

Optogenetic manipulation of the cholinergic system: Potential implications for the treatment of memory deficits in Alzheimer's disease

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ABSTRACT

Successful treatment of psychiatric disorders and neurodegenerative diseases depends crucially on our fundamental knowledge of how information is processed and stored by the brain. Understanding the interaction between neuromodulation and signal encoding would essentially aid the progress of their treatment. Given the reciprocal connections between cholinergic and hippocampal networks, it is important to investigate their functional effect on the symptoms of memory impairment. The convergence between neuromodulatory models and behavioral research is finally possible due to tremendous growth of physiological data about neuronal signal processing, as well as external control over of local circuits via electrical microstimulation and optogenetically triggered activation. Optical neural control therapy is an emerging tool in neuroscience that targets the fundamental understanding and treating of a wide range of brain-based disorders. Although this methodology is currently developed on a basic neuroscience level, the latest advances in optogenetics are successfully applied for primate research and pre-clinical testing.

Key words: acetylcholine, Optogenetics, Episodic memory, Alzheimer's disease, Medial septum.

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INTRODUCTION

The field of electrophysiology has developed in the last 50 years the backbone of neurocognitive understandings about the brain function. These fundamental findings brought the Nobel Prize 2014 for Physiology to the founders of the hippocampal place and the entorhinal grids cells. Very recent progress in neuroscience techniques, such as optogenetics, will allow us to translate the fundamental neuroscience knowledge into applications that treat neurocognitive disorders. Experiments performed so far only in vitro are now able to be tested in freely behaving animals, where we could relate neuronal communication to cognitive processes. In this review, I will first address the role of acetylcholine in memory formation and the involvement of cholinergic system in Alzheimer's disease. Then, I will discuss the current methodologies that permit localized brain activation and cell-specific stimulation. Finally, I will review the latest advances in optogenetics, which suggest a novel experimental approach in the treatment of the neurodegenerative diseases.

CHOLINERGIC MODULATION OF EPISODIC MEMORY NETWORKS

The cholinergic system has evolved into a network with two principle components: (1) the nuclei of the basal forebrain, which include (i) the nucleus basalis

magnocellularis or (basal nucleus of Meynert in primates), (ii) the medial septal nucleus and the diagonal band of Broca complex, (2) the brainstem nuclei, which include (i) the pedunculopontine tegmental nucleus and (ii) laterodorsal tegmental nucleus [1–4]. The cholinergic signaling in the brain also includes interneurons in the basal ganglia [5,6]. Nucleus basalis of the basal forebrain forms extensive cholinergic projections to the cortex [7,8]. It mediates cortical oscillatory synchrony [9–11] and is involved in arousal, sensorimotor integration and locomotion [12,13]. The pedunculopontine and laterodorsal tegmental nuclei regulate the activity in the nigro-striatal system and the brainstem reticular formation [14–16]. A major output from medial septum targets the hippocampal formation [17,18]. Thus, control of septo-hippocampal processing would allow regulation of the mnemonic properties of hippocampus. The involvement of medial septum in the episodic memory is well documented [19–21]. Medial septum is a key structure that generates large-scale synchronization of brain oscillations across the limbic circuits that encode episodic memory. Synchronous oscillatory activity particularly in the theta range (5–12 Hz) functionally links remote neuronal populations or limbic areas, providing a temporal window for synaptic plasticity [22]. Lesions of the septal inputs impair hippocampal theta rhythm by reducing its rate and decreasing its power [23–26]. Theta rhythm appears to serve a critical role for spatial and non-spatial mnemonic functions of

the limbic system [27,28]. The septo-hippocampal axis integrates sensorimotor signals and theta rhythm [29]. This temporal coupling incorporates intrinsic theta and extrinsic sensorimotor signals on each theta cycle.

The functional properties of rodent hippocampal formation are expressed by the spatial properties of the place cells." Hippocampal place cells fire in response to a rodent's spatial location [30], using sensory stimuli as a directional reference to provide a rodent's orientation in space. One of the first indications that memory modulates place fields is the finding that even after visual cues are removed, place cells firing persisted [31]. Cells in the human hippocampus are also shown to fire in correlation with spatial orientation tasks [32]. Place cells have been recorded from young and aged rats that often differ in their spatial learning abilities [33–36]. Place cells of aged animals often fail to remap in the face of salient environmental [36,37], or task change [38,39], compared to young rats. The reduced degree of remapping is believed to underlie the age-related impairments in spatial processing and episodic memory formation [40]. Importantly, the injection of cholinergic agonist (carbachol) in medial septum improved the development of a spatial representation of aged rats in novel environment [41]. Carbachol-induced activation of medial septal facilitated the formation of place fields in aged rats, whereas this protocol impaired the same process in young animals exposed to novel environment [41]. The findings indicate a mechanism of age-related cognitive impairment, which is mediated by the cholinergic septo-hippocampal processing. The link between age-related regulation of cholinergic system on hippocampus-mediated episodic memory is supported by the fact that the cholinergic modulation of the hippocampus is reduced during aging in rats [42], as well as humans [43]. Cholinergic activation of medial septum successfully enhances the memory performance in scopolamine-treated or aged memory-impaired rats [44–46]. Aged rats with behavioral deficits exhibit altered electrophysiological properties of septal activity [47]. Injection of cholinergic agonists in medial septum increases hippocampal theta power [25,44,45], while selective cholinergic lesions in medial septum are known to attenuate hippocampal theta [25,48]. Non-selective inactivation of medial septum decreases the firing rate of hippocampal neurons and reduces their ability to spike in trains [29,49,50]. Immunotoxic lesions of the septo-hippocampal cholinergic neurons failed to remap in different environments [51]. Hippocampal place fields that are formed internally as a result of network interactions during wheel running [52] are abolished by the suppression of septal activity [53], but see [54]. Similarly, the grid cells are not sustained during septal inactivation [50,55]. The cholinergic input is proposed to augment the effect of afferent inputs on neuronal spiking, mediating the storage of spatial information in the hippocampal network [56]. Septal acetylcholine, thus, could enhance attention to sensory stimuli during the encoding of new memories [57,58]. In summary, several findings indicate that the acetylcholine facilitates hippocampus-dependent memory formation [5,59].

IMPAIRED ACETYLCHOLINE PROCESSING IN ALZHEIMER'S DISEASE

It is accepted that a severe loss of cortical cholinergic innervation is part of advanced Alzheimer's disease (AD) [60]. AD is characterized by deposition of extracellular beta-amyloid peptide-containing plaques in cerebral cortical regions of Alzheimer patients, paralleled by the presence of intracellular neurofibrillary tangles in the cortical pyramidal neurons. Basal forebrain cholinergic dysfunction is a consistent feature of AD, which has been related to the cognitive deficits observed in patients with Alzheimer's disease. Aging is considered as the leading risk factor for AD. Aging is characterized by the occurrence of neurofibrillary degeneration and cell loss in the basal forebrain (Ch4–nucleus basalis complex) [61], and a concurrent loss of cholinergic innervation in the cortical areas [62]. The most prominent loss of cholinergic innervation is observed in the temporal lobes, including the hippocampus and the entorhinal cortex, where up to 80% of cholinergic projections could be degenerated [63]. Alterations in acetylcholine synthesis indicate high-affinity choline transport attenuation in post mortem AD brains [64]. Cholinergic cells are additionally impaired by increased choline flux across their membranes during exposure of beta-amyloid [65]. Under acute conditions, amyloid peptides might suppress the choline uptake and inhibit intrinsic acetylcholine release [66]. Similar impairments of acetylcholine signaling are observed in the transgenic mice, expressing AD-like amyloid pathology [67]. Injection of the amyloid beta (A β) peptide in hippocampal formation impairs working memory in rodents [68–70]. A line of research using A β oligomers shows potent disruption impairment of learning and memory function in animals [71–73]. In humans AD is closely linked to the gradual development of memory loss [74]. The preclinical period of isolated memory loss is also known as mild cognitive impairment [75]. The memory attenuation in AD patients is result of disrupted septo-hippocampal axis and abolished cholinergic innervation of the medial temporal lobe. The neurofibrillary degeneration and related cell death in Ch4–nucleus basalis complex is followed by severe and selective loss of cortical cholinergic innervation of hippocampo-entorhinal networks [76,77]. The characteristic memory loss in AD is accounted for the high degree of anatomical and physiological dysfunction of the hippocampus and entorhinal cortex [78]. Degenerated cholinergic regulation of hippocampal networks severely affects the anatomical connectivity between neurons by impairing the synaptic plasticity. For memory to occur, these modifications must persist long enough to contribute to long-term memory storage. This definitely appears to be the case for the forms of synaptic plasticity known as long-term potentiation and long-term depression. Extensive research has been conducted to establish the contribution of synaptic plasticity to spatial learning [79–83] and validate it as a mechanism encoding spatial learning [84]. Synaptic plasticity is shown to be blocked by direct exogenous A β application and in AD transgenic mouse models with abnormally high A β levels [85,86].

Several studies confirm that synaptic plasticity is already impaired during early stages of neurodegeneration in AD transgenic mice, even prior the emergence of learning and memory deficits [87,88]. The cholinergic loss is not considered to be the primary pathogenetic factor of AD *per se* and the current view is that cholinergic therapies are providing symptomatic treatment for senile and presenile forms of AD. Nevertheless, cholinomimetics remain major component of our strategy to influence the cognitive and neurodegenerative changes of AD. The aim of AD treatment is to support cholinergic system either by means of muscarinic agonists or by inhibitors of acetylcholinesterase, which increase the concentration of acetylcholine in the brain. The application of acetylcholinesterase inhibitors is currently the standard therapy for mild and advanced dementia in AD. This approach influences the onset and progression of AD and is associated with improvement in a number of behavioral symptoms including depression, psychosis and agitation [89]. The pharmacological agents currently regarded as standard treatment for AD include donepezil, rivastigmine, galanthamine, and tacrine, which are acetylcholinesterase inhibitors [90]. However, the benefit from their use is limited and there is no medication that clearly intervenes the progression of the disease. The difficulties with establishing reliable and long-term therapeutic solution for AD suggest that we have to diversify our treatment approach and consider novel experimental strategies. One of them is chronic selective activation of cholinergic neurons in the basal forebrain, which diverge to several cortical areas, including the structures that mediate the episodic memory formation. Thus, the activation of the Ch4–nucleus basalis complex can act as a cholinergic relay for modulating the function of all cortical areas including the hippocampus and entorhinal cortex [91,92]. The ability to activate selectively the cholinergic neurons would allow us to dissect the circuits involved in the development of neurodegenerative diseases.

ELECTRICAL AND OPTICAL METHODS FOR BRAIN STIMULATION

An alternative method to the classic pharmacological treatment of brain dysfunction involves microstimulation of the neural circuits engaged in neurodegenerative and psychiatric disorders [93–95]. In the recent years, electrical stimulation via implanted electrodes, known as deep brain stimulation (DBS), was demonstrated as a successful technique in the treatment of many neurological disorders, and seemed promising in treating depression. Currently, DBS has been implied in the treatment of Parkinson's disease, depression, dystonia, obsessive-compulsive disorder, and Tourette's syndrome [96–99]. DBS is an important tool in basic and clinical neuroscience that reduces the neurological symptoms related mainly to dysfunctional dopaminergic system. Although DBS gained sufficient popularity its application is restricted because of the invasive nature and the side effects related to the non-selective effect of electrical microstimulation.

Microstimulation depolarizes different types of neurons, including inhibitory GABA-ergic neurons, which might lead to unexpected decrease of the population response [100–102]. Such network effect is likely to be involved in the disproportional response to DBS, where the neuronal inhibition is largely independent of the amount of the induced activation [101]. The difficulties with the regulation of inhibitory neuron recruitment during microstimulation [102] demonstrate that the involvement of DBS methodology for therapeutic purposes is limited due to the insufficiently unclear mechanism of how electric stimulation impacts neural circuit dynamics. The disadvantage of electrical stimulation is also related to the possible distant recruitment of neurons through direct axonal stimulation [103]. The depolarizations of cell bodies, apical and distal dendrites, axons and axonal collaterals show the non-physiological manner of activation of the neuronal circuits and the lack of high spatial resolution after electrical stimulation. This methodology also evokes a highly synchronous activation of the neuronal populations, which might superimpose an additional synaptic plasticity effect [104,105]. Optogenetics, an emerging experimental technique, is able to overcome these disadvantages. Optical stimulation is a novel method, which is being developed in the last 15 years and is becoming a tool for selective activation or inhibition of specific types of neurons [106]. A major advantage of optical stimulation is the possibility to affect only neurons of a particular type [107,108]. The investigated neurons are genetically engineered to express light-sensitive proteins known as opsins, which controlling the flow of ions through the cellular membrane in response to light [109,110]. The main classes of opsins include (1) channelrhodopsins (light-gated inward nonspecific cation channels) that depolarize neurons in response to light, (2) halorhodopsins (light-driven inward chloride pumps) that hyperpolarize neurons in response to light, and (3) archaerhodopsins (light-driven outward proton pumps), which hyperpolarize neurons in response to light. To target the neurons of interest, genetic construct containing the opsin gene, along with genetic elements that control its expression, a specific “promoter” sequence is packed in a viral vector. The viral vectors with genes caring the promoter for the specific cell type, the opsins and the fluorescent protein (such as GFP) are inserted in lentiviral, adeno-associated or herpes simplex viruses [111,112]. Additionally, transgenic mice lines are able to express some of these molecules under a specific promoter [113]. To obtain neuronal expression of rhodopsins in the regions of interest, the viruses encoding rhodopsin-fluorescent protein is injected in the brain of the animals. After few weeks necessary for the viral expression, the infected cells are activated with light delivered through an optical fiber threaded through an animal's skull. Compared to electrical microstimulation, the optogenetic technique allows accurate physiological effect specific to the neurons of interest. Neurons of a specific type can be identified among the numerous recorded neurons from their response to light, and subsequently, their firing pattern can be analyzed in relation to the firing of other neurons,

local field potential patterns, and the animal's behavior. In addition, the impact of their activation or inhibition on the rest of the network can be monitored. Optical stimulation of genetically targeted neurons expressing light-sensitive channelrhodopsin (ChR2) can be used as a rapid activator of neuronal firing with potential cell-type selectivity [111,114–116]. ChR2, a transmembrane protein derived from the green algae *Chlamydomonas*, contains a chromophore which, upon absorption of blue light, undergoes a conformational change that causes the transmembrane channel to open. The influx of cations caused by the opening of the channel leads to neuronal depolarization and generation of action potentials. A major advantage of optical stimulation is the possibility to affect selectively several classes of neurons [111].

CURRENT ADVANCES AND POTENTIAL BENEFITS OF THE OPTOGENETICS

The latest advances in optogenetic tools allow us to relate cellular function to behavioral contexts. Importantly, the behavioral correlate of each cell type is differentially sensitive to the type of task rodents perform [117–123]. Application of the optogenetic technique allows the association of a particular behavioral phenotype with interrogation of a specific neural circuit [124–126]. Optogenetics has been already implied in the study of Parkinson's disease circuit investigation and therapeutic approach [127]. Optical activation and inhibition of cholinergic, and dopaminergic cells, in the mesolimbic tegmentum and nigro-striatal system elucidate the current understanding of the mechanisms underlying parkinsonian motor and non-motor symptoms [128]. The cell-specific control of neural circuits is currently validated in experimental animal models of Parkinson's disease [129,130]. Optogenetic stimulation is still not investigated in Alzheimer's disease patients or animal models due to the invasive approach of this technique. However, the revolutionary development of optogenetics is already overcoming these obstacles and raising the possibility of using optogenetic manipulations directly in humans for a wide range of brain disorders. Optical neural control therapy aims to fast restore impaired neural signals in pre-clinical testing trials. Here, I will summarize some of the recent advances in optogenetics that will lead to potential implementation of this methodology in clinical neuroscience. Independent activation of two distinct neural populations in mammalian brain is now possible after the recent development of two channelrhodopsins, Chronos and Chrimson [131]. Importantly, Chrimson is a red-light-sensitive opsin, capable of mediating neural activity in response to light of a 735-nanometer wavelength [131]. The ability to evoke neuronal activity in response to the infrared range, together with the reduced toxicity of this opsin, holds potential for eventual therapeutic use in humans. The optogenetic regulation of the cholinergic system and its effect on behavior are now possible in rodents due to the development of transgenic lines. The successful expression of the light-sensitive ChR2 from the

cholinergic neurons in the basal forebrain of rodents shows the functional effect of septal cholinergic activation on the hippocampal formation in behaving mice [132]. The selective photostimulation of choline acetyltransferase (ChAT) cells of ChAT-ChR2-EYFP transgenic mice [133], reveals the behavioral correlate of basal forebrain activation in behavioral transitions. Recently developed genetically validated ChAT::Cre rat line [134] allows to examine in details the cholinergic septal activity during exploratory behavior (Figure 1A-C) and to evaluate their effect on the hippocampal function (Figure 1D). The injection of Cre-dependent opsin-expressing viral vectors in Cre-driver mouse and rat lines results in selective expression of ChR2 in specific cell types [134–137]. Recent optogenetic data demonstrate that activation of septal cholinergic neurons evokes potent network effect in medial septum of ChAT::Cre rat, which is behavior and frequency dependent [138]. Optical stimulation is a powerful tool for establishing a causal link between the activity of cholinergic system and behavior and understanding the neural circuits suitable for optogenetic targeting of neurological disorders such as Alzheimer's disease. Before applying optogenetics in clinical neuroscience it is necessary to characterize the extent, efficiency, tolerance and pattern of opsin expression in non-human primate cortex to minimize potential risks. Another challenge is the amount of laser power applied through the optical fiber in the brain, which can lead to thermal damage [139,140]. ChR2 expression in excitatory pyramidal neurons in macaque frontal cortex was achieved *in vivo* and allowed optical stimulation combined with electrophysiological recordings [109]. This finding demonstrated neuronal activation within the infected area with millisecond temporal precision in primates. The verification of the safety and efficacy of opsins application in primates is a key step toward potential clinical translational path for optical neural prosthetics. Adeno-associated virus serotype 5 (AAV5) is safe for use in non-human primates [141,142] and can be used as a safe and effective viral vector for delivering opsins into the brains of non-human primates [143]. AAV5 is tolerated by the human immune system [144], shows very low neutralizing factor seroprevalence in humans [145], and diffuses more readily in brain tissue [146]. The ability to optically regulate aberrant neuronal activity will allow precise, side-effect-free treatments for neural disorders. The approach of synthetic neurobiology is now possible after the findings that opsins are safely expressed over many months despite repeated viral injections and repeated illumination sessions without immune attack in the primate brain [109,143,147]. The chronic implantation of optical fiber in the brain anchoring the fiber ferrule to the skull ensures that the same neuronal population is repeatedly activated or suppressed over multiple behavioral sessions. However, the permanent implantation of fibers is an invasive methodology, the application of which is more sophisticated in primates and humans. One of the main issues with application of optogenetics in primates involves the damage caused by transdural delivery of viruses and light to the brain. The thickness of

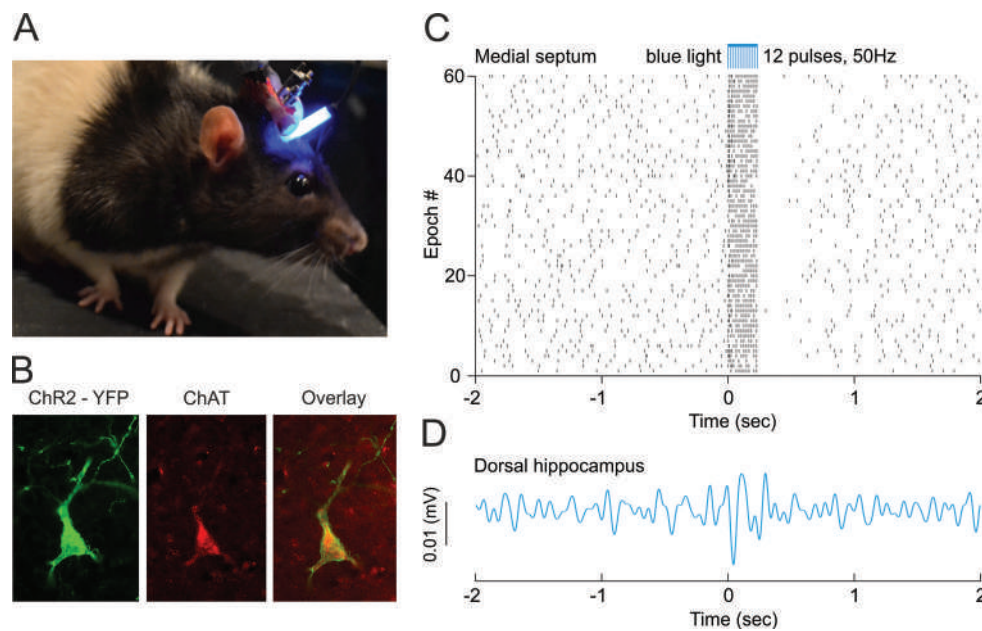


Figure 1: Optogenetic laser stimulation of ChAT cells in transgenic rats. **A.** Rat from (Chat)::Cre line is injected with adeno-associated virus carrying channelrhodopsin-yellow fluorescent protein (ChR2-YFP) viral vector and implanted with recording electrodes and optogenetic fiber in the medial septum. **B.** Colocalization of ChAT staining and ChR2-YFP expression in the medial septum. High-magnification view of ChR2-YFP expression in ChAT-positive septal cell body after injection of cre-dependent virus in the medial septum of ChAT::Cre rat. **C.** Blue laser light triggers neuronal spiking in medial septum. Raster plot from 60 repetitions of optically evoked time-locked responses of ChAT cell. Time 0 indicates the delivery of the first train of the stimulation protocol (50 Hz, 5 ms pulse duration, 12 pulses, 473 nm). **D.** Hippocampal local field responses are regulated by septal ChAT stimulation. Sample band-pass filtered (4–15 Hz) event-related potential is recorded in dorsal CA1 in parallel to the septal optogenetic stimulation. Adapted from (Tsanov and Mamad, 2014).

native dura of in primates requires the application of large-diameter cannulae for virus injection and optical fiber placement, which causes significant damage to the brain tissue. An important advance resolving this obstacle is the recent study that applied optogenetics in the behaving monkey after replacement of the native dura with a transparent artificial dura [148]. This approach allowed the use of fine glass micropipettes to inject virus and transdural illumination with minimal invasive damage of the brain tissue. The neuronal damage related to the lowering of the optical fibers into the brain was substantially reduced, permitting the precise inspection of the underlying cortical micro-vasculature during the optogenetic intervention [148]. Currently, the optogenetic modulation of neural activity in primates is successfully applied in several laboratories [109,143,148–151] and this progress is crucial to our understanding of complex behavior and higher cognitive function. The application of optogenetics to the non-human primate is a key step in ultimately developing this methodology for use in humans. The successful application of optogenetics in primates points the way toward the potential use of optical control in a new generation of therapies for the improvement of human health. The application of optogenetics in primates is a revolutionary development, but a serious challenge remains—the invasive nature of this technique suggests surgical procedures and risk of post-surgical complications in humans. The side effects of optical fiber implantation could be related to brain lesion, neural morphology changes, glial inflammation and motility, or infections [152–154]. Less invasive strategies that do not

require an implanted optical device would crucially increase experimental convenience in optogenetics for clinical testing. A recent study provides cutting-edge methodology for non-invasive application of optogenetics [155]. The authors engineered and characterized Jaws, a spectrally shifted cruxhalorhodopsin derived from the species *H. salinarum* (strain Shark), which mediates strong red light-driven optogenetic neural inhibition. Red light penetrates deeper into tissue than other visible wavelengths, enabling a non-invasive transcranial inhibition of neurons in brain structures up to 3 mm deep [155]. These data show that Jaws can noninvasively mediate transcranial optical inhibition of neurons deep in the brains of awake rodents. Optogenetic inhibition of the electrical activity of neurons is particularly advantageous for the dissection of network components. For the identification of neuron types, light suppression of should be the preferred method since it avoids the synchrony-induced spike superimposition [156–158]. Additionally, Jaws enabled significantly more inhibition of stimulus-evoked neural activity than possible with previous halorhodopsins. This approach demonstrates the ability of novel optogenetic tools to affect larger tissue volumes, allowing the causal analysis of neural circuit component contributions to brain functions and behaviors. Jaws is capable of powerful optical hyperpolarization and suppression of visually evoked neural activity in mice cone photoreceptors, restoring the sensitivity to light in retinas of retinitis pigmentosa model mice [155]. This outstanding approach serves as a potential therapy for patients with cone photoreceptor atrophy [155]. The optogenetics

methodology would allow the resensitization of the cone cells to light in patients with retinitis pigmentosa [159]. By genetically expressing light-activated hyperpolarizing ion pumps in the cone photoreceptors, the response to light and vision would be restored in patients with photoreceptor degeneration. The non-invasive optogenetic inhibition mediated by Jaws offers a powerful tool for the regulation of selective neuronal activity in for basic and clinical neuroscience. Finally, the methodological advances in optogenetics should lead to further the development of therapies for various neurological and neuropsychiatric diseases in humans.

CONCLUSION

Fundamental findings in the last 15 years led to the development of the optogenetic techniques, allowing selective manipulation of neuronal activity. The selective neuronal activation or inhibition can be restricted to the cholinergic neurons and regulation of the cholinergic system permits efficient control of the signal processing in the hippocampal formation. Stimulation-induced regulation of these regions would help us understand the mechanisms of their dysfunction and would aid the therapy of neurodegenerative brain dysfunctions. The latest advances in optogenetics demonstrate the enormous potential of optogenetic tools in the treatment of neurological disorders.

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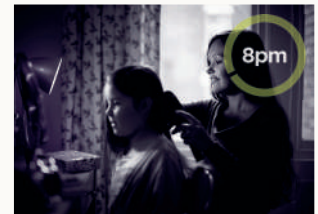
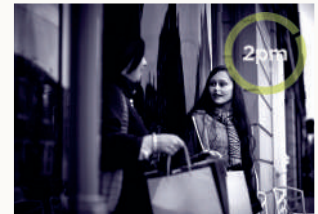


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Dosage and Administration: Children (aged 6 years and over) and adolescents: For all patients the starting dose is 30mg taken once daily in the morning. The dose may be increased by 20mg increments, at approximately weekly intervals. ELVANSE should be administered orally at the lowest effective dosage. The maximum recommended dose is 70mg/day; higher doses have not been studied. **Administration:** ELVANSE may be swallowed whole, or the capsule opened and the entire contents emptied and mixed with a soft food such as yoghurt or in a glass of water or orange juice. If the contents include any compacted powder, a spoon may be used to break apart the powder in the soft food or liquid. The contents should be stirred until completely dispersed. The patient should consume the entire mixture of soft food or liquid immediately; it should not be stored. **Long-term Use:** Pharmacological treatment of ADHD may be needed for extended periods. The physician who elects to use

ELVANSE for extended periods (over 12 months) should re-evaluate the usefulness of ELVANSE at least yearly, and consider trial periods off medication to assess the patient's functioning without pharmacotherapy, preferably during times of school holidays. **Contraindications:** Hypersensitivity to sympathomimetic amines or any of the excipients, concomitant use of monoamine oxidase inhibitors or within 14 days after MAOI treatment, hyperthyroidism or thyrotoxicosis, agitated states, symptomatic cardiovascular disease, advanced arteriosclerosis, moderate to severe hypertension, glaucoma. **Warnings:** Stimulants including ELVANSE have a potential for abuse, misuse, dependence or diversion for non-therapeutic uses that physicians should consider when prescribing these products. Stimulants should be prescribed cautiously to patients with a history of substance abuse or dependence. Monitor cardiovascular status carefully as sudden cardiac or unexplained death has been reported. Monitor psychiatric status as treatment may exacerbate symptoms of behaviour disturbance and thought disorder in patients with pre-existing psychotic disorders. Particular care should be taken when using stimulants to treat ADHD patients with comorbid bipolar disorder because of concern for possible induction of mixed/manic episode. ELVANSE is associated with worsening or emergence of aggressive behaviour, onset or exacerbation of tics, worsening of Tourette's syndrome, worsening of pre-existing anxiety, agitation or tension. Use with caution in epileptics as may increase frequency of seizures. **Precautions:** Monitor weight, growth, blood pressure. Difficulties with accommodation and blurring of vision have been reported with stimulant treatment. ELVANSE should be used with caution in patients who use other sympathomimetic drugs. The least amount of ELVANSE feasible should be prescribed or dispensed in order to minimise the risk of possible overdose by the patient. **Interactions:** Extended-release guanfacine and venlafaxine, ascorbic acid and other agents that acidify urine, sodium bicarbonate and other agents that alkalinise urine,

monoamine oxidase inhibitors, antihypertensives, narcotic analgesics, chlorpromazine, haloperidol, lithium carbonate. **Pregnancy and Lactation:** Elvanse should only be used during pregnancy if the potential benefit justifies the potential risk to the foetus. Elvanse should not be used during breast-feeding. **Driving:** Caution is advised. **Adverse Effects:** Very common: Decreased appetite, insomnia, headache, upper abdominal pain, weight decreased. Common: Anxiety, tic, affect lability, aggression, dizziness, restlessness, tremor, somnolence, tachycardia, palpitation, dyspnoea, dry mouth, diarrhoea, constipation, nausea, vomiting, rash, irritability, fatigue, pyrexia, feeling jittery, upper abdominal pain, blood pressure increased. Consult SmPC in relation to less common side effects. **Pharmaceutical Precautions:** Store below 25°C. **Date of Revision:** January 2015. **Name and Address of MA Holder:** Shire Pharmaceutical Contracts Limited, Hampshire International Business Park, Chineham, Basingstoke, Hampshire RG24 8EP, United Kingdom. Further information is available on request. ELVANSE is a registered trade name.

Adverse events should be reported to Shire at: globalpharmacovigilance@shire.com

References:

1. Wigal SB et al. Child Adolesc Psychiatry Ment Health 2009; 3(1): 17.
2. Elvanse® SmPC. Shire. January 2015.
3. Biederman J et al. Clin Ther 2007; 29: 450-463.

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