



PGC-1 α Signaling Increases GABA(A) Receptor Subunit α 2 Expression, GABAergic Neurotransmission and Anxiety-Like Behavior in Mice

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Peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) is a master regulator of mitochondria biogenesis and cell stress playing a role in metabolic and degenerative diseases. In the brain PGC-1 α expression has been localized mainly to GABAergic interneurons but its overall role is not fully understood. We observed here that the protein levels of γ -aminobutyric acid (GABA) type A receptor- α 2 subunit (GABA α 2) were increased in hippocampus and brain cortex in transgenic (Tg) mice overexpressing PGC-1 α in neurons. Along with this, GABA α 2 expression was enhanced in the hippocampus of the PGC-1 α Tg mice, as shown by quantitative PCR. Double immunostaining revealed that GABA α 2 co-localized with the synaptic protein gephyrin in higher amounts in the striatum radiatum layer of the hippocampal CA1 region in the Tg compared with Wt mice. Electrophysiology revealed that the frequency of spontaneous and miniature inhibitory postsynaptic currents (mIPSCs) was increased in the CA1 region in the Tg mice, indicative of an augmented GABAergic transmission. Behavioral tests revealed an increase for anxiety-like behavior in the PGC-1 α Tg mice compared with controls. To study whether drugs acting on PPAR γ can affect GABA α 2, we employed pioglitazone that elevated GABA α 2 expression in primary cultured neurons. Similar results were obtained using the specific PPAR γ agonist, N-(2-benzoylphenyl)-O-[2-(methyl-2-pyridinylamino) ethyl]-L-tyrosine hydrate

(GW1929). These results demonstrate that PGC-1 α regulates GABA α 2 subunits and GABAergic neurotransmission in the hippocampus with behavioral consequences. This indicates further that drugs like pioglitazone, widely used in the treatment of type 2 diabetes, can influence GABA α 2 expression via the PPAR γ /PGC-1 α system.

Keywords: GABA-A receptors, PGC-1 α , PPAR γ , pioglitazone, gene expression, neurotransmission, anxiety, behavioral test

INTRODUCTION

Peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) is an important regulator of mitochondrial biogenesis, metabolism, and cell stress (Puigserver and Spiegelman, 2003; St-Pierre et al., 2006; Scarpulla, 2011). PGC-1 α interacts with nuclear receptors, such as the peroxisome proliferator-activated receptor γ (PPAR γ), affecting the expression of metabolic and other genes (Handschin and Spiegelman, 2006). PGC-1 α itself is regulated by various factors in a cell and tissue-specific manner (Fernandez-Marcos and Auwerx, 2011; Lindholm et al., 2012; Martinez-Redondo et al., 2015). PPAR γ is present in the nervous system, and stimulation of the receptor exerts neuroprotective effects by reducing inflammation and oxidative stress in the brain (Austin and St-Pierre, 2012; Chen et al., 2012; Zolezzi et al., 2013). It has further been shown that thiazolidinedione drugs acting via PPAR γ , such as pioglitazone and rosiglitazone, can modulate behavior in experimental animals (Stopponi et al., 2011, 2013; de Guglielmo et al., 2017). However, the underlying mechanisms of how PPAR γ signaling influences behavior and neuronal connectivity are not fully understood.

Previous studies demonstrated that PGC-1 α is particularly expressed in interneurons in the brain, including those positive for the calcium-binding protein, parvalbumin (Cowell et al., 2007; Lucas et al., 2010). PGC-1 α was further shown to regulate the expression of parvalbumin as well as other genes in the brain (Lucas et al., 2010, 2014), and mice lacking PGC-1 α exhibited changes in inhibitory neurons and neurotransmission in brain cortex (Dougherty et al., 2014), striatum (McMeekin et al., 2018) and in cerebellum (Lucas et al., 2015). Recently, a role for PGC-1 α in excitatory neurons of the hippocampus was reported (McMeekin et al., 2020), enlarging the functions of this coactivator to encompass several cell populations and neuronal circuits in the brain (Mortensen et al., 2012; Engin et al., 2018).

To investigate the role of PGC-1 α in GABAergic transmission, we have here studied transgenic (Tg) mice overexpressing PGC-1 α in neurons (Mudò et al., 2012; Mäkelä et al., 2016a). In the PGC-1 α Tg mice, PGC-1 α expression and protein levels are substantially increased in brain areas, such as hippocampus and frontal cortex, as compared with wild-type mice. Behavioral analyses performed revealed an increase in anxiety-like behavior

in these mice compared with wild-types. Electrophysiological recordings showed that there was an increase in the frequency of spontaneous and miniature inhibitory postsynaptic currents (mIPSCs) in hippocampal neurons in PGC-1 α Tg mice, indicative of an altered GABAergic neurotransmission. Using immunoblotting and qPCR we observed that the expression of GABA α 2 subunit was enhanced in the hippocampus of Tg mice compared with Wt animals. These results demonstrate that stimulation of the PPAR γ /PGC-1 α system is involved in the regulation of GABA α 2 expression and GABAergic neurotransmission in hippocampus with effects on behavior.

MATERIALS AND METHODS

Animals Experiments

PGC-1 α transgenic (Tg) mice with overexpression of PGC-1 α under the Thy1 promoter in brain neurons were generated as previously described (Mudò et al., 2012). They were backcrossed over several generations with C57Bl6/J mice that also served as wild-type (Wt) controls (Mäkelä et al., 2016a). The PGC-1 α Tg and wild-type mice were bred in the same room and kept under identical conditions during growth and for experiments. Experiments were approved by the ethics committees and carried out in accordance with the European Communities Council Directive (86/609/EEC). Every attempt was made to reduce sample size and to minimize pain and suffering of animals. The number of animals used is given under the different experimental settings below.

Immunohistochemistry

Eight Wt and eight PGC-1 α Tg male mice aged 4 months were deeply anesthetized with pentobarbital (120 mg/kg, i.p.) and transcardially perfused with PBS (pH 7.6) followed by 4% PFA in 1 \times PBS (Sokka et al., 2007). Whole brains were removed from the skull and fixed in 4% PFA for 24 h, immersed in 30% sucrose for 3 days and cryofrozen in isopentane. 20 μ m thick sections from a region 1.5–2.1 mm posterior to Bregma were cut in the coronal plane on a cryostat (CM3050S, Leica Biosystems, Wetzlar, Germany). Sections were washed for 5 min in PBS, treated with 0.3% hydrogen peroxidase in PBS for 20 min, washed in PBS, and incubated for 1 h min with normal horse serum (Vector Lab) and 0.01% Triton X-100 in PBS. Primary GABA α 2 antibody (diluted 1:1000, sc-7350, Santa Cruz Biotechnology) was added overnight at 4°C, followed by secondary biotin-conjugated antibodies (1:700, no. 31820, Thermo Fisher) for 2 h at room temperature (RT). Sections were further incubated for 30 min at RT with ABC reagent (VECTASTAIN® Elite® ABC-Peroxidase Kit, PK-6100,

Abbreviations: BDNF, brain-derived neurotrophic factor; EPM, elevated plus-maze; GABA, γ -aminobutyric acid; GABA $_A$ receptor, inotropic GABA receptor; GABA $_B$, metabotropic GABA receptor; GABA α 1, γ -aminobutyric acid (GABA) type A receptor- α 1 subunit; GABA α 2, γ -aminobutyric acid (GABA) type A receptor- α 2 subunit; mIPSCs, miniature inhibitory postsynaptic currents; PGC-1 α , Peroxisome proliferator-activated receptor- γ coactivator-1 α ; PPAR γ , peroxisome proliferator-activated receptor γ ; sIPSCs, spontaneous inhibitory postsynaptic currents; Tg, transgenic mice; Wt, wild-type mice.

Vector Laboratories). Diaminobenzidine (DAB) reaction was performed with DAB substrate (Peroxidase Substrate Kit, SK-4100, Vector Laboratories) and stopped using double-distilled water. Sections were then mounted onto slides, dehydrated, cleared in xylene, and mounted with DPX (Biolab). Images were generated using 3DHISTECH Panoramic 250 FLASH II digital slide scanner at Genome Biology Unit, HiLIFE and the Faculty of Medicine, University of Helsinki. Control samples were without primary antibodies.

Double-Staining of GABAR α 2 and Gephyrin in CA1 Region of Hippocampus

Brain sections from five Wt and PGC-1 α Tg male mice aged 4 months were prepared as above and treated with 0.01 M sodium citrate buffer with 0.05% Tween-20 (pH 6) for 20 min at +95°C for antigen retrieval. Sections were washed and tissue permeabilized in 1% BSA, 10% normal donkey serum and 0.2% Tween-20 in PBS for 1h at RT. Primary rabbit anti-GABAR α 2 (1:1,000, no. 224 103, Synaptic Systems) and mouse anti-Gephyrin antibodies (1:1,000, no. 147 011, Synaptic systems) were added and sections incubated for 20 h at +4°C, washed and Alexa Fluor 488-conjugated donkey anti-rabbit (1:700, no. A-21206, Invitrogen, Thermo Fisher Scientific Inc., Carlsbad, CA, United States) and Alexa Fluor 594-conjugated donkey anti-mouse (1:600, no. A-21203, Invitrogen, Thermo Fisher Scientific Inc., United States) secondary antibodies were added for 2 h at RT. Microscopic slides were treated as above.

The colocalization of GABAR α 2 and gephyrin was examined in the stratum radiatum (SR), stratum lacunosum-moleculare (SLM) in the CA1 and molecular layer in the dentate gyrus region of the hippocampus. Images were acquired from a single optical section using a Leica SP8 inverted confocal microscope (Leica, Germany) with a 63 \times glycerol-immersion objective (numerical aperture, 1.3). Image deconvolution was done with Huygens Professional. Colocalization of GABAR α 2 and gephyrin was assessed using the ImarisColoc tool of Imaris 9.3 (Bitplane, Oxford Instruments, Oxfordshire, United Kingdom). The intensity thresholds for both channels were automatically defined based on an algorithm developed earlier (Costes et al., 2004). We analyzed first the percentage of colocalized pixels out of total pixel number and then the individual channels to arrive at the percentage of gephyrin signals above threshold that colocalize with GABAR α 2 (Figure 3). Data are from five mice in each group, using the average of two hippocampal sections per animal with four images per section.

Immunoblotting

Hippocampus and frontal cortex were dissected from seven Wt and seven PGC-1 α Tg male mice aged 4 months and lysed using ice-cold RIPA buffer (150 mM NaCl, 1% Triton-X-100, 0.5% sodium deoxycholate, 1% sodium dodecyl sulfate (SDS), 50 mM Tris-HCl pH 7.4) supplemented with protease inhibitor cocktail (Roche) and phosphatase inhibitors (PhoStop; Roche) (Hyryskyluoto et al., 2014; Mäkelä et al., 2016a). Equal amounts of protein were subjected to SDS-PAGE and blotted onto nitrocellulose filters (Amersham

Biosciences, Buckinghamshire, United Kingdom), which were incubated for 1 h in 5% skimmed milk or BSA, in TBS-T (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween 20), and then with primary antibodies overnight at 4°C. These included antibodies against GABAR α 2 (diluted 1:1,000; no. 822-GA2C, PhosphoSolutions, Aurora, CO, United States), GABAR α 1 (diluted 1:1,000; no. 812-GA1N, PhosphoSolutions, United States), PGC-1 α (1:2000; Merck Millipore, Billerica, MA, United States), and β -actin that was used as control (1:1,000; no 2066, Sigma). After washing the filter was incubated with horseradish peroxidase-conjugated secondary antibodies (1:2,500; Jackson ImmunoResearch Laboratories, West Grove, PA, United States), followed by detection using enhanced chemiluminescence (Thermo Fisher Scientific, Waltham, MA, United States). Quantifications were done using the ImageJ software and the levels of GABARs and PGC-1 α were calculated using β -actin as control.

Quantitative PCR

Hippocampus and frontal cortex were dissected from five Wt and five PGC-1 α Tg male mice aged 4 months, and RNA prepared using the RNeasy lipid tissue kit (Qiagen), followed by cDNA synthesis using Superscript VILO cDNA synthesis kit (Invitrogen). qPCR amplification was performed using the Light Cycler 480 II instrument (Roche Diagnostics, Basel, Switzerland) as described (Mäkelä et al., 2016a; Pham et al., 2019). Reactions were run in 96-well plate format in a final volume of 10 μ l, and contained 2 μ l cDNA and 100 μ M forward (F) and reverse primers (R) in 1 \times SYBR Green Master Mix (Roche). The reaction was carried out at 95°C for 10 min followed by at 95°C for 15 s, 60°C for 20 s, and 72°C for 10 s using 40 cycles. Each sample was run in triplicates on a 96-well plate, and water was used as a negative control. Expression levels were calculated based upon the $2^{-\Delta\Delta CT}$ method using *Gapdh* as control. Primers are shown in Table 1.

Neuronal Cultures

Neurons were prepared from brain of embryonic, E17-old rats and plated onto poly-ornithine (Sigma) coated six-well plates (Do et al., 2013; Mäkelä et al., 2016a; Srinivasan et al., 2020). Experiments had an ethical approval. Cells were cultured for up to 10 days in Neurobasal medium, and subsequently stimulated for 1–2 days with 1–10 μ M pioglitazone or using the PPAR γ agonist, N-(2-benzoylphenyl)-O-[2-(methyl-2-pyridinylamino) ethyl]-L-tyrosine hydrate (GW1929) (Mäkelä et al., 2016b). Immunoblotting was done essentially as described above employing cell lysates made using RIPA buffer consisting of 150 mM NaCl, 1 mM EDTA, 1% (v/v) NP-40, 0.25% (w/v) sodium deoxycholate, 50 mM

TABLE 1 | List of primer sequences.

Gene	Forward primer	Reverse primer
GABAR α 2	ACAAAAAGAGGATGGGCTTG	TCATGACGGAGCCTTTCTCT
GABAR α 1	TGAGCACACTGTCCGGAAGA	CAGCAGTCGGTCCAAAATTCT
GAPDH	GGGTTCTATAAATACGGACTGC	CCATTTTGTCTACGGGACGA

Tris-HCl, pH 7.4, and 1% (w/v) SDS. 30 μ g of protein was run on a SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and proteins were transferred to nitrocellulose membrane (Amersham Biosciences, Buckinghamshire, United Kingdom). The membranes were blocked in 5% skimmed milk-Tris-buffered saline/0.1% (v/v) Tween 20 (TBS-T) for 1 h at RT, and primary antibody was added and incubated overnight at +4°C. These included anti-GABAR α 2 (diluted 1:1,000), anti-PGC-1 α (1:2,000), and anti- β -actin (1:1,000) that was used as control. After washing the filter was incubated with horseradish peroxidase-conjugated secondary antibodies (1:2,500; Jackson ImmunoResearch Laboratories, West Grove, PA, United States), followed by detection using enhanced chemiluminescence (Thermo Fischer Scientific, Waltham, MA, United States). Quantifications were done using the ImageJ software and the levels of GABARs and PGC-1 α were calculated using β -actin as control.

Electrophysiology

Electrophysiological recordings were done essentially as described (Comhair et al., 2018). Four Wt and four PGC-1 α TG male mice aged 2 months were deeply anesthetized with isoflurane, decapitated and the brain was quickly removed. Parasagittal hippocampal slices (300 μ m-thick) were prepared using a vibratome (Leica VT1200S) in ice-cold dissection ACSF of following composition: 117 mM choline chloride, 2.5 mM KCl, 0.5 mM CaCl₂, 1.25 mM NaH₂PO₄, 7 mM MgSO₄, 26 mM NaHCO₃, 15 mM glucose, and 95% O₂/5% CO₂. Slices were left to recover for 1 h at 34°C in recording ACSF with the MgSO₄ concentration raised to 3 mM. After a recovery period, slices were transferred to the submerged recording chamber on the visually guided patch-clamp setup. Recordings were done at 30°C in ACSF of the following composition: 124 mM NaCl, 3 mM KCl, 1.25 mM NaH₂PO₄, 1 mM MgSO₄, 26 mM NaHCO₃, 2 mM CaCl₂, 15 mM glucose, and 95% O₂/5% CO₂. Whole-cell voltage clamp recordings from CA1 pyramidal neurons were performed with glass microelectrodes (4–6 M Ω), filled with solution of the following composition: 120 mM CsCl, 0.022 mM CaCl₂, 4 mM MgCl₂, 10 mM HEPES, 0.1 mM EGTA, 5 mM QX-314, 4 mM MgATP, 0.5 mM Na₂GTP. Membrane voltage was kept at -70 mV. Passive membrane properties (series resistance, input resistance, and capacitance) were monitored by injection of 5 mV voltage steps, and if the change in series resistance during the experiment exceeded 30%, the recording was discarded. Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in the presence of AMPA and NMDA receptor inhibitors (20 μ M CNQX and 5 μ M L-689,560). For miniature inhibitory postsynaptic current (mIPSC) recordings, tetrodotoxin (TTX, 1 μ M) was added to the solution. Data were acquired using a Multiclamp 700A amplifier (Axon Instruments, United States) and digitized at 20 kHz. Recordings were done with WinLTP 2.30 (WinLTP Ltd. and University of Bristol, United Kingdom) and analyzed in the Mini Analysis program 5.6.6 (Synaptosoft, United States). Automatic threshold search was used to detect synaptic events, and all events were verified manually. Amplitude, frequency and kinetics of events were calculated using build-in Mini Analysis algorithms.

Behavioral Analyses

The mice were housed in an individually ventilated cage system (Mouse IVC Green Line; 391 mm \times 199 mm \times 160 mm; air inlet and outlet valves located in the cage lid, on top of the cage; the rate of air change was set at 75 times per hour with airspeed at the maximum level of 0.05 m/s; half of the cage covered by a wire bar food hopper; Tecniplast, Italy). Cage enrichment was provided by bedding (aspen chips, 4 HP, Tapvei, Estonia), nesting material (equal amounts of aspen strips, PM90L, Tapvei, Estonia and Sizzle Nest paper strands, Dates and Group, United Kingdom) and an aspen brick (100 mm \times 20 mm \times 20 mm, Tapvei, Estonia). Food (Global Diet 2916C, pellet 12 mm, Envigo, IN, United States) and water (filtered and UV-irradiated) were available *ad libitum*. Room temperature was 22 \pm 2°C and relative humidity 50 \pm 15%. The lights were on from 6:00 AM to 6:00 PM and experiments were carried out during this period. The cages were cleaned once per week, and animals were weighed before moving to a new cage. In total, 24 wild-types (Wt) and 20 PGC-1 α transgenic (Tg) mice were examined using a battery of behavioral tests. Female (F) and male (M) mice were analyzed separately using four different groups: Wt F (n = 15), Wt M (n = 9), Tg F (n = 9), and Tg M (n = 11), and at the beginning of the experiments the mice were 169–170 days of age. Behavioral data was analyzed by two-way ANOVA or repeated measures (RM) two-way ANOVA, followed by Tukey's unequal N HSD *post hoc* test as indicated under the respective tests.

Video Tracking

Mice were video-tracked by Noldus EthoVision XT 10 system (Noldus Information Technology, Wageningen, Netherlands). The distance traveled by the subjects and the time spent in pre-defined zones was recorded as well as defined behavior (vertical counts or rearings, grooming, head-dips).

Open Field

Mice were analyzed in an open field arena of 30 cm \times 30 cm (Med Associates, St. Albans, VT, United States). The animal was released in the corner of the field; horizontal and vertical activity was recorded during the 30 min testing period by the software. The illumination in the open field was \sim 150 lx. The peripheral zone was defined as a 6 cm wide corridor along the wall.

Elevated Plus-Maze

The maze consisted of two open arms (30 cm \times 5 cm) and two closed arms (30 cm \times 5 cm, inner diameter) connected by central platform (5 cm \times 5 cm) and risen to 40 cm above the floor. The floor of each arm was light gray, and the closed arms had slightly transparent (15 cm high) side- and end-walls made of Plexiglas. The illumination level in all arms was \sim 150 lx. The mouse was placed in the center of the maze facing one of the enclosed arms and observed for 5 min. The latency to the first open arm entry, number of open and closed arm entries (four paw criteria) and the time spent in different zones of the maze were measured. The number of head dips, rearings, and fecal boli was counted manually after the trial from video recordings.

Light–Dark Box

The test was carried out in the open field arena (30 cm \times 30 cm, Med Associates, St. Albans, VT, United States) equipped with infrared light sensors detecting horizontal and vertical activity. The dark insert (non-transparent for visible light) was used to divide the arena into two halves, and an opening in the wall (a door with a width of 5.5 cm and height of 7 cm) allowed free movement from one compartment to another. Illumination in the center of the light compartment was \sim 550 lx. The animal was placed in the dark compartment and allowed to explore the arena for 10 min. Distance traveled, number of rearing, latency to enter the light compartment, and time spent in different compartments were recorded.

T-Maze Spontaneous Alternation

The T-maze was made of gray PVC. Each arm measured 30 cm \times 10 cm; a removable central partition extended from the center of the black goal wall of the T to 7 cm into the start arm. This modification prevented the mouse from seeing or smelling the non-chosen arm during the sample run, thus minimizing interfering stimuli. The entrance to each goal arm was fitted with a guillotine door. Each trial consisted of an information-gathering, sample run, followed immediately by a choice run. For the sample run, a mouse was placed in the start arm, facing away from the choice point with the central partition in place, the door on start arm closed and doors to goal arms opened. After opening the door on the start arm, the mouse was allowed to choose a goal arm and confined there for 10 s by lowering the respective guillotine door. Then, the central partition was removed, the mouse replaced to the start arm (door closed), the doors to both goal arms were opened again. This was followed by the opening of the start door, and the choice run was started. Alternation was defined as entering the opposite arm to that entered in the sample trial (whole body, including tail). Three trials were run per day with an inter-trial interval at least 1 h, on two consecutive days (six trials altogether).

Statistical Analyses

The number of animals is given under the specific subheadings. For behavior experiments, the effect of gender was particularly considered, and statistical differences when noted were given separately. Values for expression data and immunoblots are given as means \pm SEM of the averages in independent experiments. Unpaired Student's *t*-test was used for imaging and electrophysiology data, and Student's *t*-test or ANOVA followed by Dunnett *post hoc* test were used for qPCR and immunoblotting data (Graph Pad Prism version 4.0, La Jolla, CA, United States). $p < 0.05$ was considered as significant. Normality distribution of data was determined with the Shapiro–Wilks normality test ($p < 0.01$). Graphs with behaviors show values for each mouse and are presented as means with 95% confidence intervals. Behavioral data was analyzed by two-way ANOVA or RM two-way ANOVA, followed by Tukey's unequal N HSD *post hoc* test where applicable (Statistica software, StatSoft, Tulsa, OK, United States).

RESULTS

GABAR α 2 Subunits Are Increased in PGC-1 α Overexpressing Transgenic Mice

We have previously shown that PGC-1 α expression and protein levels are increased in brains of PGC-1 α Tg mice in brain regions, including substantia nigra and hippocampus, compared with wild-type mice (Mudò et al., 2012; Mäkelä et al., 2016a). Furthermore, the PGC-1 α Tg mice are protected against adverse effects of the neurotoxin, 6-hydroxydopamine, and against glutamate-mediated excitotoxicity in the hippocampus (Mudò et al., 2012; Mäkelä et al., 2016a). Proteomic analyses revealed alterations in several proteins associated with mitochondria and oxidative phosphorylation in the PGC-1 α Tg mice (Mäkelä et al., 2016a). In these experiments, we observed an increase in the level of GABAR α 2 subunit prompting further investigations. qPCR analyses revealed that the expression of GABAR α 2 was increased in hippocampus of the PGC-1 α Tg mice compared with Wt using *Gapdh* as control (Figure 1A). In frontal cortex, the variation between different animals was rather large in PGC-1 α Tg mice and the change did not reach statistical significance ($p = 0.058$, $n = 7$) (Figure 1A). Immunoblotting experiments showed that the protein levels of GABAR α 2 were significantly increased in the hippocampus and frontal cortex of Tg mice compared with Wt and using β -actin as control (Figures 1B,C). In contrast, the level of GABAR α 1 was not altered in the PGC-1 α Tg mice as shown by immunoblotting (Figures 1D,E). Altogether, these results show that GABAR α 2 subunit level is increased in hippocampus and parts of the cortex in the Tg mice as compared with Wt.

Previous studies have shown that GABAR α 2 subunits are expressed widely in the brain, including the hippocampus (Mortensen et al., 2012; Engin et al., 2018). Immunohistochemistry showed that GABAR α 2 immunoreactivity was increased in the hippocampus, as well as in parietal cortex and in the ventral retrosplinal cortex of PGC-1 α Tg mice (Figure 2). In the present work, we focused here on the hippocampus, where higher magnifications revealed that GABAR α 2 is expressed by neurons, as exemplified by immunopositive cells in the dentate gyrus, and in the CA1 and CA3 subregions of the hippocampus (Figure 2B).

Double Staining of GABAR α 2 and Gephyrin in the CA1 Region of the Hippocampus

Gephyrin is a synaptic protein involved in clustering of GABA_A receptors in postsynaptic neurons that is important for GABAergic signaling (Tyagarajan and Fritschy, 2014; Choi and Ko, 2015; Pizzarelli et al., 2020). In immunoblots the total level of gephyrin did not change between the groups (data not shown). Pyramidal neurons in the CA1 region take part in the hippocampal circuitry and have apical dendrites in the SR, and SLM (Figure 3A). GABAergic interneurons are also present in these layers. Double immunostaining experiments revealed a colocalization of GABAR α 2 immunoreactivity with gephyrin in the CA1 region of hippocampus (Figure 3B). Estimation of the percentage of double GABAR α 2/Gephyrin positive pixels out of

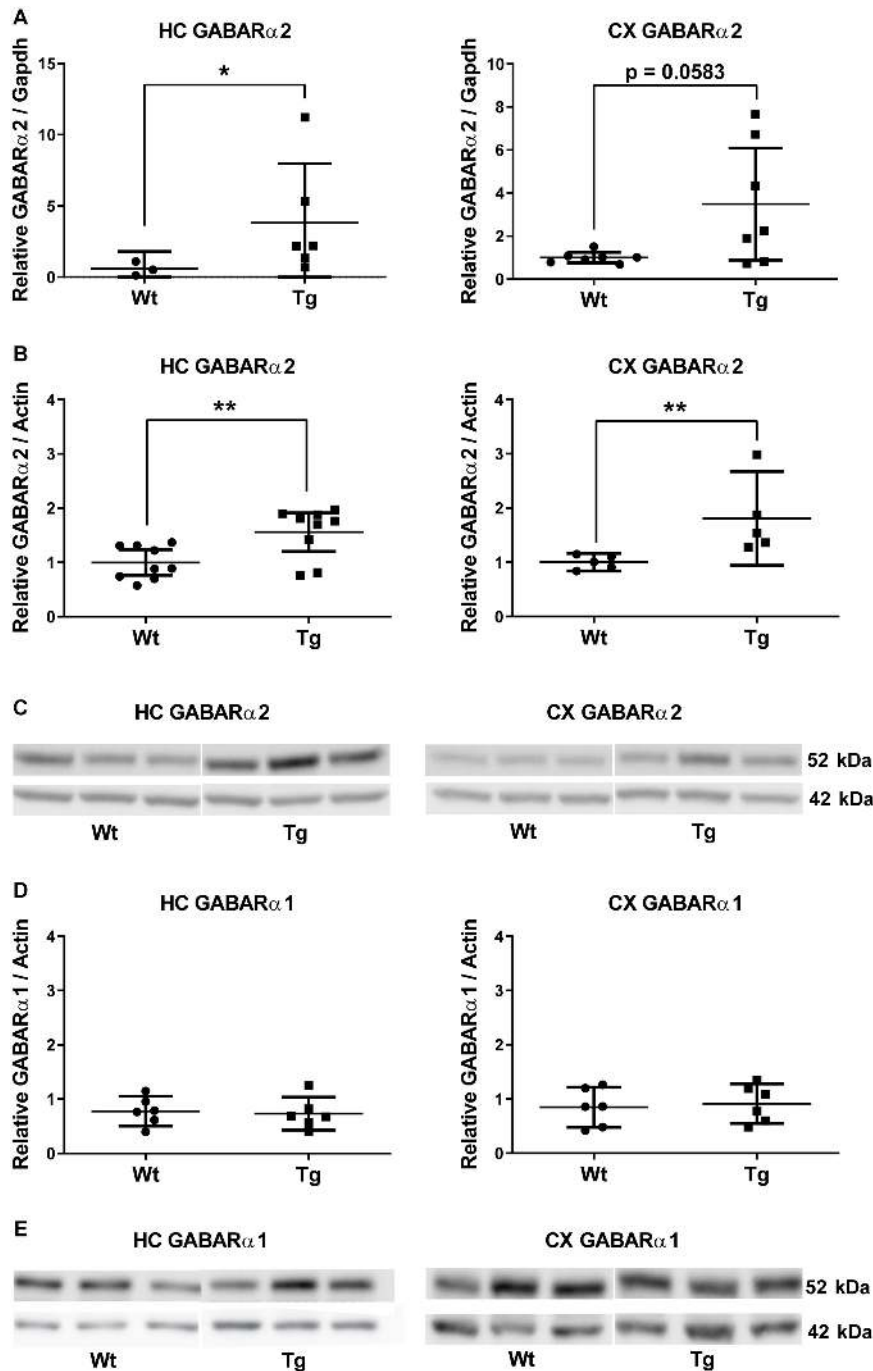
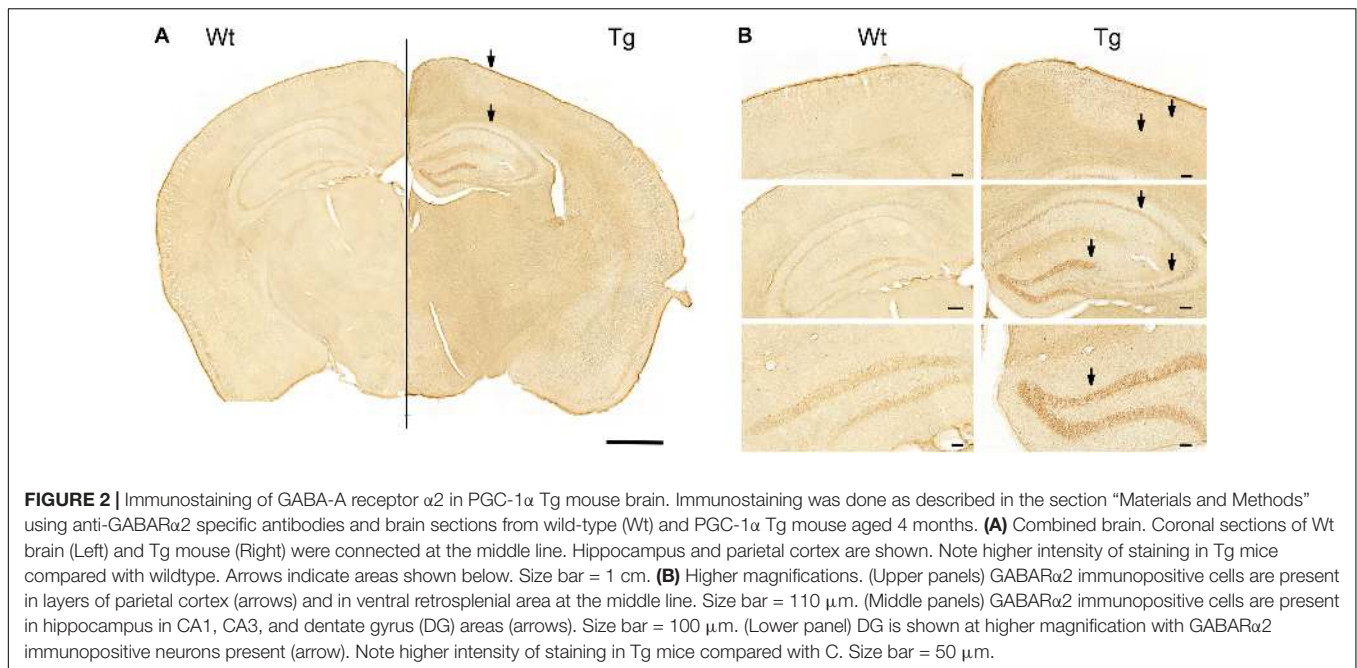


FIGURE 1 | GABA-A receptor α 2 subunit expression is increased in PGC-1 α transgenic mice. **(A)** Quantitative PCR was performed as described in the section “Materials and Methods” using cDNA from the hippocampus (HC) and frontal cortex (CX) of 4-month old wild-type (Wt) and PGC-1 α transgenic mice (Tg). Quantifications were done as described in the section “Materials and Methods” using *Gapdh* expression as control and Student’s *t*-test. Values are means \pm SEM, $n = 7$. Left, HC; GABAR α 2 mRNA levels increased in the hippocampus of Tg mice compared with Wt animals. * $p < 0.05$. Right, CX. In the frontal cortex the difference was not significant between groups. $p = 0.0583$. **(B)** Immunoblottings using lysates from the hippocampus (HC) and frontal cortex (CX) of 3-month old Wt and PGC-1 α Tg mice were done using anti-GABAR α 2 antibody and anti- β -actin antibody as control. GABAR α 2 is shown at 52 kDa (upper lanes) and β -actin at 42 kDa (lower lanes). Quantification of GABAR α 2 levels was done using β -actin as control and Student’s *t*-test. Values are means \pm SEM. $n = 9$ for HC, $n = 6$ for CX. ** $p < 0.01$ for Tg vs. Wt. **(C)** Representative immunoblots showing GABAR α 2 at 52 kDa and β -actin as a control at 42 kDa. **(D)** Immunoblottings were done using anti-GABAR α 1 antibody and anti- β -actin antibody as control. Left, HC; Right, CX. GABAR α 1 levels were quantified using β -actin as control and Student’s *t*-test. Values are means \pm SEM. $n = 7$ for HC, $n = 6$ for CX. There was no significant difference between groups. **(E)** Representative immunoblots showing GABAR α 1 at 52 kDa (upper lane) and β -actin as a control at 42 kDa (lower lanes).



the total number of positive pixels showed no obvious change in the SR, in the SLM, or in the molecular layer (ML) between Tg and Wt mice using Students *t*-test (for statistics, see **Figure 3C**, left and **Figures 3D,E**). Analyzing the gephyrin channel above threshold showed a significant increase in the percentage of gephyrin signals colocalized with GABA $\alpha 2$ in the SR layer in Tg mice compared with Wt ones using Students *t*-test (Wt: 16.09 ± 2.973 ; Tg: 29.62 ± 4.367 ; $p = 0.03$; **Figure 3C**, right). In contrast, a similar analyses revealed no significant changes between Wt and Tg mice in the ML (Wt: 36.3 ± 6 ; Tg: 41.1 ± 2 ; $p = 0.12$), or in the SLM (Wt: 46.6 ± 1.2 ; Tg: 46.5 ± 1.0 ; $p = 0.97$) sublayers, suggesting a preferential involvement of neurons in the SR layer in the Tg mice.

GABAergic Neurotransmission Is Altered in CA1 Hippocampal Region of PGC-1 α Transgenic Mice

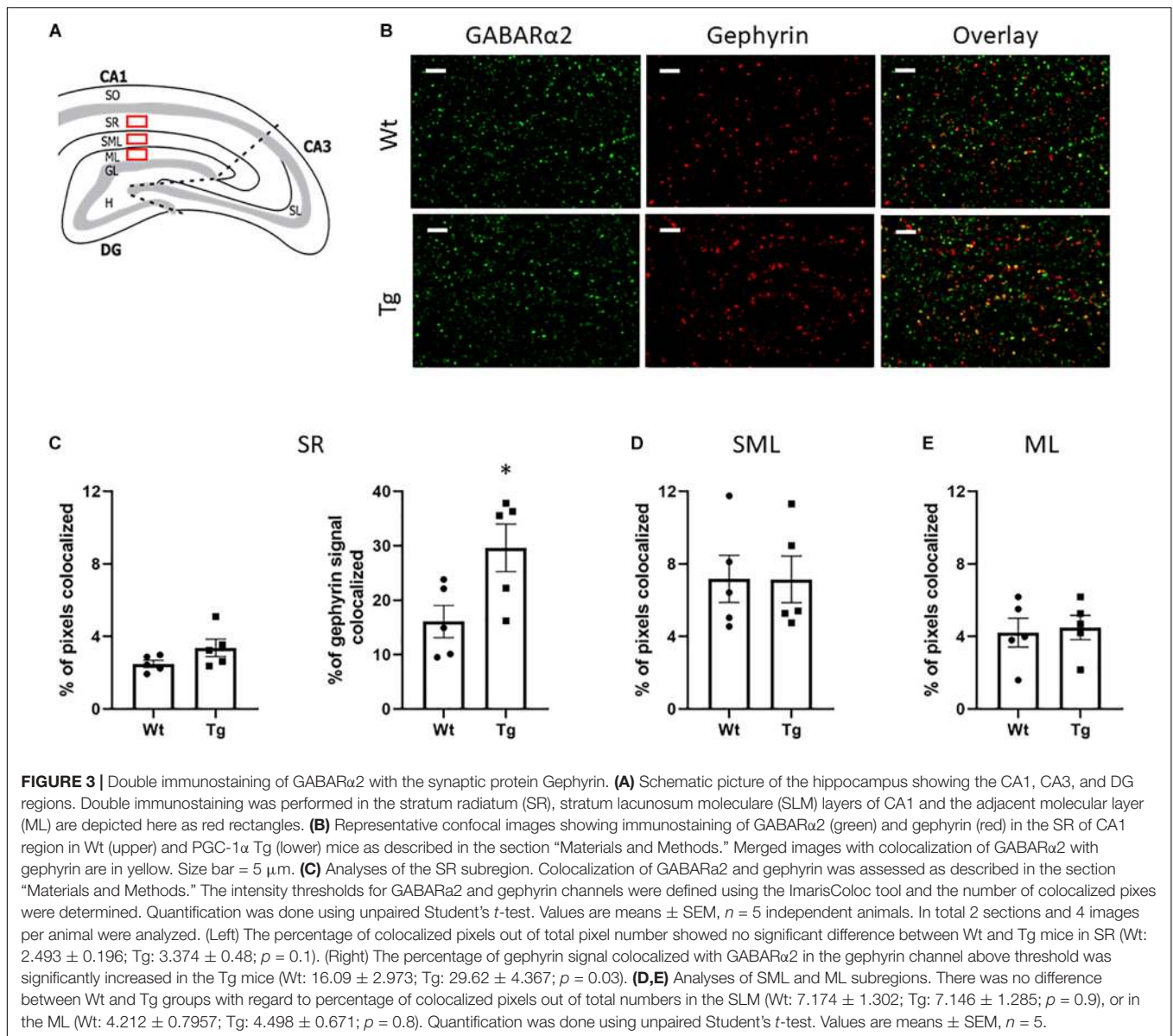
To investigate whether the alterations in GABA $\alpha 2$ observed in the PGC-1 α Tg mice were associated with changes in neurotransmission, we performed patch-clamp recordings in hippocampal slices from control and PGC-1 α Tg mice. Data showed that the input resistance and capacitance of cells examined did not differ between Wt and Tg animals (full statistics in **Table 2**). GABAergic synaptic events were recorded from CA1 pyramidal neurons revealing that the frequency of mIPSCs (Wt: 3.24 ± 0.48 Hz; Tg: 6.27 ± 0.94 Hz, $t = 2.7$, $df = 13$, $p = 0.02$; **Figures 4A,B**) and sIPSCs (Wt: 5.55 ± 0.98 Hz; Tg: 9.28 ± 1.03 Hz; $t = 2.6$, $df = 14$, $p = 0.02$; **Figures 4C,D**) was higher in the Tg mice compared with Wt. The amplitude of mIPSCs (Wt: 20.31 ± 1.97 pA; Tg: 25.62 ± 1.98 pA; $t = 1.9$, $df = 13$, $p = 0.08$; **Figures 4A,B**) and sIPSCs (Wt: 21.31 ± 2.41 pA; Tg: 31.80 ± 4.98 pA; $t = 1.9$, $df = 14$, $p = 0.08$; **Figures 4C,D**) followed the same trend, but differences did not reach significance.

This demonstrates that the GABAergic neurotransmission is accentuated in the Tg mice, possibly reflecting an increased release probability and enhanced number of GABAergic synapses in the hippocampal neurons. We further noted that the kinetics of the events were the same in the two groups (full statistics in **Table 2**), which indicates that the channel opening and desensitization of the receptor are not affected in the transgenic mice. Altogether, these results demonstrate that GABAergic neurotransmission is enhanced in the hippocampus of PGC-1 α Tg mice compared with Wt ones.

Increased Anxiety-Like Behavior in PGC-1 α Transgenic Mice

Changes in GABA α receptor subunits have been linked to anxiolytic and other types of behavior in experimental animals (Smith et al., 2012; Engin et al., 2018; Skilbeck et al., 2018; Rudolph and Moss, 2019). We were therefore interested to study the behavior of Tg mice with overexpression of PGC-1 α in brain neurons. To accomplish this, PGC-1 α Tg and Wt mice were subjected to an extensive battery of tests. For this, genotype (Gt) related and sex-specific data are shown separately as indicated in the figures. Full statistics for the animal performance in the different tests are given in the **Figure 5**, and were analyzed by two-way ANOVA or RM two-way ANOVA, followed by Tukey's unequal N HSD *post hoc* test.

In the open field test, there were no significant differences between the mice in parameters such as vertical counts, latency to enter the central area; distance traveled in the periphery or the center (data not shown). In the elevated plus-maze (EPM) test, PGC-1 α Tg mice showed a preference for closed areas compared with Wt mice, as evident from the reduced ratio of distance traveled in open vs. closed area (**Figure 5A**). The preference for closed areas of the Tg mice was not significantly different

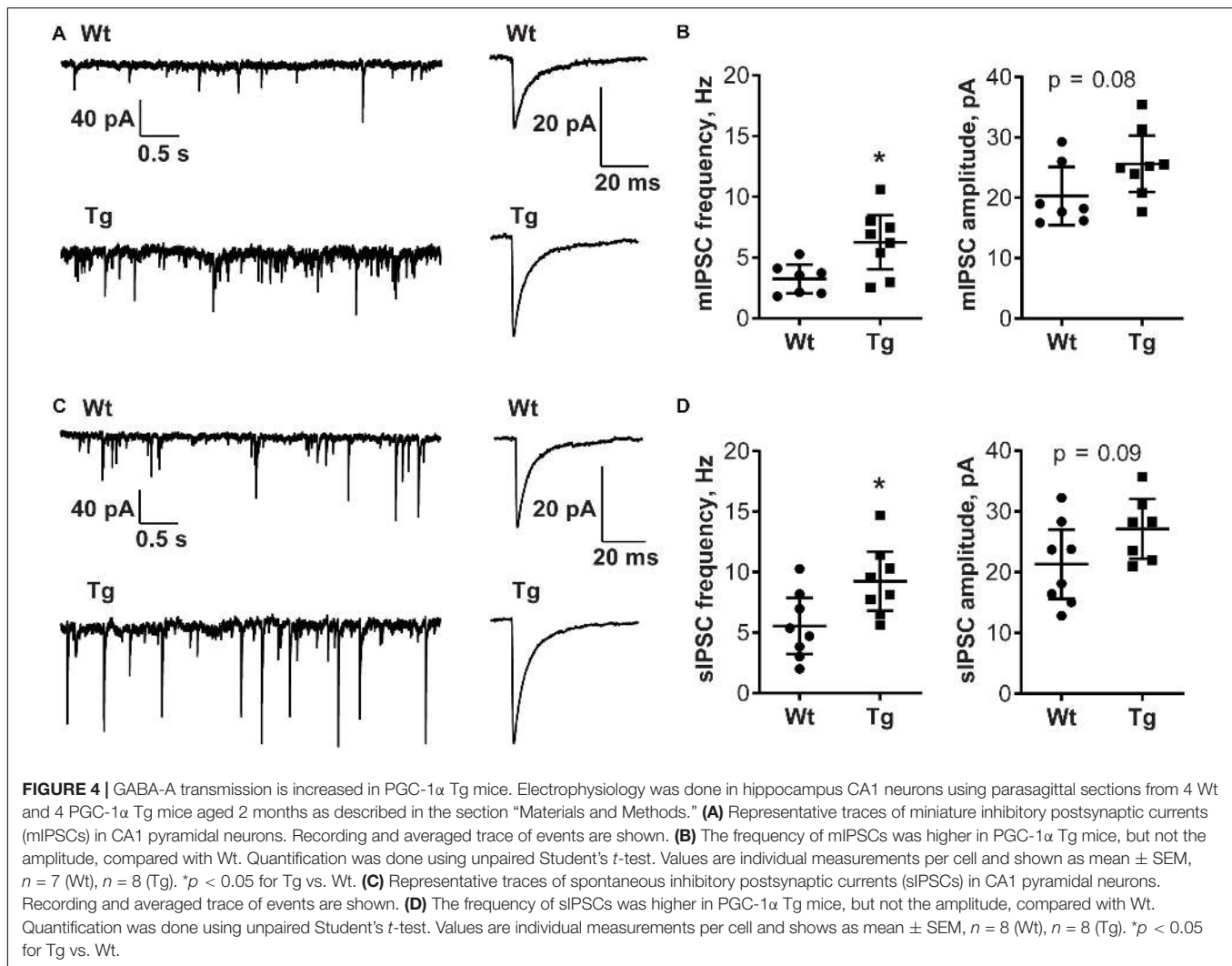


with regard to the time-related ratio (Figure 5B). The number of vertical counts was increased in the transgenic mouse group (Figure 5C). There was also a higher defecation rate among PGC-1α Tg mice (Figure 5D), notably in the Tg male group (*p* = 0.027).

In the light–dark box (LDB) test, Tg mice showed a preference for the dark area, both for the ratio of traveled distance (Figure 5E), and the time spent in light vs. dark (Figure 5F). *Post hoc* analysis revealed lower values for the Tg mice in the distance traveled (*p* = 0.00012), and the ratio of time spent (*p* = 0.0007)

TABLE 2 | Passive membrane properties and kinetics of mIPSCs and sIPSCs in CA1 pyramidal neurons of control and PGC-1α transgenic mice.

	Wt	PGC-1α Tg	Statistics (unpaired Student’s <i>t</i> -test)
Input resistance, MΩ	198.7 ± 39.33 (<i>n</i> = 7)	261.9 ± 64.71 (<i>n</i> = 8)	<i>t</i> = 0.80, <i>df</i> = 13, <i>p</i> = 0.44
Capacitance, pF	229.3 ± 37.92 (<i>n</i> = 7)	249.0 ± 35.83 (<i>n</i> = 8)	<i>t</i> = 0.38, <i>df</i> = 13, <i>p</i> = 0.71
mIPSC rise (T10/90, ms)	0.40 ± 0.02 (<i>n</i> = 7)	0.44 ± 0.03 (<i>n</i> = 8)	<i>t</i> = 0.97, <i>df</i> = 13, <i>p</i> = 0.35
mIPSC decay (τ, ms)	5.0 ± 0.48 (<i>n</i> = 7)	5.08 ± 0.44 (<i>n</i> = 8)	<i>t</i> = 0.15, <i>df</i> = 13, <i>p</i> = 0.88
sIPSC rise (T10/90, ms)	0.38 ± 0.03 (<i>n</i> = 8)	0.38 ± 0.02 (<i>n</i> = 8)	<i>t</i> = 0.07, <i>df</i> = 14, <i>p</i> = 0.94
sIPSC decay (τ, ms)	4.78 ± 0.31 (<i>n</i> = 8)	4.53 ± 0.31 (<i>n</i> = 8)	<i>t</i> = 0.58, <i>df</i> = 14, <i>p</i> = 0.57



in the light area. The total number of vertical counts revealed Gt effect (**Figure 5G**), as did the ratio of vertical counts in light vs. dark showed (**Figure 5H**). The Tg mice made more vertical counts ($p = 0.031$), however, these occurred mostly in a dark area ($p = 0.00016$).

Quantifying the total distance there was a difference between sexes with the female in both groups being more active in the EPM ($F(1, 40) = 9.38$, $p = 0.004$), and in the LDB test ($F(1, 40) = 17.55$, $p = 0.0002$). However, the changes in preference for dark area observed between Wt and Tg mice were similar for female and male mice (**Figure 5**). Together the results show an increased anxiety-like behavior independent of gender in the PGC-1 α Tg group compared with Wt.

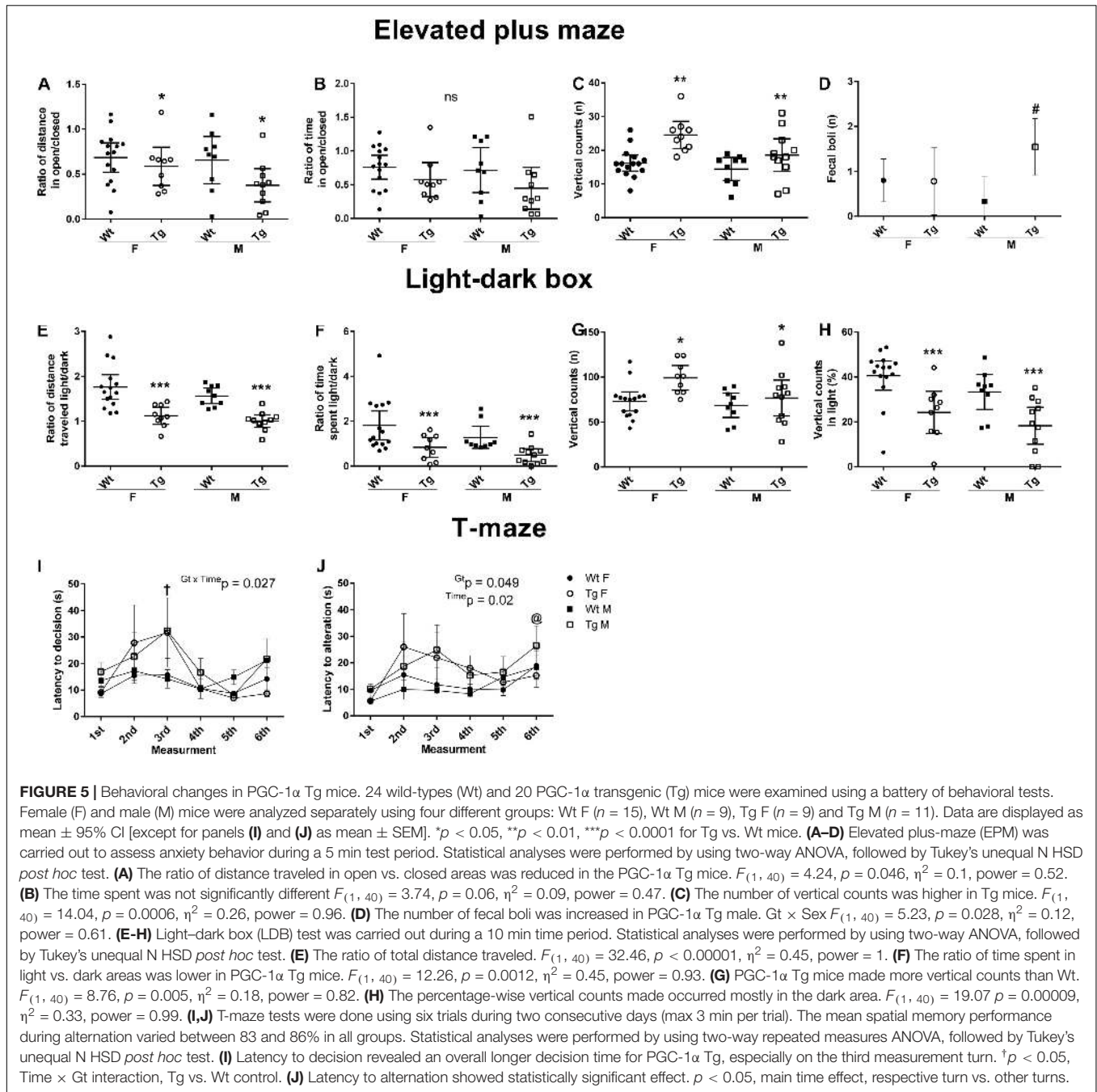
PGC-1 α Transgenic Mice Exhibit Changes in Learning Behavior

Spontaneous alternation in T-maze is a natural behavior for rodents used to assess cognitive activity (Deacon and Rawlins, 2006). The mean alternation rate of six trials varied between 83 and 86%, indicating good working spatial memory performance

in all groups (**Figure 5I**). However, the Tg mice took a significantly longer time to complete the trials, indicating a possible difference in decision making (**Figure 5J**). *Post hoc* analysis revealed that the Tg mice were different from Wt on the third turn ($p < 0.05$). Latency to alternation showed a time and genotype effect ($p < 0.05$). Taken together these results indicate that the Wt and Tg mice showed changes in learning ability related to latency measurements in the T-maze test.

Pioglitazone Elevates GABAR α 2 Expression in Neurons

The results above indicate that overexpression of PGC-1 α affects GABAR α 2 subunit expression and behavioral parameters in the PGC-1 α Tg mice. PGC-1 α acts in conjunction with nuclear transcription factors, such as PPAR γ . To investigate whether the activation of PPAR γ may influence GABAR α 2 subunit expression, we employed the drug pioglitazone that acts on PPAR γ . Primary cortical neurons were prepared from embryonic rat brain, cultured and stimulated with pioglitazone followed by immunoblotting. Data revealed that 10 μ M pioglitazone



significantly increased GABAR α 2 subunit level in the neurons after 1 day (Figure 6A). Pioglitazone further elevated PGC-1 α in the neurons, as shown for the 38 kDa neuronal isoform (Martinez-Redondo et al., 2015) (Figure 6A). This indicates a likely positive feedback by which pioglitazone can increase GABAR α 2 expression in the neurons. Finally, treatment with the PPAR γ agonist GW1929 also elevated GABAR α 2 in the neurons after 2 days (Figure 6B), showing an involvement of PPAR γ receptors. Altogether the results show that stimulation of PPAR γ can increase GABAR α 2 subunit expression in primary brain neurons.

DISCUSSION

PGC-1 α plays a physiological role in the regulation of genes contributing to the control of mitochondria functions, cell viability, and general metabolism (see Introduction for references). Impairments in PGC-1 α -mediated gene networks are observed in human diseases, including neurodegenerative disorders exemplified by Huntington's disease (St-Pierre et al., 2006; Soyal et al., 2012; McMeekin et al., 2018) and Parkinson's disease (Zheng et al., 2010; Clark et al., 2011; Mudò et al., 2012; Jiang et al., 2016; Mäkelä et al., 2016b). As shown previously,

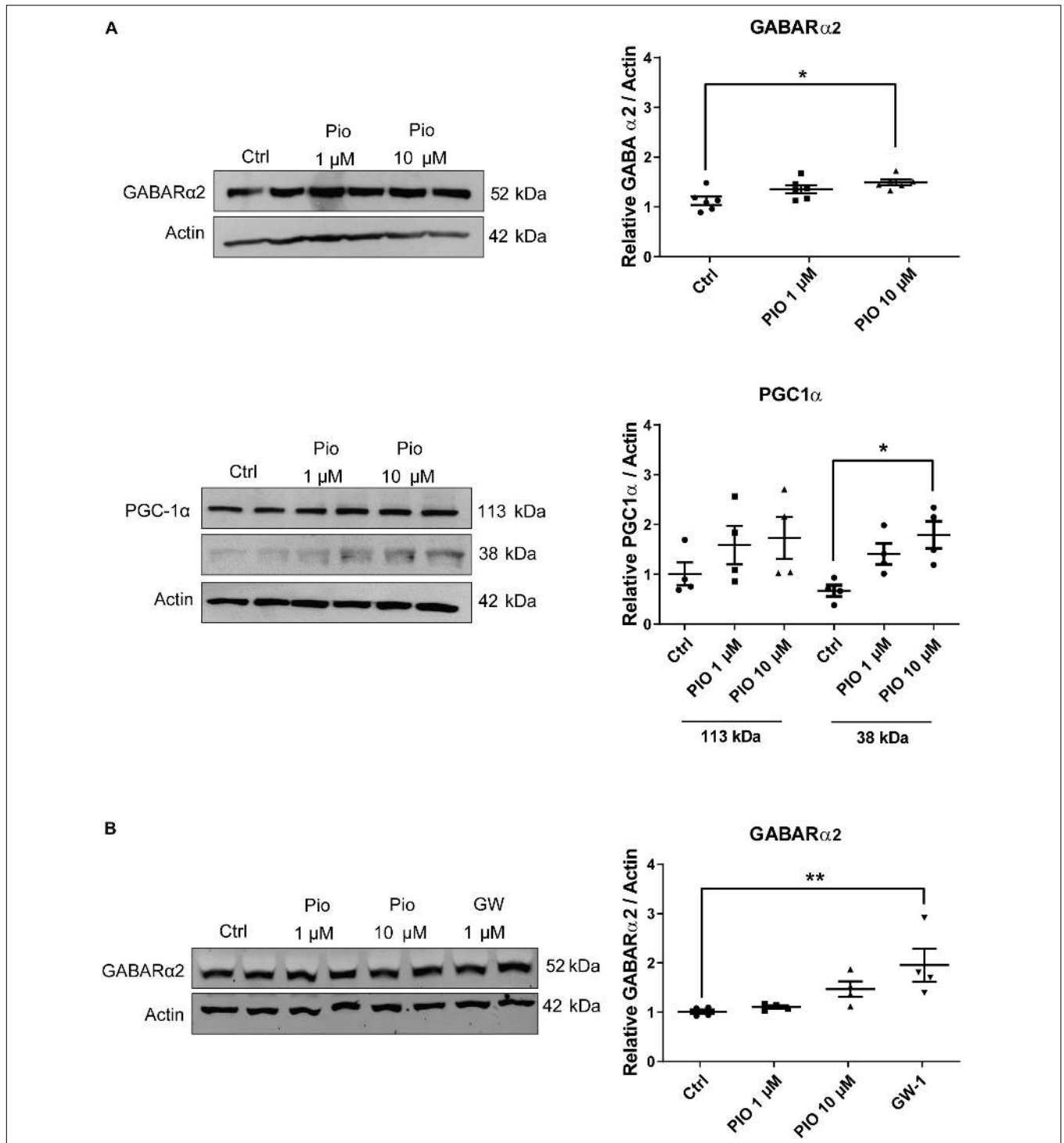


FIGURE 6 | Pioglitazone increases GABA-A receptor α 2 and PGC-1 α in primary neurons. **(A)** Primary neurons prepared from embryonic E17 rat brain cortex and cultured for 5 days as described in the section “Materials and Methods.” Cells were then stimulated with 1 or 10 μ M pioglitazone (PIO) for 24 h followed by immunoblotting using anti-GABAR α 2 or anti-PGC-1 α antibodies and anti- β -actin antibody as control. Quantifications were done by Student’s *t*-test and ANOVA followed by Dunnett *post hoc* test. (Left panel) Immunoblots of GABAR α 2 (upper lane) and PGC-1 α (lower lanes). (Right panel) Quantification using β -actin as control revealed an increase in GABAR α 2 by 10 μ M PIO. Note two isoforms of PGC-1 α in the neurons shown as 113 and 38 kDa bands of which the latter was significantly increased by 10 μ M PIO. Values are means \pm SEM, *n* = 4. **p* < 0.05 for 10 μ M PIO vs. controls. **(B)** Neurons were stimulated with 1 or 10 μ M PIO, or 2 μ M PPAR γ agonist N-(2-benzoylphenyl)-O-[2-(methyl-2-pyridinylamino) ethyl]-L-tyrosine hydrate (GW-1) for 2 days followed by immunoblotting. Quantifications were done using β -actin as control and Student’s *t*-test and ANOVA followed by Dunnett *post hoc* test. (Left) Immunoblots. (Right) Quantification of GABAR α 2. Values are means \pm SEM, *n* = 4. ***p* < 0.01 for GW-1 treatment vs. controls. Note, the values for 10 μ M PIO in these 2-day treated cultures did not reach statistical significance.

PGC-1 α can have effects in the brain also on neurotransmission, neuronal connectivity, and behavior (Lucas et al., 2010, 2014; Dougherty et al., 2014; McMeekin et al., 2016, 2020).

PGC-1 α is expressed at low levels in the wild-type hippocampus but increased substantially in the PGC-1 α Tg mice, as shown previously (Mäkelä et al., 2016a). In the present work, we observed that the transgenic overexpression of PGC-1 α in brain neurons increased GABA α 2 subunit levels in the hippocampus. This was accompanied by an enhanced GABAergic neurotransmission as studied in the CA1 region of hippocampus, and an increased anxiety-like behavior observed in PGC-1 α Tg mice. The role described here for PGC-1 α in modulating GABA α 2 expression and GABAergic neurotransmission may be important for brain disorders characterized by GABAergic dysfunctions (Lewis et al., 2008; Ramamoorthi and Lin, 2011; McMeekin et al., 2016; Engin et al., 2018).

γ -aminobutyric acid is the major inhibitory neurotransmitter in the brain, and it is present in local interneurons and GABAergic projection neurons (Korpi and Sinkkonen, 2006; Mortensen et al., 2012; Chua and Chebib, 2017). There are two classes of GABA receptors; the ionotropic GABA $_A$ receptors which are ligand-gated ion channels, and the metabotropic GABA $_B$ receptors which represent G-protein coupled receptors. GABA $_A$ receptors are pentameric structures composed of different subunits (Sieghart and Sperk, 2002; Mortensen et al., 2012; Chua and Chebib, 2017). GABA $_A$ receptors containing GABA α 2 subunits are widespread in different parts of the brain including the hippocampus (Mortensen et al., 2012; Engin et al., 2018). Immunoblotting experiments revealed that GABA α 2 subunit levels were increased in hippocampus and in the frontal cortex in the PGC-1 α Tg compared with Wt, whilst those of GABA α 1 were unchanged. Using anti-GABA α 2 antibodies, we noted that there are intensively labeled immunopositive neurons in the CA1, CA3, and DG regions in the hippocampus of TG mice. In addition, cells in the parietal cortex and in the ventral retrosplenial areas were immunostained for GABA α 2 in the TG mice, warranting more studies on these brain areas in the future.

Previous studies have shown that endogenous PGC-1 α is expressed in the brain by different neurons including interneurons (Cowell et al., 2007; Lucas et al., 2010). In our previous study, we noted an expression of PGC-1 α in dopamine neurons in the Thy1-mediated overexpression of PGC-1 α in the Tg mice (Mudò et al., 2012). We have previously shown that PGC-1 α expression is elevated in the hippocampus in principal neurons in the PGC-1 α Tg mice compared with Wt animals (Mäkelä et al., 2016a). Here we further show that cultured cortical neurons express PGC-1 α (Figure 6). Of note, recent studies have shown that deletion of PGC-1 α in hippocampal pyramidal neurons resulted in neuronal hyperexcitability in the hippocampus (McMeekin et al., 2020). This is in line with our findings here that overexpression of PGC-1 α in the PGC-1 α Tg mice caused an increase the inhibitory GABAergic drive in the hippocampal neurons. Pyramidal neurons and inhibitory interneurons are functionally coupled in hippocampus indicating that overexpression of PGC-1 α in one of the cell types could influence the other one. Preliminary double labeling studies indicated that PGC-1 α in the Tg mice is expressed in pyramidal neurons in the hippocampus that are also positive for GABA α 2.

However, we cannot exclude that some interneurons in the PGC-1 α TG hippocampus have also increased PGC-1 α expression. Future experiments, employing cell-specific markers and double immunolabeling with PGC-1 α *in vivo* and in neuron cultures from Tg animals will be helpful to address this question.

As demonstrated by previous data the number, localization and subunit composition of GABA $_A$ receptors are altered in different physiological states, such as during brain development (Yu et al., 2006; Möhler, 2007), following stress (Hsu et al., 2003; Skilbeck et al., 2018), in epilepsy (Raol et al., 2006; Lund et al., 2008), in alcohol dependence (Blednov et al., 2013), in anxiety (Smith et al., 2012; Engin et al., 2016; Rudolph and Moss, 2019), and in neuropsychiatric disorders, including schizophrenia (Lewis et al., 2008; Ramamoorthi and Lin, 2011; Engin et al., 2018).

The GABA $_A$ subunits exhibit distinct electrophysiological and pharmacological properties important for their actions in brain circuitry (Sieghart and Sperk, 2002; Korpi and Sinkkonen, 2006; Mortensen et al., 2012; Chua and Chebib, 2017; Engin et al., 2018). In particular, the GABA α 2 subunit is considered as the preferential target for the anxiolytic-like effects of benzodiazepine (Smith et al., 2012; Rudolph and Moss, 2019). GABA $_A$ receptors containing GABA α 2 subunits further contribute to fast network oscillations in the hippocampus that is crucial for memory encoding and retrieval (Heistek et al., 2013), and influence behavioral responses linked to depression and anxiety (Vollenweider et al., 2011; Engin et al., 2016). In view of these findings, there is an increased interest in designing novel drugs specific to GABA α 2 subunit for various brain disorders (Engin et al., 2012).

In the present work, we observed that the expression of GABA α 2 subunit was increased in the hippocampus of the PGC-1 α Tg compared with Wt, whilst that of GABA α 1 were unchanged. To study the physiology of this, we performed electrophysiology experiments in the CA1 region revealing that the frequency, but not the amplitude of sIPSCs and mIPSCs was enhanced in the hippocampus in Tg compared with Wt mice. Changes in the frequency of spontaneous and miniature synaptic currents may be caused both by alterations in release probability (via presynaptic mechanism), and by changes in the number of functional synapses (Glasgow et al., 2019). To investigate this further we examined the extent to which the GABA α 2 subunit is associated with gephyrin in postsynaptic clusters in the CA1 region of hippocampus. Gephyrin is a large scaffolding protein in the postsynaptic compartment of inhibitory neurons (Tyagarajan and Fritschy, 2014; Chooi and Ko, 2015). Reduced GABA $_A$ receptors clustering is known to enhance anxiety-like behavior (Crestani et al., 1999). Using an assay for colocalization of pixels developed earlier (Costes et al., 2004), we analyzed the gephyrin channel above threshold. Results showed that there is an increase in the number of GABA α 2 pixels in the SR layer in the TG compared with Wt animals. This indicates a higher number of GABA α 2 subunit in association with postsynaptic gephyrin structures in the CA1 region of the hippocampus in PGC-1 α Tg mice compared with Wt. Interestingly, in the ML and SML sublayers of CA1 no such increases in the percentage of gephyrin signals colocalized with GABA α 2 were noted. This may suggest that the dendritic interneurons, which innervate pyramidal cells

in SR, are particularly affected in the Tg mice. These may include, among others, parvalbumin-positive bistratified cells, cholecystinin-positive Shaffer collateral-associated cells and somatostatin-positive neurogliaform cells (Booker and Vida, 2018). The precise modulations of the hippocampal circuitry in the PGC-1 α Tg warrant further studies.

Our observations showing an increase in the expression of GABA α 2 and its co-localization with gephyrin favor the possibility that the elevated frequency of mIPSCs is related to the higher number of active GABAergic synapses on CA1 pyramidal cells. However, we cannot exclude a function of PGC-1 α in enhancing the release probability in GABAergic synapses in the CA1 region. It is possible that overexpression of PGC-1 α alters the excitation/inhibition balance in hippocampus as well as in other brain regions by increasing the number of inhibitory synapses on principal neurons with behavioral consequences and will require further studies. In particular in the hippocampus, the roles of ventral and dorsal hippocampus separately would be important to study in more details.

To measure possible changes in behavior in the Tg mice, we focused on the hippocampus, which is an important area for learning, memory processing, stress responses and anxiety-like behavior (Engin et al., 2016; Padilla-Coreano et al., 2016; Jimenez et al., 2018). Results obtained with the EPM and LDB tests indicated that the PGC-1 α Tg showed an increase in anxiety-like behavior. Thus, the Tg mice were found to spend less time in the open area compared with the Wt mice and showed a higher number of vertical counts in the dark. These findings demonstrate an enhanced anxiety-like behavior in the PGC-1 α Tg mice compared with Wt.

Previously, it has been shown that intrahippocampal infusion of GABA-A receptor agonist inhibit anxiety reactions (Engin and Treit, 2007). This is seemingly at variance with present findings of an increased anxiety-like behavior in the PGC-1 α Tg mice. As shown by Zhang et al. (2014), ventral and dorsal hippocampus play contrasting roles in anxiety regulation in mice. Thus, anxiety-related behavior is regulated by ventral hippocampus-amygdala-medium prefrontal cortex circuitry (Adhikari, 2014). Specific lesions or inhibition of ventral hippocampus impair acquisition of conditioned freezing in fear conditioning paradigm (Maren and Holt, 2004), and severely affect behavior related to unconditioned fear (e.g., entrance to open arms in an elevated plus maze test (Kjelstrup et al., 2002). Anxiety evokes an increase in the synchrony of mPFC and ventral hippocampus EEG in the theta range, and inhibition of hippocampal input to the prefrontal cortex produced an anxiolytic effect (Padilla-Coreano et al., 2016). Stimulation of inputs from basolateral amygdala to the CA1 area of ventral hippocampus evokes anxiety-like behavior (Pi et al., 2020). Increased inhibition of the CA1 pyramidal cells in the PGC-1 α Tg animal model may disturb the function of mPFC-vHIPP-amygdala circuit, causing anxiety-like behavior.

In addition, the contribution of other brain areas to the observed behavioral phenotype cannot be excluded. In this work, we have shown increased inhibition in the CA1 region and anxiety phenotype in the mouse behavior. As such these data do not establish a direct causal relationship between the altered inhibition in hippocampal circuitry caused by PGC-1 α overexpression and the observed anxiety-like behavior.

Additional experiments including rescue of initial PGC-1 α levels are needed to demonstrate causality links between gene expression and behavior. In particular, the specific roles of ventral and dorsal hippocampus in the anxiety-linked behavior in PGC-1 α Tg mice would be important to study in more detail in the future.

To investigate spatial learning in the Tg mice, we employed the T-maze test. In this test, Wt and Tg mice behaved rather similarly, however, the time to reach a decision in the T-maze was relatively longer in the Tg mice. This may indicate difficulties in decision-making, possibly related to increased anxiety. To decipher the behavior of Tg mice compared with Wt in more detail, tests such as Barnes maze and other learning tests could be valuable to perform. There is also drugs available acting on specific GABA α receptors that could be helpful in this context. For example, clinical studies employing the GABA α 2 specific compound, MK-077, have revealed an improvement in cognition and gamma-band oscillations in the frontal cortex of schizophrenia patients (Lewis et al., 2008). Whether such alterations are also found in brains of PGC-1 α Tg mice will require further investigations.

In the brain, there is a complex regulation of the different GABA receptors by various agents and factors (Korpi and Sinkkonen, 2006). Brain-derived neurotrophic factor (BDNF) was shown to increase the expression of GABA α 4 subunits in the neurons via stimulation of the early response factor 3 (Roberts et al., 2006). BDNF, however, reduced GABA α 1 subunit expression through a Janus kinase/signal transducer and activator of transcription pathway (Lund et al., 2008). BDNF was reported also to induce PGC-1 α in cultured hippocampal neurons (Cheng et al., 2012). In the present work, we noted that the expression of GABA α 2 subunit was increased in the Tg mice related to enhanced levels of PGC-1 α in brain neurons. This was corroborated in experiments using the drug pioglitazone and the agonist, GW1929 to stimulate PPAR γ in primary hippocampal neurons. Thus, pioglitazone and GW1929 both increased the mRNA levels of GABA α 2 subunits in the neurons within 1–2 days.

The precise mechanisms for the upregulation of GABA α 2 in the neurons warrant further investigations including the use of gene promoter constructs. In our study, we have focused on PPAR γ but it is clear that PGC-1 α as a transcriptional coactivator can interact with other transcription factors as well and this may vary between tissues. We are currently studying which of several transcription factors PGC-1 α preferentially interacts with in brains of control and TG mice. Furthermore, it is likely that other genes than GABA α 2 are altered in the PGC-1 α Tg mice and may contribute to the observed changes in GABAergic transmission and behavior as studied in the present paper. In future work, we will investigate these gene products in detail, including calcium-dependent proteins involved in neurotransmitter release in the brain.

From the clinical point of view, thiazolidinedione drugs, like pioglitazone, are used in the treatment of metabolic diseases such as type-2 diabetes. An increasing amount of data suggests that such drugs and compounds acting on PPAR γ -PGC-1 α signaling may have beneficial effects in different brain diseases (Patrone et al., 2014; Carta and Simuni, 2015;

Mäkelä et al., 2016b). Adding to this we show here that pioglitazone can increase expression of GABA α 2 subunits in brain neurons with possible functional consequences for inhibitory neurotransmission that will require further investigations. In conclusion, the role described here for PGC-1 α in increasing GABA α 2 subunit expression and GABAergic neurotransmission may be important in different neuropsychiatric disorders and other brain diseases.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethics Committee and the Koe-eläinkeskus University of Helsinki.

AUTHOR CONTRIBUTIONS

LK and DL designed the project. TV, SM, DP, AS, CP, EV, TTi, OE, ML, TTa, LK, VV, and DL designed the experiments.

REFERENCES

- Adhikari, A. (2014). Distributed circuits underlying anxiety. *Front. Behav. Neurosci.* 8:112. doi: 10.3389/fnbeh.2014.00112
- Austin, S., and St-Pierre, J. (2012). PGC1 α and mitochondrial metabolism—emerging concepts and relevance in ageing and neurodegenerative disorders. *J. Cell Sci.* 125(Pt 21), 4963–4971. doi: 10.1242/jcs.113662
- Blednov, Y. A., Benavidez, J. M., Black, M., Chandra, D., Homanics, G. E., Rudolph, U., et al. (2013). Linking GABA(A) receptor subunits to alcohol-induced conditioned taste aversion and recovery from acute alcohol intoxication. *Neuropharmacology* 67, 46–56. doi: 10.1016/j.neuropharm.2012.10.016
- Booker, S. A., and Vida, I. (2018). Morphological diversity and connectivity of hippocampal interneurons. *Cell Tissue Res.* 373, 619–641. doi: 10.1007/s00441-018-2882-2
- Carta, A. R., and Simuni, T. (2015). Thiazolidinediones under preclinical and early clinical development for the treatment of Parkinson's disease. *Expert Opin. Investig. Drugs* 24, 219–227.
- Chen, Y. C., Wu, J. S., Tsai, H. D., Huang, C. Y., Chen, J. J., Sun, G. Y., et al. (2012). Peroxisome proliferator-activated receptor gamma (PPAR- γ) and neurodegenerative disorders. *Mol. Neurobiol.* 46, 114–124. doi: 10.1007/s12035-012-8259-8
- Cheng, A., Wan, R., Yang, J. L., Kamimura, N., Son, T. G., Ouyang, X., et al. (2012). Involvement of PGC-1 α in the formation and maintenance of neuronal dendritic spines. *Nat. Commun.* 3:1250. doi: 10.1038/ncomms2238
- Choi, G., and Ko, J. (2015). Gephyrin: a central GABAergic synapse organizer. *Exp. Mol. Med.* 47:e158. doi: 10.1038/emmm.2015.5
- Chua, H. C., and Chebib, M. (2017). GABA α Receptors and the Diversity in their Structure and Pharmacology. *Adv. Pharmacol.* 79, 1–34.
- Clark, J., Reddy, S., Zheng, K., Betensky, R. A., and Simon, D. K. (2011). Association of PGC-1 α polymorphisms with age of onset and risk of Parkinson's disease. *BMC Med. Genet.* 12:69. doi: 10.1186/1471-2350-12-69
- Comhair, J., Devoght, J., Morelli, G., Harvey, R. J., Briz, V., Borrie, S. C., et al. (2018). Alpha2-Containing Glycine Receptors Promote Neonatal Spontaneous Activity of Striatal Medium Spiny Neurons and Support Maturation of

TV, SM, DP, AS, CP, VS, JN, MU, MK, RM, TTi, and VV performed the experiments. TV, SM, AS, ML, VV, and DL wrote the manuscript. All authors analyzed the experiments, edited and approved the manuscript.

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- Glutamatergic Inputs. *Front. Mol. Neurosci.* 11:380. doi: 10.3389/fnmol.2018.00380
- Cowell, R. M., Blake, K. R., and Russell, J. W. (2007). Localization of the transcriptional coactivator PGC-1 α to GABAergic neurons during maturation of the rat brain. *J. Comp. Neurol.* 502, 1–18. doi: 10.1002/cne.21211
- Costes, S. V., Daelemans, D., Cho, E. H., Dobbin, Z., Pavlakis, G., and Lockett, S. (2004). Automatic and quantitative measurement of protein-protein colocalization in live cells. *Biophys. J.* 86, 3993–4003. doi: 10.1529/biophysj.103.038422
- Crestani, F., Lorez, M., Baer, K., Essrich, C., Benke, D., Laurent, J. P., et al. (1999). Decreased GABA α -receptor clustering results in enhanced anxiety and a bias for threat cues. *Nat. Neurosci.* 2, 833–839. doi: 10.1038/12207
- Deacon, R. M., and Rawlins, J. N. (2006). T-maze alternation in the rodent. *Nat. Protocol* 1, 7–12.
- de Guglielmo, G., Kallupi, M., Scuppa, G., Demopulos, G., Gaitanaris, G., and Ciccocioppo, R. (2017). Pioglitazone attenuates the opioid withdrawal and vulnerability to relapse to heroin seeking in rodents. *Psychopharmacology* 234, 223–234. doi: 10.1007/s00213-016-4452-1
- Do, H. T., Bruelle, C., Tselykh, T., Jalonen, P., Korhonen, L., and Lindholm, D. (2013). Reciprocal regulation of very low density lipoprotein receptors (VLDLRs) in neurons by brain-derived neurotrophic factor (BDNF) and Reelin: involvement of the E3 ligase Mylip/Idol. *J. Biol. Chem.* 288, 29613–29620.
- Dougherty, S. E., Bartley, A. F., Lucas, E. K., Hablitz, J. J., Dobrunz, L. E., and Cowell, R. M. (2014). Mice lacking the transcriptional coactivator PGC-1 α exhibit alterations in inhibitory synaptic transmission in the motor cortex. *Neuroscience* 271, 137–148. doi: 10.1016/j.neuroscience.2014.04
- Engin, E., and Treit, D. (2007). The role of hippocampus in anxiety: intracerebral infusion studies. *Behav. Pharmacol.* 18, 365–374. doi: 10.1097/FBP.0b013e3282de7929
- Engin, E., Liu, J., and Rudolph, U. (2012). alpha2-containing GABA(A) receptors: a target for the development of novel treatment strategies for CNS disorders. *Pharmacol. Ther.* 136, 142–152.
- Engin, E., Smith, K. S., Gao, Y., Nagy, D., Foster, R. A., Tsvetkov, E., et al. (2016). Modulation of anxiety and fear via distinct intrahippocampal circuits. *eLife* 5:e14120. doi: 10.7554/eLife.14120

- Engin, E., Benham, R. S., and Rudolph, U. (2018). An Emerging Circuit Pharmacology of GABAA Receptors. *Trends Pharmacol. Sci.* 39, 710–732.
- Fernandez-Marcos, P. J., and Auwerx, J. (2011). Regulation of PGC-1 α , a nodal regulator of mitochondrial biogenesis. *Am. J. Clin. Nutr.* 93, 884S–890S.
- Glasgow, S. D., McPhedrain, R., Madranges, J. F., Kennedy, T. E., and Ruthazer, E. S. (2019). Approaches and limitations in the investigation of synaptic transmission and plasticity. *Front. Synaptic Neurosci.* 11:20. doi: 10.3389/fnsyn.2019.00020
- Handschin, C., and Spiegelman, B. M. (2006). Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. *Endocr. Rev.* 27, 728–735.
- Heistek, T. S., Ruiperez-Alonso, M., Timmerman, A. J., Brussaard, A. B., and Mansvelter, H. D. (2013). α 2-containing GABAA receptors expressed in hippocampal region CA3 control fast network oscillations. *J. Physiol.* 591, 845–858. doi: 10.1113/jphysiol.2012.243725
- Hsu, F. C., Zhang, G. J., Raol, Y. S., Valentino, R. J., Coulter, D. A., and Brooks-Kayal, A. R. (2003). Repeated neonatal handling with maternal separation permanently alters hippocampal GABAA receptors and behavioral stress responses. *Proc. Natl. Acad. Sci.* 100, 12213–12218. doi: 10.1073/pnas.2131679100
- Hyrskyluoto, A., Bruelle, C., Lundh, S. H., Do, H. T., Kivinen, J., Rappou, E., et al. (2014). Ubiquitin-specific protease-14 reduces cellular aggregates and protects against mutant huntingtin-induced cell degeneration: involvement of the proteasome and ER stress-activated kinase IRE1 α . *Hum. Mol. Genet.* 23, 5928–5939. doi: 10.1093/hmg/ddu317
- Jiang, H., Kang, S. U., Zhang, S., Karuppagounder, S., Xu, J., Lee, Y. K., et al. (2016). Adult Conditional Knockout of PGC-1 α Leads to Loss of Dopamine Neurons. *eNeuro* 3, 183–116. doi: 10.1523/ENEURO.0183-16.2016
- Jimenez, J. C., Su, K., Goldberg, A. R., Luna, V. M., Biane, J. S., Ordek, G., et al. (2018). Anxiety Cells in a Hippocampal-Hypothalamic Circuit. *Neuron* 97, 670.e–683.e. doi: 10.1016/j.neuron.2018.01.016
- Kjelstrup, K. G., Tuvnes, F. A., Steffenach, H. A., Murison, R., Moser, E. I., and Moser, M. B. (2002). Reduced fear expression after lesions of the ventral hippocampus. *Proc. Natl. Acad. Sci. U S A.* 99, 10825–10830. doi: 10.1073/pnas.152112399
- Korpi, E. R., and Sinkkonen, S. T. (2006). GABA(A) receptor subtypes as targets for neuropsychiatric drug development. *Pharmacol. Ther.* 109, 12–32.
- Lewis, D. A., Cho, R. Y., Carter, C. S., Eklund, K., Forster, S., Kelly, M. A., et al. (2008). Subunit-selective modulation of GABA type A receptor neurotransmission and cognition in schizophrenia. *Am. J. Psychiatr.* 165, 1585–1593. doi: 10.1176/appi.ajp.2008.08030395
- Lindholm, D., Eriksson, O., Mäkelä, J., Belluardo, N., and Korhonen, L. (2012). PGC-1 α : a master gene that is hard to master. *Cell. Mol. Life Sci.* 69, 2465–2468. doi: 10.1007/s00018-012-1043-0
- Lucas, E. K., Markwardt, S. J., Gupta, S., Meador-Woodruff, J. H., Lin, J. D., Overstreet-Wadiche, L., et al. (2010). Parvalbumin deficiency and GABAergic dysfunction in mice lacking PGC-1 α . *J. Neurosci.* 30, 7227–7235. doi: 10.1523/JNEUROSCI.0698-10.2010
- Lucas, E. K., Dougherty, S. E., McMeekin, L. J., Reid, C. S., Dobrunz, L. E., West, A. B., et al. (2014). PGC-1 α provides a transcriptional framework for synchronous neurotransmitter release from parvalbumin-positive interneurons. *J. Neurosci.* 34, 14375–14387. doi: 10.1523/JNEUROSCI.1222-14.2014
- Lucas, E. K., Reid, C. S., McMeekin, L. J., Dougherty, S. E., Floyd, C. L., and Cowell, R. M. (2015). Cerebellar transcriptional alterations with Purkinje cell dysfunction and loss in mice lacking PGC-1 α . *Front. Cell Neurosci.* 8:441. doi: 10.3389/fncel.2014.00441
- Lund, I. V., Hu, Y., Raol, Y. H., Benham, R. S., Faris, R., Russek, S. J., et al. (2008). BDNF selectively regulates GABAA receptor transcription by activation of the JAK/STAT pathway. *Sci. Signal.* 1:ra9. doi: 10.1126/scisignal.1162396
- Maren, S., and Holt, W. G. (2004). Hippocampus and Pavlovian fear conditioning in rats: muscimol infusions into the ventral, but not dorsal, hippocampus impair the acquisition of conditional freezing to an auditory conditional stimulus. *Behav. Neurosci.* 118, 97–110. doi: 10.1037/0735-7044.118.1.97
- Martinez-Redondo, V., Pettersson, A. T., and Ruas, J. L. (2015). The hitchhiker's guide to PGC-1 α isoform structure and biological functions. *Diabetologia* 58, 1969–1977.
- McMeekin, L. J., Lucas, E. K., Meador-Woodruff, J. H., McCullumsmith, R. E., Hendrickson, R. C., Gamble, K. L., et al. (2016). Cortical PGC-1 α -Dependent Transcripts Are Reduced in Postmortem Tissue From Patients With Schizophrenia. *Schizophr. Bull.* 42, 1009–1017. doi: 10.1093/schbul/sbv184
- McMeekin, L. J., Li, Y., Fox, S. N., Rowe, G. C., Crossman, D. K., Day, J. J., et al. (2018). Cell-Specific Deletion of PGC-1 α from Medium Spiny Neurons Causes Transcriptional Alterations and Age-Related Motor Impairment. *J. Neurosci.* 38, 3273–3286. doi: 10.1523/JNEUROSCI.0848-17.2018
- McMeekin, L. J., Bartley, A. F., Bohannon, A. S., Adlaf, E. W., van Groen, T., Boas, S. M., et al. (2020). A Role for PGC-1 α in Transcription and Excitability of Neocortical and Hippocampal Excitatory Neurons. *Neuroscience* 435, 73–94. doi: 10.1016/j.neuroscience.2020.03.0
- Mortensen, M., Patel, B., and Smart, T. G. (2012). GABA Potency at GABA(A) Receptors Found in Synaptic and Extrasynaptic Zones. *Front. Cell Neurosci.* 6:1. doi: 10.3389/fncel.2012.00001
- Mudò, G., Mäkelä, J., Di Liberto, V., Tselykh, T. V., Olivieri, M., Piepponen, P., et al. (2012). Transgenic expression and activation of PGC-1 α protect dopaminergic neurons in the MPTP mouse model of Parkinson's disease. *Cell. Mol. Life Sci.* 69, 1153–1165. doi: 10.1007/s00018-011-0850-z
- Mäkelä, J., Mudò, G., Pham, D. D., Di Liberto, V., Eriksson, O., Louhivuori, L., et al. (2016a). Peroxisome proliferator-activated receptor- γ coactivator-1 α mediates neuroprotection against excitotoxic brain injury in transgenic mice: role of mitochondria and X-linked inhibitor of apoptosis protein. *Eur. J. Neurosci.* 43, 626–639. doi: 10.1111/ejn.13157
- Mäkelä, J., Tselykh, T. V., Kukkonen, J. P., Eriksson, O., Korhonen, L. T., and Lindholm, D. (2016b). Peroxisome proliferator-activated receptor- γ (PPAR γ) agonist is neuroprotective and stimulates PGC-1 α expression and CREB phosphorylation in human dopaminergic neurons. *Neuropharmacol.* 102, 266–275. doi: 10.1016/j.neuropharm.2015.11.020
- Möhler, H. (2007). Molecular regulation of cognitive functions and developmental plasticity: impact of GABAA receptors. *J. Neurochem.* 102, 1–12. doi: 10.1111/j.1471-4159.2007.04454.x
- Padilla-Coreano, N., Bolkan, S. S., Pierce, G. M., Blackman, D. R., and Hardin, W. D. (2016). Direct Ventral Hippocampal-Prefrontal Input Is Required for Anxiety-Related Neural Activity and Behavior. *Neuron* 89, 857–866.
- Patrone, C., Eriksson, O., and Lindholm, D. (2014). Diabetes drugs and neurological disorders: new views and therapeutic possibilities. *Lancet Diabetes Endocrinol.* 2, 256–262.
- Pham, D. D., Bruelle, C., Thi, Do, H., Pajanoja, C., Jin, C., et al. (2019). Caspase-2 and p75 neurotrophin receptor (p75NTR) are involved in the regulation of SREBP and lipid genes in hepatocyte cells. *Cell Death Dis.* 10:537. doi: 10.1038/s41419-019-1758-z
- Pi, G., Gao, D., Wu, D., Wang, Y., Lei, H., Zeng, W., et al. (2020). Posterior basolateral amygdala to ventral hippocampal CA1 drives approach behaviour to exert an anxiolytic effect. *Nat. Comm.* 11:183. doi: 10.1038/s41467-019-13919-3
- Pizzarelli, R., Griguoli, M., Zacchi, P., Petrini, E. M., Barberis, A., Cattaneo, A., et al. (2020). Tuning GABAergic Inhibition: Gephyrin Molecular Organization and Functions. *Neuroscience* 439, 125–136. doi: 10.1016/j.neuroscience.2019.07.036
- Puigserver, P., and Spiegelman, B. M. (2003). Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr. Rev.* 24, 78–90.
- Ramamoorthi, K., and Lin, Y. (2011). The contribution of GABAergic dysfunction to neurodevelopmental disorders. *Trends Mol. Med.* 17, 452–462.
- Raol, Y. H., Lund, I. V., Bandyopadhyay, S., Zhang, G., Roberts, D. S., Wolfe, J. H., et al. (2006). Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy. *J. Neurosci.* 26, 11342–11346. doi: 10.1523/JNEUROSCI.3329-06.2006
- Roberts, D. S., Hu, Y., Lund, I. V., Brooks-Kayal, A. R., and Russek, S. J. (2006). Brain-derived neurotrophic factor (BDNF)-induced synthesis of early growth response factor 3 (Egr3) controls the levels of type A GABA receptor alpha 4 subunits in hippocampal neurons. *J. Biol. Chem.* 281, 29431–29435. doi: 10.1074/jbc.C600167200
- Rudolph, U., and Moss, S. J. (2019). Modulating anxiety and activity. *Science* 366, 185–186. doi: 10.1126/science.aaz3176
- Scarpulla, R. C. (2011). Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. *Biochim. Biophys. Acta* 1813, 1269–1278.

- Sieghart, W., and Sperk, G. (2002). Subunit composition, distribution and function of GABA(A) receptor subtypes. *Curr. Top Med. Chem.* 2, 795–816. doi: 10.2174/1568026023393507
- Skilbeck, K. J., Johnston, G., and Hinton, T. (2018). Long-lasting effects of early-life intervention in mice on adulthood behaviour, GABAA receptor subunit expression and synaptic clustering. *Pharmacol. Res.* 128, 179–189. doi: 10.1016/j.phrs.2017.09.021
- Smith, K. S., Engin, E., Meloni, E. G., and Rudolph, U. (2012). Benzodiazepine-induced anxiolysis and reduction of conditioned fear are mediated by distinct GABAA receptor subtypes in mice. *Neuropharmacology* 63, 250–258. doi: 10.1016/j.neuropharm.2012.03.000
- Sokka, A. L., Putkonen, N., Mudo, G., Pryazhnikov, E., Reijonen, S., Khiroug, L., et al. (2007). Endoplasmic reticulum stress inhibition protects against excitotoxic neuronal injury in the rat brain. *J. Neurosci.* 27, 901–908. doi: 10.1523/JNEUROSCI.4289-06.2007
- Soyal, S. M., Felder, T. K., Auer, S., Hahne, P., Oberkofler, H., Witting, A., et al. (2012). A greatly extended PPARC1A genomic locus encodes several new brain-specific isoforms and influences Huntington disease age of onset. *Hum. Mol. Genet.* 21, 3461–3473. doi: 10.1093/hmg/dds177
- Srinivasan, V., Bruelle, C., Scifo, E., Pham, D. D., Soliymani, R., Lalowski, M., et al. (2020). Dynamic Interaction of USP14 with the Chaperone HSC70 Mediates Crosstalk between the Proteasome, ER Signaling, and Autophagy. *iScience* 23:100790. doi: 10.1016/j.isci.2019.100790
- St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jäger, S., et al. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 127, 397–408. doi: 10.1016/j.cell.2006.09.024
- Stopponi, S., Somaini, L., Cippitelli, A., Cannella, N., Braconi, S., Kallupi, M., et al. (2011). Activation of nuclear PPAR γ receptors by the antidiabetic agent pioglitazone suppresses alcohol drinking and relapse to alcohol seeking. *Biol. Psychiatry* 69, 642–649. doi: 10.1016/j.biopsych.2010.12.010
- Stopponi, S., de Guglielmo, G., Somaini, L., Cippitelli, A., Cannella, N., Kallupi, M., et al. (2013). Activation of PPAR γ by pioglitazone potentiates the effects of naltrexone on alcohol drinking and relapse in msP rats. *Alcohol. Clin. Exp. Res.* 37, 1351–1360. doi: 10.1111/acer.12091
- Tyagarajan, S. K., and Fritschy, J. M. (2014). Gephyrin: a master regulator of neuronal function? *Nat. Rev. Neurosci.* 15, 141–156. doi: 10.1038/nrn3670
- Vollenweider, I., Smith, K. S., Keist, R., and Rudolph, U. (2011). Antidepressant-like properties of alpha2-containing GABA(A) receptors. *Behav. Brain Res.* 217, 77–80.
- Yu, Z. Y., Wang, W., Fritschy, J. M., Witte, O. W., and Redecker, C. (2006). Changes in neocortical and hippocampal GABAA receptor subunit distribution during brain maturation and aging. *Brain Res.* 1099, 73–81. doi: 10.1016/j.brainres.2006.04.118
- Zhang, W. N., Bast, T., Xu, Y., and Feldon, J. (2014). Temporary inhibition of dorsal or ventral hippocampus by muscimol: distinct effects on measures of innate anxiety on the elevated plus maze, but similar disruption of contextual fear conditioning. *Behav. Brain Res.* 262, 47–56. doi: 10.1016/j.bbr.2013.10.044
- Zheng, B., Liao, Z., Locascio, J. J., Lesniak, K. A., Roderick, S. S., Watt, M. L., et al. (2010). PGC-1 α , a potential therapeutic target for early intervention in Parkinson's disease. *Sci. Transl. Med.* 2:52ra73. doi: 10.1126/scitranslmed.3001059
- Zolezzi, J. M., Silva-Alvarez, C., Ordenes, D., Godoy, J. A., Carvajal, F. J., Santos, M. J., et al. (2013). Peroxisome proliferator-activated receptor (PPAR) γ and PPAR α agonists modulate mitochondrial fusion-fission dynamics: relevance to reactive oxygen species (ROS)-related neurodegenerative disorders? *PLoS One* 8:e64019. doi: 10.1371/journal.pone.0064019

Conflict of Interest: TV and JN were employed by Quretec Ltd., Tartu, Estonia and TT and MU by Protobios LCC, Tallinn.

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