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About the Journal of Young Investigators

The Journal of Young Investigators (JYI) is always looking for new ways to help undergraduates share their research with a broader audience. An important part of this process is highlighting how specific experiments and projects fit into the expansive body of existing literature. Integration and assessment of peer-reviewed work is critical to scientific progress. With this in mind, JYI launched its first special edition. We invited and recruited a number of undergraduate authors to submit mini-reviews analyzing recent work in a specific field of science.

From Parkinson's Disease to non-hermitian wave mechanics, the mini-reviews in the special edition cover a variety of topics. Nonetheless, the authors herein provide the background and details which allow a general audience to understand the relevance of the work and progress happening in specific fields. Given our audience and mission, JYI places high value on this quality of submitted work. We hope you enjoy reading each piece as much as we enjoyed reviewing and selecting them.

The special edition was made possible by the strong work of motivated undergraduate students and their advisors. Whether you are a JYI staff member, an author of this edition, or an advising faculty member, JYI appreciates the time and effort you dedicate to make our initiatives possible. Thank you.

JYI is a student-led initiative to broaden the undergraduate scientific experience, allowing students to participate in the scientific review and publication processes of its peer-reviewed undergraduate journal. Incorporated as a non-profit, student-run corporation, JYI represents over 50 different academic institutions from over half a dozen countries. JYI has been featured in *EurekAlert!*, *Chemical Engineering News*, *Science*, and *The Chronicle on Higher Education*. An article highlighting JYI appeared in The New York Times in 1999.

About the Cover

Visualization of an optical light shone onto demyelinating cells of a multiple sclerosis patient to stimulate growth through long-term potentiation. Controlled and specific, continuous photostimulation is suggested to combat and decrease MS symptomology while enhancing neural networks.

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Journal of Young Investigators
Celebrating 20 years of undergraduate research
REVIEW ARTICLE

A New Perspective on Parkinson's Disease: Pathology Begins in the Gastrointestinal Tract

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Gastrointestinal dysfunction has a high prevalence in the preclinical phase of Parkinson's Disease. This review analyzes recent reports that show abnormalities in the gastrointestinal tract of Parkinson's patients compared to controls, suggesting that the disease originates in the gut. The enteric nervous system, which is composed of myenteric and submucosal plexuses, is susceptible to degeneration in Parkinson's disease. Evidence regarding Parkinsonian-related loss of myenteric dopamine neurons in the outer plexus of the enteric nervous system is currently controversial. The diseased submucosal plexus composition, located in the inner enteric plexus has not yet been derived. The dual-hit hypothesis suggests a submucosal role in disease propagation from the gastrointestinal tract to midbrain regions. Parkinson's patients have an altered gut microbiota composition and varying bacterial concentrations that are correlated with distinct disease phenotypes. These unique bacteria produce short chain fatty acids, which can permeate across the blood brain barrier and indirectly stimulate reactive microgliosis, thus generating a proinflammatory environment that stimulates α-synuclein aggregation. The abnormal Parkinson's gut microbiota composition has shown to be sufficient in inducing the diseased state. A few reports suggest that diseased microbiome replacement with healthy microbiota via fecal transplant can improve the disease phenotype. Future therapeutic development should target the gut microbiome and its interaction with the enteric nervous system to provide means for an early diagnosis and possible treatment innovations.

INTRODUCTION

Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder in the elderly population, following Alzheimer's disease (Lin et al., 2014). PD is a chronic disorder, characterized primarily by motor deficits including resting tremor, rigidity, bradykinesia, and postural instability (Burke & O'Malley, 2013; Choi et al., 2016; Lohr & Miller, 2014; Miller et al., 1999; Taylor et al., 2014). Although dopaminergic atrophy in the substantia nigra pars compacta mediates the presence of these motor deficits, the clinical indicators do not appear until over 70% of dopamine (DA) nerve terminals in the striatum have atrophied, suggesting the presence of compensatory mechanisms (Bezard et al., 2013). In disease propagation, alpha-synuclein proteins bind ubiquitin ligands and accumulate in damaged cells (Rao & Gershon, 2016). Alpha-synuclein aggregation leads to Lewy body formation, the characteristic pathological marker. It is currently unclear whether dopaminergic atrophy leads to alpha-synuclein aggregation or if it is the aggregates that lead to cell death.

Few causative factors have thus far been supported, though some environmental toxins have been shown to cause disease symptomology (Pan-Montojo & Reichmann, 2014). For example, exposure to the herbicide Paraquat can result in dopaminergic degeneration and Lewy body formation in the substantia nigra by generating high levels of oxidative stress (Pan-Montojo & Reichmann, 2014). In addition, the production of the synthetic opioid drug MPPP can generate an accidental compound MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), which quickly induces a Parkinsonian state when its toxic metabolite inhibits complex I of the electron transport chain (Pan-Montojo & Reichmann, 2014). The possibility of an endogenous neurotoxic mechanism that was acquired in early life has been contemplated for many years (Gibb & Lees, 1988). It is thought that this potential pathogen is transported from the gastrointestinal (GI) tract to the brain via the vagus nerve over the course of twenty years (Syensson et al., 2015). Svensson and colleagues (2015) examined a cohort of patients who underwent vagotomies. They found that patients who received a truncal vagotomy (i.e., the surgical severance of both vagal trunks) had a lower risk of Parkinson's disease compared to a

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matched comparison group from the general population (Syensson et al., 2015). Additionally, the basal ganglia are stable throughout the presymptomatic stage (Bezard et al., 2013). It thus appears that the disease origin lies outside the conventional diseased structure (Bezard et al., 2013).

Prior to subcortical disturbance, insoluble alpha-synuclein aggregates have been found throughout the GI tract, causing disrupted gastric motility in transgenic mice (Rao & Gershon, 2016). In the absence of central nervous system (CNS) pathology, genetic mouse models show an increased GI transit time (i.e., longer duration of ingested substance movement through the GI tract compared to the norm) with decreased colonic motility compared to controls (Rao & Gershon, 2016). Increased GI transit time can result in constipation (Rao & Gershon, 2016). Gastrointestinal deficits are some of the most prevalent non-motor symptoms in PD patients and they have shown to affect more than 65% of patients in various cultures (Bugalho et al.,2016; Cheon et al., 2008). Further, the environmental toxins and genetic mutations that initiate PD progression are also associated with enteric nervous system (ENS) deficits. The ENS controls GI tract behavior through innervations in the submucosal and myenteric plexuses surrounding the gut epithelium (Jenkins & Tortora, 2006; Rao & Gershon, 2016). Activity in the ENS is mediated by autonomic input. Disease indicators within the ENS may precede CNS symptoms and pathology, and thereafter propagate to the midbrain region based on the finding that gastrointestinal deficits are present prior to subcortical degeneration (Rao & Gershon, 2016). ENS manifestations could therefore be useful in the future diagnosis and treatment of PD (Rao & Gershon, 2016).

GI dysfunction has a high prevalence in the preclinical phase of PD. Recent hypotheses suggest PD origination in the gut, rather than the brain (Mukherjee et al., 2016; Sampson et al., 2016). This review assesses recent studies that report alterations in the GI tract of PD patients, and as well will describe future therapeutic development in the ENS and its surrounding structures.

Gut-Brain Axis

The autonomic nervous system (ANS) is a component of the peripheral nervous system. Both divisions control the ENS, making the ENS the largest component of the ANS (Rao & Gershon, 2016). The dorsal motor nucleus of the vagus nerve is the main excitatory parasympathetic input to the GI tract (Hawkes et al., 2007; Mukherjee et al., 2016). Sympathetic nerves also innervate the GI tract, resulting in inhibitory control (Figure 1) (Hawkes et al., 2007).

The ENS contains over 100 million neurons that are intrinsically arranged into microcircuits, which allow the ENS to control the GI tract behaviour without CNS input (Rao & Gershon, 2016). However, neurotransmitter molecules and signalling pathways allow for communication between the two nervous systems (Rao & Gershon, 2016). As such, CNS disease processes frequently have ENS manifestations. Similarly, diseases that originate in the gut routinely have central features. For example, coeliac disease, a

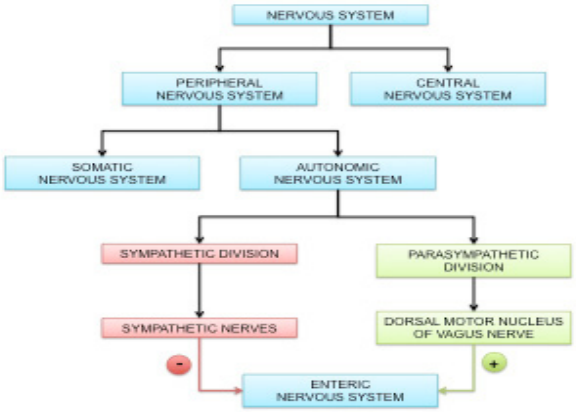

Figure 1. Flow diagram outlining how the nervous system interacts with the gastrointestinal tract. The nervous system has two components: central, which includes the brain and spinal cord, and peripheral, which comprises all the nerves that extend throughout the body. The peripheral nervous system is further divided into somatic and autonomic nervous systems. The autonomic nervous system includes sympathetic and parasympathetic divisions. The sympathetic division controls the fight or flight response, while the parasympathetic division mediates the rest and digest response. The sympathetic division acts through sympathetic nerves to inhibit enteric nervous system (ENS) activity. The parasympathetic division acts primarily through the dorsal motor nucleus of the vagus nerve to stimulate ENS activity.

systemic autoimmune disorder that is triggered by gluten ingestion, often has neurological manifestations, the most common symptom being ataxia (Hadjivassiliou et al., 2010).

The ENS is susceptible to degeneration in PD (Annerino et al., 2012; Fasano et al., 2015; Singaram et al., 1995). Alpha-synuclein aggregations in Lewy bodies are a characteristic PD pathology marker. Aggregates have traditionally been found in the CNS, although growing evidence supports their presence in the ENS as well (Annerino et al., 2012; Fasano et al., 2015; Singaram et al., 1995). The aggregation distribution pattern is most concentrated in the submandibular salivary gland and lower oesophagus, and becomes progressively less concentrated through the stomach, small intestine, colon, and rectum (Fasano et al., 2015). This pattern appears to follow visceromotor projection neuron innervation (Fasano et al., 2015). These neurons originate in the dorsal motor nucleus of the vagus nerve and innervate the extent of the GI tract (Fasano et al., 2015). Communication via vagus nerve input is dominant in the superior regions, while sympathetic innervation is dominant in the inferior GI regions (Fasano et al., 2015). The vagus nerve could thus potentially be acting as a 'highway' between gut and brain PD pathologies. It is thought that the disease spreads in a prion-like fashion from the ENS to the CNS, only infecting dopaminergic cells (Hawkes et al., 2007). The vagus nerve has a direct connection with the medulla, which is inferior to the substantia nigra pars compacta. Since this region is dopamine-rich, most of the disease symptomology and pathology stems from sub-

stantia nigral degeneration (Mukherjee et al., 2016).

The neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is used to model idiopathic PD (Smeyne & Jacson-Lewis, 2005). The MPP+ metabolite causes dopaminergic atrophy by interfering with complex I of the electron transport chain within mitochondria, thereby thwarting energy production (Smeyne & Jacson-Lewis, 2005). Animal models have shown up to a 40% loss of enteric DA neurons within ten days of MPTP administration (Rao & Gershon, 2016). Thus, CNS PD-indicative pathology is also present in the ENS. In the case of PD, major unanswered question is whether the CNS or ENS pathology developed first.

The dual-hit hypothesis suggests that PD begins in the gut and then propagates to the brain. Accordingly, a pathogen infects the brain via both nasal and gastric routes after nasal secretions, containing this unknown pathogen in the saliva, are swallowed (Mukherjee et al., 2016). This viral, neurotropic pathogen is thought to cross the gut epithelial lining. In theory, the pathogen reaches the preganglionic parasympathetic motor neurons of the vagus nerve via transsynaptic transmission through axons from the submucosal plexus, allowing retrograde transport into the medulla (Mukherjee et al., 2016). The process then has downstream effects that allow propagation from the posterior regions to the anterior regions, ultimately infecting the substantia nigra pas compacta (Mukherjee et al., 2016). It is therefore proposed that a neurotropic pathogen initiates PD and is transported to the substantia nigra via the vagus nerve.

Myenteric and Submucosal Plexuses

The submucosal plexus innervates the submucosa (Jenkins & Tortora, 2006). Adjacent to the submucosa is the mucularis, a circular layer of muscle, which is innervated by the myenteric plexus (Figure 2) (Jenkins & Tortora, 2006). The two plexuses are interconnected (Rao & Gershon, 2016). They are incorporated into 7.5 metres of bowel in a human adult (Rao & Gershon, 2016). Enteric neurons are unevenly distributed throughout the myenteric and submucosal plexuses (Rao & Gershon, 2016). Irregularity of neuronal density throughout the length of the bowel makes quantification of the total neuronal density via paraffin sectioning highly susceptible to sampling error (Rao & Gershon, 2016). Relatively few neurons exist in any one segment (Rao & Gershon, 2016).

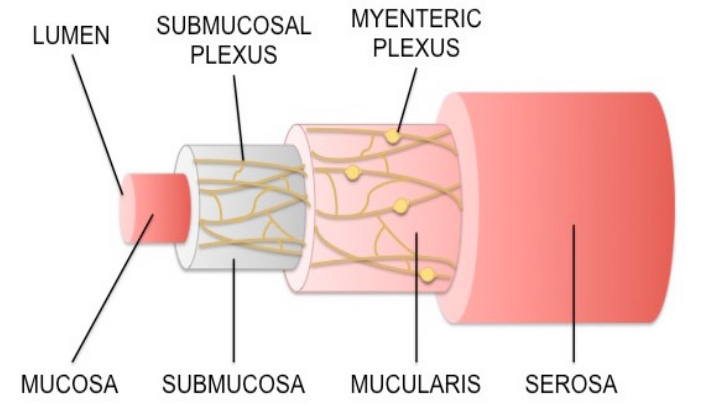

Figure 2. Enteric nervous system innervations throughout the gastrointestinal tract. The submucosal plexus innervates the submucosa, which surrounds the mucosa. The mucosa is a mucous layer that lines the gut epithelium. The mucularis encompasses the myenteric plexus and surrounds the submucosa. The serosa, a layer of muscle, surrounds the two plexuses.

Singaram and colleagues (1995) analyzed colon samples from eleven patients with severe PD as well as multiple non-diseased patients. They found that dopaminergic neurons from the colonic myenteric plexus were significantly reduced in PD patients (Singraram et al., 1995) and are correlated to the presence of Lewy bodies in myenteric neurons (Singraram et al., 1995). These results are controversial to other studies, likely due to the technology advances in recent endeavours (Rao & Gershon, 2016). For example, Annerino and colleagues (2012) found no differences in the total density of myenteric neurons between controls and patients with advanced PD. Although neurons containing tyrosine hydroxylase (TH), a dopamine precursor molecule, are most abundant in the stomach, coexistence of Lewy bodies with TH is rare (Annerino et al., 2012). This suggests that myenteric Lewy body formation is not localized to dopaminergic neurons. However, Lewy body pathology in myenteric neurons is synchronous with parasympathetic input from dorsal motor neurons of the vagus nerve, which is known to modulate ENS activity (Annerino et al., 2012). Thus, the researchers suggest that myenteric neuron loss is likely not a characteristic of PD (Annerino et al., 2012). Further investigation of the myenteric plexus is required to elucidate controversial evidence.

Constipation

Constipation is primarily caused by slowed colonic transit in PD patients (Smeyne & Jackson, 2015). Gastric motility takes twice as long in PD-affected individuals (Smeyne & Jackson, 2015. The slowed process is caused by a motor deficit in the GI tract. Lewy bodies accumulate in vasoactive intestinal peptide (VIP) neurons, which amount to nearly 50% of all myenteric neurons (Annerino et al., 2012; Smeyne & Jackson, 2015; Stirpe et al., 2016). Lewy body-filled VIP interneurons disinhibit motor neurons in the GI tract distal smooth muscles, resulting in reflex relaxation impairment (Stirpe et al., 2016). Constipation appears to be more severe in patients who have had PD for many years (Tan et al., 2016).

Biomarkers for constipation are being considered as a prodromal PD diagnostic marker. This non-motor symptom could allow for early diagnosis up to ten years sooner than classical diagnostic measures (Stirpe et al., 2016). However, there is a 15-20% prevalence of constipation in the general population, of which only 30-60% are comorbid for both constipation and PD (Postuma & Berg, 2016). Thus, constipation has relatively low prognostic integrity. Constipation has been found to play a role in negatively affecting the absorption of levodopa (Ogawa et al., 2012; Postuma & Berg, 2016; Stirpe et al., 2016; Tan et al., 2016). L-3,4 dihydroxyphe-

nylalanine (L-dopa or levodopa) is the immediate DA precursor molecule (Bianchine et al., 1971). Since DA levels are depleted in midbrain regions of PD patients, levodopa is administered for subsequent DA synthesis (Bianchine et al., 1971). However, the effects of constipation disrupt oral levodopa absorption, and thus motor fluctuations have been observed (Stirpe et al., 2016). A 2012 case study showcased a 69-year-old man who had both PD and intractable constipation for nine years, and unexpectedly presented with parkinsonism-hyperpyrexia syndrome (PHS) (Ogawa et al., 2012). A sudden cessation of dopaminergic activity within the CNS has been shown to cause PHS (Newman et al., 2009). The man in the case study did not discontinue his medication, alter his dosage, or change prescriptions. His physicians concluded that the sudden onset of PHS was triggered by his constipation (Ogawa et al., 2012). Levodopa is absorbed in the small intestine (Ogawa et al., 2012). As such, ingested levodopa is unable to be absorbed for an acute period due to decreased gastric motility and thus cannot be used in the CNS, which can lead to PHS. Hence, PD patients who are comorbid for constipation can experience worsened Parkinsonian symptoms that fluctuate over time due to levodopa malabsorption.

Small Intestinal Bacterial Overgrowth

Compromised gut motility is thought to lead to small intestinal bacterial overgrowth (SIBO) [24]. The small intestine typically contains over one thousand bacteria cells in healthy adults. If the concentration of bacteria cells exceeds one hundred thousand, SIBO syndrome exists [29]. Anaerobic bacteria have a tendency to metabolize sugar molecules into short-chain fatty acids, carbon dioxide, and hydrogen [30]. When SIBO patients exhale, the concentration of hydrogen in their breath is elevated [30]. On the contrary, when healthy humans fast and are at rest, they do not exhale hydrogen as it is produced during anaerobic respiration only [31]. Thus, anaerobic bacteria from the gut generate hydrogen in SIBO syndrome [31].

There are two hydrogen breath tests that can indicate SIBO presence, including the glucose load test and the lactulose test [30]. The glucose load test is more diagnostically accurate and assesses proximal bacterial overgrowth [30]. The lactulose test is less accurate, but can diagnose distal bacterial overgrowth, which is more common [30]. There is currently no universally accepted standard concentration of hydrogen that elicits a definite diagnosis of SIBO [30,32]. Studies report a wide variation of SIBO prevalence in the PD population, ranging from 25.3% to 67% [24,33,34]. Variation between studies could be due to a specific bacterial overgrowth that causes diverse diagnostic interpretation. *Methanobrevibacter smithii* is a gut bacterium that is present in 15-30% of the general population [32]. It converts four hydrogen atoms into one molecule of methane [32]. People with this bacterium tend to exhale less hydrogen as a result, despite the presence of SIBO, causing a false-negative interpretation.

Once bacterial overgrowth is established, it appears to aid with gut motility by allowing the intestine walls to interact with bacterial metabolites that stimulate movement through the intestines [24]. SIBO is correlated with less severe constipation in PD individuals and some researchers suggest that SIBO could offer some benefit in individuals comorbid for PD and constipation [24].

Alternatively, SIBO induces an inflammatory response in the gut mucosa [24,33]. Mucosal inflammation may interfere with intestinal permeability, allowing toxins to permeate the gut epithelium, leading to microglial activation [20]. SIBO is thus generating a proinflammatory environment, which reduces levodopa absorption and aids alpha-synuclein aggregation in the CNS, leading to worse Parkinsonian symptoms [16,20]. Gut motility is reduced because dopamine levels are low, causing decreased movement through the GI tract. Levodopa malabsorption results from this increased GI transit time. The inflammatory environment mediates alpha-synuclein aggregation via cytokine release and interaction with the proteins. Alpha-synuclein is a regulator of dopamine synthesis through its interaction with TH [35]. However, the overexpression of alpha-synuclein reduces activity in the TH promoter region, leading to an overall reduction in TH levels, thereby further reducing dopamine synthesis [35]. As a result, motor fluctuations have a higher prevalence in diseased individuals who also have SIBO [34]. The bacterial overgrowth is a predictor of worse motor function, independent of disease duration [24].

Gut Microbiota

The diseased gut microbiome is different than that of healthy controls (Scheperjans et al., 2015). Upon PD-derived human gut bacteria implantation, mice develop motor impairment, which is not seen with control-derived human gut microbiota exposure (Sampson et al., 2016). Thus, the composition of gut microbiota in mouse models is sufficient to induce the diseased phenotype. Since these studies have shown that human PD gut bacteria implantation causes Parkinsonian-like phenotypes in mice, it may be important to begin similar studies humans. Sampson and colleagues (2016) suggest that there are certain gut microbiota control pathways that stimulate alpha-synuclein aggregation and inhibit aggregate degradation. Other researchers suggest that reduced beneficial bacterial abundance sanctions an inflammatory outbreak, leading to alpha-synuclein aggregate profusion (Unger et al., 2016). Nonetheless, varying gut bacteria concentrations account for the diverse disease phenotypes (Scheperjans et al., 2015).

PD patients have greater Enterobacteriaceae abundance than healthy controls (Table 1) (Forsyth et al., 2011; Unger et al., 2016). The presence of this bacterium is correlated with increased intestinal permeability in PD patients (Forsyth et al., 2011). A compromised intestine wall may expose the ENS to pro-apoptotic factors, possibly driving the PD atrophic sequence (Forsyth et al., 2011). Enterobacteriaceae overabundance is positively correlated with worse postural instability and increased gait difficulty (Mukherjee et al., 2016). Concentration of this bacterial family might be a critical indicator of PD pathology.

Some studies report reduced levels of gut *Lactobacillaceae* in

Table 1. Variation in the gut composition of control individuals compared to PD patients and the associated symptoms for each microbiota or organic material concentration difference.

Microbiota and Organic Materials	Concentration Difference	Associated Symptoms	References
Acetate	-		[37]
Bacteroidetes	-		[37]
Blautia	+		[43]
Bradyrhiociaceae	+		[36, 44]
Butyrate	-	Reduced GI motility	[37]
Clostridiales incertae sedis IV	+		[36, 44]
Coprococcus	+		[43]
Enterobacteriaceae	+	Increased axial motor symptoms More abundant in patients with non-tremor dominant subtype	[36, 37, 44]
Faecalibacterium prausnitzii	-	Reduced GI motility	[37, 43]
Lactobacillaceae[1]	+ -	Reduced ghrelin concentration Reduced inflammation	[36, 44] [37]
Prevotellaceae	-	Increased gut permeability Reduced vitamin concentrations (e.g., thiamine and folate) Reduced ghrelin concentration	[36, 37]
Propionate	-		[37]
Proteobacteria	+		[43]
Roseburia	+		[43]
Ruminococcaceae	+	Abundance is not related to PD, but to reduced Prevotellaceae levels	[36, 44]
Short chain fatty acids	-		[37]
Verrucomicrobiacea	+		[36, 44]

[1]There are between studies differences in *Lactobacillaceae* concentration levels in diseased individuals compared to controls.

diseased individuals (Table 1) (Mukherjee et al., 2016). Since various species promote anti-inflammatory effects, reduced levels reinforce inflammatory probability (Unger et al., 2016). *Lactobacillaceae* appear to modulate the intestinal barrier whereby intestinal inflammation is associated with gut microbiota changes (Mukher-jee et al., 2016; Sampson et al., 2016). The microbiome change might contribute to the misfolding of alpha-synuclein as the proin-flammatory environment mediates protein misfolding via cytokine release and interaction with alpha-synuclein proteins (Sampson et al., 2016). Moreover, reduced *Lactobacillaceae* concentrations in PD individuals reduce dopamine synthesis, as these species are major producers of the neuroactive compound (Borre et al., 2014).

Other studies report *Lactobacillaceae* concentration increases. Both *Lactobacillaceae* abundance and *Prevotellaceae* reduction are correlated with reduced ghrelin concentration (Table 1) (Scheperians et al., 2015). Ghrelin is a gut hormone that primarily signals hunger, but is also responsible for regulating dopaminergic effects in the nigrostriatal pathway (Bayliss et al., 2016; Scheperians et al., 2015). It is thought to be neuroprotective (Scheperians et al., 2015). Reduced ghrelin concentrations leads to dopamine dysregulation, which facilitates central degeneration (Scheperians et al., 2015). Ghrelin has two forms: des-acylated, which accounts for over 90% of circulating ghrelin, and acylated, which constitutes less than 10% (Bayliss et al., 2016). Chronic des-acylated

ghrelin administration increases corticosterone levels (Bayliss et al., 2016). Prolonged exposure to stress is known to have a role in PD development (Bayliss et al., 2016). In contrast, in vivo MPTP exposure shows that acylated ghrelin has neuroprotective effects (Bayliss et al., 2016). The acylated isoform inhibits microglial activation through interaction with the microglial activator MMP-3 in substantia nigral dopamine neurons, preventing successive inflammation (Moon et al., 2009). Accordingly, ghrelin has potential for therapeutic innovation.

Butyrate concentrations are significantly depleted in diseased individuals (Table 1) (Unger et al., 2016). When levels are low, sodium-butyrate concentrations are also low. Sodium-butyrate is a histone deacetylase inhibitor (i.e., a compound that prevents removal of acetyl groups from histone complexes). The compound is dopamine neuroprotective (Unger et al., 2016). A *Drosophila* PD model experiment demonstrated motor impairment prevention upon sodium-butyrate treatment (St. Laurent et al., 2013). In addition, butyrate beneficially impacts the ENS, promoting colonic contractility by interacting with the colon mucosa (Unger et al., 2016). Thus, reduced butyrate concentrations in PD patients leads to GI dysmotility and indirectly increases dopamine neuron histone deacetylation.

Faecalibacterium prausnitzii is the bacterium that produces butyrate as a metabolite (Unger et al., 2016). Levels are reduced in PD patients (Table 1) (Unger et al., 2016). It is a beneficial gut bacterium that has anti-inflammatory properties (Unger et al., 2016). As such, reduced levels may lead to a compromised intestinal epithelium (Mukheriee et al., 2016; Sampson et al., 2016). Entacapone is a drug that is commonly used to treat PD symptoms (Unger et al., 2016). Its use is negatively correlated with *Faecalibacterium prausnitzii* abundance, and consequently butyrate abundance (Unger et al., 2016). It is currently unclear whether the diseased state has a pathological *Faecalibacterium prausnitzii* reduction or whether the medication is causing the decreased abundance.

Mice that are genetically manipulated to overexpress alpha-synuclein show both fine and gross motor deficits, and have GI dysfunction (Sampson et al., 2016). Their gut motility is significantly reduced, resulting in constipation (Sampson et al., 2016). The transgenic mice display low levels of GI microglial activity, increased alpha-synuclein inclusions, and increased motor deficits (Sampson et al., 2016). These symptoms are eliminated in mice whose gut bacteria are diminished by antibiotics (Sampson et al., 2016). However, when the germ-free mice are treated with short chain fatty acids (SCFAs), the diseased state is restored (Sampson et al., 2016). SCFAs are metabolites from the bacterial breakdown of carbohydrates (e.g., butyrate, acetate, propionate) (Borre, et al., 2014; Sampson et al., 2016). When germ-free mice that overexpress alpha-synuclein are treated with SCFAs, their microglia are significantly larger in diameter, and have fewer branches that are shorter in length compared to wildtype mice treated with SCFAs (Sampson et al., 2016). Accordingly, SCFAs potentially mediate gut-brain immune signalling, and their concentration alteration in the diseased state may foster gut-brain dysregulation.

Sampson and colleagues (2016) propose a potential pathway for the pathophysiology of PD from the gut to the brain. Gut microbiota produce SCFAs and the metabolites cross the blood brain barrier and indirectly activate microglia (Sampson et al., 2016). When CNS alpha-synuclein aggregates come in contact with microglial cells, they also activate them (Sampson et al., 2016). Reactive microgliosis involves the release of proinflammatory molecules (Forsyth et al., 2011). Proinflammatory environments promote alpha-synuclein aggregation, driving a feed-forward inflammatory cascade that results in cell death and disease propagation (Figure 3) (Sampson et al., 2016).

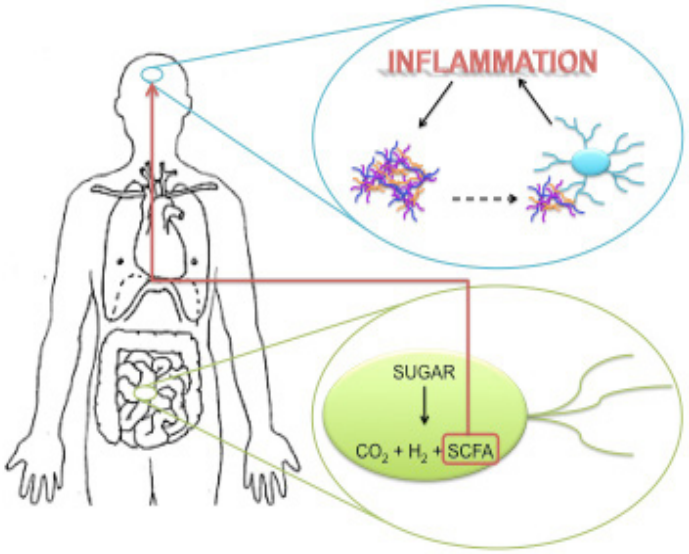

Figure 3. Proposed pathway of disease propagation from the gut to the brain. Anaerobic gut bacteria metabolize sugar into carbon dioxide, hydrogen, and short chain fatty acids (SCFAs). The SCFAs cross the blood brain barrier and indirectly activate microglia. When alpha-synuclein aggregates in the brain come in contact with microglial cells, they also activate them. Microglial hyperactivation leads to inflammation. Proinflammatory environments promote alpha-synuclein aggregation, driving a feed-forward inflammatory cascade. (Nygaard, 2010; Sampson et al., 2016)

Discussion and Future Directions

It is evident from recent studies that the gut is involved in Parkinson's disease. Constipation is an early non-motor symptom, which has sparked intrigue to analyze the gut-brain axis and moreover the ENS. Reports have shown a strong implication of gut bacteria in disease pathology. Microbiome alterations appear to play a significant role in the disease phenotype. However, the diseased state does not elicit a noteworthy effect on the phenotype of myenteric neurons [21]. Thus, the specific myenteric neuron subtype is not correlated with pathology susceptibility. In addition, the exact compartment of alpha-synuclein aggregation commencement is unknown [21]. Annerino and colleagues (2012) examined the myenteric plexus in a subset of patients with advanced PD and found

aggregation throughout the entirety of the GI tract. Future endeavours should use a cohort with a wide range of diseased states, including those who are likely to develop PD. A timeline for gut PD pathology development has not yet been produced. Furthermore, the timing of ENS inception compared with CNS propagation has not been discovered. Such evidence would be sufficient in answering the sought after answer regarding whether the gut or brain is affected first.

The dual-hit hypothesis suggests that a viral neurotropic pathogen initiates Parkinsonian degeneration after entering the body through nasal mucous [15]. The secretions drain into the stomach and are thought to penetrate the epithelial lining [15]. Submucosal axons are believed to mediate pathogen transmission to the vagus nerve [15]. The neurotoxin is assumed to propagate into the medulla and ultimately infect the midbrain region [15]. Although this hypothesis has not yet been evidentially supported, it could be the critical connection between gut and brain interdependence in PD pathology. To our knowledge, the diseased submucosal plexus has yet to be examined. Since the dual-hit hypothesis suggests a role of submucosal axons and since pathology in myenteric neurons is controversial [19,21], there is a demand to expose the accurate function of the submucosal plexus in disease propagation.

A prominent barrier in quantifying and analyzing gut dopaminergic cells lies in the method of identification. TH immunoreactivity is prominently used to detect dopamine neurons [8]. The problem with using this method in the gut is that TH coexists in dopamine and norepinephrine neurons [8]. The gut is innervated by large amounts of noradrenergic sympathetic axons that extend from the extrinsic prevertebral ganglia to the bowel [8].

Staining for both TH and dopamine β-hydroxylase, an enzyme that converts dopamine into norepinephrine would thus be advantageous. Future research must address this issue and consider other identification techniques.

Reduced *Faecalibacterium prausnitzii* levels result in low butyrate concentrations in PD individuals [37]. Butyrate concentrations can be measured from fecal samples [37], which is a non-invasive tool that could potentially be used as a diagnostic marker. However, it is currently unclear whether the reduced bacterial levels are pathologically derived or whether they are triggered by Entacapone use [37]. In pursuance of potentially using butyrate quantification as a diagnostic strategy, it is first critical to elucidate whether Entacapone is causing the concentration change.

Reduced *Prevotellaceae* abundance is also observed in PD patients [15,36,37,43,44], though the observation is not exclusive to PD as it is also present in autism and type I diabetes [36]. Fecal samples could however be used to exclude a PD diagnosis [36]. If fecal *Prevotellaceae* levels are high, PD is likely not diagnostically appropriate. However, if levels are reduced, PD remains a diagnostic candidate. Since this method will not definitively reveal a PD diagnosis, further strategies will need to be implemented.

Although partially invasive, a colon biopsy, whereby researchers are looking for alpha-synuclein aggregates, has potential as a diagnostic approach [23]. The ENS is certainly more accessible than the brain. However, current microbiome knowledge and imaging is not yet sufficiently advanced to use this strategy. For instance, current studies have not yet evaluated the differences in gut microbiomes of individuals with PD, constipation, and those who are comorbid for the two. This comparison would be useful in identifying specific bacterial concentrations in the diseased gut alone, furthermore having potential to illuminate PD specific biomarkers. Future endeavours should certainly focus on accumulating correlative evidence surrounding this notion.

Not only can the gut microbiome be used to potentially diagnose PD, but moreover it may be informative in devising treatment options. Recent studies suggest the importance of ghrelin investigation in neuroprotective efforts against PD progression [40]. Acylated ghrelin has been administered experimentally with promising results in slowing PD progression [40]. It would thus be valuable to consider the use of acylated ghrelin as a potential therapeutic technique, especially during the early stages of neurodegenerative diseases.

Sampson and colleagues (2016) have shown that the diseased microbiome is sufficient to induce Parkinsonian symptoms. It may alternatively be possible for the removal of the diseased microbiome to also remove disease symptoms. One patient with PD has shown symptom improvement in response to fecal transplant [45]. Further, three patients with multiple sclerosis who underwent a fecal transplant regained walking ability [46]. Gut bacteria, possibly through the normal metabolism of carbohydrates into SCFAs, thus have a strong impact on motor functioning. Future investigation of the interaction between the microbiome and PD pathology in order to develop more effective therapies is encouraged.

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Journal of Young Investigators
Celebrating 20 years of undergraduate research
REVIEW ARTICLE

Application of Neuroscience Principles for Evidence-based Design in Architectural Education

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We spend approximately 90% of our time within a built environment, whether it is in our homes, offices, schools, city parks, or public spaces. This bears significance, as we are equally shaped by both our genetic makeup as well as our environment, which brings into question of how we experience space, and in turn how these experiences impact our behaviour. To gain a greater understanding of these impacts, neuroscience seeks to root out the principles of biological mechanisms involved in consciousness, spatial navigation and environmental stressors. However, the use of these principles is not discussed extensively within the curriculum of undergraduate or graduate architecture programs in North America. This paper aims to highlight that such information is critical to the advancement of evidence-based design by acknowledging the role of conscious awareness within space. An observable shift in the design community in collaborative neuroscience research can be seen in multi-sensory and virtual reality labs being constructed into design firms within North America. This review stipulates that architecture students should be prepared for the changes in quantifiable research and perceptual data collection coming to their field by examining the importance of neuroscience research in perception and consciousness. Architecture students who can interpret scientific research will not only have the advantage of a greater understanding of the human condition within space, but they will also be able to evolve the standard of design.

INTRODUCTION

Architects have long sought to inspire creativity, ingenuity, worship, community and awe using the tools at their disposal. Homo faber, "Man the Maker", crafts his environment, thereby controlling his fate. As a result of human ingenuity, we now spend over 90% of our time within a built environment crafted to suit our needs (Janda & Janda, 2017). Design is inspired by societal reform and scientific exploration expressed as an art form in itself. If architecture is an expression of creativity as a mean to reflect on the human condition, one might argue that such a reflection can also be found within neuroscientific exploration of the mind. As we come to understand the biological mechanisms of perception, consciousness and their residual impacts on mental and physical health, there is question of how our environment might in turn affect those mechanisms.

Perception of space relies upon conscious awareness: the ability to receive and comprehend exterior and interior stimuli through the use of the Global Workspace Theory. A good example of the interjection of neuroscience and architecture can be found in spatial navigation research. Scientific authors are capable of identifying floor patterns that are most and least useful to way-finding. Studies have also found that computer game and virtual reality architects may play an integral role in retaining memory and attention in elderly populations (Optale et al., 2010). Architectural students can benefit from a greater understanding of the impact of environmental stressors on biological mechanisms. Chronic stress response is one of the most pressing design problems as it may increase the risk of psychobiological disorders such as immune deficiencies, irritable bowel syndrome, depression, and anxiety (Hammen, 2015).

Neuroscience research permits an objective review of the usability and mental health impacts of space. "An informed architect could use this research as a means for evidence-based design (EBD), a concept which seeks to ratify design standards of the built environment by incorporating research from multiple disciplines into the design process." However, of the 113 post-secondary architecture institutions in North America, only the New School of San Diego offers students a certification program, which applies neuroscience principles to evidence-based practice ("Certificate in Neuroscience for Architecture" 2017). Although EBD is currently taught in many programs geared towards renewable/sustainable/green buildings, a truly multidisciplinary approach to EBD involves neuroscientific, psychological and economic research to guide design, a method commonly used in healthcare facilities today (Ulrich, Zimring, & Zhu, 2008).

Students in architecture are entering a field which is now exploring ways to make use of neurobiological data analysis involv-

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ing environmental stimuli to achieve EBD. This review stipulates that architecture students will benefit from a greater understanding of conscious perception towards health-centric design. This will also allow them to collaborate with multisensory and virtual reality labs along with other cutting edge design firms and academic institutions merging neuroscience research into design.

Neurological Influences of the Environment on Health

Consciousness is perhaps the most important existential question that has yet to be solved. It is described in a number of ways in relation to philosophical, psychological, and neuroscientific interpretation. It is broadly defined as "the mind", the perceptual awareness of external and internal stimuli, which influence cognitive activity (Searle, 2000). Consciousness is often considered separate from other neurological mechanisms that humans share due to its qualitative and subjective nature. The majority of consciousness research has been conducted on the verbal and behavioural assessment of participants. These findings can be questionable as we all experience our environment, our personalities, and our memories differently thereby increasing the chance of error in experimental findings. However, consciousness is an entirely biological phenomenon and subjective ontology challenges, but does not prevent an objective scientific research. The breadth of this field touches upon multisensory interpretation, memory recall, attention and various cognitive mechanisms; however, this section will focus on the conscious effects of our environment on way-finding and stress.

Through the use of electroencephalography (EEG) and functional magnetic resonance imaging (fMRI), neuroscientists attempt to identify the neuronal pathways, which produce the conscious experience. The Global Workspace Theory (GWT) is widely accepted in the scientific community to be representative of conscious and unconscious processes (Prakash et al., 2008). It is similar to the concept of working memory in that GWT proposes experience to be momentary and subjective. Multisensory stimuli (conscious) are initially interpreted by various cognitive processes (unconscious), which is referred to as the "receiving process". This information can then be used to produce a movement, emotion, or behaviour. GWT lends itself quite readily to computational modeling and can distinguish the brain regions impacted by competition of sensory modalities (e.g., a video and its audio being out of sync).

Way-Finding and Spatial Awareness

There are a multitude of research initiatives involving the brain and behaviour within the environment. Way-finding will be used as a practical example to showcase the power of design as a psychobiological influencer on behaviour and health.Way-finding is the neurophysiological experience of self while navigating the environment. This biological mechanism allows us to locate ourselves within space by taking in information from visual and auditory cues while simultaneously utilizing stored spatial memories. The environmental cues paired with spatial memory then allow for a decision to be made via limb movement and body axis direction (Macagno, 2014). Way-finding is a particularly relevant tool in design as spatial alignment efficiency can either aid navigation or cause confusion and unease within the built environment.

Way-finding can be categorized by the activation of neuronal pathways that create a cognitive mapping system. Nobel Prize laureate John O'Keefe and Lynn Nadel researched hippocampal cell signalling in rats. They found that specific groups of neurons, termed "place cells", fired when rats either tasked with location recall or object recall (O'Keefe & Nadel, 1978). The authors stipulated that place representations within the hippocampus were activated together depending on the physical and perceived distances between places. Later on these findings formed the basis of the theory of "grid cells", place-modulated cells in the presubiculum and hippocampus which fire in a crystal-like fashion in conjunction with head-direction neurons (Boccara et al., 2010). The discovery of hippocampal involvement of place cells and grid cells has offered a fascinating insight on the way we understand geometric boundaries, spatial memory and directional movement. These findings may be especially significant to architects when considering the effects of floor misalignment on the perception of our environment. Designers may also take advantage of research involving the deterioration of the aging hippocampus which impacts spatial memory in the geriatric population.

One of the ways that a building is considered a success is in the functional ease of navigation. There is a positive correlation between the perceived figural complexity of space and how the actual space reflects that perception (Weisman, 1981). If misaligned, the spatial structure of the built environment can be known to cause cognitive dissonance with way-finding. A study performed in 2004 by Werner and Schindler studied this effect via the use of a computer program simulating various aligned, misaligned, connected and disconnected floor plans (Figure 1). Fifty-six participants familiarized themselves with a digital interface and an assigned floor plan. They were then instructed to find five target objects within the span of five minutes (Werner & Schindler, 2004). The authors found that misalignment to a central reference frame reduced the speed of accuracy in finding objects by 25% as compared to aligned floor plans. When participants were asked to point in the direction they believed an object might be according to a specific floor plan their pointing error was on average greater for misaligned than aligned floor plans (Werner & Schindler, 2004). These findings represent an exciting example of how neuroscience can interject itself into architectural education. The orientation of a floor plan can directly impact the usability and positive experience within space, this information can help disseminate patterns of design that are more efficient, thereby reducing spatial dissonance.

Finding our position within space is directly impacted by our ability to recall the layout and landmarks of our environment, and this ability deteriorates significantly with age. From approximately 50 years onwards, MRI scans reveal a decline in white matter volume, reaching up to 26% in reduction at 90 years old (Gunning-Dixon, Brickman, Cheng, & Alexopoulos, 2009). This

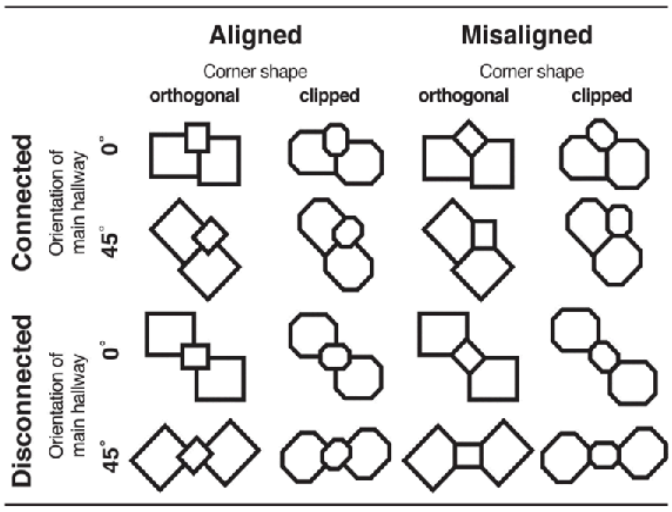

Figure 1. 16 floor plans, the top two rows are connected (at 0° and 45°), the two rows at the bottom are disconnected floor plans (at 0° and 45°) (Werner & Schindler, 2004). Findings suggest that aligned floorplans reduce effort to establish spatial navigation.

cell loss is detrimental to hippocampal functioning, the principle region responsible for spatial memory in regards to navigation and orientation. However, research suggests that enriched environments have positive neurological benefits for the geriatric community. Researchers took a novel approach of using virtual reality (VR) to determine the possible effects on cognition and memory (Optale et al., 2010). The participants, a median age of 80 years old, were placed in either a VR memory training experimental group or control group for 6 months. The VR training involved simulated visuo-auditory environments and focused on way-finding scenes. The authors found that participants treated with VR memory training had better long term memory and cognitive functioning than the control group which they theorized may have been caused by a boost in attention capabilities. Way-finding and memory training using virtual reality environments designed using perception based learning programs can be used to reinvigorate spatial memory recall.

How we design our built environment influences the ways that we behave within that environment. There should be an educational focus on tested principles in way-finding to promote evidence-based design. Using research, we can disseminate the floor plans that generate the greatest positive response to navigation within space. When considering aging populations architects can assist virtual reality programmers in creating enriched environments that can improve spatial memory.

Impact of Environmental Stressors on Health

The stressors within our environment can shape our health. This is particularly true of how chronic stress can cause immune deficiencies and increase susceptibility to psychobiological disorders. The hypothalamic-pituitary-adrenal (HPA) axis is the pathway which mediates stress response. As a survival tactic, HPA axis activation causes physiological changes such as disruption of the digestive system, vasodilation and the release of adrenaline. The HPA axis secretes glucocorticoid cortisol, an anti-inflammatory response hormone, to suppress inflammatory cytokine production. This can be accomplished by inhibiting pro-inflammatory gene promoters, blocking in cell cascades effects, and antagonizing protein-protein interactions which mediate cytokine production (Slavich & Irwin, 2014). Under chronic stress circumstances we can observe a glucocorticoid resistance, as immune cells become less sensitive to anti-inflammatory mechanisms, causing an inability to properly regulate rising cytokine levels (Schleimer, 1993). Inflammation also causes an up regulation of enteroendocrine cells which produce serotonin, permeating the sympathetic response of the HPA axis (Spiller et al., 2000). Therefore, chronic stress can cause immune response irregularities, which can in turn increase susceptibility to common viral attacks and functional disorders, and diminish our capacity to mitigate future stress responses.

The immune system is the body's first line of defense against viral, pathogenic and bacterial infections. This is accomplished by first detecting the agent, and then sending neural and endocrine signals to the brain. These signals up regulate the creation of inflammatory response cytokines IL-6, IL-1, and TNF-α to the affected area in an effort to contain the infection (Slavich & Irwin, 2014). Genetic predispositions towards inflammation and stress response inhibition combined with the impact of environmental stressors have been connected to an increased risk to functional disorders such as irritable bowel syndrome (IBS) (Drossman, Camilleri, Mayer, & Whitehead, 2002). It is estimated that approximately 10-25% of the population is affected at some point but only 30% of those suffering from IBS are likely to seek out treatment (Drossman et al., 2002). Overexposure of proinflammatory cytokines and cortisol may also increase susceptibility to heightened states of anxiety and depression. Furthermore, deregulation of the HPA axis can impact synaptic plasticity as well as dopaminergic and serotonin output within the striatal areas, the amygdala, and the hippocampus (Hammen, 2015).

Furthermore, maladaptive stress response can be transmitted through transgenerational epigenetic modification. In rodent and primate models, we can observe prevalence in genetic expression instigated by stress response across multiple generations (Franklin et al., 2010). Studies have shown that unpredictable home environments in rats can reduce 5HT1A receptor expression in the dorsal raphe nucleus in descendants (Franklin et al., 2010). This change in receptor expression is akin to the pathogenesis seen in antisocial behaviours and personality disorders (Gudsnuk & Champagne, 2012). Vulnerability to stress induced psychobiological disorders is perhaps the most urgent health contingency that neuroscience research can address in the greater development of evidence-based design architecture.

EVIDENCE-BASED DESIGN

Evidence-based design (EBD) involves the use of clinical research in the design concept of the built environment to improve health,

productivity, and economic outcomes. It is a relatively new approach as it prioritizes objective and quantifiable results. EBD utilizes mounting research from neuroscience, environmental psychology, architecture, and behavioural economics to produce a framework of desired outcomes from our buildings. This section will review the process of EBD in addition to the architecture curriculums integrating of neuroscience and architecture. Furthermore, there will be an observation of the benefit of EBD in the design of health care facilities.

EBD and Neuroscience

There are a multitude of comparisons in the way science and design curriculums measure feasibility of findings and outcomes. Scientific concepts must be grounded within specific methods intrinsic to their validity. Publication of findings is critical to the advancement of research as it allows for objective review. The architectural approach differs as the interpretation of design is often subjective when considering the cultural and artistic ramifications of a structural landmark. Design is often led by trend or form, a novel build is often praised for its avant-garde design and emphasis on function can be perceived as a detriment to creativity. However, neuroscience research involving perceptual stimuli and its impacts on behaviour and health can be used to improve the current practice. As in scientific exploration, architecture may see its greatest advancements once design research is integral from building conception to measured impact and publication of findings.

A truly progressive curriculum expands onto scientific dialogue, which seeks to validate how to best enhance the human experience and eliminate the designs that are not beneficial. Students must understand the components which are conducive to those human experiences in order to conduct an evidence-based practice of responsible design. One post-secondary curriculum which stands out amongst others in terms of neuroscience and architecture integration is the New School of Architecture in San Diego. This is the first educational institution in the world to offer a certificate program in Neuroscience for Architecture ("Certificate in Neuroscience for Architecture" 2017). The courses focus on four areas that involve evidence based design practice. Students learn about environmental psychology, which is the quantifiable relationship between environment and behaviour. There is also an overview of the neurological components responsible for sensory and cognitive responses, which permit human experience within space. Students have access to neuroscience seminars focus on how to best improve health care facilities, educational, spiritual, and corporate environments using neuroscience principles. The integration of these concepts is further solidified with studio time geared towards applying these principles towards the built environment.

This program was developed by fellow of AIA and founder of the Academy of Neuroscience for Architecture (ANFA) John Eberhard, along with Dr. Eve Edelstein, PhD in Neuroscience and MA in Architecture. Both have extensive backgrounds in research and practical application of neuroscience within the built environment. Eberhard is the author of such behavioural neuroscience and architecture books such as "*Inquiry by Design*"(with John Zeisel, 2006), "*Architecture and the Brain*" (2007), and "*Brain Landscape*" (2008). His involvement in promoting neuroscience based EBD led to the creation of ANFA, a nexus of both fields in collaboration and research. Dr. Edelstein is the world's first PhD Neuroscientist with a master in Architecture. She has contributed to over 43 scientific papers involving the impact of the environment on the body and brain. As a faculty member of the New School, Dr. Edelstein is educating architecture students on the concept of the built environment as a psychobiological influencer on behaviour and health. She is also the founder of Innovative Design Science, which is a design firm that specializes in implementing neuroscience research, virtual reality mock-ups, on-site design studies, as well as pre- and post-occupancy evaluations of the build. Students in San Diego are privileged to take part in a new approach towards architectural education, as they will come to understand the benefits of neuroscience and EBD in creating better buildings for its occupants.

The Role of EBD in Healthcare

EBD is most commonly used in the design of healthcare facilities. This may be due to the higher risks associated with hospitals which demand informed design to minimize loss. The concept of EBD first emerged in the 1960s as American and British health care providers measured the impact of spatial alignment and wayfinding of floor layouts on staff productivity (Clipson & Johnson, 1987). Today, its method has been widely adopted by health care providers across North America. Notably, the US military Health System has constructed over 70 hospitals totalling $6 billion dollars in construction (Ulrich, Zimring, & Zhu, 2008). EBD in healthcare focuses primarily on four components: mental health improvement for patients and staff, patient recovery, staff productivity, and the use of evidence-based metrics.

The Center for Health and Design has provided a universal list of guidelines to perform EBD in healthcare facilities. The first step involves a literature review of neurological, psychological, architectural and economic research in relation to the problems the project is attempting to solve. Financial operations also need to be considered in association to multi-year investment returns and cost-effectiveness of design options. SWOT analysis is used as a decision-making tool in the placement of technical and safety healthcare features. Furthermore, the design team is heavily involved with patients and staff in regards to surveys, simulations, and pre- and post-occupancy evaluations. The goal of this method is to acquire as much information as possible in regards to healing environments to guide the construction of the facility.

The impact of EBD in healthcare is that of a measureable improvement in health outcomes, which leads to a reduced chance of infection and medical error, thereby reducing the length and cost of a patient's stay. Researchers performed a meta-analysis of healthcare facility layouts and patient recovery time (Ulrich et al., 2008). The findings suggested that single patient rooms reduced the chance of infection, allowed for better communication

with staff, and length of stay (Ulrich et al., 2008). The Agency for Healthcare Research and Quality (AHRQ) is currently leading the way in EBD by lobbying for the health and economic research in hospital design. The center advocated the use of EBD as a means to reduce avoidable incidences by using single patient room layouts, acuity-adaptable rooms and accessible nursing stations (Shoemaker & Kazley, 2010). The AHRQ also mentioned that too often there are no clear, measurable or expected outcomes for large design projects. Although EBD demonstrates these features readily, it is not widely adopted throughout the field of design (Shoemaker & Kazley, 2010). These concepts are beneficial to more than just the healthcare community as the use of EBD can transfer over to all building types and occupancy groups to improve living standards.

An understanding of scientific analysis impacts the ability for designers to implement the important research of perceptual awareness, behavioral interaction and consciousness into the conception of various structures. Educating architecture students in neuroscience allows for an EBD approach to be implemented in all areas of design. If students are more experienced with research and foster a greater awareness of the use of measurable impacts on health, they will be equipped with the knowledge to push design standards forward.

CONCLUSION

The future of architectural design will depend upon the advancement of evidence-based design and the inclusion neuroscientific research regarding the human experience within space. Studies involving consciousness and the Global Workplace Theory can be used to teach design students about neural correlates which permit conscious awareness (Mallgrave, 2010). A fitting example of neuroscience in architecture is found in the research involving spatial navigation and way-finding. The alignment of floor plans and their feasibility can be monitored and designed to permit the greatest ease in locating one's self within a build environment. The aging population may also be presented with virtual reality experiences designed by computer game architects to improve memory and attention capacity (Optale et al., 2010). Neuroscience research may also be used to study the effect of environmental stressors on mental health. Students, receiving an overview of chronic HPA axis activation and its role in psychobiological disorders such as IBS, depression, anxiety and transgenerational modifications, have a responsibility to minimize the impact of stress in our daily lives through responsible design. Should students be more aware of the influence of design on the health mechanisms that allow conscious interpretation to take place, they would be more capable to participate in evidence-based design practices.

There is only one architectural program in North America which offers a Certificate in Neuroscience for Architecture; it is offered at the New School of San Diego. Students come into contact with the benefits of EBD with courses on environment and behavior, an overview of the conscious response within space, neuroscience seminars, as well as studio time dedicated to the merger of both disciplines ("Certificate in Neuroscience for Architecture" 2017). EBD is currently taught in many Architectural programs, but the course work only relates to the use of energy efficiency research to increase sustainability rather than neurobiological research to enhance perceptual experience. However, the application of neuroscience principles in EBD is widely accepted in one area of architecture today, healthcare facilities. The multidisciplinary approach involves extensive background research, patient and staff health, and economic feasibility by implementing design standards that will reduce the length and cost of a patient's stay. The methods used in the design of healthcare facilities and their measurable outcomes can be applied to any building type. Designers working specifically within EBD using neuroscience research demonstrate that the methods can be taught in architectural programs to promote responsible design.

The limitations of the present research involve the subjective nature of the conscious experience. The greatest challenge to the application of neuroscience as a tool in EBD involves its acceptance within the architectural community. Neuroscience and EBD are generally found within healthcare design as the planning, financial, and life risk implications are extensive. The design process is much greater and more time consuming than other builds on average. However, students stand to benefit from scientific incorporation within design, if only to have a better understanding of the impact of their work.

The future directions of the merger of these two fields involve the use of interactive labs funded by governmental agencies and architecture firms in collaboration with academic institutions. Public access to design research will improve social welfare by eliminating the design standards that are not conducive to occupant health and wellbeing. Architecture firms will need to look at the impact of their builds and become accountable for their health impacts. This may be accomplished by performing post-hoc analyses, animal and human lab research, retrieving foot traffic sensor data, satisfaction surveys and virtual reality prototyping. Before, we could look at architecture as a balance between form and function, which mostly based on what we feel rather than what we can prove as the science was not present. Allowing design to go on without accountable measures of perceptual adaptation when they are now becoming available through research negates advancement within the field and students should be ready for the changes to come in their profession.

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Journal of Young Investigators
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REVIEW ARTICLE

Therapeutic Potential of Optogenetic Treatment for Individuals with Multiple Sclerosis

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Multiple Sclerosis (MS) is a chronic neuroautoimmune condition characterized by neurodegeneration and demyelination throughout the central nervous system. While the pathology of MS is largely unknown, its symptoms are well defined. Current MS therapies such as intravenous corticoid injection, disease modifying treatments (DMTs) and neuro-rehabilitation exist; however most are ineffective as they do not manage symptoms efficeiently, leading to many adverse side effects. Optogenetic stimulation of demyelinated regions may serve as the needed therapy to effectively treat symptoms given the advances achieved in its rapid mechanisms and accurate cell-type-specific delivery strategies. In fact, the hallmark of optogenetic technology is the fast and accurate activation of specific neurons. Current evidence supports optogenetics as a means of controlling or enhancing neural circuitry involved in specific symptoms. This is done by targeting specific cells implicated in their respective neural circuits and activating them, or activating interneurons that inhibit the target pathway. Moreover, continuous photostimulation has been found to strengthen neuronal circuitry by promoting long-term potentiation (LTP). This review analyzes several studies that utilize optogenetics to alleviate MS-related symptoms such as cognitive impairment, visual impairment, bladder/bowel dysfunction, and tremors by controlling their specific pathways. It will also assess how these studies may translate to MS patients. Possible challenges in creating such a treatment will also be discussed. Given literature on the application of optogenetic treatment in neurodegenrative models is limited, this review presents a theoretical means of creating optogenetics treatment for MS and other neurodegenerative disorders.

Introduction

Multiple Sclerosis (MS) is a chronic autoimmune disease that leads to focal and diffuse neurodegenration and myelination throughout the nervous system (Kolasinski et al., 2012; Siffrin, Vogt, Radbruch, Nitsch, & Zipp, 2010). In its most common form, relapse-remitting MS, it is characterized by high inflammation levels that lead to a continuous cycle of relapse and remission (Raffel, Wakerley, & Nicholas, 2016). These relapses, called exacerbations, may come in the form of new or worsening of old symptoms that are largely neurological such as visual impairment and imbalance that worsen over days or weeks, then recover spontaneously (Wingerchuk et al., 2014). Other common symptoms are cognitive impairment, loss of bladder control, leg weakness and sensory symptoms (Raffel et al., 2016).

Genetic and environmental factors both have a role in MS development; however, a specific link to the disease has not been found (Harbo, Gold, & Tintoré, 2013). Genetically, MS is best characterized by a mutation on the human leukocyte antigen (HLA) gene locus, which causes abnormal antigen recognition of T cells leading to attacks on myelin proteins (Raffel et al., 2016). These findings have not been conclusive, as many other genes involved in immunological roles have also been found to play a role in contributing to MS. Environmental risk factors include smoking ,sunlight exposure, and vitamin D deficiency (Raffel et al., 2016).

Currently, there are no treatments that cure MS (Ziemssen et al., 2016). Instead, treatments target symptom management to increase patients' quality of life. These include high doses of corticosteroids such as methylprednisolone (Jongen et al., 2016), Disease modifying Treatments (DMTs) such as interferon β-1a, interferon β-1b, alemtuzumab, fingolimod and natalizumab (Carrithers et al., 2014; Gajofatto & Benedetti, 2015), and neuro-rehabilitation (Dasari, Wootla, Warrington, & Rodriguez, 2016). All of these treatment options have adverse effects or are not particularly effective in the long term (Jongen et al., 2016; Ontaneda, Fox, & Chataway, 2015; Schäcke, Döcke, & Asadullah, 2002; Ziemssen et al., 2016). There is a considerable need for new treatment options that are more effective, while reducing the adverse side-effects. A potential therapy for MS-affected individuals may be the therapeutic application of optogenetics.

Optogenetics is a novel method that utilizes photoreceptors to selectively activate neurons (Hegemann & Nagel, 2013). The genetic code of these receptors is delivered either virally or non-virally to be expressed on the cells of interest. Once expressed,

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light is shone directly on these cells through an optic fiber inserted into the brain or spinal cord to be activated. Advances in this technology allow the photoreceptors to be selectively expressed on specific cell types, and in turn enable the control of specific neural pathways. Although largely used in research applications (Namboodiri & Stuber, 2016), literature describing the therapeutic potential in neurodegenerative diseases is lacking. More recently, there has been a spike in literature demonstrating the therapeutic applications of optogenetics in the context of various disorders and symptoms. For example, evidence has shown optogenetic stimulation enhances cognitive function (Goshen, 2014), visual ability (Jazayeri & Remington, 2016), bladder/bowel dysfunction (Stamp et al., 2017), and tremors (Tønnesen, 2013) through neuromodulatory effects.

While many of these studies have been conducted on specific symptoms, the focus of the optogenetic treatment has not been on any neurodegenerative models that express similar symptomology. Indeed, the current literature on the topic does not present any research on the translation of optogenetic treatment into any neurogenerative models expressing similar symptomology, requiring further research in this field (Ahmad, Ashraf, & Komai, 2015; Bordia, Perez, Heiss, Zhang, & Quik, 2016; Bryson, Machado, Lieberam, & Greensmith, 2016; Jazayeri & Remington, 2016). This review paper will assess the potential use of optogenetics in the development of therapies for MS related symptoms such as cognitive impairment, visual impairment, bladder/bowel dysfunction, and tremors.

Development of Optogenetic Mechanisms

Initial studies investigating the use of photoreceptors invovled metabotropic photoreceptors of vertebrate and invertebrate eyes (Zemelman, Lee, Ng, & Miesenbö Ck, 2002). However, these systems were too complex to manipulate and the delay between light exposure and action potential firing was highly variable ranging from a few hundred milliseconds to tens of seconds (Lyon, 2013). Focus shifted to ionotropic microbial opsins as they exhibit fast, direct light-dependent ion conduction across the cell membrane (Mudiayi, Wong, & Gruber, 2015). Furthermore, microbial opsins allow reversible control of neurons on the timescale of individual action potentials, which was lacking in earlier methods (Boyden Zhang, Bamberg, Nagel, & Deisseroth, 2005). From a therapeutic perspective, however, the difference in seconds and milliseconds between the speed of activation of these cells is not significant. The rapid control of these cells does not necessarily affect the overall efficiency of the treatment. It is critical to understand that most optogenetic developments were directed as a means to enhance research tools. For example, creating faster optical controls allow remote control of individual spikes or synaptic events and enabling genetically targeted photostimulation with finer temporal resolution (Boyden et al., 2005). While the increased control speed is welcomed, it holds no major significance other than that symptoms would be halted seconds earlier. However, as it stands today, most studies use the microbial channelrhodopsin-2 (Nagel et al., 2003), warranting focus on this protein as a primary candidate for developing an optogenetic treatment.

ChR2 is isolated from the genome of the single celled algae *Chlamydomonas reinhardtii* (Nagel et al., 2003). ChR2 application was further developed by inserting the protein via viral vectors into mammalian hippocampal (HPC) neurons (Boyden et al., 2005). Once imbedded, high-speed optical switching photostimulates neurons, impressively responding in one to two milliseconds. Furthermore, neural activity was controlled by simply switching the optical blue light on or off (Boyden et al., 2005). This control can also be used by activating inhibitory circuitries, creating an antagonist effect on the region or function of interest. Molecularly, once the optical light is shone on the brain region of interest, photostimulation increased ion transport across the cellular membrane by either opening an ion channel or by actively pumping ions (Mudiayi et al., 2015).

Current Delivery Strategies and Therapeutic Obstacles

Non-viral delivery methods of expressing ChR2 in cells include in utero electroporation, transgenic models, chemical lipofection, and laser-assisted cellular poration (Boyden et al., 2005; Carter & de Lecea, 2011; Mohanty & Lakshminarayananan, 2015). Although beneficial for research, these methods are not viable translational strategies for human treatment. Moreover, these methods possess limitations such as not being able to specifically target cells, posing a risk to cellular components or the foreign DNA, and association with axonal pathology (Bryson et al., 2016; Mohanty & Lakshminarayananan, 2015). Due to the lack in cell specific targeting and the lack of lateralization to humans of non-viral methods, it seems viral delivery methods provide the most sensible means of creating an optogenetic treatment, especially when studied in non-human primate models.

The most common method of expressing ChR2 in a nervous system is to infect neurons with a deficient virus replication, typically an adeno-associated virus (AAV) or lentivirus (LV), containing the transgene of interest driven by a short promoter or enhancer element (Carter & de Lecea, 2011). AAVs are small viruses that efficiently transduce neurons while inducing minimal immune responses in the host brain (Blits et al., 2010). LV vectors are derived from a genus of retroviruses that cause chronic diseases characterized by long incubation periods such as the human immunodeficiency virus (HIV; Dull et al., 1998). In both methods, once ChR2 is expressed in the region of interest, illuminating the neurons with blue light at a bandwidth of 450–490 nm induces rapid depolarizing currents. However, literature has shown a difference in efficacy when used in non-human primates. One study has shown transduction with AAV yields positive functional and behavioral results, but not LV, indicating that AAV may be a more effective viral delivery method in primates compared to LV (Gerits et al., 2012). Moreover, in a recent breakthrough study,

successful cell-type-specific expression of ChR2 in midbrain dopamine neurons of wild-type Rhesus macaques utilized AAVs, not LVs (Stauffer et al., 2016). A vector delivering Cre recombinase under the control of a tyrosine hydroxylase (TH) promoter fragment and a vector delivering a Cre-recombinase-dependent ChR2 were mixed and injected to attain cell-type-specific expression of ChR2 (Stauffer et al., 2016). The TH promoter can be substituted in the first vector to other neuron-subtype-specific promoters to optogenetically control other neuron types in a monkey brain. For example, in an MS patient with lesions in the spinal cord affecting γ-aminobutyric acid (GABA) neurons, the Cre-recombinase vector being develived would require a glutamate decarboxylase (GAD) promoter fragment. This is because GAD is the enzyme that catalyzes the decarboxylation of glutamate to GABA and is only found in GABAergic cells, ensuring ChR2 expression is limited these cells. Further applications of this technique can be found in treatment of various MS symptoms utilizing the specific pathways and neural circuitry they operate through.

Evidence of Therapeutic Potential for Multiple Sclerosis Related Symptoms

Photostimulation may serve as a factor in dealing with MS symptoms such as cognitive impairment (Goshen, 2014), visual impairment (Jazayeri & Remington, 2016), bladder/bowel dysfunction (Stamp et al., 2017), and tremors (Tønnesen, 2013) by controlling their specific pathways. This especially possible once coupled with stem cell therapy (Bryson et al., 2016). However, while optogenetics may treat these symptoms, this review does not intend to demonstrate the effect of photostimulation on the autoimmune function of the disorder. To our knowledge, literature documenting immunomodulation using optogenetics is lacking, and what has been published only discusses proof-of-concept and designs for future development (Tan, He, Han, & Zhou, 2017). The approaches involve optogenetic control of immune responses with novel tools that modulate lymphocyte trafficking, inflammasome activation, dendritic cell (DC) maturation, and antitumor immunity (Tan et al., 2017). Further information on the theoretical methods involving the combination of optogenetics and immunoengineering, termed optoimmunoengineering, can be found in the review conducted by Tan et al. (2017)

The most common cognitive impairment seen in MS is visual learning and memory (Chiaravalloti & Deluca, 2008). Evidence has shown the problem lies in the initial learning of the memories as memory recall in MS patients is equal to healthy individuals, indicating that long-term memory systems are relatively intact (Chiaravalloti & Deluca, 2008). The theoretical construct suggested to treat cognitive impairment in MS patients is to utilize induced pluripotent stem cells to derive oligodendrocyte progenitor cells and mature oligodendrocytes for remyelination of regions displaying degeneration that process working memory. While theroretically, stem cell transplantation should be able to resolve the issue with neuronal loss, clinical trials are showing unsuccessful results that are not entirely understood (Zhang et al., 2011). Recent studies suggest there is limited success due to the complexity involved with degrading glial scarring (Mallory, Grahn, Hachmann, Lujan, & Lee, 2015). It is hypothesized phototimulation of oligodendrocytes after differentiation would strengthen the remylination process as well as neural circuitry within that brain region through long-term potentiation (Lignani et al., 2013; Takeuchi et al., 2016). Takeuchi et al. (2016) were able to demonstrate optogenetic stimulation of the locus coeruleus (LC) enhances consolidation of everyday memory. The study electrophysiologically recorded LC firing rates in novel enivronments and stimuli and recreated this effect with photostimulation alone. Moreover, LTP was observed with repeated stimulation. This demonstration of LTP due to optogenetics is significant in providing contact for how optogenetics may affect neural circuitry with repeated stimulation. In this function and in the rest of the symptoms that will be discussed, we hypothesize that not only will optogenetics control the symptom by decreasing or inhibiting its presence, photostimulation may also lead to enhanced management of symptoms without the need for simulation in the long term.

Visual impairment is often seen in MS patients, commonly manifesting as optic neuritis, which is an acute inflammatory disorder of the optic nerve typically presenting with sudden monocular visual loss and eye pain (Garcia-Martin et al., 2017). Macaques were used to study visual information processing mechanisms in the lateral geniculate nucleus (LGN) and primary visual cortex (V1) by administering an AAV with an effective CamKII promoter into koniocellular cells (K-cells) at the LGN (Klein et al., 2016). The LGN is made up of K-cells, parvo cells and magno cells, each distinct in their circuitry, function and biochemistry, despite all passing through the V1. K-cells however were used as they are especially different form the other two cells. The vectors used were able to target K-cells, nearby CamKII-positive cells, as well as transduce distant layer 6 pyramidal cells of V1 and retinal ganglion cells (Klein et al., 2016). Measurements were conducted using average local field potential (LFP) responses across stimulation trials and current source-density (CSD) profiles were calculated for the visual flicker and optogenetic conditions to assess V1 laminar activation. Of the total population of LGN neurons recorded, the authors identified 23% as being directly affected by the optogenetic stimulation, in comparison to the ~10% observed in the literature (Klein et al., 2016). Although less than 50% of the cells were activated, the authors were able to confirm that at the neuronal circuit level, the amount of selectively recruited K-cells was sufficient to drive short-latency activity in the supra-granular layers of downstream area V1 (Klein et al., 2016).

In translating these findings for an MS treatment, a more enhanced outcome may be observed if all cells of LGN are recuited. This is especially since this study only sought to assess the ability of optogenetic stimulation in the visual cortex to understand the visual pathway, not with the intention of developing a therapy for visual impairment. No mention was made on the level of enhancement observed in the visual ability of the monkeys, warranting further studies on optogenetic stimulation of this brain region.

Moreover, an optogenetic treatement for this function coupled with stem cell therapy may provide a means to alter visual impairment in MS. Similar to the function described in treating cognitive impairment, photostimulation of these pathways may treat visual impairment by strengthening and expanding the neural circuitry and remyelination processes. Interestingly, clinical trials are being conducted for a fascinating treatment option for retinitis pigmentosa (RP) using ChR2 and the concept of optogenetic stimulation (Birch, 2016).

Bladder and bowel dysfunctions are commonly seen in MS cases, causing some of the most distressing symptoms with as many as 75% MS of patients presenting the symptom (Andretta, Simeone, Ostardo, Pastorello, & Zuliani, 2014). The most frequent bladder symptoms seen are storage symptoms such as urinary frequency, urgency, and urge incontinence (Andretta et al., 2014). Voiding symptoms such as hesitancy, incomplete voiding and urinary retention are present as well, although to a lesser extent (Andretta et al., 2014).

Enteric neural cells from fetal or postnatal mouse bowels expressing ChR2 were transplanted into the distal colon of 3-4 week old wild-type mice (Stamp et al., 2017). The transplanted neural cells were able to differentiate into multiple functional types of neurons, integrating and providing functional innervation of the smooth muscle of the bowel wall (Stamp et al., 2017). In the study, optogenetics was used to to selectively stimulate graft-dervied neurons to identify that enteric neural cells isolated from the embryonic and postnatal bowels, giving rise to functional inhibitory motor neurons, excitatory motor neurons, and interneurons following transplantation into the distal colon of recipient mice (Stamp et al., 2017). While optogenetic stimulation was not the primary cause of the functional recovery, when used as a tool, photostimulation can control how these new cells operate. In a disorder that leads to a loss of function due to continuous degeneration, optogenetic stimulation can speed the recovery period as cells are continuously destroyed. Moreover, we hypothesize continuous photostimulation will lead to LTP and strengthening of these networks faster than without the stimulation. These findings are significant as they illuminate a fundamental limitation in almost all the current gold-standard nerve-targeted treatment approaches which do not specifically address isolated neural circuits and lead to undesirable side effects such as unwanted bowel movements or sexual function (Park et al., 2017).

Tremors are believed to occur in up to 75% of MS patients appearing in various forms such as postural, kinetic, proximal, distal tremors, and internal tremors (IT; Ayache et al., 2015). Dysfunction in inhibitory cerebellar efferent projections likely play a role in the generation of tremors during posture or movement in MS patients, however, additional lesions of other cerebral pathways might be involved (Ayache et al., 2015). Optogenetic treatment for tremors would function similarly to those documented in Parkinson's disease (PD). Cell-type specific targeting of dopaminergic neurons in the substantia nigra pars compacta (SNc) has shown to be a viable means of treating denervation of striatal target areas (Stauffer et al., 2016; Tønnesen, 2013). This process is thoroughly described in the study by Stauffer et al. (2016). Applying photostimulation to the regions found to underlie tremor development and motor dysfunction in MS, such as gait, would be a feasible means of treating these symptoms, given its documented applications in similar cases (Kravitz et al., 2010).

Future Directions and Conclusion

Optogenetics has been reported to be a revolutionary technique in neurobiology research. As such, the objective of this review is to assess the potential use of optogenetics in developing therapies for MS related symptoms that include cognitive impairment, visual impairment, bladder/bowel dysfunction, and tremors. Studies for each of these symptoms have been discussed and analyzed and two primary conclusions have been found. Intially, optogenetics remains a tool to enhance treatment. For example, in the study by Stamp et al. (2017), the primary function of the study was to understand how implanted enteric cells functionally integrate into endogenous cells. Optogenetics was merely a tool to control this function to further study it. While it was not used as a treatment, this function of controlling the circuitry may be manipulated into a therapy for those with bladder dysfunction and other illnesses observed in MS.

Secondly, while this control does require manually intiating photostimulation, continuous stimulation may lead to enhancement in circuitry through neuroplastic and LTP effects as seen in Takeuchi et al. (2016). We hypothesize the manual function of turning on photostimulation in the brain may work similarly to a pacemaker where optic fibers are connected internally in the body. Of course these concepts remain theoretical and require extensive research to validate this possibility.

Ultimately, furthering this therapeutic tool is limited by the knowledge present for specific symptoms as well as their mechanisms (Bryson et al., 2016; Jazayeri & Remington, 2016; Mohanty & Lakshminarayananan, 2015; Mudiayi et al., 2015). Just as optogenetics may be used as a therapeutic tool, it can be utilized to understand these symptoms as it is currently used as an investigative tool (Carter & de Lecea, 2011; Mohanty & Lakshminarayananan, 2015; Mudiayi et al., 2015). A first step to identifying how photostimulation can lead to therapeutic effects could ultimately be using optogenetics to understand individual symptoms by experimenting with its circuitry and inhibiting or activating symptom pathways. Once this knowledge has been attained, more specific and accurate targets can be found to control MS symptomology.

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Journal of Young Investigators
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REVIEW ARTICLE

Chronic Traumatic Encephalopathy: Connecting Mechanisms to Diagnosis and Treatment

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Chronic traumatic encephalopathy is a progressive neurodegenerative disease that has been linked to the incidence of repetitive mild traumatic brain injuries. As chronic traumatic encephalopathy has no formal diagnosis or treatment, current research is striving to better understand its neuropathology in order to develop effective diagnostic and treatment strategies. This review will outline recent research findings in the understanding of the neuropathological mechanisms of chronic traumatic encephalopathy, and connect these findings to advancements in the diagnosis and treatment of the disease. With the emergence of more sophisticated technology, neuroimaging techniques have shown promise as prospective diagnostic tools. Functional neuroimaging techniques that allow for the observation of task-related brain activity such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) imaging have provided significant insight into the progression of chronic traumatic encephalopathy. Additionally, a branch of magnetic resonance imaging (MRI) called diffusion tensor imaging (DTI) is currently being used to assess white matter integrity, which is often compromised in cases of repetitive mild traumatic brain injury and may be indicative of an increased risk for developing chronic traumatic encephalopathy and other neurodegenerative diseases. Several forms of pharmacotherapy, including lithium treatment and monoacylglycerol antagonists, have been suggested to target the common neuropathological markers of chronic traumatic encephalopathy. Recent research suggests that a combination of pharmacotherapy and cognitive therapy may effectively reduce symptoms and improve the quality of life in individuals with chronic traumatic encephalopathy.

INTRODUCTION

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease that is commonly observed in professional athletes, military veterans, and other individuals who have been subjected to repetitive brain injuries. Approximately 42 million people worldwide suffer from brain injury every year, which increases their risk of developing chronic traumatic encephalopathy later in life (Gardner & Yaffe, 2015).

The main symptoms associated with the disease are profound memory loss, motor deterioration, unexplained aggression, depression, and suicidality. These cognitive and behavioral symptoms are also accompanied by biological changes in the brain. Similar to Alzheimer's disease, CTE is primarily characterized by an accumulation of tangles of protein, although the distribution of these tangles throughout the brain is unique to each disease (Walker & Tesco, 2013).

Significant attention was directed towards chronic traumatic encephalopathy when Dr. Bennet Omalu discovered the disease in a brain autopsy of former National Football League athlete Mike Webster, whose cognitive abilities had drastically declined following his retirement. Numerous indicators of significant brain deterioration were observed in Webster's autopsy, which was suggested to be accountable for his cognitive dysfunction in his later years (Omalu et al., 2005). Since this initial autopsy, 96% of professional athletes who have been examined for CTE by autopsy have been tested positive for the disease. Although CTE appears to be most prevalent among American football athletes, it is not restricted to this group of individuals. It is suggested that any individual who has been subjected to extensive brain injury throughout their life, including victims of abuse, can develop CTE (Baugh et al., 2012).

Although many great strides have been made in the progression of research on CTE, there is still much that remains unclear about the disease. Currently, there is no formal diagnosis that can be made while the individual is still alive. A post-mortem diagnosis can be performed by an autopsy, which allows for the identification of neuropathological markers of the disease. These markers include the presence of TAR DNA-binding protein 43 (TDP-43), a diffuse spread of hyperphosphorylated tau protein, and enlarged ventricles (Gavett, Stern, & McKee, 2011). Further-

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more, there are no established treatment or rehabilitation protocols for individuals who are suspected to have the disease. The goal of current research on chronic traumatic encephalopathy is to investigate prospective solutions to these gaps in knowledge, and this review will discuss recent findings in this area.

Diagnosing Chronic Traumatic Encephalopathy

Although there have been numerous suggested guidelines for diagnosing CTE in vivo based on neuropsychological observations and life history, there are currently no widely accepted pre-mortem diagnostic criteria for the disease. The emergence of more sophisticated technology is creating opportunities for advancing in vivo diagnostic methods, particularly with regards to advancements in neuroimaging techniques. These advancements enable more detailed evaluations of the features of CTE, which are greatly beneficial for further understanding the neuropathological correlation of the disease and how they are unique from other neurodegenerative diseases.

Traditional structural neuroimaging techniques such as computed tomography (CT) and MRI that are often used in clinical assessments to evaluate gross anatomical changes are unable to effectively detect many of the pathological features of the disease that can only be observed at the cellular level, such as the aforementioned TDP-43 and hyperphosphorylated tau protein. However, DTI has become a popular tool for assessing brain injury as it is specialized for detecting abnormalities in brain white matter, which are common in cases of CTE as a result of the mechanical stress on axons following injury (Sundman, Doraiswamy, & Morey, 2015). Assessing white matter integrity can provide insight into the severity of brain damage, and may be a useful tool in identifying individuals at risk of developing CTE.

In addition to DTI, functional neuroimaging techniques have demonstrated great promise in establishing connections between the neuropathology and symptomatology of CTE. Functional neuroimaging uses various techniques to assess brain activity, typically during the performance of a specific task. Positron emission tomography, or a PET scan, uses radioisotopes to measure the amount of glucose being taken up by regions of the brain, which is indicative of activity level. Barrio et al. (2015) used PET scans to investigate the differences in brain activity between retired football players who exhibited symptomatology associated with CTE, confirmedly diagnosed Alzheimer's patients, and normal controls. The study employed the radioisotope [F-18]FDDNP because it has a high affinity for insoluble protein aggregates, which are trademark features of both CTE and Alzheimer's disease. The neuroimaging results revealed significantly different signaling patterns across all three conditions. In the group of retired football players suspected to be tested positive for CTE, tau protein aggregates were observed to congregate in subcortical areas and limbic structures, namely in the amygdala. In contrast, the results of the Alzheimer's group indicated tau protein aggregates predominantly in medial temporal regions, with minimal involvement of subcortical structures. These findings are highly valuable as they not only demonstrate that neuroimaging techniques can potentially be used in the detection of chronic traumatic encephalopathy in vivo, but also to distinguish the neuropathology of CTE from other neurodegenerative disorders.

Another functional neuroimaging technique that has shown promise in identifying features of CTE is fMRI, which measures the levels of oxygen concentration in regions of the brain in response to a certain task or external stimuli. Brain regions with increased oxygen concentrations are suggested to respond with increased activity in comparison to regions with lower oxygen concentrations. Ford, Giovanello, and Guskiewicz's experiment (2013) was the first to use fMRI to assess differences in brain activity during memory tasks in football players who had been subjected to multiple concussions. The study compared two groups: football players who had experienced more than three concussions during their careers in sports and football players who had experienced less than three concussions. The results indicated no significant differences in performance on memory tasks between the two groups, although notable differences in neural activity were observed in the fMRI results. The low-frequency concussion group displayed more neural activity during relational memory tasks in the parahippocampal gyrus and the inferior parietal cortex. Previous research has suggested that these regions are associated with relational memory tasks in typical individuals. In contrast, the high-frequency concussion group recruited more neural activity from regions of the prefrontal cortex for the same relational memory tasks. The authors hypothesized that this may be due to the fact that the brain regions that are typically active during relational memory tasks were damaged in the high-frequency concussion group, thus explaining the lack of activity in these regions. Further investigation into the long-term effects of this differential neural activity during memory tasks is warranted, as this study did not observe any significant differences in functionality. These findings suggest that the restructuring of neural connections are likely to occur following repetitive brain injury, and support the hypothesis that there are discrepancies in the neurobiology of repetitive versus acute brain injury. This may have implications for the in vivo imaging of neural functioning in individuals who have been subjected to repetitive brain injury and who are suspected to test positive for CTE.

Prospective Treatment Methods for Chronic Traumatic Encephalopathy

Just as there is a lack of pre-mortem diagnostic criteria for CTE, the same is true for treatment methods. The prospective treatment methods for CTE are predominantly preventative in nature by aiming to target and alleviate the adverse neurobiological outcomes of brain injury before they can become pathological and manifest as neurodegeneration. The investigation of pharmacological agents as candidates for the treatment of concussive brain injury has been a popular area of research, and there are several studies that have begun to investigate the use of pharmacological agents to directly target the neuropathology of chronic traumatic encephalopathy itself, rather than the preliminary features of brain injury.

Zhang and colleagues observed that pharmacologically inhibiting the enzyme monoacylglycerol lipase, which plays an important role in degrading endocannabinoid neurotransmitters, significantly reduced the release of pro-inflammatory cytokines and suppressed the phosphorylation of tau protein in a mouse model with repetitive brain injury (Zhang, Teng, Song, Hu, & Chen, 2015). Endocannabinoid neurotransmitters such as 2-arachidonoylglycerol are known to have anti-inflammatory properties, and these findings suggest implications of the endocannabinoid system in the management and reversal of CTE-like neuropathology. Additional research has demonstrated that administering the pharmacological agent JZL184, another monoacylglycerol lipase inhibitor, to rats which had undergone experimental mild traumatic brain injury significantly reduced neuroinflammation, glutamate excitotoxicity, and behavioural impairments associated with brain injury (Mayeux, Katz, Edwards, Middleton, & Molina, 2017). These animal studies provide evidence for the neuroprotective role of the endocannabinoid system in cases of repetitive brain injury. Despite these promising findings, these types of drugs have yet to progress into human clinical trials as enhancing neurotransmission in the endocannabinoid system may have adverse effects including cognitive and sensorimotor impairments (Di Marzo, 2008).

Another experiment using a mouse model of traumatic brain injury revealed that administering lithium significantly reduced tau neuropathology in the thalamus and improved performance on spatial learning tasks (Yu, Zhang, & Chuang, 2012). Although this study chiefly focused on the ability of lithium to reduce the presence of beta-amyloid plaques in the brain, a feature that is more consistent with Alzheimer's disease, the fact that an attenuation of tau neuropathology was also observed suggests that these findings may also be relevant to CTE. Additional research on using lithium as a treatment for brain injury and CTE is warranted as lithium is known to have psychotropic effects, including producing symptoms of dysphoria and cognitive slowing (Moncrieff, Cohen, & Porter, 2013). Considering that cognitive disturbances and depression, which are often accompanied by dysphoria, are symptoms of brain injury and CTE, these effects of lithium may pose obstacles for its usage as a treatment method. These experiments are notable as they indicate that neurodegenerative markers of CTE may have the capacity to be reversed pharmacologically.

DISCUSSION

There have been many relevant research findings concerning the underlying mechanisms of CTE and implications for diagnosis and treatment. Functional neuroimaging techniques have drastically altered the way that the components of the disease can be investigated, and research has demonstrated a potential role for certain pharmacological agents in the treatment of the disease. In particular, several researchers are focusing on agents that enhance the anti-inflammatory properties of the endocannabinoid system.

Several limitations exist in terms of investigating prospective treatment methods for CTE. For one, the lack of a standardized pre-mortem diagnostic criteria for the disease makes it difficult to formulate a suitable treatment. Furthermore, there are obstacles with translating the existing pharmacological treatment research to human populations due to the known side effects of the proposed drugs. The aforementioned findings are promising, however there is still much that is largely unknown. There are some established hypotheses that aim to explain how brain injury can predispose an individual to developing CTE, although the specific mechanisms by which this occurs remain unclear. Moreover, there is currently no pre-mortem diagnostic protocol for the disease, despite the fact that there is substantial evidence suggesting that in vivo diagnoses are possible. Future research on the relationship between repetitive brain injury and disease progression is warranted, in addition to further investigation into the formation of a standardized diagnostic criteria and effective treatment methods for the disease.

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Journal of Young Investigators
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REVIEW ARTICLE

Non-Hermitian Wave Mechanics: An Unorthodox Way into Embedded Systems

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This review outlines an unconventional but timely formulation of quantum dynamics of systems in contact with an environment. This alternative approach to traditional quantum mechanics is generic and is currently gaining attention in a number of fields as, for example, quantum scattering and transport, optical waveguides, devices embedded in an environment, oscillatory classical systems, RLC circuits and other open systems with loss and gain. Here we briefly outline this formulation in which the condition of space-time reflection (PT-symmetry) plays a central role. If PT-symmetry is broken upon parametric change, real energy levels generally turn complex. At the onset of such a symmetry breaking levels coalesce at "Exceptional Points" (EP).

Introduction

In 1926, Erwin Schrödinger formulated his famous non-relativistic equation for matter waves. In this form quantum mechanics (QM) has since then remained a never-ending success. It expands the classical Newtonian mechanics for particle orbitals into the world of quantum matter as atoms, molecules, solid matter, micro- and nano-scale devices, etc., in which particles acquire wave properties. For this reason it is also referred to, particularly in the early years of the new theory, as wave mechanics (WM) with reference to common wave phenomena present in acoustics, electromagnetism, vibrational structures as membranes and drums, hydrodynamics and more. The predictive power of QM is, as well known, overwhelming.

In short, traditional QM as above rests solidly on a number of postulates as (Schiff, 1968):

(a) A physical system is represented by a wave function $\Phi(\boldsymbol{r},t)$ which holds all information of a system;
(b) Physical observables, as for example momentum $\boldsymbol{p}$, are represented by Hermitian operators meaning that associated eigenvalues are real numbers and equal possible outcomes of measurements;
(c) The operator representing energy, the sum of kinetic energy T and potential energy V, is the usual Hamiltonian

$$H = T + V = \frac{p^2}{2m} + V(\boldsymbol{r}) = \frac{-\hbar^2}{2m}\nabla^2 + V(\boldsymbol{r}), \qquad (1)$$

where m is the mass of a particle which moves under the influence of a real potential $V(\boldsymbol{r})$ ($\hbar$ is the reduced Planck constant $h/2\pi$). When $V(\boldsymbol{r})$ does not depend on time t the eigenvalues E_n of the Hermitian Hamiltonian H are the energy levels of a system.
(d) The time evolution of the wave function is given by the time-dependent Schrödinger equation

$$i\hbar\frac{\partial\Phi}{\partial t} = \frac{-\hbar^2}{2m}\nabla^2\Phi + V(\boldsymbol{r})\Phi. \qquad (2)$$

For the case above one then has

$$\Phi_n(\boldsymbol{r},t) = \psi_n(\boldsymbol{r})\exp(-iE_nt/\hbar) \qquad (3)$$

where $\psi_n(\boldsymbol{r})$ is the n:th stationary solution $H\psi_n = E_n\psi_n$ with real eigenvalue E_n.

In this review we will introduce an extension (PT-symmetry) to the well known Hermitian QM and describe its implications on QM as well as analogous classical systems. After reviewing the background and current state of the field we discuss some open problems and suggest further studies with the goal to inspire new and clever ideas.

A New Paradigm: Non-Hermitian QM and Parity-Time (PT) Symmetry

Measurements in QM return the eigenvalues of observables; for example, a measurement of a particle's energy yields an eigenvalue of the Hamiltonian. The important assumption of Hermitian operators guarantees that eigenvalues are real and that QM is consistent with measurements. However, more lately it has been argued that the requirement of Hermiticity may be too taxing. Can the energy levels be real also for a Hamiltonian that is complex, i.e., a non-Hermitian one? Under certain circumstances, the answer is yes. Bender and Boettcher (1998) showed how this happens when a system is symmetric under the combined PT operations of parity, or mirror symmetry, (P) and time-reversal (T). These symmetry operations translate to $\boldsymbol{p}\rightarrow\boldsymbol{-p},\boldsymbol{r}\rightarrow\boldsymbol{-r}$ for parity and $\boldsymbol{p}\rightarrow\boldsymbol{-p},\boldsymbol{r}\rightarrow\boldsymbol{r},\boldsymbol{i}\rightarrow\boldsymbol{-i}$

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for time reversal. Enforcing this symmetry implies for the potential to satisfy $V(\boldsymbol{r})=V^*(-\boldsymbol{r})$ and thus there is a balanced flow, i.e., gain versus loss is harmonized (Bender, 2005, 2007; Weigert, 2004).

To get an understanding of the role of the complex potential $V(\boldsymbol{r})= V_{Re}(\boldsymbol{r})+ iV_{Im}(\boldsymbol{r})$ consider the simple case of a pair of nearby even and odd states that are localized, for example, to the interior of a closed cavity (Figure 1). Let the solutions for the "unperturbed" case $V_{Im}(\boldsymbol{r})=0$ be E_1 and E_2. Under a parametric change such that $V_{Im}(\boldsymbol{r})\neq 0$ the two levels will interact according to the 2×2 matrix equation

$$(\mathrm{E}_1 - E)c_1 + iV_{int}c_2 = 0, \quad (4)$$

$$iV_{int}c_1 + (\mathrm{E}_2 - E)c_2 = 0, \quad (5)$$

where V_{int} is the interaction matrix element between the initial states *1* and *2*, i.e., $V_{int}= \langle 1 | V_{Im} | 2 \rangle = \langle 2 | V_{Im} | 1 \rangle$; c_1 and c_2 are the mixing coefficients for the two states. The eigenvalues of the mixed states are

$$E_{1,2} = \frac{(\mathrm{E}_1 + \mathrm{E}_2) \pm \sqrt{(\mathrm{E}_1 - \mathrm{E}_2)^2 - 4V_{int}^2}}{2}. \quad (6)$$

The modified eigenvalues are evidently real as long as energy gap between states 1 an 2 is larger than $|2V_{int}|$. There is a balance between gain and loss. However, as the gap becomes equal to abs($2V_{int}$) on further parametric increase a profound change takes place. The eigenvalues coalesce into a common value referred to as an exceptional point (EP); beyond this point the eigenvalues become complex. Rewriting Eq. (6) as

$$E_{1,2} = E \pm \frac{i}{2}\Gamma. \quad (7)$$

The time-dependent solutions in Eq. (2) are now

$$\Phi_{1,2}(\mathbf{r},t) = \psi(\mathbf{r})\exp\left(-\frac{iEt}{\hbar} \pm \frac{\Gamma t}{2\hbar}\right). \quad (8)$$

Beyond the exceptional point there may thus be either exponential decay or growth of the states. The outline above is a rather elementary one but points to the existence of EPs into which states, may coalesce on parametric change. If we consider the exponentially decaying states, which would apply to fermions because of the Pauli principle that forbids double occupancy, one should thus have the possibility of switching a state on and off by playing with V_{int}.

In the next section we will discuss the specific example of a quantum in contact with an environment. There will be a number of states and for this reason one will have to use more refined methods than above to solve the Schrödinger equation, in this case numerical methods based on finite differences. As we will find the occurrence of EPs is a more complicated story than above, they may come and go with the gain/loss parameter V_{int}.

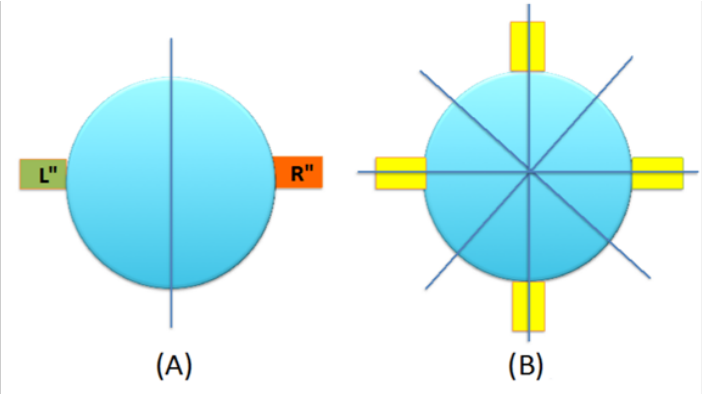

Figure 1. Schematic picture of two-dimensional circular dots. (A) shows the case with two opposite ports with complex potentials $V_L(x) = V_R^*(-x)$. The interior potential is real and may be set equal to zero. The potential in the exterior region may be set to infinity, i.e., wave functions are confined to the circular area and ports. The vertical line is the line of reflection. The two ports serve as source and drain. Because of PT-symmetry, gain and loss can balance each other. (B) shows a dot with several ports with the possibility of combining the corresponding potentials according to the different symmetry lines and PT invariance. The flow of particles between the ports may thus be monitored by flexible pairings of the potentials in the different sections, i.e., the system will act a bit like a switchboard. While retaining PT-symmetry, the imaginary part of the potential may be chosen differently for the pairs giving rise to a more complex two-dimensional landscape of EPs. Obviously we may also consider more ports than just four.

A Two-Dimensional Quantum Dot in Contact with an Environment

There is a rich variety of quantum dots fabricated from different materials for different purposes. They may be three- or two-dimensional objects embedded in solid materials, colloidal nanocrystals, etc., with intriguing physics and vast applications. A common feature is, as already the name indicates, that states are confined within a dot are quantized because of its smallness, typically in the nanometer regime. Research, basic and applied, remains very dynamic and there is a rich literature with many good monographs, see for example (Klimov 2010) and more.

Here we will focus on a particular kind of quantum dots that may be created in layered semi-conductor hetero-structures like $Ga_{1-x}Al_xAs/GaAs$. Because of a mismatch between the band-gaps of the two materials and modulation doping with donor atoms there will be an effectively two-dimensional electron gas that resides at the interface. A smart step is to add metallic top layer/gate which makes it possible to vary the density of electrons, even to deplete it. Another smart step is to use lithography to shape the electron gas into small structures like one-dimensional wires, dots of various geometries, combinations of such objects into networks, etc., as for example described by Ferry, Goodnick, & Bird (2009).

Here we present a schematic model of a circular two-dimensional quantum dot embedded in a hetero-structure (Figure

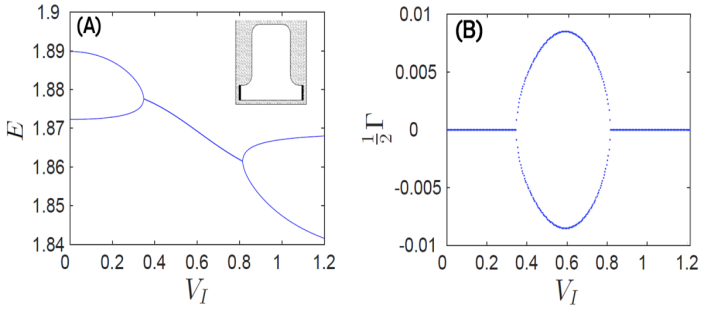

Figure 2. Parametric variation of complex energy levels in arbitrary units for two nearby interacting quantum states in an embedded quantum dot with two opposite ports mimicking source and drain. V_I defines the imaginary potential within the two "leads", $+iV_I$ in one of them and $-iV_I$ in the other as V_L and V_R in Figure 1A. Here (A) shows the real part of the two energy levels and (B) the imaginary part. The calculations refer to the dot in the inset: $V_I \neq 0$ is in dark areas at the two ends. The real part of the interior potential is set to zero (Tellander & Berggren, 2017).

1). The dot contains a number of electrons, usually small, that may be varied via the top gate. There are also pairs of ports that serve as emitters and collectors. In Figure 1A, for example, we may let the left port L be purely imaginary with $V_L=iV_{Im}$ and $V_R=-iV_{Im}$ for the other port R. Evidently there will be a current flowing through the dot. Related configurations have been elaborated for an electron/microwave billiard (Berggren et al., 2010) and, most recently, for interacting Bose-Einstein condensates (Schwartz et al., 2017).

As shown in Figure 2, the pair of levels may change under the parametric change, and we recover the EP discussed in the previous section. In addition we find, however, that there is another EP on further increase of the interaction, i.e., the state with real eigenvalues is restored. The calculations are more cumbersome than the analytic analysis above; a convenient approach is to turn to numerical finite difference methods described previously (Tellander & Berggren, 2017). Indeed, this method allows for a greater number of states, than just two as was discussed above. With a larger number of states one can expect more EPs to appear in the spectrum. However, the EPs only seem to appear over a finite range of V_{Im} (Tellander & Berggren, 2017) which means that the spectrum can, as in Figure 2, be divided into three regions:the left region where V_{Im} is less than the critical values and all eigenvalues are real, the finite critical region where many EPs exist and the rightmost part of the spectrum where most of the eigenvalues are again real. This crossover between different dynamical regimes is called a dynamical crossover and is of great importance for experimental studies of non-Hermitian QM. In the region of many EPs, the transmission through the system should be enhanced and the states that remain complex in the right region of the spectra are believed to be associated to superradiant modes (a collection of emitters, such as atoms, that radiates strongly due to coherence) studied in atomic physics. Whether superradiance really can be viewed as a dynamical crossover is an unanswered question (Rotter and Bird, 2015).

A system with more gates (Figure 1B) allows for a more direct measurement of EPs and has the possibility to settle the long-lived discussion in the field about the geometric phase obtained by a state when an EP is encircled in the parameter space. This phase is geometric in the sense that it is independent of the path that encircles the EP; compare with Cauchy's theorem for complex curve integrals or the classical experiment using Foucault's pendulum to prove that the earth rotates around its own axis. The system in Figure 1B can have one independent imaginary potential for each pair of leads and the parameter space is therefore two-dimensional. This system could therefore be transported around an EP and the phase change of the wave function could be extracted. Similar experiments in analogous systems such as microwave (Dembowski, 2001) and exciton-polariton (Gao, 2015) billiards have been preformed but a pure quantum experiment is still in the future.

Summary and Outlook

Above we have outlined in a schematic way how quantum states and currents in a biased PT-symmetric cavity in contact with surrounding reservoirs may be emulated by means of complex potentials for source and drain. This is, for example, of considerable computational convenience when modelling transport in real devices at small source-drain bias. This idea is already found to work well for the analogue case of two-dimensional microwave billiards (Berggren et al., 2010). There is still, however, a challenge to design and implement real semiconductor devices with the above characteristics.

The physics associated with PT-symmetry is common for a number of wave phenomena and there is a rich and rapidly expanding literature. This includes, for example, electromagnetic systems, in particular in the fields of optics and photonics for which many new possibilities have opened up. Complex potentials in terms of complex refractive indices enter here in a natural way. Promising cases for further studies are therefore co-axial waveguides, microwave billiards and more. In classical mechanics the same kind of behavior may be realized by means of a driven and a damped pendulum coupled to each other. Also in electronics when two RLC−circuits are inductively coupled, one with amplification and one with attenuation, a PT-symmetric system is obtained with EPs that may be studied in details. This shows that PT-symmetry phenomena are ubiquitous in wave physics as well as electrical systems. For recent updates and reviews see (Christodoulides et al., 2017; Konotop et al., 2016; Rotter & Bird, 2015) which shows that the present field is an expanding one within fundamental science and technology. Most recently it has also been shown how the formalism for non-Hermitian quantum physics with gain and loss may be used to analyse a very different kind of system, namely photosynthesis (Eleuch & Rotter, 2017).

Finally, it is exciting to find that there is a much older field of physics with its very own traditions and literature that relates to vibrations in string instruments like violins, cellos and pianos

(Gough, 1981; Weinreich, 1977, 1979). One thus talks about wolf-notes which are unfortunate facts of life for, for example, cellists who may have to struggle with and tame "wolf cellos." Wolf notes refer to unwanted interactions of different modes and how these coalesce into damped degenerate states at certain frequencies. The similarity with EPs that appear in non-Hermitian quantum systems as described above for a quantum dot and illustrated in Figure 2 is obvious. We therefore wish to name such features "*quantum wolves*."

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