Neuropharmacology 113 (2017) 434–444

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

DMSO modulates CNS function in a preclinical Alzheimer's disease model

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ARTICLE INFO

Article history:
Received 18 April 2016
Received in revised form
15 October 2016
Accepted 18 October 2016
Available online 20 October 2016

Keywords:
DMSO
Aβ
Spines
Spatial memory
Anxiety
Odor habituation

ABSTRACT

DMSO has a widespread use as a vehicle for water-insoluble therapeutic drug candidates but may also exert disease-relevant pharmacological effects by itself. However, its influence on the CNS has hardly been addressed. Here we examined the brain structure and function following chronic exposure to low DMSO dose at a paradigm with flawed synaptic connectivity in a preclinical transgenic mouse model for Alzheimer's disease (APP_{SDL} mice). DMSO treatment increased spine density in a region-specific manner in the hippocampus of APP_{SDL} mice *ex vivo* and *in vivo*. Moreover, DMSO exhibited clear influence on the behavior of this mouse line by enhancing hippocampal-dependent spatial memory accuracy, modulating hippocampal-independent olfactory habituation and displaying anxiolytic effect. Despite that most of the action of DMSO was observed in animals with elevated Aβ levels, the drug did not exert its function via decreasing the oligomeric Aβ species. However, challenging organotypic hippocampal slice cultures with NMDA receptor antagonist MK-801 recapitulated the effect of DMSO on spine density, indicating a tuning influence of DMSO on receptor signalization. Our findings demonstrate that DMSO should be considered as a true bioactive compound, which has the potential to be a beneficial adjuvant to counteract Aβ-mediated synaptotoxicity and behavioral impairment.

1. Introduction

Dimethyl sulfoxide (DMSO) is widely used in preclinical and clinical research, as it enhances the entrance of water-insoluble drug candidates into the central nervous system (Broadwell et al., 1982). An ideal vehicle is expected to be biocompatible, reliable, and devoid of biological actions *per se*. However, within the last decades, pharmacological effects of DMSO have been reported, and some of its biological activities have shown to be beneficial against various pathologies (Santos et al., 2003; Yu and Quinn, 1994).

As for many pharmaceutical compounds, the mechanism of action of DMSO appears to be dependent of both the treatment duration and the dose employed. While a consensus exists that DMSO doses of 10% or higher are toxic *in vivo* (Hanslick et al., 2009), the effects of low DMSO doses both *in vitro* (Hanslick et al., 2009; Kahler, 2000) and *in vivo* are still controversially judged (Galvao et al., 2014). Intriguingly, particularly low DMSO concentrations appear to profoundly influence neural network activities (Nakahiro et al., 1992; Nasrallah et al., 2008; Obregon et al., 2005; Tamagnini et al., 2014; Tsvyetlynska et al., 2005). Moreover, chronic DMSO treatment has been shown to attenuate spatial memory deficits induced by ischemia (Farkas et al., 2004) suggesting that prolonged DMSO administration has protective actions for the central nervous system (CNS) under pathological conditions.

Memory deficits have also been associated with abnormal Aβ levels in humans as well as in transgenic mice mimicking amyloidosis-related disorders (Brouillette et al., 2012; Lesne et al., 2006; Shankar et al., 2008). Synaptic failure is considered to be the immediate cause of cognitive decline and memory dysfunction in Alzheimer's disease (AD) (Dorostkar et al., 2015). Dendritic spines are anatomical substrates for memory storage (Tackenberg et al., 2009) and spine loss represents an early feature of AD (Penazzi et al., 2016; Selkoe, 2002), occurring long before the pathological plaque deposition of Aβ. Previous studies showed that

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http://dx.doi.org/10.1016/j.neuropharm.2016.10.020

NMDA receptors (NMDARs) are involved in Aβ-mediated synaptic deficits (Lacor et al., 2007; Shankar et al., 2007) and a drug acting as NMDAR antagonist is currently used to attenuate clinical symptoms during mild cognitive impairment (MCI) (Emre et al., 2010). Elevated Aβ levels further correlate with anxiety-like behavior (Lee et al., 2004; Porter et al., 2003) or atypical olfactory-behavior (Alvarado-Martinez et al., 2013; Cheng et al., 2013; Wesson et al., 2010). These pathological phenotypes represent also relevant criteria for the early diagnosis of neuropathology, such as AD or Parkinson's disease (PD) (Baba et al., 2012; Berendse and Ponsen, 2006; Jimbo et al., 2011).

To date, only anxiolytic effects of DMSO have been reported (Matheus et al., 1997) but effects on memory have not been studied. Therefore the present study was designed to determine the potential effect of chronic, low, non-toxic DMSO dose on hippocampal plasticity and hippocampal-dependent behavior, and whether this treatment may be beneficial to rescue Aβ-mediated presymptomatic CNS alterations. We first aimed to analyze the consequence of DMSO (0.01%) treatment on spine density using *ex vivo* hippocampal slice cultures prepared from APP_{SDL} mice and non-transgenic controls. APP_{SDL} mice are a model for the long prodromal phase of AD, since they develop Aβ plaques only after 18 months of age (Blanchard et al., 2003). Furthermore, using this model, we also determined whether spine modulation by DMSO involves NMDARs. Secondly, we hypothesized that the genotype- and region-specific changes observed *ex vivo* may also occur in the hippocampus of old male transgenic and non-transgenic mice after a prolonged treatment with a low DMSO (1%) dose. At last, we assessed whether such plastic changes may influence hippocampus-dependent and -independent behavior, as well as basal home cage and spatial memory-induced neuronal activities.

2. Material and methods

2.1. Animals

General health assessment and behavioral testing were carried out on male transgenic APP_{SDL} mice and their wild-type littermates. Mice were 17–18 months old at the start of the testing. In addition, adult 7–8 months old male mice from both genotypes were incorporated into the behavioral assessment to evaluate the behavioral performances of the old mice. For dendritic spine analysis, double heterozygous transgenic EGFP/APP_{SDL} mice were generated by crossing transgenic homozygous EGFP mice (GFP M line) (Feng et al., 2000) with heterozygous APP_{SDL} mice. Mice were individually housed per cage in a sterile room with 12/12-h light-dark cycle. The room was maintained under constant temperature and humidity conditions. Water and food were available *ad libitum*.

All animal studies were conducted in accordance to National Institutes of Health guidelines and German animal care regulations and approved by the ethical committee on animal care and use of Lower Saxony, Germany.

2.2. Drug treatment

Dimethyl sulfoxide (Merck KGaA, Darmstadt, Germany) was provided to mice *ad libitum* at 1% (v/v), mixed in water. The drug treatment was administrated during 14 days prior behavioral testing and was maintained all along the behavioral procedures, thus ending after 20 days of treatment.

2.3. Organotypic hippocampal slice cultures and Sindbis virus infection

Organotypic hippocampal slice cultures were generated from postnatal days 7 (P7) mouse pups (Fig. 1B) that were cultured, infected with Sindbis virus expressing EGFP, and fixed as previously described (Bakota et al., 2012; Sundermann et al., 2012). For treated conditions, 0.01% DMSO, 50 μM MK-801 or both (Sigma Aldrich, Munich, Germany) were applied in low-serum Nb-N1 medium. For high-resolution imaging, pyramidal neurons located in the hippocampal regions CA1 and CA3 were selected.

2.4. Spine analysis in the mouse brain

After 14 days of treatment mice were transcardially fixed with 4% paraformaldehyde in PBS (w/v). The brains were removed, post-fixed overnight at 4 °C and then transferred to PBS. Hundred μm thick coronal sections were sliced on an automated vibratome (Leica VT1200S, Leica Biosystems), floating sections were collected, mounted with a Confocal-matrix (Micro-Tech-Lab, Graz, Austria) and coverslipped. For imaging, neurons were randomly selected from the dorsal hippocampus (approximately −1.58 mm from Bregma) for each hippocampal subfield: proximal CA3 (e.g. close to the DG), CA2 and intermediate part of CA1 (Supplemental Fig. S2). Borders of the CA regions were defined with the help of the nomenclature by Franklin and Paxinos (Franklin and Paxinos, 2007) and Kohara and collaborators (Kohara et al., 2014).

2.5. Spine imaging and quantification

For both fixed organotypic and mouse brain slices, image acquisition and quantification of EGFP expressing dendritic segments were performed as described earlier (Sundermann et al., 2012; Tackenberg and Brandt, 2009).

2.6. Design and behavioral procedures

To minimize the number of mice used in the experiments, the same set of adult and old male mice from both genotypes were used for all behavioral testing. The testing began with an elevated-plus maze test on day 14, followed by the olfactory behavior test on day 15 and the Barnes maze test that started on day 17 of the DMSO treatment period. Except for mice that did not participate in the behavioral tasks (i.e., home cage condition), mice were daily handled during 14 days, prior to the start of the behavioral procedures, to habituate them to the experimenter blinded to the treatment condition and the genotype of mice.

See Supplementary information for the behavioral procedures in detail.

2.7. Immunohistochemistry

c-fos immunohistochemistry was performed on coronal brain sections of 50 μm thickness from 17 to 18 months old wild-type and APP_{SDL} mice that performed the Barnes maze task and from additional mice of both genotypes that did not participate in the task (i.e., home cage, basal condition).

Mouse brain sections were produced from brain tissue blocks neuroanatomically defined as follows: the anterior part of the dorsal hippocampus is between −1.58 mm and −1.74 mm from Bregma; the lateral parietal cortex (LPar) and the retrosplenial granular cortex (RSGc) between −1.46 mm and −2.06 mm.

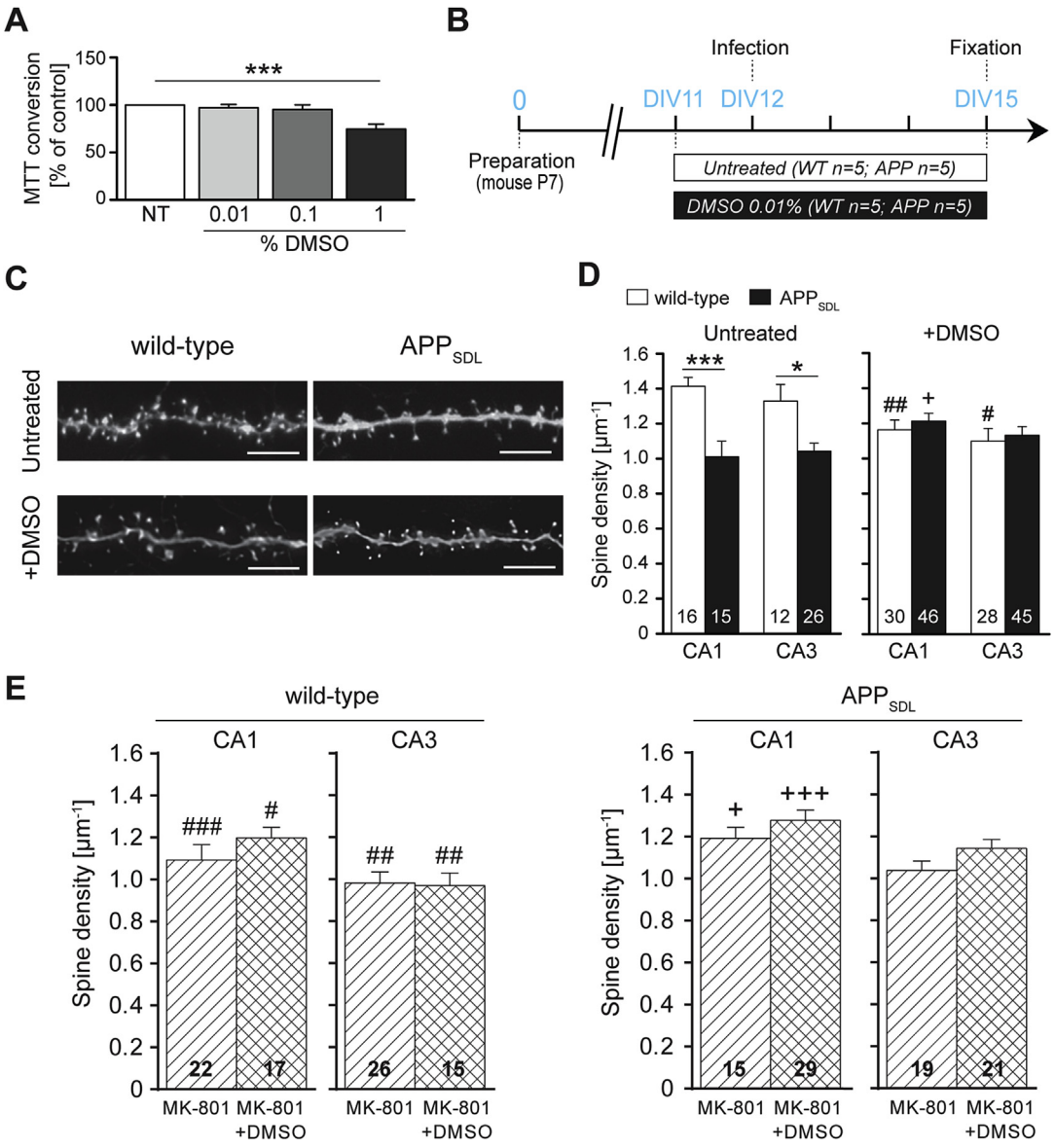

Fig. 1. Chronic DMSO treatment modulates spine density in a genotype-specific manner in organotypic hippocampal slice cultures. (A) PC12 cell viability assay using MTT reduction method with increasing DMSO concentrations. One way ANOVA showed significantly decreased cell viability after 4 days treatment with 1% DMSO compared to untreated culture. N = 4 independent experiments run in triplicates. (B) Time line of the experimental design. Organotypic hippocampal slices were treated with 0.01% DMSO, 50 µM MK-801 or both during 4 days. (C) Representative high-magnification micrographs of dendritic segments from CA1 neurons, imaged in both untreated and DMSO treated slices of wild-type and APP_{SDL} mice. Scale bar, 5 µm. (D) Decreased spine density on dendritic segments from untreated APP_{SDL} slices was significantly attenuated by DMSO in a region-specific manner, whereas the treatment reduced spine density on dendrites from wild-type mice in both hippocampal regions; CA1 and CA3 regions, n = 5 mice/group. (E) Blockage of NMDAR with the antagonist MK-801 alone or MK- 801/DMSO decreased spine density from apical dendritic segments imaged from wild-type neurons located in the CA1 and CA3 regions but specifically increased spine density on dendritic segments from APP_{SDL} neurons located in CA1 region. N = 3–6 mice/group. Numbers in bars represent the number of dendritic segments analyzed in each experimental condition. All values are represented as mean ± S.E.M. *P < 0.05 and ***P < 0.001, (D and E) wild-type versus APP_{SDL}, same experimental condition. #P < 0.05, ##P < 0.01 and ###P < 0.001, significantly decreased compared to untreated, same genotype. + P < 0.05 and +++ P < 0.001, significantly increased compared to untreated, same genotype. Factorial ANOVA, post-hoc Fisher's LSD for multiple comparisons. For detailed statistics see Supplementary Tables 1 and 2.

2.8. c-fos imaging and quantification

Quantification of the c-fos positive cell density was performed on every third coronal section using the Machine Learning Based Image Segmentation (MLBIS) method (Penazzi et al., 2014). See Supplemental information and Supplemental Fig. S3 for details.

2.9. Other methods and complementary information

For a detailed description of the methods regarding animal lines, general health assessment, oligomeric Aβ amount determination, MTT assay, immunohistochemistry and quantification, see Supplementary information.

2.10. Statistical analysis

Statistical analysis was conducted using Statistica software (StatSoft Inc, Tulsa, OK, USA). One-way, factorial and repeated-measures ANOVA as well as Student's t-test were used in this study, followed by either post-hoc Fisher's LSD for multiple comparisons, Tukey's test or Newman-Keuls. Detailed information of the test applied is described in Supplementary information. All

results are expressed as mean ± S.E.M. The level of significance is set at $P < 0.05$.

3. Results

3.1. DMSO modulates dendritic spine density in a genotype-specific manner in ex vivo hippocampal organotypic cultures

We first determined a potential toxic effect of DMSO treatment using a MTT reduction assay, an indicator of cell viability. As a neural model, PC12 cells were exposed to 0.01%, 0.1% or 1% of DMSO during 4 days (Fig. 1A). MTT conversion was significantly altered at 1% DMSO but not at 0.1% or 0.01% (Fig. 1A, Supplementary Table 1). To explore the effect of non-toxic dose of DMSO on dendritic spine density, 0.01% DMSO was applied for 4 days to organotypic hippocampal slice cultures from wild-type and APP_{SDL} mice (Fig. 1B). In the absence of DMSO, spine density was significantly reduced on dendritic segments from neurons of APP_{SDL} mice in both CA1 and CA3 regions (Fig. 1C and D, left, Supplementary Table 2), in agreement with previous results (Tackenberg and Brandt, 2009). DMSO treatment increased spine density in the CA1 region of APP_{SDL} mice and decreased spine density in both hippocampal regions of wild-type mice (Fig. 1C and D, right, Supplementary Table 2). This suggests that chronic, low, non-toxic dose of DMSO modulates spines in a region- and genotype-specific manner, abolishing the differences in hippocampal spine density between APP_{SDL} and wild-type mice *ex vivo*.

Previous studies reported that DMSO suppresses NMDA-induced ion currents and Ca^{2+} influx (Lu and Mattson, 2001) therefore we tested the effect of MK-801, a non-competitive NMDA receptor antagonist on hippocampal spine density of wild-type and APP_{SDL} mice (Fig. 1E, Supplementary Table 2). Treatment of slice cultures with 50 μM MK-801 during 4 days recapitulated the decrease of spine density in CA1 and CA3 regions of wild-type mice and the increase in spine density in the CA1 region of APP_{SDL} mice, closely mimicking the effect of DMSO. MK-801 together with DMSO did not result in further changes. This finding suggests that DMSO modulates spine density via its action on NMDA receptors.

3.2. DMSO treatment modulates hippocampal spine density in a genotype-specific manner in vivo

We next aimed to evaluate whether systemic administration of chronic, low, non-toxic DMSO also influences hippocampal spine density in old (17–18 M) wild-type and APP_{SDL} male mice *in vivo*. At this age, analysis of hippocampal and neocortical regions from APP_{SDL} brain did not reveal the presence of Aβ plaques (data not shown), confirming this mouse line as a presymptomatic AD model. The distinct mossy fiber terminal layer is one of the main anatomical references to identify the CA2 hippocampal subfield. Due to the visibility of the entire mossy fiber tract in the GFP M mouse line (Feng et al., 2000) we could include also the analysis of the CA2 hippocampal pyramidal cells. To rule out a potential toxic effect of chronic DMSO, water intake as well as body and organ weights were measured. Body weight of untreated and treated mice from both genotypes remained stable during the 14 days of treatment (Supplemental Fig. S1A). Except for the significant difference occurring between untreated and treated wild-type mice in the weight of the right kidney, no further changes were observed in organ weight. Daily water intake of mice from both genotypes was not influenced by DMSO treatment (Supplemental Figs. S1B and C). These data strongly suggest that chronic treatment with 1% DMSO induces neither toxic nor diuretic effects in old mice.

The same DMSO treatment was provided to old (17–18 M) EGFP and EGFP/APP_{SDL} male mice (Fig. 2A). Dendritic segments from CA1, CA2 and CA3 pyramidal neurons were imaged in the dorsal hippocampus (Supplemental Fig. S2, Fig. 2B). In the absence of DMSO, spine density was decreased on dendrites of CA1 and CA2 neurons from EGFP/APP_{SDL} mice compared to control EGFP mice (Fig. 2C, Supplementary Table 3). DMSO treatment did not alter spine density on CA1 neurons but increased spine density on dendrites of CA2 and CA3 neurons of EGFP/APP_{SDL} mice (Fig. 2C, Supplementary Table 3). Therefore paralleling our previous observations from *ex vivo* cultures, *in vivo* DMSO treatment results in a region- and genotype-specific spine change that tends to abolish Aβ-mediated decrease in spine density. However, the effects of DMSO differ between the two systems. One aspect is that DMSO reduces spine

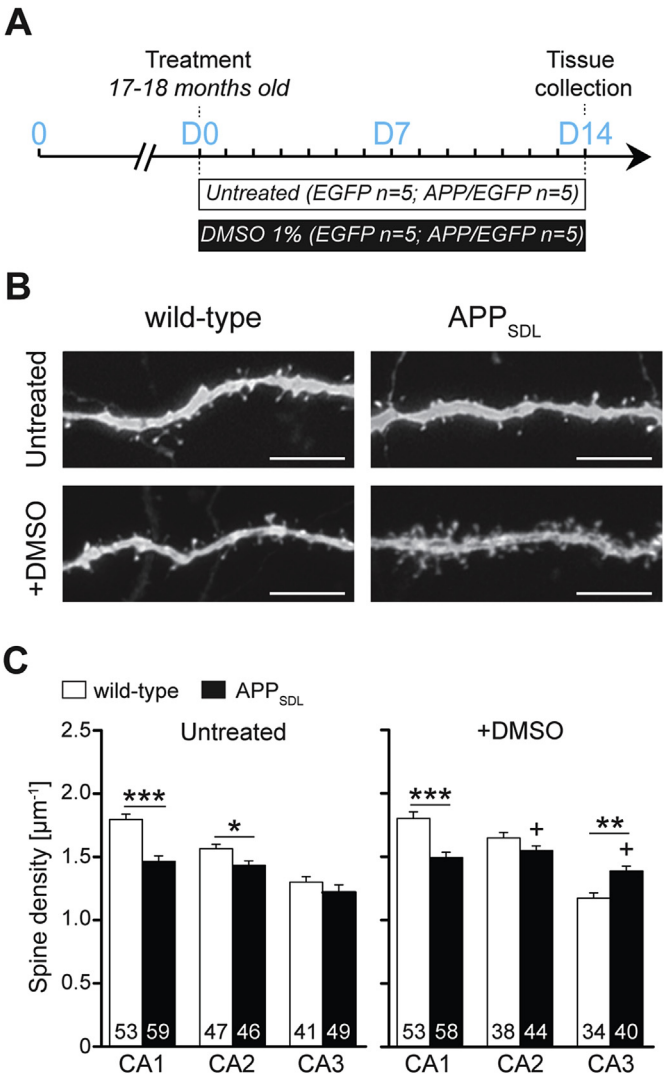

Fig. 2. Chronic DMSO treatment modulates spine density in a genotype- and region-specific manner *in vivo*. (A) Time line of the experimental design. EGFP and EGFP/APP_{SDL} male mice (17–18 months) received *ad libitum* either water (untreated) or 1% DMSO in drinking water during 14 days. (B) Representative high-magnification micrographs of dendritic segments from CA3 neurons imaged from untreated and DMSO treated wild-type and APP_{SDL} mice. Scale bar, 5 μm (C) DMSO treatment significantly increased spine density on neurons from APP_{SDL} mice in CA2 region and on CA3 neurons compared to their untreated counterpart, n = 5 mice/group. No significant change occurred on APP_{SDL} neurons from CA1 region that displayed reduced spine density compared to wild-type mice under untreated condition. Numbers in bars represent the number of dendritic segments analyzed in each experimental condition. All values are represented as mean ± S.E.M. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, wild-type versus APP_{SDL}, same experimental condition. + $P < 0.05$, significantly increased compared to untreated, same genotype. Factorial ANOVA followed by post-hoc Fisher's LSD for multiple comparisons. For detailed statistics see Supplementary Table 3.

density on dendrites of control mice *ex vivo*, whereas in the *in vivo* experiment, this does not seem to happen. The difference may be caused by the progressive age-related changes of the plastic properties of neurons or the respective treatment conditions.

3.3. DMSO treatment tends to improve spatial memory accuracy

Potential consequences of chronic DMSO treatment on hippocampal-dependent spatial memory were tested in old (17–18 M) wild-type and APP_{SDL} mice using the Barnes maze task (Barnes, 1979). Untreated adult (7–8 M) mice from both genotypes were included as additional reference groups for the evaluation of the performance of old mice. Adult and old mice of both genotypes successfully learned to locate the target hole, as demonstrated by the gradual decrease in spatial errors (Fig. 3A, Supplementary Table 4) and escape latency (Fig. 3B, Supplementary Table 4). A probe test was conducted one day after the last acquisition day. All mice retrieved the target location, as indicated by their selective preference for the target hole compared to chance level (Fig. 3C, Supplementary Table 4). Adult wild-type and APP_{SDL} mice accurately remembered this position as they visited significantly more the target hole than the immediately adjacent holes. In contrast, old untreated wild-type and APP_{SDL} mice displayed a broader search pattern that extended to the immediately adjacent holes, indicating a non-specific effect of aging on spatial memory accuracy. Interestingly, drug effect in old mice was close to reach significance ($P = 0.08$, see Supplementary Table 4). Detailed analyses confirmed that DMSO treated APP_{SDL} mice significantly focused their search on the target hole whereas vehicle treated APP_{SDL} mice evenly explored target and neighboring holes. This suggests that DMSO treatment tends to increase spatial memory accuracy in APP_{SDL} mice.

We further examined whether DMSO treatment modulates brain activation during spatial memory retrieval using the neuronal activity marker c-fos (Bullitt, 1990). Semi-automatic detection and quantification of c-fos-positive cell nuclei (Penazzi et al., 2014) (Supplemental Fig. S3) was performed in the DG, CA3 and CA1 regions of the dorsal hippocampus and in the lateral parietal (LPar) and retrosplenial granular (RSGc) cortices of untreated and treated old wild-type and APP_{SDL} mice, both in basal home cage condition and after spatial memory retrieval. Under basal condition, APP_{SDL} mice displayed hyperactivated DG and CA1 neurons and reduced neuronal activation in the CA3 region (Fig. 4A, Supplementary Table 5). DMSO treatment normalized this Aβ-mediated alteration of basal neuronal activation in CA1 and DG to the level of wild-type mice. In the neocortex, no differences were observed in basal neuronal activation in the regions analyzed. However, DMSO increased neuronal activation in the RSGc of wild-type mice (Fig. 4 B, Supplementary Table 5). Taken together, DMSO showed a region- and genotype-specific inhibitory effect on neuronal activation

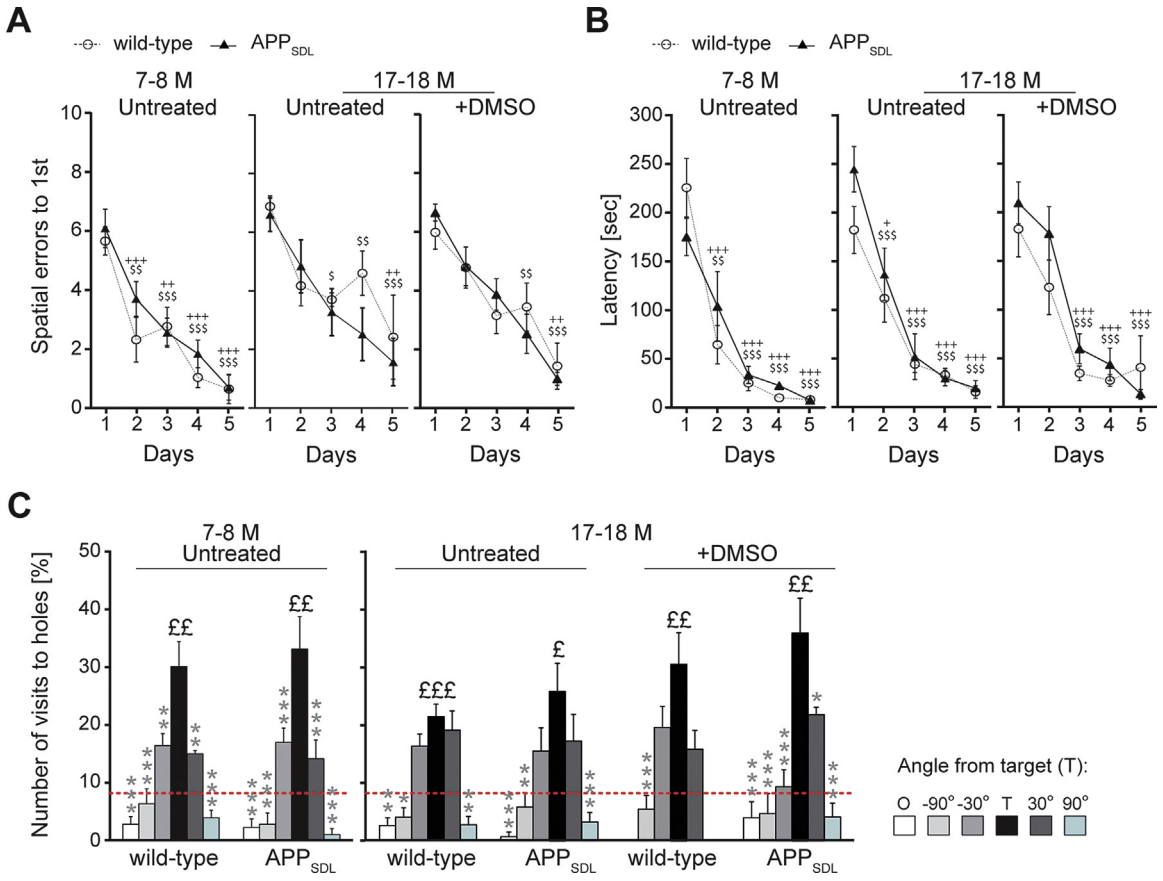

Fig. 3. Chronic DMSO treatment modulates spatial memory accuracy and retrieval induced neuronal activation. (A–C) Barnes maze test. (A) Across training sessions, mice showed significant decrease in number of spatial errors before the first target exit hole visit and (B) reduced escape latency. (A,B) Data are expressed as daily average of three trials. Wild-type mice: + $P < 0.05$, ++ $P < 0.01$ and +++ $P < 0.001$, versus day 1; APP_{SDL} mice: $ $P < 0.05$, $$ $P < 0.01$ and $$$ $P < 0.001$, versus day 1. (C) During the probe trial, adult mice presented a specific visit of the target hole. Old, untreated wild-type and APP_{SDL} mice showed a significantly decreased precision in their search pattern and DMSO treatment specifically improved the memory accuracy performance of old APP_{SDL} mice compared to their untreated counterparts. Opposite (O) and target (T) holes. Chance level is indicated by the red line. All values are represented as mean ± S.E.M. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, versus target exit hole. £ $P < 0.05$, ££ $P < 0.01$ and £££ $P < 0.001$, versus chance level. (A–C) Adult untreated: wild-type ($n = 6$) and APP_{SDL} mice ($n = 7$). Old treated and untreated: wild-type and APP_{SDL} mice ($n = 7$ mice/group). Repeated measures ANOVA followed by post-hoc Newman-Keuls multiple comparison test. (C) For chance level, Student's t-test. For detailed statistics see Supplementary Table 4.

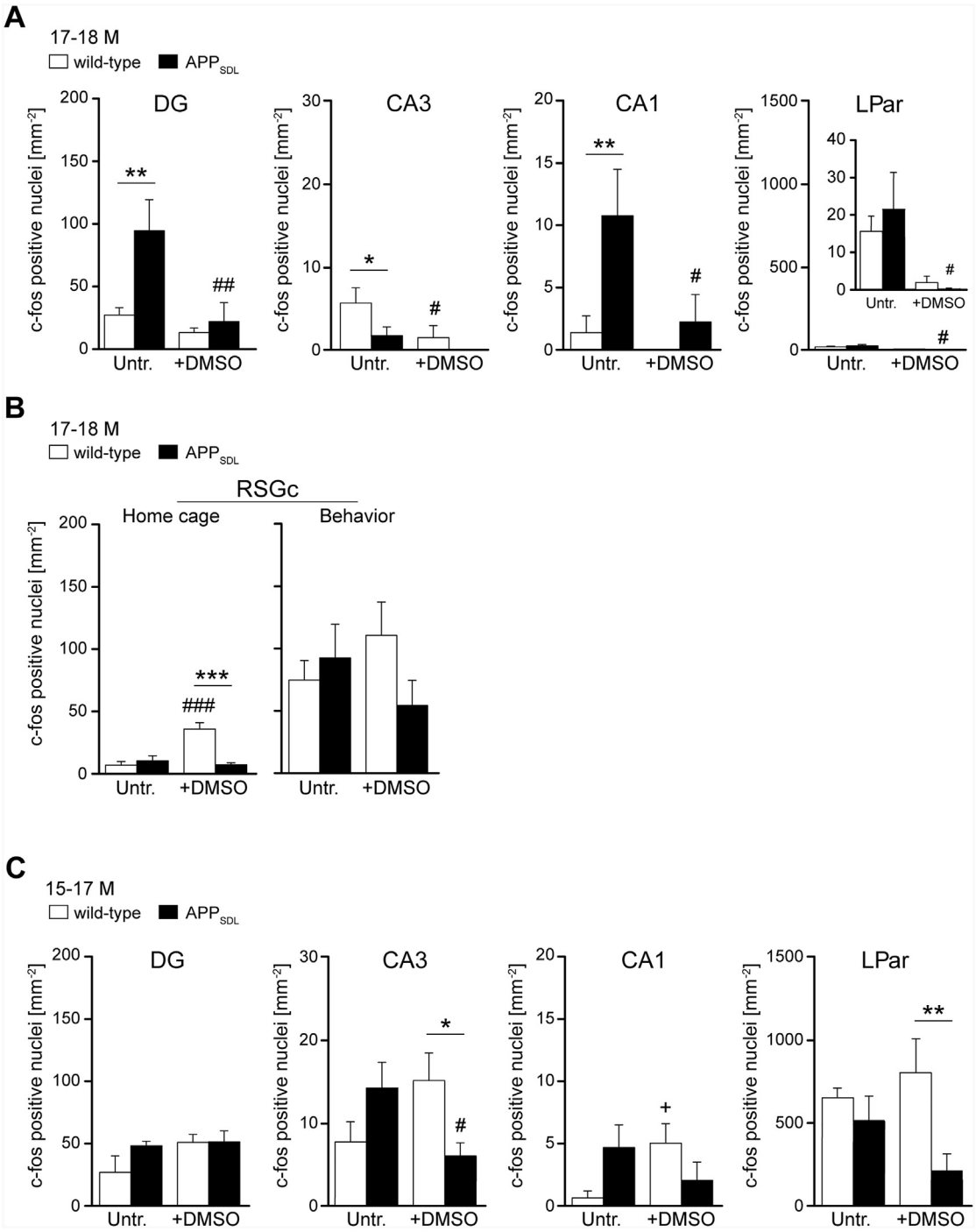

Fig. 4. Chronic DMSO treatment modulates neuron activation in a genotype- and region-specific manner. (A) DMSO treatment affects the expression of c-Fos under basal home cage condition in a genotype-specific manner in the DG, CA3, CA1 of the dorsal hippocampus and LPar. Insert at right panel shows rescaled graph of the same. (B) DMSO treatment also affects c-fos positive cell nuclei in the retrosplenial granular cortex (RSGc) under basal home cage condition (A,B left) n = 4–5 mice/group. (B, right) n = 6–7 mice/group. *P < 0.05, **P < 0.01 and ***P < 0.001, wild-type versus APP_{SDL} mice, same experimental condition. #P < 0.05, ##P < 0.01 and ###P < 0.001, significantly decreased compared to untreated same genotype. (C) DMSO treatment modulates spatial memory retrieval-related c-fos positive cell density in a region and genotype-specific manner by decreasing c-fos density in the CA3 region of APP_{SDL} mice, by increasing c-fos density in the CA1 region of wild-type mice and by inducing a significant difference between both genotypes in the LPar region. No significant effect occurred in the DG. Old treated and untreated wild-type and APP_{SDL} mice (n = 6–7 mice/group). All values are represented as mean ± S.E.M. *P < 0.05 and **P < 0.01, wild-type versus APP_{SDL}, same experimental condition. #P < 0.05, significantly decreased and + P < 0.05 significantly increased compared to untreated, same genotype. Factorial ANOVA followed by post-hoc Fisher's LSD for multiple comparisons. For detailed statistics see Supplementary Table 5.

abolishing Aβ-mediated neuronal hyperactivation in the hippocampus of APP_{SDL} mice.

The spatial memory retrieval resulted in comparable neuronal activation between wild-type and APP_{SDL} mice in all hippocampal and neocortical regions analyzed (Fig. 4B and C, Supplementary Table 5) suggesting that Aβ-mediated alteration of neuronal activation was specific to baseline condition. DMSO treatment significantly reduced neuronal activation in the CA3 region of APP_{SDL} mice but raised the level of neuronal activity in the CA1 region of wild-type mice compared to untreated mice from the same genotype

(Fig. 4C, Supplementary Table 5). In the LPar, DMSO induced a significant difference in c-Fos expression between wild-type and APP_{SDL} mice that was not apparent in untreated condition. No changes were induced by DMSO in the RSGc of mice from both genotypes (Fig. 4B, Supplementary Table 5). Therefore, chronic DMSO treatment modulates both basal and spatial memory retrieval induced neuronal activation in a region- and genotype-specific manner.

3.4. DMSO treatment restores olfactory sensitivity

Influence of chronic DMSO treatment on olfactory behavior was determined in old (17–18 M) wild-type and APP_{SDL} male mice utilizing an odor habituation task (Fig. 5A). Again, untreated adult (7–8 M) mice served as an additional control for the evaluation of the performance of old mice. No difference was observed in nose poke (NP) duration between the two genotypes of adult mice. However, all along the odor sampling period, NP duration of old APP_{SDL} mice was significantly higher (Fig. 5B, Supplementary Table 6), a difference abolished by DMSO treatment, which normalized NP duration to the level of age-matched wild-type mice and adult mice of the same genotype. In contrast, NP number was only increased in old wild-type mice, which did not differ any longer from the level of adult mice after DMSO treatment (Fig. 5C, Supplementary Table 6). We examined the investigation time during the first five NPs, as an indicator of odor habituation. Only old, untreated APP_{SDL} mice displayed a delayed habituation, highlighted by the absence of a gradual decrease between the first and fourth NP duration. Interestingly, for the first three NPs, the olfactory performance of old APP_{SDL} mice was normalized to the level of old wild-type mice by chronic DMSO (Fig. 5D, Supplementary Table 7), indicating that Aβ-induced decreased olfactory habituation was attenuated by DMSO treatment.

3.5. DMSO treatment attenuates anxiety-related behavior

DMSO has been previously reported to display anxiolytic properties (Matheus et al., 1997). Whether chronic DMSO treatment modulates anxiety-like behavior in old wild-type and APP_{SDL} male mice was determined using the elevated-plus maze test. As before, untreated adult (7–8 M) mice served as reference control for the evaluation of the performance of old mice. Adult APP_{SDL} mouse behavior was indistinguishable from wild-type mice in terms of distance travelled (Fig. 6A, Supplementary Table 8), number of entries (Fig. 6B, Supplementary Table 8), percentage of entries in open arms (Fig. 6C, Supplementary Table 8) and percentage of time spent in open arms (Fig. 6D, Supplementary Table 8). In contrast, old APP_{SDL} mice significantly differed from old wild-type mice with respect to the distance travelled, the number of entries and the percentage of time spent in open arms, indicating a genotype-specific effect of aging on anxiety-like behavior. DMSO treatment induced a significant decrease of the number of entries for old wild-type mice and abolished the differences in the percentage of entries and time spent in open arms for APP_{SDL} mice, which furthermore did not differ from chance level anymore. In contrast, time spent in open arms by old treated wild-type mice was significantly lower than chance level after DMSO treatment. These data suggest that the age-related increase of anxiety-like behavior in old APP_{SDL} animals is attenuated by chronic DMSO treatment. On the opposite, DMSO treatment may display anxiogenic effects in old wild-type animals.

4. Discussion

DMSO has already been tested in several clinical trials more than 3 decades ago (Swanson, 1985), and some of its biological activities have shown to be beneficial against various pathologies, such as dermatologic, urinary, pain or inflammatory disorders (Santos et al., 2003; Yu and Quinn, 1994). Nevertheless the effect on the CNS structure and function had been remarkably ignored. In the present study, we demonstrate for the first time that chronic treatment with low, non-toxic DMSO dose displays region- and genotype-specific modulating actions on (i) hippocampal spine density, both *ex vivo* and *in vivo*, (ii) hippocampal-dependent and independent behavior, and (iii) neuronal activity, abolishing the differences observed between old APP_{SDL} and wild-type male mice.

After intravenous and cutaneous application, DMSO shows a fast (within few hours) and approximately equal distribution in soft tissues, including brain. There is no evidence for accumulation of DMSO in the brain tissue and the elimination occurs within 12–36 h in experimental animals (Denko et al., 1967; Kolb et al., 1967). However at high doses, DMSO presents diuretic properties (Suominen and Uusi-Penttila, 1968), resulting in altered water consumption. Here, we have shown that chronic treatment with 1% DMSO neither modified daily water intake nor resulted in a dramatic change in organ weight, suggesting that 14 days of chronic treatment with 1% DMSO is generally not harmful for old mice. Low concentrations of DMSO have been shown to induce neurochemical (Matsumoto et al., 1985; Nasrallah et al., 2008; Obregon et al., 2005) and neuroelectrical changes (Lu and Mattson, 2001; Nakahiro et al., 1992; Tamagnini et al., 2014; Tsvyetlynska et al., 2005), which consequently might result in plastic synaptic changes. Yet, to date structural changes in synaptic connectivity and their potential effect on behavior after systemic, chronic administration of DMSO were barely investigated.

Under physiological conditions, naturally secreted Aβ peptides are known to regulate CNS function (Cirrito et al., 2005). However, increased concentrations of soluble Aβ (Wei et al., 2010) cause a marked decrease in spine density, which has been shown to be reversible (Smith et al., 2009). In our transgenic APP_{SDL} mouse model, Aβ resulted in a region-specific decrease of spine density on hippocampal neurons, in both organotypic slice cultures and in the brain, which was region-specifically counteracted by DMSO treatment. The intrinsic properties of neurons from these hippocampal subfields have been reported to differ in many aspects, including their NMDA and AMPA receptor content (Martens and Wree, 2001), determining their sensitivity to Ca^{2+} (Grishin et al., 2004). Low DMSO dose has been shown to decrease Ca^{2+} responses to glutamate, protecting hippocampal neurons from excitotoxic death (Lu and Mattson, 2001). We and others have shown that Aβ-induced spine pathology involves NMDA receptor activation (Tackenberg and Brandt, 2009). Obtaining memory facilitating effects from an NMDA receptor antagonist may sound counterintuitive. However, improvement of memory performances by a NMDA receptor antagonist had been repeatedly shown in a wide range of disease models associated with memory disturbances (Marszalek-Grabska et al., 2016; Scholtzova et al., 2008) but also in non-diseased animals depending on the type of task, timing and dose provided (Mondadori and Weiskrantz, 1993; Puma and Bizot, 1998). Here we provide evidence suggesting that spine density modulation by DMSO is indeed achieved by its action on NMDA receptors, since an NMDAR antagonist, MK-801 recapitulated the effect of DMSO on spine density in organotypic slice cultures.

Modulation of cell signaling by low concentrations of DMSO could also explain the differential regulation of neuronal activation in various brain regions, as determined by the c-Fos expression analysis. The most obvious examples include the subregions that elicit Aβ-related hyperactivity. These data are consistent with recent work reporting that changes in neuronal activation occur independently of plaque deposition due to the presence of soluble

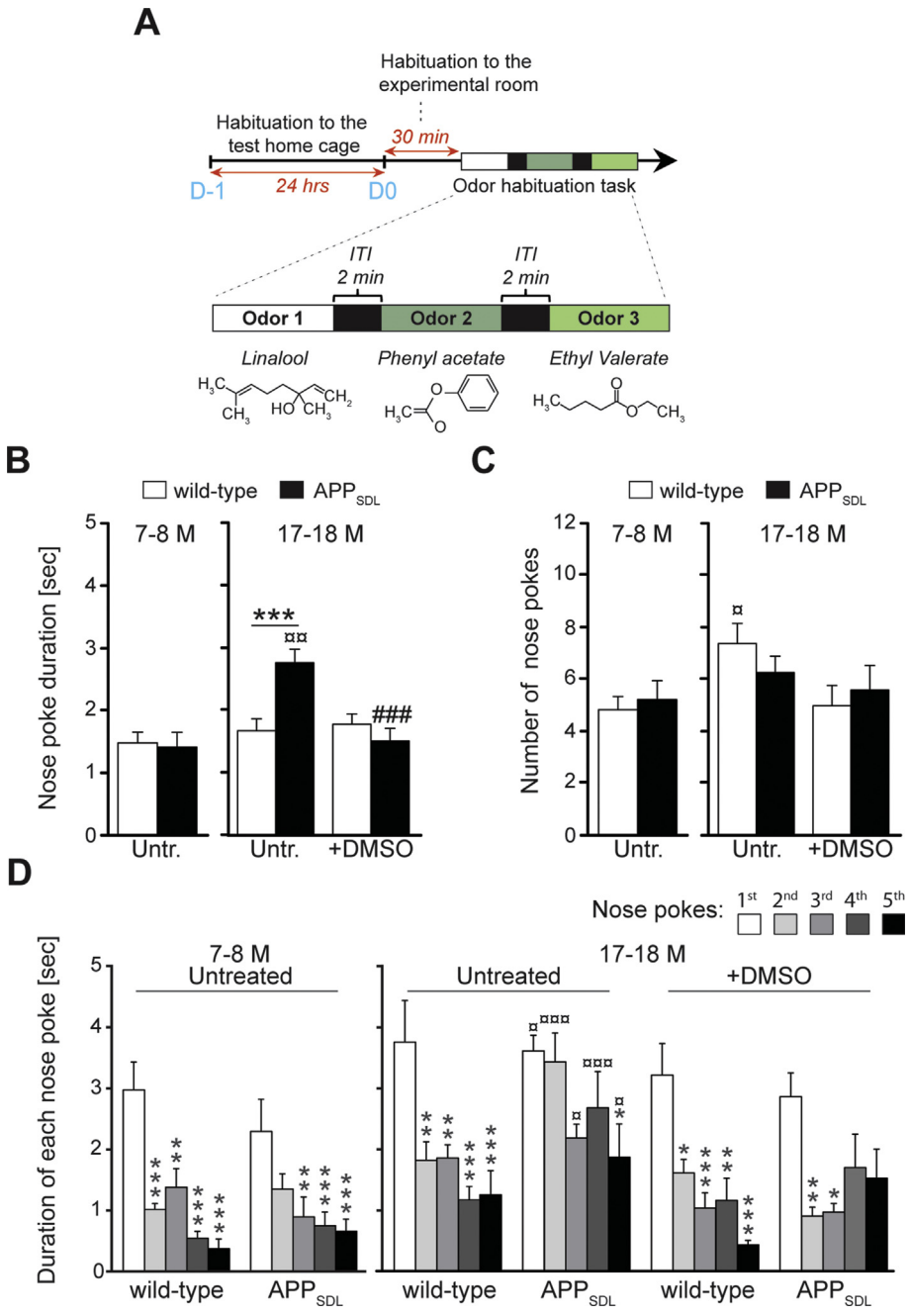

Fig. 5. Chronic DMSO treatment restores olfactory habituation of old transgenic mice. (A) Time line of the experiment. (B) Mean of nose poke (NP) duration does not vary between adult mice of both genotypes. NP duration of untreated APP_{SDL} mice significantly increased with aging compared to adult mice of the same genotype but is reduced by DMSO treatment. (C) Mean number of nose pokes is significantly increased for old untreated wild-type mice compared to adult mice of the same genotype, a difference abolished by DMSO treatment. (D) Progressive decrease in the duration of the first five nose pokes occurs for adult mice, indicating habituation to new odorants. Odor habituation of old untreated APP_{SDL} mice significantly differs from the performance of adult mice from the same genotype, and from old untreated wild-type mice, whereas DMSO treatment significantly attenuates olfactory deficits of APP_{SDL} mice. Adult wild-type (n = 6) and APP_{SDL} mice (n = 7), old wild-type and APP_{SDL} mice (n = 6–7 mice/group). All values are represented as mean ± S.E.M. (B) *P < 0.05, **P < 0.01, wild-type versus APP_{SDL}, same experimental condition. ##P < 0.01 significantly decreased compared to untreated, same genotype. (D) *P < 0.05, **P < 0.01 and ***P < 0.001, versus first nose poke. (B–D) ¤ P < 0.05, ¤¤ P < 0.01 and ¤¤¤ P < 0.001, versus adult animal of the same genotype. (B,C) Adult mice, one way ANOVA and old untreated and treated mice, factorial ANOVA. (D) Repeated measures ANOVA. (F) Factorial ANOVA. (B,C and F) post hoc Fisher's LSD for multiple comparisons. (D) post hoc Tukey's test. For detailed statistics see Supplementary Tables 6 and 7.

Aβ (Busche et al., 2012). Moreover, excessive hippocampal activation has also been reported in cognitively impaired individuals at high risk to convert to AD (Dickerson et al., 2005). Interestingly, pharmacological reduction of hippocampal hyperactivation in such individuals improved their memory performances (Bakker et al., 2012). Here we showed that DMSO exerts a beneficial influence through the normalization of this pathological trait. It should however be noticed that DMSO is a very interesting molecule: it contains a hydrophilic and a hydrophobic part, and in the same time it is a dipole molecule with polar character. Already early studies described DMSO being effective in reversibly altering the configuration of proteins (Rammler and Zaffaroni, 1967). Later on *in silico* molecular dynamics analysis as well as cell studies pointed to a concentration dependent membrane altering effect of DMSO (de

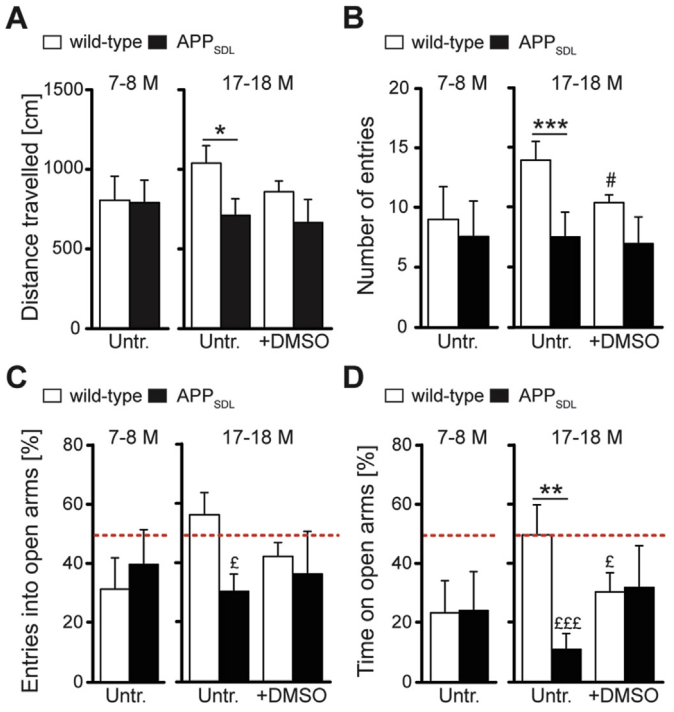

Fig. 6. Chronic DMSO treatment is anxiolytic for old APP_{SDL} mice but anxiogenic for old wild-type animals. (A–D) Adult mice of both genotypes did not show a significant difference in any parameter measured during elevated-plus maze. (A) A significant difference, which was abolished by DMSO treatment, was apparent between old treated and untreated wild-type and APP_{SDL} mice regarding the distance travelled, (B) the number of entries and (D) the time spent in open arms. (C) No significant difference was evident in percentage of entries into the open arms between the two genotypes. (C,D) Chance level is indicated by the red line. (A–D) Adult wild-type (n = 6) and APP_{SDL} mice (n = 7), old wild-type and APP_{SDL} mice (n = 7 mice/group). All values are represented as mean ± S.E.M. (A–D) *P < 0.05, **P < 0.01 and ***P < 0.001, wild-type versus APP_{SDL} mice, same experimental condition. (B) #P < 0.05 significantly decreased compared to untreated, same genotype. (C,D) £ P < 0.05 and £££ P < 0.001 versus chance level (50%). (A–D) Adult mice, one-way ANOVA; old untreated and treated mice, factorial ANOVA. (C–D) Student 's *t*-test for chance level. (A–D) post hoc Fisher's LSD test for multiple comparisons. For detailed statistics see Supplementary Table 8.

Menorval et al., 2012; Gurtovenko and Anwar, 2007). DMSO has therefore pleiotropic actions, implying that part of the beneficial effects might also be attributed to a more global influence of this compound. Previously, high DMSO concentrations have been proposed to alter Aβ aggregation (Shen and Murphy, 1995). However, our investigation of the oligomeric Aβ (oAβ) amount in both, OB and hippocampus of APP_{SDL} mice showed no difference due to DMSO used at low dose (Fig. 7, Supplementary Table 9), indicating that the beneficial effect of DMSO is not caused by decreased levels of oAβ and confirming that the mode of action of DMSO is highly concentration dependant.

DMSO-mediated changes on spines are likely to affect synaptic plasticity underlying hippocampal-dependent learning and memory. Indeed, age-related decrease in spatial memory accuracy was attenuated with 1% DMSO treatment for APP_{SDL} mice. However, DMSO might modulate also hippocampal-independent behavior. Altered olfactory function has been associated with amyloidosis-related pathologies and might represent an informative biomarker to diagnose the earliest stage of neuropathologies, such as AD or PD (Alvarado-Martinez et al., 2013; Jimbo et al., 2011; Wesson et al., 2010). This has been further confirmed by a very recent study in humans, where it has been suggested that olfactory tests have the potential for screening for MCI and MCI that is likely to progress to AD (Roberts et al., 2015). Here, we found that old

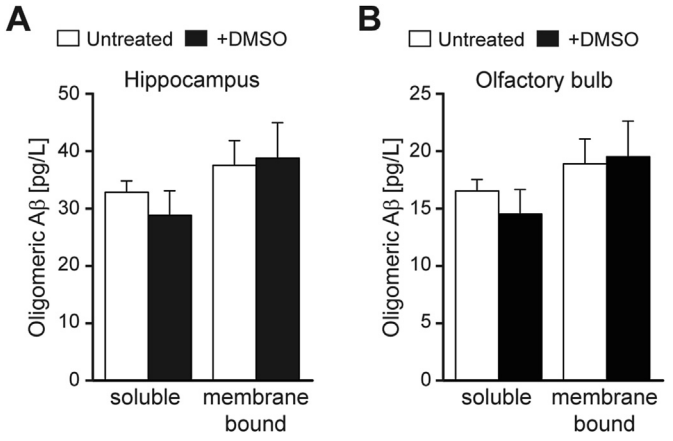

Fig. 7. Chronic DMSO treatment does not change the oligomeric Aβ level. Student's *t*-test confirmed that the levels of soluble and membrane bound Aβ oligomers were not changed between untreated and DMSO treated APP_{SDL} mouse brain tissue of the (A) the hippocampus and (B) olfactory bulb. All values are represented as mean ± S.E.M. For detailed statistics see Supplementary Table 9.

APP_{SDL} mice displayed decreased olfactory habituation compared to wild-type mice. Interestingly, chronic DMSO normalized APP_{SDL} olfactory performances to the level of adult mice. Differences in anxiety-like behaviors between APP_{SDL} and wild-type mice develop with aging. Consistent with previous work (Matheus et al., 1997), DMSO treatment displayed a moderate anxiolytic effect in old APP_{SDL} mice. Intriguingly, chronic DMSO appeared to be anxiogenic for old wild-type mice by mildly decreasing the time spent in open arms.

4.1. Conclusions

Currently, DMSO continues to be one of the most used vehicle in the evaluation of potential hydrophobic drug candidates in basic biological research. Our data highlight unexpected effects of systemic administration of chronic, low, non-toxic DMSO with a differential effect in wild-type and APP_{SDL} mice. Thus, DMSO should be considered as a true bioactive compound whose mechanisms of action might be beneficial against some neurodegenerative conditions. Consequently, the effects of chronic DMSO on synaptic plasticity and behavior are important to be considered for the development of appropriate therapies for neurodegenerative diseases.

Conflict of interest

RB serves as a scientific advisor and owns stock in KineMed Inc., Emerville (USA). Other authors declare no conflict of interest.

Acknowledgements

We thank Vanessa Herkenhoff for technical assistance. The work was supported by the 'Incentive Award of the Faculty of Biology/Chemistry' of the University of Osnabrück (to L.B.).

Appendix A. Supplementary data

Supplementary data related to this chapter can be found at http://dx.doi.org/10.1016/j.neuropharm.2016.10.020.

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