Triiodothyronine Treatment reverses Depression-Like Behavior in a triple-transgenic animal model of Alzheimer’s Disease

Andréa V. Maglione1,2, Bruna P. P. do Nascimento2,3, Miriam O. Ribeiro3, Talytha J. L. de Souza1, Renata E. C. da Silva1, Monica A. Sato4, Carlos A. A. Penatti5, Luiz R. G. Britto6, Janaina S. de Souza1, Rui M.B. Maciel1, Rodrigo Rodrigues da Conceição1, Roberto Laureano-Melo7, Gisele Giannocco1

Received: 25 January 2022 / Accepted: 19 July 2022 / Published online: 11 August 2022
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Abstract
Alzheimer disease’s (AD) is a neurodegenerative disorder characterized by cognitive and behavioral impairment. The central nervous system is an important target of thyroid hormones (TH). An inverse association between serum triiodothyronine (T3) levels and the risk of AD symptoms and progression has been reported. We investigated the effects of T3 treatment on the depression-like behavior in male transgenic 3Xtg-AD mice. Animals were divided into 2 groups treated with daily intraperitoneal injections of 20 ng/g of body weight (b.w.) L-T3 (T3 group) or saline (vehicle, control group). The experimental protocol lasted 21 days, and behavioral tests were conducted on days 18–20. At the end of the experiment, the TH profile and hippocampal gene expression were evaluated. The T3-treated group significantly increased serum T3 and decreased thyroxine (T4) levels. When compared to control hippocampal samples, the T3 group exhibited attenuated glycogen synthase kinase-3 (GSK3), metalloproteinase 10 (ADAM10), amyloid-beta precursor-protein (APP), serotonin transporter (SERT), 5HT1A receptor, monocarboxylate transporter 8 (MCT8) and bone morphogenetic protein 7 (BMP-7) gene expression, whereas augmented superoxide dismutase 2 (SOD2) and Hairless gene expression. T3-treated animals also displayed reduced immobility time in both the tail suspension and forced swim tests, and in the latter presented a higher latency time compared to the control group. Therefore, our findings suggest that in an AD mouse model, T3 supplementation promotes improvements in depression-like behavior, through the modulation of the serotonergic related genes involved in the transmission mediated by 5HT1A receptors and serotonin reuptake, and attenuated disease progression.

Keywords Alzheimer’s disease · Triiodothyronine · Depression · hippocampus

Introduction
Alzheimer’s disease (AD) is the most common cause of dementia in the aging population. It has been reported that among AD cases, less than 1% of the patients are under 60 years of age and more than 40% are over the age of 85 (Reitz et al., 2011). It is an irreversible and severe neurological disorder, which can slowly progress for years (Stelzmann et al., 1995). Pathologically, AD is characterized by extensive neuronal loss accompanied by accumulated neurofibrillary tangles and amyloid plaques in the brain (Karch and Goate, 2015). In addition to the well-documented cognitive decline, natural AD progression includes functional losses and behavioral/psychological changes that are present in up to 90% of these patients (Masters et al., 2015). While patients commonly present depression, AD-related behavioral impairment is highly heterogeneous (Burns, 1990; Teri et al., 1992; Cummings, 2000). These cognitive symptoms are likely due to the fact that the serotonergic system is involved in mood and memory and the serotonergic projections to the hippocampus regulate synaptic plasticity processes (Fernandez et al., 2017).

The onset and progression of AD are due to a combination and an accumulation of genetic and environmental factors (Van Rensburg et al., 2006; Letra et al., 2014). For instance, mutations in the genes encoding for beta-amyloid precursor protein (APP), apolipoprotein E (apoE), presenilin 1 (PSEN1) and presenilin 2 (PSEN2) have been consistently associated with AD onset (Lambert et al., 2002; Xia et al., 2015). Since those genes are located on different chromosomes, the disease onset likely occurs through a common
studies have demonstrated that APP gene and elucidated. Moreover, peripheral and central administra-
which the decreased serum free T3 levels increase the risk of developing this neurodegenerative disease have not been
Although this study is very relevant, the mechanisms by 
AD has been demonstrated by Quinlan et al. (2004; Cortés et al., 2018). It should be pointed out that T3 alone or in combination with other antidepressants has been successfully used for the treatment of major depression (Cooper-Kazaz and Lerer, 2008; Kelly and Lieberman, 2009; Touma et al., 2017). Previous studies shown that HT1A receptors are mainly concentrated in the limbic system, particularly the hippocampus (dentate gyrus and CA1), lateral septum, and amygdala, in cingulate and entorhinal cortices, and in the dorsal and median raphe nuclei, which are regions implicated in spatial learning and memory (Barnes and Sharp, 1999; Lanfumey and Hamon, 2000; Glikmann-Johnston et al., 2015). Gamma aminobutyric acid (GABA) plays an important role in the regulation of neuronal excitability, acting as the main inhibitory neurotransmitter in the brain (Roberts and Kuriyama, 1968; Owens and Kriegstein, 2002; Mody and Pearce, 2004). GABA is converted primarily from glutamic acid by the action of glutamic acid decarboxylase (GAD), binds to GABA A and GABA B receptors, and undergoes reuptake to presynaptic nerve terminals with the aid of transporter proteins called GAT-1, GAT-2 GAT-3, and BGT-1 (Fuhrer et al., 2017). Even though earlier reports have shown that GABAergic neurons are relatively resistant in AD, further research has suggested the involvement of GABAergic terminal atrophy in the progression of the disease (Hardy et al., 1987a, b). In addition, Furher et al. (2017) have demonstrated an impaired expression of GABA transporter in the hippocampus in human Alzheimer disease (Fuhrer et al., 2017). We hypothesized that T3 treatment could reverse the depression-like behavior through molecular changes in the hippocampus of transgenic AD mice. To understand the molecular mechanism involved, we analyzed genes related to GABA and Serotonergic systems, neuroplasticity and AD progression in control and T3 treated 3xTg-AD mice. In addition, we evaluated genes that can be positively or negatively regulated by TH to verify the hippocampal thyroid status.

Experimental procedure

Animals

Six-months-old male transgenic 3xTg-AD (APPswe, PS1m146v, tauP301L) mice, weighing between 20 and 35 g, were employed for the study. This animal model of AD expresses three dementia-related transgenes, APPswe, PS1M146V, and tauP301L, and the progression of the
Experimental protocol

Hypertriiodothyronemia was induced with intraperitoneal (IP) injections of triiodothyronine (T3) (L-T3, Sigma-Aldrich, St. Louis, MO, USA) at the dose of 20 ng/g of b.w., which is a replacement dose as previous described by Bianco et al. (2014). Animals were weighed 4 days per week for dose adjustment according to the body weight. This dose was considered ten folds higher than the physiological doses calculated considering a daily T3 production of roughly 2.0 ng/g of b.w. (Bianco et al., 2014). Stock solutions of T3 (1 µg/µL) in 40 mM sodium hydroxide, pH = 10 were prepared and frozen until the day of use (Bianco et al., 2014). The solutions were adjusted to pH = 7.7 and IP injections were administered using a 1.0 ml syringe. Control animals received injections of 0.9% NaCl solution (saline, vehicle) that had the same volume as the injections received by the T3 group. The treatment consists in chronic administration of T3 (21 days) since previous studies demonstrate a modulation of serotonergic system in this period (Lifschytz et al., 2011). On days 18–20, behavioral tests were performed (Fig. 1).

Behavioral tests

The behavioral tests included open field, tail suspension and forced swimming, with a random double-blind distribution. All tests were performed at 24-hour intervals and the testing order was determined according to the degree of invasiveness, starting with the open field, followed by the tail suspension and lastly forced swimming test. Each experimental group contained 4 or 6 mice. All tests were carried out between 9:00 AM and 12:00 PM, and the researcher present during each test remained outside the experimental area, only entering the room between trials. Each test was recorded with an overhead camera and behavioral parameters were later analyzed by at least two researchers.

Open-Field Test

The open-field test arena was constructed in acrylic, with a white floor labeled with black symmetrical squares and walls to prevent animal escape. The animals were placed in the center of the arena for 5 min, and the total number of squares crossed, center ratio, time in center zones and rearing were assessed. Exploratory activity is related to the total distance traveled during the test, vertical activity is associated with the number of rearing, and anxiety-like responses are linked to the center ratio (Roth and Katz, 1979).

Tail suspension test

This tail suspension test is based on the observation that when rodents are placed in an inescapable situation, they initially exhibit escape-oriented movements and then assume an immobile posture. In this test, the plight involves hemodynamic stress applied by hanging the animals by the tail (Thierry et al., 1986). In our protocol, the mice were suspended in a rectangular box with a suspension bar of sufficient size, in a location along the bar that was away from the walls of the box (Can et al. 2011). The test was videotaped...
for 5 min, and the immobility time and latency to the first immobility episode were recorded.

**Forced swim test**

This forced swim test is based on the development of an immobile posture immediately following a stressful situation (Porsolt et al., 1977) and involves individually placing of each mouse in a water-filled polypropylene cylinder (radius = 30 cm; depth = 50 cm) at 25°C. The animals are unable to escape or touch the bottom of the cylinder. Animals were submitted to the test for 5 min and immobility time and latency to the first immobility episode were recorded. It is known that immobility time is indicative of low resilience and highly associated with depression-like behavior (Porsolt et al., 1979).

**Euthanasia process**

Two days after the last behavior test, animals were anesthetized with ketamine (100 mg/ml) and xylazine (20 mg/ml) administered by IP injection in a dose of 0.2 ml of ketamine and 0.1 mL of xylazine per 100 g of b.w.. Blood samples were collected from the left ventricle, centrifuged at 3500 rpm at 4°C (Excelsa II 206 BL, Sao Paulo, SP, Brazil) for 15 min and stored in a freezer at -20°C. These samples were later used for determining serum T3 and total T4 levels. Hippocampal samples were collected, immediately frozen in liquid nitrogen and stored at -80°C for use later on in the gene expression experiments by Real-Time quantitative PCR (qPCR).

**Total RNA isolation and real-time PCR**

Total RNA from brain tissue corresponding to the murine hippocampus was isolated using the TRizol® reagent (Invitrogen, Carlsbad, CA, USA), according to the RNA extraction protocol provided by Invitrogen. RNA concentrations were determined spectrophotometrically by recording the absorbance readings at 260 and 280 nm (NanoDrop® ND-1000 UV-Vis, Delaware, USA). Approximately 2 µg of total RNA from each sample was used to perform the reverse transcriptase reactions. First-strand cDNAs were synthesized using the MML-V reverse transcriptase (Invitrogen, Carlsbad, CA, USA). From the obtained cDNA, cycle curves were performed with each primer set. Real-Time qPCR was performed using the EVA Green RT-PCR assay (Solis Biodyne, European Union) and run on an ABI PRISM 7700 Sequence Detector (ABI Applied Biosystems). The primers sequences were manufactured by Integrated DNA Technologies (IDT, Sao Paulo, SP, Brazil) and are listed in Table 1. We used GABA1 and GABA2 genes to evaluate the GABAergic system; TPH2, HT1RA e SLC6A4 genes to evaluate the serotonergic system; BDNF, NTF3 and NGF genes to evaluate neuroplasticity; APP, GSK3, ADAM10 and BACE1 genes to evaluate the Alzheimer’s disease progression and SOD2, MCT8, SLCO1C1, BMP-7 and HR genes to evaluate the neuronal influence of T3 in the hippocampus.

**Serum T3 and T4 measurements**

Serum T3 and total T4 levels were measured using the Elecsys® Kit (Roche Diagnostics, Germany), following the instructions of the manufacturer. Negative controls with Cal set were used before making the measurements and the obtained results were used for calibration. The values were measured automatically and based on a standard curve. For T3, the lower limit of detection for the kit is 1.25 nmol/l and the upper limit is 8.50 nmol/l. For T4, the lower limit of detection is 5.40 nmol/l and the upper limit is 320.0 nmol/l. None of the samples used in the data analyses had values that were outside of these ranges.

**Data Analysis**

The RT-PCR data were analyzed using Microsoft Excel and the samples were normalized based on the respective controls. The difference between samples was normalized according to the variation in the mean of CT (ΔCT), which was subsequently used to calculate the relative expression levels of genes of interest. The results from these experiments are expressed in arbitrary units (Livak and Schmittgen, 2001). All results are presented as mean±SEM. The assumption of normal data distribution was determined using the Shapiro-Wilk test. Parametric comparisons were performed with data that passed the normality test. In these cases, between-group comparisons were analyzed with the Student’s unpaired t-test. Significance level was set at p<0.05 and the t value is presented to measure the size of the difference relative to the variation in the sample data. On the other hand, data that did not have a normal distribution were compared using the Mann-Whitney test. The Grubbs’ test was employed for detecting outliers. Cohen’s d analysis was used to evaluate the effect sizes between the groups, which is represented by the difference between means divided by the standard deviation. Effect sizes were interpreted as: small (0.2<d<0.5), moderate (0.5<d<0.8) and large (d>0.8). Relationships between variables were identified with Pearson correlation analyses. Significance level was set at p<0.05. All statistical analyses were performed with GraphPad Prism 6 statistical software (La Jolla, CA, USA).
and a significant decrease in total T4 (14.9 ± 1.07 µg/dl vs. 80.9 ± 3.02 µg/dl, p < 0.0001, t = 20) levels when compared to control animals.

**Results**

## Serum hormone measurements

The serum hormone levels of controls and T3-treated mice are summarized in Table 2. We observed that mice administered T3 exhibited a significant increase in serum T3 (5.31 ± 0.53 ng/dl vs. 2.68 ± 0.25 ng/dl, p = 0.004, t = 4), and a significant decrease in total T4 (14.9 ± 1.07 µg/dl vs. 80.9 ± 3.02 µg/dl, p < 0.0001, t = 20) levels when compared to control animals.

### Behavioral tests

#### Open-Field Test

The open-field test showed no statistically significant differences in the total number of squares crossed (7.40 ± 3.47 vs. 19.20 ± 7.26, p = 0.18, t = 1.46), center ratio (0.48 ± 0.07 vs. 0.31 ± 0.021, p = 0.07, t = 2.0), time in center zones (48.00 ± 19.79 s vs. 26.20 ± 13.22 s, p = 0.37, t = 0.95) or rearing (4.50 ± 1.04 vs. 8.40 ± 1.69, p = 0.11, t = 1.84) when comparing the control and T3-treated groups (Fig. 2A-D). However, the Cohen’s d analysis showed that T3 had a

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**Table 1** List of primers used for RT qPCR

| Gen Bank     | Coded Protein          | Function               | Primers                                                                 |
|--------------|------------------------|------------------------|--------------------------------------------------------------------------|
| GAD1 (NM_008077.5) | Glutamate decarboxylase 1 (GAD67) | GABA synthesis | F: 5'-CTCAGGCGTGATGTCAGATGTTTAC-3' R: 5'-AAAGGACGATCATAGAGATTGGTGTG-3' |
| GAD2 (NM_008078.2) | Glutamate Decarboxylase 2 (GAD65) | GABA synthesis | F: 5'-TCACATATGCTCCACCATGAGTAC-3' R: 5'-GCCCTGTAAGTCAATCTCCCTAC-3' |
| TPH2 (NM_173391.3) | Tryptophan hydroxylase (TPH2) | Serotonin synthesis | F: 5'-AGTCTACATCCTCCCAACTCTGCT-3' R: 5'-CATCTGTCAGCAACATCTCCAGTC-3' |
| HT1A (NM_008308.4) | 5HT1a receptor | Serotonin G1 protein-coupled receptor | F: 5'-GTGAGAGGAACACCTGAAGGC-3' |
| SLC6A4 (NM_010484.2) | Serotonin transporter (SERT) | Serotonin Reuptake | F: 5'-CCCAGTGAATAGCTCCTCCAG-3' |
| Bdnf (NM_001048139.1) | Brain-derived neurotrophic factor (BDNF) | Neuroplasticity | F: 5'-TCAACTAAGTCCCACCCTAAG-3' |
| NTF3 (NM_001164035.1) | Neurotrophin 3 (NTF3) | Maintenance of the nervous system | F: 5'-GGAGTTTGCCGGAAGACTCTC-3' |
| APP (NM_001198823.1) | Amyloid Beta Precursor Protein (APP) | Formation of amyloid plaques | F: 5'-TCCATGTGCCAAGGAACACTTC-3' |
| GSK3β (NM_001031667.1) | Glycogen synthase kinase-3 β (GSK3β) | Regulates beta-amyloid peptides | F: 5'-TGGCTTGACAGCGAGGAAAA-3' |
| ADAM10 (NM_007399.3) | A disintegrin and metalloproteinase 10 (ADAM10) | Cleavage of TNF-alpha and E-cadherin | F: 5'-GGAGTTTGCCGGAAGACTCTC-3' |
| BACE1 (NM_011792.5) | β-Site APP cleavage enzyme 1 (BACE1) | Formation of amyloid beta peptide | F: 5'-GGAGTTTGCCGGAAGACTCTC-3' |
| SOD2 (NM_013671.3) | Superoxide Dismutase 2 (SOD2) | Positively regulated by TH | F: 5'-GTGTGCTCTGGTAATTTTCTA-3' |
| MCT8 (NM_009197.2) | Monocarboxylate transporter 8 (MCT8) | Negatively regulated by TH | F: 5'-GTGTGCTCTGGTAATTTTCTA-3' |
| BMP-7 (NM_007557.3) | Bone Morphogenetic Protein 7 (BMP-7) | Negatively regulated by TH | F: 5'-GTGTGCTCTGGTAATTTTCTA-3' |
| HR (NM_021877.3) | Hairless (HR) | Positively regulated by TH | F: 5'-GTGTGCTCTGGTAATTTTCTA-3' |
| RPL19 (NM_009078.2) | Ribosomal Protein L19 (RPL19) | Housekeeping (Internal Control) | F: 5'-GTGTGCTCTGGTAATTTTCTA-3' |

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**Table 2** Serum thyroid hormone levels in control and T3-treated AD mice. Values are expressed as the mean ± SEM. n = 6 animals/group. Different letters in the same column indicate p < 0.05 as determined by the Student’s t-test

|                      | Total Serum T4 (µg/dl) | Total Serum T3 (ng/dl) |
|----------------------|------------------------|------------------------|
| Control              | 80.9 ± 3.02<sup>a</sup> | 2.68 ± 0.25<sup>a</sup> |
| T3-Treated           | 14.9 ± 1.07<sup>b</sup> | 5.31 ± 0.53<sup>b</sup> |
The Cohen’s d analysis indicated that T3 induced a strong effect on immobility time (1.91) (Table 3). In addition, Pearson correlation analyses showed that immobility time was strongly correlated with SERT (r = 0.80), HTR1a (r = 0.93), GSK3β (r = 0.95), ADAM10 (r = 0.95) gene expression, as well as serum T4 levels (r = 0.74) (Table 4).

Forced swim test

As shown in Fig. 2E, in the forced swim test, which is a classic test for evaluating depression-like behavior, the T3-treated animals exhibited longer latency (9.75 ± 5.60 s vs. 105.80 ± 32.16 s, p = 0.03, t = 2.94) and a shorter immobility time (124.50 ± 9.94 s vs. 53.00 ± 9.95 s, p = 0.002, t = 5.08) than control mice. Cohen’s d analysis indicated that T3 treatment had a strong effect on latency to immobility (2.08) and immobility time (3.59) (Table 3). Moreover, Pearson correlation analyses detected a strong correlation between immobility time and SERT (r = 0.78), SOD2 (r = 0.87) and GSK3β (r = 0.86) gene expression, as well as serum T4 levels (r = 0.91) (Table 4).

Tail suspension test

In the tail suspension test (Fig. 2E), the T3 treated animals had a latency time that was similar to control mice (36.25 ± 12.76 s vs. 63.75 ± 29.04 s, p = 0.42, t = 0.87). In contrast, the T3-treated group exhibited a significant reduction in immobility time when compared to control mice (132.50 ± 18.31 s vs. 64.50 ± 17.19 s, p = 0.03, t = 2.71). The Cohen’s d analysis indicated that T3 induced a strong effect on immobility time (1.91) (Table 3). In addition, Pearson correlation analyses showed that immobility time was strongly correlated with SERT (r = 0.80), HTR1a (r = 0.93), GSK3β (r = 0.95), ADAM10 (r = 0.95) gene expression, as well as serum T4 levels (r = 0.74) (Table 4).

**Fig. 2** The results presented above show the behavioral parameters of 6-month AD male mice treated with saline (White) and T3 (20 ng/g of b.w., Black) (n = 4–5). The evaluated parameters were: total squares crossed in the open field test (A), center ratio in the open field test (B), time in center area in the open field test (C), rearing in the open field test (D), latency to immobility and time of immobility in the forced swim test and tail suspension test (E). Data represent mean (± SEM) analyzed by Mann-Whitney test. *p < 0.05 and **p < 0.01 compared to control group. s: seconds

**Table 3** Effect size between groups and behavioral parameters

| Behavioral Parameter | Cohen’s d |
|----------------------|-----------|
| Total squares crossed | 1.01      |
| Center ratio         | 1.34      |
| Time in center zone  | 0.62      |
| Rearing              | 1.27      |
| Latency to immobility| 0.61      |
| Immobility time      | 1.91      |
| Latency to immobility| 2.08      |
| Immobility time      | 3.59      |

Numbers in italics represent a moderate magnitude of effect, whereas numbers in bold represent a large magnitude of effect.

Strong effect on the total number of squares crossed (1.01), center ratio (1.34) and rearing (1.27) (Table 3).

Strong effect on the total number of squares crossed (1.01), center ratio (1.34) and rearing (1.27) (Table 3).
Table 4 Pearson correlation matrix between serum hormones measurement, hippocampal gene expression and behavioral parameters assessed in forced swimming test and tail suspension test. Abbreviations: total squares crossed (TSC), center ratio (CR), time in center area (TCA), immobility in tail suspension test (ITST), immobility in forced swim test (IFST)

|       | GAD  | SERT | TPH2 | NGF  | NTF3 | Hairless | T3  | T4  | TSC | CR  | TCA | Rear | ITST | IFST |
|-------|------|------|------|------|------|----------|-----|-----|-----|-----|-----|------|------|------|
| r     |      |      |      |      |      |          |     |     |     |     |     |      |      |      |
| GAD   | 0.33 | 1    |      |      |      |          |     |     |     |     |     |      |      |      |
| 65    |      |      |      |      |      |          |     |     |     |     |     |      |      |      |
|       | -0.23| 0.29 | 1    |      |      |          |     |     |     |     |     |      |      |      |
|       | -0.08| 0.38 | 1    |      |      |          |     |     |     |     |     |      |      |      |
|       | 0.16 | *0.66| 1    |      |      |          |     |     |     |     |     |      |      |      |
|       | *0.69| 0.08 | 0.41 | *0.59| 1    |          |     |     |     |     |     |      |      |      |
| NGF   | 0.04 | 0.51 | 0.35 | 0.55 | 0.29 | 1        |     |     |     |     |     |      |      |      |
|       | 0.77 | -0.21| 0.03 | 0.07 | 0.13 | 1        |     |     |     |     |     |      |      |      |
|       | 0.47 | 0.19 | *-0.63| -0.27| -0.19 | 0.36 | -0.34 | 0.43 | 1    |     |     |      |      |      |
|       | -0.06| 0.28 | *0.62 | 0.46 | 0.49 | 0.28 | 0.48 | 0.01 | *-0.66| 1    |     |      |      |      |
|       | 0.57 | 0.35 | -0.36| -0.15| -0.1 | *0.65 | -0.2  | *0.75| -0.1 | 1    |     |      |      |      |
|       | 0.19 | 0.31 | *0.76 | 0.34 | 0.65 | 0.36 | 0.36 | 0.14 | -0.55| -0.14| 1    |      |      |      |
| Hairless | 0.41 | 0.27 | -0.51| -0.18| -0.24| 0.45 | -0.21| 0.59 | -0.45| -0.42| 1    |      |      |      |
|       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       | -0.15| *0.64| 0.21 | 0.53 | 0.26 | 0.005  | -0.28| 0.25 | -0.28| 0.1  | -0.22| 1    |      |      |
|       | 0.03 | *0.57| 0.48 | 0.4  | 0.02 | -0.46  | *0.68 | -0.28| 0.52 | -0.51| *0.61| 1    |      |      |
|       | 0.08 | *0.63| *0.69| 0.4  | 0.02 | -0.54  | -0.27 | -0.46| *0.65| 1    |      |      |      |      |
| T3    | 0.18 | -0.57| **-0.62||**-0.53| -0.31 | 0.06 | 0.67 | **-0.15| *-0.77| 0.43 | -0.56| *-0.71| *-0.71| 1    |
|       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       | 0.89 | 0.78 | 0.87 |      |      |      |      |      |      |      |      |      |      |      |
| T4    | -0.09| 0.43 | 0.58 | **0.8 | 0.3  | 0.34  | -0.28 | **-0.64| -0.56| *0.73 | *0.79 | 0.62 | *0.74 | **-0.74| 1    |
|       |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|       | 0.86 |      |      |      |      |      |      |      |      |      |      |      |      |      |
| TSC   | 0.33 | 0.07 | -0.35| 0.02 | -0.24| 0.17  | -0.16| 0.35 | 0.3  | -0.03| 0.57 | -0.03| 0.55 | -0.11| -0.41| 0.27 | -0.57| 1    |
| CR    | -0.14| 0.04 | 0.61 | 0.11 | 0.41 | -0.13 | 0.06 | -0.29| -0.5 | 0.15 | -0.58| 0.5  | *-0.69| 0.04 | 0.51 | 0.36 | -0.2 | 0.57 | -0.6 | 1    |
| TCA   | -0.52| -0.11| 0.22 | 0.13 | 0.02 | -0.3  | 0.24 | -0.23| -0.51| -0.01| -0.6 | 0.04 | -0.56| 0.43 | 0.07 | 0.13 | -0.14| 0.33 | -0.59| 0.55| 1    |
| ITST  | 0.49 | *0.79| *0.8 | *0.74| 0.67 | 0.29  | -0.42| 0.7  | -0.1 | -0.29| *0.72| -0.68| *0.75 | -0.19| 0.37 | -0.06| -0.19| 1    |
| IFST  | -0.04| 0.27 | *0.78| 0.48 | 0.67 | 0.13  | 0.4  | -0.28| **-0.870.44| *-0.72| 0.63 | *-0.85| 0.55 | **0.86| 0.65 | -0.6 | **0.91 | *-0.71| *0.74 | 0.41 | *-0.79 | *-0.76| 1    |

r, Pearson coefficient, and correlations (two-tailed) that are significant at the 0.05(*), 0.01(**) or 0.001(***).
GSK3β (1.50) and ADAM10 (1.67) in T3-treated mice (Table 5). Pearson correlation analyses identified a strong correlation between serum T3 levels and the expression of serotonergic system-related genes, including SERT ($r = 0.89$) and HTR1a ($r = 0.78$). Likewise, serum T3 levels were also strongly correlated with MCT8 ($r = 0.87$) and BMP7 ($r = 0.77$) gene expression levels, which have also been shown to be regulated by thyroid hormones (Table 4).

We also observed that in AD-related genes, a positive correlation was found between serum T3 levels and ADAM10 ($r = 0.71$) and GSK3β ($r = 0.71$) expression levels; and serum T4 levels were correlated with ADAM10 ($r = 0.74$) and GSK3β ($r = 0.84$) expression. A strong correlation between HT1r expression and ADAM10 ($r = 0.74$) and GSK3β ($r = 0.74$) expression was also observed (Table 4).

**Discussion**

The findings of this study showed that T3 supplementation in the transgenic AD mouse model (APPsw, PS1m146v, tauP301L, 3xTg-AD) promotes improvements in depression-like behavior. These positive effects appear to be mediated through the modulation of the serotonergic pathway, since the immobility time on Tail Suspension Test presented a strong correlation with SERT and HTR1a expression, whereas immobility time on Forced Swim Test presented a strong correlation with SERT expression. The Open Field
Test does not show a correlation with genes related to serotonergic pathway in Pearson analysis, although the Cohen’s d analysis showed that T3 had a strong effect on the total number of squares crossed, center ratio and rearing. This result of Open Field can be explained by freezing and limited exploratory behavior, characteristics of this animal model as related symptom of Alzheimer’s Disease. Other studies demonstrated that 3xTg-AD mice showed hypoactivity in Open Field Test (Sterniczuk et al., 2010; Filali et al., 2012).

Previous studies have shown that thyroid hormones play important roles in a variety of organs. An association between the central nervous system and AD as well as hormonal disturbances, such as in hypo- and hyperthyroidism, has been previously reported (O’Barr et al., 2006; Gessl et al., 2012; Chaker et al., 2016). In the current study, we showed that the IP injections of T3 increased serum T3 and decreased T4 levels. We also showed that T3-treated animals presented changes in gene expression levels that were suggestive of a hyperthyroid status in the hippocampus. These results do not only demonstrate the clinical accuracy of our hypertriiodothyronemia model but are also in line with previous studies (Hamidi et al., 2012; Gessl et al., 2012; Chaker et al., 2016). In the present study, T3 treatment attenuated 5-HT1A and SERT gene expression in this AD mouse model, suggesting that serotonergic neurotransmission is influenced by T3, which could contribute to the observed antidepressant action. This proposal is further reinforced by the strong correlation between serum T3 levels and the expression of genes involved in these processes, and the strong correlation between the serotonergic system-related genes and the antidepressant parameters associated with each test, especially the tail suspension test.

Interestingly, the beneficial aspects of serotonergic neurotransmission modulation are not restricted to the improvement of AD-affected behaviors, but are also involved in delaying disease progression. According to Noristani et al. (2012), a high tryptophan diet reduces CA1 intraneuronal β-amyloid in the 3xTg-AD mouse model, suggesting that enhanced tryptophan intake and consequent increase in 5-HT neurotransmission could effectively reduce plaque formation in AD (Noristani et al., 2012). In addition, fluoxetine stimulates type 2A protein phosphatases (PP2A), consequently activating Wnt/β-catenin signaling and inhibiting

### Table 5: Effect size on gene expression between the control and T3-treated groups

| Coded Protein | Cohen’s d |
|---------------|-----------|
| GAD 65        | 0.33      |
| GAD 67        | 0.13      |
| TPH2          | 0.50      |
| SERT          | 1.84      |
| HTR1a         | 1.32      |
| BDNF          | 0.45      |
| NGF           | 0.53      |
| NT3F          | 0.03      |
| SOD2          | 1.79      |
| MCT8          | 1.97      |
| SLCO1C1       | 0.76      |
| BMP7          | 2.48      |
| Hairless      | 1.71      |
| BACE1         | 0.36      |
| GSK3β         | 1.50      |
| ADAM10        | 1.67      |

Numbers in italics represent a moderate magnitude of effect, whereas numbers in bold represent a large magnitude of effect.
the hippocampal GSK3β activity. Fluoxetine treatment also reduces APP cleavage and Aβ generation, thus suggesting that serotonergic targets could be exploited in both AD prevention and treatment (Huang et al., 2018).

In this context, the modulation of 5-HT receptors, especially 5-HT2A, 5-HT2C, and 5-HT4 receptors, may be beneficial for AD management (Thathiah and De Strooper, 2011). The beneficial effects of 5-HT receptor modulation in promoting the non-amyloidogenic pathway appears to be mediated by a direct interaction with α-secretase ADAM10 or β-secretase BACE1 (Cochet et al., 2013). Additionally, a recent review proposed that the role of 5HT1A, in terms of synaptic plasticity, changes with aging, transitioning from a stimulated to inhibited state (Duda et al., 2018). This same review also provided evidence for serotonin-mediated inhibition of GSK3β expression, via 5HT1 receptors (Duda et al., 2018). Moreover, 5-HT1A receptor activation was shown to increase the inhibitory serine-phosphorylation of GSK3β (Li et al., 2004). On the other hand, the dopaminergic system can reduce GSK3β serine-phosphorylation through D2 receptor activation, which is due to the inactivation of Akt by protein phosphatase-2 in a β-arrestin2-driven protein complex (Beaulieu et al., 2005). In a mechanism similar to β-arrestin2 recruitment, 5-HT2A receptor activation reduces serine-phosphorylation of GSK3, thereby increasing its activity (Li et al., 2004). Consistent with these observations, GSK3β inhibitors have been shown to reduce depressive-like behavior in animal models of depression (Duda et al., 2018).

In our study, the T3-treated group exhibited a significant reduction in GSK3β and ADAM10 gene expression. This is particularly relevant to AD as GSK3 is a kinase that contributes to the abnormal phosphorylation during tau protein-mediated microtubule stabilization, a process that leads to neurofibrillary tangle formation (Beurel et al., 2015). Studies have shown that β-amyloid peptide formation results in abnormal APP processing and leads to the induction of tau phosphorylation and GSK3 activation (Kuruva and Reddy, 2017). Based on these assumptions, GSK3β inhibitors have great potential for treating AD. Indeed, in different AD animal models, different GSKβ inhibitors promoted lower levels of tau phosphorylation and amyloid deposition, rescuing neuronal loss and restoring memory deficits (Serenó et al., 2009; Griebel et al., 2019). Notably, our results identified a strong correlation between 5HT1A, GSK3β and ADAM10 gene expression, as well as with serum T3 and T4 concentrations. Thus, the T3-induced GSK3 and ADAM 10 down-regulation may be indirectly mediated by the serotonergic system.

Herein, the expression of BACE1 is decreased in T3-treated animals. This aspartyl protease is responsible for the amyloidogenic cleavage of APP and it is increased in the brain of patients with AD (Sathya et al., 2012). The T3-treated animals had also reduced ADAM10 gene expression levels. Once translated, this metalloproteinase plays an important role in glutamatergic synapse modulation (Marcello et al., 2017). Indeed, several studies have shown that ADAM10 expression is reduced in patients with moderate to severe AD (Colciaghi et al., 2004; Kim et al., 2009) and it has been speculated that the attenuation ADAM10 expression would consequently increase N-cadherin levels, ultimately resulting in more AMPA receptors and larger, more stable synapses (Malinverno et al., 2010). Moreover, a recent review showed that T3 increases N-cadherin levels in stem-cells repressing EGF-EGFR and cAMP-PKA signaling (Izaguirre and Casco, 2016). The Pearson correlation analyses detected a positive association between ADAM10 gene expression and the behavioral parameters of the tail suspension test, as well as with serum T3 and T4 levels. Thus, the results from the present study indicate that T3-mediated ADAM10 attenuation, increases N-cadherin levels, and likely, resulting in better glutamatergic synapses and improved depressive behavior.

Another important aspect that needs to be discussed is mitochondrial dysfunction in AD. Extensive studies have demonstrated mitochondrial degradation by autophagy and impaired oxidative phosphorylation in the brains of AD patients that is induced by reactive APP and β-amyloid (Perez Ortiz and Swerdlow, 2019). These deleterious effects are also accompanied by impaired mitochondrial metabolism and increased reactive oxygen species production (Zhao and Zhao, 2013). Further, mitochondrial SOD1 deficiency has been shown to induce amyloid β protein oligomerization and memory loss, whereas SOD2 overexpression has decreased hippocampal oxidative damage (Murakami et al., 2011). Increased SOD2 expression also prevented memory deficits in the Tg2576 AD mouse model (Massaad et al., 2009). Notably, in the present study, T3 treatment significantly increased the hippocampal SOD2 expression, which could slow AD progression. GSK3β also plays a key role in the regulation of mitochondrial function and the observed downregulation of GSK3β induced by T3 treatment could represent another mechanism for reducing the impacts of mitochondrial dysfunction in AD.

A recent study revealed that T3 supplementation was capable of reversing mnemonic deficits and the progression of neuroinflammation in hypothyroid rats. In that study, T3 replacement reduced the expression of Aβ42 peptide and proinflammatory markers, including TNFα (Chaalal et al., 2019). Furthermore, GSK3β activation induces NF-κB signaling, consequently increasing the transcription of TNFα and other pro-inflammatory cytokines (Maixner and Weng, 2013). These factors promote neuroinflammation by activating JNK and p38 MAPK signaling cascades. Taking together
presubiculum, are the least resistant to the effects of several stressors (Grieve et al. 2005; Kalpouzos et al., 2009; La Joie et al., 2010). In the current study, we have performed the gene expression in the whole hippocampus and we did not have additional data to provide the information about what cells in the sub-regions of the hippocampus were affected by T3 treatment, which is a limitation of this study. In our study, we have performed the expression of AD-related and behavior-related genes, however we did not carry out the protein analysis by Western Blotting and immunohistochemistry to evaluate the alteration in the target genes, which is also a limitation of this study and that will still require further investigation.

In conclusion, our data suggest that T3 therapy improves the depression-like behavior observed in an AD transgenic mouse model and that these effects are mediated through the modulation of the serotonergic related genes involved in the transmission mediated by 5HT1A receptors and serotonin reuptake. The benefits of T3 treatment are not only restricted...
to improving AD-related depressive behavior but are also applicable to disease progression. Further studies will be necessary to assess the impact of T3 treatment in order to reduce neuroinflammation and how T3 reduces depression and downregulates AD-related genes.

Acknowledgements We would like to thank the São Paulo State Research Foundation [FAPESP JSS# 2018/22763-0, 2017/07053-3 and GG# 2017/23169-1] and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the master’s degree scholarship to Andrea Vancetto Maglione.

Author contributions: all authors contributed to the study conception and design. Material preparations, data collection and analysis were performed by Andrea Vancetto Maglione, Bruna Nascimento, Talythia Souza, Janaina Souza, Rodrigo Rodrigues da Conceição, Roberto Laureano e Gisele Giannocco. The first draft of the manuscript was written by Andrea Vancetto Maglione and Gisele Giannocco and all authors commented on previous version of the manuscript. All authors read and approved the final manuscript.

Funding This work was supported by the FAPESP project number 2017/23169-1. Andrea Vancetto Maglione has received research support with master degree scholarship by CAPES.

Data Availability the datasets generated and analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Competing interests the authors have no relevant financial or non-financial interests to disclose.

Ethics approval all procedures were performed in this manuscript are in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Animals Ethics Committee of the Universidade Federal de São Paulo (UNIFESP), under protocol # 1880171017.

Consent to participate not applicable.

Consent to publish not applicable.

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Authors and Affiliations

Andréa V. Maglione1 · Bruna P. P. do Nascimento2,3 · Miriam O. Ribeiro3 · Talytha J. L. de Souza1 · Renata E. C. da Silva1 · Monica A. Sato4 · Carlos A. A. Penatti5 · Luiz R. G. Britto6 · Janaina S. de Souza1 · Rui M.B. Maciel1 · Rodrigo Rodrigues da Conceição1 · Roberto Laureano-Melo7 · Gisele Giannocco1

Rodrigo Rodrigues da Conceição rodriguescontato1@hotmail.com
Gisele Giannocco ggiannocco@gmail.com
Andréa V. Maglione andreia@maglione.com.br
Bruna P. P. do Nascimento bruna.pascarelli@gmail.com
Miriam O. Ribeiro miriam.ribeiro@mackenzie.br
Talytha J. L. de Souza talythaleitte@gmail.com
Renata E. C. da Silva renataelen@live.com
Monica A. Sato monica.akemi.sato@gmail.com
Carlos A. A. Penatti carlos.a.a.penatti@me.com
Luiz R. G. Britto britto@icb.usp.br
Janaina S. de Souza ninasenasouza@gmail.com
Rui M.B. Maciel rui.macial@unifesp.br
Roberto Laureano-Melo
laureanomelor@gmail.com

1 Dept. Medicine, Laboratory of Endocrinology and Translational Medicine, Universidade Federal de São Paulo, UNIFESP/EPM, São Paulo, Brazil

2 Laboratory of Translational Medicine, Universidade Federal de São Paulo, UNIFESP/EPM, São Paulo, Brazil

3 Developmental Disorders Program, Center of Biological Science and Health, Mackenzie Presbyterian University, São Paulo, Brazil

4 Dept. Morphology and Physiology, Faculdade de Medicina do ABC, Centro Universitário FMABC, Santo André- Brazil, São Paulo, Santo André, Brazil

5 Laboratory of Human Physiology, Universidade Nove de Julho, São Paulo, Brazil

6 Institute of Biomedical Sciences, Universidade de São Paulo, São Paulo, Brazil

7 Laboratory of Physiopharmacology and Behavior, Universidade de Barra Mansa, Rio de Janeiro, Brazil