Investigating the reliability and clinical utility of minimally-invasive portable electroretinography and optical coherence tomography techniques in children undergoing vigabatrin therapy

by:

Xiang Ji

A thesis submitted in conformity with the requirements for the degree of Master of Science
Institute of Medical Science
University of Toronto

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Abstract
The antiepileptic drug for Infantile Spasms – vigabatrin, has been associated with retinal toxicity in over one third of children treated. Young and non-verbal children cannot cooperate with conventional adult-oriented monitoring devices, therefore a safe and reliable technique to monitor for vigabatrin retinal toxicity is missing. This study evaluated the tolerability, reliability and clinical feasibility of two alternative, handheld systems for assessment of retinal function: the RETeval ERG and Envisu OCT. We found that non-sedated children showed poor tolerability on the RETeval ERG. Under sedation the same children showed high tolerability on the handheld OCT. Responses from both techniques demonstrated good reliability, however the assessments of longitudinal changes and clinical feasibility were limited by the small sample size. We suggest that the RETeval device is used in situations where sedation for clinical ERG is not possible, and the Envisu OCT is used alongside the clinical ERG to monitor for vigabatrin retinal toxicity prospectively.
Acknowledgements

This thesis was a collective effort of many inspiring and kind people who I was lucky enough to be surrounded with every day. First of all, many thanks to my supervisor, Dr. Carol Westall for guiding me through my project with her patience, knowledge and care. The support that I have received from her has certainly exceeded all expectations of a PI. Not only did I learn a tremendous amount of clinical research knowledge from her as a world-renowned electrophysiologist, but I have also internalized the techniques of human interaction so that I am better able to appreciate others around me who may represent different cultures or beliefs.

My deepest gratitude goes out to Cynthia VandenHoven and Leslie McKeen who are imaging specialists at the eye clinic. Without their contributions of acquiring OCT imaging from all my participants, my project would not have been possible. Occasionally, they took time away from personal tasks/clinical duties to ensure the quality of my research and the consistency of my results - Thank you so much!

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Thank you to all parents and guardians of children who have given me their trusts for me to complete the study with their beloved children. Your kindness and your contribution to the future of science is so admirable and deeply appreciated.

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Lastly, I would like to thank my parents, grandparents and my friends for your kind emotional support throughout my project. With you around, I feel more motivated and confident to move forward each and every day.
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List of Abbreviation

IS - Infantile spasms
TSC - Tuberous sclerosis complex
Electroencephalography - EEG
Magnetic resonance imaging - MRI
Positron emission tomography - PET
Serotonergic neurons - 5HT
Cholinergic neurons - ACh
Noradrenergic Neurons - NE
Hypothalamic Pituitary-Adrenal Axis - HPA Axis
Lenticular Nuclei - LN
Adrenocorticotropic hormone - ACTH
Vigabatrin - γ-vinyl GABA
GABA-transaminase - GABA-T
Central nervous system - CNS
Cerebrospinal fluid - CSF
Visual fields - VF
Electroretinography - ERG
Oscillatory potentials - OPs
Vigabatrin-related visual field loss - VRVFL
Support, Help and Resources for Epilepsy - SHARE (vigabatrin monitoring program)
Risk Evaluation and Mitigation Strategy - REMS (vigabatrin monitoring program)
Visual Electrophysiology Unit - VEU
International Society for Clinical Electrophysiology of Vision - ISCEV

Dark-adapted - DA (ERG parameter)

Light-adapted - LA (ERG parameter)

Inner plexiform layer - IPL

Handheld ERG (HH-ERG)

Intraclass correlation coefficient - ICC

Optical coherence tomography - OCT

Spectral-domain OCT - SDOCT

Retinal nerve fiber layer - RNFL

Retinal ganglion cell - RGC

General anesthesia - GA

Handheld SDOCT - HH_OCT

Retinopathy of prematurity - ROP

Shaken baby syndrome - SBS

Optic pathway gliomas - OPG

Postmenstrual age - PMA

Research Ethics Board - REB

Within normal limits - WNL

Right eye – OD; Left eye - OS

Pearson’s correlation coefficient - PCC

Optic nerve head - ONH

Optic pathway glioma - OPG

Neurofibromatosis Type I - NF1
Purpose and Rationale

The purpose of this study was to assess the tolerability, reliability and clinical feasibility of two minimally-invasive, portable techniques (RETeval ERG and Envisu OCT) to monitor for retina changes in children < 3 years of age undergoing vigabatrin treatment. This study also aimed to compare the responses from both techniques to the current gold-standard clinical sedated ERG. Early detection of vigabatrin-associated toxicity using techniques with good tolerability, reliability and feasibility may lead to better clinical decisions regarding the course of treatment.

Vigabatrin is an effective antiepileptic drug for children with Infantile Spasms, however, it has been associated with irreversible retinal toxicity as manifested by bilateral concentric visual field loss, reduction of the light-adapted 30-Hz flicker ERG amplitude, and attenuation of the peripapillary retinal nerve fiber layer thickness. The lives of these children would be drastically improved if the additional burdens of retinal toxicity were to be diminished. Currently, reliable monitoring techniques to detect early signs of vigabatrin-induced retinal toxicity in young, non-verbal children are limited, as this particular cohort of patients cannot cooperate with adult-oriented techniques such as the table-top OCT and Goldmann kinetic perimetry. The current gold-standard ERG requires sedation, which could be a source of unease and potential invasiveness for parents and children, respectively. These ideas are supported by Dr. William Good, Editor of the Journal of the American Association for Pediatric Ophthalmology and Strabismus who commented that “It’s time to develop better vision function tests in nonverbal patients”.

The RETeval ERG is proposed to be a non-invasive and efficient technique for young children as it uses skin electrodes as opposed to corneal electrodes used on clinical ERG, and it can be
performed without the need for sedation or pupillary dilation. The Envisu OCT can be performed in infants laying supine at multiple angles with the flexibility of its eye-piece (light stimulus). It is also quick with image acquisition that can significantly reduce time under sedation.

**Outcome Variables**

For the RETeval ERG: ERG light-adapted 30-Hz flicker amplitude (μV) and implicit time (ms).

For the Envisu OCT: mean peripapillary RNFL thickness (μm), and RNFL thickness in the superior, inferior, nasal and temporal quadrants of the optic nerve head.

**Hypothesis and Research Aims**

We hypothesize that handheld Envisu OCT would show high tolerability as it is highly flexible and is performed under sedation. The RETeval ERG would show moderate to high tolerability as only the short 30-Hz flicker response protocol (less than 10 seconds per trial) is assessed.

However, given that the RETeval is performed whilst children are prepped for sedation children who are non-verbal may not show complete testing compliance. We predict that both RNFL measures with handheld OCT and 30-Hz flicker amplitude measures with handheld ERG will show high reliability. Previous studies demonstrate high intra- and inter-visit reliability of both techniques in older cohorts with other retinal conditions. We also hypothesize that longitudinal changes on the handheld ERG and OCT will match that of the clinical ERG for some children, and that responses from both techniques will show a moderate to strong positive association with the clinical ERG flicker amplitude.

**Research Aim 1:** Assess the tolerability of both handheld devices in children undergoing vigabatrin treatment for IS. We will calculate how many children are able to complete one trial
of the handheld RETeval (30-Hz flicker) and Envisu OCT (RNFL thickness) evaluations in at least one eye.

**Research Aim 2:** Intra-visit and inter-visit reliabilities of both devices will be represented by intraclass correlation coefficient (ICC) statistics. Intra-visit reliability of RNFL measures using Envisu and 30-Hz flicker measures using RETeval will be calculated from two repeated responses of the same eye of one participant within a single testing session. The inter-visit reliability will be assessed from two responses across consecutive study visits of the same participant. The ICCs will be compared between RETeval ERG and Envisu OCT to determine which technique is more reliable for our particular cohort of children.

**Research Aim 3:** The clinical feasibility of the handheld ERG and OCT techniques will be determined through evaluations of the 30-Hz flicker and RNFL thickness changes assessed over time; these results will be compared for agreement with prospective assessments of the clinical flicker response. To establish the longitudinal study design, handheld OCT and ERG techniques will be performed at baseline visit and at several follow-up visits. The responses of both devices at the first visit will also be compared with that of the clinical ERG to determine the degree of association using $R^2$ and $\omega^2$. 
General Introduction
Introduction

1. Infantile Spasms

Infantile spasms (IS), or West Syndrome, is a catastrophic childhood seizure disorder first publicly described by Dr. William James West in 1841, from his own son (West, 1841). This condition is reported to affect approximately 2-5 children per 10,000 new births globally (Riikonen & Donner, 1979; Sidenvall & Eeg-Olofsson, 1995; Rantala & Putkonen, 1999; Wong & Trevathan, 2001; Wheless et al., 2012), and the overall prevalence rate is between 1.5 to 2 children per 10,000 individuals (Cowan & Hudson, 1991; Trevathan et al., 1999), with the male population slightly more susceptible in disease development (Riikonen & Donner, 1979).

Clinically infantile spasms are characterized by clusters of extensor or flexor spasms, or both that occur in the first year of life. The seizures respond clinically only to corticosteroids, ACTH, or vigabatrin. Infantile spasms is termed a catastrophic childhood seizure disorder because of the high percentage of children who have significant global developmental delay, impairment of cognition, and evolution into other types of medically refractory epilepsy as they grow older (Jeavons et al., 1973; Matsumoto et al., 1981; Wong & Trevathan, 2001; Partikian & Mitchell, 2010). As well, there is an increased mortality rate in children with IS (Trevathan et al., 1999). Beginning with the occurrence of initial seizure symptoms in the first twelve months of life, the spasms resolve in more than one third of children when approaching adulthood (Riikonen, 2001). However, 20-50% retain seizure symptoms and progress to develop a condition called Lennox-Gastaut syndrome (Ohtahara et al., 1976; Lombroso, 1983; Trevathan et al., 1999), which contributes in part, to the increase in overall mortality rate. Most cases of IS are believed to be associated with symptomatic causes such as metabolic insults, infections, CNS malformations,
Down syndrome, and tuberous sclerosis complex (TSC) (Koo et al., 1993; Partikian & Mitchell, 2010); however a relatively smaller percentage of individuals with IS are associated with genetic predisposition, unknown or idiopathic etiologies (Watanabe, 1998; Fejerman et al., 2000).

Electroencephalography and Infantile Spasms
Children with IS exhibit unique waveforms on electroencephalography (EEG) examinations named hypsarrhythmia, often displaying signs of arrhythmic, high-voltage slow waves and spikes that change randomly over time (Gibbs & Gibbs, 1952; Gibbs et al., 1954; Wong & Trevathan, 2001; Pavone et al., 2014). The disorganized EEG pattern was first described in 1950s by Gibbs as ‘chaotic and disorganized’, and ‘variable in duration and topography’. This distinct and diffuse abnormality on the EEG can help health professionals make more accurate diagnosis of IS in children, and this is often used in conjunction with characteristic clinical manifestations including episodes of bilateral contractions of flexors and extensors in the neck, trunk, arms and legs (Kellaway et al., 1979; Fusco & Vigevano, 1993).

Brains Regions Implicated In Infantile Spasms
Although the EEG system could display relevant neural signals in a timely manner, it lacks the spatial resolution to localize the source of spasms, as IS could originate both multifocally and focally. With appropriate imaging techniques such as magnetic resonance imaging (MRI) and positron emission tomography (PET) (Van Bogaert et al., 1993; Chugani & Conti, 1996), specific brain regions were studied in more details (Table 1). In particular, many brainstem structures can influence central executive and motor functions through wide cortical projections, and these include the pons, the serotonergic group, the cholinergic group and the noradrenergic
neurons. Other implicated areas such as the hypothalamic-pituitary-adrenal (HPA) axis and the lenticular nuclei involved in endocrine homeostasis and metabolism may also be involved in IS.

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<th>Subcortical Brain Structures Hypothesized</th>
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<tr>
<td>Brainstem (Pons)</td>
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</tr>
<tr>
<td>Serotonergic Neurons (5HT)</td>
<td>Pranzatelli, 1994</td>
</tr>
<tr>
<td>Cholinergic Neurons (ACh)</td>
<td>Pranzatelli, 1994</td>
</tr>
<tr>
<td>Noradrenergic Neurons (NE)</td>
<td>Pranzatelli, 1994</td>
</tr>
<tr>
<td>Hypothalamic Pituitary-Adrenal (HPA) Axis</td>
<td>Baram, 1993</td>
</tr>
<tr>
<td>Lenticular Nuclei (LN)</td>
<td>Chugani et al., 1992</td>
</tr>
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Table 1. Brain structures hypothesized as the source of infantile spasms.

**Treatment Approaches for Infantile Spasms**
Due to undesirable outcomes for IS patients following confirmed diagnosis, a reliable treatment plan is important, and should be administered during the earliest possible time window. The first-line treatment options for children with IS can be vigabatrin (Sabril®, Lundbeck Pharmaceuticals, USA), adrenocorticotropic hormone (ACTH), oral corticosteroids (Bitton et al., 2012; Riikonen, 2014). A study at The Hospital for Sick Children, Toronto, Canada, compared the short-term effectiveness of ACTH to that of steroid prednisolone in patients following failed initial vigabatrin therapy for IS (Jones et al., 2015). This non-randomized, retrospective study recommended the use of the more expensive ACTH as a second-line option substituting for vigabatrin, as it was more effective than prednisolone in alleviating the symptoms of spasms in the short term. However, it was recommended that ACTH or the ACTH-steroid combination be used with extreme caution, because they can cause life-threatening adverse effects. These can range from severe irritability and heightened blood pressure, to heart failure and hyperactive immune response (Vigevano & Cilio, 1997). In a recent multicenter randomized open label trial,
O’Callaghan and colleagues found that the combination of prednisolone or tetracosactide with vigabatrin was significantly more effective in seizure cessation in infants than hormonal treatment alone (O’Callaghan et al., 2017).

Figure 1. The structural diagrams of vigabatrin (γ-vinyl GABA) (left) and GABA (right).
2. Vigabatrin

An alternative approach to combat infantile spasms began with the introduction of vigabatrin (Figure 1) in 1974. Vigabatrin, \((\gamma\text{-vinyl GABA})\), is an anticonvulsant drug classified as a competitive and irreversible inhibitor of the GABA-transaminase (GABA-T) enzyme in the central nervous system (CNS) (Lippert et al., 1977; Jung et al., 1977). The role of the GABA-T protein is to break down and reduce levels of \(\gamma\)-aminobutyric acid (GABA) neurotransmitters through oxidative deamination in the brain. Therefore, vigabatrin would elevate intracellular GABA concentrations. The overall outcome following the oral administration of vigabatrin is a hugely elevated amount of GABA in the cerebrospinal fluid (CSF) for up to one week until new GABA-T molecules are manufactured (Ben-Menachem et al., 1988). Since GABA is the main inhibitory neurotransmitter in the CNS, the heightened levels of neuronal firing in IS was anticipated to decline (Wheless et al., 2007). Within the CNS, vigabatrin is very versatile given its water solubility and ability to compete with GABA molecules for covalent attachments to the enzymatic active sites of GABA-T. This substitution is highly specific to influence GABA, and vigabatrin does not impact the behaviours of other neurotransmitters, with the sole exception of glycine (Halonen et al., 1988), which acts similarly to GABA. This is mainly due to the fact that vigabatrin closely mimics GABA in the brain as its structural analogue, and the two molecules only differ through a vinyl functional group located on the \(\gamma\) carbon of vigabatrin (Figure 1). Although it is well understood that vigabatrin elevates GABA concentration, the mechanism of action remains unclear. Using a mouse knockout model of TSC, the authors proposed that vigabatrin may function by ways of inhibiting the mammalian rapamycin (mTOR) signaling pathway, which is dysregulated in TSC (Zhang et al., 2013). The inhibition of mTOR has shown therapeutic importance in disorders involving GABA metabolism (Vogel et al., 2017).
Properties of Vigabatrin

Table 2 lists several pharmacological properties of vigabatrin that deem it a more favorable drug choice for many with IS. These include favorable pharmacokinetics, high selectivity, ease of oral administration, high tolerance in patients, and low risk of acute drawbacks (Schechter, 1989).

<table>
<thead>
<tr>
<th>Pharmacological Property</th>
<th>Description</th>
<th>References</th>
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<tbody>
<tr>
<td>Half-Life</td>
<td>5-7 hours</td>
<td>Schechter, 1989</td>
</tr>
<tr>
<td>Administration</td>
<td>Orally administered, easily given</td>
<td>Schechter, 1989</td>
</tr>
<tr>
<td>Selectivity</td>
<td>Highly selective for GABA-T</td>
<td>Schechter, 1989</td>
</tr>
<tr>
<td>Stereochemistry</td>
<td>Only S(+)-enantiomer biologically active</td>
<td>Haegele &amp; Schechter, 1986</td>
</tr>
<tr>
<td>Drug Absorption</td>
<td>Fast with max absorption at 2 hours</td>
<td>Schechter, 1989</td>
</tr>
<tr>
<td>Blood Protein Interaction</td>
<td>Minimal</td>
<td>Rimmer &amp; Richens, 1989</td>
</tr>
<tr>
<td>Side Effects</td>
<td>Headache, fatigue, irritability, dizziness, visual toxicity, hypotonia, hyperactivity</td>
<td>Hoke et al., 1993, Aicardi et al., 1996</td>
</tr>
</tbody>
</table>

Table 2. The known pharmacological properties of vigabatrin in the CNS.

Vigabatrin has a very desirable pharmacokinetic profile with its bioavailability at greater than 90%, minimal liver metabolism, and negligible binding with blood proteins (Rimmer & Richens, 1989; Gidal et al., 1999). Given these properties, the half-life for vigabatrin in humans is between 5-7 hours in the blood plasma (Schechter, 1989), and after 1 day with negligible input from the liver, majority of the drug are eliminated while unchanged in the urine following
excretion and filtration in the kidneys (Haegele & Schechter, 1986). Vigabatrin is not only highly specific when targeting the GABA-T enzymes, but it is also highly stereoselective prior to interactions due to its enantiomeric nature (Gidal et al., 1999). Enantiomers are mirror images of one another, and in the case of vigabatrin, it presents itself as a racemic mixture of S (+) - and R (-) - enantiomers that are equal in proportions, but not in the level of impact to GABA-T. Only the S (+) - enantiomer is assumed to be biologically active (Haegele & Schechter, 1986; Jacqz-Aigrain et al., 1997), and able to elevate GABA concentrations in the CSF in a dose-dependent fashion. Vigabatrin is taken orally and quickly absorbed within the body by the digestive system (Schechter, 1989), and it is easier to use with less side effects than ACTH or corticosteroids. Because of this advantage over ACTH or high dose corticosteroids, vigabatrin is currently the first-line option for every newly diagnosed patient with IS at our hospital, The Hospital for Sick Children (Bitton et al, 2012; Jones et al., 2015). Nevertheless, vigabatrin has been shown to associate with visual toxicity, and this information will be discussed later in Section 3.

**Clinical Relevance of Vigabatrin**

In Canada, vigabatrin has been in clinical use since 1994, and in the U.S, it is only approval in the treatment of IS by the U.S Food and Drug Administration (FDA). Vigabatrin is prescribed as second-line therapy for adults with refractory complex partial seizures (Ben-Menachem et al., 1989), and as a first-line therapy for children with IS, especially those with TSC as an etiology (Chiron et al., 1991; Aicardi et al., 1996; Chiron et al., 1997; Mytinger & Joshi., 2012; Bitton et al., 2012). At the Hospital for Sick Children in Toronto, vigabatrin is given as first-line option despite varying etiologies (Bitton et al., 2012), due to the aforementioned advantages over ACTH and other steroids.
Vigabatrin as Second Line Treatment for IS

In 1989, Ben-Menachem et al used lumbar puncture techniques in adults (21-53 years) with complex partial seizures to show that, a single add-on of vigabatrin administration at 50 milligram per kilogram was sufficient to elevate GABA concentrations in the CSF and reduce seizure symptoms. When the time gap between consecutive doses was shortened to once per day, this effect was even more pronounced. Two years later in 1991, the add-on effect of vigabatrin in children was explored by Chiron and colleagues, where they found that 43% of 68 patients were completely rescued from spasms 5 months post therapy. These improvements were highlighted in children with symptomatic TSC, where more than 70% of them were spasms-free following vigabatrin treatment.

Vigabatrin as First-Line Monotherapy for IS

Vigabatrin is the first-line treatment in children with IS (Bitton et al., 2012). A prospective randomized study found the efficacy of vigabatrin to exceed that of hydrocortisone (a steroid hormone) (Chiron et al., 1997). In this study 11 children with TSC became seizure free with vigabatrin treatment and of those treated with hydrocortisone five out of 11 became seizure free (Table 3). Additionally, all 7 children who have switched to vigabatrin from hydrocortisone due to unwanted drawbacks and ineffectiveness were also seizure free. Aside from its clinical efficacy, vigabatrin has surpassed hydrocortisone in reducing the time needed to achieve seizure free status and showed less acute side effects. In a mouse knockout model of TSC, vigabatrin was able inhibit the activities of mTOR signaling, which is a pathway frequently dysregulated in TSC (Zhang et al., 2013). This may explain the unique efficacy of vigabatrin in controlling for seizures in children with TSC etiology. In addition, children diagnosed with TSC should receive
frequent serial EEG monitoring as early as possible, since early findings of abnormal EEG waves may guide treatment to reduce the risks of developing spasms and cognitive deficits (Whitney et al., 2017).

<table>
<thead>
<tr>
<th></th>
<th>Vigabatrin</th>
<th>Hydrocortisone</th>
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<tbody>
<tr>
<td><strong>Dosage</strong></td>
<td>150 mg/kg daily</td>
<td>15 mg/kg daily</td>
</tr>
<tr>
<td><strong>Number of Spasm-Free</strong></td>
<td>All 11 infants</td>
<td>5/11 infants</td>
</tr>
<tr>
<td><strong>Time Taken to Become Spasm-Free</strong></td>
<td>3 and half days</td>
<td>13 days</td>
</tr>
<tr>
<td><strong>Number to Experience Side-Effects</strong></td>
<td>8 children</td>
<td>17 children</td>
</tr>
</tbody>
</table>


Vigevano and Cilio (1997) found that the efficacy of ACTH was greater than that of vigabatrin in a study where the proportion with seizure cessation was compared between 23 children treated with vigabatrin and 19 treated with ACTH. Using a randomized control study these authors found that a greater proportion of infants became seizure free with ACTH (74%) than vigabatrin (48%) (Table 4). However, given the advantages of ACTH, they still insisted in recommending vigabatrin as the initial treatment, particularly for infants with TSC. In a 2012 American Academy of Neurology evidence-based guideline on the treatment of IS, Go and colleagues recommended the use of ACTH over vigabatrin for children with unknown causes of IS in hopes of preserving their normal development (Go et al., 2012). In addition, a randomized control study showed that vigabatrin as a first-line treatment was able to achieve complete seizure resolution (clinically and in terms of EEG) in 38/68 children (Bitton et al., 2012), and the remaining children became seizure-free following the addition of synthetic ACTH, suggesting that the
simultaneous use of both drugs may be more beneficial and effective for children with IS than either drugs alone.

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Vigabatrin</th>
<th>ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Spasm-Free (%)</td>
<td>11/23 (48%)</td>
<td>14/19 (74%)</td>
</tr>
<tr>
<td>Time Taken to Become Spasm-Free</td>
<td>1-14 days</td>
<td>2-12 days</td>
</tr>
<tr>
<td>% to Experience Side-Effects</td>
<td>13%</td>
<td>37%</td>
</tr>
</tbody>
</table>


Vigabatrin for Children without TSC

Is vigabatrin also an effective first option for children without TSC? A randomized open study by Lux and colleagues in 2004 found that vigabatrin is less effective in stopping seizures in children with IS and without TSC than hormonal first-line methods such as prednisolone or synthetic ACTH (intramuscular tetracosactide). Twenty of 52 children (54%) were completely seizure free with vigabatrin treatment, and 40 out of 55 children (73%) were seizure free following either one of two hormonal treatments. In a more recent retrospective study from our hospital, The Hospital for Sick Children, Jones and colleagues reported a success rate of 30% in 61 children (2 months - 2 years) with IS and without TSC (Jones et al., 2015) (Table 5). The 18 first-line responders (30%) showed significant improvement from seizure symptoms in terms of clinical findings and EEG reports, and as for the remaining 43 children who did not respond initially to vigabatrin, they were administered ACTH and prednisolone as second options; however, the results were not impressive, especially for prednisolone which had a small 10% success. One of the interesting findings was that those children with healthy development at the
time they were diagnosed with IS were more likely to respond to initial vigabatrin treatment, as this was seen in successful responders where 13 out of 18 children had normal developments.

<table>
<thead>
<tr>
<th>% of Total Children (/61)</th>
<th>Successful Vigabatrin First-Line Treatments (N=18)</th>
<th>Unsuccessful Vigabatrin First-Line Treatments (N=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with Normal Development At Diagnosis (%)</td>
<td>30%</td>
<td>70%</td>
</tr>
<tr>
<td></td>
<td>13/18 (72%)</td>
<td>10/43 (23%)</td>
</tr>
</tbody>
</table>


Since vigabatrin is particularly effective for children with IS and TSC, and is not as effective for children without TSC, the question that remains is should vigabatrin be used in conjunction with other hormonal treatments to achieve better seizure control in children without TSC?

O’Callaghan and colleagues found that the combination therapy of vigabatrin and prednisolone or tetracosactides is significantly better at controlling seizures than hormonal therapy alone (p=0.002), as 72% of children receiving combined approach and 57% receiving single therapy became seizure-free 14 and 42 days post treatment (O’Callaghan et al., 2017). In addition, these children on combined therapy showed faster response to treatment (median response time = 2 days) than those treated with hormone therapy (median response time = 4 days; p<0.001). In this study, the effectiveness of the combination therapy may be due to a synergistic effect between the treatments (O’Callaghan et al., 2017), and further studies are needed to confirm this hypothesis.
<table>
<thead>
<tr>
<th>Study</th>
<th>% Of Spasms Cessation</th>
<th># of Patients</th>
<th>First-line Comparisons</th>
<th>Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Aicardi et al., 1996</td>
<td>68%</td>
<td>192</td>
<td>N/A</td>
<td>99 mg/kg daily</td>
</tr>
<tr>
<td>^Chiron et al., 1997</td>
<td>100%</td>
<td>11</td>
<td>Hydrocortisone at 15 mg/kg daily (N=11; 45% seizure-free)</td>
<td>150 mg/kg daily</td>
</tr>
<tr>
<td>^Vigevano &amp; Cilio, 1997</td>
<td>48%</td>
<td>23</td>
<td>ACTH at 10 IU daily (N=19; 74% seizure-free)</td>
<td>Between 100-150 mg/kg daily</td>
</tr>
<tr>
<td>#Fejerman et al., 2000</td>
<td>40%</td>
<td>116</td>
<td>N/A</td>
<td>Between 50-200 mg/kg daily</td>
</tr>
<tr>
<td>^Lux et al., 2004</td>
<td>54%</td>
<td>52</td>
<td>1. Prednisolone at 40 mg daily (N=30; 70% seizure-free). 2. Tetracosactide at 40 IU every other day (N=25; 76% seizure-free).</td>
<td>Between 100-150 mg/kg daily</td>
</tr>
<tr>
<td>*Jones et al., 2015</td>
<td>30%</td>
<td>61</td>
<td>N/A</td>
<td>Between 50-150 mg/kg daily</td>
</tr>
<tr>
<td>^Bitton et al., 2012</td>
<td>56%</td>
<td>68</td>
<td>Synthetic ACTH at 150 IU every 2 days (N=30; 80% seizure-free).</td>
<td>Between 100-150 mg/kg daily</td>
</tr>
</tbody>
</table>

Table 6. The effectiveness of vigabatrin as first-line options for infantile spasms studied from retrospective (represented by *), prospective (represented by #) and randomized controlled studies (represented by ^).

Summary

Overall, vigabatrin has shown remarkable clinical efficacy both as first and second line treatment options for children with infantile spasms due to many different causes. These results from retrospective, prospective and randomized trial studies are summarized in Table 6. In particular,
initial vigabatrin therapy had substantially more success in treating seizure symptoms of children with an underlying TSC etiology, and it carried fewer risks of life-threatening adverse side effects as compared to ACTH and other steroid counterparts. However, in the cases of children without TSC, the effectiveness of vigabatrin varied greatly from 9% to 54% (Lux et al., 2004; Jones et al., 2015), possibly due to the specific differences in research methodology and variety of etiologies. Although vigabatrin is the first-line treatment in children with IS (Aicardi et al., 1996; Bitton et al., 2012; Riikonen, 2014), we should be cautious given the findings of retinal toxicity that exist amongst select individuals receiving this therapy. More recently in a large multicenter study, O’Callaghan and colleagues found that the combination of vigabatrin and hormonal treatments is significantly more effective in seizure control than hormonal therapy alone, suggesting that the synergistic effect between the drugs may be clinically relevant for children with IS and without TSC.

3. Vigabatrin-Associated Visual Toxicity

While vigabatrin does not have the side effects of ACTH and high dose corticosteroid therapy for IS, the potential retinal toxicity poses a major concern. A systematic review, published in 2010, included 32 observational studies, published between 1999-2009. (Maguire et al., 2010). The review, which included 1,678 patients treated with vigabatrin for partial epilepsy, revealed that the associated visual field deficits occurred more frequently in adults than in children (Maguire et al., 2010). Specifically, more than half of adults and nearly one third of children receiving vigabatrin on average showed visual deficits, and the prevalence rate increased with cumulative dose (Lawden et al., 1999; Malmgren et al., 2001), mean dose (Wild et al., 2007), older age, male gender (Wild et al., 1999; Wild et al., 2007), and treatment duration (Miller et
al., 1999; Hardus et al., 2001; Malmgren et al., 2001; Wild et al., 2007; Riikonen, 2014). Hence, the authors suggested the use of vigabatrin to be only “reserved for patients with epilepsy for whom there is no other alternative”. Nevertheless, vigabatrin is an important drug with substantial clinical efficacy for children suffering from IS, and it is currently the first-line option at the Hospital For Sick Children, therefore a better understanding of the associated retinal toxicity could reduce this additional burden and significantly improve their quality of life.

**Visual Field Loss in Adults and Older Children**

A common phenomenon of vigabatrin-related retinal toxicity is bilateral concentric constriction of the visual fields (VFs). This was first noted in 1997 from three clinical cases in adults by Eke and colleagues and reported to the Committee on Safety of Medicines. Information from the three patients with complex partial epilepsy and temporal lobe epilepsy are listed in Table 7 (patients 1-3). Specifically, the patients have developed worse peripheral VFs in both eyes after two to three years post vigabatrin treatment (dosage ranging between 2000-4000 mg/day), while their central visions and other functional markers remained stable as seen from electroretinography (ERG) and funduscopy. It was to note that a 44-year-old patient with the highest dosage (4000 mg/day) experienced a larger deficit within the nasal fields, and a 46-year-old female patient showed reductions in oscillatory potentials (OPs).
<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (Seizure Type)</th>
<th>Other Drugs Taken Concurrently</th>
<th>Vigabatrin Dosage</th>
<th>Clinical Findings/Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22 (complex-partial)</td>
<td>Carbamazepine &amp; Sodium Valproate</td>
<td>2000 mg daily add-on</td>
<td>-Constriction of VFs after 3 years -Reduced OPs</td>
</tr>
<tr>
<td>2</td>
<td>44 (temporal lobe)</td>
<td>Phenytoin &amp; Sodium Valproate</td>
<td>4000 mg daily add-on</td>
<td>-Constriction of VFs after 2 years (nasal quadrant more affected) -Reduced OPs</td>
</tr>
<tr>
<td>3</td>
<td>46 (complex-partial)</td>
<td>Carbamazepine</td>
<td>3500 mg daily add-on</td>
<td>-Constricted VFs bilaterally 38 months later -Reduced OPs</td>
</tr>
<tr>
<td>4</td>
<td>58 (complex-partial &amp; secondary generalized)</td>
<td>Carbamazepine</td>
<td>4500 mg daily add-on</td>
<td>-Reduced VFs in 4 months -Reduced OPs</td>
</tr>
<tr>
<td>5</td>
<td>22 (complex-partial &amp; temporal lobe)</td>
<td>N/A</td>
<td>2000 mg daily monotherapy</td>
<td>-Blurred vision -Reduced OPs</td>
</tr>
<tr>
<td>6</td>
<td>29 (complex-partial)</td>
<td>Carbamazepine</td>
<td>4500 mg daily add-on</td>
<td>-Reduced VFs -Reduced OPs</td>
</tr>
<tr>
<td>7</td>
<td>67 (complex-partial)</td>
<td>N/A</td>
<td>4000 daily monotherapy</td>
<td>-Reduced VFs (temporal more affected) -Reduced OPs</td>
</tr>
</tbody>
</table>

Since the work by Eke and colleagues, many other studies using static and kinetic perimetry testing have also reported on the adverse VF losses following vigabatrin treatment in adults; these reports are listed in Table 8. In 1998, Krauss and colleagues reported on four individuals with complex partial epilepsy (22-67 years), out of a total 38 patients, who exhibited retinal complications. Of these, three had notable peripheral VF loss and all showed significant abnormalities of ERG oscillatory potentials (Table 7, patients 4-7). This finding was consistent with other ERG studies (Eke et al., 1997; Arndt et al., 1999; Kälviäinen et al., 1999; Miller et al., 1999), and has supported the idea that amacrine cells (interneurons in the retina) were greatly disturbed by vigabatrin, possibly due to their highly GABAergic nature. A 67-year-old patient with complex partial seizures (patient #7 in table 7) had larger deficits in the temporal fields than the nasal fields following vigabatrin monotherapy. Miller and colleagues (1999) also described similar temporal field loss in their patients with bilateral VF losses, however, other studies by Lawden et al and Wild et al showed opposite findings of relative temporal sparing and nasal deficits in patients with vigabatrin-related VF losses (VRVFL), respectively.

<table>
<thead>
<tr>
<th>Study</th>
<th>Age (Years)</th>
<th>Average Dosage</th>
<th>Perimetry Findings</th>
<th>ERG Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arndt et al., 1999</td>
<td>8-65</td>
<td>2769 mg/day add-on</td>
<td>-12/20 with VF constriction -7/12 asymptomatic</td>
<td>10 patients with OPs deficits</td>
</tr>
<tr>
<td>Daneshvar et al., 1999</td>
<td>28-52</td>
<td>3000-4000 mg/day</td>
<td>-12/12 with bilateral VF constriction -8/12 asymptomatic</td>
<td>-4/10 with reduced b-wave on scotopic ERG -1/10 with reduced 30-Hz flicker</td>
</tr>
<tr>
<td>Kälviäinen et al., 1999</td>
<td>19-73</td>
<td>N.S monotherapy</td>
<td>-13/32 with bilateral VF constriction -All 13 patients asymptomatic</td>
<td>-Reduced OPs in severe cases (9/13) of VF constriction -Reduced amplitude of cone</td>
</tr>
</tbody>
</table>
In 1999, Daneshvar reported that the ERG deficits in patients treated with vigabatrin were confined to reductions in the scotopic b-wave amplitudes, and not solely the aforementioned OPs. This observation was consistent with Miller et al., 1999, and gave rise to the idea that the Muller cells, rather than amacrine cells, were involved in VRVFL, since Muller cells contribute to scotopic b-waves (Miller & Dowling, 1970), and that vigabatrin has been shown previously to elevate GABA concentrations in these cells from animal studies. The authors postulated that a smaller presence of Muller cells in the peripheral retina as compared to central retina is vulnerable to vigabatrin insult, and this could cause peripheral visual field loss following drug intake, while greatly preserving central vision. In addition, Miller et al (1999) found a strong positive correlation between reductions in cone-dominant flicker amplitudes on the ERG and the

<table>
<thead>
<tr>
<th>Study</th>
<th>Dosage</th>
<th>ERG Deficits</th>
<th>ERG Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lawden et al., 1999</td>
<td>12-67</td>
<td>Max 1.5-5 mg/day</td>
<td>-12/31 with bilateral VF constriction (temporal field unaffected)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-8/12 asymptomatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Normal ERG results</td>
</tr>
<tr>
<td>Miller et al., 1999</td>
<td>22-73</td>
<td>3300 mg/day monotherapy</td>
<td>-Approximately 50% with bilateral VF constriction (temporal more affected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>than nasal)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-22/32 asymptomatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Reduced OPs in all (32) patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Reduced rod &amp; cone b-waves</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Reduced cone flicker response</td>
</tr>
<tr>
<td>Wild et al., 1999</td>
<td>34.2 +/- 11.2</td>
<td>1000-2400 mg/day</td>
<td>-42/145 with VRVFL (nasal more affected)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-41/42 asymptomatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-Reduced OPs in select patients with available ERG</td>
</tr>
<tr>
<td>Malmgren et al., 2001</td>
<td>17-58</td>
<td>0.02 - 11.4 kg cumulative dose</td>
<td>-19/99 with visual field defects</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-All asymptomatic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 8. Studies showing bilateral visual field constrictions with vigabatrin treatment. N.S represents not specified dosage information. Patients without ERG testing were deemed “N/A”. OPs= electroretinogram oscillatory potentials.
degree of VFs loss on kinetic perimetry, suggesting the ERG flicker responses could be a useful biomarker of retinal toxicity for children taking vigabatrin that could be acquired in a timely fashion.

An important note to take away from these earlier studies is that majority of patients who exhibited VRVFL clinically did not experience any symptoms except for when central vision was noticeably disturbed. From the reports listed from Table 8, 58%-100% of patients with vigabatrin-associated VF constrictions were asymptomatic, and in the rare symptomatic cases, the visual complaints involved patients inadvertently running into furniture (Wild et al., 1999). The highly asymptomatic and irreversible nature could make timely VRVFL detection very difficult (Malmgren et al., 2001), especially for infants whose visual system is still developing as are the communication skills required to report visual complications. Therefore, given the prevalence of VRVFL and its correlations with ERG abnormalities, regularly scheduled monitoring sessions are suggested to be in place beginning from drug administration to assess the risks of vigabatrin treatment in a longitudinal manner.

**Vigabatrin-Associated Retinal Toxicity in Young Children**

Due to the presence of permanent retinal toxicity, it is recommended that physicians ensure they inform the patients, or parents of patients in the case of pediatrics, the risks of adverse deficits following vigabatrin treatment (Mackay et al., 2004; Sabril (vigabatrin) Official US Site). Healthcare providers in the USA are required to adhere to the guidelines of the Support, Help and Resources for Epilepsy (SHARE) program before initiating therapy. The SHARE program was developed and updated by the vigabatrin (Sabril) manufacturer, Lundbeck Pharmaceuticals,
following the FDA request for a Risk Evaluation and Mitigation Strategy (REMS) aiming to minimize adverse side effects (Postmarket Drug Safety Information for Patients and Providers). This strategy is well implemented in the Department of Ophthalmology and the Visual Electrophysiology Unit (VEU) at the Hospital for Sick Children. Specifically, patients undergoing vigabatrin participate in periodic monitoring sessions for toxicity detection every 3-4 months starting at baseline or within 4 weeks following initial dose and continue scheduled visual assessment even after drug cessation.

**Visual Field Testing in Children Treated with Vigabatrin**

VRVFL occurs more frequently in adults and older children than younger children (Vanhatalo et al., 2002; Wild et al., 2007; Maguire et al., 2010). In 2007, a large, multi-center prospective study of toxicity risk factors compared the frequency of VRVFL between children who were 8-12 years of age and their older counterparts (Wild et al., 2007). The findings agreed with earlier studies such that the younger cohort had less individuals with VRVFL overall. This trend remained consistent for patients who were either still undergoing vigabatrin therapy at the time of visual field testing, or for those who were weaned off from treatment for at least 6 months. This maintenance of visual deficits following drug cessation implies VRVFL is irreversible in many children. However, the results from this report could not be applied to infants with IS, as this particular cohort of young children was not selected for inclusion.

Goldmann perimetry testing of visual fields in very young children undergoing vigabatrin therapy has shown very little success over the years due to issues of compliance and developmental delays (Wohlrab et al., 1999). Perimetry is not possible in children under three years of age and is tolerated better in older children (Gaily et al., 2009). Wohlrab and colleagues
(1999) investigated VRVFL in 153 children with partial epilepsy and IS and reported a substantially low visual field testing tolerability of only 8% (12/153 children tested). They found that 5/12 (42%) children on vigabatrin therapy who completed Goldmann perimetry exhibited bilateral concentric visual field loss. Interestingly, all participants were asymptomatic with vigabatrin, and this was consistent with studies in adults and older children. Nevertheless, the visual field outcomes in children undergoing treatment during the first 12 months of life remained unclear for nearly 10 years (Gaily, 2012). In 2009 (vigabatrin was approved by FDA for treatment of infantile spasms), Gaily et al (2009) reported the first study of visual field assessment in 16 children who started vigabatrin during infancy (average age of initiation: 7.6 months). Due to aforementioned concerns with testing cooperation, Goldmann perimetry was performed once these children reached between 6 to 12 years of age. It was found that only 1/16 children (6%) developed mild VRVFL, and this result was much lower than the estimated 34% by Maguire et al., 2010. Wohlrab and colleagues found similar visual field outcomes in 1/15 children who were previously treated during infancy (drug initiation between 2.5-12 months). It was to note that both reports used kinetic perimetry to assess VRVFL given its effectiveness and reliability in school-age children (Vanhatalo et al., 2002). The findings from these studies are listed in Table 9.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Age of Vigabatrin Onset (Months)</th>
<th>Age of Kinetic Perimetry (Years)</th>
<th>Visual Fields Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaily et al., 2009</td>
<td>7.6</td>
<td>6-12</td>
<td>1/16 with mild VRVFL</td>
</tr>
<tr>
<td>Wohlrab et al., 2009</td>
<td>2.5-12</td>
<td>&gt;6.5</td>
<td>1/15 with VRVFL</td>
</tr>
<tr>
<td>Riikonen et al., 2014</td>
<td>4-30</td>
<td>5-22</td>
<td>11/32 with VRVFL</td>
</tr>
</tbody>
</table>

Table 9. Studies of VRVFL for children undergoing vigabatrin therapy during infancy and later tested on perimetry during school-age.
Riikonen et al., 2014 found a 34% prevalence rate of VRVFL when children previously treated with vigabatrin were tested during school age (Table 9). This finding was in contrast to Gaily et al (2009) and Wohlrab et al (2009). Moreover, this study by Riikonen and colleagues showed the significance of treatment duration, such that the number of children experiencing VRVFL increased 7-fold when duration was lengthened from less than 1 year (9%) to longer than 2 years (63%). This was consistent with previous reports (Miller et al., 1999; Hardus et al., 2001; Malmgren et al., 2001; Wild et al., 2007). Those who had an IS etiology of TSC showed higher frequency of mild and severe VRVFL (60%), and this was possibly due to longer treatments needed to suppress their seizure symptoms (Riikonen et al., 2014).

**Summary**

Vigabatrin is associated with bilateral concentric visual field constriction in both adults and children when tested with static and kinetic perimetry. The frequency of VRVFL is higher in adults and older children than younger children. The overall risk of visual deficits increases progressively with treatment duration and dosage. Many children treated with vigabatrin during infancy for IS show characteristic VRVFL when tested with kinetic perimetry several years later. The asymptomatic as well as the irreversible nature of the condition makes timely detection very challenging, especially for infants with IS who are unable to express any subjective visual deficits. Therefore, regular monitoring sessions is advisable for both adults and children undergoing vigabatrin therapy, and this is in accordance with the SHARE program developed by Lundbeck Pharmaceuticals in response to REMS as proposed by the FDA (Sabril (vigabatrin) Official US Site). However, visual field testing has been proven to be very difficult in young patients with minimal attention span and was more appropriate for children who were at least
school-age. Another approach to achieve periodic monitoring in infants is with electroretinography, as many adults and older children with VRVFL have also shown functional deficits on the ERG, such as Oscillatory Potentials, b-wave, and flicker amplitude reductions. At the Hospital for Sick Children, electroretinography is usually performed under full sedation in children to enhance compliance.

4. Vigabatrin-Associated Electrophysiological Changes

An electroretinogram (ERG) is the electrical responses following light stimulation to different cellular components in the retina. The ERG waveforms are displayed graphically with voltage (microvolts, uV) plotted against time (milliseconds, ms). ERGs are usually implemented clinically under the guidelines and standards of the International Society for Clinical Electrophysiology of Vision (ISCEV), which was updated and published in *Documenta Ophthalmologica* in 2015 (McCulloch et al., 2015). The ISCEV standards highlighted six steps in completing full-field ERG testing. The steps are labelled according to the stimulus strength (flash strength in cd.s.m\(^{-2}\)) and the state of light or dark adaptation. These include both scotopic (dark-adapted, DA) and photopic (light-adapted, LA) measurements (Figure 2). The DA testing involves adapting to a fully darkened environment for at least 20 minutes prior to 0.01 ERG (low intensity ERG), and these precede the LA steps which involve 10 minutes of exposure to light prior to 3.0 single-flash ERG. The protocols begin with evaluating the rod-dominant responses (0.01 cd.s.m\(^{-2}\) ERG) and proceed to assess cone-rod mixed responses (DA 3.0 cd.s.m\(^{-2}\) ERG, strong flash 10.0 cd.s.m\(^{-2}\) ERG and DA 3.0 OPs), and eventually ending with cone-isolated responses (LA 3.0 cd.s.m\(^{-2}\) ERG, LA 3.0 flicker).
ERG and VRVFL

Conventional visual field assessments can be unreliable in young children due to difficulties of compliance (Wohlrab et al., 1999). Given the issues of vigabatrin-associated visual toxicity, scheduled monitoring is carried out with other well-tolerated techniques, such as the ERG. Changes on the ERG correlate with VRVFL in adults and older children; these include oscillatory potentials, cone/rod b-waves, and flicker response deficits (Table 8; Page 15). Multiple studies have reported OPs deficits in patients with VRVFL (Eke et al., 1997; Arndt et al., 1999; Kälviäinen et al., 1999; Miller et al., 1999), particularly, Kälviäinen and colleagues have shown OPs deficits only in cases of severe VRVFL, suggesting the role of amacrine cells in vigabatrin toxicity. Daneshvar et al., 1999 found changes in dark-adapted b-wave amplitudes in individuals with VRVFL, and this implied the possible involvement of Muller cells in addition to amacrine cells. Cone flicker amplitude reduction also associated strongly with the degree of
VRVFL (Daneshvar et al., 1999; Miller et al., 1999; Harding et al., 2000), proposing the idea that bipolar cells and the rest of inner retina, including photoreceptors, were affected by vigabatrin.

**Vigabatrin and OPs**

DA and LA OPs are small rising phase wavelets of the scotopic (cone-rod mixed) and photopic (cone) b-waves, respectively (Morong et al., 2003). These OPs are produced in the inner plexiform layer (IPL) of the inner retina and are stratified into two subgroups of early and late OPs, each with different outcomes following vigabatrin treatment in children (Morong et al., 2003; Westall et al., 2003). In a longitudinal report from 2002, Westall and colleagues studied the ERG changes in children (1.5-180 months old) receiving treatment at the VEU of Hospital For Sick Children, and found that cone b-waves followed an increase-then-decrease response (quadratic pattern) in children undergoing monotherapy and add-on treatment. Moreover, the LA OPs derived from cone b-waves were mostly affected in the early portion (OP2 and OP3) but not the later phase (OP4). This suggested that the ON-type bipolar cells were implicated in toxicity, as the early phases were generated by presence of light, and the later stages were activated by the offset of light. Since OP2 and OP3 are ON-types, they are most likely influenced by GABA, and OP4 is an OFF-type thus insensitive to GABA. These findings were consistent with another longitudinal ERG study in infants (1.5-24 months) at 6 months intervals, where the authors found notable declines in the earlier OPs amplitudes as compared to later OPs during treatment (Morong et al., 2003). It was to note that the LA OPs assessment in both studies were an addition made to the existing ISCEV standards.
Now the issue to be addressed was whether these OPs changes on the ERG were indicative of vigabatrin toxicity or other non-toxic physiological responses by the inner retina. To answer these questions, the same group of Westall and colleagues (2003) conducted ERG observations in 17 children after cessation of vigabatrin treatment. When vigabatrin was discontinued, the earlier phase of the LA OPs elevated dramatically in a dosage-independent manner compared to the OP response with ongoing vigabatrin treatment ($p = 0.000075$). This spontaneous recovery implied that OP deficits seen during therapy were a physiological reaction to increased GABA concentrations, rather than a vigabatrin-associated toxicity response. The amacrine cells in the IPL of the retina give rise to OPs through inhibitory feedback mechanisms and are highly GABAergic in nature (Krauss et al., 1998; Wachtmeister, 1998). Therefore, a vigabatrin-related GABA surge disrupted this feedback circuit to depress early OPs, and that cessation of treatment kept GABA levels in check to restore early OPs activities. The later OPs were not disturbed by vigabatrin in the same way. The later phase of the LA OPs probably reflect activity related to hyperpolarizing cells; these depolarize to the offset of stimulus (OFF-cells) and maybe sensitive to glycine instead of GABA; glycine is abundant in the peripheral areas of the IPL in animal studies (Pourcho, 1980). Interestingly, the OPs response following discontinuation remained subnormal, which may have been the result of abnormal OPs prior to drug administration due to unclear reasons.

**Vigabatrin and 30-Hz Flicker ERG**

Overall, the study by Westall et al (2003) showed that the initial OPs reduction in children was not a consistent marker of retinal toxicity as recovery occurred following drug withdraw. The LA cone b-waves and the 30-Hz LA flicker responses may be better ERG functional biomarkers of
visual deficits as they have remarkable correlations with VRVFL. (Harding et al., 2000) (Figure 3). Specifically, in two consecutive reports of children aged 3-15 years from 2002 to 2003, Harding and colleagues proposed that flicker assessment was the most capable ERG parameter to accommodate VFs measurements given its high specificity at 71% and sensitivity at 75% (Harding et al., 2002; Spencer & Harding, 2003). These studies are quoted extensively in support of the suggestion that these LA cone responses are the most consistent marker of visual toxicity. However, the sample size was small and some caution in interpreting these data is advised. Data from Westall and colleagues support the notion that the LA 30 Hz flicker is reduced with vigabatrin treatment (Figure 3) (Westall et al., 2002; Morong et al., 2003; Westall et al., 2003; McCoy et al., 2011; Westall et al., 2014). In contrast to the LA OPs, 30-Hz flicker ERG did not show improvements following drug discontinuation (Westall et al., 2003), suggesting the changes seen during treatment were most likely due to toxicity.

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Figure 3. 30-Hz flicker (left) and LA cone (right) responses tested at 6 months intervals in a 5 months old child undergoing vigabatrin therapy. Control data are acquired from a healthy 30 months old individual. Image from McCoy, B., Wright, T., Weiss, S., Go, C., & Westall, C. A. (2011). Electoretinogram changes in a pediatric population with epilepsy: is vigabatrin acting alone? Journal of Child Neurology, 26(6), 729–33. Please see appendix for copyright license.
A retrospective report from McCoy and colleagues (2011) at The Hospital for Sick Children used LA 30-Hz flicker as a marker to compare the toxic effect of vigabatrin between children undergoing vigabatrin monotherapy and those receiving vigabatrin with other antiepileptic drugs in combination. LA 30-Hz ERG data were collected from 160 children (1 month-18 years) who were tested on at least three occasions since baseline. The definition of toxicity was electroretinogram reduction from baseline on at least 2 consecutive occasions. Retinal toxicity was detected in 18 of the 160 patients (11.25%) with higher percentage affecting those treated with additional antiepileptic medication than vigabatrin alone (P < 0.002).

A few years later, Westall et al., 2014 published a more focused study in 146 infants with IS aged 3-35 months who were too young to cooperate with VFs assessments. Significant declines in the 30-Hz flicker amplitude at follow-up indicative of toxicity were seen in 21% of patients, and the levels of reduction were higher with longer duration of treatment with no improvement post drug withdraw. Importantly, the risk factors identified from ERG findings appeared to be confined to duration, and there were negligible contributions from total dosage, biological sex, or age at treatment onset. Here, the LA flicker demonstrated tremendous clinical utility in quantifying visual deficits in children undergoing vigabatrin in a longitudinal fashion. In these studies the flicker responses were age-corrected to accommodate for developmental confounders.

**Developmental Effects on the ERG in Pediatric Cohorts**

Since retinal monitoring is important for young children with IS receiving vigabatrin treatment, knowledge of interpretation of the ERG results is crucial in this particular cohort. During the time that infantile spasms is treated, the ERG is undergoing considerable developmental changes (Westall et al., 1999; Fulton et al., 2003; Sergott & Westall., 2011). A joint study between The
Children’s Hospital in Boston and The Hospital for Sick Children in Toronto reported on the ERG responses in visually-healthy infants and young children and found significant developmental changes from comparisons to adult population (Fulton et al., 2003). Figure 4 clearly shows that the adult amplitudes are much higher than infants for the 0.01 rod-isolated, 3.0 rod-cone mixed, and 3.0 cone-isolated parameters. In fact, this large difference was true for all infants younger than 5 weeks old, and especially for 26% of them who have yet to develop a measurable rod response. Moreover, for this particular young group, the median detectable b-wave amplitudes of the three tested responses were only 15% to 46% of those seen in adults. The LA 3.0 flicker ERG on the other hand reaches adult standards after about 5 years of development, which is similar to the responses from cone b-waves (Westall et al., 1999).

Figure 4. Comparison of ERG responses between a 4 months old infant and an adult participant. Both participants were normal. The ISCEV standard 0.01 DA rod, 3.0 DA rod-cone combined, and LA 3.0 cone responses were compared. Image from Fulton, A. B., Hansen, R. M., & Westall, C. A. (2003). Development of ERG responses: The ISCEV rod, maximal and cone responses in normal subjects. Documenta Ophthalmologica, 107(3), 235–241. Please see appendix for copyright license.
**Novel Portable ERG Technique in Young Children**

The current gold standard to perform clinical evaluations of retinal function in children lies within the full-field ERG technique, which sometimes requires sedation using chloral hydrate and pupil dilation for infants who cannot provide full compliance. At the Hospital for Sick Children in Toronto, Ontario, the ISCEV ERG protocols are completed by the VEU using a large Espion E2 Color Dome (Diagnosys LLC, Lowell, MA) that is connected to the disposable active electrodes placed in front of the cornea. The clinical ERG in very young children introduces discomfort through corneal contacting electrodes, and pupil dilation, and it gives rise to potential invasiveness through full sedation, as some studies have shown adverse side-effects following sedation procedures (Malviya et al., 2000; Maxwell et al., 2003).

A novel handheld ERG (HH-ERG), RETeval (LKC technologies, Gaithersburg, MD, USA), has the ability to record ISCEV ERG responses while eliminating the need of pupil dilation (mydriasis-free) and corneal electrodes. The device’s pre-set algorithm allows it to accommodate changes in pupil diameters of up to 6.5 mm (Kato et al., 2015), and to use skin electrodes placed underneath the lower eyelid. The 30-Hz flicker response recorded using this technique showed remarkable clinical utility in several studies of retinal conditions in older population while bypassing sedation (Maa et al., 2016; Fukuo et al., 2016; Nakamura et al., 2016). Moreover, ERG responses from this mydriasis-free device demonstrated remarkable within-visit reliability as measured from intraclass correlation coefficient (ICC) in young adults (aged 21.4 ± 0.9 years) (Asakwa et al., 2017). In particular, the ICC was the highest for the amplitude (uv) and implicit time (ms) of the LA cone-dominant 30-Hz flicker response (ICC = 0.92 and 0.91, respectively). Given its clinical efficacy and intravisit reliability, this device is worth investigating in infants who are usually uncooperative to conventional methods.
Summary

The full-field ERG is a better tolerated monitoring method in young children with IS than VFs assessment using perimetry. The change in LA OPs during vigabatrin treatment may only be a result of drug-related physiological changes in the inner plexiform layer of the retina, and not due to toxicity as previously proposed. The LA 30-Hz flicker on the other hand may represent a more reliable marker of retinal deficits as its amplitude reduction strongly associates with VRFVL in children, and this decline persists even after treatment discontinuation. To fully appreciate retinal changes in very young children undergoing vigabatrin therapy, we must take their normal development into account, since many ERG responses do not match adult levels until weeks or years later. ERG responses mature at varying rates, such that they are reflective of the developmental speeds of different retinal layers. Overall, response amplitude increases, and implicit time decreases with age.

A portable ERG device (RETeval) designed to eliminate the need for pupillary dilation is now available and may be better tolerated than sedated ERG in providing recordings. The 30-Hz flicker response of this portable device is quick to record and has reasonable clinical utility and reliability. However, now the question becomes whether this handheld technique is comparable to the current gold standard ERG in providing reliable assessment of retinal function in infants without the use of mydriatics, corneal electrodes and sedation.

5. Optical Coherence Tomography

Another imaging technique in the field of ophthalmology is the optical coherence tomography (OCT), a contactless, non-invasive, and efficient device that utilizes low-coherence interferometry to provide real-time, cross-sectional, structural information of the retina (Gabriele...
et al., 2008; Scott et al., 2009; Hahn et al., 2011; Terry et al., 2016). The mechanism works in analogous ways to ultrasound, where the echo time delays and the reflected lights, as opposed to sound, are used to build false-colour images of the retina, cornea, and optic disc that appear black and white (Srinivasan et al., 2006). Since its introduction in the 1990s at the Massachusetts Institute of Technology, OCT has been an invaluable addition to the clinical assessment of both common and rare ophthalmological diseases, such as glaucoma, diabetic macular edema, age-related macular degeneration, and ocular albinism (Gabriele et al., 2008; Brennen et al., 2009; Chong et al., 2009; Kampougeris et al., 2013).

**Time-Domain OCT vs. Spectral-Domain OCT**

In the 1990s, the Stratus device (Carl Zeiss Meditec, Dublin, CA) became the first clinical-ready time-domain OCT. At the time, it represented a revolutionary change as all retinal layers could be imaged and visualized simultaneously in a patient without the need of staining. However, time-domain OCT had a subpar tissue resolution of about 10 um, and slow imaging speed of 400 A scans/second (Srinivasan et al., 2006; Hahn et al., 2011), which makes it unreliable especially when the participants have slight eye movements. The motion artifacts occur frequently affecting quality due to movement limitations in the reference mirror of the time-domain OCT that is required to obtain A scans. Fortunately, with the introduction of the FDA-approved Fourier, or spectral-domain OCT (SDOCT), improvements are made over the time-domain OCT in terms of efficiency with 40-100 times faster speeds, and resolution with less than 5 um of tissue resolution (Srinivasan et al., 2006; Gabriele et al., 2008; Gabriele et al., 2011). Given the substantial speed of image acquisitions, the reference mirror is no longer required on the SDOCT, thus artifacts related to eye movement are greatly diminished. Consequently, the SDOCT became a preferred
option by many healthcare professions due to tremendous clinical efficacy, and to date, several SDOCT devices are commercially available. Notably, the table-top Cirrus SDOCT (Carl Zeiss Meditec Inc, Dublin, CA), and the handheld SDOCT (Bioptigen, Inc., Research Triangle Park, NC) are well-tolerated by children in the eye clinic at The Hospital for Sick Children. In addition, these machines can collect and process in vivo images at an astonishing rate of up to 20,000 A scans every second while maintaining a less than 5 microns resolution (Gabriele et al., 2008).

**OCT in Participants Undergoing Vigabatrin Therapy**

Reductions in the peripapillary retinal nerve fiber layer (RNFL) thickness occur in children undergoing vigabatrin treatment (Miller et al., 1999; Buncic et al., 2004). Buncic and colleagues in 2004 described 3 children (2.5-15 years of age) who exhibited unique inverse RNFL atrophy around the optic disc from funduscopy, with this thinning most noticeable near the nasal retinal quadrant with relative macular sparing. Since this loss was supported by concurrent findings of ERG 30-Hz flicker deficits following vigabatrin treatment, it suggested that RNFL could be an excellent structural biomarker to accommodate ERG response in the clinical diagnosis of retinal toxicity. Moreover, RNFL thickness attenuation that is recorded and quantified with OCT associates strongly with VRVFL (Table 10) (Choi & Kim, 2004; Wild et al., 2006; Lawthom et al., 2009; Clayton et al., 2010; Moseng et al., 2011; Clayton et al., 2012), and this correlation further supports the incorporation of RNFL and OCT clinically. Wild et al used traditional time-domain OCT to show that the mean RNFL loss from 360-degree scans of the optic disc was present in all patients (42.9 ± 8.9 years of age) who had VRVFL (100% specificity and 100% sensitivity). Lawthom et al reported similar findings of RNFL thickness reduction in all
participants (41.5 ± 11.1 years of age) with VRVFL, and subsequently within these patients, they found a nasal-dominant pattern of RNFL deficits with relatively-unaffected temporal quadrant and some involvements from the superior and inferior quadrants. In a subgroup of participants who were on vigabatrin but were devoid of VRVFL, 25% of them showed abnormal RNFL, implying that the OCT technique is more sensitive than conventional perimetry to detect nerve fiber deficits which may have preceded functional abnormalities.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Mean Age In Years</th>
<th>OCT Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi &amp; Kim, 2004</td>
<td>18</td>
<td>-Peripheral RNFL deficits with temporal sparing</td>
</tr>
<tr>
<td>Wild et al., 2006</td>
<td>42.9 ± 8.9</td>
<td>-Significant mean RNFL decline in Ps with VRVFL vs. controls (P &lt; 0.001)</td>
</tr>
<tr>
<td>Lawthom et al., 2009</td>
<td>41.5 ± 11.1</td>
<td>-All Ps with VRVFL showed RNFL decline overall &amp; in N quadrant</td>
</tr>
<tr>
<td>Clayton et al., 2011</td>
<td>46.0 ± 11.6 (SDOCT) 46.1 ± 10.6 (time-domain OCT)</td>
<td>-Strong correlation between RNFL thickness and peripheral visual fields for SDOCT (r = 0.769) &amp; time-domain OCT (r = 0.814)</td>
</tr>
<tr>
<td>Moseng et al., 2011</td>
<td>54.3 ± 9.5</td>
<td>-Significant mean RNFL decline in Ps with VRVFL vs. control (P &lt; 0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-S, N, I quadrants affected most</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-T quadrant unaffected</td>
</tr>
<tr>
<td>Clayton et al., 2012</td>
<td>45.9 ± 11.4</td>
<td>-N &amp; S 30-degree quadrant more affected in Ps undergoing vigabatrin vs. control (P &lt; 0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-↑ c. dose correlates with more RNFL decline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-T quadrant spared even with</td>
</tr>
<tr>
<td></td>
<td></td>
<td>↑ c. dosage</td>
</tr>
</tbody>
</table>
Clayton and colleagues in 2011 found a strong positive relationship between visual field size from Goldmann kinetic perimetry and RNFL thickness from both time-domain OCT ($r = 0.768$), and the SDOCT ($r = 0.814$). In these adults undergoing vigabatrin treatment, OCT was a tolerable and reproducible approach, especially with SDOCT which showed lower variability across repeated scans as compared to its time-domain counterpart. This linear association between RNFL attenuation and VRFVL suggested the involvement of retinal ganglion cell (RGC) axons in retinal toxicity (Ravindran et al., 2001). The axons of RGCs give rise to the retinal nerve fibers that leaves the eye at the optic disc while carrying visual signals towards CNS. In addition, the superior and inferior 90-degree quadrants were more affected than the nasal quadrant, and the temporal quadrant was relatively spared. One year later, the same group evaluated the characteristic pattern of retinal deficits in vigabatrin-exposed patients, and they reported that peripapillary RNFL attenuation around the optic disc became worse with increased cumulative doses (Clayton et al., 2012). Importantly, they demonstrated that even with a very large vigabatrin dose (>1000 g) and severe VRFVL, the temporal quadrant of the retina remained relatively unaffected. This temporal-specific finding agrees with many studies in literature (Wild et al., 2006; Lawthom et al., 2009; Clayton et al., 2010; Moseng et al., 2011; Kjellstrom et al., 2014; Wright et al., 2017; Peng et al., 2017), and is consistent with the sparing of central visual functions following vigabatrin treatment (Daneshvar et al., 1999; Clayton et al., 2010).
OCT in Young Children Undergoing Vigabatrin Treatment

While OCT-oriented studies in adults receiving vigabatrin treatment are abundant, similar reports are limited in awake young children due to compliance issues (Avery et al., 2014; Origlieri et al., 2016). In 2016, a prospective study by Origlieri and colleagues evaluated OCT changes in a young pediatric cohort of 19 children (5.0 years; range 1.1-13.9 years) with and without TSC. They found that participants with TSC (higher dose group at >1500 mg/day of vigabatrin) showed RNFL thickness reductions in the superior, nasal and inferior quadrants ($P <0.05$), and this pattern of attenuation with temporal sparing was consistent with previous adult studies.

Conventional time-domain OCT was used in this study, and due to its table-mounted set up, most cases of retinal examination were performed under general anesthesia (GA) to ensure compliance. Another study by Wright and colleagues performed SDOCT in children who were previously treated with vigabatrin and diagnosed with retinal toxicity (n=10) (Wright et al., 2017). Notably, all children who showed ERG deficits and could tolerate OCT testing (n=4; age at testing $14.7 \pm 5.7$ years) showed RNFL attenuations. The decline was predominant in the superior and inferior quadrant; however the temporal quadrant was largely spared (Figure 5).

This correlation between the 30-Hz response and RNFL thickness strongly suggested that OCT was a useful biomarker of retinal toxicity in children <18 years of age.
Figure 5. Mean and quadrant specific RNFL thickness values obtained from SDOCT. Gray boxes (Group II) represent RNFL thickness of individuals without prior 30-Hz ERG deficits, and white boxes (Group I) represent RNFL thickness of individuals diagnosed with flicker deficits. Both right eye (OD) and left eye (OS) results are shown. Both Group I and II were treated with vigabatrin during infancy. Image from Wright, T., Kumarappah, A., Stavropoulos, A., Reginald, A., Buncic, J. R., & Westall, C. A. (2016). VIGABATRIN TOXICITY IN INFANCY IS ASSOCIATED WITH RETINAL DEFECT IN ADOLESCENCE: A Prospective Observational Study. Retina (Philadelphia, Pa.), 858–866. Please see appendix for copyright license.

Visual Assessment in Children with Handheld OCT

To date, the use of SDOCT for optic nerve assessment in infants are limited (Vinekar et al., 2015), due to testing difficulties seen amongst this particular pediatric group (Muni & Lee., 2009; Gerth et al., 2009; Rootman et al., 2012). Specifically, traditional OCT examinations required children to sit upright and to steadily rest their heads on the chin rest for an extended period of time (Muni & Lee, 2009). With the introduction of a handheld SDOCT (HH-OCT) device (Envisu, Leica Microsystems, NC, USA), infants who were too young to undergo
conventional testing could now be imaged while lying supine and under sedation or GA (Hahn et al., 2011). The HH-OCT is highly flexible allowing the child to be imaged at multiple angles, and with its fast (17,000 A scans/s) and high-resolution (<5 microns tissue resolution) protocols (Gabriele et al., 2008; Hahn et al., 2011), reliable cross-sectional optic disc scans can be taken in very young children for the first time. This technique has shown remarkable clinical efficacy in accommodating the monitoring process of many pediatric ophthalmological diseases (Mallipatna et al., 2015), and some of the common conditions are summarized in Table 11.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Condition(s)</th>
<th>Age</th>
<th>Sedation Status</th>
<th>Clinical Feasibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chong et al., 2009</td>
<td>Ocular albinism</td>
<td>4-13 years</td>
<td>Under GA for strabismus surgery</td>
<td>Positive</td>
</tr>
<tr>
<td>Gerth et al., 2009</td>
<td>Healthy - Maculopathy - Retinal dystrophy - Posttraumatic choroidal neovascularization</td>
<td>7 months - 9.9 years</td>
<td>10/30 sedated (7 months - 3.7 years)</td>
<td>Positive</td>
</tr>
<tr>
<td>Chavala et al., 2009</td>
<td>ROP (PS, retinoschisis, &amp; retinal detachment)</td>
<td>37 weeks -38 weeks PMA</td>
<td>1 patient w/o sedation 2 patients w/ GA</td>
<td>Positive</td>
</tr>
<tr>
<td>Scott et al., 2009</td>
<td>SBS</td>
<td>6 - 14 months</td>
<td>Under GA</td>
<td>Positive</td>
</tr>
<tr>
<td>Avery et al., 2014</td>
<td>OPG</td>
<td>1.8 - 12.6 years</td>
<td>All under sedation</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Table 11. Studies of ophthalmological conditions using HH-OCT in infants and young children. All reports showed positive clinical feasibility of the HH-OCT in distinguishing between healthy and diseased structural morphologies. ROP refers to retinopathy of prematurity, SBS refers to shaken baby syndrome, and OPG denotes optic pathway gliomas (sporadic/neurofibromatosis type I). GA refers to OCT examination under general anesthesia. PMA refers to postmenstrual age.
The Bioptigen SDOCT also demonstrated distinguishable reliability (Avery et al., 2014). In a cohort of 88 participants from the study by Avery and colleagues, 59 children (age range 0.8 - 13 years) with OPG were stratified into the intravisit group, and 29 children (age range 1.8-12.7 years) who have completed follow-up assessments with the handheld OCT were put into the intervisit group. The high ICCs in both groups suggested that this portable technique could acquire RNFL thickness measurements in children with excellent reliability.

**Summary**

Implementation of the OCT has provided clinicians with a more detailed visualization of the retina in various ophthalmological diseases. The SDOCT in particular is capable of providing higher resolution scans of the retina in a quick and efficient manner. Global and quadrant-specific RNFL thickness attenuation as quantified by SDOCT and time-domain OCT has been shown to positively associate with VRVFL and LA 30-Hz flicker deficits, suggesting its validity as a structural biomarker for patients with vigabatrin-related retinal toxicity. Notably, the nasal quadrant of the peripapillary RNFL was the most affected following vigabatrin treatment, and the temporal quadrant was mostly preserved. Given their young age and cognitive delays in certain circumstances, children with IS being treated with vigabatrin who are < 3 years of age are unable to provide full compliance to testing on the tabletop SDOCT. Children with non-compliance on conventional techniques were often sedated or anesthetized for an extended period of time, and these procedures were a source of unease for parents. The handheld SDOCT with its maneuverable light stimulus could be an excellent alternative to monitoring for retinal toxicity in infants lying supine. The time under sedation or GA could be significantly reduced due to its high scanning efficiency and clinical efficacy.
Format of thesis

Chapters I and II are independent studies using each of the outcome variables, Chapter I: RETeval ERG, and Chapter II: Envisu OCT.

Chapter I: Measuring Light-adapted 30-Hz Flicker

Electroretinograms using RETeval in Patients with Infantile Spasms
Abstract

Background
The anti-epileptic drug vigabatrin has been associated with reduction in light-adapted 30-Hz flicker electroretinogram (ERG) amplitude. We evaluated the tolerability, intra-visit reliability, and clinical feasibility of RETeval™, a handheld ERG device which does not require pupillary dilation for assessing the 30-Hz flicker in children under three years of age.

Methods
In this prospective study, 28 children were recruited for assessment with the RETeval™ 30-Hz flicker ERG before the standard ophthalmological and sedated-ERG protocols used for monitoring potential vigabatrin-associated retinal damage. Intra-visit reliability was evaluated from two repeated measurements of the same participant within one testing session using the intraclass correlation coefficient (ICC) statistics. The association between the RETeval™ and the clinical ERG LA 30-Hz flicker responses was determined using omega squared (ω²).

Results
Overall, 32% of participants tolerated the RETeval™ exam and had successful awake recordings. The intra-visit ICCs for the RETeval™ 30-Hz flicker amplitude (µV) were high: 0.81 (OD) and 0.86 (OS), while the implicit times (ms) were 0.79 (OD) and 0.42 (OS). The RETeval™ 30-Hz flicker response showed a positive association with the 30-Hz flicker response on clinical ERG for amplitudes (ω² = 0.71).

Conclusions
This is the first study to assess the utility of RETeval™ device in children under three years of age undergoing vigabatrin treatment. The RETeval™ LA 30-Hz flicker ERG has moderate to poor tolerability. When testing was successful, RETeval™ demonstrated high intra-visit reliability with responses consistent to the standard clinical ERG. RETeval™ may be beneficial for assessment of retinal toxicity in young children treated with vigabatrin.
Background

Infantile Spasms (IS) in West Syndrome is a devastating childhood disorder affecting approximately 2-5 per 10,000 children. (Riikonen & Donner, 1979; Sidenvall & Eeg-Olofsson, 1995; Rantala & Putkonen, 1999). IS presents as recurrent seizures, often leading to cognitive declines and premature mortality (Wong & Trevathan, 2001). An effective anti-epileptic drug in infants ≤ 2 years of age is vigabatrin (SABRIL®, Lundbeck, Deerfield, IL) (Chiron et al., 1997; Jones et al., 2015), an irreversible pharmacological inhibitor of the GABA-transaminase protein in the central nervous system (Schechter, 1989). This drug has been associated with retinal toxicity causing bilateral concentric visual field loss (Eke et al., 1997; Lawden et al., 1999). Visual field loss occurs in about half of adults and one third of children treated with vigabatrin (Maguire et al., 2010). These visual field complications are correlated with other functional and structural deficits including attenuation of the light-adapted (LA) 30-Hz flicker electroretinogram (ERG) response (Harding et al., 2000), and reduction in circumpapillary retinal nerve fibre layer thickness using optical coherence tomography (OCT) (Clayton et al., 2012). Specifically, the cone flicker response is a strong predictor for visual field deficits with a sensitivity of 100% and specificity of 75% (Harding et al., 2000). Children undergoing vigabatrin therapy are monitored for retinal deficits on a regular basis, since vigabatrin is associated with retinal toxicity. Early detection of toxicity could provide more information allowing physicians and parents to make more well-informed decisions regarding the course of treatment.

Currently, reliable paediatric visual-assessment techniques are limited (Good, 2011). Children who are ≤ 3 years of age cannot cooperate effectively with adult-oriented approaches such as the Goldmann perimetry (Wohlrab et al., 1999) and table-mounted OCT (Origlieri et al., 2016). The clinical LA 30-Hz flicker ERG is typically the tool for monitoring vigabatrin-associated effects.
in young children (Westall et al., 2003). Clinical ERG testing follows standards recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV) (McCulloch et al., 2015), which require pupillary dilation and electrode placement in close proximity to the cornea. In our research setting, the clinical ERG protocol is conducted under sedation with the child wrapped in a blanket. Sedation could be time-consuming and a potential unease for parents. The RETeval™ system (LKC Technologies, Gaithersburg, MD, USA) is a non-invasive, handheld ERG system not requiring dilation. The RETeval™ uses skin electrodes and can record LA 30-Hz flicker ERG responses in addition to other standard protocols. It has the ability to accommodate for changes in pupil diameter up to 6.5 mm by constantly altering the flash luminance (cd·s/m²) to stabilize retinal illuminance (Td·s) (Kato et al., 2015). RETeval™ has been used in studies of pediatric patients with nystagmus (Grace et al., 2017), diabetic retinopathy (Al-Otaibi et al., 2017), pre- and post-cataract surgery (Miura et al., 2016), and in healthy participants (Asakawa et al., 2017). This prospective, observational study aims to evaluate the tolerability, intra-visit reliability, and clinical feasibility of the LA 30-Hz flicker ERG response administered by the RETeval™ system in non-sedated, vigabatrin-treated infants.

**Methods**

**Research Ethics Approval**

This study was approved by the Research Ethics Board (REB) at SickKids Hospital and adhered to the guidelines of the tenets of the Declaration of Helsinki for clinical research involving human participants (Please see Appendix for approval letter).
Participant Recruitment

Children with IS undergoing vigabatrin therapy were recruited during their scheduled appointment to monitor for retinal toxicity using the clinical ERG at the Ophthalmology clinic. The nurses and orthoptists were part of the circle of care for the child. A member of this team introduced the research personnel to the parents and/or guardians who were then approached for informed consent for their child to participate in the research study.

Inclusion criteria were children ≤ 3 years of age who had been diagnosed with IS and were about to be treated or undergoing treatment with vigabatrin. Exclusion criteria were other known retinal diseases or medications other than vigabatrin known to affect the retina. Sex was not a determining factor for inclusion in this study.

Clinical Evaluation

Patients whose parents and/or guardians had given written approved consent received a clinical Ophthalmology intake assessment which consisted of identifying the presence or absence of nystagmus or strabismus, and an evaluation of visual acuity using Teller or Cardiff acuity cards.

RETeval™ ERG

The LA 30-Hz flicker ERG protocol using the RETeval™ was conducted by a graduate student trained in the use of the device (Xiang Ji). Children were placed into a comfortable supine position and the skin underneath both eyes was gently scrubbed with Nuprep®. A disposable RETeval™ sensor strips electrode (LKC Technologies Inc., Gaithersburg, MD, USA) was placed over the skin two mm below the lateral half of the lower eyelids of both eyes. The experimenter held the device in such a way that the hand-piece cusped around the eyes of the child (Figure 6).
A built-in automatic sensor tracked the pupils for imaging stabilization, and a built-in camera and display provided live videos allowing the tester to monitor for eye movements and blinking responses. The parents or guardians were present during the test and helped encourage the child to look towards the device; also a small toy and/or sounds were placed close to the device to attract the child’s attention. If fixation ceased or if the child closed his/her eyes the trial was halted and repeated. If it was not possible to get the child’s attention testing ceased. Children had previously been exposed to light during the clinical intake and did not receive additional light adaptation. The test was conducted in each eye using a pre-set protocol (85 Td·s @ 28.3-Hz flicker with 848 Td background). The RETeval™, which housed the flickering light, was placed over one eye and testing always commenced with the right eye followed by the left eye. Overall, two repetitions were attempted on each eye to allow assessment of intra-visit reliability of the RETeval™ response. The device was repositioned following each trial. In the case of non-compliance or if the duration of the procedure was over 5 minutes, testing was discontinued.

**Clinical ERG**

Following the RETeval™ ERG, the child was given mydriatic eye drops of 1% tropicamide (Mydriacyl®; Alcon Laboratories Inc, Texas, USA) and 2.5% phenylephrine (Mydfrin®; Alcon Laboratories Inc, Texas, USA) by the nurses to dilate the pupils. Chloral hydrate was administered orally to achieve a level of sedation that would last approximately 2 hours (weight dependent chloral hydrate dosage: 80 mg/kg up to 1g maximum). The clinical ERG was performed in accordance with ISCEV standards (McCulloch et al., 2015). Following the administration of a topical anaesthetic, corneal electrodes (ERG-JET™, Fabrinal Eye Care, La Chaux-de-Fonds, Switzerland) were positioned over the cornea of each eye, and the clinical ERG
was conducted using Ganzfeld illumination (Espion E2 Color Dome, Diagnosys LLC). The clinical ERG LA 30-Hz flicker amplitude (µV) was used to compare with the RETeval™ 30-Hz flicker ERG response for cross-platform association.

**Neuro-ophthalmologist Evaluation**

Dilated fundus examinations were performed by a neuro-ophthalmologist while the child was under sedation following the clinical ERG. The optic disc was assessed to detect any visual signs (atrophy) of retinal toxicity.

**Data Analysis**

For RETeval™ testing, intra-visit reliability was evaluated from two repeated measurements of the same participant within one testing session using the intraclass correlation coefficient (ICC) statistics (Statistical Package for Social Studies software, IBM, Chicago, IL). The association between the RETeval™ LA 30-Hz flicker ERG and the clinical ERG LA 30-Hz flicker responses was determined using the partial omega squared ($\omega^2$) estimated in PROC GLM (SAS 9.4, Cary, NC); this statistical method accounted for the within subject correlation as data from all eyes were included in the analysis. The partial omega squared represents the proportion of shared variance between the two measures after controlling for the random subject effect.

**Results**

**Tolerability in infants**
Between November 2016 and March 2018, 28 children (N = 28) were recruited; of these 9 (13.6 ± 6.7 months, range 6-27 months) completed the RETeval™ 30-Hz flicker ERG protocol (32%). The demographics data for these children are listed in Table 12. Seven of these nine children (77.8%) completed the intra-visit assessments, with at least one eye having two repetitions of the 30-Hz flicker ERG response. Two of the 9 participants completed one follow-up visit and one was successfully assessed on 3 visits (1 baseline + 2 follow-ups). For the clinical ERG, all 28 children (100%) completed the LA 30-Hz flicker ERG protocol under sedation.

**Intra-visit reliability**

The intra-visit reliability of the RETeval™ 30-Hz flicker ERG response was assessed by the agreement between two repeated measurements within a single visit using the ICC statistics. Seven right eyes from seven participants and five left eyes from five participants provided two repetitions for intra-visit calculations. Because left and right eyes within the same participant are not independent, right and left eyes were analyzed separately and the reliability of ICCs in the right eyes was assessed using data in the left eyes. The intra-visit ICCs for the RETeval™ 30-Hz flicker ERG amplitude (µV) were 0.81 and 0.86 for the right and left eyes, respectively. The intra-visit ICCs for the 30-Hz flicker implicit time (ms) were 0.79 and 0.42 for the right and left eyes, respectively. Representative diagrams (Figure 7a, b) from a 13-month old child show intra-visit agreement of the RETeval™ 30-Hz flicker response. The waveform morphology of the RETeval™ closely resembles the clinical ERG (Figure 7c, d) within a span of 100 ms; the difference is in the response amplitude.

Intra-visit reliability data are shown in Figure 8; the values of the 30-Hz flicker responses on two trials are plotted against each other. Within this specific infant cohort, the flicker response
amplitudes of both the clinical ERG and the RETeval™ ERG were not associated with change in age (Figure 9).

**Clinical Feasibility**

The clinical feasibility of the RETeval™ 30-Hz flicker response was determined through comparisons with the clinical ERG. Nine out of 28 patients (32%) had at least one measurable RETeval™ recording, and altogether 14 eyes (n = 14) (11 from the first-visit and 3 from follow-up visits) contributed to the calculation of cross-platform correlation. The amplitude of the RETeval™ 30-Hz flicker response showed a positive association with the 30-Hz flicker response on clinical ERG ($\omega^2 = 0.71$, Figure 10a). No correlation was found between the implicit times of the RETeval™ and the clinical flicker ERGs ($\omega^2 = 0$, Figure 10b).

Longitudinal testing on the RETeval™ was possible in 2/4 children (n = 3 eyes) who returned for the follow-up clinical ERG assessment. In particular, one child (ID316) had the left and right eyes tested on the RETeval™ at the first and follow-up visits. In this participant, the RETeval™ 30-Hz flicker amplitude at visit 2 increased in both eyes compared to baseline, with the left eye showing a larger increase than the right eye (2% OD, 16% OS) (Figure 11a). The clinical ERG in patient 316 showed an 8% response reduction in the left eye and 56% increase in the amplitude in the right eye compared with baseline (Figure 11b). The second participant (ID313) had only the right eye tested with the RETeval™ at the first and two follow-up visits (Figure 11a). During this patient 316’s second visit, the RETeval™ 30-Hz flicker amplitude decreased from baseline by 11%, compared with a 16% decrease for the clinical ERG. However, during the third visit, the RETeval™ 30-Hz flicker amplitude increased from baseline by 27% (Figure 11a) while the clinical ERG decreased from baseline by 40% (Figure 11b).
Discussion

The aim of the present study was to investigate the tolerability, reliability, and to some degree, clinical feasibility of the RETeval™ ERG for recording the 30-Hz flicker response in non-sedated infants undergoing vigabatrin therapy.

Tolerability

Tolerability, as measured by the number of participants who were able to complete the RETeval™ 30-Hz flicker ERG protocol, was low to moderate. Testing may have been unsuccessful because these children had been fasting for their sedated exams and often showed irritability and fatigue. Children were sometimes asleep upon arriving at the Ophthalmology clinic and were woken for the RETeval™ ERG protocol which could not proceed without constant pupil detection (at least 10 seconds). Moreover, to ensure the proper flow of clinical procedure, the research team was often given time constraints, which resulted in early forfeiting of the handheld ERG evaluation in some children. We believe that the success rate would see an improvement if these children were tested without the requirement for sedation.

Reliability

The intra-visit reliability established in the present study for the RETeval™ 30-Hz flicker protocol was moderate and comparable to previous reports using the RETeval™ device. Intra-visit reliability for the RETeval™ flicker ERG is strong in healthy adults between 20-24 years old (ICC=0.92 for amplitude and 0.91 for implicit time) (Asakawa et al., 2017). Concurrently, we collected RETeval™ data on a larger cohort between 11 months – 69 years (N=92, median age =20), and found an ICC of 0.82 for amplitudes and 0.53 for implicit time using the
RETeval™ 30-Hz flicker response (Liu et al., 2018). In the present infant cohort, the mean age was 13.6 months. The mild reduction in our reliability assessment as compared to other studies may be attributed to experimenter and patient dependent factors, including increased body movement of the infants during recording, poor contact or adhesiveness of the skin electrodes, and excessive shifting of the eyes causing loss of pupil detection. The waveform morphology of the RETeval™ flicker response is repeatable and resembles the waveforms of the clinical ERG. The reduced response magnitude in comparison to the clinical ERG is attributed to the skin electrode placement as compared with corneal electrodes used in the clinic.

**Clinical efficacy**

Our study exhibited a strong positive correlation between the RETeval™ and the clinical ERG 30-Hz flicker amplitudes. This strong correlation suggests that the RETeval™ may be useful for retinal assessment when the clinical ERG cannot be easily employed such as when the child does not cooperate, in children with medical contraindications to sedation or when sedation is unavailable.

Further evidence of clinical efficacy of the RETeval™ 30-Hz flicker response comes from the its reduction in adults with known retinal diseases causing cone dysfunctions (Nakamura et al., 2016). The RETeval™ flicker amplitude is significantly reduced in adult patients with known retinal diseases causing cone dysfunctions (Nakamura et al., 2016). Likewise, in children with nystagmus and retinal dystrophy (5.6±2.7 years), the RETeval™ 30-Hz flicker response amplitude is reduced compared with controls with nystagmus and no retinal dystrophy (Grace et al., 2017). Nevertheless, reports of cone-dominant ERG changes in infants using the RETeval™ system are limited. To the best of our knowledge, the present study is the first that employs the RETeval™ 30-Hz flicker response to evaluate potential retinal defects in infants < 3 years of age.
undergoing vigabatrin therapy for IS. Longitudinal testing in this study did not identify any participants with vigabatrin associated retinal toxicity on the clinical ERG. Vigabatrin associated retinal toxicity has been previously defined by our lab as a > 40% reduction in amplitude in the LA flicker response from baseline on two consecutive follow-up visits (Westall et al., 2014). This definition was used to avoid the misclassification of toxicity after a single test session when reduction in amplitude may have been artefactual. As a result, comparisons of diagnostic accuracy could not be made between the clinical ERG and the RETeval™ flicker responses in our cohort of patients. For the child with three longitudinal assessments (ID313), the progressive amplitude reduction seen across three visits on the clinical ERG was consistent with the RETeval™ ERG at visit 2 but not at visit 3; RETeval™ demonstrated a 27% increase in flicker amplitude from baseline whereas the clinical sedated ERG showed a 40% reduction in amplitude. This discrepancy at visit 3 is likely related to ocular artefacts during sedated-ERG whereby the well-known Bell’s phenomenon, which sometimes occurs under sedation, may have resulted in the upward shift of the eye and subsequent artefactual reduction in ERG amplitude.

**Limitations**

There are several limitations for this study. Firstly, given the issues of non-compliance, the sample size was small, which may restrict the generalizability and the power of our results. The age-expected normal range for the RETeval™ LA 30-Hz flicker amplitude is currently unknown for children younger than 3 years of age. Non-compliance, especially following fasting periods for the clinical ERG, substantially reduced the tolerability of the RETeval™ flicker in infants. Furthermore, the skin electrodes used by the RETeval™ system were originally designed for older children and adults and were sometimes difficult to be positioned at appropriate markers on
the infant’s face. As a result of the electrode size issue, testing was sometimes forfeited due to excessive signal noise, or failed because the electrodes fell off from the skin underneath the lower eyelids.

**Conclusion**

This study was the first to demonstrate the effectiveness of the RETeval™ LA 30-Hz flicker protocol in infants undergoing vigabatrin treatment. Within this very young cohort of children, the RETeval™ system demonstrated moderate to poor tolerability, however when testing was successful, it showed adequate intra-visit reliability. The RETeval™ 30-Hz flicker response was comparable to the clinical ERG in that there was a high correlation between the flicker ERG amplitudes and a resemblance of the waveform morphology. The American Association for Pediatric Ophthalmology and Strabismus recommends against sedated-ERG testing every 3-6 months as a means of monitoring for vigabatrin toxicity in young children, as serial ERG assessments can be “inconvenient, costly and potentially dangerous” (American Association for Pediatric Ophthalmology and Strabismus, Policy Statements 2017). RETeval™ is a quick and sedation-free technique, therefore it may prove to be a viable option for some children whose parents or physicians require more information on possible toxicity than might be provided by the fundus examination alone.

**Literature Search**

We conducted a comprehensive and systematic review of the literature using OVID which included databases Embase Classic and Embase (1947-2018 Week 26). Inclusion criteria were
studies with the keywords RETeval OR handheld ERG OR handheld electroretinography OR handheld electroretinogram OR mydriasis-free ERG OR mydriasis-free (63 publications) AND Infantile Spasms (also including subject heading Infantile Spasm; 6890 publications). There were no studies to date that met the inclusion criteria.

Table and Figures

Table 12. Demographics information and clinical ERG interpretations for 9 children tested with the RETeval 30-Hz flicker ERG.

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex (M/F)</th>
<th>Age at Testing (months)</th>
<th>Etiology</th>
<th>Other Medications</th>
<th>Current Daily Dose at Testing (body weight)</th>
<th>Clinical LA 30 Hz Flicker ERG Findings</th>
</tr>
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<tbody>
<tr>
<td>313</td>
<td>F</td>
<td>6 (1\textsuperscript{st} Visit) 10 (2\textsuperscript{nd} visit) 14 (3\textsuperscript{rd} visit)</td>
<td>Unknown</td>
<td>None</td>
<td>1000 mg (9.9 kg at visit 3)</td>
<td>All visits WNL Third visit amplitude reduced by 40% from first visit</td>
</tr>
<tr>
<td>314</td>
<td>M</td>
<td>13</td>
<td>Neonatal Hypoxic-ischemic Encephalopathy</td>
<td>Keppra Vitamin D</td>
<td>900 mg (7kg)</td>
<td>WNL</td>
</tr>
<tr>
<td>315</td>
<td>M</td>
<td>11</td>
<td>Unknown</td>
<td>Phenobarbitol</td>
<td>Discontinued</td>
<td>WNL</td>
</tr>
<tr>
<td>316</td>
<td>F</td>
<td>27 (1\textsuperscript{st} Visit) 34 (2\textsuperscript{nd} visit)</td>
<td>TSC</td>
<td>1. Oxcarbazepine 2. Keppra</td>
<td>1500</td>
<td>First visit reduced from normal range for OD Second visit WNL</td>
</tr>
<tr>
<td>317</td>
<td>F</td>
<td>12 (1\textsuperscript{st} Visit) 16 (2\textsuperscript{nd} visit)</td>
<td>Intraparenchymal Hemorrhage with Ventricular Extension</td>
<td>1. Vitamin D 2. Phenobarbital</td>
<td>Discontinued after first visit 1200 mg at visit 1 (9.2 kg at visit 1)</td>
<td>All visits WNL</td>
</tr>
<tr>
<td>324</td>
<td>F</td>
<td>8 (1\textsuperscript{st} Visit)</td>
<td>Periventricular Leukomalacia</td>
<td>Pyridoxine</td>
<td>1800 mg (12 kg at visit 2)</td>
<td>All visits WNL</td>
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<tr>
<td>325</td>
<td>M</td>
<td>7</td>
<td>Unknown</td>
<td>None</td>
<td>1000 mg (8.3 kg)</td>
<td>WNL</td>
</tr>
<tr>
<td>341</td>
<td>M</td>
<td>22</td>
<td>Right Posterior Cerebral Infarction</td>
<td>1. Keppra 2. Clobazam</td>
<td>1500 mg (10.8 kg)</td>
<td>WNL</td>
</tr>
<tr>
<td>344</td>
<td>F</td>
<td>16</td>
<td>Unknown</td>
<td>1. ACTH 2. Keppra</td>
<td>1400 mg (12.4 kg)</td>
<td>WNL</td>
</tr>
</tbody>
</table>

* N/A = not applicable; these children were not tested on the RETeval 30-Hz flicker ERG at a follow-up visit. WNL is within normal limits; refers to the clinical 30-Hz flicker ERG response as compared with the age-expected normative range. First visit (baseline) is the first time the RETeval 30 Hz flicker ERG was performed. OD and OS are right and left eyes, respectively.
Figure 6. RETeval™ handheld ERG being operated on an infant under sedation. Real-time pupil tracking is shown on the screen of the device during testing. The LA 30-Hz flicker ERG waveforms are displayed on screen immediately following test completion. Repeated trials are shown as superimposed traces to provide instant appraisal of waveform reproducibility.
Figure 7. RETeval™ 30-Hz flicker ERG response in a 13-month child (ID 314) with Infantile Spasms (A = OD, B = OS). Clinical ERG LA 30-Hz flicker response (C = OD, D = OS) of the same participant (ID 314) is measured on the date of RETeval™ testing. Two same-session repeated trials are superimposed to demonstrate intra-visit reliability.
Figure 8. RETeval™ 30-Hz flicker ERG response amplitude (A, C) and implicit time (B, D) measured from 12 eyes of 7 study participants. Consistency between the first and second trial amplitudes and implicit times are compared to the line of perfect agreement. A, B = OD; C, D = OS.
Figure 9. Correlations for (A) RETeval™ 30-Hz flicker amplitude and the (B) clinical ERG 30-Hz flicker amplitude with age at testing in 9 children with successful RETeval™ ERG recording. No age-dependent correlations were found for either system.
Figure 10. Correlations between the RETeval™ 30-Hz flicker amplitude and the clinical ERG 30-Hz flicker response in terms of amplitude (A) and implicit time (B).
Figure 11. Longitudinal testing in two children with the RETeval™ LA 30-Hz flicker (A) and clinical LA 30-Hz flicker (B) protocols.
Format of thesis

Chapters I and II are independent studies using each of the outcome variables, Chapter I: RETeval ERG, and Chapter II: Envisu OCT.

Chapter II: The Clinical Feasibility of Noninvasive Portable OCT Technique in Children < 3 years Undergoing Vigabatrin Treatment
Abstract

Purpose
Vigabatrin (VGB) is an effective drug for infantile spasms; however, it has been associated with retinal toxicity which may manifest as retinal nerve fiber layer (RNFL) thinning measured from optical coherence tomography (OCT). Children who are ≤ 3 years of age cannot fully comply with adult-oriented procedures such as table-top OCT which require stable upright postures for a prolonged period of time. We propose that Envisu, a handheld (HH) OCT, would be a suitable monitoring technique in infants given its time efficiency and its flexibility. In this prospective observational study, we aim to assess the tolerability, intra- and inter-visit reliabilities, and to some degree clinical efficacy of the Envisu OCT in evaluating peripapillary RNFL thickness in children undergoing vigabatrin treatment.

Methods
Twenty-nine children (10.1 ± 6.0 months old, range 4-27 months) with infantile spasms undergoing vigabatrin therapy were recruited. Seventeen children from this group completed follow-up assessments. We performed HH-OCT in sedated children lying supine and the RNFL thickness was evaluated with segmentation software. Eighteen optic disc scans (n=18) underwent segmentation analysis by two independent graders who were masked from all participant identifiers. The intra-visit and inter-visit reliabilities were calculated for RNFL thickness measurements from the handheld OCT using intraclass correlation coefficient (ICC) statistics. For the follow-up group, each child’s initial baseline was used as control for subsequent comparisons.

Results
The HH-OCT was successfully tolerated in all children. Amongst the four quadrants of both eyes, superior and temporal were consistently the RNFL quadrants with the highest and lowest thickness respectively. The superior and the inferior quadrants RNFL measurements compared between two independent raters showed high inter-rater ICC values of 0.89 and 0.82, respectively. High intra-visit reliability was shown with average ICC of 0.90 (range 0.82-0.98) for OD and 0.83 (range 0.75-0.89) for OS, across 4 retinal divisions. Inter-visit ICCs for the superior, nasal, inferior and temporal quadrants were 0.90, 0.82, 0.86, and 0.98, respectively. During longitudinal assessment, differences in RNFL thickness between baseline and the first follow-up visit were not statistically significant.

Conclusions
In this cohort, RNFL thickness measures using the HH-OCT provided reasonable reliability within a single visit, between consecutive visits, and between independent examiners of RNFL segmentation supporting its use as an adjunctive tool in the clinical setting. Further long-term follow-up is required to better understand RNFL thickness declines in this specific population and its association with vigabatrin toxicity.
### Background

Infantile Spasms (IS) or West Syndrome is first described by William James West in 1841 (West, 1841), and is a childhood seizure disorder affecting 2-5 per 10,000 children (Riikonen & Donner, 1979; Sidenvall & Eeg-Olofsson, 1995; Rantala & Putkonen, 1999). Patients with IS frequently exhibit characteristic electroencephalography (EEG) waveforms called hypsarrhythmia (Gibbs & Gibbs, 1952; Gibbs et al., 1954; Wong & Trevathan, 2001; Pavone et al., 2014), which are accompanied clinically by bilateral flexor and extensor contractions of the limbs, trunk and neck (Kellaway et al., 1979; Fusco & Vigevano, 1993). The prognosis for IS in children is considered poor given this condition often manifests as recurrent seizures, mental retardation, cognitive delays and premature mortality (Jeavons et al., 1973; Matsumoto et al., 1981; Wong & Trevathan, 2001; Partikian & Mitchell, 2010). Vigabatrin (γ-vinyl GABA; SABRIL®, Lundbeck, Deerfield, IL) is an effective anti-epileptic drug used as first-line monotherapy in children ≤ 2 years of age with IS (Jones et al., 2015). It is a competitive and irreversible pharmacological inhibitor of the GABA-transaminase enzyme, which functions by increasing GABA (γ-aminobutyric acid) concentrations in the central nervous system (Lippert et al., 1977; Jung et al., 1977). Unfortunately, vigabatrin has been associated with retinal toxicity causing bilateral concentric visual field loss in as many as one third of the children treated (Maguire et al., 2010; Riikonen et al., 2014). The prevalence of vigabatrin-associated retinal toxicity increases with cumulative and daily dose (Lawden et al., 1999; Wild et al., 2007), treatment duration (Miller et al., 1999; Hardus et al., 2001), older age and male gender (Wild et al., 1999). These visual field complications are correlated with structural and functional biomarkers, such as attenuation of the peri-papillary retinal nerve fiber layer (RNFL) thickness (Buncic et al., 2004) measured from optical coherence tomography (OCT) (Lawthom et al.,
2009; Clayton et al., 2010; Moseng et al., 2011; Clayton et al., 2012), and reduction of the light-adapted (LA) 30 Hz flicker response measured from standard electroretinography (ERG) (Harding et al., 2000; Westall et al., 2003; McCoy et al., 2011; Westall et al., 2014). It is important for children undergoing vigabatrin therapy to be continually assessed for retinal defects, as early detection of toxicity may allow physicians and guardians to reach a more informed decision regarding the course of treatment. At present, reliable visual-monitoring techniques for infants undergoing vigabatrin treatment are limited (Good, 2011), as children who are ≤ 3 years of age cannot fully comply with adult-oriented procedures such as the Goldmann visual field perimetry (Wohlrab et al., 1999; Gaily et al., 2009) and table-top OCT (Muni & Lee., 2009; Gerth et al., 2009; Rootman et al., 2012; Origlieri et al., 2016) which require stable upright postures for a prolonged period of time. Wohlrab and colleagues demonstrated a very poor tolerability for visual field testing in 153 children with partial epilepsy or IS, as only 8% (12/153) of those tested were able to complete the procedure. Given this issue, infants treated with vigabatrin for IS during the first year of life could only be assessed for visual toxicity on perimetry during school-age (> 5 years of age) (Gaily et al., 2009; Wohlrab et al., 2009; Riikonen et al., 2014). Wright et al., 2017 showed a positive association between RNFL thickness declines from the table-top spectral-domain OCT (SDOCT) and 30 Hz LA flicker response reductions from the clinical ERG. However, the OCT was performed during adolescence after many years following their ERG assessments. Therefore, the use of SDOCT for optic disc assessments that is concurrent with ERG functional evaluations in infants remains limited.

Envisu (Leica Microsystems, NC, USA) is a novel handheld SDOCT system with a maneuverable eye-piece which acquires volumetric scans of the optic disc while allowing young
children who cannot sit upright to be resting in a supine position (Hahn et al., 2011). Many studies have shown the effectiveness of this device to serve as a structural biomarker for various retinal conditions (Chong et al., 2009; Gerth et al., 2009; Chavala et al., 2009; Avery et al., 2014). Notably, Avery and colleagues have demonstrated its remarkable intra- and inter-visit reliabilities in evaluating RNFL thickness in patients with optic pathway glioma and neurofibromatosis type I. Nevertheless, the median age of participants in their study was 5.1 years (range 0.8 – 13 years), and the results could not be generalized to the infant cohort. We propose that Envisu OCT would be a suitable monitoring technique in infants given its time efficiency requiring only 10 minutes of sedation, and its flexibility allowing the scan to be taken at multiple angles. In this prospective observational study, we assessed the tolerability, intra- and inter-visit reliabilities, and to some degree the clinical efficacy of the Envisu OCT in evaluating peripapillary RNFL thickness in children undergoing vigabatrin treatment.

**Methods**

**Research Ethics**

The Research Ethics Board (REB) at the Hospital for Sick Children (Sickkids) has given approval for the present study while following the tenets of the Declaration of Helsinki for clinical research (Please see Appendix for approval letter).

**Recruitment**

*Inclusion criteria*

Children ≤ 3 years of age who had been diagnosed with IS by the Neurology department at SickKids Hospital. These children were undergoing or expected to begin vigabatrin treatment
either as a monotherapy or as an add-on treatment alongside other medication. Sedation for the clinical ERG (standard of care to monitor for vigabatrin-associated retinal toxicity) occurs prior to assessment with the handheld OCT.

Exclusion criteria

This study excluded children who had other known retinal conditions or had been taking medications other than vigabatrin known to affect the retina. Any child who developed vigabatrin-related retinal defects within one year of treatment were removed from between-visit reliability assessments. Sex was not a determining factor for inclusion in this study.

Intake Evaluation

Patients whose parents and/or guardians provided written approval were first tested for visual acuity (Teller and/or Cardiff acuity cards), and identification for the presence of strabismus or nystagmus as part of their pre-sedation procedure by an orthoptist. Following this clinical routine, the pupils of the children were dilated with mydriatic eye drops of 1% tropicamide (Mydriacyl®; Alcon Laboratories Inc, Texas, USA) and 2.5% phenylephrine (Mydfrin®; Alcon Laboratories Inc, Texas, USA).

Gold-Standard Clinical ERG

A topical anesthetic was orally administered to achieve a deep level of sedation for approximately 2 hours, with the dosage determined in a weight-dependent manner (chloral hydrate given according to body weight at 80 mg/kg up to 1g maximum). The sedated 6-step photopic and scotopic ERG was then performed for 30 minutes by an orthoptist from the
Sickkids Visual Electrophysiology Unit (VEU) according to the standards of The International Society for Clinical Electrophysiology of Vision (ISCEV). Corneal electrodes (ERG-JET™, Fabrinal Eye Care, La Chaux-de-Fonds, Switzerland) were placed in front of the cornea and the ERG light stimuli were delivered within a Ganzfeld (Espion E2 Color Dome, Diagnosys LLC). The photopic 30 Hz flicker response (amplitude in microvolts) was the only ERG parameter reported for this study, as it is an effective biomarker for vigabatrin-associated retinal toxicity (Harding et al., 2000).

**Neuro-ophthalmologist Evaluation**

Dilated fundus examinations were performed by a neuro-ophthalmologist while the child was under sedation. The optic disc was assessed to detect any visual signs (atrophy) of retinal toxicity due to vigabatrin treatment, and refractive error was assessed by an optometrist.

**Handheld Envisu OCT Assessment**

While the child was under sedation, handheld SDOCT was performed by an imaging specialist using the Envisu system. This system acquires 36,000 A-scans every second at a 3.5-micron tissue resolution and a 2.45 mm scan depth. The image acquisition process was similar to previous published studies (Scott et al., 2009; Avery et al., 2014). The adjustable eye-piece (camera probe) that was attached via a 1.3-meter fiber-optic cable allowed the imaging technicians to work with maximum flexibility, such that retinal scans were taken at multiple angles while the child was lying supine (Figure 12). Differences in axial length affected the working distance between the OCT camera and the cornea, and this was accounted for by adjustments to the reference arm length prior to testing. Refractive error as reported by an
optometrist was compensated for by altering the focus on the eye-piece. The technician held the eyelids open with the index finger to reveal the pupil while positioning the camera over the eye. The optic nerve head (ONH) scan was initiated by a foot pedal, and two repeated measurements for both right (OD) and left (OS) eyes could be completed in less than 3 minutes. In our study, we made a slight adjustment to increase the physical size of the volumetric scan (up to 12mm x 12mm at a depth of 2.45mm) to ensure maximum coverage around the ONH. Each scan with an area of 12mm x 12mm centered on the optic disc was obtained in a few seconds using the non-isotropic 1000 A-scans x 100 B-scans sampling method. The real-time horizontal and vertical B-scans of the optic disc displayed on the on-board computer software (InVivoVue 2.4 OCT Management Software) allowed an instant appraisal of image quality, which could be enhanced by the imaging specialist through repeated trials. Following imaging completion, horizontal B-scans along with an en-face fundus-like image allowed the technicians to examine the scans qualitatively for motion artifacts and signal noises.

**OCT Segmentation**

The raw files (.OCT) were exported from the InVivoVue 2.4 software for RNFL thickness analysis. Custom-built software, OCT Explorer Iowa Reference Algorithms (Retinal Image Analysis Lab, Iowa Institute for Biomedical Imaging, Iowa City) was used for segmentation to determine the circumpapillary RNFL thickness in the superior, nasal, inferior and temporal quadrants around the optic disc. In cases of subpar image quality, the software’s automatic segmentation mechanism was insufficient, and manual tracing was done with guidance from experienced neuro-ophthalmologists. A 3.4mm diameter circle containing 256 A-scans was
placed by the software on top of the geometric center of the ONH (centered around the ONH) and each of the 4 retinal quadrants had an equal 64 A-scans that were processed.

**Inter-rater Consistency**

Optic disc scans with a lower signal to noise ratio required extensive manual adjustments using the OCT Explorer segmentation software. To assess inter-rater consistency, RNFL segmentation analysis was compared with another independent grader who was masked from all participant identifiers including demographic information, dose, and baseline or follow-up status (visit number).

**Statistical Analysis**

Intra-visit reliability was calculated from two repeated optic disc scans of the same eye of one participant within a single testing session. The inter-visit reliability was assessed from two scans across consecutive study visits of the same participant. The intra-visit and inter-visit reliabilities were calculated for RNFL thickness measurements from the handheld OCT using intraclass correlation coefficient (ICC) statistics with the Statistical Package for Social Studies software (SPSS, IBM, Chicago, IL). Inter-rater ICC was calculated to assess consistency between two independent graders performing RNFL segmentations using the OCT Explorer software. The association between peripapillary RNFL thickness from Envisu OCT and 30 Hz LA flicker response from clinical ERG was determined from descriptive statistics with $R^2$. Paired-sample t-test was used to compare the mean RNFL values across two longitudinal visits.


**Longitudinal Assessments**

Intra-visit reliability of the Envisu OCT device to evaluate peripapillary RNFL thickness was assessed during the child’s first handheld OCT session from two repeated images of the same eye. The first handheld OCT sessions were not always consistent with the first clinical ERG visits (baseline testing), as recruitment often occurred during one of their required follow-up clinical visits. Inter-visit reliability and longitudinal changes were assessed across two OCT scans of the same eye; one from the first visit, and another from the follow-up visit in 3-4 months. An age-matched control cohort was not established, instead, RNFL thickness from the first visit served as each child’s baseline measurements.

**Results**

**Tolerability**

Twenty-nine children (10.1 ± 6.0 months old, range 4-27 months) with Infantile Spasms undergoing vigabatrin therapy were recruited during their scheduled clinical ERG appointment at the SickKids Ophthalmology clinic. The handheld OCT was subsequently tested on all children given full device availability. The handheld OCT was available for testing 29 participant visits. Complete OCT imaging, in one or both eyes, was possible in all 29 participants. Fourteen children (14.7 ± 5.8 months old at second visit, range 9-34 months) completed one follow-up session with the handheld OCT. Three children (15.7 ± 1.2 months old at third visit, range 14-17 months) completed three follow-up OCT visits. Imaging was successful in all children in at least one eye (n=3). Demographics information for all participants is listed in Table 13.
RNFL Thickness across Quadrants

The mean peripapillary RNFL thickness across the four 90-degree retinal quadrants (superior, nasal, inferior and temporal) was calculated for both right and left eyes (Table 14). The mean RNFL thickness values across all quadrants for each eye were nearly identical; 82.78 µm for OD and 81.57 µm for OS. Amongst the four 90-degree quadrants of both eyes, superior and temporal were consistently the RNFL quadrants with the highest and lowest thickness respectively (Figure 13, Table 14).

Intra-visit Reliability

Twenty-nine children imaged with the handheld OCT contributed 28 right eyes (n=28) and 25 left eyes (n=25) for intra-visit reliability calculation. The average intra-visit ICC for peripapillary RNFL thickness in OD was 0.90 (range 0.82-0.98), with the superior and temporal quadrants having the highest values. The average intra-visit ICC for OS was 0.83 (range 0.75-0.89) with the superior quadrant having the lowest value. The ICCs for all four 90-degree retinal quadrants are listed in Table 14. Intra-visit reliability expressed as plots of second trial against first trial RNFL thickness is shown in Figure 14.

Inter-visit Reliability

Fourteen children (n=14) who have successfully completed one follow-up visit on the handheld OCT contributed one eye for inter-visit reliability analysis. Ten of the 14 eyes tested were from right eye, and the remaining 4 eyes were left eye. Inter-visit ICCs for the superior, nasal, inferior and temporal quadrants were 0.90, 0.82, 0.86, and 0.98, respectively. Inter-visit reliability for all 4 retinal quadrants are expressed as plots of second trial against first trial RNFL thickness in Figure 15.
Inter-rater Reliability

Eighteen optic disc scans (n=18) underwent segmentation analysis by two independent graders. Specifically, the superior and the inferior quadrants RNFL measurements were compared and showed high inter-rater ICC values of 0.89 and 0.82, respectively. The inter-rater reliability for both quadrants is shown in Figure 16.

Longitudinal Assessment

Fourteen children (n=14) completed one follow-up visit with handheld OCT testing at a mean interval of 5.7 months (range 3-10 months) following the baseline visit. The difference was not significant between the mean RNFL thickness values of visit 2 and baseline for all quadrants (Figure 17). Within the superior quadrant (Figure 18), 5/14 children showed increase in RNFL thickness from baseline (range 1.1%-11.1%), whereas the remaining group showed either no change (1/14) or declines (8/14) from baseline (range 1.7%-17.5%). Specifically, four of these children showed reductions of greater than 10% (Table 15, Figure 18). The nasal quadrant also had five children showing increases from baseline (range 1.3%-13.5%), one child showing no change, and 8/14 children showing declines (range 1.1%-20.8%). Three of these children had reductions of >10% (Table 15, Figure 18). The inferior and temporal quadrants demonstrated less individual changes in RNFL thickness from baseline as only one child from each group showed reductions of >10%.

Three children (n=3) completed three consecutive visits on the handheld OCT every 3-5 months. The first child (ID302) showed large RNFL thickness reductions across the superior, nasal and inferior quadrants in visits 2 and 3 in comparison to baseline (Figure 19A). In particular, during visit 3, the nasal quadrant RNFL thickness reduced by 35% from baseline, followed by the
superior quadrant at 27%, and the inferior quadrant by 14%. The temporal quadrant was relatively spared across the three visits, showing only a 4% reduction compared to baseline. The second child (ID327) showed RNFL deficits in the nasal and inferior quadrants, which reduced by 12% and 16% from baseline respectively at visit 3 (Figure 19B). The superior and the temporal quadrants remained relatively unchanged. The third child (ID 332) was unaffected across all quadrants except for the temporal-specific 16.2% decline observed in visit 2 (Figure 19c). However, the thickness value in the temporal quadrant at visit 3 increased by 39% from visit 2, and by 16.5% from visit 1. Overall, the temporal quadrant was consistently spared in all children during the third visit. Representative single B-scans within the superior optic disc across 3 consecutive visits for participant ID302 are shown in Figure 20.

**Comparisons with Clinical ERG**

There were no associations between peripapillary RNFL thickness and LA 30 Hz flicker ERG amplitudes for all 4 retinal quadrants of both eyes. At follow-up, three children (n=3) showed notable declines in the 30 Hz flicker response on the gold-standard clinical ERG with respect to the first visit (Table 13), although the flicker ERG amplitude was within normal range. The first child (ID312) at visit 2 showed a 23.7% reduction on the right eye flicker ERG response and a 12.4% RNFL thickness attenuation in the nasal quadrant. The second child (ID313) had a 40.2% reduction in flicker response of the right eye, and this was accompanied by a 6.1% decline in RNFL thickness in the superior quadrant. The right eye flicker ERG response was reduced by 26.2% in the third child (ID324), and the superior-specific RNFL thickness declined by 4.5%.
Discussion

Tolerability

To the best of our knowledge, this was the first study to assess the reliability and longitudinal changes of handheld OCT in young children (< 3 years old) undergoing vigabatrin therapy. Previous studies incorporating the OCT to investigate vigabatrin-associated retinal toxicity were mostly restricted to adults and older children who could tolerate table-mounted imaging protocols. Studies of younger children on vigabatrin have involved electrophysiological testing using the LA 30 Hz flicker ERG, table-top OCT, and visual fields testing after drug discontinuation during adolescence (Westall et al., 2003; Westall et al., 2014; Wright et al., 2017). RNFL thickness attenuation is associated with vigabatrin toxicity, therefore reliable retinal monitoring should occur regularly. Given issues of testing compliance, the use of SDOCT for optic disc assessment in infants are currently limited. In this study, we demonstrated that the handheld SDOCT was well tolerated in infants under sedation, as all 29 children who were imaged on the system during their first visit were able to complete the protocol with at least one eye. This was consistent for all 14 children during their second follow-up visit and for all 3 children during the third follow-up visit. The high tolerability may be attributed to the OCT device’s flexibility allowing infants to be imaged while supine, its rapid scanning speed, and its high tissue resolution (Gabriele et al., 2008; Hahn et al., 2011).

In our cohort of infants receiving vigabatrin treatment (aged 1-27 months), the handheld OCT was a more appropriate monitoring technique to evaluate circumpapillary RNFL thickness under sedation, as these children were too young to sit upright and cooperate with table-mounted OCT or Goldmann perimetry. It was to note that all participants in our study underwent handheld OCT assessment under sedation due to the standard protocol at SickKids Hospital requiring sedation.
for clinical ERG. The use of sedation by our group was necessary to maintain tolerability, and this was consistent with earlier studies documenting the clinical feasibility of handheld OCT to distinguish between healthy and diseased retinal morphologies (Scott et al., 2009; Gerth et al., 2009; Chong et al., 2009).

**Mean RNFL Thickness**

In the present study, the mean RNFL thickness values from our infant cohort (aged 1-27 months) during the first visit in the superior and inferior quadrants are higher than the nasal and temporal quadrants. No differences were seen between both eyes. The normative range of peripapillary RNFL thickness in healthy children < 3 years of age has not been studied; therefore, there are no reference values in this population. In comparison to studies of RNFL using Cirrus (table-top) SDOCT in healthy children aged 4 to 13 years (Elia et al., 2012; Barrio-Barrio et al., 2013; Al-Haddad et al., 2014), the mean RNFL thickness is reduced in our study population in the superior and inferior quadrants, while the temporal and nasal quadrants are consistent. It is to note that all of our mean values fall within the 5\textsuperscript{th} and 95\textsuperscript{th} percentiles of normal RNFL thickness (age 4-9 years) except for the inferior quadrant, which is slightly lower than the 5\textsuperscript{th} percentile. This difference could be due to the young age of our participants, as some may show slower or abnormal development in the retina or be attributed to vigabatrin-associated retinal toxicity targeting the superior and inferior quadrants. In addition, normative RNFL data established from table-top OCT may not be representative of the true baseline values used as reference for the handheld OCT.
Reliability

In a previous study, conventional table-top SDOCT has shown good reliability (ICCs > 90%) in evaluating the peripapillary RNFL thickness across different retinal quadrants (Clayton et al., 2011). Due to aforementioned concerns regarding testing compliance, the same reliability was not demonstrated in a pediatric population. In the present study, we reported that the peripapillary RNFL thickness measured from handheld OCT in all four quadrants provided good reliability (ICCs 0.75-0.98) in infants under sedation. This was the case for the intra-visit testing (n=29) as well as the inter-visit testing (n=14). Good reliability from both study groups suggests that RNFL assessments with handheld OCT are reliable within a single session or between consecutive sessions in infants (1-34 months) undergoing vigabatrin treatment. It is to note that ICCs calculated from this study may not be applicable to infants < 3 years of age with other retinal conditions affecting the RNFL, as ICCs are population-specific, and the mechanisms of RNFL attenuations are not always universal (Avery et al., 2014).

Our intra-visit and inter-visit ICCs are comparable to the values published by Avery and colleagues in children (mean age 5.1 years, range 0.8 to 13 years) with optic pathway glioma or Neurofibromatosis Type 1 who had normal vision. ICCs from both studies were based on non-isotropic volume (1000 A scans x 100 B scans) of RNFL thickness analysis. For the intra-visit and inter-visit cohorts, the ICCs in our study are slightly reduced across all quadrants in comparison to the aforementioned study. The slight reduction in reliability measures could have resulted from differences in the mean age of participants between the two studies, as our study focused on a much younger cohort (mean age 0.84 years compared to 5.1 years). Our infant cohort is more difficult to assess using the handheld OCT given physical restrictions of their smaller head and eye dimensions which may limit available working areas accessible by the eye-
piece. This could create additional motion artifacts affecting the signal to noise ratio and overall quality of the optic disc scan.

The inter-visit ICCs did not show notable changes from intra-visit ICCs across all retinal quadrants, suggesting that the overall RNFL thickness was relatively preserved in 14 children who have completed follow-up assessments. However, longitudinal attenuations within individuals may still be observed.

Segmentation analysis of the RNFL thickness occasionally required manual tracing due to poor image quality. Eighteen optic disc scans were compared between two independent graders in two quadrants (superior and inferior), and good reliability was demonstrated through inter-grader ICCs. This consistency suggests that RNFL thickness measured from handheld OCT are reliable even when manual tracing was required.

**Longitudinal Assessment**

The ability of the handheld OCT to assess longitudinal changes in the peripapillary RNFL thickness would be beneficial for infants taking vigabatrin for IS, as scheduled monitoring may detect structural biomarkers of retinal toxicity which could then affect the course of treatment. RNFL attenuations are implicated in vigabatrin toxicity based on assessments using the table-top OCT from both adults and young children. Significant reductions in RNFL thickness have been observed when comparing vigabatrin-treated individuals to aged-matched vigabatrin-naïve and/or healthy counterparts (Wild et al., 2006; Moseng et al., 2011). In the present study, an age-matched normative range for healthy children could not be established due to the requirement of sedation for handheld OCT. Instead, each child’s first visit on the handheld OCT served as his/her baseline result for comparison with follow-up sessions. For the 14 children who
completed one follow-up handheld OCT evaluation, the mean RNFL thickness between the visits were not significantly different for all four quadrants, however large declines were observed in some patients, especially in those with > 10% reductions in one of the quadrants. Ten to fifteen percent decline in average RNFL thickness is considered clinically significant and represent progression of disease (Avery et al., 2014).

Looking closely at individual changes showed that most attenuations occurred within the superior, nasal, and to some degree, the inferior quadrants. The temporal quadrant was relatively spared. In the superior and nasal quadrants 8 children showed RNFL decline from the initial visit. The mean reduction was approximately 10% for superior and 9% for inferior.

Findings in literature regarding which retinal quadrant is most consistently associated with vigabatrin toxicity remain equivocal. Some studies in adults and older children demonstrate characteristic nasal-dominant RNFL loss or “inverse” optic disc atrophy (Buncic et al., 2004; Lawthom et al., 2009), while other studies using the OCT show attenuations in the superior and inferior quadrants (Clayton et al., 2011; Kjellstrom et al., 2014; Origlieri et al., 2016; Wright et al., 2017). The present study support these findings from older cohorts showing declines in RNFL thickness in the superior, nasal, and inferior quadrants. Attenuations in these three quadrants may be attributed to the origin of nerve fiber projections originating from the peripheral retina which is more vulnerable to vigabatrin insult. One post-mortem study from a 41-year-old male receiving vigabatrin for complex partial epilepsy confirmed the loss of ganglion cells and nerve fiber layer in the peripheral retina (Ravindran et al., 2001). Vigabatrin is largely associated with toxicity in the periphery causing bilateral concentric field loss (Lawden et al., 1999; Miller et al., 1999; Wild et al., 1999).
The results from three children who completed three consecutive handheld OCT sessions are in accordance with a nasal-specific pattern of RNFL decline with temporal sparing. Temporal sparing has been well-documented throughout studies in both adults and children undergoing vigabatrin therapy (Wild et al., 2006; Lawthom et al., 2009; Clayton et al., 2010; Moseng et al., 2011; Kjellstrom et al., 2014; Wright et al., 2017; Peng et al., 2017). The relative preservation of temporal RNFL may be attributed to nerve axons projecting to the temporal optic disc through papillomacular bundles and their origins in the central retina (responsible for central vision), which may be unaffected by vigabatrin (Lawthom et al., 2009; Wright et al., 2017). Furthermore, attenuations of nasal RNFL thickness with temporal sparing are more consistent with the pattern of peripheral visual field loss seen in vigabatrin-treated patients. This is because the temporal optic disc is associated with the central macular visual field which is relatively preserved, and the nasal optic disc corresponds more to the surroundings of the visual fields (Garway-Heath et al., 2000), which show characteristic deficits following drug treatment. Nevertheless, we cannot definitively rule out the involvement of central retina including the fovea in vigabatrin-associated RNFL loss, as deficits may progress centrally with time (Peng et al., 2017). RNFL loss was more pronounced during the third visit in two of these children, suggesting that future longitudinal OCT monitoring should include long-term follow-up assessments.

**Comparison with Clinical ERG**

Previous comparisons between RNFL thickness and flicker response in children taking vigabatrin during infancy were published by performing the table-top OCT after drug discontinuation during adolescence when tolerability was sufficient (Kjellstrom et al., 2013; Wright et al., 2017). In the present study, we were able to examine flicker response and OCT
findings that were acquired during the same visit. Given the restricted range of RNFL thickness values within each retinal quadrant and the relatively small sample size, no correlation was found between thickness and 30 Hz flicker amplitude. Individually, 3 children showed notable flicker response reductions between two consecutive visits that were accompanied by mild RNFL loss within the nasal and superior quadrants. However, these ERG deficits were not seen in children who showed > 10% RNFL attenuations from baseline. It was to note that the flicker amplitude was the only ERG parameter examined as it is a good functional biomarker for vigabatrin-associated toxicity consistent with visual field loss (Harding et al., 2000). In this study, more children showed only RNFL attenuations within one or more quadrants than both ERG and OCT deficits. This might be a result of greater variability in ERG flicker amplitude results or the possibility that the RNFL measures using handheld OCT is more sensitive than the clinical flicker ERG response in detecting early retinal changes following vigabatrin treatment. The latter would imply that structural declines in RNFL may represent primary damage to the neuroretina which precedes functional deficits such as ERG abnormality or visual field loss. This idea is further supported by the findings that individuals receiving vigabatrin treatment could demonstrate RNFL decline without exhibiting functional visual field deficits (Wild et al., 2006; Lawthom et al., 2009).

Limitations

Due to recruitment specificities, the relatively small sample size in this study may restrict the generalizability and the power of our results. The first handheld OCT assessment sometimes occurred during one of the follow-up clinical ERG visits as opposed to the baseline visit. Since handheld OCT evaluation requires sedation for clinical ERG to proceed, some children had
fewer or no follow-up OCT testing. Given the limited number of longitudinal visits (only three visits including baseline in n=3 children), this study was unable to continue following patients who showed RNFL thickness declines, and it is unknown whether these participants will progress to exhibit deficits consistent with retinal toxicity. Our definition of vigabatrin-associated toxicity is dependent on serial clinical ERG assessments. Three patients showed LA 30 Hz flicker ERG amplitude reductions from baseline, however these findings were within age-expected normal range and therefore were not classified as having vigabatrin toxicity. As a result of the few patients who showed clinical ERG amplitude reductions from baseline, RNFL findings could not be correlated with the clinical ERG.

**Conclusions**

To the best of our knowledge, this is the first study to assess the reliability of the handheld OCT and use a longitudinal design measuring RNFL thickness in children with IS < 3 years of age who are taking vigabatrin. RNFL thickness measures using the handheld OCT provided good reliability within a single visit, between consecutive visits, and between independent examiners of RNFL segmentation. Further long-term follow-up is required to gain a better understanding of RNFL thickness decline in this specific population and its association with vigabatrin toxicity.
## Tables and Figures

**Table 13.** Demographic and clinical 30 Hz flicker ERG information for n=29 participants tested on the handheld OCT.

<table>
<thead>
<tr>
<th>Sub ID</th>
<th>Age at Testing (months)</th>
<th>Sex (M/F)</th>
<th>Etiology</th>
<th>Other Medications</th>
<th>Current Daily Dose (body weight)</th>
<th>Clinical 30 Hz ERG Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>302</td>
<td>6 (1&lt;sup&gt;st&lt;/sup&gt; Visit) 11 (2&lt;sup&gt;nd&lt;/sup&gt; Visit) 14 (3&lt;sup&gt;rd&lt;/sup&gt; Visit)</td>
<td>M</td>
<td>Unknown</td>
<td>None</td>
<td>1200 mg (9.8 kg @ visit 3)</td>
<td>Visit 1 - reduced amplitude in OD Visit 2 - normal, increase from visit 1 Visit 3 - normal, no change</td>
</tr>
<tr>
<td>304</td>
<td>4 (1&lt;sup&gt;st&lt;/sup&gt; Visit) 14 (2&lt;sup&gt;nd&lt;/sup&gt; Visit)</td>
<td>M</td>
<td>TSC</td>
<td>1. Phenobarbitol 2. Vitamin D</td>
<td>1000 mg (12.8 kg @ visit 2)</td>
<td>Visit 1 - normal Visit 2 - normal, no change</td>
</tr>
<tr>
<td>305</td>
<td>17</td>
<td>F</td>
<td>Focal cortical dysplasia</td>
<td>None</td>
<td>1200 mg (10 kg)</td>
<td>Normal</td>
</tr>
<tr>
<td>306</td>
<td>23</td>
<td>F</td>
<td>TSC</td>
<td>1. Oxcarbazepine 2. Topiramate</td>
<td>1600 mg (16.7 kg)</td>
<td>Normal</td>
</tr>
<tr>
<td>307</td>
<td>7</td>
<td>M</td>
<td>Periventricular Leukomalacia and Hypoglycemia at Birth</td>
<td>Clobazam</td>
<td>1250 mg (10 kg)</td>
<td>Normal</td>
</tr>
<tr>
<td>308</td>
<td>9 (1&lt;sup&gt;st&lt;/sup&gt; Visit) 16 (2&lt;sup&gt;nd&lt;/sup&gt; Visit)</td>
<td>F</td>
<td>Intra-parenchymal Hemorrhage with Ventricular Extension</td>
<td>1. Vitamin D 2. Phenobarbitol</td>
<td>1200 mg (8.77 kg @ visit 1) Discontinued following visit 1</td>
<td>Visit 1 - normal Visit 2 - normal, no change</td>
</tr>
<tr>
<td>No.</td>
<td>1st Visit</td>
<td>2nd Visit</td>
<td>Gender</td>
<td>Diagnosis</td>
<td>Treatment</td>
<td>Dose</td>
</tr>
<tr>
<td>-----</td>
<td>-----------</td>
<td>-----------</td>
<td>--------</td>
<td>-----------</td>
<td>-----------</td>
<td>------</td>
</tr>
<tr>
<td>309</td>
<td>9</td>
<td>18</td>
<td>F</td>
<td>Left Middle Cerebral Artery Infarction</td>
<td>Keppra</td>
<td>1000 mg (10.5 kg @ visit 2)</td>
</tr>
<tr>
<td>312</td>
<td>9</td>
<td>13</td>
<td>M</td>
<td>Unknown</td>
<td>Vitamin D</td>
<td>1200 mg (9.5 kg @ visit 2)</td>
</tr>
<tr>
<td>313</td>
<td>6</td>
<td>14</td>
<td>F</td>
<td>Unknown</td>
<td>None</td>
<td>1000 mg (9.9 kg @ visit 2)</td>
</tr>
<tr>
<td>314</td>
<td>6</td>
<td>M</td>
<td>M</td>
<td>Neonatal Hypoxic-ischemic Encephalopathy</td>
<td>Keppra</td>
<td>900 mg (7 kg)</td>
</tr>
<tr>
<td>315</td>
<td>11</td>
<td>M</td>
<td>M</td>
<td>Unknown</td>
<td>Discontinu ed</td>
<td>Normal</td>
</tr>
<tr>
<td>316</td>
<td>27</td>
<td>34</td>
<td>F</td>
<td>TSC</td>
<td>1. Oxcarbazepine 2. Keppra</td>
<td>1500 mg (15 kg @ visit 2)</td>
</tr>
<tr>
<td>318</td>
<td>9</td>
<td>15</td>
<td>M</td>
<td>Unknown</td>
<td>None</td>
<td>1000 mg (10.3 kg @ visit 2)</td>
</tr>
<tr>
<td>324</td>
<td>8</td>
<td>12</td>
<td>F</td>
<td>Periventricular Leukomalacia</td>
<td>Pyridoxine</td>
<td>1800 mg (12 kg @ visit 2)</td>
</tr>
<tr>
<td>325</td>
<td>7</td>
<td>M</td>
<td>M</td>
<td>Unknown</td>
<td>None</td>
<td>1000 mg (8.3 kg)</td>
</tr>
<tr>
<td>#</td>
<td>First Name</td>
<td>Gender</td>
<td>Diagnosis</td>
<td>Medications</td>
<td>Dosage</td>
<td>Weight</td>
</tr>
<tr>
<td>----</td>
<td>------------</td>
<td>--------</td>
<td>-----------</td>
<td>-------------</td>
<td>--------</td>
<td>--------</td>
</tr>
</tbody>
</table>
| 327| M          | Unknown| Polymicrogyria in the Left Frontal Lobe | 1. Phenobarbitone  
2. Clobazam  
3. Topiramate  
4. Keppra | 1500 mg (9.8 kg) | Normal |
| 332| F          | Periventricular Leukomalacia | 1. Prednisolone  
2. Vitamin D | 900 mg (7.8 kg @ visit 3) | Visit 1 - normal  
Visit 2 - normal, no change  
Visit 3 - normal, no change |
| 337| F          | Mutation of TUBBB2A Gene | 1. Prednisolone  
2. Topiramate  
3. Vitamin D | 1000 mg (10.3 kg @ visit 2) | Visit 1 - normal  
Visit 2 - normal, no change  
Visit 3 - normal, no change |
| 340| F          | Trisomy 21 | None | 300 mg (9.1 kg @ visit 2) | Visit 1 - normal  
Visit 2 - normal, no change |
| 341| M          | Right Posterior Cerebral Infarction | 1. Keppra  
2. Clobazam | 1500 mg (10.8 kg) | Normal |
| 343| M          | TSC | ACTH | 1000 mg (8.7 kg) | Normal |
| 344| F          | Unknown | 1. Keppra  
2. ACTH | 1400 mg (12.4 kg) | Normal |
| 346| F          | Unknown | None | 1500 mg (9.6 kg) | Normal |
| 347| F          | Polymicrogyria in the Left Frontal Lobe | 1. Phenobarbitone  
2. Clobazam  
3. Topiramate  
4. Keppra | 1500 mg (9.8 kg) | Normal |
| 351| M          | Right Schizencephaly | 1. Keppra  
2. Prednisolone | 1000 mg (not available) | Normal |
Table 14. Intra-visit ICC and mean peripapillary RNFL thickness values for four 90-degree retinal quadrants around the optic disc.

<table>
<thead>
<tr>
<th></th>
<th>Superior</th>
<th>Nasal</th>
<th>Inferior</th>
<th>Temporal</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD (Intra-visit ICC)</td>
<td>0.98</td>
<td>0.82</td>
<td>0.85</td>
<td>0.93</td>
</tr>
<tr>
<td>OS (Intra-visit ICC)</td>
<td>0.75</td>
<td>0.83</td>
<td>0.86</td>
<td>0.89</td>
</tr>
<tr>
<td>OD Mean Thickness (µm)</td>
<td>109.4 ± 33.4</td>
<td>69.7 ± 14.8</td>
<td>89.5 ± 16.7</td>
<td>62.5 ± 14.6</td>
</tr>
<tr>
<td>OS Mean Thickness (µm)</td>
<td>104.4 ± 20.6</td>
<td>69.1 ± 11.3</td>
<td>91.8 ± 16.3</td>
<td>61.0 ± 13.3</td>
</tr>
</tbody>
</table>

* N=28 eyes were assessed for OD, and n=25 eyes were assessed for OS.

* Normal clinical 30 Hz flicker ERG response is based on comparisons to age-expected normative range. N/A refers to participants without follow-up testing on the handheld OCT. Weight information is not available in one child (ID351). Vigabatrin was discontinued at the time of most recent handheld OCT testing in 2 children (ID308, ID315).
Figure 12. Imaging technician operating the handheld Envisu OCT on an infant under sedation. The eye-piece connected to the fiber-optic cable allows flexibility as children can be imaged in a supine position. Real-time optic disc images can be displayed on the on-board computer screen for instant appraisal of quality and location of scan.
Figure 13. Mean peripapillary RNFL thickness across four 90-degree retinal quadrants in both eyes (n=28 for OD; n=25 for OS). Hollow-circles (°) and asterisk (*) refer to the outliers and extreme outliers respectively of each quadrant.
Figure 14. Intra-visit reliability expressed as plots of second trial RNFL thickness against first trial RNFL thickness for all four 90-degree retinal quadrants of OD (A) and OS (B). The least squares regression line represents perfect agreement between the two trials and is used as reference.
Figure 15. Inter-visit reliability expressed as plots of second visit RNFL thickness against first visit RNFL thickness for all four 90-degree retinal quadrants. A total of 14 children (n=14) have completed two consecutive visits on the handheld OCT. Four eyes (4/14) were from analyzed OS and the remaining eyes (10/14) were from OD. The least squares regression line represents perfect agreement across two visits and is used as reference.
Figure 16. Inter-rater reliability expressed as plots of second grader RNFL thickness against first grader RNFL thickness for the superior (A) and inferior (B) quadrants. A total of 18 optic disc scans (n=18) were compared between two independent raters. The least squares regression line represents perfect agreement and is used as reference.

Figure 17. Longitudinal RNFL thickness assessments across two visits (visit 1 = baseline, visit = first follow-up) for all four retinal quadrants. Paired-sample t-tests (two-tailed) showed that the
differences in RNFL thickness between baseline and the first follow-up visit were not statistically significant. P-values for all quadrants are shown.

![Graph showing changes in RNFL thickness](image)

**Figure 18.** Percent change from baseline during visit 2 for n=14 children who have completed one follow-up testing on the handheld OCT. All four circumpapillary quadrants are shown. Triangles represent the interval in months between two consecutive handheld OCT visits.

**Table 15.** Longitudinal RNFL thickness changes across two consecutive visits on the handheld OCT.

<table>
<thead>
<tr>
<th>Superior</th>
<th>Nasal</th>
<th>Inferior</th>
<th>Temporal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Participants Showing RNFL Thickness Declines (/14)</td>
<td>8</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Number Showing Declines &gt;10%</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>
**Figure 19.** Longitudinal RNFL thickness measurements for all quadrants across 3 consecutive visits in n=3 children. (A) ID302 (6 months at visit 1), (B) ID327 (9 months at visit 1), (C) ID332 (10 months at visit 1). Follow-up intervals were between 3-5 months.
1. **Baseline – 6 months old.** Superior quadrant thickness: 120.74 µm

2. **Visit 2 – 11 months old.** Superior quadrant thickness: 103.47 µm
3. Visit 3 – 14 months old. Superior quadrant thickness: 88.27 µm

Figure 20. Representative single horizontal B-scan derived from the superior quadrant of participant ID302 across 3 consecutive visits. Red and yellow lines border the RNFL and show its relative thickness in each specific B-scan. The blue and green intersection on the B-scans corresponds to the location within the periphery of the optic disc (3.4 mm circle placed around the geometric center of the ONH). The overall superior quadrant RNFL thickness for 3 visits is shown.
General Discussion

The present study investigated the tolerability, reliability and clinical feasibility of the handheld ERG and OCT techniques to assess the retina in children undergoing vigabatrin treatment for infantile spasms. To the best of our knowledge, a prospective observational study with either portable devices in children receiving vigabatrin therapy as young as our cohort (< 3 years of age) has never been done. Specifically, our study evaluated the reliability and longitudinal changes of the peripapillary RNFL thickness measured from handheld OCT, and the LA 30-Hz flicker amplitude measured from handheld ERG. RNFL attenuations and 30-Hz flicker response reductions are structural and functional biomarkers, respectively, that have been associated with vigabatrin-related retinal toxicity in adults and older children. The key findings from this study were: (1) RETeval ERG showed moderate to poor tolerability in non-sedated infants; (2) the amplitude and timing of the RETeval 30-Hz flicker response were positively associated with the clinical ERG; (3) the RETeval 30-Hz flicker and handheld OCT had good intra-visit reliability; and (4) the handheld OCT had moderate inter-visit reliability, with some results associated with longitudinal changes of the clinical 30-Hz flicker ERG responses.

Tolerability of Handheld ERG and OCT Techniques

The present study was the first to evaluate the tolerability of the handheld ERG and OCT to monitor for retinal changes associated with vigabatrin in infants younger than 3 years of age. Previous studies incorporating functional and structural assessments of vigabatrin-associated neuroretina changes have been successful in adults and older children tested with conventional systems such as the table-top OCT (Lawthom et al., 2009; Clayton et al., 2010), clinical sedated-ERG (Harding et al., 2000), and visual field perimetry (Lawden et al., 1999; Wild et al., 1999).
Data from very young children receiving vigabatrin treatment are important; however they are limited given the aforementioned lack of testing compliance. In this study, tolerability was measured as the proportion of participants who were able to cooperate with and complete the handheld RETeval flicker and OCT optic disc protocols. Tolerability at 32% for the RETeval system was considered poor within our non-sedated infant population. The lack of success with RETeval testing would be, at least partially, because the children were being prepped for sedation. Given the requirement of clinical ERG as part of the standard of care for patients undergoing vigabatrin treatment, parents had been instructed that their children needed to fast since the previous evenings. This would result in fatigue and irritability in the children and compliance with RETeval diminished. For RETeval, constant pupil detection (at least 10 seconds) is required for the flicker protocol to proceed to completion. Another challenge was that the research team was often given strict time limits to ensure the proper flow of clinical vigabatrin-monitoring procedures, which resulted in the early forfeiting of RETeval flicker testing in several children. We suggest that the RETeval tolerability in awake infants would be moderately improved if children are not required to undergo sedation, as fasting-related fatigue and irritability would no longer be factors for non-compliance.

The handheld OCT showed remarkable tolerability within the same cohort of infants. It is important to note that, in contrast to the handheld ERG, the handheld OCT was performed under sedation, and therefore we cannot make direct comparisons between the tolerabilities of the two systems. The high tolerability was consistent in children across the first, second and third handheld OCT visits, suggesting the contributions from the system’s fast imaging speed, and characteristic flexibility of the eye-piece (delivering the light stimulus) allowing acquisition in
supine positions (Gabriele et al., 2008; Hahn et al., 2011). This study marks the first time that the SDOCT was used for optic disc assessment in conjunction with the clinical 30-Hz flicker ERG in vigabatrin-treated children < 3 years of age. Previous RNFL evaluations of young children receiving vigabatrin during infancy were achieved with electrophysiological testing accompanied by table-mounted OCT or perimetry which were tested many years after drug discontinuation during adolescence when tolerability was sufficient (Wright et al., 2017).

It is important for young children with IS to receive frequent and reliable monitoring for potential vigabatrin-associated retinal toxicity, as this drug is effective, and additional burdens of visual deficits should be avoided. In the current study, all handheld OCT sessions were performed under sedation, which in our group was necessary to maintain testing tolerability, as participants were too young to maintain an upright posture to cooperate with table-mounted OCT or perimetry. The use of sedation to maintain compliance on the handheld OCT was consistent with previous studies showing its clinical feasibility to distinguish between normal and abnormal retinal morphologies (Scott et al., 2009; Gerth et al., 2009; Chong et al., 2009). Our findings of good tolerability for the handheld OCT is consistent with the hypothesis; however for the RETeval ERG, there was insufficient compliance provided by our young and non-verbal cohort during awake testing.

**Mean RNFL Thickness**

Within our current study cohort (aged 1-27 months), the mean RNFL thickness at the first visit in the superior and nasal quadrants were higher compared to the nasal and temporal quadrants. The normative values of circumpapillary RNFL thickness from healthy children matching our specific age group are currently missing. In comparison to previous RNFL studies using table-
top SDOCT in typically developing children (aged 4 to 13 years) (Elia et al., 2012; Barrio-Barrio et al., 2013; Al-Haddad et al., 2014), the mean RNFL values from the current study are reduced in the superior and inferior quadrants, while data from the temporal and nasal quadrants are similar to those in these published studies. This difference could be due to the young age of our participants, as some of them may have slower retinal development in the optic disc. This could also be due to vigabatrin-associated retinal toxicity affecting specifically the superior and inferior quadrants.

**Reliability**

The intra-visit reliability for implicit times and amplitudes of the RETeval 30-Hz flicker ERG were moderate to strong with ICCs ranging between 0.42 - 0.86. The ICC values were reduced in comparison to a previous report demonstrating high ICC values of above 0.9 across both RETeval ERG parameters in healthy controls (Asakawa et al., 2017). Our concurrent RETeval study in an adult population (N = 92, median age = 20 years, range 11 months - 69 years) of healthy controls and individuals with various retinal diseases showed strong intra-visit reliability (ICC = 0.82 for amplitudes and ICC = 0.53 for implicit time) (Liu et al., 2018). The reduced measures of reliability in our study was most likely the result of a much younger age group (mean age 13.6 months) as compared with the aforementioned studies (mean age > 20 years). Within the infant cohort, experimenter and patient dependent factors may have produced a lower signal to noise ratio through one or both trials of the RETeval flicker protocol. These include excessive body and head movements of the child during ERG recording due to low compliance, poor contact of the skin electrodes due to inadequate cleansing of the skin underneath causing reduced electrode adhesiveness, and loss of constant pupil detection due to excessive shifting of
the eyes. Although reliability was demonstrated to be moderate to strong, which largely agrees with our original hypothesis, the relatively small sample size of children with successful RETeval assessments in this study may limit its power and overall generalizability. The research aim for assessing reliability of the RETeval response overestimated the level of compliance, as our results suggest that the skin electrodes and the general imaging mechanisms used by the RETeval system to acquire retinal responses may not be ideal for this particular population of non-sedated infants. The electrodes were sometimes too large to be placed fully beneath the child’s lower eyelids due to the smaller size of their heads, and the protocols requiring approximately 10 seconds of pupil illumination demanded nearly perfect cooperation, which within this young group was rarely achieved.

In a previous study, measures of the circumpapillary RNFL thickness using table-mounted SDOCT demonstrated remarkable reliability (ICCs > 90%) for all retinal quadrants (Clayton et al., 2011). The same degree of reliability could not be shown amongst the pediatric population given the limitations of non-compliance. In this study, we were able to report good reliability (ICCs range 0.75 - 0.98) for RNFL measures across all quadrants using the handheld OCT in infants under sedation. This level of reliability was consistent for both the intra-visit and the inter-visit groups, suggesting that handheld OCT RNFL evaluations in our population (1-34 months) are reliable within a single visit and between consecutive sessions. It is worth mentioning that reliability measures from the present study may not be generalized to another infant cohort with other retinal conditions causing changes in RNFL thickness, because ICC values depend on specific study populations and the basis of RNFL deficits are not always universal across different retinal diseases (Avery et al., 2014).
Overall, our reliability measures were comparable to the published values by Avery et al., 2014 from an older cohort (mean age 5.1 years, range 0.8-13 years) with OPG and NF1. Based on ICCs calculated with the non-isotropic volume (1000 A scans x 100 B scans) of RNFL thickness analysis, our values for the 4 retinal quadrants were slightly lower, suggesting the importance of mean age differences between the participants of the two studies. Our report focused on a much young infant cohort (mean age 0.84 years) whereas the earlier study included an older population (mean age 5.1 years). RNFL thickness in infants was likely more difficult to assess using the handheld OCT due to their physical developmental limitations. The smaller dimensions of their heads and eyes may restrict the size of the available working area accessible by the handheld OCT eye-piece, which would introduce undesirable motion artifacts affecting the overall quality of the optic disc scan in one or both trials. Furthermore, there were no notable changes between the intra-visit ICCs and the inter-visit ICCs, suggesting that the overall RNFL thickness may be relatively spared in children who have completed at least one longitudinal assessments. Nevertheless, no changes in the overall inter-visit ICC values does not represent an absence of retinal toxicity, as specific attenuations within individual participants and quadrants may still be significant.

Given the occasional low-quality optic disc scans due to the difficulties of imaging within this population, RNFL analysis (segmentation) required manual tracing to complete the calculation of thickness values. Eighteen scans were compared for the accuracy of RNFL thickness values between two independent graders in the superior and inferior retinal quadrants. Good reliability was demonstrated through the inter-rater ICCs. This consistency suggests that RNFL thickness measured from handheld OCT can be reliable even when experimenter adjustments were introduced.
The reliability measures for both portable systems were moderate to high. The values were slightly reduced from published results in older groups, however they were more consistent with the original hypothesis which accounted for the previously-described difficulties of acquiring high quality ERG and OCT data in our participants given their young age. In comparison, RNFL assessments using the handheld OCT demonstrated better reliability than flicker responses measured with the RETeval ERG within our group of children. Therefore, the handheld OCT performed under sedation may serve as a reliable structural biomarker to monitor for retinal changes in children (aged 1 month - 3 years) receiving vigabatrin treatment for IS. Nevertheless, this reliability cannot be generalized to similarly-aged infant cohorts with other retinal conditions causing RNFL loss, as the basis of retinal changes can be different.

**Comparison with Clinical ERG**

The clinical LA 30-Hz flicker response has been routinely assessed in children undergoing vigabatrin treatment. The clinical flicker ERG amplitude is a reliable functional biomarker of vigabatrin-associated retinal toxicity in children with high sensitivity (100%) and specificity (75%) (Harding et al., 2000). Moreover, the flicker response deficits have been shown to persist even after vigabatrin discontinuation (Westall et al., 2003). The 30-Hz flicker amplitudes from the present study showed a positive association ($\omega^2 = 0.71$) with the clinical 30-Hz flicker response. This strong correlation suggests that the handheld RETeval is comparable to the clinical ERG in evaluating the cone-dominated flicker response in infants < 3 years of age. This correlation was based on a very limited sample size of children who were able to tolerate non-sedated RETeval testing, and is not representative of the entire infant population undergoing vigabatrin treatment or with other retinal conditions. A study with a much larger sample size may demonstrate the true association. However, the preliminary results are in accordance with the
hypothesis. They show great prospects for the use of RETeval to achieve retinal monitoring alongside the clinical ERG, or in situations where the clinical ERG cannot be performed, such as when children have development concerns, parents opting out for more non-invasive diagnostic testing, and patients having particular contraindications to sedation. In contrary to the hypothesis, the RETeval 30-Hz flicker implicit time did not show any association with that of the clinical ERG \( (\omega = 0) \), possibly due to the small range of the RETeval and clinical ERG implicit time values between our participants and the small sample size of children who could tolerate handheld ERG. The implicit time measured using RETeval is therefore not a useful biomarker for infants undergoing vigabatrin treatment.

Previous comparisons between OCT RNFL thickness and ERG flicker amplitude in children undergoing vigabatrin during infancy was done by performing the table-top OCT many years following drug withdrawal and clinical sedated-ERG when tolerability was sufficient (Wright et al., 2017). Significant positive associations between the superior and inferior RNFL thickness with the clinical 30-Hz flicker response were shown in an earlier adult study (aged 30-78 years) (Kjellstrom et al., 2014). In this study, we were able to compare the results of the clinical ERG and the SDOCT that were acquired during the same visit. In contrast to the aforementioned work and to our hypothesis, no correlations were found in this study between RNFL thickness and 30-Hz flicker amplitude for all four 90-degree retinal quadrants. Given the young age (1-27 months at OCT visit 1) and limited sample size of our study cohort, the differences between RNFL thicknesses of participants are smaller than previous reports, resulting in a narrow range of values and no associations with the clinical ERG. A larger study consisting of an adequate sample size may see a more widespread range of RNFL values between participants.
Three children demonstrated flicker amplitude reductions from baseline on the clinical ERG, and these findings were accompanied by RNFL loss within the superior and nasal quadrants. It is to note that most participants in this study showed RNFL deficits without clinical ERG abnormalities, suggesting that RNFL measures using the handheld OCT is a more sensitive marker than flicker ERG in detecting early signs of retinal toxicity. It also implies the possibility that declines in RNFL thickness represent primary damage to the neuroretina, which precedes functional deficits seen on the clinical flicker ERG.

**Longitudinal Assessments**

Assessment of cone-dominant ERG abnormalities using the RETeval flicker response has been previously described in an older cohort of 35 patients (age 33.1 ± 17.9 years) and 50 normal controls (aged 28.5 ± 13.0 years) with cone dysfunction (Nakamura et al., 2016). The study reported a significant (nearly 8-fold) flicker amplitude reduction in patients with cone dysfunction as compared to age-matched healthy controls. Nevertheless, reports of cone-dominant ERG changes in infants were limited given issues of non-compliance. The present study was the first to evaluate the clinical feasibility of the 30-Hz flicker response using a handheld RETeval ERG in infants. For two children with successful baseline and follow-up RETeval recordings, their concurrent longitudinal clinical ERG results did not identify vigabatrin-associated retinal toxicity, which was previously defined by our group as having more than 40% flicker response reductions from baseline across two consecutive follow-up visits. This definition was used to avoid the misclassification of toxicity after a single test session when reduction in amplitude may have been artefactual. Comparisons of diagnostic accuracy to identify vigabatrin toxicity between the two ERGs was not possible given this small sample size, and the absence of toxicity based on the clinical system. A closer examination at participant ID
313 with 3 handheld ERG assessments showed that, the progressive clinical ERG amplitude
decline across visits was consistent with the RETeval response at visit 2 and not at visit 3, which
instead reported a notable increase in response from baseline. This discrepancy may have
resulted from the use of a less sensitive RETeval skin electrode in comparison to the clinical
corneal electrodes, as reduced sensitivity may not have detected the decline in flicker response at
visit 3. It was also likely related to ocular artifacts during sedated-ERG whereby the well-known
Bell’s phenomenon, which sometimes occurs under sedation, may have resulted in the upward
shift of the eye and subsequent artefactual reduction in ERG amplitude.
RNFL defects were previously represented by thickness reductions in older vigabatrin-treated
individuals when compared to age-matched healthy, or vigabatrin-naïve controls (Wild et al.,
2006; Moseng et al., 2011). We suggest that serial handheld OCT assessments are more
appropriate for our group of children as vigabatrin toxicity should be reflected through
progressive RNFL thickness declines from baseline. In this study, an age-matched control group
could not be established given that the handheld OCT was performed under sedation for clinical
ERG, and thus most parents of treatment-naïve children would not provide informed consent.
For children who completed one follow-up OCT testing in addition to baseline, the mean
difference in RNFL thickness values between the two visits was not significant, however notable
attenuations could be observed in some children. Most RNFL changes in these patients occurred
in the superior, inferior, and nasal quadrants, while the temporal quadrant was relatively spared.
It is unclear which retinal quadrant is the most consistently affected in vigabatrin toxicity, as
some studies demonstrate nasal-dominant RNFL loss (Buncic et al., 2004; Lawthom et al.,
2009), while other studies show equal deficits in the superior and inferior quadrants (Clayton et
al., 2011; Kjellstrom et al., 2014; Origlieri et al., 2016; Wright et al., 2017). In the present study,
results from the inter-visit cohort (n=14) who completed one follow-up testing showed relatively larger deficits in the superior quadrant, followed by the nasal and inferior quadrants. Reductions within these quadrants may be because the nerve fibers projecting to these quadrants of the optic disc originate from more peripheral areas of the retina that are more vulnerable to vigabatrin insult. Nevertheless, the small sample size of this group may limit the power of our findings.

Results from three children in the inter-visit cohort who completed 3 visits on the handheld OCT showed nasal-dominant RNFL reductions with temporal sparing. The relative preservation of the temporal quadrant is consistent with our original hypothesis as well as many studies in the literature using table-top OCTs in older vigabatrin-treated participants (Wild et al., 2006; Lawthom et al., 2009; Clayton et al., 2010; Moseng et al., 2011; Kjellstrom et al., 2014; Wright et al., 2017; Peng et al., 2017). This phenomenon may be attributed to the origin of the nerve axons projecting to the temporal side of the optic disc. These nerve axons originating near the central retina project through papillomacular bundles and are responsible for central vision, which is relatively unaffected by vigabatrin. In addition, vigabatrin-associated peripheral visual field loss is more consistent nasal-dominant RNFL deficits and temporal sparing, as the nasal quadrant of the optic disc corresponds to the periphery of the visual fields and the preservation of the temporal quadrant is in accordance with the relatively unaffected central macular visual fields (Garway-Heath et al., 2000).

Limitations

The RETeval ERG 30-Hz flicker showed poor tolerability in children < 3 years of age when performed awake, possibly due to the requirement of fasting for sedation which exacerbates irritability and fatigue. Reduced testing compliance resulting in a small sample size means that
the power and generalizability of this study’s measure of reliability and clinical feasibility are largely limited. Retinal response recording using RETeval requires constant pupil detection for up to 10 seconds, and this was not possible in most children due to excessive movement artifacts. The RETeval skin electrode was originally designed for use in older patients, and were too large to be positioned at correct markers on the child’s face. As a result, some testing could not be completed due to excessive electrode signal noise, or electrode falling off from the skin surface due to poor adhesion.

Longitudinal assessments of 30-Hz flicker response using RETeval ERG and RNFL measures using handheld OCT showed limited feasibility in children younger than 3 years of age. For the RETeval, the research aim was flawed, because we did not take into account of the low compliance caused by fasting and fatigue due to the requirement for sedation. As a result, only 2 children were able to complete one follow-up RETeval testing. Since our lab’s definition of vigabatrin toxicity depends on serial flicker ERG assessments including several follow-up sessions, these two participants provided limited information regarding toxicity and thus clinical utility.

For the handheld OCT, high tolerability was achieved in children under sedation for clinical ERG; however the reliance on sedation suggests that OCT could only be performed during the child’s clinical ERG visit. Hence, the first-ever (baseline) handheld OCT testing may fall on one of the child’s follow-up or exit clinical ERG appointments as opposed to the baseline ERG visit, meaning that some children would have fewer or no follow-up OCT testing in addition to the baseline visit. This study is unable to continue following specific individuals who have shown RNFL deficits on the OCT during the initial visits to detect whether they will progress to exhibit attenuations consistent with true retinal toxicity. Furthermore, peripapillary RNFL thickness
reductions measured in a longitudinal fashion may be masked by the child’s normal development, thus creating another layer of complexity while interpreting changes on the handheld OCT.

Conclusion

To the best of our knowledge, this study was the first to assess the tolerability, reliability and clinical feasibility of the RETeval ERG and the Envisu OCT in children < 3 years of age undergoing vigabatrin treatment. Within this young cohort, the RETeval system demonstrated poor tolerability and limited clinical feasibility; however, when testing was successful, it showed good intra-visit reliability, and strong positive association with the clinical ERG, suggesting that the handheld ERG may be used in young children when sedation for the clinical system is not possible. The Envisu OCT demonstrated remarkable tolerability under sedation and good reliability within a single visit and between consecutive visits. A future long-term study with multiple follow-up OCT visits are needed to gain a better understanding of RNFL thickness reduction that occurs in children undergoing vigabatrin treatment.

Future Directions

The power of the results from this study is limited by the small sample size. A larger study is required to properly assess the reliability and clinical feasibility of the two handheld techniques in children younger than 3 years of age. For the RETeval ERG, future testing should be performed in an environment where sedation for clinical ERG is not required. This may substantially reduce the level of irritability and fatigue which pose additional challenges to non-sedated testing compliance. With increased compliance, we are able to complete multiple follow-
up assessments on the RETeval ERG, and observe changes in the LA 30-Hz flicker amplitude in a longitudinal fashion as a result of vigabatrin treatment. Also, with increased sample size, our original research aim to assess RETeval’s clinical feasibility becomes more realistic, as we would be able find true associations between the RETeval and the clinical ERGs.

The RETeval ERG is small and portable, meaning that it could be easily transported to and be used by eye clinics around the world that are currently lacking a proper sedation or visual electrophysiology team due to financial constraints and resource limitations. With its tolerability expecting to improve without the need for sedation, the RETeval system may be a valuable substitute for children in facilities where clinical ERG cannot be performed.

The RETeval skin electrode is too large for use on an infant, and this was a notable issue for our study cohort, because several cases of testing were forfeited or failed due to the sensor strips falling off from the skin surface. If the supplier of RETeval could implement a much smaller, infant-appropriate skin electrode, tolerability may also see an improvement.

Future handheld OCT studies that have a longer duration may improve the issue of children not completing follow-up visits for assessment of longitudinal changes in RNFL. Recruitment criteria should be amended, such that the first OCT (baseline) testing will always occur during the first (baseline) clinical ERG visit. Consequently, not only will we be able to evaluate its clinical feasibility from comparisons with the gold-standard clinical ERG, but we will have more information regarding longitudinal changes in RNFL as a result of vigabatrin treatment over the entire course of drug treatment or even longer.

In contrast to the table-top OCT, the Envisu handheld OCT does not have an on-board software for automatic segmentation and signal to noise ratio appraisal, therefore manual adjustments during segmentation analysis are sometimes required to quantify RNFL thickness values across
different retinal quadrants. If the quality assurance algorithm could be added to the existing Envisu system, each optic disc scan taken could be scored qualitatively to minimize experimenter errors or bias. With this, the imaging specialist will be able to retake the image or proceed to completion without the need to assess for image quality by simple visualization.
References


CSF biochemistry and seizure control in epileptic patients. British Journal of Clinical Pharmacology, 27(1 S), 79S–85S.


Appendix A - Research Ethics Board Approval Letter

Research Ethics Board (REB) Amendment Approval Letter

2016-11-07

Carol Westell
Ophthalmology

REB number: 1000015923
Study Title: Vigabatrin and Infantile Epilepsy

Thank you for the amendment application submitted on 2016-10-31. This amendment to the above referenced study was reviewed through a delegated process (not by Full Board review). Any concerns arising from this review have been documented and resolved.

The Hospital for Sick Children Research Ethics Board has reviewed and approved this amendment application, including the study documents listed below:

1. Research Assent Form - Controls, version October 21, 2016 [Final Research Assent Form - Controls.pdf (1.0)]
2. Research Assent Form - Controls, version October 21, 2016 [Final Research Assent Form - Patients.pdf (1.0)]
3. Research Consent Form, (For Parent(s) of Controls), version October 21, 2016 [Final REB Vigabatrin Parental Control Consent Form.pdf (1.0)]
4. Research Consent Form, version October 21, 2016 [Final REB Vigabatrin Patient Consent (handheld OCT).pdf (1.0)]
5. Protocol, version October 21, 2016 [Final_Protocol_Vigabatrin.pdf (1.0)]
6. Amendment Form Re-EVAL October 2016.pdf (1.0)
7. Control Group Recruitment Letter, version November 4, 2016 [recruitment_letter_controls_clean_copy.doc (1.0)]
8. Treatment Group Recruitment Letter, version November 4, 2016 [recruitment_letter_JS_clean_copy.doc (1.0)]

During the course of this investigation, any significant deviations from the approved protocol and/or unanticipated developments or significant adverse events should immediately be brought to the attention of the REB.

Arbela Mancias-Eno

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Tel. (416) 813-8179  Fax. (416) 811-6315

REB # 1000015923  Amendment Delegated, Page 1 of 2

The SickKids REB operates in compliance with the Tri-Council Policy Statement; ICH Guidelines for Good Clinical Practice E6(R1); Ontario Personal Health Information Protection Act (2004); Part C Division 5 of the Food and Drug Regulations; Part 4 of the Normal Health Products Regulations, and the Medical Devices Regulations of Health Canada. The approval and the views of the REB have been documented in writing. The REB has reviewed and approved the clinical trial protocol and informed consent form for the trial. All investigational drug trials at SickKids are conducted by qualified investigators.

Furthermore, members of the Research Ethics Board who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.
Appendix B - Research Ethics Board Approval Parent Consent for Patients

Research Consent Form
(OCT examination of children taking Vigabatrin)

Title of Research Project:
Vigabatrin and Infantile Epilepsy

Investigator(s):

Responsible Individual: Dr. Carol Westall (PI) 416 813 6516
Research Coordinator: Ms. Sabrina Dhaliwal 416 813 7654 ext. 204170
Ophthalmologist: Dr. J. Raymond Buncic
Ophthalmologist: Dr. Arun Reginald
Ophthalmologist: Dr. Michael Wan
Graduate Student: Mr. Patrick Ji
Research Volunteer Ms. Naima Rahman

Purpose of the Research:

The drug Vigabatrin is used to help control seizures. In some people the drug may cause problems with vision. This might be related to small changes to the retina. The retina is the inner lining of the eye that makes a picture of what we see (like the sensor in a camera).

We want to better understand what is happening to the eyes in children taking Vigabatrin. As part of clinical care your child will be receiving a standard (sedated) electroretinogram (ERG). This test lets us detect changes in how the retina is working. We want to study different tests, hand held flicker ERG and Ocular Coherence Tomography (OCT) can give the same, or better, information about your child’s eye.

Description of the Research:

We will study children who will be receiving an electroretinograms to monitor the effects of Vigabatrin. This will include children about to start taking Vigabatrin as well as those already receiving treatment. Your child will probably be scheduled to receive ERG testings every 4 months. We would like to test your child with the additional OCT test at every visit. We expect data collection to go on for 2 years.

As part of your child’s routine visit to the ophthalmology clinic they will receive several tests of their vision. These will probably include a test of their visual acuity (how well they can see), as well as the ERG and photographs of your child’s eye.

In addition we will perform the hand held (non-sedated) ERG to test the response to a gentle flickering light. This short 20 second protocol will be performed twice on the child under
normal room lighting and will require a tape/ band aid to be placed under the eye. The hand held device does not require any sedation procedure or pupil dilation. Your child will probably be asleep (sedated) for the standard-sedated ERG test and the photography. If your child is sedated for the standard ERG a nurse will be present at all times to monitor your child for safety. The standard ERG and photography require your child’s eyes to be dilated.

After ERG is complete, while your child is still asleep, we will perform one additional test, Ocular Coherence Tomography (OCT). This test is routinely performed by eye doctors but until recently could not be used for infants. We will perform the OCT in the same room as the ERG and the nurse will be present at all times. The OCT is similar to an ultrasound that uses light, it lets us look at the structure of the retina. A hand held probe will be held very close to, but not touching, your child's eye. The OCT imaging will require about 5 minutes per eye for a total of 10 minutes. If your child starts to wake up during this OCT imaging we will stop the test.

We will compare the information from the OCT test with the results of the ERGs. We will also review your child’s health record to gain additional information. This will include:

1. your child's age and sex
2. the results of other vision tests
3. information about medications they are taking
4. detailed descriptions of the type and cause of the seizures they have experienced.

**Potential Harms:**

We know of no harm that taking part in this study could cause you or your child. The OCT uses light to image the retina. The amount of light used is known to be safe. There is no ionizing radiation involved in this test.

**Potential Discomforts or Inconvenience:**

The additional OCT test will add about 10 minutes to the total testing time for your child.

**Potential Benefits:**

**To individual subjects:**
Your child will not benefit directly from participating in this study. If there are any unexpected abnormalities detected from the OCT we will tell your child’s eye doctor. The results from the OCT will be included in your child’s hospital record.

**To society:**
Knowledge gained from this study will hopefully allow doctors to optimize Vigabatrin therapy to ensure patients receive the maximum benefit whilst minimizing visual problems. Better control of the risks associated with this powerful therapy will make its use in a wider population more feasible.
Confidentiality:
We will respect your privacy. No information about who you are (your child is) will be given to anyone or be published without your permission, unless required by law. For example, the law could make us give information about you if a child has been abused, if you have an illness that could spread to others, if you or someone else talks about suicide (killing themselves), or if the court orders us to give them the study papers.

Sick Kids Clinical Research Monitors or the regulator of the study may see your health record to check on the study. By signing this consent form, you agree to let these people look at your records. We will put a copy of this research consent form in your patient health record and give you a copy as well.

The data produced from this study will be stored in a secure, locked location. Only members of the research team (and maybe those individuals described above) will have access to the data. This could include external research team members. Following completion of the research study the data will be kept as long as required then destroyed as required by Sick Kids policy. Published study results will not reveal your identity.

Reimbursement:
We will reimburse you for all your reasonable out of pocket expenses for being in this study eg., meals, babysitters, parking and getting you to and from Sick Kids. If you stop taking part in the study, we will pay you for your expenses for taking part in the study up until that point.

Participation:
If you choose to let your child take part in this study you can take your child out of the study at any time. The care your child gets at Sick Kids will not be affected in any way by whether your child takes part in this study.

New information that we get while we are doing this study may affect your decision to take part in this study. If this happens, we will tell you about this new information. And we will ask you again if you still want to be in the study.

During this study we may create new tests, new medicines, or other things that may be worth some money. Although we may make money from these findings, we cannot give you or your child any of this money now or in the future because you or your child took part in this study.

If your child becomes ill or are harmed because of study participation, we will treat your child for free. Your signing this consent form does not interfere with your legal rights in any way. The staff of the study, any people who gave money for the study, or the hospital are still responsible, legally and professionally, for what they do.

Sponsorship:
The sponsor of this research is Sick Kids Hospital.
**Conflict of Interest:**
Some of the people doing this study may have a conflict of interest. That means that they may benefit personally, financially, or in some other way from this study.

Dr. Westall (Principal Investigator) has received or may receive for research related to the present study money, or one or more of the following other benefits: speaker’s fees, travel assistance, industry-initiated research grants, investigator-initiated research grants, consultant fees, honoraria, gifts, intellectual property rights such as patents, etc. from sponsor(s) that have activities related to the present study.

**Consent:**
“By signing this form, I agree that:
1) You have explained this study to me. You have answered all my questions.
2) You have explained the possible harms and benefits (if any) of this study.
3) I know what I could do instead of having my child take part in this study. I understand that I have the right to refuse to let my child take part in the study. I also have the right to take my child out of the study at any time. My decision about my child taking part in the study will not affect my child’s health care at Sick Kids.
4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my child’s medical records will be kept private except as described to me.
6) I understand that no information about my child will be given to anyone or be published without first asking my permission.
7) I agree, or consent, that my child___________________ may take part in this study.”

______________________________
Printed Name of Parent/Legal Guardian

______________________________
Parent/Legal Guardian's signature & date

______________________________
Printed Name of person who explained consent

______________________________
Signature of Person who explained consent & date

______________________________
Printed Witness’ name (if the parent/legal guardian Witness’ signature & date does not read English)

If you have any questions about this study, please call Sabrina Dhaliwal at 416-813-7654 ext.204170.

If you have questions about your rights as a subject in a study or injuries during a study, please call the Research Ethics Manager at 416-813-5718.
### Patient Information

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Consent obtained: ☐ Yes ☐ No

### Intake (Scoring)

#### Visual Acuity

**ETDRS, Chart 2**

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**ETDRS, Chart 3**

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#### Colour Vision

**Mollon-Reffin Minimalist**

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Notes:

#### Contrast Sensitivity

**M&S Smart System II**

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#### Visual Acuity

**Cardiff**

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Notes:

**Teller**

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Notes:
1. ERG

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<th>Full ERG Protocol: □</th>
<th>Light Adapted Protocol: □</th>
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Light adapted protocol: Using DTL electrodes, No long flash required. To be performed binocularly.

2. Ophthalmic Examination

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EOM: □ Normal □ Other

Notes: ____________________________________________________________

3. Imaging – Fundus Photography and OCT

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<td>OD □ OS □ OU</td>
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</table>

Protocol: OCT Bioptigen Disc for RFNL (If possible for both eyes, if not then chosen eye)
Appendix D - Research Ethics Board Approval Outcome Measures Form

**Outcome Measures Form**
Date:
Subject ID:
Age:
Diagnosis:
Comorbidities:

**Vigabatrin**
Date Vigabatrin Initiated:
Duration on Vigabatrin:
Current Dose:
Cumulative Dose:
Date off Vigabatrin:

**OCT**
Graded level of sedation at the beginning of test:
Nurse who graded the sedation:

Was the optic disc visualized (Y/N):
How was the image quality <done later:

Individual who graded the image:

**Times:**
Time of Dilation first drops: second drops:
Time when sedative administered
Time when child sedated

Time ERG Started:
Time ERG Finished:

Time OCT Started:
Time OCT Finished (Please note if incomplete):

Reimbursement and receipt:
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Institution name Institute of Medical Science, University of Toronto
Expected presentation date Sep 2018
Portions Figure 1
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Expected presentation date Sep 2018
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Appendix F— List of Specific Contributions

1. Dr. Carol Westall (Electrophysiologist, Optometrist) - Principal Investigator

2. Dr. Thomas Wright (Electrophysiologist, Research Associate) - involved in the original research design of the handheld ERG and OCT projects. He assisted me with training on the OCT RNFL segmentation software (OCT Explorer), and with specific segmentation analysis through the handheld OCT project. He acted as an independent grader of RNFL analysis for comparisons of inter-grader consistency.

3. Cynthia VandenHoven and Leslie MacKeen (Imaging Specialists) - acquired OCT optic disc scans with the handheld OCT for both intra-visit and inter-visit cohorts, and visually assessed the quality of images.

4. Dr. Annie Dupuis (Biostatistician) - involved in the original statistical research design of the handheld ERG and OCT projects. She also assisted with verification of the intraclass correlation statistics used in both studies.

5. Michelle McFarlane and Sabrain Dhaliwal (Clinical Research Coordinators) - assisted with patient recruitment and data collection for the handheld ERG and OCT projects. Updated research consent forms and protocols to stay up to date with Research Ethics Board at Sickkids Hospital.

6. Henry Liu and Naima Rahman (Medical Student, Summer Student) - assisted with patient recruitment, handheld ERG data analysis and masking the segmentation grader from knowing participant information to minimize bias.

7. Vision Science Research Program (University of Toronto) - full research funding award for two years of my master’s degree (~$27,000/year).