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Memory recovery through gene therapy with an antibody fragment selective for Aß oligomers in a model of Alzheimer's disease in rats

Recuperación de la memoria mediante terapia génica con un fragmento de anticuerpo selectivo para oligómeros de Aß en un modelo de Alzheimer en ratas

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Abstract

Strong evidence supports the hypothesis that synapse damage and memory impairment in early Alzheimer Disease (AD) might be due to synaptic failure caused by amyloid beta oligomers (AßOs). The preclinical efficacy of a single-chain variable fragment (scFv) antibody NUsc1 that selectively targets a subpopulation of AßOs has been demonstrated; NUsc1 prevented inhibition of AßO-induced long-term potentiation in hippocampal slices and short-term memory impairment in mice. Since specific targeting of AßOs by NUsc1 can be a substantial improvement in target engagement and efficacy of AD therapy, an adeno-associated virus (AAV) vector was developed to drive neuronal expression of NUsc1 within the brain. AAV-NUsc1 rescued Short-Term Memory (STM) for object and conspecific interaction in mouse models of AD. In the McGill-R-Thy1-APP (Tg+/–) heterozygous transgenic McGill-R-Thy1-APP (Tg+/–) rat model of AD, progressive amyloid pathology is accompanied by cognitive impairment involving long-term memory (LTM) decline. LTM in a Novel-Object-Recognition (NOR) task was impaired in 4-month-old (Tg+/–) male rats, suggesting that they are unable to form/evoke such discriminative memories. Hence, it was investigated if AAV-NUsc1 treatment could rescued this memory. 10-12 weeks-old either Tg or wild type male rats were i.c.v. infused with AAV-NUsc1. Two months later, short-term exploratory behavior, habituation to an Open Field (OF), object discrimination and LTM for objects were assessed. AAV-NUsc1 treated Tg rats were able to successfully perform the task 24 h after training, denoting recovery of LT discrimination capacity and LTM formation. Wild type rats successfully performed the task either treated or not with AAV-NUsc1. In addition, exploration and short-term habituation to an open field was preserved in Tg+/– rats either treated or not. Our present and previous results suggest that AAV-NUsc1 represents a significant advance in gene therapy, supporting the feasibility of immunotherapy using viral vector-mediated NUsc1 gene delivery as a potential therapeutic approach in AD.

Keywords: LTM; Alzheimer's Disease; gene therapy; scFv; single chain antibody fragment; Aß oligomer

Resumen

Evidencias conspicuas respaldan la hipótesis de que la presencia de oligómeros beta-amiloides (AßO) ocasiona un deterioro sináptico y de la memoria en etapas tempranas de la Enfermedad de Alzheimer (AD). Se ha demostrado que el anticuerpo de cadena única y Fragmento variable (scFv) NUsc1, que une selectivamente una subpoblación de AßO, evitó el deterioro de la memoria a corto plazo, inducido por AßO en ratones. Dado que la selectividad de NUsc1 mejora sustancialmente la detección del AßO, y consecuentemente su eficacia terapéutica para AD, se ha desarrollado un vector derivado de Virus Adenoasociado para expresar NUsc1 (AAV-NUsc1) en el cerebro. AAV-NUsc1 rescató la Memoria de Corto Plazo (STM) para el reconocimiento de objetos e interacción con congéneres en ratones modelo de AD. La rata McGill-R-Thy1-APP transgénica heterocigota (Tg+/–) modelo de AD, sufre una patología amiloide progresiva acompañada de deterioro cognitivo, que afecta la Memoria de Largo Plazo (LTM) de Reconocimiento de Objetos (NOR) evitando su formación/evocación. Evaluando si el tratamiento con AAV-NUsc1 podría rescatar la LTM de reconocimiento en ratas macho (Tg+/–) de 4 meses. Ratas macho Tg y de genotipo salvaje (Wt) de 10-12 semanas fueron infundidas i.c.v con AAV-NUsc1. Dos meses más tarde, se evaluaron: el comportamiento exploratorio a corto plazo, la habituación a un Campo Abierto (OF), la discriminación y LTM para objetos. 24 h después del entrenamiento se observó que las ratas Tg tratadas con AAV-NUsc1 recuperaron la capacidad de expresar una LTM y de reconocer objetos novedosos. De manera similar, las ratas Wt tratadas con AAV-NUsc1 y las del grupo control, realizaron la tarea con éxito. La exploración y habituación al OF fueron similares para ratas Tg+/– y Wt, tratadas y control. Nuestros resultados sugieren que AAV-NUsc1 representa un avance significativo en terapia génica, respaldando la viabilidad de la inmunoterapia mediada por vectores virales aportando el gen de NUsc1 como posible enfoque terapéutico para AD.

Palabras clave: Memoria de larga duración MLD; enfermedad de Alzheimer; terapia génica; scFv: fragmento de anticuerpo de cadena simple; oligómeros Aß

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Introduction

Strong evidence supports the hypothesis that synapse damage and memory impairment in early Alzheimer Disease (AD) might be caused by synaptic failure due to Amyloid Beta Oligomers (AβOs) action leading to synapse dysfunction (Ferreira & Klein, 2011; Mucke & Selkoe, 2012; Selkoe & Hardy, 2016). In AD, both brain and Cerebrospinal Fluid (CSF) appear to accumulate AβOs (Fukumoto et al., 2010; Georganopoulou et al., 2005; Gong et al., 2003; Larson & Lesné, 2012), which seemed to be prominent in various transgenic animal models of brain amyloidosis, including those showing little or no amyloid plaque (Chabrier et al., 2014; Tomiyama et al., 2010). Furthermore, brain infusion of AβOs leads to cognitive deficits in wild-type mices, inducing major features of AD pathology, including brain inflammation, synapse elimination, tau hyperphosphorylation and selective neurons death (Ferreira & Klein, 2011).

Recently, it was demonstrated the efficacy of NUsc1, an AβO-specific single-chain variable-Fragment (scFv) antibody (chosen from a human-derived antibody library by phage display), to selectively bind and neutralize AβOs (Sebollela et al., 2017) in preclinical studies (Selles et al., 2022). Interestingly, NUsc1 shows little or no reactivity to non-toxic Aβ monomers or to insoluble amyloid fibrils; instead, NUsc1 targets an AβOs specific subpopulation that binds to the neuronal surface and is highly toxic to synapses (Sebollela et al., 2017; Selles et al., 2022; Velasco et al., 2012). ScFv antibodies lacking the Fc domain show a reduced capacity to activate cellular immune and/or inflammatory responses (Holliger & Hudson, 2005; Huang et al., 2013; Monnier et al., 2013). These results appear particularly appealing for experimental immunotherapy, since the use of immunoglobulins in clinical trials produced significant brain inflammation, as previously reported (Ferrer et al., 2004; Fuller et al., 2014; Nicoll et al., 2003; Orgogozo et al., 2003). In addition, as the nucleotide sequence coding for a scFv is shorter than for a native immunoglobulin, the corresponding transgene is easier to be incorporated and delivered within an Adeno-Associated Virus (AAV)-derived vector. This experimental approach consisted of a serotype 9 neurotropic vector derived from an AAV with the scFv (single-chain variable fragment) antibody transgene, under control of the neuron-specific promotor Synapsin-1 (AAV-NUsc1). Experimental gene-therapy with AAV-NUsc1 vector was recently shown to rescue short-term social and non-social memory in AD mouse models (Selles et al., 2022).

Although transgenic mice models provide valuable information on AD pathology and are useful to evaluate some putative treatments, frequent failure to translate the results to human beings calls for better animal models. Rat brains present more analogies with human brains, leading to more social and complex behavior. I.e., rats display a larger behavioral repertoire than that of mice, allowing more refined cognitive functions assessment. As Do Carmo and Cuello (2013) pointed out, rats could be considered closer to humans than mice from both a genetic and a physiological point of view, particularly regarding some proteins like ApoE and tau, that are relevant in AD (Tran et al., 2013).

In this work, the McGill-R-Thy1-APP rat, a transgenic animal model carrying the human amyloid precursor protein APP_{751} gene, was used. This gene carries the following mutations of familial AD: the double Swedish (K670N; M67IL) and the Indiana (V717F) mutations, under the promotor of murine thymocyte antigen (Thy 1.2), thus restricting transgene expression to neurons (Leon et al., 2010) and leading to a slow-progressing AD-like pathology. In heterozygous (Tg+/–) McGill-R-Thy1-APP young rats, Aß pathology is mainly intracellular, with scarce amyloid plaques that increase with age. This progressive amyloid accumulation and deposition is accompanied by cognitive decline. The performance of Tg+/– rats was previously assessed at various ages in different tasks (Leon et al., 2010; Iulita et al., 2014; Habif et al., 2021). Habituation to an Open Field (OF) is preserved in Tg+/– rats at 3-, 4-, and 6-month of age (Habif et al., 2021). At variance, 4 month-old Tg+/– male rat performance was significantly impaired for long-term memory in a novel object-recognition task. These results suggest that Tg+/- male rats appear unable to either consolidate or evoke these type of memories (Habif et al., 2021).

Here, a comparative analysis was made of the performance of male Tg+/– and wild type (Wt) male rats for short-term intra-session exploratory behavior and habituation to an open field (OF), object discrimination, and long-term memory for objects, under AAV-NUsc1 treatment.

Material and Methods

Animals

McGill-R-Thy1-APP (Wistar) Tg rats (Leon et al., 2010), were originally produced in the Department of Pharmacology and Therapeutics, McGill University (Montreal, Canada), by A.C.Cuello's team. A colony was established in the IBCN, School of Medicine, University of Buenos Aires (Buenos Aires, Argentina). By crossbreeding Tg+/+ or Tg+/– rats with Wt Wistar rats, heterozygous Tg+/– rats, as well as their Wild type (Wt) littermates, were obtained. Heterozygous Tg+/– rats were identified by performing PCR amplification of DNA to reveal the presence of the human (h) APP transgene (Gene ID: NM_000484.4) using the primers: hAPP-Fw: 5′-AGGACTGACCACTCGACCAG-3′ and hAPP-Rv: 5′-CGGGGGTCTAGTTCTGGAT-3′. *Rattus norvegicus* peptidylprolyl isomerase G–(Ppig) (Gene ID: XM_006234324.3) was choose as a housekeeping control gene, and the following primers were used: CycloFw:5′-TACAACAG TAGAACAAGGGAGCGAAG-3′ and CycloRv: 5′-ATCCCTC CTTCTTCTCCTCCTATCTTT-3′ (Habif et al., 2021). RNA was extracted with Trizol (Invitrogen) from tissue obtained from an ear biopsy. This RNA was used as template for synthesis of cDNA that was then submitted to real-time PCR amplification.

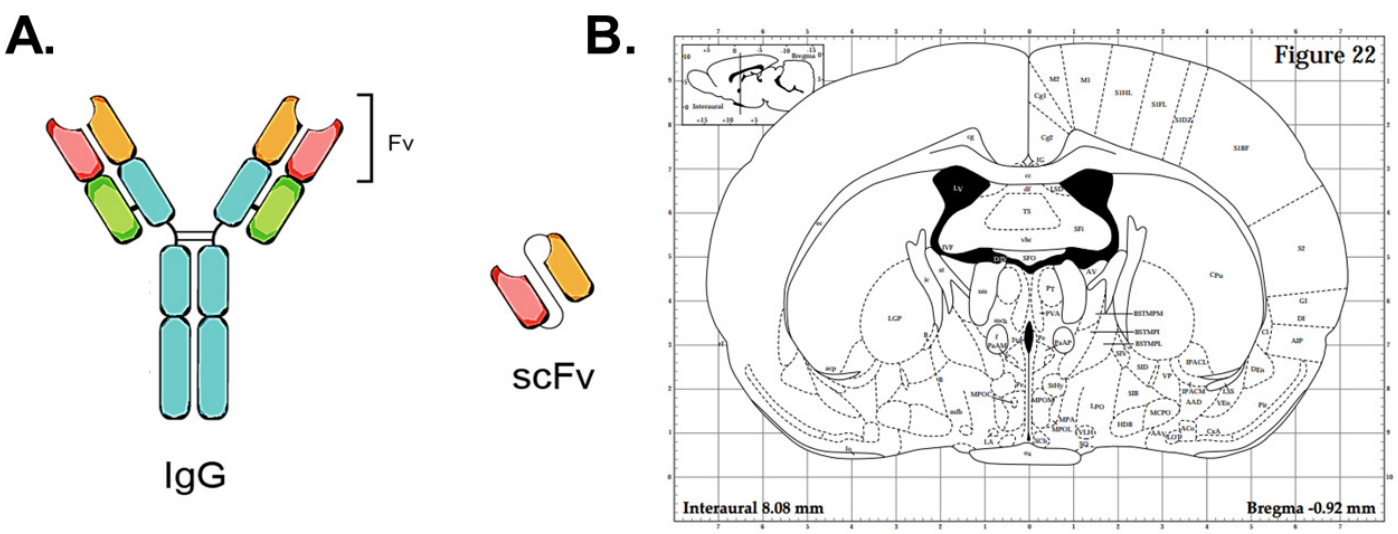
Adult male animals (200–250 g) were maintained in a special room under an inverted light cycle of 12 h light: 12 h darkness, at 25◦C and with *ad libitum* access to food and water, in groups of three to four animals per cage.

All the experiments with animals were developed in accordance with "Institutional Committee for Care and Use of Laboratory Animals" (CICUAL), of the School of Medicine, University of Buenos Aires (Argentina), as well as in compliance with the "Guide to the Care and Use of Experimental Animals" of the Canadian Council on Animal Care (Canada) and the "National Institutes of Health" (NIH) (USA).

AAV-NUsc1 vectors for gene therapy

A neurotropic serotype 9 AAV vector bearing the transgene of an engineered antibody (Figure 1A) scFv NUsc1, with the neuron-selective promotor of Synapsin-1 and a Signal Peptide (SP) for antibody exportation was developed to be used as experimental gene therapy in humanized models of Alzheimer disease. For details on the construction and expression of the transgene see (Selles et al., 2022).

FIGURE 1. *A) Scheme showing an IgG and a scFv derived antibody (without Fc), showing the heavy and light polypeptide chains linked by an artificial peptide. B) Diagram showing the lateral ventricles (black) where the AAV-NUsc1 vector was infused.*



Source: A. Modified from Ahmad et al. (2012); B) Modified from Paxinos & Watson (2013).

Vector Treatment

AAV-NUsc1 vector (3×10^9 particles of AAV-NUsc1 in a volume of 2 µL) was infused i.c.v. through stereotaxic surgery (AP: 0,096 LL: 0,18 DV: 0.45 according to coordinates of the Paxino & Watson, 2013; Figure 1B) under i.p. ketamine (50%, Kensol®): xilacine (2%, Xilol®), in a 3:0.5 proportion (75 mg: 5 mg/kg of body weight), in 10-12 weeks-old Wt and Tg +/– rats. Immediately after surgery, and in the following two days, rats were intramuscularly injected with enrofloxacin 5%, 5 mg/kg once a day.

Behavioral Tasks

Two months after infusion, male Tg+/– rats and their Wt littermates at 4.5 to 5-month of age, either treated or not (controls) with the AAV-NUsc1 vector, were trained in the behavioral tasks along one week. Rats were first left to freely explore an Open Field (OF). Thereafter, they were trained in a task for Novel Object Recognition (NOR) in the same arena, adapted to assess Long-Term Memory (LTM).

Open Field (OF)

Both spontaneous exploratory activity and habituation to the OF were individually assessed. In the first day, each rat was exposed to a 75.0 cm long × 75.0 cm wide × 50.0 cm high open field, with twenty-five squares (15 cm x 15 cm each) drawn on the floor, with visual cues on its walls (there were two opposite plain walls and two striped, one with vertical stripes and the other with horizontal stripes). Each rat was left to freely explore this OF for 5min (OF session duration). Then, the animal was immediately returned to its home cage with food and water ad libitum.

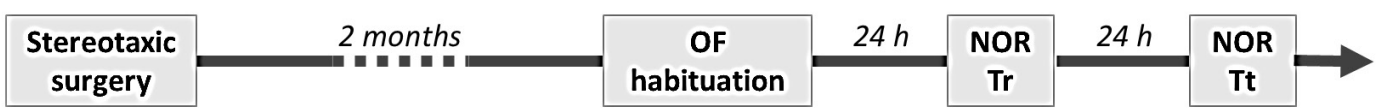
As an indication of "horizontal" exploratory activity, the times an animal crossed the lines designed on the floor was recorded minute by minute; this number of crossings was compared within the session as an indication of habituation to and recognition of the arena. The number of rearings was an indicator of "vertical" exploration. When the total number of crossings (as well as of rearings) was significantly lower in the last minute than in the 1st minute of the session, the animal was considered habituated to the arena, at least for the short-term.

For the OF task, the number of both crossings and rearings (discontinuous variables) were recorded. As both number of crossings and rearings did not follow a Gaussian distribution (D'Agostino & Pearson's normality test), these data were analyzed with nonparametric statistics (Mann Whitney), and the results were expressed as medians with interquartile (P25/P75) ranges (Figure 3).

Novel object recognition (NOR)

The NOR task was performed in the same OF arena. Objects were made of acrylic and had different colors, shapes, textures and size. During sessions, objects were fixed to the floor to avoid possible displacement. Previous tests were carried out to evaluate objects exploration; none of the objects evoked any significant preference (not shown).

FIGURE 2. *Schematic representation of the temporal course of the experimental procedures.*



Source: Authors.

Each rat was placed in the OF where two identical objects A and A' were previously included; the animal was kindly left on the floor watching to and close to the wall opposite to the objects location. During this first 5 minutes' session, referred as the training session, the time the animal spent exploring objects A and A'; which is considered as exploratory behavior whenever the rat touched the object with its limbs, directed its nose towards it, sniffed it. To avoid the interference of olfactory cues, the OF and the objects were cleaned with 70% ethanol thoroughly in the intervals between trials. The test session was performed 24 h after training, with previous replacement of one of the familiar objects by a new one B, while the location of the remaining familiar object was alternated for each animal/session, to avoid location preference.

During the training session, time spent exploring the familiar (either A or A') and the novel object (B), and the latency to start exploring any of the objects were recorded. Total time spent exploring objects was calculated afterwards. Preference Index (PI) in the training session was expressed as the ratio of the difference between the time spent exploring A minus the time exploring A' (A-A') and total time exploring both objects (A+A'). The minimum for total objects-exploration time along a training session to accept a rat performance to be appropriate for our records was set at 10 seconds (Akkerman et al., 2012).

For the test session, the Preference Index (PI) was defined as the ratio between time exploring the novel object B minus time exploring the familiar A, (B-A), and the total time exploring both objects (A + B) (Figure 4A).

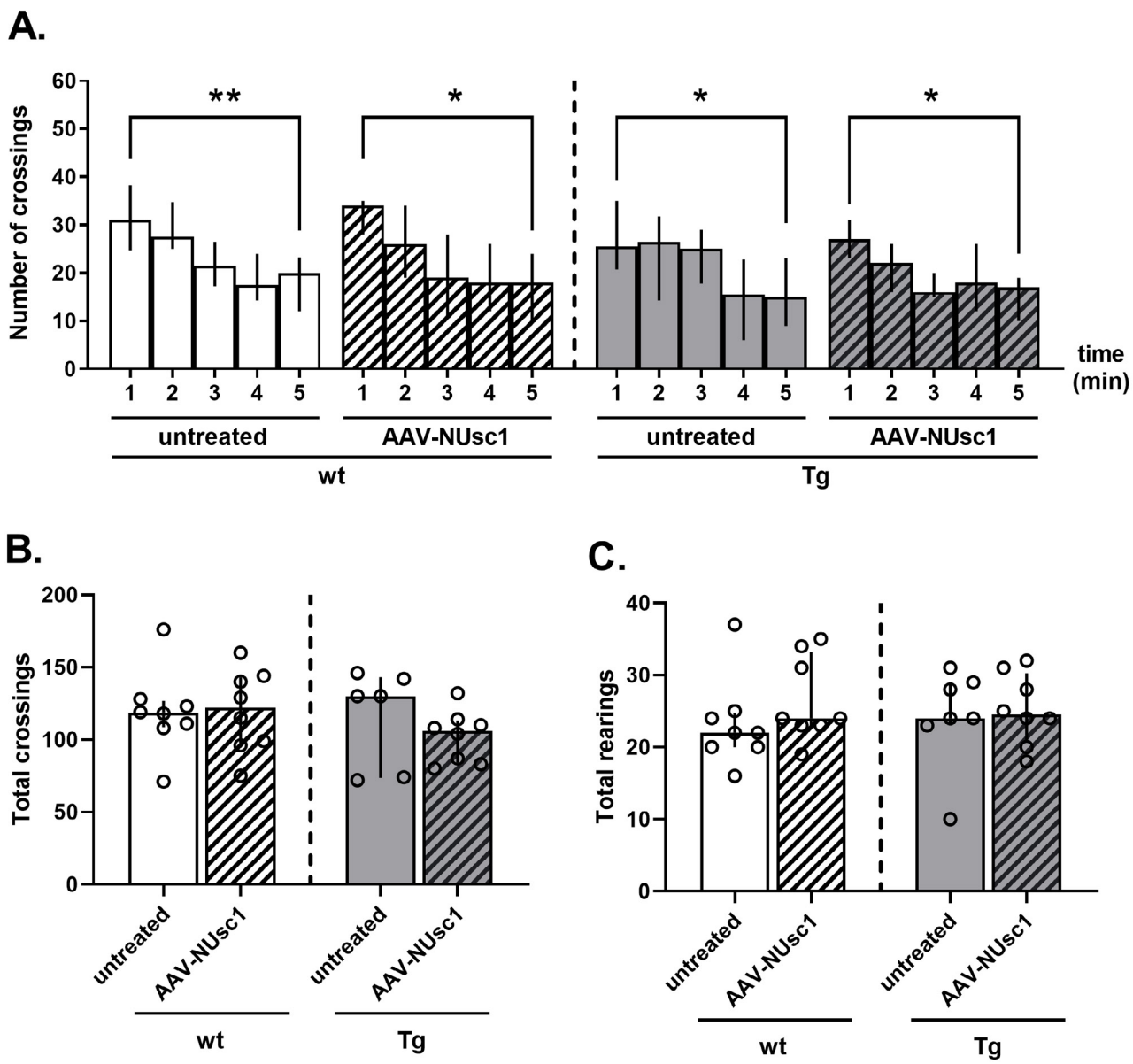
To assess whether animals explored the new objects in the NOR test session for a significantly longer time than in the training session, and longer than what would be expected by chance (50%), Student's t-Test was carried out. To evaluate the latency to approach the objects as well as the total exploration time, Two-way ANOVA with Sidak's multiple comparisons test was employed. Significance was set at $p < 0.05$. All data analysis was performed using the GraphPad Prism program (version 8.0.2).

Results

OF task

All four groups of animals showed a statistically significant lower number of crossings (considered as horizontal exploration) in the last minute compared with the 1st minute of the session (Figure 3A). In addition, they showed a significant decrease in the number of rearings (interpreted as vertical exploration) during the session (Figure 3C), denoting recognition of, and habituation to the environment (habituation and working memory mainly). Total exploration time was not significantly different between all four groups (Figure 3B), and there were no significant differences in between neither the number of crossings nor the number of rearings for the genotypes and treatment assessed during the 5 min session (Figure 3B), denoting that motility and spontaneous exploration were similar.

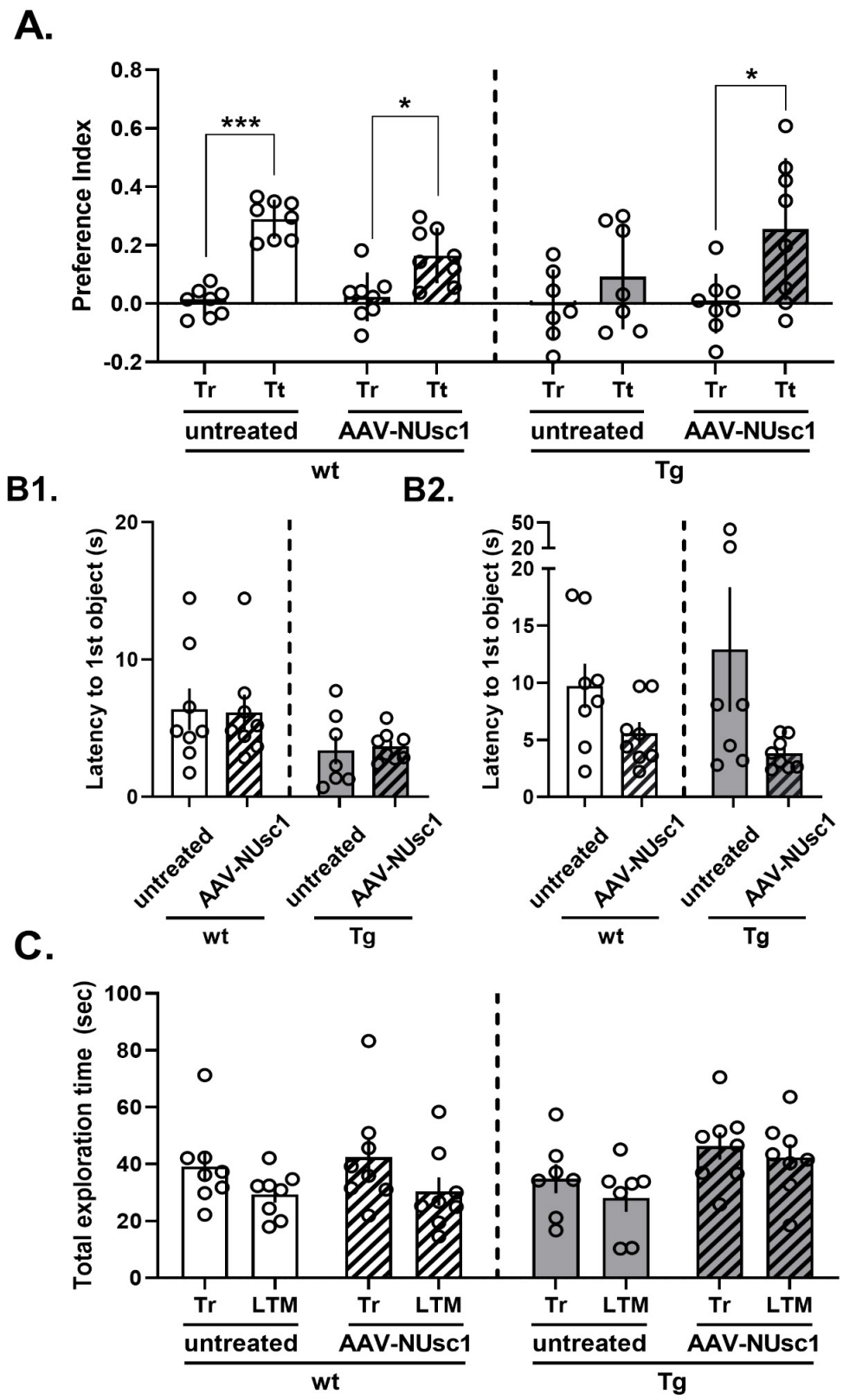
FIGURE 3. *Exploration and habituation to an OF.*



Bar diagrams show median and (25; 75) interquartile ranges for Wt (white bars) and heterozygous Tg+/– McGill-R-Thy1-APP male rats (dark gray bars), of untreated-control (plain bars) and AAV-NUsc1 vector-treated groups (striped bars). A) Progression of number of crossings. Parameters were recorded during each minute of the 5 min session. Differences were compared by Wilcoxon matched pairs signed rank test; $^{*}P < 0.05$, $^{**}P < 0.01$. B) Total number of crossings. C) Total number of rearings. Parameters were recorded during the whole session. B & C: Data were compared by Kruskal-Wallis test. (Wt control $n = 8$; Wt vector treated $n = 8$; Tg control $n = 7$; Tg vector treated $n = 8$).

Source: Authors.

FIGURE 4. *Novel Object Recognition Task.*



A) Preference indexes. B) Latencies to first object: 1. Training session; 2. Test session. C) Total exploration times. Bar diagrams representing mean of performance with standard error, of Wt (white bars) and heterozygous Tg+/– McGill-R-Thy1-APP male rats (dark gray bars) (Tr, training session; Tt; test session), for control (untreated; plain bars) and AAV-NUsc1 vector-treated groups (AAV-NUsc1, striped bars). For B. Paired t test, $^{*}p < 0.05$; $^{***}p < 0.000$. (Wt Ctrl $n = 8$; Wt Vc $n = 8$; Tg Ctrl $n = 7$; Tg Vc $n = 8$). For C, two-way ANOVA followed by Sidak's multiple comparisons, #$p < 0.05$.

Source: Authors.

NOR task

For all groups the Preference Index (PI) of the Training session (Tr) was not significantly different from "0" (chance) with two identical objects A-A', showing that rats similarly explored both objects, indicating no object/side preference (Figure 4A). PI of test session (Tt) was significantly higher than chance (0) and their respective PI at training (Tr), for both control and vector treated Wt groups, since they explored the new object B for significantly longer time than A. In spite of a wider data dispersion for Tg rats than for Wt animals, there was a significant difference to chance, with a significantly higher test PI than training PI for AAV-NUsc1 vector-treated Tg group (Figure 4A). At variance, non-treated Tg rats did not explore the new object B for longer than A; and the PI was not significantly different from chance suggesting that they did not remember the familiar object and could not discriminate the new one. This indicates that Wt and Tg AAV-NUsc1-treated animals recognized and remembered the familiar object, denoting Long-Term Memory (LTM) formation. Both latencies to explore the first object and total exploration time in the training session were similar for Wt and Tg vector treated animal groups, discarding eventual neophobic or anxiety-like behaviors during training (Figure 4B1; Figure 4C). In the test session, two-way ANOVA showed a significant effect of treatment on latencies to start exploring the first object, with longer latency for non-treated Tg rats (control) compared to vector-treated Tg group, while both Wt groups were not statistically different (Figure 4B2) (two-way ANOVA of vector-treatment factor interaction, $p = 0.0267$; without significant difference by Sidak's multiple comparisons, $p = 0.0604$). This suggests a trend toward a shorter latency to start exploring the first object by vector-treated Tg rats, similar to that of Wt animals. There also seemed to be a trend toward a higher total exploration time in the test session for AAV-NUsc1-treated Tg rats compared to control Tg rats (two-way ANOVA also revealed a significant effect of vector treatment factor, with $p = 0.0467$; though without significant differences by Sidak's multiple comparisons test, with $p = 0.064$ for comparison among groups; and $p = 0.0555$ by t test among Tg groups).

Discussion

Memory deficits in McGill-R-Thy1-APP Tg rats

Alzheimer Disease (AD) is likely a multifactorial neurodegenerative pathology, manifested through a progressive deterioration of cognitive functions, with noticeable memory impairment. There is neither cure for that condition, nor effective disease-modifying treatment. Therefore, McGill-R-Thy1-APP rats appear to be a useful model to help clarify AD-like brain alterations related to amyloid burden (Iulita et al., 2014; Leon et al., 2010), as well as to evaluate experimental drug treatments aimed to either delay or halt cognitive decline (Pimentel et al., 2015).

McGill-R-Thy1-APP rats learning and memory deficits were documented by various authors (Galeano et al., 2014; Iulita et al., 2014; Leon et al., 2010); though Long-Term Memory (LTM) has been far less investigated. Habif et al. (2021) have shown that Tg+/– male rats display significant deficits in LTM performance for discrimination of objects at 4 month of age. These LTM deficits appear independent of navigation memory deficits, since it was shown in Leon et al. (2010) that 3-month-old Tg+/– rats performance in the MWM was not different from that of Wt rats. Although in the MWM, 13-month-old Tg+/– rats displayed a slower learning curve compared to Wt rats, those differences in memory retrieval disappeared at the end of an intensive training (5 sessions, one per day); therefore, it seems that spatial memory for navigation was preserved in Tg+/- rats, at least up to 13 months of age (Leon et al., 2010).

It was previously shown that 3-, 4-, 6-month-old Tg+/– male rats display similar habituation to the environment as wild type animals (Habif et al., 2021), though they show LTM impairment at 4 months of age . This was unexpected as it had previously been reported that 3-month-old Tg+/– rats achieved the criteria for recognition of new objects shortly after training (STM) (Iulita et al., 2014). Also, Pimentel et al. (2015) showed that at 9-month, Tg+/+ rats (without discrimination between females and males) were able to learn and remember objects discrimination after 51 min (following the 1st session) (Pimentel et al., 2015), forming a STM of objects. Thus, it allows to discard the possibility that performance could be affected either by a deficit in acquisition or in some sensory modality. Although it cannot be discarded the possibility that the appearance of some neophobic behavior might be interfering with animal interaction with the novel object, affecting NOR performance. This was not the case for STM of NOR in Tg rats at 9 months of age, as reported by Pimentel et al. (2015), nor for memory of the environment in the OF in 5-month-old heterozygous male rats (current results).

Interestingly, España et al. (2010) reported the development of fear behavior consistent with a decrease in exploration and an increase of anxiety at 6 months of age in three transgenic mice models of AD exposed to an intense bright light. Nevertheless, neither signals of anxiety or fear, nor other signals of neophobia were evident in our heterozygous rats, since there was no freezing behavior during the exposure to a totally new OF and in the 1st exposure to novel objects in the NOR task. Moreover, NOR task-STM performance was similar between Wt and Tg+/– rats (Habif et al., 2021).

AAV-NUsc1 vector: The gene therapy tool

The scFv antibody NUsc1 displays very little or no detectable reactivity neither with monomers of Aß peptides nor with insoluble fibrils of amyloid (Sebollela et al., 2017; Velasco et al., 2012), whilst it binds a subpopulation of Aß oligomers. These AßOs attach to the neuron's surface, being highly toxic to synapses. Selles et al. have recently reported that recombinant NUsc1 infusion into the ventricles (i.c.v.) prevented AßOs induced memory impairment in mice and avoided long-term potentiation inhibition in hippocampal slices of mice (Selles et al., 2022). They also showed that NUsc1 could be

expressed from an *ad hoc* developed AAV vector; in cultures of rat hippocampal neurons transfected with that AAV vector, NUsc1 inhibited the binding of AßO to nerve cells surface and impeded the loss of dendritic spines induced by AßOs. Remarkably, the same AAV vector expressing NUsc1 impeded deficits in STM, in young mice that were previously infused i.c.v. with AßOs. Furthermore, aged APPswe/PS1DE9 mice, a well-known AD animal model, were rescued from similar memory deficits by AAV-NUsc1 (Selles et al., 2022).

Here, it has been shown that AAV-NUsc1, rescued LTM for objects in a Tg model of AD-like amyloid brain pathology, the McGill-R-Thy1-APP rat (Figure 4A). A trend toward a longer latency to start exploring the first object has also been found by control Tg rats than by AAV-NUsc1-treated Tg rats (Figure 4B2). This effect could be due to some sort of neophobia, lack of discrimination due to memory impairment, or disorientation. Moreover, it seems to be a trend toward an increase for the total exploration time at the test session for vector-treated Tg rats compared to control Tg rats. The effective LTM recovery, together with the observed trends, suggest that AAV-NUsc1 might have a wide and robust effect reversing cognitive deficit in McGill R-Thy1-APP rats.

A number of clinical trials for AD involving Aß targeted immunoglobulins are currently under way. Recently, the FDA approved an antibody targeting multiple Aß assemblies (Aducanumab) (Sevigny et al., 2016). Also, a monoclonal antibody (Lecanemab) that works by binding to and eliminating toxic Aß protofibrils was recently reported to slow by 27% the cognitive decline in AD patients (Van Dyck et al., 2022). However, considerable controversy remains regarding the actual targets *in vivo* of Aducanumab and of other Aß assemblies-targeted IgGs (monomers, low or high molecular weight oligomers, fibrils and amyloid plaques) and their benefits to cognitive function in AD patients.

NUsc1 artificial antibody specifically targeting Aß oligomers might be a relevant improvement in both target engagement and efficacy. To our knowledge, Selles et al. (2022) is the first report on a gene therapy experimental treatment to achieve sustained expression of a single-chain antibody fragment scFv in neurons, which binds and neutralizes a highly toxic AßO subpopulation; with the advantage of exhibiting a very low reactivity with monomers and fibrils. Hence, it leads to substantial protection from AßO-induced STM deficit in Wt mice, and to a significant reversion of age-dependent STM impairment in APPswe/PS1DE9 mice.

Taking into account that the McGill R-Thy1-APP Tg rat model involving STM and LTM decline has been well characterized by this group and others, treating them at 10-12 weeks of age with the AAV-NUsc1 vector to find out if there would be some protective effect on LTM deficits two months later. Importantly, AAV-NUsc1-treated Tg rats were able to successfully perform the NOR task 24 h after training, denoting recovery of long-term discrimination capacity and LTM formation. Therefore, the current findings comprise another relevant positive result of the AAV-NUsc1 gene therapy approach for AD. Furthermore, the i.c.v. infusion of AAV-NUsc1 and its expression (for two months) had no impact on control Wt rats' performance neither in the OF nor in the NOR test.

Here is a summary of the main advantages of this treatment: 1) A single infusion of AAV-NUsc1 vector led to NUsc1 sustained brain expression. 2) Local expression and secretion from neurons of a scFv devoid of Fc would less likely be able to induce adverse immune/inflammatory processes. 3) NUsc1 specifically targets an AβOs subpopulation that is highly toxic for synapses and neurons (Sebollela et al., 2017; Velasco et al., 2012). 4) Although the AAV-NUsc1 vector has been infused i.c.v., capsid modifications could be introduced to allow intravenous or intranasal administration, leading to an AAV vector that would be able to cross the blood-brain barrier (Fischell & Fishman, 2021).

AAV vectors are commercially available in different serotypes for use in gene therapy (Dunbar et al., 2018), and are currently being tested in clinical trials. Taken together, our results suggest that AAV-NUsc1 represents a significant advance in immuno-gene therapy for AD.

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Declaration of Interests

A patent application covering the use of AAV-NUsc1 in Alzheimer's disease (Compositions and Methods for Treating Alzheimer's Disease) has been approved with the Patent Number US 11,541,086 B2 from Jan 3 2023; filed by Northwestern University. Inventors: W.L.K., D.A.J., S.T.F., A. Sebollela, and M.C.S.

Authors Contribution

Conceptualization: STF and DAJ.
Funding acquisition: STF, ALE and DAJ.
Investigation on single chain selective antibodies: A. Sebollela, WLK, and STF developed the recombinant NUsc1 scFv.
Investigation: DAJ, ALE, A. Salvetti, and STF conceived plasmid constructs and vector development. Investigation and Methodology: ALE, A. Salvetti, MCC: developed the AAV plasmid and vector.

Investigation on the in vitro and in vivo effects of the vector: NCC, MVO, MH, MCS, DAS performed research assays.
Formal analysis of results: NCC, MVO, MH, MCS, DAS, DAJ. Supervision, analysis and discussion of results: DAJ and STF.
Project administration: DAJ and STF.
Writing – Original draft: Preparation and presentation of the initial draft: DAJ, MVO, MH, NCC, STF with contribution from other authors. Writing – Critical review, correction or comments: A. Sebollela, MCS, WLK, ALE, A. Salvetti.

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