NEUROSCIENCE
RESEARCH ARTICLE
M. Groleau et al. / Neuroscience 386 (2018) 79–90

Stimulation of Acetylcholine Release and Pharmacological Potentiation of Cholinergic Transmission Affect Cholinergic Receptor Expression Differently during Visual Conditioning

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Abstract—Cholinergic stimulation coupled with visual conditioning enhances the visual acuity and cortical responses in the primary visual cortex. To determine which cholinergic receptors are involved in these processes, qRT-PCR was used. Two modes of cholinergic enhancement were tested: a phasic increase of acetylcholine release by an electrical stimulation of the basal forebrain cholinergic nucleus projecting to the visual cortex, or a tonic pharmacological potentiation of the cholinergic transmission by the acetylcholine esterase inhibitor, donepezil. A daily visual exposure to sine-wave gratings (training) was paired with the cholinergic enhancement, up to 14 days. qRT-PCR was performed at rest, 10 min, one week or two weeks of visual/cholinergic training with samples of the visual and somatosensory cortices, and the BF for determining mRNA expression of muscarinic receptor subtypes (m1, m2, m3, m4, m5), nicotinic receptor subunits (α3, α4, α7, β2, β4), and NMDA receptors, GAD65 and ChAT, as indexes of cortical plasticity. A Kruskal–Wallis test showed a modulation of the expression in the visual cortex of m2, m3, m4, m5, α7, β4, NMDA and GAD65, but only β4 within the basal forebrain and none of these mRNA within the somatosensory cortex. The two modes of cholinergic enhancement induced different effects on mRNA expression, related to the number of visual conditioning sessions and receptor specificity. This study suggests that the combination of cholinergic enhancement and visual conditioning is specific to the visual cortex and varies between phasic or tonic manipulation of acetylcholine levels.

Key words: cholinergic system, acetylcholinesterase inhibitors, muscarinic receptors, nicotinic receptors, sensory training, visual cortex.

INTRODUCTION

Acetylcholine (ACh) modulates the functioning of the visual cortex (V1) through muscarinic (mAChRs) and nicotinic (nAChRs) cholinergic receptors with regards to attention, learning, and memory processes. It particularly sustains the tuning of visual processing and building long-term cortical responses in the visual cortex of rodents and primates (Groleau et al., 2015; Coppola et al., 2016; Herrero et al., 2017). This contributes to the improvement of visual capacities such as visual acuity and contrast sensitivity, and behavioral guidance (Kang et al., 2014b; Gritton et al., 2016). Moreover, the consistent electrical or pharmacological activation of the cholinergic projections to V1 during visual training in the rat increases (i) the amplitude of visual evoked potentials in response to the stimulus (Kang and Vaucher, 2009; Bhattacharyya et al., 2013; Kang et al., 2015; Chamoun et al., 2016), (ii) the behavioral visual discrimination (Aggelopoulos et al., 2011; Soma et al., 2013a; Kang et al., 2014a), (iii) the recovery of visual function after an optic nerve crush (Chamoun et al., 2017). This effect is obtained whenever the activation is provided by phasic – electrical – (Kang et al., 2015) or tonic stimulation, using the acetylcholinesterase inhibitor donepezil (DPZ), that builds up extracellular ACh (Chamoun et al., 2016), which is administered during the visual training.

The cholinergic receptor subtypes, located on different neuronal elements in the visual cortex, mediate distinct and sometimes opposing roles in the modulation of V1 neurons (Thiele, 2013; Groleau et al., 2015; Kang et al., 2015). The metabotropic mAChRs m1 and m2 are the subtypes most abundantly found in the visual cortex of the rodent (Levey et al., 1991). M1 has been shown involved in the facilitation of feed-forward processing, reducing the thalamic suppression within V1

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Abbreviations: ACh, acetylcholine; ChAT, choline acetyltransferase; DPZ, donepezil.

https://doi.org/10.1016/j.neuroscience.2018.06.023
0306-4522/

(Levey et al., 1991; Gil et al., 1997; Wess, 2003; Krnjevic, 2004; Thiele, 2013). Moreover, it has been associated with long-term potentiation effects (Wess, 2004; Origlia et al., 2006), in coordination with NMDA receptors (Kirkwood et al., 1999; Kang and Vaucher, 2009) in V1. M2, mostly present on the presynaptic cell, has been shown to increase cortical activity during visual processing (Kang et al., 2015), possibly through the disinhibition of pyramidal cells by GABAergic cell inhibition. The GABAergic neurons are highly associated with cortical plasticity mechanisms (Iwai et al., 2003; Jiao et al., 2006), with the GABA-synthesizing enzyme GAD65 expression being linked to plasticity of the visual cortex (Hensch et al., 1998). Although less present in the rodent's visual cortex, the m3 subtype has been demonstrated to control V1 sensitivity (Groleau et al., 2014) and plasticity (Origlia et al., 2006). On the other hand, the ionotropic pentameric nAChRs have been associated with attentional processes (reviewed by Metherate, 2004). The $\alpha4\beta2$ receptors, the main nAChRs in the visual cortex, enhance thalamo-cortical transmission, likely through its location on presynaptic terminals of the thalamic fibers (Lavine et al., 1997), and is distributed throughout all of the cortical layers (reviewed by Lucas-Meunier et al., 2003; Aztiria et al., 2004). The $\alpha7$ receptor is also abundant in the cortex (reviewed by Metherate, 2004), and seems to be involved in visual acuity (Origlia et al., 2012) and more generally, in neuronal plasticity (Nordman and Kabbani, 2012; Sadahiro et al., 2016).

In an attempt to isolate the involvement of specific AChRs in visual processing and plasticity, we previously demonstrated that 1 mg/kg DPZ administered during a 2-week visual training produced an upregulation of the cholinergic receptors m3, m4, m5 and $\alpha7$ at the end of the experiment, but not of the other subunits, including m1, m2 and $\alpha4\beta2$ (Chamoun et al., 2016). We have also pharmacologically shown that M2 and the nAChRs contributed to enhance the cortical response to visual stimulation. This was done by blocking this response using antagonists injected within V1 during a 1-week visual training (Kang et al., 2015). The expression for these receptors at this time point was not measured. In the present study, we examined the expression of the different AChRs in the cholinergic enhancement of visual training at four time points. This was performed in the visual cortex and somatosensory cortex (taken as a control), as well as in the basal forebrain (BF) which contains the cellular body of cholinergic neurons projecting to V1. Moreover, we wanted to see whether plasticity makers—NMDA receptors and GAD65—were also regulated during the training. In addition, the expression of the ACh-synthesizing enzyme, choline acetyltransferase (ChAT), was examined as an index of the ACh yield. We examined the effect of the two different paradigms of cholinergic enhancement previously used: electrical (Kang et al., 2014a) and DPZ (Chamoun et al., 2016) potentiation of the cholinergic neurons, paired to a daily (up to 14 days) exposure of the rat to a 3D visual stimulation. The electrical stimulation was located in the horizontal limb of the diagonal band of Broca (HDB), the BF nucleus that projects to V1. The identification of the cholinergic receptor subtypes expressed at the 4 different time points of the stimulation (at rest, once, 7, 14 days) was performed by the quantitative real-time polymerase chain reaction technique (qRT-PCR). The results show that consistent coupling of visual training with cholinergic enhancement regulates the expression of mAChRs and nAChRs mainly within V1, and is specific to the mode of cholinergic stimulation.

EXPERIMENTAL PROCEDURES

Animal preparation

All procedures were carried out in accordance with the guidelines of the Canadian Council for the Protection of Animals, and were accepted by the Ethics Committee of the Université de Montréal. A total of 75 adult male Long Evans rats (200–225 g) were separated into 12 groups (Fig. 1). The animals were maintained in a 12-h light/dark normal daylight cycle with *ad libitum* access to food and water.

Electrode implantation for HDB stimulation

Animals from the groups that received the HDB electrical stimulation were unilaterally implanted with a tungsten-stimulating electrode. Animals were anesthetized with isoflurane (induction 5%, maintain 3%), and placed in a stereotaxic apparatus. Core body temperature was maintained at 37 °C using a thermostatically controlled heating pad (FHC, Bowdoinham, ME, USA). A hole in the skull was created with a dental drill to access HDB (mm from Bregma: AP −0.3, ML +2.0, DV −9.0), and two stainless steel screws were installed adjacent to the insertion site to help secure the implanted electrode with dental cement. The skin was then sutured, and local anesthesia (2%, Astra Zeneca, Mississauga, Canada) was topically administered. Carprofen (Rimadyl 5 mg/kg) was injected s.c. (anti-inflammation agent) after the surgery and injected again 24 h later.

Visual exposure procedure

Visual exposure consisted of a sine wave grating (0.12 cycle/degree, orientation 30°, phase converting at 1 Hz) displayed (Vpixx software, v 2.79, VPixx technologies Inc., Saint-Bruno, QC, Canada) on 3 monitors constituting a 3D environment at 21 cm of the animal's head (one computer in front of the rat and two lateral monitors [LG], luminance 37 cd/m^2). The rats were awake and restrained in a hammock. To avoid any effect of stress, all of the animals were habituated with 10-min restraint in the hammock for 3 consecutive days prior to being stimulated or sham-stimulated. The training sessions consisted of 10-min visual exposures (while the rat was restrained) presented once a day at the same time for 1, 7 or 14 days.

HDB electrical stimulation

The electrical stimulation of the HDB was performed during the 10-min visual stimulation session

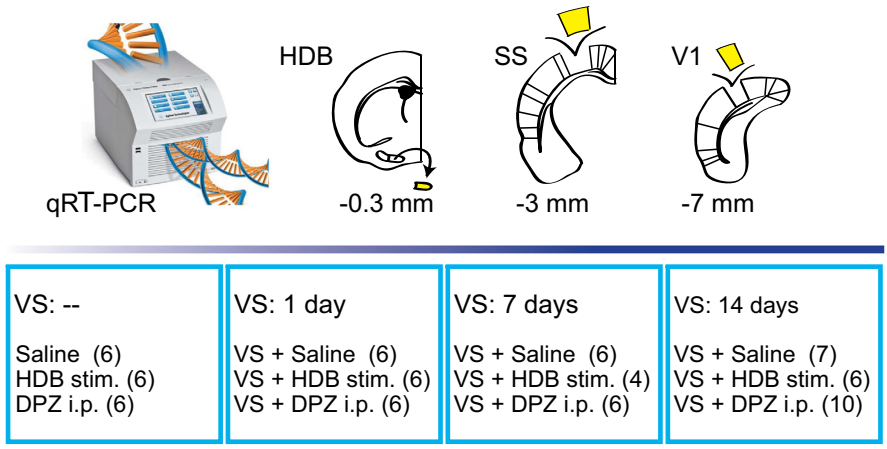

Fig. 1. Experimental groups. mRNA expression measured by qRT-PCR – represented by the machine – was performed on three regions sampled in twelve experimental groups. The regions, i.e. basal forebrain (BF), somatosensory cortex (SS) and primary visual cortex (V1) are represented by coronal sections at −0.3, −3 and −7 mm from Bregma (upper panel). Three treatments were administered, i.e. saline, electrical stimulation of the basal forebrain (HDB stim), or donepezil (DPZ) during daily 10-min visual stimulation (VS). mRNA expression was performed at rest without VS or after 1, 7, 14 days of VS (blue boxes). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Kang et al., 2014a, 2015) (train of pulses 100 Hz, 0.5 ms, 50 μA, 1 s on/1 s off, Pulsemaster A300, WPI, Sarasota, FL, USA).

Donepezil treatment

DPZ (Sigma–Aldrich, St-Louis, MO, USA) was used at a dose of 1 mg/kg in a sterile 0.9% NaCl solution (Soma et al., 2013b; Chamoun et al., 2016). It was administered i.p. 30 min daily before the visual exposure or the sacrifice, to reach the maximum effect of the drug (Soma et al., 2013b). Animals that did not receive DPZ injections received a vehicle injection (saline).

Measurement of cholinergic receptors expression by quantitative qRT-PCR

qRT-PCR has been described previously (Pouliot et al., 2012; Chamoun et al., 2016). Briefly, rats were heavily anesthetized with isoflurane and sacrificed by decapitation immediately after the last training session. 1 mm^3 of the visual cortex (Bregma AP −7, ML ±2 mm), somatosensory cortex (Bregma AP −3, ML ±2 mm) and BF (AP −0.3 mm ML +2 mm, DV −9 mm) were dissected within 1 min on a cold plate and put in RNA later stabilization reagent (QIAGEN, Valencia, CA, USA). The primer pairs (mAChR [m1–m5], nAChR [α3, α4, α7, β2, β4] plus NMDA, GAD65 and ChAT) were designed by Vector NTI software, based on the NCBI (National Centre for Biotechnology Information) BLAST database (Chamoun et al., 2016) (Table A.1). A commercial kit (RNeasy®Lipid Tissue, QIAGEN, Valencia, CA, USA) was used to extract total RNA from the cortices. Rat 18S ribosomal RNA was used as a housekeeping control and cDNA was synthesized from 250 ng of total ARN in a total reaction volume of 20 μl (QunatiTect Rev. Transcription Kit, Qiagen Toronto, On, Canada). SYBR Green-based qRT-PCR using Mx3000P Q-PCR System (Stratagene, La Jolla, CA, USA) was performed. Target genes and reference genes were amplified and duplicated in the same run. The relative quantification of gene expression was determined using the MxProTM Q-PCR software version 3.00 (Stratagene, La Jolla, CA, USA). The quantification was analyzed by the $2-\Delta\Delta Ct$ method, and normalized by respective 18S values (Livak and Schmittgen, 2001; Pouliot et al., 2012; Chamoun et al., 2016).

Statistical analysis

Statistical analyses were calculated using SPSS 17.0 (SPSS Inc., Chicago, IL, USA). The comparison of the receptor expressions was assessed using the non-parametric Kruskal–Wallis test with a significance level of $p < 0.05$. The statistical analysis was not corrected for multiple comparisons since this correction could lead to robust under-evaluation of changes for a large number of comparisons (Rothman, 1990), as required for the statistical analysis of an array of mRNA. All of the data and statistical results are presented instead. The data sets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

RESULTS

Change of expression in the AChRs was virtually exclusive to the visual cortex (Fig. 2, Tables B.1–B.3). There was no change in the somatosensory cortex and only β4 subunit was upregulated in the BF. The expression of each mRNA examined did not differ from basal levels measured in saline conditions without any visual stimulation at any time point in the control condition (without cholinergic potentiation). The global pattern of changes elicited by the HDB electrical

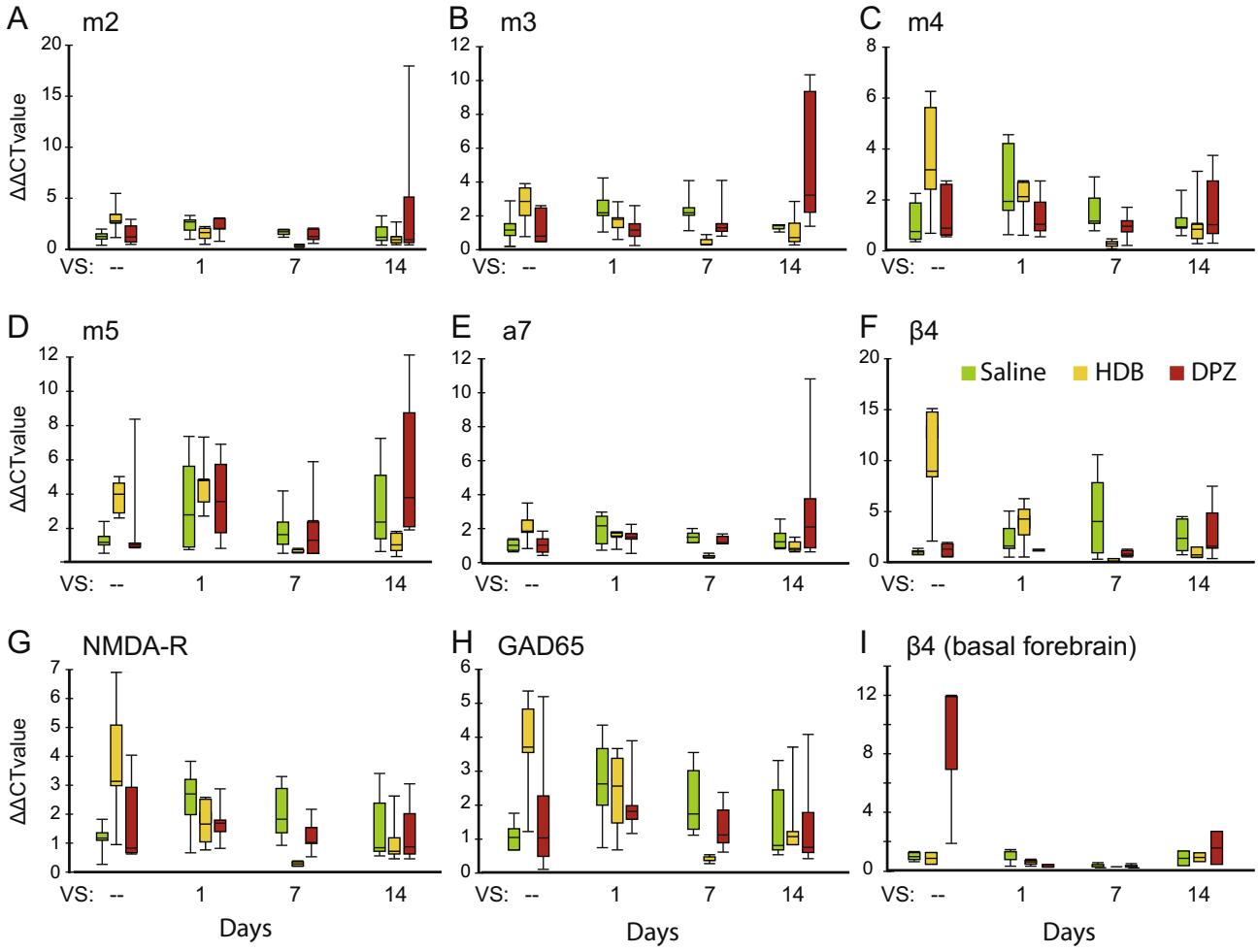

Fig. 2. Representation of the significant changes in the mRNA expression at 0, 1, 7, 14 days of visual stimulation combined with saline or Donepezil injection and HDB electrical stimulation in the primary visual cortex and somatosensory cortex (I). (A–H): in the visual cortex, the main change in mRNA expression occurred in (A) m2, (B) m3, (C) m4, (D) m5 mAChRs, as well as (E) α7 and (F) β4 nAChR subunits and plasticity markers (G) NMDA receptor and (H) GAD65 were increased by the electrical stimulation of the HDB, and reduced by the combination of the electrical stimulation and visual training. m3 was increased at the end of the visual training coupled with DPZ administration. (I) In the somatosensory cortex, only the β4 nAChR subunit was increased by the electrical stimulation of the HDB, but not during the subsequent combination of the electrical stimulation and visual training. Receptor expression was demonstrated as the ratio receptor mRNA/18S mRNA ΔΔCT value (median and ICR); VS: –, no visual stimulation; VS: 1, 7, 14 days: 10-min visual stimulation, daily, for 1, 7 or 14 days; Saline: Saline Treatment; HDB: HDB electrical stimulation; DPZ: 1 mg donepezil daily i.p. administration.

stimulation was different compared to DPZ stimulation. The electrical stimulation elicited a strong increase in receptor expression when it was administered without visual training, but the receptors were expressed at basal levels a consecutive HDB stimulation coupled with visual stimulation. The DPZ stimulation elicited a late increase in receptor expression. These two global pictures were independent of the receptor subtypes.

The muscarinic receptors expression changes in the primary visual cortex according to the stimulation type and the timing

There is a significant difference depending on the stimulation type, and timing of sampling for some muscarinic receptors tested in the visual cortex (Fig. 2A–D, Table B.1). The muscarinic receptors m2 ($H(11) = 21.160$, $p = 0.032$), m3 ($H(11) = 25.394$, $p = 0.008$), m4 ($H(11) = 22.127$, $p = 0.023$) and m5 ($H(11) = 23.025$, $p = 0.018$) subtypes, but not m1 ($H(11) = 18.299$, $p = 0.075$) show a significant difference in expression in function of the timing. Electrical stimulation without visual stimulation increases the level of the m4 mRNA expressions, but these levels returns to baseline when combined with visual training and were even decreased below basal level at 7 days of training. No changes were seen for the m3 concerning the electrical stimulation, however, the m3 mRNA expression was increased after 14 days of training combined with DPZ administration. No significant changes were seen within the somatosensory cortex (Table B.2) for any of the mAChRs: m1 ($H(11) = 7.282$, $p = 0.776$), m2 ($H(11) = 11.112$, $p = 0.434$), m3 ($H(11) = 14.776$, $p = 0.193$), m4 ($H(11) = 8.480$, $p = 0.670$), and m5 ($H(11) = 11.956$, $p = 0.367$). Within the BF (Table B.3), no change is observed for the mAChR for

any of the subtypes: m1 ($H(11) = 17.890$, $p = 0.084$), m2 ($H(11) = 10.486$, $p = 0.487$), m3 ($H(11) = 13.728$, $p = 0.248$), m4 ($H(11) = 9.458$, $p = 0.580$) or m5 ($H(11) = 14.800$, $p = 0.192$).

The nicotinic receptor expression changes in the primary visual cortex according to the stimulation type and the timing for the α7 and β4 subunits

There was an altered nicotinic receptor mRNA expression for the α7 ($H(11) = 24.938$, $p = 0.009$) and β4 nAChR subunit ($H(11) = 31.699$, $p = 0.001$) within V1 (Fig. 2E–F, Table B.1), where the electrical stimulation of the HDB without any visual exposure increases the receptor expression. No significant changes were seen for α3 ($H(11) = 12.000$, $p = 0.364$), α4 ($H(11) = 17.203$, $p = 0.102$), and β2 ($H(11) = 18.452$, $p = 0.072$) subtypes over time. In the somatosensory cortex, no changes were seen for α3 subunit ($H(11) = 17.615$, $p = 0.091$), α4 ($H(11) = 10.319$, $p = 0.502$), α7 ($H(11) = 8.348$, $p = 0.682$), β2 ($H(11) = 11.680$, $p = 0.388$), and β4 ($H(11) = 10.825$, $p = 0.458$) subunits (Table B.2). Within the BF, an alteration was also observed with the β4 subunit ($H(11) = 20.463$, $p = 0.039$), where the DPZ-treated group presents a high expression of the receptor with no visual exposure, which is not observed following repetitive visual exposure (Fig. 2I, Table B.3). No changes were seen for α3 ($H(11) = 14.826$, $p = 0.191$), α4 ($H(11) = 14.060$, $p = 0.230$), α7 ($H(11) = 9.286$, $p = 0.596$), and β2 ($H(11) = 8.340$, $p = 0.683$) subtypes.

The plasticity marker expression changes in the primary visual cortex according to the stimulation type and the timing, but not the synthetic enzyme of ACh

In V1 (Fig. 2 G-H, Table B.1), the expression of some plasticity markers was changed. NMDAR ($H(11) = 25.900$, $p = 0.007$) and GAD65 ($H(11) = 27.329$, $p = 0.004$), but not the ChAT ($H(11) = 17.497$, $p = 0.094$). For the NMDAR and GAD 65, the expression of their mRNA is increased by electrical stimulation of HDB without visual exposure, and reduced upon combination with visual exposure that peaks at 7 days of exposure. Within the somatosensory cortex (Table B.2), there was no change in the plasticity marker expression for the (NMDAR ($H(11) = 14.692$, $p = 0.197$), GAD65 ($H(11) = 10.669$, $p = 0.471$) and ChAT ($H(11) = 9.840$, $p = 0.545$). Finally, in the BF (Table B.3), there was no change observed for the NMDAR ($H(11) = 9.529$, $p = 0.573$), GAD65 ($H(11) = 6.935$, $p = 0.804$) or the ChAT ($H(11) = 9.007$, $p = 0.621$).

DISCUSSION

In this study, we administered 1 mg/kg DPZ or HDB electrical stimulation at rest or combined with daily visual exposure for 1, 7 or 14 days. At each of these time points, the pattern of cholinergic receptors mRNA expression was assessed by qRT-PCR in V1, the somatosensory cortex, and the BF. The main change in the expression of cholinergic receptors and plasticity markers occurred in V1. This confirms the effect of combining cholinergic enhancement with visual exposure is predominant in V1 for both tonic and phasic conditions. While the same long-lasting, physiological effect was seen for HDB electrical stimulation (Kang et al., 2014a) and DPZ injection (Chamoun et al., 2016), the receptor expression pattern over time differs between HDB electrical stimulation and DPZ administration.

The main changes are seen in the visual cortex, while other regions investigated are scarcely affected

The robust result of this study underlines that changes in receptors mRNA are observed mostly in V1, whereas discrete changes were seen in the other regions investigated. The somatosensory region was used as a control for cortical activation as it is not related to vision, but receives cholinergic projections from other BF subpopulations. It did not show any alteration of the cholinergic receptors or plasticity markers following the visual exposure combined with electrical stimulation of the HDB, or DPZ treatment. This confirms that changes are mostly elicited in the visual cortex, which is activated by both the cholinergic and thalamo-cortical projections, and represents the first cortical structure for visual processing. This finding does not exclude that changes could be seen in additional cortical areas that were not investigated.

The BF was examined because this area contains cholinergic neurons, as well as GABAergic and glutamatergic neurons (review by (Brashear et al., 1986; Gritti et al., 1997)), projecting to the cortex. Thus, changes occurring at the terminals of presynaptic basalo-cortical fibers could be detected within the BF (as the mRNA is located within the cell nucleus, and only the proteins migrate to the axon terminals). The expression of β4 subunit was altered in this structure, though not the m2 which is supposed to be located on the presynaptic cholinergic fibers. The expression of the plasticity markers was also not affected. The β4 subunit was increased with one acute administration of DPZ without visual exposure. The increase in β4 observed in the cholinergic nucleus could have been elicited by the use of the receptor at a presynaptic level in the cortex, or a use of receptors at the surface of the perikarya of cholinoceptive BF neurons. The precise cellular location of this receptor subtype has not yet been identified, so both alternatives are possible. The BF interneurons established a complex crossed innervation (Zaborszky and Duque, 2000), therefore this change in β4 expression could indicate a strong activation of the BF by the visual training or the attention load related to it. The α3β4 is also located at a postsynaptic level, so this receptor might be activated by a cholinergic activation of cholinoceptive BF neurons. It was surprising that the m2 mRNA was not increased in the BF, since this receptor is known as the principal presynaptic muscarinic receptor in the cortex, modulating the cortical ACh release. Moreover, m2 has been pharmacologically demonstrated to participate in the enhancement effect of electrical HDB stimulation coupled with visual exposure (Kang et al., 2015). It is

possible that the change in this receptor was not detected because only a subset of the BF neurons project to V1 (although data from the whole BF were collected for experimental reasons).

Changes of expression in the visual cortex

The main change in mRNA expression occurred in the visual cortex with m2–m5 mAChRs and α7 and β4 nAChR subunits. Plasticity markers were increased by the electrical stimulation of the HDB at rest and reduced after 7 days of the combination of electrical stimulation with visual training. This confirms previous pharmacological results showing m2–m4 receptor involvement in the enhancement of the electrophysiological responses by HDB stimulation coupled to visual stimulation (Kang et al., 2015). m3 and m5 were increased at the end of the visual training when coupled with DPZ administration. Thus, intra-cortical cells adapted the expression of these receptors in response to the increased cholinergic transmission, as well as visual experience.

The β4 subunit, usually associated with α3 to form the α3β4 nAChR, was upregulated by an electrical stimulation, followed by a down-regulation along with HDB electrical stimulation and visual training. This receptor is identified as an excitatory receptor, mostly present on the pyramidal cells (Lucas-Meunier et al., 2009). The α7 receptor mRNA variation in our paradigm is not surprising, given that α7 is involved in the cholinergic enhancement processes, and more generally in plasticity mechanisms (Sadahiro et al., 2016). The inhibitory m2 subtype is largely present on GABAergic neurons (reviewed by (Groleau et al., 2015)) or presynaptic basalo-cortical fibers, and it can work as a suppressor of GABA release (Salgado et al., 2007; Nunez et al., 2012). The variation of this subtype indicates a modulation in the functions of the inhibitory system. The m4, even if it has a low prevalence in the visual cortex (Flynn et al., 1995; Zhang et al., 2002), is an inhibitory receptor present on GABAergic cells (Volpicelli and Levey, 2004). Its activation could thus produce the disinhibition of the pyramidal cells, as shown in the somatosensory cortex (Eggermann et al., 2014). Therefore, the regulation of all of these receptors indicates a strong excitation of the cortex elicited by the electrical stimulation of the HDB. However, the decrease in the expression of these receptors in function of timing and visual experience suggests an adaptation and a reduction in the effect of the electrical stimulation with time, or with glutamatergic thalamo-cortical excitation. Alternatively, other mediation pathways might be triggered by this first stimulation, such as Hebbian long-term potentiation (LTP) changes in the intracellular compartment. Such LTP-like changes have been evidenced in the visual cortex (Origlia et al., 2006) during HDB and visual stimulation (Origlia et al., 2006; Kang and Vaucher, 2009). Increased expression of NMDAR and GAD65 in the present study supports this idea. On the other hand, the expression of the m3 and m5 mAChR is upregulated at the end of the 2-week training when coupled with DPZ. This excitatory receptor might rather be involved in long-term or after-effects of this potent double stimulation. The m3 mAChR is present on GABAergic interneurons (Amar et al., 2010), and it appears to modulate the inhibitory drive. The m5 subtype is mainly found on endothelial cells (Elhusseiny and Hamel, 2000), and controls cortical perfusion and oxygenation. Therefore, it seems that the treatments performed may have influenced the regulation of blood flow. However, it was shown that cholinesterase inhibitors enhance neuronal efficiency rather than having a vascular effect (Silver et al., 2008; Ricciardi et al., 2013).

The m1 receptor mRNA stability in our paradigm is also surprising, given that m1 is widely found in V1. However, this is consistent with previous results showing that pharmacologically blocking M1 transmission during coupled visual/cholinergic stimulation had no impact on the potentiation of visually evoked-potential (Kang et al., 2015). Moreover, the stability of this subtype is also observed when the cholinergic system is activated in vitro (Cabadak et al., 2011). This could result from their specific low-rate turnover or high concentration in endogenous pools. The α4β2 receptors expression not detected in this study, which may indicate that the cell bodies expressing this receptor are located in other brain areas not targeted by our sampling. The α4β2 receptor is mainly located on GABAergic neurons (Lucas-Meunier et al., 2009) and on thalamo-cortical terminals. We did not examine the thalamus or other feedback cortical areas. In addition, change at the level of GABAergic perikarya might be too faint to be detectable by the sampling method. Therefore, the absence of the regulation of α4β2 does not necessarily indicate an absence of activity. The expression of ChAT mRNA is also not affected. Therefore, the synthesis of ACh seems to remain constant even in the case of cholinergic activation.

Comparison of the electrical and pharmacological activation of the cholinergic system

The electrical stimulation seems to elicit strong changes when displayed alone, but not with concomitant visual stimulation. On the other hand, DPZ administration seems to induce changes at the end of the experiment, suggesting long-term changes or a late restoration effect. The two modes evoked different subtypes of receptors.

The electrical stimulation of BF neurons directly induced the post-synaptic overexpression of the cholinergic receptors in V1, without visual stimulation. On the contrary, DPZ must be combined with visual processing in order to alter expression of the receptors. Therefore the electrical effect could be rapidly down-regulated, whereas DPZ induces sustained effects which are more constant, but do not required high turnover rates of receptors. These differential effects could be elicited by the levels of ACh release evoked by the 2 modes of stimulation: a short and intense transient release with electrical stimulation, and a supposedly minor release of ACh with spontaneous activation of the cholinergic fibers due to visual stimulation, but prolonged action due to DPZ. The rapid and strong electrical release of ACh in the cortex could induce a fast response at the level of the mRNA receptor

expression. Variations occurring later, at 1 or 2 weeks, could therefore correspond to involvement of the receptors in plasticity mechanisms, such as with the m3. It is known that ACh has a dose-dependent effect on the neocortex, eliciting either excitation or depression of neuronal activity (Oldford and Castroalamancos, 2003). Moreover, it has been shown that high levels of ACh could favor afferent thalamo-cortical input to the visual cortex, sustaining the encoding of sensory information while low levels of ACh would favor strong cortico-cortical feedback and cortical consolidation (Hasselmo and McGaughy, 2004). Therefore, electrical vs pharmacological stimulation of the cholinergic fibers might set the circuit dynamics differently. Our study supports this proposition by showing involvement of distinct receptors in the two stimulation paradigms, although the gross long-lasting physiological effect observed with these two means of cholinergic potentiation is a similar enhancement to the effect observed during visually evoked potentials and behaviorally-assessed visual acuity (Kang et al., 2014a; Chamoun et al., 2016).

In addition, the different pattern of mRNA expression during DPZ systemic administration might be due to the involvement of other brain structures or neuromodulatory systems that might influence the mRNA expression within V1. For example, the dorsolateral geniculate nucleus is sensitive to ACh (McCormick, 1992), and its function and output to the cortex might have been affected by the AChEI. Also, the interaction between the cholinergic system and other modulatory systems is complex (Shimegi et al., 2016). More specifically, the serotonin and norepinephrine systems have diffuse projections to V1 and interact with the cholinergic system. Both the locus coeruleus and raphe dorsalis, which contain norepinephrine and serotonin cell bodies, are innervated by cholinergic fibers (Shute and Lewis, 1967), and AChEI might have also influenced this cholinergic activity. In turn, both serotonin and norepinephrine can affect the glutamatergic or GABAergic signaling in the cortex (Hasselmo and Bower, 1992). Moreover, it has been suggested that serotonin can inhibit the release of ACh in the cortex (Maura et al., 1989). However, since the ChAT was not affected in our samples, even if an indirect effect of the serotoninergic or noradrenergic system exists, the impact of the AChEI seems to mainly target the cholinergic receptors in the visual cortex.

Since the overall physiological and behavioral enhancement induced by two weeks of combined visual exposure and HDB stimulation (Kang et al., 2014a) or DPZ (Chamoun et al., 2016) is similar, the difference between the differential expressions in the mRNA of these cholinergic receptors and plasticity markers does not seem to play a critical role at the integrated functional level. It is therefore possible to assume that the cortical circuit involved in visual information integration compensates for punctual discrete variations in the mRNA of cholinergic receptors and plasticity markers.

In conclusion, we showed that the consistent coupling of visual training with cholinergic enhancements regulates the expression of mAChRs and nAChRs specifically within V1. The electrical stimulation seems more potent in terms of mRNA changes for m2–m5, α7 and β4, and the visual exposure coupled to DPZ induces late m3 mRNA regulation. Since the cholinergic receptors are usually associated with long-term changes related to plasticity processes, these alterations could sustain perceptual learning as seen in previous studies.

ACKNOWLEDGMENTS

We are profoundly grateful to Frédéric Huppé-Gourgues for his help with the statistical analyses. We would like to thank the Centre for Interdisciplinary Research in Rehabilitation of Greater Montreal for their resource support.

AUTHOR CONTRIBUTIONS

M.G. and E.V. designed the experiments and interpreted the results. M.G. and M.C. performed the experiments, M. G. analyzed the data. M.G. and E.V. wrote the paper. All authors approved the final manuscript.

FUNDING

This work was supported by the Canadian Institutes for Health Research (CIHR); Grant number: MOP-111003 (EV). Natural Sciences and Engineering Research Council of Canada (NSERC); Grant number: 238835-2011 (E.V.). M.G. received financial support from the School of Optometry and FRSQ Vision Research Network.

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APPENDIX A.

See Table A.1, B.1–B.3

Table A1. Primer list

18S	Forward	5′-TCAACTTTCGATGGTAGTCGCCGT-3′
	Reverse	5′-TCCTTGGATGTGGTAGCCGTTTCT-3′
m1	Forward	5′-AGCAGCTCAGAGAGGTCACAGGCA-3′
	Reverse	5′-GGGCCTCTTGACTGCATTTGGGGA-3′
m2	Forward	5′-CAAGACCCAGTATCTCCGAGTCTG-3′
	Reverse	5′-CGACGACCCAACTAGTTCTACAGT-3′
m3	Forward	5′-ACAGAAGCGGAGGCAGAAAACTTT-3′
	Reverse	5′-CTTGAAGGACAGTAGAGTAGC-3′
m4	Forward	5′-AAGGAGAAGAAGGCCAAGACTCTG-3′
	Reverse	5′-GCGAGCAATGCTGGCAAACTTTCG-3′
m5	Forward	5′-TGTAGCAGCTACCCCTCTTCAGAG-3′
	Reverse	5′-AGCAGCAGCTGGAGACAGAAAGTA-3′
α3	Forward	5′-ATTGCCGAAAACATGAAAGC-3′
	Reverse	5′-CCAGGATGAAAACCCAGAGA-3′
α4	Forward	5′-GACCACCTCAAGGCAGAAGA-3′
	Reverse	5′-CCCAGAAGGCAGACAATGAT-3′
α7	Forward	5′-TATCACCACCATGACCCTGA-3′
	Reverse	5′-CAGAAACCATGCACACCAGT-3′
β2	Forward	5′-TGCGAAGTGAGGATGATGAC-3′
	Reverse	5′-ACGGTCCCAAAGACACAGAC-3′
β4	Forward	5′-AGCGATGACCGAGATCAAAG-3′
	Reverse	5′-ACAAACACGAACACCCACAG-3′
NMDA	Forward	5′-CTGGCCT(G/C)AGTGACAAGAAGTTCC-3′
	Reverse	5′-CAGATGAAGGTGATGAGGCTGAGG-3′
GAD65	Forward	5′-TCTTTTCTCCTGGTGGTGCC-3′
	Reverse	5′-CCCCAAGCAGCATCCACAT-3′
ChAT	Forward	5′-AAAAGGCTCCCCAAAAGATG-3′
	Reverse	5′-TTCAGCCAGTATTCAGAGACCC-3′

Table B1. Expression of the cholinergic receptors in the primary visual cortex V1

Receptor	Visual treatment	VS: –	VS: 1 day	VS: 7 days	VS: 14 days
Muscarinic receptor subtype					
m1	Saline	0.91 (0.61–2.00)	1.71 (0.86–2.96)	1.67 (1.46–2.03)	1.72 (0.95–2.39)
	HDB	2.26 (0.92–5.65)	1.52 (0.37–1.82)	0.49 (0.39–0.53)	1.14 (0.80–3.48)
	DPZ	1.06 (0.63–2.37)	1.91 (0.37–2.19)	1.24 (0.76–2.86)	1.75 (0.72–4.51)
m2	Saline	1.17 (0.30–1.91)	2.64 (0.94–3.28)	1.65 (1.13–1.91)	1.16 (0.36–3.29)
	HDB	2.67 (1.03–5.43)	1.57 (0.43–2.14)	0.31 (0.13–0.37)	0.91 (0.43–2.74)
	DPZ	1.18 (0.39–2.82)	1.96 (0.76–3.01)	1.18 (0.52–2.00)	0.89 (0.35–18.4)
m3	Saline	1.14 (0.23–2.87)	2.15 (1.05–4.220	2.17 (1.20–4.19)	1.41 (1.04–1.45)
	HDB	2.87 (0.76–3.88)	1.79 (0.67–2.84)	0.28 (0.26–0.84)	0.74 (0.29–2.85)
	DPZ	1.01 (0.48–2.56)	1.13 (0.27–2.55)	1.28 (0.81–4.11)	3.20 (1.40–10.2)
m4	Saline	0.90 (0.49–2.31)	2.01 (0.76–4.53)	1.25 (0.87–2.94)	1.04 (0.69–2.48)
	HDB	3.18 (0.77–6.11)	2.16 (0.66–2.78)	0.30 (0.16–0.46)	0.93 (0.42–3.25)
	DPZ	1.04 (0.68–2.74)	1.15 (0.69–2.79)	1.04 (0.29–1.75)	1.11 (0.45–7.49)
m5	Saline	1.04 (0.38–2.13)	2.60 (0.55–7.31)	1.51 (0.39–4.11)	2.24 (0.48–7.21)

(*continued on next page*)

Table B1 (*continued*)

Receptor	Visual treatment	VS: –	VS: 1 day	VS: 7 days	VS: 14 days
	HDB	3.86 (2.44–4.87)	4.70 (2.52–7.16)	0.45 (0.34–0.63)	0.82 (0.20–1.61)
	DPZ	0.80 (0.48–8.25)	3.45 (0.63–6.86)	1.10 (0.31–5.60)	3.66 (1.76–12.2)
Nicotinic receptor subunit					
α3	Saline	0.95 (0.70–1.54)	2.66 (0.47–7.10)	1.37 (0.10–1.66)	1.25 (0.43–7.20)
	HDB	4.45 (0.53–5.07)	1.87 (0.42–3.36)	0.27 (0.24–3.23)	0.90 (0.21–1.78)
	DPZ	0.95 (0.57–4.08)	1.09 (0.31–5.13)	0.91 (0.58–2.52)	0.80 (0.27–25.69)
α4	Saline	0.92 (0.63–2.26)	3.10 (1.42–5.45)	1.65 (1.15–2.19)	1.04 (0.42–4.58)
	HDB	1.61 (0.58–4.14)	1.58 (1.21–2.69)	1.15 (0.37–5.12)	1.40 (0.90–4.49)
	DPZ	0.66 (0.53–3.14)	2.65 (1.41–18.71)	1.01 (0.77–3.16)	0.83 (0.31–11.5)
α7	Saline	1.03 (0.68–1.40)	2.12 (0.73–2.88)	1.48 (1.18–1.94)	1.20 (0.84–2.48)
	HDB	1.79 (0.86–3.37)	1.69 (0.83–1.75)	0.36 (0.30–0.47)	0.85 (0.64–1.48)
	DPZ	0.73 (0.43–1.85)	1.44 90.65–2.13	1.15 (1.08–1.67)	2.08 (0.66–9.74)
β2	Saline	0.99 (0.73–1.53)	1.67 (0.94–2.79)	1.45 (0.90–1.79)	0.60 (0.40–1.39)
	HDB	1.13 (0.79–1.97)	1.33 (0.88–1.87)	0.98 (0.43–1.72)	1.13 (0.95–1.53)
	DPZ	0.81 (0.62–2.05)	1.65 (1.27–3.00)	1.09 (0.83–1.91)	0.62 (0.34–7.76)
β4	Saline	1.00 (0.79–1.34)	1.60 (0.49–5.07)	4.03 (0.38–10.6)	2.41 (0.77–4.47)
	HDB	9.11 (2.05–15.3)	4.25 (0.43–6.33)	0.28 (0.18–0.37)	0.70 (0.40–1.50)
	DPZ	1.04 (0.38–6.71)	1.25 (0.34–2.01)	0.85 (0.53–3.52)	1.61 (0.37–14.3)
Plasticity markers					
NMDA R	Saline	1.11 (0.31–0.81)	2.66 (0.68–3.78)	1.79 (0.93–3.25)	0.83 (0.58–3.35)
	HDB	3.11 (0.97–6.79)	1.67 (0.80–2.57)	0.32 (0.26–0.37)	0.72 (0.47–2.61)
	DPZ	0.97 (0.63–3.98)	1.68 (0.83–2.83)	1.04 (0.54–2.16)	0.89 (0.45–3.01)
GAD65	Saline	0.97 (0.67–1.73)	2.57 (0.74–4.34)	1.71 (1.07–3.55)	0.76 (0.54–3.32)
	HDB	3.71 (1.23–5.33)	2.56 (0.67–3.63)	0.34 (0.28–0.52)	1.07 (0.50–3.69)
	DPZ	1.24 (0.10–5.18)	1.78 (1.12–3.86)	1.14 (0.57–2.35)	0.77 (0.44–4.05)
ChAT	Saline	0.98 (0.61–1.68)	1.44 (0.53–3.93)	1.89 (0.95–3.58)	1.14 (0.65–1.78)
	HDB	3.44 (1.07–5.15)	3.29 (0.54–6.77)	0.57 (0.33–0.99)	1.09 (0.29–4.34)
	DPZ	1.09 (0.54–5.18)	1.39 (0.39–2.17)	1.14 (0.61–6.03)	1.19 (0.26–2.13)

Receptor expression (receptor mRNA/18S mRNA) ΔΔCT value, expressed as median (MIN-MAX); VS: –, no visual stimulation; VS: 1, 7, 14 days: 10-min visual stimulation, daily, for 1, 7 or 14 days; Saline: Saline Treatment; HDB: HDB electrical stimulation; DPZ: 1 mg donepezil daily i.p. administration.

Table B2. Expression of the cholinergic receptors in the somatosensory cortex

Receptor	Visual treatment	VS: –	VS: 1 day	VS: 7 days	VS: 14 days
Muscarinic receptor subtype					
m1	Saline	0.99 (0.82–1.25)	1.01 (0.04–1.92)	0.83 (0.32–1.62)	1.00 (0.27–1.32)
	HDB	1.11 (0.60–1.30)	0.76 (0.53–1.32)	0.67 (0.46–1.68)	1.00 (0.60–1.83)
	DPZ	1.10 (0.93–2.54)	0.94 (0.38–1.47)	1.16 (0.72–1.45)	1.09 (0.76–1.56)
m2	Saline	1.22 (0.46–1.60)	1.06 (0.23–1.48)	0.76 (0.42–1.46)	1.01 (0.44–1.19)
	HDB	0.79 (0.36–1.28)	0.68 (0.49–2.18)	1.09 (0.67–1.68)	1.16 (0.61–2.24)
	DPZ	1.75 (0.63–2.41)	1.39 (0.23–2.00)	1.04 (0.30–1.33)	1.37 (0.69–1.97)
m3	Saline	1.01 (0.38–1.94)	0.91 (0.18–2.50)	0.43 (0.11–1.57)	0.95 (0.18–1.03)
	HDB	0.67 (0.28–1.00)	0.44 (0.11–0.82)	0.82 (0.35–1.58)	0.54 (0.21–0.92)
	DPZ	1.10 (0.28–2.86)	1.00 (0.15–2.00)	0.63 (0.28–1.21)	0.73 (0.32–1.96)
m4	Saline	1.00 (0.58–1.80)	1.50 (0.38–3.24)	0.92 (0.57–2.41)	1.18 (0.03–1.60)
	HDB	1.52 (0.78–2.36)	1.01 (0.72–2.60)	1.64 (0.48–3.21)	1.42 (0.76–3.03)
	DPZ	1.85 (0.87–4.81)	1.09 (0.57–2.48)	1.16 (0.86–2.50)	2.42 (1.05–4.81)
m5	Saline	1.62 (0.17–1.96)	3.32 (0.68–6.01)	2.30 (1.64–3.30)	1.19 (0.83–1.67)
	HDB	2.86 (0.59–6.47)	2.04 (0.90–4.87)	1.49 (0.35–3.30)	2.29 (0.66–4.45)
	DPZ	3.91 (0.97–5.79)	1.68 (0.25–2.91)	1.23 (0.11–3.36)	2.50 (1.52–8.33)
Nicotinic receptor subunit					
α3	Saline	1.01 (0.66–1.53)	1.24 (0.64–3.75)	0.52 (0.40–0.86)	0.81 (0.16–1.06)
	HDB	0.69 (0.46–1.79)	0.81 (0.42–1.24)	0.96 (0.93–1.29)	0.71 (0.28–1.19)
	DPZ	1.76 (0.55–4.13)	0.68 (0.16–1.95)	0.77 (0.68–1.00)	1.16 (0.75–1.83)
α4	Saline	1.04 (0.75–1.16)	1.07 (0.35–1.84)	0.70 (0.57–1.36)	0.97 (0.13–1.80)
	HDB	0.82 (0.46–1.03)	0.73 (0.54–1.02)	0.46 (0.33–1.29)	0.74 (0.56–1.28)
	DPZ	0.92 (0.49–4.76)	0.81 (0.30–1.02)	0.87 (0.63–1.01)	0.90 (0.57–1.51)

Table B2 (*continued*)

Receptor	Visual treatment	VS: –	VS: 1 day	VS: 7 days	VS: 14 days
α7	Saline	1.05 (0.57–1.38)	1.07 (0.48–1.57)	0.89 (0.61–0.95)	1.08 (0.29–1.22)
	HDB	0.91 (0.55–1.96)	1.01 (0.51–1.66)	1.05 (0.75–1.60)	0.83 (0.55–1.62)
	DPZ	1.12 (0.69–2.63)	0.92 (0.30–1.12)	0.92 (0.71–1.12)	1.18 (0.78–1.41)
β2	Saline	0.98 (0.80–1.27)	1.17 (0.51–1.43)	0.78 (0.63–1.35)	1.14 (0.15–1.82)
	HDB	0.86 (0.50–1.18)	1.01 (0.77–1.26)	0.61 (0.43–1.19)	0.79 (0.69–0.96)
	DPZ	0.86 (0.70–3.52)	0.76 (0.39–0.93)	0.86 (0.66–1.17)	0.75 (0.56–1.59)
β4	Saline	1.69 (0.11–4.10)	0.51 (0.34–1.07)	0.42 (0.10–2.64)	2.09 (0.03–4.75)
	HDB	0.77 (0.17–2.11)	1.07 (0.31–6.19)	1.87 (0.26–22.8)	1.09 (0.16–2.75)
	DPZ	0.65 (0.02–1.41)	0.54 (0.12–4.39)	0.39 (0.15–0.77)	1.09 (0.42–3.60)
Plasticity markers					
NMDA R	Saline	1.01 (0.43–1.75)	1.16 (0.15–2.35)	0.61 (0.25–0.94)	0.74 (0.13–1.31)
	HDB	0.63 (0.47–1.04)	0.48 (0.16–1.39)	0.91 (0.49–1.61)	0.68 (0.54–1.77)
	DPZ	1.32 (0.93–2.03)	1.31 (0.23–2.31)	0.92 (0.40–1.12)	1.00 (0.63–1.48)
GAD65	Saline	1.01 (0.36–2.00)	0.92 (0.23–1.54)	0.61 (0.30–1.09)	0.75 (0.17–0.96)
	HDB	0.59 (0.30–1.05)	0.70 (0.33–1.25)	0.92 (0.46–1.58)	0.81 (0.42–1.49)
	DPZ	1.15 (0.60–2.29)	1.44 (0.13–1.86)	0.84 (0.22–1.07)	0.97 (0.65–1.63)
ChAT	Saline	1.13 (0.44–1.92)	0.83 (0.43–2.52)	0.80 (0.41–0.96)	0.88 (0.10–1.43)
	HDB	0.60 (0.46–2.24)	0.99 (0.45–1.62)	1.65 (0.90–1.86)	0.99 (0.54–1.36)
	DPZ	1.50 (0.26–1.92)	1.38 (0.46–3.18)	1.39 (0.39–1.56)	0.98 (0.95–1.24)

Receptor expression (receptor mRNA/18S mRNA) ΔΔCT value, expressed as median (MIN-MAX); VS: –, no visual stimulation; VS: 1, 7, 14 days: 10-min visual stimulation, daily, for 1, 7 or 14 days; Saline: Saline Treatment; HDB: HDB electrical stimulation; DPZ: 1 mg donepezil daily i.p. administration.

Table B3. Expression of the cholinergic receptors in the basal forebrain

Receptor	Visual treatment	VS: –	VS: 1 day	VS: 7 days	VS: 14 days
Muscarinic receptor subtype					
m1	Saline	1.25 (0.51–1.58)	0.96 (0.44–2.22)	0.69 (0.21–1.83)	0.18 (0.28–1.89)
	HDB	0.97 (0.21–1.73)	0.32 (0.23–0.44)	0.29 (0.23–0.34)	3.17 (1.26–5.07)
	DPZ	2.38 (2.00–3.98)	1.03 (0.79–1.63)	0.71 (0.51–2.98)	0.40 (0.23–0.58)
m2	Saline	0.96 (0.85–1.23)	0.75 (0.68–2.46)	0.92 (0.40–1.35)	0.93 (0.62–1.24)
	HDB	0.89 (0.38–1.40)	0.78 (0.51–1.14)	0.57 (0.54–0.60)	0.74 (0.58–0.90)
	DPZ	0.47 (0.37–0.56)	0.82 (0.53–1.14)	0.25 (0.16–0.46)	1.24 (0.94–1.54)
m3	Saline	1.14 (0.70–1.25)	0.74 (0.70–0.75)	0.83 (0.58–1.44)	2.13 (1.64–2.63)
	HDB	0.70 (0.55–0.86)	0.68 (0.66–0.76)	0.73 (0.45–1.01)	0.65 (0.62–0.69)
	DPZ	0.43 (0.40–0.66)	0.90 (0.60–1.10)	0.17 (0.15–0.58)	1.35 (1.09–1.61)
m4	Saline	1.19 (0.68–1.23)	1.12 (1.26–1.74)	0.73 (0.31–2.77)	1.51 (0.60–3.10)
	HDB	1.88 (0.58–3.18)	0.63 (0.36–0.69)	0.38 (0.29–0.46)	3.42 (1.24–5.59)
	DPZ	1.36 (0.39–2.00)	0.99 (0.94–1.57)	0.90 (0.60–3.10)	0.77 (0.56–0.98)
m5	Saline	1.02 (0.82–1.19)	0.63 (0.11–0.83)	0.36 (0.30–0.86)	2.16 (0.72–3.61)
	HDB	0.70 (0.57–0.83)	0.96 (0.89–1.91)	1.12 (0.69–1.55)	1.17 (1.08–1.26)
	DPZ	0.50 (0.46–0.60)	1.20 (1.00–1.21)	0.64 (0.12–1.48)	0.77 (0.20–1.34)
Nicotinic receptor subunit					
α3	Saline	0.97 (0.50–2.07)	0.84 (0.42–1.15)	0.81 (0.66–1.00)	1.81 (0.90–2.73)
	HDB	0.93 (0.92–0.93)	1.69 (1.34–2.76)	0.78 (0.28–1.29)	0.84 (0.69–0.99)
	DPZ	0.67 (0.46–0.69)	0.59 (0.55–1.02)	0.38 (0.21–1.98)	14.1 (0.53–27.6)
α4	Saline	0.92 (0.67–1.63)	0.49 (0.39–0.64)	1.51 (0.42–2.72)	1.37 (1.13–1.62)
	HDB	0.59 (0.50–0.69)	0.96 (0.55–1.13)	0.65 (0.35–0.96)	0.76 (0.71–0.81)
	DPZ	0.47 (0.19–1.41)	0.84 (0.46–0.87)	0.50 (0.36–0.81)	9.74 (1.08–18.4)
α7	Saline	1.09 (0.75–1.22)	0.84 (0.47–1.35)	1.21 (0.59–6.52)	1.84 (0.97–2.70)
	HDB	1.15 (0.90–1.39)	1.23 (1.06–1.34)	1.97 (1.66–2.27)	0.95 (0.83–1.06)
	DPZ	0.69 (0.58–1.43)	1.27 (0.92–1.38)	1.02 (0.83–1.02)	1.00 (0.50–1.49)
β2	Saline	0.93 (0.92–1.17)	0.96 (0.93–1.47)	0.63 (0.60–1.28)	1.28 (0.73–1.84)
	HDB	0.83 (0.78–0.88)	0.87 (0.76–0.88)	0.98 (0.69–1.28)	0.79 (0.58–1.00)
	DPZ	0.78 (0.49–1.04)	0.94 (0.67–1.18)	0.69 (0.63–0.95)	1.45 (1.04–1.86)
β4	Saline	1.06 (0.69–1.37)	1.35 (0.35–1.45)	0.33 (0.25–0.50)	0.87 (0.40–1.35)
	HDB	0.87 (0.51–1.24)	0.63 (0.33–0.82)	0.30 (0.29–0.31)	0.94 (0.64–1.23)
	DPZ	12.1 (1.92–12.2)	0.31 (0.30–0.49)	0.30 (0.18–0.50)	1.60 (0.46–2.74)

(*continued on next page*)

Table B3 (*continued*)

Receptor	Visual treatment	VS: –	VS: 1 day	VS: 7 days	VS: 14 days
Plasticity markers					
NMDA R	Saline	0.92 (0.89–1.22)	0.47 (0.43–1.28)	0.79 (0.70–0.88)	1.09 (0.85–1.33)
	HDB	1.05 (0.95–1.15)	0.77 (0.73–1.04)	0.73 (0.21–1.24)	1.76 (1.30–2.21)
	DPZ	1.10 (0.68–1.55)	0.91 (0.76–0.96)	0.22 (0.15–0.68)	1.60 (0.84–2.35)
GAD65	Saline	1.02 (0.91–1.08)	0.50 (0.44–1.07)	0.53 (0.32–0.72)	1.23 (0.48–1.97)
	HDB	0.87 (0.86–0.89)	0.82 (0.12–1.16)	0.67 (0.17–1.16)	0.99 (0.80–1.18)
	DPZ	0.57 (0.47–0.96)	0.98 (0.69–1.17)	0.21 (0.17–1.00)	0.65 (0.28–1.03)
ChAT	Saline	0.92 (0.88–1.24)	2.32 (2.02–2.87)	3.71 (0.76–5.19)	0.69 (0.51–0.87)
	HDB	3.40 (0.07–6.73)	1.47 (0.13–2.39)	0.12 (0.09–0.15)	1.34 (0.96–1.71)
	DPZ	1.27 (0.08–2.15)	1.58 (0.15–2.00)	0.21 (0.21–0.24)	0.78 (0.00–1.57)

Receptor expression (receptor mRNA/18S mRNA) ΔΔCT value, expressed as median (MIN-MAX); VS: –, no visual stimulation; VS: 1, 7, 14 days: 10-min visual stimulation, daily, for 1, 7 or 14 days; Saline: Saline Treatment; HDB: HDB electrical stimulation; DPZ: 1 mg donepezil daily i.p. administration.

(Received 30 January 2018, Accepted 18 June 2018)
(Available online 27 June 2018)