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Photobiomodulation reduces gliosis in the basal ganglia of aged mice

Nabil El Massri [a], Tobias W. Weinrich [b], Jaimie Hoh Kam [b], Glen Jeffery [b], John Mitrofanis [a,*]

[a] Department of Anatomy F13, University of Sydney, Sydney, NSW, Australia
[b] Institute of Ophthalmology, University College London, London, England

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ABSTRACT

This study explored the effects of long-term photobiomodulation (PBM) on the glial and neuronal organization in the striatum of aged mice. Mice aged 12 months were pretreated with PBM (670 nm) for 20 minutes per day, commencing at 5 months old and continued for 8 months. We had 2 control groups, young at 3 months and aged at 12 months old; these mice received no treatment. Brains were aldehyde-fixed and processed for immunohistochemistry with various glial and neuronal markers. We found a clear reduction in glial cell number, both astrocytes and microglia, in the striatum after PBM in aged mice. By contrast, the number of 2 types of striatal interneurons (parvalbumin$^+$ and encephalopsin$^+$), together with the density of striatal dopaminergic terminals (and their midbrain cell bodies), remained unchanged after such treatment. In summary, our results indicated that long-term PBM had beneficial effects on the aging striatum by reducing glial cell number; and furthermore, that this treatment did not have any deleterious effects on the neurons and terminations in this nucleus.

1. Introduction

A characteristic feature of the central nervous system in aging is an activation of glial cells (Lynch et al., 2014; Soreq et al., 2017). For example, many previous studies have reported a marked increase in the number of glial cells due to aging, both astrocytes and microglia, across the central nervous system (Beach et al., 1989; Unger, 1998). For both types of glial cells, there is also an increase in their size and immunohistochemical expression of various markers (Beach et al., 1989; Begum et al., 2013; Conde and Streit, 2006; Cotrina and Nedergaard, 2002; Unger, 1998).

A further feature of aging is that, in contrast to the increase in glial cell number, there is a progressive loss of neurons. This loss manifests after a series of intrinsic molecular changes within the neurons leading to dysfunction and ultimately death. Furthermore, such changes render aging neurons more susceptible to insult, whether by environmental toxin or genetic mutation, such as in Alzheimer's or Parkinson's disease. In fact, aging is a major risk factor for both these neurodegenerative disorders (Balaban et al., 2005; Kujoth et al., 2005; Linnane et al., 1989; Salvadores et al., 2017).

There is general agreement that a pivotal part of the intrinsic change leading to glial and neuronal aging and death is dysfunction of the mitochondria. Mitochondria drive neuronal function by producing adenosine triphosphate and with aging, this ability diminishes. This is associated with an increase in toxic reactive oxygen species, oxidative stress, and subsequent neuronal death (Balaban et al., 2005; Kujoth et al., 2005; Linnane et al., 1989; Salvadores et al., 2017).

In view of these key features of aging, the development of treatments that target a reduction of gliosis and the protection of mitochondria in neurons have generated much interest (Chaturvedi and Beal, 2008). In this context, recent studies have shown that relatively short-term exposure to red to infrared light (λ = 600–1070 nm), or photobiomodulation (PBM), not only reduces gliosis markedly (Begum et al., 2013; El Massri et al., b, 2016a,) but also improves mitochondrial function (Begum et al., 2013; Eells et al., 2004; Gkotsi et al., 2014; Hamblin, 2016; Karu, 2010; Khan and Arany, 2015; Rojas and Gonzalez-Lima, 2011; Sivapathasuntharam et al., 2017), in both aging and disease.

In this study, we explored whether long-term PBM had any impact, beneficial or deleterious, on gliosis and/or neuronal survival in aging. We chose the caudate-putamen complex, or striatum, of the basal ganglia for investigation mainly because of our long-standing interest in Parkinson's disease (El Masri et al., 2016a,b; Shaw et al., 2010), and that it represents a central "hub" of functional neurotransmission for many other neural centers,

* Corresponding author at: Department of Anatomy, University of Sydney, Anderson-Stuart Building F13, Sydney 2006, Australia. Tel.: +61 29 351 2500; fax: +61 29 351 2813.
E-mail address: john.mitrofanis@sydney.edu.au (J. Mitrofanis).

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from the cerebral cortex to the thalamus and to all the other nuclei in the basal ganglia (Parent and Hazrati, 1995). Furthermore, except for features of the dopaminergic system (Darbin, 2012), few studies have explored the glial and neuronal organization of the striatum, together with the greater basal ganglia, in aging. Indeed, no previous study has explored the effects of PBM in this key brain area in aging. To this end, we examined several cellular structures of the striatum, namely, the 2 types of glial cells (glial fibrillary acidic protein $[GFAP]^+$ astrocytes and ionized calcium-binding adapter molecule 1 $[IBA1]^+$ microglia), 2 types of neurons (parvalbumin $[Pv]^+$ and encephalopsin $[Eno]^+$), and 1 type of termination (tyrosine hydroxylase $[TH]^+$). In general, by using these striatal structures as examples, we aimed to gain insight into the overall age-related changes evident in the basal ganglia and the impact after PBM.

2. Materials and methods

2.1. Subjects

Male C57BL/6 mice (n = 16) were housed on a 12-hour light/dark cycle with unlimited access to food and water. Animals were aged at 3 (young) or at 12 (aged) months old (examining animals at only 1 stage for young [3 months] and using 12 months as aged is common [e.g., Vacano et al., 2018]). All experiments were approved by the Animal Ethics Committee of the University College London and Home Office–licensed procedures conforming to the UK Animal Licence Act (1986). We had 3 groups of mice; 3m (aged 3 months, n = 5; young controls, with no PBM), 12m (aged 12 months, n = 6; old controls, with no PBM), and 12m + PBM (aged 12 months, n = 5; PBM treated).

2.2. Photobiomodulation

Animals in the 12m + PBM group were treated with PBM (670 nm) for 20 minutes per day. This treatment occurred in the morning. Treatment commenced when the animals were 5 months old and continued for 8 months, up until the animals reached 12 months of age. Our rationale for commencing treatment at 5 months was that at this age, mice in the wild are considered "old", but in other respects "normal". We hence have used fully mature mice (5 months) and mapped progress through to an older age (12 months), recording any changes to this progress with or without PBM.

2.3. Immunohistochemistry and cell analysis

Mice had their brains aldehyde-fixed (4% buffered paraformaldehyde), cryoprotected, and sectioned coronally using a freezing microtome (El Massri et al., 2016a,b; Shaw et al., 2010). Sections of striatum were incubated in normal goat serum (KPL) and then in either rabbit anti-GFAP (1:500, ab7260; Abcam; to label astrocytes), rabbit anti-IBA1 (1:1000, ab178846; Abcam; to label microglia), rabbit anti-TH (1:500, T8700; Sigma; to label dopaminergic terminals), rabbit anti-Eno (1:500, ab75285; Abcam; to label striatal neurons), or mouse anti-Pv (1:3000, P3088; Sigma; to label striatal neurons) followed by biotinylated goat anti-rabbit or anti-mouse IgG and then streptavidin-peroxidase complex (71-00-19; KPL). In addition, sections of the midbrain were incubated in anti-TH to label the dopaminergic cells that project to the striatum, and these were processed further as described previously. Finally, all sections were reacted in a 3,3′- diaminobenzidine tetrahydrochloride solution (D3939; Sigma) and then coverslipped. For controls, sections were processed as described previously except that no primary antibody was used. These control sections were immunonegative. For cell analysis, the number of immunoreactive cells in the striatum (and the midbrain) was estimated using the optical fractionator method (Stereo Investigator; MBF Science), as described previously (El Massri et al., 2016a,b; Shaw et al., 2010). We also measured the density of TH^+ terminals in the striatum. Bright-field images of TH^+ terminals were captured under standard illumination conditions for each section. Each image was then processed in an identical manner using ImageJ software (NIH). For each image, color threshold was adjusted to a set level, when the TH^+ terminals were distinguished from background. The mean gray value was then measured for each image. The resulting values in the striatum provided a reliable and replicable measure of the density of TH^+ terminals in each image (El Massri et al., 2016a). For comparisons in the number of cells and density of terminations between groups, a 1-way analysis of variance (ANOVA) test was performed, in-conjunction with a Tukey multiple comparison test was used (GraphPad Prism).

3. Results

Our results will explore the age-related changes, and the effects of PBM, in the striatum. The changes in glia, neurons, and terminal patterns will be considered separately.

3.1. Glia

There were marked changes evident in both types of glial cells following long-term PBM in the striatum of aged mice. Fig. 1A shows a graph of the estimated total number of $GFAP^+$ astrocytes in the striatum in the different experimental groups. There were clear differences in cell number between the different groups (Fig. 1A; ANOVA: $F = 14$; $p < 0.001$). There was a ~60% increase in the number of $GFAP^+$ astrocytes between the 3m and 12m groups (Tukey-Kramer: $p < 0.0001$). In the 12m + PBM group, the number of astrocytes was much lower than the 12m group (Tukey-Kramer: $p < 0.01$), being similar to the 3m group (Tukey-Kramer: $p > 0.05$). In terms of morphology and overall immunoreactivity, $GFAP^+$ cells of the 12m group (Fig. 1C) tended to be much larger and more strongly labeled than those of the 3m (Fig. 1B) and 12m + PBM (Fig. 1D) groups. They appeared "activated". For the $IBA1^+$ microglial cells, there were differences in cell number between the different groups also (Fig. 1E; ANOVA: $F = 11$; $p < 0.001$). Although there were no differences in the number of $IBA1^+$ microglia between 3m and 12m groups (Tukey-Kramer: $p > 0.05$), there was a ~50% reduction in cells between the 12m (and 3m) and 12 + PBM groups (Fig. 1E; Tukey-Kramer: $p < 0.01$). In terms of morphology, we found no differences evident among $IBA1^+$ microglia of the 3m (Fig. 1F), 12m (Fig. 1G), and 12m + PBM (Fig. 1H) groups; we encountered no "activated", amoeboid-like cells, with all cells having the classical resting-state morphology. In summary, we found that long-term PBM had a major effect on the number of glial cells in the striatum of older animals.

3.2. Neurons

In contrast to the findings on the glial cells, we found no major changes after long-term PBM in the 2 distinct neuronal types we examined, namely those that express Pv or Eno. For Pv^+ cells, previous studies have reported that these form a subgroup of the GABAergic (γ-aminobutyric acid) interneurons in the rodent striatum (Kawaguchi et al., 1995). Overall, there were clear differences in the numbers of Pv^+ cells between the experimental groups (Fig. 2A; ANOVA: $F = 31$; $p < 0.0001$), due mainly to the ~50% reduction in their number between the 3m and 12m groups (Tukey-Kramer: $p < 0.0001$). There were no major differences, however, between