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Locus coeruleus degeneration exacerbates olfactory deficits in APP/PS1 transgenic mice

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Abstract

Neuronal loss in the locus coeruleus (LC) is 1 of the early pathological events in Alzheimer's disease (AD). Projections of noradrenergic neurons of the LC innervate the olfactory bulb (OB). Because olfactory deficits have been reported in early AD, we investigated the effect of induced LC degeneration on olfactory memory and discrimination in an AD mouse model. LC degeneration was induced by treating APP/PS1 mice with N-(2-chloroethyl)-N-ethyl-bromo-benzylamine (DSP4) repeatedly between 3 and 12 months of age. Short term odor retention, ability for spontaneous habituation to an odor, and spontaneous odor discrimination were assessed by behavioral tests. DSP4 treatment in APP/PS1 mice resulted in an exacerbation of short term olfactory memory deficits and more discrete weakening of olfactory discrimination abilities, suggesting that LC degeneration contributes to olfactory deficits observed in AD. Importantly, DSP4 treatment also increased amyloid β (Aβ) deposition in the olfactory bulb of APP/PS1 mice, which correlated with olfactory memory, not with discrimination deficits.

Keywords: Alzheimer's disease; Locus coeruleus; Noradrenaline; Olfaction; Odor discrimination; Odor memory

1. Introduction

The locus coeruleus (LC) is the main neuromodulatory nucleus that synthesizes noradrenaline (NA) and innervates cortical areas (Berridge and Waterhouse, 2003; Foote et al., 1983). NA produced by the LC plays a key role in general arousal, selective attention, and memory (Sara, 2009). Loss of LC neurons observed in various neurodegenerative diseases, including Alzheimer's disease (AD) (Marien et al., 2004) is correlated to the severity of dementia (Bondareff et al., 1987). Alterations of the noradrenergic system therefore have been proposed to contribute to clinical signs of AD (Marien et al., 2004). While the noradrenergic deficit is a well-established feature of AD, its importance for cognition during the course of AD and the mechanisms by which NA influences AD pathogenesis have long remained unknown. Recently, a new approach combining LC lesion in an AD mouse model revealed that NA depletion increases Aβ deposition, inflammation, and hippocampal cognitive deficits (Heneka et al., 2002, 2003, 2006; Kalinin et al., 2007). These findings underline that changes in the noradrenergic system aggravate the course of disease.

The olfactory bulb (OB) is a cortical sensory structure, directly receiving inputs from the olfactory sensory neurons located in the nasal cavity. Interestingly, the OB is a major efferent target of the LC as in rodents, about 40% of LC neurons project to different layers of the OB (McLean et al., 1989; Shipley et al., 1985). Accordingly, NA is strongly involved in olfactory perception and memory. For instance, odor discrimination ability is modulated by NA in a complex way as assessed by experimental manipulation target-

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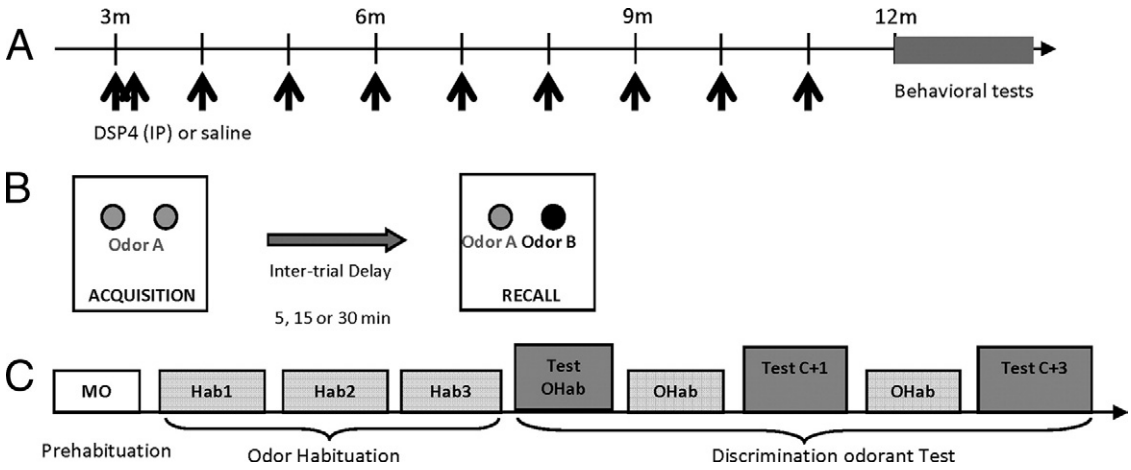


Fig. 1. Experimental design. (A) Experimental time course. Mice received an intraperitoneal injection of N-(2-chloroethyl)-N-ethyl-bromo-benzylamine (DSP4) (50 mg/kg in saline) or equal volume of saline solution every month from 3 months to 11 months, and a supplementary injection 1 week after the first. Behavioral tests started when mice were 12 months old. (B) Odor retention task was conducted on a hole-board apparatus allowing automatic recording of odor investigation. During a first trial (acquisition), mice were exposed to the same odor in 2 holes. After a delay of 5, 15, or 30 minutes, mice were exposed to the first odor (familiar odor) in 1 hole, and a new 1 (novel odor) in the second hole during a second trial (recall). Each mouse performed the task twice and each member of the pair of odorants served alternatively as familiar and novel odor. A delay of several days was kept between the 2 tests. (C) Habituation/discrimination test. The odor was impregnated in a swab, placed in a tea ball, and hung in a clean cage. Mice were first habituated to the apparatus during a prehabituation trial with mineral oil (MO). Then mice were exposed to the habituation odor during 3 successive trials (Hab1, Hab2, Hab3) and finally to 3 presentations of test odorants (Test OHab, Test C+1, Test C+3) in a random order, separated by a presentation of habituation odor (OHab) between each test odorant. Experiments were repeated using 2 odors sets.

ing adrenoreceptor types α or β (Doucette et al., 2007; Mandairon et al., 2008). NA infusion in the OB has been shown to restore odor habituation and spontaneous discrimination after LC lesion (Guerin et al., 2008). NA is also critical for maternal recognition in sheep (Levy et al., 1990), and participates in different forms of olfactory learning, from short term odor recognition (Veyrac et al., 2007) to associative conditioning (Bouret and Sara, 2004; Brennan et al., 1998; Moriceau and Sullivan, 2004). Finally, NA regulates adult olfactory neurogenesis (ongoing formation of new neurons in the OB), which is involved in odor memory formation (Lazarini et al., 2009; Nissant et al., 2009) and altered in neurodegenerative diseases (Curtis et al., 2007).

About 90% of patients with early AD exhibit olfactory dysfunction, including detection and identification deficits. These dysfunctions are severe and appear early in the pathological process (Hawkes, 2003). In line with these functional impairments, senile plaques and neurofibrillary tangles are encountered in the OB of AD brain (Attems et al., 2005; Kovacs et al., 2001), suggesting that it is among the first sites undergoing pathological changes in AD.

Altogether, these data suggest that early NA deficiency is part of and contributes to the vulnerability of the OB to pathological changes in AD and decline of cellular and cognitive aspects of OB functioning. In this study we investigated how LC lesioning with a specific neurotoxin (Fritschy and Grzanna, 1989) in a transgenic mouse model of AD at 3 months of age affects OB structure, as well as olfactory memory and discrimination at 12 months of age.

2. Methods

2.1. Animals, LC lesion and experimental groups

Male and female amyloid precursor protein (APP)/presenilin 1 (PS1) double transgenic mice (Jankowsky et al., 2001) were used (B6C3-Tg(APPswe, PSEN1dE9) 85Dbo/J, the Jackson Laboratory, Bar Harbor, ME, USA). The animals have been backcrossed for at least 8 generations. Twenty-two APP/PS1 hemizygous mice and 24 wild-type littermates, 3 months of age, were used. Animals were housed in individual cages under standard conditions with food and water ad libitum. The study design contained 4 mice treatment groups: APP/PS1 transgenic mice treated with N-(2-chloroethyl)-N-ethyl-bromo-benzylamine (DSP4) or with saline, and their nontransgenic littermate controls treated with DSP4 or with saline. Treatments were done intraperitoneally using 50 mg/kg DSP4 or saline at 3 and 3.2 months and then 1 injection per month during 8 months (Jardanhazi-Kurutz et al., 2010) (Fig. 1A). Mice were last treated 1 month prior to behavioral testing.

This DSP4 treatment induces a persistent degeneration of noradrenergic neurons in the rat LC (Fritschy and Grzanna, 1989) and mouse (Heneka et al., 2006; Jardanhazi-Kurutz et al., 2010) and depletes at least 80% of cortical NA in mice (Heneka et al., 2006; Marien et al., 2004). Four experimental groups were thus constituted: wild-type mice, DSP4 treated wild-type mice, APP/PS1, and DSP4-treated APP/PS1 mice.

Table 1
Odor sets used for habituation/spontaneous discrimination test

Odor set	Prehabituation	Habituation phase	Discrimination phase		
		Hab1 - Hab3	Hab odor	C+1 odor	C+3 odor
Acetate esters	Mineral oil	Propyl acetate 6.3 × 10-5	Propyl acetate 6.3 × 10-5	Butyl acetate 2.2 × 10-4	Hexyl acetate 2.3 × 10-3
Aldehydes	Mineral oil	Pentanal 6.6 × 10-5	Pentanal 6.6 × 10-5	Hexanal 2.2 × 10-4	Octanal 1.5 × 10-3

Odors are diluted (vol/vol) in mineral oil producing a vapor-phase pressure of 1 Pa. The table gives the volume of pure odorant to be diluted in 1 volume of mineral oil.
Key: Hab, habituation.

2.2. Behavioral testing

2.2.1. Odor recognition task

This task allows assessment of retention of a memory trace associated with a nonrewarded exposure to an odor (Veyrac et al., 2007). Briefly, mice were exposed to 2 identical odors in the acquisition session and 1 identical and a novel odor in the recall session. Intertrial delays of 5, 15, and 30 minutes were used (Fig. 1B). The order of the tests at the 3 delays was randomized within each group.

2.2.1.1. Odorants. Three pairs of 2 odorants were used in this task: pure (+) limonene and pure (−) carvone; anisol (1%) and amyl acetate (1%), both diluted in mineral oil; pentanal (10%) and propyl acetate (10%) both diluted in mineral oil. Concentrations were chosen on the basis of our previous studies (Mandairon et al., 2009; Veyrac et al., 2009) to ensure a proper level of diffusion of the odor within the hole, assessed by the experimenter.

2.2.1.2. Data analysis. The time spent exploring each hole in every trial was automatically recorded by capacitive captors located in the holes (Mandairon et al., 2009) and averaged within groups. To analyze recognition of a previously encountered odor, investigation times were analyzed by an analysis of variance (ANOVA) for repeated measures for different interval times, followed by novel and familiar odor intragroup comparisons using paired *t* tests. More time spent investigating the novel odor over the familiar odor indicates that the latter, already presented during acquisition, is remembered.

In order to assess global exploratory behavior, mean exploration of the 2 holes during acquisition was calculated for each group, and compared by unpaired *t* tests.

The cumulative percentage of mice reaching a given level of preference for the novel odor was calculated for each group. Between group comparisons of these values were done by Kolmogorov-Smirnov tests.

2.2.2. Habituation/discrimination test

This olfactory test measures the nonassociative memory through habituation and discrimination by assessing cross-habituation between structurally and perceptually similar odorants (Cleland et al., 2002; Guerin et al., 2008; Mandairon et al., 2006). Tests were performed as described by Guerin et al. (2008). Briefly, a swab was impregnated with an odorant and put in a mesh ball hanging in a clean cage. Mice were first habituated to the apparatus during a prehabituation trial with mineral oil (MO). Then mice were exposed to the habituation odor during 3 successive trials (Hab1, Hab2, Hab3) and finally to 3 presentations of test odors (Test OHab, Test C+1, Test C+3) in a random order, separated by a presentation of habituation odor (OHab) between each test odor. Investigation time in each trial is defined as duration of active sniffing with the nose less than 1 cm from the mesh ball.

2.2.2.1. Odor sets. According to previous literature validating the test (Cleland et al., 2002), all odorants were dissolved in mineral oil in order to reach a vapor-phase partial pressure of 1 Pa and prepared freshly for each experiment. Two sets of 3 odorants were used: a set of acetate esters and 1 of aldehyde odorants (Table 1). Within each set, the 3 odorants differed by the length of their carbon chain and correlatively differed in perceptual proximity. Odors become more perceptually different with the length of the carbon chain of the odorant (Cleland et al., 2002).

2.2.2.2. Data analysis. Investigation times during habituation and test trials were averaged across animals. Habituation and discrimination were analyzed by ANOVA for repeated measures followed by Fisher least significant difference (LSD) post hoc tests. Habituation was identified as a significant reduction of investigation time between Hab1 and Hab3 (Fig. 1C). Discrimination was identified as significant increase of investigation time during presentation of a test odorant, compared with the habituation odor (Cleland et al., 2002).

To compare habituation and discrimination performance independently of overall variations in investigation times, a habituation index and a discrimination index were calculated for each group (Guerin et al., 2009). The habituation index was calculated as 1 - (Hab3/Hab1), where Hab3 was the investigation time of the odor during the third habituation trial and Hab1 the investigation time during the first presentation of the habituation odor (Fig. 1C). The discrimination index was defined as 1 - (Test OHab/Test C+1 or Test C+3), where Test C+1 or Test C+3 was the time the test odor was investigated, and Test OHab, the time the

habituation odor was investigated. Between group differences in indexes were analyzed by classical ANOVA for habituation and ANOVA for repeated measures for discrimination, followed by Fisher LSD post hoc tests. In order to assess global exploratory behavior, mean exploration of the habituation odor during the habituation phase was calculated for each group, and compared by unpaired Student *t* tests.

2.3. Immunohistochemistry

Animals were treated, brains collected and immunohistochemistry for the norepinephrine transporter (NET) performed as described by Jardanhazi-Kurutz et al. (2010). Frontal OB sections taken at same anteroposterior level for the different animals were processed simultaneously for immunohistochemistry for the NET. Pictures of the granule cell layer, the main target of noradrenergic fibers in the OB, were taken on a BX61 fluorescence microscope using a 40× objective (both Olympus, Hamburg, Germany) and analyzed with Morpho Expert (Explora Nova, La Rochelle, France). The total area of NET positive fibers was measured in series of images of the granule cell layer (10 images per animal; 4–5 animals per experimental group). Area measurements were calculated automatically in Morpho Expert by a simple luminance thresholding algorithm. Results were expressed as the ratio between the NET positive area and the total area of interest and analyzed by ANOVA and post hoc pairwise comparisons.

2.4. Thioflavin S staining

Eight sections with an interval of 70 μm were stained with thioflavin S according to standard procedures and mounted in mounting medium adapted to immunofluorescence (Mowiol 4-80, Merck, Darmstadt, Germany) and containing the antifading agent 1,4-Diazabicyclo[2.2.2]octan (Sigma, München, Germany). The number of plaques and total area occupied by thioflavin S positive plaques were assessed by a self written script programmed on NIH ImageJ 1.42 using the maximum entropy threshold plug-in and the area occupied by thioflavin S positive plaques in different layers of the OB was assessed by Morpho Expert (Explora Nova, La Rochelle, France). Results were analyzed by unpaired *t* tests.

2.5. Nissl staining (cresyl violet) and morphometric measurements

A series of 6 sections equally distributed along the anteroposterior axis of the OB (and taken at the same anteroposterior level for each animal) with an interval of 280 μm was stained with cresyl violet. The different layers of the OB were then delineated using the mapping software Mercator V1.85 (Explora Nova, La Rochelle, France). The area of each layer was assessed and analyzed by ANOVA followed by Fisher LSD post hoc tests.

2.6. Statistics

Student *t* tests were performed using Prism 5 (GraphPad, San Diego, CA, USA), Kolmogorov-Smirnov tests using StatView 5.0.1 (SAS Institute, Inc., Cary, NC, USA), and ANOVA, followed by Fisher LSD post hoc test using SYSTAT 7.0.1 (SSI, Richmond, CA, USA).

3. Results

3.1. DSP4 treatment strongly reduces noradrenergic fibers in the OB

To assess the effect of the DSP4 treatment on noradrenergic innervation of the OB, noradrenergic fibers in the OB were labeled using immunohistochemistry for the NET as a marker of noradrenergic terminals (Jardanhazi-Kurutz et al., 2010). Areas occupied by NET positive fibers were quantified in the granule cell layer, the main target of noradrenergic fibers in the OB (McLean et al., 1989) (Fig. 2A). Wild-type and APP/PS1 mice showed no difference in NET positive fibers. As expected, DSP4 treatment strongly decreased NET positive fibers in both wild-type and APP/PS1 mice, indicating that the treatment effectively induced degeneration of noradrenergic fibers in the OB (Fig. 2B).

3.2. DSP4 treatment increased Aβ deposition in the OB

The number of amyloid plaques and the area labeled by thioflavin S were assessed in the OB. Amyloid plaques were found in the OB of APP/PS1 mice. The area labeled by thioflavin S was increased by DSP4 treatment (Fig. 2C, D). The same result was found for the number of plaques (3-fold increase, unpaired *t* test, $p < 0.01$, data not shown). Amyloid plaques were found essentially in the granular layer of the OB where DSP4 treatment increased the total thioflavin S-labeled area (Fig. 2E).

3.3. Structural integrity of OB

To further analyze the structural integrity of the OB, the cross-sectional area of each layer was delineated and measured on a series of sections regularly distributed along the rostrocaudal axis (see Methods) (Fig. 2F). We found that the overall laminar organization of the OB was preserved in APP/PS1 and DSP4 treated mice (Fig. 2G). The area of the glomerular layer, which receives inputs from the nasal cavity, was similar in all groups, suggesting that the peripheral olfactory input was not altered by transgene expression or DSP4 treatment. In contrast, the area of the granule cell layer, which contains the granular interneurons (Shepherd et al., 2007), was reduced in APP/PS1 mice and APP/PS1 DSP4-treated mice. Finally, the area of the external plexiform layer in which granule cell interneurons project their main dendritic trees, was reduced only in APP/PS1 DSP4-treated mice, indicating that DSP4 treatment had aggravated the deleterious effect of the APP/PS1 transgene on the

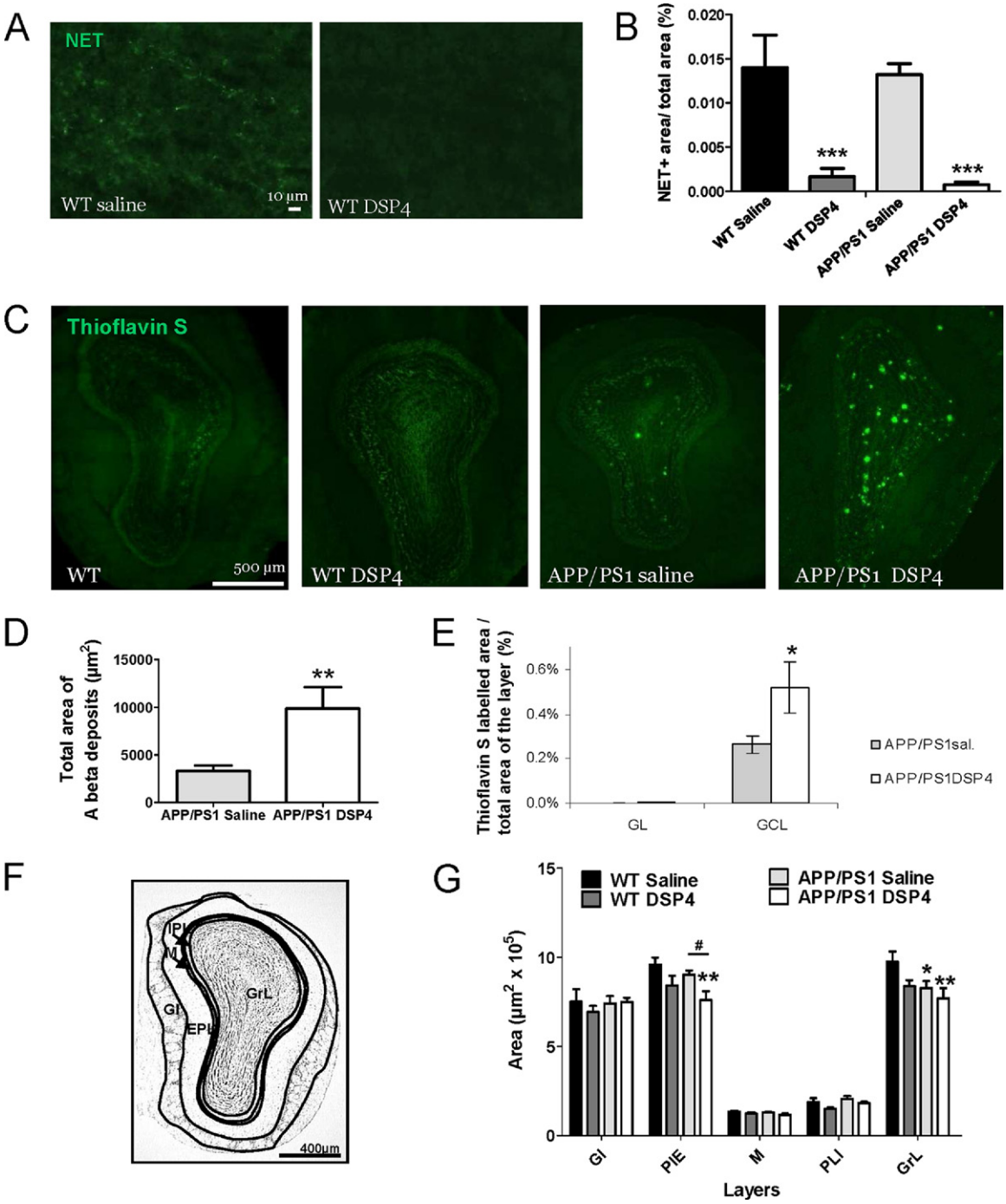


Fig. 2. Noradrenaline (NA) depletion downregulates norepinephrine transporter (NET), increases Aβ deposition, and decreases the area of granule cell layer in the olfactory bulb (OB) in APP/PS1 mice. (A) NET was immunolabelled on frontal sections of the OB. Bar = 500 μm. (B) NET immunoreactive area in the OB of wild-type and APP/PS1 mice (1-way analysis of variance [ANOVA], $F(3,14) = 15.71$, $p < 0.001$, followed by post hoc LSD test, $^{***}p < 0.001$). (C) Amyloid deposits were detected in the OB. (D) Plaque area for OB sections (unpaired t test: $^{**}p < 0.01$). (E) Thioflavin S-labeled area in GCL and GL of the OB (unpaired t test, $^{*}p < 0.05$). (F) Example of morphometric area assessment of the OB layers on Nissl-stained section (see Methods). EPL, external plexiform layer; GL, glomerular layer; GrL, granular cell layer; IPL, internal plexiform layer; M, mitral cell layer. (G) The area of granule cell layer is decreased in APP/PS1 and APP/PS1 N-(2-chloroethyl)-N-ethyl-bromo-benzylamine (DSP4) treated mice (ANOVA $F(3,15)=3.26$, $p < 0.05$, followed by LSD post hoc test, $^{*}p < 0.05$; $^{**}p < 0.01$). The area of external plexiform layer is reduced in APP/PS1 DSP4 treated mice (ANOVA $F(3,15) = 3.56$, $p < 0.05$, followed by least significant difference (LSD) post hoc test, $^{**}p < 0.01$ with respect to control; $\#p < 0.05$ with respect to APP/PS1 saline).

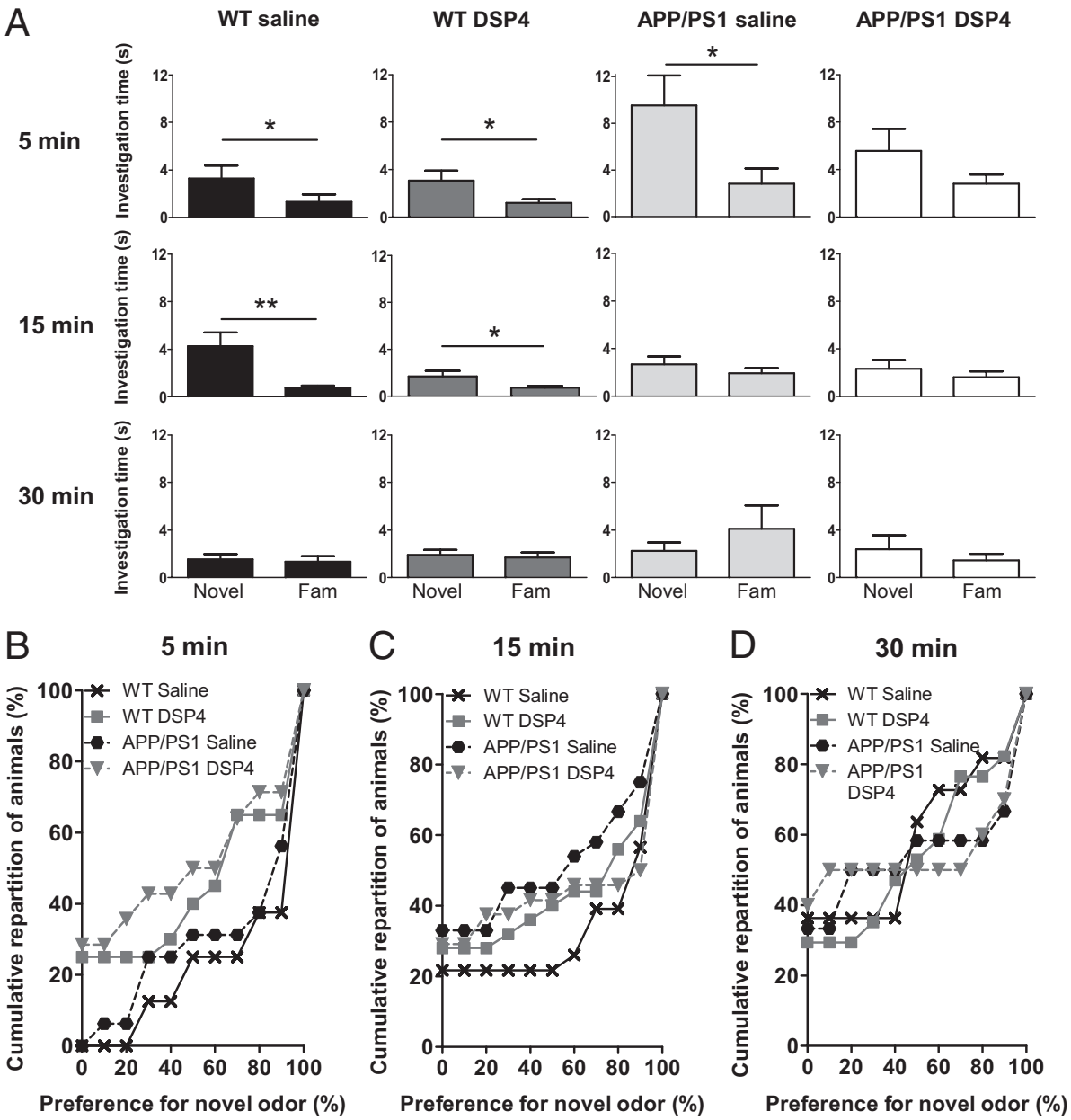


Fig. 3. Impairment of odor retention in APP/PS1 mice after N-(2-chloroethyl)-N-ethyl-bromo-benzylamine (DSP4) treatment. (A) Mean investigation time (seconds) (± SEM, n = 18/group) of novel (Novel) and familiar (Fam) odors during recall at indicated times after acquisition (analysis of variance [ANOVA] for repeated measures, time effect $F(2,118) = 3.92$, $p = 0.02$, odor effect $F(1,59) = 4.99$, $p = 0.029$ followed by least significant difference (LSD) post hoc test, $^{*}p < 0.05$, $^{**}p < 0.01$). (B, C, D) Cumulative distribution of mice according to their performance in 5-minute (B), 15-minute (C), and 30-minute delayed (D) odor recognition task. Distribution of mice according to their memory performance is changed for APP/PS1 DSP4 treated mice compared with wild-type in the 5-minute delay task (2-sample Kolmogorov-Smirnov, wild-type saline versus APP/PS1 DSP4, $p < 0.01$). Wild-type DSP4 treated, APP/PS1 saline and APP/PS1 DSP4 treated mice were different from controls in the 15-minute delay task (p-values < 0.05). No difference was found in the 30-minute delay task.

bulbar layer, the latter containing most synaptic contacts between mitral and granule cells.

3.4. Recognition memory deficits in APP/PS1 mice are exacerbated by DSP4 treatment

To assess the retention time of an olfactory memory trace, we used an olfactory recognition test. Exploration of a novel odor over a previously presented one indicates memory of the latter. Wild-type animals explored the novel odor more than the familiar one 5 and 15 minutes after its first presentation, but not after 30 minutes (Fig. 3A). Similarly, wild-type DSP4-treated mice remembered the previously presented odor for 15 minutes. In contrast, APP/PS1 mice explored the novel odor more 5 minutes after the

acquisition trial but not after 15 minutes. So, APP/PS1 mice showed a deficit in retention of the familiar odor. Interestingly, this deficit was aggravated in APP/PS1 DSP4-treated mice, which did not recognize the odor already 5 minutes after acquisition (Fig. 3A).

In order to perform between group comparison of performance, we used an index of preference for novel odor (see Methods). We represented the data as cumulative percentage of mice reaching a given level of preference for the novel odor (Fig. 3B–D). This representation of the data gives an indication of how easily the task was performed by individuals of each group. Five minutes post acquisition, only APP/PS1 DSP4 treated mice performed differently from wild-type mice (Fig. 3B). Indeed, the proportion of APP/PS1 DSP4 treated mice showing a low level of performance (preference for novel odor below 50% for instance) was higher than in the wild-type group, confirming the memory deficit in APP/PS1 DSP4-treated mice after 5 minutes posttrial delay. Fifteen minutes after acquisition, APP/PS1 mice, APP/PS1 DSP4 and wild-type DSP4 treated mice distribution was shifted toward lower level of performances compared with wild-type mice (Fig. 3C). This latter finding, combined with exploration time data (Fig. 3A) showing that the wild-type DSP4 treated group was able to remember the familiar odor, indicated that DSP4 treatment did not prevent odor recognition, but nevertheless lowered the quality of performance after this delay. In summary, APP/PS1 mice showed a memory retention deficit compared with wild-type mice 15 minutes after acquisition. This deficit was further exacerbated in APP/PS1 DSP4 treated mice, which did not remember the familiar odor after 5 minutes.

3.5. DSP4 treatment aggravates deficits in fine odor discrimination in APP/PS1 mice

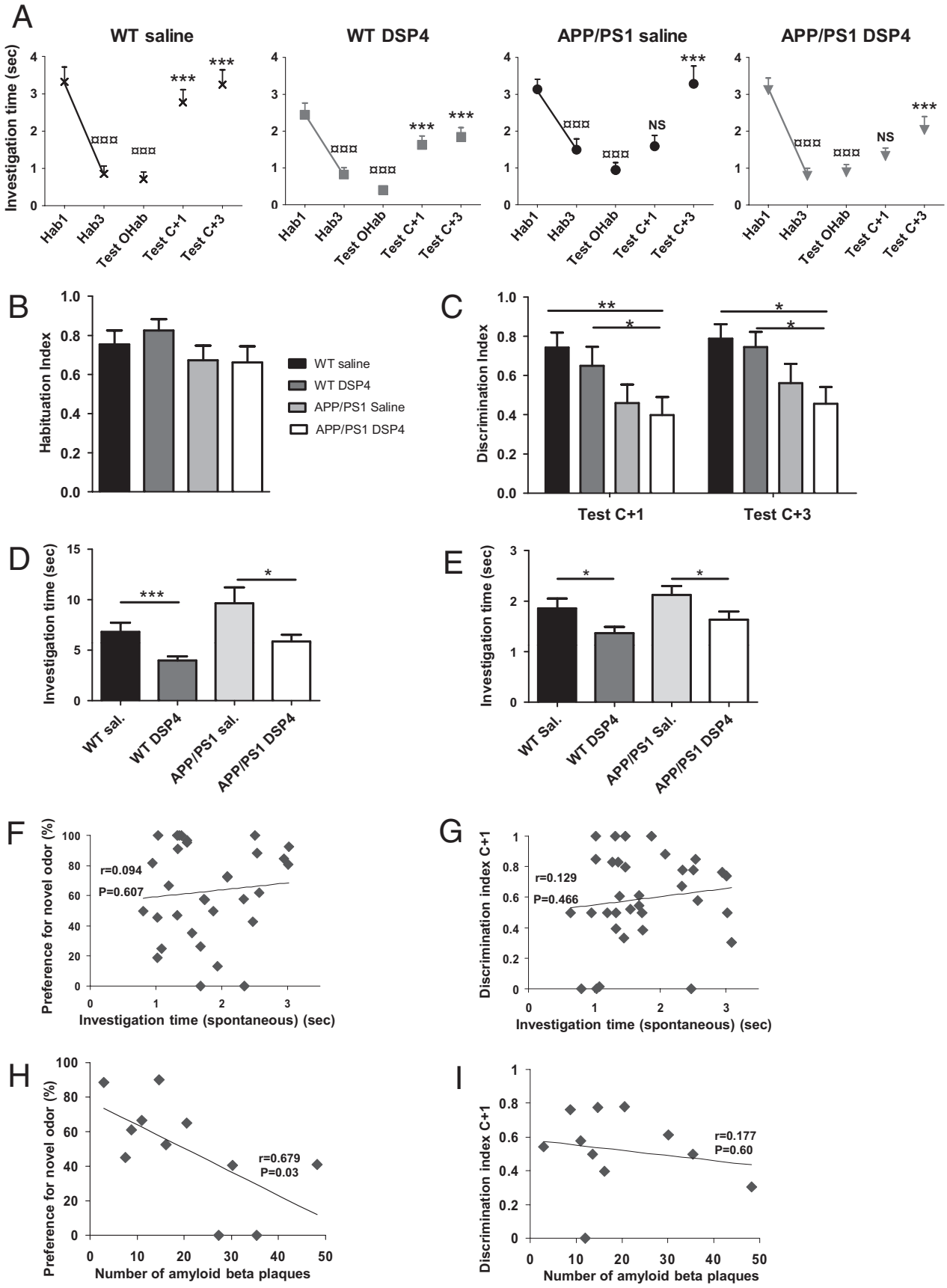
Olfactory discrimination was measured using a habituation-dishabituation test. In this task, memorization of the habituation odor across the 3 habituation trials ensure that investigation of the test odors actually reflects the ability to discriminate the test odors from the habituation odor. To assess fine discrimination, we used odorants with a graded similarity with the habituation odorant, differing by their carbon chain length (see Methods). Investigation time of the habituation odor similarly decreased in all groups indicating that the habituation odor was detected and that habituation occurred in the same manner in all groups (Fig. 4A). This finding was confirmed by the calculation of the habituation index (Fig. 4B). This index allows direct between group comparisons of the level of habituation independently of overall differences in investigation time. Habituation index was similar in all groups.

To measure discrimination performance, investigation time of the test odors (Test C+1 and Test C+3) was compared with investigation time of habituation odor during the odor discrimination test (Test OHab). In wild-type saline and wild-type DSP4 mice, investigation time increased for the test odor with a carbon chain length carrying 1 (Test C+1) or 3 more carbons (Test C+3) compared with the habituation odorant (Test OHab) (Fig. 4A), indicating that both test odors were discriminated from the habituation odor. In APP/PS1 saline and APP/PS1 DSP4 treated mice, the C+3, but not the C+1 odor was investigated longer than the habituation odor (Fig. 4A). This result means that APP/PS1 mice discriminated the C+3, but not C+1 odor, from the habituation odor, regardless of the DSP4 treatment. In conclusion, APP/PS1 mice showed a deficit in fine discrimination because they could not discriminate the 2 closest odors.

To reveal more subtle differences in discrimination ability between groups, we used a discrimination index, depicting the strength of discrimination (Fig. 4C). Of note, DSP4 treatment of wild-type mice did not alter the index of discrimination (Fig. 4C). APP/PS1 mice showed a decline in discrimination index, but this decline did not reach statistical significance compared with wild-type or wild-type DSP4 treated mice. In contrast, in APP/PS1 DSP4 treated mice the index of discrimination was decreased compared with wild-type saline and wild-type DSP4 treated, for easy (C+3) and difficult (C+1) discrimination. In conclusion, APP/PS1 DSP4 treated mice had the worst discrimination performance, while DSP4 treatment alone did not affect the discrimination index.

3.6. DSP4 treatment induces a reduction in spontaneous odor investigation

Because NA has been implicated in novelty-related exploratory behavior (Aston-Jones and Bloom, 1981; Sara, 2009), we looked for any global effect of DSP4 treatment on odor exploration and its relationship with olfactory performance. We calculated global exploration of odor during the acquisition trial for the odor retention task and during the habituation trials for the habituation/discrimination test. In both cases, the overall investigation time of odors was lower in DSP4 treated mice compared with saline treated mice (Fig. 4D, E). However, we also found that olfactory performance was unrelated to the level of spontaneous exploration. Indeed, 5 (Fig. 4F), 15, and 30 minutes (data not shown) following acquisition, no correlation was found between investigation of novel odor during recall (memory performance) and exploration time during acquisition. Similarly, discrimination index for the C+1 (Fig. 4G) or C+3 odor (data not shown) did not correlate with exploration during the habituation phase. In summary, these data indicate that reduced spontaneous investigation of the odors in DSP4 treated animals cannot account for the observed differences in olfactory memory or discrimination.


A
WT saline
WT DSP4
APP/PS1 saline
APP/PS1 DSP4
Investigation time (sec)
Hab1
Hab3
Test OHab
Test C+1
Test C+3
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NS
B
Habituation Index
WT saline
WT DSP4
APP/PS1 Saline
APP/PS1 DSP4
C
Discrimination Index
**
*
Test C+1
Test C+3
D
Investigation time (sec)
WT sal.
WT DSP4
APP/PS1 sal.
APP/PS1 DSP4
E
Investigation time (sec)
WT Sal.
WT DSP4
APP/PS1 Sal.
APP/PS1 DSP4
F
Preference for novel odor (%)
r=0.094
P=0.607
Investigation time (spontaneous) (sec)
G
Discrimination index C+1
r=0.129
P=0.466
Investigation time (spontaneous) (sec)
H
Preference for novel odor (%)
r=0.679
P=0.03
Number of amyloid beta plaques
I
Discrimination index C+1
r=0.177
P=0.60
Number of amyloid beta plaques

3.7. Amyloid load is inversely correlated with odor retention but not with discrimination performance

Linear regression between Aβ load and olfactory performance was calculated. A significant correlation was found between the number of plaques and the percentage of novel odor investigation after a 5-minute delay, when APP/PS1 DSP4 treated mice showed a memory deficit (Fig. 4H). There was also a significant correlation of performance with the total plaque area ($p < 0.05$, data not shown). In contrast, no significant correlation was found between number of plaques or area of plaques and discrimination of the C+1 (Fig. 4I) or C+3 odor ($p > 0.05$ in all cases).

4. Discussion

In the present study, we showed that long term DSP4 treatment known to induce loss of noradrenergic neurons of the LC (Heneka et al., 2010; Jardanhazi-Kurutz et al., 2010), exacerbated olfactory memory in APP/PS1, compared with wild-type mice, suggesting that LC degeneration contributes to memory deficits observed in AD. We further showed that this DSP4 treatment increased Aβ deposition in the granule cell layer (GCL) of the OB of APP/PS1 mice and that the olfactory memory deficit was correlated with Aβ plaque area and number. For odor recognition we thus used distant odorants of a different molecular family that animals of all groups were able to discriminate. The differences between groups in outcome of the recognition test can therefore be confidently interpreted to reflect differences in short term olfactory memory. Previously, DSP4 treatment has been shown to reduce spatial memory in APP23 mice (Heneka et al., 2006). The simple form of olfactory memory assessed here by the odor recognition task does not depend on the hippocampus as suggested for object recognition tasks (Mumby, 2001) and more specifically for olfactory discrimination tasks which can be learned and retained in hippocampus-lesioned animals (Jonasson et al., 2004; Kaut and Bunsey, 2001; Kaut et al., 2003). Our findings thus suggest that the aggravating effect of NA depletion on cognitive function is not specific for hippocampus-dependent memory. In line with this conclusion, our data also demonstrate that DSP4 treatment increased Aβ deposition in the OB of APP/PS1 mice. This increase was similar in proportion to the previously reported effect of DSP4 treatment on Aβ plaque load in the hippocampus (Heneka et al., 2006).

In the present work, the effect of chronic treatment with DSP4 on short term olfactory memory in wild-type animals was more subtle than that of acute DSP4 treatment reported previously (Guerin et al., 2008; Veyrac et al., 2007). The possibility that chronic DSP4 treatment triggers slowly developing compensatory mechanisms aimed at restoring normal noradrenergic function may explain the differences observed. In our study, repeated DSP4 injections blocked effectively noradrenergic sprouting responses (Fritschy and Grzanna, 1992) as evidenced by low noradrenergic fiber density at the end of the treatment. However, increased expression of noradrenergic receptors (Berridge and Waterhouse, 2003) and compensatory noradrenergic up regulation, as seen in postmortem AD brains (Szot et al., 2006, 2007) cannot be excluded and could at least partially account for the almost normal olfactory memory in the wild-type DSP4-treated animals. By contrast, compensation mechanisms may have been impaired in the APP/PS1 mice, which developed clear memory deficits in response to DSP4 treatment.

In our study, DSP4 treatment did not affect the formation of habituation memory in wild-type or APP/PS1 mice after a 5-minute delay between odor presentations. This may appear contradictory with the finding that recognition memory was impaired at the same delay, because habituation memory relies on short term memory of the presented odor. It should be mentioned that in the recognition task, animals get 1 chance to choose between 2 odors, 1 of which is novel, while the habituation task allows memory formation across several trials. It might be that the habituation task is too easy

Fig. 4. N-(2-chloroethyl)-N-ethyl-bromo-benzylamine (DSP4) treatment does not impair habituation capacity in APP/PS1 mice, but aggravates discrimination deficits. (A) Mean investigation time during habituation phase (Hab1, Hab3) and discrimination (Test OHab, Test C+1, Test C+3). Each group habituated to the habituation odor (HabO) (analysis of variance [ANOVA] for repeated measures, wild-type (WT) saline: $F(3,42) = 25.47$, $p < 0.0001$; WT DSP4 treated: $F(3,60) = 20.22$, $p < 0.0001$; APP/PS1 saline: $F(3,57) = 12.98$, $p < 0.0001$; APP/PS1 DSP4 treated: $F(3,63) = 21.39$, $p < 0.0001$, followed by least significant difference (LSD) post hoc tests, ¤¤¤p-values < 0.001). Regarding discrimination, wild-type saline (ANOVA followed by LSD post hoc test; $F(2,26) = 22.1$, $p < 0.0001$) and DSP4 treated ($F(2,36) = 12.54$, $p < 0.001$), mice investigated C+1 and C+3 odor more than habituation odor ($p < 0.001$ for difference between Test Ohab and C+1 or C+3). APP/PS1 saline ($F(2,38) = 11.74$, $p < 0.001$) and APP/PS1 DSP4 treated mice ($F(2,40) = 4.27$, $p < 0.05$) investigated C+3 odor more than habituation odor ($p < 0.001$), but not C+1 odor ($p > 0.05$). ***$p < 0.001$. (B) Habituation index showed no significant differences between groups (ANOVA $F(3,75) = 1.14$, $p > 0.05$). (C) Discrimination index is reduced for both test odors (C+1 and C+3) in APP/PS1 DSP4-treated mice compared with wild-type saline and wild-type DSP4 treated mice, respectively (ANOVA for repeated measures $F(3,71) = 4.90$, $p < 0.01$ followed by post hoc LSD tests *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). (D) DSP4 treatment reduced investigation time in wild-type and APP/PS1 mice during acquisition of odor recognition (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). (E) Investigation time during habituation phase (all habituation trials pooled) of habituation/discrimination test. Wild-type DSP4 treated and APP/PS1 DSP4 treated mice explored the habituation odor less than wild type and APP PS1 saline mice (unpaired t test, *$p < 0.05$). (F) Lack of correlation between investigation time during acquisition and odor retention performance (expressed as a percentage of novel odor investigation). (G) Lack of correlation between investigation time during habituation and discrimination performance (discrimination index for C+1). (H) Aβ load is correlated with odor retention performance 5 minutes after acquisition (linear regression between the number of amyloid plaques and odor retention performance, $r = 0.679$, $p < 0.03$). (I) Aβ load is not correlated with discrimination index.

to detect a deficit in olfactory memory compared with the recognition test.

Not only was olfactory memory impaired in APP/PS1 mice but also discrimination of closely related odors, which was further aggravated by DSP4 treatment. In contrast to olfactory memory fine discrimination of odors, although impaired, was not correlated with Aβ plaque load. Both processes apparently are not affected by the same pathogenic (toxic) Aβ species, APP processing products and/or brain areas.

Adult neurogenesis, targeting principally the GCL of the OB is involved in olfactory memory (Lazarini et al., 2009; Sultan et al., 2010) and discrimination (Gheusi et al., 2000; Moreno et al., 2009). A reduction in neurogenesis consistent with the reduction of GCL area, could contribute to the olfactory deficits reported here. The likelihood that Aβ interferes with neurogenesis in the GCL in APP/PS1 mice is strengthened by the finding that preparations of fibrillar Aβ inhibit stem cell proliferation (Haughey et al., 2002). The plexiform layer that contains dendrites of the interneurons of the GCL tends to be reduced in the APP/PS1 mice and more so in combination with DSP4, suggesting that the full development of adult-born neurons may be most sensitive to DSP4 treatment.

The increase of Aβ due to DSP4 treatment could also contribute more directly to deleterious effects of DSP4 on olfactory performances in APP/PS1 mice through alteration of synaptic functioning (Sakono and Zako, 2010) and/or increase in glial activation reported following DSP4 treatment (Heneka et al., 2006) in APP23 mice.

Altogether our data indicate that Aβ pathology combined with DSP4 treatment did not induce a general failure of olfactory function but because of the use of highly sensitive olfactory tests, revealed selective olfactory impairments which mimic those found in human disease regarding recognition memory (Lehrner et al., 1997; Nordin and Murphy, 1998; Nordin et al., 1996) and discrimination (Djordjevic et al., 2008). Furthermore, short term olfactory memory deficits, which were found to correlate with Aβ load could be a useful marker for the progression of the pathology. Our data validate the use of olfactory signs as indexes for the progression of AD-like pathology and strongly support a contribution of NA depletion to olfactory deficits in AD.

Disclosure statement

There are no actual or potential conflicts of interest.

All appropriate approval and procedures were used concerning animal experimentation.

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