 doi:10.20944/preprints202209.0412.v1

Review

Intertwining Neuropathogenic Impacts of Aberrant Circadian Rhythm and Impaired Neuroregenerative Plasticity in Huntington's Disease: Neurotherapeutic Significance of Chemogenetics

Sowbarnika Ravichandran[1], Ramalingam Suhasini[2], Sudiksha Madheswaran Deepa[1], Divya Bharathi Selvaraj[1], Jemi Feiona Vergil Andrews[1], Viruthachalam Thiagarajan[2,3,*] and Mahesh Kandasamy[1,3]

[1]Laboratory of Stem Cells and Neuroregeneration, Department of Animal Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli-620024, Tamil Nadu, India
[2]Photonics and Biophotonics Lab, School of Chemistry, Bharathidasan University, Tiruchirappalli-620024, Tamil Nadu, India
[3]Faculty Recharge Programme, University Grants Commission (UGC-FRP), New Delhi-110002, India.
* Correspondence: vthiags@gmail.com; v.thiagarajan@bdu.ac.in(V.T.); pkmahesh5@gmail.com, mahesh.kandasamy@bdu.ac.in(M.K.)

Abstract: Huntington's disease (HD) causes progressive movement disorders and cognitive deficits. Besides, sleep disturbances and emotional distress are prominent clinical signatures of HD. The experimental subjects and HD human brains display altered regenerative plasticity resulting from aberrant neurogenic and nonneurogenic areas. Sleep disorders, emotional disruption, and cognitive deficits have been linked to impaired cell cycle events of neural stem cells (NSC) in neurodegenerative disorders. In a physiological state, circadian clock gene pathways play important roles in the regulation of the proliferation and differentiation of NSC, whereas the irregular circadian clock pathway is attributed to impairment in the neurogenic process. The recent advancement of chemogenetic-based approaches represents a potential scientific tool to rectify the abnormal circadian clock which may aid in mitigating neurogenic failure in the brain. Notably, GABAergic vasoactive intestinal peptide (VIP)-expressing neurons in the brain plays a key role in the regulation of neuroplasticity and circadian rhythm. Thus, this conceptual review article addresses the potential link between sleep disorder and aberrant neurogenic events in HD and proposes chemogenetic kindling of vasoactive intestinal peptide (VIP) -expressing GABAergic neurons in the brain as a therapeutic strategy for reprogramming the clock gene pathways in mitigating the neurodegenerative failure in HD.

Keywords: Huntington's disease; circadian rhythm; clock genes; adult neurogenesis; chemogenetics

 doi:10.20944/preprints202209.0412.v1

Introduction

Huntington's disease (HD) is an incurable autosomal dominant hereditary neurodegenerative disorder [1]. An abnormal polyglutamine (poly Q) expansion in the huntingtin (HTT) protein resulting from more than 40 CAG repeats in the exon1 of the *HTT* gene has been linked to progressive degeneration of medium spiny GABAergic neurons primarily in the basal ganglia [1,2]. Besides, HD has been characterized by reactive astrogliosis and microglial activation responsible for neuroinflammation in the very functional areas of the brain including the striatum and hippocampus [3]. The neuropathogenic events in HD lead to irreversible dyskinetic movement, cognitive decline, and mood disorders [4]. Moreover, induced stem cell quiescence and reactive neuroblastosis, and impaired neuronal differentiation in the hippocampus have become increasingly evident in experimental animal models of HD [5–9]. Ample research reports indicate that ongoing hippocampal neurogenesis plays an important role in learning, memory, and mood stabilization in the physiological brain [10]. Whereas neurological deficits, memory loss, and mood disorders observed in many neurodegenerative disorders have been attributed to a neurogenic failure in the hippocampus [11,12]. Therefore, boosting the hippocampal neurogenesis represents a valid regenerative therapeutic approach in HD. In order to maintain good mental health, sufficient sleep is an essential physiological function, while prolonged sleep deprivation or chronic sleep disturbances are potent risk factors for escalating mood disorders and memory loss [13]. The expression of the m*HTT* gene interrupts the molecular pathways of clock genes leading to the aberrant circadian rhythm in HD [14–16]. In a physiological state, circadian clock gene pathways play important roles in the regulation of the proliferation and differentiation of NSC, whereas impairment in the neurogenic process is attributed to the irregular circadian clock pathway [17,18]. Therefore, abnormal

 doi:10.20944/preprints202209.0412.v1

regulation of hippocampal neurogenesis and irregular circadian rhythm can collectively contribute to intertwining pathogenicity leading to mood disorders and cognitive deficits in HD. Considering the facts, it can be proposed that the reversal of irregular circadian rhythm might contribute to brain repairs and upregulating regenerative plasticity in HD.

GABAergic vasoactive intestinal peptide (VIP)-expressing neurons in the suprachiasmatic nucleus (SCN) of the hypothalamus plays a key role in the regulation of circadian rhythm, while degeneration or functional defects in the VIP neurons of SCN due to neuropathogenic events and improper sensory inputs can trigger abnormal circadian rhythmicity [19–21]. These VIP neurons play an important role in the control of GABAergic transmission responsible for synaptic plasticity of the pyramidal neurons in the hippocampus [22,23]. Thus, dysregulation of GABAergic transmission resulting from mHTT protein might be overlapped with altered expression and functions of VIP leading to neuro regenerative failure in HD. Therefore, the implementation of strategies that aids in the restoration or activation of the VIP neurons in the brain could contribute in rectifying sleep disorder and regenerative failure in HD. Chemogenetics has been established potent molecular tool to specifically regulate the intracellular signalling pathways [24,25]. Therefore, this article describes the potential overlap between the pathological sleep discord and aberrant neurogenesis noticed in the brains of subjects with HD and emphasizes chemogenetic activation of GABAergic neurons expressing VIP in the brain as a therapeutic strategy to rectify the aberrant clock gene pathways by which neurodegenerative failure is expected to be reversed in HD.

 doi:10.20944/preprints202209.0412.v1

Regulation of circadian rhythm in physiological state

Circadian rhythm represents a biological chronometer of the living system that regulates, intertwines, overlaps, and synchronizes various physiological, biochemical, cellular, and genetic events, in response to the gut-brain axis, atmospheric temperature, and different sensory inputs from light and dark conditions [26–28]. In mammals, the periodic modulation of circadian rhythm has been tightly linked to both the photic and non-photic stimuli [29]. In the eyes, retinal ganglion cells (RGCs) express the photopigment known as melanopsin, a key photoreceptor that mediates the non-image-forming functions of the light and pupillary light reflexes [30]. Similarly, to rods and cone cells, RGCs are also intrinsically photosensitive units that play a key role in transmitting photic signals from the eyes to SCN through optic chiasma [31] (Fig 1). SCN is compartmentalized into the dorsal shell and ventral core subdivisions and receives inputs from three afferent pathways, namely the retinohypothalamic tract, the genicular-hypothalamic tract, and a compact serotonergic plexus [32]. Eventually, the efferent projections of SCN target the pineal gland through the VIP [33]. VIP is a major neuropeptide that is widely expressed in the gut, pancreas, and brain [34]. In particular, the VIP neurons are highly present in various regions of the brain including the cerebral cortex, amygdala, septum, hippocampus, thalamus, and hypothalamus [35,36]. VIP acts through VPAC1 and 2 receptors to stimulate the secondary messengers cAMP, and PKA signaling cascade and presynaptically enhance gamma-aminobutyric acid (GABA) release in the neuronal population brain [36]. VIP receptors are widely present in the GABAergic interneurons of the hippocampus and VIP-mediated enhancement of synaptic transmission to CA1 pyramidal cells involves inhibition of GABAergic interneurons that controls the synaptic plasticity of the pyramidal neurons [36]. In the SCN, VIP neurons present contribute an important role in synchronizing the circadian cycle [37]. VIP-secreting neurons are mainly located in the ventrolateral area of the SCN, which

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 September 2022 doi:10.20944/preprints202209.0412.v1

receives the environmental input from the optic chiasm through the retinohypothalamic tract and play a vital role in regulating the circadian cycle [20,38,39]. The release of VIP from neurons of SCN regulates the biosynthesis of melatonin in the pineal gland [40]. VIP acts through VPAC2 receptors to stimulate the secondary messengers cAMP, and PKA signalling cascade and presynaptically enhance the release of GABA release in the neuronal population of SCN [36]. The synergistic coactivation of VIP and GABAergic pathways in the brain has been identified as a key step in priming the molecular oscillation responsible for the circadian rhythm [19]. Thus, inactivation or defects in VIP neuronal pathway appears to be a key determinant of circadian rhythm dysfunction seen in many pathogenic conditions resulting from GABAergic dysfunction including HD.

Fig 1

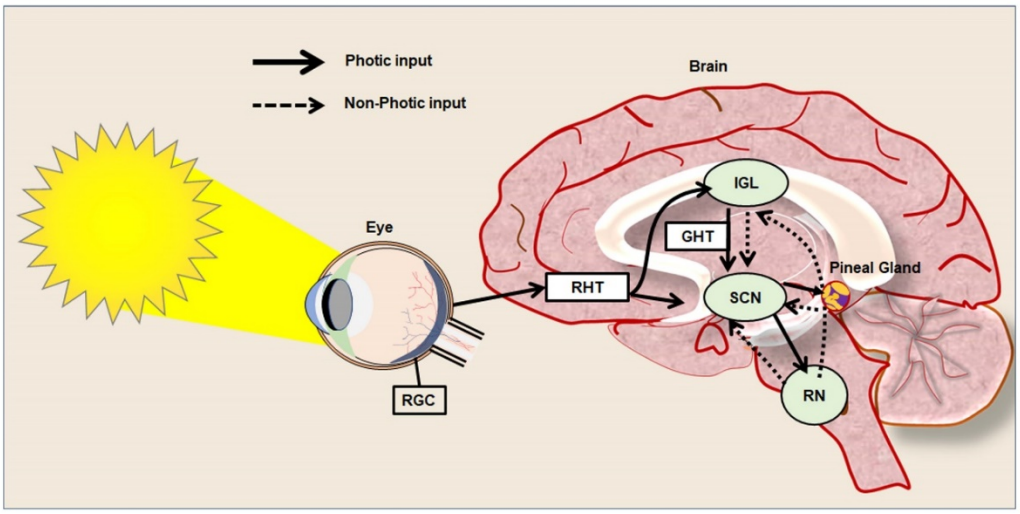


Fig 1 Photic and non-photic input of circadian rhythm in the healthy brain.

The digital diagram represents the photic and non-photic inputs from the retina to the brain and neural pathways among the hypothalamus, pineal gland, and raphe nucleus that regulate circadian rhythm in a healthy brain. The straight line represents the photic inputs and the dotted line represents the non-photic inputs.

Neuropathogenic input of abnormal regulation of clock genes in HD

 doi:10.20944/preprints202209.0412.v1

While HD patients have been found to display decreased activity during day time, they show increased activity during the night time thereby [41]. Polysomnographic and actigraphic findings in HD patients indicate frequent eye and leg movements during sleep [42]. Several neuroimaging studies of the hypothalamus have revealed prominent neuropathological alterations in SCN in corroboration with abnormal sleep-wake cycles in HD [41]. Aziz NA et al. reported that there is a delay in the release of melatonin from the pineal gland in HD patients due to abnormal neurotransmission in SCN [43]. The drosophila model of HD has been noticed to exhibit sedentary behaviours as a reflection of impaired circadian rhythm [44]. Experimental data gathered from the sheep model of HD reveals that the sleep disorder resulting from abnormal circadian rhythm is an early sign of the onset of the disease [16]. Kuljis DA et al. indicated that the expression of the m*HTT* in the brain is responsible for sleep disorder in a bacterial artificial chromosome-based transgenic mouse model of HD [45,46]. Also, the R6/2 mouse model of HD exhibits progressive disruption in the circadian rhythm leading to reduced physical activity and sluggish behavior [47]. Loh DH et al. observed progressive deterioration of motor function in association with altered sleep patterns due to defects in the circadian rhythm in the Q175 mouse model of HD [15]. Besides, experimental subjects with HD have been reported to display depression in association with altered circadian rhythm leading to a considerable level of a progressive form of memory deficits [48]. Considering the aforementioned facts, insights into mHTT-mediated dysregulation of circadian clock genes pathway in HD has become an important scientific quest.

Circadian rhythms have been known to be regulated by key clock genes such as Period1 and 2 (Per1/2), Cryptochrome1/2 (Cry1/2), Brain and muscle Arnt-like protein 1 (Bmal1), and Circadian Locomotor Output Cycle Kaput (CLOCK) [49–51]. Bmal1 functions as a transcriptional activator in the heterodimeric form in

 doi:10.20944/preprints202209.0412.v1

the cytoplasm and it enters the nucleus and binds with the promoter region of Per and Cry called E-box to regulate the expression of various genes [52,53]. Recently, a gene knockout study in embryonic stem cells (ESCs) indicates that the Bmal1/CLOCK gene regulates the transcription of REV-ERBα/β, which play an important role in neuronal growth, lipid metabolism, and inflammatory processes [54]. Cry1 and Bmal1/CLOCK also modulate the feedback loop of D-box binding protein and interleukin-3-regulated protein which is also important for the regulation of neuroplasticity [55,56]. Clock genes have also been involved in the non-circadian phenotypes such as regulation of immune cells, metabolic pathways, and their loss of function which leads to abnormal aging and progression of malignant disorders [49]. Notably, the genetic ablation of Per gene in the drosophila model has been reported to induce mitochondrial dysfunction and oxidative stress leading to prominent neurodegeneration in the brain [57]. Besides, the Per mutant mouse model has been reported to display abnormal mitotic events due to defects in tumor suppressor genes thereby, indicating the roles of circadian clock genes in cell cycle control [58]. In addition, an experimental mouse model with conditional deletion of the Bmal1 gene in the excitatory forebrain neurons has been reported to exhibit cognitive impairments [59]. Besides, several experimental studies reported that aberrant expression or dysfunction of clock genes leads to cognitive impairment, movement, and mood-related disorders in many neurodegenerative conditions including HD [60].

HD has been characterized by dysfunctions in the transcriptional regulation of clock genes, which in turn are considered to be an initial trigger for various neuropathogenic changes and altered mental states [41,48,61]. The abnormal sleep patterns noticed in the fly model of HD have been reported to be linked with alteration in the transcription of clock genes [14]. Abnormal sleep-wake disorders noticed in the R6/2 mouse model of HD

 doi:10.20944/preprints202209.0412.v1

have been reported to be associated with aberrant expressions of Per2 and Bmal1 in the striatum and SCN [47,48]. Moreover, R6/2 mouse models have also been characterized by low levels of VIP expression and its receptor VIPR2 in the brain [62]. Alteration in the metabolic events in the liver of R6/2 mouse has been reported to be associated with abnormal expression of Cry1, D site of albumin promoter Binding Protein (DBP), and Per2 [47]. Further, Bmal1 knockout mouse has been characterized by gliosis, neuronal loss, degeneration of presynaptic terminals, and decreased neural connectivity upon 3-nitropropionic acid-induced acute HD condition [63,64]. Notably, the supplement of sleeping pills in the R6/2 mouse has been reported to revert the function of Per2 resulting in significant improvement in cognitive performance [65,66]. While the involvement of clock genes in neuroplasticity has been increasingly noticed, prolonged sleep disruption and expression of the mutant *HTT* gene have been known to interfere with the regulation of neuro regenerative plasticity [67] (Fig 2). Therefore, regulation of neurogenesis in stem cell niches of the brain can be expected to be linked with the expression of the clock genes.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 September 2022 doi:10.20944/preprints202209.0412.v1

Fig 2

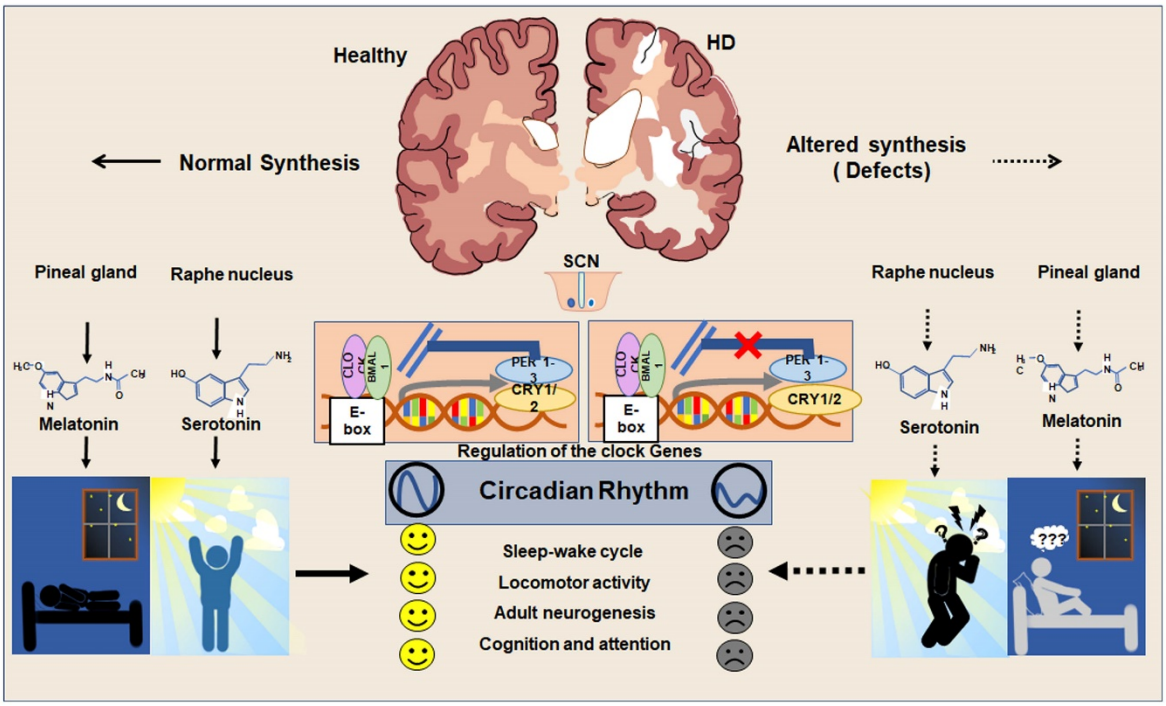


Fig 2 Regulation of circadian clock in Healthy and HD brains.

In a healthy brain, normal secretion of melatonin and serotonin takes place to ensure the proper sleep-wake cycle and neuroplasticity. In the HD brain, the secretions of melatonin and serotonin are altered and the regulation of clock genes is impaired, resulting in depression, cognitive deficits, and impaired neurogenesis

Potential overlap between altered clock gene pathway and dysregulation of neuro regenerative plasticity in HD

Experimental evidence indicates that mitosis of NSCs in the adult brain mostly occurs at night in experimental animals due to nocturnal behavior [18,68]. HD has extensively been characterized by aberrant neurogenesis in the hippocampus, striatum, and OB [5,6,8,12,69]. Abnormal neurogenic events such as stem cell quiescence, reactive neuroblastosis, and defect in maturation and integration of new-born neurons have been well documented in the experimental models of HD [5–8,12]. The occurrence of adult neurogenesis has also been

 doi:10.20944/preprints202209.0412.v1

recognized in other brain regions including the cortex, striatum, amygdala, spinal cord, hypothalamus, and the brain stem [70,71]. Among them, understanding the regulation and functional roles of neurogenesis in the hypothalamus has become increasingly important as it appears to take part in many neurophysiological pathways that overlap with many physiological functions [72]. However, direct evidence for the regulation of neurogenesis in the hypothalamus in HD remains limited. While regulation of neurogenesis in the hippocampus has been known to be drastically affected by many neurodegenerative diseases including HD, reports on modulation of neurogenesis in the hypothalamus under the influence of pathogenic conditions remain limited. An immunohistochemical study by Gabery S et al. revealed that the decline in the number of vasopressin- and oxytocin- expressing neurons noticed in the hypothalamus of the post-mortem human HD brain might be due to mutant *HTT*-mediated impaired neurogenesis [73]. Depression and anxiety-like behaviours have been reported to occur in subjects with HD due to the dysregulation of neurogenesis and abnormal neuroplasticity in the hypothalamus [74,75]. The hypothalamic neurogenesis has been noticed to be controlled indirectly by the clock genes like Bmal1, Per1, and Per2, while the glial differentiation was found to be high in the neurospheres derived from mice lacking Cry1 and Cry2 genes [18]. The Bmal1 deficient mice show premature aging, neurodegeneration, and cognitive deficits along with a reduced level of NSC proliferation and impaired migration of neuroblasts in the brain [76]. Reduced cell proliferation and a lower number of secondary neurospheres have also been observed from the NSC isolate with the absence of both Cry1 and Cry2 circadian clock proteins [18]. Moreover, deletion of Per2 has also been reported to induce the cell cycle exit of NSCs [77]. Thus, defects in the regulation of the clock gene pathway seen in HD can be attributed to aberrant neurogenesis in different brain regions including the hypothalamus. Therefore, correcting the defective clock gene pathway could be one of the valid strategies to boost neuro regenerative

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 September 2022 doi:10.20944/preprints202209.0412.v1

plasticity, thereby compensating for neurodegeneration in HD. Recently, optogenetics and chemogenetics-based technologies have been considered to have the advantage to modulate the aberrant clock gene pathways in the brain [78]. Therefore, the implementation of tailored scientific strategies that rectify the clock gene pathway and promote neuroregenerative plasticity might provide a valid treatment option for HD (Fig 3).

An overview and significance of optogenetics and chemogenetics-based experimental interventions for neuronal activities

Optogenetics refers to the cutting-edge technology of implementing light to examine and regulate gene expression as well as desired cellular functions in intact living systems [79]. A specific wavelength of light has been used to activate or deactivate a subset of the neuronal populations that are genetically modified to produce light-responsive proteins called opsins [80]. In particular, the primary light-sensitive opsins like channelrhodopsin and halorhodopsin have extensively been implemented for the experimental exploitation in animals for which, implantation of a regulatable light delivery device in the specific brain area is mandatory to attain high spatiotemporal resolution [81]. While degeneration of GABAergic medium spiny neurons has been ascertained as the unique underlying cause of HD, selective optogenetic stimulation provoked GABAergic transmission in the somatostatin-positive striatal interneurons in R6/2 mice [82]. Using the optogenetic method, Cepeda et al. revealed that abnormal GABAergic transmission in the striatum of the transgenic animal models of HD occurs due to defects in multiple neuronal populations [83]. Another study found that wireless optogenetic stimulation of GABAergic neurons, upon electrographic detection of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 September 2022 doi:10.20944/preprints202209.0412.v1

spontaneous hippocampal seizures resulted in shorter seizure durations in patients with temporal lobe epilepsy [84]. Though optogenetics can be proposed to correct the abnormal GABAergic transmission in HD, optogenetic methods have been associated with certain practical difficulties when it comes to an implication in humans as it involves neuro-invasive experimental procedures like implantation of optical fiber into the brain. The effective operation of the optical fiber generates heat that could be detrimental to the tissue [85]. Eventually, penetration of light to the region located deep in the brain tissue is limited by the scattering effect and this issue may be overcome by using longer wavelengths of light [86] (Fig 3). Yet another limitation of optogenetics is the non-specific transfection of optogenes in neurons and the fact that implantation of optrodes requires prolonged anesthesia that could also lead to some adverse effects [87]. Therefore, the recent advancement in scientific strategies like chemogenetics appears to have an alternative to optogenetics as it overcomes key limitations associated with conventional invasive neurosurgeries

Fig 3

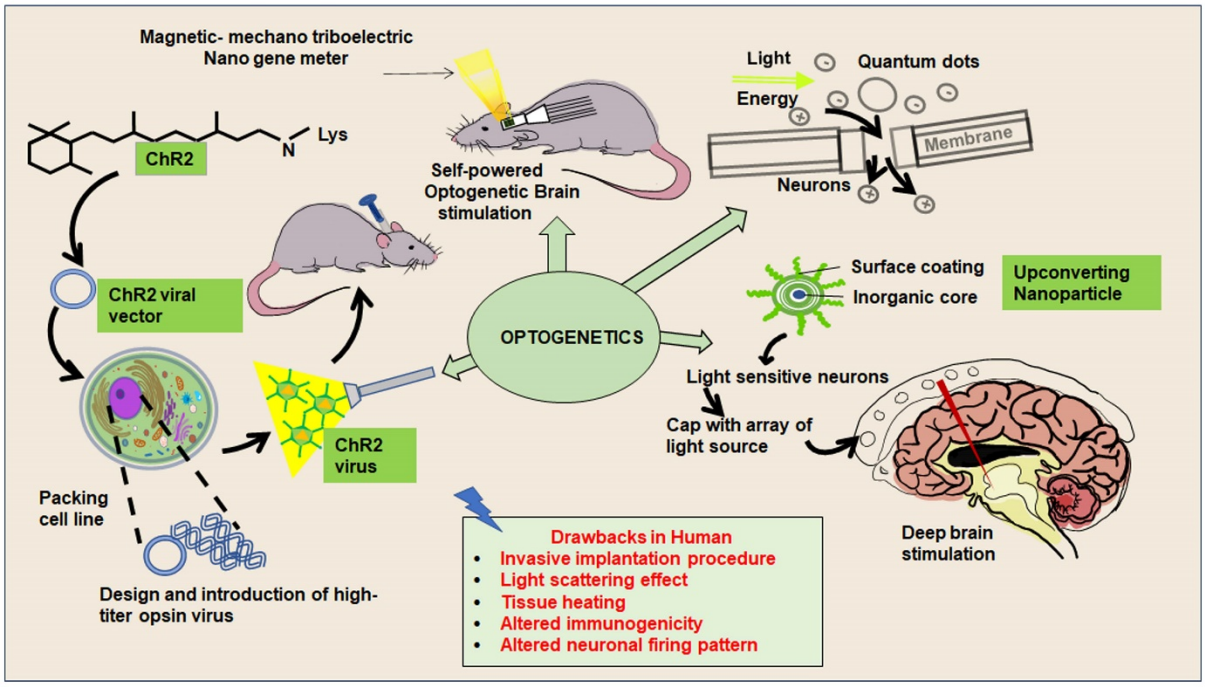


Fig 3 Optogenetic modulation of neurons in the experimental brains

 doi:10.20944/preprints202209.0412.v1

The graphical illustration describes various optogenetic approaches to modulate neurons in experimental brains. Magnetic-mechano triboelectric nano gene meter is inserted into mice to stimulate neurons. Channelrhodopsin-2 viral vector injected into the brain which is modulated by blue light led to neuronal excitation, the introduction of Quantum dots (QDs) and upconversion nanoparticles (UCNPs) which are illuminated by NIR lights into the deep brain region can stimulate the neuronal activity, and in response to this, light-gated ion channels are opened upon the emission of visible light of specific wavelength by QDs and UCNPs.

Chemogenetics involves the administration of inert exogenous ligands that selectively target the non-immunogenic synthetic receptors or enter through ion channels to influence the desired signalling pathways in a given cell type [88]. Unlike optogenetics, chemogenetics does not require the invasive implantation of a light device. Different types of synthetic genetic adducts, cytoplasmic enzymes, and membrane-spanning receptors have been utilized for the operation of chemogenetics [89]. The genetically engineered proteins such as receptors activated solely by a synthetic ligand (RASSL) and designer receptors exclusively activated by designer drugs (DREADDs) are modified forms of G protein-coupled “designer” receptors that have low affinity for their endogenous ligand, but a high affinity for selective exogenous ligands such as clozapine-N-oxide (CNO) or salvinorin B (SALB) [88,90,91]. Recently, Deschloroclozapine (DCZ), is highly selective for hM3Dq and hM4Di DREADDS with good brain concentration profiles, thus providing good chances for multiplexed/bimodal control of physiological systems along with side-effect-free brain theranostics. These exogenous ligands can be administered locally or systemically to induce or deactivate the cellular activities, metabolism, and downstream signalling cascades through the modulation of the activities of DREADDs and RASSL [88]. Especially, the efficacy of different types of DREADDs is currently being pursued to identify the neural pathways related to cognition, motor functions, emotions, drug addiction, and drug abuse in various experimental models including non-human primates [88,91,92]. For the modulation of neurotransmission in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 September 2022 doi:10.20944/preprints202209.0412.v1

the brain, the mutant forms of human muscarinic acetylcholine receptors such as Gq-coupled human M3 muscarinic DREADD (hM3Dq), human M4 muscarinic DREADD (hM4Di), Gs-DREADD (GsD), Rq (R165L) and kappa-opioid-receptors (KORD) have been used as DREADDs which can be regulated by respective exogenous ligands [93].

Chemogenetics-mediated neuromodulation by stereotactic delivery of adeno-associated virus (AAV) vectors containing hM3D (Gq) or Human synapsin **(**hSyn)-hM4D(Gi) into different sites of basal ganglia significantly improved motor performance in 6-OHDA model of Parkinson's disease (PD) [94]. A prominent experimental suppression of the hyper neural activities by infusion of AAV vectors encoding hM4Di-DREADD into the subarachnoid space and hippocampus followed by CNO treatment has been reported to decrease the Aβ aggregation in a transgenic animal model of Alzheimer's disease (AD) [95]. Functional neuroimaging and behavioural studies revealed that injection of an adenoviral vector carrying a hM3Dq DREADD into the vitreous induced abnormal neural activates and anxiety-like symptoms in association with altered signatures of circadian rhythm in Opn4Cre/+ mice expressing Cre-dependent melanopsin in the RGCs [96]. While the synthetic ligands have been known to cross the blood-brain barrier (BBB), intracranial injections of recombinant AAV vectors encoding Gq-DREADDs have initially been considered for selective neuronal transfection in the human brain [97]. To overcome stereotactic injection-related adverse issues, non-invasive AAV delivery by microbubble-enhanced focused ultrasound (FUS) waves used at specific locations in the brain has been proposed [98]. Besides, a non-invasive in vivo retrograde gene delivery strategy modulates neuronal subpopulation in the brain from the periphery using AAV vectors encoding chemogenetic receptors [99]. Furthermore, in chemogenetic platforms, positron emission tomography (PET) has been used for the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 September 2022 doi:10.20944/preprints202209.0412.v1

non-invasive measurement of the expression and anatomic site of chemogenetic receptors as well as detection of radiolabelled clozapine and other ligands [25]. The chemogenetic approach has an advantage over deep brain stimulation, which is presently used to treat symptoms of parkinsonism, in which it eliminates the need for a permanent stimulating electrode implant while maintaining scalable control over neuromodulation via the dosage of the chemogenetic effector drug [25]. Considering the aforementioned facts, FUS and retrograde gene delivery strategy-based chemogenetics can be non-invasively implemented to restore the defects in the circadian rhythm (Fig 4).

Fig 4

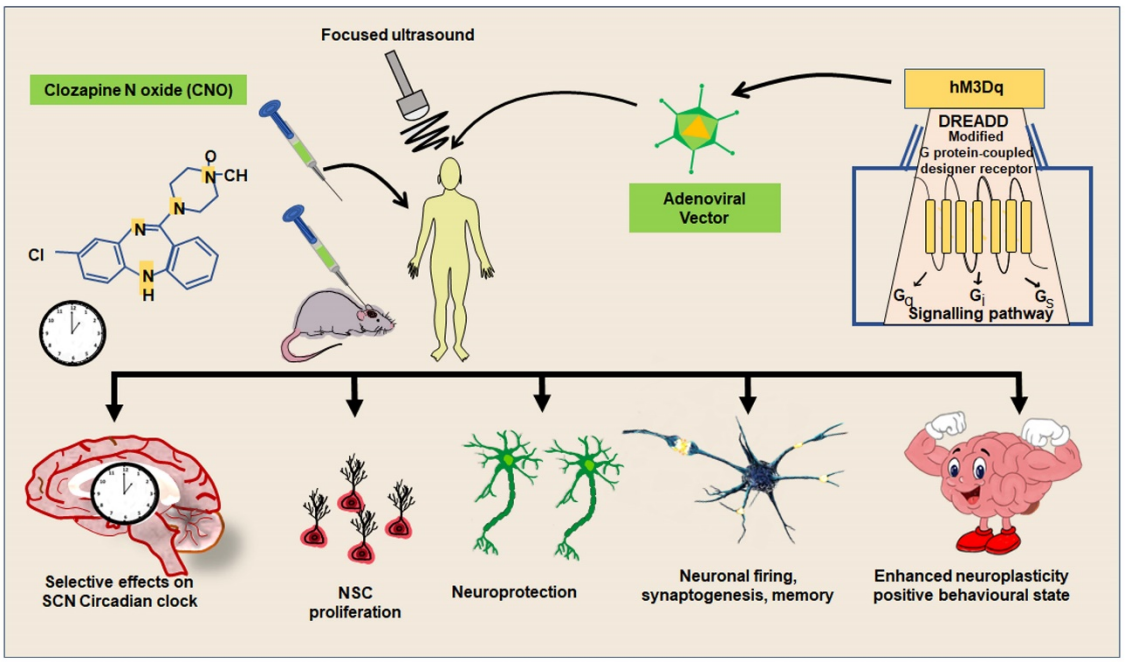


Fig 4 The regulation of neuronal activity by chemogenetics

The figure indicates the different strategies of chemogenetics. hM3Dq DREADD is inserted into the viral vector and surgically injected into the brain. The entry of DREADD into specific brain regions can be improved by focused ultrasound. The DREADD-infected neurons can be activated by CNO, which is given orally or via injection. This can result in increased neuronal firing, NSC proliferation, and neuroplasticity thereby improving mental health and behavioral outcomes along with selective effects on the circadian clock.

Discussion

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 September 2022 doi:10.20944/preprints202209.0412.v1

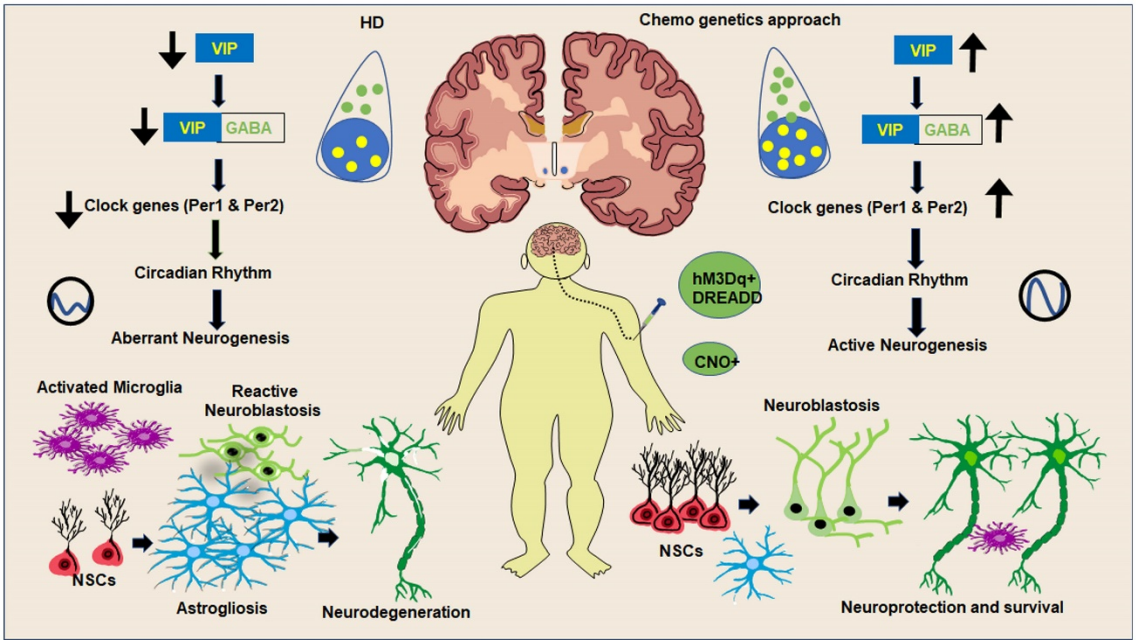
Chemogenetic-innervation of VIP neurons as a subtle therapeutic strategy to rectify aberrant clock gene pathways and GABAergic system to boost neurogenesis in HD

GABAergic synaptic input plays a significant role in refining and regulating circadian rhythms in SCN [21,100]. Interestingly, VIP neurons that are present in the SCN receive direct glutamatergic input from the RGCs of the retina and transmit the photic inputs to other neurons through the co-secretion of VIP and GABA[101]. VIP has been known to synchronize circadian rhythm by regulating the expression of the core clock genes, Per1 and Per2, in the SCN of the brain [15]. Similarly, GABA appears to play a crucial role in the regulation of clock gene expression in the SCN [21,100]. While VIP has been reported to enhance the synaptic transmission of GABA in different areas in the normal brain, the expression of *mHTT* has been reported to decrease the synthesis of VIP, thereby leading to the defect in the GABAergic input in the brain of subjects with HD [62]. To note, an in-situ hybridization study of the post-mortem human HD brain indicated reduced transcriptional levels of VIP in SCN [102]. Considering the aforementioned facts, it can be presumed that decreased levels of VIP responsible for abnormal GABAergic neurotransmission in the SCN of the brain might be an underlying basis for the dysregulation of the circadian rhythm. Recent experimental evidence strongly advocates that VIP expression prevents neurodegeneration by mitigating microgliosis and producing neurotrophic factors in the brains of neurodegenerative experimental models [103,104]. Therefore, the restoration of the VIP-mediated signalling pathway in the brain might mitigate the sleep disorders seen in many diseases including HD. The VIP expressing neurons have also been known to co-express the muscarinic acetylcholine receptors [105,106]. Therefore, the implementation of chemogenetic receptors like hM3Dq-DREADD can be used to activate the VIP neurons in the SCN, through which the aberrant GABAergic inputs and dysregulation of the clock gene pathway in the SCN can be restored in HD. Many studies have

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 September 2022 doi:10.20944/preprints202209.0412.v1

demonstrated the implementation of chemogenetics in pre-clinical models of neurological disorders including PD and epilepsy [107,108]. The ligands of chemogenetic approaches like clozapine and clozapine N-oxide (CNO) are clinically approved for human trials [109]. AAV vectors are approved for phase I and phase II clinical trials by the Recombinant DNA Advisory Committee and the Food and Drug Administration (FDA) [110]. While the chemogenetic regulation of neural transmission has been a scientific intense focus, the implementation of recombinant AAV vectors encoding Gq-DREADDs for the regulation of circadian rhythm in HD has not been proposed yet. Further, the proposed chemogenetic approach to re-establish the circadian rhythm via the activation of VIP neurons can be expected to facilitate the enhanced neurogenesis in the brains of the subjects with HD, thereby the neurodegeneration seen in different areas including the hippocampus, olfactory, and hypothalamus can be replenished. With careful use of chemogenetics-based treatment/methodology would be expected to result in providing a potent spatial and temporal resolution for exact manipulation of the brain functions related to specific behaviour. Thus. the chemogenetic approach can effectively be translated to treat sleep disorders, movement disorders, and dementia in HD

Fig 5



 doi:10.20944/preprints202209.0412.v1

Fig 5 Possible therapeutic implication of chemogenetics in HD

The graphical representation illustrates the neuropathological abnormalities and defects in the VIP neurons and GABergic system, abnormal expression of clock genes, and aberrant hypothalamic neurogenesis in HD (Right side). The figure indicates the hypothesis that the activation of VIP neurons by chemogenetics through the retrograde route might restore the GABAergic inputs neuroregeneration in HD (Left side).

Conclusion and Future directions

HD has been characterized by neurodegeneration, desynchrony of the circadian rhythm, and impaired neurogenesis. The abnormal expression of mutant *HTT* genes induces hypothalamic pathology through the aberrant expression of clock genes. The altered circadian rhythm appears to be associated with the disturbed sleep-wake cycle, hormonal imbalance, and depression which lead to a considerable decline in cognitive performance, eventually leading to impaired neuro regenerative plasticity. Recent advancements in chemogenetic and optogenetic-based approaches represent a conceivable strategy to rectify the aberrant expression of clock genes in the brain. While defects in VIP neurons appear to be a key pathogenic event associated with abnormal GABAergic transmission, implementing chemogenetics could be a promising therapeutic attempt to enhance the activity of the VIP neurons in the hypothalamus as well as hippocampus by which it could be possible to realign the clock genes and circadian rhythm that are impaired in HD. Eventually, this approach could effectively aid in reinstating neuro regenerative plasticity in HD as the expression of clock genes facilitate cell cycle regulation of NSCs provoking neurogenic process in the brain. Like other treatments, limitations that arise from unknown adverse effects related to chemogenetics could not be ignored completely. Thus, the implementation of chemogenetics under the proper safety guidelines would

 doi:10.20944/preprints202209.0412.v1

be an achievable target in humans. The proposed approach could be translated to treat many other human diseases that are connected to abnormal sleep-wake cycles and aberrant neuro regenerative plasticity.

Declaration of Conflicting Interests:

The authors declare that there is no conflict of interest.

Authors' contribution

MK and VT conceived the idea, and hypothesis and contributed to the framework of the manuscript and illustrations. SR, RS, JFVA, SMD, DBS, VT, and MK wrote the initial draft. All authors contributed to the entire revision of the article and made critical comments and suggestions. All authors discussed the content and contributed to the final manuscript.

Funding

M.K. gratefully acknowledges a research grant (SERB-EEQ/2016/000639), an Early Career Research Award (SERB-ECR/2016/000741) from Science and Engineering Research Board (SERB) under the Department of Science and Technology (DST), Government of India. V.T gratefully acknowledges a research funding from the Department of Science and Technology, Nanomission, Government of India (Grant No. DST/NM/NB/2018/10(G)), Science and Engineering Research Board, Department of Science and Technology, India [Grant No. YSS/2014/00026] University Grants Commission, India [Grant No. F. 4-5(24-FRP)/2013(BSR)]. MK acknowledges MOE-RUSA 2.0 Biological Sciences, and VT acknowledges MOE-RUSA 2.0 Physical Sciences, Bharathidasan University for the financial support. J.F.V.A was supported

as Project Assistant from the project grant SERB-EEQ/2016/000639. RS was supported as JRF and SRF from the SERB (YSS/2014/00026). Divya Bharathi Selvaraj is the recipient of RUSA 2.0 project fellowship (Ref.No.BDU/RUSA 2.0/TRP/BS/Date 22/04/2021).

Acknowledgment

M.K and V.T have been supported by the University Grants Commission-Faculty Recharge Programme (UGC-FRP), New Delhi, India. The authors would like to thank Anaswara TS for skilled assistance in the digital artwork. The authors acknowledge UGC-SAP and DST-FIST for the infrastructure of the Department of Animal Science and Department of Chemistry, Bharathidasan University.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Not applicable.

References

[1] N.S. Moily, L.N. Kota, R.M. Anjanappa, S. Venugopal, R. Vaidyanathan, P. Pal, M. Purushottam, S. Jain, M. Kandasamy, Trinucleotide repeats and haplotypes at the huntingtin locus in an Indian sample overlaps

with European haplogroup a, PLoS Curr. 6 (2014). https://doi.org/10.1371/currents.hd.a3ad1a381ab1eed117675145318c9a80.

[2] M. Jimenez-Sanchez, F. Licitra, B.R. Underwood, D.C. Rubinsztein, Huntington's Disease: Mechanisms of Pathogenesis and Therapeutic Strategies, Cold Spring Harb Perspect Med. 7 (2017). https://doi.org/10.1101/cshperspect.a024240.

[3] N. Manickam, R.K. Radhakrishnan, J.F. Vergil Andrews, D.B. Selvaraj, M. Kandasamy, Cell cycle re-entry of neurons and reactive neuroblastosis in Huntington's disease: Possibilities for neural-glial transition in the brain, Life Sci. 263 (2020) 118569. https://doi.org/10.1016/j.lfs.2020.118569.

[4] F.O. Walker, Huntington's disease, Lancet. 369 (2007) 218–228. https://doi.org/10.1016/S0140-6736(07)60111-1.

[5] M. Kandasamy, S. Couillard-Despres, K.A. Raber, M. Stephan, B. Lehner, B. Winner, Z. Kohl, F.J. Rivera, H.P. Nguyen, O. Riess, U. Bogdahn, J. Winkler, S. von Hörsten, L. Aigner, Stem cell quiescence in the hippocampal neurogenic niche is associated with elevated transforming growth factor-beta signaling in an animal model of Huntington disease, J. Neuropathol. Exp. Neurol. 69 (2010) 717–728. https://doi.org/10.1097/NEN.0b013e3181e4f733.

[6] Z. Kohl, M. Kandasamy, B. Winner, R. Aigner, C. Gross, S. Couillard-Despres, U. Bogdahn, L. Aigner, J. Winkler, Physical activity fails to rescue hippocampal neurogenesis deficits in the R6/2 mouse model of Huntington's disease, Brain Res. 1155 (2007) 24–33. https://doi.org/10.1016/j.brainres.2007.04.039.

[7] M. Kandasamy, L. Aigner, Reactive Neuroblastosis in Huntington's Disease: A Putative Therapeutic Target for Striatal Regeneration in the Adult Brain, Front Cell Neurosci. 12 (2018) 37. https://doi.org/10.3389/fncel.2018.00037.

[8] M. Kandasamy, M. Rosskopf, K. Wagner, B. Klein, S. Couillard-Despres, H.A. Reitsamer, M. Stephan, H.P. Nguyen, O. Riess, U. Bogdahn, J. Winkler, S. von Hörsten, L. Aigner, Reduction in subventricular zone-derived olfactory bulb neurogenesis in a rat model of Huntington's disease is accompanied by striatal invasion of neuroblasts, PLoS ONE. 10 (2015) e0116069. https://doi.org/10.1371/journal.pone.0116069.

[9] S.E. Lazic, H. Grote, R.J.E. Armstrong, C. Blakemore, A.J. Hannan, A. van Dellen, R.A. Barker, Decreased hippocampal cell proliferation in R6/1 Huntington's mice, Neuroreport. 15 (2004) 811–813. https://doi.org/10.1097/00001756-200404090-00014.

[10] T. Toda, S. Parylak, S.B. Linker, F.H. Gage, The role of adult hippocampal neurogenesis in brain health and disease, Mol Psychiatry. 24 (2019) 67–87. https://doi.org/10.1038/s41380-018-0036-2.

[11] M. Kandasamy, M. Anusuyadevi, K.M. Aigner, M.S. Unger, K.M. Kniewallner, D.M.B. de Sousa, B. Altendorfer, H. Mrowetz, U. Bogdahn, L. Aigner, TGF-β Signaling: A Therapeutic Target to Reinstate Regenerative Plasticity in Vascular Dementia?, Aging Dis. 11 (2020) 828–850. https://doi.org/10.14336/AD.2020.0222.

[12] M. Kandasamy, R. Reilmann, J. Winkler, U. Bogdahn, L. Aigner, Transforming Growth Factor-Beta Signaling in the Neural Stem Cell Niche: A Therapeutic Target for Huntington's Disease, Neurol Res Int. 2011 (2011) 124256. https://doi.org/10.1155/2011/124256.

[13] S. Banks, D.F. Dinges, Behavioral and Physiological Consequences of Sleep Restriction, J Clin Sleep Med. 3 (2007) 519–528.

[14] A. Faragó, N. Zsindely, L. Bodai, Mutant huntingtin disturbs circadian clock gene expression and sleep patterns in Drosophila, Scientific Reports. 9 (2019) 7174. https://doi.org/10.1038/s41598-019-43612-w.

[15] D.H. Loh, T. Kudo, D. Truong, Y. Wu, C.S. Colwell, The Q175 Mouse Model of Huntington's Disease Shows Gene Dosage- and Age-Related Decline in Circadian Rhythms of Activity and Sleep, PLOS ONE. 8 (2013) e69993. https://doi.org/10.1371/journal.pone.0069993.

[16] Z. Voysey, S.V. Fazal, A.S. Lazar, R.A. Barker, The sleep and circadian problems of Huntington's disease: when, why and their importance, J Neurol. 268 (2021) 2275–2283. https://doi.org/10.1007/s00415-020-10334-3.

[17] A.A.H. Ali, C. von Gall, Adult Neurogenesis under Control of the Circadian System, Cells. 11 (2022) 764. https://doi.org/10.3390/cells11050764.

[18] A. Malik, R.V. Kondratov, R.J. Jamasbi, M.E. Geusz, Circadian Clock Genes Are Essential for Normal Adult Neurogenesis, Differentiation, and Fate Determination, PLOS ONE. 10 (2015) e0139655. https://doi.org/10.1371/journal.pone.0139655.

[19] J. Fan, H. Zeng, D.P. Olson, K.M. Huber, J.R. Gibson, J.S. Takahashi, Vasoactive Intestinal Polypeptide (VIP)-Expressing Neurons in the Suprachiasmatic Nucleus Provide Sparse GABAergic Outputs to Local Neurons with Circadian Regulation Occurring Distal to the Opening of Postsynaptic GABAA Ionotropic Receptors, J Neurosci. 35 (2015) 1905–1920. https://doi.org/10.1523/JNEUROSCI.2661-14.2015.

[20] D. Ono, K. Honma, S. Honma, Roles of Neuropeptides, VIP and AVP, in the Mammalian Central Circadian Clock, Frontiers in Neuroscience. 15 (2021). https://www.frontiersin.org/articles/10.3389/fnins.2021.650154 (accessed September 26, 2022).

[21] D. Ono, K.-I. Honma, Y. Yanagawa, A. Yamanaka, S. Honma, Role of GABA in the regulation of the central circadian clock of the suprachiasmatic nucleus, J Physiol Sci. 68 (2018) 333–343. https://doi.org/10.1007/s12576-018-0604-x.

[22] M.R. Groen, O. Paulsen, E. Pérez-Garci, T. Nevian, J. Wortel, M.P. Dekker, H.D. Mansvelder, A. van Ooyen, R.M. Meredith, Development of dendritic tonic GABAergic inhibition regulates excitability and plasticity in CA1 pyramidal neurons, J Neurophysiol. 112 (2014) 287–299. https://doi.org/10.1152/jn.00066.2014.

[23] G.F. Turi, W.-K. Li, S. Chavlis, I. Pandi, J. O'Hare, J.B. Priestley, A.D. Grosmark, Z. Liao, M. Ladow, J.F. Zhang, B.V. Zemelman, P. Poirazi, A. Losonczy, Vasoactive Intestinal Polypeptide-Expressing Interneurons in the Hippocampus Support Goal-Oriented Spatial Learning, Neuron. 101 (2019) 1150-1165.e8. https://doi.org/10.1016/j.neuron.2019.01.009.

[24] E.J. Campbell, N.J. Marchant, The use of chemogenetics in behavioural neuroscience: receptor variants, targeting approaches and caveats, Br J Pharmacol. 175 (2018) 994–1003. https://doi.org/10.1111/bph.14146.

[25] C.J. Magnus, P.H. Lee, J. Bonaventura, R. Zemla, J.L. Gomez, M.H. Ramirez, X. Hu, A. Galvan, J. Basu, M. Michaelides, S.M. Sternson, Ultrapotent chemogenetics for research and potential clinical applications, Science. 364 (2019) eaav5282. https://doi.org/10.1126/science.aav5282.

[26] S. Reddy, V. Reddy, S. Sharma, Physiology, Circadian Rhythm, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2022. http://www.ncbi.nlm.nih.gov/books/NBK519507/ (accessed September 26, 2022).

[27] C.A. Czeisler, J.J. Gooley, Sleep and circadian rhythms in humans, Cold Spring Harb. Symp. Quant. Biol. 72 (2007) 579–597. https://doi.org/10.1101/sqb.2007.72.064.

[28] V.S. Ayyar, S. Sukumaran, Circadian rhythms: influence on physiology, pharmacology, and therapeutic interventions, J Pharmacokinet Pharmacodyn. 48 (2021) 321–338. https://doi.org/10.1007/s10928-021-09751-2.

[29] E. Challet, P. Pévet, Interactions between photic and nonphotic stimuli to synchronize the master circadian clock in mammals, Front. Biosci. 8 (2003) s246-257. https://doi.org/10.2741/1039.

[30] C. La Morgia, V. Carelli, M. Carbonelli, Melanopsin Retinal Ganglion Cells and Pupil: Clinical Implications for Neuro-Ophthalmology, Front Neurol. 9 (2018) 1047. https://doi.org/10.3389/fneur.2018.01047.

[31] M.T.H. DO, K.-W. YAU, Intrinsically Photosensitive Retinal Ganglion Cells, Physiol Rev. 90 (2010) 1547–1581. https://doi.org/10.1152/physrev.00013.2010.

[32] V. Reghunandanan, R. Reghunandanan, Neurotransmitters of the suprachiasmatic nuclei, J Circadian Rhythms. 4 (2006) 2. https://doi.org/10.1186/1740-3391-4-2.

[33] A.P. Patton, M.H. Hastings, The suprachiasmatic nucleus, Current Biology. 28 (2018) R816–R822. https://doi.org/10.1016/j.cub.2018.06.052.

[34] M. Iwasaki, Y. Akiba, J.D. Kaunitz, Recent advances in vasoactive intestinal peptide physiology and pathophysiology: focus on the gastrointestinal system, F1000Res. 8 (2019) F1000 Faculty Rev-1629. https://doi.org/10.12688/f1000research.18039.1.

[35] I. Gozes, D.E. Brenneman, VIP: molecular biology and neurobiological function, Mol Neurobiol. 3 (1989) 201–236. https://doi.org/10.1007/BF02740606.

[36] D. Cunha-Reis, A. Caulino-Rocha, VIP Modulation of Hippocampal Synaptic Plasticity: A Role for VIP Receptors as Therapeutic Targets in Cognitive Decline and Mesial Temporal Lobe Epilepsy, Frontiers in Cellular Neuroscience. 14 (2020). https://www.frontiersin.org/articles/10.3389/fncel.2020.00153 (accessed September 26, 2022).

[37] D.K. Welsh, J.S. Takahashi, S.A. Kay, Suprachiasmatic Nucleus: Cell Autonomy and Network Properties, Annu Rev Physiol. 72 (2010) 551–577. https://doi.org/10.1146/annurev-physiol-021909-135919.

[38] J.R. Jones, T. Simon, L. Lones, E.D. Herzog, SCN VIP Neurons Are Essential for Normal Light-Mediated Resetting of the Circadian System, J Neurosci. 38 (2018) 7986–7995. https://doi.org/10.1523/JNEUROSCI.1322-18.2018.

[39] E.D. Herzog, T. Hermanstyne, N.J. Smyllie, M.H. Hastings, Regulating the Suprachiasmatic Nucleus (SCN) Circadian Clockwork: Interplay between Cell-Autonomous and Circuit-Level Mechanisms, Cold Spring Harb Perspect Biol. 9 (2017) a027706. https://doi.org/10.1101/cshperspect.a027706.

[40] A. Aulinas, Physiology of the Pineal Gland and Melatonin, in: K.R. Feingold, B. Anawalt, A. Boyce, G. Chrousos, W.W. de Herder, K. Dungan, A. Grossman, J.M. Hershman, H.J. Hofland, G. Kaltsas, C. Koch, P. Kopp, M. Korbonits, R. McLachlan, J.E. Morley, M. New, J. Purnell, F. Singer, C.A. Stratakis, D.L. Trence, D.P. Wilson (Eds.), Endotext, MDText.com, Inc., South Dartmouth (MA), 2000. http://www.ncbi.nlm.nih.gov/books/NBK550972/ (accessed November 8, 2020).

[41] R. Herzog–Krzywoszanska, L. Krzywoszanski, Sleep Disorders in Huntington's Disease, Front Psychiatry. 10 (2019). https://doi.org/10.3389/fpsyt.2019.00221.

[42] C. Piano, A. Losurdo, G. Della Marca, M. Solito, G. Calandra-Buonaura, F. Provini, A.R. Bentivoglio, P. Cortelli, Polysomnographic Findings and Clinical Correlates in Huntington Disease: A Cross-Sectional Cohort Study, Sleep. 38 (2015) 1489–1495. https://doi.org/10.5665/sleep.4996.

[43] N.A. Aziz, H. Pijl, M. Frölich, J.P. Schröder-van der Elst, C. van der Bent, F. Roelfsema, R.A.C. Roos, Delayed onset of the diurnal melatonin rise in patients with Huntington's disease, J Neurol. 256 (2009) 1961–1965. https://doi.org/10.1007/s00415-009-5196-1.

[44] E. Gonzales, J. Yin, Drosophila Models of Huntington's Disease Exhibit Sleep Abnormalities, PLoS Curr. 2 (2010). https://doi.org/10.1371/currents.RRN1185.

[45] D. Kuljis, A.M. Schroeder, T. Kudo, D.H. Loh, D.L. Willison, C.S. Colwell, Sleep and circadian dysfunction in neurodegenerative disorders: insights from a mouse model of Huntington's disease, Minerva Pneumol. 51 (2012) 93–106.

[46] D.A. Kuljis, L. Gad, D.H. Loh, Z. MacDowell Kaswan, O.N. Hitchcock, C.A. Ghiani, C.S. Colwell, Sex Differences in Circadian Dysfunction in the BACHD Mouse Model of Huntington's Disease, PLoS ONE. 11 (2016) e0147583. https://doi.org/10.1371/journal.pone.0147583.

[47] E.S. Maywood, E. Fraenkel, C.J. McAllister, N. Wood, A.B. Reddy, M.H. Hastings, A.J. Morton, Disruption of Peripheral Circadian Timekeeping in a Mouse Model of Huntington's Disease and Its Restoration by Temporally Scheduled Feeding, J Neurosci. 30 (2010) 10199–10204. https://doi.org/10.1523/JNEUROSCI.1694-10.2010.

[48] N.A. Aziz, G.V. Anguelova, J. Marinus, G.J. Lammers, R.A.C. Roos, Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease, Parkinsonism Relat. Disord. 16 (2010) 345–350. https://doi.org/10.1016/j.parkreldis.2010.02.009.

[49] T.S. Andreani, T.Q. Itoh, E. Yildirim, D.-S. Hwangbo, R. Allada, Genetics of Circadian Rhythms, Sleep Med Clin. 10 (2015) 413–421. https://doi.org/10.1016/j.jsmc.2015.08.007.

[50] A. Charrier, B. Olliac, P. Roubertoux, S. Tordjman, Clock Genes and Altered Sleep–Wake Rhythms: Their Role in the Development of Psychiatric Disorders, Int J Mol Sci. 18 (2017). https://doi.org/10.3390/ijms18050938.

[51] G. Mazzoccoli, V. Pazienza, M. Vinciguerra, Clock genes and clock-controlled genes in the regulation of metabolic rhythms, Chronobiol. Int. 29 (2012) 227–251. https://doi.org/10.3109/07420528.2012.658127.

[52] X. Zheng, X. Zhao, Y. Zhang, H. Tan, B. Qiu, T. Ma, J. Zeng, D. Tao, Y. Liu, Y. Lu, Y. Ma, RAE1 promotes BMAL1 shuttling and regulates degradation and activity of CLOCK: BMAL1 heterodimer, Cell Death Dis. 10 (2019). https://doi.org/10.1038/s41419-019-1346-2.

[53] R. Ye, C.P. Selby, Y.-Y. Chiou, I. Ozkan-Dagliyan, S. Gaddameedhi, A. Sancar, Dual modes of CLOCK:BMAL1 inhibition mediated by Cryptochrome and Period proteins in the mammalian circadian clock, Genes Dev. 28 (2014) 1989–1998. https://doi.org/10.1101/gad.249417.114.

[54] R. Ikeda, Y. Tsuchiya, N. Koike, Y. Umemura, H. Inokawa, R. Ono, M. Inoue, Y. Sasawaki, T. Grieten, N. Okubo, K. Ikoma, H. Fujiwara, T. Kubo, K. Yagita, REV-ERBα and REV-ERBβ function as key factors regulating Mammalian Circadian Output, Scientific Reports. 9 (2019) 10171. https://doi.org/10.1038/s41598-019-46656-0.

[55] J.S. Takahashi, Transcriptional architecture of the mammalian circadian clock, Nat Rev Genet. 18 (2017) 164–179. https://doi.org/10.1038/nrg.2016.150.

[56] K.H. Cox, J.S. Takahashi, Circadian clock genes and the transcriptional architecture of the clock mechanism, J. Mol. Endocrinol. 63 (2019) R93–R102. https://doi.org/10.1530/JME-19-0153.

[57] N. Krishnan, K. Rakshit, E.S. Chow, J.S. Wentzell, D. Kretzschmar, J.M. Giebultowicz, Loss of circadian clock accelerates aging in neurodegeneration-prone mutants, Neurobiol Dis. 45 (2012) 1129–1135. https://doi.org/10.1016/j.nbd.2011.12.034.

[58] C.C. Lee, Tumor suppression by the mammalian Period genes, Cancer Causes Control. 17 (2006) 525–530. https://doi.org/10.1007/s10552-005-9003-8.

 doi:10.20944/preprints202209.0412.v1

[59] K.H. Snider, H. Dziema, S. Aten, J. Loeser, F.E. Norona, K. Hoyt, K. Obrietan, Modulation of learning and memory by the targeted deletion of the circadian clock gene Bmal1 in forebrain circuits, Behav. Brain Res. 308 (2016) 222–235. https://doi.org/10.1016/j.bbr.2016.04.027.

[60] F. Rijo-Ferreira, J.S. Takahashi, Genomics of circadian rhythms in health and disease, Genome Medicine. 11 (2019) 82. https://doi.org/10.1186/s13073-019-0704-0.

[61] L. Moumné, S. Betuing, J. Caboche, Multiple Aspects of Gene Dysregulation in Huntington's Disease, Front Neurol. 4 (2013) 127. https://doi.org/10.3389/fneur.2013.00127.

[62] J. Fahrenkrug, N. Popovic, B. Georg, P. Brundin, J. Hannibal, Decreased VIP and VPAC2 receptor expression in the biological clock of the R6/2 Huntington's disease mouse, J. Mol. Neurosci. 31 (2007) 139–148. https://doi.org/10.1385/jmn/31:02:139.

[63] E.S. Musiek, Circadian clock disruption in neurodegenerative diseases: cause and effect?, Front Pharmacol. 6 (2015). https://doi.org/10.3389/fphar.2015.00029.

[64] E.S. Musiek, M.M. Lim, G. Yang, A.Q. Bauer, L. Qi, Y. Lee, J.H. Roh, X. Ortiz-Gonzalez, J.T. Dearborn, J.P. Culver, E.D. Herzog, J.B. Hogenesch, D.F. Wozniak, K. Dikranian, B.I. Giasson, D.R. Weaver, D.M. Holtzman, G.A. FitzGerald, Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration, J Clin Invest. 123 (2013) 5389–5400. https://doi.org/10.1172/JCI70317.

[65] P.N. Pallier, E.S. Maywood, Z. Zheng, J.E. Chesham, A.N. Inyushkin, R. Dyball, M.H. Hastings, A. Jennifer Morton, Pharmacological Imposition of Sleep Slows Cognitive Decline and Reverses Dysregulation of Circadian Gene Expression in a Transgenic Mouse Model of Huntington's Disease, J Neurosci. 27 (2007) 7869–7878. https://doi.org/10.1523/JNEUROSCI.0649-07.2007.

[66] P.N. Pallier, A.J. Morton, Management of sleep/wake cycles improves cognitive function in a transgenic mouse model of Huntington's disease, Brain Res. 1279 (2009) 90–98. https://doi.org/10.1016/j.brainres.2009.03.072.

[67] C. Fernandes, N.B.F. Rocha, S. Rocha, A. Herrera-Solís, J. Salas-Pacheco, F. García-García, E. Murillo-Rodríguez, T.-F. Yuan, S. Machado, O. Arias-Carrión, Detrimental role of prolonged sleep deprivation on adult neurogenesis, Front Cell Neurosci. 9 (2015). https://doi.org/10.3389/fncel.2015.00140.

[68] S. Tamai, K. Sanada, Y. Fukada, Time-of-Day-Dependent Enhancement of Adult Neurogenesis in the Hippocampus, PLoS One. 3 (2008). https://doi.org/10.1371/journal.pone.0003835.

[69] M. Kandasamy, L. Aigner, Neuroplasticity, limbic neuroblastosis and neuro-regenerative disorders, Neural Regen Res. 13 (2018) 1322–1326. https://doi.org/10.4103/1673-5374.235214.

[70] D.M. Feliciano, A. Bordey, L. Bonfanti, Noncanonical Sites of Adult Neurogenesis in the Mammalian Brain, Cold Spring Harb Perspect Biol. 7 (2015) a018846. https://doi.org/10.1101/cshperspect.a018846.

[71] G. Ming, H. Song, Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions, Neuron. 70 (2011) 687–702. https://doi.org/10.1016/j.neuron.2011.05.001.

[72] S. Yoo, S. Blackshaw, Regulation and function of neurogenesis in the adult mammalian hypothalamus, Prog Neurobiol. 170 (2018) 53–66. https://doi.org/10.1016/j.pneurobio.2018.04.001.

[73] S. Gabery, G. Halliday, D. Kirik, E. Englund, Å. Petersén, Selective loss of oxytocin and vasopressin in the hypothalamus in early Huntington disease: a case study, Neuropathol. Appl. Neurobiol. 41 (2015) 843–848. https://doi.org/10.1111/nan.12236.

 doi:10.20944/preprints202209.0412.v1

[74] S. Hult Lundh, N. Nilsson, R. Soylu, D. Kirik, Å. Petersén, Hypothalamic expression of mutant huntingtin contributes to the development of depressive-like behavior in the BAC transgenic mouse model of Huntington's disease, Hum. Mol. Genet. 22 (2013) 3485–3497. https://doi.org/10.1093/hmg/ddt203.

[75] P. Pla, S. Orvoen, F. Saudou, D.J. David, S. Humbert, Mood disorders in Huntington's disease: from behavior to cellular and molecular mechanisms, Front Behav Neurosci. 8 (2014). https://doi.org/10.3389/fnbeh.2014.00135.

[76] A.A.H. Ali, B. Schwarz-Herzke, S. Mir, B. Sahlender, M. Victor, B. Görg, M. Schmuck, K. Dach, E. Fritsche, A. Kremer, C. von Gall, Deficiency of the clock gene Bmal1 affects neural progenitor cell migration, Brain Struct Funct. 224 (2019) 373–386. https://doi.org/10.1007/s00429-018-1775-1.

[77] P. Bouchard-Cannon, L. Mendoza-Viveros, A. Yuen, M. Kærn, H.-Y.M. Cheng, The Circadian Molecular Clock Regulates Adult Hippocampal Neurogenesis by Controlling the Timing of Cell-Cycle Entry and Exit, Cell Reports. 5 (2013) 961–973. https://doi.org/10.1016/j.celrep.2013.10.037.

[78] K. Vlasov, C.J. Van Dort, K. Solt, Optogenetics and Chemogenetics, Methods Enzymol. 603 (2018) 181–196. https://doi.org/10.1016/bs.mie.2018.01.022.

[79] W. Boesmans, M.M. Hao, P. Vanden Berghe, Optogenetic and chemogenetic techniques for neurogastroenterology, Nat Rev Gastroenterol Hepatol. 15 (2018) 21–38. https://doi.org/10.1038/nrgastro.2017.151.

[80] J.P. Newman, M. Fong, D.C. Millard, C.J. Whitmire, G.B. Stanley, S.M. Potter, Optogenetic feedback control of neural activity, Elife. 4 (2015) e07192. https://doi.org/10.7554/eLife.07192.

[81] S.D. Klapper, A. Swiersy, E. Bamberg, V. Busskamp, Biophysical Properties of Optogenetic Tools and Their Application for Vision Restoration Approaches, Front. Syst. Neurosci. 10 (2016). https://doi.org/10.3389/fnsys.2016.00074.

[82] S.M. Holley, L. Galvan, T. Kamdjou, A. Dong, M.S. Levine, C. Cepeda, Major Contribution of Somatostatin-Expressing Interneurons and Cannabinoid Receptors to Increased GABA Synaptic Activity in the Striatum of Huntington's Disease Mice, Front Synaptic Neurosci. 11 (2019). https://doi.org/10.3389/fnsyn.2019.00014.

[83] C. Cepeda, L. Galvan, S.M. Holley, S.P. Rao, V.M. André, E.P. Botelho, J.Y. Chen, J.B. Watson, K. Deisseroth, M.S. Levine, Multiple Sources of Striatal Inhibition Are Differentially Affected in Huntington's Disease Mouse Models, J Neurosci. 33 (2013) 7393–7406. https://doi.org/10.1523/JNEUROSCI.2137-12.2013.

[84] K. Hristova, C. Martinez-Gonzalez, T.C. Watson, N.K. Codadu, K. Hashemi, P.C. Kind, M.F. Nolan, A. Gonzalez-Sulser, Medial septal GABAergic neurons reduce seizure duration upon optogenetic closed-loop stimulation, Brain. 144 (2021) 1576–1589. https://doi.org/10.1093/brain/awab042.

[85] S.F. Owen, M.H. Liu, A.C. Kreitzer, Thermal constraints on in vivo optogenetic manipulations, Nat Neurosci. 22 (2019) 1061–1065. https://doi.org/10.1038/s41593-019-0422-3.

[86] A. Malyshev, R. Goz, J.J. LoTurco, M. Volgushev, Advantages and Limitations of the Use of Optogenetic Approach in Studying Fast-Scale Spike Encoding, PLoS One. 10 (2015). https://doi.org/10.1371/journal.pone.0122286.

[87] M.C. Berthoud, C.S. Reilly, Adverse effects of general anaesthetics, Drug Saf. 7 (1992) 434–459. https://doi.org/10.2165/00002018-199207060-00005.

 doi:10.20944/preprints202209.0412.v1

[88] B.N. Armbruster, X. Li, M.H. Pausch, S. Herlitze, B.L. Roth, Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand, Proc Natl Acad Sci U S A. 104 (2007) 5163–5168. https://doi.org/10.1073/pnas.0700293104.

[89] O. Keifer, K. Kambara, A. Lau, S. Makinson, D. Bertrand, Chemogenetics a robust approach to pharmacology and gene therapy, Biochem Pharmacol. 175 (2020) 113889. https://doi.org/10.1016/j.bcp.2020.113889.

[90] B.R. Conklin, E.C. Hsiao, S. Claeysen, A. Dumuis, S. Srinivasan, J.R. Forsayeth, J.-M. Guettier, W.C. Chang, Y. Pei, K.D. McCarthy, R.A. Nissenson, J. Wess, J. Bockaert, B.L. Roth, Engineering GPCR signaling pathways with RASSLs, Nat Methods. 5 (2008) 673–678. https://doi.org/10.1038/nmeth.1232.

[91] B.L. Roth, DREADDs for Neuroscientists, Neuron. 89 (2016) 683–694. https://doi.org/10.1016/j.neuron.2016.01.040.

[92] S.M. Sternson, B.L. Roth, Chemogenetic tools to interrogate brain functions, Annu Rev Neurosci. 37 (2014) 387–407. https://doi.org/10.1146/annurev-neuro-071013-014048.

[93] P.D. Whissell, S. Tohyama, L.J. Martin, The Use of DREADDs to Deconstruct Behavior, Front Genet. 7 (2016) 70. https://doi.org/10.3389/fgene.2016.00070.

[94] F. Assaf, Y. Schiller, A chemogenetic approach for treating experimental Parkinson's disease, Mov Disord. 34 (2019) 469–479. https://doi.org/10.1002/mds.27554.

[95] P. Yuan, J. Grutzendler, Attenuation of β-Amyloid Deposition and Neurotoxicity by Chemogenetic Modulation of Neural Activity, J Neurosci. 36 (2016) 632–641. https://doi.org/10.1523/JNEUROSCI.2531-15.2016.

[96] N. Milosavljevic, A.E. Allen, J. Cehajic-Kapetanovic, R.J. Lucas, Chemogenetic Activation of ipRGCs Drives Changes in Dark-Adapted (Scotopic) Electroretinogram, Invest Ophthalmol Vis Sci. 57 (2016) 6305–6312. https://doi.org/10.1167/iovs.16-20448.

[97] J.L. Gomez, J. Bonaventura, W. Lesniak, W.B. Mathews, P. Sysa-Shah, L.A. Rodriguez, R.J. Ellis, C.T. Richie, B.K. Harvey, R.F. Dannals, M.G. Pomper, A. Bonci, M. Michaelides, Chemogenetics revealed: DREADD occupancy and activation via converted clozapine, Science. 357 (2017) 503–507. https://doi.org/10.1126/science.aan2475.

[98] B. Baseri, J.J. Choi, Y.-S. Tung, E.E. Konofagou, Multi-Modality Safety Assessment of Blood-Brain Barrier Opening Using Focused Ultrasound and Definity Microbubbles: A Short-Term Study, Ultrasound Med Biol. 36 (2010) 1445–1459. https://doi.org/10.1016/j.ultrasmedbio.2010.06.005.

[99] B.K. Kaspar, D. Erickson, D. Schaffer, L. Hinh, F.H. Gage, D.A. Peterson, Targeted retrograde gene delivery for neuronal protection, Mol Ther. 5 (2002) 50–56. https://doi.org/10.1006/mthe.2001.0520.

[100] D. Ono, K.-I. Honma, Y. Yanagawa, A. Yamanaka, S. Honma, GABA in the suprachiasmatic nucleus refines circadian output rhythms in mice, Commun Biol. 2 (2019) 232. https://doi.org/10.1038/s42003-019-0483-6.

[101] M.A. Ma, E.H. Morrison, Neuroanatomy, Nucleus Suprachiasmatic, in: StatPearls, StatPearls Publishing, Treasure Island (FL), 2020. http://www.ncbi.nlm.nih.gov/books/NBK546664/ (accessed November 8, 2020).

[102] D.J. van Wamelen, N.A. Aziz, J.J. Anink, R. van Steenhoven, D. Angeloni, F. Fraschini, R. Jockers, R.A.C. Roos, D.F. Swaab, Suprachiasmatic Nucleus Neuropeptide Expression in Patients with Huntington's Disease, Sleep. 36 (2013) 117–125. https://doi.org/10.5665/sleep.2314.

[103] M. Delgado, D. Ganea, Neuroprotective effect of vasoactive intestinal peptide (VIP) in a mouse model of Parkinson's disease by blocking microglial activation, FASEB J. 17 (2003) 944–946. https://doi.org/10.1096/fj.02-0799fje.

[104] G. Deng, L. Jin, The effects of vasoactive intestinal peptide in neurodegenerative disorders, Neurol Res. 39 (2017) 65–72. https://doi.org/10.1080/01616412.2016.1250458.

[105] C. Eva, J.L. Meek, E. Costa, Vasoactive intestinal peptide which coexists with acetylcholine decreases acetylcholine turnover in mouse salivary glands, J Pharmacol Exp Ther. 232 (1985) 670–674.

[106] C. Liu, M.U. Gillette, Cholinergic regulation of the suprachiasmatic nucleus circadian rhythm via a muscarinic mechanism at night, J Neurosci. 16 (1996) 744–751.

[107] C. Alcacer, L. Andreoli, I. Sebastianutto, J. Jakobsson, T. Fieblinger, M.A. Cenci, Chemogenetic stimulation of striatal projection neurons modulates responses to Parkinson's disease therapy, J Clin Invest. 127 (n.d.) 720–734. https://doi.org/10.1172/JCI90132.

[108] D. Kätzel, E. Nicholson, S. Schorge, M.C. Walker, D.M. Kullmann, Chemical-genetic attenuation of focal neocortical seizures, Nat Commun. 5 (2014) 3847. https://doi.org/10.1038/ncomms4847.

[109] A. Lieb, M. Weston, D.M. Kullmann, Designer receptor technology for the treatment of epilepsy, EBioMedicine. 43 (2019) 641–649. https://doi.org/10.1016/j.ebiom.2019.04.059.

[110] S. Daya, K.I. Berns, Gene Therapy Using Adeno-Associated Virus Vectors, Clin Microbiol Rev. 21 (2008) 583–593. https://doi.org/10.1128/CMR.00008-08.