



*Original Contribution*

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## ATORVASTATIN AND ROSUVASTATIN IMPROVE LEARNING AND MEMORY IN RATS AFTER LONG-TERM TREATMENT

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### ABSTRACT

**PURPOSE:** The aim of the present study was to investigate the effect of 90-day treatment with Atorvastatin and Rosuvastatin on learning and memory processes of rats without brain damage.

**METHODS:** Male Wistar rats were treated orally for 90 days with atorvastatin and rosuvastatin in a dose 10 and 20 mg/kg body weight in parallel with vehicle-treated group. After this period learning ability and memory retention were evaluated using active avoidance test – automatic reflex conditioner (shuttle box) and two passive avoidance tests – step-through и step-down. The following behavioral reactions were investigated with the active avoidance test: conditioned responses (avoidance), unconditioned responses (escapes) and intertrial crossings. The passive avoidance tests were used to observe the latency of reaction.

**RESULTS:** In the active avoidance test groups receiving atorvastatin in a dose 10 and 20 mg/kg body weight and rosuvastatin in a dose 10 mg/kg body weight showed increased number of conditioned responses compared to the control group. In the step down passive avoidance test animals treated with atorvastatin in a dose 10 and 20 mg/kg body weight had increased latency of reaction during the learning session and in the short-term memory retention test compared to the control group.

**CONCLUSIONS:** Atorvastatin (10 and 20 mg/kg body weight) and rosuvastatin (10 mg/kg body weight) improve cognitive function in rats after 90-day treatment.

**Key words:** Statins; Neuroprotective effect; Active avoidance; Passive avoidance

### INTRODUCTION

In the past decade data has emerged for the neuroprotective effect of statins (1). Clinical trials have reported that statins treatment slow the development of cognitive decline in Alzheimer patients (2) and reduce the risk of dementia in the elderly (3, 4). Experimental studies have shown their beneficial effects on cognitive function in animal models of vascular dementia, amnesia and after traumatic brain injury (1, 5). On the other hand statin administration is associated with cognitive impairment in patients, and was reduced upon withdrawal of the drug (6, 7).

The aim of the present study was to investigate the effect of 90-day treatment with atorvastatin and rosuvastatin on the processes of learning and memory in rats without brain damage.

### MATERIAL AND METHOD

In the present study 40 male Wistar rats with mean weight 180 – 200 g were used. The animals were kept under standard laboratory conditions (temperature 22 ±1.0<sup>0</sup> C, humidity 45%, light/dark cycle 12/12 hours) and had received water and food *ad libitum*. Our study has been approved by the Local ethics committee of Medical University-Plovdiv and by the Bulgarian Food Safety Agency. Animals were treated orally for 90 days. They were divided into 5 groups (n = 8).

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**Group I** – 8 animals, treated orally with saline (1ml/kg body weight)

**Group II** – 8 animals, treated orally with atorvastatin (10 mg/kg body weight)

**Group III** – 8 animals, treated orally with atorvastatin (20 mg/kg body weight)

**Group IV** – 8 animals, treated orally with rosuvastatin (10 mg/kg body weight)

**Group V** – 8 animals, treated orally with rosuvastatin (20 mg/kg body weight)

We used the drug preparations atorvastatin (Atoris) produced and distributed by KRKA (Slovenia) and rosuvastatin (Crestor), manufactured and distributed by Astra Zeneca.

After the 90-day period cognitive function and memory retention were evaluated using active avoidance test and two passive avoidance tests.

An automatic reflex conditioner for active avoidance was used – shuttle box (Ugo Basile, Italy). The learning session consists of five consecutive days. Each day 30 trials were performed with the following parameters: 6 s light and buzzer (670 Hz and 70 dB) accompanied in the last 3 s by electric shock on the floor of the chamber (0.4 mA). Between every trial there is 12 s intertrial pause. A memory retention test was performed 7 days later – on the 12<sup>th</sup> day. The parameters automatically counted were as follows: number of conditioned responses (avoidances), number of unconditioned responses (escapes) and number of intertrial crossings.

Automatic set-up for passive avoidance “step through” was used (Ugo Basile, Italy). The learning session consisted of 2 consecutive days, followed by short-term memory retention test on the 3<sup>th</sup> day. Long-term memory retention test was performed on the 9<sup>th</sup> day. Each day 3 trials were performed with a 30-min pause between them. The test parameters were as follows: door delay 7 s, electrical stimulation on the floor of the chamber for 9 s with intensity 0.4 mA. Learning criterion was the latency of reactions of 180 s in the light chamber.

Automatic set-up for passive avoidance “step-down” was also used which represents wire cage with plastic platform (Ugo Basile, Italy). The learning session consisted of 2 consecutive days, followed by memory retention sessions –

short-term memory retention test was performed on the 3<sup>rd</sup> day and on the 7<sup>th</sup> day long-term test. The sessions consisted of 2 trials each day (electrical stimulation duration of 10 s at intensity 0.4 mA) with 60-min interval between trials. The latency of reactions was accepted as criterion for learning and memory retention – the rat remaining on the platform for 60 s.

Data were analysed by statistical software SPSS 17.0. For every parameter mean and standard error was calculated using  $p < 0.005$ . The following statistical analyses were used: one way ANOVA; Independent samples T-test; Paired samples T-test.

## RESULTS

### Active avoidance

In the active avoidance test the number of conditioned responses of the control group did not differ significantly during the learning session and in the memory retention test compared to the first day of the experiment. The group treated with atorvastatin in a dose 10 mg/kg body weight had increased number of avoidances on the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> day of the learning session as well as in the memory retention test on day 12, compared to the same day control group. The animals receiving atorvastatin in a dose 20 mg/kg body weight had increased number of conditioned responses on the 2<sup>nd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 12<sup>th</sup> day compared to the same day vehicle-treated group. The group treated with rosuvastatin (10 mg/kg body weight) showed increased number of avoidances on the 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 12<sup>th</sup> day of the study, compared to the same day control group. Animals treated with the higher dose rosuvastatin did not differ significantly during the learning session and the memory retention test compared to the group, receiving saline (**Figure 1**).

In the shuttle box active avoidance test the control group showed no significant difference in the number of unconditioned responses during the experiment compared to the first day. Rats, treated with atorvastatin (10 mg/kg body weight) had increased number of escapes on the 1<sup>st</sup> and 2<sup>nd</sup> day of the study compared to the same day control group. Animals receiving atorvastatin (20 mg/kg body weight) showed increased number of unconditioned responses on the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>th</sup> day compared to the same day control group. The group treated with

rosuvastatin (10 mg/kg body weight) showed increased number of escapes on day 1, 2 and 4, compared to the animals receiving saline. Rats receiving the higher dose of rosuvastatin had no

significant change in the number of unconditioned responses during the learning session and in the memory retention test compared to the control group (Figure 2).

\*Statistically significant vs control group to the same day to  $p < 0.05$

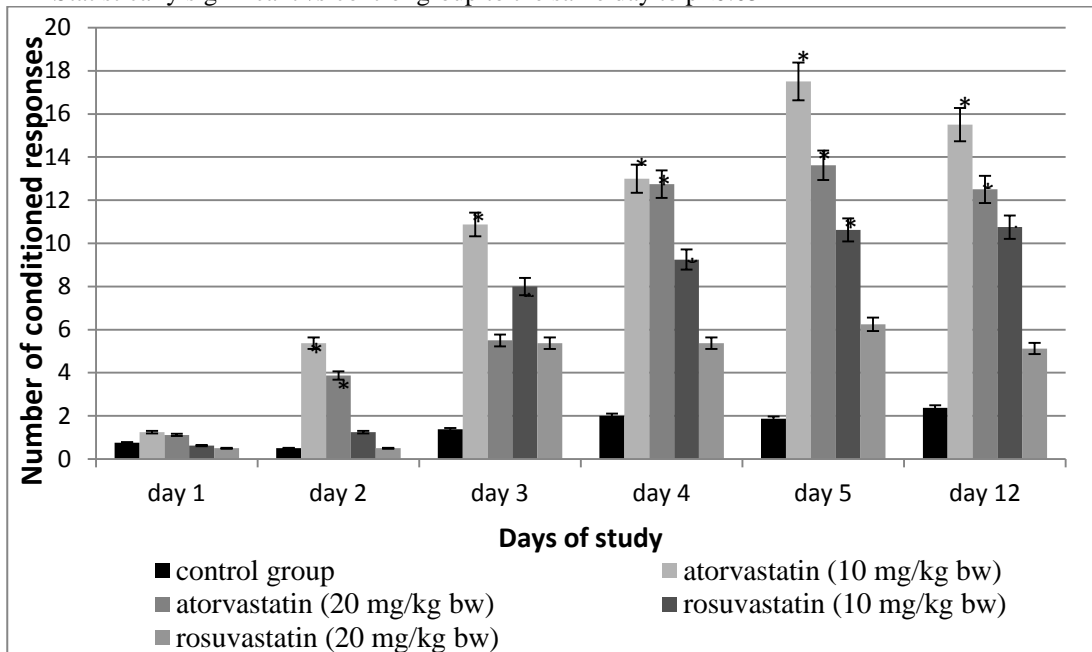
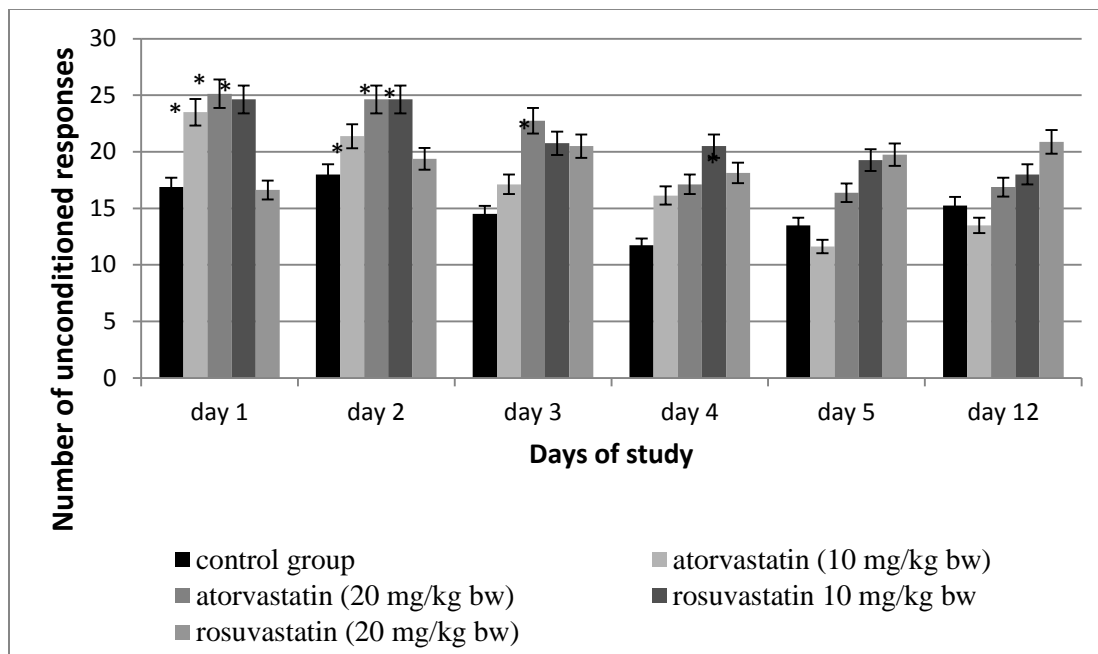


Figure 1. Number of conditioned responses in shuttle box active avoidance test after 90-day treatment with atorvastatin and rosuvastatin



\* Statistically significant vs control group to the same day to  $p < 0.05$

Figure 2. Number of unconditioned responses in shuttle box active avoidance test after 90-day treatment with atorvastatin and rosuvastatin

In the active avoidance test animals treated with atorvastatin and rosuvastatin in both doses showed no significant difference in the number

of intertrial crossing, which allows us to accept data from the other two parameters for reliable (Table 1).

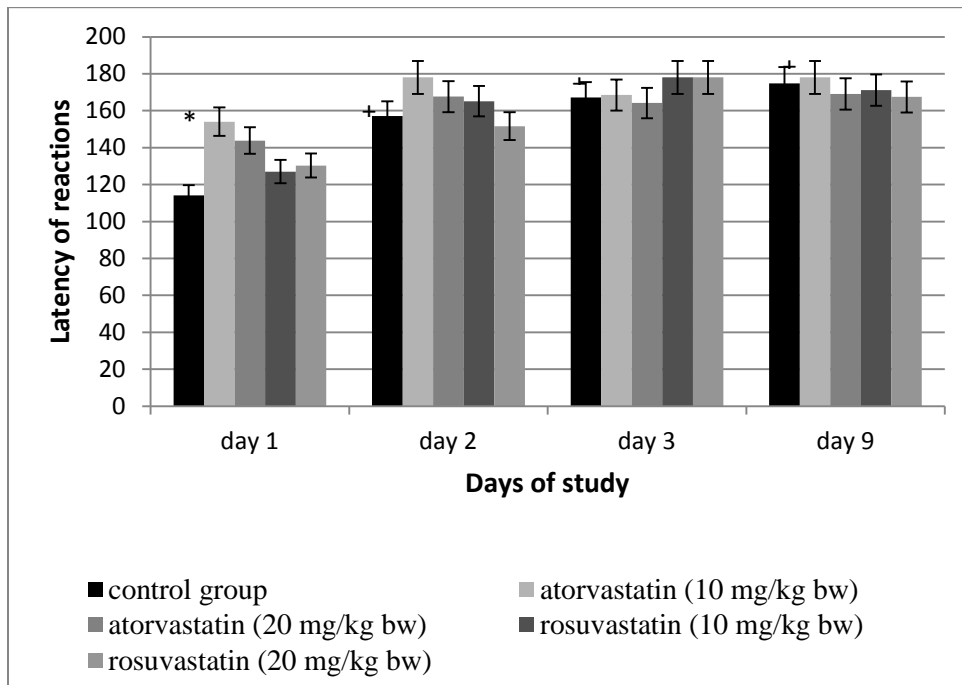
**Table 1.** Number of intertrial crossings in shuttle box active avoidance test after 90-day treatment with atorvastatin and rosuvastatin

Days of study	Control group (saline) mean±SEM	Atorvastatin (10 mg/kg bw) mean±SEM	Atorvastatin (20 mg/kg bw) mean±SEM	Rosuvastatin (10 mg/kg bw) mean±SEM	Rosuvastatin (20 mg/kg bw) mean±SEM
Day 1	14.12±2.85	11.37±1.73	10.75±2.85	11.50±2.01	13.12±1.96
Day 2	17.62±2.58	18.00±1.71	13.75±2.64	14.12±1.74	13.87±2.25
Day 3	11.87±2.50	14.62±1.99	15.50±2.0	16.12±2.24	15.00±2.08
Day 4	11.62±2.87	14.25±2.60	12.50±2.32	13.25±2.11	15.75±2.13
Day 5	11.12±2.75	13.75±1.66	12.62±2.89	14.25±1.19	13.62±1.70
Day 12	16.62±4.01	13.87±1.52	13.25±1.93	15.12±0.54	15.37±1.51

**Passive avoidance**

In the step through passive avoidance test group treated with saline showed increased latency of reactions on the 2<sup>nd</sup>, 3<sup>rd</sup> and 9<sup>th</sup> day compared to the first day of the experiment. Animals receiving atorvastatin (10 mg/kg body weight)

had increased the latency period on day 1, compared to the same day control group. The other statin-treated groups had no significant difference in the latency of reactions, compared to the vehicle-treated rats (Figure 3).



+ Statistically significant vs 1<sup>st</sup> day of the control group to p<0.05; \* Statistically significant vs control group to the same day to p<0.05

**Figure 3.** Latency of reaction in seconds in passive avoidance test step through after 90-day treatment with atorvastatin and rosuvastatin

In the step down passive avoidance test the control group showed increased latency of reactions on the 2<sup>nd</sup>, 3<sup>rd</sup> and 7<sup>th</sup> day of the experiment, compared to the first day. Rats receiving both doses of atorvastatin showed increased the latency period on the 2<sup>nd</sup> and 3<sup>rd</sup>

day, compared to the same day vehicle-treated group. Animals treated with rosuvastatin in a dose 10 and 20 mg/kg body weight had no significant difference in the latency of reactions, compared to the control group (**Table 2**).

**Table 2.** Latency of reactions in seconds in passive avoidance test step down after 90-day treatment with atorvastatin and rosuvastatin

Days of study	Control group (saline) mean±SEM	Atorvastatin (10 mg/kg bw) mean±SEM	Atorvastatin (20 mg/kg bw) mean±SEM	Rosuvastatin (10 mg/kg bw) mean±SEM	Rosuvastatin (20 mg/kg bw) mean±SEM
Day 1	27.19±5.76	28.31±4.24	27.41±7.10	25.80±6.61	34.65±7.99
Day 2	45.55±4.03 <sup>+</sup>	59.60±0.31*	60.00±0*	50.26±3.90	48.76±4.31
Day 3	47.71±5.14 <sup>+</sup>	60.00±0*	60.00±0*	55.33±3.05	53.66±3.38
Day 9	60.00±0 <sup>+</sup>	60.00±0	56.68±3.31	52.73±4.75	58.71±1.28

<sup>+</sup>Statistically significant vs 1<sup>st</sup> day of the control group to p<0.05; \* Statistically significant vs control group to the same day to p<0.05

## DISCUSSION

In the available literature there is insignificant data concerning the influence of statin treatment on processes of learning and memory in rats, without brain damage. Most studies suggest a beneficial effect of statin treatment in animals with neurophaty on learning and memory performance. However, data from few studies showed no improvement of statin treated control animals compared to untreated controls. This suggest that statins can protect the brain against damage but still remains uncertain whether they can improve cognitive function in intact animals (1).

Baytan et al. study the effect of 45-day treatment with simvastatin on spatial memory, evaluated by Barnes maze test. The results showed impairment of spatial memory in animals receiving simvastatin in a dose 10 mg/kg body weight, but not 30 mg/kg body weight (8). Results from our study differ from those of Baytan et al as we report improvement of learning performance and long-term memory retention in the active avoidance test. Difference in the effect of simvastatin and fluvastatin on spatial memory has been reported. Fluvastatin after 4-week treatment in rats did not alter spatial memory (9). The difference in the results of the neuroprotective effect of statin in animals without brain damage could give us the option to

choose the most suitable drug preparation according to the aim of the treatment.

Douma et al investigate the influence of simvastatin on cognitive function in rats that underwent olfactory bulbectomy, which leads to severe cognitive impairment with deficits in learning and memory. Simvastatin did not improve cognitive performance in the open field test, step through passive avoidance test and the object-location task. However, simvastatin improve cognition in intact rats (10). In our study cognitive function of the rats was not improved in the step through passive avoidance test rather than in the active avoidance and step down passive avoidance tests. Improvement of cognition could be due to modulation of signaling pathways implicated in synaptic plasticity and memory formation (11). Increased levels of NMDA\* receptors following chronic treatment with simvastatin have been reported (12). These receptors play an important role in learning and memory (13). In the memory formation process NO\*\* serves as retrograde messenger, which modulates synaptic function and affects short and longterm memory (14). It has been reported that statins increase eNOS\*\*\* expression and inhibit iNOS\*\*\*\*, which results in increased levels of NO without leading to overproduction (1). These factors could be part of the complex mechanism involved in the improvement of cognitive performance by statin treatment.

## CONCLUSION

The analysis of the influence of long-term oral treatment with statins on learning and memory performance give us a reason to conclude that atorvastatin in a dose 10 and 20 mg/kg body weight and rosuvastatin (10 mg/kg body weight) improve learning performance and long-term memory retention in the active avoidance test. In the step down passive avoidance test atorvastatin (10 and 20 mg/kg body weight) improve processes of learning and short-term memory retention after 90-day application.

## ABBREVIATIONS

\*NMDA – N-methyl-D-aspartate

\*\*NO – nitric oxide

\*\*\*eNOS – endothelial nitric oxide synthase

\*\*\*\*iNOS – inducible nitric oxide synthase

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