Neurochemical Research (2023) 48:2104–2115
https://doi.org/10.1007/s11064-023-03870-7

ORIGINAL PAPER

NMDA Receptor Activation and Ca^{2+}/PKC Signaling in Nicotine-Induced GABA Transport Shift in Embryonic Chick Retina

Arthur Cardoso Souto[1] · Matheus Heidemann Tempone[2] · Lyslie Azeredo Coutinho Gonçalves[1] · Vladimir Pedro Peralva Borges-Martins[1] · Maria Carolina Peixoto-Rodrigues[3] · Ana Clara Oliveira Damascena[1] · Gabriel Ferraz[4] · Alex Christian Manhães[5] · Newton Gonçalves Castro[4] · Ricardo Augusto de Melo Reis[2] · Ana Lucia Marques Ventura[6] · Regina Célia Cussa Kubrusly[1]

Received: 15 August 2022 / Revised: 23 December 2022 / Accepted: 21 January 2023 / Published online: 15 February 2023

Abstract

Nicotinic receptors are present in the retina of different vertebrates, and in the chick retina, it is present during early development throughout to post-hatching. These receptors are activated by nicotine, an alkaloid with addictive and neurotransmitter release modulation properties, such as GABA signaling. Here we evaluated the mechanisms of nicotine signaling in the avian retina during the development of neuron-glia cells at a stage where synapses are peaking. Nicotine almost halved [^{3}H]-GABA uptake, reducing it by 45% whilst increasing more than two-fold [^{3}H]-GABA release in E12 embryonic chick retinas. Additionally, nicotine mediated a 33% increase in [^{3}H]-D-aspartate release. MK-801 50 µM blocked 66% of nicotine-induced [^{3}H]-GABA release and Gö 6983 100 nM prevented the nicotine-induced reduction in [^{3}H]-GABA uptake by rescuing 40% of this neurotransmitter uptake, implicating NMDAR and PKC (respectively) in the nicotinic responses. In addition, NO-711 prevented [^{3}H]-GABA uptake and release induced by nicotine. Furthermore, the relevance of calcium influx for PKC activation was evidenced through fura-2 imaging. We conclude that the shift of GABA transport mediated by nicotine promotes GABA release by inducing transporter reversal via nicotine-induced EAA release through EAATs, or by a direct effect of nicotine in activating nicotinic receptors permeable to calcium and promoting PKC pathway activation and shifting GAT-1 activity, both prompting calcium influx, and activation of the PKC pathway and shifting GAT-1 activity.

Keywords Nicotine · GABA · Acetylcholine · Nicotinic receptors · Glutamate · GABA transport

Introduction

Pharmacologically, nicotine acts as an agonist of nicotinic acetylcholine receptors (nAChR), with a greater affinity than acetylcholine, the endogenous ligand [1]. Nicotine increases dopamine release in the mesolimbic pathway of the reward system, which is associated with drug reinforcement [2, 3]. In the retina, dopamine release has been reported to be modulated by different concentrations of nicotine up to 1 mM [4]. In addition to dopamine, the activation of presynaptic nAChR promotes the release of other neurotransmitters, explaining the widespread neuromodulator effects of nicotine [5, 6].

One of those neurotransmitters is γ-aminobutyric acid (GABA), the main inhibitory neurotransmitter of the central nervous system (CNS) [7]. Early on during development, GABA is present in various CNS regions [8, 9], and, on the vertebrate retina, this neurotransmitter is found early on in

✉ Regina Célia Cussa Kubrusly
reginakubrusly@id.uff.br

1 Laboratório de Neurofarmacologia, Departamento de Fisiologia e Farmacologia, Universidade Federal Fluminense, Inst Biomédico - Rua São João Batista, 187, Sala 416, Centro, Niterói, CEPRJ 24020-005, Brazil

2 Laboratório de Neuroquímica. IBCCF, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

3 Laboratório de Neuroplasticidade, Departamento de Neurobiologia, Instituto de Biologia, Universidade Federal Fluminense, Niterói, RJ, Brazil

4 Laboratório de Farmacologia Molecular, ICB, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

5 Laboratório de Neurofisiologia, IBRAG, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

6 Laboratório de Neuroquímica, Departamento de Neurobiologia, Universidade Federal Fluminense, Rio de Janeiro, RJ, Brazil

subpopulations of horizontal, ganglion, amacrine, and Müller glia cells [10–12]. GABA decreases nicotine-induced (10–100 μM) increase in EPSP in chick embryo ganglion cells [13].

In the avian retina, GABA transport is mediated by its high-affinity transporters, GABA transporters (GATs) [14, 15]. GATs are sodium symporters found in different cell types responsible for the transport of GABA together with Na^+ and Cl^- [16]. They canonically operate along the Na^+ gradient uptaking GABA from the synaptic cleft, however, they can release this neurotransmitter by a reversal of this gradient both in the brain [17] and in the retina [18]. These transporters are essential to GABA homeostasis and are modulated by a plethora of chemical mediators and signaling proteins such as kinases, phosphatases, second messengers, hormones, or changes in the Na^+ gradient [19, 20]. Phosphorylation in tyrosine residues increases GAT-1 density in the cell membrane [20, 21] while tonic phosphorylation of serine residues by protein kinase C (PKC) removes GAT-1 from the cell membrane [22, 23].

Beyond its importance during development, GABA is also essential in modulating the retinal tissue excitability in the adult retina [24]. Concurrently, the vertebrate retina has already been described as having a GABA + cell with nAChR in its soma, axon, and dendrites [25, 26].

Cholinergic cells, in the avian and rodent retina, are limited to a subpopulation of amacrine cells known as starburst amacrine cells [27]. The appearance of this neuronal subtype starts very early in the embryonic retina development, at day 4 (E4), and plays a crucial role in the correct morphology and functioning during tissue development [11]. Moreover, the fundamental machinery for cholinergic signaling is also found early during retinal development, including its synthesizing and degrading enzymes, choline acetyltransferase (ChAT), and acetylcholinesterase (AChE), respectively, specific subunits of nicotinic receptors (e.g. α3, α8, β2) and ACh itself [10, 11].

The avian retina is a reliable developmental model that has been studied for at least half a century in terms of neurochemistry, with a peculiar mechanism to induce GABA and excitatory amino acid release (EAA). GABA and aspartate release in this retina depends on amino acid transporters to mediate the release of the neurotransmitter in a Ca^{2+} independent and Na^+ dependent manner [12, 28]. GABA release has been induced by different addictive players such as caffeine [15] or by activation of the endocannabinoid system [29]. In this sense, the mechanisms of nicotine on GABA release were not fully evaluated on avian retina cells, although nicotinic and glutamatergic receptors have been identified on this tissue [25, 30]. In the present study, we aimed to evaluate the effects of acute exposure to nicotine over GABA transport, in an ex vivo model of chicken embryo retinas, at 12 days of the embryonic stage (E12). This period of development represents the beginning of synaptogenesis [10], which is critical to the maturation of the GABAergic system and upon which nicotine and cholinergic activation might have long-term effects.

Material and Methods

Ethics Statement

The following procedures were approved by the Animal Care and Use Committee of Federal University of Rio de Janeiro protocol 038/19 and follow the guidelines of the Brazilian Society for Neuroscience and Behavior (SBNeC) and Brazilian Law No. 11794/ 2008.

Animals and Retinal Dissection

Fertilized White Leghorn eggs (*Gallus gallus domesticus*) were obtained from a local hatchery and staged as previously described [31]. Embryonic retinas (E12) were dissected and separated from the other ocular tissues in CMF solution, at 37 ℃. Subsequently, retinas were placed in p35 plastic dishes (Corning, Glendale, Arizona) containing 1 mL of Hanks' 4 solutions with Ca^{+2} and Mg^+, for neurochemical assays. Altogether, 82 embryos (164 retinas) were used.

Nicotine Treatment

Assays were performed on ex vivo retinal tissue. Thus, after being dissected and placed on multiwell dishes, retinas were acutely treated with nicotine (50 μM) for 30 min in a water bath (37 ℃) before they were used for neurochemical experiments and quantification of protein density or calcium transients. Specific treatments for each experiment will be detailed below.

Materials

For neurotransmitter uptake and release assays, tritiated GABA ([^{3}H]-GABA) with the specific activity of 35 Ci/mmol and [^{3}H]-D-aspartate 12 Ci/mmol were obtained from PerkinElmer (Massachusetts, USA). Mecamylamine hydrochloride (non-selective antagonist of nAChRs), MK-801 (dizocilpine) maleate (NMDARs non-competitive blocker), NMDA (NMDARs agonist), NO-711 (1-[2-[[(diphenylmethylene)imino]oxy]ethyl]-1,2,5,6-tetrahydro-3 pyridinecarboxylic acid) hydrochloride (selective GAT-1 inhibitor), nicotine (+)-bitartrate, Gö 6983 (2-[1-(3-dimethylaminopropyl)-5-methoxyindol-3-yl]-3-(1H-indol-3-yl) maleimide) (a pan-PKC inhibitor against for PKCα, PKCβ, PKCγ, PKCδ and PKCζ), rabbit anti-GAT-1 antibody (HPA013341) and

bovine serum albumin (BSA) were obtained from Sigma / RBI (St Louis, MO, USA).

Nicotine (1, 10, or 50 µM) was administered acutely (10, 15, or 30 min) to ex vivo retina tissue (E12). NO-711 (50 µM) and Gö 6983 (100 nM) were added 5 min before incubation with nicotine, in specific uptake experiments. [^{3}H]-GABA was incubated together with the previously specified drugs for 60 min with the retina, immediately after 30 min of treatment with nicotine. Additionally, after [^{3}H]-GABA uptake 5 min pulses with Hanks' solution or MK-801 (50 µM) were performed for the release assay. Nicotine (50 µM), NMDA (100 µM), and mecamylamine (3 µM) were further utilized in retinal cell culture for calcium imaging assays.

Solutions and Medium

Dulbecco's Modified Eagle's Medium (DMEM), 1% fetal calf serum (FCS), and gentamicin (Invitrogen, Life Technologies, Rockford, IL). Trypsin 0.25% solution (Gibco). B27 supplement (Invitrogen) (25 µg final concentration) was added to 50 mL of DMEM for enriched neuronal cultures. Saline buffer (Ca^{2+} and Mg^{2+} free solution): 76.55 g/L NaCl, 3.05 g/L KCl, 1.65 g/L Na_2HPO_4, 0.610 g/L KH_2PO_4, 21.95 g/L glucose, and 7.90 g/L $NaHCO_3$; Cells were seeded in 15 mm glass coverslips pretreated with poly-L-lysine (10 µg/mL) and laminin (10 µg/mL).

Hanks 4 mM glucose solution (NaCl 128 mM; KCl 4 mM; $MgCl_2$ 1 mM; $CaCl_2$ 3 mM; HEPES 20 mM; glucose 4 mM; pH = 7.4); *Modified Hanks 4 w/o* Na^+ (Tris HCl 128 mM; KCl 4 mM; $MgCl_2$ 1 mM; $CaCl_2$ 3 mM; glucose 4 mM; pH = 7.4); *CMF solution* (NaCl 131 mM; KCl 4.1 mM; Na_2HPO_4 0.92 mM; KH_2PO_4 0.45 mM; $NaHCO_3$ 9.4 mM; glucose 12 mM; pH = 7.4); *Krebs solution (*132 mM NaCl, 4 mM KCl, 1.4 mM $MgCl_2$, 2.5 mM $CaCl_2$, 6 mM glucose, 10 mM HEPES, pH = 7.4); *Loading solution* 5 µM fura-2/AM (Molecular Probes), 0.02% pluronic F-127 (Molecular Probes) in Krebs solution.

[^{3}H]-GABA Uptake & Release

[^{3}H]-GABA uptake and release were performed as described before [15]. Briefly, after the pre-exposure drug treatments, each retina was incubated for 1 h in 1 mL of Hanks 4 solution or Tris–HCl solution pH 7.4 at 37 °C containing 0.5 µCi of [^{3}H]-GABA (35 Ci/mmol = 35.106 µCi) and 20 µM of non-radioactive GABA as a carrier without changing the initial medium.

For the uptake assay, both control and nicotine-treated retina groups were analyzed alone or in the presence of NO-711 (50 µM) or Gö 6983 (100 nM) before [^{3}H]-GABA loading. After a 60 min incubation with [^{3}H]-GABA, the solution was removed, and the tissue was washed three times with 3 mL of cold Hanks (4 mM) solution. This process was sufficient to wash out the radioactivity not taken up by the cells. Then, 1 mL of Milli-Q water (Millipore) was added to disrupt cell membranes, followed by a freeze–thaw cycle. For the release assay, after the [^{3}H]-GABA was incorporated by the cell, the medium was removed, and the tissue was washed twice with 1 mL Hanks 4 solution. Afterward, the retina was superfused with 0.5 mL of Hanks 4 alone (basal), NO-711 (50 µM) or with MK-801 (50 µM) at 37 °C for 5 min, and both the supernatant and cell lysate were collected.

Cell radioactivity was determined using a liquid scintillation counter and results were either normalized by protein concentration, estimated with Lowry protein assay using BSA as standard, in the uptake assay, or to the percentage of total radioactivity in the release assay, as described previously [28].

Neuron-Enriched Chick Cell Culture

Neuron-enriched chick cell cultures were prepared according to Kubrusly et al. [29]. Briefly, 8-day-old chick embryo (E8) retinas were dissected, cleared of pigmented epithelium, and placed on saline. Trypsin was added to the medium and incubated at 37 °C for 10 min. After a brief centrifugation, the pellet was suspended in 2 mL of DMEM/F12 medium plus 1% fetal calf serum (FCS), plus B27 supplement, and mechanically dissociated by pipetting the tissue. Cells were plated on 15 mm coverslips (Marienbad, German) previously treated with poly-L-lysine and laminin. The plates were then transferred to a humidified atmosphere of 95% air/5% CO_2 in an incubator for 4 days (C4).

[^{3}H]-D-Aspartate Release

[^{3}H]-D-Aspartate release was evaluated as described before [32]. Briefly, each retinal culture (E8C4) was incubated for 1 h in 1 mL of Hanks 4 solution pH 7.4 at 37 °C containing 1 µCi of [^{3}H]-D-aspartate (12 Ci/mmol) and 10 µM of non-radioactive D-aspartate as a carrier, with or without prior addition of nicotine. After the [^{3}H]-D-aspartate was incorporated by the cells, the medium was removed, and the cultured well was washed twice with 1 mL Hanks 4 solution. Afterward, the cells were superfused with 0.5 mL of Hanks 4 (basal) at 37 °C and incubated for 5 min. Cellular and supernatant radioactivity was assayed as described in [^{3}H]-GABA.

Western Blot

Western Blot was performed as described before [15]. Briefly, both retinas were extracted and homogenized together in a RIPA buffer containing a cocktail of protease

inhibitors. The homogenate was used to assess the expression of GAT-1 protein in control and nicotine-treated groups. Protein concentration was estimated [33] and the samples were diluted in a buffer composed of 10% glycerol (v/v), 1% ß-mercaptoethanol, 3% SDS and 62.5 mm Tris base which were boiled for 5 min.

Approximately 30 µg of protein from each sample were used in electrophoresis in 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (ECL-Hybond) by a semi-dry transfer method. Membranes were washed with a Tween 20 Tris-buffered saline (TTBS) and blocked for 2 h with TTBS plus 1% BSA. After blocking, membranes were lightly washed with TTBS and incubated with primary anti-GAT-1 antibodies (1:500 in TTBS; Sigma-Aldrich) overnight at 4 °C. On the following day, the primary antibodies were removed, and the membranes were washed three times with TTBS to remove unconjugated antibodies. Following this, incubation was carried out with anti-rabbit secondary antibodies conjugated to peroxidase (1:5000 in TTBS; Sigma-Aldrich), for 2 h at room temperature. After incubation, the membrane was again washed three times with TTBS (10 min each), and the probe was detected using an ECL kit (Amersham).

Membranes were re-probed with mouse anti-β-tubulin antibody (T5201) (1:25,000 in TTBS, Sigma-Aldrich) for 1 h at room temperature, washed 3 times with TTBS, and incubated with a secondary anti-mouse antibody conjugated to peroxidase (1:5000 in TTBS; Sigma-Aldrich) for 45 min at room temperature, followed by three new TTBS washes. Immunostaining was detected with the ECL kit. The intensity of the bands was analyzed using ImageLab 5.2.1 software (Bio-Rad Laboratories Inc).

Calcium Imaging

Variations of free intracellular calcium ($[Ca^{2+}]i$) levels were evaluated in neuron-enriched cell cultures [34], following the application of KCl or ATP using a previously described method [35]. Briefly, cells in culture were loaded for 40 min with 5 µM fura-2/AM (Molecular Probes) and 0.02% Pluronic F-127 (Molecular Probes) in Krebs solution, in an incubator with 5% CO2 at 37 °C. After a 10-min post-loading period at room temperature in Krebs solution, to obtain complete hydrolysis of the probe, 15 mm coverslips with the cells were mounted in an RC-20 chamber on a P-5 platform (Warner Instruments, Hamden, CT) for rapid perfusion on the stage of an inverted fluorescence microscope (Eclipse Ti-U; Nikon). Cells were continuously perfused with Krebs solution (273 mOsm) and stimulated with 50 µM nicotine, 100 µM NMDA, 50 mM KCl (372 mOsm), or 100 µM ATP, with or without 50 µM MK-801 or 3 µM mecamylamine. The solutions were prepared immediately before the assays and were added to the cells by a fast-transition system (approximately 1 s). The variations in $[Ca^{2+}]i$ were evaluated at intervals of 500 ms, by quantifying the ratio of fluorescence emitted at 510 nm following alternate excitation at 340 and 380 nm, using a Lambda DG4 illumination system (Sutter Instrument, Novato, CA), a 20× objective, a 510 nm band-pass filter (Semrock, Rochester, NY) and an Evolve 16-bit cooled EMCCD camera (Photometrics, Tuscon, AZ). Acquired images were processed using MetaFluor software (Molecular Devices, Sunnyvale, CA). The time courses of the fura-2 fluorescence ratio of individual cells were analyzed to identify responding cells in each experiment based on a 30% increase threshold of the $[Ca^{2+}]i$ level induced by the stimulus.

Statistical Analysis

Data were processed using GraphPad Prism 8.4.3 (GraphPad Software, LLC) and expressed as % of the total or as mean ± standard error of the mean (SEM). For comparisons between pairs of measurements, Student's two-tailed unpaired t-test was performed. For the analysis of three or more experimental groups, a one-way or two-way ANOVA was used, followed by a Bonferroni posttest. Values were considered statistically significant when $p < 0.05$; "n" is equal to the number of animals per group in each experiment.

Results

To evaluate if a single exposure to nicotine alters GABA uptake levels of the chick retina at E12 when synapses are peaking, dissected retinas were acutely treated with basal (Hanks 4) or nicotine, before a [^{3}H]-GABA uptake assay. As previously established, control tissue shows drastically reduced [^{3}H]-GABA uptake in the absence of Na^+, at low temperature (4 ℃), and in the presence of NO-711 (50 µM), consistent with GAT-1-mediated transport [28, 29]. The addition of 50 µM nicotine reduced [^{3}H]-GABA uptake by approximately 45% compared to the control group, an effect not observed with the exposure to 1 µM or 10 µM nicotine (Fig. 1a). Therefore, 50 µM nicotine was chosen to carry out the subsequent experiments.

Additionally, we assessed the time required for nicotine's effect on GABA uptake by pre-incubation for 5, 10, 15, and 30 min before the addition of [^{3}H]-GABA (Fig. 1b). Most of the reduction in GABA uptake due to nicotine (50 µM) occurred between 5 and 10 min, with minor reduction at longer exposure times. Thirty min of exposure were standardized for the following experiments.

Our next step was to evaluate whether GAT-1 total protein levels at E12 chicken retinas are changed by exposure to nicotine or saline. As shown in Fig. 2a–b, no significant

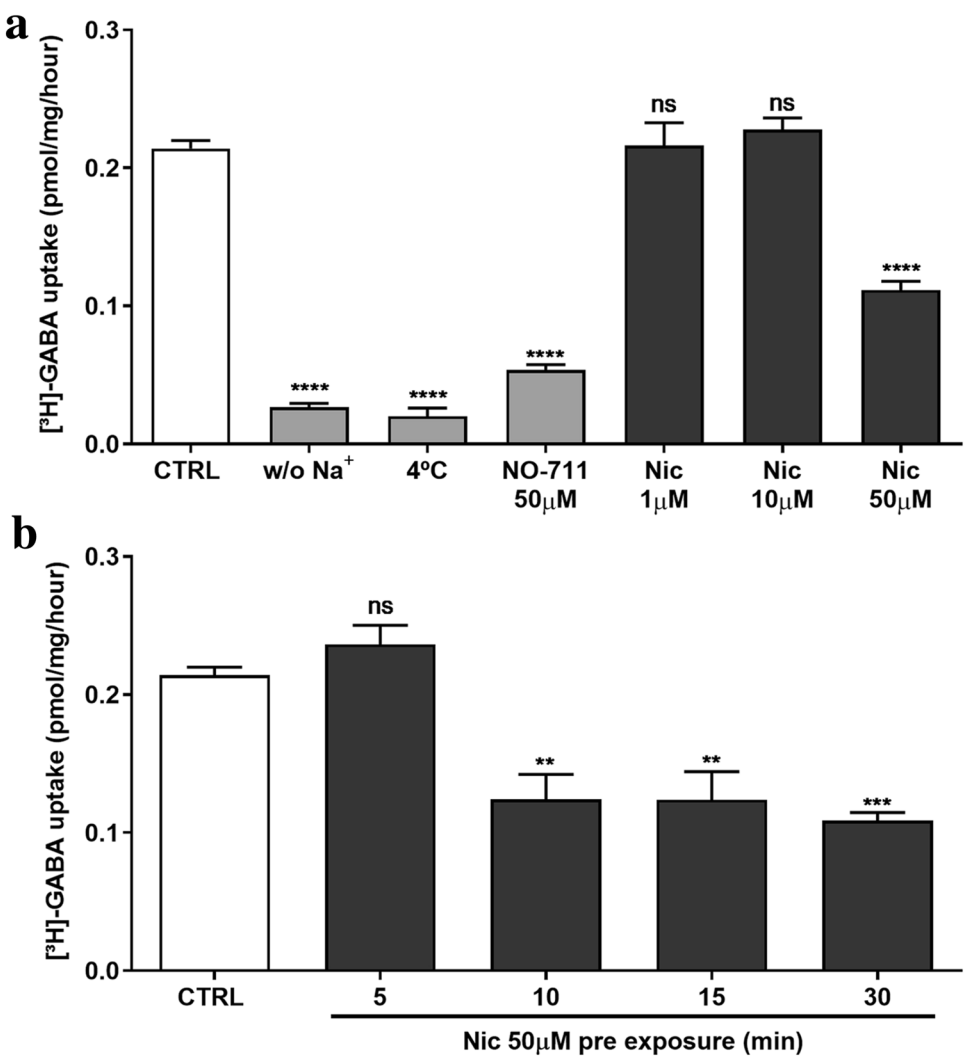

Fig. 1 Concentration and time-dependent modulation of retinal [^{3}H]-GABA transport by nicotine. **a** [^{3}H]-GABA uptake was prevented by the replacement of sodium by Tris in the incubation medium, at low temperature, or in the presence of GAT-1 inhibitor NO-711 50 µM. Furthermore, after a 30 min incubation period on E12 retinal tissue, nicotine 50 µM (but not 1 or 10 µM) reduced [^{3}H]-GABA uptake by approximately 45% (control = 0.214 ± 0.005; n = 5; w/o Na^+ = 0.026 ± 0.002; n = 3; 4ºC = 0.020 ± 0.006; n = 3; NO-711 = 0.053 ± 0.003; n = 3; nicotine 1 µM = 0.2160 ± 0.016; n = 3; nicotine 10 µM = 0.227 ± 0.008; n = 5; nicotine 50 µM = 0.111 ± 0.006 pmol/mg/h; n = 5). **b** A reduction in [.^{3}H]-GABA uptake is seen after 10 min in the presence of nicotine 50 µM (control = 0.214 ± 0.005; n = 5; nicotine 5' = 0.236 ± 0.013; n = 5; nicotine 10' = 0.124 ± 0.018; n = 6; nicotine 15' = 0.123 ± 0.020; n = 6; nicotine 30' = 0.111 ± 0.006 pmol/mg/h; n = 6). Data are represented as mean ± S.E.M. **p < 0.01; ***p < 0.001; ****p < 0.0001

difference was seen between control and nicotine-exposed retinas, suggesting that the decrease of [^{3}H]-GABA uptake in the presence of nicotine was related to the modulation of GAT-1 activity, and not to its expression or turnover. Furthermore, we also observed in Fig. 2c that [^{3}H]-GABA release is mainly mediated by GAT-1 since its blockade by selective GAT-1 inhibitor NO-711 (50 µM) prevented both basal and nicotine-mediated release. Since nicotine promotes excitatory amino acid release in other areas [36] and GABA release is activated by NMDA receptors in the embryonic retina [12, 28], we investigated whether nicotine affects [^{3}H]-GABA release in an NMDA receptor-dependent manner. In contrast with the uptake, pre-exposure to nicotine 50 µM more than doubled the amount of [^{3}H]-GABA released in 5 min compared to the control group (Fig. 3a). The addition of the NMDA receptor channel blocker MK-801 (50 µM) during the 5 min release pulse prevented the effect of nicotine. As a control, the addition of MK-801 did not affect basal levels of [^{3}H]-GABA release.

Both L- and D-aspartate induce [^{3}H]-GABA release by chick retinal cells through NMDA receptor activation [28]. We next considered the possibility that it affected [^{3}H]-GABA release indirectly, through a glutamatergic agonist. We evaluated if nicotine affects [^{3}H]-D-aspartate release in retinal cell cultures at a developmental stage equivalent to E12. As shown in Fig. 3b, nicotine (50 µM for 30 min before [^{3}H]-D-aspartate loading) increased [^{3}H]-D-aspartate release, corroborating the idea that nicotine's effect on GABA transport depends on stimulation of the glutamatergic system.

We then pursued the identification of a cellular pathway that could be associated with the nicotinic effect on GAT-1. Because NMDA receptor activation modulates GAT-1 activity [15, 32, 37] and GABA transporters might be regulated by phosphorylation [20–23], we hypothesized that an NMDA receptor-mediated calcium influx and PKC activation pathway could be related to nicotine's effects in [^{3}H]-GABA uptake and release. Thus, the [^{3}H]-GABA uptake assay in retinal explants was performed in the presence of 100 nM

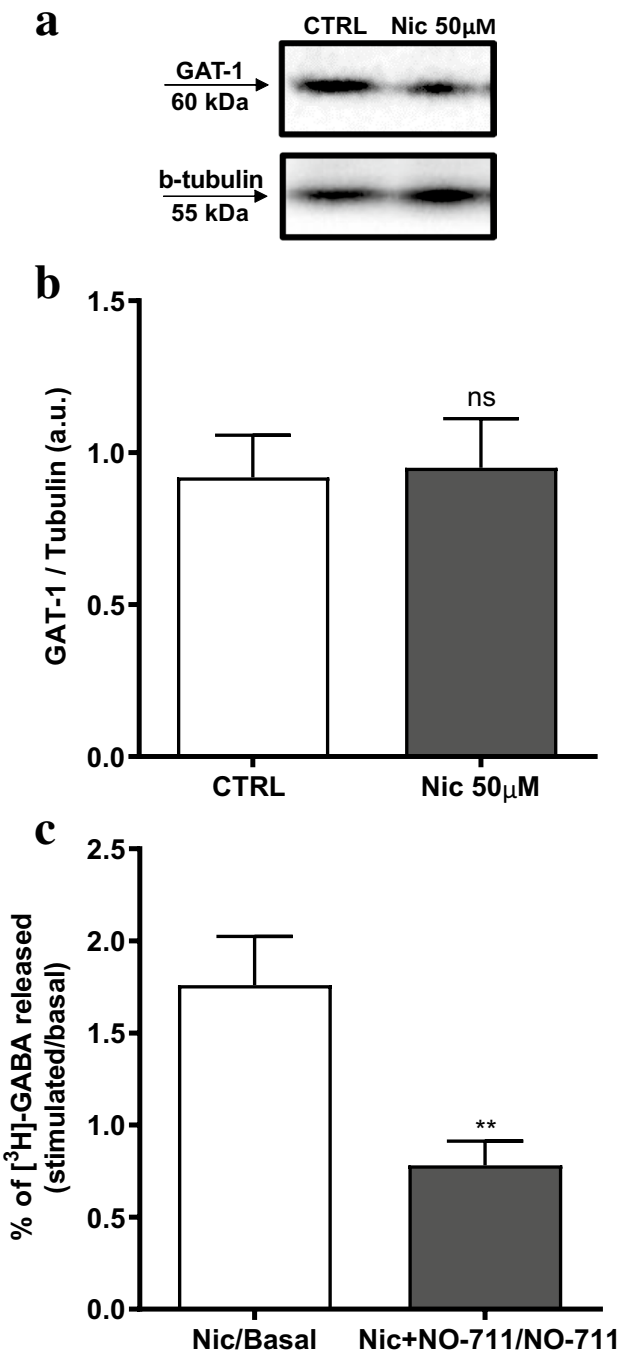

Fig. 2 Lack of nicotine modulation of GAT-1 protein density in the retina **a** Western Blot of 60 kDa GAT-1 transporters in E12 retina, after 30 min of incubation with basal or nicotine 50 µM. **b** Densitometry analysis demonstrating no difference in the GAT-1 protein levels (relative to β-tubulin) between the control and nicotine-treated group (control = 0.918 ± 0.139; n = 3; nicotine = 0.950 ± 0.161 a.u.; n = 3). Data are represented as mean arbitrary units (a.u.) ± S.E.M. **c** [^{3}H]-GABA release in both control and nicotine treated groups are prevented by NO-711 on E12 chicken retinas (Nic/Basal = 1.76% ± 0.26; n = 4; Nic + NO-711/NO-711 = 0.78% ± 0.13; n = 11; % of [^{3}H]-GABA released (stimulated/basal)). Data are represented as % of stimulated/basal (a.u.) ± S.E.M. **p < 0.01

Gö 6983, a pan-specific PKC inhibitor (Fig. 3c). Gö 6983 alone did not modify the basal [^{3}H]-GABA uptake; however, it abolished the inhibitory effect of nicotine. These results indicate that the effects of nicotine on GABA transport are dependent on excitatory amino acid release, activation of NMDA receptors, and activation of PKC.

As some nicotinic and NMDA receptors are highly permeable to Ca^{2+} ions, ratiometric calcium imaging was performed in mixed retinal neuro-glial cultures at E8C6 (from E8 retinas kept for six days in vitro). Retinal cells can be segregated in terms of Ca^{2+} signaling because neurons respond to 50 mM KCl while glial cells respond to ATP [34, 35], as shown in Fig. 4a-b. Nicotine only activated retinal cells that were also stimulated by 50 mM KCl (Fig. 4c), a feature associated with Tuj-1 or $Beta_{III}$ tubulin antibody labeling in our cultures [35], hallmarks for a neuronal phenotype. Approximately 13% of retinal neurons responded to nicotine (156 out of 1223 cells). On the other hand, nicotine did not affect cells that were selectively activated by 1 mM ATP (627 cells), which were shown before to be labeled by 2M6 or GFAP antibodies, characteristics of glial cells (Fig. 4c) [29, 35]. Calcium shifts induced by brief perfusion of 50 µM nicotine were completely blocked by 3 µM mecamylamine (Fig. 4d), indicating that the response was mediated by nicotinic receptor channels. Likewise, NMDA only activated cells that were also stimulated by KCl (presumably neurons), and neither of these responses was blocked by mecamylamine (Fig. 4d–e). Approximately 62% of the retinal neurons (161 out of 259 cells) responded to 100 µM NMDA (in the absence of Mg^{2+}), while there was no response in cells that responded to ATP (92 glial cells). As expected, the NMDA response was blocked by 50 µM MK-801 (Fig. 4g). However, the responses to nicotine were also completely blocked in the presence of 50 µM MK-801 (Fig. 4f). Data are quantified in Fig. 4h and i.

Discussion

In the present work, we investigated how acute exposure to nicotine acts on GABA transport in chicken embryos' retinas and retinal neuron-glia cells in culture. Nicotine decreased GABA uptake and increased GABA release, apparently through PKC-dependent modulation of GAT-1 activity. As demonstrated by release experiments and single-cell imaging in culture, nicotine activates at least two mechanisms that might lead to PKC activation. Single-cell imaging revealed that nicotine activates nicotinic receptors (blocked by mecamylamine), eliciting calcium transients in a population of retinal neurons, which might directly promote PKC activation. As expected, NMDA receptors also induced Ca^{2+} influx, blocked by MK-801. Since, nicotine promotes

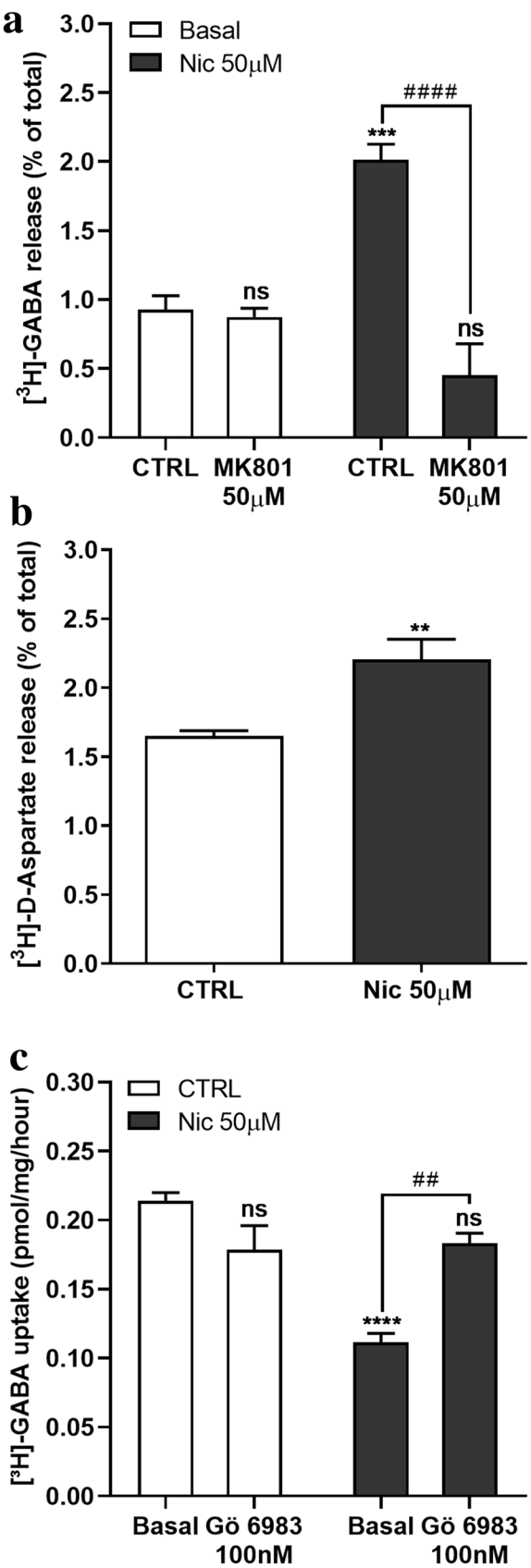

Fig. 3 Nicotine increases [^{3}H]-GABA release via NMDA receptor activation followed by PKC mobilization. **a** [^{3}H]-GABA release is two-fold increased 30 min after nicotine 50 μM exposure on E12 chicken retinas, and this effect is prevented by MK-801, a NMDA selective antagonist (Control = 0.92% ± 0.10; n = 8; MK-801 = 0.87% ± 0.10; n = 3; Nicotine = 2.01% ± 0.11; n = 3; Nicotine + MK-801 = 0.68% ± 0.10 [^{3}H]-GABA release (% of total), n = 3). **b** Nicotine induces an enhancement of [^{3}H]-D-aspartate release in cultured retinas, at an equivalent age when compared to ex vivo tissue experiments (Control = 1.64% ± 0.04; n = 5; Nicotine = 2.20% ± 0.14 [^{3}H]-GABA release (% of total), n = 3) **c** Addition of Gö-6983 100 nM in ex vivo retinal explants, 5 min prior to release assay, reverts nicotine's effect of [^{3}H]-GABA release increment to basal values (Control = 0.214 ± 0.005; n = 5; Go 6983 = 0.178 ± 0.017; n = 6; Nicotine = 0.111 ± 0.006; n = 5; Nicotine + Go 6983 = 0.143 ± 0.011 [^{3}H]-GABA uptake (pmol/mg/h); n = 4). Data are represented as mean ± S.E.M. **p < 0.01; ***p < 0.001; ****p < 0.0001 from control and $^{\#\#}$p < 0.01; $^{\#\#\#}$p < 0.0001 from basal nicotine

excitatory amino acid release, this might lead to NMDA receptor-induced calcium influx and further PKC activation.

GATs are bidirectional electrogenic symporter carriers that depend on sodium/chloride gradient for promoting GABA transport [38]. In chicken retinas, it is possible to observe mainly three out of the four types of GABA transporters: GAT-1, GAT-3, and GAT-4 [38]. GAT-3 and GAT-4 are mostly expressed in glial cells, while GAT-1 is in neurons, as also observed in other CNS areas [39]. The replacement of sodium ions by Tris, or the reduction to lower temperatures (4ºC), abolished [^{3}H]-GABA uptake in embryonic avian retina cells, NO-711, a selective GAT-1 inhibitor [28] also blocked both uptake and release, demonstrating the relevance that GAT-1 has in the GABA uptake of chicken embryos' retina over other types of GABA transporters.

In addition to these findings, a reduction in approximately 45% of [^{3}H]-GABA uptake was observed when retinas were incubated with 50 μM nicotine for 30 min, which was not observed at lower concentrations such as 1 or 10 μM. Interestingly, this effect was shown to occur after 10 min of the addition of nicotine, but not after 5 min. Clearly, brief exposure to 50 μM nicotine raises intracellular calcium in a subpopulation of retinal neurons, but longer exposures could have additional effects, possibly associated with nicotinic receptor desensitization [40]. The increase of GABA and dopamine release by nicotine have already been shown in even higher concentrations of nicotine in the retina [4]. Our results on the effects on GABA uptake suggest that signaling cascades are involved by activation of other pathways or neurotransmitters release, as we are going to discuss.

We have previously demonstrated that excitatory amino acids, such as aspartate induces [^{3}H]-GABA release via NMDA receptors activation in the avian retina cells [12]. In addition, in that same study, we demonstrated that short exposure to caffeine (20 min) in embryonic retina slices of chicks was able to promote phosphorylation of NMDA receptors, increasing the activity of these receptors, and

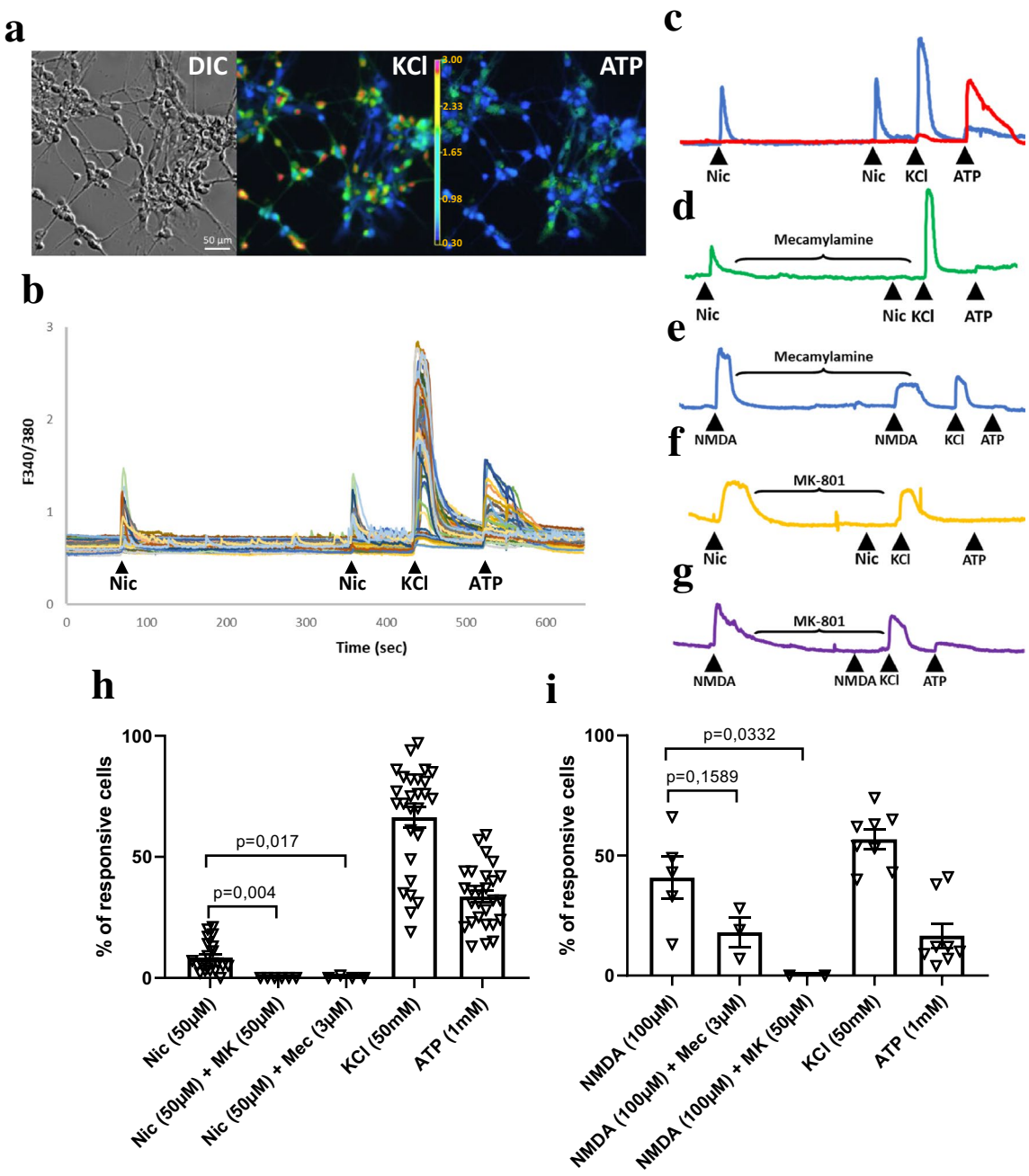

Fig. 4 Nicotine-induced calcium shifts on cultured chick retina. **a** Retinal cells in culture are shown in a bright field (left panel), KCl fluorescence (middle panel), and ATP fluorescence (right panel). **b** A typical graph is shown, each line represents a single selected cell, for the responses of nicotine, KCl, and ATP. **c** Nicotine activated retinal cells that were stimulated by 50 mM KCl, the typical response of neurons; on the other hand, nicotine did not affect cells that were selectively activated by 1 mM ATP, red line. **d** Calcium shifts induced by nicotine were blocked by 3 µM mecamylamine. **e** NMDA activated cells that were also stimulated by KCl, and both responses were not blocked by mecamylamine. **f** NMDA response was blocked by 50 µM MK-801. **g** The responses of nicotine were blocked in the presence of 50 µM MK-801. Data are quantified in **(h)** and **(i)**

evoking [^{3}H]-GABA release more efficiently. Therefore, intracellular pathways are activated by calcium influx mediated by NMDA receptors which are efficiently regulated [12]. It is widely known that in the striatum [41], hippocampus [42], thalamocortical synapses at the prefrontal cortex [43], ventral tegmental area, and accumbens [44] or other areas involved with reward circuitry [45], nicotine has been shown to induce glutamate release. We have shown that nicotine can also induce endogenous excitatory amino acid release in the avian retinal circuitry, therefore, activation of

glutamatergic receptors is a possible mechanism controlling [^{3}H]-GABA release invoked by nicotine. As demonstrated, nicotine increases [^{3}H]-GABA release in the avian retina, and pre-incubation of retina cells with MK-801, an NMDA receptor antagonist, prevented the nicotine effect on [^{3}H]-GABA release. Nonetheless, it is important to note that MK-801 has been reported to inhibit α7 nAChR in humans in lower concentrations than used in this work, showing eightfold less potent inhibition than mecamylamine [46]; this might need further verification in our model.

Since GAT-1 protein levels were not changed, transport activity may have been modulated by nicotine through post-translational mechanisms. GATs are regulated by protein kinases such as tyrosine kinase, which phosphorylate tyrosine residues that increase GAT-1 levels in the membrane [21], or PKC, which phosphorylates serine residues of the same transporter, constitutively withdrawing it from the plasma membrane [22, 23]. Those sites are mainly found on the intracellular loops 1 and 2, which can regulate the transporter activity and expression [47, 48]. Thus, these transporters are constantly being recycled to/from the cytoplasm or moving laterally through it [38]. Indeed, an increase in PKC activity was shown to reduce GABA uptake in neurons [22]. Moreover, in previous studies from our group, it has been stated that PKA also plays a role in modulating GAT-1 transporter in chicken retinas by increasing [^{3}H]-GABA release in a mechanism dependent on the reversal of this transporter activity [12, 15]. Also, from another study of our lab, it was possible to evaluate that this type of post-translation modification is not exclusively of GABA transporters, but also EAATs can have their activity modulated by internal phosphorylation by PKA, which reduced [^{3}H]-D-aspartate uptake [39].

We observed that the selective PKC inhibitor nearly abolished the effect of nicotine in chick retinal cells, so we suggest that the entry of calcium ions through NMDA receptors might activate the PKC pathway and regulate GAT-1 activity in retinas exposed to nicotine. Indeed, in the chick retina, calcium imaging reveals that neurons are selectively stimulated by 50 mM KCl while glia cells are activated by ATP [29, 34]. When nicotine was added to this same system, only cells that were activated to KCl responded to nicotine, but not the ones responsive to ATP. This further implied the neuronal localization of nAChR. Conversely, other immunocytochemical studies showed that nicotinic α4 and β2 subunits are located on the dendritic arbors of the retinal ganglion cells (RGCs) in the adult rabbit [49] related to the direction selectivity of the retina. The effect of nicotine was inhibited

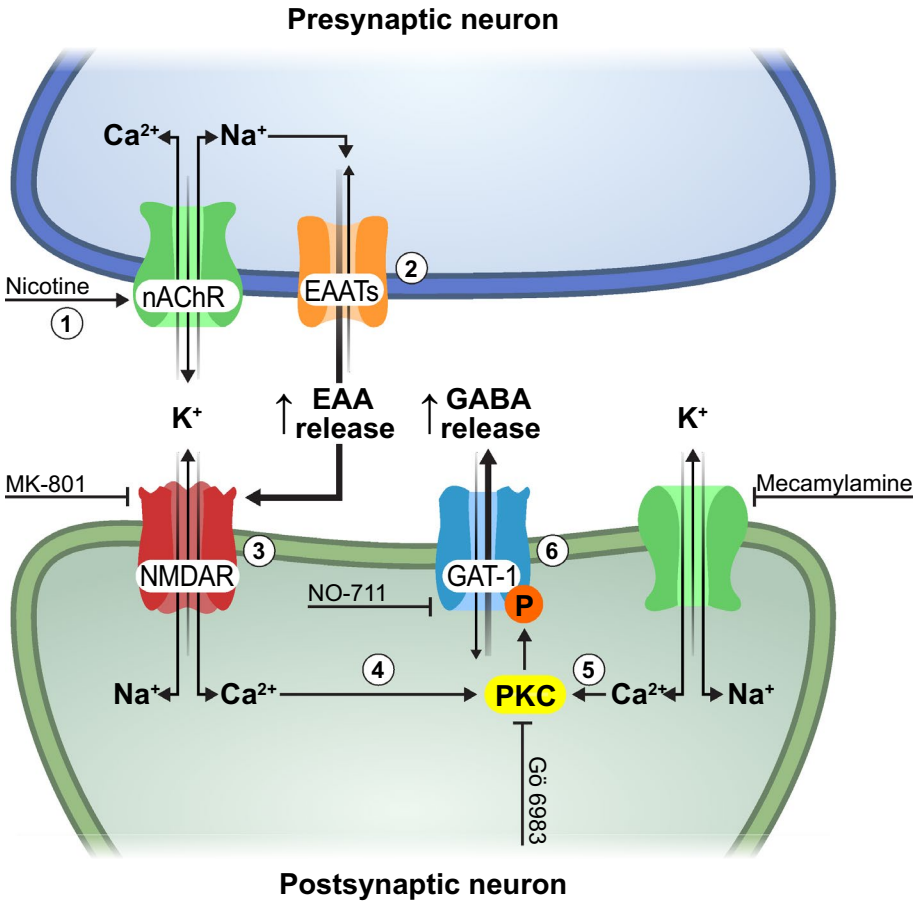

Fig. 5 Proposed model of nicotine-mediated increase of both GABA and EAA release in the chick retina. We suggest that the nicotine effect on GABA and EAA release occurs as follows: **1** nicotine binds to presynaptic nAChR, promoting sodium influx. **2** Sodium leads to EAA release via its high-affinity transporters (EAATs). **3** Increased EAA in the synaptic cleft will enhance NMDAR activation on the postsynaptic neuron, resulting in sodium and calcium influx. **4** Calcium activates the PKC pathway. **5** Another possible pathway is through postsynaptic nAChR activation, increasing calcium influx and PKC activity. **6** PKC modulates GAT-1 activity by phosphorylation. As a result, GABA release is enhanced by a mechanism of transport reversion. Both NMDAR, PKC, GAT-1, and nAChR roles are impaired by MK-801, Gö 6983, NO-711, and mecamylamine respectively

by mecamylamine, a non-competitive antagonist of nicotinic acetylcholine receptors that functions as a channel blocker.

Therefore, our results point to the following two non-exclusive hypotheses that possibly explain the effect of nicotine on the modulation of GABA transport. In the first hypothesis, nicotine, a nAChR agonist, modulates indirectly the GAT-1 transporter by targeting first nicotinic receptors on presynaptic neurons, prompting sodium influx which will promote EAA release, mainly glutamate, via its high-affinity transporters (EAATs) [50]. This increase of glutamate release in the synaptic cleft will increase NMDAR activation on the postsynaptic neuron—as well as possibly AMPA receptors—which will prompt this channel activity [28]. The NMDAR is known to be permeable to both sodium and calcium, the latter can also take part as a second messenger via the PKC pathway (Fig. 5). On the other hand, the second hypothesis relies on the direct effect of nicotine. Figure 4 shows that mecamylamine (a non-selective nicotinic receptor antagonist), and MK-801 (NMDA receptor antagonist) completely blocked nicotine-mediated calcium influx. However, it is important to note that MK-801 has been reported to inhibit α7 nAChR in humans in lower concentrations than used in this work, showing eight fold less potent inhibition than mecamylamine [46]; this might need further verification in our model. Therefore, it is possible that MK-801 blocked nicotinic receptors highly permeable to calcium, such as α7 receptors, preventing ion influx and inhibiting a potential direct effect of nicotine in activating PKC (Fig. 5).

Independently of the mechanism, for both hypotheses, the increase of calcium influx will promote PKC activation and the possible phosphorylation of a plethora of targets, such as GAT-1, which can promote an increase of this transporter activity and opening [38]. Finally, this modulation via PKC of the GAT-1 will promote an increase in GABA release by reversion of transport. Furthermore, both NMDAR, PKC, and GAT-1 roles are also implied by the blockade of the increase in GABA release respectively by MK-801 (NMDAR blocker) and Gö 6983 (PKC inhibitor) (Fig. 5), whereas GAT-1 role on GABA transport on chick retina has already been reported [12].

Retinal nicotinic and glutamatergic receptors are highly coupled during a period of development in which the retina is rapidly maturing to induce calcium shifts and modify critical periods with retinal waves. Indeed, several synaptic and extra-synaptic factors regulate retinal waves. Cholinergic waves are generated parallel to glutamatergic events, as shown with knockout vesicular glutamate transporter type 1, demonstrating that the two wave-generating circuits are linked [51]. Our data reinforce that nicotinic and glutamatergic receptors run in parallel to increase calcium shifts in the embryonic avian retina. Regarding the diseased retina, interactions between the cholinergic, GABAergic, and glutamatergic systems are present in neurodegenerative disorders [52]. Indeed, it has been shown that an α7-nicotinic cholinergic receptor agonist, PNU-282987, modulates GABAA and NMDA receptors. Authors using whole-cell patch-clamp recordings from retinal ganglion cells from control and glaucomatous retinal slices showed that activation of α7-nicotinic receptor increases the amplitude of currents elicited by GABA and reduces the amplitude of currents elicited by NMDA [53]. Therefore, retinal nicotinic receptors might be targets of agents that control the diseased retina.

Thus, as evidenced, several factors and neurotransmitters work together for the correct formation of the CNS, including, the fine maintenance of the balance between inhibitory and excitatory stimuli. Nicotine, a widely used abuse drug, can modify both EAA and GABA transport during this critical period of development of the avian retina. Although further studies are needed, the elucidation of the possible pathways and interactions with different neurotransmitters systems is essential to develop possible countermeasures and to understand the effects that this drug has on our CNS.

Acknowledgements RAMR and NGC are supported by CNPq and FAPERJ (grant numbers E-26/202.668/2018, E-26/010.002215/2019, E-26/010.001652/2019, 307698/2019-0, 426342/2018-6 and 312157/2016-9), and INCT-INNT (National Institute for Translational Neuroscience). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001.

Author Contributions ACS: conceptualization, methodology, formal analysis, investigation, writing–original draft preparation MHT: methodology, formal analysis, investigation, writing. LACG, VPPBM, MCPR, ACOD: conceptualization, methodology, formal analysis, investigation, writing. GF: methodology, formal analysis, investigation. ACM, NGC, RAMR: conceptualization, resources, writing–reviewing and editing. ALMV, RCCK: conceptualization, resources, writing–reviewing and editing.

Funding ACS and VPPBM have received funding from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Finance Code 001. RCCK, MHT, NGC, RAMR have received funding from Conselho Nacional de Desenvolvimento Científico e Tecnológico. RCCK, MCPR, NGC, RAMR have received funding from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro.

Data Availability Not appliable.

Declarations

Conflict of interest The authors declare that there are no competing financial or non-financial interests.

Ethical Approval The research was carried out following the guidelines approved by the Animal Care and Use Committee of Federal University of Rio de Janeiro protocol 038/19 and follow the guidelines of the Brazilian Society for Neuroscience and Behavior (SBNeC) and Brazilian Law No. 11794/ 2008.

References

1. Israel MR, Morgan M, Tay B, Deuis JR (2018) Toxins as tools: fingerprinting neuronal pharmacology. Neurosci Lett 679:4–14. https://doi.org/10.1016/j.neulet.2018.02.001
2. Ávila-Ruiz T, Carranza V, Gustavo LL et al (2014) Chronic administration of nicotine enhances NMDA-activated currents in the prefrontal cortex and core part of the nucleus accumbens of rats. Synapse 68:248–256. https://doi.org/10.1002/SYN.21726
3. Fowler CD, Turner JR, Imad Damaj M (2020) Molecular mechanisms associated with nicotine pharmacology and dependence. Handb Exp Pharmacol 258:373–393. https://doi.org/10.1007/164_2019_252
4. Neal MJ, Cunningham JR, Matthews KL (2001) Activation of nicotinic receptors on GABAergic amacrine cells in the rabbit retina indirectly stimulates dopamine release. Vis Neurosci 18:55–64. https://doi.org/10.1017/S0952523801181058
5. Wonnacott S (1997) Presynaptic nicotinic ACh receptors. Trends Neurosci 20:92–98. https://doi.org/10.1016/S0166-2236(96)10073-4
6. Reid MS, Fox L, Ho LB, Berger SP (2000) Nicotine stimulation of extracellular glutamate levels in the nucleus accumbens: neuropharmacological characterization. Synapse 35:129–136. https://doi.org/10.1002/(SICI)1098-2396(200002)35:2%3c129::AID-SYN5%3e3.0.CO;2-D
7. Sears SMS, Hewett SJ (2021) Influence of glutamate and GABA transport on brain excitatory/inhibitory balance. Exp Biol Med 246:1069–1083. https://doi.org/10.1177/1535370221989263
8. Popova E (2015) GABAergic neurotransmission and retinal ganglion cell function. J Comp Physiol A 201(3):261–283. https://doi.org/10.1007/S00359-015-0981-Z
9. Wang L, Shen X, Wu Y, Zhang D (2016) Coffee and caffeine consumption and depression: a meta-analysis of observational studies. Aust N Z J Psychiatry 50:228–242. https://doi.org/10.1177/0004867415603131
10. da Calaza K, C, Gardino PF, (2010) Neurochemical phenotype and birthdating of specific cell populations in the chick retina. An Acad Bras Cienc 82:595–608. https://doi.org/10.1590/S0001-37652010000300007
11. Thangaraj G, Greif A, Bachmann G, Layer PG (2012) Intricate paths of cells and networks becoming "Cholinergic" in the embryonic chicken retina. J Comp Neurol 520:3181–3193. https://doi.org/10.1002/CNE.23083
12. Ferreira DDPDP, Stutz B, de Mello FGG et al (2014) Caffeine potentiates the release of GABA mediated by NMDA receptor activation: Involvement of A1 adenosine receptors. Neuroscience 281:208–215. https://doi.org/10.1016/j.neuroscience.2014.09.060
13. Sorimachi M, Rhee JS, Shimura M, Akaike N (1997) Mechanisms of GABA- and glycine-induced increases of cytosolic Ca2+ concentrations in chick embryo ciliary ganglion cells. J Neurochem 69:797–805. https://doi.org/10.1046/J.1471-4159.1997.69020797.X
14. Govindpani K, Calvo-Flores Guzman B, Vinnakota C et al (2017) Towards a better understanding of GABAergic remodeling in Alzheimer's disease. Int J Mol Sci. https://doi.org/10.3390/ijms18081813
15. Borges-Martins VPP, Ferreira DDP, Souto AC et al (2019) Caffeine regulates GABA transport via A1R blockade and cAMP signaling. Neurochem Int. https://doi.org/10.1016/j.neuint.2019.104550
16. Eskandari S, Willford SL, Anderson CM (2017) Revised ion/substrate coupling stoichiometry of GABA transporters. Adv Neurobiol 16:85–116. https://doi.org/10.1007/978-3-319-55769-4_5
17. Bernath S, Zigmond MJ (1988) Characterization of [^{3}H] GABA release from striatal slices: evidence for a calcium-independent process via the GABA uptake system. Neuroscience 27:563–570. https://doi.org/10.1016/0306-4522(88)90289-8
18. Calaza KC, Gardino PF, de Mello FG (2006) Transporter mediated GABA release in the retina: role of excitatory amino acids and dopamine. Neurochem Int 49:769–777. https://doi.org/10.1016/J.NEUINT.2006.07.003
19. do Nascimento JL, Ventura AL, Paes de Carvalho R, (1998) Veratridine- and glutamate-induced release of [3H]-GABA from cultured chick retina cells: possible involvement of a GAT-1-like subtype of GABA transporter. Brain Res 798:217–222
20. Whitworth TL, Quick MW (2001) Substrate-induced regulation of γ-aminobutyric acid transporter trafficking requires tyrosine phosphorylation. J Biol Chem 276:42932–42937. https://doi.org/10.1074/jbc.M107638200
21. Law RM, Stafford A, Quick MW (2000) Functional regulation of γ-aminobutyric acid transporters by direct tyrosine phosphorylation. J Biol Chem 275:23986–23991. https://doi.org/10.1074/jbc.M910283199
22. Beckman ML, Bernstein EM, Quick MW (1999) Multiple G protein-coupled receptors initiate protein kinase C redistribution of GABA transporters in hippocampal neurons. J Neurosci. https://doi.org/10.1523/JNEUROSCI.19-11-J0006.1999
23. Wang D, Quick MW (2005) Trafficking of the plasma membrane gamma-aminobutyric acid transporter GAT1. Size and rates of an acutely recycling pool. J Biol Chem 280:18703–18709. https://doi.org/10.1074/jbc.M500381200
24. Yang XL (2004) Characterization of receptors for glutamate and GABA in retinal neurons. Prog Neurobiol 73:127–150. https://doi.org/10.1016/J.PNEUROBIO.2004.04.002
25. Hamassaki-Britto DE, Brzozowska-Prechtl A, Karten HJ et al (1991) GABA-like immunoreactive cells containing nicotinic acetylcholine receptors in the chick retina. J Comp Neurol 313:394–408. https://doi.org/10.1002/CNE.903130213
26. Dmitrieva NA, Lindstrom JM, Keyser KT (2001) The relationship between GABA-containing cells and the cholinergic circuitry in the rabbit retina. Vis Neurosci 18:93–100. https://doi.org/10.1017/S0952523801181083
27. Masland RH (2012) The neuronal organization of the retina. Neuron 76:266–280. https://doi.org/10.1016/j.neuron.2012.10.002
28. Kubrusly RCC, de Mello MCF, de Mello FG (1998) Aspartate as a selective NMDA receptor agonist in cultured cells from the avian retina. Neurochem Int 32:47–52. https://doi.org/10.1016/S0197-0186(97)00051-X
29. Kubrusly RCC, Günter A, Sampaio L et al (2018) Neuro-glial cannabinoid receptors modulate signaling in the embryonic avian retina. Neurochem Int 112:27–37. https://doi.org/10.1016/j.neuint.2017.10.016
30. Loureiro-dos-Santos NE, Prado MAM, De Melo Reis RA et al (2002) Regulation of vesicular acetylcholine transporter by the activation of excitatory amino acid receptors in the avian retina. Cell Mol Neurobiol 22:727–740. https://doi.org/10.1023/A:1021809124814
31. Hamburger V, Hamilton HL (1992) A series of normal stages in the development of the chick embryo. Dev Dyn 195:231–272. https://doi.org/10.1002/aja.1001950404
32. de Freitas APAP, Ferreira DDPDDP, Fernandes A et al (2016) Caffeine alters glutamate–aspartate transporter function and expression in rat retina. Neuroscience 337:285–294. https://doi.org/10.1016/j.neuroscience.2016.09.028
33. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3
34. de Melo Reis RA, Freitas HR, de Mello FG (2020) Cell calcium imaging as a reliable method to study neuron-glial circuits. Front

Neurosci 14:975. https://doi.org/10.3389/FNINS.2020.569361/BIBTEX
35. Freitas HR, Ferraz G, Ferreira GC et al (2016) Glutathione-induced calcium shifts in chick retinal glial cells. PLoS One 11:e0153677. https://doi.org/10.1371/JOURNAL.PONE.0153677
36. Castillo-Rolón D, Ramírez-Sánchez E, Arenas-López G et al (2021) Nicotine increases spontaneous glutamate release in the rostromedial tegmental nucleus. Front Neurosci 14:1323. https://doi.org/10.3389/FNINS.2020.604583/BIBTEX
37. Martins RS, de Freitas IG, Sathler MF et al (2018) Beta-adrenergic receptor activation increases GABA uptake in adolescent mice frontal cortex: modulation by cannabinoid receptor agonist WIN55,212–2. Neurochem Int 120:182–190. https://doi.org/10.1016/j.neuint.2018.08.011
38. Scimemi A (2014) Plasticity of GABA transporters: an unconventional route to shape inhibitory synaptic transmission. Front Cell Neurosci 8:128. https://doi.org/10.3389/fncel.2014.00128
39. Sathler MF, Stutz B, Martins RS et al (2016) Single exposure to cocaine impairs aspartate uptake in the pre-frontal cortex via dopamine D1-receptor dependent mechanisms. Neuroscience 329:326–336. https://doi.org/10.1016/j.neuroscience.2016.05.022
40. Loring RH, Zigmond RE (1990) Pharmacological and biochemical properties of nicotinic receptors from chick retina. Eur J Neurosci 2:863–872. https://doi.org/10.1111/J.1460-9568.1990.TB00397.X
41. Ryu IS, Kim J, Seo SY et al (2017) Behavioral changes after nicotine challenge are associated with α7 nicotinic acetylcholine receptor-stimulated glutamate release in the rat dorsal striatum. Sci Rep 7:1–14. https://doi.org/10.1038/s41598-017-15161-7
42. Damborsky JC, Griffith WH, Winzer-Serhan UH (2015) Neonatal nicotine exposure increases excitatory synaptic transmission and attenuates nicotine-stimulated GABA release in the adult rat hippocampus. Neuropharmacology 88:187–198. https://doi.org/10.1016/j.neuropharm.2014.06.010
43. Lambe EK, Picciotto MR, Aghajanian GK (2003) Nicotine induces glutamate release from thalamocortical terminals in prefrontal cortex. Neuropsychopharmacology 28(2):216–225. https://doi.org/10.1038/sj.npp.1300032
44. Lenoir M, Kiyatkin EA (2013) Intravenous nicotine injection induces rapid, experience-dependent sensitization of glutamate release in the ventral tegmental area and nucleus accumbens. J Neurochem 127:541. https://doi.org/10.1111/JNC.12450
45. Alasmari F, Al-Rejaie SS, AlSharari SD, Sari Y (2016) Targeting glutamate homeostasis for potential treatment of nicotine dependence. Brain Res Bull 121:1. https://doi.org/10.1016/J.BRAINRESBULL.2015.11.010
46. Briggs CA, McKenna DG (1996) Effect of MK-801 at the human α7 nicotinic acetylcholine receptor. Neuropharmacology 35:407–414. https://doi.org/10.1016/0028-3908(96)00006-8
47. Bennett ER, Kanner BI (1997) The membrane topology of GAT-1, a (Na+ + Cl-)-coupled γ-aminobutyric acid transporter from rat brain. J Biol Chem 272:1203–1210. https://doi.org/10.1074/jbc.272.2.1203
48. Zafar S, Jabeen I (2018) Structure, function, and modulation of γ-aminobutyric acid transporter 1 (GAT1) in neurological disorders: a pharmacoinformatic prospective. Front Chem 6:397. https://doi.org/10.3389/fchem.2018.00397
49. Lee JS, Kim HJ, Ahn CH, Jeon CJ (2017) Expression of nicotinic acetylcholine receptor α4 and β2 subunits on direction-selective retinal ganglion cells in the rabbit. Acta Histochem Cytochem 50:29–37. https://doi.org/10.1267/AHC.16024
50. Stutz B, Yamasaki EN, De Mello MCF, De Mello FG (2011) Exchange of extracellular l-glutamate by intracellular d-aspartate: the main mechanism of d-aspartate release in the avian retina. Neurochem Int 58:767–775. https://doi.org/10.1016/J.NEUINT.2011.03.001
51. Blankenship AG, Ford KJ, Johnson J et al (2009) Synaptic and extrasynaptic factors governing glutamatergic retinal waves. Neuron 62:230–241. https://doi.org/10.1016/J.NEURON.2009.03.015
52. Stone TW (2021) Relationships and interactions between ionotropic glutamate receptors and nicotinic receptors in the CNS. Neuroscience 468:321–365. https://doi.org/10.1016/J.NEUROSCIENCE.2021.06.007
53. Zhou X, Zong Y, Zhang R et al (2017) Differential modulation of GABAA and NMDA receptors by an α7-nicotinic acetylcholine receptor agonist in chronic glaucoma. Front Mol Neurosci 10:422. https://doi.org/10.3389/FNMOL.2017.00422/BIBTEX