N-Acetyl-D-Mannosamine Treatment Alleviates Age-Related Decline in Place-Learning Ability in Dogs

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ABSTRACT. This study aimed to investigate the therapeutic effects of *N*-acetyl-D-mannosamine (ManNAc) on age-related cognitive dysfunction in dogs. ManNAc was administered to 5 dogs with low cognitive levels for 2 months, and the cognitive ability and active-resting cycle were periodically assessed for improvement. ManNAc treatment significantly reduced the number of error trials in the place-learning test, especially in the first month of administration. Three ManNAc-treated dogs also showed improvement in the active-resting cycle. In conclusion, ManNAc treatment appears to alleviate age-related cognitive dysfunction.
KEY WORDS: active-resting cycle, age-related cognitive dysfunction, cognitive ability, *N*-acetyl-D-mannosamine.

Dogs are generally considered "seniors" when they reach 7 years of age or greater with a risk for cognitive dysfunction [14] with impairments in any of the 4 behavioral categories: orientation in the immediate environment, social interactions with human family members, learning and memory and the active-resting cycle [8]. Furthermore, aged dogs display a wide range of individual variability in cognitive functioning and also display age-related neuropathologies similar to those of humans, such as amyloid plaque deposition, and thus, dogs are regarded as a model for age-related cognitive decline in humans [1]. Progression of age-related cognitive dysfunction is becoming important as the dog population ages, and we recently developed a convenient cognitive test, which can be performed within a day for simple detection of cognitive decline [6].

The neural mechanisms responsible for the various cognitive abilities are not fully understood. Recent studies have demonstrated that newly generated neurons in the hippocampus play a pivotal role in spatial cognitive ability [3] and have also revealed the biological roles of neurogenesis in the mouse and rat hippocampus in the adult brain [9, 10, 13]. *N*-acetyl-D-mannosamine (ManNAc) is the isomer of *N*-acetyl-D-glucosamine and the precursor of sialic acids, which are the most abundant terminal monosaccharides on glycoconjugates on eukaryotic cell surfaces and are involved in a variety of cellular functions [12]. We revealed in mice that 4 weeks of ManNAc treatment alleviated the age-related decline in learning ability and memory in the place-recognition task with an increase of neurogenesis in the hippocampus [4]. ManNAc was also reported to increase the duration of rapid eye movement (REM) sleep and improve the active-resting cycle after a few days of administration [12]. Therefore, ManNAc is a potential therapeutic agent for improving not only cognitive function but also quality of life (QOL), as correction of impaired active-resting cycles is important for maintaining QOL in aged humans [2]. Therefore, this study aimed to investigate the ability of ManNAc treatment to improve age-related cognitive dysfunction in dogs.

The subjects were 5 Labrador Retrievers (1 male and 4 females, aged 93.60 ± 11.98 months; Table 1) that lived at the Japan Guide Dog Association facility (Fujinomiya, Japan). They were among the older dogs in the facility and, being retired or used only for demonstrations, were able to remain in the facility throughout the experiment. Each dog was fed twice a day (7:30, 17:00) and was cared for by the facility staff. The retired dogs took a walk and light exercise about an hour twice a day. The dogs for demonstrations were trained about an hour twice a day. All dogs underwent medical examination, including blood tests, and were found to be in good health.

The subjects of this study received 2-month courses of treatment with both ManNAc and glucose (control, an equivalent calorie value) in a crossover design with a 1-month washout period. One capsule containing 250 mg ManNAc or glucose was administered orally to each subject once a day for 2 months. Place-learning tests were performed 6 times on each subject. Tests were conducted just before the first and second treatments to obtain baseline data and were repeated once after the first month and again after the second month of each treatment. Motor activity was measured on all days from 1 week before the first treatment until the end of the second treatment.

The tests were conducted in a kennel (2 × 3 m) at the Japan Guide Dog Association facility. We adopted the method previously developed for dogs [6]. All tests were recorded

Table 1. Subjects' characteristics and the number of error trials in the baseline sessions

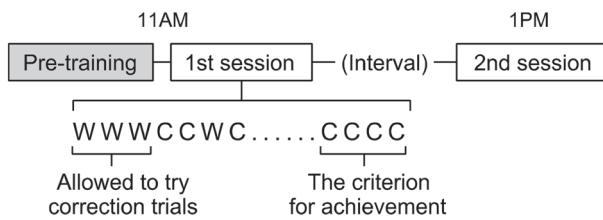| Name	Age (months)	Sex	Status	Number of error trials in the baseline sessions	
				ManNac	Glucose
Bart	129	Male, Neutered	Retired	23	28
Kayla	114	Female, Spayed	Retired	23	24
Biscay	84	Female, Spayed	For demo	24	19
Vila	75	Female, Spayed	For demo	44	34
Ink	66	Female, Spayed	For demo	–	–

Fig. 1. The place-learning test procedure. The pre-training period continued until the dog spontaneously approached or touched a bowl's flap. In the first session, the first 3 trials were used for correction, if the dog selected a wrong bowl. The first session ended when the dog selected the correct bowl in 4 consecutive trials. The second session was conducted the same way. C: correct selection; W: wrong selection. This figure was adapted from [6].

using a digital video recorder. One experimenter and 1 handler participated in each study. Three stainless-steel bowls (17×6 cm) with flaps were placed 30 cm apart. A string (2 m) was attached to the rim of each flap for opening the flap. A commercial dog treat (approximately 1 cm^2) was used as a reward stimulus. The handler had the dog sit and wait 1.5 m away from the bowls (the standby position) before presenting the food reward. Each place-learning test consisted of a pre-training session and 2 test sessions (Fig. 1). During the interval between the 2 test sessions (approximately 1 hr), the dog was removed from the experimental kennel and returned to its own kennel.

Pre-training: This step was conducted to teach the dogs that the bowls could contain reward stimuli. The handler placed a piece of food in each bowl. When a subject spontaneously approached or touched a bowl flap, the handler immediately gave the dog a verbal reward (such as "Good!"), and the experimenter rewarded the dog by opening the flap. The first session began as soon as the dogs learned how to obtain the food.

Testing: The testing consisted of 2 sessions. The first session began at approximately 11 AM and the second at 1 PM. In each session, the food stimuli were placed in all bowls (1 piece/bowl) while the dog watched in the standby position, and the bowl flaps were closed. The dog was then released and allowed to select a bowl spontaneously (1 trial). In both sessions, the dog was allowed to eat only the food in a specific bowl (the "correct bowl"), which the experimenter chose randomly, and the position of the correct bowl was changed in the second session. As a general rule of scoring,

if the dog directly approached or touched the correct bowl, the trial was scored as correct. The handler immediately gave a verbal reward, and the experimenter quickly opened the bowl flap and allowed the dog to eat the food. If the dog did not immediately select the correct bowl, the trial was scored as wrong. The handler quickly returned the dog to the standby position and commanded it to stay until the next trial (20 sec). The criterion for finishing the session was a correct score in 4 consecutive trials. The administration study analyzed the values obtained by subtracting the sum of baseline of each treatment from the sums of the error trials conducted 1 and 2 months after the start of corresponding treatment (the index of error trials).

An acceleration meter (MSR145 Modular Signal Recorder, MSR Electronics GmbH, Seuzach, Switzerland) was attached to each dog's collar to monitor its motor activity. Triaxial motor activity data were recorded at 4-sec intervals and were summed with an application (MSR 4.16). Data were calculated as the variation from the resting values. The 4-sec data points were summed over each 15-min interval. These data were divided into daytime (7:00–19:00) and nighttime (19:00–7:00) data due to the facility's schedule. The ratio of the daytime activity to nighttime activity in each day was calculated and averaged over each week (as the index of active-resting amplitude) for comparison with a higher index of active-resting amplitude indicating a better active-resting cycle.

Statistical analyses for cognitive ability and motor activity were performed by 2-way analysis of variance (ANOVA) with repeated measures; if a significant difference was found, the Bonferroni method was used for post-hoc analysis. As the sample size was limited (n=4 or 5), the level of statistical significance was set at 10%.

To compare the index of error trials, we analyzed the first and second sessions separately using 2-way ANOVA with repeated measures on the factors "administration" (ManNAc and glucose) and "times" (baseline, 1st month and 2nd month) and found a significant main effect of "administration" (f (1)=29.66, *P*=0.01) for the first session. The post-hoc test showed that the index of error trials in the first session was significantly lower during ManNAc treatment than during glucose treatment (1st month: *P*=0.05, 2nd month: *P*=0.02; Fig. 2a). The treatment had no significant effect on the result for the second session (Fig. 2b). Next, 2-way ANOVA with repeated measures was conducted on the total of the index of error trials and showed a statistically signifi-
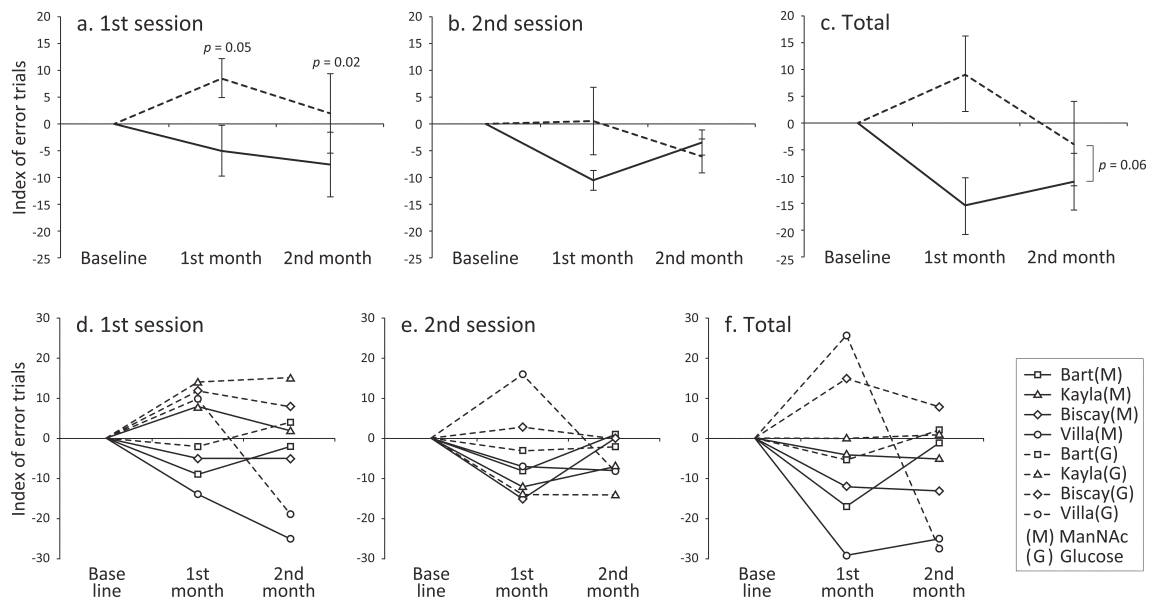
Fig. 2.   Changes in the index of error trials during treatment. The average of the index of error trials values for the first session
(a) and overall (c) declined significantly during ManNAc treatment. For Biscay and Villa, the index of error trials values was
clearly lower than the baseline throughout ManNAc treatment, especially for the first session (d, f). For the second session, all
dogs showed lower index of error trials values after the first month of ManNAc treatment, although the index values of 2 dogs
returned to near-baseline after the second month (e).

cant main effect of "administration" (f (1)=8.16, P=0.07).
The post-hoc Bonferroni test found the significant difference
between ManNAc and glucose treatment (P=0.06, Fig. 2c).
The data for each dog are shown in Fig. 2 panels d, e and f.
All dogs showed a tendency towards decreased index of er-
ror trials values in the second session during the first month
of ManNAc treatment, although 1 dog (Kayla) showed an
increased index of error trials in the first session (Fig. 2d and
2e). We also examined the order effect and variability over
time in the baseline scores and found no significant differ-
ence in both. Overall, the index of error trials values of all
dogs decreased during ManNAc treatment (Fig. 2f).

A 2-way ANOVA with repeated measures was conducted
for the factors "administration" (ManNAc and glucose)
and "weeks" (baseline, 1st, 2nd, 3rd, 4th, 5th, 7th and 8th
week) on the index of active-resting amplitude and showed
a statistically significant main effect of "administration" (f
(1)=8.75, P<0.01). The post-hoc Bonferroni method found
that the index of motor activity was significantly higher
during ManNAc treatment than during glucose treatment
(P<0.01) and was also significantly higher during the 2nd,
3rd and 4th individual weeks of ManNAc treatment than dur-
ing the corresponding weeks of glucose treatment (P=0.06,
0.05 and 0.07, respectively; Fig. 3a).

We also conducted within-subject comparison for each
dog using 2-way ANOVA with repeated measures and found
that 3 dogs showed significantly higher index values during
ManNAc administration than during glucose administration
(Bart: administration f (1)=65.54, P<0.01, administration *
week f (8)=2.333, P=0.037; Villa: administration f (1)=10.98,

P=0.02; Ink: administration f (1)=24.119, P=0.004; Fig. 3b).
One dog showed a significant main effect of "week" (Kayla;
week f (7)=2.31, P=0.05). Only Biscay showed the opposite
tendency with a higher index of motor activity during glu-
cose treatment than during ManNAc treatment (administra-
tion f (1)=110.65, P<0.01).

The most remarkable finding of the present study is that
the number of error trials in dogs that had demonstrated
age-related declines in cognitive ability temporarily but sig-
nificantly decreased during ManNAc treatment. The present
results are consistent with those of studies in mice [4, 12]
and suggest the possibility that ManNAc may be a useful
therapy for age-related cognitive decline in dogs. ManNAc
can rescue the sialylation of glycoproteins in GNE-knockout
embryonic stem cells [11] and induce neural proliferation
and dendrite outgrowth *in vitro* [5]. ManNAc treatment
decreased the number of total error trials by Biscay, Kayla
and Villa throughout the 2-month treatment period, and this
effect was most pronounced in Biscay and Villa. On the
other hand, the effect of ManNAc was only temporary in
Bart, as his number of error trials decreased after 1 month
but returned to baseline after 2 months. These individual dif-
ferences in the response to ManNAc require a further study
of parameters including metabolic function and metabolic
clearance ability. We also did not find the noticeable effects
of ManNAc in the 2nd session, especially in the 2nd month.
The 2nd session was more difficult than the 1st one, because
of the need for reverse learning; therefore, the beneficial ef-
fects of ManNAc might appear only in the easier session.
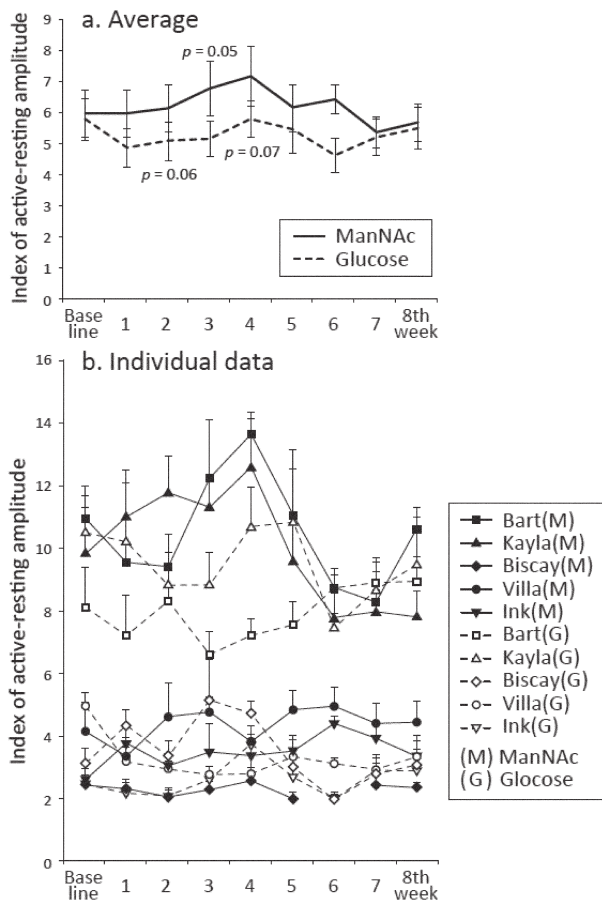These results might be because of insufficient pharmaco-

Fig. 3. Changes in the index of active resting amplitude during treatment. The average of the index of active resting amplitude values was significantly higher during ManNAc treatment than during glucose treatment (a). Bart, Villa, Kayla and Ink showed significantly higher values throughout ManNAc treatment than during glucose treatment. By the post-hoc test, 4 dogs showed significantly higher index of active-resting amplitude values during the following weeks of ManNAc treatment: Bart: weeks 1 (P=0.05), 3 (P=0.05) and 4 (P<0.01); Kayla: weeks 1 (P=0.09) and 2 (P=0.04); Villa: weeks 3 (P<0.01) and 6 (P=0.02); Ink: weeks 1 (P=0.04), 2 (P=0.07) and 6 (P<0.01). In Biscay, the index was lower during ManNAc treatment than during glucose treatment in weeks 1 (P=0.02), 2 (P=0.04), 3 (P=0.09) and 4 (P=0.02). Black marks indicate significant differences between ManNAc and glucose treatment according to the post-hoc test (b).

logical properties of ManNAc to show strong therapeutic benefits or of the shortage of dosage. The other possibility is the drug tolerance as a cause of decline of beneficial effect over the time.

The daytime motor activity was more than twice the nighttime activity in all dogs regardless of the treatment. The ratio of active-resting cycle disorders increased significantly in dogs aged ≥13 years [7]. The subjects of the present study were younger (5–10 years old) than the dogs in the previous studies. Therefore, signs of the active-resting cycle disorder might not have been apparent in the subjects of the pres-

ent study; however, 4 of them (all except Biscay) showed a significant higher index of active-resting amplitude during ManNAc treatment. In this study, we could not determine whether ManNAc improved age-related dysfunction, because we did not detect clear dysregulation of active-resting cycle. The findings of the present study suggest that age-related cognitive dysfunction might precede disturbances in the active-resting cycle in dogs and that the quality of sleep might be improved by ManNAc treatment regardless of age, as in the previous study in rodents [12]. The relationship between the effects of ManNAc on cognitive ability and on nighttime motor activity would be an interesting topic for a future study.

In conclusion, although further investigation with a larger sample size is needed, ManNAc alleviated age-related cognitive dysfunction at least temporarily in all dogs examined.

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