Optogenetic modulation of GABAergic system improves Aβ1-42-induced memory deficits

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Introduction

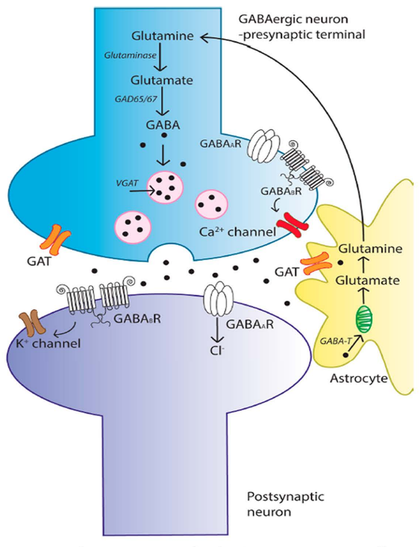


Figure 1. Overview of GABAergic signalling system. Adapted from Govindpani et al (2017)

Accumulation of beta amyloid (Aβ), neurofibrillary tangles and disrupted excitatory neurotransmission are considered the major factors underlying the Alzheimer's disease (AD) pathogenesis. The loss of memory and cognitive deficits are largely caused by extensive neurodegeneration primarily in the hippocampus. Recently, the role of the γ-aminobutyric acid (GABA)ergic system in the progression of AD and its therapeutic potential have gained considerable attention. Evidence suggests the remodelling of GABAergic system in AD and its contribution to E/I imbalance causing cognitive impairments in AD; in particular, A β-induced elevation of extracellular GABA level and disruption of inhibitory function in the hippocampus. However, how GABAergic alteration affects AD pathogenesis is still not well understood. One of the latest tools that allow selective modulation of GABAergic system with high spatiotemporal precision is optogenetics. It involves transducing targeted cells with opsin (light-sensitive protein) and delivering light only to the desired region to activate/inhibit their activity. Optogenetics aid in investigation of the therapeutic potential of GABAergic modulation in AD.

Aim: To examine if modulation of GABAergic inhibition in the hippocampus has efficacy to improve cognitive deficits in an Aβ1-42 induced AD mouse model using optogenetics *in vivo*.

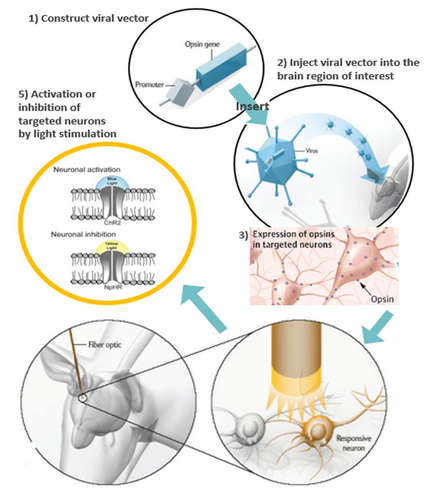
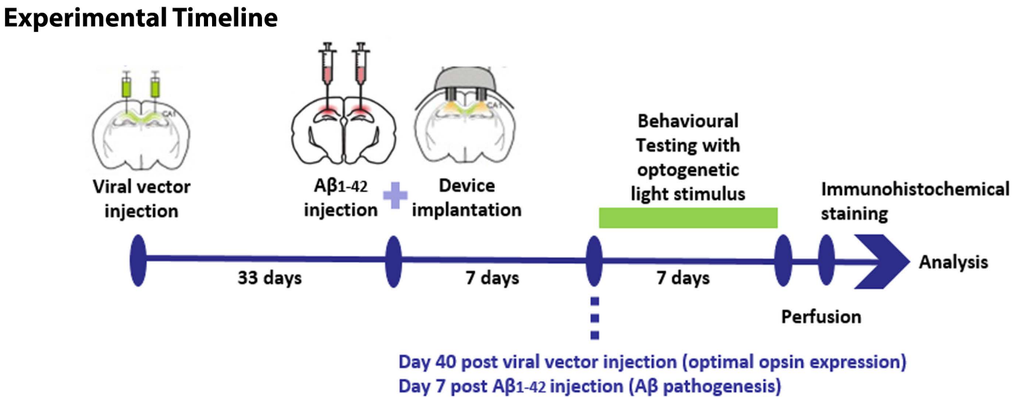


Figure 2. Steps involved in optogenetic control of specific cells expressing opsin. Adapted from Deisseroth (2010) and (2015)

Methods

Animals: 6 to 8 weeks old male wild type C57BL/6J mice were used in this study.

Experimental Timeline



Viral expression of opsin in GABAergic cells

GABAergic cell-specific expression of opsin was achieved by bilaterally injecting lentivirus (0.5µl each hemisphere) containing a gene encoding inhibitory opsin eNpHR3.0 tagged with EYFP under the GABAergic neuron specific promoter GAD67 (LV.GAD67.eNpHR3.0.EYFP) into the hippocampal CA1 region (AP -2.0; ML ±1.3; DV -1.0, -1.25, -1.5).

Aβ-induced AD mouse model

Animals received bilateral intra-hippocampal injection of 1 µl of 20 µM neurotoxic Aβ1-42 (AP -2.0; ML ±1.3; DV -1.9, -2.4, -2.9). By day 7 post Aβ1-42 injection, animals exhibit learning and memory impairments and neuropathological changes.

Optic device implantation

The optical control was achieved through the implantation of an ultraminiaturised bilateral implant device with 590nm uLED (powered and controlled wirelessly) into the CA1 region of the hippocampus (AP -2.0; ML ±1.3; DV -1.05).

Behavioural tests

Novel object alteration (NOA; Figure 3) and novel object recognition tests (NOR; Figure 4) were performed to examine animal's long-term spatial and recognition memory. Data was presented as the discrimination index.

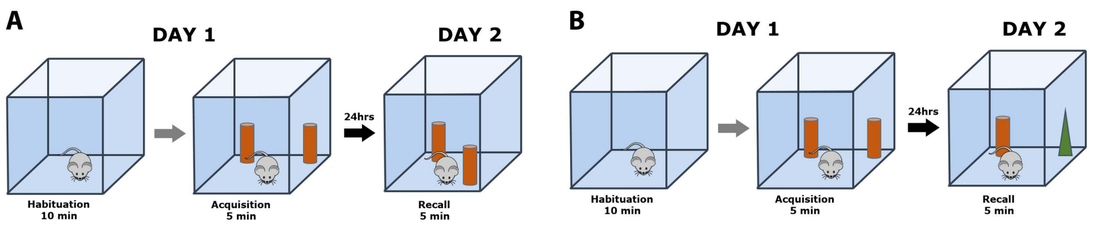


Figure 3. Schematic representation of the novel object alteration (NOA; A) and novel object recognition (NOR; B) test.

Optogenetic stimulation

Animals were given continuous yellow light (590 nm) stimulus over the period of 5 min during the acquisition and recall phases of the behavioural testing.

Immunohistochemistry

Free-floating fluorescent immunohistochemistry (f-IHC) was performed to examine the changes in the expression of NeuN, pTau and GABAA receptor (GABAAR) subunits α1, α2, α5, β3 and γ2. The integrated density of the stainings were compared between each experimental group.

Conclusion

- An acute optogenetic inhibition of GABAergic cells in the hippocampus during the learning and recall phases of the cognitive behavioural testing improved Aβ1-42 induced long-term spatial recognition memory in vivo.
- In AD, there is an increase in the ambient level of GABA and changes in the GABAAR activity which leads to excessive tonic inhibition and dampening of LTP contributing to memory impairment. Our findings suggests that the improvement of cognitive deficits achieved by optogenetic inhibition of GABAergic cells may be possibly mediated by reduction of the increased levels of extrasynaptic GABA and pTau, and restoration of the changes in GABAAR subunit containing cells induced by Aβ1-42.
- Our study support the critical role of GABAergic system in AD pathogenesis and advance our understanding of the potential for GABAergic modulation as a therapy for AD.

Results

An acute optogenetic inhibition of GABAergic cells in the CA1 region during behavioural testing significantly improved Aβ1-42-induced long-term spatial and recogniton memory deficits

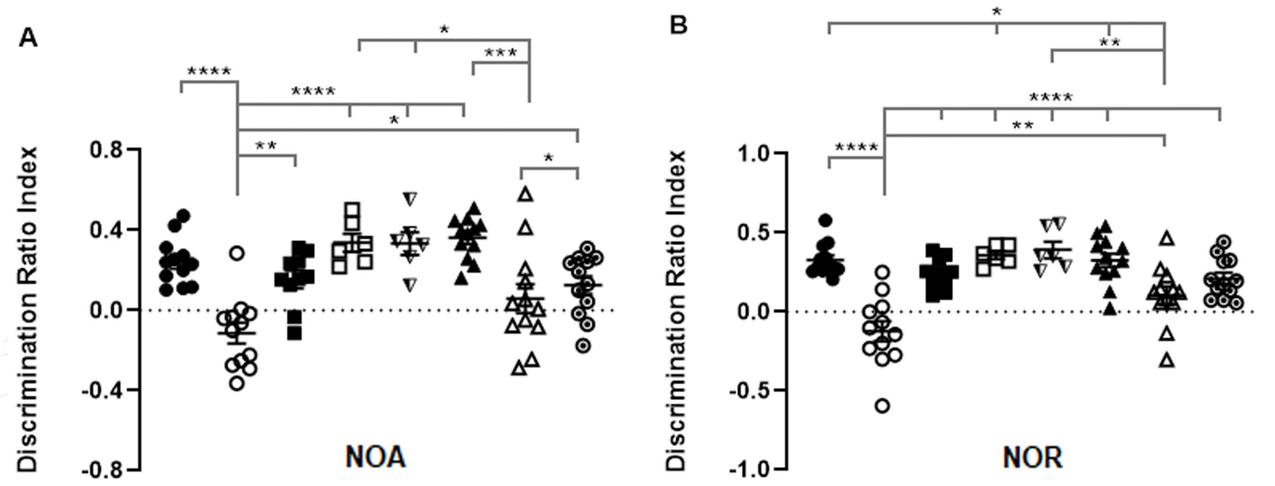


Figure 5. Graphs showing the discrimination ratio for different experimental groups during NOA (A) and NOR (B) tests. A positive value indicates more time investigating the novel location or novel object while a negative value indicates more time spent with the familiar location or familiar object. A discrimination index of zero indicates equal time spent with both novel and familiar location or objects. ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, One-way ANOVA with Tukey's multiple comparison post-hoc test. n = 6 to12 each group.

Optogenetic treatment reduced pTau level in the CA1 hipocampal region

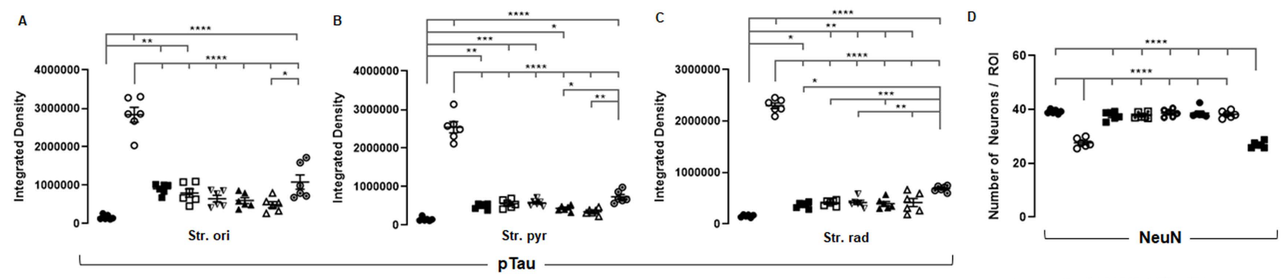


Figure 5. Quantification of pTau level in the CA1 layers stratum oriens (str. ori; A), stratum pyramidale (str. pyr: B) and stratum radiatum (str. rad; C) and number of neurons in the pyramidale layer in the CA1 region (D) in different experimental groups. Data is presented as mean integrated density ± SEM, ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, One-way ANOVA with Tukey's multiple comparison post-hoc test. n = 6 to12 each group.

Optogenetic treatment ameliorated Aβ1-42-induced changes in the levels of GABAAR subunits α5, and γ2 in all layers of the CA1 region and α2 subunit in the Str. ori layer of the CA1 region

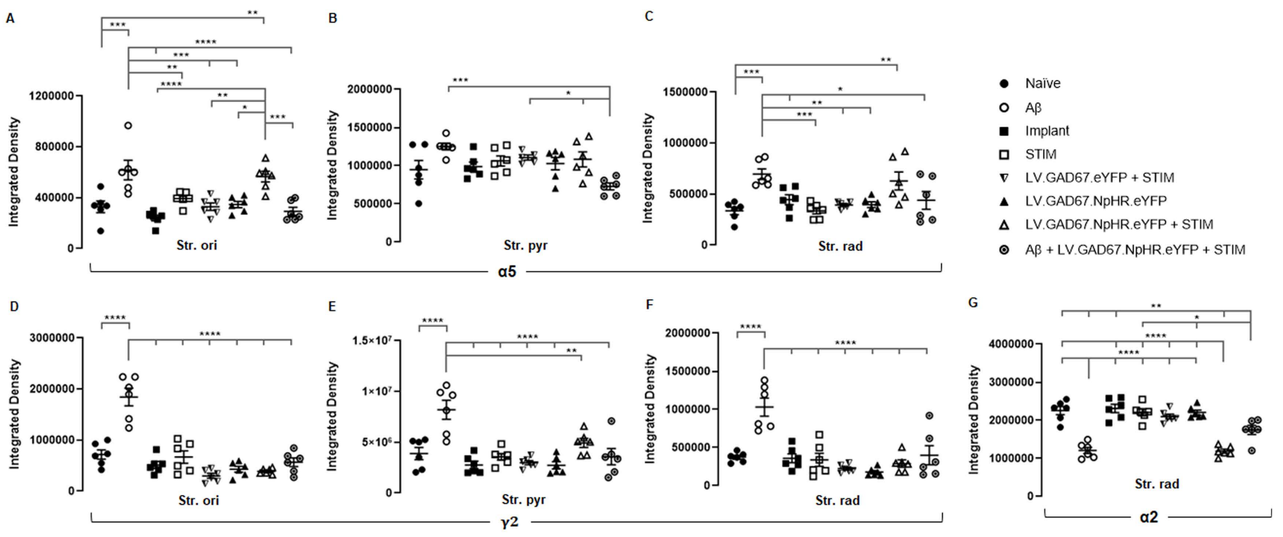


Figure 5. Quantification of the level of GABAAR subunits α5 (A-C), γ2 (D-F) and α2 (G) in the layers of the CA1 region between different experimental groups. Data is presented as mean integrated density ± SEM, ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05, One-way ANOVA with Tukey's multiple comparison post-hoc test. n = 6 to12 each group.

Acknowlegements

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