ASSESSMENT OF T-REFLEX IN ADULT PATIENTS WITH EARLY GUILLAIN-BARRÉ SYNDROME AT GROOTE SCHUUR HOSPITAL

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DECLARATION OF INDEPENDENT WORK

I, Sebastian Wessels, hereby declare that this research project submitted to the Central University of Technology for the degree MASTER OF HEALTH SCIENCES IN CLINICAL TECHNOLOGY is my own independent work and that it has not been submitted to any institution by me or any other person in fulfilment of the requirements for the attainment of any qualification.

Signature

9/1/2019

Date

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ABBREVIATIONS AND SYMBOLS

<	Smaller than
>	Larger than
AIDP	Acute inflammatory demyelinating polyneuropathy
AMAN	Acute motor axonal neuropathy
CIDP	Chronic inflammatory demyelinating polyneuropathy
CMAP	Compound muscle action potential
CV	Conduction velocity
EMG	Electromyograph
G1	Active electrode
G2	Reference electrode
GBS	Guillain-Barré syndrome
H-reflex	Hoffmann Reflex
m/s	Meters per second
ms	Milliseconds
mV	Millivolt
NCS	Nerve conduction studies
SNAP	Sensory nerve action potential
T-reflex	Electromyographically recorded deep tendon reflex
uV	Microvolt

DEFINITIONS

Antidromic	Conducting nerve impulses in a direction opposite to the usual direction (Merriam-Webster, 2019).
Artifactual	Impulses related to electrical, environmental origin. i.e. Not generated biologically (Merriam-Webster, 2019).
Axonal	The appendage of the neuron that transmits impulses away from the cell body (Merriam-Webster, 2019).
Corticospinal	Relating to the cerebral cortex and the spinal cord (Merriam-Webster, 2019).
Demyelinating	Causing or characterised by the loss or destruction of myelin (Merriam-Webster, 2019).
Electromyograph	An instrument that converts the electrical activity associated with functioning skeletal muscle into a visual record or into sound (Merriam-Webster, 2019).
Goniometer	An instrument for measuring solid angles (Merriam-Webster, 2019).
Neuropathology	The pathology of the nervous system (Merriam-Webster, 2019).
Orthodromic	Conducting impulses in the normal direction (Merriam-Webster, 2019).
Prone	Lying face downward (Merriam-Webster, 2019).
Supine	Lying on the back, face or front upward (Merriam-Webster, 2019).
Supraspinal	Situated above the spine of the scapula (Merriam-Webster, 2019).

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ABSTRACT

Guillain-Barré Syndrome (GBS) is a polyneuropathy involving both proximal (spinal root) and peripheral nerves, and is characterised clinically by acute progressive weakness associated with reduced or absent deep tendon reflexes. Electromyographically recording the deep tendon reflex (T-reflex) has proven to be useful when confirming the diagnosis of many peripheral neuropathies. However, research on the T-reflex in GBS remains limited. The primary aim of this study was to describe the T-reflex in patients with GBS compared with healthy subjects.

This is a prospective, controlled, non-blinded, cohort study. Adults with GBS (GBS cohort) and healthy subjects (Control cohort) were recruited concurrently. During all electrophysiological evaluations, both T-reflexes and conventional NCS (including F-responses), were performed on all subjects.

The T-reflex was successfully recorded in 28 subjects (14 GBS- and 14 control cohort). In patients with GBS, T-reflex responses had significantly prolonged latencies, slowed conduction velocities and increased duration. Furthermore, T-reflex responses were abnormal in all subjects with GBS manifesting the clinical presentation, CSF features and abnormalities on conventional nerve conductions studies consistent with this diagnosis. In contrast to normal control patients, the T-reflex response had indistinct onset and offset and polyphasic rather than biphasic or triphasic morphology. However, no significant differences in the T-reflex were identified between the various subtypes of GBS. Significantly, the T-reflex was more often recordable than the more widely utilised conventional F-response. Thus, the results of this study strongly suggest that the T-reflex is sensitive to the electrophysiological changes associated with GBS, but that it is not specific for the subtype of GBS. Given that the T-reflex is painless and easy to perform, the results of this study

suggest that it should be more widely utilised, together with conventional nerve conduction studies, in the assessment of patients with GBS.

KEYWORDS: T-reflex, electromyographic reflex, deep tendon reflex, stretch reflex, nerve conduction studies, nerve conduction velocity, reflex velocity, Guillain-Barré syndrome, acute inflammatory demyelinating polyneuropathy, acute motor axonal neuropathy.

CHAPTER 1:

INTRODUCTION

It is important, when a patient presents with symptoms and signs of weakness, to determine the anatomical structure or structures involved. For example, pathology of the central nervous system (CNS), peripheral nervous system (PNS), neuro-muscular junction (NMJ) or muscle may all result in weakness. It is also important to establish the aetiology of that weakness, which may include auto-immune inflammation, metabolic derangements, infection, ischaemia, and trauma, etc. This diagnostic process typically involves careful history taking and clinical neurological examination, as well as investigations such as imaging of the brain and spinal cord, cerebrospinal fluid (CSF) analysis, various blood tests and neurophysiological testing of peripheral nerves and muscles.

Where spinal root or peripheral nerve pathology is suspected in a weak patient, neurophysiological testing may be extremely helpful. It generally involves both motor and sensory nerve conduction studies (NCS), as well as electromyography (EMG). NCS and EMG may confirm axonal- or demyelinating pathology involving the spinal roots or peripheral nerves, myopathy or dysfunction at the neuromuscular junction. However, NCS and EMG do not directly assess tendon reflexes.

With regards to the clinical examination, tendon reflexes are often helpful as these are increased where pathology involves the central nervous system and depressed or absent if pathology involves the peripheral nervous system.

A tendon reflex may be elicited by an abrupt, brief stretching of a muscle tendon, typically generated by the tap of a tendon hammer. This results in reflex contraction of the muscle associated with that tendon. A common example is the knee jerk which, together with other tendon

reflex testing, is performed in virtually all clinical neurological examinations. It is perhaps surprising, therefore, that although electromyographic recording of the deep tendon reflex (T-reflex) is easy to perform and has been shown to be useful in the diagnosis of neurological conditions such as chronic demyelinating polyradiculoneuropathy (CIDP) (Kuruoglu and Oh, 1994) and Charcot-Marie-Tooth disease (García *et al.,* 2015), it is not widely utilised.

The T-reflex can be electromyographically recorded using a specially adapted tendon hammer in conjunction with commercially available electromyography machines. The resulting trace can be displayed on a monitor and objectively evaluated for various parameters using commercially available software. A mechanical tap on the tendon delivered with this hammer stimulates the T-reflex and, simultaneously, initiates the recording cycle. The resulting compound muscle action potential (CMAP) is recorded by a surface recording electrode (G1) and a reference electrode (G2) placed at standardised locations on the target muscle. The resulting CMAP can be quantified with regards to various parameters such as latency, conduction velocity, duration and amplitude.

Conventional NCS and EMG have proven very useful when confirming the diagnosis of many polyneuropathies such as Guillain-Barré syndrome (GBS), which is also referred to as acute demyelinating polyradiculoneuropathy (AIDP). In contrast, research into the utility of the T-reflex in the diagnosis of certain polyneuropathies, such as GBS, remains limited.

This prospective study examined the neurophysiological T-reflex in a cohort (n=14) of adult patients in the early stages of GBS as compared with a cohort of age- and gender-matched normal controls (n=14). The primary findings of this study are that the T-reflex is simple to perform, produces easily interpretable and reproducible results in adult control patients and is sensitive for the identification of early electrophysiological changes which occur in the neural pathways of GBS patients. However, although this study confirmed that neurophysiological T-reflex analysis is sensitive in identifying early neuropathic changes in GBS patients, it lacked specificity with respect

to identifying the various subtypes of GBS, and especially distinguishing between the demyelinating vs. axonal forms of this disease. Nevertheless, this study suggests that the use of T-reflex analysis may be useful in conjunction with conventional NCS and EMG in the diagnosis and assessment of patients with suspected GBS, particularly where NCS and EMG may be difficult to perform, such as in an Intensive Care Unit environment.

1.1. Relevance of study

Although several neurophysiological tools may be employed to investigate peripheral nerve pathways, conventional NCS are probably the most widely used. However, because NCS require the direct application of multiple small electric shocks over peripheral nerves, this causes discomfort in many patients undergoing the test. In contrast, T-reflex analysis, which typically involves a tap on the relevant tendon, is much better tolerated. Furthermore, with the exception of F-response testing, standard NCS are limited to the assessment of distal segments of motor and sensory pathways, whereas the T-reflex assesses integrity of the entire sensory-motor reflex pathway.

As mentioned, the clinical observation of decreased or absent tendon reflexes is key to confirming the diagnosis of GBS. However, clinical assessment of tendon reflexes may be subject to considerable inter-observer variation (Lani, 1990). Electromyographic recording of the T-reflex provides additional information of both proximal and distal peripheral nerve function, which is easily reproducible, diagnostically useful and arguably more objective. Although previous research has compared the T-reflex in normal subjects vs. subjects with various pathologies affecting peripheral nerves, information in the literature regarding the T-reflex in patients with GBS is scant. Furthermore, standardised protocols do not exist and there is little consensus on how best to

perform electrophysiological T-reflex testing. In particular, there is some disagreement (Péréon *et al.,* 2004; Boët *et al.,* 2013) as to how best to calculate the conduction velocity of the T-reflex.

1.2. Aims

The primary aim of this study was to describe the neurophysiological features of the T-reflex in GBS patients vs. healthy control subjects in terms of measurable parameters such as latency, amplitude, duration and conduction velocity in order to identify those parameters which may be useful for further investigation.

1.2.1. Objectives

The objectives of this study included the following:

- Produce normative (control) data from healthy subjects age and gender matched to the GBS cohort and compare this with normative data published in the literature
- Within the GBS cohort, determine the relative proportions of demyelinating, axonal and equivocal forms of GBS using classification guidelines published in the literature
- Compare the usefulness of electrophysiological T-reflex testing vs. conventional NCS in patients with GBS and in differentiating between various subtypes of GBS (e.g. demyelinating, axonal, etc.)
- Evaluate a novel method of calculating the T-reflex conduction velocity. Determine whether or not abnormalities in T-reflex responses occur earlier in the course of GBS than abnormalities in conventional NCS

CHAPTER 2:

LITERATURE REVIEW

Guillain-Barré syndrome (GBS), also known as acute inflammatory demyelinating polyradiculoneuropathy (AIDP), was first described in 1916 by French neurologists Georges Guillain, Jean-Alexander Barré and Andre Strohl (Hughes and Cornblath, 2005). For many years, the underlying pathogenesis of GBS was regarded as exclusively due to demyelination of peripheral nerves. However, more recently, a primary axonal form of GBS has been identified. This is referred to as acute motor axonal neuropathy (AMAN) (Uncini and Kuwabara, 2012).

Although the incidence and prevalence of GBS in South-Africa have not yet been published, a combined regressive model based on North American and European populations suggests the incidence of GBS progressively increases with age from 0.62 per 100 000 children younger than 9 years of age to 2.66 per 100 000 adults older than 80 per year (Sejvar *et al,*. 2011).

2.1. Pathogenesis of Guillain-Barré syndrome

The pathogenesis of GBS is thought to involve auto-antibodies directed against antigens expressed in the myelin covering peripheral nerves. These auto-antibodies may be the result of “molecular mimicry” in which an immune response induced by a preceding infection, such as Campylobacter Jejuni, Cytomegalovirus, Epstein Barr virus or Mycoplasma pneumonia, produces circulating antibodies which cross-react with myelin-related antigens on the surface of peripheral nerves (Hughes and Cornblath, 2005).

2.2. Diagnosis and clinical features of GBS

A diagnosis of GBS is based on the patient's presenting clinical features (symptoms and physical signs) and is supported by electrodiagnostic and laboratory test results. GBS typically presents as roughly symmetrical ascending weakness starting in the feet and distal lower limbs. This steadily progresses over hours, days or weeks to also affect upper limbs and motor cranial nerves. Bulbar muscles may also be affected. Facial weakness is common and extraocular movements may occasionally be involved. In severe cases, complete tetraplegia and respiratory failure may occur. Hypo- or areflexia is commonly seen early in the course of the disease (Albers and Kelly, 1989). Importantly, respiratory function should be carefully monitored using serial vital capacity measurements, as ventilator support may be required. Minor sensory symptoms and signs are often associated. Dysautonomia, including cardiac dysthymias and labile blood pressure, are common. Urinary retention and ileus are unusual but well documented.

The maximum severity of symptoms and signs (nadir) generally occurs within 4 weeks of onset and, by convention, where progression of clinical features persists beyond 8 weeks, a diagnosis of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is regarded as more appropriate (Albers and Kelly, 1989; Sander and Latov, 2003, Van den Bergh and Piéret, 2004).

Clinical features typically required to make the diagnosis of GBS include lower motor neuron symptoms and signs such as hypotonic weakness affecting more than one limb, hyporeflexia / areflexia and progression of symptoms over fewer than eight weeks. Features that support the diagnosis may include relative symmetry of symptoms and signs, associated mild sensory symptoms and signs affecting the peripheries (toes and fingers), motor cranial nerve involvement, relative sparing of sphincter involvement and absence of fever or other constitutional symptoms. Notably, a history of a respiratory or diarrheal illness 10 to 14 days preceding onset of the clinical features of GBS symptoms from which the patient has fully recovered also supports the diagnosis

of GBS (Hughes and Cornblath, 2005; Doorn *et al.,* 2008; Sejvar *et al.,* 2011; Walling and Dickson, 2013).

Analysis of cerebrospinal fluid (CSF) in patients with GBS characteristically reveals raised protein concentration in the absence of inflammatory cells, although this increase in protein may be delayed for some days after the onset of symptoms. Up to 5 lymphocytes are acceptable but polymorphs should not normally be present. Where polymorphs or more than 5 lymphocytes are present in the CSF, infectious and other causes should be excluded before a diagnosis of GBS is made. In South Africa, HIV testing should always be performed as the immune dysregulation associated with HIV infection has been shown to increase the risk of developing GBS, especially at the time of HIV-seroconversion (Brannagan and Zhou, 2003; Wagner and Bromberg, 2007).

Pooled intravenous immunoglobulin or plasma exchange administered early (within two weeks of onset of GBS symptoms) has been shown to reduce the risk of ICU admission and ventilation, and also to reduce the duration of in-patient admission (Cornblath and Hughes, 2009). It is important to diagnose GBS early in the course of the disease so that appropriate treatment can be initiated as soon as possible after onset of symptoms, the use of electrodiagnostic tools (e.g. NCS and EMG) to support the clinical diagnosis may be extremely helpful (Gordon and Wilbourn, 2001; Albert *et al*., 2011).

Over the past decade, significant advances have been made in understanding the neurophysiological abnormalities and immunopathology present in peripheral nerves of patients with GBS and this has led to a number of discrete subtypes of GBS being identified (Uncini and Kuwabara, 2012). These subtypes include the typical demyelinating form (AIDP), an acute motor axonal neuropathy (AMAN), acute motor-sensory axonal neuropathy (AMSAN), a pure acute large fibre sensory neuropathy, acute pan-dysautonomia and even forms which may involve the central nervous system such as Bickerstaff encephalitis and Miller-Fisher syndrome, amongst others

(Vucic *et al,.* 2009). The axonal forms of GBS (e.g. AMAN) tend to evolve more rapidly, are more severe and are associated with a poorer prognosis than the more common demyelinating forms (Uncini and Kuwabara, 2012). Furthermore, patients with AMAN more often develop respiratory decompensation necessitating intubation and mechanical ventilation (Uncini and Kuwabara, 2012).

2.3. Electrodiagnosis of GBS

Standard NCS are frequently helpful when making the diagnosis of GBS. However, it is well recognised that it may be as long as 10 days after the onset of GBS symptoms before electrophysiological abnormalities become apparent when using conventional NCS (Gordon and Wilbourn, 2001; Uncini *et al*., 2010; Uncini and Kuwabara, 2012; Yuki *et al*., 2012, Rajabally *et al*., 2015). A possible explanation for this is that standard motor and sensory NCS primarily evaluate the distal nerve segments and not the proximal spinal motor roots where demyelination in GBS may initially occur. Furthermore, even F-responses, which do assess proximal nerve roots, may only be affected when sufficient damage has occurred to those proximal motor roots (Mauricio *et al*., 2014).

In 1985, Albers *et al.* defined electrodiagnostic criteria which support the diagnosis of GBS as: a) decreased conduction velocity, b) increased distal latency, c) temporal dispersion of motor signals, e) the presence of a conduction block, and f) prolonged F-responses. These diagnostic criteria were later amended to include clear limits for conduction velocity, distal latency, conduction blocks and the F-response latency, as well as more specific criteria for temporal dispersion (Asbury and Cornblath, 1990). Electrodiagnostic criteria now also exist for AMAN, although final consensus on these has yet to be established (Ho *et al*., 1995; Hadden *et al*., 2004). Ho *et al.* (1995) recommend that there should be reduced distal signal amplitude involving at least two motor nerves without any evidence of demyelination for the diagnosis of AMAN, while Hadden *et al.* (1998) propose allowing

at least some features of demyelination. The diagnostic criteria proposed by Ho *et al.* (1995) and Hadden *et al.* (2004) are currently the most widely used electrodiagnostic criteria for differentiating between the demyelinating and axonal forms of GBS in both research and clinical practice.

Rajabally *et al.* (2015) propose that, by adapting the criteria of Hadden *et al.* (2004) and Ho *et al.* (1995), a single electrophysiological study rather than serial studies may be sufficient to allow accurate diagnosis of GBS and its subtypes. Rajabally's modified criteria were developed using sensitive and specific cut-off values for demyelination, as well as by incorporating new knowledge regarding electrophysiology of GBS. Rajabally's adapted criteria may establish a high specificity for differentiating between demyelinating and axonal polyneuropathy (Rajabally *et al*., 2015).

2.4. Electromyographic recorded deep tendon reflex (T-reflex)

Evaluation of spinal reflexes is diagnostically useful when assessing function of the peripheral nerves and spinal cord. These spinal reflexes can be assessed clinically or electromyographically and include the Hoffman-reflex (H-reflex) and T-reflex (Kim and Yoon, 2003). While clinical evaluation of a tendon reflex performed by a neurologist is subjective, electromyographic recording of this reflex may allow more objective assessment of parameters such as latency and quantification of the response.

Both H- and T-reflexes depend on the monosynaptic projections of the 1a afferent sensory fibres onto alpha-motor neurons in the spinal cord (Seynnes *et al.,* 2008). However, the T-reflex has an advantage over the H-reflex in that mechanical stimulation rather than electrical stimulation is used to obtain the reflex muscle response. Thus, the T-reflex may be regarded as causing less discomfort to fatigued or pain-sensitive patients than the H-reflex, and some even regard it as more practical and easier to perform (Kuruaglu and Oh, 1994).

T-reflexes are stimulated mechanically using a specially adapted tendon hammer. As the hammer strikes the tendon, it triggers the start of the recording via a micro switch embedded in the head of the hammer. The resulting muscle response is recorded from surface electromyographic (EMG) electrodes placed over the relevant target muscle. In this way the latency (in ms) and amplitude (in mV) of a tendon reflex response can be determined (Péréon *et al*., 2004). The tap delivered by the hammer on a tendon produces a brief stretch of the muscle associated with that tendon which, in turn, activates the muscle spindle sensory nerve endings (stretch receptors) within that muscle. This induces action potentials in peripheral afferent 1a sensory fibres, which travel to, and enter, the spinal cord via the dorsal spinal roots. In the cord, the afferent 1a sensory axons synapse directly with, and generate action potentials in, the efferent α-motor neurons which innervate the relevant target muscle, thus completing the reflex circuit (Pierrot-Deseilligny and Mazevet, 2000).

Recorded T-reflex responses typically have bi- or triphasic morphology, depending on electrode placement and the muscle involved. These represent compound muscle action potentials (CMAPs) which are usually quantified in terms of amplitude and latency, duration and conduction velocity (Péréon *et al*., 2004).

Of note, corticospinal and other supra-spinal pathways modulate the T-reflex responses in terms of both amplitude and latency. Furthermore, several protocols have been proposed for eliciting the T-reflex in previous research (see Table 2.1), which vary with regards to the subject's position and electrode placement etc. At present, no widely accepted standardised protocol exists for performing the T-reflex and this, in turn, limits comparison and correlation between studies (Voerman *et al.,* 2005). This issue is dealt with in more detail below.

2.5. Recording the T-reflex

Review of protocol recommendations for performing the T-reflex published in the literature reveals significant differences with respect to the generation and recording of this reflex. These include numerous variations regarding equipment used, the positioning of the subjects and which T-reflex parameters should be measured. Table 2.1 and Table 2.2 summarise some of these differences in 25 relevant papers dealing with the T-reflex. Correlation of results between papers is difficult. Similarly, comparison and appropriate use of previously published normal values for the T-reflex are problematic due to differences which exist in the reflex hammers used (commercially available or self-made), populations of control subjects and methodological inconsistencies such as using measurements from both legs of the same person (Frijns *et al*., 1997).

Despite the differences summarised above, there appears to be general consensus regarding the following technical parameters when recording the T-reflex: a) the time base should be set to 5ms, b) the sensitivity set to 5mV/division, c) the band pass filter set to 10Hz and d) the digitised sampling rate should be at least 2000 Hz (Ozmerdivenli *et al.,* 2002). Various specially adapted reflex hammers are available including a hammer with a spring contact, a hammer with a micro switch and a hammer with a piezo-electric element (Frijns *et al*., 1997). Depending on which hammer is used, the delay from hammer tap to the start of the sweep recording varies and this may explain some differences in the T-reflex latencies reported in previous studies.

In 5 of the studies referred to below, the T-reflex was performed with the subject in the prone position; in 8 studies the subjects were tested supine, in 2 studies standing upright and in 7 studies sitting on an adjustable chair. Many studies did not report the subjects' position. In addition, researchers used several methods to elicit the T-reflex, including various specially adapted hammers and pendulums. Furthermore, they variously observed T-reflex responses electromyographically and by means of video, accelerometers and goniometers.

Table 2.1. An overview of normal responses in various standard T-reflexes published in the literature, excluding articles in which T-reflexes were quantified according to degree of flexion at a joint.

Author	n	Muscles	Normal latency (ms)	Normal amp (mV)	CV (m/s)*
Kuruaglu and Oh (1994)	40	Medial Gastrocnemius	32.1 (3.0)	3.0 (2.0)	
		Rectus femoris	17.2 (2.0)	1.4 (0.9)	
Frijns *et al.* (1997)	102	Rectus femoris	21.0 (1.5)	1.77 (1.2)	
		Soleus	35.2 (2.6)	4.05 (2.1)	
Morita *et al.* (1998)	17	Soleus	38.2 (2.6)		
Ozmerdivenli *et al.* (2002)	20	Gastrocnemius	31.97 (1.11)	3.1 (0.2)	
Kim and Yoon (2003)	50	Soleus	31 (2.20)	4.30 (3.30)	
Maffiuletti *et al.* (2003)	6	Soleus		3.6 (2.2)	
	6	Gastrocnemius		2.1 (1.2)	
Péréon *et al.* (2004)	60	Rectus femoris	19.9 (1.7)		67.8 (3.6)
	78	Soleus	33.0 (2.5)		61.2 (2.6)
	23	Triceps	14.0 (1.0)		65.1 (3.1)
	23	Biceps	13.4 (0.9)		65.4 (3.0)
	7	Flexor carpi radialis	20.2 (1.5)		58.6 (2.6)
Ertekin *et al.* (2006)	12	Adductor magnus	16.2 (0.5)	0.6 (0.1)	
	12	Quadriceps	19.1 (0.5)	1.2 (0.2)	
Ališauskiene *et al.* (2007)	100	Vastus medialis	22.4 (2.1)	32.7 (5.2)	
Grosset *et al.* (2007)	55	Gastrocnemius			
Sharma *et al.* (2007)	38	Biceps brachii	8.9 (1.9)	1.5 (1.1)	
		Rectus femoris	17.4 (2.4)	0.6 (0.6)	
		Gastrocnemius	30 (2.4)	2.1 (2.7)	

**CV=Conduction velocity, first standard deviation in parenthesis where available*

Table 2.1(cont.). An overview of normal responses in various standard T-reflexes published in the literature, excluding articles in which T-reflexes were quantified according to degree of flexion at a joint.

Author	n	Muscles	Normal latency (ms)	Normal amp (mV)	CV (m/s)*
Seynnes *et al.* (2008)	8	Soleus		0.2 (0.0)	
		Gastrocnemius lateralis		0.3 (0.1)	
Tataroglu *et al.* (2009)	20	Vastus medialis	22.4 (1.9)		
Nikolaev (2010)	20	Biceps	17 (1.3)		
	20	Carporadialis	18.8 (1.4)		
Min *et al.* (2012)	21	Biceps brachii	15.6 (2.0)	4.6 (1.7)	
Boët *et al.* (2013)	50	Soleus		7.1 (0.7)	60.7
Dafkin *et al.* (2013)	15	Extensor quadriceps	57.6 (19.5)		
Tetsunaga *et al.* (2013)	80	Biceps brachii	16.1 (1.7)	0.7 (0.4)	
		Brachioradialis	20.7 (1.6)	0.2 (0.2)	
		Triceps brachii	17.1 (1.6)	0.4 (0.2)	
		First dorsal interosseous	32.6 (3.8)	0.2 (0.1)	
Uysal *et al.* (2014)	25	Quadriceps femoris	17.9 (1.9)	6.4 (2.9)	
García *et al.* (2015)	28	Biceps brachii	12.9		
Gürbüz *et al.* (2015)	25	Patella	17 (1.9)	6.4 (2.9)	
Pope and Defreitas (2015)	30	Rectus femoris		0.4 (0.3)	
Yong-Wook (2015)	50	Rectus femoris		0.7 (0.3)	
Karacan *et al.* (2016)	15	Soleus		1.1 (0.5)	
Mildren *et al.* (2016)	15	Soleus		1.9 (0.4)	

**CV=Conduction velocity, first standard deviation in parenthesis where available*

Table 2.2(a). Placement of recording electrodes for upper limb reflexes by various researchers.

Reflex	Author	Active electrode (G1) Placement	Reference electrode Placement
Biceps brachii	Péréon *et al.* (2004)	Midway along a line drawn between elbow and shoulder	Equidistant between the active electrode (G1) and the elbow
	Sharma *et al.* (2009)	Halfway between the acromial process and elbow crease	4 cm distal to the active electrode (G1)
	Nikolaev (2010)	Centre of biceps brachii muscle	Not specified
	Min *et al.* (2012)	Midway along the biceps brachii muscle	5 cm distal to active
	Tetsunaga *et al.* (2013)	Over the motor point of the target muscle	Over the lateral epicondyle of the humerus
	García *et al.* (2015)	Belly of biceps brachii muscle	3-5 cm proximal to the biceps brachii muscle
Brachio-radialis	Nikolaev (2010)	Not specified	Not specified
	Tetsunaga *et al.* (2013)	Over the motor point of the target muscle	Over the lateral epicondyle of the humerus
Triceps	Tetsunaga *et al.* (2013)	Over the motor point of the target muscle	Over the olecranon
	Péréon *et al.* (2004)	Midway between elbow and shoulder	Equidistant between the active electrode (G1) and the elbow

cm = centimetre

Accelerometers were typically fitted to the reflex hammer in order to quantify the force of the stimulation (Tetsunaga *et al.,* 2013), with goniometers and EMG electrodes placed over the relevant muscle to measure the resulting T-reflex response (Chandrasekhar *et al.,* 2013). For example, in the case of ankle T-reflexes, electromyographic recordings were measured from G1 electrodes placed over soleus- or gastrocnemius muscles. Responses were measured over the quadriceps muscle for the patella (knee) T-reflex, and over the triceps brachii and biceps brachii muscles of the upper limbs. Previous studies have concluded that there is no significant difference in T-reflex responses between the right and left side (Kim and Yoon, 2003). An overview of published normal responses of the T-reflex in the literature is summarised in Table 2.1.

Table 2.2(b). Placement of recording electrodes for lower limb reflexes by various researchers.

Reflex	Author	Active electrode (G1) Placement	Reference electrode Placement
Patellar reflex	Kuruaglu and Oh (1994)	On the rectus femoris muscle, halfway between the anterior superior iliac spine and the superior border of the patella	5 cm distal to the active electrode (G1)
	Frijns *et al.* (1997)	Belly of the rectus femoris muscle midway along a line connecting the anterior superior iliac spine with the upper margin of the patella	5 cm distal to the active electrode (G1)
	Péréon *et al.* (2004)	Midway between the anterior superior iliac spine and the upper margin of the patella	Equidistant between the active electrode (G1) and the patella
	Ertekin *et al.* (2006)	Vastus medialis (Needle)	Vastus medialis (Needle)
	Ališauskiene *et al.* (2007)	On the thigh, along a line joining the superior edge of the patella to the contralateral anterior superior iliac spine at a distance from the superior edge of the patella equal to the quarter of the distance between the ipsilateral anterior superior iliac spine and the superior edge of the patella	On the patella
	Sharma *et al.* (2009)	On the rectus femoris muscle, half way between the anterior superior iliac spine and the superior border of the patella	5 cm distal to active electrode (G1)
	Tataroglu *et al.* (2009)	On the belly of vastus medialis	Tendon of vastus medialis just above the patella
	Dafkin *et al.* (2013)	5 cm above the superior margin of the patella	5 cm below active electrode (G1)
	Uysal *et al.* (2014)	Midway along the quadriceps femoris	Biceps femoris
	Gürbüz *et al.* (2015)	Over rectus femoris muscle	Not specified
	Pope and Defreitas (2015)	On the rectus femoris muscle	Tibial tuberosity
	Yong-Wook (2015)	Over rectus femoris muscle	On the patella

cm = centimetre

Table 2.2(b)(cont). Placement of recording electrodes for lower limb reflexes by various researchers.

Reflex	Author	Active electrode (G1) Placement	Reference electrode Placement
Soleus reflex	Kuruaglu and Oh (1994)	Over the gastrocnemius muscle equidistant from the crease of the popliteal fossa and the medial malleolus	5cm distal to the active electrode (G1)
	Frijns *et al.* (1997)	On the soleus muscle midway along a line connecting the popliteal fossa with the medial malleolus	5 cm below the active electrode (G1)
	Morita *et al.* (1998)	Over the soleus muscle	Between soleus and stimulation site
	Ozmerdivenli *et al.* (2002)	Proximal to the medial gastrocnemius muscle halfway between the midpoint of the popliteal fossa and upper border of the medial malleolus	Along the same line 5 cm distal to the active electrode (G1)
	Kim and Yoon (2003)	Midpoint along the line connecting the popliteal fossa and the medial malleolus of the tibia	5 cm distal to the active electrode (G1)
	Maffiuletti *et al.* (2003)	Medial and lateral gastrocnemius electrodes fixed lengthwise over the middle of the muscle belly	5 cm distal to where the two heads of the gastrocnemius join the Achilles tendon
	Péréon *et al.* (2004)	In line with the Achilles tendon, midway between the two ends of the fibula	Over the tendon equidistant between the active electrode (G1) and the heel (calcaneus)
	Grosset *et al.* (2007)	Over the belly of both gastrocnemius muscles	2 cm below the insertion of the gastrocnemii on the Achilles tendon for the soleus
	Sharma *et al.* (2009)	Over the medial gastrocnemius muscle half way between the popliteal fossa and the medial malleolus	5 cm distal to the active electrode (G1)
	Boët *et al.* (2013)	On the calf, posteriorly, along a line between the centre of the popliteal fossa and the Achilles tendon, equidistant between the two ends of the fibula bone	Over the tendon at an equal distance between the active electrode (G1) and the heel-bone
	Karacan *et al.* (2016)	On the medial soleus muscle belly	On the lateral malleolus
	Mildren *et al.* (2016)	Over the soleus muscle	Over tibialis anterior

cm = centimetre

Placement of recording EMG electrodes in previous studies lacks consistency. The location of electrodes is typically dependent on muscle shape, especially where a muscle is composed of several distinct capita (Péréon *et al*., 2004). The general principle is to place the active recording electrode (G1) over the belly of the target muscle and the reference electrode (G2) a few centimetres away over the tendon of that muscle (Frijns *et al*., 1997; Péréon *et al*., 2004; Chandrasekhar *et al.,* 2013; Tetsunaga *et al.,* 2013). Table 2.2 summarises the placement of the active and reference electrodes according to previous studies. In addition to the reflexes contained in Tables 2.1 and 2.2, some researchers have recorded additional T-reflexes in the forearm and thighs (Ertekin *et al.,* 2006).

2.6. Measuring the T-reflex response

The morphology of T-reflex compound muscle action potential (CMAP) response varies according to the target muscle involved. For instance, T-reflex traces recorded from rectus femoris and biceps brachii muscles are typically biphasic (Figure 2.1) whereas those recorded from soleus, triceps brachii or gastrocnemius tend to be tri-phasic (Figure 2.2. and Figure 2.3). As is the case with the standard motor nerve conduction CMAP responses, T-reflex responses are usually quantified with respect to latency, amplitude, conduction velocity and occasionally duration.

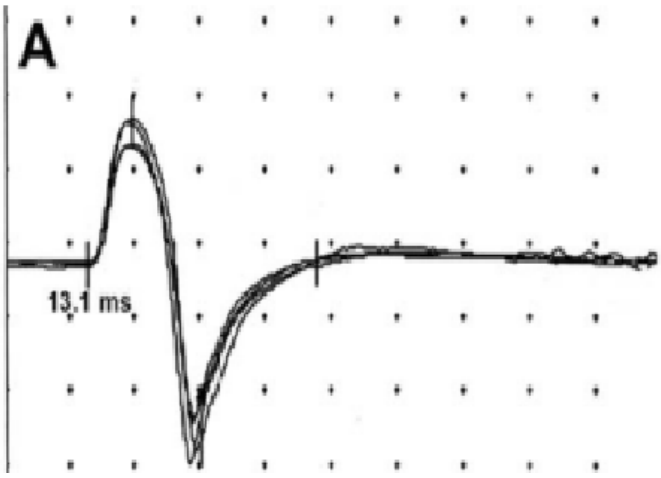

Figure 2.1. Biceps brachii T-reflex CMAP response from normal subject (adapted from García et al., 2015)

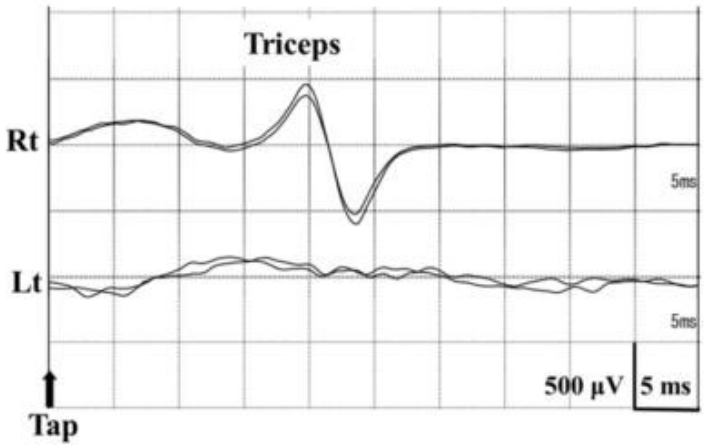

Figure 2.2. T-reflex CMAP response recorded from both triceps brachii muscles in a patient with left (Lt) C7-radiculopathy and normal response on the right (Rt) (adapted from Tetsunaga et al., 2013)

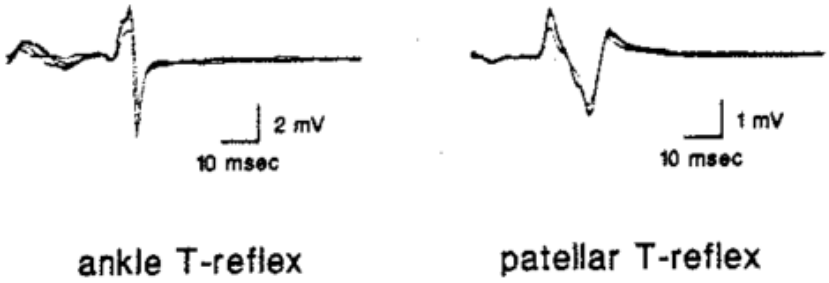

Figure 2.3. Ankle and patellar T-reflexes recorded from a normal subject (adapted from Kuruaglu and Oh, 1994)

The amplitude of the T-reflex (usually measured peak-to-peak) is related to the degree of reactive contraction of muscle and this is affected by intensity of stimulus (striking strength), muscle tone, joint position vestibular stimulation, alertness of the subject, temperature and other factors (Verhagen *et al.,* 1988; Kameyama *et al.,* 1989; Oh, 1993). In fact, for this reason, some researchers have chosen not to measure the amplitude of the T-reflex response because of its variability.

Latency of the T-reflex CMAP response is measured from the start of the recording (i.e. the time the hammer strikes the tendon) to the initial onset negativity of the CMAP response. This latency is dependent on: a) the time taken to accommodate the tendon–muscle compliance in order to launch the discharges in spindle sensors within the muscle, b) propagation of the signal along afferent sensory Ia fibres, c) monosynaptic delay within the spinal cord, d) propagation of the signal along efferent α motor neuron pathways and, e) synaptic delay across the neuromuscular junction at the target muscle (Péréon *et al*., 2004; Boët *et al.,* 2013). Considering this pathway, it is unsurprising that the latency of the T-reflex strongly correlates with a subject's height (Frijns *et al*., 1997; Ozmerdivenli *et al.,* 2002; Péréon *et al*., 2004; Ališauskiene *et al.,* 2007; Sharma *et al.,* 2007) Consequently, one approach has been to normalise the latency recorded according to height of the subject by use of the formula-latency index = (height in cm ÷ latency in ms)² (Sharma *et al.,* 2007). Other researchers have employed "side-to-side comparisons" (i.e. internal controls) in order to

identify abnormalities. Unfortunately, the side-to-side approach is unhelpful in cases of diffuse polyneuropathy (peripheral neuropathy).

Because numerous factors may influence the latency and the amplitude of the T-reflex response, Pèrèon *et al.* (2004) has suggested eliciting and displaying several T-reflex traces in cascade form in order to identify and track the most reproducible latency. Pèrèon has also validated the process of averaging several traces (with good baseline) in order to measure the amplitude of the T-reflex CMAP response.

The duration of a T-reflex response is measured by calculating the difference between the onset latency and the time when the deflection returns to baseline (Kuruaglu and Oh, 1994).

As mentioned above, conduction velocity of the T-reflex is often referenced to the height of the subject. More specifically, the distal latency and subject's height may be used to determine average peripheral nerve conduction velocity by using the following formula (Péréon *et al*., 2004):

$$PNCV = Subject's\ height(m) \times \frac{K}{Latency\ (\mathrm{sec})}$$

K is a constant derived from the correlation between the subject's height and the spine-to-active electrode distance in normal subjects. Published normal values for the T-reflex, excluding age groups younger than 10 years, are summarised in Table 2.3. Boët *et al.* (2013) referred to Péréon *et al.* (2004) but described the formula as:

$$\mathrm{CV}\ (\mathrm{ms}^{-1}) = \mathrm{Height}\ (\mathrm{m}) \,/\, \mathrm{Latency}\ (\mathrm{sec})$$

Boët *et al.* (2013) does not include a constant in his formula and does not indicate individual conduction velocities of the subjects in his study.

Table 2.3. Normal conduction velocity values according to age for the T-reflex as described by Péréon et al. (2004)

Computed proximal nerve conduction velocities according to age group (m/s, mean (S.D.)				
	K constant	**10–18 years**	**19–49 years**	**50–80 years**
Rectus femoris	8	69.0 (1.9)	67.8 (3.6)	66.7 (3.4)
Soleus	12	60.9 (3.7)	61.2 (2.6)	58.1 (2.8)
Triceps	5.4		65.1 (3.1)	64.3 (3.1)
Biceps	5.2		65.4 (3.0)	64.8 (3.1)
Flexor carpi radialis	7		58.6 (3.7)	57.4 (2.9)
T-reflex latencies according to age group (ms, mean (SD)				
		10–18 years	**19–49 years**	**50–80 years**
Rectus femoris		18.0 (1.0)	19.9 (1.7)	20.1 (1.6)
Soleus		30.6 (2.7)	33.0 (2.5)	34.1 (2.4)
Triceps			14.0 (1.0)	14.3 (1.1)
Biceps			13.4 (0.9)	13.6 (1.1)
Flexor carpi radialis			20.2 (1.5)	20.7 (1.5)

2.7. Physiological factors affecting the T-reflex response

Numerous factors influence the latency, amplitude and conduction velocity of the T-reflex recording.

2.7.1. Positioning

When recording the T-reflex, it is important to understand the salience of both body and limb positioning. The T-reflex can be reliably recorded with the subject sitting upright in an arm chair,

standing upright or lying down, either supine or prone. Each position has advantages and disadvantages. For instance, in the standing position, assessment of the ankle T-reflex during static postural activation is possible without the need to control for postural sway (Mildren *et al.,* 2016). Body positioning is less important than limb positioning when performing the T-reflex (Pope and Defreitas, 2015). Positioning of the limbs is critical as the greater the angle of the limb/muscle being tested, the greater the stretch of the relevant muscle/tendon and, consequently, the larger the mean amplitude of the T-reflex response being elicited (Yong-Wook, 2015). Chandrasekhar *et al.* (2013) have quantified the extent to which the reflex response is affected in terms of knee angles.

2.7.2. Age

Age is also an important factor influencing the T-reflex response. By way of example, the velocity and amplitude of patellar and ankle T-reflex responses increase with age from birth to 18 years and, subsequently, remain stable during adulthood until about 50 years of age (Péréon *et al*., 2004). More particularly, nerve conduction velocity of the patellar and ankle reflexes increases logarithmically from birth to 18 years of age, with a dramatic increase occurring soon after birth. Teen values occur around the age of 6 (Péréon *et al*., 2004). There is also a decline in the magnitude of reflex response with increasing age during adulthood, which is especially marked after the age of 50 (Chandrasekhar *et al.,* 2013).

2.7.3. Height

The height of a subject (which is related to limb length) is another factor which affects the T-reflex. This should be measured meticulously as there is a strong correlation between the latency of the T-reflex and the height of the subject (Kuruaglu and Oh, 1994; Péréon *et al*., 2004). Péréon *et al.* (2004), Sharma *et al.* (2009), Boët *et al.* (2013) and Kuruaglu and Oh (1994) have all used subject

height rather than limb length measurements in order to calculate T-reflex conduction velocity, which is interesting as limb length measurements are routinely used when calculating standard F-responses.

2.7.4. Pre-synaptic Inhibition

Pre-synaptic inhibition of monosynaptic reflexes was first described in a study of a cat in 1957. (Eccles *et al.,* 1962). It is reported to be mediated via inhibitory interneurons on 1a afferent pre-synaptic terminals, resulting in a reduction of the neurotransmitter release and, as a result, reduction in post-synaptic motor neuron depolarisation. This mechanism is thought to be selective enough to affect different collaterals from the same muscle spindle afferent independently (Rudomin and Schmidt, 1999). Moreover, pre-synaptic inhibition can alter afferent signals which, in turn, can lead to different patterns of modulation of reflex motor neuron excitability (Misiaszek, 2003). It is for these reasons that it is not possible to determine the level of alpha-motor neuron excitability by measuring the amplitude of an electromyographic recorded reflex.

Importantly, pre-synaptic inhibition, which suppresses T-reflex activation, is affected by many factors including afferent feedback from other peripheral receptors and descending supra-spinal signals (Zehr and Stein, 1999). It can also be increased by stimulation of the nerves supplying the relevant antagonist muscle. With regard to the soleus muscle, for example, pre-synaptic inhibition may be observed when the peroneal nerve, which innervates the ipsilateral tibialis anterior muscle, is stimulated (Zehr, 2002). Pre-synaptic inhibition can also be induced by afferent activation (Karacan *et al.,* 2016). A simple change in leg posture is sufficient to alter the level of pre-synaptic inhibition and the effects of pre-synaptic inhibition can be reduced by performing the Jendrassik maneuver (Misiaszek, 2003; Passmore and Bruno, 2012).

2.7.5. Post-synaptic depression

The activity at the synapse between 1a sensory afferents and α-motor neurons is an important factor affecting transmission through the reflex arc (Misiaszek, 2003). Post-activation depression results from reduced neurotransmitter release from pre-synaptic terminals that have recently been activated (Kohn *et al.,* 1997). Thus, recent activation of the 1a afferent is thought to result in reduction of available pre-synaptic neurotransmitter stores in the 1a afferent terminals. When this depletion is pronounced, the pre-synaptic neurotransmitter stores are insufficient to generate a post-synaptic action potential (Kohn *et al.,* 1997). Contracting the target muscle shortens the length of the muscle and this will activate the muscle spindle stretch receptors. This activation of stretch receptors causes inactivation of the 1a afferents, which are involved when eliciting the reflex, and suppression of the T-reflex response amplitude will result due to post activation depression (Hultborn *et al.* 1996; Pierrot-Deseilligny and Mazevet, 2000).

Passive or active shortening of the muscle may decrease latency, because the end-plate zone is shifted proximally into the limb, while the location of the active surface electrode remains unchanged. This, in turn, results in an artefactual increase in peripheral nerve conduction velocity (Uysal *et al.,* 1999). Therefore, it is critical that the limb is positioned in a normalised fixed flexion angle of the relevant joint before the active and reference electrodes are applied. Furthermore, repeated and prolonged passive muscle stretching reduces sensitivity of the muscle spindles, which results in reduced activation of the large-diameter afferents (Avela *et al.,* 1999). Interestingly, Ozmerdivenli *et al.* (2002) have shown that chronic athletic training can alter the amplitude of the T-reflex response, which may represent enhanced adaptation.

2.8. Pathological factors influencing the T-Reflex response

Initially the use of the T-reflex was limited mainly to electrophysiological assessment to assist in the diagnosis of compressive radiculopathies. Side-to-side comparisons of the latencies and amplitudes of the same T-reflex allowed confirmation of unilateral spinal root lesions (radiculopathies) with high degrees of sensitivity and specificity (Tetsunaga *et al.,* 2013). In contrast, definition of normative values of the T-reflex from healthy control groups was required in order to assess subjects for diffuse peripheral polyneuropathy.

The fact that the T-reflex is dependent on both proximal (i.e. root) and distal (i.e. peripheral nerve) function means that electromyographic recording of this reflex is likely to be useful in the assessment of demyelinating polyradiculoneuropathies which typically affect both proximal and distal nerve function. While most consensus criteria for the diagnosis of both axonal and demyelinating polyradiculoneuropathy require clinically depressed or absent deep tendon reflexes (Notermans *et al.,* 1994; Vucic *et al.,* 2009), this clinical feature is not always present, and it has been estimated that up to 35% of the patients with polyneuropathy may initially present with preserved clinical tendon reflexes (Sharma *et al.,* 2009).

Kuruaglu and Oh (1994) were two of the first researchers to investigate the use of the T-reflex in demyelinating polyneuropathies and, more specifically, in patients with acquired chronic demyelinating polyradiculoneuropathy (CIDP). They found the mean T-reflex latency of the patients with CIDP was approximately 150% that of normal controls. Although 7 out of 22 of the patients with CIDP in their study had brisk or normal reflexes, 6 of these had abnormal T-reflex responses. Consequently, Kuruaglu and Oh (1994) concluded that the T-reflex is a useful indicator of demyelinating peripheral neuropathy.

In 2009, Sharma *et al.* studied the T-reflex and its diagnostic value in patients with polyneuropathy. The study included three patient cohorts: (1) predominantly demyelinating polyneuropathies, which included acute and chronic inflammatory demyelinating polyneuropathy; (2) chronic axonal polyneuropathies; and (3) small fibre polyneuropathies. The results of their study confirmed that T-reflex latencies at all sites were significantly prolonged in patients with demyelinating polyneuropathy compared with patients with chronic axonal polyneuropathy or small fibre polyneuropathy. They concluded that the T-reflex is more sensitive than the H-reflex in distinguishing axonal from demyelinating polyneuropathy (Sharma *et al.,* 2009).

More recently, in 2015, García *et al.* analysed the utility of the T-reflex in Charcot-Marie-Tooth (CMT) disease type 1a, which is an autosomal dominant form of inherited demyelinating polyneuropathy. The majority (72.9%) of the 62 adult patients with this condition in their study had absent bicep tendon reflexes. Nevertheless, the T-reflex was unrecordable in only 4 patients. In other words, T-reflex responses with increased distal latency and reduced conduction velocity could be recorded in the CMT1a patients who were clinically areflexic. Similarly, this study showed that electrophysiological T-reflex testing could identify features of demyelinating polyneuropathy in subjects with clinically preserved tendon reflexes. Garcia *et al.* concluded that the T-reflex is a simple but effective technique for identifying the demyelinating polyneuropathy associated with CMT1a and that it may be useful in patients who are unable to tolerate the electrical stimulation associated with standard nerve conduction studies (García *et al.,* 2015).

The utility of the T-reflex is not limited to evaluation of efferent motor pathways. For instance, T-reflex latencies are significantly prolonged in patients with small fibre polyneuropathy, which suggests possible sub-clinical involvement of large myelinated sensory fibres (Sharma *et al.,* 2007). Brachioradialis and biceps T-reflex latency increase reliably in C7-C8 sensory radiculopathy and C5-C6 sensory radiculopathy respectively (Nikolaev, 2010). This implies that the T-reflex may

be a useful method to study sensory or motor root conduction separately where pathology is limited to sensory or motor root components.

The amplitude of the T-reflex is influenced by both central (e.g. synaptic efficiency) and peripheral factors (Ertekin *et al.,* 2006). Nevertheless, the T-reflex represents an effective tool for quantifying spasticity. For example, adductor muscle spasticity is a frequent complication in patients with upper motor neuron pathology such as stroke. The effect of any treatment (invasive or non-invasive) for adductor spasticity can be easily monitored with acceptable inter-and intra-observer reliability (Min *et al.,* 2012) by evaluating the adductor T-reflex (Ertekin *et al.,* 2006). Furthermore, a double peak morphology of the T-reflex CMAP ("notching phenomenon") has been observed in the biceps reflex of patients with spasticity affecting this muscle and it has been suggested that this notching phenomenon may represent a biological marker of spasticity (Gürbüz *et al.,* 2015).

As the preceding review of the literature shows, a good deal is now known about the physiology of the T-reflex and the factors, both physiological and pathological, which affect it. It is perhaps surprising, therefore, that the T-reflex has not been utilised more widely as a tool to assist in the diagnosis of polyneuropathies, mononeuropathies and radiculopathies. This study seeks to describe changes in the parameters which occur in T-reflexes in subjects with Guillain-Barré Syndrome/acute demyelinating polyradiculopathy (GBS), and thus the utility of the T-reflex in patients with this condition.

CHAPTER 3:

METHODOLOGY

3.1. Study Location

The study took place in the Neurophysiology Laboratory (E8) at Groote Schuur Hospital (GSH). GSH is a large tertiary referral hospital located in Cape Town, in the Western Cape Province of South Africa.

3.2. Study Design

A prospective, controlled, non-blinded, cohort study was performed after ethics approval was granted by the University of Cape Town's Human Research Ethics committee (attached in APPENDIX A). Data was collected according to the layout of the study design in Figure 3.1.

Between March 2016 and September 2017 (recruitment period), all patients with clinically suspected GBS were prospectively and sequentially recruited into the study cohort at the time of their referral to the Division of Neurology at GSH for clinical and electrophysiological evaluation.

T-reflex (latency, duration, conduction velocity and amplitude of the T-reflex response) and standard NCS (motor conduction velocity, motor distal latency, motor signal amplitude, and latency of the F-response) testing were performed on all enrolled subjects within two weeks of onset of GBS symptoms. Informed consent included permission to undergo sequential electrophysiological testing.

During the recruitment period, age and gender matched subjects were also recruited into a control cohort. T-reflexes were recorded to determine normative values, and NCS was performed to ensure that none of the control subjects has subclinical neuropathy or radiculopathy. Statistical analysis commenced after recruitment of both cohorts were completed.

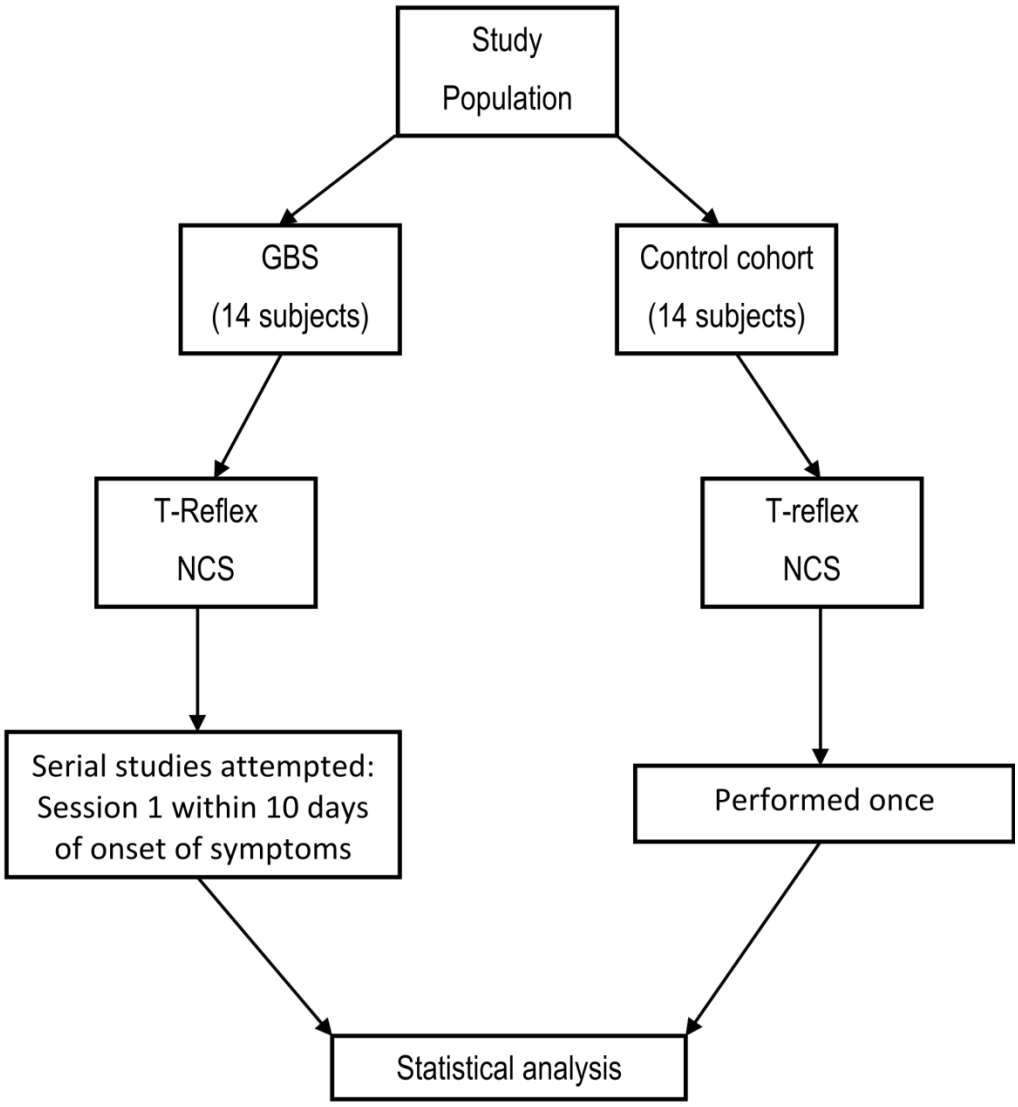

Figure 3.1. Layout of study design

3.3. Study population

3.3.1. Study group

The study population from which the study cohort was recruited was confined to adult patients referred to Groote Schuur Hospital in Cape Town. Control subjects were demographically matched as far as possible with respect to age and gender.

The GBS cohort consisted of patients in whom a provisional clinical diagnosis of Guillain-Barré syndrome / AIDP had been made and who had been referred to the GSH Neurophysiology Laboratory for electrophysiological evaluation. Some of the subjects enrolled into the GBS cohort also participated in the International Guillain-Barré Syndrome Outcome Study (IGOS) (HREC reference: 267/2014) which was being conducted concurrently at UCT/Groote Schuur Hospital.

NCS on the GBS cohort were performed according to best clinical practice at the GSH Neurophysiology Laboratory, and the results of these studies were also collected prospectively for the IGOS study.

3.3.2. Control group

The control cohort consisted of healthy adult volunteers who were not known to have any neurological- or medical conditions associated with neuropathy.

3.4. Inclusion and exclusion criteria

3.4.1. Inclusion Criteria

Adult patients (18 years and older) of both genders were eligible for inclusion in the study and control cohorts, subject to the inclusion and exclusion criteria outlined below.

3.4.1.1. Guillain-Barré Syndrome

Inclusion criteria for enrolment to the GBS cohort were all adult patients referred to the GSH Division of Neurology and admitted to GSH with a provisional clinical diagnosis of GBS made by an attending specialist neurologist (either axonal or demyelinating forms), and with onset of GBS symptoms less than two weeks prior to enrolment. Requirements for the clinical diagnosis of GBS were progressive weakness affecting more than one limb with abnormal clinical lower motor neuron signs (e.g. reduction in muscle tone, hypo/areflexia), with or without bulbar involvement, and with or without mild sensory symptoms/signs. Cerebrospinal fluid analysis which showed raised protein concentration but no inflammatory cells strengthened the clinical diagnosis in many of the subjects enrolled in the study group, but was not a requirement for inclusion.

3.4.1.2. Control cohort

The inclusion criteria for the Control cohort included subjects 18 years or older with no known neurological deficits or medical conditions associated with neuropathy, age and gender matched to subjects in the study cohort.

3.4.2. Exclusion criteria

3.4.2.1. Guillain-Barré Syndrome Cohort

Patients with depression of consciousness and other subjects unable to provide informed consent were excluded, as were patients known to be infected with drug-resistant tuberculosis.

Subjects enrolled in the GBS cohort were also excluded if the attending neurologist subsequently changed their provisional diagnosis of GBS during the course of their admission to hospital. Patients excluded by this means was replaced by recruiting additional subjects with GBS.

Other exclusion criteria included confirmed or suspected alcohol or drug abuse and patients with known polyneuropathy predating the onset of GBS symptoms. Patients with the Miller Fisher or Bickerstaff's Encephalitis variants of GBS were also excluded.

3.4.2.2. Normal Control Cohort

Subjects known to suffer from any neurological conditions or medical conditions known to be associated with neuropathy (e.g. diabetes, abuse of alcohol, exposure to neurotoxins, autoimmune illnesses, etc.); or with an abnormal neurological examination were excluded from the control cohort.

3.5. Research procedures and data collection methods

3.5.1. Nerve conduction studies (NCS)

Standard electrodiagnostic NCS were performed according to the best practice protocols routinely used in the GSH Neurophysiology Laboratory as detailed below. The skin temperature of the appropriate limb was maintained above 32 degrees celsius for each respective study.

3.5.1.1. Sensory nerve conduction studies

The median and ulnar sensory nerves were evaluated orthodromically, while superficial peroneal and sural nerves were evaluated antidromically.

3.5.1.1.1. Median sensory

The stimulation site was between the metacarpal bones of the second and third digit on the dorsal palm (palmer sensory studies). The active electrode (G1) was placed 8 centimetre (cm) proximal to the stimulation site on a line between the tendon of flexor carpi radialis and the tendon of flexor pollicis longus, while the reference electrode (G2) was placed on the same line 3.5cm proximal to the active electrode (G1).

3.5.1.1.2. Ulnar sensory

The stimulation site was situated on the palm of the hand between the metacarpal bones of the fourth and fifth digits. The active electrode (G1) was placed 8cm proximal to the stimulation site

along the line of the tendon of flexor carpi ulnaris and the tendon of palmaris longus, while the reference electrode (G2) was placed on the same line 3.5cm proximal to G1.

3.5.1.1.3. Sural sensory

The active electrode (G1) was placed half way along a line between the lateral malleolus and the heel, while the recording electrode (G2) was placed 3.5cm distal to the active electrode (G1) along a line drawn towards the fifth metatarsal bone. The stimulation site was 14cm proximal to the active electrode on a line extended from the active electrode and the head of fibula.

3.5.1.2. Motor nerve conduction studies

All motor nerves were recorded orthodromically, with the active electrode (G1) placed at the appropriate site over the belly of the target muscle and the reference electrode (G2) placed just distal to the active electrode. The following motor nerves were recorded: median, ulnar, tibial, and peroneal.

3.5.1.2.1. Median motor

The active (G1) electrode was placed over the belly of abductor pollicis brevis and the reference electrode (G2) placed on the metacarpophalangeal joint of the thumb. The stimulation site was 8cm proximal to the G1 electrode between the tendon of flexor carpi radialis and the tendon of flexor pollicis longus. The proximal stimulation site was at the antecubital fossa.

3.5.1.2.2. Ulnar motor

The active recording electrode (G1) was placed over the belly of the abductor digiti minimi muscle and the reference electrode (G2) on the metacarpophalangeal joint of the fifth digit. The distal stimulation site was 8cm proximal to the G1 electrode between the flexor carpi ulnaris tendon and the palmaris longus tendon.

The proximal stimulation site was 3-4cm distal to the medial epicondyle and 3-4cm proximal to the medial epicondyle respectively.

3.5.1.2.3. Peroneal motor

The recording electrode (G1) was placed over extensor digitorum brevis and the reference electrode (G2) on the metatarsophalangeal joint of the fifth digit. The distal stimulation site is 8cm proximal to the recording electrode, adjacent to the fibula bone. The proximal stimulation sites are immediately proximal and immediately distal to the fibular head.

3.5.1.2.4. Tibial motor

The recording electrode (G1) was placed over the abductor halluces muscle, at the point 1cm medial and 1cm distal to the navicular bone. The reference electrode (G2) was placed over the mataphalangeal joint of the great toe. The distal stimulation site was 8cm proximal to the active electrode, ventral to the medial malleolus. The proximal stimulation site was in the popliteal fossa.

3.5.1.2.5. F-response

The placement of the recording electrodes (G1 and G2) was the same as the motor nerve conduction studies for a specific nerve. The stimulation site was the same as the distal stimulation site for the equivalent motor nerve conduction study, except that the anode of the stimulator is arranged proximal to the cathode. Ten sequential stimulations were recorded and the first repeatable, reliable latency was marked.

3.5.2. Electromyographically recoded deep tendon reflex (T-reflex)

The T-reflex is not routinely performed at the Neurology Laboratory in Groote Schuur Hospital. Consequently, this was performed according to protocols described by other researchers (Sharma *et al.,* 2009; Péréon *et al*., 2004). The commercially available electronic tendon (reflex) hammer was acquired from SleepNet/BreathNet in association with Natus neurology (part number: 842-116700).

The biceps brachii, triceps brachii, rectus femoris and soleus T-reflexes were recorded unilaterally on the least affected side in GBS subjects and on one side in control subjects. Subjects were placed in a supine position during the recording of all T-reflexes. Where necessary, electrode placements were adjusted to mitigate initial positivity of the T-reflex response. Repeated stimulation (tendon taps) of the same T-reflex were performed at random time intervals (in order to avoid the effect of anticipation) and the results were averaged. A minimum of 3 stimulations were required, although the ideal number of stimulations for each T-reflex is estimated to be ten. A maximum of 12 tendon taps were performed unless the patient complained of discomfort, in which case this number was reduced.

The patient's height and limb lengths were measured. Upper limb length was measured from the placement of the active electrode (G1) of each respective T-reflex to the suprasternal notch. Lower limb length was measured from the placement of the active electrode (G1) of each respective T-reflex to the xiphoid process.

Several recordings of each T-reflex were made. These were superimposed onto one another and an average computed digitally before the various parameters such as latency, duration, amplitude and conduction velocity etc. were calculated (Figure 3.2). Latency and duration were measured using the superimposed T-reflex responses. Latency was measured using a point from where the onset of the first negative deflection of the T-reflex response most frequently occurred. The duration of the T-reflex response was measured from the same point of first negative deflection of the T-reflex response (onset latency) to the point where this deflection returned to baseline. Amplitude was measured using the computed average T-reflex responses, from the most negative peak to the most positive peak. Figure 3.2. Illustrates the marking and measurements of a recorded reflex.

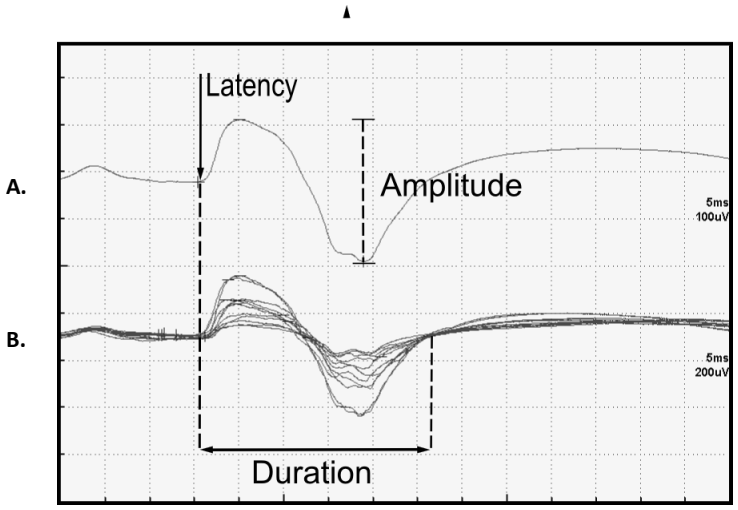

Figure 3.2. Example of T-reflex recordings generated in the biceps brachii muscle in a normal subject. The top tracing (A) represents a digitally averaged T-reflex response derived from repeated superimposed T-reflex responses (B).

The conduction velocity of each reflex was calculated according to two separate formulas:

1. A formula proposed by Pèrèon *et al.* (2004) in which the estimated proximal nerve conduction velocity (PNCV) is calculated using the subject's height and a constant, K, derived from the correlation between the subject's height and the spine-to-active electrode (G1) distance in normal subjects. The values for K are: 8 for rectus femoris; 12 for soleus; 5.4 for triceps; 5.2 for biceps; and 7 for flexor carpi radialis (Pereon, et al., 2004).

$$PNCV = Subject's\ height(m) \times \frac{K}{Latency\ (\mathrm{sec})}$$

2. A formula, devised by Wessels, the co-investigator in this study, in which respective limb length (d, measured in centimetres) is multiplied by 2, and then divided by the latency (l, in milliseconds) with the quotient multiplied by 10 in order to produce proximal nerve conduction velocity (PNCV, meters per second).

$$Nerve\ conduction\ velocity\ = \frac{2d}{l} \times 10$$

3.5.2.1. Biceps brachii T-reflex

The biceps brachii reflex was recorded with the subject in the supine position, with the elbow flexed at 130 degrees. The active electrode (G1) was placed on the belly of the biceps brachii muscle, halfway between the acromion process and the elbow crease. The reference electrode (G2) was placed midway between the active electrode (G1) and the elbow. The ground electrode was placed

between the reference electrode (G2) and the stimulation site. This reflex was omitted if the patient had an intravenous line inserted in the region of the cubital fossa.

3.5.2.2. Triceps brachii T-reflex

The triceps brachii reflex was recorded with the subject in the supine position, with the elbow flexed at 130 degrees. The active electrode (G1) was placed on the body of the triceps brachii muscle, halfway between the top of the shoulder (humerus-clavicular joint) and the lateral olecranon process. The reference electrode (G2) was placed midway between the active electrode (G1) and the stimulation site. The ground electrode was placed between the stimulation site and the reference electrode (G2).

3.5.2.3. Patellar reflex

The patellar reflex was recorded with the subject in the supine position, with a pillow placed under the knee to ensure 135 degrees flexion of the knee joint. The active electrode (G1) was placed on the body of the rectus femoris muscle, midway between the anterior superior iliac spine and the superior border of the patella. The reference electrode (G2) was placed halfway between the active electrode (G1) and the superior border of the patella. The ground electrode was placed between the reference electrode and the stimulation site (patella tendon).

3.5.2.4. Ankle reflex

This reflex was recorded with the subject in the supine position, with the hip joint flexed and externally rotated, and the knee joint flexed at 90 degrees. The ankle was passively dorsiflexed

before the reflex was stimulated by a tap on the Achilles tendon. The active electrode (G1) was placed on the body of the gastrocnemius muscle in line with the Achilles tendon, halfway between the two ends of the fibula bone. The reference electrode (G2) was placed in line with the Achilles tendon, midway between the active electrode (G1) and the medial malleolus. The ground electrode was placed between the reference electrode and the stimulation site on the Achilles tendon.

3.6. Statistical analysis

A professional statistician from the University of Cape Town was recruited to assist with the analysis of the data in this study. Statistical analysis was performed using Stata version 15 (StataCorp). 2017. Stata Statistical Software: Release 15. College Station, TX: (StataCorp).

The following statistical tests were performed to assess the comparison of T-reflex parameters between the control and GBS cohort. Kernel density plots were inspected to evaluate the assumption of normality. The Kolmogorov-Smirnov test was performed to determine whether or not equality of distributions existed in the respective T-reflex parameters between the GBS- and control cohorts.

The standard t-test could not be performed on any of the acquired data because data from all T-reflex parameters collected was not normally distributed. The Wilcoxon rank-sum test (also known as the Mann-Whitney *U*-test) is not sensitive to normal distribution and was therefore performed to compare data from subjects in the GBS vs. Control cohorts. The null hypothesis of the Wilcoxon rank-sum test requires equality of variance. If the Kolmogorov-Smirnov test rejects equality of variance in all parameters, the alternative hypothesis has to be considered. Subsequently, the results of the Wilcoxon rank-sum test were interpreted in terms of the mean ranks.

The Sign test was used to compare T-reflex conduction velocities calculated using limb length (CV-limb) vs. T-reflex conduction velocities calculated using height (CV-height) in the control cohort in order to test the equality of matched pairs of observations in terms of their medians.

A paired Bonett-Seier test (Bonett and Seier, 2003) was performed to evaluate the equality of variance between CV-limb and CV-height. Bonett and Seier (2003) derived an adjusted test of the Pitman-Morgan test to evaluate equality of variance in paired-data using the mean absolute deviation from the median which is thought to be more appropriate for small samples from non-normal distributions.

3.7. Patient safety

All test procedures in this study are standard procedures performed on a regular basis at the GSH neurophysiology laboratory, with the exception of T-reflex testing. Although electrophysiological T-reflex testing is not a standard procedure in the neurophysiological laboratory at Groote Schuur Hospital, it has been validated internationally. Standard NCS and T-reflex tests performed in this study are non-invasive and not associated with any known side effects. The NCS apparatus at the GSH neurophysiology laboratory is standard equipment manufactured by Nihon Khoden and Natus Neurology Inc. Isolation procedures (the wearing of disposable gloves and apron and mask) were followed to protect the subjects from communicable infections.

3.8. Human research and ethics committee

Ethic approval for this study was granted by the University of Cape Town Human Research Ethics Committee (HREC) on 8 February 2016 (HREC reference number: 852/2015). This study conforms to the requirements and principles set by the HREC. All participants of the study were recruited voluntarily and did not receive any financial reimbursement for participation.

CHAPTER 4:

RESULTS

4.1. Population data

All subjects in this study were adults (18 years and older) serially recruited after being referred to Groote Schuur Hospital with a provisional, and later confirmed, diagnosis of GBS. Fourteen subjects (10 male and 4 female) were enrolled into the GBS cohort, and 14 healthy subjects (9 male and 5 female) enrolled into the control cohort. In addition, 4 subjects were approached but not enrolled into the GBS cohort respectively for the following reasons: 1 subject did not meet the age criteria, 1 subject had a depressed level of consciousness and 2 subjects' diagnoses were changed to CIDP rather than GBS during the course of the study. Only 1 subject was approached but not enrolled into the control group because he had electrophysiological evidence of subclinical polyneuropathy on standard NCS. Median age was 40.5 years in the GBS cohort (23-68 years) and 42.5 years in the control cohort (22-69 years). Average age of all 28 subjects in both cohorts was 41.1 years.

Table 4.1. Average and mean ages of subjects enrolled in the GBS and control cohorts.

	Total (n)	Female (n)	Male (n)	Min age	Max age	Median age	Mean age	SD
GBS	14	4	10	23	68	40.5	41.36	12.98
CONTROL	14	5	9	22	69	42.5	41.43	14.17
TOTAL	28	9	19	22	69	40.5	41.39	13.33

A total of 36 electrophysiological studies were performed on these 28 subjects, because 7 of the 14 subjects in the GBS cohort agreed to undergo a second study. Only 1 GBS subject agreed to

undergo a third study. (Table 4.2). Four GBS subjects were recruited within a week of onset of symptoms, 9 were recruited between 7 and 9 days after onset of symptoms, and 1 was recruited on day 10 after onset of symptoms.

Table 4.2. Day after onset of symptoms on which electrophysiological studies were performed on GBS subjects, including 1st, 2nd and 3rd studies.

Day after onset of symptoms	Session 1 (n subjects)	Session 2 (n subjects)	Session 3 (n subjects)	Total (n subjects)
Day 3	1	0	0	1
Day 5	2	1	0	3
Day 6	1	0	0	1
Day 7	4	0	0	4
Day 8	3	0	0	3
Day 9	2	0	1	3
Day 10	1	1	0	2
Day 12	0	1	0	1
Day 14	0	3	0	3
Day 15	0	1	0	1
TOTAL	14	7	1	22

T-reflex responses (CMAPs) recorded in the normal subjects typically had bi- or tri-phasic morphology. They also demonstrated distinct onset and offset. Moreover, responses for a specific T-reflex tended to be reproducible as demonstrated when several traces were superimposed (Figure 4.1 A). In contrast, T-reflex recordings in GBS patients were often polyphasic with delayed onset, gradual offset, markedly increased duration and a more variable baseline (Figure 4.1 B). Furthermore, it was often difficult to confidently identify the offset of the response (CMAP) in these subjects.

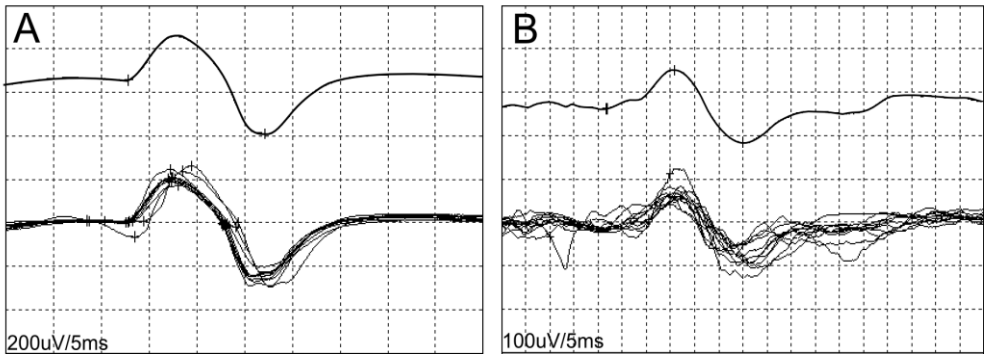

Figure 4.1. Example of biceps brachii T-reflex recording of a healthy subject (A) and of a patient diagnosed with GBS (B).

4.2. Statistical analysis of study data

All parameters for every T-reflex recorded in this study showed deviation from normality (Figure 4.2). In addition, the difference in shape between the two groups suggests that there is no equality of variance for any of these parameters.

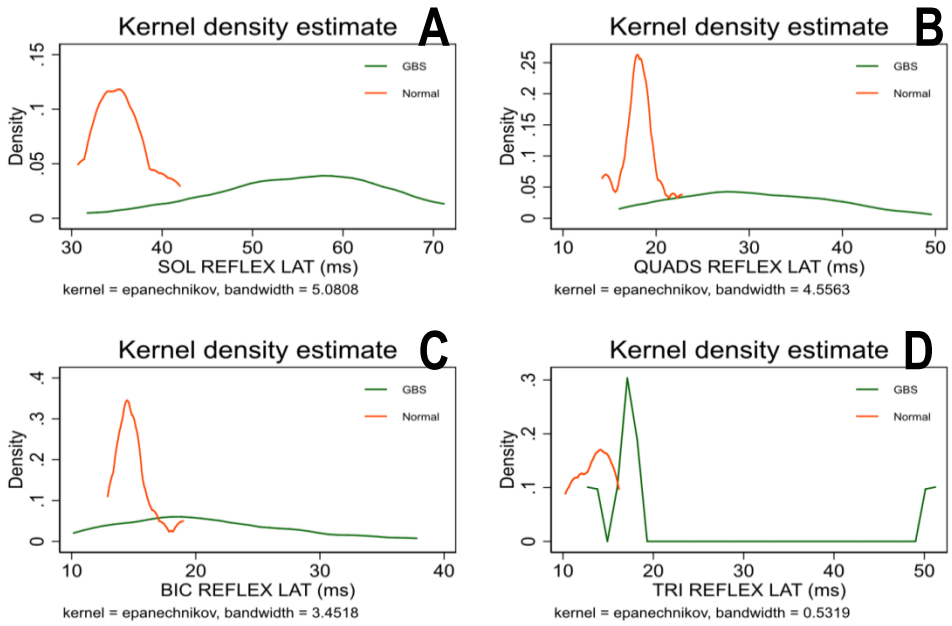

Figure 4.2. Kernel density estimate line graphs for T-reflex latency recorded from the: A) Soleus, B) Quadriceps, C) Biceps brachii and D) Triceps brachii muscles.

Results of the Kolmogorov-Smirnov test indicate that the distributions of all respective parameters of all T-reflexes were unequal between the GBS vs. Control cohorts, except for duration of biceps brachii and duration of triceps brachii T-reflexes (Table 4.3).

The results of the Wilcoxon rank-sum test show statistically significant differences for the mean ranks of all parameters of all T-reflexes except for duration of the triceps brachii T-reflex. In all cases, the mean ranks for the control cohort were more favourable compared with the mean rank of the GBS cohort.

Table 4.3. Summary of data collected during the first electrophysiological study for all parameters recorded for each T-reflex.

T-Reflex	Parameter	n	Median	Mean	D	K-S p value	Mean Rank	z	Prob>|z|
Soleus	Latency (msec)	11 (14)	56.35 (35.00)	54.90 (35.50)	0.9091	<0.001	19.6 (7.8)	4.004	0.001
Quadriceps	Latency (msec)	10 (14)	30.65 (18.23)	30.64 (18.17)	0.8571	<0.001	19.1 (7.8)	3.865	<0.001
Biceps brachii	Latency (msec)	10 (13)	19.90 (14.60)	21.02 (15.00)	0.6231	0.013	16.4 (8.6)	2.729	0.0063
Triceps brachii	Latency (msec)	5 (13)	17.60 (13.90)	23.15 (13.30)	0.8	0.007	14.6 (7.5)	2.514	0.012
Soleus	Duration (msec)	11 (14)	40.00 (16.15)	49.63 (15.69)	1	<0.001	20 (7.5)	4.215	<0.001
Quadriceps	Duration (msec)	10 (14)	36.98 (24.17)	41.48 (23.90)	0.7286	0.002	17.8 (8.9)	2.928	0.0034
Biceps brachii	Duration (msec)	10 (13)	30.30 (24.10)	31.04 (23.36)	0.4682	0.114	15.6 (9.2)	2.233	0.0256
Triceps brachii	Duration (msec)	5 (13)	21.50 (22.00)	24.37 (20.21)	0.2615	0.865	5 (9.3)	0.246	0.8053
Soleus	Amplitude (mV)	11 (14)	0.23 (2.06)	0.43 (2.20)	0.7143	0.001	7 (17.6)	-3.559	<0.001
Quadriceps	Amplitude (mV)	10 (14)	0.12 (1.50)	0.25 (1.48)	0.7571	0.001	6.3 (16.9)	-3.632	<0.001
Biceps brachii	Amplitude (mV)	10 (13)	0.28 (1.69)	0.46 (1.55)	0.7462	0.002	6.7 (16.1)	-3.287	0.001

Triceps brachii	Amplitude (mV)	5 (13)	0.20 (1.88)	0.36 (2.15)	0.8	0.007	3.6 (11.8)	-2.908	0.0036
Soleus	CV-limb (m/s)	11 (14)	32.76 (54.08)	35.23 (54.69)	0.9091	<0.001	6.5 (18.1)	-3.887	<0.001
Quadriceps	CV-limb (m/s)	10 (14)	28.74 (55.22)	31.76 (55.83)	1	<0.001	5.5 (17.5)	-4.099	<0.001
Biceps brachii	CV-limb (m/s)	10 (13)	28.80 (46.51)	28.99 (45.94)	0.7462	0.002	6.3 (16.4)	-3.535	<0.001
Triceps brachii	CV-limb (m/s)	5 (13)	45.45 (61.22)	41.91 (59.90)	0.8462	0.005	4.2 (11.5)	-2.612	0.009
Soleus	CV-Height (m/s)	11 (14)	36.25 (56.75)	38.03 (57.58)	0.9091	<0.001	6.7 (17.9)	-3.777	<0.001
Quadriceps	CV-Height (m/s)	10 (14)	44.01 (75.45)	46.83 (75.13)	1	<0.001	5.5 (17.5)	-4.099	<0.001
Biceps brachii	CV-Height (m/s)	10 (13)	44.01 (60.20)	45.01 (59.39)	0.6462	0.01	7.4 (15.5)	-2.853	0.0043
Triceps brachii	CV-Height (m/s)	5 (13)	51.24 (68.51)	48.43 (71.55)	0.8	0.007	3.6 (11.8)	-2.908	0.0036

Test statistic D and exact p-value (K-S) of the Kolmogorov-Smirnov test; as well as the z-value and Prob>|z| of the Wilcoxon rank-sum test are included. Control cohort data inputs are highlighted in blue and placed in parenthesis. Latency and duration measured in milliseconds (msec), amplitude measured in millivolts (mV) and CV-limb and CV-height measured in meters per second (m/s)

4.3. Study data compared with previous research

Data collected from the control cohort in this study was insufficient to confidently calculate normative values. Consequently, normative ranges for T-reflex responses published in the literature were assessed and those reported by Péréon were used in this study. Péréon *et al.* (2004) divided T-reflex normative data into separate categories according to age (Péréon *et al*., 2004). Only adult population groups (19-49 and 50-80 years of age respectively) from the Péréon data are shown in Table 4.4 and 4.5.

Table 4.4. Normative T-reflex latency data published by Péréon et al,. (2004)

T-Reflex	Age group	(n)	Mean (msec)	Std. error	95% conf. interval
Soleus	19-49	78	33	0.28	32.44-33.56
Soleus	50-80	40	34.1	0.78	33.33-34.87
Quadriceps	19-49	60	19.9	0.22	19.46-20.34
Quadriceps	50-80	20	20.1	0.36	19.35-20.85
Biceps	19-49	23	13.4	0.19	13.01-13.79
Biceps	50-80	5	13.6	0.49	12.23-14.97
Triceps	19-49	23	14	0.21	13.57-14.43
Triceps	50-80	5	14.3	0.49	12.93-15.67

The majority of T-reflex latency data in the control cohort (43 of 56 latencies) correlated with the normative latency ranges published by Péréon *et al.* (2004). In the Control cohort, 5 T-reflex latencies (2 quadricep- and 3 tricep brachii) were faster, and 8 T-reflex latencies (2 soleus, 4 biceps brachii and 2 triceps brachii) were slower than the normative latency range published by Péréon *et al.* (2004). In the GBS cohort, 50 T-reflex latencies were prolonged, while only 6 T-reflex latencies (1 soleus, 3 quadriceps, 1 bicep brachii and 1 triceps brachii reflex) were within the normative range (Table 4.5.).

Table 4.5. T-reflex latency data derived from the first electrophysiological study in all subjects (GBS and Control cohort) compared with normative latency data published by Péréon et al. (2004).

Cohort	T-Reflex	< NR (n)	Within NR	> NR (n)	Total
GBS	Soleus	0 (0)	1 (7.14)	13 (92.86)	14 (100)
Control	Soleus	0 (0)	12 (85.71)	2 (14.29)	14 (100)
GBS	Quadriceps	0 (0)	3 (21.43)	11 (78.57)	14 (100)
Control)	Quadriceps	2 (14.29)	12 (85.71)	0 (0)	14 (100)
GBS	Biceps	0 (0)	1 (7.14)	13 (92.86)	14 (100)
Control	Biceps	0 (0)	10 (71.43)	4 (28.57)	14 (100)
GBS	Triceps	0 (0)	1 (7.14)	13 (92.86)	14 (100)
Control	Triceps	3(21.43)	9 (64.29)	2 (14.29)	14 (100)

Percentages are included in parenthesis. NR = normative range published by Péréon et al. (2004).

4.4. Conventional NCS and the T-reflex

All subjects in the GBS cohort fulfilled the diagnostic requirements for GBS according to Hadden's and Rajabally's criteria (Uncini and Kuwabara, 2012; Rajabally *et al*., 2015). Each subject in the GBS cohort was electrophysiologically classified into subtypes as demyelinating, axonal, equivocal or inexcitable based on the results of standard nerve conduction studies (Table 4.6) using, respectively, both Hadden's and Rajabally's criteria, which are included as appendices B and C. There was some discrepancy when applying these two criteria to classify subtypes in the GBS cohort, with more variation noted with Rajabally's than Hadden's criteria (Table 4.6). All subjects in the GBS cohort had abnormal T-reflex recordings. However, there was no correlation between any specific T-reflex parameter abnormality and GBS subtypes as defined by either classification (Table 4.6).

During each electrophysiological study, conventional NCS (including F-wave testing), and T-reflexes were performed on all subjects. In the GBS cohort, T-reflex responses were elicited more often than F-wave responses (Table 4.7). In the lower limbs of GBS subjects, soleus T-reflex responses were elicited in 11 (78.57%) GBS subjects vs. tibial F-wave responses in only 5 (35.71%) subjects in this cohort. Quadriceps T-reflex responses were elicited in 10 (71.43%) GBS subjects. In the upper limbs, 10 (71.43%) biceps brachii T-reflex responses were elicitable vs. only 5 (35.71%) median F-wave responses, and only 6 (42.86%) ulnar F-wave responses. Also, in the upper limbs of subjects in the GBS cohort, some T-reflex responses were more easily elicited than others. For example, biceps brachii T-reflex responses were elicited in 10 GBS subjects (71.43%) vs. triceps brachii T-reflex responses in only 5 subjects (35.71%).

Table 4.6. Summary of GBS subtype classification respectively according to Hadden's and Rajabally's electrodiagnostic criteria in each subject, for each neurophysiological testing session.

Pt nr	Session (n)	Hadden's criteria	Rajabally's criteria	Abnormal T-reflex parameters (total)			
				Soleus (n)	Quadriceps (n)	Biceps brachii (n)	Triceps brachii (n)
1	1	Axonal	Axonal	5	4	5	X
1	2	Inexitable	Inexitable	3	5	3	X
2	1	Axonal	Axonal	X	X	X	X
2	2	Inexitable	Axonal	X	2	1	0
3	1	Demyelinating	Equivocal	2	4	4	1
3	2	Demyelinating	Equivocal	X	X	X	X
4	1	Axonal	Axonal	5	4	2	4
4	2	Equivocal	Axonal	X	X	X	X
5	1	Demyelinating	Axonal	5	2	2	1
5	2	Demyelinating	Axonal	5	3	4	0
5	3	Demyelinating	Axonal	5	1	2	1
6	1	Demyelinating	Demyelinating	X	3	X	X
6	2	Demyelinating	Demyelinating	5	4	4	4
7	1	Demyelinating	Demyelinating	5	4	4	X
8	1	Demyelinating	Demyelinating	5	X	X	X
9	1	Demyelinating	Equivocal	X	X	2	X
10	1	Demyelinating	Demyelinating	5	4	5	5
10	2	Demyelinating	Demyelinating	5	X	X	X
11	1	Demyelinating	Equivocal	5	5	X	X
12	1	Demyelinating	Demyelinating	5	5	4	X
13	1	Demyelinating	Demyelinating	5	X	2	3
14	1	Demyelinating	Axonal	5	5	1	X

"n" indicates total number of abnormal parameters for each T-reflex. "X" indicates that none of the parameters were recordable/measurable

Table 4.7. Responses elicitable in the GBS cohort: T-reflexes and F-waves

	Elicitable in 1st study (n=14)	Elicitable in 2nd study (n=7)
T-Reflexes		
Soleus	11(78.57%)	3 (42.86%)
Quadriceps	10 (71.43%)	4 (57.14%)
Biceps	10 (71.43%)	4 (57.14%)
Triceps	5 (35.71%)	3 (42.86%)
F-Waves		
Tibial	4 (28.57%)	2 (14.29%)
Median	5 (35.71%)	2 (14.29%)
Ulnar	6 (42.86%)	2 (14.29%)

Percentages in parenthesis

4.5. T-Reflex Conduction Velocity (CV): CV-limb vs. CV-height

Conduction velocities of all four T-reflexes (soleus, quadriceps brachii, biceps brachii and triceps brachii) calculated using limb length (CV-limb) were all consistently slower than respective conduction velocities calculated using subject height (CV-height). This difference was significant when assessed with the one-sided sign test (Table 4.8). The p-values for the Bonett-Seier test for soleus, biceps brachii and triceps brachii T-reflexes were all > 0.05 and thus the variance of the data collected from CV-limb vs. CV-height does not differ. However, the p-value for the quadriceps T-reflex is < 0.05 and thus the data collected for quadriceps reflex CV-limb and CV-height is different. (Table 4.8 and Figure 4.3).

Table 4.8. Comparative statistics: CV-limb vs. CV-Height.

T-Reflex	p (sign)	Mean abs. dev. x	Mean abs. dev. y	z	p (Bonett-Seier)
Soleus	<0.001	3.990	4.185	0.346	0.728
Quads	<0.001	3.587	5.660	-1.994	0.046
Bicep	<0.001	4.097	3.884	0.218	0.827
Triceps	<0.001	5.232	7.641	-1.063	0.287

p (sign) = p value for the one-sided sign test; mean abs. dev of x = the mean absolute deviation of x; mean abs. dev of y = the mean absolute deviation of y; p (Bonett-Seier) = p value for the paired Bonett-Seier test.

Using Boxplot graphs, the margin box for the quadriceps CV-limb is shown to be smaller compared to the margin box of quadriceps CV-height, implying significance (Figure 4.2 B). This also appears to be true for triceps brachii, although an outlying plot affects the Bonett-Seier test analysis (Figure 4.3 D).

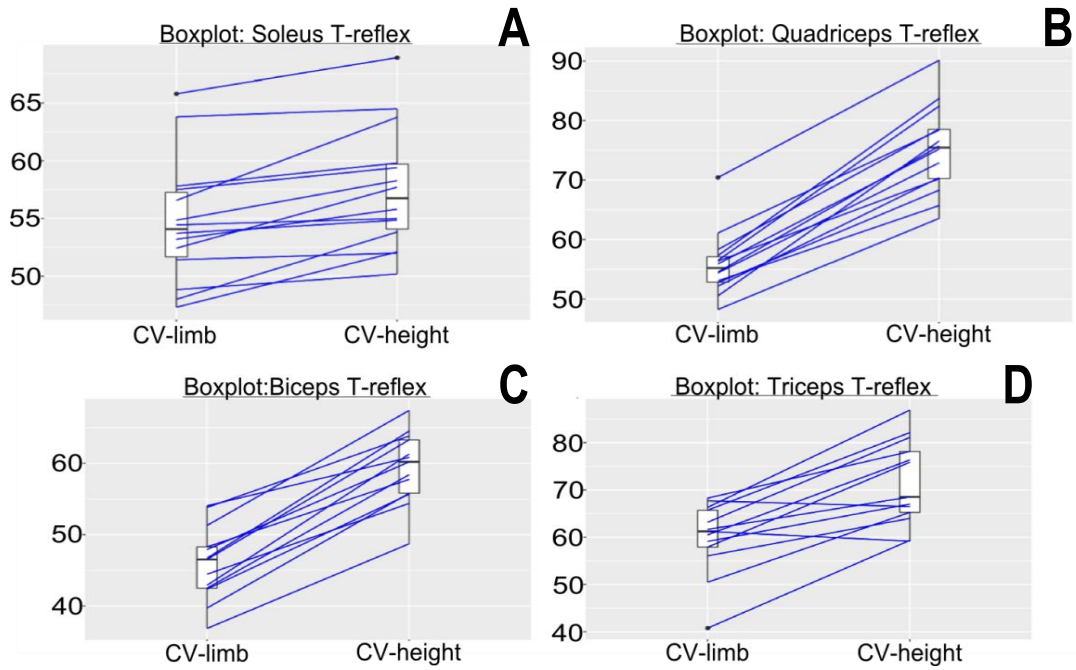

Figure 4.3. Boxplots displaying CV-limb vs. CV-height data for A) soleus, B) quadriceps, C) biceps and D) triceps brachii T-reflexes. Each blue line connects values of the same participant across the two measurements.

CHAPTER 5:

DISCUSSION AND CONCLUSION

5.1. Background

The incidence of Guillain-Barré Syndrome (GBS) in North America and Europe is reported to progressively increase with age from 0.62 per 100 000 children younger than 9 years of age to 2.66 per 100 000 adults older than 80 per year (Sejvar *et al*., 2011). However, the incidence and prevalence in South Africa has not been studied. GBS manifests clinically as a polyradiculoneuropathy involving both proximal (spinal root) and peripheral nerves, and is characterised by progressive clinical weakness which develops over days or weeks. Typically, motor nerves are more affected than sensory nerves, and clinical hypo- or areflexia is required in order to make the diagnosis (Doorn *et al*., 2008). Traditionally, deep tendon reflexes are clinically evaluated by a neurologist. Conventional nerve conduction studies (NCS), which are currently used to provide electrophysiological evidence to support the clinical diagnosis of GBS, are limited to some extent in that they primarily evaluate peripheral nerve function with only F-responses providing some information on the motor nerve pathway of the spinal root function (Lo *et al*., 2008). In contrast, electromyographic testing of the T-reflex provides information regarding both proximal (spinal root) and peripheral (sensory and motor) nerve function (Voerman *et al*., 2005). Moreover, NCS require the application of electric stimuli (i.e. shocks) to the nerves being assessed which causes discomfort to the subject, while the T-reflex is a painless diagnostic tool in which the stimulus is simply a tap on the appropriate tendon. T-reflexes are also easy to perform and provide reproducible results. Despite the obvious benefits of T-reflex testing, little consensus exists in the literature on how best to perform this test. Furthermore, research specifically examining the T-reflex in the assessment and diagnosis of GBS remains limited.

5.2. Discussion

The primary aim of this prospective, controlled, non-blinded, cohort study was to describe the T-reflex in subjects with early GBS. To this end, the T-reflex was successfully performed in 28 subjects (14 patients with GBS and 14 age and gender matched normal controls). Because of the small number of subjects enrolled, specialised statistical analysis was required in order to demonstrate significance. More particularly, specialised rank-sum tests confirmed significant, non-biased differences between study and control cohorts.

5.2.1. The T-reflex in early GBS

Because data was not normally distributed, the Wilcoxon rank-sum test was used to evaluate respective statistical significance of differences observed in various T-reflex parameters measured in GBS vs. Control cohorts. On the basis of Kolmagorov-Smirnov analysis, the results of the Wilcoxon rank-sum test were interpreted in terms of the mean ranks in order to evaluate significance.

Using this statistical approach, latencies and durations of all T-reflexes tested in the GBS cohort were shown to have significantly larger rank values than the equivalent T-reflexes in the Control cohort (Table 4.3). In other words, all T-reflex latencies and durations were significantly prolonged in the GBS cohort vs. the respective T-reflexes in the Control cohort, with the exception of the duration of the triceps brachii T-reflex. Furthermore, conduction velocities had significantly smaller rank values in the GBS cohort vs. the Control cohort, which confirms a significant reduction in velocity in the GBS cohort. (Table 4.3). A literature search identified only one other study, published as a poster abstract, which has found similar results (Allen *et al*., 2011). However, the authors provided no details on methodology or number of subjects enrolled. In addition, García *et*

al. (2018) and Alvarez-Paradelo *et al*. (2016) respectively published case reports in which they address the potential usefulness of the T-reflex in the diagnosis of GBS.

Although data in the literature on the utility of T-reflexes in GBS are scant, the findings of the present study are comparable to previous publications involving the T-reflex response in other demyelinating polyneuropathies such as chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) and inherited Charcot-Marie-Tooth Disease Type 1a. Kuruaglu and Oh (1994); Sharma *et al*. (2009), and García *et al*. (2015) concluded the latency, conduction velocity and, less so, the duration of the T-reflex are useful indicators of demyelinating polyneuropathy. The rank values for T-reflex amplitudes in the GBS cohort in this study were stochastically smaller than in the Control cohort. This observation must be interpreted with care because large intra- and inter-subject variability of T-reflex amplitudes were present in spite of superimposing and averaging several traces, as suggested by Pèrèon *et al.* (2004).

In this study, T-reflex responses (CMAPs) recorded in the normal subjects typically showed distinct onset and offset, as well as bi- or tri-phasic morphology. Moreover, responses for a specific T-reflex tended to be reproducible as demonstrated when several traces were superimposed (Figure 4.1 A). These findings are consistent with the research of Kuruaglu and Oh (1994), Tetsunaga *et al*. (2013) and García *et al*. (2015). In contrast, T-reflex recordings performed on GBS patients in this study were often polyphasic with delayed onset, gradual offset, markedly increased duration and a more variable baseline, and it was often difficult to confidently identify the offset of the response (CMAP) in these subjects (Figure 4.1 B). Although these T-reflex characteristics in subjects with GBS have not formally been described in the literature, they are comparable with examples of T-reflex recordings in CIDP patients published by Kuruaglu and Oh (1994).

5.2.2. Nerve conduction studies and the T-reflex

As mentioned previously, information provided by conventional motor and sensory NCS is largely limited to distal segments of peripheral nerves, with only F-responses permitting electrophysiological evaluation of the proximal motor pathways (i.e. spinal motor roots). Notably, T-reflex testing has the advantage of allowing simultaneous evaluation of proximal and distal segments, as well as both motor and sensory components of a reflex arc, although separate evaluation of these motor and sensory components is not possible.

Electrodiagnostic evaluation of proximal nerve pathways (i.e. spinal roots) provides useful information when considering a diagnosis of GBS. However, all electrodiagnostic tests, even when performed according to strict technical protocol, may be negatively affected by external factors such as disruptive artefacts, or the inability to acquire useable traces. In this study, T-reflexes were recordable in most of the GBS subjects. In contrast, the majority of F-responses were not elicitable in these subjects. A possible explanation for this is that action potentials recorded with F-responses are small and fragmented, whereas T-reflex responses are larger and more robust because they represent a compound muscle action potential (CMAP).

Furthermore, while F-responses provide useful information on proximal motor nerve/root function, they may be unrecordable or unreliable in the case of severe segmental demyelination or in electrically active environments such as intensive care units. Moreover, because the T-reflex signals are larger than F-responses, they tend to be less susceptible to environmental artefacts and provide more information in the case of demyelinating polyneuropathy. However, direct comparison of the respective usefulness of conventional NCS vs. T-reflexes is problematic because these tests respectively involve evaluation of different elements of peripheral nerve function.

All GBS subjects in this study were categorised according to the two electrodiagnostic classifications respectively suggested by Hadden *et al.* (1998) and Rajabally *et al.* (2015) (Table 4.6). Both these classifications are used to identify the subtypes of GBS using the results of conventional NCS and therefore represent a useful method by which to interpret the NCS results. Electrodiagnostic categories include: normal, demyelinating, axonal, inexcitable and equivocal. The demyelinating category requires prolonged motor nerve conduction latencies, reduced conduction velocities and prolonged or absent F-responses. The axonal category requires reduced motor- and sensory action potential amplitudes in the absence of any demyelinating features. Where no NCS responses are elicitable, these are categorised as inexcitable. NCS results which are abnormal but cannot be classified as axonal, demyelinating or inexcitable are classified as equivocal. Of interest is the fact that subtype classification according to Hadden *et al.* (1998) vs. Rajabally *et al.* (2015) differed in 9 of the 22 conventional nerve conduction studies performed on subjects in the GBS cohort (Table 4.6). The increased sensitivity to axonal subtype of GBS supports validity of the electrodiagnostic criteria proposed by Rajabally *et al*. (2015).

Analysis of the results from the GBS cohort confirmed abnormalities in several parameters in most of the T-reflexes tested, irrespective of whether they were categorised as axonal, demyelinating, inexcitable or equivocal. Although abnormalities in all parameters of the T-reflex in GBS subjects were statistically different from those of control subjects, there were no abnormalities identified in the various parameters of T-reflex testing, which unequivocally predicted any of these NCS-based GBS subtypes. These findings suggest that the T-reflex is sensitive to identifying early electrophysiological changes associated with GBS, but that it is not specific enough to distinguish between axonal or demyelinating subtypes of the syndrome.

5.2.3. CV-height vs. CV-limb

Although several methods have been published describing how to perform the T-reflex (Table 2.2), these differ substantially from one another and, currently, no standardised protocols exist. This is especially true for calculating T-reflex conduction velocity in a supine subject. Consequently, two methods were used to determine T-reflex conduction velocity in this study: a) a formula using the height of the patient as suggested by Péréon *et al.* (2004) and Boët *et al.* (2013) (CV-height), and b) a formula devised by Wessels, the co-investigator in this study, using limb length measured from the active electrode (G1) to the spine without the relative constant (CV-limb). The suggested formula using measure limb length, rather than height, to calculate conduction velocity in the T-reflex has not yet been described in the literature.

It has been suggested that the height adjusted normal values should be used to evaluate latency and conduction velocity (Kuruaglu and Oh 1994; Frijns *et al*., 1997; Péréon *et al*., 2004; Voerman *et al*., 2005; Sharma *et al.* 2007; Boët *et al*., 2013). However, in this study, using the subject's height to calculate T-reflex conduction velocity (CV-height) produced faster velocities in shorter reflexes (quadriceps, triceps brachii and biceps brachii) vs. a longer reflex (soleus) when compared with respective conduction velocities calculated using limb length (CV-limb). This difference in conduction velocity values is similar when comparing the CV-height with conduction velocities published by Péréon *et al*. (2004) and implies a systemic error. A likely reason for this is the human error which occurs when measuring the height of a subject lying supine rather than standing upright. In this study, the height of all subjects was measured in the supine position. CV-limb were consistently slower than CV-height for all T-reflexes. The paired Bonett-Seier test revealed that the variance of the two methods is similar, except in the case of the quadriceps reflex. This implies that the two methods are equally accurate. However, because it is important to calculate the conduction velocity of the T-reflex accurately in the context of length-dependent polyneuropathy such as GBS, it may be more appropriate to use CV-limb.

The findings of this study confirm that electrophysiological changes in the T-reflex represent a sensitive biomarker of GBS-related neuronal dysfunction and that assessment of the T-reflex may be useful, in addition to conventional NCS, in the assessment of patients with suspected GBS.

5.3. Limitations of study

Although most patients referred sequentially to the GSH neurophysiology laboratory agreed to be enrolled in the study, GBS is a rare condition and thus the number of subjects enrolled was low. This has meant that statistical analysis of differences observed between conventional nerve conduction study and T-reflex results was difficult.

For comparative purposes, it would have been useful to include additional cohorts in this study, enrolling subjects with polyneuropathies other than GBS (e.g. small fibre sensory neuropathy, CIDP, and diabetic neuropathy etc.).

The delay between the onset of GBS symptoms and performing the first electrophysiological study (i.e. conventional NCS and T-reflexes) varied considerably between subjects (Table 3.1 and Table 3.2). Because of this, the small sample size and the fact that clinical disease severity differed between subjects, it was not possible to accurately correlate changes in T-reflex parameters and the progression of symptoms in the GBS cohort in this study. More particularly, it was not possible to identify at which stage of GBS the T-reflex reflex first shows evidence of neuropathy. Furthermore, it was not possible to determine if the T-reflex first becomes abnormal at, before, simultaneously with or after abnormalities elicited by conventional NCS.

It would have been useful to perform serial T-reflexes and NCS at regular intervals on subjects in the GBS cohort in order to observe the comparative evolution of the T-reflex changes paralleling clinical progression of the disease. This was not possible because most subjects in the GBS cohort chose not to undergo serial neurophysiological assessments unless this was deemed necessary by the treating neurologist.

The Hoffman-reflex (H-reflex) was not included in this study protocol, and this may have been useful. In theory, the H-reflex evaluates similar reflex pathways to the T-reflex. However only the soleus H-reflex can be reliably recorded in adults. The appropriate protocol used to record the soleus H-reflex requires the patient to lie prone (Kim and Yoon, 2003), which is impractical in patients with severe weakness and patients who are ventilated, which is frequently the case with GBS patients.

5.4. Recommendations

Further investigations with larger sample sizes are required in order to identify the most useful T-reflex variables to use as biomarkers for the diagnosis of GBS and other polyneuropathies.

Recommendations for future studies include:

- Future studies should examine the T-reflex analysis vs. conventional NCS in GBS with increased participant numbers in order to identify any significant differences in the sensitivities of these two tests in early GBS. It would be useful to know whether or not T-reflex changes occur earlier in the course of the disease than conventional NCS.
- The results of this study suggest that unilateral analysis of the biceps brachii, popliteal and ankle T-reflexes are probably the most reproducible of the T-reflexes to test in GBS, although this supposition requires confirmation in future studies. The triceps brachii T-reflex appears to

be less reliable in this study, probably because evaluation is complicated by the anatomy of the muscles.

- Placement of the recording electrodes should be simple and related to easily identifiable anatomical landmarks in order to produce reproducible T-reflex traces.
- This study suggests that parameters of the T-reflex, which are likely to be most reliable in assessment of GBS patients include latency, duration and conduction velocity but not signal amplitude. This should be confirmed in future studies in which larger numbers of participants are enrolled.
- This study suggests that conduction velocity should be calculated using the distance between the active electrode (G1) and the suprasternal notch when recording the biceps brachii T-reflex, and between the active electrode (G1) and the xiphoid process when recording the ankle and patellar T-reflexes.
- All subjects should lie supine when the T-reflex is recorded. This ensures objective comparison of the T-reflex between different cohorts, even if patients are bedridden or ventilated.
- This study also suggests that several traces should be recorded and superimposed for each T-reflex and that the average or median should be used to mitigate confounding factors such as stimulation intensity.

CHAPTER 6:

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APPENDICES:

APPENDIX A

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
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09 February 2016

HREC REF: 852/2015

Dr L Tucker
Neurology
E-8
NGSH

Dear Dr Tucker

PROJECT TITLE: ASSESSMENT OF THE T- REFLEX IN ADULT PATIENTS WITH EARLY GUILAIN-BARRE SYNDROME AT GROOTE SCHUUR HOSPITAL (Masters-candidate-S Wessels)

Thank you for your response letter dated 20 January 2015, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 28 February 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the following student, Sebastian Wessels will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical

HREC 852/2015

APPENDIX B

Hadden *et al*.'s electrodiagnostic criteria for Guillain-Barré syndrome (Hadden *et al*., 2004)

1. Normal
(All the following in all nerves tested)
DML ≤100% ULN
F-wave present with latency ≤100% ULN
MCV ≥100% LLN
Distal CMAP ≥100% LLN
Proximal CMAP ≥100% LLN
Proximal CMAP/distal CMAP ratio >0.5
2. Primary demyelinating
(At least one of the following in each of at least two nerves, or at least two of the following in one nerve if all others inexcitable and distal CMAP ≥10% LLN)
MCV <90% LLN (85% if Distal CMAP <50% LLN)
DML >110% ULN (120% if Distal CMAP <100% LLN)
Proximal CMAP/distal CMAP ratio <0.5 and distal CMAP ≥20% LLN
F-response latency >120% ULN
3. Primary axonal
None of the above features of demyelination in any nerve (except one demyelinating feature allowed in one nerve if distal CMAP <10% LLN)
Distal CMAP <80% LLN in at least two nerves
4. Inexcitable
Distal CMAP absent in all nerves (or present in only one nerve with distal CMAP <10% LLN)
5. Equivocal
Does not exactly fit criteria for any other group
CMAP, compound muscle action potentials; DML, distal motor latency; LLN, lower limit of normal; MCV, motor conduction velocity; ULN, upper limit of normal.

APPENDIX C

Modified electrodiagnostic criteria for GBS (Rajabally *et al*., 2015)

1. Normal
(All the following in all nerves tested)
DML ≤100% ULN
F-wave present with latency ≤100% ULN
MCV ≥100% LLN
Distal CMAP ≥100% LLN
Proximal CMAP ≥100% LLN
Proximal CMAP/distal CMAP ratio >0.5
2. Primary demyelinating
(At least one of the following in each of at least two nerves, or at least two of the following in one nerve if all others inexcitable and distal CMAP ≥10% LLN)
MCV <90% LLN (85% if Distal CMAP <50% LLN)
DML >110% ULN (120% if Distal CMAP <100% LLN)
Proximal CMAP/distal CMAP ratio <0.5 and distal CMAP ≥20% LLN
F-response latency >120% ULN
3. Primary axonal
None of the above features of demyelination in any nerve (except one demyelinating feature allowed in one nerve if distal CMAP <10% LLN)
Distal CMAP <80% LLN in at least two nerves
4. Inexcitable
Distal CMAP absent in all nerves (or present in only one nerve with distal CMAP <10% LLN)
5. Equivocal
Does not exactly fit criteria for any other group