Dopaminergic neurotransmission dysfunction induced by amyloid-ß transforms cortical long-term potentiation into long-term depression and produces memory impairment

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Abstract

Alzheimer's disease (AD) is a neurodegenerative condition manifested by synaptic dysfunction and memory loss, but the mechanisms underlying synaptic failure are not entirely understood. Although dopamine is a key modulator of synaptic plasticity, dopaminergic neurotransmission dysfunction in AD has mostly been associated to noncognitive symptoms. Thus, we aimed to study the relationship be-tween dopaminergic neurotransmission and synaptic plasticity in AD models. We used a transgenic model of AD (triple-transgenic mouse model of AD) and the administration of exogenous amyloid-b (Ab) oligomers into wild type mice. We found that Ab decreased cortical dopamine levels and converted in vivo long-term potentiation (LTP) into long-term depression (LTD) after high-frequency stimulation delivered at basolateral amygdaloid nucleuseinsular cortex projection, which led to impaired recognition memory. Remarkably, increasing cortical dopamine and norepinephrine levels rescued both high-frequency stimulation -induced LTP and memory, whereas depletion of catecholaminergic levels mimicked the Ab-induced shift from LTP to LTD. Our results suggest that Ab-induced dopamine depletion is a core mechanism underlying the early synaptopathy and memory alterations observed in AD models and acts by modifying the threshold for the induction of cortical LTP and/or LTD.

Keywords:Amyloid-beta; Alzheimer's disease; Long-term depression; Long-term potentiation Dopamine; Recognition memory impairment

Introduction

Alzheimer's disease (AD) is a neurodegenerative condition characterized by memory loss and synaptic failure, which has become a concern for public health as the population peak ages. To find effective interventions for AD, novel targets based on the identification of altered molecular mechanisms underlying AD-related pathophysiology are required.

Dopamine is a key neuromodulator of synaptic plasticity (Lisman et al., 2011; Pignatelli and Bonci, 2015). It has been shown that enhancement of dopamine activity improve memory in humans and animals (Calabresi et al., 2007; Nitsche et al., 2009). In line with this, we reported that acute stimulation of dopaminergic

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neurotransmission improved deficits in object recognition memory in a triple-transgenic mouse model of AD (3 Tg-AD; Guzmán-Ramos et al., 2012a). Other studies in AD models have also shown that pharmacologic stimulation of dopaminergic transmission im-proves memory performance (Ambrée et al., 2009; Himeno et al., 2011). Moreover, AD patients showed unexpected positive effects of dopaminergic drugs on cognitive performance and cortical plasticity, strengthening the hypothesis of dopamine involvement in AD (Koch et al., 2011, 2014; Martorana and Koch, 2014; Martorana et al., 2009). However, a direct link between AD-associated synaptic plasticity impairment and dopaminergic neurotransmission failure has yet to be demonstrated.

Experimental evidence suggests that increased production and deposition of amyloid-b (Ab) has a causal role in early synaptic failure in AD pathogenesis (Shankar et al., 2008; Townsend et al., 2006). Thus, we sought to evaluate the effects of Ab on dopami-nergic neurotransmission, and its outcome in synaptic plasticity and memory, which allow us to understand memory alterations and the contribution of aging to the pathology. To this aim, we ntegrate several techniques like behavioral tasks, free-moving microdialysis, in vivo electrophysiologic recordings, pharmaco-logic manipulations, and histologic and immunohistochemical an-alyses in the 3 Tg-AD model, that shows a progressive deposition of hyperphosphorylated tau tangles and intracellular and extra-cellular Ab depositions (Billings et al., 2005) and in a nonfamilial AD model induced by the administration of exogenous Ab oligomers in wild type (WT) mice.

We focused on the insular cortex (IC), a neocortical region affected early in AD patients (Suto et al., 2014) and animal models (Guzmán-Ramos et al., 2012a), that is known to play a key role in dopamine-dependent memory, particularly in the conditioned taste aversion, a robust learning paradigm in which animals associate novel taste to gastric malaise, resulting in a reduced consumption of the associated taste (coded as aversive) in subsequent presentations (Bermúdez-Rattoni, 2004). This learning paradigm requires dopamine release in the IC during the presentation of novel taste (Guzmán-Ramos et al., 2010), and it is a relevant memory model regarding synaptic facili-tation, as in vivo high-frequency stimulation (HFS) applied to the basolateral amygdaloid nucleus (BLA) induces long-term potentia-tion (LTP) in the IC and enhances conditioned taste aversion retention (Escobar and Bermúdez-Rattoni, 2000). Moreover, this synaptic facilitation requires functional (N-methyl-D-aspartate) NMDA re-ceptors and involves the same molecular mechanisms in the IC during novel taste learning (Escobar and Bermúdez-Rattoni, 2000; Jones et al., 1999), Through these experimental approaches, here we show that dopaminergic neurotransmission dysfunction induced by Ab in the 3 Tg-AD model, or by administration of exogenous Ab oligomers in WT mice transforms LTP into long-term depression (LTD) in the BLA-IC pathway and produces memory impairment.

2. Methods and materials

2.1. Animals

Homozygous 3 Tg-AD (http://jaxmice.jax.org/strain/004807. html; Oddo et al., 2003) and B6129SF2/J WT (http:// jaxmice.jax. org/strain/101045 html) male mice were used at 3 to 4-month old (3-month) and 10 to 11-month old (10-month). Experiments were performed in accordance with the current rulings in Mexican law (NOM-062-ZOO-1999) and with the approval of the local Animal Care Committee (FBR30-14). Mice were individually housed in a 22 Ce24 C environment with a 12/12 light or dark cycle with water and food ad libitum, except when stated otherwise.

2.2. Surgical procedures

Mice were placed on a stereotaxic apparatus with a mouse adaptor and maintained under anesthesia with w0.5% isoflurane. A micro-dialysis guide cannulae (CMA/7 Microdialysis, Solna, Sewden) or two 23-ga stainless steel cannulae (9-mm long; Small Parts, Logansport, IN, USA) were implanted aiming to the IC (dorsoventral [DV] 2.75 mm; anteroposterior [AP] þ1.1 mm; mediolateral [ML] 3.3 mm) and secured with small screws and dental acrylic. Dummy cannulae (33 ga, 12 mm) were inserted into the guide cannulae to prevent clogging. Local antibiotics were applied (polymyxin B and iodine). In all cases, we performed histologic analyses after mice sacrifice.

2.3. Behavioral tasks

Novel and aversive taste recognition: behavioral experiments were performed between 10:00 AM and 2:00 PM. After 7 days of surgery recovery, the implanted mice were deprived from water and trained to drink in microdialysis cages (system for freely moving animals CMA-Microdialysis, Solna, Sewden), through two bottles (20 min/d). The mean water consumption of consecutive five days was considered baseline. On the conditioning day, mice were allowed to drink only 0.3% saccharin solution (Sigma-Aldrich, St Louis, MO, USA) through a single bottle during 20 minutes and then 30 minutes later received an intraperitoneal 0.4 M LiCl (7.5 mL/Kg). Saccharin intake was measured, and the percent consumption of saccharin compared to daily water intake represented an eval-uation of novel taste recognition. Twenty-four hour later, mice were allowed to choose between water presented in one bottle and 0.3%saccharin solution presented in another bottle to test conditioned taste aversion memory. Preference index was obtained from two-bottle memory test, it is defined as mL of saccharin/(mL of saccharin þ mL of water) 100, consumed in the test; hence, 50 indicates equal preference. Two-bottle taste preference test: saccharin solutions were prepared in drinking water using reagent-grade chemicals purchased from Sigma-Aldrich. All taste stimuli were presented at room temperature. Male 3 Tg-AD and WT mice at 3-month and 10-month (n ¼ 5 for each group) were used for two-bottle taste preference test as previously described (Bachmanov et al., 2001; Golden et al., 2011). Mice were given access for 48 hours to 2 bottles, one containing drinking tasteless water and the other a saccharin solution; the food was provided ad libitum. After 24 hours, the intake of each fluid was recorded, and the bottle positions were switched to minimize any positional effect. After another 24 hours, the fluid intake was again recorded. Saccharin was presented in an ascending concentration series: 0.01%; 0.02%, 0.1%, 0.3%, 1% and 3% (Bachmanov et al., 2001; Golden et al., 2011).

2.4. Microdialysis and capillary electrophoresis

Microdialysis membranes (CMA/7 Microdialysis, Solna, Sewden) of 2 mm were inserted in the guide cannula placed in the IC. Ringer solution (118 mM NaCl, 4.7 mM KCl, and 2.5 mM CaCl2) or high-potassium Ringer solution (110 mM KCl) were perfused at a rate of 0.25 mL/min with an automated microinfusion pump (Carnegie Medicine, Stockholm, Sweden). After 60 minutes of stabilization, samples were collected every 16 minutes. The first 3 samples were taken as baseline levels and then, we proceeded to conditioned taste aversion acquisition (Fig. 1A). Samples were stored at 80 C until analysis. Samples were derivatized with 5-furoylquinoline-3-carbaldehyde and analyzed by capillary electrophoresis coupled to laser-induced fluorescence detector (Beckman-Coulter PACE/MDQ, Glycoprotein System CA, USA) as previously described (Guzmán-Ramos et al., 2010, 2012b).

2.5. Ab oligomers preparation and characterization

The Ab1-42, ultrapure, treated with NaOH peptide was obtained from Millipore (Temecula, CA, USA). The powder was suspended in 1% NH4OH at a concentration of 1 mg/mL, and the solution was sonicated for one minute to allow conversion to monomers (Fezoui et al., 2000). The Ab1-42 oligomers were prepared by diluting stock solution in phosphate buffered saline (PBS) and incubating for 24 hours at 4 C. Oligomer solutions were maintained at 4 C and used within 5 hours of preparation (Lambert et al., 1998). The so-lution of Ab oligomers was prepared at a concentration of 4 mM, determined by bicinchoninic acid. A volume of 0.5 mLof4mM so-lution was intracortically administered in each experiment (see in the following). Routine characterization of oligomer preparations was performed by western blot and size-exclusion capillary elec-trophoresis that showed the presence of low-molecular-weight Ab oligomers (dimers, trimers, tetramers, and higher molecular mass oligomers; See Supplementary Fig. 1). For this, western blot was carried out by polyacrylamide gel in 12% polyacrylamide precast gels (Invitrogen, Carlsbad, CA, USA) at 100 V for 105 minutes and

bilaterally for behavioral experiments and unilaterally for electro-physiologic recordings. A volume of 0.5 mL per hemisphere was delivered at a rate of 0.25 mL/min. PBS 0.1 M, pH 7.4 or nomifensine (25 mM; Sigma-Aldrich) were administered 20 minutes before HFS and recordings or before novel taste presentation. The Ab1-42 peptide was obtained for Millipore (Temecula, CA) and reverse sequence of Ab42-1, inactive control for the Ab, was obtained from Bachem (Bubendorf, Switzerland). Both peptides prepared at a final concentration of 4 mM and 0.5 mL were injected 24 hours before behavioral protocol or electrophysiologic recordings. A 6-hydroxydopamine (OHDA) (4 mg/mL in 2% ascorbic acid; Sigma-Aldrich) or vehicle was injected 24 hours before electrophysio-logic recordings. The tracer fluoro-gold (FG; 2% w/v diluted in 0.9%saline; Sigma-Aldrich) was injected unilaterally into the right IC (total volume of 0.3 mL) of anesthetized 3 Tg-AD mice or in WT mice that had 24 hours previous surgery, during which Ab1-42 oligomers or control peptide were injected into the right IC. FG and Ab1-42 oligomers were injected exactly in the same anatomic site. Animals were sacrificed 10 days after FG injection for histologic analysis.

2.7. In vivo electrophysiologic recordings

A constant current stimulation (150e200 mA monophasic pulses, 0.25 ms duration) was delivered to the BLA (AP 1.46 mm, ML þ2.79 mm, DV e4.75 mm 0.5). IC-evoked responses were recorded unilaterally by using a monopole stainless steel electrode placed in the IC (DV 2.75 mm; AP þ1.1 mm; ML 3.3 mm) in anesthetized animals with pentobarbital (47.5 mg/kg i.p). The IC responses were measured by obtaining the field excitatory post-synaptic potentials (fEPSP) slope measured 1e2 ms after the fEPSP onset. The current intensity that elicited a 50% maximal response was determined and used for all subsequent stimulation. Low-frequency responses were evoked once every 20 seconds throughout a 20 minutes baseline period. LTP was tested if the fEPSP slope was stable, defined by fEPSP amplitudes that differed from the mean of the 20 minutes baseline period by 5% or less. To induce, LTP 10 trains of 100 Hz/1 s were delivered, after which, IC-evoked responses were collected for an additional hour. Inputeoutput (I/O) curves were assessed to determine the synaptic excitability for a range of stimulation intensities, using multiples of threshold intensity (1e10). Threshold intensity was defined as the stimulation intensity required to produce a 0.1 mV response amplitude. The I/O curves were plotted by the relationship of the fEPSP amplitude (mV) to the raw shock strength (mA). In addition, to investigate changes on synaptic strength induced by Ab oligomers, electrodes were implanted and secured with Loctite super glue adhesive (Henkel, Düsseldorf, Germany) in BLA and IC. To verify the correct positions of the electrodes, simultaneous evoked test pulses were applied during the implantation procedure. fEPSP were evoked throughout the baseline period, after which Aâ oligomers were administered in the IC through the recording electrode and IC-evoked responses were collected for additional 20 minutes. Twenty four hour later, animals were anesthetized and basal transmission, I/O and LTP were tested.

2.8. Histologic and immunohistochemical analyses

Animals were killed with an overdose of pentobarbital, and the brains fixed by cardiac perfusion with 0.9% NaCl followed by 4%paraformaldehyde, pH 7.4. Brains were removed and postfixed for 48 hours before being transferred into 30% sucrose. The tissue was embedded in Tissue-Tek (VWR, Chicago, IL, USA) and cut coronally using a cryostat (Leica Biosystems, Richmond, USA) into 35-mm thick free-floating sections. Trizma-buffered saline 0.1 M, pH 7.4 was used to dilute immunoreagents and for washing brain sections between incubations. Free-floating sections were washed for 30 minutes, then placed in 5% bovine serum albumin (BSA, Sigma) for 60 minutes, and incubated at 4 C on 5% BSA, 0.1% Triton X-100 with the following primary antibodies: mouse monoclonal anti-body against Ab 1e16 (1:1000; 6E10, Covance, Emeryville, CA, USA) and a rabbit polyclonal antibody against tyrosine hydroxylase (TH; 1:1000; Pel-Freez, Rogers, AR, USA). For Ab immunodetection, epitopes were previously exposed with 86% formic acid for 5 minutes. Negative controls with no primary antibodies were pre-pared. Sections were then washed for 60 minutes in trizma-buffered saline followed by incubation at room temperature for 2 hours in 5% BSA, 0.1% Triton X-100 with secondary antibodies: goat antimouse IgG conjugated to fluorescein isothiocyanate (1:250; Millipore, Darmstadt, Germany) and goat antirabbit IgG conjugated to CY3 (1:250; Millipore, Darmstadt, Germany). Sec-tions were again washed and mounted on Super-frost Plus micro slides (VWR, Leuven, Belgium). Quantification of Ab, density of THþ fibers and number of FG-labeled neurons were characterized by capturing images using an Olympus FV10i confocal microscope (Olympus, Miami, FL, USA), equipped with ultraviolet and/or visible light LD lasers. A 10 objective was used. For quantification, a total of 5e6 mice were used. Five consecutive sections were imaged for the IC and ventral-tegmental area (VTA) and 9 images were acquired per section. Each confocal image corresponded to a field of 424.6 424.6 mm and was analyzed with ImageJ (NIH). We used the feature Hessian plugin to analyze cortical fibers (Sathyanesan et al., 2012)and the triangle function to adjust threshold limits for intracellular Ab accumulation and FG-labeled cells.

2.9. Statistical analyses

Multifactorial analysis of variance (ANOVA) was performed for neurotransmitter levels, one-way ANOVA for behavioral scores and Student's t test for histologic analysis. Data obtained from in vivo electrophysiologic recordings were analyzed by repeated measures two-way ANOVA (RM2wANOVA, groups factor) for the fEPSP slopes of baseline (20 minutes) and after-tetanus (last 20 minutes). I/O curves relationship was analyzed by comparing nonlineal regres-sion curves with extra-sum-of-squares F test analysis for Y0, plateau and K parameters. In all cases, Bonferroni's post hoc analysis was used, and $p < 0.05$ was considered statistically significant.

3. Results

3.1. Taste memory impairment is related to lack of dopamine release during novel taste recognition in 3 Tg-AD model

We first evaluated the performance of 3 Tg-AD mice on taste recognition memory test in 3-month and 10-month 3 Tg-AD and WT mice. Pairing of the novel taste saccharin with malaise induced by LiCl resulted in significant reductions in the preference index for saccharin during memory test in WT 3-month, WT 10-month, and 3 Tg-AD 3-month mice but not in 3 Tg-AD 10-month mice, which failed to show an aversion to saccharin [$F(3,24) ¼ 19.96$; $p < 0.01$; Fig. 1C]. Poor performance in memory test was associated to dif-ferences in saccharin consumption during novel taste presentation [$F(3,25) ¼ 11.31$, $p < 0.01$; Fig. 1B]. Thus, to determine an impaired neurochemical activity during the presentation of a new taste and LiCl administration, we performed in vivo microdialysis in the same groups evaluated for gustatory recognition memory (Fig. 1A). Dopamine release, measured in the IC, revealed significant differ-ences among groups [$F(3,89) ¼ 7.530$, $p < 0.01$]. When novel taste was presented, significant increase of cortical dopamine levels was evident as compared with baseline levels in 3-month WT, 3-month 3 Tg-AD, and 10-month WT mice ($p < 0.05$). However, 10-month 3 Tg-AD mice did not show any increase in the extracellular con-centration of dopamine [Factor genotype: $F(1,89) ¼ 4.209$, $p < 0.05$; factor age: $F(1,89) ¼ 7.704$, $p < 0.01$; Fig. 1D]. Conversely, extra-cellular glutamate levels showed no significant difference among groups [$F(3,90) ¼ 0.911$, $p ¼ 4.388$]. The injection of LiCl produced an increase of extracellular glutamate in all tested groups compared to baseline fractions [$F(3,103) ¼ 18.722$, $p < 0.01$], indicating that the visceral signaling is unimpaired regardless of age or genotype (Fig. 1E).

Increased consumption of novel taste in transgenic animals could be explained by sensorial gustative impairments. Thus, we evaluated taste preferences and taste sensitivity curves in the 3 Tg-AD and WT mice at both ages. Data for each group were analyzed using two-way ANOVA with strain as the between group-factor and concentration as the within group-factor. Taste preference test show comparable taste sensitivities and gustatory curves for all groups [$F(3,122) ¼ 0.598$, $p ¼ 0.67$; Fig. 1F and G], which rules out the possibility of loss of taste perception and reflects that impaired release of dopamine in 10-month 3 Tg-AD mice roots the loss of novel taste recognition, and thus impairs aversive taste memory formation.

3.2. Ab accumulation is related to low cortical catecholaminergic levels and axonal loss in 3 Tg-AD mice

We next sought to determine whether dopamine release could be stimulated in 3 Tg-AD mice. High potassium stimulation significantly increased release of dopamine and norepinephrine levels compared with baseline in 3-month WT mice [$F(3,91) ¼ 7.891$ $p < 0.01$ for dopamine and $F(3,101) ¼ 4.720$, $p < 0.05$ for norepinephrine]; 10-month WT mice $F(3,98) ¼ 8.345$, $p < 0.01$ for dopamine and $F(3,98) ¼ 3.581$, $p < 0.05$ for norepinephrine] and in 3-month 3 Tg-AD [$F(3,97) ¼ 4.934$, $p < 0.05$ for dopamine and $F(3,97) ¼$ for norepinephrine]. Interestingly, the 10-month 3 Tg-AD animals showed a significantly lower increase in dopamine and norepinephrine levels than the 10-month WT group ($p < 0.05$ for dopamine and $p < 0.01$ for norepinephrine; Fig. 2A and B). These results suggest that catecholamine release is impaired in 10-month 3 Tg-AD mice, but it is still susceptible to be stimulated by high potassium depolarization. Analysis of baseline neurotransmitter levels confirmed that dopamine concentration was lower in the IC of 10-month 3 Tg-AD mice compared with other groups [$F(3,23) ¼ 6.341$, $p ¼ 0.002$; see Supplementary Fig. 2A]. Baseline norepi-nephrine levels were not different among groups [$F(3,23) ¼ 0.20$, $p ¼ 0.8918$; Supplementary Fig. 2B].

Then, we performed immunofluorescence analyses to evaluate the effect of Ab pathology progression on the expression of TH as a marker of catecholamine function. Histologic analysis revealed that TH expression is not significantly affected by aging in 10-month WT mice in comparison with 3-month WT mice (p ¼ 0.15; Fig. 2C and D). The WT mice at both ages did not show any Ab immune-positive signal. Interestingly, the density of TH immunoreactive (THþ) cortical terminals in 10-month 3 Tg-AD mice was less profuse than in 3-month 3 Tg-AD mice. Quantification analysis show that at 3-month intracellular Ab accumulation is low in the IC, and THþ terminals are plentiful. At 10-month, 3 Tg-AD mice showed an extensive accumulation of intracellular Ab, which coincides with reduction of density of THþ axons in 10-month 3 TgAD mice ($p < 0.01$), at approximately 50% relative to the analyzed area and compared with 3-month 3 Tg-AD group (Fig. 2E). Atrophy of THþ fibers related to Ab has been described in APPswe/PS1DE9 mouse model of amyloidosis previous to neurodegeneration in the VTA (Liu et al., 2008). Thus, we confirmed cortical axonal loss from VTA neurons by detection of retrogradely transported FG in neuronsexpressing TH in VTA. Fig. 2F and G shows a decreased number of FGþ neurons in the VTA of 10-month 3 Tg-AD mice in comparison with 3-month 3 Tg-AD mice ($p < 0.01$).

Altogether, our results show that the dopaminergic and norad-renergic systems are impaired in 10-month 3 Tg-AD because of the Ab-induced loss of THþ axons. The dopaminergic system is strongly affected in 10-month 3 Tg-AD mice, as denoted by the reduced basal levels and the impaired release induced by activity and high potassium stimulation. On the other hand, the noradrenergic sys-tem seems to be less affected in 10-month 3 Tg-AD mice, which have an altered potassium-induced release. The impairment in gustatory recognition memory might be related to the lack of dopamine release as we previously reported that dopamine, but not norepinephrine, release in the IC is related to novel taste presen-tation (Guzmán-Ramos et al., 2010).

3.3. Ab pathology impairs synaptic plasticity in the BLA-IC pathway by switching HFS-induced LTP to LTD in the 3 Tg-AD model

To further understand how intracellular Ab accumulation leads to memory impairment, we evaluated whether synaptic plasticity in the BLA-IC pathway is affected by age-related Ab pathology. We used an experimental protocol of HFS to induce in vivo LTP in anesthetized animals and measured fEPSP of IC-evoked responses in 3-month and 10-month 3 Tg-AD mice (Fig. 3A). Electrophysio-logic recordings show that 3-month 3 Tg-AD mice display LTP in the BLA-IC pathway [$F(1,5) ¼ 97.09$, $p < 0.01$], whereas a clear LTD is shown in 10-month 3 Tg-AD mice after HFS [$F(1,4) ¼ 86.19$, $p < 0.01$; Fig. 3B and C]. We also evaluated the impact of aging in syn-aptic plasticity in the BLA-IC pathway performing the same experimental protocol of HFS to induce in vivo LTP in control mice. The 3-month WT mice group showed a clear synaptic potentiation [$F(1,5) ¼ 137.9$, $p < 0.01$], but the induction of LTP was blocked in 10-month WT mice [$F(1,4) ¼ 12.17$, $p ¼ 0.02$; Fig. 3B and C]. This stresses the role of aging in AD-synaptic failure but also highlights the role of dopamine and norepinephrine in the Ab-related syn-aptopathy. We found that aging-mediated mechanisms seem to disrupt LTP to the point of flattening changes in synaptic plasticity, whereas Ab not only impaired LTP but induced LTD after HFS related to a catecholaminergic neurotransmission dysfunction. To analyze whether the change in the ability to induce synaptic plas-ticity is accompanied by a change in basal synaptic communication through the BLA-IC pathway, we conducted I/O curve experiments. Nonlineal regression analyses showed a significant reduction of I/O curves in 10-month mice when compared with 3-month 3 Tg-AD mice ($p < 0.01$), which reflects a decrease of basal synaptic efficacy in 3 Tg-AD mice, as accumulation of cortical intracellular Ab pro-gresses (Fig. 3D). Synaptic transmission resulted also affected by aging in control mi ce as shown in the I/O curves [$F(3,14) ¼ 471.7$, $p < 0.01$; Fig. 3D].

3.4. Blockade of catecholamine's reuptake restores synaptic plasticity and memory

We previously showed that administration of 0.5 mL of 0.25-mM nomifensine increased IC levels of dopamine in 3 Tg-AD mice and restored memory impairment (Guzmán-Ramos et al., 2012a). In addition, it has been reported that treatment with dopaminergic (Jürgensen et al., 2011) and noradrenergic (Li et al., 2013) receptors agonists ameliorates Ab-mediated effects on synaptic plasticity in in vitro models. Therefore, we asked whether restoration of dopa-mine cortical levels could retrieve both synaptic dysfunction and memory. For this, we administered the catecholaminergic reuptake inhibitor nomifensine. Nomifensine or vehicle was administered by bilateral intracortical injection in the IC, 20 minutes before HFS

BLA-CI pathway, could have a behavioral impact. Nomifensine or vehicle was administered in the IC in two 10-month 3 Tg-AD mice. Intracortical administration of nomifensine was performed 20 mi-nutes before saccharin presentation (novel taste) followed by LiCl injection, and taste memory was assessed 24 hours later. Blockade of dopamine reuptake improved novel taste and aversive taste recognition [One-way ANOVAs for novel taste recognition $F(1,15) = 11.57$, $p < 0.01$, and $F(1,15) = 26.076$, $p < 0.01$, for aversive taste memory; Fig. 4D]. These results confirm that increasing extracel-lular dopamine levels can restore synaptic plasticity and memory function.

3.5. Intracortical administration of Ab1-42 in WT animals induces loss of THþ terminals, synaptic plasticity alterations and memory deficits

To verify that the switch from LTP to LTD could be attributed to Ab deposition in the 3 Tg-AD model, we assessed the effect of an intracortical infusion of Ab1-42 oligomers, or the reverse peptide Ab42-1 in 3-month and 10-month WT mice. We administered 0.5 mL of a 4-mM solution of each peptide intracortically, as this dose and peptide were previously reported to decrease cortical dopamine levels in rats (Trabace et al., 2007). We performed in vivo BLA-IC electrophysiologic recordings 24 hours after peptide administra-tion (Fig. 5A and B). WT mice administered with reverse peptide (Ab42-1) reached LTP [$F(1,5) = 166.5$, $p < 0.01$], but 3-month [$F(1,7) = 9.3$, $p < 0.01$]and 10-month WT mice administered with Ab1-42 oligomers [$F(1,6) = 95.0$, $p < 0.01$] had a decrease in synaptic efficacy instead of LTP after HFS. A trend toward stronger LTD was shown by 10-month WT mice compared with 3-month WT mice, both administered with Ab1-42 oligomers. The I/O ramp indicates that synaptic transmission in the BLA-IC pathway is affected after infusion of Ab1-42 ($p < 0.01$; Fig. 5C). To investigate whether Ab oligomers change the basal synaptic strength, we implanted elec-trodes in the BLA-IC pathway to determine intra-animal basal transmission changes before, immediately after and 24 hours after the Ab infusion. We found a mild trend toward decrease in basal fEPSP comparing before (0.53 0.02 mV) and 24 hours after (0.47 0.02 mV) peptide infusion ($p = 0.81$, $n = 3$). This implies that syn-aptic strength, measured as basal transmission, is not profoundly affected by Ab oligomers after 24 hours, but its deleterious effect on BLA-IC synapses is evident by increasing the stimulation current in I/O curves.

Histologic analysis revealed that administration of Ab1-42 olig-omers induced a significant loss of THþ axons density in the IC of 3-month WT mice as shown by the reduction in TH expression ($p < 0.01$; Fig. 5D). In addition, the IC injection of the retrograde tracer FG, 24 hours after the Ab1-42 oligomers administration resulted in a decreased number of FGþ neurons in the VTA compared with control peptide injection ($p < 0.01$; Fig. 5E). This implies that Ab impairs the VTA-IC dopaminergic pathway. Behaviorally, the group receiving Ab1-42 oligomers increased novel saccharin consumption and failed to show aversion during memory test in comparison with reverse peptide [One-way ANOVAs ($F(1,8) = 8.22$; $p < 0.05$, for novel taste recognition and $F(1,8) = 15.77$; $p < 0.01$), for preference index; Fig. 5F]. In summary, Ab1-42 but not Ab42-1 decreased dopaminergic neurotransmission and produced synaptic dysfunc-tion and memory impairment, similar to the effects observed in the 10-month 3 TgAD mice.

3.6. Cortical dopamine levels modify LTP and/or LTD-induction threshold

To corroborate that the Ab-induced conversion from LTP to LTD after HFS was because of a decreased dopaminergic neurotransmission, we administered 0.25 mg of 6-OHDA intra-cortically in 3-month WT mice. This neurotoxin selectively dam-ages catecholaminergic terminals, decreases dopamine levels in the IC and impairs taste aversion, as previously described (Fernandez-Ruiz et al., 1993). Twenty-four hour after 6-OHDA administration, HFS was delivered in the BLA-IC pathway. Fig. 6A and B shows that WT mice administered with vehicle, displayed LTP after HFS [$F(1,4) = 927.1$, $p < 0.01$], but the 6-OHDA administered group presented a sustained LTD [$F(1,4) = 1029.0$, $p < 0.01$]. The I/O ramp did not show an affected synaptic transmission in the BLA-IC pathway after injection of 6-OHDA ($p = 0.02$; Fig. 6C). Immuno-histochemical analyses confirmed that 3-month WT mice admin-istered with 6-OHDA had a significant loss of THþ axons in the IC at 24 hours (Fig. 6D).

Summary graphs (Fig. 7A and B) show that a reduced cortical dopamine levels induced by Ab in 10-month 3 Tg-AD mice, by Ab1-42 oligomers or by 6-OHDA administered in WT mice, produce a switch from LTP to LTD after HFS, while aging only blocks LTP. Remarkably restoring dopamine levels with nomifensine can reverse Ab-induced conversion from LTP to LTD, allowing the in-duction of LTP (Fig. 5E).

4 Discussion

We report here that Ab accumulation in an AD transgenic model or Ab1-42 oligomers administered in WT mice caused the loss of THþ terminals and reduced cortical dopamine release. This dopa-mine dysfunction switched the induction of LTP into LTD in the BLA-IC pathway and produced recognition memory impairments. Remarkably, the administration of a catecholaminergic reuptake blocker reverted Ab-induced synaptic dysfunction. Moreover, the depletion of cortical dopaminergic levels by 6-OHDA, mimicked the conversion of LTP to LTD. All in all, these results suggest that Ab induces cortical plasticity dysfunction and memory deficiency through an impaired control of dopamine neuromodulation.

Dopamine basal concentration and dopamine release were reduced in 10-month 3 Tg-AD mice when they were presented with a novel gustatory stimulus. This is consistent with our previ-ous findings of impaired dopaminergic release when 10-month 3 Tg-AD mice were exposed to novel objects in a recognition memory task (Guzmán-Ramos et al., 2012a). Conversely, glutamate release remained unaffected during gustatory memory test. In addition, a strong stimulation by high potassium showed an impaired dopamine and norepinephrine release. Accordingly, it has been demonstrated that Ab1-42 oligomers administered to adult rats decreased dopamine baseline levels and reduced dopamine release in the cerebral cortex after high potassium stimulation (Itoh et al., 1996; Trabace et al., 2007). We also assessed the Ab-linked dopa-minergic dysfunction by histologic analyses that showed loss and atrophy of THþ terminals related to Ab deposition in the 3 Tg-AD model and a decreased labeling of the retrograde tracer FG in the VTA. Moreover, the exogenous administration of Ab1-42 oligomers mimicked the loss of cortical THþ terminals in WT mice, which strongly suggests that an Ab-induced loss of catecholaminergic immunopositive fibers could be a hallmark of early stage AD histopathology. This is consistent with the reduction of THþ terminals found in AD patients (Booze et al., 1993a, 1993b) and other AD mouse models (Francis et al., 2012; Liu et al., 2008).

Our results show that both Ab accumulation in the 3 Tg-AD model and Ab1-42 oligomers administration in the IC impair aversive taste memory. This is consistent with other reports in different transgenic mouse models of AD (Devi and Ohno, 2010; Janus et al., 2004; Pistell et al., 2008; Ramírez-Lugo et al., 2009), but we found that aversive taste memory impairment is due a dysfunctional recognition of the novel taste. Only scant studies have investigated presence of Ab but a significant LTD, which was related to low cortical levels of dopamine. This goes well in line with recent find-ings proposing that the main synaptic defect in AD-mouse models is because of their inability to undergo normal metaplasticity, showing an altered LTP and/or LTD induction threshold across different ages in the APPswe; PS1DE9 transgenic model (Megill et al., 2015).

The altered LTP and/or LTD induction reported here is related to low cortical levels of dopamine. In line with this, it has been proposed that the dopamine control of the threshold for LTP and/or LTD induction depends on sustained tonic dopamine release regulated by cortical afferents rather than phasic dopamine release presinaptically controlled by neuron firing (Grace, 1991; Sheynikhovich et al., 2013). This proposed role of tonic dopamine is different to the role of the hippocampal phasic dopamine shown to control the temporal persistence of LTP (O'Carroll and Morris, 2004; Takeuchi et al., 2014). In addition, experiments in prefron-tal cortical slices have shown that switching from LTD to LTP de-pends on dopamine levels before synaptic stimulation (Kolomiets et al., 2009; Matsuda et al., 2006), showing that tonic dopamine release modulates synaptic AMPA and NMDA receptors trafficking, which in turn modify the threshold for LTP and/or LTD induction (Sheynikhovich et al., 2013). Similarly, it has been shown in vivo that under conditions of chronic D1 receptors blockade, HFS of the medial prefrontal cortex actually induces LTD instead of the expected LTP (Coppa-Hopman et al., 2009).

The IC has a high density of dopaminergic fibers arising princi-pally from the ipsilateral VTA and substantia nigra (Ohara et al., 2003), whereas noradrenergic fibers arise from locus coeruleus, subcoeruleus, and other hindbrain nuclei (Robertson et al., 2013). In addition, noradrenergic terminals could also release dopamine in the IC (Smith and Greene, 2012). Here, we show that Ab induces the loss of catecholaminergic terminals and decreases tonic and phasic dopamine levels which may lead to a modification of the threshold to generate cortical plasticity and may also impair the temporal persistence of synaptic plasticity. This idea was corroborated in our experiments by abrogating dopaminergic neurotransmission by 6-OHDA, which induced loss of THþ axons and mimicked synaptic plasticity alterations found in the AD models.

Remarkably, we found that administration of nomifensine in the 3 Tg-AD mice reinstated the ability to induce LTP after HFS. This is consistent with an in vitro study using Ab oligomers (Jürgensen et al., 2011) and studies in AD patients (Koch et al., 2014;

Martorana and Koch, 2014) suggesting that AD-related plasticity dysfunction may be ameliorated by dopaminergic activity stimu-lation. Moreover, a feasible mechanism for this was showed by Jürgensen et al., who demonstrated in hippocampal neurons that the selective activation of dopamine D1/D5 receptors prevents the reduction of surface located AMPA and NMDA receptors by decreasing the GluR1 AMPA receptor subunit phosphorylation at Ser845, which regulates the membrane location of the receptor. Nomifensine, in this work, increases dopamine levels in the IC of 10-month 3 Tg-AD mice by inhibiting its reuptake (Guzmán-Ramos et al., 2012a), which may lead to an increased activation of D1/D5 receptors that restores synaptic AMPA and NMDA receptors trafficking damaged by Ab oligomers. It is important to emphasize, that although we found that Ab impairs the dopaminergic control of LTP and/or LTD induction threshold in the BLA-IC pathway, we would expect that this phenomenon may also occur in other brain areas given that; first, it has been shown that dopamine controls the threshold for LTP and/or LTD induction in the prefrontal cortex (Sheynikhovich et al., 2013) and hippocampus (Abraham et al., 2001); and second, dopaminergic terminals have been found lost in the hippocampus, motor, and somatosensory cortices in AD models and patients (Booze et al., 1993b; Liu et al., 2008). This suggests that the dopaminergic system may constitute a target for the development of novel therapeutic approaches for AD.

The fact that age is the major risk factor for AD is reflected in our experiments showing an impaired induction of LTP in middle-aged mice. This is consistent with findings of LTP impairment in middle-aged animals in the neocortex (Lalo et al., 2014) and hippocampus (Rex et al., 2005, 2006) and could reflect that the plasticity in the BLA-IC pathway affected by age renders these pathways vulnerable in AD. Interestingly, 10-month WT mice showed a higher synaptic impairment with a single dose of Ab1-42 oligomers in the BLA-IC pathway, this is in the same vein with reports showing that AD-prone areas are also highly vulnerable to normal age changes (Fjell et al., 2014) and stresses the importance of studying aging as an important factor in Ab mechanisms of synaptic failure.

5 Conclusions

We have established a novel relationship between catecholaminergic neurotransmission and AD by demonstrating that Ab-induced pathologic alterations in cortical synapses and memory are mediated by dopamine dysfunction. One of the implications of this interaction is that dopamine may constitute a target for the development of novel therapeutic approaches for AD treatment. Our work represents an in vivo evidence of the dopaminergic control of LTP and/or LTD induction threshold and its implication in AD, outlining the important function of cortical dopamine under-lying the mechanism of synaptic plasticity and memory formation in the healthy and diseased brain.

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