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Research article

# Chronic administration of *N*-acetyl-D-mannosamine improves age-associated impairment of long-term potentiation in the senescence-accelerated mouse

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## HIGHLIGHTS

- Chronic administration of ManNAc improves the attenuated LTP in 6-month-old SAMP8.
- Chronic administration of ManNAc improves the attenuated LTP in 14-month-old SAMR1.
- ManNAc increases the synaptic transmission of not 6-month-old but 14-month-old SAM.

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## ABSTRACT

*N*-Acetyl-D-mannosamine (ManNAc), a precursor of a sialic acid, is recently reported to improve the cognitive function in aged animals. However, the effect of chronic administration of ManNAc on impaired synaptic transmission and plasticity with age still remain unknown. In this study, we electrophysiologically determined the effect of chronic administration of ManNAc on deteriorated synaptic transmission and plasticity using hippocampal slices from senescence-accelerated mouse prone 8 (SAMP8) which shows age-related impairment of learning and memory. Oral administration of ManNAc for 8 weeks improved the field excitatory postsynaptic potentials (fEPSPs) in both SAMP8 and SAM resistant 1 (SAMR1), the control strain of SAMP8, at 14 months of age, but not at 6 months of age. On the other hand, ManNAc administration improved long-term potentiation (LTP), representative of long-term synaptic plasticity, of 6 month-old SAMP8 but not of age-matched SAMR1. In addition, ManNAc improved LTP of 14 month-old SAMR1 but not of age-matched SAMP8. At the same time, we checked the PPR but ManNAc did not affect the PPRs at either before or after high-frequency stimulation for LTP induction. These results indicate that chronic administration of ManNAc improves the age-dependent attenuation of synaptic transmission and LTP, and shows the availability of ManNAc treatment as potential therapeutic application for cognitive dysfunction.



## 1. Introduction

*N*-Acetyl-D-mannosamine (ManNAc) is the precursor of *N*-acetylneuraminic acid (Neu5Ac), a representative sialic acid existing the most abundantly in the body. In in vitro experiments, ManNAc treatment facilitates the neurite elongation as well as a sialic acid does [1], and modulates the expression of orexin neurons [2]; therefore, it is considered that ManNAc promotes the neuronal networks and is expected for improvement of brain function. Recently, it was reported that oral administration of ManNAc ameliorated the ability for spatial learning and memory in aged mice and dogs [3,4]. As long-term potentiation (LTP), a typical form of synaptic plasticity at the hippocampus is considered to be a basic mechanism of learning and memory at a cellular level [5], ManNAc may alter the LTP as well as impaired cognitive function in aged animals. However, functional effects of chronic administration of ManNAc on LTP have been poorly investigated.

Senescence-accelerated mouse (SAM) shows a senescence-related phenotype such as a short lifespan and rapid advancement of senescence. SAM Prone 8 (SAMP8) is one of the SAM strains and represents an impairment of learning and memory with the passive avoidance test and the water maze test compared with SAM

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Resistant 1 (SAMR1), the control strain [6,7]. An earlier study reported that SAMP8 showed attenuated LTP compared to SAMR1 [8]. In their study, 2, 6, 12 month-old SAMP8 were used and attenuated LTPs could be observed at 6 and 12 months old, suggesting that significant senescence of cognitive function in SAMP8 against SAMR1 starts from 6 months old along with age-related decline of learning ability [9–13]. Thus, SAMP8 is a suitable animal model of aging not only for behavioral studies but also for electrophysiological investigations although detailed molecular mechanism corresponding to age-dependent cognitive dysfunction still remains unclear. Here, we aimed to test if the chronic treatment of ManNAc improves the decreased LTP with aging, using 6 month-old and 14 month-old SAMP8.

## 2. Materials and methods

### 2.1. Animals

We used 6 and 14 month-old male SAMP8, and age-matched male SAMR1 as control. Animals were purchased from Japan SLC Inc. (Shizuoka, Japan). They were housed three to five animals per cage under 12 h light/dark cycles in a temperature-controlled room (24 °C). The mice were randomly assigned to either control groups or ManNAc supplemented groups. They could freely access to food and water (or water including 0.5% ManNAc for ManNAc supplemented groups) for 8 weeks. All animal experiments were performed in accordance with the Institute of Ethical Guidelines under the protocols approved by the Animal Experimental Expert Committee of the University of Tokyo.

### 2.2. Slice preparation

Hippocampal slices were made as described previously [14]. Briefly, mice were deeply anesthetized with urethane (1.0 g/kg) and decapitated. The brains were removed from the skulls under the ice-cold sucrose-rich slicing solution (SRSS) containing 85 NaCl, 2.5 KCl, 1.25 $NaH_2PO_4$, 25 $NaHCO_3$, 25 glucose, 75 sucrose, 0.5 $CaCl_2$, 4 $MgCl_2$, and 0.6 ascorbic acid (in mM). Slices were made with a DSK-Linear Slicer Model Pro 10 (Dosaka EM, Kyoto, Japan) at 350 μm and incubated in SRSS that was slowly returned to room temperature (around 24 °C) and replaced to an artificial cerebrospinal fluid (ACSF) containing 125 NaCl, 2,4 KCl, 1.2 $NaH_2PO_4$, 25 $NaHCO_3$, 25 glucose, 1 $CaCl_2$, and 2 $MgCl_2$ (in mM) at the speed of 1 mL/min. Both SRSS and ACSF were equilibrated with 95% $O_2$/5% $CO_2$.

### 2.3. Electrophysiological recordings

After 1 h, individual slices were transferred to a recording chamber and perfused continuously with ACSF containing 125 NaCl, 2.4 KCl, 1.2 $NaH_2PO_4$, 25 $NaHCO_3$, 25 glucose, 2$CaCl_2$, and 1 $MgCl_2$ (in mM) at the speed of 1–3 mL/min at room temperature. Glass electrodes were pulled from borosilicate glass and filled with 2 M NaCl for recording electrodes and ACSF for stimulating electrodes, respectively. Electric stimuli were delivered from Electronic Stimulator SEN-3401 and Isolator SS-203J (Nihon Kohden, Tokyo, Japan) with Schaffer collateral stimulation. Field EPSP (fEPSP) was recorded positioned in stratum radiatum distantly more than 100 μm from pyramidal cells in CA1 region of hippocampus.

### 2.4. Stimulation protocols

Control test pulses were given every 20 s. Pulse intensity was set for generating 1/3–1/2 of fEPSP induced by maximum intensity. Baseline stimuli were delivered more than 10 min after fEPSP reached to the steady state. For paired-pulse stimulation, 2 pulses were delivered with 40 ms intervals for 5 min before measurement of control fEPSPs ('before tetanus' in Fig. 3) and after LTP recordings ('after tetanus' in Fig. 3). Paired-pulse ratio (PPR) was calculated by dividing second fEPSP slopes by first fEPSP slopes. For induction of LTP, high-frequency stimulation (HFS), in which 100 Hz stimuli for 1 s were delivered three times at 10 s intervals, were adopted. Recordings were continued for 60 min after stimulation. We calculated the degree of LTP by averaging the slopes of control fEPSPs measured just before the HFS for 10 min and comparing it to the average slopes of fEPSPs between 50 and 60 min after the HFS.

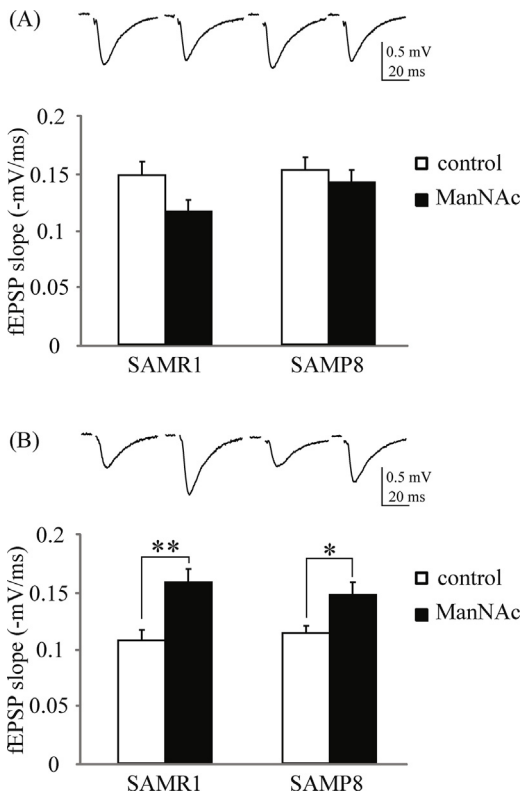


**Fig. 1.** Effect of chronic administration of ManNAc on fEPSPs in SAMP8 and SAMR1. (A) Representative traces of fEPSPs (top) and summary (bottom) recorded from 6 month-old SAMP8 and SAMR1. Water-administered SAMR1 ($n = 11$), ManNAc-administered SAMR1 ($n = 12$), water-administered SAMP8 ($n = 17$), and ManNAc-administered SAMP8 ($n = 15$) were used. (B) Representative traces of fEPSPs (top) and summary (bottom) recorded from 14 month-old SAMP8 and SAMR1. Water-administered SAMR1 ($n = 17$), ManNAc-administered SAMR1 ($n = 15$), water-administered SAMP8 ($n = 24$), and ManNAc-administered SAMP8 ($n = 20$) were used. Statistical analysis was carried out using ANOVA and the significant level for the statistical tests was set at 0.01 (**) or 0.05 (*).

### 2.5. Statistical analysis

Data were collected and analyzed with pClamp10.2 (Axon Instruments, Union City, CA). All values were represented as mean ± SE; $n$ values are the number of recordings from individual slice preparations (i.e., slices were not used for more than a single recording).

### 2.6. Chemicals

All chemicals were purchased from Wako chemical (Osaka, Japan) except urethane (Sigma–Aldrich, St. Louis, MO) and ManNAc. ManNAc was provided by Drs. Shiota and Hayaikawa [2].