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**ORIGINAL ARTICLE**

# Beneficial effect of 6 weeks lasting handling of adult rats on spatial memory in experimental model of neurodegeneration

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**ABSTRACT**

Handling is a form of experience which can result in physiological changes depending on the period of postnatal age when performed. There is a lot of evidence about the positive effect of neonatal handling, but a lack dealing with handling of adult rats. Behavioral changes and memory deficits are present in dementia-like disorders. In the present work, we tested whether 6 weeks lasting handling of young adult rats could revert memory impairment induced by trimethyltin (TMT) (7.5 mg/kg, intraperitoneally). Testing rats in Morris water maze revealed significant effect of TMT as well significant effect of handling. We observed improvement of spatial memory also between healthy, non-degenerated rats as well as degenerated rats, represented by shorter latency onto the platform. In our paper, we report beneficial effect of handling on spatial memory that is in compliance with published works about beneficial effect of cognitive therapy and training in patients with early stage of Alzheimer´s disease and dementia.

**KEY WORDS:** trimethyltin; handling; memory; neurodegeneration

## Introduction

Life experiences and environmental factors can modulate animal physiology, behavior and memory (Del Arco *et al.,* 2007; Levine, 2001; Winterfeld, 1998). In 2003 Frick and Fernandez demonstrated beneficial effect of enrichment on spatial memory in adult female mice. Another intervention, handling, was broadly investigated in neonates. In adult and old rats, early postnatal handling has been shown to reduce anxiety, decrease levels of prolactin and corticosterone following stress, prevent age-related loss of hippocampal CA1 and CA3 pyramidal cells and deterioration of working memory and recognition memory (Boufleur *et al.,* 2013; Fenoglio *et al.,* 2005; Ferré *et al.,* 1995; Meaney *et al.,* 1988; Meerlo *et al.,* 1999; Stamatakis *et al.,* 2008; Valée *et al.,* 1999; Viau *et al.,* 1993). The above mentioned neonatal handling mediated memory improvement was accompanied by increase in dendritic length and dendritic spine density in the cortex (Richards *et al.,* 2012). Positive effect of early postnatal handling has been shown on prenatal stress, alcohol exposure and malnutrition in experimental rats (Raineki *et al.,* 2014). Much less is known about the consequence of handling of adult rats (Costa *et al.,* 2012; Deutsch-Feldman *et al.,* 2015).

In our study, we focused on the effect of late postnatal handling on spatial memory in experimental model of dementia induced by trimethyltin (TMT). TMT has a harming effect on the hippocampus that together with memory damage makes TMT a useful tool for studying perspective neuroprotective drugs (Geloso *et al.,* 2011). With the perspectivity of stannin, a key protein involved in TMT sensitivity, TMT is responsible for disruption of calcium homeostasis, production of reactive oxygen species, increment in levels of malondialdehyde, astrocyte activation and reactive gliosis, as well as the spread of neuronal apoptosis leading to functional deterioration of synaptic transmission (Corvino *et al.,* 2011; Gasparova *et al.,* 2012; Kaur *et al.,* 2013; Piacentini *et al.,* 2008; Thompson *et al.,* 1996). It is learning and cognition decline that is a typical hallmark of dementia (Gutiérrez-Rexach & Schatz, 2016; Popa-Wagner *et al.,* 2015). In patients with

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cognitive impairment tactile stimulation and cognitive exercise improved memory (Gates *et al.,* 2011; Scherder *et al.,* 1995).

Our hypothesis to test the effect of handling on neurodegeneration-induced rats was based on our previous observation when 28 days lasting oral administration of *aqua pro injectione* (vehiculum) to rats showed better achievement in Morris water maze test compared to our control groups of rats in experiments without such regular manipulation with animals. We wanted to verify whether handling could be beneficial not only for unaffected control rats but even for neurodegeneration induced rats. We decided to test rat spatial memory in Morris water maze, a behavioral test best suitable for testing spatial memory and learning (Vorhees & Williams, 2006) on the model of TMT-induced neurodegeneration with aspect of handling. In the current study we report beneficial effect of late handling on learning and spatial memory in intact and degenerated adult rats.

## Materials and methods

### Animals

Male Wistar rats (n=32), 8 weeks old, weighing 197±1.3 g at the beginning of the 8-week lasting experiment came from the breeding station Dobra Voda of the Institute of Experimental Pharmacology and Toxicology (Slovak Republic, reg. No. SK CH 24011). All procedures with animals were performed in compliance with the principles of laboratory animal care issued by EU Directive 2010/63/EU for animal experiments, proved and controlled by the State Veterinary and Food Administration of Slovakia and the Ethical Committee of the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences. The rats were housed in plastic cages (4 rats per cage) with pelletful food (KKZ-P/M) and water *ad libitum*.

### Experimental procedure

After 7-day adaptation, the rats were randomly allocated into 4 groups (n=8/group):

- Control handled group (C-H),
- Control non-handled group (C-NH),
- TMT handled group (TMT-H),
- TMT non-handled group (TMT-NH).

Control rats received a single i.p. dose of saline (with 0.1% DMSO). TMT rats were affected by a single i.p. dose of TMT chloride (7.5 mg/kg; dissolved in 0.1% DMSO; Sigma-Aldrich, USA) in the volume of 0.2 ml/100 g of rat weight. Four weeks prior to the TMT or vehicle administration, handling was performed on the C-H and TMT-H groups. Altogether handling lasted for 6 weeks (7 days per week), including 2 weeks after TMT/vehicle administration, and ended one week before the Morris water maze test. Handling consisted of tactile stimulation of rats, 10 minutes per cage/day. The rats were also allowed to enter the nearest table next to their cage during handling. Non-handled groups of rats were left undisturbed in the next room.

### Morris water maze

The testing in Morris water maze was performed with the hidden platform positioned on the same place during five subsequent days without probe trial on last day according to Gasparova *et al.* (2014). Briefly, the rats were tested in the range of the 21$^{st}$ till the 25$^{th}$ day (beginning at 7 a.m.) after TMT/or saline administration. The platform was hidden 0.5 cm under the water (23 °C) surface of the pool with a diameter of 180 cm. Each rat was placed in succesive steps into each of the 4 quadrants of the pool every day. The rat had 60 seconds to find the platform. After finding the platform, the animal was left on the platform for 20 seconds. After completion of the 4th quadrant, each animal was carefully dried and placed under a lamp. The data were collected by a camera located above the pool and connected to the computer with the ANY-maze videotracking software (Stoelting Europe, Ireland).

### Statistics

Values are means ± SEM. Data obtained from Morris water maze were analyzed using Statistica 7.0 software, Fisher´s least significant difference test. Body mass gain was analyzed using two factorial ANOVA for independent samples.

## Results

Six weeks lasting handling resulted in significant reduction of body weight gain in both handled groups compared to respective non-handled groups. Body weight gain of handled TMT-treated group did not differ from handled saline-treated group (Figure 1). The observed body weight gain reduction was the effect of handling in the mentioned corresponding groups ($F(1,28)=20.93$; $p<0.001$).

Percentual expression of body weight gain of individual groups calculated as a difference between the body weight on the 1st day of handling and after 6 weeks of handling, at the end of the experiment. C-NH group, C-H group, TMT-NH group, TMT-H group. Data are expressed as mean ± SEM, $^{*}p<0.05$.

In the Morris water maze test, data revealed significant positive effect of handling ($F(1,28)=10.05$; $p<0.01$) (Figure 2A) and negative effect of treatment with TMT ($F(1,28)=15.23$; $p<0.001$) (Figure 2B) on escape latency to the platform.

The effect of handling is expressed as cumulative mean value of escape latency to the platform from both non-handled (NH) *vs.* handled (H) groups (A) and the effect of treatment is expressed as cumulative mean value from both non-treated (C) *vs.* both TMT-treated groups (TMT) (B). Data are expressed as mean ± SEM, $^{**}p<0.01$, $^{***}p<0.001$.

The trend of improved spatial memory of handled groups is shown in Figure 3A. Note that the escape latency for TMT-H group is similar to the C-NH group. Total mean escape latency during 5 consecutive days revealed significant amelioration of spatial memory between C-NH and C-H and TMT-NH and TMT-H (Figure 3B).

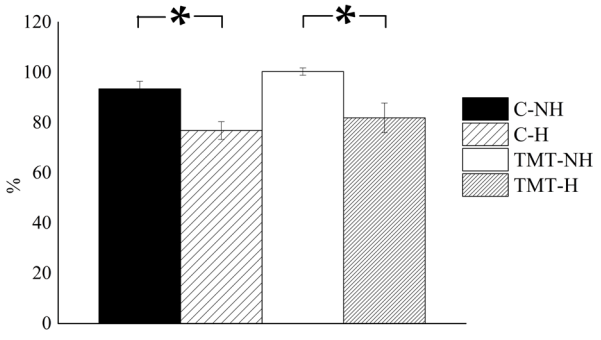


**Figure 1.** Effect of 6 weeks handling and treatment on body weight gain. Data are expressed as mean ± SEM, *$p$<0.05 compared to non-handled group.

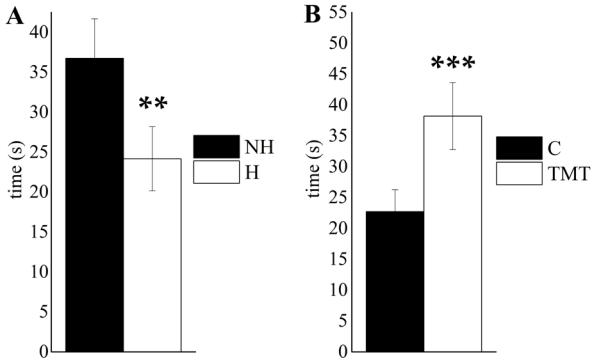


**Figure 2.** Effect of handling and treatment on latency to find platform in Morris water maze. Data are expressed as mean ± SEM, **$p$<0.01; ***$p$<0.001 compared to control.

## Discussion

Diagnostical guidelines characterize Alzheimer dementia (AD) as a continuum dividing it to preclinical stage without symptoms, mild cognitive impairment (MCI) and finally AD (Albert *et al.,* 2011). Garrett and Valle (2014) doubt about this division because of the difficulty in diagnosis, non-uniformity and the confession that memory is malleable, as shown by cognitive training programs in humans (Garrett & Valle, 2014; Kurz *et al.,* 2009). We rate TMT-induced neurodegeneration as a mild cognitive impairment with no neurofibrillary tangles and senile plaques.

In the rat, we show a modulatory effect of long-lasting postnatal handling on spatial memory and learning. Beyond the positive effect of handling on neurodegeneration-induced rats, we also report improvement of spatial memory in control, healthy rats compared to control non-handled group. Costa *et al.* (2012) showed anxiety reducing effect of handling with improved memory tested in elevated plus-maze test.

Beneficial effect of cognitive training was already observed in patients with MCI and AD (Belleville *et al.,* 2006) and here we demonstrate the beneficial effect of postnatal handling on memory of adult animals with induced neurodegeneration. Along the effect on memory, we also observed significant decrease in body weight of handled rats. On the contrary, Panagiotaropoulos *et al.* (2004) and Vallée *et al.* (1996) observed neonatal handling mediated increase in body weight of rats as well as increased food intake in adulthood. Another study using young adult rats revealed no effect of handling of young rats on body weight (Deutsch-Feldman *et al.,* 2015). The reason of discrepancies may origin from the period of development when handling was performed, as well as the duration of handling, that was in our experiment much longer than in the studies mentioned.

In conclusion, we report ameliorated spatial memory in healthy non-treated rats and rats with TMT-induced neurodegeneration when handled for 6 weeks. Because of lack of studies about the effect of handling in adulthood, the explanation of the precise mechanism is still shrouded in mystery. There is a suggestion that the effect of the procedure used in the present experiment and positive effect of cognitive training and tactile stimulation in patients with MCI and AD-like dementia can be based on similar mechanisms. Further investigation is necessary for complete description of this phenomenon.

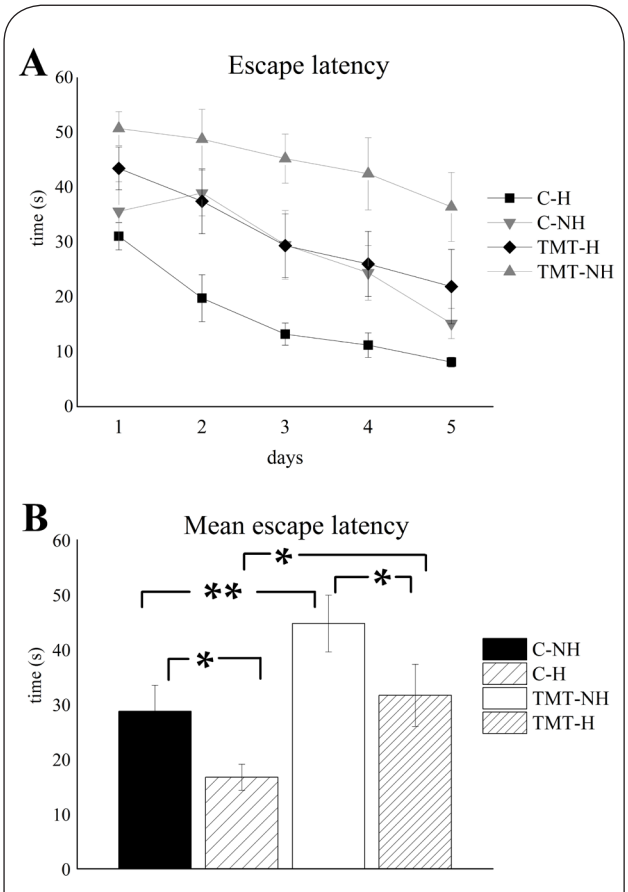


**Figure 3.** Escape latency to the platform. (A) Escape latency to the platform for individual days of testing and mean latency (B) calculated from 5 consecutive days for the given group. Data are expressed as mean ± SEM, *$p$<0.05, **$p$<0.01.

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