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The Perception, Aetiology and Clinical Assessment of Restless Legs Syndrome and Periodic Limb Movements

Samantha Elizabeth Kerr

A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree Doctor of Philosophy.

Johannesburg, 2013

Declaration

I, Samantha Kerr, declare that this thesis is my own work, except to the extent indicated in the acknowledgements or contributions sections. It is being submitted for the degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg.

This thesis is submitted in the format, approved by the Faculty, of published work with accompanying introduction and conclusion.

Part of this work was previously submitted for examination as a Masters Degree and examined and approved but not awarded since the MSc was subsequently upgraded to the present PhD Thesis. This work has not otherwise been submitted for any other degree or examination in this or any other university.

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(signature of candidate)

Date

January 2013

Abstract

Restless Legs Syndrome (RLS) and Periodic Limb Movements (PLM) are common neurological disorders for which the underlying aetiology is not fully understood. Currently RLS and PLM are thought to be caused by a central deficiency of dopamine or other functional abnormalities of the central nervous system. The work included in this thesis investigated different new methods of assessing the sensory and motor features of RLS and PLM, in an attempt to extend our understanding of their aetiology and improve the accuracy of diagnosis of these conditions. The first two studies in the thesis described and characterized the sensations of RLS symptoms, and whether they are influenced by the presence of pain, in an English speaking South African population. The most frequently cited descriptors were different to those used in the current RLS diagnostic criteria. Inclusion of the most commonly used RLS descriptors in the diagnostic criteria may help to improve the accuracy of RLS diagnosis. Patients who experienced painful RLS had greater McGill Pain Questionnaire scores and used different terms to describe their RLS to those that did not have painful RLS sensations. The third project quantified the responses of the Hoffman and patellar reflexes in RLS patients using electromyography and kinematics. The RLS patients exhibited hyporeflexia in the evening compared to the morning, and compared to control participants. This data suggests that RLS is not the result of a global state of hyperexcitability, as the literature suggests, but may reflect more discrete functional abnormalities of the spinal cord. A diurnal variation in the patellar reflex was found, supporting the notion of circadian variations of spinal excitability in RLS patients. The final investigation assessed the sensory qualities (discomfort and pain) of RLS in conjunction with motor activity evoked by using the Suggested Immobilization Test. Despite rating significant levels of discomfort, the majority of the RLS patients did not exhibit

PLM; possibly suggesting a disconnect between the sensory and motor components of RLS. In conclusion, it is the major finding of this thesis that inclusion of new assessment techniques for the measurement of sensory and motor features of RLS and PLM provides both new insights and potential clinical tools enhancing our understanding of these disorders.

Acknowledgements

Firstly, I would like to thank my supervisors, Warrick McKinon and Alison Bentley, for their continual guidance, motivation and support, both academically and personally. To Warrick, your dedication, limitless patience and encouragement through the ups and downs of the process was infinitely appreciated. To Alison, thank you for your enthusiasm, looking after me and for giving me the opportunity to expand my professional horizons by participating in field work and numerous conferences.

Thanks to Dr Dave Anderson for your involvement and sacrificing time after your consulting hours to assist me.

I am grateful for the funding provided by the Wits Dial.a.Bed sleep laboratory, the University of the Witwatersrand Medical Research Endowment fund and for the travel grants received from the Movement Disorder Society, the University of the Witwatersrand Deans discretionary fund, the International Student Congress of Medical Sciences, the Society of Neuroscience in Africa, the World Federation of Neurology and the International Brain Research Organisation.

Thanks to members of the School of Physiology, particularly the members of the sleep lab, for their support and to the late Willie de Bruin for the construction of equipment and the constant loaning of his tools.

Special thanks to the subjects involved in my research for your interest and willingness to ‘grin and bear it’ through the discomfort of some of the procedures.

To my family: Dad, Mum, Chris and Amy, who have supported and believed in me through this journey and made me endless cups of tea.

Lastly, to all my friends, and especially: Mark Bailey, Mary Borland, Emma Digby, Maryke du Plooy, Nicol Janse van Rensburg, Jessica Roberts, Suzanne Paidas, Cathy and Mark Tew, and the Muirfielders. Thank you for your encouragement, inspiration, hugs and laughter (sometimes with me and other times at my expense). For so willingly being my guinea pigs, making me smile and your understanding when I just couldn’t. You made the difference.

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Abbreviations

3OMD	3-ortho-methyldopa
5H1AA	5-hydroxyindolacetic acid
ADHD	Attention Deficit Hyperactivity Disorder
CSF	Cerebrospinal fluid
CNS	Central nervous system
CPG	Central pattern generator
L-DOPA	L-dihydroxyphenyalanine
EMG	Electromyography
FIT	Forced Immobilization Test
H-reflex	Hoffmann reflex
HVA	Homovanillic acid
IRLS	International Restless Legs Scale
IRLSSG	International Restless Legs Syndrome Study Group
JHRLSS	John Hopkins Restless Legs Syndrome severity scale
MPQ	McGill Pain Questionnaire
NINDS	National Institute of Neurological Disorders and Strokes
PLM	Periodic Limb Movements
PLMD	Periodic Limb Movements Disorder
PLMS	Periodic Limb Movements during sleep
PLMW	Periodic Limb Movements during wakefulness
PSG	Polysomnography
QST	Quantitative sensory testing
REM	Rapid eye movement
RLS	Restless Legs Syndrome
SIT	Suggested Immobilization Test
WASM	World Association of Sleep Medicine
VAS	Visual analogue scale

Preface

Restless legs syndrome and periodic limb movement disorder are common sleep-related movement disorders that can have debilitating consequences for those that suffer from them. Despite extensive research, our understanding of these disorders and our ability to assess both the sensory and motor components associated with these disorders has been limited by the few techniques used to assess the signs and symptoms. Expansion of the available techniques could add significantly to the knowledge base on these disorders. The sections that follow describe how this body of work intends to contribute to the understanding and assessment of these disorders.

Chapter 1 introduces the topic of restless legs syndrome (RLS) and periodic limb movements (PLM) followed by a general background to these disorders. The literature review then explores the current assessment of RLS and PLM investigating sensory and motor components separately, and then together. The rationale and objectives of the entire thesis follows.

Chapter 2 and 3 include projects which use new techniques to investigate the sensory features of RLS. The difficulty patients experience in describing their RLS sensations has resulted in a diverse range of words used to describe the condition. To date these descriptors have not been fully categorised. Chapter 2 contains a published paper which explored the descriptors used by a cohort of South African RLS patients. Chapter 3 contains a paper which analyses whether the words selected by the same sample of RLS patients were influenced by the presence of pain.

Chapter 4 looks at the motor aspects of RLS and PLM. Although the origins of RLS and PLM are unknown, spontaneous sensations and motor events suggest that there may be a state of spinal

hyperexcitability in these patients. The published paper in this chapter evaluates the state of spinal excitability in RLS patients using the Hoffmann (H-reflex) and patellar reflexes.

Chapter 5 interrogates the relationship between the sensory and motor features of RLS and PLM simultaneously using the Suggested Immobilization Test (SIT). The immobility of the SIT provokes RLS discomfort, possibly pain and motor restlessness and as such is an ideal candidate for exploring the relationship between these three features. This relationship is the focus of the paper (currently under review) included in this chapter.

Chapter 6 summarizes and discusses each of the thesis objectives, their subsequent results and the contribution of these to the understanding of RLS and PLM. Suggestions for future directions that have emerged as a result of this research are offered.

Chapter 7 includes the references used for the literature review in Chapter 1. The references for Chapters 2, 3, 4 and 5 are included as part of each paper.

Chapter 8 includes the following appendices: The RLS questionnaire used to screen all the RLS patients recruited for the studies; ethical clearance certificates; all scales, questionnaires and word lists used in the studies incorporated in this thesis. Also included in the appendix are other publications that have emanated from or in conjunction with the work included in this thesis but do not constitute part of the thesis' body of work.

Written outputs emanating from the work contained in this thesis

Published papers

1. Kerr S, Bentley A, Anderson D and McKinon W. Reflex testing reveals circadian variation of spinal excitability in restless legs syndrome patients.
   Sleep and Biological Rhythms 2011; 9: 157–164.
2. Kerr S, McKinon W and Bentley A. Descriptors of Restless Legs Syndrome sensations. *Sleep Medicine* 2012; 13(4): 409-413.

Submitted papers

1. Kerr S, Bentley A and McKinon W. Does the presence of pain influence the descriptors used for the sensory discomfort in Restless Legs Syndrome.

2. Kerr S, McKinon W and Bentley A. The relationship between sensory and motor components of Restless Legs Syndrome during the Suggested Immobilization Test. Submitted to the *International Journal of Neuroscience*.

Other written outputs in conjunction with the work contained in this thesis
(co–authored by the thesis author during the period of PhD candidature)

1. Dafkin C, Green A, Kerr S and McKinon W. The Patellar Reflex: Does activity of quadriceps femoris muscles reflect leg movement?
   Neurological Research (in press, 2012. DOI: 10.1179/1743132812Y.0000000011)

2. Dafkin C, Green A, Kerr S, Veliotes D and McKinon W. The Accuracy of Subjective Clinical Assessments of the Patellar Reflex.
   Muscle and Nerve (in press 2012. DOI:10.1002/mus.23487)

Conference presentations

International conferences

'Neurophysiological and biomechanical characteristics of Restless legs Syndrome'. Oral presentation at the International Student Congress of Medical Sciences, Groningen, the Netherlands, June 2009. Awarded best presentation in the Neurology session.

'Neurophysiological and biomechanical characteristics of Restless legs Syndrome'. Poster presentation at the International Congress of Parkinson's Disease and Movement Disorders, Paris, France, June 2009.

'Relationship between discomfort, pain and motor activity associated with Restless Legs Syndrome during the Suggested Immobilization Test'. Poster presentation at the European Sleep Research Society Congress, Lisbon, Portugal, September 2010.

'Circadian Rhythms in Restless Legs Syndrome'. Oral presentation at the Society of Neuroscience in Africa Congress, Addis Ababa, Ethiopia, February 2011.

'Descriptors of Restless Legs Syndrome sensations'. Poster presentation at the World Congress of Neurology, Marrakech, Morocco, November 2011.

'The relationship between sensory and motor components of Restless Legs Syndrome during the Suggested Immobilization Test'. Poster presentation at the Society for Neuroscience annual meeting, New Orleans, USA, October 2012.

Local South African conferences

'Neurophysiological characteristics of Restless legs Syndrome'. Oral presentation at the South African Pharmacology and Neurosciences Congress, Grahamstown, South Africa, October 2008.

'Discrete spinal cord functional abnormalities revealed by reflex testing in patients with Restless Legs Syndrome'. Oral presentation at the Physiological Society of Southern Africa Conference, Stellenbosch, South Africa, September 2009.

'Neurophysiological characteristics of Restless legs Syndrome'. Oral presentation at the Clinical Neurophysiology Society of South Africa Congress, Johannesburg, South Africa, October 2009.

'Neurophysiological and biomechanical characteristics of Restless legs Syndrome' Poster presentation at the University of the Witwatersrand Postgraduate Symposium, Johannesburg, South Africa, November 2009.

'Discrete spinal cord functional abnormalities revealed by reflex testing in patients with Restless Legs Syndrome'. Oral presentation at the South African Sleep Society Congress, Stellenbosch, South Africa, February 2010.

Related experiences

21st IBRO-ISN School of Neuroscience in Africa

"*Cell death mechanisms and neurodegenerative disorders*"

Fayoum, Egypt, December 2009.

25th IBRO-ISN School of Neuroscience in Africa

"Onslaughts on the central dopaminergic system""

Durban, South Africa, November 2010.

Contributions to the research

As part of the required declaration of this dissertation, I acknowledge contributions by various individuals to this work as detailed below.

The experimental design for all work described in this thesis was devised by myself in conjunction with my supervisors, Warrick McKinon and Alison Bentley.

Drafting of the written works was undertaken by myself with guidance from my supervisors.

Warrick McKinon and I were responsible for the laboratory modifications, camera mounting, construction of the light augmentation rings and timing device, development of some kinematics analysis tools, kinematics calibration frame and design and construction of the patellar hammer.

Other than the H-reflex testing performed by Dr Anderson, all data collection and analysis was completed by me.

Algorithms for the entire kinematics process were written by Warrick McKinon, myself or obtained from freely available (acknowledged) sources.

CHAPTER 1

LITERATURE REVIEW



“I feel as if my leg is like a candy cane

that is slowly being eaten from the inside out by an army of ants”.

Quoted description of Restless Legs Syndrome and image: as provided by a patient with RLS participating in the studies contained within this thesis.

1. RESTLESS LEGS SYNDROME AND PERIODIC LIMB MOVEMENTS

Restless legs syndrome (RLS) and periodic limb movement disorder (PLMD) are related neurological disorders presenting with spontaneous sensory (RLS) and motor (PLM) activity. It is estimated that up to 10% of the general population (Garcia-Borreguero *et al.*, 2006) suffer from RLS and PLM and the consequences affect both sufferers and their partners. Although often occurring together, and suspected to be manifestations of the same disorder, RLS and PLM can present independently of each other. In the sections below I first introduce the reader to the concept of RLS and PLM, comment on the prevalence of these disorders and provide a description of their pathophysiology and circadian variations and followed by a review of previous work done to quantify and qualify these disorders. As will be discussed below, our understanding of these disorders is largely observational. Much research remains before we have a full understanding of, and are therefore able to effectively treat RLS and PLM.

1.1. DEFINITIONS AND DIAGNOSIS

The accurate diagnosis of RLS and PLM is crucial for determining prevalence statistics (as discussed hereafter), administering the correct treatment which has lifelong implications and differentiating RLS from other disorders that have similar presentations to it. The diagnosis is dependent on the definitions of RLS and PLM and thus it is critical that these definitions are correct and accurate.

1.1.1. Restless legs syndrome

Restless legs syndrome (RLS) is a sleep-related movement disorder characterized by an urge to

move in response to uncomfortable or painful sensations experienced in the legs and occasionally the arms. The irresistible urge to move results in voluntary leg movements to relieve the discomfort (Allen & Earley, 2001b). Symptoms are exacerbated in the evening primarily during periods of inactivity such as long distance travel, or preparing to sleep, and improve in the morning (Hening *et al.*, 1999b).

Published descriptions of the sensations of RLS include: "parasethesia and dysesthesia", "tingling, burning, jittery, and prickling" and even as "ants or Coca-Cola in the bones and veins" (Earley *et al.*, 2000b). Symptoms have also been described as painful in up to 80% of RLS patients (Winkelmann *et al.*, 2000) and have been suggested to be a subclinical form of pain (Bentley *et al.*, 2007). The alleviation of RLS symptoms with analgesic medications, amongst other treatments, further supports the concept that pain pathways are involved in the sensory symptoms of RLS (Hening *et al.*, 1999a).

Various different strategies (e.g. vigorous leg movements, stretching, flexing, walking, rubbing and massage) are employed by RLS patients to ease their discomfort. Relief comes almost instantaneously with such movements and symptoms can remain at bay for up to an hour after cessation of movement, depending on the severity of the disorder. However, because symptoms occur just preceding sleep, these strategies often delay sleep onset (Montplaisir *et al.*, 2005).

RLS was first recognized as a problem and described in the 17th century. An English physician, Sir Thomas Willis, described RLS symptoms in 1685 as: "Wherefore to some, when being abed they betake themselves to sleep, presently in the arms and legs leapings and contractions of the tendons, and so great a restlessness and tossing of their members ensue that the diseased

are no more able to sleep than if they were in a place of the greatest torture" (as cited in Wetter & Pollmacher, 1997). Subsequently, in the 19th century RLS was deemed a psychiatric disorder known as "anxietas tibiarum" owing to the bizarre descriptions of symptoms (Ekbom, 1945). RLS was clinically accepted and formally described by Karl Ekbom in 1945 giving rise to the name of 'Ekbom syndrome' or the more commonly used 'restless legs syndrome' (Ekbom, 1945; Wetter & Pollmacher, 1997; Allen & Earley, 2001b). In 1990, RLS was formally classified as a sleep disorder in the International Classification of Sleep Disorders (Thorpy, 1990). The diagnostic features, all based on patients' symptoms, have subsequently been modified, re-defined and validated by the International Restless Legs Syndrome Study Group (IRLSSG) in 1995 (Walters, 1995) and later revised in 2003 (Allen *et al.*, 2003).

Currently, RLS is characterized and clinically diagnosed according to the following four essential criteria (Walters, 1995; Allen *et al.*, 2003):

1. "An urge to move the legs, usually accompanied or caused by uncomfortable and unpleasant sensations in the legs"
2. "The urge to move or unpleasant sensations begin or worsen during periods of rest or inactivity such as lying or sitting"
3. "The urge to move or unpleasant sensations are partially or totally relieved by movement, such as walking or stretching, for at least as long as the activity continues"
4. "The urge to move or unpleasant sensations are worse in the evening or night than during the day, or only occur in the evening or night"

Positive answers to all four of these questions are required for the diagnosis of RLS. Recently the four diagnostic criteria of RLS have been summarized into a single question for rapid

screening in a neurological clinical practice as follows: “When you try to relax in the evening or sleep at night, do you ever have unpleasant, restless feelings in your legs that can be relieved by walking or movement?” (Ferri *et al.*, 2007).

In addition to the essential criteria for the diagnosis of RLS, there are supportive non-essential clinical features that may be useful in resolving uncertainties regarding the diagnosis. These supportive features are: a positive family history of RLS; positive response to dopaminergic treatment and the presence of PLM (during wakefulness or sleep) (Allen *et al.*, 2003). First degree relatives are 3-5 times more likely to experience RLS than people without RLS (Allen *et al.*, 2003). Initially, many RLS sufferers exhibit a positive therapeutic response to treatment with either L-dopa or dopamine-receptor agonists. As previously noted, PLM are commonly associated with RLS and have a suspected common aetiology and therefore their appearance may confirm the diagnosis of RLS (Allen *et al.*, 2003; Trenkwalder & Paulus, 2010). Complicating the accurate diagnosis of RLS is that there are different RLS phenotypes.

1.1.1.1. Restless legs syndrome phenotypes

There are various classifications and subgroups proposed for RLS which may suggest that RLS is a heterogeneous disorder. RLS patients can be classified according to whether their RLS is idiopathic or secondary, early or late onset or familial or sporadic. According to Allen and Earley the aetiology of RLS may differ between groups with different expressions of the disorder, thus making it difficult to accurately define (Allen & Earley, 2000).

The most accepted distinction is between primary and secondary RLS. Primary or idiopathic RLS involves RLS symptoms in the absence of other medical problems known to be associated

with RLS. The secondary or symptomatic form of RLS is essentially 'acquired', occurring in association with other conditions such as iron deficiency anaemia, uraemia, neuropathies, pregnancy and renal failure (Wetter & Pollmacher, 1997; Patrick, 2007; Whittom *et al.*, 2007). Despite the underlying differences causing the RLS, there do not appear to be any differences in the physical presentation between primary and secondary RLS.

RLS used to be described as a disorder affecting 'older' people (Allen & Earley, 2001b). Subsequent research has indicated that there are two distinct phenotypes, namely: early and late onset (Walters *et al.*, 1996). The critical onset age (years) quoted in the literature ranges from 20's to mid 40's depending on methods used to determine age-of-onset (Allen & Ritchie, 2008). The cut-off between early and late onset is determined as the trough between the peaks of the bimodal distribution when the onset of RLS is plotted as a frequency distribution. Earlier studies set the cut-off for late onset at 45 years (Allen & Earley, 2000; Polydefkis *et al.*, 2000) but subsequent reports indicate that the division between early and late onset appears in the mid 30's (Whittom *et al.*, 2007; Allen & Ritchie, 2008). Various problems complicate the determination of age-of-symptom-onset. Accurate recall of the symptoms can be difficult to determine and may reflect a change in the severity of the disorder rather than a variance in age-of-symptom-onset (Allen & Ritchie, 2008). Additionally, RLS in children is often misdiagnosed as attention deficit hyperactivity disorder (ADHD) or growing pains (Montplaisir *et al.*, 2005). Early onset RLS is more slowly progressing, more likely to have a familial component, has no relationship to iron status, and has a female preponderance when compared to late onset RLS (Allen & Earley, 2000; Winkelmann *et al.*, 2002; Hanson *et al.*, 2004; Whittom *et al.*, 2007).

Another division amongst RLS patients is the split into familial, those having first degree relatives with RLS; and sporadic, with no close relatives suffering from RLS. Approximately 60-65% of RLS patients indicate a positive family history (Winkelman, 2006). There may be a 5-fold risk increase for first degree relatives of patients with early onset RLS (Allen & Ritchie, 2008). A positive family history of the disorder is often used as supporting confirmation in the diagnosis of RLS (Montplaisir *et al.*, 2005). Genetics studies to locate specific genes that are responsible for RLS are still in their infancy. Various candidate genes have been identified and investigated, and thus far it has been suggested that the genetic component is complex, possibly with multiple genes playing a role, with the genes for early and late onset RLS possibly occurring on different chromosomes (Zucconi *et al.*, 2007).

The different phenotypes of RLS indicate that there may be multiple factors contributing to this disorder. Looking at other components of the disorder may help differentiate whether it is a homogenous or heterogeneous disorder.

1.1.2. Periodic limb movements

Involuntary movements, known as periodic limb movements (PLM), are commonly associated with RLS. According to Allen and Earley (2001) 85% of patients with RLS also express PLM, possibly suggesting a similar origin for the two.

PLM are characterized by involuntary leg movements or muscle twitches occurring mainly during sleep (PLMS) but which can also occur during restful wakefulness (PLMW). A further distinction is made for periodic limb movement disorder (PLMD) which is defined as "… a clinically significant sleep disruption from PLMS that cannot be accounted for by another sleep

disorder" (Allen & Earley, 2001b). In the literature, PLMD is generally referred to as PLM, despite PLM actually describing a sign of PLMD. For consistency with the literature, I will refer to the disorder as PLM with recognition that the presence of PLM does not imply the disorder.

PLM were first noted in 1943 by Allison (as cited in Coccagna *et al.*, 2004) and were originally called 'nocturnal myoclonus' by Symonds in 1953, who incorrectly assumed that they were a form of epilepsy (Symonds, 1953). In the early 1980s the limb movements were characterized and called "PLM" by Coleman (Coleman, 1982).

The uni- or bilateral repetitive leg movements are characterized by extension of the big toe; partial dorsiflexion of the ankle, the knee and occasionally the hip (Coleman *et al.*, 1980). Smith (1985) reported, based on video image analysis, that PLM during sleep resembled the Babinski sign (Smith, 1985). The Babinski sign, upon plantar stimulation, is characterised by dorsiflexion of the big toe and fanning of the other toes (Van Gijn, 1996). The particular sequence of muscle activations of PLM prompted Lugaresi to conclude that PLM resemble the flexor withdrawal reflex (cited in Vetrugno *et al.*, 2007a).

PLM are diagnosed by an increase in activity on surface electromyography (EMG) of either one or both of the anterior tibialis muscles, the muscle most commonly involved in PLM (Hening, 2004a). Polysomnography (PSG) is the gold standard method used in the basic diagnosis of PLM, particularly PLMS. PSG is required to rule out movements related to respiratory events, and differentiate PLMS from other possible sleep disorders (Hening, 2004a).

According to the World Association of Sleep Medicine (WASM) (Zucconi *et al.*, 2006), the defining criteria, based on Coleman's original observations, for pathological leg movements

involved in a PLM sequence consist of electromyographic activations fulfilling the following parameters:

1. The EMG amplitude increases by at least 8μV above baseline
2. Each individual burst has a duration of 0.5 to 10 seconds
3. Each EMG activation must be separated by at least 5 but not more than 90 seconds.
4. There are four or more EMG bursts meeting the above criteria.

Hundreds of these PLM, clustering into episodes, may be present during sleep and are generally more numerous in, but not exclusive to, the first half of the night (Wetter & Pollmacher, 1997). The leg movements may or may not be associated with arousals depending on the duration of the movement and the patient's sleep stage (Wetter & Pollmacher, 1997). The severity of the PLM is determined by calculating the number of PLM per hour of total sleep time, termed the PLM index (Wetter & Pollmacher, 1997; Hornyak *et al.*, 2006). A PLM index of greater than 5 is considered pathological (Hornyak *et al.*, 2006). PLM are commonly associated with various other sleep disorders including sleep apnoea, rapid eye movement (REM) sleep behaviour disorder, insomnia, hypersomnia, and narcolepsy (Vetrugno *et al.*, 2007a). PLM are also known to be present in association with non-sleep related disorders such as congestive heart failure, spinal cord injury, end-stage renal failure and hypertension (Wetter & Pollmacher, 1997; Vetrugno *et al.*, 2007a).

PLM, however, are also known to manifest in healthy individuals, particularly the elderly, with between 30% and 50% of people over the age of 60 years having a PLM index greater than 5 without associated RLS or other sleep disorders (Ancoli-Israel *et al.*, 1985; Dickel & Mosko, 1990).

1.2. PREVALENCE AND IMPORTANCE OF RESTLESS LEGS SYNDROME AND PERIODIC LIMB MOVEMENTS

To date epidemiological studies of RLS have primarily focused on Western (mainly Caucasian) societies with less attention paid to non-Western populations and specific groups commonly associated with the presence of RLS. Garcia-Borreguero et al (2006) extensively reviewed the epidemiological studies of RLS from 1993 to 2005 and reported that the most common prevalence is between 2.5 and 10% of the general population (Garcia-Borreguero *et al.*, 2006), making it one of the most common neurological disorders (Cervenka *et al.*, 2006). A recent paper examining previous (pre 2010) epidemiological studies suggests that the frequency of RLS increases as a function of increasing distance from the equator, particularly in North America and Europe (Koo, 2011). RLS is more prevalent in the elderly (two to three fold increase from young adults to those over 60 years old) and with an apparent gender bias of 2:1 (female: male) across all ages (Garcia-Borreguero *et al.*, 2006).

Non-western population studies are limited and report a wide range of prevalence of RLS in comparison to Western based studies, however methodological issues bring the validity of some of these findings into question (Garcia-Borreguero *et al.*, 2006). The prevalence of RLS in the general population of Asian countries that have been studied has a broad range, between 0.9% and 12.0% (South Korea); 1.0% and 11.4% (Japan); 1.6% (Taiwan); and 2.1% (India) (Ohayon *et al.*, 2012). The higher values observed in these studies were based on research using a single screening question (not defined by the IRLSSG diagnostic criteria) and thus may not be an accurate reflection of the prevalence of RLS in these populations. Very few studies have been conducted in native South American and African populations. One study in Ecuador reported a low prevalence of RLS between 0.8% and 3.2% (Ohayon *et al.*, 2012), whereas

another in Argentina reported a much higher prevalence of 20.2% (Persi *et al.*, 2009). One population based study in Northern Tanzania reported an extremely low prevalence of 0.01% however the authors did indicate this may not be a true reflection of the population as "preoccupation with daily survival" may have distracted the people in this remote, rural community from noticing and reporting the non-life threatening symptoms of RLS (Winkler *et al.*, 2010). A small study in South Africa indicated a prevalence of RLS in 23.3% of an aged population (Venter *et al.*, 2001). This study was, however, conducted in a presumably Caucasian dominant community and may not reflect the true population dynamics of the country (an ethnic breakdown of the sample was not reported). A study of individuals of African American ancestry reported a prevalence of 4.7% in their sample (Lee *et al.*, 2007).

The IRLSSG established diagnostic criteria for RLS in 1995, but some epidemiological studies do not use these criteria or were conducted before the criteria were established and thus the diagnosis of RLS may vary between studies, which itself may confound attempts to try accurately describe prevalence. Further confounding factors in the epidemiological studies are the lack of distinction between primary and secondary RLS. Acquired RLS has a higher prevalence in various metabolic, neurological and other conditions. There are specific medical situations where the prevalence of RLS is greater, for example: during pregnancy between 19% (Goodman *et al.*, 1988; Suzuki *et al.*, 2003) and 26% (Manconi *et al.*, 2004) of women experience RLS and between 7% (Bhowmik *et al.*, 2003) and 83% (Holley *et al.*, 1991) of patients in end stage renal failure also have RLS. The RLS diagnostic criteria do not differentiate between different RLS phenotypes and therefore the prevalence data may be an inaccurate reflection of the population.

The prevalence of PLMS is particularly difficult to ascertain because sufferers are often unaware of the condition and rely on bed partners to report these nocturnal leg activities. Unlike RLS that can be evaluated by questionnaires using the diagnostic criteria, the assessment of PLMS is largely and most accurately dependent on EMG in conjunction with PSG based on the WASM scoring criteria (Zucconi *et al.*, 2006). Scofield et al. (2008) performed PSG on a sample of 592 American individuals and discovered a prevalence of PLMS of 7.6% in this population. Predominantly more Caucasian than African American individuals (9.3% vs. 4.3%) presented with >5 PLMS per hour (Scofield *et al.*, 2008). PSG recordings on 100 elderly people showed that up to 50% of the group had PLM without an associated sleep disorder (Dickel & Mosko, 1990). High costs, and the practicalities of EMG and PSG measurements, hinder large scale epidemiological studies.

Telephone interview surveys and actigraphy (measure of limb acceleration) studies offer alternate, much more practical methods facilitating large scale epidemiological studies. Despite the advantages of utilizing telephonic and actigraphy for determining prevalence statistics, there have been relatively few studies using these techniques, possibly related to methodological problems. The largest prevalence study to date (n=18980), by telephone interview survey using the International Classification of Sleep Disorders criteria found a prevalence of 3.9% in Europe (Ohayon & Roth, 2002). Pathological PLMS (PLM index greater than 5) were present in 37% of volunteers in a small actigraphy study conducted in the United Kingdom (Morrish *et al.*, 2002). Despite the high co-morbidity between RLS and PLM there does appear to be a difference in the reported prevalence of RLS and PLM. Whether this is due to methodological errors in determining the epidemiology or indicates that perhaps these are independent disorders is yet to be determined.

The clinical significance and impact of RLS and PLMS is unclear, some patients cite severe sleep complaints and associated fatigue where others report inconsequential symptoms. The sensations and relief-seeking leg movements of RLS generally occur just prior to sleeping or can awaken patients from their sleep. This frequently delays sleep onset, causes sleep disruption and subsequent excessive daytime sleepiness, and even depression (Allen & Earley, 2001b). The total sleep time of RLS patients has been shown to be remarkably reduced with a mean sleep efficiency of only 50% (compared to over 80% in healthy sleepers) (Allen & Earley, 2000). Other studies have shown that RLS patients do not report fatigue and daytime sleepiness (based on Epworth Sleepiness Scale scores) despite significant levels of sleep loss (Bassetti *et al.*, 2001; Saletu *et al.*, 2002; Gamaldo *et al.*, 2009). Differences in RLS severity, patient ages, and RLS phenotypes (e.g. age of RLS onset) could possibly account for the different outcomes. If RLS is a heterogeneous disorder, then differences in the patient samples could lead to conflicting results.

Besides the sleep related consequences of RLS and PLM, or possibly even related to them, there are also reports that these patients are at risk of cardiovascular problems, depression and anxiety disorders. The sleep fragmentation and sleep deprivation accompanying RLS and PLM puts these patients at greater risk of developing cardiovascular problems possibly due to sympathetic nervous system hyperactivity (Winkelman *et al.*, 2008). The association of RLS and PLM with cardiovascular risk factors makes these two disorders a far greater public health problem than previously recognised (Winkelman *et al.*, 2008; Schlesinger *et al.*, 2009; Walters & Rye, 2009).

Evidence from patients with insomnia indicates a high prevalence of depression associated with the reduced sleep quality thus it follows that RLS patients that may have delayed sleep

onset and decreased sleep efficiency could also be at risk of depression (Allen & Earley, 2001b; Tsuno *et al.*, 2005). Depressive and anxiety disorders have also been shown to have an increased prevalence amongst RLS patients (Earley & Silber, 2010). Although treatment for the symptoms of RLS and PLM is currently available, we still lack a full understanding of these disorders and their underlying pathophysiology.

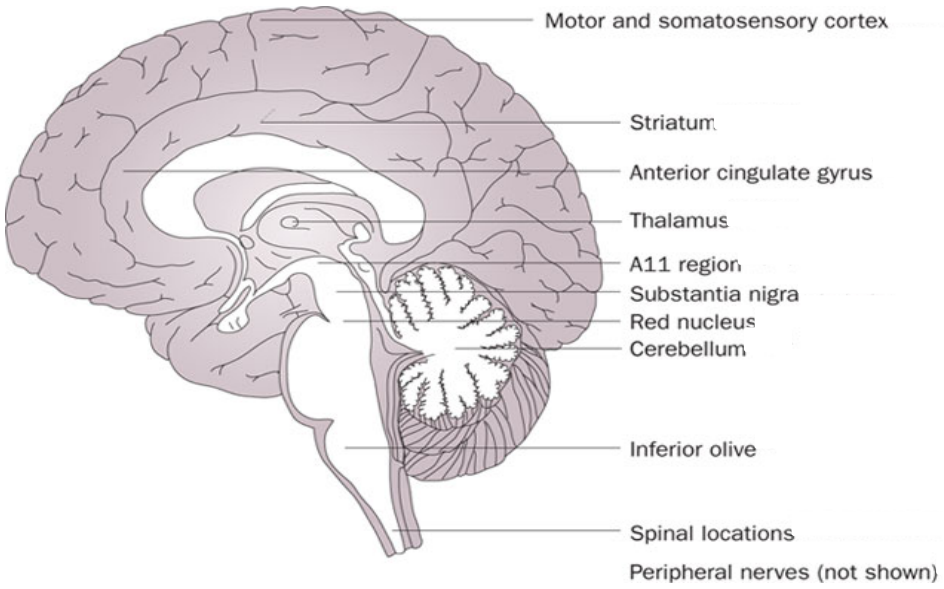
1.3. AETIOLOGY OF RESTLESS LEGS SYNDROME AND PERIODIC LIMB MOVEMENTS

The aetiology of RLS and PLM is yet to be fully resolved and there are numerous potential theories. A detailed exploration of all the theories concerning the aetiology of RLS and PLM would require extensive study beyond the scope of this literature review. A brief overview of the main theories concerning the aetiology of RLS and PLM follows hereafter, with particular reference to those of relevance to the current thesis.

The combined sensorimotor nature of RLS and PLM suggests that there is either an abnormality in a single region where both sensory and motor areas coincide or there may be many regions involved in the pathogenesis of these disorders, extending from the limbs themselves to the cerebral cortex. The circadian bound nature of the symptoms also needs to be considered in determining the pathophysiology of RLS and PLM. Various different chromosomes have possible links to RLS, which might support a multifactorial pathophysiology (Winkelman, 2006).

One theory that does seem to account for all the features of the diagnostic criteria is that of decreased dopamine in the central nervous system. Dopamine plays a regulatory role in both sensory and motor functions (Winkelman, 2006). Traditionally the research approaches used

to explore the dopamine hypothesis of RLS and PLM have included: study of the role of the neurotransmitter, dopamine, itself; identification of specific structures or functions of the nervous system, cortical and subcortical, particularly related to the dopaminergic system (identified in Figure 1) and iron metabolism (Allen & Earley, 2001b). The involvement of each of these research approaches, and their relation to one another, in the development of our understanding of the pathogenesis of RLS and PLM will be discussed below and will lead into the concept of central sensitization followed by considerations of the circadian variations of symptoms.



Trenkwalder, C. & Paulus, W. (2010) Restless legs syndrome: pathophysiology, clinical presentation and management *Nat. Rev. Neurol.* 6, 337-346

Figure 1: Areas of the CNS putatively implicated in the pathogenesis of RLS and PLM (Trenkwalder & Paulus 2010)

1.3.1. Abnormalities of the dopamine system

The serendipitous discovery that RLS and PLM respond exceptionally well to dopamine agonist treatment indicates that RLS and PLM may be caused by an abnormality of the dopaminergic

linked pathways. The dysfunction is suspected to be central rather than peripheral because dopamine antagonists that can cross the blood-brain barrier exacerbate RLS symptoms, whereas those that cannot cross the blood-brain barrier produce no noticeable symptoms (Winkelman, 2006). Evidence suggests that abnormalities in the dopaminergic linked pathways, reduced dopamine uptake and binding, and/or hypoactive dopaminergic neurotransmission are possible causes of the symptoms of RLS and PLM (Cervenka *et al.*, 2006; Winkelman, 2006).

Figure 2 represents the dopamine synthetic and metabolic pathway and shows relevant relationships with factors that are regulated by or regulate dopamine and are featured in the RLS and PLM literature. Homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5H1AA) are cerebrospinal fluid (CSF) monoamine metabolites. HVA is the major dopamine metabolite and is a measure of dopamine metabolism and 5H1AA is the main metabolite of serotonin. The ratio between them (HVA: 5H1AA) is an indication of the serotonergic modulation of dopaminergic activity. 3-ortho-methyldopa (3OMD) is the product of a minor alternative pathway for the metabolism of L-dopa (L-dihydroxyphenyalanine), the pre-cursor molecule of dopamine.

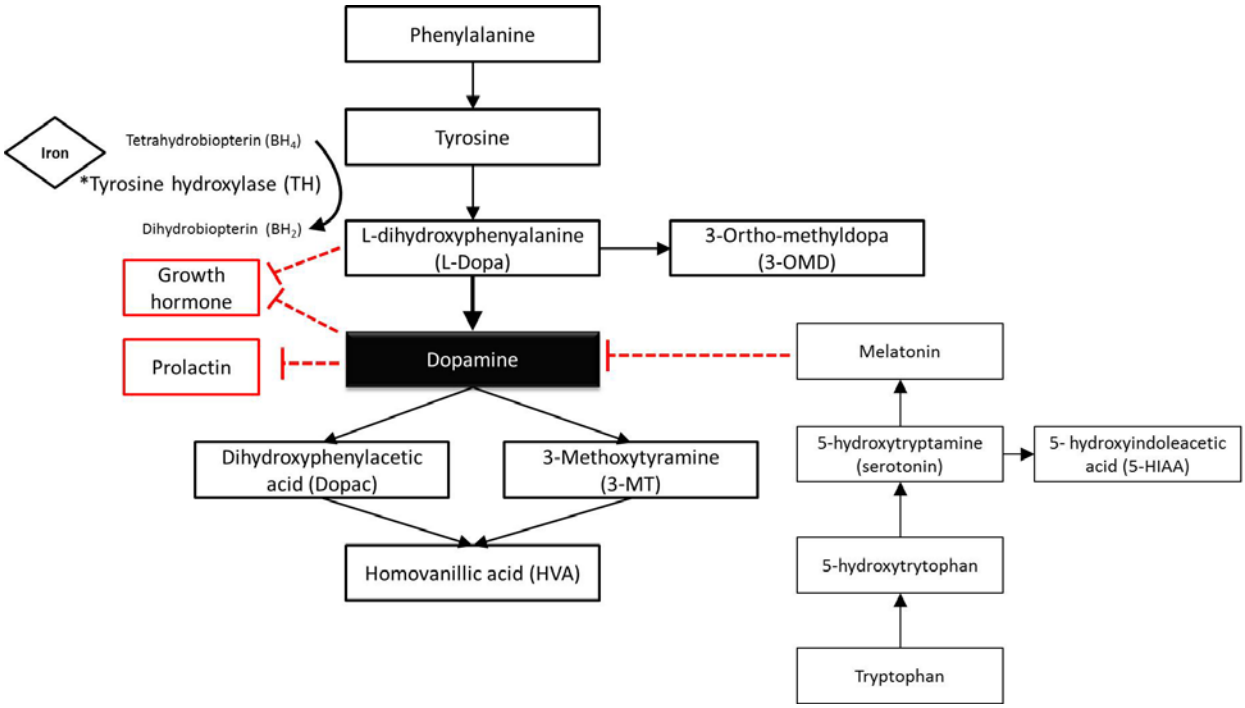


Figure 2: Schematic diagram of the dopamine synthesis and breakdown pathway including interactions with relevant inputs and outputs that may play a role in the pathophysiology of RLS and PLM. The dotted red lines indicate inhibition. *The conversion of tyrosine to L-Dopa by the iron dependent tyrosine hydroxylase is the rate limiting enzymatic step.

Despite the positive response following dopaminergic treatment indicating that there is an abnormality of the dopaminergic system in RLS and PLM patients, there is relatively little evidence of a dopaminergic deficiency in these patients. A study conducted in a single patient with severe RLS in 1985 reported high CSF dopamine and HVA concentrations (Montplaisir et al., 1985). Subsequently, focusing specifically on factors directly involved in the metabolism of dopamine (represented in Figure 2), CSF levels of HVA, 3OMD, levodopa and serotonergic metabolites were no different between patients with RLS and control subjects (Earley et al., 2001, Stiasny-Kolster et al., 2004d). Further research focusing on the CSF levels of dopamine and its metabolites has been conducted however these studies were done in the context of the circadian variation and are discussed in section 1.3.4. Neuroimaging studies of specific dopaminergic pathways have also provided evidence of the abnormalities of the dopamine system in RLS and PLM patients.

Both the A9 (nigrostriatal) and A11 spinal dopaminergic systems have been implicated in the pathophysiology of RLS given their involvement in movement generation and circadian rhythms respectively. The nigrostriatal A9 cell group is one of the major ascending dopaminergic inputs to the striatum and is suspected to be involved in the initiation of voluntary movement (Kandel et al., 2000). Degeneration of these pathways with reduced dopamine is associated with the tremors, rigidity and akinesia characteristics of Parkinson's disease (Rye, 2004). Therapeutic agents used to treat Parkinson's disease have also been successful in the alleviation of RLS symptoms. The major source of dopamine in the basal ganglia (responsible for control of motor function) is the A9 nigrostriatal cell group (Vetrivelan *et al.*, 2010). Loss of dopamine in the A9 pathway may result in involuntary movements (Obeso *et al.*, 2002) such as those seen in PLM and thus this pathway is implicated in the aetiology of PLM.

There is relatively little consistent evidence that the A9 pathway is affected in patients with RLS and PLM. Staedt and colleagues originally reported a deficiency in striatal D2 dopamine receptors (Staedt *et al.*, 1993; Staedt *et al.*, 1995). Various neuroimaging studies using SPECT and PET have examined receptor binding potentials of radioligand dopamine D2-receptors to explore processing of sensory stimuli. Striatal D2-receptor binding potentials were reported to be increased (Cervenka *et al.*, 2006), decreased (Turjanski *et al.*, 1999; Michaud *et al.*, 2002c) and no different (Eisensehr *et al.*, 2001; Tribl *et al.*, 2004) in RLS patients compared to control subjects. Striatal dopamine transporter (DAT) has also been shown to be normal (Eisensehr *et al.*, 2001; Michaud *et al.*, 2002c), downregulated (Earley *et al.*, 2011) and upregulated (Kim *et al.*, 2012) in RLS patients compared to control subjects. Nigrostriatal presynaptic dopaminergic function has also been shown to be normal (Trenkwalder *et al.*, 1999b) and hypofunctional (decreased F-dopa uptake) (Turjanski *et al.*, 1999; Ruottinen *et al.*, 2000) in RLS patients

compared to control subjects. Many of these neuroimaging studies had small sample sizes and were conducted during the symptom free daytime period. Taking into account possible methodological problems, it appears that there may be dopaminergic hypofunction in RLS patients however further research of the role of the nigrostriatal pathway in the aetiology of RLS and PLM is required.

Dysfunction of the A11 diencephalospinal dopaminergic pathway, the only significant descending spinal dopamine system, has also been implicated in the pathophysiology of RLS. Abnormality in the spinal cord could produce RLS and PLM sensorimotor symptoms as the spinal cord is the primary input of sensory afferents and final output stage of motorneurones (Paulus & Schromburg, 2006). The A11 system is involved in sensorimotor integration and antinociception at the level of the spinal cord (Barraud *et al.*, 2010). Clemens et al (2006) proposed two mechanisms whereby the A11 pathway hypofunction may result in alterations of the peripheral sensory information that reaches the thalamus and ultimately the cortex (Clemens *et al.*, 2006). The first hypothesis suggests that peripheral sensory input is altered at the level of the spinal cord as the A11 pathway is known to modulate sensory input to the dorsal horn. A loss of dopamine would result in abnormal sensory information being sent to the thalamus. The same group proposed an alternative mechanism which also occurs at the level of the spinal cord, but involves the A11 pathway modulation of sympathetic output to the muscles. With hypofunction of the A11 pathway, there will be an increased sympathetic outflow to the periphery, changing the sensory information returned to the spinal cord (Clemens *et al.*, 2006). The A11 dopaminergic cells are also involved in pain control (dopamine inhibits neurons in the dorsal horn where pain information is received). Dysfunction of this pathway may disturb the gate control mechanism for pain and therefore allow transmission of the painful symptoms of RLS (Moller *et al.*, 2010). Also, further supporting the argument in

favour of A11 pathway involvement in the aetiology of RLS and PLM is that the A11 pathway is anatomically connected to the suprachiasmatic nucleus which is involved in the control of circadian rhythms (Trenkwalder & Paulus, 2004).

Contrary to the prevalent idea that all RLS patients have a dopamine deficiency, Allen et al (2009) suggest that in fact RLS patients with abnormally elevated CSF 3OMD and HVA concentrations have increased dopamine synthesis and tyrosine hydroxlase expression associated with a significant deficiency of central iron (Allen *et al.*, 2009). These RLS patients with abnormally increased CSF 3OMD levels also presented with significantly more PLMS/hour than RLS patients with normal 3OMD levels suggesting the former group had a greater severity of RLS. The RLS patients with normal 3OMD levels exhibited CSF HVA concentrations and ferritin levels that were significantly slightly lower than control subjects. The authors do suggest that there may be a biphasic pattern of dopaminergic status related to the severity or phase of the disorder. There may be increased production of dopamine in patients with severe RLS symptoms (more PLM and discomfort) associated with decreased concentrations of central iron and patients with less severe RLS symptoms may possibly have decreased dopamine concentrations and less central iron loss (Allen *et al.*, 2009).

This pattern of dopaminergic status, where RLS symptoms will present if dopamine concentrations are too low or too high, is similar to the dopamine paradox of symptom augmentation demonstrated in Figure 3. Kim and colleagues propose a similar idea in their recent paper where they suggest that RLS may be due to dopaminergic dysregulation, rather than simple increased or decreased dopaminergic neurotransmission. They suggest that upregulation of dopaminergic neurotransmission may cause severe RLS whereas downregulation may result in less severe RLS (Kim *et al.*, 2012).

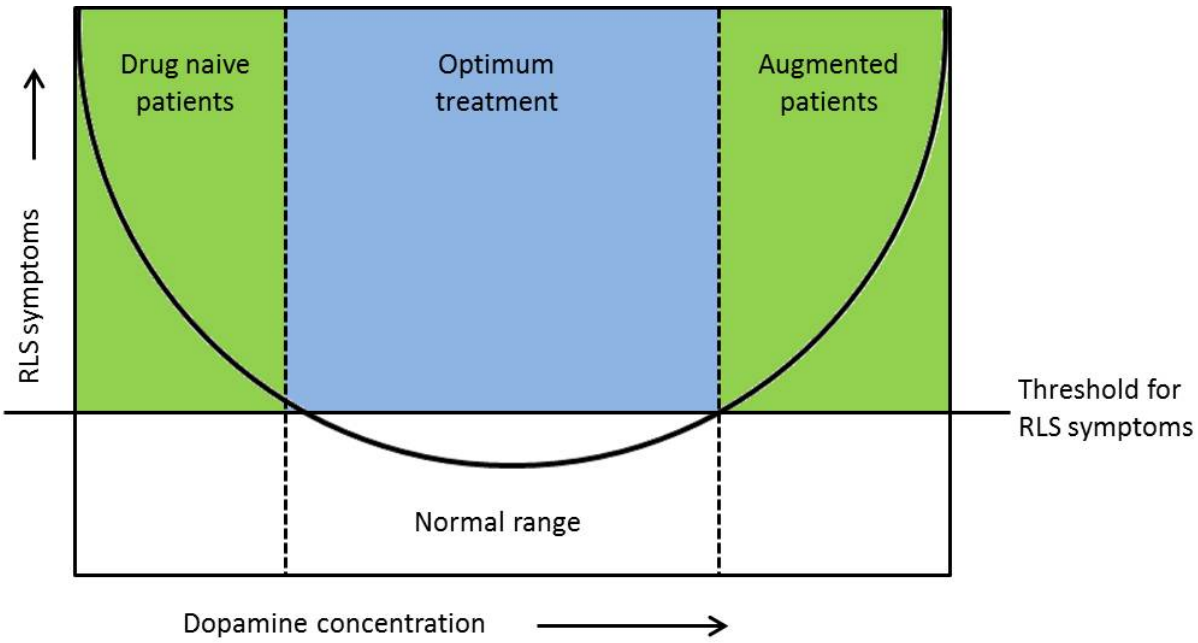


Figure 3: The U-shaped dopamine paradox curve. RLS symptoms present as a function of the dopamine concentration. Drug naive patients, who have less than optimal dopamine concentrations, occur on the left of the curve. Patients on optimal treatment, where dopamine concentrations are within the normal range and do not present with RLS symptoms, occur in the middle. In patients with symptom augmentation on the right of the curve, there is an exacerbation of symptoms with an abundance of dopamine (Paulus & Trenkwalder, 2006).

There does not seem to be a consistent pattern of abnormality in the dopaminergic systems and most of the evidence supporting dopaminergic involvement is based on pharmacological studies with a lack of conclusive physiological data. The conflicting literature regarding the dopaminergic system may reflect different phases of the disorders progression, differences in underlying pathology or heterogeneity within patient samples (for example disease severity, family history and age-of-onset) which may complicate the results. Many of the studies focusing on the role that dopamine plays in RLS and PLM were conducted during symptom free daytime periods, which may explain some of the conflicting literature and emphasize the importance of taking into account the circadian variation of symptoms (discussed in section 1.3.4).

1.3.2. Iron deficiency

Iron appears to play a central role in the dopaminergic aetiology of RLS. The conversion of tyrosine to dihydroxyphenyalanine (DOPA) by the enzyme tyrosine hydroxylase (the rate limiting enzyme), in the dopamine pathway, is iron dependent (iron is a cofactor for the hydroxylation of tyrosine) (Figure 2). Iron is also reported to be involved in the regulation of dopamine D2 binding sites and iron deficiency is accompanied by a significant reduction in the D2 receptor binding capacity (Ashkenazi *et al.*, 1982; Beard, 2003). The greatest iron concentrations in the brain are found primarily in components of the basal ganglia (substantia nigra, globus pallidus, and putamen) and the red nucleus (Krieger & Schroeder, 2001), areas which have been implicated in the pathophysiology of RLS and PLM.

Evidence supporting the role that iron plays in the pathophysiology of RLS and PLM includes studies using neuroimaging techniques, autopsy data, analysis of CSF and serum levels of ferritin and transferrin. In the body iron is bound to the proteins, ferritin and transferrin, to facilitate storage and transportation in a non-reactive form. Ferritin concentration is the best serum marker of iron stores. Transferrin is an iron binding blood plasma protein that is responsible for transferring iron from the gut or storage sites to other sites in the body or within the central nervous system (CNS) (Patrick, 2007). Measuring ferritin and transferrin concentrations provide a more accurate reflection of the overall iron status of the body than measuring the highly variable serum levels of iron alone.

Impaired iron metabolism and reduced iron concentrations have been detected in RLS patients particularly in the substantia nigra and the putamen measured using both neuroimaging techniques and immunohistochemical evaluation of brain tissue harvested during autopsy

(Allen *et al.*, 2001; Connor *et al.*, 2003; Baier & Trenkwalder, 2007; Patrick, 2007). Also, serum ferritin levels (Allen, 2004) have been negatively correlated with the severity of RLS symptoms (O'Keeffe *et al.*, 1994; Sun *et al.*, 1998; Clardy *et al.*, 2006). Disorders or conditions associated with iron deficiency, e.g. anaemia, pregnancy and end stage renal disease, have a high prevalence of RLS. Moreover, treating the underlying iron deficiency is known to result in resolution of the RLS symptoms (Allen & Earley, 2007).

RLS patients exhibit an altered brain iron acquisition profile (Connor *et al.*, 2011). In epithelial cells extracted during autopsy from the choroid plexus (the site of CSF production) of RLS patients, iron and ferritin staining were reduced whereas levels of transferrin and its receptor were upregulated (Connor *et al.*, 2011). CSF extracted via lumbar puncture from RLS patients has also been shown to have decreased ferritin and increased transferrin levels compared to controls (Earley *et al.*, 2000a; Mizuno *et al.*, 2005). Increased transferrin levels are indicative of decreased iron availability. The endothelial cells of the blood brain barrier express the transferrin receptors and thus upregulation of these receptors may be to increase the central uptake of iron. There was also reduced activity of iron regulatory protein and decreased transferrin levels in the microvasculature derived from the motor cortex suggesting that there are problems of brain iron acquisition in RLS patients (Connor *et al.*, 2011).

Overall, the data supports the concept that there is a brain iron deficiency in RLS patients. Imaging studies of the dopaminergic pathways which report decreased D2 binding are consistent with the insufficient brain iron hypothesis, and thus, some but not all of the dopamine studies are aligned with the hypothesis that central iron deficiency can account for the dopaminergic dysfunction reported for RLS patients. Further research is required to determine the relationship between iron and dopamine in the aetiology of RLS and PLM.

1.3.3. Central sensitization

If the hypothesis of insufficient brain iron in RLS and PLM patients which results in reduced dopaminergic function is true, this would then impact the A11 diencephalospinal pathway, the only significant descending spinal dopaminergic system. These descending pathways modulate the activity of spinal interneurones (both inhibitory and excitatory) and particularly those pathways using monoamines (for example dopamine depresses monosynaptic reflexes) (Clemens & Hochman, 2004). Thus, a deficiency of dopamine would alter the spinal cord function resulting in increased excitability of involuntary motor reflexes (central sensitization).

Central sensitization is caused by increased neuronal membrane excitability or reduced neuronal inhibition. Decreases in stimulation thresholds result in previously subthreshold inputs becoming stimulatory and thus there is an increased output of action potentials (Latremoliere & Woolf, 2009). Spontaneous action potentials will occur if the resting membrane potential is depolarised above the threshold potential. The spontaneous nature of the sensory and motor features of RLS and PLM has directed investigators towards the concept that these features may arise due to central sensitization. In particular, hyperexcitability of the spinal cord (central sensitization) has been proposed as the final common pathway responsible for the sensorimotor features of RLS and PLM and has some supporting evidence in pain and reflex studies.

Central sensitization presents, in some circumstances, as hyperalgesia (increased sensitivity to pain). Hyperalgesia can be tested using Quantitative Sensory Testing (QST). Some authors have shown that the function of the peripheral nociceptive pathways is normal in RLS patients (Han *et al.*, 2007; Tyvaert *et al.*, 2009) whilst CNS pain processing is amplified by RLS (Han *et al.*,

2007; Tyvaert *et al.*, 2009; Edwards *et al.*, 2011). Stiasny-Kolster et al. (2004) showed that RLS patients exhibit static mechanical hyperalgesia in response to punctate mechanical stimuli (Stiasny-Kolster *et al.*, 2004c). Schattschneider et al. (2004), using quantitative thermotest assessments, found that temperature perception in both idiopathic and secondary RLS was impaired but there were no differences in hot or cold pain thresholds between the two groups. Also, quantitative measures of the peripheral C fibre axon reflex showed significantly reduced flare responses and vasodilation in secondary but not idiopathic RLS indicating possible small fibre neuropathy in the former group (Schattschneider *et al.*, 2004). Thus they demonstrate that in idiopathic RLS the altered temperature perception is not due to a peripheral mechanism but rather an impairment of central somatosensory processing.

Areas of brain activation during RLS sensory discomfort (i.e. areas that are affected by RLS and PLM) have been assessed using various neuroimaging techniques (magnetic resonance imaging, single photon emission computed tomography, positron emission tomography and voxel-based morphometry). The identified brain areas of interest are similar to those areas associated with pain, and include the thalamus, cortex (various sites), cerebellum, hippocampus, amygdala and basal ganglia (Tracey, 2005). It has been further proposed that there is a deficiency in endogenous opioid inhibition of ascending spinothalamic pathways resulting in altered processing at the thalamic level and the feeling of abnormal sensations (Walters *et al.*, 2009). The identification of these areas may further support the notion that pain pathways are associated with the pathophysiology of RLS and PLM. However, further research is required to elucidate the exact contribution of the pain pathways to RLS and PLM.

Also of central (spinal) origin is the concept that PLM are possibly generated by the spinal central pattern generators (CPG) for gait. CPGs generate rhythmic, repetitive and stereotyped

movements (Lacquaniti *et al.*, 1999) which are characteristic traits of PLM. Increased excitability of the spinal cord could possibly trigger spontaneous movements such as those seen in PLM. CPGs can also act independently of supraspinal control (Lacquaniti *et al.*, 1999). The occurrence of PLM in patients following spinal cord injury indicates that their origin would have to be at the level of the spinal cord, making hyperexcitability of CPGs an attractive possibility for the creation of PLMS (Vetrugno *et al.*, 2007a).

Examining patients with spinal cord lesions, a condition of known spinal hyperexcitability due to interruption of descending supraspinal inhibition (Sheean, 2002), for evidence of RLS and PLM also contributes to the idea that these syndromes are generated from a spinal origin. Disorders involving upper motor neuron lesions, that isolate the spinal cord from supraspinal inhibitory control, are classical examples of spinal hyperexcitability (Nielsen *et al.*, 2007). Quatrale et al (2003) proposed sleep related disinhibition of descending central inhibitory pathways as a possible mechanism of spinal hyperexcitability in RLS and PLM patients and thus it would be expected that there would be commonality of spinal excitability between RLS and spasticity (as seen in spinal cord lesion patients) (Quatrale *et al.*, 2003). Isolated case studies of small numbers of patients with spinal cord injuries have indicated that RLS and PLM have developed following spinal cord insult of various causes. Ten patients with myelopathy had associated PLM (Yokota *et al.*, 1991), 3 patients had PLM accompanying a focal thoracic spinal cord lesion (Lee *et al.*, 1996), 3 patients following spinal cord lesions diagnosis presented with RLS (Hartmann *et al.*, 1999), and RLS developed in one patient after a vascular injury of the spinal cord (Tings *et al.*, 2003). The evidence of PLM in patients with spinal cord injury strongly supports that PLM are of a spinal cord origin.

PLM may have spinal circuitry in common the Babinski reflex and the flexor reflex. Video image analysis in the 1980s revealed that PLMS resembled the Babinski sign (Smith, 1985) however no objective measure has been done since then to confirm this observation. The importance of the similarity between PLM and the Babinski sign is that clinically, the presence of the Babinski sign may be indicative of upper motor neuron lesions. Disorders involving upper motor neuron lesions, that isolate the spinal cord from supraspinal inhibitory control, are classical examples of spinal hyperexcitability (Nielsen *et al.*, 2007). More recently, similarities between the flexor reflex and PLM have been demonstrated and the authors suggest that the two may share a common, spinal generator (Bara-Jimenez *et al.*, 2000).

Various neurophysiological studies, particularly using the Hoffmann reflex (H-reflex) (discussed in detail in section 2.2.3.2.) have been conducted supporting the concept of spinal hyperexcitability as the origin of RLS and PLM. The first study showing spinal hyperexcitability in RLS patients was performed by Wechsler et al (1986) who found an H/M ratio of 98% in two RLS patients and concluded that this was evidence of spinal hyperexcitability. However, the other four RLS subjects in their study did not exhibit this increased H/M ratio, these results were not compared to control subjects, and statistical analysis was not performed, thus casting doubt over these deductions (Wechsler *et al.*, 1986). Subsequently, various researchers testing the H-reflex demonstrated impaired H-reflex excitability curves and vibratory inhibition depression, indicative of spinal disinhibition (Martinelli *et al.*, 1987; Rijsman *et al.*, 2005) as well as decreased inhibition of 1b interneurones (neurones modulating spinal locomotor rhythm generators) (Scaglione *et al.*, 2008) which all point towards spinal hyperexcitability in RLS and PLM patients.

Examination of a different reflex, the nociceptive flexor (withdrawal) reflex, has also shown spinal hyperexcitability in RLS and PLM patients. PLM patients' reflex thresholds were decreased during sleep compared to wakefulness which is a reversal of the natural state-dependent changes in spinal excitability. The patients with PLM also exhibited lower thresholds, and thus increased excitability, compared to control subjects during wakefulness and during sleep (Bara-Jimenez *et al.*, 2000). Overall these data indicate spinal hyperexcitability in PLM patients which is maintained during sleep.

Despite the many arguments supporting the role spinal hyperexcitability may play in RLS and PLM, not all the literature shows data supporting the concept of global spinal hyperexcitability. Several studies examining the H-reflex showed no differences between RLS and control subjects for either H-latency; H-amplitude or H/M ratio (Bucher & Trenkwalder, 1996; Akyol *et al.*, 2003; Rijsman *et al.*, 2005; Scaglione *et al.*, 2008). Also, in other neurophysiological tests, patients with RLS and PLM exhibited no differences compared to controls in motor and sensory nerve conduction, F-wave, blink reflex, flexor reflex responses and mixed nerve silent periods (Wechsler *et al.*, 1986; Akyol *et al.*, 2003). These inconsistencies require further exploration before the aetiology of central sensitization in RLS and PLM can be confirmed.

A simplified version of the possible descending control pathways that could exist and the potential consequences of 'losing' the descending pathway are presented in Table 1. The descending pathways are either inhibitory or excitatory and will synapse with an inhibitory or excitatory neuron. Loss of the descending input to the subsequent neuron will change its excitability state. Most of the literature cites that there is disinhibition of inhibitory pathways in RLS and PLM patients which would result in hyperexcitability (increased excitability of the excitatory neurons). It is possible that loss of a descending inhibitory pathway could also cause

long term upregulation of receptors on secondary inhibitory neurons resulting in increased inhibitory outputs, which would not present as spinal hyperexcitability. The conflicts in the literature regarding the state of spinal excitability could possibly be explained by the hypothesis that these are complex neuronal interactions, and that the proposition of global spinal hyperexcitability, alone, might be too simplistic as a cause for RLS and PLM.

Table 1: Representation of the descending spinal networks that exist and the possible outputs if there was loss of the descending pathway

Descending pathway	**Output neuron**	**Expected output if there was loss of the descending pathway**
Inhibitory	Excitatory	Increased excitability
Inhibitory	Inhibitory	Increased inhibition
Excitatory	Excitatory	Decreased excitability
Excitatory	Inhibitory	Decreased inhibition

It should be noted that if the various changes in dopamine, iron and spinal cord excitability can explain the sensory and motor disturbances in RLS and PLM, the circadian variations which form part of the diagnostic criteria would also have to be explained by these same mechanisms.

1.3.4. Circadian rhythms of RLS

The higher prevalence of RLS symptoms at night is not only due to patients' increased likelihood of immobility but involves an independent circadian factor (Trenkwalder *et al.*, 1999a). Both the intensity of sensory RLS symptoms and the number of PLM/hour peak around midnight and are at a minimum in the morning as assessed by numerous suggested immobilization tests (SIT) throughout the day and night (Trenkwalder *et al.*, 1999a). The

number of PLM peak on the falling phase of the 24 hour temperature curve just before the minimum and the fewest symptoms occur on the rising phase occurring after the minimum (Hening *et al.*, 1999b; Trenkwalder *et al.*, 1999a; Michaud *et al.*, 2004).

The evidence for circadian variations in dopamine concentrations which would account for the circadian variation in RLS are however not clear and unequivocal. In healthy subjects, plasma and urinary excretion of free dopamine follow a circadian pattern of peaking during the day with the trough occurring in the evening (Sowers & Vlachakis, 1984; Kawano *et al.*, 1990). Two studies showed no differences in cerebrospinal fluid concentrations of dopamine metabolites between RLS and control subjects sampled in the morning (Earley *et al.*, 2001) or the evening (Stiasny-Kolster *et al.*, 2004d). However, Earley et al (2006) showed increased concentrations of CSF tetrahydrobiopterin (BH4) (BH4 is enzymatic cofactor in the biosynthesis of both serotonin and dopamine), HVA: 5HIAA and 3OMD in RLS patients in the morning compared to the evening (which were not present in control subjects) indicating greater than normal diurnal fluctuations (Earley *et al.*, 2001; Earley *et al.*, 2006). Dopamine, and its metabolite HVA, have also been shown to peak in the morning and early afternoon in RLS and Parkinson's disease patients (Poceta *et al.*, 2009).

The diurnal variations of serum iron concentrations in healthy subjects have been well documented (Sinniah *et al.*, 1969; Statland *et al.*, 1976; Scales *et al.*, 1988; Dale *et al.*, 2002). Blood iron concentrations in the evening decrease to almost half the daytime serum levels in healthy individuals (Scales *et al.*, 1988). No consistent diurnal pattern for transferrin and ferritin concentrations have been shown (Dale *et al.*, 2002). To my knowledge, the relationship between the natural variation of iron, ferritin and transferrin plasma and CSF levels and RLS has not been investigated (Baier & Trenkwalder, 2007).

Research has also focused on peptide hormone secretions that are either regulated by dopamine (prolactin and growth hormone) or that regulate the secretion of dopamine (melatonin) (the relationships between dopamine and these hormones are demonstrated in Figure 2). Measurements of these hormones provide an indirect measure of the dopaminergic system. Dopamine inhibits the release of prolactin and growth hormone. One study found normal plasma levels of prolactin and growth hormone in RLS patients. However following L-dopa administration the authors found greater night time inhibition of prolactin compared to controls suggesting that there may be circadian changes to the sensitivity of the postsynaptic dopamine receptors (Garcia-Borreguero *et al.*, 2004b). Another study found that although prolactin and growth hormone secretions fluctuated throughout the day, there were no differences between the 24 hour patterns in RLS patients compared to healthy controls, indicating that the hypothalamic-pituitary axis dopamine receptors or those particular dopamine pathways may not be involved in RLS (Wetter *et al.*, 2002).

Melatonin exerts an inhibitory effect on the secretion of dopamine and its peak secretion coincides with the night time worsening of sensory and motor features of RLS (Michaud *et al.*, 2004). Melatonin secretion profiles however are no different between RLS patients and healthy controls and thus the relationship between increased melatonin secretion and increased RLS symptoms remains uncertain (Tribl *et al.*, 2003; Michaud *et al.*, 2004).

While studies focusing on dopamine, iron and related hormones have shown some evidence of circadian rhythms, variations in spinal excitability have not been examined in a RLS context. In fact, very few RLS neurophysiology studies have taken time of day into account. Rijsman et al (2005) examined the H-reflex in the late afternoon, a period when RLS symptoms become apparent, and reported impaired H-reflex excitability curves and vibratory inhibition

depression (Rijsman *et al.*, 2005). Hyperexcitability of the flexor reflex (lower thresholds and greater spatial spread) in PLM patients was shown in the evening (Bara-Jimenez *et al.*, 2000). Bucher et al (1996) chose to look at a symptom-free period and no differences were seen in the H-reflex parameters.

In summary, although the exact site of the disturbance is not clear, there do appear to be disturbances in the dopaminergic pathways and iron metabolism. While both the sensory and motor components demonstrate circadian variations the evidence for the mechanisms of these underlying circadian variations remains unclear. The lack of information on the sensory and motor components of RLS and PLM may be compromised by the limited range of assessment tools used previously.

2. THE ASSESSMENT OF RLS AND PLM

The essential components of the RLS diagnostic criteria are that a patient experiencing RLS feels an “urge to move” in response to “uncomfortable and unpleasant sensations” in their legs. From these agreed diagnostic criteria it is clear that there is a sensory and motor component to RLS. The sensory component is characterized by the feeling of abnormal sensations in the legs accompanied by an urge to move, and the motor component includes sensory relief from physical activity and the involuntary leg movements during wakefulness (PLMW) and sleep (PLMS). The objective and subjective assessment of these different aspects will be the focus of the sections to follow.

2.1. ASSESSMENT OF SENSORY FEATURES

The human somatosensory system involves the relay of sensory information from somatic receptors in the body (soma) to intra cerebral nuclei and ultimately to the somatosensory cortex of the brain via spinal or cranial nerve pathways. Sensation begins when external or internal stimuli stimulate a receptor. The type of receptor that is stimulated varies with the sensory modality (e.g. pain stimulates nociceptors). Depending on the type of receptor, the ascending spinal pathway occurs via either the dorsal columns (most mechanoreception and proprioception) or spinothalamic tract (crude mechanoreception, temperature and pain). Tracts ascend the spinal cord to the thalamus where basic processing occurs. Information is then relayed to the primary somatosensory cortex for further processing and finally to the association cortices to fully characterize the dimensions and location of the sensation. Inputs from the limbic system (emotional), cerebellum (primitive response) and the prefrontal cortex (rationalization) are also involved in the processing of sensory information.

Dysfunctions of the somatosensory system can occur at numerous points within the complex networks and various tools have been developed which allow for the investigation of the different sites of dysfunction. This review will focus specifically on assessment techniques of sensory function that have been employed or show useful potential in the assessment of the qualities and severity of RLS sensations. As these sensations have been described both as non-painful and painful, aspects of both types of sensations will be explored.

2.1.1. Assessing the quality of RLS (non-painful) sensory features

Characterising and classifying the quality of sensations may be helpful in determining the RLS diagnosis, the monitoring of RLS severity and in distinguishing RLS from RLS mimics (RLS-like disorders) such as leg cramps, positional discomfort and peripheral neuropathy (Hening et al., 2009). The key to providing differential diagnoses for RLS and conditions that mimic RLS may lie in characterising the diagnostic descriptors for each condition in order to distinguish them. Also, characterising the RLS descriptors may help to determine if there are different pathophysiological mechanisms for different RLS phenotypes. If the mechanisms are different for the various RLS phenotypes, then there may be differences in the expression of symptoms. Characterising these differences of expression may in turn reflect the underlying differences in phenotype.

The study of non-painful sensation experienced by humans, its perception and emotional responses have been extensively studied perhaps due to the commercial opportunities which arise with the knowledge of how potential customers perceive commercial products. The application of such knowledge has been utilized by the lay media to optimize the optimal human sensory experience (i.e. flashy visuals for advertising or choosing clothing textures for enhancing the tactile experience). Substantial funding is contributed to researching the qualities of these non-painful sensations to enhance the users experience and for commercial benefits such as perfumes (smell) and food (taste). In a clinical setting, non-painful sensations are perceived to be of less clinical relevance than painful sensations, and therefore have attracted less scientific focus. The majority of the sensations experienced by RLS patients are non-painful and it is perhaps for the reasons mentioned above that less research has been devoted to their interrogation.

At present, there are no specific tools to assess the quality of the sensory features of RLS. Anecdotal descriptions of RLS such as “tingling”, “painful” and “pins and needles” are cited in the literature and up until very recently, there has been no formal assessment or comprehensive study assessing the scope of the sensations of RLS. Even the terms included in the diagnostic criteria have not been validated in a large scale study. Patients express difficulty in describing the unusual sensations of RLS, however the diagnosis of RLS is primarily based on the subjective descriptions of sensations. As they stand, the diagnostic criteria are unable to exclude conditions that mimic RLS which brings the prevalence statistics into doubt (Hening *et al.*, 2009). The perception and communication of sensations are limited by an individual’s personal experience of these sensory occurrences, thus making sensory evaluations more subjective than is desirable in an assessment methodology. There is a diverse range of possible descriptors which lack characterization and classification and yet these terms are used for diagnostic purposes.

There is very little data describing the quality of sensations in RLS. One recent study asked RLS patients to spontaneously describe their RLS sensations (Karroum *et al.*, 2012). Patients described sensory and affective descriptors similar to the anecdotal terms used in the literature however very few used ‘unpleasant and uncomfortable’, the terms included in the diagnostic criteria. The authors also challenged the idea that there may be a purely motor form of RLS as 95% of RLS patients in their study could report sensory descriptors. Further, the authors suggest that the most commonly used descriptors by their cohort of patients correspond to those used on validated neuropathic pain screening tools (Karroum *et al.*, 2012).

The unusual sensations experienced by patients with RLS were previously described as parasethesias and dysesthesias (Hening *et al.*, 1999a). Dysesthesias are defined by the

European Federation of Neurological Societies as “abnormal and unpleasant” sensations whereas parasethesias are “abnormal but not unpleasant” sensations (Cruccu *et al.*, 2004). The RLS diagnostic criteria of “unpleasant and uncomfortable” sensations presumable arise from these definitions for dysesthesia and parasethesia. Despite the entrenched use of these terms, to date no studies have compared the sensations of dysesthesia and parasethesia with RLS and thus it is not certain whether these terms accurately describe RLS. Currently there are no descriptive terms for parasethesia and dysesthesia either. Parasethesia and dysesthesia are terms usually used to describe spontaneous sensations associated with neuropathic pain. Given the similar nature of the spontaneous sensations of RLS, the relationship between RLS and pain has also been investigated (and will be discussed in section 2.1.3.1.).

2.1.2. Quantifying the severity of RLS sensations

While relatively little work has been done to qualify the non-pain sensations of RLS, there are various approaches to quantify these sensations and their impact on sufferers. There are three validated scales assessing the subjective severity of RLS, the International Restless Legs Scale (IRLS) (Appendix D) (Walters *et al.*, 2003; Scaglione *et al.*, 2008), the John Hopkins Restless Legs Syndrome Severity Scale (JHRLSS) (Allen & Earley, 2001c) and the Visual Analogue Scale (VAS) (Tergau *et al.*, 2001; Tribl *et al.*, 2005).

The IRLS is a 10-point scale, designed by the International Restless Legs Syndrome Study Group, that reflects on “subjective assessment of the primary [diagnostic] features, intensity and frequency of the disorder, associated sleep problems, and probes the impact of symptoms on patients mood and daily functioning” (Allen *et al.*, 2003; Walters *et al.*, 2003). Each of the 10 items in the IRLS is graded from 0-4 based on the perceived severity or frequency. The IRLS is a

subjective scale that asks patients to reflect on the severity of their RLS in the past week. The IRLS severity score has been used to assess the efficacy of numerous different treatment options including: Ropinirole (Trenkwalder *et al.*, 2004a; Walters *et al.*, 2004), Pergolide (Trenkwalder *et al.*, 2004b), Cabergoline (Stiasny-Kolster *et al.*, 2004a; Oertel *et al.*, 2006; Trenkwalder *et al.*, 2007), Pramipexole (Winkelman *et al.*, 2006) and Rotigotine (Stiasny-Kolster *et al.*, 2004b; Trenkwalder *et al.*, 2008).

The JHRLSS, designed at John Hopkins University (as the name would suggest), was the first published clinical severity rating scale. It is a far simpler evaluation method than the IRLS, comprising of a single question related to the time of RLS symptom commencement. There is a choice of never, mild, moderate and severe as subjective intensity ratings based on a 4 point scale corresponding to the time of symptom commencement. As symptoms tend to dissipate for most patients in the early morning, the time that they begin reflects the duration of time that patients endure restless legs. Thus the earlier the symptoms start, the more severe the RLS (Allen & Earley, 2001c). The JHRLSS is not used extensively to determine pharmacological treatments efficacy however it is used successfully as a clinical assessment tool for the severity of RLS.

The Visual Analogue Scale (VAS) is a numeric rating scale commonly used in pain assessment studies (further discussed in section 2.1.3). An adaptation of this scale can be used to assess the severity of RLS with “not severe” as the one anchor and “most severe” as the other anchor (Tergau *et al.*, 2001). The current intensity of RLS discomfort can also be assessed using the VAS, with “no discomfort” and “extreme discomfort” as the two anchoring statements (Michaud *et al.*, 2002a). The VAS provides an instantaneous assessment of the RLS severity at the time of the assessment. VAS score data is entirely dependent on the patient’s

interpretation of severity and could reflect anything from the intensity of the actual symptoms to the impact of the sensations on mood and function. The VAS has successfully been used to assess the improvement of subjective RLS symptoms as a measure of severity, following administration of: Apomorphine (Tribl *et al.*, 2005), Piribedil (Evidente, 2001) and Alpha-Dihydroergocryptine (Tergau *et al.*, 2001). Michaud et al (2002) used the VAS to assess the intensity of RLS discomfort at regular intervals and provide an overall quantifiable measure of the intensity of sensory discomfort of RLS (Michaud *et al.*, 2002a).

The severity rating scales mentioned above have been used widely and are a critical assessment tool for RLS. One of the problems with these scales is that the measures have no connection to the quality or severity of the sensations themselves. For example, the IRLS rating scale primarily assesses the impact of RLS on the patients sleep and daily functioning but provides relatively little information regarding the intensity of the RLS symptoms themselves. The presumption is that increasing severity of RLS leads to increased impact. Also, given the emerging association between pain and RLS, these rating scales lack specific questions about painful symptoms.

2.1.3. Assessing the quality of and quantifying painful sensations

Pain is a component of the sensory profile of many RLS patients and the pain assessment tools that have been used in previous RLS studies range from simple pain rating scales through to multidimensional qualitative and quantitative approaches.

Direct estimation methods, such as numeric and verbal rating scales are frequently used tools in the assessment of pain severity and intensity particularly in settings that require an

instantaneous response (Williams *et al.*, 2000). The Visual Analogue Scale (VAS) is one of the most commonly used of these methods. This scale is a one dimensional, quantitative measurement tool consisting of a single 100mm line that is anchored at either end with the extremes of a pain experience (i.e. “no pain” and “worst pain ever experienced”). Patients are asked to mark a point between the two anchor points that reflects their present pain. The continuous nature of the scale is preferable to numeric or verbal scales which force patients to translate their feeling into a numerical value, or choose a single word from a pre-defined list (Carlsson, 1983). The two anchors are adaptable, making the VAS a versatile tool for assessing various different subjective components. One of the main disadvantages of the VAS is that it is a unidimensional tool whereas pain is largely recognised to be multidimensional.

The McGill Pain Questionnaire (MPQ) (Appendix E) was originally developed by Melzack (Melzack, 1975) to overcome the problems associated with the previous unidimensional description and measurement of pain. There are 20 groups of 78 words on the MPQ and each of these is grouped according to the quality of the words and ranked within each group in increasing order of intensity. Both the description of the pain (sensory, affective and evaluative) and the relative intensity can be defined using the MPQ. A VAS recording the present pain is also included in the MPQ. This allows for a multi-dimensional analysis of pain compared to the one dimensional assessment provided by simple rating scales.

The qualitative properties and quantitative assessment obtained from the MPQ provide different, but related, information regarding pain. Descriptive data is useful in assessing the quality and type of pain, whereas the severity of the pain can be calculated either by summing the total number of descriptors selected, or by calculating the sum of the ranks of each descriptor chosen within the 20 groups. Since its development in 1975, the MPQ has been

extensively used in its original and translated formats (Melzack, 1975; Costa *et al.*, 2009) and the shortened version has also been validated for situations when time constraints are imposed (Melzack, 1987).

The MPQ is particularly useful for assessing clinical pain and determining the therapeutic outcomes of pain treatment studies, providing quantitative measurements that can be statistically tested (Melzack, 1975). The verbal qualities of pain descriptors on the MPQ can also been used to discriminate between different types of pain. Wilkie et al (2001) reported that the descriptor choices from the MPQ were different between patients with nociceptive and neuropathic pain. Being able to distinguish between different types of pain is important when deciding the optimum pain management therapies for each type of pain (Wilkie *et al.*, 2001). For example, in postoperative pain, a patient complaining of a throbbing pain is indicative of pain arising from deep tissue damage whereas a patient, who complains of a sharp pain, may be indicating a more superficial source. The qualities of the pain lend themselves to indicating their potential location and provide guidance towards the appropriate analgesics which would be different for each type of pain (Fortin *et al.*, 1992).

The subjective scales mentioned (VAS and MPQ) can be used to assess clinical pain as is, or can be applied to induced pain states, such as those provoked using Quantitative Sensory Testing (QST). QST is a non-invasive psychophysical assessment and quantification of the somatosensory function of small and large sensory fibres. QST measures the responses to mechanical (static and dynamic) and thermal stimuli of varying intensities, normally assessing the threshold and tolerance to the stimuli. QST can target specific receptors, peripheral nerve fibres or neuroanatomical pathways depending on the testing modality (pain, pressure, touch, vibration or temperature) and as such is not restricted to the assessment of pain. Unlike nerve

conduction studies which only provide reliable measures of large myelinated sensory afferent nerves, QST is a useful tool for assessing sensory function and impairment of large and small, myelinated and unmyelinated fibres (Chong & Cros, 2004; Hansson *et al.*, 2007; Bachmann *et al.*, 2010). In the context of RLS and studies of patients with PLM, QST has focused specifically on testing the function of the pain pathway.

2.1.3.1. Pain assessment tools and RLS

The previously discussed pain assessment tools have been used in RLS and PLM studies in an attempt to qualify and quantify the pain sensations of RLS and elucidate the role of the pain pathways in RLS and PLM. In particular, the VAS has been used in conjunction with the MPQ (Bentley *et al.*, 2007) and as an outcomes measure in QST studies (Edwards *et al.*, 2011).

A small number of RLS studies have included the MPQ as part of their assessment. von Spiczak et al (2005) used the pain scores from the affective component of the MPQ as an outcome measure to assess the role of opioids in RLS. These pain scores were inversely correlated with opioid receptor binding in some areas serving the medial pain system (orbitofrontal cortex and anterior cingulate gyrus) (von Spiczak *et al.*, 2005). Subsequently, the MPQ was tested in its ability to assess non-painful RLS sensations. The quality and severity of RLS sensations could be measured using the MPQ and there was a good correlation between the severity score from the IRLS and the severity score calculated from the MPQ. The word choices in RLS patients from the MPQ were unique as they were different to the words selected by patients with either neuropathic or nociceptive pain (Bentley *et al.*, 2007). Similarly, Karroum et al (2012) also found that RLS patients could use the French version of the MPQ to report their sensations with a correlation between the IRLS severity score and the MPQ sum of intensity scale values.

However, unlike the previous study, Karroum and colleagues report that RLS patients selected words that were similar to those in validated neuropathic pain screening tools except that the RLS patients also selected words describing cold and numbness which are not neuropathic pain terms (Karroum *et al.*, 2012). This discrepancy between the two studies may be due to Bentley and colleagues having statistically compared the RLS descriptors with previously reported neuropathic pain descriptors whereas Karroum and associates appear to have purely reported visually observed similarities.

QST has been successfully implemented as a means to assess the central somatosensory processing of pain and temperature related information in RLS patients. RLS patients demonstrated central sensitization of nociceptive processing and increased pain sensitivity as measured by the increased response to static and dynamic mechanical stimuli (gentle stroking stimuli and pin prick) (Stiasny-Kolster *et al.*, 2004c; Bachmann *et al.*, 2010). Psychophysical testing (pressure algometer, hot and cold painful stimuli) also revealed lower pain thresholds and elevated indices of temporal summation among RLS patients further supporting that there is amplified pain processing in RLS patients (Edwards *et al.*, 2011). Assessment of painful and non-painful thermal and mechanical perceptions and pain thresholds revealed altered sensory modality profiles in primary and secondary RLS patients (Schattschneider *et al.*, 2004; Bachmann *et al.*, 2010). QST is a useful tool in the assessment of RLS and PLM and has contributed towards the central sensitization theory as the aetiology of these two disorders.

Whilst pain assessment tools have successfully been used to assess RLS sensory features they are limited by the obvious pitfall that not all RLS sensations are painful. The MPQ has been used to assess the non-painful sensations of RLS, however it is restricted to pain related words and may be unable to assess the full scope of RLS sensations. The non-painful sensations have not been successfully assessed and there is no validated measuring instrument for assessment

of the non-painful RLS sensations. The combination of sensory and motor features of RLS and PLM and the possibility that there is a common aetiology suggests that mechanisms responsible for the sensory features may be involved in the motor phenomena of PLM and vice versa. Thus assessment of motor features may provide insight into our understanding of both disorders.

2.2. ASSESSMENT OF MOTOR FEATURES

Movement has various levels of complexity, from simple reflexes to complex voluntary movements, in order of increasing complexity and can be either automatic or volitional (Askenasy *et al.*, 1987). The simplest automatic movement, the reflex, can involve a single sensory and motor neuron and requires no conscious control for its initiation. Central pattern generators and rhythmic movements, also at a spinal level, involve some conscious control and may be voluntarily initiated. Complex voluntary movements, as the name implies, are under conscious control and involve multiple different features in the nervous system. The complex motor pathways initiate in the motor cortex, and via descending spinal motor tracts, synapse with the alpha motor neuron, sending action potentials to the neuromuscular junction to cause activation of the muscle and subsequent voluntary movement.

The complete range of tools for the assessment of movements is extensive and beyond the scope of this literature review, therefore the sections that follow will only describe techniques used previously or those that could be used to assess motor features of RLS and PLM. First, electrophysiology assessment, which records the electrical signal generated in a muscle which has been stimulated by the motorneurone, will be discussed. Following which the thesis will review assessment techniques of the actual movement that may be elicited from the electrical

activation of the muscle (biomechanics). The uses of both electrophysiology and movement in the assessment of the most simple movement, the reflex (as a reflection of spinal cord motor function) will then be discussed with particular focus on the excitability state of the spinal cord, changes in which have been implicated in the generation of RLS and PLM.

2.2.1. Electrophysiological assessment

Electrophysiology is a means by which researchers are able to record the electrical properties of biological cells and tissues. The most common electrophysiological measure used in the study of RLS and PLM is electromyography (EMG). The electrical signal (action potentials) generated in a muscle in response to motorneurone activity or other electrical events is recorded. EMG involves recording electrical activity as a potential difference, between the two recording electrodes, representing the neuromuscular activation of the muscle. Recordings are made either by surface electrodes placed on the skin over the belly of the muscle or needle electrodes inserted into the muscle. Recordings made via surface electrodes are more common than the more invasive needle electrode recordings. Needle electrode recordings are however more accurate than surface electrode measurements. The most common type of recording technique is bipolar, where two electrodes record from the muscle and a third is placed in an electrically neutral grounding site (Kamen, 2004).

EMG recordings can be done to reflect the activity of a single muscle or multiple muscles simultaneously. Single muscle recordings are useful for determining if a particular muscle is involved in an activity and to establishing the frequency and magnitude of that particular muscles use. Simultaneous recordings from multiple muscles provide information regarding

the relationships of one muscle relative to another. The timing sequence of multiple muscles performing a task (for example during walking) can be determined (Kamen, 2004).

The electrical activity recorded by electromyography emanates from action potentials which originate in the muscle under the skin. The EMG recording can be affected by many factors and as such cannot be used as a direct measure of the number of action potentials. Confounding factors that influence EMG readings include: variations in muscle fibre type (where action potentials may elicit variations in EMG amplitude with changes in the surface area of a muscle fibre/sarcomere); bidirectional movement of action potentials from the neuromuscular junction (where surface EMG can vary according to the direction of flow of the action potentials); placement and variations in the electrical conductivity of recording and reference sites; positioning of the recording electrodes relative to the motor endplate region, skinfold thickness and electrode impedance. Other sources of electrical activity (both external and other body originated electrical activity e.g. electrocardiogram), may confuse the EMG signal as they may not be fully accounted for by using a reference electrode (Kamen & Caldwell, 1996). Despite measurement difficulties, recording and analysis of electrical signals involved in neuromuscular activation are successfully used to assess motor activity in RLS and PLM patients and PLM are defined by activity sequences recorded via surface EMG.

2.2.1.1. Electrophysiology and RLS and PLM

Periodic limb movements, as the name implies, have to do with 'limb movements' however EMG as part of PSG record the electrical activity of the muscle which is an indirect reflection of the actual motion (muscle electrical activity may not necessarily result in movement, see below). The EMG recording on the PSG specifically records from a single muscle (most

commonly the anterior tibialis in PLM studies) and the EMG activations must fulfil specific defining criteria (see section 1.1.2.) to be considered PLM. The severity of the condition is calculated based on the number of PLM per hour. As EMG is a recording of muscle activation and not the actual leg movement per se, activations can occur without subsequent movement. A measurable electrical signal may not cause great enough contraction of the muscle to produce a visible movement which may result in overestimation of PLM during PSG (Kazenwadel *et al.*, 1995). Alternatively, muscle activation in two opposing muscles (where EMG activity is registered), may not result in movement to the opposing nature of agonist and antagonist muscles.

The anterior tibialis muscle was originally recommended as the muscle of choice for EMG recordings used to detect and quantify PLM, as dorsiflexion of the ankle (a result of anterior tibialis muscle contraction) is one of the defining features of PLM (Guilleminault *et al.*, 1975). Although the anterior tibialis is the most frequent initiating muscle in the PLM motor sequence, other muscles: the gastrocnemius, biceps femoris, rectus femoris and extensor digitorum brevis, have also been shown to begin the muscle contraction sequence (Provini *et al.*, 2001; de Weerd *et al.*, 2004). The complex muscle recruitment patterns for other muscles involved in the PLM sequence are reported to be both inconsistent, even for different sequences within the same patients (Provini *et al.*, 2001; Plazzi *et al.*, 2002), as well as regular and recognisable, albeit specific to individuals (de Weerd *et al.*, 2004). These results indicate that not all PLM follow a fixed, stereotypical pattern as was previously assumed. Despite evidence of other muscles being involved in the PLM motor pattern, the anterior tibialis muscle is consistently the only muscle recorded to detect and quantify PLM (Provini *et al.*, 2001). By only recording from the anterior tibialis muscle there may therefore be a marked underestimation of the occurrence of PLM.

EMG and movement analysis are complementary, but not necessarily interchangeable, measurement tools (see the published papers co-authored by the thesis author in appendices H and I for further discussion regarding the relationship between the two measurements). The simultaneous use of both electrophysiological and biomechanical methods is particularly useful in gait analysis (Vaughn *et al.*, 1999). Assessing the mechanics of the movement generated by electrical muscular activity requires a different form of analysis to electrophysiology.

2.2.2. Biomechanical assessment

Movement, in general, can either be translational or rotational. Translational motion (also known as linear movement) occurs along a straight or curved line for example the movement of the body walking along a path. Rotational or angular motion describes the circular movement around a fixed point axis of rotation. Segmental movement of body parts falls into the category of rotational motion. For example, in the motion of the lower limb during the patellar reflex, the knee joint is the axis of rotation. The measurement of movement describes the displacement of one joint relative to another.

Modern biomechanics is a new science originating from the 1970-80s era and the word is derived from two concepts, that of life (from the Greek *bios*) and mechanics. Mechanics is defined as “the study of motion of objects, and the related concepts of force and energy” (Giancoli, 1998) and therefore biomechanics is essentially the study of the motion (and related force and energy) of biological organisms and systems. There are various subdivisions within the field of biomechanics, however, and the two areas of relevance to this review are

accelerometry and the category of kinematics (a division of dynamic mechanics), defined as "the study of time and space factors of motion of a system" (Kreighbaum & Barthels, 1996).

Accelerometry is a low-cost tool for the assessment of human movement in laboratory and home environment based studies. Accelerometers are small, lightweight, non-invasive devices which can be placed over various joints to measure movement of specific limbs or to provide a general whole body activity record. These devices measure limb acceleration, detecting both the frequency and intensity of movement, in one axis (uniaxial) or three orthogonal axes (triaxial) (Mathie *et al.*, 2004). Activity monitoring (actigraphy) using accelerometry provides a less expensive, less intrusive and ambulatory home-based method for assessing movement. One of the main advantages of actigraphy is that it is ambulatory and can record continuously for extended periods of time. The use of actigraphy, however, also has various limitations. Uniaxial accelerometers only provide information about movement in a single plane and therefore may not be a true reflection of total movement. The orientation and site of placement of the accelerometry device is critical as these relate to the plane of movement and determine the type of data recorded (e.g. whole body movement vs. segmental movement). Actigraphy cannot specify the type of movement recorded, only that movement has occurred. This is particularly important in PLM detection as respiratory related movements may also be detected by actigraphy and may be difficult to distinguish from PLM (Hening, 2004a).

Kinematics provides an objective way to describe the movement of the body or a body part within a particular frame of reference. Kinematics can be used to quantify the displacement, velocity and acceleration of movement in three dimensions (Kreighbaum & Barthels, 1996; Giancoli, 1998). The kinematics tool that I will particularly focus on in this review is video kinematic motion analysis.

Video kinematic motion analysis involves capturing and tracking motion as visual data, and uses three dimensional Euclidian geometry to objectively quantify movement. Non-invasive reflective markers are placed on areas that are most useful to capture motion e.g. on the joints. The movement of markers is then recorded during the motion to be analysed and the data used to back extrapolate the movement of the joint under study. In this way an accurate and objective measurement of the motion is obtained. An unavoidable limitation of the non-invasive kinematics method is that, due to skin movement artefact, the marker movement does not always accurately represent the movement of the underlying structures.

2.2.2.1. Biomechanics and RLS and PLM

Actigraphy has been involved in the study of sleep/wake patterns for the last 20 to 30 years and more recently specifically used to detect and screen for PLMS and other sleep disorders (Sadeh, 2011). In patients with RLS and PLM, actigraphy offers an alternative means to detect PLM instead of the gold standard of EMG as part of PSG. The defining criteria for PLM are based on muscle activation and do not depend upon actual movements whereas actigraphy records actual leg movements. Whether the activation or movement is a more important factor in causing the sleep disturbances associated with PLM is yet to be determined.

The use of actigraphy as a screening and even diagnostic tool for PLM has been the subject of several studies to determine the reliability and sensitivity of accelerometers in detecting PLM. Two review papers concerning the role of actigraphy in sleep studies report on research indicating that actigraphy is accurate in detecting PLM but that it is not a suitable replacement for the diagnosis of PLM using EMG on PSG (as there may be muscle activations without subsequent movement which are not detected by actigraphy) (Sadeh *et al.*, 1995; Ancoli-Israel

et al., 2003). A later study also showed that although actigraphy could accurately detect the presence of PLM (with greater sensitivity and specificity as PLM index scores increased), Bland Altman analysis indicates that actigraphy and EMG as a part of PSG are not interchangeable (King *et al.*, 2005). Actigraphy may however be useful in follow up studies, screening for PLM and for determining treatment efficacy (Sadeh *et al.*, 1995; Ancoli-Israel *et al.*, 2003). New actigraphy devices specifically tailored to detect PLMS are being developed which may assist in overcoming some of the problems encountered with using accelerometers for the detection of PLM (Sadeh, 2011).

The use of biomechanics (particularly three dimensional motion analysis) for assessment of motor function is a fairly novel tool in the investigation of RLS and PLM. One kinematic gait analysis study, in conjunction with EMG, has been carried out focusing on RLS patients (Paci *et al.*, 2009). The authors found that RLS patients had an abnormal EMG activation of the gastrocnemius muscles when walking. Kinematic analysis indicated that the timing of this abnormal activation occurred when there should have been relatively less activation of the gastrocnemius muscle (relative silence) at that phase of the stance. This same activation was not detected in control participants, possibly indicating that RLS patients may have poor control of the relative silence. The activation had no detectable effect on gait kinematics but the authors suggest that it could be supportive as diagnostic criteria in complicated cases (Paci *et al.*, 2009).

The use of biomechanical techniques and electrophysiology in the assessment of reflexes, as a reflection of spinal cord motor function, is the focus of the next section.

2.2.3. Assessment of spinal reflexes

Spinal reflexes are a combination of sensory and motor pathways. Both excitatory and inhibitory descending pathways influence the excitability state of motorneurones and lesions of the CNS can result in either hyperactive or hypoactive reflexes. Thus, although spinal reflexes are a combination of sensory and motor pathways, changes in the excitability state of the spinal cord will influence the motor output and hence assessment of spinal reflexes falls under assessment of motor features.

2.2.3.1. General principles of reflexes

Reflexes are relatively stereotypical involuntary muscular contractions initiated in response to a sensory stimulus. Reflexes have a sensory and motor component with one or multiple synapses that have excitatory and inhibitory possibilities. Although there are many different types of reflexes present in the body this review focuses specifically on somatic spinal reflexes.

Abnormal reflex responses potentially indicate underlying changes in the state of the spinal cord (Paulus & Schromburg, 2006), thus increased spinal excitability may cause exaggerated reflex responses and decreased excitability would present as weak or absent reflexes. Different reflexes utilize different neuronal contributions and changes in these reflexes can be used to assess alterations of the spinal cord excitability state at various levels. For example, the Achilles tendon (ankle) reflex reflects the connections at spinal segments S1 and S2, and the biceps reflex traverses spinal segments C5 and C6 (Wang & Cymet, 2005). Muscles inverted at the spinal segments L4-S1 levels are activated during PLM (Provini *et al.*, 2001). This thesis will particularly focus on spinal reflexes occurring in the lower limbs (spinal segments L2-S2), the

site of RLS and PLM. Specific lower limb reflexes included in this thesis to investigate spinal excitability are: the Hoffmann reflex (H-reflex) and the patellar reflex. The H-reflex has been the most commonly used in RLS and PLM studies and the patellar reflex, a monosynaptic reflex, is the most widely used in clinical practise.

Common clinical measurements pertaining to reflexes are the latencies and amplitudes of electromyographic readings of muscle activity in the reflex path (Figure 4). Latency, the conduction time for the afferent and efferent impulses, defined as the period between the time point of stimulation (electrical or mechanical) and the time point of the initial voltage deflection in the recording (usually electromyographic) signal. Latency is physiologically determined by various components: the conduction speed of the sensory and motor neurons, synaptic delay, length of the reflex arc, amount of neurotransmitter released and distribution of action potentials (Pomfrett, 2005). Amplitude is a measure of the potential difference (in mV) between the positive and negative peak recorded by electromyography (Voerman *et al.*, 2005) and is an indication of the electrical signal output by the spinal cord - a measure of motorneurone activation. The amplitude is largely affected by the quantity of neurotransmitter released from the neurons. Neurotransmitter release is influenced by presynaptic inhibition and postactivation depression (Misiaszek, 2003).

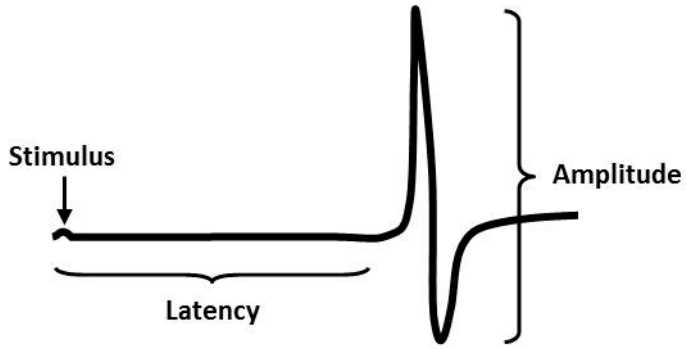


Figure 4: Schematic representation of an electromyographic recording of a biphasic action potential recorded during a reflex showing the parameters of amplitude and latency measured to quantify the reflex.

2.2.3.2. Hoffmann reflex

The H-reflex was first described by Piper in 1912 and later characterised and named after Hoffmann in 1918 who described the M- and H- waves (Schieppati, 1987). The H-reflex is elicited by percutaneous electrical stimulation of a mixed (both sensory and motor axons) peripheral nerve and recorded by bipolar surface electrodes placed over the relevant muscle that is being innervated by the stimulated nerve (Zehr, 2002). The H-reflex is mainly used in the detection of neuropathies and radiculopathies where the H-wave is either delayed or absent. The H-reflex can be tested on numerous different muscles, particularly those in the extremities, thereby varying the spinal segments being tested. Most common, and of relevance to this review, is examination of the lower limbs. H-reflexes performed on the peroneal and tibial nerves measure functioning of spinal segments L5, S1 and S2.

The H-reflex is often referred to as a monosynaptic reflex. However, despite the largely monosynaptic circuitry, conflicting literature indicates that there are also oligosynaptic and polysynaptic contributions to the H-reflex (Misiaszek, 2003). In part, the percutaneous electrical stimulation method used to evoke the H-reflex also has the potential to activate

surrounding neurons (including group 1b afferents innervating Golgi tendon organs and cutaneous fibres) which provide other neural input (Misiaszek, 2003).

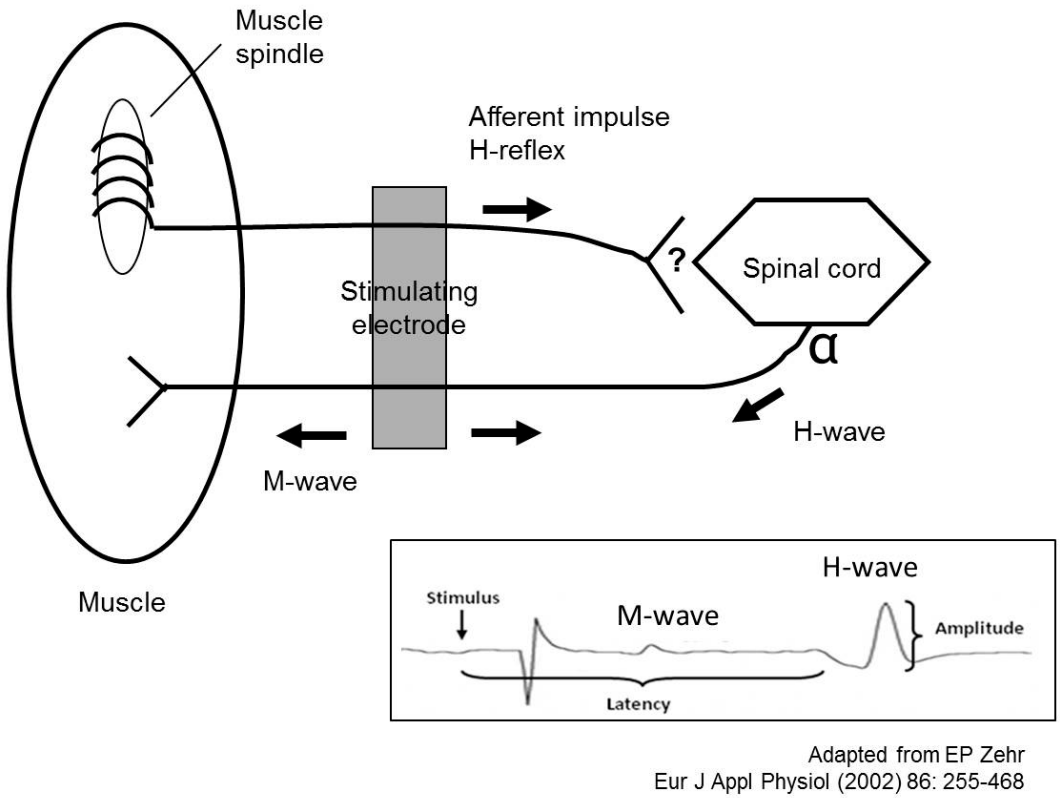


Figure 5: Schematic representation of the pathway involved in the H-reflex and the associated electromyographic tracing of the M-wave and the H-reflex. The question mark indicates possible polysynaptic neuronal contributions. α represents the alpha motor neuron.

The mixed nature of the nerve prevents external electrical stimulation from exciting either just the sensory or just the motor nerve therefore both are depolarized during the H-reflex procedure (Figure 5). The stimulation point of the H-reflex bypasses the muscle spindle by directly activating the sensory afferent and action potentials are propagated in both directions in the nerve: towards the spinal cord and towards the muscle. Sensory afferent information is transmitted via type 1a nerve fibres to the spinal cord where these fibres synapse directly onto alpha motor neurons. The resulting reflex response is termed the H-reflex (or H-wave). The action potentials travelling in the opposite direction (towards the muscle) are a result of direct

stimulation of axons of the alpha motorneurones and cause direct contraction of the muscle. This direct motor response is termed the M-wave (M standing for muscle) and has a shorter latency than the H-wave because of the close proximity of the stimulation site to the muscle (Zehr, 2002).

The procedure of inducing the H-reflex involves stimulating the mixed nerve with gradually increasing intensities. The larger diameter of the sensory 1a-afferents allows them to depolarize at lower stimulation intensities than the α-motorneurones thus the threshold of the H-wave is lower than that of the M-wave. Therefore, as the stimulation intensity is increased from sub-threshold, the H-wave will appear without an M-wave but, at greater stimulation intensities, the smaller diameter α-motorneurones are recruited and the M-wave becomes apparent (Zehr, 2002). Propagation of action potentials in the α-motorneurones occurs in both directions, resulting in antidromic 'traffic' in the axon of the motorneurone going towards the spinal cord as well as orthodromic traffic towards the peripheral muscle. As the action potential 'traffic' towards the spinal cord increases it prevents the signal from the spinal cord from reaching the muscle and recording electrodes. The H-wave therefore decreases with increasing stimulation intensity until it is eventually blocked and only the M-wave is visible. At maximal stimulation intensity the M-wave should be a measure of activation of the entire motorneuronal pool. The relationship between the amplitudes of the H- and M-waves measured at increasing stimulus intensities is expressed as the H/M ratio and is indicative of the excitability of the motor neurons. A recruitment curve is a representation of the amplitudes of the H- and M-wave plotted against stimuli intensity and depicts the relationship between the two as the electrical stimulus intensity is increased (Voerman *et al.*, 2005).

The normal and abnormal values for the soleus H-reflex measures of latency, H/M ratio and amplitude were extensively reviewed by Voerman et al (2005). The mean H-wave latency in healthy subjects was from 28.2 - 31.2ms, amplitude was between 1.44 and 10.05mV and H/M ratio from 0.06 – 0.66. In cases of upper motor neuron lesions the mean range of latency was between 28.6 – 32.2ms, amplitude between 4.9 to 7.21mV and H/M ratio from 0.32 – 0.9 (Voerman *et al.*, 2005). Clearly, for all three measures there is a distinct overlap between normal and so called abnormal values, however given these results were taken from a range of studies, different methodologies should be taken into consideration as these could account for the differences observed. Despite the range in potential values the H-reflex is a widely used experimental neurophysiological tool and can be useful in determining changes in the spinal cord if methodologies are consistent. Conditioning stimulation, where electrical or vibratory stimulation is applied elsewhere in the body (i.e. the Achilles tendon), leads to facilitation or suppression of the H-reflex (Misiaszek, 2003) and is often used to standardise the H-reflex response.

Due to the different methodologies and related issues of stimulating and measuring the H-reflex, an alternative reflex, reflecting similar neuronal circuitry, may be useful in assessing spinal excitability. The patellar reflex, although monosynaptic, is often considered as the mechanical counterpart of the H-reflex.

2.2.3.3. Patellar reflex

The patellar or knee jerk reflex is a deep tendon, stretch reflex initiated by a hammer strike on the patellar tendon causing extension of the lower leg (Wang & Cymet, 2005). Muscle spindles in the quadriceps muscle detect the stretch of the muscle and this sensory information is

transmitted via type 1a afferent nerve fibres to the dorsal root ganglia. In the spinal cord 1a afferent fibres synapse directly onto alpha motor neurons that innervate the same muscle, the quadriceps, from which the sensory stimulation arose (Figure 6). The simple reflex arc initiated by the muscle stretch activates the quadriceps muscle to contract causing extension of the lower leg. Interneuron in the spinal cord activated by incoming sensory information inhibit contraction of the antagonist group of hamstring muscles, and relay information regarding the reflex to higher brain regions involved in movement and sensation.

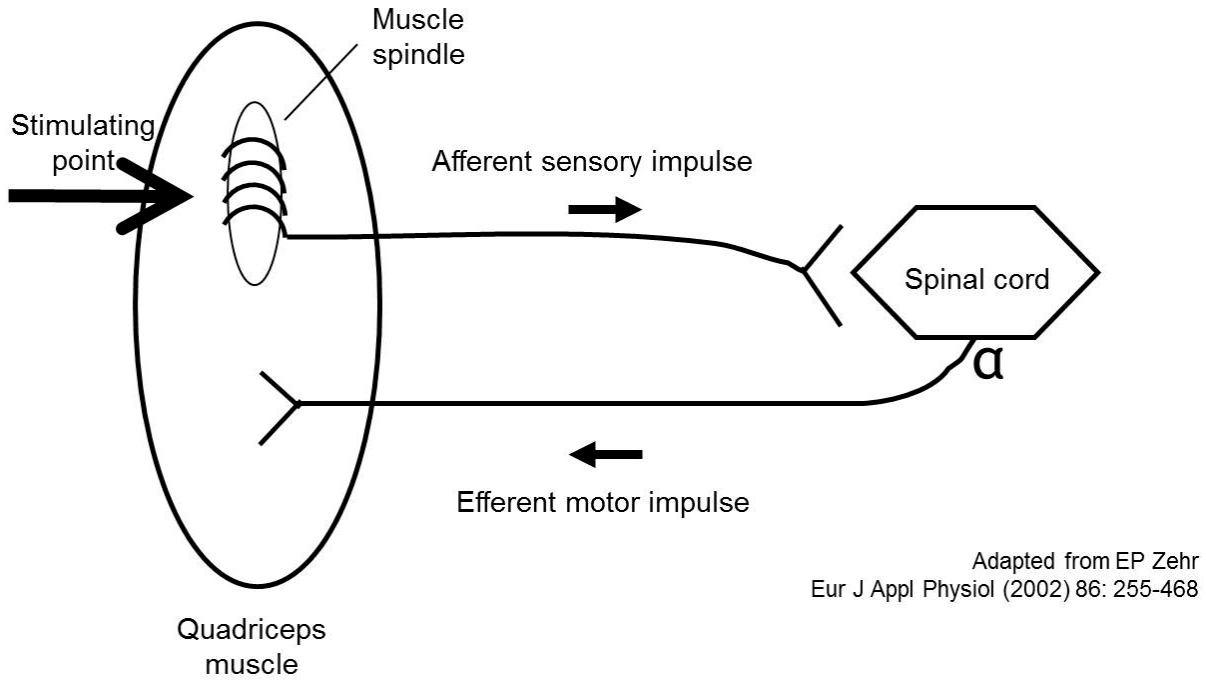


Figure 6: Schematic representation of the monosynaptic patellar reflex arc. α represents the alpha motor neuron.

The patellar reflex is commonly used in clinical practice as an indicator of the state of spinal excitability, and is the only true monosynaptic reflex. The importance of the monosynaptic reflex is that the simple reflex arc is an accurate and independent reflection of spinal excitability. The patellar reflex arc exclusively traverses spinal segments L2, L3 and L4 (Wang & Cymet, 2005) therefore demonstrating the excitability of this particular region and testing the functionality of the femoral nerve.

Measuring the amplitude of the reflex has only been done at a clinical level. Accordingly, the Mayo Clinic Scale (Manschot *et al.*, 1998) and the National Institute of Neurological Disorders and Strokes (NINDS) myotatic reflex scale (Hallett, 1993) were developed to evaluate muscle reflexes (Table 2).

Table 2: The Mayo Clinic Scale and the National Institute of Neurological Disorders and Strokes (NINDS) myotatic reflex scale for evaluating muscle reflex responses

Mayo Clinic Scale (Manschot *et al.*, 1998)		**NINDS myotatic reflex scale** (Hallett, 1993)	
-4	Absent	0	Absent reflex
-3	Just elicitable	1	Small, less than normal trace response or requires reinforcement
-2	Low	2	Normal (lower half of range)
-1	Moderately low	3	Normal (upper half of range)
0	Normal	4	Enhanced reflex often including clonus
1	Brisk		
2	Very brisk		
3	Exhaustible clonus		
4	Continuous clonus		

Clearly, both the NINDS and Mayo Clinic scales are dependent on observer interpretation and are therefore highly subjective and require the experience of clinical judgement. The two extremes of both scales are more easily identifiable but points in between are less distinguishable. This is acceptable in clinical practise, but for reporting quantified results in research papers a more precise measure is required. The relationship between the NINDS or Mayo Clinic scale and EMG measurements of reflex response is reported by some as fair (Stam

& van Crevel, 1990) but others conclude that there is great variability (Zhang *et al.*, 2000). In research that I have co-authored, we found strong correlations between both the subjective NINDs and Mayo Clinic scale and objective biomechanical and EMG measures of the patellar reflex (Dafkin *et al.*, 2012, Appendix I).

An objective measure of the patellar reflex would eliminate potential confounders based on clinicians' judgment. Mamizuka et al (2007) designed a more objective measure by attaching a tri-axial accelerometer to the ankle and recording the time delay (29.6 ± 6.0ms) and acceleration time (150.8 ± 19.5ms) of the reflex. The authors included a comparison between healthy and spastic subjects providing normal and hyperexcitable spinal cord data, but the standard deviation for each group was greater than the differences between the groups (Mamizuka *et al.*, 2007). We found, in recent research that I have co-authored, that there is strong positive correlation between kinematically measured patellar reflex variables (particularly change in knee angle) and EMG measures of reflex amplitude (Dafkin *et al.*, 2012, Appendix H).

The range of patellar reflex latencies and amplitudes in normal healthy subjects on EMG have been investigated by various researchers (Stam & Tan, 1987; Frijns *et al.*, 1997). Electromyographic recordings of the right rectus femoris muscle showed the mean patellar latency to be 21 ± 1.5 ms at rest and 20.8 ± 1.5ms when facilitated by the Jendrassik manoeuvre. The mean patellar EMG amplitude was 1.8 ± 1.2 mV at rest and 2.4 ± 1.4 mV when facilitated by the Jendrassik manoeuvre (Frijns *et al.*, 1997). Similar patellar reflex latencies of 19.9 ± 1.7ms (Pereon *et al.*, 2004), and similar quadriceps amplitude of 1.4 ± 0.2mV were reported by other studies (Stam & Tan, 1987). The reported mean latencies differ slightly between studies and even within studies when testing different patellar hammer models

(Frijns *et al.*, 1997). These differences are attributed to variations in the way latency is measured. Reflex latency is measured (in ms) from the point of tendon stimulation referred to as the 'starting point'. The 'starting point' may vary depending on the delay between striking the tendon and the actual start of the timing measurement which is reported to be different for different patellar hammer models. Other factors that influence the latency are the striking force of the patellar hammer and underlying muscle tone (Pereon *et al.*, 2004). Despite these small variations, using the reflex latency appears to be more reliable than reporting reflex amplitude. The EMG amplitude of the patellar reflex was reported to differ despite a consistent 'striking' which produced a similar reflex latency each time (repeated 12 times) (Frijns *et al.*, 1997). The reflex amplitude is often used diagnostically however given its potential for wide variability, it should be used cautiously.

2.2.3.4. Assessment of spinal reflexes in RLS and PLM

The spinal cord is implicated in the production of RLS and PLM sensorimotor symptoms as it is the primary input of sensory afferents and final output stage of motorneurones (Paulus & Schromburg, 2006). The spontaneous sensations and movements of RLS and PLM suggest an increased excitability of the neurones in the spinal cord. The evidence for this is limited and is dependent on studies undertaken using reflexes.

The majority of research examining the state of spinal excitability in RLS and PLM patients has involved the H-reflex. Evidence supporting a state of spinal hyperexcitability that has been obtained previously includes: an increased H/M ratio in RLS patients (Wechsler *et al.*, 1986), impaired H-reflex excitability curves (Martinelli *et al.*, 1987; Rijsman *et al.*, 2005); depressed vibratory inhibition (Achilles tendon vibration) (Rijsman *et al.*, 2005) and decreased 1b

interneuron inhibition compared to healthy controls (Scaglione *et al.*, 2008). However, not all the H-reflex data supports the concept of global spinal hyperexcitability in RLS and PLM patients. Several studies examining the H-reflex showed no differences between RLS and control subjects for either H-latency; H-amplitude or H/M ratio (Bucher & Trenkwalder, 1996; Akyol *et al.*, 2003; Rijsman *et al.*, 2005; Scaglione *et al.*, 2008). The obvious difference between the H-reflex data for and against spinal hyperexcitability in RLS and PLM patients is the different measurement parameters. The majority of the evidence indicating spinal hyperexcitability in RLS and PLM patients was detected under the influence of conditioning stimulation.

Another reflex that has been tested in RLS and PLM patients is the nociceptive flexor reflex, whose motor sequence has been noted to be similar to PLM. One study demonstrated state dependent lower threshold and greater spatial spread of the reflex in PLM patients which was proposed to indicate spinal hyperexcitability (Bara-Jimenez *et al.*, 2000). Another study however, showed that the flexor reflex amplitudes were normal for RLS and PLM patients (although these were not compared to a control group included in the study) (Wechsler *et al.*, 1986).

No other spinal reflexes have been tested in RLS and PLM patients. Brainstem reflexes have been tested but are cranial nerve reflexes and therefore do not fall under the field of spinal reflexes. Therefore, reflex testing primarily using the H-reflex has been inconclusive. Focusing research on other reflex pathways, for example the patellar reflex, may be helpful in elucidating the aetiology of RLS and PLM and particularly spinal hyperexcitability.

Given the circadian component of the RLS diagnostic criteria, it should follow that any changes in spinal excitability in RLS patients should exhibit circadian variations. For example, it would be expected that there would be a state of spinal hyperexcitability in the evening when RLS symptoms are present and that there would be decreased excitability in the morning when symptoms dissipate. Surprisingly, this hypothesis has not been tested in RLS patients.

In normal subjects, the literature regarding circadian variations of the natural state of spinal excitability, as tested by the various different reflexes, is conflicting. The H-reflex and the stretch reflex have both been shown to have increased evening amplitudes compared to the morning (Dowman & Wolpaw, 1989; Lagerquist *et al.*, 2006) but in other hands also to demonstrate no circadian variations (Castaingst *et al.*, 2004; Guette *et al.*, 2005; Lagerquist *et al.*, 2006). The evidence of naturally increased spinal excitability in the evening would support the hypothesis that spinal hyperexcitability in the evening is responsible for the symptoms in RLS patients.

2.3. MIXED SENSORY AND MOTOR ASSESSMENT

Whilst the sensory and motor features of RLS and PLM can be assessed separately, as has been the focus of this review to this point, there has been relatively little focus on the assessment of both features in conjunction and their relationship to each other. Given the high co-morbidity between RLS and PLM, and the likely possibility that they have similar origins, it would be useful to assess the sensory and motor features simultaneously and the Suggested Immobilization Test fulfils this requirement.

2.3.1. Immobilization tests

The Suggested Immobilization Test (SIT) was developed by Montplaisir et al (1998) as an objective diagnostic criterion to quantify motor restlessness (PLMW) associated with RLS. The test exploits the diagnostic criterion that RLS symptoms are most often present during periods of rest. In the SIT test protocol, subjects are required to sit up in bed at a 45° angle with their legs outstretched and remain vigilant for the full 60 minutes of the test. Bilateral EMGs of the anterior tibialis muscles are recorded and patients are asked to limit their voluntary movements as much as possible during the suggested immobilization (Montplaisir *et al.*, 1998). The EMG recordings are scored according to criteria established by Michaud (Michaud *et al.*, 2001) and are presented as the PLMW index (the number of leg movements per hour of immobility). The immobility provokes RLS symptoms as well as involuntary movements making the SIT an ideal test to assess simultaneous presentation of the sensory and motor features of RLS and the relationship between them. Sensory symptoms can be assessed in conjunction with the motor component by asking subjects to complete a discomfort visual analogue scale (VAS) every 5 minutes for the hour of the test (Michaud *et al.*, 1999). The mean discomfort score represents the average value of all the 5 minute measures (Michaud *et al.*, 2002b).

The Forced Immobilization Test (FIT), also developed by Montplaisir and colleagues, is similar to the SIT except that the patient's legs are strapped into a restrictive device to physically prevent voluntary movements (Montplaisir *et al.*, 1998). Surprisingly, the results observed for the FIT showed fewer leg movements than the SIT despite a greater degree of immobility. The authors speculate that the nature of the FIT immobilization would not allow relief from the discomfort and thus there were fewer leg movements, possibly suggesting that there is an

element of conscious input into PLM. Following the validation of the SIT, it was favoured for further studies and the FIT is rarely utilised now.

The SIT has been modified in various studies to be performed over different lengths of time. Initially the test was performed for 30 minutes (Brodeur *et al.*, 1988), but the standard protocol has become 1 hour (Michaud *et al.*, 2002a; Michaud *et al.*, 2002b; Garcia-Borreguero *et al.*, 2004a; Haba-Rubio & Sforza, 2006; Aksu *et al.*, 2007). Several studies have shown that in RLS patients, RLS discomfort and number of PLMW increase patients after 30 minutes of the SIT (Michaud *et al.*, 2002a; Aksu *et al.*, 2007), justifying the argument that the SIT should be carried out for over 30 minutes.

The SIT has a diverse range of applications. It has been used diagnostically to discriminate between RLS patients and control subjects (Montplaisir *et al.*, 1997; Michaud *et al.*, 2002a; Michaud *et al.*, 2002b); to assess the relationship between sensory and motor features of RLS (Pelletier *et al.*, 1992; Michaud *et al.*, 2002a; Michaud *et al.*, 2002b; Birinyi *et al.*, 2005; Aksu *et al.*, 2007); to determine the effects of rest duration over time (Michaud *et al.*, 2002a; Birinyi *et al.*, 2005); as a measure of severity of RLS (Garcia-Borreguero *et al.*, 2004a; Haba-Rubio & Sforza, 2006; Aksu *et al.*, 2007); to examine the association between leg movements during wakefulness and sleep (Garcia-Borreguero *et al.*, 2004a; Michaud *et al.*, 2002a; Michaud *et al.*, 2002b); to assess the success of pharmacological treatments (Brodeur *et al.*, 1988; Tribl *et al.*, 2005; Vetrugno *et al.*, 2007b); and to assess circadian variations of symptoms (Trenkwalder *et al.*, 1999a).

As part of the proposition that the SIT is an objective, diagnostic criterion for RLS it requires a feature that allows it to discriminate RLS patients from control participants and the SIT PLMW

index has been proposed for such diagnostic purposes (Montplaisir *et al.*, 1997; Michaud *et al.*, 2002a; Michaud *et al.*, 2002b). The exact number of PLMW however, required for distinguishing between RLS and control subjects, varies between studies.

Montplaisir et al (1998) originally demonstrated that a SIT PLMW index of greater than 40 was able to differentiate RLS patients from control participants (Montplaisir *et al.*, 1998). However, both Michaud et al (2002) and Haba-Rubio and Sforza (2006) proposed that a much lower PLMW index of 12 successfully divided RLS and control participants. Cut-off points for these studies were statistically calculated based on receiver-operator curve analysis and defined as "the value that most minimized the misclassification of patients and controls" (Montplaisir *et al.*, 1998; Michaud *et al.*, 2002b). The discrepancy shown between the two cut off points may be because of underlying differences in the presentation and severity of RLS. Also, the PLMW index is a measure of PLM, a feature which not all RLS patients present with (Allen & Earley, 2001b).

Interestingly, the cut off criteria suggested above do not always hold. Using the proposed cut offs, the participants in the study by Garcia-Borreguero et al (2004) would have been excluded as RLS patients based on their mean PLMW index (11.8 ± 5.4), however these were all confirmed RLS patients (Garcia-Borreguero *et al.*, 2004a). In another study the control participants had a morning PLMW index of 33.6 ± 62.2 and an evening PLMW index of 15.5 ± 22.5, both greater than the PLMW index of 12 as a cut off. Also, the standard deviation of the morning value implies that some of the control participants would clearly have PLMW index scores of greater than 40. However these control participants did not fulfil any of the RLS essential diagnostic criteria (Gamaldo *et al.*, 2009). Healthy control participants can exhibit PLM (during wakefulness and sleep) without underlying pathology (Ancoli-Israel *et al.*, 1985;

Dickel & Mosko, 1990) (as has been previously discussed in section 1.1.2). Although a good index of motor restlessness, the SIT is not yet reliable enough to separate true RLS patients from controls and some other features may be needed to improve the diagnostic accuracy of this technique.

One such additional feature of the SIT test is exploration of discomfort exacerbation with rest. Accordingly, Michaud et al (2002) showed that the SIT mean discomfort score was better able to discriminate RLS patients from controls than the PLMW index (Michaud *et al.*, 2002b). The SIT mean discomfort score showed higher sensitivity for diagnosing RLS than the PLMW index (82 ± 8% vs. 62 ± 10%) and both had the same specificity (84 ± 10%). The SIT mean discomfort score was able to correctly classify 82.7% of patients and controls whereas the PLMW index only had an accuracy of 69.3%. Further studies are required to confirm the use of the SIT discomfort score as a diagnostic tool for RLS, however it shows promise and greater reliability than the PLMW index.

Another way to explore the connection between the sensory and motor features is to look at their relationship to each other during the SIT. The immobility of the SIT invokes both the sensory and motor features of RLS. Given the high co-morbidity between RLS and PLM it would seem likely that there is a relationship between the sensory and motor features however the related literature is inconsistent. Whether the sensory drives the motor or the other way around or they are independent features is debatable. The PLMW index plateaus after 35 minutes of the SIT, but the discomfort levels continue to increase throughout the test (Michaud *et al.*, 2002a). Whether the plateau observed is as a result of the legs being unable to produce more PLMW ("ceiling effect"), or that there is actually a dissociation between the sensations and movements at this point, requires further exploration (Birinyi *et al.*, 2005). This

trend is apparent for RLS patients who have high numbers of PLMW, however those with fewer PLMW show a simultaneous increase in both PLMW and discomfort (Birinyi *et al.*, 2005).

Some studies have focused on the temporal relationship of the sensory features in relation to the movements during the SIT and the FIT. Weak positive correlations between discomfort and the PLM index have been shown in some studies (Michaud *et al.*, 2002b; Aksu *et al.*, 2007) and no relationship was shown in a further study (Michaud *et al.*, 2002a). Nearly half of all the PLMW occur independently of associated sensations (Pelletier *et al.*, 1992), and almost half of the sensations are not associated with movements (Birinyi *et al.*, 2005). Nearly 75% of the sensations occur after the movement with the remainder occurring before the PLMW (Pelletier *et al.*, 1992; Birinyi *et al.*, 2005). Both concluded that there was a dissociation between the sensory and motor features and thus it could not be determined whether the sensations drive movements or vice versa.

The relationship between the severity of RLS and its sensory and motor features also appears to be undefined. The SIT PLMW index correlated with the IRLS severity score in one study (Garcia-Borreguero *et al.*, 2004a), however this relationship was not demonstrated in other studies (Haba-Rubio & Sforza, 2006; Aksu *et al.*, 2007). The validity of the finding that severity correlated with the PLMW index is not obvious. Looking at the distribution of the PLMW in the study by Garcia-Borreguero and colleagues, they are clearly left skewed, with a possible outlier at the extreme high end of PLMW possibly forcing the correlation (Garcia-Borreguero *et al.*, 2004a). The same analysis of the studies that indicated no relationship between the SIT PLMW index with the IRLS severity score could not be performed because these studies did not include the relevant data.

Only one study has assessed the relationship between RLS severity and sensory discomfort and found a positive correlation between the two (Aksu *et al.*, 2007). The relationship between the severity and discomfort is not surprising as the mean discomfort score measured using the VAS scale in the SIT is both a reflection of the discomfort and the current intensity and severity of RLS. The current, commonly used, IRLS severity scale does not reflect the actual current intensity of the symptoms which is the focus of the measurements made with the SIT, so the two may be measuring different factors that both influence the severity of RLS. Further research is required to ascertain the usefulness of the SIT as a measurement tool to assess the severity of RLS.

The SIT has been proposed as a more convenient and time efficient method of assessing PLM compared to the overnight PSG recording of PLMS and PLMW; however its use requires further validation. Only a few studies have been conducted focusing on the relationships between the SIT PLMW and the PSG PLMS and PSG PLMW however the findings from these methods are not consistent. SIT PLMW index was shown to marginally correlate with PSG PLMS and PLMW (Garcia-Borreguero *et al.*, 2004a), and also to have no relationship with PSG PLMS and PLMW (Michaud *et al.*, 2002a; Michaud *et al.*, 2002b). Clearly, these relationships require further exploration before the SIT can be used in place of PSG screening of PLM.

The SIT has also been used to assess the success of treatment interventions and objective evaluation of augmentation. The original paper describing the SIT assessed the efficacy of L-Dopa in treating RLS and showed a reduction in the number of PLM the evening after the administration of the L-Dopa (Brodeur *et al.*, 1988). Apomorphine, a combined opioidergic and dopaminergic agonist, administered to RLS patients resulted in a rapid and marked improvement in sensory and motor features as measured by the VAS and PLMW index from

the SIT (Tribl *et al.*, 2005). A case study reported by Vetrugno et al (2007) used the SIT to evaluate the augmentation of symptoms exacerbated by Tramadol, and then assessed the patient again with the SIT to show an improvement of symptoms following a change in treatment (Vetrugno *et al.*, 2007b).

The inconsistent relationships between SIT measurements (PLMW and sensory features), RLS severity and PLMS may all reflect that RLS is a heterogeneous disorder and underlying differences in population samples may account for the major differences noted. Therefore future studies may need to be more selective in subject selection to create a more homogenous sample before we can obtain consistent results.

In conclusion, numerous techniques have been employed to assess both the sensory and motor features of RLS and PLM and our knowledge of these disorders has been advanced by the assessment techniques that are available. However, there are still gaps in our understanding of the aetiology, perception and clinical assessment of RLS and PLM and further research using new techniques is still required to fully understand these disorders and provide effective treatment for them.

3. RATIONALE FOR THE PRESENT STUDIES

RLS and PLM have distinct sensory and motor features and presumably share a common aetiology given their high co-morbidity. Despite knowledge that dopamine agonists, and to a lesser extent opioids, effectively treat the symptoms of RLS and PLM, the aetiology of both is unclear. Extensive research has been conducted on these two disorders, particularly in recent years, however there is still a lack of reliable assessment tools available to investigate them.

Currently, the diagnosis of RLS is based primarily on a patient's description of their symptoms. This is a subjective measure and patients themselves indicate difficulty in describing their sensations. The terms which patients use to describe their sensations have not been characterised nor have the sensory descriptions patients use been compared to the descriptors included in the diagnostic criteria. Effective descriptions about RLS sensations may contribute to more accurate diagnosis, which may, in turn, improve our understanding of their aetiology. Therefore my first study focused on the descriptors used by RLS patients to describe their sensations. English speaking South African participants fulfilling all four of the RLS diagnostic criteria completed a semi structured interview, comprising the following: a spontaneous description of their sensations, completing the IRLS and MPQ, followed by the selection of terms relevant to their sensations from a literature derived list of RLS words and phrases. The most frequently offered and selected descriptors were determined and compared to the terms used in the diagnostic criteria.

The first study (above) set the foundations for the second study, which focused on whether the presence of painful RLS sensations influenced the choice of descriptors chosen. A subgroup of RLS patients experience painful RLS (up to 80% of patients), but whether these patients have

any underlying pathological difference is unknown. The same set of participants included in study one were categorised based on their self-reported presence or absence of painful RLS symptoms, confirmed by differences in their MPQ scores. The word choices between the painful and non-painful groups were compared to determine if pain influenced how a patient perceived and reported their sensations. Defining the word choices of the different phenotypes, may help improve the diagnosis of RLS and assist in the elucidation of RLS aetiology.

There are clear sensory and motor components to the terms that patients use to describe their RLS sensations i.e. “feels like bubbles in my veins” (purely sensory), whereas “it’s just an urge to move” relates to movement. For my third study, the focus of the thesis shifted from assessment of the sensory features to assessing the motor component of RLS. In this third study I specifically focused on the motor output of the spinal cord.

Evidence in the literature to date suggests that these disorders are caused by functional abnormalities of the central nervous system and, of particular interest to my third study, hyperexcitability of the spinal cord. Due to the contradictory data from currently used techniques I wanted to examine the excitability state of the spinal cord of RLS patients using a new technique. And, in accordance with the diagnostic criteria stating that symptoms should worsen in the evening, I also wanted to investigate whether the state of spinal excitability in RLS patients differed between the asymptomatic morning and symptomatic evening periods. Considering the idea that PLM and RLS are possibly the product of state dependent disinhibition of inhibitory pathways (Quatrale et al. 2003) and that circadian influences appear to play a determining role in their expression (Hening et al. 1999b), it is surprising that time factors have not been taken into account in previous studies of reflex activity in these patients.

I used two different reflexes to assess differences related to different spinal locations and stimuli. The two reflexes (patellar and H-reflex) share largely similar neuronal circuitry but traverse the spinal cord at different levels and are stimulated via different methods. The patellar reflex was chosen due to its monosynaptic nature (thus limiting other neuronal influences) and the H-reflex was used as the electrical counterpart of the patellar reflex (despite conflicting literature about oligosynaptic inputs) which has been used in previous RLS studies. Using kinematics in conjunction with electrophysiology, I hoped to accurately determine if in fact there is a state of hyperreflexia and spinal hyperexcitability in patients with RLS, which would present during the evening period when symptoms peak.

The purpose of using both kinematics and electrophysiology to assess spinal reflexes was to simultaneously measure the electrical muscle activation and the consequent movement. Clinical assessment uses either EMG (for muscle activation) or visual rating scales of reflexes (for movement) (e.g. NINDS) however the relationship between these is yet to be quantified. It would be a natural assumption that the one would relate to the other but, to my knowledge, this has not been assessed for the patellar reflex and particularly not in the unusual case of RLS. Subsequently, this information in this group of patients should be particularly important in assessing the use of actigraphy as a screening/recording device for PLM. As a development from this research, a subsequent project has assessed the relationship between electrical muscle activation and the movement during the patellar reflex in healthy people. Two publications co-authored during the period of PhD candidature are attached as Appendix H and Appendix I.

Finally, because RLS is described as a sensorimotor disorder it is essential to assess both the sensory and motor features together, but very little of this interactive research has been done.

The Suggested Immobilization Test (SIT) provokes RLS discomfort and motor restlessness thereby facilitating the simultaneous assessment of both these features. Despite many RLS patients reporting painful sensations, these have not been previously assessed during the SIT.

In my fourth study the relationship between the discomfort, pain and motor activity during the SIT was assessed in RLS patients. Continuing from the idea in study two that painful RLS may be a different presentation of the disorder, I specifically asked patients to distinguish between RLS pain and RLS discomfort on separate Visual Analog Scales for each sensation. Concurrent bilateral EMGs of the tibialis anterior (the most commonly activated muscle during PLMs) were also recorded.

Thus the four studies look at new types of assessment for the sensations alone, the motor activity alone as well as the combination of sensory and motor function during the SIT.

4. AIMS OF THE RESEARCH

Overall the aim of the thesis is to investigate different aspects and assessments of the sensory and motor features of RLS and PLM, subjectively and objectively. The specific objectives for each of the studies are:

1. To describe and characterize the sensations of RLS symptoms in an English speaking South African population.
2. To examine how the presence of pain influenced the descriptor choices used by RLS patients.
3. To objectively quantify the H-reflex and patellar reflex in RLS patients compared to control participants at two different times of the day.
4. To explore the relationship between sensory symptoms, both pain and discomfort, and motor activity during the Suggested Immobilization Test in patients with RLS.

CHAPTER 2

Paper one: Kerr S, McKinon W and Bentley A.

Descriptors of Restless Legs Syndrome sensations.

***Sleep Medicine 2012; 13(4): 409-413*.**

Sleep Medicine 13 (2012) 409–413

Contents lists available at SciVerse ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

Descriptors of restless legs syndrome sensations

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ARTICLE INFO

Article history:
Available online 17 March 2012

Keywords:
Restless legs syndrome
Terminology
Semantics
Sleep disorder
Diagnosis
Sensations

ABSTRACT

Background: Restless legs syndrome (RLS) is characterised by an urge to move in response to unusual sensations in the legs. Patients experience difficulty describing their RLS sensations, resulting in a diverse range of descriptors which have not been fully categorised. The purpose of this study was to describe RLS sensations and to evaluate the accuracy of current diagnostic descriptors.
Methods: Forty-one RLS participants completed an interview which involved: providing spontaneous descriptions of RLS sensations, completing the McGill Pain Questionnaire (MPQ), and selecting descriptors from a list of previously published RLS terms (prompted descriptors).
Results: The most frequent spontaneous descriptors were: "irritating" (17%), "painful" (17%), and "urge to move" (24%); prompted descriptors were: "restless" (88%), "uncomfortable" (78%), and "need to stretch" (76%); and MPQ words were: "tingling" (56%) and "jumping" (54%).
Discussion: The most frequently cited descriptors in this study differ from the terminology used in the RLS diagnostic criteria. Inclusion of these frequently used descriptors may improve the diagnostic accuracy of RLS. Our data emphasise the need for an international, large scale, multicultural study to determine the most accurate diagnostic descriptors to define RLS more clearly.
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1. Introduction

Restless legs syndrome (RLS) is a condition characterised by an urge to move in response to unusual sensations normally experienced in the legs. In the 19th century RLS was deemed a psychiatric disorder known as "anxietas tibiarum" by Wittmaack owing to the bizarre descriptions of symptoms given by those experiencing RLS [1]. Karl Ekbom described RLS clinically in 1945, giving rise to the name of "Ekbom syndrome," or the more commonly used "restless legs syndrome" [2–4]. RLS was formally classified as a sleep disorder in the International Classification of Sleep Disorders in 1990 [5]. More recently, diagnostic criteria based on patients' subjective symptoms have been developed and validated by the International Restless Legs Syndrome Study Group (IRLSSG) in 1995 [6] and, later, revised in 2003 [7]. The critical components of these criteria are "an urge to move" and "uncomfortable and unpleasant sensations" in the legs, although some patients report the urge to move independent of any associated sensations [7]. It is unclear where the terminology used to describe the sensory and motor components in the current diagnostic criteria originate and, to our knowledge, there has never been a comprehensive study done assessing the scope of the sensations experienced by RLS patients.

In previous studies RLS sensations were classified as parasethesia or dysesthesia or described by patients anecdotally as "tingling, burning, jittery, and prickling," and even as "ants or coca-cola in the bones and veins" [8]. Parasethesia and dysesthesias are usually symptoms of neuropathic pain and, so, the relationship between RLS and pain has been investigated. To this end, RLS symptoms have been described as painful in 50% to 80% of RLS patients [9]. It has been suggested that the sensations may represent a subclinical form of pain as the McGill Pain Questionnaire (MPQ) can be used to assess the severity and quality of these RLS symptoms [10]. The alleviation of RLS symptoms with analgesic medications, amongst other treatments, further supports the concept that pain pathways are involved in the sensory symptoms of RLS [11]. RLS does not however fit neatly into existing pain models and the relationship between RLS and pain sensations needs further investigation.

The aetiology of RLS is still largely unknown. While it is obvious to researchers in this field that the sensations of RLS are unique, very little work has been done to look for any common sensory features to define the sensations more precisely. The purpose of the study was to characterise the range of descriptors used by an English speaking South African population to describe their RLS sensations.

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http://dx.doi.org/10.1016/j.sleep.2011.11.020

2. Methods

The methods are divided into two parts. The first part describes the compilation of a list of RLS descriptors, which is subsequently included in a structured interview, described in part 2.

2.1. Part 1

We conducted a comprehensive literature search including peer-reviewed articles and relevant blogs and websites and extracted all words, phrases, and expressions used in the literature to describe RLS. The majority of the descriptors from the peer reviewed articles were obtained from a small number of papers that had extensive lists of terms [7,12–15]. Descriptors that were synonyms were condensed into one single descriptor (e.g., "got to move" and "have to move"). These descriptors were split into two separate lists, one for RLS words ($n = 113$) and the other for RLS phrases ($n = 31$). The order of the words and phrases were randomized on several copies of the lists so that each participant had a different arrangement to avoid biasing any of the descriptors.

2.2. Part 2

2.2.1. Participants

Participants were recruited on a voluntary basis by local advertisement. They completed a screening questionnaire which included the four essential RLS diagnostic criteria questions using the exact wording as defined by the International Restless Legs Syndrome Study Group (IRLSSG) [7] and basic demographic data and confirmed, on history, the exclusion of any known secondary causes or mimics of RLS. Participants who answered all four of the diagnostic questions in the affirmative and had no history of secondary RLS or RLS mimics were included in the study. Participants were questioned at the interview stage to again confirm the absence of secondary RLS and RLS mimics. All participants were fluent in English. Ethical clearance (clearance number M070452) was obtained from the University of the Witwatersrand Human Research Ethics Committee and participants signed a written informed consent form. All data from participants were coded in order to preserve participant anonymity.

2.2.2. Study design

All RLS participants were given an interview booklet and were interviewed by the first author, who has experience in diagnosing RLS and who asked them to complete the following tasks:

1. Describe their RLS symptoms in their own words (spontaneous descriptors). The interviewer recorded these responses using both a digital recorder and by making written records.
2. Complete the International Restless Legs Syndrome Severity Scale.
3. Complete the McGill Pain Questionnaire (MPQ). Participants were asked to select the most relevant word per group that related to their RLS sensations and to leave out any groups that did not feature relevant words.
4. Identify all words and phrases from the lists compiled in part 1 of the study which could describe their RLS sensations (prompted descriptors). Participants were told to select as many words and phrases as they felt were relevant to the description of their RLS sensations.

2.2.3. Data organisation and analysis

2.2.3.1. Descriptors. The spontaneous descriptors that the participants used to describe their own RLS sensations were split into two lists, one for words and the other for phrases. Descriptors that were synonyms were condensed into one single descriptor (e.g., got to move and have to move) and the frequency of choice of each descriptor was determined. All the duplicate terms were subsequently removed to provide the sum of spontaneous descriptors offered. The number of new descriptors contributed by this participant group was calculated. The frequencies for each of the chosen prompted descriptors (words and phrases) and words on the MPQ were also calculated. The mean (±SD) number of words and phrases selected was calculated for the spontaneous, prompted, and MPQ descriptors.

Phrases were divided into primarily sensory or primarily motor in description and the number of each determined. Manual stepwise regression was used to obtain the minimum number of words that could be used to describe 100% of participants' sensations from the list of prompted words (starting with the most frequently selected word).

3. Results

3.1. Participant information

Forty-one participants, fulfilling all four of the essential RLS diagnostic criteria, took part in the study. (Six participants who did not meet all four of the diagnostic criteria were excluded). The characteristics of the participants, their RLS history, and their severity scores are presented in Table 1. There was a broad range for age of onset, level of pain, and severity of RLS. A positive family history of RLS was reported by 41% of the participants. The majority of the participants were treatment naive, 10% of the participants had previously tried dopaminergic treatment for their RLS (discontinued due to adverse side effects or not obtaining repeat prescriptions), and 27% had tried other treatments (mainly over the counter medications). None of the participants were currently receiving RLS treatment. All the participants had a minimum level of a completed secondary education.

3.2. Descriptors

The participants spontaneously provided 62 words and 39 phrases in total. Seventeen of these descriptors were not present in the list of previously published terms (part 1). The most frequent spontaneously offered and selected prompted words and phrases as well as the words selected from the MPQ are shown in Tables 2 and 3. The mean number, per participant, of spontaneous words was 2.6 ± 2.0, prompted words was 21.8 ± 12.5, and MPQ words was 10.1 ± 4.3. The mean number of spontaneous phrases per participant was 2.4 ± 1.9 and prompted phrases was 8.5 ± 5.1.

For the spontaneous phrases there was an equal number given for the motor and sensory descriptors. Of the top 10 prompted phrases, 80% were motor descriptors, including the first six choices, despite there being an almost equal split of sensory and motor descriptors on the phrases list. The number of descriptors, both

Table 1
Characteristics of RLS participants (Mean [SD]).

	RLS participants	Range
Number	41	–
Male: female (*n*)	10:31	–
Age (years)	47.0 (13.2)	24–74
Duration of RLS (years)	17.6 (12.4)	1–48
Age of RLS onset (years)	29.8 (13.6)	10–72
RLS severity scale score	20.0 (6.4)	4–30
MPQ score	18.8 (9.9)	4–51

Table 2
The eight most frequent words given by RLS participants ($n = 41$) when asked to describe their RLS sensations spontaneously, prompted with words from a literature derived list of RLS descriptors, and asked to choose words from the McGill Pain Questionnaire (MPQ).

TOP "8" words (% of participants that offered/chose the word)					
Spontaneous words (%)		Prompted words (%)		MPQ words (%)	
Irritating	17	Restless	88	Tingling	56
Painful	17	Uncomfortable	78	Jumping	54
Crawling	15	Twitchy	63	Nagging	51
Uncomfortable	15	Unpleasant	59	Tiring	44
Discomfort	12	Irritating	56	Annoying	41
Restless	10	Nagging	56	Tugging	39
Tingling	10	Fidgety	46	Dull	34
Twitching	10	Jerky	46	Gnawing	29

words and phrases, required to include all participants are shown in Fig. 1. Four words and four phrases were sufficient to cover all the participants as far as descriptive words and phrases are concerned.

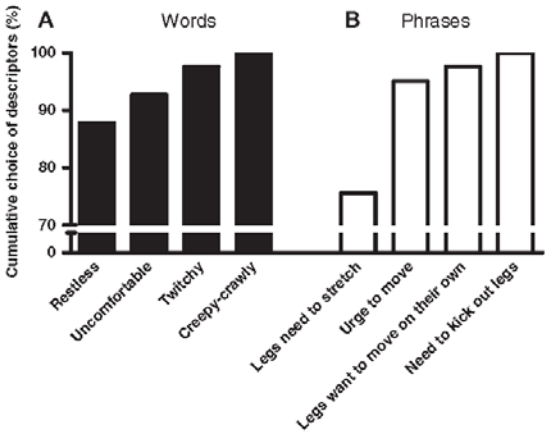

Fig. 1. The minimum words required from a literature derived list of RLS descriptors to cover. 100% of the population. The most frequently selected word (A) and phrase (B) is on the left and the additional words and phrases added to the right.

4. Discussion

In this cohort of English speaking South African RLS participants, with a broad range of ages, symptom duration, age-of-onset, and severity of RLS, there appears to be some common themes when participants describe RLS associated sensations. Participants only provided a small number of descriptors when asked to describe their RLS, but selected a much larger sample of descriptors when provided with a selection of established terms. Different words were chosen spontaneously, when prompted, and from the MPQ. Spontaneous and prompted phrases, however, were similar. The participants spontaneously described an equal number of sensory and motor phrases, however, upon prompting, the motor phrases were the dominant choice.

These findings represent the results from a small sample of English speaking South African RLS participants. A larger, more culturally diverse, sample size may have provided a better representation of the RLS population as a whole, although there is no evidence that RLS descriptors change between populations. We felt that it was necessary to restrict the initial study to English speaking participants to avoid the confounding variables associated with multiple language translations and back translations. The findings could have been biased by the fact that the word "restless" is the most frequently chosen descriptor which emanates from the participants familiarity with the phrase "restless legs syndrome". Other words chosen could also have been influenced by prior knowledge of RLS descriptors. Although the study included a range of RLS severities, the majority of our participants suffered from mild to moderate RLS and this may have influenced the choice of descriptors, although the relationship between descriptors and severity of RLS is unknown. Another limitation may be the use of history to exclude RLS mimics and secondary RLS, although six potential mimics were excluded. The data would indicate that this group of RLS participants are quite consistent in their choice of words and phrases, thus reducing the chance of mimics occurring in the sample. The words chosen on the MPQ must also be viewed with caution as, unlike the other selections, participants are restricted in the number of words they can choose – 20 being the maximum. Thus, other words may have been valid but in the same group as the word chosen and therefore not available for selection.

Table 3
The 10 most frequently selected phrases chosen by RLS participants ($n = 41$) when asked to describe their RLS sensations spontaneously and when selecting words from a literature derived list of RLS descriptors (prompted).

TOP "10" phrases (% of participants that offered/chose the phrase)			
Spontaneous phrases		Prompted phrases	
Have/urge to move	24	Legs need to stretch	76
Need to stretch	17	Just an urge to move	73
Pins and needles	12	Legs want to move on their own/ won't be still	66
Legs refuse to sit still	10	Need to kick out your legs	59
Have to walk	7	Legs need to walk/jog	59
Worms inside leg	7	The got to moves	51
Columns of ants marching up and down	5	Anxiety in your legs	49
Feels as if you've hit your funny bone	5	Feels like your legs want to jump off your body	34
Feels like want to run/ exercise/use muscle	5	Legs have too much energy/are full of energy	34
Muscles aren't relaxed	5	Nervous legs	32
Spontaneous tapping	5		
There's something deep inside the leg	5		
Want to get up and go	5		

The word "restless" was only offered spontaneously by 10% of the participants, but when these same participants were provided with a choice of pre-selected descriptors, restless was the most frequently chosen word (88%) to describe their RLS sensations. Given the name *restless* legs syndrome, it is assumed that patients would experience restlessness; however, the word restless is not included in the diagnostic criteria. The official IRRLSSG diagnostic criteria, asks whether a patient experiences an "uncomfortable" or "unpleasant" sensation [7]. Uncomfortable was chosen by 78% of participants in this cohort and unpleasant by only 59%. These two words were selected together by 56.1% of participants. The words "uncomfortable" and "unpleasant" are not frequently offered when participants spontaneously described their symptoms. One RLS diagnostic criterion requires a patient to feel the "urge to move" [7]. The relevance of this criterion is reflected by its selection as the most frequent spontaneously offered phrase and the second most commonly chosen prompted phrase. The phrase "need to stretch" is not included in the diagnostic criteria; however, it was as frequently selected, both spontaneously and when prompted, as the phrase "urge to move."

There appear to be some differences between the terms used in the diagnostic criteria and those that patients use. It is unclear where the wording in the IRRLSSG diagnostic criteria originates. We suspect that the terms "uncomfortable" and "unpleasant" are derived from the original definition of RLS as "parasethesia" or "dysesthesia." Dysesthesia are defined by the European Federation of Neurological Societies as "abnormal and unpleasant" sensations, whereas parasethesia are "abnormal but not unpleasant" sensations [16]. Ekbom's original description of RLS using his original 34 cases of patients with RLS reported their sensations as "crawling," "unpleasant," "irritating," and "disagreeable," but rarely as painful, and that patient's found it "impossible to keep their legs still" [3]. Previously, far greater emphasis has been placed on the sensory components than the urge to move. Current literature indicates that the need to move is far more useful diagnostically than the RLS sensations and, as such, diagnostic criteria state that the urge to move may or may not be accompanied by uncomfortable sensations [7]. This may also be due to the lack of extensive work trying to classify the sensory discomfort more accurately.

A relationship between the sensations of RLS and those of pain was not confirmed by these data. The only common words between those selected from the MPQ and the spontaneous and prompted words were "tingling" and "nagging," which were not in the top five words chosen in either the spontaneous or the prompted word group. This may indicate that, although the MPQ may be valid to measure the severity of RLS, the descriptors in that questionnaire are not sufficient as diagnostic criteria [10]. These data also question the prevalence data of patients commonly reporting "pain" as a descriptor, although a composite of words may indeed feel like pain. In the early literature reporting on RLS, Ekbom repeatedly indicated that sufferers of RLS have difficulty describing the sensations they experience [3] and this problem persists with patients today. A great deal more work is required to tease out the relationship between RLS sensations and pain.

The phrasing of the diagnostic criteria, although a purely semantic exercise, may influence a clinician's diagnosis of a patient. Popat et al. pointed out that "using a consistent set of questions (with respect to wording, response choices, and algorithm for classifying RLS status)" is essential in being able to compare RLS epidemiological studies [17]. Using the published wording of the IRRLSSG should ensure that researchers are using a standard set of descriptors to diagnose RLS, but these descriptors may not be sufficiently accurate. Physicians may be primed to relate to the current wording and may not recognise other, more unusual, yet valid, descriptors.

An extensive study done in numerous countries in the Northern hemisphere (Europe and USA) using the descriptors "uncomfortable" and "unpleasant" (included in the diagnostic criteria) reported a marked under-diagnosis of RLS [18]. These researchers concluded that true RLS could be misinterpreted as a variety of other conditions such as varicose veins, neuropathy, leg cramps, chronic venous insufficiency, or damage to the lumbar spine [18]. Alternatively, it has been suggested that other conditions are diagnosed as RLS as the four diagnostic criteria, as they stand, are not able to exclude RLS mimics [19]. Creating a better word selection so that patients can better describe their symptoms could assist clinicians in distinguishing RLS from RLS mimics and a differential diagnosis could possibly be made based on the word choice of patients. The same process done here for RLS mimics should be done for the conditions that may mimic RLS to bring clarity to the situation. Having a list of validated descriptors for each condition, including paraesthesia and dysethesia, would assist the primary care doctor in differentiating the conditions and would thus improve the diagnostic accuracy of RLS.

The descriptors selected in this study reflect the choices of an English speaking South African population. The descriptors may differ between different cultural and language groups, as well as between primary and secondary RLS. There is a need to perform a number of studies in English speakers of different cultural backgrounds and geographical areas to determine the consistency of the English descriptors shown in this study in order to improve the diagnosis of RLS. Patient descriptors also need to be assessed in other languages as well as changes that may occur after treatment and with augmentation.

In conclusion, our participants had a wide selection of words to describe their RLS, with some unique to individual participants, but a few common descriptors which covered the whole sample could be found. Expanding the word choice in the diagnostic criteria may improve the accuracy of RLS diagnosis and exclude mimics more easily.

Conflict of Interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: doi:10.1016/j.sleep.2011.11.020.

Acknowledgement

Funding provided by the Wits Dial.a.Bed sleep laboratory.

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CHAPTER 3

Paper two: Kerr S, McKinon W and Bentley A.

Does the presence of pain influence the descriptors used for the sensory discomfort in Restless Legs Syndrome?

Does the presence of pain influence the descriptors used for the sensory discomfort in Restless Legs Syndrome?

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Running title: Pain descriptors of RLS

Keywords: Restless legs syndrome, sensations, pain, McGill Pain Questionnaire, severity, descriptors

ABSTRACT

Context and Objectives: Restless Legs Syndrome (RLS) is characterised by unusual sensations in the legs which are described as painful in 50-80% of RLS patients. The purpose of this study was to examine whether the presence of pain changed the words used to describe the sensations of RLS.

Methods: RLS participants (n=41) selected descriptors of their RLS sensations from a list of previously published RLS terms, completed the McGill Pain Questionnaire (MPQ) and the International Restless Legs Syndrome Severity Scale. Participants were divided according to a self-reported presence or absence of painful RLS sensations. The most frequently selected words were compared between the two groups.

Results: The participants with painful RLS had higher MPQ scores than the non-painful RLS participants (median (interquartile range) 25 (15-27) vs. 15.5 (11-22) P=0.041). Apart from the first three words: “restless”, “uncomfortable” and “twitchy”, the overall word choice was different between the two groups (χ^2 = 76.96, P<0.0001). Non-painful RLS was described as “nagging” whereas painful RLS was characterised by “cramping” and “painful”.

Conclusion: Descriptors of RLS sensations are changed by the presence of pain which may indicate an aetiological difference in the patient who has painful RLS.

INTRODUCTION

Restless legs syndrome (RLS) is a condition characterised by an urge to move in response to unusual sensations normally experienced in the legs. The descriptors used in the diagnostic criteria lack formal characterisation and do not take the presence of pain into consideration. The original descriptions of RLS sensations as parasethesia (non-painful) and dysesthesia (painful) did allow for these two groups but these terms have now been discontinued.

Varying numbers of RLS patients describe their symptoms as painful (1) and the McGill Pain Questionnaire (MPQ) has been used to quantify and qualify these sensations (2,3). The alleviation of RLS symptoms with analgesic medications, amongst other treatments, indicates that pain pathways may be involved in the sensations associated with RLS (4). RLS patients have also been shown to have amplified nociceptive processing and increased pain sensitivity (5,6). The presence of pain in patients with RLS may also confound the description of RLS sensations. The purpose of this pilot study was to determine if the presence of pain changed the preferred description of RLS sensations in an English speaking South African sample of patients with RLS.

METHODS

Participants

Participants were recruited on a voluntary basis by local advertisement and were asked to answer a screening and basic demographics questionnaire. Participants were included in the study if they answered all four essential diagnostic RLS questions as defined by International

Restless Legs Syndrome Study Group (IRLSSG) in the affirmative (7), had no history of known secondary causes of RLS and were fluent in English. Ethical clearance (clearance number M070452) was obtained from the University of the Witwatersrand Human Research Ethics Committee and participants signed a written informed consent form.

Study design

The participants were each given a randomly arranged list of RLS terms (n=113) derived from the literature and the internet (8) and were asked to select as many words as they wanted to describe their RLS sensations. Each participant was also asked to complete the International Restless Legs Syndrome Study group (IRLSSG) Severity Scale and to complete the McGill Pain Questionnaire (MPQ). On the MPQ, participants were told to select one word per group that was relevant to their RLS sensations and leave out groups that had no relevant words. Participants were allocated to the painful or non-painful RLS groups based on the answer to the question "Would you describe your RLS sensations as painful?"

Data analysis

All data were non parametric and are represented as median (interquartile range) unless otherwise stated. The characteristics (e.g. MPQ scores) of the painful and non-painful groups were compared using a Mann-Whitney test. Spearman's correlations were performed between the MPQ score and the age-of-onset; IRLS severity score and RLS duration. The five most frequently selected words for both the painful and non-painful groups were compared using a Chi^2 test. Post-hoc analysis for individual words was done using a Fisher's exact test with Bonferroni correction.

RESULTS

Participant information

Forty one participants (76% females) fulfilling all four of the essential RLS diagnostic criteria, were included in the study. The characteristics of all the participants, as well as when divided according to the presence or absence of painful RLS sensations are shown in Table 1. Most of the participants (63%) were treatment naive, 10% had previously tried dopaminergic therapy and 27% had tried over the counter remedies. No patients had taken treatment for their RLS in the week prior to the study.

Painful compared to non-painful RLS

The participants who stated that their RLS sensations were painful had higher scores (greater levels of pain) on the MPQ than the participants who had non-painful RLS (Table 1). There were no other significant differences between the two groups. The word choices overall were significantly different between participants with painful and non-painful RLS (Chi^2 = 76.96, $p<0.0001$) (Figure 1). The most frequently selected words for both groups were “restless”, “uncomfortable” and “twitchy”. “Nagging” was more likely to be chosen by patients with non-painful RLS and “cramping” and “painful” more likely to be chosen by patients with painful RLS (Figure 1). “Painful” was only the fifth most frequently selected word by the pain group and was not selected by the non-painful group.

There were no significant correlations between the MPQ score and: age-of-onset (r=0.2141, p=0.1789); IRLSSG severity score (r=0.1056, p=0.5109) or duration of RLS symptoms (r=-0.1846, p=0.2480).

DISCUSSION

In this group of RLS sufferers 25% complained that their RLS was painful which was validated by the higher MPQ pain score in this group of patients. The presence of pain and the MPQ score was not associated with RLS duration, age of RLS onset, RLS severity or presence of positive family history. The first three words chosen were common for both groups. After those three participants with non-painful RLS were more likely to describe their sensations as “nagging” whereas participants with painful RLS chose “cramping” and “painful” to describe their sensations.

The participants in this pilot study represent a small sample of English speaking South Africans with RLS and the findings may differ in other cultures and language groups. The majority of our participants suffered from mild to moderate RLS and patients with severe RLS may choose different words.

Despite dividing the participants according to the presence or absence of pain, the most frequently selected word in the painful RLS group was not in fact “painful” although the proportion of participants selecting this word was significantly higher than in the non-painful group. The first three words chosen were the same for both groups which implies that the presence of pain would not influence the diagnosis of RLS. After the first three words there are significant differences in word choices between the two groups.

This is the first study to show differences in the descriptors when patients with RLS have painful sensations. Different descriptors may indicate a difference in aetiology as occurs in postoperative pain (9). This may imply that the presence or absence of pain indicates two phenotypes of RLS. The potential impact of this finding on the aetiology, treatment, course and measurement of RLS requires further study.

The presence of pain, whether subjective or by the MPQ, in this group of people with RLS did not correlate with severity of the RLS measured by the IRLSSG Severity scale. This severity scale primarily measures the impact of RLS on a patient's daily life (7), does not measure the severity of the actual sensations and has no specific pain related questions. Thus either other measures need to be devised to assess the severity of the actual sensations which may need to include a question on the severity of pain as well, or the presence of painful sensations does not increase the impact of RLS on daily life.

In conclusion, the word choice in patients with RLS appears to vary according to the presence or absence of painful RLS sensations which may support the idea of RLS as a disorder with multiple phenotypes. Future research should take cognisance of patients presenting with painful RLS as this may be an important confounding factor. More research needs to tease out possible relationships between RLS and pain.

Acknowledgements

Funding provided by the Wits Dial.a.Bed sleep laboratory.

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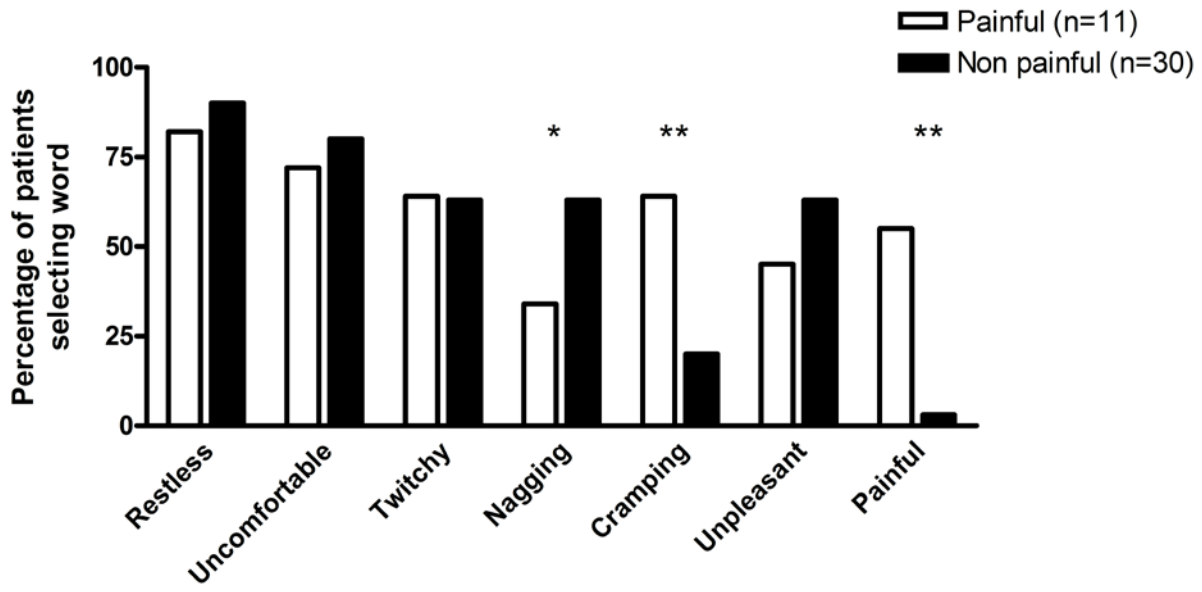
Table 1: Characteristics of RLS participants divided by the presence or absence of painful symptoms

	Total	**Non-painful**	**Painful**	**P value**
Number	41	30	11	-
Male: female (n:n)	11:30	8:22	3:8	1.00
Age (years)	50 (39-58)	49 (31-58)	51 (44-59)	0.47
Duration of RLS (years)	15 (6-28)	16 (7-29)	12 (4-31)	0.36
Age of RLS onset (years)	25 (18-38)	25 (18-36)	35 (16-50)	0.39
RLS severity	20 (16-25)	19 (15-25)	23 (16-28)	0.20
MPQ score	17 (11.5-25)	15.5 (11-22)	25 (15-27)	*0.04
Family history (%)	39	40	36.4	0.72

All data represented as median (interquartile range). P values represent comparisons between painful and non-painful groups using the Mann-Whitney test. Gender and family history comparisons using Fishers exact test.



Post hoc analysis for word choice comparing painful to non-painful sensations by Fishers exact test with correction for multiple comparison: *P=0.0006 nagging; **P=0.0003 cramping and painful

Figure 1: Word choices of RLS patients with painful or non-painful RLS sensations.

CHAPTER 4

Paper three: Kerr S, Bentley A, Anderson D and McKinon W (2011).

Reflex testing reveals circadian variation of spinal excitability in restless legs syndrome patients.

Sleep and Biological Rhythms 2011; 9: 157–164.

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Sleep and Biological Rhythms 2011; **9**: 157–164 doi:10.1111/j.1479-8425.2011.00501.x

ORIGINAL ARTICLE

Reflex testing reveals circadian variation of spinal excitability in restless legs syndrome patients

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Abstract

Restless legs syndrome (RLS) is a condition characterized by night-time exacerbation of symptoms, suggesting a possible circadian aetiology. It is hypothesised that RLS is caused by a central deficiency of dopamine causing spinal hyperexcitability. The study objective was to compare spinal reflex responses in RLS patients at two different times and with healthy participants. Standard electromyographic (EMG) techniques were used to quantify patellar and H-reflexes in RLS patients ($n = 11$) and matched control subjects ($n = 9$). Kinematic analysis was performed on patellar reflexes to measure knee angular velocity and displacement. Both reflexes were tested in the evening and the following morning. RLS patients had a significantly attenuated evening quadriceps EMG amplitude during patellar reflex testing compared to morning measurements ($p = 0.0078$) and compared to evening measurements in the control group ($p = 0.040$). Also, the RLS patients had significantly less knee angular displacement in the evening compared to morning measurements ($p = 0.018$). There were, however, no significant differences in any of the H-reflex measurements. Our data confirm a circadian variation in RLS aetiology but found no evidence of global spinal hyperexcitability as measured by patellar and H-reflex assessment. Differences in the findings from patellar and H-reflex assessments may indicate discrete (non-uniform) changes in the excitability of the spinal cord function amongst RLS patients.

Key words: H-reflex, kinematics, patellar reflex, restless legs.

INTRODUCTION

Restless legs syndrome (RLS) is a sleep-related movement disorder characterized by an urge to move the legs, often associated with uncomfortable or painful sensations. The urge to move results in voluntary leg movements to relieve the discomfort.[1] Symptoms are exacerbated in the evening, primarily during periods of inactivity, and improve in the morning.[2] RLS has been described as painful in 80% of RLS patients[3] and the sensations can be described using the McGill pain questionnaire.[4]

Garcia-Borreguero *et al.* (2006) extensively reviewed the epidemiological studies of RLS ranging from 1993 to 2005 and indicated that the most commonly reported prevalence is between 2.5 and 10% of the general Western population.[5] Non-Western population studies are limited and appear to indicate a very low prevalence of RLS (2–4%) in comparison to Caucasian based studies.[5,6]

Despite the finding that dopamine agonists effectively treat RLS symptoms, implying a central process, the

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Accepted 16 May 2011.

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aetiology of RLS remains unclear. RLS is thus defined by spontaneous sensations with an urge to move. When this urge is resisted, spontaneous limb movements may occur. This phenomenon is best illustrated by the suggested immobilisation test (SIT), whereby the immobilisation provokes symptoms of RLS[7]. The spinal cord, as the site for primary input of sensory afferents and final output of motor neurones to the legs, has been implicated as a central site where RLS dysfunction may occur.[8] The spontaneous sensations and limb movements typical of RLS could be explained by hyperexcitability of both the sensory and motor neurones within the spinal cord. Hyperexcitability in the spinal cord is not an isolated phenomenon: it affects motor, sensory and autonomic systems.

The spinal cord controls the structures coordinating spinal reflexes, and, as such, the state of spinal cord excitability may be assessed using the characteristics of the reflex responses.[9] The patellar reflex is one of the most commonly assessed reflexes, as it is an easily elicitable monosynaptic reflex that is an accurate, independent reflection of spinal excitability without the influence of other neuronal input. The patellar reflex is often considered as the mechanical counterpart of the H-reflex; however, the nature of stimulation of the latter reflex bypasses the muscle spindle, providing a measure of neuronal activity independent of the sensitivity of muscle spindle and potential changes within the muscle.[10]

The body of evidence supporting the argument for spinal hyperexcitability in RLS patients primarily comes from research on the H-reflex: impaired H-reflex excitability curves, vibratory inhibition depression (indicative of spinal disinhibition)[11,12] and decreased inhibition of 1b interneuron (neurones modulating spinal locomotor rhythm generators).[13] A study that tested the nociceptive flexor reflex, showed sleep-related disinhibition (hyperexcitability) of spinal cord reflex circuitry in patients with RLS and periodic limb movements (PLM) compared to healthy control subjects.[14] RLS patients also exhibited hyperalgesia in response to pinprick, indicating central sensitization.[15,16] The spontaneity of sensations may have a similar origin to neuropathic pain, also being generated by hyperexcitable sensory neurons. The evidence for this hyperexcitability is not, however, certain and most of these changes in spinal excitability were observed independently of time of day despite circadian variation being a diagnostic criterion.

Other measures of spinal cord excitability have not been evaluated and it is unclear whether the hyperexcitability proposed is of a global or more defined nature. Thus, the objective of this study was to use the patellar reflex in conjunction with the H-reflex to accurately determine whether there is a state of global spinal hyperexcitability in patients with RLS, and whether this state is subject to circadian variations. Electromyographic and kinematic techniques were used to determine differences between the muscle activation (EMG) and the actual movement (kinematics) of the reflex.

METHODS

Subjects

Volunteers were recruited by local advertisement and screened using a questionnaire including four diagnostic questions to diagnose RLS[17]. Control participants were included in the study if they answered the four questions negatively and reported no sleep or neurological disorders. Control participants were matched for gender and age. RLS patients completed the International Restless Legs Syndrome Study Group (IRLSSG) severity scale, documenting the perceived severity of their symptoms.[18] Subjects were asked to refrain from caffeine intake on the day of the study. Ethical clearance (clearance number M070452) was obtained from the local Ethics Committee and subjects were informed of all experimental procedures prior to signing a written informed consent form. All data from participants were coded in order to preserve participant anonymity.

H-reflex testing

All reflex testing was performed on the right leg and was stimulated and recorded using the Neuropak S1 (Nihon Kohden, MEB-9400 K, Japan). The H-reflex testing was performed by a single experienced clinical investigator between 17.30 and 18.30 and again between 08.00 and 09.30 the following day. Subjects lay in a relaxed, semi-reclined supine position. One surface recording electrode was placed over the peroneus longus muscle (approximately 14 cm distal to the proximal border of the popliteal fossa inferior to patellar border) and another 5 cm distal to the first one.

The peroneal nerve was electrically stimulated lateral and distal to the popliteal fossa with the cathode placed proximal to the anode. Stimulation was delivered at a rate of 1 Hz (1 ms duration). Intensity was increased in 5 mA increments from a sub-maximal value for the H-reflex to a super-maximal value for the M-wave.[19] Measurements of amplitude and latency for both the

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M-wave and H-reflex were obtained. For comparisons between subjects, the influence of stature was accounted for by dividing each subject's H-latency by height (corrected latency).

Patellar reflex testing

Patellar hammer construction

A free-standing axle-mounted patellar hammer was constructed for the study. In order to allow for remote activation of the device, a perspex extension with an attached electromagnet was fixed adjacent to the hammer. The hammer was released from the same height and position every time for each subject to ensure that its motion was reproducible.

Electromyography

For the patellar reflex, disposable silver chloride surface electrodes (Bluetrode ASF 40 × 40 mm) were attached to the quadriceps femoris muscle (active electrode 10 cm superior to patellar border, reference electrode 5 cm proximal to the active electrode) and grounding electrodes proximal to the lateral femoral epicondyle. Surface electrodes were attached to a multi-channel recording system and subjected to standard filtering and processing techniques (Cadwell Easy® Ambulatory 2, Version 2.0.2, Cadwell Laboratory Inc, Kennewick, WA). Data were exported into EDFbrowser 1.04 (Free software, Leiden University Medical Centre, Netherland) for further analysis.

Kinematics

Four digital cameras (Pixellink E-PLA741) were positioned at a height of 2.53 m at approximately 0°, 45°, 270° and 315° relative to the forward facing orientation of the subject. A removable image calibration frame (0.9 m^3), with twelve calibration points, was constructed around the patellar hammer. The calibration points were of known x (length), y (height) and z (depth) coordinates, providing reference values for measurements of the reflex. Before each experimental night the calibration frame was digitally recorded for 10 frames.

Reflective biomechanical markers (2 mm diameter) were attached to the right leg on the medial and lateral femoral epicondyle, the medial and lateral malleolus, the lateral mid-thigh and the head and foot of the patellar hammer. Digital recording of each reflex was simultaneously recorded at 50 frames s^{-1} from each camera and was digitally stored for subsequent kinematic analysis.

Image processing and analysis was performed using Matlab7 (Mathworks, Natick, USA). A custom Matlab centroid-based automated image thresholding and marker-tracking algorithm was used to identify marker centroids in each frame. Each marker was reconstructed in three dimensions using a modified direct linear transform algorithm[20] and reconstruction algorithm.[21] Raw marker location data was used to avoid measurement artifacts due to smoothing (the measurement frequency used was less than that of other systems).

Patellar reflex protocol

The patellar reflex testing was performed between 18.30 and 19.30 after H-reflex testing and again the following morning between 07.00 and 08.00 before H-reflex testing. Subjects were seated in an upright position with a back support (standardized body position) with their lower legs hanging freely. The optimal site for eliciting the patellar reflex over the patellar tendon was determined using a conventional clinical reflex hammer. This spot was marked and used for subsequent trials.

The electromagnetic release of the patellar hammer, EMG recordings and video capture was operated from a position outside the view of the cameras. Five repeated measures with a minimum inter-stimulus interval of 30 s were recorded in the evening and the following morning.

Three-dimensional kinematic reconstruction of the patellar reflex began with construction of a plane defined by the lateral mid-thigh marker and the two knee markers (thigh plane). The leg shank axis was constructed as the line joining the midpoint of a line drawn between the knee markers and a similar line between the ankle markers (Fig. 1). Knee angular displacement was measured as the maximum angle between the thigh plane and the leg shank axis (in degrees) after the patellar reflex. Knee angular velocity was determined as the change in knee angular displacement over time (degrees s^{-1}).

Measurements obtained for the patellar reflex experiments were: amplitude of the quadriceps muscle activity (EMG); maximum knee angular displacement; and maximum knee angular velocity (kinematic analysis).

Data analysis

Parameters of the patellar reflex data from each trial for each subject were averaged. Data obtained for the reflex

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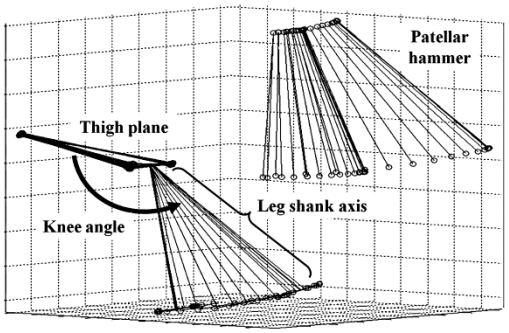

Figure 1 Three-dimensional representation of the patellar reflex following the patellar hammer strike in one of the control subjects. Each successive line depicts the position of the patellar hammer and leg shank at 20 ms intervals. Circles indicate the marker positions.

analyses in this study are treated as non-parametric since inspection of the data did not suggest they were normally distributed. The only exception was that ages of the two groups were compared using an unpaired Student's *t*-test. For each of the H- and patellar reflex parameters, morning results were compared to evening results using the Wilcoxon matched pairs signed rank test. Comparisons between the RLS and control groups at each time point were analyzed using the Mann-Whitney test. Data are represented as median and interquartile range (unless otherwise stated).

RESULTS

Subjects

Eleven RLS patients and nine healthy age- and gender-matched control subjects were used in the study (Table 1). All subjects were right-hand dominant and had no evidence of neurological disorders. The mean duration of RLS symptoms was 18.5 ± 10.7 years and mean severity on the IRLSSG severity scale was 20.9 ± 4.33 (RLS group). Five of the RLS patients had a family history of RLS. Nine of the eleven RLS patients were treatment-naïve, and the two that were not stopped their RLS medication (Pexola and Sinemet) a week before the night of the study.

Electromyographic analysis

H-reflex

The control and the RLS group were not significantly different for any morning or evening H-reflex parameter (latency, amplitude and H/M ratio) (Table 2). There was also no circadian variation in either the RLS or control group in any of the H-reflex parameters.

Table 1 Characteristics of RLS patients and control subjects

	RLS patients	Control subjects
Number	11	9
Male : female (*n*)	4:7	3:6
Age (years)	48.1 ± 10.9	46.8 ± 12.6
RLS (*n*)	11	0
Duration of RLS (years)	18.5 ± 10.7	NA
Family history (%)	45	NA
Age of RLS onset (years)	30.2 ± 13.8	NA
RLS severity	20.9 ± 4.33	NA
Right hand dominance (%)	100	100
Height (m)	1.7 ± 0.1	1.6 ± 0.1

[Mean (± SD)]. *NA = not applicable to control subjects.*

Patellar reflex

In the RLS patient group the quadriceps EMG amplitude in the evening was significantly lower when compared to the morning measurement ($p = 0.0078$) and the control group evening measurement ($p = 0.040$). No other comparisons were significantly different (Fig. 2).

Kinematic analysis

Kinematic analysis of the patellar reflex was performed on a subset of the subjects as technical issues prevented data being collected for all subjects (for kinematic reconstruction each marker for each subject needed to be visible from at least two cameras at all times). The subset sample size in each group for knee angular displacement was: RLS ($n = 7$) and control (AM: $n = 6$; PM: $n = 5$); and for knee angular velocity was: RLS (AM: $n = 5$; PM: $n = 6$) and control (AM: $n = 6$; PM: $n = 5$).

Knee angular displacement of the patellar reflex was significantly smaller in the RLS group than in the control group in the evening, but not the morning (Table 3). Knee angular velocity was not significantly different between the RLS and control subjects or between times of the day in the RLS group (Table 3). Comparison between morning and evening was not done for knee angular velocity in the control group, as there was insufficient paired data to be statistically relevant.

DISCUSSION

Neurophysiological and kinematic analysis in our RLS patients revealed that the evening quadriceps EMG

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Table 2 H–reflex parameters for restless legs syndrome patients (RLS, $n = 10$) and control (CONT, $n = 7$) subjects

Parameter	Time	RLS	CONT	p value
Latency (ms)	Morning	35.00 (30.35–36.55)	33.68 (31.43–39.23)	NA
	Evening	33.28 (31.40–36.95)	31.40 (30.15–36.85)	NA
Corrected latency (ms/m)	Morning	20.20 (18.86–23.05)	20.44 (19.97–21.50)	0.89
	Evening	20.15 (17.89–22.33)	19.62 (19.03–22.47)	1.00
Amplitude (mV)	Morning	0.13 (0.09–0.21)	0.14 (0.07–0.24)	0.96
	Evening	0.15 (0.08–0.29)	0.12 (0.07–0.20)	0.67
H/M ratio	Morning	5.95 (3.80–11.55)	8.20 (1.80–18.50)	0.42
	Evening	10.20 (4.40–15.50)	10.00 (3.80–19.20)	0.84

Comparisons between RLS and control participants were conducted using a Mann-Whitney test. Morning versus evening comparisons conducted using a Wilcoxon signed rank test were insignificant for $p < 0.05$. Data represented as medians and interquartile ranges. NA = analysis not applicable.

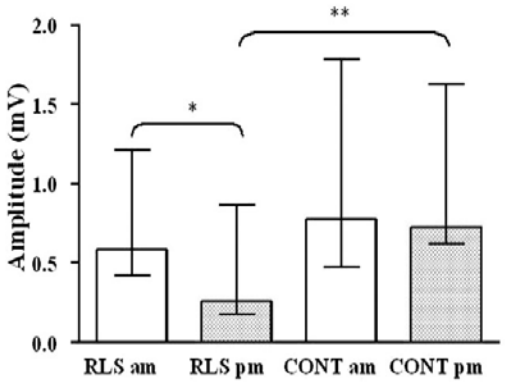

Figure 2 Morning (AM) and evening (PM) patellar reflex EMG amplitudes. Restless legs syndrome (RLS, $n = 9$) and control (CONT, $n = 9$) subjects. *$p = 0.0078$ (RLS AM vs. RLS PM, Wilcoxon signed rank test), **$p = 0.040$ (RLS PM vs. CONT PM, Mann-Whitney test). Data represented as median and interquartile range.

amplitude and knee angular displacement from the patellar reflex were smaller than in the control group as well as smaller than the morning values. Knee angular velocity in the patellar reflex and all of the H-reflex parameters (latency, amplitude and H/M ratio) did not differ between the groups or different times of the day. Our data suggests that RLS patients exhibit spinal hypoexcitability where patellar reflexes are concerned but not for the H-reflex data since no difference in spinal excitability between the two groups was found. Our data thus do not support the hypothesis that RLS patients exhibit spinal hyperexcitability in comparison to healthy control subjects.

Although our sample size (11 RLS, 9 control) lies within a similar range (5–24 subjects) as previous studies looking at reflex testing in such a group of patients (RLS subjects),[11–13,22–24] our inability to find more pronounced changes in circadian variation or intergroup differences may be related to insufficient sample size. Also, patients were not recruited from hospital populations and this may account for differences in the results from other studies. Patients who have sought out medical treatment for their RLS may have a greater severity than those who do not feel the problem warrants medical advice. The patients in our sample were primarily treatment-naive and thus did not suffer from effects of residual medication. It is also possible that the tests used, H-reflex and patellar reflex, were not sufficiently sensitive to detect subtle circadian variations. An important constraining factor in this study was the timing of the reflex tests. Had the H- and patellar reflex testing been done later in the evening during the worst phase of RLS symptoms, our results may have been more pronounced. However, according to the John Hopkins Restless Legs Severity Scale (JHRLSS) symptom onset in patients with a moderate severity of RLS is

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Table 3 Comparison of biomechanical characteristics of the patellar reflex between patients with restless legs syndrome (RLS) and controls (CONT)

Parameter	Time	RLS	CONT	*p* value
Knee angular displacement (degrees)	Morning	137.0 (117.7–144.7)	149.5 (133.3–161.7)	0.101
	Evening	138.4 (132.9–146.2)	150.5 (138.6–158.9)	*0.018
Knee angular velocity (degrees/s)	Morning	183.1 (130.7–221.2)	181.4 (160.7–191.5)	0.178
	Evening	235.0 (164.6–355.3)	236.6 (178.4–354.2)	0.114

Comparisons between RLS and control participants were conducted using a Mann-Whitney test. Morning versus evening comparisons conducted using Wilcoxon signed rank test were insignificant for $p < 0.05$. Data represented as median and interquartile range. *$p < 0.05$

around 18.00.[25] The patients in this study had a moderate severity of RLS (rated on the IRLSSG severity scale) and thus were likely to have been symptomatic during the experimental procedure. The H-reflex was stimulated at an unconventional site due to technical problems in eliciting the tibial nerve soleus H-reflex in the first couple of patients, and thus was shifted to the peroneal nerve. Both these nerves traverse the spinal cord at the level of the sacrum and therefore should reflect the same extent of excitability.

This is the first study to look at changes in the patellar reflex in patients with RLS. The results from the patellar reflex testing in the present study would suggest that, if hyperexcitability existed in RLS patients, it is unlikely to be a global spinal phenomenon. To date, research has either indicated spinal hyperexcitability or no change in the state of spinal excitability in RLS patients as compared to controls. By using the patellar reflex, which has not been examined before in RLS patients, the discovery that this mono-synaptic reflex exhibits hypoexcitability when compared to controls is novel.

The lack of changes in the H-reflex parameters studied here (H-latency, H-amplitude and H/M ratio) are consistent with other studies which also found no differences between RLS and control subjects for either H-latency;[13,23,24] H-amplitude[13] or H/M ratio.[12,13,23,24] RLS and PLM patients also exhibited no differences compared to controls in motor and sensory nerve conduction, F-wave, blink reflex and mixed nerve silent periods.[23] Other studies, using the H-reflex, have shown spinal hyperexcitability in patients with RLS[11–13] but these studies used different parameters of the H-reflex. Thus, in agreement with existing data and for the parameters that we selected, neurophysiological testing using the H-reflex does not reveal changes in spinal excitability in RLS patients.

The different results in the two reflexes may be partly explained by the anatomy of the spine. The H-reflex is more complex than the monosynaptic patellar reflex, as the circuit also contains spinal interneurones. The afferent and efferent neurones innervating both these lower limb reflexes are expected to share similar properties and thus the differences observed between these two reflexes may lie within this inter-neuronal pool. If there is a state of spinal hyperexcitability in patients with RLS, the data presented here imply that the hyperexcitability is not necessarily restricted to excitatory neurones and possibly includes inhibitory neurones as well.

A secondary hypothesis was made, that a circadian variability in RLS symptoms would be matched by the reflex parameters measured. Thus an increase in the RLS symptoms in the evening would be matched by hyperreflexia. This hypothesis is also not supported by the data, which showed a 45% decrease in the evening amplitude of the quadriceps EMG and a smaller evening knee angular displacement in these patients compared to morning values.

The normal circadian variation of reflexes in healthy subjects depends on the reflex measured. Increased evening reflex amplitudes have been reported in the soleus muscle H-reflex[26] and the spinal stretch reflex in primates.[27] Conversely, in humans, increasing thresholds (reflecting reduced reflexes for the same stimulus) for the flexor reflex throughout the day (from 06.00 to 00.00) have been reported.[28] However, no circadian variation was demonstrated in the flexor capri muscle H-reflex,[26] the soleus muscle H-reflex,[29] the Achilles tendon reflex[30] or the patellar reflex.[31] Thus, while inconclusive, and dependent on muscle and reflex type, the state of the spinal cord has been shown to become both more and less excitable towards the evening. The lack of circadian fluctuation in the H-reflex and patellar

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reflex in the literature concurs with the control group in the current study.

Very few studies have examined the circadian variation in spinal reflexes in patients with RLS and few neurophysiology studies done on patients with RLS have taken time of day into account. Two studies, focusing on similar H-reflex parameters to this study, looked at RLS patients in the late afternoon[12] and during a symptom-free period,[23] and also found no circadian changes.

We found that the patellar reflex may reflect spinal hypoexcitability in the evening compared to the morning. If there are no changes in the neuronal component properties of the patellar tendon, which are known to exhibit diurnal changes, this may provide the answer. However, patellar tendon stiffness decreases from morning to evening making the tendon more compliant in the evening[32] likely leading to an increased reflex in the evening. Although, if the cause of patellar hyporeflexia in the RLS patients was due to changing properties of the tendon, then the same variation would be expected in the control group. Alternatively, changes in muscle tone could account for the circadian variation observed. Increased evening tibial muscle tone in RLS patients compared to decreased muscle tone in control participants was reported by Veldi *et al*. (2008) suggesting that the muscle relaxation time is too short.[33] This echoes patients' descriptions of their RLS sensations as an inability to relax the muscle.

In summary, the results of this study suggest that the symptoms of RLS do not result from global spinal hyperexcitability. The finding of hyporeflexia in patients with RLS suggests an abnormality more complex than that currently described in the literature. These results do show that RLS patients display diurnal fluctuations in spinal reflexes, supporting the circadian changes in RLS. Further research into more varied and specific spinal pathways is required to explain these findings.

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CHAPTER 5

Paper four: Kerr S, Bentley A and McKinon W.

The relationship between sensory and motor components of Restless Legs Syndrome during the Suggested Immobilization Test

Submitted to the *International Journal of Neuroscience*

The relationship between sensory and motor components of Restless Legs Syndrome during the Suggested Immobilization Test

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Running title: Sensory and motor features of RLS

ABSTRACT

The Suggested Immobilization Test (SIT) was developed as an objective, diagnostic tool to quantify motor restlessness (periodic limb movements, PLM) associated with Restless Legs Syndrome (RLS). There has been limited research exploring the relationship between discomfort, pain and motor activity associated with RLS during the SIT. Sixteen RLS patients and eight control participants completed the International Restless Legs Syndrome Study Group Severity Scale (IRLS) and the SIT with bilateral recordings of tibialis anterior EMG (SIT PLM index), as well as pain and discomfort visual analogue scales every five minutes for the 60 minute duration of the SIT. The mean discomfort score had the ability to discriminate between RLS and control participants (P=0.003) however the PLM index did not (P=0.752). RLS patients were then split according to the presence or absence of PLM and according to the IRLS severity scores. Both RLS groups had similar levels of discomfort which were greater than the controls however the RLS patients with PLM had higher pain scores (P<0.01, Friedman test) than both the RLS without PLM and controls. Patients with severe RLS had significantly higher discomfort and pain scores than patients with mild RLS. Despite rating significant levels of discomfort, the majority of the RLS patients did not exhibit PLM possibly suggesting a disconnect between the sensory and motor components of RLS. This suggests that the SIT is a good objective diagnostic measure only in a specific group of patients with RLS.

Keywords: Pain, discomfort, periodic limb movements, severity

INTRODUCTION

Restless legs syndrome (RLS) is a sleep related movement disorder characterised by an urge to move often in response to uncomfortable or painful sensations experienced in the legs. Literature indicates that the need to move is more useful diagnostically than the "uncomfortable and unpleasant" sensations, which may or may not accompany the urge to move [1]. The link between the sensations and the motor component is further confirmed by relief after voluntary movement [2] and the exacerbation of symptoms during periods of inactivity or sleep [3]. One way of assessing this link is by using the Suggested Immobilization Test (SIT). The SIT provokes RLS sensory symptoms (as per the diagnostic criteria) as well as involuntary movements known as Periodic Limb Movements (PLM) [4]. To date, the only sensation measured during the SIT has been discomfort [5-7]. RLS symptoms have also been described as painful in between 40 and 80% of RLS patients [8]. The success of opioid treatment, the ability of patients to measure RLS symptoms on a scale for pain [9] and that RLS patients have also been shown to have amplified nociceptive processing and increased pain sensitivity [10,11] implicate the pain pathways in the pathophysiology of the sensory symptoms of RLS [9,12].

Very little research has been published on the relationship between the sensory and motor components of RLS and PLM during the SIT and that which is available shows contradictory results. Therefore, the objective of this study is to explore the relationship between sensory symptoms, including pain and discomfort, and motor activity during the SIT.

METHODS

Screening and participant selection

Participants with RLS were recruited on a voluntary basis by local advertisements. They completed a questionnaire with the four essential RLS diagnostic criteria questions as defined by the International Restless Legs Syndrome Study Group (IRLSSG) [13]. To confirm the diagnosis of RLS subjects had to answer all four RLS diagnostic questions in the affirmative to be included in the RLS group. They provided basic demographic data and excluded, on history, any known secondary causes of RLS or evidence of other sleep, pain or neurological disorders. No physical examination of the patients was conducted. Control participants answered the four RLS diagnostic questions in the negative, were clear of other sleep disorders and could be age and gender matched to the RLS participants. The RLS patients then completed the International Restless Legs (severity) Scale (IRLS). Each participant was questioned regarding their family history of RLS and a positive family history was recorded if the participant could accurately recall a family member reporting RLS fulfilling all four of the diagnostic criteria. Participants were asked to refrain from caffeine intake on the day of the study. Ethical clearance (clearance number M070452) was obtained from the local Ethics Committee and participants signed a written informed consent form. All data from participants were coded in order to preserve participant anonymity.

Suggested Immobilization Test

RLS patients and control participants completed the same Suggested Immobilization Test (SIT) regime for an hour in the evening starting between 20:00 and 21:30pm [4]. The participants sat upright (with a back support) with their legs outstretched resting on the bed. Silver chloride

surface electrodes (Bluetrode ASF 40x40mm) were attached bi-laterally to the tibialis anterior (active electrode 10cm inferior to patellar border, reference electrode 5cm distal to that) and ground electrodes attached proximal to the femoral epicondyle. Participants were asked to try and limit their voluntary movements as much as possible for the duration of the test (but could move if they needed to) and were monitored to ensure they did not fall asleep. Periodic limb movements (PLM) were scored according to the criteria established by Michaud [14] and the SIT PLM index was calculated for the hour of immobility. No concomitant or overnight EEG recordings were taken.

Visual Analogue rating Scales (VAS)

Sensory symptoms were assessed every five minutes for the hour long duration of the SIT using two Visual Analogue Scales of 100mm each; one for pain and the other for discomfort. The anchors for the pain VAS were 'no pain' and 'most severe pain' and the anchors for the discomfort scale were 'no discomfort' and 'most severe discomfort'. Participants were asked to make a mark on the line between the two anchors which corresponded to their perceived level of pain or discomfort and to differentiate feelings of pain from discomfort. The length of the line segment from the no pain or no discomfort anchor to the mark was measured in mm and recorded as the pain or discomfort score. The mean pain and discomfort scores were calculated as the average value of the respective scores recorded every 5 minutes.

Data analysis

All the data was treated as non parametric and is presented as median and interquartile range (unless otherwise stated). Groups were compared using either Mann Whitney or Kruskal-Wallis

with a Dunn's multiple comparison post hoc test. The VAS scores were plotted over the hour and compared using the Friedman's test with Dunn's multiple comparison posthoc test. Correlations between variables were done using Spearman's rank correlations.

RESULTS

Participant demographics

Sixteen RLS patients, fulfilling all four of the essential RLS diagnostic criteria, and eight healthy age matched control participants were included in this study (Table 1). Thirteen of the sixteen RLS patients were treatment-naïve, one had discontinued dopaminergic treatment (Pramipexole) due to adverse side effects months before the study and two stopped their RLS medication (Pramipexole and Levodopa) a week before the night of the study. The RLS patients had a typical gender split and a wide range of RLS severities, duration of symptoms and age of onset. There were no significant differences between these variables.

During the SIT, three RLS patients had PLMs (index 42 ± 16), while the other thirteen RLS patients did not display PLMs. Seven of the eight control participants did not exhibit PLM. Subsequently, the RLS patients were split according to the presence (RLS with PLM) or absence (RLS without PLM) of PLM. Table 2 presents the characteristics of the RLS patients split according the presence or absence of PLM. The RLS with PLM patients had a longer duration of RLS ($P=0.036$) and higher PLM index ($P<0.01$) compared to the RLS without PLM patients. There

were no significant differences in any other demographic variables between the RLS patients with PLM compared to those without PLM.

The RLS patients were also divided according to the IRLS severity scores. Patients were assigned to the mild RLS group if they scored 20 or less and the severe group if they scored over 20. Six patients were included in the severe RLS group and had significantly greater RLS severity than the ten patients with mild RLS (P=0.0002). There were no other significant differences between the two groups (Table 2).

PLM index

There was no significant difference between the PLM index of the total RLS group and control participants (P=0.752, Mann Whitney test) or between the RLS patients without PLM and control participants (P >0.05). The PLM index of the RLS patients with PLM was significantly greater than the PLM index of both RLS patients without PLM (P < 0.01) and control participants (P < 0.001). The PLM index was not significantly different between the mild and severe RLS groups (P=0.4923).

Discomfort and pain curves

The RLS patients as a group compared to the control subjects had significantly greater discomfort but not greater pain scores during the SIT (P=0.003 and P=0.131 respectively, Friedman test, Dunn's Multiple Comparison posthoc test). When the RLS group was divided according to the presence or absence of PLM the level of discomfort was not significantly

different from each other but both groups experienced significantly more discomfort than the control participants (Figure 1A).

The RLS patients with PLM had significantly greater pain scores than the control participants (Figure 1B) and the RLS patients without PLM experienced significantly less pain than the RLS patients with PLM. There was no significant difference between the pain scores of the RLS patients without PLMs and the control participants (Figure 1B).

The severe RLS group had significantly greater discomfort and pain scores than both the mild RLS group and the control participants (Figure 2 A and B). The mild RLS group experienced significantly greater discomfort but not pain compared to the control participants (Figure 2 A and B).

In the RLS patients with PLM, there is a rapid increase in the discomfort score after 35 minutes and the pain score from 40 minutes. In the severe RLS group, the discomfort score progressively increases but this same pattern of evolution is not seen in the mild RLS group or in the pain scores for either group.

Correlations

Correlations between subjective measurements and motor activity were performed on the combined group of RLS patients only due to the small numbers in the RLS with PLM group. There was a significant relationship between the mean discomfort and mean pain scores for

RLS patients(r= 0.6407, P= 0.0075) as well as between the mean discomfort and RLS severity score (r= 0.5335, P= 0.0333). There was no correlation between the RLS severity score and the mean pain score (r= 0.3832, P= 0.1429). Motor activity (PLM index) could not be used in correlation analysis due to the lack of PLM in the majority of subjects.

DISCUSSION

In this cohort of RLS patients, the majority of the patients had no PLM during the SIT despite rating increasing levels of RLS discomfort. Subsequently the RLS patients were divided according to the presence or absence of PLM. The RLS patients without PLM were distinguished from the RLS patients with PLM by significantly lower pain scores and a significantly shorter duration of RLS symptoms. Patients with significantly greater severity of RLS also scored significantly higher discomfort and pain scores compared to patients with mild RLS and the severity score correlated to the mean discomfort score but not the mean pain scores. There was, however, a positive correlation between pain and discomfort in all RLS patients during the SIT. To our knowledge, this is the first study to have included a VAS pain scale to record pain symptoms during the SIT.

The main and obvious caveat of this study is that of the extremely small sample size of the RLS patients with PLM and the wide interquartile range of the results. We are therefore cautious not to draw conclusions based on this small sample. Our results may differ from those observed in other SIT studies based on differences in the population dynamics. On average, the RLS participants in this study appear to be younger, with a relatively early onset of RLS

resulting in a longer RLS duration than reported in other studies. Of the other studies that reported severity ratings, the severity of RLS in our participants is on a par with these studies.

There is controversy regarding patients who have RLS symptoms without PLM. In some studies the PLM index has been shown to discriminate RLS patients from control participants [5,6,15]. The presence of PLM in the diagnosis of RLS is considered essential by some authors, but other studies confirm our data by showing that not all RLS patients have evident PLM [2,4,15]. The PLM index criteria for distinguishing between RLS and control subjects also do not seem to be well defined and ranges from 40 PLM/hour [4] to 12 PLM/hour [16] to differentiate between RLS subjects and healthy controls. Discrepancy between the cut off points may be because of the heterogeneity of RLS patient groups and the unpredictable appearance of PLM during the SIT as seen in our group of participants. A lot more research is required to clarify the PLM criteria during the SIT in patients with RLS.

Regarding the presence of PLM to diagnose RLS should not be supported as PLM are a common but non-essential, supportive RLS diagnostic feature whereas the sensory component (i.e. discomfort) is an essential part of the diagnostic criteria. The discomfort scores in our study better distinguished the RLS group from control subjects compared to the PLM index, similar to a previous study [5]. There is evidence that the number of PLM plateau after 35 minutes (at approximately 10 PLM/5 minute interval) of the SIT whereas the sensory symptoms continued to increase, implying a dissociation between the sensory and motor events [6,17]. We could not test this in our patient sample due to insufficient numbers of PLM. The discomfort score

which distinguished all RLS patients (with and without PLM) from controls may therefore be a more useful measure than the PLM index in diagnosing RLS.

The disconnect between the discomfort of RLS and PLM during the SIT in our study does not seem to occur with pain and PLM, as pain increased with increasing PLM numbers for the RLS patients with PLM. While one study did show increasing discomfort and increasing PLM numbers throughout the SIT, without any correlation between these two variables [6], the same group also showed that there is a correlation between the mean SIT discomfort and PLM [5]. The authors speculate that the discrepancy between the discomfort and PLM may be due to patients not voluntarily moving in response to discomfort (as per the SIT instructions), indicating a possible level of voluntary control over PLM. These studies lacked any measurement of pain during the SIT and did not differentiate between pain and discomfort.

Our participants with RLS but without apparent PLMs had a significantly shorter duration of RLS (11.5 years (range 2-30)) than those with PLM group (32 years (range 20-32)). It is possible that in our population PLM and pain become more common features as the disorder progressed. In contrast, two other articles focusing on the SIT where patients with RLS had PLM much shorter durations of RLS symptoms were reported of 4.9 ± 4.1 years [7], 2007) and 9.1 ± 2.1 years [18]. The relationship between PLM, pain and duration of RLS symptoms deserves further exploration.

There appear to be complex relationships between the sensory and motor features and measures of severity in RLS. We showed a correlation between the level of discomfort and the

severity which has been noted in one other study [7]. Despite the correlation between the mean pain and discomfort scores the mean pain score did not correlate with the severity in this study as shown in previous studies[9]. The lack of relationship between pain and severity may be because while patients can experience severe discomfort which scores highly on the severity scale not all RLS patients experience painful symptoms. The differences could be due to the severity scale not reflecting the intensity of the actual sensory symptoms but rather a measure of their impact and not accounting for painful RLS. The presence of pain, as an additional sensory symptom, may not exacerbate the impact of the severe discomfort of RLS. However, the patients with severe RLS reported significantly greater pain during the SIT than both the patients with mild RLS and the control participants. These differences in pain were most apparent in the latter part of the SIT (Figure 2B) and thus comparisons at different time points could be more sensitive than using the mean pain score.

These conflicting relationships are possibly the result of differences in underlying pathophysiology for RLS and further support the proposal that the presence or absence of PLM in patients with RLS may indicate two different phenotypes. The RLS patients with PLM seem to have a different demographic profile particularly regarding the gender split and family history of RLS to the RLS patients without PLM. It is important that these under-investigated intricacies, relating RLS to PLM and pain, are explored further to develop a fuller understanding of the aetiology of RLS.

In conclusion, these results suggest that the motor component of the SIT is not a good, stand alone, objective measure for RLS as not all RLS patients who fulfil the diagnostic criteria for

moderate RLS exhibit PLM on the SIT. The possibility of different phenotypes in RLS related to PLM and pain should be explored.

Acknowledgements

Funding provided by the Wits Dial.a.Bed sleep laboratory and University of the Witwatersrand Medical Faculty Research Endowment fund. **Declaration of interest**: The authors have nothing to disclose related to the content of this manuscript.

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Table 1: Characteristics of Restless Legs Syndrome (RLS) patients and healthy control participants. Data are represented as median (range).

	Control	**RLS patients**
Sample size	8	16
Male: female (n:n)	4:4	5:11
Age (years)	51 (25-57)	45 (24-59)
Duration of RLS (years)	n.a.	15 (2-32)
Age of RLS onset (years)	n.a.	25 (12-56)
RLS severity	n.a.	19.5 (6-29)
Family history (%)	0	44
Treatment (n)	n.a.	3
SIT PLM index	0 (0-7)	0 (0-59)

SIT, Suggested Immobilization Test. PLM, Periodic Limb Movements.

Table 2: Characteristics of Restless Legs Syndrome (RLS) patients with and without Periodic Limb Movements (PLM) and mild and severe RLS. Data are represented as median (range).

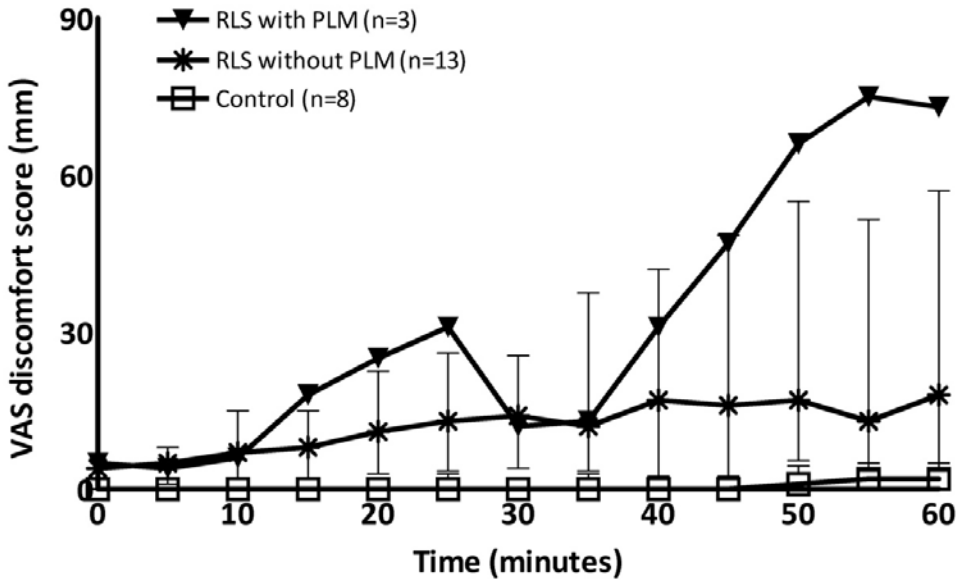
	RLS patients			
	with PLM	Without PLM	Mild	Severe
Sample size	3	13	10	6
Male: female (n:n)	2:1	3:10	2:4	3:7
Age (years)	55 (53-56)	40 (24-59)	39.5 (24-58)	54.5 (30-56)
Duration of RLS (years)	32 (20-32)	11.5 (2-30)*	12.5 (2-33)	18.5 (5-32)
Age of RLS onset (years)	25 (12-56)	23 (21-36)	20.5 (12-56)	35 (21-45)
RLS severity	23 (15-29)	19 (6-24)	14.5 (6-20)	22.5 (21-29)**
Family history (%)	66	38	50	33
Treatment (n)	1	2	2	1
SIT PLM index	39 (27-59)	0*	0 (0-39)	0 (0-59)

SIT, Suggested Immobilization Test. PLM, Periodic Limb Movements.

* P<0.05 compared to RLS with PLM

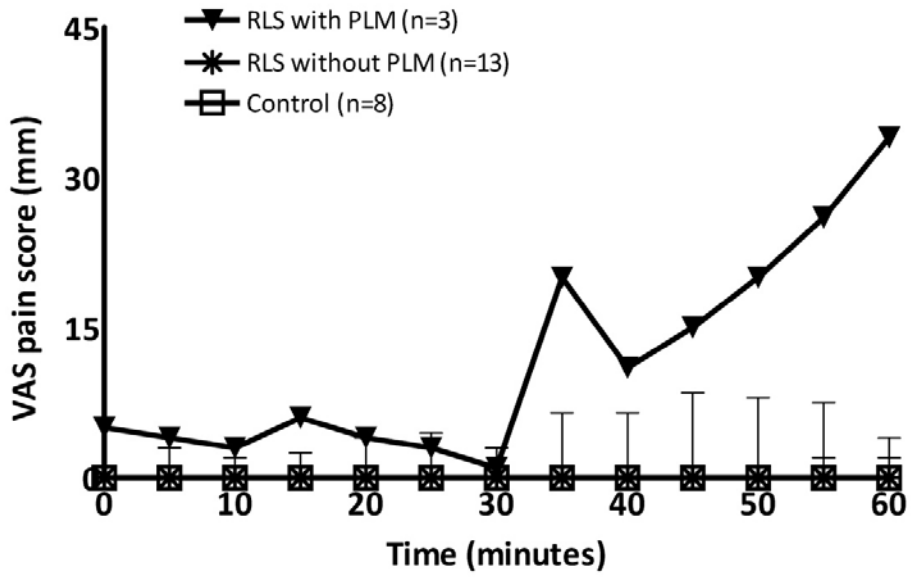
** P=0.0002 compared to Mild RLS

A.



RLS without PLM vs. control (P<0.050); RLS with PLM vs. control (P<0.001). Friedman test, Dunn's Multiple Comparison posthoc test.

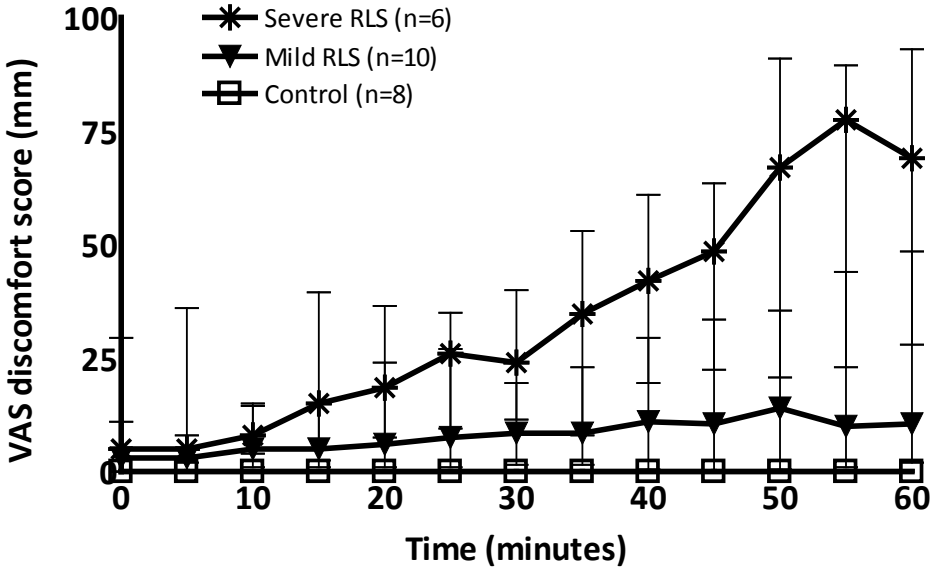
B.



RLS with PLM vs. RLS without PLM (P<0.010); RLS without PLM vs. control (P<0.001), Friedman test, Dunn's Multiple Comparison posthoc test.

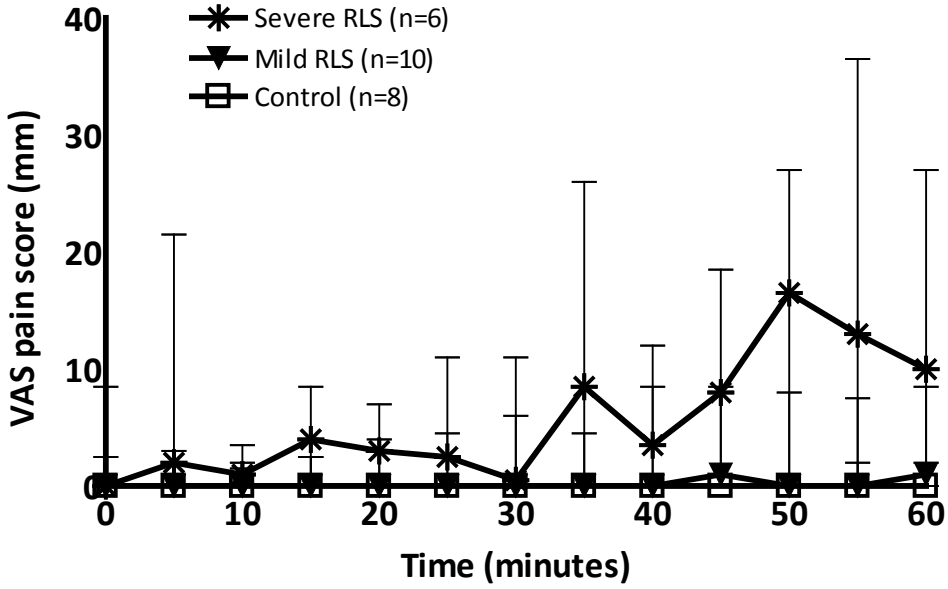
Figure 1: The median (interquartile range) discomfort scores (A) and pain scores (B) recorded on a 100mm Visual Analogue Scale (VAS) every five minutes during the Suggested Immobilization Test for Restless Legs Syndrome (RLS) patients with and without Periodic Limb Movements (PLM) and healthy control participants. Error bars are absent from the RLS with PLM due to the small sample size of this group.

A.



P<0.05 mild RLS vs. severe RLS and mild RLS vs. control; P<0.001 severe RLS vs. control, Friedman test, Dunn's Multiple Comparison posthoc test.

B.



P<0.05 mild RLS vs. severe RLS; P<0.001 severe RLS vs. control, Friedman test, Dunn's Multiple Comparison posthoc test.

Figure 2: The median (interquartile range) discomfort scores (A) and pain scores (B) recorded using a Visual Analogue Scale (VAS) every five minutes during the Suggested Immobilization Test for Restless Legs Syndrome (RLS) patients with mild and severe RLS and healthy control participants.

CHAPTER 6

CONCLUSIONS

Restless Legs Syndrome (RLS) and Periodic Limb Movements (PLM) are well documented sleep related movement disorders with established diagnostic criteria. These common disorders affect a noteworthy proportion of the general population however the prevalence data may be confounded by various other conditions known to 'mimic' RLS and the descriptors used for RLS have not been formally characterized which could lead to inaccuracies in the diagnosis. Some reports indicate that the symptoms of RLS and PLM can have devastating consequences on the quality of life and wellbeing of sufferers. Although effective treatment is available, we still do not understand the mechanisms behind the signs and symptoms. Current treatments, however, are not always effective in all patients and there is also a great risk of symptom augmentation. To fully understand these disorders, and therefore provide a platform for even more effective treatments, there is a need for further research possibly employing new assessment tools to fill in the knowledge gaps and to provide tools for the improved diagnosis and monitoring of these conditions. Therefore, in this thesis I have investigated some aspects of the assessment of sensory and motor features of RLS and PLM with the overall objective of contributing to the understanding of their aetiology and improving the accuracy of diagnosis. Thus, the primary aims of this thesis were: to describe and characterize RLS sensations both as a tool to determine the aetiology and for diagnostic purposes; to objectively quantify two spinal reflexes as a reflection of spinal excitability in RLS patients and assess the circadian variations thereof, and lastly to focus on the Suggested Immobilization Test (SIT), a means of simultaneously assessing both the sensory and motor components of RLS and PLM, facilitating investigation of the relationship between them and possibly providing clues to their aetiology.

6.1. Sensory assessment - Descriptors of RLS sensations

RLS is diagnosed based on the patient's subjective, self-reported symptoms. However, many patients experience difficulty in expressing the nature of their sensations and as such they could be misdiagnosed if a clinician is primed to relate to only those descriptors included in the diagnostic criteria. Therefore, my first study investigated the word choice of 41 English speaking South African RLS patients who spontaneously provided descriptions of their RLS sensations, selected terms from pre-determined lists of RLS descriptors derived from the literature and then selected terms from the McGill Pain Questionnaire. The patients struggled to spontaneously describe their sensations, often resorting to bizarre similes such as "like the feeling you get at the onset of a sneeze, only in your legs" to portray the feeling. Patients were able to provide a much larger sample of descriptors when selecting from a large selection of terms previously generated and reported by RLS sufferers. While the "unassisted" spontaneous descriptors given by patients may be the most accurate descriptors of the sensations they are experiencing, patients that struggle to communicate about their sensations may benefit from a prompted list of words (Williams *et al.*, 2000; McDonald & Weiskopf, 2001). Thus, suspected RLS patients may be able to communicate more effectively with their health care professional if they are provided with an appropriate list of terms known to be associated with RLS as identified by a substantial number of patients with confirmed RLS.

Further highlighting the difficulty in precisely describing RLS sensations is that the RLS patients chose different words spontaneously, when prompted and from the MPQ. The MPQ, which contains only pain related words, was included in the study based on the results of Bentley et al (2007) who found that the sensory symptoms of RLS could be assessed using a qualitative

pain questionnaire. The relationship between pain and RLS was not confirmed by my first study. Only two words, 'tingling' and 'nagging' were found in common between those selected from the MPQ, those spontaneously offered and those selected from the established list. However, in the follow on study (paper two), there were clear differences in the choice of words between RLS patients with painful and non-painful sensations. The words "cramping" and "painful" were favoured by the patients with painful RLS. In a clinical setting, a patient complaining of cramping and painful sensations in their legs may be diagnosed with a condition that mimics RLS such as leg cramps or peripheral neuropathy, resulting in a marked under-diagnosis of RLS (Hening *et al.*, 2004b). Coupling these words (painful and cramping) with the other commonly selected word 'restless', may assist in determining the RLS diagnosis. These results are consistent with Ekbom's original proposal that there may be two main forms of RLS, one characterized by painful symptoms and the other by non-painful parasethesia (Ekbom, 1945).

Alternatively, the RLS prevalence statistics could be overestimated as the diagnostic criteria for RLS, as they stand, are unable to exclude conditions that mimic RLS (Hening *et al.*, 2009). There is a clear need for improved assessment of RLS and conditions that mimic this condition in order to provide better differential diagnoses. The key may lie in the diagnostic descriptors and differences in the word choices defined by each condition which could potentially aid in distinguishing each of these. Future research is required to characterise the descriptors of the conditions that mimic RLS to facilitate the differentiation of these conditions from RLS.

The phrasing of the diagnostic criteria, although a purely semantic exercise, may influence a clinician's diagnosis of a patient. Physicians may be primed to relate to the current wording

and may not recognize other, more unusual yet valid, descriptors. Also, different phenotypes may influence a clinician's diagnosis of RLS and have implications for the possible treatment options provided. For example, patients who have painful RLS may respond better to opioid treatment than those without painful RLS symptoms. Thus, it is important that the clinicians are provided with the most accurate set of descriptors (which are possibly RLS phenotype specific) for RLS which will enable them to recognize RLS and optimize the patient's treatment according to the RLS phenotype. More research is needed investigating the relationship between pain and RLS and particularly the responses to different treatments.

The choice of descriptors used by patients with RLS may be affected by different languages and cultural influences which in turn may also affect prevalence estimations. The terms patients use to describe their symptoms may be culture specific. For example, a North American patient may be more inclined to select the phrase "Elvis legs" than an African patient from a rural community who may be unfamiliar with the pop icon reference. The diagnosis of RLS may be improved by overcoming language and cultural barriers and obtaining differential diagnostic terms for conditions mimicking RLS. There is a definite need to repeat this study in different English speaking populations and translations into other languages. Ultimately, an international, large scale, multicultural study is required to determine the most accurate diagnostic descriptors for RLS which could be used throughout the world.

One of the essential components of the diagnostic criteria is that the patient experiences an "urge to move" in response to "uncomfortable and unpleasant sensations" in their legs. The 'urge to move' can present independently of associated uncomfortable sensations but in some patients the sensory and motor component cannot be separated (Allen *et al.*, 2003). The

current emphasis is that the urge to move is more diagnostically useful than the actual sensations, which is supported by my research showing that motor phrases were the dominant choice. Karroum et al (2012) simultaneously published similar research to paper one included in this thesis but in their discussion they challenge the idea that there is a pure motor form of RLS. The majority of patients in their study could provide sensory verbal descriptors to adequately describe their condition. In fact, the authors only present the sensory and affective descriptors provided and do not report if the RLS patients spontaneously offered any movement-related descriptors (Karroum *et al.*, 2012). Further research is required to determine whether in fact a pure motor form of RLS does exist.

Whether they occur with the sensations or independently, the motor symptoms are a common feature in RLS. RLS symptoms are relieved by motor activity and exacerbated by inactivity, which emphasizes the contribution of the motor system in RLS. As such, the motor component of RLS is the focus of the next section.

6.2. Motor assessment - Spinal excitability in RLS patients

My second study focused on the assessment of two spinal reflexes, the H-reflex and the patellar reflex, as a reflection of spinal excitability in RLS patients measured at two different times of the day. For simplicity in addressing the issue of spinal excitability, I shall first discuss the differences observed in spinal excitability between RLS and control subjects independently of circadian timing, and in the section to follow address the circadian variations in spinal excitability.

Using neurophysiology and kinematics I found that the patellar amplitude and knee angular displacement were smaller in the RLS group compared to the control group, but that the knee angular velocity and all of the H-reflex parameters (latency, amplitude and H/M ratio) showed no difference between the two groups. The patellar reflex data indicates that RLS patients exhibit spinal hypoexcitability in comparison to healthy control subjects. However, the H-reflex data contradicts this and suggests that there is no difference in spinal excitability between these two groups. The study does not exclude the possibility that changes in the patellar reflex could be due to changes outside the spinal cord, such as in the muscle spindle or nerve fibres, or that methodological problems contributed to these findings. My original hypothesis and that of others in the literature, that RLS patients would exhibit spinal hyperexcitability in comparison to healthy control subjects, is not supported by my data.

The possible reasons why the two reflexes showed different results include that there are variable influencing factors in both stimulating and recording each reflex or that there are different spinal segments involved. Segmental spinal excitability changes may account for the differences noted between the H-reflex and patellar data in this thesis. The H-reflex of the lower limb tests the functionality of the peroneal or tibial nerve and primarily traverses spinal segments L5, S1 and S2 whereas the patellar reflex is innervated by the femoral nerve which reflects spinal segments L2-L4. Thus the two reflexes reflect the function of different spinal segments.

Tendon reflexes are subject to the influence of gamma motor neurons and changes in muscle spindle sensitivity which are in themselves segmental. Although speculative, my data would therefore suggest that RLS does not cause global hyperexcitability of the spinal cord but that

any affects are rather more localised. Given the differences in reflexes reflecting different spinal levels, it is important for this to be taken into account in research that assesses spinal excitability in RLS.

The parameters of the H-reflex that I focused on, namely H-latency, H-amplitude and H/M ratio have fairly consistent findings in the context of RLS patients. Concurring with my findings for the H-reflex, several other studies have shown that there were no differences between RLS and control subjects measuring different aspects of the H-reflex (Bucher & Trenkwalder, 1996; Akyol *et al.*, 2003; Rijsman *et al.*, 2005; Scaglione *et al.*, 2008). Thus, added to the results from my H-reflex data, neurophysiological testing of these parameters does not reveal changes in spinal excitability in RLS patients, which is contrary to the widely accepted spinal hyperexcitability theory.

The body of evidence supporting spinal hyperexcitability in RLS and PLM patients that is reviewed in chapter 1 (section 1.3.3. and section 2.2.3.4.) primarily focused on results obtained from the H-reflex (Wechsler *et al.*, 1986; Martinelli *et al.*, 1987; Rijsman *et al.*, 2005; Scaglione *et al.*, 2008) and the flexor reflex (Bara-Jimenez *et al.*, 2000) showing excitability of the H-reflex and disinhibition of the spinal circuitry. Different H-reflex parameters and the use of a different reflex (patellar) could possibly account for the apparent conflict between my data and that in the literature. Other studies, also reviewed in chapter 1, have also shown that not all the evidence indicates that RLS and PLM patients exhibit spinal hyperexcitability, as is the case in my study.

It has been shown in studies looking at spinal excitability states in spasticity (which is normally characterised by hyperreflexia), that hyperexcitability may depend on the site of the lesion and it may not be a global spinal phenomenon. Zhang et al (2000) demonstrated in spastic multiple sclerosis patients that the patellar reflex has increased gain and decreased contraction time which is consistent with spinal hyperexcitability (Zhang *et al.*, 2000). However, Salazar-Torres et al (2004) looked at the biceps brachii stretch reflex in spastic stroke subjects and found decreased reflex amplitude compared to controls. Thus, cerebral lesions (stroke) rather than the spinal cord are not always associated with hyperreflexia. Therefore, similar to what might be occurring in RLS and PLM, the evidence from these previous two studies indicates that hyperexcitability may not be a global spinal phenomenon.

The results from the patellar reflex testing in my study concur with the proposition that hyperexcitability may not be a global spinal phenomenon in RLS patients. Thus far, research has either indicated spinal hyperexcitability or no changes in the state of spinal excitability in RLS patients. By introducing the use of the patellar reflex, which has not been examined in the context of RLS and PLM patients, the discovery that it is hyporeflexive in these subjects is new to the field. Not only is this contrary to the spinal hyperexcitability concept, but it suggests an abnormality opposite to that which has been described in the RLS literature, but that has been demonstrated in other conditions.

Given the conflicting neurophysiology literature specifically in the field of RLS and PLM, the use of kinematics analysis was introduced to provide an objective measure of visually observed reflex parameters. The only previous work using biomechanics to assess the patellar reflex in any context used accelerometry and not video kinematics. Mamizuka et al (2007) using a

triaxial accelerometer determined that the patellar reflex in spastic patients had greater peak angular speed than healthy control subjects (Mamizuka *et al.*, 2007). Unfortunately, the differences in the averages were smaller than the standard deviations which could be attributed to a possible experimental flaw and thus, although promising, the use of accelerometry for the assessment of reflexes requires further validation.

The novel measures that I have introduced to look at the state of spinal excitability have provided interesting, but as yet unexplainable, results. I can only speculate why my results (and that of some other authors) are contrary to the widely accepted, but not conclusively proven, global spinal hyperexcitability theory. Possibly RLS patients either have spinal hypoexcitability of certain components and thus similar global excitability to control subjects. Whether the lower patellar amplitude necessarily reflects a significant reduction in excitability (hypoexcitability) as opposed to excitability at the lower end of the range but still within normal limits needs to be investigated. Future studies should examine the patellar amplitude of RLS and PLM patients after treatment and in a much larger sample of patients and compare these results to conditions of known hyporeflexia to determine if in fact RLS and PLM patients do exhibit hypoexcitability of the patellar reflex. Conversely, the patellar reflex data I obtained could be reflecting hyperexcitability of inhibitory neurones which would cause attenuation in the patellar amplitude. However, this could not be definitively determined by the current study and until the spinal mechanisms are delineated in RLS we may not be able to determine this possibility. Assuming that my results are an accurate reflection and not a consequence of methodological problems, this research suggests that the aetiology of RLS and PLM is not as simple as a global spinal hyperexcitability in these patients. The hyperexcitability paradigm that has been established in the literature may be specific to discreet connections which are

different to the specific monosynaptic patellar reflex circuits that were tested in paper two. Investigating these different circuits, which may be more or less excitable is worth pursuing. Perhaps researching segmental excitability and intra-segmental excitability of different pathways within the same set of RLS patients could help determine the global state of spinal excitability in these patients. Further research into more specific spinal pathways, possibly using drugs, autopsy studies or neuroimaging techniques to isolate particular pathways, is required to specifically elucidate the state of spinal excitability in patients with RLS and PLM.

6.2.1. Circadian variations of spinal excitability in RLS patients

Symptoms of RLS follow a circadian pattern and if one assumes that a change in the state of spinal excitability drives RLS symptoms, then this state should follow a circadian variation to reflect the change in RLS symptoms throughout the day. Thus a secondary aim of my third study was to assess the diurnal changes in spinal excitability. The previous section showed some changes in spinal excitability in RLS patients independent of circadian factors, which may determine whether symptoms are dominant or absent.

Taking into account the time of day, the RLS patients' patellar amplitude in the evening was smaller than the morning and compared to the control subjects. The RLS patients' patellar amplitude had an approximate decline of 45% from morning to evening thus displaying a circadian variation. The control group however, did not exhibit a circadian rhythm for the patellar reflex. All the other reflex parameters showed no difference in the morning or evening between groups, and no circadian variation within either group. Therefore, during the time when symptoms are expressed, there are differences between RLS patients and control

subjects, although not in the direction expected, but when symptoms are absent, differences are insignificant between RLS patients and controls. Some of the patellar reflex (amplitude and knee angular displacement) data indicates that the RLS patients had altered spinal excitability during the symptom dominant evening period but expressed as hypoexcitability and not as hyperexcitability. However, not all of my data agree with this as the H-reflex and other measures of the patellar reflex show that there are no circadian changes in spinal excitability in RLS patients.

There is evidence suggesting that some spinal reflexes have inherent circadian fluctuations whereas others do not. Although inconclusive, and somewhat dependent on muscle and reflex type, the state of the spinal cord has been shown to become both more and less excitable throughout the day therefore indicating that excitability changes are not a global spinal phenomenon. Further studies are required to determine the exact circadian variations of spinal reflexes and what factors contribute to the presence or absence of these circadian patterns.

This is the first study looking at circadian changes of reflexes within the same set of RLS patients and shows circadian variations in the patellar reflex but not in the H-reflex. The possibility that properties of the patellar tendon change throughout the day could perhaps play a role in the differences observed in the patellar reflex. Pearson et al (2006) found that patellar tendon stiffness decreased from morning to evening and therefore the tendon should be more compliant in the evening (Pearson & Onambele, 2006). This, however, would not reflect as a smaller reflex as was the case in my research. Also, if the cause of the change in patellar reflex in the RLS patients was due to a change in the properties of the tendon then the same variation would be expected in the control group.

Circadian variation in spinal excitability has not been examined in patients with RLS and very few neurophysiology studies on patients with RLS actually take time of day into account. Looking at the literature at reflexes that have been tested during the time when symptoms are present, spinal hyperexcitability has been indicated. Impaired H-reflex excitability curves and vibratory inhibition depression, indicative of spinal disinhibition, and lower thresholds and greater spatial spread of the flexor reflex in PLM patients have been demonstrated in the late afternoon and during the evening (between 21:00 and 00:00) (Bara-Jimenez *et al.*, 2000; Rijsman *et al.*, 2005). These results are in comparison to control subjects but were not made between morning and evening measurements made within the same set of patients.

As I have previously indicated, the H-reflex parameters I tested did not detect changes in spinal excitability. It is possible that the tests I used are not sensitive enough to detect subtle circadian variations, or that a far larger sample size might have shown differences. Despite this, there was no statistically significant circadian variation of the H-reflex in either controls or patients with RLS. For the first time however, my results show that RLS patients show diurnal fluctuations in the spinal excitability state as reflected by the patellar reflex. During the period when RLS symptoms are predominant, there is an altered spinal excitability reflected by the patellar reflex data, supporting the idea that RLS is a disorder involving fluctuations in spinal excitability. Future studies should focus on examining circadian variations using more sensitive measures of spinal excitability as well as multiple reflexes within the same patients. Again, researching the segmental excitability and intra-segmental excitability and whether these change throughout the day (in accordance with the circadian pattern of symptoms) may help elucidate the pathophysiology of RLS and PLM.

6.3. Sensorimotor assessment – Suggested Immobilization Test

My fourth study looked at the relationship between the sensory (discomfort and pain) and motor features of RLS and PLM during the Suggested Immobilization Test (SIT). All the RLS patients reported significant increases in their levels of discomfort throughout the test, however only three of the sixteen patients presented with simultaneous PLM. The SIT has been proposed as an objective diagnostic test for RLS however, whether the motor activity (PLM index) or measure of discomfort (mean discomfort score) is better able to distinguish RLS patients from control subjects, is questionable.

The PLM index is an objective measure of the number of PLMs presented during the SIT and has been shown to successfully distinguish RLS patients from controls (Montplaisir *et al.*, 1997; Michaud *et al.*, 2002a; Michaud *et al.*, 2002b). The exact, and minimum, number of movements required to distinguish RLS patients from controls is debatable. One study has shown that a PLM index of 12 is sufficient (Haba-Rubio & Sforza, 2006), whereas another has proposed a much higher number of 40 (Montplaisir *et al.*, 1998). A further problem associated with this method is that not all RLS patients present with PLM, as was seen in the majority of participants in my fourth paper. According to the above criteria, using a PLM index of either 12 or 40, only three of the RLS patients included in my study would have been diagnosed with RLS (despite all the patients fulfilling the essential diagnostic criteria). PLM are a common, but non-essential, supportive RLS diagnostic feature whereas the sensory component (i.e. discomfort) is an essential part of the diagnostic criteria.

The SIT can also be used to assess the relationship between sensory and motor features of RLS. RLS sensory symptoms are exacerbated during inactivity and are relieved by voluntary movement, suggesting a link between the two however the association is unclear. There is some evidence of a disconnect between the two as shown by escalating discomfort levels concurrent with a plateau in the number of PLM and a lack of correlation between sensory and motor features (Michaud *et al.*, 2002a; Birinyi *et al.*, 2005). We could not perform correlation analysis between sensory and motor components on our sample as the majority of the patients did not present with PLM. That, in itself, suggests that there is a disconnect at a particular level of PLM between these components as all the RLS patients had increasing levels of discomfort without associated motor activity.

The lack of PLM in the majority of the RLS patients prompted me to divide the RLS patients according to the presence or absence of PLM. Subsequent analysis revealed that the RLS patients with PLM reported greater levels of pain during the SIT and had increasing numbers of PLM throughout the SIT compared to the patients with RLS without PLM and the control participants. I do recognise that the small sample size limited the analysis options and as such, any deductions (relating to the RLS with PLM patients) are made with some caution. These data do suggest however that there is a relationship between pain and PLMs. Perhaps RLS patients with pain are more prone to develop PLM or those with PLM are more likely to be associated with pain. It would be interesting to see if the descriptors selected by patients with painful RLS or PLMW on the SIT included more movement related terms. Also, perhaps painful RLS symptoms only present as the disorder progresses, given that there was a longer duration of symptoms in this group of patients. The relationship between pain, discomfort and PLM warrants further exploration. The complex associations between the sensory and motor

features of RLS and the appearance of PLM and pain in only some patients imply that RLS is a heterogeneous disorder.

6.4. Concluding statement

Further assessment of the sensory and motor features, both in conjunction and isolation, are required. New assessment techniques are essential to advance the field and tease out some of the current contradictory results in RLS and PLM research. Each of the studies included in this thesis introduce a new perspective or technique for the assessment of RLS and PLM. The results from the studies included in this thesis indicate that RLS is a more complex disorder than previously imagined. The diverse range of descriptors, including both sensory and motor terms, which are influenced by pain and possibly by other differences in underlying mechanisms, suggest that RLS is a heterogeneous disorder with different phenotypes in addition to those already described. The hypoexcitability of the patellar reflex shown in this thesis, which is contrary to the theory in the literature of spinal hyperexcitability as the aetiology of RLS and PLM, indicates that excitability changes of the spinal cord may not be a global phenomenon in these patients. Furthermore, the distinction between painful and non-painful RLS based on the SIT PLM index and the patient's word choice supports that RLS and PLM are heterogeneous disorders with multiple phenotypes. The discrepancies noted in the literature in each of these topics and between my results and the literature may arise from the assumption that there is one common pathophysiology for both the disorders. Generally the concept of different RLS phenotypes, although previously described, has been ignored when selecting patients to include in studies of this type. Much more research, using different

techniques including those introduced in this thesis, is required to tease out the different phenotypes.

A statement made in a paper by Bachmann et al (2010) sums up the importance of recognising the different phenotypes of RLS and the implications thereof, “Differentiation of restless legs subtypes is of utmost clinical relevance as it enables a differentiated therapeutic approach for patients with RLS” (Bachmann *et al.*, 2010). Healthcare providers and future researchers should take cognizance of the potential clinical and research implications of possible differences in underlying pathologies of RLS and PLM to overcome therapeutic difficulties and research complicated by multiple phenotypes. In conclusion, including new assessments of the sensory and motor features (as presented in this thesis) together with effective communication about the type of RLS sensations may improve the accuracy of diagnosis and strengthen our understanding of the RLS and PLM aetiology.

CHAPTER 7

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CHAPTER 8

APPENDICES

Appendix A: Restless Legs Syndrome questionnaire

1. What is your age? _____ years

2. What sex are you? male / female (circle correct one)

3. What is your height? _____ m / feet and inches (circle correct one)

4. What is your weight? _____ kg / lb. (circle correct one)

5. What ethnic group do you belong to? _____

6. If you are a male - what is your collar size? _____ cm / inches (circle correct one)

7. Have you had a regular bedpartner for the last 2 months? yes / no (circle correct one)

8. Has your bedpartner commented on you doing any of the following while asleep? (circle correct one)

	Most nights	Sometimes	Almost never	Never
a. Snoring heavily and regularly?	1	2	3	4
b. Episodes where you appear to hold your breath?	1	2	3	4
c. Restless sleep?	1	2	3	4
d. Regular kicking of your legs while asleep?	1	2	3	4

9. If you do not have a regular bed partner, have you ever been told that you do any of the following? (circle correct one)

a. Severe snoring? Yes / No

b. Stop breathing while asleep? Yes / No

c. Very restless while sleeping? Yes / No

d. Legs twitching regularly while asleep?Yes / No

10. a. Do you ever get an uncomfortable sensation in your lower legs (below the knees) which urges you to move your legs after sitting still for only 15 minutes. This uncomfortable sensation may come on before falling asleep, while watching TV or during a long trip. Yes / No (circle correct one)

If you answered yes to the previous question -

b. Does the sensation go away (even partly) when you move your legs?
Yes / No (circle correct one)

c. Is the sensation worse at night – compared to the day-time?
Yes / No (circle correct one)

d. Does the sensation occur only when your legs are resting e.g. lying down or sitting still? Yes / No (circle correct one)

e. Is there anything eg something that you eat or drink that makes the sensation worse? Explain.

_____

f. How many days per week does this sensation occur? _____ days

g. At what age did this sensation start? _____ years

h. Has the type of sensation changed over time? Explain.

_____

i. Is the sensation staying the same / getting better / getting worse with time? (circle correct one)

j. Do you know of anyone in your family who has the same sensation in their legs? Yes / No
(circle correct one)

k. How are they related to you i.e. your mother and/or sister?

_____

l. Have you ever used medication to treat this uncomfortable sensation?
Yes / No (circle correct one)

m. If you answered yes to question k. please list the medication tried and any effect of the medication.

_____

_____

12. As a child, did you ever have pains in the shins at night-time?
Yes / No / Can't remember (circle correct one)

13. If you answered yes to Q**12**:

At what age did you first have these pains?

_____ years old / can't remember

Was there anything that relieved the pain? Yes / No / Can't remember

If yes what was it - _____

At what age did this pain in the legs go away?

_____ years old / Don't remember / Never went away

14. As a child were you ever diagnosed with a learning problem?
Yes/No (circle correct one)

15. If you answered yes to Q**14** – indicate which of the following by placing a cross next to the correct one.

Attention Deficit Disorder ADD
General concentration problem
Other- specify _____

16. Do you have a twitching or jumping sensation in your legs while awake and sitting still?
Yes / No (circle correct one)

17. If you answered yes to Q**16**,

a. how often do these twitches occur? (circle the correct answer)

less than once a week / once a day / more than once a day.

b. What age were you when these twitches started? _____ years

c. Are the twitches getting worse over time / getting better over time / staying the same since they started? (circle the correct one)

18. Do you have difficulty falling asleep at night more than 3 nights per week?
i.e. takes more than 30 minutes? Yes / No (circle correct one)

If you answered yes to Q**18**, What do you suspect the reason to be?
_____
_____

19. Do you wake up during the night and find it difficult to go back to sleep?
Yes / No (circle correct one)

If you answered yes to Q**19**

How many nights per week? _____ nights

What do you suspect the reason to be?_____

20. Do any of your relatives have a sleep problem? Yes / No

If you answered yes to Q **20**

What is your relationship to them i.e. your mother?

_____

21. How many cigarettes do you smoke per day? (circle correct one)

none / 1-15 per day / 16-30 per day / more than 31 per day

22. How many units of alcohol do you drink per week? (one unit would be one single tot of spirits / one beer / one glass of wine

_____ units per week

23. How many cups of caffeine do you drink per day? This includes coffee, tea, Cola drinks or energy drinks. Explain.

_____

24. How likely are you to doze off or fall asleep in the following situations after you've had your usual nights sleep: (circle one number for each)

	Would never doze	Slight chance	Moderate chance	High chance
a. Sitting and reading	0	1	2	3
b. Watching television	0	1	2	3
c. Sitting inactive in a public place	0	1	2	3
d. Passenger in a car for an hour without a break	0	1	2	3
e. Lying down to rest in the afternoon	0	1	2	3
f. Sitting and talking to someone	0	1	2	3
g. Sitting quietly after lunch with no alcohol	0	1	2	3
h. In a car, while stopped for a few minutes in the traffic	0	1	2	3

25. Are you on any regular medication? Yes / No(circle correct one)

If yes – please could you list the medical conditions that you are currently treated for in the space below.

_____

Could you please list all medication that you are currently taking including the dosage and number of tablets or sprays per day.

_____

_____

_____

26. Have you ever been diagnosed or treated for the following conditions? Please circle the correct answer

a. Iron deficiency anemia yes / no If yes: What age_____ yrs

Were you treated and what were you given? _____

_____

Did the treatment make any difference to the restless legs? Yes / no

b. Back problems – either chronic back pain or disc problems or injury in an accident? Yes / no

If yes: What age did it start? _____ yrs

Did this occur before the onset of restless legs syndrome?
Yes / no

c. Renal failure yes/no

If so what age were you?_____yrs

How long did the renal failure last for? _____ Yrs/months

<u>For women only:</u> Were you pregnant while you had restless legs? Yes/no

If you answered yes: How did the restless legs change during the pregnancy – (you can indicate more than one option)

They started during the pregnancy
They stayed the same during the pregnancy
They got worse as the pregnancy progressed
They disappeared after the pregnancy and restarted later on.

Appendix B: Ethical clearance certificate (paper one and two)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Samantha Kerr

CLEARANCE CERTIFICATE	**M10343**
PROJECT	Characteristics of Restless Legs Syndrome Sensations
INVESTIGATORS	Dr Samantha Kerr.
DEPARTMENT	School of Physiology
DATE CONSIDERED	26/03/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 04/05/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr A Bentley

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Appendix C: Ethical clearance certificate (paper three and four)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Kerr

CLEARANCE CERTIFICATE	**PROTOCOL NUMBER M070452**
PROJECT	Biomechanical and Electrophysiological Characteristics of Lower Limb Reflexes in Patients with Restless Legs Syndrome and....
INVESTIGATORS	Ms S Kerr
DEPARTMENT	School of Physiology
DATE CONSIDERED	07.05.04
DECISION OF THE COMMITTEE*	APPROVED UNCONDITIONALLY

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 07.05.28 **CHAIRPERSON** .....
(Professors PE Cleaton-Jones, A Dhai, M Vorster, C Feldman, A Woodiwiss)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Bentley A Dr

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10005, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix D: International RLS study group severity rating scale (IRLS)

In the past week…

1. Overall how would you rate the RLS discomfort in your legs or arms?

□ Very severe □ Severe □ Moderate □ Mild □ None

2. Overall how would you rate the need to move around because of your RLS symptoms?

□ Very severe □ Severe □ Moderate □ Mild □ None

3. Overall, how much relief from your RLS arm of leg discomfort did you get from moving around?

□ No relief □ Mild relief □ Moderate relief □ Either complete or almost complete relief
□ No RLS symptoms to be relieved

4. How severe was your sleep disturbance due to your RLS symptoms?

□ Very severe □ Severe □ Moderate □ Mild □ None

5. How severe was your tiredness or sleepiness during the day due to your RLS symptoms?

□ Very severe □ Severe □ Moderate □ Mild □ None

6. How severe was your RLS on the whole?

□ Very severe □ Severe □ Moderate □ Mild □ None

7. How often did you get RLS symptoms?

□ Very often (this means 6-7 days per week)
□ Often (this means 4-5 days per week)
□ Sometimes (this means 2-3 days per week)
□ Occasionally (this means 1 day per week)
□ Rarely (this means less than 1 day per week)

8. When you had RLS symptoms, how severe were they on average?

□ Very severe (this means 8 hours or more per 24 hour day)
□ Severe (this means 3-8 hours per 24 hour day)
□ Moderate (this means 1-3 hours per 24 hour day)
□ Mild (this means less than 1 hour per 24 hour day)
□ None

9. Overall, how severe was the impact of your RLS symptoms on your ability to carry out your daily activities, for example having a satisfactory family, home, social, school or work life?

□ Very severe □ Severe □ Moderate □ Mild □ None

10. How severe was your mood disturbance due to your RLS symptoms – for example being angry, depressed, sad, anxious or irritable?

□ Very severe □ Severe □ Moderate □ Mild □ None

Appendix E : McGill Pain Questionnaire

PRI: S _____ A _____ E _____ M _____ PRI (TOTAL) _____ PPI _____
(1-10) (11-15) (16) (17-20) (1-20)

1	2	3	4
1. Flickering	1. Jumping	1. Pricking	1. Sharp
2. Quivering	2. Flashing	2. Boring	2. Cutting
3. Pulsing	3. Shooting	3. Drilling	3. Lacerating
4. Throbbing		4. Stabbing	
5. Beating		5. Lancinating	
6. Pounding			

5	6	7	8
1. Pinching	1. Tugging	1. Hot	1. Tingling
2. Pressing	2. Pulling	2. Burning	2. Itching
3. Gnawing	3. Wrenching	3. Scalding	3. Smarting
4. Cramping		4. Searing	4. Stinging
5. Crushing			

9	10	11	12
1. Dull	1. Tender	1. Tiring	1. Sickening
2. Sore	2. Taut	2. Exhausting	2. Suffocating
3. Hurting	3. Rasping		
4. Aching	4. Splitting		
5. Heavy			

13	14	15	16
1. Fearful	1. Punishing	1. Wretched	1. Annoying
2. Frightful	2. Gruelling	2. Blinding	2. Troublesome
3. Terrifying	3. Cruel		3. Miserable
	4. Vicious		4. Intense
	5. Killing		5. Unbearable

17	18	19	20
1. Spreading	1. Tight	1. Cool	1. Nagging
2. Radiating	2. Numb	2. Cold	2. Nauseating
3. Penetrating	3. Drawing	3. Freezing	3. Agonising
4. Piercing	4. Squeezing		4. Dreadful
			5. Torturing

Appendix F: List of RLS descriptive words

Aching	Fizzy	Pulsating	Traction
Afflictive	Fluttery	Queasy	Trembling
Agitated	Frustrated	Quivering	Troublesome
Agonizing	Funny	Raw	Tugging
Alive	Ghostly	Restless	Twinging
Anesthetized	Gnawing	Scratchy	Twitchy
Animated	Grabbing	Searing	Uncomfortable
Antsy	Heavy	Shaky	Uneasy
Apprehensive	Hurting	Sharp	Unnerved
Asleep	Hyper	Shock-Like	Unpleasant
Awkward	Irritating	Skittish	Unquiet
Biting	Itchy	Slithering	Unyielding
Bizarre	Jerky	Smarting	Uptight
Burning	Jiggly	Sore	Wearisome
Buzzing	Jittery	Squirming	Weird
Caustic	Jumpy	Stinging	Wiggly
Clutched	Lively	Straining	Wired
Cramping	Mysterious	Strange	Worming
Creepy-Crawly	Nagging	Suffering	Worried
Dead	Numb	Supernatural	Wrenching
Disagreeable	Ouchies	Tearing	Wriggling
Distressing	Overwrought	Tender	Writhing
Drawing	Painful	Tense	Yanking
Effervescent	Panicky	Thorny	
Electric	Peculiar	Throbbing	
Energetic	Piercing	Tickling	
Excited	Pounding	Tight	
Exploding	Pressured	Tingling	
Fidgety	Prickling	Tired	
Fiery	Pulling	Toiling	

Appendix G: List of descriptive phrases

Ants/spiders/restless grasshoppers in the legs
Anxiety in your legs
Blood racing through your legs
Crazy legs
Elvis legs
Feels like your legs want to jump off your body
Giggly legs
Hyped-up
Jimmy legs
Just an urge to move
Legs are in a tizzy
Legs have too much energy/are full of energy
Legs need to stretch
Legs need to walk/jog
Legs want to move on their own/won't be still
Like being stung by 20 mosquitoes and not being able to scratch
Like insects biting the inside of your legs
Like jumping beans in your legs
Like something is poking your legs
Like something popping inside your legs
Need to handle your legs
Need to kick out your legs
Nervous legs
Pepsi/cola/soda bubbles in the veins
Pins and needles
Runaway legs
The got to moves
The heebie jeebies
Water moving through your legs
Worms under the skin
Wound up

Appendix H: Author proofs of the published paper co-authored by the thesis author during the period of PhD candidature

The patellar reflex: does activity of quadriceps femoris muscles reflect leg movement?

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Objectives: The assessment of spinal reflexes has traditionally been performed by clinicians with minimal need for recording equipment, where doctors rely on their training and may use established subjective reflex rating scales. With advances in technology, it is now possible to assess reflexes objectively. This study compared two objective methods of assessing patellar reflex magnitude, duration, and latency, namely electromyography (EMG) of the quadriceps muscles and kinematic assessment of the leg movement around the knee joint.
Methods: Reflexes of 24 healthy participants were assessed and seven variables were found to describe each reflex. These were the change in knee angle, the velocity of the reflex, the time to maximum knee angle, the biomechanical movement latency, the EMG maximum amplitude, the negative peak duration, and the EMG latency. Spearman's rank correlation tests were run in order to compare all of the variables.
Results: The results showed that there were positive correlations between EMG maximum amplitude and the change in knee angle (R^2=0.75; P<0.0001) as well as the EMG maximum amplitude and the velocity of the reflex (R^2=0.30; P=0.0058). There was also a negative correlation between EMG maximum amplitude and the biomechanical movement latency (R^2=0.35; P=0.0024).
Discussion: The results show that there is a relationship between muscle activity and the actual visual movement of the leg assessed using kinematics. This relationship is closest between kinematic measurements and EMG measures of reflex amplitude.

Keywords: Electromyography, Kinematics, Patellar reflex

Introduction

Historically the use of tendon hammers has facilitated an easy way for neurologists to assess spinal reflexes with no recording apparatus needed other than the doctor him/herself. More recently however, electromyography (EMG) methods have made it possible to objectively quantify such reflexes. For this reason, EMG is used clinically and during research of spinal reflexes. Studies have used comparisons between EMG measurements and subjective rating scales in order to assess the accuracy of these scales.[1] Comparisons can also be done between EMGs of the same subject recorded at different times assessing changes in reflex activity.[2] There are problems with EMG however. The most reliable EMG readings are made from needles extending into a muscle bed (invasive) and are prone to error induced by muscle movement, which itself may alter electrode placement. Surface EMG is therefore also used to assess muscle activity and is less invasive but has questionable reproducibility in clinical settings[3] which could be caused by many factors such as: placement of electrodes, the layers between the electrode and the muscle (e.g. subcutaneous fat), and the influence of electrical activity in surrounding muscles.

With the emergence of kinematic techniques, it is now possible to obtain accurate and objective measurements of movement. Despite the coexistence of both EMG and kinematic methods, and despite an exhaustive search by the current authors to find such evidence, the relationship between EMG (muscle activity) and actual movements observed during clinical reflex testing is largely missing. One study that comes close to comparing clinical measurements to objective kinematic measurements showed that the biomechanics of gait are correlated with performance on a clinical motility scale.[4] A second study used a tri-axial accelerometer in an attempt to quantify and characterise the acceleration of the lower leg following patellar tendon reflexes.[5]

In the current study, we aimed to quantify the relationship between kinematic and EMG measurements

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DOI 10.1179/1743132812Y.0000000011

Neurological Research ner2699.3d 1/3/12 17:13:11
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of one of the most extensively tested deep tendon reflexes, the patellar (knee-jerk) reflex. In so doing, we aim to investigate the relationship between muscle activity in the quadriceps muscles and the kinematically quantified movement of the leg during the reflex.

Methods

Ethical approval

Ethical approval was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (M10266) and each volunteer gave written informed consent.

Procedure

The patellar reflex was elicited on the right legs of 24 healthy subjects. Subjects were seated comfortably with their legs hanging freely in order to allow forward leg movement. Each subject was then struck with a manual reflex hammer on their patellar tendon so that the optimum spot for eliciting the reflex was found. Reflexes were elicited using an automated axle mounted patellar hammer applied to the optimum spot for the patella tendon.

Kinematic measurements

Reflexes were recorded using five high speed cameras (E-PLA741; Pixellink, Ottawa, Canada). Images from the five high speed cameras were simultaneously recorded at a frame rate of 50 frames/second. Two retroreflective markers were placed on the head and the foot of the patellar hammer. A further five retroreflective markers were positioned on each subject's lateral thigh, medial and lateral epicondyles of the femur, the medial malleolus of the tibia, and the lateral malleolus of the fibula. Calibration of measurement volume involved the recording of a removable calibration frame with known three-dimensional coordinates.

Models of the hammer and the leg were reconstructed in three dimensions using a modified direct linear transform algorithm[6] and reconstruction algorithm.[7] The starting knee angle, maximum knee angle, time taken to reach the maximum angle, and hammer velocity at time of strike were determined. This allowed four objective biomechanical variables to be calculated: the change in knee angle (from its initial point to maximum angle), the velocity of the reflex (maximum angle/time to maximum angle), and the biomechanical latency (time taken between hammer strike and initiation of movement).

EMG measurements

EMG recordings were taken simultaneously with the kinematic recordings. Two electrodes were positioned over the quadriceps muscle (5 cm above the superior margin of the patellar and 5 cm proximal to the first). A third grounding electrode was positioned on the upper half of the left leg. Three objective variables were taken from the EMG recordings (using PowerLab, ADI instruments, Sydney, Australia): maximum amplitude of the reflex, the negative peak duration during the reflex, and the EMG latency (time taken between the hammer strike and the beginning of muscle activation, corrected for the height of the subject[8]).

Statistical analysis

The non-parametric nature of all data was confirmed using Lilliefors test. Spearman's rank correlations assessed the relationship between the biomechanical variables and the EMG variables.

Results

Participants

Twenty-four healthy participants (7 males and 17 females) had an average age of 24.3 years (range: 19–42 years). Participants had an average height of 1.63 m (1.47–1.84 m) and an average weight of 63.1 kg (44.2–83.7 kg). The kinematic and EMG measurements for each variable for each participant are shown in Table 1.

The strongest positive relationship was found when the change in knee angle and the EMG maximum amplitude were correlated (Fig. 1). The results of other Spearman's rank correlation tests are shown in Table 2. The movement velocity of the reflex and the EMG maximum amplitude were positively correlated (R^2=0.30; P=0.0058); EMG maximum amplitude and the biomechanical movement latency were shown to be negatively correlated (R^2=0.35; P=0.0024). No other significant relationship between kinematic movement and muscle activity was shown.

Discussion

The seven variables that were used in this study to describe the patellar reflex all measured different reflex properties. Aside from the obvious distinction, where EMG data describe muscle activity and kinematic measurements assess the related movement, the kinematic variables: change in knee angle and the velocity of the reflex, along with the EMG maximum amplitude are variables that assess reflex amplitude. The time taken to reach the maximum angle and the negative peak duration assessed reflex duration. Finally, the biomechanical movement latency and the EMG latency reflect the latency of the reflex.

Of the three EMG variables considered in this study, only the maximum amplitude of the reflex was found to be correlated to kinematically quantified measurement variables. EMG maximum amplitude was positively correlated with both change in angle of the knee during the reflex and the velocity of the reflex. Reflex amplitude has been identified as an objective measure of the patellar reflex.[9,10] Previous

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studies have shown reflex amplitude to display considerable interindividual and intraindividual variability, as was seen in our subjects, as well as being influenced by numerous factors.[3,8,9] Our data would suggest that both EMG maximum amplitude and the change in angle of the knee during the reflex are equally good descriptors of reflex amplitude. This is expected as the EMG maximum amplitude has been shown to represent the force of the muscle contraction during a reflex.[11] The velocity of a reflex is a measure of how fast the leg moves during that reflex. This will be directly dependent on synchronization of the motor units in the muscle, the corresponding EMG amplitude, and the magnitude of muscle contraction.[10]

In addition, EMG maximum amplitude was also negatively correlated to the biomechanical movement latency. This latter finding, where the magnitude of muscle activation is related to earlier movement of the leg may be due to larger muscle activations generally resulting in greater force of movement which is transferred into perceptible movement earlier than lesser muscle activations.

The remaining EMG variables: the negative peak duration and EMG latency were not correlated with any kinematic variables. The lack of relationships may reflect that the different variables assess different distinct reflex aspects. EMG latencies have been assessed in previous studies[4,9] and have been found to be consistent even over multiple reflexes on the same individual.[9] The lack of a detectable relationship between the EMG latency and a discernable movement of the leg may be due to a variety of confounding variables. These include leg mass, an independent variable, confounding the relationship between muscle activation and movement.

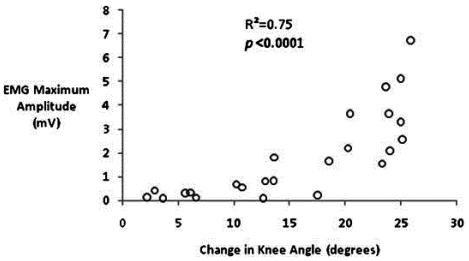

Figure 1 The results of the Spearman's rank correlation comparing the electromyographic (EMG) variable maximum amplitude and the biomechanical variable change in knee angle.

The time taken from muscle activation to physical leg movement (the electromechanical delay) may also be an independent confounding variable. The ability to discern reflex muscle activity or reflex related movement with background muscle activity or movement would have a significant impact on the relationship (this factor would be particularly evident at smaller reflex magnitudes). Percentage of muscle fibre types of the individual[12] (the ratios of fast twitch and

Table 1 The biomechanical and electromyographical characteristics of the 24 reflexes

	Biomechanical measurements				Electromyographical measurements		
Participant	Change in angle (°)	Time to maximum angle (millisecond)	Velocity of the reflex (degree/millisecond)	Movement latency (millisecond)	Maximum amplitude (mV)	Negative peak duration (millisecond)	Electromyographical latency (millisecond/m)
1	2.2	360	0.01	180	0.16	15	52.98
2	2.9	400	0.01	140	0.43	21	27.59
3	5.6	460	0.01	40	0.34	17.5	84.46
4	6.6	340	0.02	160	0.13	15	91.84
5	24.9	440	0.06	60	3.34	17.5	67.07
6	25.0	380	0.07	40	5.15	17.5	57.58
7	12.6	100	0.13	80	0.11	12.5	60.98
8	18.5	360	0.05	20	1.69	15	38.04
9	23.9	380	0.06	40	3.68	15	47.77
10	20.4	340	0.06	20	3.66	15	72.67
11	13.5	320	0.04	20	0.85	20	50.60
12	3.6	360	0.01	160	0.12	15	47.62
13	10.7	540	0.02	80	0.56	15	31.98
14	25.1	740	0.03	100	2.60	17.5	47.06
15	23.6	360	0.07	60	4.81	15	85.44
16	20.2	380	0.05	60	2.22	12.5	47.30
17	17.5	40	0.44	160	0.26	17.5	44.91
18	13.6	440	0.03	80	1.82	15	76.47
19	25.8	400	0.06	60	6.76	15	73.90
20	12.8	480	0.03	120	0.83	12.5	81.67
21	6.1	500	0.01	220	0.35	12.5	176.04
22	23.9	780	0.03	60	2.12	10	58.28
23	10.2	400	0.03	40	0.70	12.5	63.61
24	23.3	440	0.05	80	1.57	17.5	53.25
Median (IQ range)	15.55 (14.38)	390 (85)	0.04 (0.04)	70 (85)	1.21 (2.44)	15 (3.13)	57.93 (27)

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Table 2 Spearman's correlation coefficients (R^2) for the comparison between biomechanical and electromyographic (EMG) variables associated with the patellar reflex

Biomechanical variables	EMG variables	Spearman's R^2 value	P value
Change in knee angle (°)	Negative peak duration (millisecond)	0.01	0.682
	Latency (millisecond)	0.00	0.990
Time to maximum angle (millisecond)	Maximum amplitude (mV)	0.05	0.312
	Negative peak duration (millisecond)	0.02	0.525
	Latency (millisecond)	0.01	0.620
Velocity of the reflex (°/millisecond)	Maximum amplitude (mV)	0.30	0.006*
	Negative peak duration (millisecond)	0.00	0.786
	Latency (millisecond)	0.00	0.974
Biomechanical movement latency (millisecond)	Maximum amplitude (mV)	0.35	0.002*
	Negative peak duration (millisecond)	0.01	0.679
	Latency (millisecond)	0.00	0.945

Note: *Significant $P<0.05$.

slow twitch fibres correlate negatively to EMG latency[12]) or finally, the fact that a myriad of factors influence the sensitivity of surface EMG measurements. Despite this disagreement, EMG latencies have been shown to be clinically important as they provide an uncomplicated means of assessing peripheral nerve conduction and have been shown to be better diagnostic tools than EMG amplitude.[8] In addition to the fact that movement latency would be difficult to accurately assess in the clinical setting, our data would suggest that movement latency would not be a substitute for EMG latency.

Conclusion

This study proposed to quantify the relationship between kinematic and EMG variables of the patellar reflex. The findings show that there is a relationship between muscle activity and the actual movement of the leg. This relationship is most closely seen between EMG maximum amplitude and the biomechanical variables change in knee angle and the velocity of the reflex.

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Appendix I: Author proofs of the published paper co-authored by the thesis author during the period of PhD candidature

THE ACCURACY OF SUBJECTIVE CLINICAL ASSESSMENTS OF THE PATELLAR REFLEX

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Accepted 4 June 2012

ABSTRACT: *Introduction*: Measurement precision and accuracy of spinal reflexes plays an essential role in the clinical neurological examination. Reflexes are conventionally assessed either electromyographically or with rating scales. In this study we compared objective kinematic T-reflex and subjective assessments of patellar reflexes in 15 normal healthy subjects. *Methods*: Randomized recordings of objectively quantified reflexes were rated by 24 medical students, 16 general practitioners, and 12 neurologists, using a visual analog scale and the NINDS and Mayo clinical reflex scales. *Results*: For all groups of raters, Spearman's rank correlations showed that subjective ratings significantly correlated with change of knee angle ($R^2 = 0.72–0.79$, $P < 0.001$) and maximum T-reflex amplitude ($R^2 = 0.84–0.94$, $P < 0.001$). Stepwise multiple regression analysis showed that all subjective rater groups relied most on the change of knee angle to assess the reflex. *Conclusions*: These findings show that subjective assessments of reflexes using reflex rating scales correlate strongly with biomechanical and electromyographic measures.

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The testing of spinal reflexes is an important diagnostic tool for gauging neurological disturbances. Variations in spinal reflexes aid in the diagnosis and localization of neurological problems.[1] One of the important spinal reflexes assessed is the patellar reflex. When quantifying the magnitude of the patellar reflex there are two popular standardized subjective scales, namely the National Institute of Neurological Disorders and Stroke (NINDS) myotatic reflex scale[2] and the Mayo clinical reflex scale.[3] The development of these scales has allowed medical professionals to standardize their assessments of reflexes,[4] but the interobserver reliability of these scales is uncertain.

Currently, electromyography is the best available method to gauge abnormalities of the patellar reflex.[5] Quadriceps muscle electromyography allows the magnitude of the reflex (T-reflex) to be assessed objectively and removes subjective variation.[6] Presently, subjective rating scales are used more widely than electromyography in clinical settings. Despite the theoretical benefits of using standardized rating scales rather than capricious methods of describing reflex magnitude, recent research has shown that there is high interobserver variation between medical professionals when they subjectively assess reflexes.[4,5,7,8]

With recent advances in digital video recording technology and computer processing it is now possible to accurately measure movement in three-dimensional space. When assessing the patellar reflex using this technology, confounding factors based on subjective judgments are excluded and variables that accurately describe the movement of the reflex can be calculated.

The objectives of this study were first to assess the ability of subjective ratings to represent objective measurements of the patellar reflex. Second, the accuracy of three different groups of raters (with different levels of experience in assessing reflexes) using three different rating scales was assessed. Third, we sought to determine which aspects of the reflex the rater relied on most when assessing the patellar reflex—that is, which objective variables best correlated with a rater's assessment. And last, we aimed to identify whether years of clinical experience in measuring reflexes affect how accurately a rater can assess a reflex when compared with the objective reflex measures.

METHODS

Ethics Approval. Ethics approval was obtained from the human research ethics committee (medical) of the University of the Witwatersrand (M10266) and written informed consent was obtained from all participating subjects and clinicians.

Procedures. Data for this study were collected in two phases. The first phase involved recording objectively the patellar reflex in 15 subjects using electromyography and biomechanical measurements as well as video recordings. The second phase encompassed the subjective assessments of these same reflexes by 3 different rating groups. The details of each phase are described in what follows.

Abbreviations: ANOVA, analysis of variance; GP, general practitioner; NINDS, National Institute of Neurological Disorders and Stroke; VAS, visual analog scale

Key words: change of knee angle, kinematics, patellar reflex, reflex rating scales, T-reflex

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Published online 00 Month 2012 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mus.23487

Phase I. Patellar reflex measurements were performed on subjects seated on a standardized chair, which allowed the legs to swing freely. Reflexes were elicited using an automated axle-mounted patellar hammer.[9,10] A removable image calibration (12-point) frame, defining a calibrated measurement volume of approximately 3 m^3, was constructed around the patellar hammer and included space allowance for the forward swinging motion of the leg as part of the patellar reflex.

The right leg patellar reflex of 15 healthy subjects was recorded using five high-speed cameras (E-PLA741; Pixellink, Ottawa, Ontario, Canada) positioned around the calibrated measurement volume. Two additional conventional digital video cameras (Model DCR-HC21E; Sony, Japan) were positioned at a right angle to the subjects' legs (side view of the reflex) and at an oblique angle to the subjects' legs (Fig. 1). Five retroreflective markers were positioned on each subject: on the lateral thigh; the medial and lateral epicondyles of the femur; the medial malleolus of the tibia; and the lateral malleolus of the fibula. A further two retroreflective markers were placed on the head and the foot of the patellar hammer. Simultaneous T-reflex recordings of the quadriceps muscles were obtained during each reflex measurement, by positioning two electrodes over the extensor quadriceps muscle (Fig. 1). The first electrode was placed 5 cm above the superior margin of the patella, and the second electrode was placed 5 cm proximal to the first. A third grounding electrode was positioned on the upper half of the contralateral leg. T-reflex recordings were made using PowerLab (26T; ADInstruments, Bella Vista, Australia).

The measurement volume was calibrated using the calibration frame (described previously) prior to each reflex recording. Subjects were seated comfortably with their legs hanging freely. Each subject was struck on their patellar tendon with a manual reflex hammer to locate the spot that would elicit the best reflex. This spot was marked, and the axle-mounted automated patellar hammer was adjusted for each subject to hit the preselected spot. As the hammer was automated, it allowed an equal force to be administered for each reflex. The reflex was recorded concurrently by the high-speed cameras and the conventional cameras. Images from the high-speed cameras were recorded simultaneously at a frame rate of 50 Hz, and the cameras were synchronized before and after the patellar reflex using an LED light as a visual cue.

All image processing and subsequent kinematic data handling were conducted using MATLAB, version 7 (The Mathworks, Inc., Natick, Massachusetts). A customized algorithm was used to track

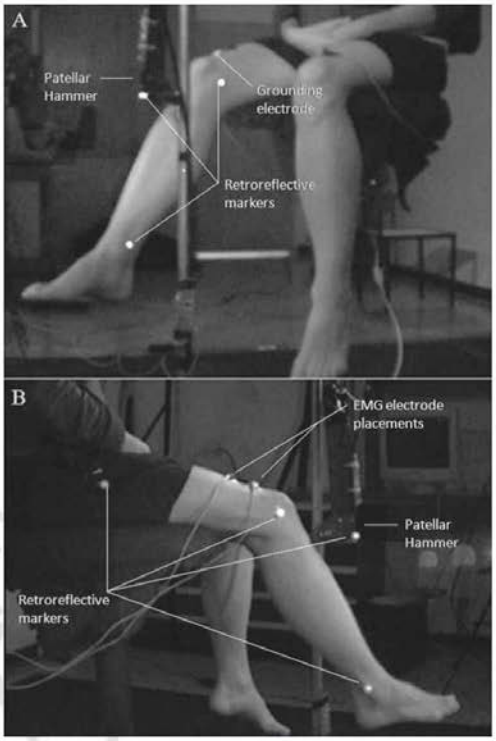

FIGURE 1. Still images from two conventional video cameras. (**A**) Side view of the reflex. (**B**) Oblique angle of the reflex.

movement of the retroreflective markers throughout the hammer drop and the subsequent reflex action. Each marker was reconstructed in three dimensions using a modified direct linear transform algorithm[11] and reconstruction algorithm[12] creating a three-dimensional (3D) model of the hammer and the leg (Fig. 2). The starting knee angle, maximum knee angle, time taken to reach the maximum angle, and hammer velocity at time of strike were determined, and the maximal angular acceleration of the knee, change in knee angle (from its initial point to maximum angle), velocity of the reflex (maximum angle/time to maximum angle), and movement latency (time taken between hammer strike and initiation of movement) could all be calculated. Three other objective variables were taken from the T-reflex recordings, including: the maximum amplitude of the T-reflex; the negative peak duration during the T-reflex; and the T-reflex latency (time taken between the hammer strike and the beginning of muscle activation, corrected for height). It should be noted the T-reflex latencies here do not include a hammer-specific delay, which is an artificial artifact

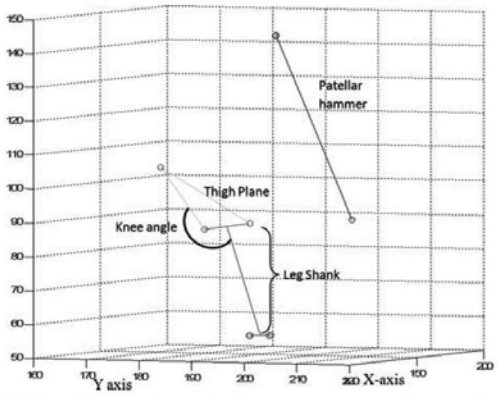

FIGURE 2. Three-dimensional representation of the patellar reflex demonstrating the starting position of the patellar hammer with the leg at rest. Circles indicate marker positions on the top and bottom of the patellar hammer, the lateral thigh, the epicondyles of the femur (knee), and the lateral and medial malleoli (ankle). The lines form the leg shank, which was taken from the midpoint of the two knee markers to the midpoint of the two ankle markers, and the thigh plane, which incorporates the thigh marker and the two knee markers.

originating from the inertial switch inherent to many electrically triggered patella hammers.

Phase II. Video footage, from the two conventional video cameras of the 15 recorded reflexes, was assessed as follows by three independent groups of raters. Medical students from the University of the Witwatersrand (ranging from second to fourth year of study), general practitioners, and neurologists were shown the 15 video recordings of the reflexes (in randomized orders) and asked to assess the reflexes using three different rating scales. These scales were the NINDS reflex scale,[3] the Mayo clinical reflex scale,[3] and a visual analog scale (VAS), ranging from an absent reflex as one anchor and clonus as the other anchor.

Each rater also completed a questionnaire documenting demographic data, number of years as a medical professional (for the medical students this was taken as their year of study), and experience at assessing the patellar reflex.

Statistical Analysis. All data are presented as median ± interquartile range (unless otherwise specified). Statistical analysis was performed using MATLAB (version 7) and GraphPad Prism (version 5.00 for Windows; GraphPad Software, San Diego, California). The nonparametric nature of all relevant data was confirmed using the Lilliefors test.

The relationship between subjective ratings and the objective variables was assessed using Spearman's rank correlations for each of the rating scales. These were performed for each individual rater, each group of raters (medical students, general practitioners, or neurologists), and the combination of all three groups.

To compare the correlations between the eight objective measurements of reflex magnitude, the three rating groups, and the three subjective rating scales, a 3 × 3 × 8 analysis of variance (ANOVA) was used. In a separate analysis, to assess which of the five kinematic (observable) variables were correlated with the subjective rating of each rater, a stepwise regression analysis was performed on each rater's data. This regression process was performed for each rater in each of the three groups and for each subjective rating scale. The resultant data showed which kinematic (observable) variables best matched the subjective rating for each rater, yielding a beta-value for each variable. These beta-values were compared for each rating scale and between subject groups to assess whether different kinematic variables were chosen for each. The latter comparison was achieved by using a 3 × 3 × 5 ANOVA followed by Kruskal–Wallis tests and Dunn's post hoc tests to locate differences should they exist.

Spearman's rank correlations were also performed to correlate experience and duration as a medical professional to rating each of the objective variables.

RESULTS

Subjects. Demographic data for 15 reflex-tested subjects are shown in Table 1. Analysis of the 15 reflexes showed a wide variation in reflex magnitude as assessed by both kinematic and electromyographic methods. It should be noted that our T-reflex latency values (57.6 ± 19.5 ms/m) varied greatly from the normal range of values (18.7 ± 1.1 ms/m) obtained by conventional automated inertial switch-based hammers.[13] The age, gender, years as a medical professional, and experience at rating the patellar reflex of the rater groups are shown in Table 2. T1 T2

Objective 1: Ability of Subjective Ratings to Represent Objective Measurements. The correlations between each rater group and objective measurements all displayed the same pattern and thus are shown as combined data. The subjective ratings were

Table 1. Demographic characteristics of the subjects ($n = 15$) used to measure patellar reflexes.

Variable	Mean	Standard deviation	Range
Age (years)	23.73	5.20	19–40
Height (cm)	164.13	10.34	147–184
Weight (kg)	64.28	10.72	49.7–83.7
Body mass index (kg/m^2)	24.01	4.44	18.5–35.8
Gender (M/F)	6/9		

Table 2. Characteristics of the three groups of subjective raters obtained from the questionnaires.

Variables	Mean	Standard deviation	Range
Students ($n = 24$)			
Age (years)	20.21	0.98	19–23
Experience at rating the reflex (years)	0.17	0.38	0–1
Years at medical school (years)	2.5	0.78	2–4
Gender (M/F)	6/18		
General practitioners ($n = 16$)			
Age (years)	46	8.57	33–67
Experience at rating the reflex (years)	18.19	9.73	6–41
Years as a medical professional (years)	19.38	8.39	9–41
Gender (M/F)	10/6		
Neurologists ($n = 12$)			
Age (years)	32.27	4.98	27–45
Experience at rating the reflex (years)	9.25	3.67	5–17
Years as a medical professional (years)	8.17	3.83	4–17
Gender (M/F)	8/4		

correlated with many of the objectively measured variables, and there were very strong correlations between the subjective assessments of the reflex for all three scales and the T-reflex maximum amplitude and change in knee angle during the reflex (Table 3). T3

Objective 2: Differences between Accuracy of Rating Scales and Rating Groups. To assess how closely each rating group and rating scale were related to the different objective measurements of the reflexes, the correlation between each individual's ratings and each objective variable, as well as for each scale, was used. The mean squared Spearman's correlation coefficients (R^2) between the objective variables and each rating scale were used to create a 3 × 3 × 8 (3 rating groups × 3 rating sales × 8 objective variables) matrix. The variables most closely related to the subjective ratings were the change in knee angle and T-reflex maximum amplitude for all 3 scales, and these are shown in Figure 3. The results of the 3 × 3 × 8 three-way ANOVA comparing all of these correlations for all eight objective variables shows that there was no significant difference between the accuracy of either the three rating groups or the three rating scales. However, there was a significant difference between how closely the rater assessments related to the different objective variables ($F = 1690.42$, $P = 0.0$; Table 4). F3 T4

Objective 3: Identification of Kinematic Properties of Reflex that Raters Rely on When Subjectively Assessing Patellar Reflex. Stepwise multiple regressions showed that all rating groups (using all rating scales) relied primarily on the change in angle of the knee to assess the reflex. Many of the raters also relied on the maximum angular acceleration. The beta-values of the regressions also showed that a few raters relied on the velocity of the reflex, movement latency, and the time to maximum angle when assessing a reflex. The 3 × 3 × 5 three-way ANOVA analysis of these beta-values (models for 3 subject groups, using 3 different rating scales, and obtaining beta-values for all 5 kinematic variables) showed that there were significant differences between which kinematic properties of a reflex the three rating groups relied on when rating a reflex (Table 5). Kruskal–Wallis tests between the beta-values of objective variables contributing to predictive models of the subjective ratings indicated that students and GPs rely significantly more on change in angle of the knee during the reflex than do neurologists. Further analysis indicates that the beta-values for variables predicting scoring T5

Extend the line

Table 3. Squared Spearman's correlation coefficients (R^2) for biomechanical and T-reflex variables correlated with mean ratings from three reflex rating scales.

	Biomechanical variables				
Scale	Maximal angular acceleration	Change in angle of the knee	Time to maximum angle	Velocity of reflex	Movement latency
NINDS reflex scale	0.00	0.79‡	0.09	0.40*	0.45†
Mayo clinical reflex scale	0.16	0.72‡	0.09	0.39*	0.40*
Visual analog scale	0.01	0.72‡	0.06	0.48†	0.47†

	T-reflex variables		
	Maximum amplitude	Negative peak duration	Latency
NINDS reflex scale	0.87‡	0.02	0.02
Mayo clinical reflex scale	0.94‡	0.04	0.00
Visual analog scale	0.84‡	0.03	0.00

*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

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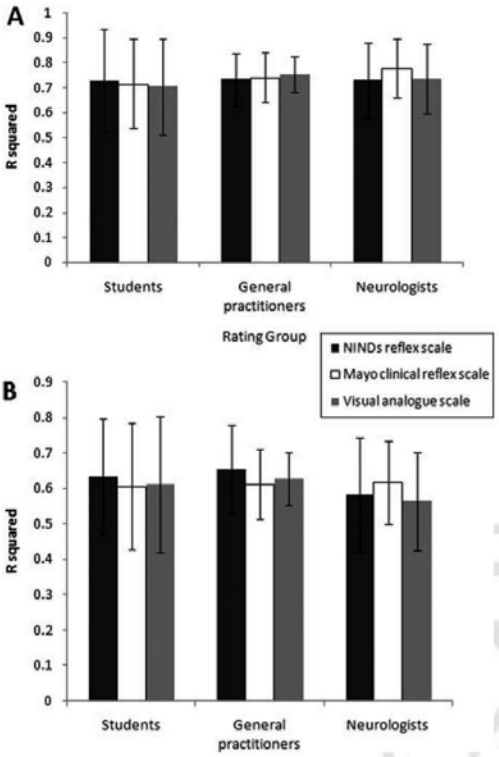

FIGURE 3. Comparisons of squared Spearman's rank correlation coefficients (R^2) values between scores for all three subjective rating scales and the change in angle of the knee during the reflex (**A**) and T-reflex maximum amplitude of the quadriceps muscle during the reflex (**B**). Data are presented as mean and standard deviation. The data show that there was no significant difference between the three rating groups or between the three rating scales.

of the NINDS reflex scale differed significantly from both the Mayo clinical scale and the VAS, and that the VAS and Mayo scales had higher beta-values, and were therefore more predictable when using objective properties of the reflex (significantly higher beta-values of $P < 0.05$).

Objective 4: Role of Experience of Rater when Assessing a Reflex. There were no correlations between the experience at rating the patellar reflex or years as a medical professional and the ratings of the reflexes as compared with the objective variables.

DISCUSSION

The principal findings of this study are first that subjective assessments of the patellar reflex are closely related to T-reflex maximum amplitude and biomechanically measured change in angle of the knee during the reflex. Second, there was no significant difference between the accuracy of the ratings of the patellar reflex (as defined by objectively measured parameters) given by students, general practitioners, and neurologists or between the accuracy of the NINDS reflex scale, the Mayo clinical reflex scale, and a VAS. Third, we showed that, when assessing a patellar reflex, an individual relies predominantly on the change in knee angle during the reflex. Last, experience at assessing reflexes was shown to have little effect on how accurately an individual assesses a reflex. It should be noted that the design of the study necessarily limited evaluators to watching videos of the reflexes and not eliciting the reflexes themselves. Although the use of such video analysis may not directly reflect clinical settings, using this approach was fundamentally necessary for standardization.

Table 4. Results of 3 × 3 × 8 unbalanced three-way ANOVA comparing squared Spearman's correlation coefficients values for three rating groups, three rating scales, and the eight objective variables.

Source	Sum of squares	Degrees of freedom	Mean squares	*F*-value
Scales	0.002	2	0.0012	0.17
Objective Variables	89.129	7	12.7327	1690.42*
Groups	0.004	2	0.0019	0.25
Scales vs. Objective Variables	0.019	14	0.0013	0.18
Scales vs. Groups	0.007	4	0.0017	0.22
Groups vs. Objective variables	0.152	14	0.0108	1.44
Scales vs. Objective Variables vs. Groups	0.063	28	0.0022	0.30
Error	8.858	1176	0.0075	
Total	105.294	1247		

*$P = 0.0$.

Objective 1: Ability of Subjective Ratings to Represent Objective Measurements. The two objective variables that were most strongly correlated with the

Table 5. Results of 3 × 3 × 5 unbalanced three-way ANOVA comparing beta-values for three rating groups, three rating scales, and the five visible (kinematic) objective variables.

Source	Sum of squares	Degrees of freedom	Mean squares	*F*-value
Scales	9.12	2	4.5617	0.84
Objective Variables	4.24	4	1.0593	0.19
Groups	23.22	2	11.6101	2.13
Scales vs. Objective Variables	30.36	8	3.7954	0.69
Scales vs. Groups	7.38	4	1.8443	0.34
Groups vs. Objective Variables	96.5	8	12.0628	2.21*
Scales vs. Objective Variables vs. Groups	29.97	16	1.8733	0.34
Error	4015.09	735	5.4627	
Total	4206.07	779		

*$P = 0.0251$.

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subjective ratings given by all raters were: the change in angle of the knee during the reflex, and the T-reflex maximum amplitude (Table 3). Change in angle is a kinematic variable and can be seen by the rater when assessing a reflex. All T-reflex variables are not visible to the rater when observing a reflex. The maximum amplitude of the T-reflex in the quadriceps muscle has been used in clinical studies, in combination with motor evoked potentials.[14] The T-reflex amplitude has also been shown to be representative of the force of the muscle contraction during the patellar reflex,[5] which influences the size and briskness of a reflex. The relationship of this variable to leg movement, which is visible to the eye, may explain why the subjective assessment of a reflex would be so strongly correlated with the T-reflex maximum amplitude. Note, however, that this study looked at T-reflex amplitude only where it may have also been useful to standardize the maximum surface electromyographic response (the Tmax/Mmax ratio).

Surface electromyography is a simple, non-invasive method of reflex assessment.[15] This study has established that it also accurately represents the subjective assessment of a reflex when clinical reflex rating scales are used. Stam and van Crevel found a close relationship between observer assessments of the briskness of the patellar reflex using the Mayo clinical scale and the T-reflex amplitude of the patellar reflex.[5] It could then be hypothesized that T-reflex maximum amplitudes of the patellar reflex would provide an unbiased description of both the size of the reflex and the briskness of the reflex. T-reflex amplitude may be more representative of muscle activation than the leg movement that results; therefore, it is possible that T-reflex amplitude is a more useful measurement than any assessment of the resulting leg movement. T-reflex amplitudes, however, vary significantly between reflexes, even in the same individual.[16] T-reflex amplitudes have also been found to have low reproducibility in clinical settings.[15] In addition, T-reflex amplitude is known to be influenced by a large number of variables such as the force of the hammer, muscle tone, and subject alertness.[6] Despite this, we have shown that, when raters recognize the size of the reflex, a subjective rating of the reflex is strongly related to T-reflex maximum amplitude. However, the relative usefulness of a subjective rating scale versus the T-reflex of potentially low reproducibility (but potentially more related to muscle activation) remains unknown.

A descriptive variable of a reflex that remains constant even during multiple repetitions will be better able to indicate a neurological disturbance.[16] T-reflex latency is just such a variable.[16] The study showed that T-reflex latency is not correlated with the subjective assessment of the reflex along with two other variables: the time taken to reach maximum angle, and the negative peak duration (Table 3). Further clinical analysis of these variables needs to be done to determine whether they could be used in a more sensitive, specific, and accurate test of patellar reflexes.

In this study we have shown that all the rating scales used are highly predictive of objective kinematic measurements of the change in knee angle during the patellar reflex. Such a comparison may support the emerging use of objective methods to assess reflexes.

Objective 2: Differences between Accuracy of Rating Scales and between Rating Groups. Previous studies have found that there is a large amount of interobserver variation in the subjective assessment of the patellar reflex.[4,5,7,8] Our results show that there was no significant difference between any of the raters in their assessments of the reflexes. This could be due to factors related to the design of the experiment.

In all previous studies the reflex was elicited by raters themselves in order to reflect the clinical setting.[4,5,7,8] Because the force, location of where the hammer hit the leg, and the path of the hammer were not controlled, a wide variation in actual reflex size would have been observed in these studies. Therefore, it is likely that the large amount of interobserver variation would have been caused by the large amount of variation between reflexes. All of these aspects were controlled for in the current study by the raters viewing the same reflex that was elicited by an automated axle-mounted patellar hammer. The standardization of our group of reflexes and perhaps the uniform presentation of the reflexes to raters, may account for the low interobserver variation.

Litvan et al. also showed a low interobserver variation.[17] They used only four neurologists with similar backgrounds and techniques,[17] which may have decreased the variation between reflexes and decreased the interobserver variation. Although it may be suggested that the degree of standardization of reflexes in our study does not imitate the clinical setting, the differences seen between our findings and findings of non-standardized application of the patellar hammer emphasize the significant role that such variation in application of the patellar reflex may play.

We have also shown that there was no difference between the accuracy of the rating scales. Clinically, only the NINDS and Mayo clinical rating scales have been validated. Manschot et al.

compared the NINDS and Mayo clinical rating scales and found that both were equally poor at showing agreement between raters.[4] We found that the opposite was true in that both of these scales were equally proficient at showing agreement between raters when compared with objective measures of the reflex (Table 3). Differences between the findings in our study and those of Manschot et al.[4] may be due to the fact that in our study the same recorded reflexes were rated using the two scales. This decreased the variation between reflexes, which decreased the variation between observers. This led to low interobserver variation between the two scales. Medical students who had no clinical experience at rating reflexes could comfortably use both of the standard rating scales, the NINDS and the Mayo scales, which highlights their ease of use.

Objective 3: Identification of Kinematic Properties of the Reflex that Raters Rely on when Subjectively Assessing Patellar Reflex. We found that the raters rely most on the change in angle of the knee when assessing the patellar reflex. We also found that students and GPs rely significantly more on the change in knee angle when assessing a reflex than do neurologists. Because we quantitatively assessed all the fundamental mechanics of each reflex, and because none of these mechanical factors were substituted by neurologists (for the decreased reliance on the change in knee angle, compared with the other raters), we speculate that neurologists may rely on a variable that was not measured in this study or an unquantifiable clinical skill. This variable could possibly be linear muscle acceleration, which is a direct measure of muscle velocity during the patellar reflex.[18]

The differences shown between the ability of objective measurements to predict the subjective ratings using different rating scales (the VAS and Mayo clinical scale are more closely predicted by individual kinematic properties of a reflex than the NINDS scale) is most probably due to the construction and properties of these scales. The NINDS reflex scale has only five options, whereas the Mayo clinical reflex scale has nine options, and the VAS has a theoretically unlimited amount of options (continuous scale). The fact that the more descriptive scales are more closely predicted is likely related to the variation in options that are available, thus yielding higher beta-values in a stepwise multiple regression.

Objective 4: Role of Experience of Rater when Assessing a Reflex. Experience of rating the patellar reflex is often suggested as a variable that could influence interobserver variation when assessing reflexes.[7,17] It has been found that training does not affect interobserver variation,[17] and that reliability of a neurological examination does not increase with experience.[7] Our results show that, although students and neurologists may be looking at different facets of a reflex (see earlier), the amount of experience an individual has rating reflexes did not correlate with how well they assessed the reflex (measured as the correlation between kinematic properties of a reflex and subjective rating). Also, the number of years raters have been medical professionals did not correlate with their ability to rate the patellar reflex. The results of previous studies[7,17] combined with the results from this study therefore suggest that experience at rating the patellar reflex does not affect how accurately an assessor rates a reflex.

In conclusion, in this study we have found that deep tendon reflexes can be reliably assessed by clinical observers using reflex rating scales and that previously reported poor interrater reliability may be due to the variability caused by viewing different reflexes and the possible inconsistent use of manual reflex hammers (unlike in this study where the same reflexes were assessed, by each rater). Subjective assessments of patellar reflexes are strongly correlated with objective kinematic and electromyographic measurements of those reflexes. Also, we found that the property of the patellar reflex that is most closely monitored when using subjective rating scales is the change in angle of the knee.

The authors thank all subjects who participated in our study for their time and patience during the recording of the reflexes. We also thank the medical professionals and students for their precious time. Funding for this study was provided by the Biomechanics Laboratory, School of Physiology, University of Witwatersrand. The financial assistance of the South African National Research Foundation (NRF) toward this research is acknowledged.

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