-month-old 3-np-treated animals (G4; \( n = 9 \)) were strongly impaired by the treatment, and the time to reach the goal zone remained unchanged despite training.
Between these groups (G2A vs. G4), time to reach the goal zone differed significantly from the second to the eighth day \((p < .01)\). Compared to the age-matched 3-np-treated group (G2A), there was a significantly better running time of the sham-treated group (G1) on day 1 \((p < .02)\), 2 \((p < .01)\), 5 \((p < .05)\), and 7 \((p < .01)\).

Regarding the comparison of running times between 9-month-old 3-np-treated groups G4 versus G3A (same age, sham-treated) and G2A, there are highly significant differences on all days, except on the first day of training. From the second day forward, running times in group G4 were lower over the complete experimental period \((p < .01\) from second day to the last day).

**M-Complex Maze Versus Radial Maze in Old Mice**

Having reached an advanced age of 22 months, groups G3B and G2B had to perform the m-complex maze task and the eight-arm radial maze task as described above. They had already performed the original complex maze task at the age of 9 and 4.5 months, respectively. Attrition over time led to the reduction from 10 to 8 mice in the old age group G3B and a reduction from 10 to 6 mice in group G2B.

The average running time on the first and last days in the m-complex maze was 121.4 ± 19.0 seconds and 85.2 ± 38.7 seconds \((p = .42)\) in G2B and 115.5 ± 27.4 seconds and 65.8 ± 10.7 seconds \((p = .11)\) in G3B animals, respectively. Neither group improved their running time to reach the goal zone of the maze over the experimental period examined.

A comparison of running time over all 40 trials showed a significant difference between these groups. Both the time needed to escape from the m-complex maze (Figure 3A, \(p < .01\)) as well as length of the path taken (Figure 3B, \(p < .01\)) favored sham-treated G3B over 3-np-treated G2B animals. In contrast to the failure to learn the m-complex maze over the experimental period, both groups significantly improved on the radial maze task in terms of the average running time to visit all eight arms (Figure 4A) as well as a reduction in the number of errors made (Figure 4B).

A comparison of group G3B (sham-treated at middle age) and G2B (3-np-treated at young age) results in the radial maze showed better performance of group G3B. Regarding all trials of the radial maze task, animals in group G3B needed an average time of 181.7 ± 10.4 seconds, and animals in group G2B needed 237.7 ± 8.6 seconds to visit all eight arms and consume the food pellets \((p < .01;\) Figure 5A). Group G2B lingered in the middle area of the radial maze for longer period than did the other groups (G3B: 22.0 ± 1.8 seconds, G2B: 38.9 ± 2.3 seconds, \(p < .01;\) Figure 5B). Group G2B was also more prone to make errors (10.6 ± 1.1 vs 8.2 ± 0.8 for group G3B, mean and standard errors; \(p = .08\)).

**DISCUSSION**

**Young and Middle-Aged Animals in a Complex Maze**

In this study we investigated whether young and middle-aged CD-1 mice are impaired in a spatial version of a complex maze after repetitive treatment with 3-np. Similar to other reports (16,33), young sham-treated animals performed slightly better than 3-np-treated young animals. In contrast, 3-np-treated 9-month-old mice were severely affected by repetitive treatment with 3-np compared to age-matched sham-treated animals, and they did not improve their running time over the course of the experiment.

Comparing groups treated with 3-np in young and in middle age demonstrates that 3-np treatment is more toxic to middle-aged than young animals. This finding suggests...
that, in a functionally relevant fashion, the hippocampus is impaired by 3-np treatment in an age-dependent fashion. The finding also conforms with previous demonstrations that the integrity of hippocampal function is mandatory for mouse spatial orientation in water mazes (34–37) as well as in the complex maze used in this study (29,31,32). Regarding escape time, sham-treated 9-month-old mice and 3-np-treated 4.5-month-old mice performed equally, which suggests a similarity between repetitive inhibition of oxidative phosphorylation and natural aging in terms of spatial learning impairments (38–40).

Old Mice in the M-Complex and Eight-Arm Radial Mazes

Compared to the quick learning of the complex maze in animals at 4.5 and 9 months, learning was impaired in advanced age in the m-complex maze. No learning success was observed over the experimental period of 8 days. However, an overall comparison of time and path length to escape from the maze showed significantly worse performance for animals treated with 3-np at the age of 4.5 months. Thus, although only minor immediate impairment of maze learning occurs by repetitive inhibition of oxidative phosphorylation with 3-np treatment, a more severe learning deficit surfaces in older age later on. Thus, 3-np may advance aging via mitochondrial oxidative damage leading to neuronal degeneration (41–43). One argument supporting this hypothesis is that the protein microenvironment of mitochondrial enzymes is altered even in the offspring of animals repetitively treated with 3-np (27). Alternatively, or perhaps in addition, repetitive treatment with 3-np may alter the regulation of neurotrophins (31).

In our nonspatial version of an eight-arm radial maze, both groups improved performance significantly over the experimental period. However, animals repetitively treated with 3-np at the age of 4.5 months showed significantly poorer performance in this task. Nonspatial tasks are regarded as hippocampus-independent (8,44), thus impairment may result from impairment induced by 3-np in other brain regions such as the striatum (45–47).

Summary

We conclude that, although only minor learning deficits resulting from repetitive inhibition of oxidative phosphorylation with 3-np at a young age appear immediately, severe learning deficits in these mice surface in old age.

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