SLEEP IN PATIENTS WITH PAINFUL DIABETIC PERIPHERAL NEUROPATHY: IMPACT OF PAIN, GLUCOSE AND PHARMACOLOGICAL INTERVENTION

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By

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Malin Eriksson
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This thesis is dedicated to my family; mum, dad and my brother Carl-Johan Eriksson. Thanks for all your love and support, I could not have done it without you. The dedication is also to my late brother Mathias Eriksson, you are in my thoughts.
ABSTRACT

Sleep problems are one of the most common complaints in patients with diabetes. Sleep problems can impact on quality of life and contribute even further to the burden of living with diabetes. The aim of this thesis was to establish if there were any differences in objective sleep between patients with painful peripheral neuropathy and a historically created healthy control group. A further aim was to investigate the impact of pain, glucose and clinical sleep disorders such as sleep apnoea and periodic limb movements on sleep in patients with painful diabetic peripheral neuropathy. In addition the study investigated three of the most common treatments for neuropathic pain in this patient group: pregabalin, duloxetine and amitriptyline, to see if there were improvements in pain and sleep.

Polysomnography was used to assess sleep in both the diabetes group and healthy control group. Diabetic patients had significantly less total sleep time, lower sleep efficiency, more wake time, increased latency to sleep and felt they were significantly sleepier during the day compared with healthy volunteers. The poor sleep in the diabetic study group was not associated with pain or nocturnal glucose levels (hyperglycaemic/hypoglycaemic events). Prevalence of periodic limb movement was high in this patient group but the number of events was considered mild and not associated with any arousals or awakenings. Sleep apnoea did however correlate with the number of awakenings and there was a correlation between an increase in lighter stage 1 sleep and an increase in apnoea/hypopnoea index.

All three treatments were effective at reducing pain, and no treatment was superior with regards to analgesic efficacy. Clear separation of the treatments was found when looking at objective sleep with pregabalin significantly improving and duloxetine worsening sleep while amitriptyline showed little change when compared with placebo. Clinical sleep variables
however suggested that pregabalin might increase the incidence of apnoeas highlighting that all factors need to be considered when deciding on the best treatment regime.

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<tr>
<td>LPS</td>
<td>Latency to persistent sleep</td>
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<tr>
<td>LSEQ</td>
<td>Leeds Sleep Evaluation Questionnaire</td>
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<tr>
<td>NAW</td>
<td>Number of awakening</td>
<td></td>
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<tr>
<td>NREM</td>
<td>Non rapid eye movement sleep</td>
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<tr>
<td>OSA</td>
<td>Obstructive sleep apnoea</td>
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<tr>
<td>PDK</td>
<td>Phosphoinositide dependent kinase</td>
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<td>PGB</td>
<td>Pregabalin</td>
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<tr>
<td>PI</td>
<td>Phosphatidylinositol</td>
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<tr>
<td>Abbreviation</td>
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<tr>
<td>PKB</td>
<td>Protein kinase B</td>
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<td>PLM</td>
<td>Periodic Limb Movement</td>
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<td>PSG</td>
<td>Polysomnography</td>
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<td>QOL</td>
<td>Quality of life</td>
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<td>QOS</td>
<td>Quality of sleep</td>
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<td>REM</td>
<td>Rapid eye moment sleep</td>
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<td>RLS</td>
<td>Restless leg syndrome</td>
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<td>ROC</td>
<td>Right outer canthus</td>
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<tr>
<td>SCN</td>
<td>suprachiasmatic nucleus</td>
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<tr>
<td>SE</td>
<td>Sleep efficiency</td>
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<td>SOL</td>
<td>Sleep onset latency</td>
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<td>SDB</td>
<td>Sleep disorder breathing</td>
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<td>SSRC</td>
<td>Surrey sleep research centre</td>
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<tr>
<td>SWS</td>
<td>Slow wave sleep</td>
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<tr>
<td>TST</td>
<td>Total sleep time</td>
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<tr>
<td>VLPO</td>
<td>ventrolateral preoptic nucleus</td>
<td></td>
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<tr>
<td>WASO</td>
<td>Wake after sleep onset</td>
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CHAPTER 1 - INTRODUCTION
CHAPTER 1 GENERAL INTRODUCTION

1.1 Diabetes mellitus

The prevalence of diabetes mellitus (DM) worldwide in 2000 was estimated to be 171 million according to the World Health Organisation, making it one of the most common chronic medical disorders seen in clinical practice (Galer et al., 2000; WHO, 1999). The number of people with diabetes is increasing rapidly and the estimated prevalence for 2010 is 221 million according to the International Diabetes federation (IDF). This increase is due to the increased prevalence of obesity and physical inactivity amongst people but also due to the growth of the world’s population. It is thought the numbers will continue to rise and have been anticipated to have reached 334 million by 2025 and 366 million by 2030 according to IDF and WHO, respectively (Wild et al., 2004).

The two most common forms of diabetes are type I DM and type II DM, with the latter being the far most common of the two and accounting for around 90% of all cases worldwide (Alberti and Zimmet, 1998; Graves and Eisenbarth, 1999; Kasuga, 2006; Lewis, 1999; Luna, 2005; Vinik and Flemmer, 2002).

1.1.1 Type I DM

Type I DM is an autoimmune disorder known to have strong genetic links, but environmental factors such as viruses may also play a role in the development of type I DM (Bresson and Herrath von, 2004). The onset may occur at any age but it most commonly occurs in childhood and adolescence. The progression of the disease may vary from slow to rapid, and rapid progression is most common in children (Bresson and Herrath von, 2004; Pozzilli and DiMario, 2001; WHO, 1999).
1.1.2 Type II DM

The onset of type II DM is more common later on in life and the risk of developing type II DM increase with age. However, its occurrence in children and adolescence is nowadays much more common and is thought to be due to the increased prevalence of obesity (Pinhas-Hamiel et al., 1999). A study by Haines et al showed that 95% of children diagnosed with type 2 DM were overweight and 83% were obese (Haines et al, 2007). Factors such as centripetal obesity and lack of exercise increase the risk of developing type II DM (WHO, 1999). In fact, a body mass index (BMI) of > 25 increases the risk of developing type II DM by 3-fold and between 60% and 90% of diabetic patients are clinically overweight (Kasuga, 2006; Petersen Falk and Shulman, 2006). However, type II DM is also known to have a strong genetic component. This has been shown in a study of identical twins, where a 100% concordance rate for type II DM was found (Barnett et al., 1981). Another study by Barnett et al. (1981) found that 48 out of 53 non-insulin-dependent diabetic identical twin pairs were concordant for diabetes. In the 5 pairs which were not concordant the diabetic twin had just recently been diagnosed. Tests on the 5 non affected twins in these pairs showed that they had higher fasting glucose levels and higher HbA1c compared to controls, indicating that they are also prone to develop type 2 DM (Barnett et al., 1981). These were very early studies and were just an indication of a possible genetic link. The genetics of type II DM are still unclear and thought to be extremely complex, but more recent studies have indicated stronger genetic links with frequent polymorphisms of many genes such as insulin, calpain 10 involved in pathways of insulin secretion and glucose metabolism, and peroxisome proliferator-activated receptor (PPAR) gamma involved in regulation of metabolism of carbohydrate, lipids and protein. There is also evidence that differences in the insulin receptor substrate-1 (IRS-1) gene may be associated with type 2 DM. IRS-1 plays a important role in the insulin signalling pathway which will be discussed later (Hansen and Pedersen, 2005;
Kovacs et al., 2003; Leahy, 2005; Malecki, 2005). Leahy (2005) has also suggested a decrease in β-cell mass through genetic factors as a possible predisposing factor for glucose intolerance and development of type 2 DM (Leahy, 2005). Further supportive evidence for a genetic aetiology is the high prevalence of type II DM in some ethnic populations. A study by Haines et al showed that the incidence rate of type 2 DM in children was far higher in south Asians and blacks, in fact 3.5 and 11 times higher, respectively, compared to Caucasians (Haines et al., 2007). Similar findings have been seen in the US with increased prevalence of type 2 DM seen in Pima Indians, Hispanics, Asians and Afro-Caribbeans compared to US Caucasians (American Diabetes Association, 2000; Zhang et al., 2009). However, this difference may be a combination of genetics as well as environmental factors and the fact that some ethnic groups appear to have higher prevalence of obesity which is known to be a risk factor in developing type 2 DM (American Diabetes Association, 2000; Wardle et al, 2006).

Type II DM was historically considered a condition of wealthy Western nations but its prevalence is now rapidly increasing world-wide. Africa has shown a projected increase of as much as 111%, and the projected increase in South East Asia is estimated to be another 40 million cases by 2025. This increase may be due to change in life style (IDF, 2003). Type II DM is a global cause of increased morbidity and it is therefore important to try to understand its genetic and metabolic aetiologies (Petersen Falk and Shulman, 2006).

1.2 Diabetes and glycaemic control

Diabetes is a serious chronic metabolic disorder characterized by persistent high blood sugar levels (hyperglycaemia) which is caused by impaired glucose homeostasis (Graves and Eisenbarth, 1999; Luna, 2005; WHO, 1999). This impairment is due to defective insulin
action, insulin secretion or both. This will be discussed in more detail later on but first one needs to understand the complexity of normal glucose homeostasis.

1.2.1 Normal glucose homeostasis

Glucose is an important source of energy for the human body. Under normal circumstances there is a balance between the glucose in the systemic circulation and tissue glucose uptake and utilization - this is known as glucose homeostasis. This homeostasis is balanced by the organised interaction between insulin secretion, tissue glucose uptake and glucose production in the liver (hepatic glucose production), and is tightly regulated as it is important to keep the plasma glucose concentrations within a narrow window (Zierath and Kawano, 2003). Normal fasting plasma glucose levels should be below 6.1 mmol/l and at 2 hour post glucose dose the levels should be below 7.8 mmol/l (Messier, 2005).

1.2.1.1 Role of Insulin

Insulin is a peptide hormone which is synthesized and secreted by the β-cells of the islets of Langerhans of the pancreas in response to increased circulating levels of glucose and amino acids. It plays an important role in metabolic processes such as carbohydrate (table 1.1), lipid and protein metabolism and is essential for maintenance of glucose homeostasis. Furthermore, insulin is important in cell proliferation and gene transcription.
Hepatocytes | Muscle cells | Adipocytes
---|---|---
↑ Glycogenesis | ↑ Glycogenesis | ↑ Glycogenesis
↑ Glycolysis | ↑ Glycolysis | ↑ Glycolysis
↑↓ Gluconeogenesis | ↑ Glucose uptake | ↑ Glucose uptake
↓ Glycogenolysis | | |

Table 1.1: Role of insulin in carbohydrate metabolism. Insulin increases glycolysis (use of glucose for energy) in adipocytes, muscle cells and hepatocytes. It also increases glucose uptake to muscle cells and adipocytes by facilitating mechanisms that translocate GLUT4 glucose transporters to plasma membrane. Furthermore, insulin also stimulates glycogenesis (synthesis of glycogen from glucose) in adipocytes, muscle cells and hepatocytes. It also inhibits hepatic glycogenolysis (breakdown of stored glycogen into glucose) and can regulate gluconeogenesis (synthesis of glucose from non-carbohydrate sources).

The different metabolic processes are mediated by insulin binding to its receptors on the plasma membrane of, for example, hepatocytes, adipocytes and skeletal muscle cells (Greenfield and Campbell, 2004; Lebovitz, 2005). The binding of insulin causes a rapid autophosphorylation of the insulin receptor activating the receptor and triggering a cascade of intracellular events (figure 1.1). The main pathway being activated is the insulin receptor substrate (IRS)/phosphatidylinositol 3 kinase (PI) pathway. This IRS/PI 3 kinase pathway is also called phosphoinositide dependent kinase (PDK)/protein kinase B (PKB) pathway as it causes activation of these two kinases (Luna, 2005). The active insulin receptor can phosphorylate IRS which becomes active. Active IRS can activate PI-3 kinase via p85 binding, which is the regulatory subunit of PI-3 kinase and acts as its adaptor. Active PI-3 kinase can then phosphorylate PI 4,5-bisphosphate to form PI 3,4,5-trisphosphate. The latter can recruit PKB to the plasma membrane of the cell and PDK-1 can then phosphorylate PKB which becomes active. This activation stimulates glycogen synthesis as active PKB phosphorylates and therefore inactivates GSK-3 (glycogen synthase kinase 3). Inactive GSK-3 can no longer inhibit glycogen synthase and glycogen synthesis can therefore take place.
Active PKB is also thought to phosphorylate several other proteins involved in the translocation of GLUT 4 transporters to the plasma membrane in cells like adipocytes and skeletal muscle cells, therefore increasing the glucose uptake into these cells (Greenfield and Campbell, 2004).

The binding of insulin to its insulin receptor will also activate the Ras/mitogen-activated protein kinase pathway (Ras/MAP kinase pathway) which is involved in the regulation of cell growth and proliferation (Luna, 2005). The adaptor protein, growth factor receptor bound protein 2 (GRB2) binds to the active insulin receptor and son of sevenless protein (SOS) can then interact and bind to the SRC homology 3 (SH3) domain on GRB2. Activated SOS is involved in the formation of active Ras as it promotes the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP). Active Ras-GTP triggers the phosphorylation cascade involving MAP kinases and these can finally phosphorylate target proteins such as transcription factors in the nucleus.
GTP
GDP
Ras activation triggers
phosphorylation cascade involving MAP
kinases
activation on DNA
Cell growth and gene expression
GLUT 4
Figure 1.1: Insulin signalling pathways in adipocytes and muscle cells. 1. IRS/PI-3 kinase pathway. Binding of insulin activates insulin receptor and triggers IRS/PI-3 kinase pathway. This results in glycogenesis and possible translocation of GLUT 4 transporters. 2. Ras/MAP kinase pathway. Binding of insulin and activation of insulin receptor can also triggers Ras/MAP kinase pathway involved in cell growth and gene expression. IRS-insulin receptor substrate, P-Phosphate group, PI-phosphatidylinositol, PDK-phosphoinositide dependent kinase, PKB-protein kinase b, SOS-Son of sevenless protein, Ras-small GTP binding protein, GRB2-adapter protein, GLUT 4-glucose transporter, MAP kinase-mitogen activated protein kinase.

1.2.1.2 Glucose transport and metabolism

1.2.1.2.1 Fasting state

In the fasting state, blood glucose levels are maintained predominantly by hepatic glycogenolysis (breakdown of stored glycogen into glucose) and gluconeogenesis (glucose formation from a non carbohydrate source). When glucose levels decrease, the body responds by secreting glucagon from the pancreatic α-cells. Glucagon stimulates glycogenolysis and
therefore more glucose is released into the blood for transport to different tissues (figure 1.2) (Heller, 1999; Lewis, 1999). The rate of hepatic glucose production during fasting correlates with the rate of tissue glucose uptake in vital tissues and organs that require glucose as their energy source such as the central nervous system (CNS). This matching regulation of hepatic glucose production and tissue glucose uptake will maintain the fasting blood glucose levels at a constant concentration.

1.2.1.2.2 Fed state

After a meal (fed state) the blood glucose levels rises in the body and this will increase the production and secretion of insulin from the pancreatic β cells. As mentioned earlier the insulin molecules will bind to receptors located on the plasma membrane of the cells, resulting in autophosphorylation of the receptors and receptor activation. This activation stimulates the synthesis and translocation of GLUT 4 glucose transporters and increases the peripheral glucose uptake. GLUT 4 is only found in insulin sensitive cells such as the skeletal muscle cells, adipocytes and cardiac muscle cells and is the major transporter responsible for the insulin dependent glucose uptake. Skeletal muscle cells account for 75% of the glucose uptake, making it the major site of glucose disposal following ingestion or infusion of glucose while the adipocytes and cardiac muscle cells metabolize a smaller percentage (Zierath and Kawano, 2003).

Activation of insulin receptors promotes the metabolic pathway which converts the excess glucose into glycogen (glycogenesis). This process occurs mainly in hepatocytes (liver cells) but also in muscle cells, and the produced glycogen is stored and used as an energy reserve. Furthermore, the increase in plasma insulin concentration suppresses hepatic glucose production by decreasing glycogenolysis. All these responses minimize hyperglycaemia (high blood glucose levels) and return glycemic levels to lower pre-meal values.
Figure 1.2: Simplified diagram of glucose homoeostasis. Fed state: 1. Levels of blood glucose increase after a meal (blue triangles), and trigger the secretion of insulin from the pancreas. 2. This results in increased insulin concentrations (red circles). 3. Increased insulin concentration aids the insulin dependent uptake of glucose into skeletal muscle cells, adipocytes and hepatocytes (green arrows). Note that glucose uptake in the brain and liver is insulin independent. In addition, increase in insulin levels inhibits hepatic glycogenolysis (glucose synthesis from stored glycogen) and no further glucose is released into the blood stream (red arrow). These mechanisms regulate blood glucose levels which return to normal levels. Fasted state: 1. Low glucose levels trigger the secretion of glucagon (green circles) from the pancreas. 2. This results in high levels of glucagon. 3. Glucagon stimulates glycogenolysis in the hepatocytes so that glucose is synthesised and released into the blood stream and transported to vital tissues and organs such as the brain.

However, in diabetes this regulation malfunctions and blood glucose levels remain high (hyperglycaemia) and this is the main hallmark of diabetes (Graves and Eisenbarth, 1999; Herman and Kahn, 2006; Lebovitz, 2005; WHO, 1999).
1.2.2 Defects of glucose homoeostasis in diabetes

The malfunction of glucose homeostasis in DM is caused by either an autoimmune destruction of the insulin secreting β cells as seen in type I DM or by insulin resistance and β cells dysfunction seen in type II DM (Herman and Kahn, 2006; Porte, 2001). Such defects result in elevated glucose levels and diabetes is defined as fasting plasma glucose levels above 7.0 mmol/l or above 11.1 mmol/l at 2 hours post glucose dose (Messier, 2005).

1.2.2.1 β cell destruction (autoimmune) in type I DM

The destruction of β cells of the pancreatic islet of Langerhans is a result of an abnormal immune response to self antigens and only occurs in type I DM. Autoimmune β cell destruction will result in no or too little insulin being released (insulin deficiency). The deficiency of insulin results in no translocation of GLUT 4 transporters to the plasma membrane of adipocytes and muscle cells, and the insulin dependent glucose uptake into these cells is therefore inhibited. The result is that more glucose remains in the blood stream, leading to impaired glucose homoeostasis (Bresson and Herrath von, 2004; Graves and Eisenbarth, 1999; Narendran et al., 2003; Pozzilli and DiMario, 2001). Another effect of insulin deficiency is the increase of glycogenolysis in hepatocytes. Normally, insulin binds to its receptors on hepatocytes, triggering an intracellular cascade leading to the activation of glucogenesis. In the absence of insulin this process is inhibited and glycogenolysis occurs instead. The produced glucose can then be transported into the blood stream via the insulin independent GLUT 2 transporters and contributing even further to the hyperglycaemic state (see figure 1.3).
Defects of glucose homoeostasis in type I DM

Figure 1.3: Simplified diagram of the defects of glucose homoeostasis in type I DM. In type I DM there is autoimmune destruction of pancreatic β-cells leading to insulin deficiency. Insulin deficiency results in no translocation of GLUT 4 transporters to the plasma membrane of adipocytes and muscle cells, and the insulin dependent glucose uptake into these cells is therefore inhibited. The result is that more glucose remains in the blood stream. Another effect of insulin deficiency is the increase of glycogenolysis in hepatocytes. The produced glucose is transported into the blood stream via the insulin independent GLUT 2 transporters, increasing the glucose concentration even more.

1.2.2.2 Insulin resistance in type II DM

Insulin resistance means that insulin is still synthesised and secreted in response to increased blood glucose and will still bind to the insulin receptor (see figure 1.4). There are however defects in the intracellular signalling pathway, making the signal weaker (Lebovitz, 2005). This resistance to insulin is also thought to be due to abnormal insulin receptors and/or a deficiency of insulin receptors on the liver, muscle and fat cells (Lewis, 1999). Insulin resistance may be present for several years before overt hyperglycaemia occurs, as initially
the β-cells can cope with glucose loads by over production of insulin. Thereafter this mechanism begins to fail and gradual β-cell dysfunction will lead to hyperglycaemia (Kahn, 2000; Kahn et al., 1999; Kasuga, 2006; Porte, 2001; Prentki and Nolan, 2006).

1.2.2.3 β- cell dysfunction in type II DM

The most characteristic dysfunction of the β cells in type II DM is impaired insulin secretion. If an individual has reduced insulin sensitivity the β cells will as mentioned earlier respond by increasing the insulin secretion. However, this protective mechanism will eventually fail as the β cells cannot cope with keeping up this increase, resulting in an underproduction of insulin and recent studies suggest that type II DM only develops in insulin resistant people once β cell dysfunction occurs, resulting in a decline in insulin secretion (hyposecretion). This leads to failure in sustaining an adequate β cell compensatory response in the wake of insulin resistance (Kahn, 2000; Luna, 2005; Prentki and Nolan, 2006).

In type II DM there is also a change in insulin to proinsulin ratios. Proinsulin is an inactive precursor of insulin and only after enzymatic processing is it converted to active insulin, which is then secreted from the β cells. In healthy individuals a small amount of proinsulin escapes this processing and is released together with the insulin. However, in type II DM patients this proinsulin processing is reduced, leading to an increased secretion of biologically inactive proinsulin. The change in insulin to proinsulin ratio is also related to β-cell dysfunction and levels of hyperglycaemia (Kahn, 2000; Kahn et al., 1999; Kasuga, 2006; Porte, 2001; Prentki and Nolan, 2006; Roder et al., 1998).
Defects of glucose homoeostasis in type II DM

Increased insulin secretion to compensate for insulin resistance

Failure of compensatory mechanism results in glycogenolysis and gluconeogenesis, and synthesised glucose is released into bloodstream contributing further to the hyperglycaemic state

Increased insulin secretion to compensate for insulin resistance

Failure of compensatory mechanism results in glycogenolysis and gluconeogenesis, and synthesised glucose is released into bloodstream contributing further to the hyperglycaemic state

Compensatory mechanism begins to fail and gradual β-cell dysfunction occurs

Inhibition of insulin dependent glucose uptake, glucose remains in the bloodstream

Failure of compensatory mechanism results in glycogenolysis and gluconeogenesis, and synthesised glucose is released into bloodstream contributing further to the hyperglycaemic state

Results of insulin resistance and β-cell dysfunction

Figure 1.4: Simplified diagram of the defects of glucose homoeostasis in type II DM. Insulin resistance is due to several different factors. It can be due to abnormal insulin receptors or due to deficiency of insulin receptors. This means that even if insulin is secreted it can’t bind to insulin receptors and therefore its signalling actions are inhibited. Another factor can be a defect in the intracellular pathway, insulin can still bind to its receptor but defects in the pathway result in no signalling. When insulin resistance occurs the β-cells compensate by over production of insulin. However, this mechanism begins to fail when the β-cells cannot cope any longer and gradual β-cell dysfunction occurs. This results in inhibition of insulin dependent glucose uptake into adipocytes and muscle cells and glucose remains in the bloodstream. Glycogenesis in hepatocytes is decreased in the absence of insulin action and glycogenolysis (breakdown of glycogen to produce glucose) occurs instead. The produced glucose can be transported into the bloodstream via GLUT 2 transporters contributing even further to the hyperglycaemic state.
1.2.3 Hyperglycaemia

Hyperglycaemia is as mentioned earlier caused by several defects in glucose homoeostasis (Herman and Kahn, 2006; Porte, 2001). Asymptomatic hyperglycaemia and insulin resistance can be present for a long period of time before overt diabetes occurs (Laakso, 1999). In this state fasting plasma glucose can still be within the normal range, but increases abnormally in the fed state. This can later lead to overt hyperglycaemia which is when a subject has consistent blood glucose levels > 7.0 mmol/L, but symptoms may not occur until higher levels of above 15 mmol/L are reached. Hyperglycaemia is a risk factor for the development of diabetic complications as chronic levels of > 7.0 mmol/L can cause damage to blood vessels and organs. Such complications will be discussed in more detail later. The definition of acute hyperglycaemia varies between studies with values from 7.8 mmol/L to above 15 mmol/L.

Symptoms of chronic and acute hyperglycaemia include excessive thirst and excessive urination, blurred vision, fatigue, dry mouth and can lead to coma. In addition, acute hyperglycaemia > 16.5 mmol/L has been shown to result in impaired cognitive function and reduced mood (Sommerfield et al., 2004).

1.2.4 Hypoglycaemia

Hypoglycaemia is defined as low blood glucose levels. Low blood glucose in diabetic patients is described as < 3.3 mmol/l or in some cases < 3.9 mmol/l. However, symptoms rarely occur until levels fall below 3.3 mmol/l. Hypoglycaemia occur if there is an inappropriate excess of insulin relative to glucose. This scenario may for example occur if a diabetic patient receives too much insulin in relation to an inadequate carbohydrate meal. One
rare example is the patient with an insulinoma (insulin secreting tumour) in whom excessive and inappropriate insulin secretion leads to spontaneous hypoglycaemia.

When plasma glucose concentration falls, there is a physiological response by the release of some hormones such as glucagon which stimulates glucose synthesis, and adrenaline which inhibits glycogen synthesis. Glucagon is secreted by the pancreatic α cells and acts by binding to membrane receptors on target cells and activating the adenylate cyclase second messenger system. This results in increased hepatic gluconeogenesis and glycogenolysis leading to an increase in plasma glucose. The action of glucagon is therefore essential in regulating glucose levels and high insulin levels inhibit glucagon release and action (Heller, 1999; Lewis, 1999). In patients with diabetes, the glucagon and adrenaline response to hypoglycaemia begins to fail after about 2 years. These defects are progressive and most patients with type I DM have a reduced glucagon response after 5 years and ~ 40% have defects in adrenaline response after 15 years (Heller, 1999). The symptoms of hypoglycaemia are sweating, trembling, clamminess and anxiety and hypoglycaemia is a dangerous condition which may have serious effects on cerebral function. The brain is highly dependent on glucose and needs a continuous and adequate energy supply to maintain normal function. When this fails a second set of symptoms can be seen, such as headache, difficulty in thinking and confusion. Hypoglycaemia can even lead to unconsciousness, coma and even death unless glucose is rapidly administered and it is the most common cause for A&E attendance in diabetic patients (Lewis, 1999).

1.2.5 Managing of glucose homoeostasis in diabetes patients

Good glycaemic control is the primary objective for diabetic patients, as this will reduce the risk of hyper/hypoglycaemia as well as the risk of diabetic microvascular and cardiovascular complications (see section 3, diabetic complications).
Patients with diabetes must therefore learn how to monitor and control their glucose levels to minimize the risk of such complications. The most common way to do so is by self monitoring of blood glucose (SMBG).

1.2.5.1 Self monitoring of blood glucose (SMBG)

An instrument for the SMBG (finger stick blood glucose readings) has been developed to help control glucose levels. This is widely used by patients with type I DM, but is also used in some patients with type II DM, especially those treated with insulin. However, it has also been shown that SMBG may be a useful tool in some type II DM patients who are on diet treatment alone and/or oral hypoglycaemic agents (Harris, 2001; Özmen and Boyvada, 2003; Welschen et al., 2005). To achieve optimal use of SMBG, patients should go through proper glucose monitoring education, focusing on specific glycaemic targets and on how to respond to different glucose readings. SMBG provides real-time information about blood glucose levels, and helps the patient understand the impact of diet, medications and physical exercise on glucose levels on a daily basis (Harris, 2001). The frequency and timing of glucose monitoring varies widely amongst patients and often depends on different treatment regimes.

1.2.5.2 Continuous glucose monitoring system (CGMS)

The continuous glucose monitoring system (CGMS) is another instrument used to determine glucose levels. It should be used occasionally and be a supplement to SMBG rather than be used on a daily basis. There are various manufacturers on the market, but the principle of how they work is similar. A CGMS will measure the glucose continuously with an interval of for example 10 seconds and then give an average of these measurements every 5 minutes. Over a 24 h period it will provide 288 glucose readings which can then be downloaded to a computer for analysis. The CGMS can be worn for a period of up to 72 hours (Caplin et al., 2003;
As the CGMS reads glucose levels continuously, it not only identifies glucose trends, but can also identify nocturnal hypoglycaemia, when glucose checks by means of SMBG are difficult and impractical (Jeha et al., 2004; Melki et al., 2006). Hypoglycaemic episodes at night are common in patients with type 1 DM and being able to detect them by means of the CGMS is a great advantage (Jeha et al., 2004). In addition, the CGMS can be an excellent tool to improve metabolic control and this can lead to lower glycated haemoglobin (HbA1c) levels (see section 1.2.5.3 below). In order to validate the CGMS system it is important that the CGMS is calibrated with a SMBG test (finger stick reading) at least four times a day.

1.2.5.3 Glycosylated haemoglobin (HbA1c) measurements

HbA1c measurements are used to assess long term glycemic control (Özmen and Boyvada, 2003). The glycosylation take place on haemoglobin 1 on the erythrocytes (red blood cells) and is an irreversible process. The HbA1c levels indicate the amount of glycated haemoglobin present and the higher the blood glucose the higher levels of glycated haemoglobin. Erythrocytes have a life cycle of around 3 months and HbA1c levels are therefore a good indicator of how a patient's glucose control is managed over a 2-3 months period. HbA1c measurements can be used in combination with for example SMBG and CGMS (Messier, 2005; Özmen and Boyvada, 2003; Salardi et al., 2002; Saudek et al., 2006; Saudek et al., 2005). The use of SMBG and/or CGMS gives valuable data for both patients and physicians and can be used to improve metabolic control and therefore reduce the HbA1c levels (Ludvigsson and Hanas, 2003).

The normal value of HbA1c in healthy people is between 4% and 5.9% while people with diabetes will often have higher values. The International Diabetes Federation (IDF) recommends HbA1c values below 6.5% for diabetic patients, while the American Diabetes
Association (ADA) recommends a value below 7%. A very high HbA$_1c$ such as between 10.9 and 15.5 % represents very poor glucose control (Messier, 2005) and is strongly associated with complications of diabetes especially microvascular complications. If patients achieve a value of below 7% they dramatically decrease their risk of such complications (American Diabetes Association; Saudek et al., 2006; Saudek et al., 2005).

1.3. Diabetic complications

Unfortunately, many diabetic patients fail to achieve adequate glycaemic control, and as mentioned earlier, this may lead to diabetic complications. Possible complications of diabetes are many and may vary in severity. Complications may include microvascular problems such as retinopathy, nephropathy and neuropathy but diabetes also increases the risk of conditions such as cardiovascular disease and stroke. Cardiovascular disease is the most common cause of mortality in patients with diabetes and is estimated to be the cause of death in ~ 65% of diabetes patients (Laakso, 1999; Lewis, 1999; Özmen and Boyvada, 2003; Vinik and Flemmer, 2002). Several studies carried out in the Scandinavian countries as well as in the UK, US and Germany have shown that hyperglycaemia and poor glycemic control increase the risk of cardiovascular complications. It has also been shown that the risk of developing cardiovascular disease is reduced if a patient has good glycaemic control, together with low LDL (low density lipoprotein) and normal blood pressure (<130/80 mmHg) (Haffner and Cassells, 2003; Idris et al., 2006; Laakso, 1999; Vinik and Flemmer, 2002).

Diabetic nephropathy is a progressive kidney disease and is another serious complication of diabetes. Patients are at higher risk of developing the syndrome if they have poor glucose control with the presence of hyperglycaemia. Diabetic nephropathy is a common cause of chronic kidney failure and end-stage kidney disease (Head, 2003; Rippin et al., 2001).
Diabetic retinopathy on the other hand affects the eyes and is the most common cause of blindness in the younger population of the Western countries (Head, 2003). Another important microvascular complication is diabetic neuropathy, which affects the nervous system. All these microvascular complications are specific to diabetes and are a significant source of mortality and morbidity in diabetic patients (Vinik and Mehrabyan, 2004).

1.3.1 Diabetic neuropathy

Up to approximately 60% of all diabetes patients develop diabetic neuropathy during the course of their disease, however the true prevalence is not known (Atli and Dogra, 2005; Boulton, 2003; Galer et al., 2000; Gore et al., 2005; Spruce et al., 2003; Tesfaye and Kempler, 2005; Tolle et al., 2006; Vinik and Mehrabyan, 2004). Diabetic neuropathies are a family of nerve disorders caused by diabetes mellitus and can be classified into different groups (peripheral, autonomic, proximal or focal) as they affect different parts of the body in different ways. The most common form of diabetic neuropathy is peripheral neuropathy, also known as distal symmetric sensorimotor polyneuropathy (Galer et al., 2000; Gore et al., 2005; Greene et al., 1999; Nicholson, 2006; Nokleby and Berg, 2005; Tesfaye and Kempler, 2005; Vinik and Mehrabyan, 2004).

1.3.1.1 Diabetic peripheral neuropathy (DPN)

The risk of developing diabetic peripheral neuropathy (DPN) is increased by the duration of diabetes and by poor glycaemic control. Other independent risk factors are smoking, drinking alcohol, hypertension and hyperlipidaemia, (Gore et al., 2005; Tesfaye and Kempler, 2005). The onset of peripheral neuropathy first occurs in the most distal extremities such as the toes. This process then extends to the feet and the legs. There is also the possibility of upper extremity involvement. This will start in the fingertips and can progress to the hands and
arms. This distinct spread of the condition is also known as “stocking-glove distribution” (Greene et al., 1990; Greene et al., 1999; Vinik and Mehrabyan, 2004). Foot ulceration and neuropathic pain are two main complications of DPN (Coppini et al., 2000; Tesfaye and Kempler, 2005; Vinik and Mehrabyan, 2004).

1.3.1.1 Painful DPN and Mechanism of Neuropathic Pain

It is as mentioned earlier acknowledged that diabetic patients are at risk of developing painful DPN although it is difficult to estimate the true prevalence of neuropathic pain as different criteria are used for its definition. Some reports estimates that 10-20% of patients with diabetes have neuropathic pain, while others suggest it is as high as 50% in patients with a long diabetes duration (Gore et al., 2005; Tesfaye and Kempler, 2005; Tölle et al., 2006; Vinik and Mehrabyan, 2004). A recent study by Davies et al (2006) shows a prevalence of painful DPN of 26%. This study was done in patients over 18 years old and the mean age for the study population was 66 years. It was found that 60% of the study population of 269 type 2 DM patients had some degree of DPN and 26 % of whole study population had painful DPN or mixed pain (painful DPN as well as non neuropathic pain).This was determined by clinical examination as well as subjective pain questionnaires (Davies et al., 2006).

Pain can be divided into nociceptive pain (figure 1.5) or neuropathic pain (figure 1.6). Nociceptive pain is normal pain caused by external stimulation leading to receptor stimulation and activation of normal pain pathways, while neuropathic pain does not require any receptor stimulation and is caused by dysfunction of the nervous system. Under normal conditions, a noxious stimulus will activate nociceptors which are nerve endings of thinly myelinated A-δ fibres and unmyelinated C-fibres, both of which transmit painful stimuli and temperature sensation. These peripheral nerve fibres will transmit the pain signal into the
dorsal horn of the spinal cord where they synapse with second-order sensory neurons. The synapse occurs in lamina I (marginal zone) and in lamina II (substantia gelatinosa).

The synapse results in the release of specific excitatory neurotransmitters from the afferent neurons (de Leon-Casasola, 2007). The main excitatory neurotransmitters thought to be involved in the pain pathway are glutamate and substance P. The post synaptic response of glutamate release is mainly mediated via N-methyl-D-aspartate (NMDA), kainite (KA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors while substance P interacts with tachykinin receptors (NK1). Glutamate and Substance P binds to AMPA and NK-1 receptors respectively and if the stimulus is strong enough AMPA receptors will depolarise the membrane and remove Mg$^{2+}$ blockage of NMDA receptors. Glutamate can bind to the unblocked NMDA receptors on second order sensory neurons of dorsal horn and Na$^+$ and Ca$^{2+}$ can be transported in. In addition, substance P binding increases sensitivity of these second order sensory neurons and up-regulates voltage gated Na$^+$ channels and facilitates NMDA mediated transmission (de Leon-Casasola, 2007; Zhuo, 2007) and pain signals can then be transmitted via the spinothalamic tract to the thalamus in the brain where the signal is perceived as pain (figure 1.5). As important as the ascending pain pathway just described, is the descending system which includes the serotonergic and noradrenergic pathways and originates from the cortex, thalamus and brainstem (Brodal, 2005). The activated descending pain modulatory neurons can project to the dorsal horn of the spinal cord and release neurotransmitters such as noradrenaline (NA) and serotonin (5H1). These neurotransmitters suppress pain by inhibiting the transmission of incoming painful stimuli from the afferent nociceptor neurons as well as inhibition of second order sensory neurons (de Leon-Casasola, 2007).
A. Noxious stimulus

Pain signal transmitted to the dorsal horn in the spinal cord via dorsal root of ganglion

Noiciceptors on thinly myelinated A-δ fibres and unmyelinated C-fibres

B. Pain signal reaches the synapse of afferent neurons and second order sensory neurons and causes release of neurotransmitters

Glutamate

AMPA

Transmission of pain signal via second order neurons to the thalamus

Substance P

NK1

NMDA

Pain signal transmitted to the dorsal horn in the spinal cord via dorsal root of ganglion

Descending Pathway—serotonergic and noradrenergic systems

Figure 1.5: Simplified overview of the mechanism of normal nociceptive pain ascending pathway. A. A noxious stimulus will activate the nociceptors of the peripheral nerve fibres. The pain signal is then transmitted by the A-δ fibres and unmyelinated C-fibres to the dorsal horn in the spinal cord. Here the afferent neurons will synapse with second order sensory neurons. The pain signal is then transmitted by these neurons to the thalamus in the brain via the spinothalamic tract. In the brain the perception and interpretation of the pain signal take place and the descending pathway can be activated which can have an inhibitory action on sensory neurons. B. The synapse between afferent neurons and second order sensory neurons takes place in the dorsal root. The afferent neurons will release excitatory neurotransmitters such as glutamate and substance P. Glutamate can then bind to AMPA and NMDA receptors on the sensory neurons, while substance P binds to NK1. These interactions will result in activation of receptors and stimulation of the sensory neurons membrane and the pain impulse can be further transmitted.
In painful DPN it is the damage of the peripheral nerves that initiates the neuropathic pain. It mainly involves the damage of the small thinly myelinated A-δ fibres and unmyelinated C-fibres which as mentioned earlier transmit painful stimuli. However, larger fibres such as myelinated A-β fibres which transmit touch, vibration and position perception may also be involved. Furthermore, motor involvement with large myelinated A-α fibres can also be involved in peripheral neuropathy (Quattrini and Tesfaye, 2003; Tesfaye and Kempler, 2005; Vinik and Mehrabyan, 2004).

The signs and symptoms of DPN vary depending on which fibres are involved but commonly there is involvement of both large and small fibres. This damage of fibres may be due to the effect of hyperglycaemia and the degree of damage correlates with the degree and duration of hyperglycaemia. However, the full mechanism of neuropathic pain in DPN is not fully understood (Benbow and Macfarlane, 1999; Boulton, 2003; Kapur, 2003; Quattrini and Tesfaye, 2003; Tesfaye and Kempler, 2005; Tolle et al., 2006). It is thought that damage will result in hyperactive nociceptors which continuously fire without noxious stimuli present. This constant stimulation may result in peripheral sensitization, meaning that there is a decrease in the threshold at which the nociceptor responds. The pain signal is then transmitted via afferent neurons to the dorsal horn where they synapse with sensory neurons as explained previously (see figure 1.6). Sprouting is a phenomenon that may also occur. This is a re-structure of afferent neurons which sprout and form new connections with the neurons in the dorsal horn. It is for example thought that A-β fibres can sprout to a region in the dorsal horn (lamina II) where nociceptive C-fibres are located (International Association for the Study of Pain, 2000). This will result in a normal non-painful sensory input carried in A-β fibres being transmitted as a pain signal (allodynia). Sprouting may also enhance the release of neurotransmitters which increase pain signal transmission. Glutamate and Substance P can as described earlier bind to AMPA and NK-1 receptors respectively and NMDA receptors are...
as a result unblocked. Glutamate can bind to NMDA receptors on second order sensory neurons and transmit the pain signal. These neurons can also become sensitized which means that they can respond at a lower threshold and to a wider peripheral area and this is called central sensitization (figure 1.6). The peripheral sensitization of nociceptors and central sensitization of second order sensory neurons is believed to play a large role in the mechanism of neuropathic pain.

The descending inhibitory pathway may also be affected by nerve injuries. Under normal circumstances serotonergic and noradrenergic systems release serotonin and noradrenaline which can inhibit both the sensory neurons in the dorsal horn as well as the release of neurotransmitters from afferent nociceptive neurons (de Leon-Casasola, 2007). Dorsal horn GABA can also assist in the inhibitory action by blocking the excitatory activity of glutamate. In the case of nerve injuries these inhibitory actions can be reduced resulting in excessive release of glutamate and substance P and second order sensory neurons can continue to transmit pain signals.
A. No stimulus

Damage of nerve fibres can result in hyperactive nociceptors which will cause nerve fibres to fire constantly without noxious stimuli

Damage of nerve fibres
and unmyelinated C-fibres, X = damage of the nerve fibres

B. Central sensitization

Damage of fibres causes constant firing

Sprouting

Glutamate↑

Transmission of pain signal via second order neurons to the thalamus

Figure 1.6: Mechanisms of pain ascending pathway and the damage seen in painful DPN.

A. In painful DPN there is damage of unmyelinated C-fibers, myelinated A-α/β and A-δ fibers which transmit noxious information and this damage leads to hyperactive nociceptors and constant firing without noxious stimulus. B. The constant firing will stimulate the release of excitatory neurotransmitters such as glutamate from afferent neurons. Possible sprouting due to injury can result in enhanced neurotransmitter release, therefore enhancing transmission of the pain signal via second order sensory neurons. In addition, A-β which normally transmits sensation of touch can sprout into lamina II where nociceptive C-fibres are located. This sprouting may result in that a normally non painful stimulus is transmitted as painful signal and this give rise to allodynia. Furthermore, there may be a defect in the inhibitory input of the descending pathway, resulting in an increased pain response. The constant stimulation of second order sensory neurons in the substantia gelatinosa of the dorsal horn can result in sensitization, which means that these neurons can respond at a lower threshold and to a wider peripheral area, leading to central sensitization.

One should be aware of that this is just a broad overview of the pain pathway and there are more mechanisms involved.
Patients with painful DPN can experience different types of neuropathic pain and symptoms may vary from patient to patient. Most commonly, the pain is characterized as burning, shooting and tingling (Benbow and Macfarlane, 1999; Gore et al., 2005; Greene et al., 1999; Quattrini and Tesfaye, 2003; Vinik and Mehrabyan, 2004; Woolf and Mannion, 1999). This type of pain can in many cases be associated with hyperalgesia (increased pain response) and allodynia which means the pain is due to a stimulus which does not normally provoke pain (Woolf and Mannion, 1999; Zhuo, 2007). This stimulation can be gentle pressure, cold, warmth or touch. Patients may for example experience pain due to the sensation of clothing touching their feet or from the sensation of sheets against the affected area (Greene et al., 1999; Vinik and Mehrabyan, 2004). It is thought that the central sensitization seen in painful DPN contributes to the occurrence of hyperalgesia and sprouting of A-\(\beta\) fibres can result in allodynia (de Leon-Casasola, 2007). Another condition which can be seen in peripheral neuropathy is hypoalgesia (Vinik and Mehrabyan, 2004). This condition is defined as diminished pain or sensation in response to normal painful stimulus and is caused by loss of small fibres. The perception of pain and sensation is impaired and patients might therefore not feel injuries such as cuts from sharp objects or burns from e.g. hot water.

Painful DPN can be either acute or chronic. Acute painful DPN usually resolves after 6 months and is relatively uncommon. It occurs in type I and type II DM patients with poor glycaemic control (Greene et al., 1999; Quattrini and Tesfaye, 2003). Chronic painful DPN often occurs in patients who have a long history of diabetes, and the neuropathic pain often persists for longer than 6 months (Nicholson, 2006; Vinik and Mehrabyan, 2004). Patients may experience extreme pain in the feet which is often worse at night (Quattrini and Tesfaye, 2003). Other symptoms may be insomnia, anxiety and a worsening of quality of life. Chronic painful DPN is very difficult to treat, although various treatments are available. The side-
effects and drug tolerance of currently available medications may however be problematic (Quattrini and Tesfaye, 2003; Vinik and Mehrabyan, 2004).

1.3.1.1.2 Foot ulceration

The loss of pain and temperature sensation caused by small fibre damage may increase the risk of injury and also increase the risk of possible foot ulceration and subsequent infections as well as gangrene (Greene et al., 1999). Foot ulceration is a serious condition which may lead to amputation of the toes and in severe cases the whole foot. In fact, neuropathy increases the risk of amputation 1.7-fold. If there is deformity the risk increases 12-fold and if there have been previous ulcerations the risk of amputation increases 36-fold (Vinik and Mehrabyan, 2004). Foot ulceration is commonly associated with discomfort and in a study by Ribu et al. 75% of patients had pain due to diabetic foot ulceration (DFU). Furthermore, 57% of these patients also reported pain in association with activity (walking/standing) and at night (Ribu et al., 2006). It is therefore important that diabetic patients receive a good education on daily foot care and check their feet regularly. Furthermore, the patients should try and achieve good glycaemic control, as this decreases the risk of nerve damage and subsequent foot ulceration.

1.4 Diabetes and the Central Nervous System

Diabetes is a complex disease with many possible complications as described previously. Diabetes has been linked with impairment of sleep, cognitive function and, as a result, a reduced quality of life (QOL). Painful DPN is one complication which can have a severe impact on all the above parameters. In a study by Gore and colleagues, patients with painful DPN are reported to have a significant disturbance in sleep and QOL when compared to both diabetic and non-diabetic controls (Gore et al. 2005).
1.4.1 Diabetes and Sleep

Sleep is an important biological function and an essential factor for human well being and to be able to function properly. Sleep deprivation can affect a person's mood and also impair psychomotor performance and cognitive function the next day, and extreme sleep deprivation can cause hallucinations and paranoia.

It is important to understand the physiology of normal sleep and the mechanisms involved in sleep-wake states in order to investigate the negative effects that diabetes and complications such as neuropathic pain and hyper/hypoglycaemia may have on sleep quality.

1.4.1.1 Physiology of normal Sleep

1.4.1.1 Sleep and wake mechanisms and neurotransmitters involved

The sleep-wake cycle is very complex and all of the mechanisms involved are not fully understood. It is known that the sleep-wake cycle is regulated by both a circadian process (process C) and homeostatic process (process S). The homeostatic process involves a person's sleep drive and is dependent on the duration of prior wakefulness and also the duration and quality of prior sleep episodes. The longer a person remains awake the stronger the urge and need to sleep become. The circadian process is an approximately 24 hour cycle which is controlled mainly by the suprachiasmatic nucleus (SCN), the master clock in the brain. The SCN receives light input from the retina via the optic nerve and is therefore under the influence of the environmental 24 hour light/dark cycle. The SCN itself can then regulate many functions such as body temperature, hormone release, and neuronal projections which activate either sleep or wake promoting neurons. One important hormone which is driven by the SCN clock is melatonin (Saper et al., 2005). Melatonin has sleep-inducing properties and is secreted during darkness; the levels rises when the sun goes down and starts to decrease.
again in the latter part of the night. There are several other hormones and neurotransmitters involved in the sleep-wake mechanism and there is a fine balance between the sleep-promoting neuronal group and wake-promoting neuronal group with the activity of one group inhibiting the activity of the other group. This has been described as a "flip-flop model" (see figure 1.7A and B) as the change from a stable wake state to sleep occurs very quickly (Saper et al., 2001; Saper et al., 2005). In wake, the major excitatory neurotransmitter glutamate is released and can directly stimulate the cerebral cortex, leading to cortical activation and wakefulness. In addition, glutamate will activate orexin-containing neurons and other wake-promoting neuronal groups located mainly in the brainstem and hypothalamus. Orexin can increase the activity of the wake-promoting neurons which will release neurotransmitters such as acetylcholine, noradrenaline, serotonin, histamine, and dopamine, which all have several actions (Saper et al., 2005). These neurotransmitters can inhibit sleep-promoting neurons in the ventrolateral preoptic nucleus (VLPO), switching this system off and they can all produce alertness and wakefulness as they promote cortical activation (Gallopin et al., 2000). In addition, hormones such as cortisol and adrenaline are also involved in stimulating wakefulness and a person is now in a stable wake state (see figure 1.7A). When the desire and need to sleep is strong enough (homeostatic control) and the main inhibitory neurotransmitter GABA is released, the switch to sleep will take place. GABA is released by GABA neurons which project to the VLPO. These neurons are active during sleep and release not only GABA but also galanin (Saper et al., 2001). Activation of these neurons will inhibit wake promoting neurons and therefore assist in the switch to sleep taking place. Another neurotransmitter which is believed to be involved in promoting sleep is adenosine (Saper et al., 2005). Adenosine levels build up over time and it is thought that when high levels are reached this will also induce sleep by inhibiting the release of wake promoting neurotransmitters. In addition, several hormones are associated with sleep. These
include melatonin, prolactin, insulin and growth hormones (GH). The levels of these hormones increase and are thought to help facilitate sleep (see figure 1.7B).

Figure 1.7A: Wake/Sleep Switch Model-Wake state. There is a fine balance between sleep promoting neuronal groups and arousal/wake promoting neuronal groups and there is a mutually inhibitory effect from both these groups. The switch between stable wake and sleep is quick and the full mechanism of the switch between these states is not known, however both the circadian process and homeostatic process plays a regulatory role in the whole wake-sleep cycle. Wakefulness is generated by signals from these 2 processes and triggers a series of responses. Glutamate which is the major excitatory neurotransmitter in the brain can directly stimulate cerebral cortex, leading to cortical activation and wakefulness. In addition, glutamate will activate orexin containing neurons. These neurons can then activate the whole arousal system which contains wake promoting neurons. These can in turn release neurotransmitters such as serotonin, noradrenaline, dopamine and also histamine, which all have several actions. They can inhibit sleep promoting neurons in the VPLO, switching this system off and they can also cause cortical activation and wakefulness by stimulating the cerebral cortex. Increased levels of hormones such as cortisol and adrenaline can also contribute to a stable wake state. VPLO= ventrolateral preoptic nucleus.
B. Under the influence of Circadian process and Homeostatic process:
- Circadian hypnotic signal via SCN and homeostatic sleep drive will promote sleep.

- Sleep promoting neurons release GABA and galanin which can inhibit wake/arousal promoting neurons and therefore block action of neurotransmitters of the arousal system.
- Increase in adenosine levels will promote sleep by inhibiting the wake/arousal promoting neurons.

Cerebral cortex

Figure 1.7B: Wake/Sleep Switch Model - Sleep state. During wake adenosine levels build up and it is thought that when high levels are reached this will induce sleep by inhibiting the release of wake promoting neurotransmitters. The levels of hormones such as insulin, prolactin, growth hormone and melatonin are increased and will further induce sleep. When the desire and need to sleep is strong enough the switch will take place and neuronal projections of GABAergic neurones will activate sleep promoting neurones in the VPLO, which in turn will release neurotransmitters such as GABA and galanin. GABA is also a major inhibitory neurotransmitter and can therefore inhibit the activity of wake promoting neurones and this inhibition will result in no action of arousal promoting neurotransmitters and therefore no cortical activation. SCN = suprachiasmatic nucleus, VPLO = ventrolateral preoptic nucleus, GABA = γ - aminobutyric acid.
1.4.1.1.2 Sleep cycle

Once sleep is initiated there will be defined periods of rapid eye movement (REM) sleep and non rapid eye movement (NREM) sleep throughout the sleep period. Each REM/NREM cycle last approximately 90-110 minutes with approximately 4-5 cycles per night (Nicolau et al., 2000).

NREM sleep consists of light sleep (stage 1 and 2) and deep sleep (stages 3 and 4). Stage 1 sleep is the interim between wake and sleep and usually accounts for 4-5% of total sleep time. Stage 2 sleep is the most common stage of sleep, making up 45-55% of total sleep time. Stage 3 is when deep sleep begins and body temperature and blood pressure decrease. When a person enters stage 4 sleep body temperature and blood pressure decreases even further and muscle activity is very limited (figure 1.8). A combination of stage 3 and 4 together is also known as slow wave sleep (SWS) or delta sleep and is thought to be the restorative phase of sleep (Tasali et al., 2007). SWS is most common during the first part of the night and decreases as the night progresses. REM sleep is on the other hand most common during the second half of the night and is the phase when the brain is more active and dreaming occurs. The first REM period normally occurs around 70 to 90 minutes after sleep onset and is relatively short. The REM periods will then increase in length as the night progresses and the last REM period can last up to 1 hour. REM sleep makes up 20-25% of total sleep time and is thought to be involved in memory consolidation (Kamel and Gammack, 2006; Najib, 2006; Vgontzas and Kales, 1999).
Figure 1.8: Sleep cycle. Normal sleep is defined by cyclic appearance of non-rapid eye movement sleep (NREM) and rapid eye movement sleep (REM) with each cycle being approximately 90-110 minutes in humans. Stage 1 is the first stage of sleep and takes up around 4-5% of total sleep time. Stage 2 is characterised by appearance of K-complexes and spindles (explained in more details in section 2.4 of general material and methods) and is the most common stage of sleep with 45-55% of total sleep time. Stages 3 and 4 are called deep sleep or slow wave sleep due to the occurrence of slow delta waves. In young healthy adults stages 3 and 4 occupy 4-6% and 12-15% of total sleep time, respectively. Stage 3 is when 20-50% of a 30 second epoch consists of slow delta waves and stage 4 is defined as above 50% delta waves in a 30 second epoch. In REM sleep the brain waves will speed up and rapid eye movement occurs and this phase is also called stage 5 and occupies around 20-25% of total sleep time. For more detailed descriptions about sleep stages and staging please see section 2.4 in general method and materials. BP= Blood pressure.

Several studies have looked at the homeostatic regulation of sleep and the effects it as on sleep stages and on the intensity of slow wave sleep. A study in healthy young subjects by Brunner and colleagues (1993) showed that after sleep deprivation (4 hours sleep for 4 nights), there was an increase in both total sleep time and REM sleep as well as a decrease in sleep onset latency in the first two recovery nights. SWS was also increased but only in the
first recovery night and slow wave activity (spectral power density in the 0.75-4.5 Hz range) in NREM sleep increased by around 20% in the first recovery night. Similar results were seen in another study by Dijk and colleagues (1991). Subjects were kept awake for 24 hours before recovery sleep started in the morning. Even though the recovery sleep was initiated at a circadian phase where NREM sleep propensity was not high one could see an increase in slow wave activity in NREM sleep during daytime recovery sleep compared with baseline nocturnal sleep. Furthermore, other studies have looked at the effect of specific SWS deprivation rather than full sleep deprivation. It has been shown that slow wave activity does not only increase after full sleep deprivation but also after specific SWS deprivation. This increase in slow wave activity could be seen in the first hour after SWS deprivation both during nocturnal sleep but also in daytime recovery sleep (Dijk and Beersma, 1989). Other studies have looked at slow wave activity and circadian rhythm and an example of this was a study by Dijk and colleagues. Subjects were scheduled to 28 hours day/night cycle so that the sleep episodes eventually occurred at different parts of the circadian 24 hour cycle. The results showed that slow wave activity during NREM sleep had a low circadian modulation and did not follow the circadian rhythm of sleep propensity (Dijk and Czeisler, 1995). This suggests that slow wave activity is a good marker of the homeostatic regulation of sleep.

1.4.1.1.3 Gender and age difference of normal sleep cycle

There are differences in human sleep patterns due to both age and gender. It is for example well known that total sleep time decreases with age. Hume et al (1998) showed that this decrease in total sleep time was partly due to increased wake periods within sleep, therefore more fragmented sleep. Older adults also have a tendency to have more arousals than younger adults (Boselli et al., 1998; Neubauer, 1999). Furthermore, the amount of SWS decreases with age, while the amount of REM is only slightly reduced (Carrier et al., 2001;
Ehlers and Kupfer, 1997; Neubauer, 1999). In addition, the prevalence of many sleep disorders increases with age and may cause a disturbance of sleep with increase awakenings, more fragmented sleep, arousals and delayed sleep onset (Hornyak and Trenkwalder, 2004; Wolkove et al., 2007).

There is also a gender difference in sleep and Dijk and colleagues were amongst the first to look at such differences in EEG spectral power. They found that young women had significantly higher power densities over a wide frequency range including delta, theta and low alpha compared with young men (Dijk et al., 1989). A later study by Carrier and colleagues (2001) showed a gender difference across the age when looking at sleep in subjects aged 20 to 60 years with women having higher power density in for example the delta frequency range compared with men. Another study carried out in depressed patients showed no gender differences in general spectral power but a significant reduction in delta activity in the depressed men compared with the women (Nissen et al., 2002). A study by Ehlers and Kupfer (1997) showed that men and women had similar amount of SWS (by visual scoring) and similar mean slow wave activity (by spectral analysis) when in their twenties (20-29yrs). However, a significant reduction in both SWS and slow wave activity occurred in men in their thirties (30-40 yrs) while this decrease was not seen in women of same age, suggesting that the gender difference seen in regards to SWS may not become apparent until the age of 30-40 yrs (Ehlers and Kupfer, 1997).

1.4.1.1.3 Normal physiological processes within a sleep episode

As mentioned previously the sleep-wake cycle involves the central nervous system and reflects changes in cerebral cortex activation. However, there are several other changes occurring between wake state and sleep state as well as during the sleep episode. Both body temperature and blood pressure decrease during NREM and then increase again during REM
Respiratory function also changes during sleep. At sleep onset and in NREM sleep stage 1 and possible stage 2 there may be evidence of periodic breathing. This means that there are oscillations in breathing amplitude. There may be clusters of breaths separated by apnoea or near apnoea events. When one enters a more steady state of NREM sleep, including stages 2, 3 and 4, these oscillations disappear and breathing occur in a more steady and regular pattern. In REM the breathing changes again and can become erratic and shallow with an increase in hypoventilation. This may result in lower levels of blood oxygen saturation and therefore greater hypoxemia than during NREM sleep (Kryger et al., 2005).

Furthermore, sleep will have an effect on endocrine and metabolic function. Hormone levels of prolactin and growth hormone are, as previously mentioned, increased during sleep while cortisol levels are decreased as its release is inhibited. In addition, glucose regulation is changed during sleep and nocturnal glucose levels increase. The increase in nocturnal glucose concentration is due to a decrease in glucose utilisation, mainly by the brain but also by a decrease in peripheral glucose use due to lower muscle tone during sleep. Blood glucose concentration rises during the first part of the night and there is a peak in the middle of the sleep episode. Levels will then drop during the latter part of the night (Boyle et al., 1994). The rise may be due to the fact that the reduction in cerebral glucose metabolism mainly occurs during slow wave sleep which is most predominant at the beginning of the night.

Another factor which is influenced by sleep is appetite. Ghrelin and leptin are two important hormones involved in regulating appetite. They have been associated with changes in hunger, ghrelin which stimulates appetite and leptin which suppresses appetite and food intake. Several studies have shown that if there is a reduction in sleep duration, e.g. during sleep restriction or sleep deprivation, levels of ghrelin increase while levels of leptin decrease,
suggesting that sleep loss will lead to increase in appetite and therefore possible weight gain (Spiegel et al., 2004; Taheri et al., 2004, van Cauter et al., 2005).

One should be aware that there are many more physiological changes that are regulated by sleep and the onset of sleep, and above is just a brief overview of normal sleep physiology and the mechanisms involved in the sleep-wake cycle.

1.4.1.2 Possible physiological changes of sleep in diabetic patients

There are many disorders that can affect people's sleep, such as insomnia, restless leg syndrome, periodic limb movements and sleep disordered breathing (including apnoeas and hypopnoeas), and they all appear to have a high prevalence in diabetic patients (Chansens, 2007). Sleep apnoea is very common in diabetics and can cause arousals and awakenings all through the night (West et al., 2006). Frequent awakenings will result in more fragmented sleep, with a reduction in total sleep time and possibly a reduction in SWS. In addition, diabetic patients with painful DPN may report that their pain interferes with their sleep leading to difficulties falling asleep and increase in wake periods (Benbow et al., 1998; Galer et al., 2000). Another factor which may affect the sleep of diabetic patients is nocturnal glucose levels (Bendtson et al., 1992).

1.4.1.2.1 Sleep Apnoea and Hypopnoea

Sleep apnoea is a disorder with complete cessation of breathing (apnoea) and/or shallow breathing (hypopnoea) during sleep. It is thought to be more common in men than women and other factors can increase the risk of sleep apnoea including age and obesity. Sleep apnoeas can be either central (CSA) or obstructive (OSA) with the latter being the far most common, resulting from partial or full collapse of the upper airway. OSA is commonly
associated with metabolic syndrome and diabetes and may be explained by the high prevalence of obesity seen in type II DM patients (Resnick et al., 2003). A study by West and colleagues showed that the prevalence of OSA was significantly higher in diabetic male population compared with healthy population and could partly be explained by body mass index (West et al., 2006). OSA seen in particularly type II diabetics has been thought to be a complication of the disease or a result of obesity but recent studies suggest that such dysfunction in breathing occurs at an early stage in subjects with pre-diabetes. A study by Punjabi et al. (2002) with overweight middle aged men with OSA showed that 12 % of subjects had undiagnosed diabetes and as much as 40% had impaired fasting glucose (Punjabi et al., 2002). Another study has suggested that SDB causes metabolic disturbance and is associated with insulin resistance and glucose intolerance, independently of risk factors such as age, gender and obesity (Punjabi et al., 2004). SDB can be a major cause of sleep disturbance, with increased arousals and awakenings resulting in excessive daytime sleepiness.

1.4.1.2.2 Periodic Limb Movements of Sleep

Restless leg syndrome (RLS) is another disorder commonly seen in type II DM patients (Lopes et al., 2005). It has been suggested that this is triggered by an abnormal sensory input from small fibers and is therefore thought to be a feature of DPN (Gemignani et al., 2007). RLS is characterized by unpleasant limb sensations such as burning and creeping. Furthermore, patients have an uncontrollable urge to move limbs when at rest and this irresistible urge to move is an effort to relieve the uncomfortable/irritating feeling (Clavadetscher et al., 2004). RLS may therefore interfere with sleep onset latency, increasing the time to fall asleep. Commonly associated with RLS are periodic limb movements of sleep (PLMS) and as many as 80 to 90% of patients with RLS also have PLMS (Alsaeedi and
Alshammari, 2005; Paulson, 2000; Rijsman et al., 2004). PLMS can occur independently without concurrent of RLS. Prevalence of PLMS increases with age and over one third of population above 65 years have some degree of periodic limb movements. PLMS do not necessarily cause interference of sleep, however if the rate of movements is high they can cause arousals and increase nocturnal awakenings, resulting in sleep fragmentation and therefore impairment of sleep (Alsaedi and Alshammari, 2005; Neubauer, 1999; Paulson, 2000).

1.4.1.2.3 Sleep and painful DPN

Sleep disturbance is very common in patients with pain and between 50-70% of people seeking help for chronic pain have reported sleep impairment (Gore et al., 2005). In a study by Galer and associates it was shown that painful DPN had a high impact on sleep and more than half of the patients experienced worsening of the pain at night time (Galer et al., 2000). This was also supported in a study by Benbow et al. (1998) where the diabetic study group had problems with their sleep as their neuropathic pain got worse at night. In addition to worse sleep quality, patients with chronic pain report a higher rate of insomnia, twice as high as seen in subjects without pain (Moldofsky, 2001; Sutton et al., 2001). Pain is thought to interfere with sleep by increasing the number of arousals, awakenings and sleep stage shifts, resulting in sleep fragmentation and worse sleep efficiency. There is evidence that pain decreases the percentage of slow wave sleep and increases the percentage of the lighter stage 1 sleep.

1.4.1.2.4 Sleep and nocturnal glucose levels

It is known that hyperglycaemia can increase the risk of cardiovascular complications as well as worsening painful DPN (Gore et al., 2005; Haffner and Cassells, 2003; Idris et al., 2006;
Laakso, 1999; Tesfaye and Kempler, 2005; Vinik and Flemmer, 2002). However, the effect on sleep is not fully known. It is thought that nocturnal hyperglycaemia is associated with sleep apnoea/hypopnea and severe oxygen desaturation, and a study by Pallayova et al. (2006) showed a strong correlation between the two. Several other studies have shown a link between obstructive sleep apnoea, sleep loss and insulin resistance (Punjabi et al., 2002; Punjabi et al., 2004; Spiegel et al., 2005).

Another concern is nocturnal hypoglycaemia. This is commonly seen in diabetic patients, especially in type 1 DM where it occurs in 29-56% of all patients (Bendtson et al., 1992). Almost 50% of all severe hypoglycaemic events occur during sleep at night and the duration of nocturnal hypoglycaemia varies between 1 and 8 hours. These severe episodes can cause coma and may also lead to cardiac arrhythmias and death (Allen and Frier, 2003). Nocturnal hypoglycaemia can also cause more common side effects, such as impaired cognitive function (Matyka et al., 2000), and it may also disrupt patients' sleep architecture. In a study by Gais et al (2003) it was reported that hypoglycaemia had an awakening effect on healthy subjects when using a hypoglycaemic clamp. Bendtson et al (1992) found that hypoglycaemia reduced the duration of deep sleep and there was an increase in short arousals. However, other studies contradict these findings and suggest that sleep architecture is not disturbed by nocturnal hypoglycaemia. In a study in type II DM adolescents Porter et al. (1996) found that there were no changes in sleep stages, fragmentation or arousals due to nocturnal hypoglycaemia. Matyka et al (2000) showed similar results in a study carried out in diabetic children. There were no significant differences in sleep architecture in children with a hypoglycaemic night compared with non hypoglycaemic night. Sleep was measured by polysomnography and glucose levels were measured by a glucose oxidase method involving overnight blood sampling. However, they did find found defects in sleep architecture in children with type I DM compared with controls. Type I DM children had more disrupted
sleep and awakenings of various length when compared with control. Such disturbances can have a significant impact on daytime functioning and these will be discussed later. But as stated above there appears to be no direct evidence to suggest that these abnormalities in sleep architecture are related to nocturnal hypoglycaemia (Matyka et al., 2000). Another study by Pillar and associates (2003) found that the arousals from sleep may be due to rapid changes in blood glucose levels even if the absolute levels not necessarily are low (Pillar et al., 2003). Rapid falls in glucose levels, as measured by continuous glucose monitoring system, were associated with awakenings from sleep while profound nocturnal hypoglycaemia was not.

Apnoeas, PLMS, pain and possible nocturnal glucose levels can all interfere with a patient’s sleep, with increased arousals and awakenings resulting in fatigue and excessive daytime sleepiness. This can lead to cognitive dysfunction and may also affect patient mood and alter quality of life.

1.4.2 Diabetes and Cognitive function

As mentioned previously a good night’s sleep is important for people to be able to function properly and loss of sleep due to pain or sleep apnoea can result in severe impairment of next day performance such as concentration, attention, psychomotor skills and memory. Daytime performance may also be affected by medications and neuropathic pain itself, as it can make it more difficult to carry out attention based tasks. To be able to assess a person’s neuropsychological function a broad battery of cognitive function tests can be used. Test batteries have generally involved assessment of psychomotor skills, attention skills and long-term, short-term and working memory (Draelos et al., 1995; Sommerfield et al., 2003).
Patients' glucose control can also affect daytime performance as episodes of both hyperglycaemia and hypoglycaemia can result in cognitive dysfunction (Cox et al., 2005; McAulay et al., 2001; Sommerfield et al., 2004; Sommerfield et al., 2003). It is thought that optimal cognitive function is achieved when the glycaemic range is between 4 and 15 mmol/l and that dysfunction occurs if values are outside this range. It is therefore important for diabetic patients to have a good glycaemic control to minimise the risk of impaired cognitive function (Cox et al., 2005).

1.4.2.1 Effect of hyperglycaemia episodes on cognitive function

Evidence for the effects of acute hyperglycaemia on cognitive function is contradictory. Several studies have reported that acute hyperglycaemia is associated with mild cognitive impairment (Cox et al., 2005; Sommerfield et al., 2004) while others have reported no changes in cognition related to the hyperglycaemic state (Draelos et al., 1995). Most studies reporting cognitive impairment have been carried out in patients with type I diabetes, but more evidence is now accumulating with respect to type II diabetic patients. In a study done by Sommerfield and associates (2004) it was reported that acute hyperglycaemia significantly impaired working memory, attention and decreased speed of information processing in patients with type II diabetes. Furthermore, acute hyperglycaemia also had a detrimental effect on patient mood (Sommerfield et al., 2004). Another study carried out by Cox and associates (2005) assessing both type I and II diabetic patients showed impairment in cognitive function at a glucose threshold of around 15mmol/l and this impairment was seen in 55% of all subjects (Cox et al., 2005). However, such decline in cognitive function due to hyperglycaemia was not seen in a study done by Draelos et al (1995). This study involved patients with type I diabetes and no changes in cognitive function were seen at glucose levels as high as 14.4 mmol/l or even as high as 21.1 mmol/l (Draelos et al., 1995).
1.4.2.2 Effect of hypoglycaemia episodes on cognitive function

Hypoglycaemic episodes can have serious effects on the human brain. One consequence of hypoglycaemia is acute neuroglycopenia which causes deterioration of cognitive function. Neuroglycopenia refers to a lack of glucose (glycopenia) in the brain and as this affects the function of neurons, it can lead to change in behaviour and altered brain function (McAulay et al., 2001; Sommerfield et al., 2003). This deterioration in cognitive function occurs when glucose levels fall to < 3.0 mmol/l (McAulay et al., 2001; McCall, 2004; Sommerfield et al., 2004; Warren and Frier, 2005). Furthermore, the onset is immediate and progressive, but reversible, as it has been observed that cognitive function returns to normal within 90 min after glucose levels return to normal (Sommerfield et al., 2004). However, severe and drawn-out hypoglycaemia can cause permanent brain damage or even lead to death. It is not clear which part/parts of the brain are affected by neuroglycopenia. However, areas of injury have been seen in neuroimaging studies in the hippocampus and in the frontal lobes of patients who suffered severe but non-fatal hypoglycaemia (Warren and Frier, 2005).

1.4.3 Diabetes and quality of life (QOL)

The complications of diabetes which have been discussed can all affect a patient’s QOL. As mentioned previously, hyperglycaemia and hypoglycaemic can both impair cognitive function enough to interfere with a patient’s daily life. Hypoglycaemia can also affect a patient’s well-being and productivity (Davis et al., 2005). Furthermore, nocturnal hypoglycaemia still remains a common problem as it not only affects the well-being of the patient but also causes concern for their carers and family members.
Painful DPN can have a substantial effect on patient’s QOL (Benbow et al., 1998; Davies et al., 2006; Galer et al., 2000). As mentioned earlier, it is common for patients to have impaired sleep as a result of worsening of the pain at night time, and painful DPN can also have a significant impact on enjoyment of life (Galer et al., 2000). Furthermore, in the same study carried out by Galer and associates it was shown that painful DPN had a moderate impact on parameters such as recreational activities, normal work, social activities, mood and mobility. The conclusion from this study is that painful DPN has a negative impact on patient’s quality of life (Galer et al., 2000). Neuropathic pain also impacts on employment and a study by Meyer-Rosberg on patients with various neuropathic conditions reported that 52% had to reduce their working hours as a direct result of pain (Meyer-Rosberg et al., 2001). A similar pattern was seen in a study carried out by Tölle et al. (2006). They observed that almost one working day per week was lost due to neuropathic pain and that pain severity was associated with reduced productivity.

1.5 Hypothesis and Aims

The aim of this research project was to characterise sleep in patients with painful DPN and investigate the effect pain, obesity and glucose control may have on sleep variables. Increase in pain may be associated with decrease in sleep quality and reduced total sleep time. One may also find an increase in sleep onset. Obesity is commonly associated with diabetes (type 2 DM in particularly) and with OSA. One may therefore expect to see a high prevalence of OSA in this study group. Furthermore, DPN are thought to increase the occurrence of PLMS. And both these conditions may have a negative effect on sleep.

Another aim was to investigate pregabalin, duloxetine and amitriptyline, all three used in the treatment of painful DPN and the effect they may have on pain, glucose levels and sleep
parameters including objective and subjective measures. Pregabalin and amitriptyline are thought to improve sleep, however, the latter may cause hangover effects in the morning. While duloxetine can possibly reduce sleep quality and efficiency due to an alerting effect of the drug. Furthermore, both duloxetine and amitriptyline are thought to cause REM suppression. Please see Aim and Hypothesis section in each result chapter for more detailed description.
CHAPTER 2 –
GENERAL METHODS AND MATERIAL
2.1 Subjects

83 diabetic patients (male and females) with painful peripheral neuropathy were recruited for this study. Recruitment and screening took place at the Royal Bournemouth Hospital and Poole General Hospital. Patients aged 18 years and above, with diagnosis of diabetes mellitus for at least a year and neuropathic pain of diabetic origin with a score above 12 on the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) were eligible for enrolment.

Patients were excluded if there was evidence of end stage disease of a major system, evidence of recurrent and/or a severe hypoglycaemic event in last 3 years, or evidence of recent ischemic event. Pregnant or breast-feeding women and patients with a history of dependence on or abuse of alcohol/recreational drugs were also excluded. Furthermore, patients were not allowed to enter the study if they had been involved in another clinical trial within the last 3 months prior to this study.

The patients were sequentially randomized into three treatment arms (pregabalin, amitriptyline, and duloxetine). The groups were matched for age and gender where possible.

2.2 Study design

The study was a double-blind, randomised, parallel groups investigation into the effects of pregabalin, duloxetine and amitriptyline on aspects of pain, sleep and next day performance. The study design was over 36 days and consisted of 3 different treatment periods and was conducted according to ICH-GCP guidelines (Good Clinical Practise) at the Surrey Clinical Research Centre (Surrey CRC), Faculty of Health and Medical Sciences, University of Surrey, UK and approved by the Essex 1 Research Ethics Committee. All patients had written
informed consent before screening. Period one included an 8-day single-blind placebo run-in with a polysomography (PSG) habituation night (time in bed 2300-0700) on (D6) to familiarize the subjects with the environment. Furthermore, subjects had a CGMS sensor inserted once arriving to the Surrey CRC (D6). This sensor was used to monitor subject’s glucose levels continuously while they were in the unit (D6-8, D20-22, and D34-36). On day 7 patients were trained on psychometric assessment and on night 7 a full PSG assessment was made to determine baseline (placebo) sleep characteristics (time in bed was again 2300-0700). On day 8 baseline (placebo) psychometric measures were made and patients were discharged at 1700 hours. Patients took their first dose of medication at home on the evening of day 8. The second treatment period consisted of 14 days of lower dosing (D9-D22) of either pregabalin 150mg twice a day, duloxetine 60mg every morning, or amitriptyline 25mg twice a day. A re-habituation night took place (D20) followed by re-familiarisation of psychometric tests on day 21. A chronic “low dose” PSG night (D21) was carried out and psychometric testing was performed on day 22. Patients were discharged at 1700 hours on day 22. Dosing with the higher dose took place on evening of day 22 and the third treatment period consisted of another 14 days of higher dosing (D23-D36) of either pregabalin 300 mg twice a day, duloxetine 60mg twice a day, or amitriptyline 25mg every morning and 50 mg very evening. Patients arrived to CRC on day 34 of treatment period 3. A re-habituation night took place (D34) followed a chronic high dose PSG night (D35). Re-familiarisation of psychometric assessment was performed on day 35 and psychometric assessment of chronic high dose took place on day 36. Patients were then discharged at 1700 hours. An overview of the study schedule can be seen in appendix 1 and an overview of the dosing schedule can be seen in figure 2.1.
Dosing Schedule

First placebo dose on the evening of study day 1

Visit 1 at the CRC: study day 6-8 for placebo. First low dose on the evening of day 8

Visit 2 at the CRC: study day 20-22 for low dose. First high dose on the evening of day 22

Visit 3 at the CRC: Day 34-36, high dose. Last high dose on the morning of day 36

Figure 2.1: Dosing Schedule and study design. An overview of the 36 days study design, with dosing schedule indicated. Subjects received placebo for 8 days, first visit to the Surrey CRC was scheduled for study days 6-8. First low dose of medication was taken on the evening of day 8 following discharge from Surrey CRC. Low dose medication was then received for 14 days in total and the last days of this period were spent in the Surrey CRC looking at low dose assessment (study days 20-22). High dose medication was taken on the evening of day 22 following discharge and then continued for 14 days. The final residential visit occurred at the end of these 14 days (study days 34-36). The final visit (visit 3) assessed high dose medication with the last high dose taken in the morning of study day 36.

The decision of dose ranges of the study medications was made after discussion with the study diabetologists and was determined by their recommendations as well as previous published literature. The dose range was chosen in regards to the medications analgesic effect.
2.3 Sleep recording

On each residential night, PSG recordings were carried out from 2300 hours to 0700 hours using the Compumedics Siesta System® (Compumedics Ltd., Australia). The patients slept in individual sleep labs. Each sleep lab was soundproof and temperature controlled with a temperature of 18°C ± 1°C.

2.3.1 Electroencephalography (EEG)

Subjects' sleep was assessed using a standard EEG montage. The head montage consisted of 4 electrodes (C3, C4, O1 and O2) and their positions were determined using the internationally standardised 10-20 system which was developed by Jasper (1958). The system is termed “10-20” due to the fact that electrodes are located either 10% or 20% of the total distance between a given pair of skull landmarks. There are four landmarks in this system, the nasion, the inion and the left and right preauricular points. The preauricular points are located just above the cartilage which covers the external ear opening. The inion is a knob that can be felt when you run the fingers from the neck and up to the skull while the nasion is located between the eyebrows at the start of the forehead. These four landmarks are then used when measuring up the electrodes placement (figure 2.2). Note that the letters F, T, P, O and C seen in figure 2.2 were used to name electrode placement and stands for frontal, temporal, parietal, occipital lobe of the brain. C is only used for identification purposes of “the central line”. All odd numbers (1, 3, 5, and 7) refer to the left hemisphere and the even numbers (2, 4, 6, and 8) refer to right hemisphere. The z refers to the midline.
Figure 2.2: The international 10-20 system of electrode placement. The system is termed "10-20" due to that the electrodes are located either 10% or 20% of the total distance between a given pair of skull landmarks. The four landmarks are nasion, inion, and left and right preauricular points. Figure taken from Malmivuo and Plonsey (1995) with permission.

2.3.1.1 Measuring of electrode placement using 10-20 system

It is only necessary to record from one pair of electrodes (C3 and C4) in order to analyse sleep according to Rechtschaffen and Kales (1968). However, it is often easier to determine alpha activity from the occipital region and an occipital channel (O1 and O2) was therefore included in this study. No further head electrodes were used in this patient group as it may cause increased pain and irritation from electrode placement. The opposite ear (A1 and A2) were used as referents and electro-oculogram (EOG) (EGO-left and EOG-right), and submental electromyogram (EMG) (EMG-left and EMG-right) electrodes were also attached. The sub mental electromyogram and electro-occularograms was used to measure chin activity and eye movement, respectively, both especially important when looking at rapid eye
movement (REM) sleep which will be discussed later on. Furthermore, a ground electrode and a reference electrode were also used. The measure and placement of electrodes were carried out in different steps explained below.

1. The distance in centimetres from the nasion to the inion was measured = nasion-inion distance. 10% of this distance was calculated. Two 10% mark (of the nasion-inion distance) were made, one starting from nasion and measuring towards the top of the skull (FPZ site) and the other one starting from inion and measuring towards the top of the skull (OZ site), using a non-toxic marker pen. Half distance = 50% of the nasion-inion distance was also marked (CZ site) (see figure 2.2A).

2. The distance in centimetres from left to right preauricular point was then measured making sure the tape measure went through the CZ mark. 10% of this ear to ear distance was calculated and this 10% measure was marked out on each side of the skull starting from left preauricular point going up (T3) and from right preauricular point going up (T4). A further 20% from these two points was calculated (30% of the whole ear to ear measurement) to make up C3 mark on left side of the skull and C4 on the right side (see figure 2.2A and B).

3. The next step was to measure the circumference of the subject’s head (as when measuring for a hat). The tape measure passed through FPZ, T3 OZ and T4. 5% of the circumference or 10% of half of the circumference was calculated. This measurement was then used to mark two marks on each side of FPZ, Fp1 and Fp2. OZ was then located and O1 were then marked on the left hand side from OZ and O2 on the right hand side (using distance calculated above), figure 2.2B. The distance between O1
and O2 should therefore be 10% of the whole circumference, and same between Fp1 and Fp2 on the forehead.

4. Finally, the distance from Fp1 to O1 was measured going through C3 and Fp2 to O2 through C4, to be able to centre C3 and C4, respectively. Half this distance indicates the centre position of C3 and C4.

For facial electrodes the A1 electrode was placed behind the left ear and A2 electrode behind right ear (figure 2.2). A ground reference was also used and was attached on the forehead on Fp1, next to FPZ site. Furthermore, the two EMG electrodes were attached under the chin (submental electromyogram) and the EOG electrodes were placed on the outer corners of the eye. The right outer canthus (ROC) was placed on the outside of the right eye, approx 1 cm out and 1 cm up (raise). The left outer canthus (LOC) was placed on the outside of the left eye approx 1 cm out and 1 cm below (lower). Finally, a reference electrode was attached on the forehead next to the ground electrode on Fp2 site.

2.3.1.2 Electrode attachment

Once the electrode placement was measured and marked out the electrodes were attached to the subject’s head. The area where the electrode is placed was cleaned using Nuprep™. This is a skin prepping gel which will help reduce the skin impedance and result in a better reading of the electrode trace. EC2 cream® was then put onto the electrode which was then placed onto the cleaned area. The EC2 paste was used to improve electrical contact. Gauze was used to fix the electrode to the skull and it took approximately 8-10 minutes for the paste to dry fully. All the facial electrodes were attached in the same way. However, Medipore™ tape or Micropore™ tape was used instead of gauze to fix the electrodes to the facial skin.
The aim was for impedances to be maintained below 5K\(\Omega\) to assure good quality recordings. This was checked by using a ohmmeter which is an instrument that measures electrical resistance.

In addition to the head and face electrodes, leg EMG leads were used (one for each leg) and these were placed over the anterior tibialis muscles and attached using adhesive tape such as Micropore\textsuperscript{TM} tape.

Respiratory band, thermistor and pulse oximeter were also used. The respiratory band was placed across the thorax to measure chest effort. If patient was female the respiratory band was placed just beneath the breasts. Nasal airflow was measured by using Oro-Nasal Airflow Thermistor which was placed between the nose and the upper lip. The thermistor works by sensing changes in temperature during breathing as air is cold when inhaling and warm when exhaling. Finally, a pulse oximeter (Ohmeda Biox 3740\textsuperscript{®}) was placed over the patient's index finger on non-dominant hand to monitor oxygen saturation (SpO\textsubscript{2}) continuously throughout the night. This is a non-invasive method of measuring oxyhemoglobin in real time. It consists of light emitting diodes (LEDs), one LED is red and the other is infrared with different wavelength and the absorption of these wavelength is different between deoxyhemoglobin and the oxygenated form. This difference in absorption will give the SpO\textsubscript{2} reading.

2.4 Sleep Staging

For nights 7, 21, and 35, overnight sleep was manually scored directly from the computer screen and all records were scored according to the standard criteria by Rechtschaffen and Kales (R&K) (1968).
2.4.1 Staging of EEG

The EEG signal obtained from the recording can be divided into different type of waves depending of its frequency and amplitude. The different brain waves produced are beta ($\beta$), alpha ($\alpha$), theta ($\theta$) and delta ($\delta$) and an example of EEG tracing of these different brain waves can be seen in figure 2.3. There are other existing brain waves but these will not be discussed as the most relevant ones for this thesis are alpha, theta and delta waves.

**Brain Waves: EEG Tracings**

Beta ($\beta$) 13-30 Hz

Alpha ($\alpha$) 8-13 Hz

Theta ($\theta$) 4-8 Hz

Delta ($\delta$) 0.5-4 Hz

![Brain Waves: EEG Tracings](image)

Figure 2.3: Brain waves - EEG tracings. The EEG signal can be divided into different brain waves depending on amplitude and frequency. Beta waves have a frequency of 13-30 Hz, alpha of 8-13 Hz, theta of 4-8 Hz and delta are the slowest waves with a frequency of 0.5-4 Hz. Figure taken from Malmivuo and Plonsey, (1995), with permission.

**Beta waves** (13-30 Hz): The beta waves have a relatively high frequency. The waves are irregular and with a small amplitude (2-20 $\mu$V). Beta waves are common in the awake and alert condition when a person is involved in mental or physical activity (Malmivuo and Plonsey, 1995).

**Alpha waves** (8-13 Hz): The Alpha waves are rhythmic and have an amplitude of 20-60 $\mu$V. Alpha waves are common in awake and relaxed condition when a person has his/hers eyes...
closed. However, a small percentage of the population have difficulty in producing any alpha waves. Alpha waves can be measured from the occipital region (Malmivuo and Plonsey, 1995; Rechtschaffen and Kales, 1968).

*Theta waves* (4-7 Hz): Low amplitude and mixed frequency waves which can be seen mainly in stage 1 and 2 sleep but also in REM sleep.

*Delta waves* (0.5-4 Hz): The delta waves are very slow waves and have a high amplitude of $\geq 75$ μV. These waves occur during deep sleep, also known as stages 3 and 4 (Malmivuo and Plonsey, 1995).

These different brain waves can then be used when staging and divide sleep into different stages. The staging montage is set up on the computer screen and different derivations are used. C3/A2, O2/A1, LOC/A2, ROC/A1, EMG1/EMG2, C4/A1 and O1/A2 were used in this study. The criteria for scoring sleep stages should always be based on tracings obtained from C4/A1 or C3/A2 and in this study C3/A2 trace were used (Rechtschaffen and Kales, 1968). In addition, LOC/A2, ROC/A1 and EMG1/EMG2 were used to determine the presence of REM sleep. Each record was staged epoch by epoch directly from the screen and one epoch is equal to 30 sec. The filter settings for the EEG channels were set to 0.3 Hz for the low frequency filter and 35 Hz for the high frequency filter as recommended by The American Academy of Sleep Medicine (AASM), 2007.

The staging of sleep was as mentioned previously, carried out according to the standard criteria by Rechtschaffen and Kales (1968) and the different stages are Stage W, Stage 1, 2, 3 and 4 and REM.

*Stage W*: This corresponds to the waking state and is characterized by alpha activity when eyes are closed. This stage is also usually but not always associated with high tonic EMG
activity and eye blinks can be seen in the EOG tracing (figure 2.4). More than 50% of one epoch has to consist of alpha activity to be scored as wake.

Figure 2.4: EEG trace of waking state. Characterized by alpha activity in C3 and O2 channels. High tonic submental EMG tracing in EMG1-EMG2 channel. Eye blinks can also be seen in EOG trace (LOC/ROC) and are circled in red above.
Stage 1: This is the transition from wakefulness to sleep and is also called light sleep (figure 2.5). There will be a decreased amount of alpha activity and there will be a slowing of the EEG with decreased amplitude and frequency. There will be no rapid eye movements or blinks and tonic EMG are usually below those of wakefulness. An epoch can be scored as stage 1 if the slowing of the EEG is more than 50%, meaning that occurrence of alpha activity is less than 50% (Rechtschaffen and Kales, 1968) and therefore more than 50% theta activity. Vertex sharp waves can be present in the central channels C3 and C4 and slow rolling eye movements (SEM) may be seen in EOG trace.

Figure 2.5: EEG trace of stage 1 sleep. The EEG trace starts to slow down and alpha waves disappears in C3 and O2 channels. EMG trace is lower than in wake and slow rolling eye movements (SEM) are seen in EOG trace (LOC/ROC channel) and are circled in red above.
Stage 2: The definition of stage 2 is the occurrence of sleep spindles and/or K complexes and the absence of high amplitude and slow activity seen in delta waves (figure 2.6). An epoch can be scored as stage 2 if the first spindle or K complex occurs in the first part of the epoch.

Figure 2.6: EEG trace of stage 2 sleep. Stage 2 sleep is characterized by the occurrence of K-complexes and/or spindles. Spindles are clearly defined at C3 channel above (marked with red circles).

A spindle is defined as a set of distinctive waves with frequency of around 11-16 Hz with duration of at least 0.5 seconds and one should be able to see 6-7 waves within that 0.5 sec. A K complex is defined as a wave with a negative sharp wave followed by positive (see figure 2.7) with a total duration of ≥ 0.5 seconds (Rechtschaffen and Kales, 1968).

Figure 2.7: EEG trace with K complex and Spindle. K complex with a sharp negative wave, immediately followed by a positive component. Sleep spindle with 6 – 7 waves within 0.5 sec.
Stage 3: Stage 3 is defined as an epoch which has at least 20% of slow delta waves, but not more than 50% (figure 2.8).

![Figure 2.8: EEG trace of stage 3 sleep. Between 20 and 50% of the epoch consist of slow delta waves and can be see above in the top C3 channel (marked yellow and circled red) and the epoch is scored as stage 3 sleep.](image)

Stage 4: The definition of stage 4 sleep is when more than 50% of an epoch consists of delta waves (figure 2.9).

![Figure 2.9: EEG trace of stage 4 sleep. More than 50% of epoch consists of slow delta waves (marked yellow and circled red) and can be seen in top C3 channel above. The epoch is staged as stage 4 sleep.](image)
REM: REM sleep is characterized by the occurrence of rapid eye movement seen in EOG trace in figure 2.10 (LOC-A2 and ROC-A1). The chin EMG tone is at its lowest levels. The EEG pattern is similar to that of stage 1 but without vertex sharp waves and may have some more alpha activity. Distinctive “saw-tooth” waves may appear, but there is normally an absence of K complexes and spindles.

![EEG trace of REM sleep](image)

**Figure 2.10: EEG trace of REM sleep.** The rapid eye movements can be seen in EOG trace of LOC-A2 and ROC-A1 (marked blue and circled in red above). In addition, low muscle tone can be seen in EMG trace and the epoch is staged as REM sleep.

### 2.4.2 Staging of Clinical Sleep variables

Other data to be analysed were clinical sleep variables such as arousals, limb movements and sleep apnoeas/hypopneas. The latter two was done by a full body PSG assessment, including leg EMG, respiratory belt, oximeter and thermistor. All staging of clinical sleep variables were carried out according to The AASM Manual of the Scoring of Sleep and Associated Events (2007).
2.4.2.1 Micro Arousals

A micro arousal was scored if there was an abrupt shift of EEG frequency including alpha, theta and/or frequencies greater than 16 Hz (but no spindles) that lasted at least 3 seconds and up to 15 seconds (see figure 2.11). In order to score an arousal there had to be at least 10 seconds of stable sleep preceding the event. In REM sleep there had to be an increase in EMG1-EMG2 trace lasting at least one second plus above criteria in order to score an arousal. Arousal scoring should incorporate information from both the central and occipital channels (The AASM Manual for the Scoring of Sleep and Associated Events, 2007). If the event was longer than 15 seconds and consisted of alpha activity the epoch was scored as wake instead.

Figure 2.11: EEG trace of stage 4 sleep with arousal. The epoch above is scored as sleep 4 as it consists of more than 50% of delta waves. A scored arousal can be seen at the end of the epoch (marked green). There is an abrupt change in EEG with alpha activity and this change is more than 3 seconds long but less than 15 seconds and can therefore be scored as an arousal. In addition, there is an increase in chin EMG during the arousal which can be seen in the EMG trace (circled red).
2.4.2.2 Sleep Apnoea and Hypopnoea

To be able to detect obstructive and/or central sleep apnoea as well as hypopnoea, IP belt, Thermistor and Pulse oximeter were used. A hypopnoea is an episode of shallow breathing or low respiratory rate compared to normal, while an apnoea is a complete cessation of breathing. To score an hypopnoea (see figure 2.12) all the following criteria needed to be met (The AASM Manual for the Scoring of Sleep and Associated Events, 2007):

1. Nasal pressure signal/airflow trace (measured by thermistor) needs to drop by $\geq 30\%$ of baseline. One would normally see a drop in the thoracic trace too (measured by respiratory belt).
2. The duration of this drop needed to be $\geq 10$ seconds.
3. There was a $\geq 4\%$ decrease in oxygen desaturation (measured by pulse oximeter) compared to pre-event baseline.
4. At least 90% of the event’s duration had to have the reduction in amplitude mentioned above.

Figure 2.12: EEG trace of stage 2 sleep and hypopnoeas. Scored hypopnoeas are marked in light blue on the airflow and thoracic channels. Each event was $\geq 10$ seconds. SAO2 channel shows oxygen saturation (SAO2) as a red line and hypopnoea associated O2 desaturations are marked in pink.
To be able to score an obstructive apnoea (see figure 2.13) the following criteria needed to be met (The AASM Manual for the Scoring of Sleep and Associated Events, 2007):

1. There was a drop in the nasal pressure signal/airflow trace by \(\geq 90\%\) of baseline with a continued or increased respiratory effort (measured by respiratory belt) throughout the entire period of absent airflow.

2. The duration of the event needed to be \(\geq 10\) seconds.

3. At least 90\% of the event’s duration had to meet the amplitude reduction criteria for apnoea.

Figure 2.13: EEG trace of stage 1 sleep with obstructive sleep apnoea. The obstructive apnoea events are marked in blue with associated oxygen desaturation marked in pink. There is a marked drop in altitude of airflow trace while there is a continued respiratory effort seen in thoracic trace. Each event was \(\geq 10\) seconds.
To be able to score a central apnoea (see figure 2.14) the following criteria needed to be met (The AASM Manual for the Scoring of Sleep and Associated Events, 2007):

1. There was a drop in the nasal pressure signal/airflow trace by $\geq 90\%$ of baseline together with an absent of respiratory effort throughout the entire period.

2. The duration of the event needed to be $\geq 10$ seconds.

3. At least 90% of the event's duration had to meet the amplitude reduction criteria for apnoea.

Figure 2.14: EEG trace of stage 2 sleep with central sleep apnoea. The central apnoea events are marked in purple with associated oxygen desaturation marked in pink. There is an absence of both airflow and respiratory effort and therefore a marked decrease of airflow trace and thoracic trace, respectively. Each event was $\geq 10$ s.

To be able to score a mixed apnoea the same criteria as above needed to be followed except that the respiratory effort was absent in the initial period of the event, followed by resumption of the effort in the latter part of the event. All scored apnoeas and hypopnoeas of a subject were added together and then divided by the total sleep time in order to get an
Apnoea/hypopnoea index (AHI). This index defined the number of apnoeas and hypopnoeas per hour of sleep. An AHI of less than 5 episodes per hour was considered normal. An AHI of 5-15 per hour of sleep is generally defined as mild, 15-30 moderate and above 30 episodes per hour of sleep is considered severe (Ruehl and et al., 2009).

2.4.2.3 Periodic Limb Movement of Sleep (PLMS)

Periodic limb movements were scored from the trace of leg EMGs (figure 2.15). In order to score a limb movement (LM) the following criteria needed to be met:

1. The minimum duration of a LM must have been 0.5 seconds with a maximum duration of 10 seconds.

2. There must have been a minimum increase in amplitude of 8 µV above resting EMG.

One needs 4 consecutive LM with a minimum duration of 5 seconds and maximum duration of 90 seconds (defined as the time between onsets of consecutive LMs) in order to score a PLMS series. PLMS series can be scored from both left and right leg EMG. However, leg movements on two different legs which are separated by less than 5 seconds are scored as one single leg movement.

Figure 2.15: Trace of SAO2, nasal airflow, thoracic chest effort, left leg and right leg EMG. Leg movements are marked in purple on the trace of the left leg, each movement was within the criteria of 0.5-10 sec. The limb movement occurs in a periodic fashion with no less than 5 seconds between each movement and not more than 90 seconds which is the criteria for PLMS.
2.5 Subjective assessments

Several subjective tests were used in this study. The Leeds sleep evaluation questionnaire (LSEQ) and sleep diaries were used for subjective assessment of sleep. The LSEQ was carried out at 0800h on day D8 for placebo, D22 for low dose and D36 for high dose, while the diaries were filled in by each patient on a daily basis throughout the study. Line analogue rating scales (LARS) were used to assess sedation and mood, while the Karolinska sleepiness scale (KSS) was used for assessment of daytime sleepiness. Both tests were performed at 0800h, 1000h, 1300h and 1600h on above days.

Subjective pain was assessed using the brief pain inventory (BPI) and visual analogue scale (VAS). The BPI was filled in by the patients on a daily basis throughout the study, while VAS was carried out at 0800h on D8, D22 and D36.

2.5.1 Leeds Sleep Evaluation Questionnaire

The LSEQ was used to assess subjective ratings of sleep quality and early morning behaviour (Hindmarch and Gudgeon, 1980; Parrot and Hindmarch, 1980; Zisapel and Laudon, 2003)). The patients marked a series of 100 mm linear analogue scales. A score of 50 units indicated no change in subjective rating when compared with prior to treatment. Improvement was at one end of the scale (<50) and worsening on the other end of the scale (>50). Four factors were assessed by LSEQ: the ease of getting to sleep, quality of sleep, ease of waking from sleep, and behaviour following awakening (Zisapel and Laudon, 2003). Appendix 2
2.5.2 Karolinska Sleepiness Scale

Patients were shown a nine-point scale and asked to choose a number between 1 (very alert) and 9 (very sleepy and great effort to keep awake) to reflect the level of sleepiness they felt at that point (Gillberg et al., 1994). Appendix 3.

2.5.3 Brief Pain Inventory

This was a short form of the brief pain inventory (BPI) and asked patients to describe and rate their pain from 0 being no pain to 10 being worse pain possible. The results gave a pain severity score. Patients were also asked if and how much their pain interfered with different aspects of their daily life such as pain interference on sleep, 0 being no interference and 10 being pain completely interfering. The results gave a pain interference score. Appendix 4.

2.5.4 Visual Analogue Scale

The VAS was used for rating subjective pain and is a common tool to assess the analgesic properties of various drugs (Langley and Sheppeard, 1985). The VAS comprises of a vertical line, 100mm long. “No pain” and “very severe pain” is written at each end of the line and patients marked the line at the point they felt best represented their pain state at that particular moment in time. Score was determined by measuring from left end of the scale to the point where patients had marked the line. Improvement was (<50) and worsening (>50).

2.5.5 Line Analogue Rating Scale

The LARS are rating scales which allow the subjective effects of a drug to be assessed (Hindmarch, 1980). The patients marked a series of 0-100mm analogue scales where a score of 50mm indicated no change in subjective feelings. Factors being assessed were; sedation, mood and co-ordination. All positive effects such as less tired, more energetic and improved
mood were at one end of the scale (<50) and all negative effects were on the other end of the scale (>50). Appendix 5.

2.6 Continuous Glucose Monitoring

The interstitial glucose levels were monitored over the period that the patients were in the Surrey CRC. This was carried out by using the CGMS® System Gold™ Continuous Glucose Monitoring (Medtronic MiniMed, Inc., CA U.S.A). This system is designed to provide continuous measurements of interstitial glucose levels over the range 2.2-22.0 m.mol/l. The CGMS system consists of two major components, a glucose sensor and a small battery operated monitor. The sensor contains the enzyme glucose oxidase which catalyses a reaction in the presence of oxygen and glucose. This reaction transfers electrons and creates an electronic current which can be measured and converted into a glucose concentration. The sensor will measure glucose levels with an interval of 10 seconds and the monitor will then give an average of these measurements every 5 minutes. The disposable glucose sensor was inserted into the subcutaneous tissue in the abdominal area of the patient on the day of admission to the Surrey CRC (D6, D20, and D34). The sensor was then worn throughout the next day and night and removed in the afternoon of day 8, 22 and 36 (in total, approximately 48 hours for each visit). This 48 hour period provided around 576 glucose readings which were downloaded onto a computer for analysis using The Medtronic Minimed CGMS® Solutions™ Software Version 3.0.

In order to validate the CGMS system it was important that the CGMS was calibrated with a SMBG test (finger stick reading) at least four times a day. This calibration was made approximately every 4 hours during wake periods.
CHAPTER 3 - RESULTS
3. A comparison of sleep architecture in diabetics and healthy volunteers

3.1 Introduction

Sleep disturbances are thought to be common in people with diabetes mellitus (DM) and diabetic patients have been reported to have a higher prevalence of sleep apnoeas and hypopnoeas compared with the healthy population and this may result in more fragmented sleep with arousals and awakenings (Foley et al., 2004; Resnick et al., 2003; Vigg 2003). In addition, patients with painful diabetic peripheral neuropathy (DPN) often report that pain has a severe impact on sleep (Benbow et al., 1998; Galer et al., 2000; Gore et al., 2005). Other complications associated with DPN such as restless leg syndrome and periodic limb movements may contribute further to poor sleep (Foley et al., 2004; Resnick et al., 2003; Vigg 2003).

3.1.1 Study Aim

Loss of sleep and poor sleep quality may impact on patients' overall well being and add to the burden of living with DM. Most data available in regards to sleep in diabetic patients are based on subjective questionnaires where patients rate their quality of sleep. Few studies have quantified sleep disturbances in these patients through polysomnography (PSG) which is considered to be the gold standard technique used in sleep research. The aim of this study was therefore to investigate the sleep architecture and sleep quality of diabetic patients with painful DPN using polysomnography in order to obtain a more objective profile of sleep problems in these patients. An additional aim was to see if there were any differences in sleep in patients with painful DPN compared with a healthy population, and if so whether this is due to the pain associated with DPN or due to a possibly higher rate of obesity seen in
diabetic patients. Obesity is known to be linked to heavy snoring and sleep apnoea which can, as mentioned earlier, disrupt sleep by leading to more nocturnal awakenings.

3.2 Methods

3.2.1 Subjects

The subjects included in the analysis were a diabetic group and a group of healthy volunteers used for comparison. The diabetic subjects were randomly selected from the 83 subjects taking part in the main diabetic study conducted at the Surrey – CRC in 2008-2009 and approved by the Essex 1 Research Ethics Committee (study description in chapter 2 general methods and materials). The historical control group was created by using healthy volunteers from the Surrey Sleep Research Centre (SSRC) database. The healthy volunteers were selected from two large studies conducted at the Surrey-CRC, approved by Ravenscourt Ethics Committee and Quorn Research Review Committee. The overnight study conditions for both the diabetic group and healthy control group were very similar (see 3.2.2) and the healthy volunteer data was therefore considered to be suitable to use as comparison with the diabetic group. All studies were conducted in accordance with the declaration of Helsinki and good clinical practice. All patients gave written informed consent prior to screening.

3.2.1.1 Diabetic Group

40 type 1 and 2 DM patients (males = 23, females = 17) with painful DPN were selected. Baseline night (equal to visit 1, night 7) was used for analysis. They had been diagnosed with DM for at least a year and had neuropathic pain of diabetic origin with a score above 12 on the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS). For more detailed exclusion/inclusion criteria please see chapter 2 section 2.1.
3.2.1.2 Healthy Control Group

40 healthy subjects (23 male and 17 females) were selected for the analysis. They were gender and aged matched (± 2 years) with the diabetic patients. Subjects had been included in the SSRC studies if in the opinion of the principal investigator they were healthy on the basis of a physical examination, medical history, vital signs, electrocardiogram, and satisfactory laboratory test results. Subjects were excluded if they had a score of >5 points on the Pittsburgh Sleep Questionnaire Inventory (PSQI) scale, sleep apnoea (AHI of > 15/hour) or periodic limb movements (associated with arousal index >15/hour) or any other chronic sleep complaints. Subjects were also excluded if they had a BMI above 33 kg/m². The exclusion criteria were set by the sponsor for the studies of which healthy subjects were selected from.

3.2.2 PSG overnight recording and sleep staging

All recordings were carried out in the sleep laboratories at the Surrey-CRC as stated in the general methods and materials (section 2.3) using the Compumedics Siesta System® (Compumedics Ltd., Australia). Time in bed was 8 hours (2300-0700) and both groups had a habituation night before the PSG study night in order to get used to the environment. The healthy control population group had previously been screened for sleep apnoea and periodic limb movements of sleep and could therefore be considered not only healthy but also without known sleep disorders. The PSG night for both groups was staged by the same sleep stager and according to the R&K criteria as stated in general methods and materials (section 2.4).

3.2.3 Karolinska sleepiness Scale

Karolinska Sleepiness Scale (KSS) was used to assess daytime sleepiness in both population groups. A brief description of KSS can be found under general methods and materials section 2.5.2.
3.2.5 Statistical methods

The subjects in the 2 datasets (diabetic group and healthy group) were matched pair wise and each one of these paired values were analysed as a dependent variable in a linear mixed model using SAS® PROC MIXED 9.1. The dataset identifier was a single categorical independent variable and dataset identifier as a repeated measure with unstructured variance-covariance matrix. The modelling was carried out as a build-up operation with potential additional independent variable being the covariate body mass index (BMI). A model entry and rejection p-value threshold of 0.05 was used in this build-up model.
3.3 Results

3.3.1 Demographics

The control group was age (± 2 years) and gender matched with the diabetic group. Mean age for the diabetic population was 64.6, ± 9.1 years and mean age for healthy volunteer population was 64.7, ± 9.1 years. Mean body mass index (BMI) for the diabetic group was 32.0 ± SD 5.8 kg/m² and 26.4 ± 2.6 kg/m² for the control group.

3.3.2 Polysomnography (PSG)

PSG variables analysed included; sleep efficiency (SE, %), total sleep time (TST, min), wake after sleep onset (WASO, min), latency to persistent sleep (LPS, min), and duration (min) and percentage of time awake throughout the night. Total duration (min) and percentage of stage 1, 2, 3, 4, rapid eye movement (REM) sleep and slow wave sleep (SWS) were also analysed. See appendix 6 for more detailed definition of sleep variables.

In regards to the statistical analysis, all PSG analysis had BMI added as an independent co-variant. However, BMI did not significantly change any of the sleep variables and the significant differences seen between the diabetic group and the healthy control group in the results below still persisted. The differences seen in sleep could therefore not be explained by the difference in BMI between the groups.

3.3.2.1 Wake time and latency to sleep

The results showed that diabetic patients had significantly more WASO compared to healthy volunteers (mean, 81 min and 57 min, respectively, P<0.05) and longer duration of stage 0 (wake) compared with the healthy control group (mean of 98 min and 67 min, respectively, P<0.01) (figure 3.1 and 3.2).
Figure 3.1: Bar graph of wake after sleep onset (WASO). Diabetic subjects (light blue) had significantly more WASO compared with healthy subjects (dark blue). n=38 for both Bars show mean ± SEM, * = P<0.05.

Figure 3.2: Bar graph of duration of stage 0 (Wake). Diabetic subjects (light blue bar) had a significantly longer duration of wake compared with healthy subjects (dark blue bar). n = 38 for each group. Bars show mean ± SEM, ** = P<0.01.
When looking at latency to persistent sleep (see definition in appendix 6), the diabetic group had a significantly longer latency to persistent sleep compared with the healthy group, (mean of 22.5 min for diabetic patients and mean 14 min for healthy volunteers, P<0.05, figure 3.3).

![Figure 3.3: Bar graph of latency to persistent sleep. Diabetic subjects (light blue bar) had significantly longer latency to persistent sleep compared with healthy volunteers (dark blue bar). n = 40 for each group. Bars show mean ± SEM, * = P<0.05.](image-url)

### 3.3.2.2 Total Sleep Time and Sleep Efficiency

PSG variables TST and SE, which gives an indication of quality and sleep continuity, were also significantly different between the two groups (figure 3.4 and 3.3 respectively). TST was less in the diabetic group compared with the healthy volunteer group (mean 383 min compared with mean 412 min, P<0.01), and diabetic patients had significantly worse SE (P<0.01) compared with the healthy volunteers (mean 79% compared with mean 86%).
Figure 3.4: Bar graph of total sleep time (TST). Diabetic subjects (light blue bar) have significantly less TST compared with healthy subjects (dark blue bar). \( n = 38 \) for each group Bars show mean ± standard error of mean (SEM), \( ** = p < 0.01 \).

Figure 3.5: Bar graph of sleep efficiency (SE). Diabetic subjects (light blue bar) have significantly worse SE compared with healthy subjects (dark blue bar). \( n = 38 \) for each group Bars show mean ± SEM, \( ** = p < 0.01 \).
3.3.2.3 Sleep architecture NREM and REM

Total sleep time can be divided into REM sleep and NREM sleep. Diabetic patients had significantly less REM sleep compared with healthy volunteers (mean of 79 min compared with 95 min, \(P<0.01\)), figure 3.6. However, there was no significant difference in duration of NREM, figure 3.7.

Figure 3.6: Bar graph of duration of REM sleep. Diabetic subjects (light blue bar) had a significantly shorter duration of REM sleep compared with healthy subjects (dark blue bar). \(n=38\) for each group Bars show mean ± SEM, ** = \(P<0.01\).
Figure 3.7: Bar graph of duration of NREM sleep. Diabetic subjects (light blue bar) had less NREM compared with healthy subjects (dark blue bar) but this was not statistically significant (P = 0.07). n = 38 for each group. Bars show mean ± SEM.

NREM sleep can be further divided into stage 1, 2, 3 and 4, and 3 and 4 can be combined to represent SWS. Inspection of the individual NREM stages showed that whilst there was no significant difference in stages 1, 2 and 3 between diabetic patients and healthy volunteers (figure 3.8-3.10) there was a significant reduction in stage 4 sleep in diabetic patients compared with the healthy volunteers (25 min for diabetics and 40 min for healthy volunteers, P<0.01, figure 3.11).
Figure 3.8: Bar graph of duration of stage 1 sleep. There was no significant difference in stage 1 sleep between diabetic subjects (light blue bar) and healthy subjects (dark blue bar) n = 38 for each group. Bars show mean ± SEM.

Figure 3.9: Bar graph of duration of stage 2 sleep. There was no significant difference in stage 2 sleep between diabetic subjects (light blue bar) and healthy subjects (dark blue bar) n = 38 for each group. Bars show mean ± SEM.
Figure 3.10: Bar graph of duration of stage 3 sleep. There was no significant difference in stage 3 sleep between diabetic subjects (light blue bar) and healthy subjects (dark blue bar) \( n = 38 \) for each group. Bars show mean ± SEM.

Figure 3.11: Bar graph of duration of stage 4 sleep. Diabetic subjects (light blue bar) had a significantly shorter duration of stage 4 sleep compared with healthy subjects (dark blue bar). \( n = 38 \) for each group. Bars show mean ± SEM, ** = \( P < 0.01 \).
When looking at duration of SWS (stage 3 and 4 together) there was no significant difference between the two groups. However, the latency to SWS was significantly increased in the diabetic patient group (mean 60 min compared with 31 min for the healthy group, P<0.01, figure 3.12).

Figure 3.12: Bar graph of SWS latency. Diabetic group (light blue bar) had a significantly longer latency to SWS compared with the healthy group (dark blue bar). n = 38 for both. Bars show mean ± SEM, P** = P<0.01.
With regards to %REM and %NREM sleep as a proportion of TST there was no significant difference between the two groups. The diabetic patient group had 79% NREM while healthy volunteers had 77% NREM sleep, figure 3.13. For REM sleep the diabetic patient group had 21% and healthy volunteers had 23%, (figure 3.14).

**Figure 3.13: NREM sleep as % of TST.** There was no significant difference in %NREM of TST between diabetic group (light blue bar) and healthy group (dark blue bar). n = 38 for both Bars show mean ± SEM.

**Figure 3.14: REM sleep as % of TST.** There was no significant difference in %REM sleep of TST between diabetic group (light blue bar) and healthy group (dark blue bar). n = 38 for both Bars show mean ± SEM.
3.2.3.1 Analysis per third of the night

When analysing sleep one can also investigate sleep architecture in thirds of the night. Only REM sleep and SWS was analysed in this way. Results showed that REM duration was not significantly different between the two groups when each third of night was compared (figure 3.15). For SWS there was a significant reduction in amount of SWS for the first third of the night in the diabetic group compared with healthy volunteer group (mean of 29 min compared with 39 min, P<0.05) and this was mainly made up by the difference in stage 4 sleep (figure 3.16). There was no difference in SWS in the second and third 3rd of the night and as mentioned earlier, no significant difference in the night in whole.

Figure 3.15: Duration of REM sleep in 3rd of the night. A. There was no statistical significant difference in amount of REM sleep between diabetic subjects (light blue bar) and healthy subjects (dark blue bar) in the first 3rd of the night. B. There was no statistical significant difference in amount of REM sleep between diabetic subjects (light blue bar) and healthy subjects (dark blue bar) in the second 3rd of the night. C. There was no statistical significant difference in amount of REM sleep between diabetic subjects (light blue bar) and healthy subjects (dark blue bar) in the third 3rd of the night.

REM1= Duration of REM sleep in first 3rd of night, REM2= Duration of REM sleep in second 3rd of night, REM3= Duration of REM in third 3rd of night. n = 38 for each group Bars show mean ± SEM.
Figure 3.16: Duration of Slow wave sleep (SWS) in 3rd of the night. A. Diabetic subjects (light blue bar) had significantly less duration of SWS compared with healthy subjects (dark blue bar) in the first 3rd of the night. B. There was no significant difference in SWS duration between the two groups in the second 3rd of the night. C. There was no significant difference in SWS duration between the two groups in the third 3rd of the night.

SWS1 = Duration of SWS in first 3rd of night, SWS2 = Duration of SWS in second 3rd of night, SWS3 = Duration of SWS in third 3rd of night. n = 38 for each group Bars show mean ± SEM, * = P<0.05.
3.3.2.4 Gender differences

There was no significant gender differences in the amount of TST or in SE. Healthy women had similar SE and TST as healthy men, while there was only a slight difference seen between diabetic women and diabetic men with diabetic women having more TST and higher SE than diabetic men, however this was still not significant (figure 3.17 for SE).

Figure 3.17: Bar graph of sleep efficiency (SE) for gender. Gender did not affect the % of SE and there was therefore no significant difference between men and women. Healthy females (dark orange bar) and healthy males (dark purple bar) had similar amount of SE, while diabetic females (light orange bar) had a slightly higher % SE compared with diabetic males (light purple bar) but this difference was still not statistically significantly. n = 23 males and 17 females for both groups. Bars show mean ± SEM.
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Figure 3.19: Bar graph of REM sleep duration for gender. Gender did not affect the amount of REM sleep and there was therefore no significant difference between men and women. Healthy females (dark orange bar) and healthy males (dark purple bar) had similar amount of wake time, while diabetic females (light orange bar) had slightly less REM sleep compared with diabetic males (light purple bar) but this was still not significantly different. \( n = 23 \) males and 17 females for both groups. Bars show mean ± SEM.

Figure 3.20: Bar graph of duration of SWS for gender. Gender did have a significant effect on SWS with women across both groups having significantly more SWS compared with the men. Healthy females (dark orange bar) and diabetic females (light orange bar) compared with healthy males (dark purple bar) and diabetic males (light purple bar) \( n = 23 \) male and 17 females in both groups Bars show mean ± SEM, \( P^{**} = P < 0.01 \).
Figure 3.21: Bar graph of duration of stage 4 sleep for gender. Gender did have a significant effect on stage 4 sleep with women across both groups having more stage 4 sleep compared with the men. Healthy females (dark orange bar) and diabetic females (light orange bar) compared with healthy males (dark purple bar) and diabetic males (light purple bar) n = 23 male and 17 females in both groups Bars show mean ± SEM, P** = P<0.01.
3.3.2.5 Age differences

Age may also affect sleep and analysis showed that both SE and TST were significantly reduced with increasing age (P<0.01) in both diabetic patients and healthy volunteers. The effect of age on SE can be seen figure 3.22 below.

Figure 3.22: Scatter plot of sleep efficiency (SE) over age. SE decreases significantly with age (P<0.01) in both diabetic subjects (light blue) and healthy subjects (dark blue).
Further analysis of both population groups showed that age had a significant impact on WASO and duration of wake (figure 3.23 for the latter), both increasing with increasing age (P<0.01 for both).

Figure 3.23: Scatter plot of duration of stage 0 (wake) with age. Duration of wake significantly increased with age in both diabetic subjects (light blue) and healthy subjects (dark blue). P<0.01
When looking at the effect of age on sleep architecture it was shown that stage 1 sleep increased with increasing age, while stage 2 sleep decreased (P<0.05 for both), (figure 3.24 and 3.25, respectively). There was no significant age effect on stages 3, 4, while the effect of age on REM sleep approached significance, P = 0.052.

Figure 3.24: Scatter plot of duration of stage 1 sleep with age. Duration of stage 1 sleep significantly increased with age in both diabetic subjects (light blue) and healthy subjects (dark blue). P<0.05

Figure 3.25: Scatter plot of duration of stage 2 sleep with age. Duration of stage 2 sleep significantly decreased with age in both diabetic subjects (light blue) and healthy subjects (dark blue). P<0.05
3.3.3 Subjective day time sleepiness

There was a significant difference in subjective day time sleepiness between the two groups (see figure 3.26) which was measured using Karolinska Sleepiness Scale (KSS). Diabetic patients were significantly more sleepy compared with healthy volunteers (mean 5.0 and 2.9, respectively, $P<0.001$). Range 1-7 for healthy subjects and 2-9 for diabetic patients (see percentage of distribution in figure 3.27).

![Figure 3.26: Bar graph of Karolinska Sleepiness Scale. Diabetic subjects (light blue bar) were significantly more sleepy compared with healthy subjects (dark blue bar). KSS = Karolinska Sleepiness Scale. Bars show mean ± SEM, *** = $P<0.001$](image)

![Figure 3.27: Bar graph of distribution of KSS scores. Diabetic subjects (light blue bars), Healthy subjects (dark blue bars) KSS = Karolinska Sleepiness Scale.](image)
3.4 Discussion

Poor sleep is commonly reported in diabetic patients and the prevalence of insomnia, restless leg syndrome, periodic limb movement and sleep disordered breathing have been previously reported as being high in this patient group (Foley et al., 2004) and they can all affect sleep and cause sleep disturbances (Resnick et al., 2003; Vigg 2003). Furthermore, obesity is commonly seen in diabetic patients, especially in patients with type 2 DM and it has been reported that obese individuals, even in the absence of sleep disordered breathing, have reduced quality of sleep with low amounts of slow wave sleep (Tasali et al., 2007).

Another factor that may affect sleep in this patient group is common diabetic complications such as painful diabetic peripheral neuropathy (DPN). Pain can have a negative effect on sleep and pain is often reported to worsen at night (Benbow et al., 1998; Galer et al., 2000; Gore et al., 2005). Night time pain can interfere with time falling asleep, increase awakenings during the night and therefore result in difficulties in maintaining sleep (Moldofsky, 2001).

In addition, sleep disturbances may also be associated with impaired glucose metabolism. One study by Trento et al. (2008) using actigraphy as an objective measure of sleep showed that HbA1c correlated inversely with sleep efficiency and positively with moving time during sleep suggesting that impaired glucose control led to an increase in wake during sleep and consequently poorer sleep efficiency. The study also compared actigraphic sleep from type 2 diabetic patients on oral agents only (M = 32, F = 17, mean age = 61.0 years, mean BMI = 27.1 kg/m²) with a matched planned healthy control group (M = 11, F=11, mean age = 58 years, mean BMI = 25.5 kg/m²; mean age, BMI, gender). The results showed that the diabetic patient group had lower sleep efficiency and higher fragmentation index and moving time compared with the healthy volunteer group. These results suggest that diabetic patients have
disturbed sleep even in the absence of exogenous insulin treatment and complications such as neuropathy or obesity. All patients with symptoms of neuropathy were excluded and no patients on exogenous insulin treatment were included in the study. The diabetic patients had a mean BMI of 27 kg/m² which is considered overweight but not obese as definition of obesity is BMI > 30. The BMI in diabetic groups was also matched with that of the healthy group (Trento et al., 2008).

The aim of the analysis presented here was to characterise sleep, using polysomnography, in diabetic patients with painful DPN and to investigate if there were any differences in sleep quality and sleep architecture in this patient group compared with a historical healthy population. The two groups were matched for age and gender and all the analyses were adjusted for BMI in order to distinguish whether potential differences could be explained by covariates such as BMI.

The results showed that there were several differences in sleep between the two groups. Diabetic patients had significantly worse SE, less TST, more WASO and a longer duration of wake during the whole night compared with healthy subjects and this indicated poorer sleep quality and worse sleep continuity. In addition, diabetic patients had a significantly longer LPS compared with the healthy control group. Poor sleep quality and sleep continuity may result in increased daytime sleepiness and indeed analysis of the KSS showed that diabetic patients were significantly sleepier during the day compared with healthy subjects and this may have an impact on patients’ overall well being.

The analysis also showed distinct differences in sleep architecture between diabetic patients and healthy volunteers. Diabetic patients overall had a significantly shorter duration of REM sleep compared with healthy subjects. This difference was observed in all thirds of the night although was only statistically significant when looking at the night as a whole. Diabetic
patients also had less SWS compared with healthy subjects, but this was only statistically significant in the first 3rd of the night. When dividing SWS into stage 3 and 4 one could see that diabetic subjects had less stage 4 sleep compared with healthy subjects. This difference was statistically significant and was observed across the whole night. The difference seen between the healthy volunteers and diabetic patients might partly be explained by the fact that the diabetic group had a mean BMI of 32 whilst the healthy volunteer group had a mean BMI of 26. Previous reports have suggested that obese individuals may have a lower amount of SWS (Tasali et al., 2007). However, adjustment for BMI did not affect the significant differences in TST, SE and SWS across the two groups nor did it affect stage 4 sleep or any other sleep stages. The loss of SWS seen in the diabetic group seems to be replaced with increased duration of the lighter stage 1 sleep and increased amount of stage 2 sleep compared with the healthy group. This increase in stage 1 sleep, at the expense of deeper stage 4 sleep leads to the finding of no overall significant change in NREM sleep across the two groups.

Despite changes in TST, duration of REM sleep and stage 4 sleep it appeared that the proportion of NREM and REM sleep as a percentage of TST was still preserved. Both the diabetic patients and healthy volunteers had a similar percentage of REM (21 and 23 % respectively) and NREM sleep (79 and 77% respectively). This suggested that although absolute amounts of sleep stages may differ, the overall sleep architecture is preserved.

Finally, the effect of age and gender on sleep in both diabetic patients and healthy volunteers was examined. Age related changes in sleep have previously been reported in healthy subjects (Boselli et al., 1998; Hume et al., 1998; Neubauer et al., 1999). Total sleep time decreases with age, partly due to increased wake periods within sleep, therefore more fragmented sleep. Older adults also have a tendency to have more arousals than younger
adults (Boselli et al., 1998; Hume et al., 1998; Neubauer, 1999). This change in sleep in healthy subjects could also be seen in this study as there was a worsening of TST and SE and increase in wake and WASO with increasing age, but the changes with age were consistent across both the healthy group as well as the diabetic group. Furthermore, the amount of SWS has been previously reported to decrease with age, while the amount of REM is thought to be only slightly reduced (Carrier et al., 2001; Ehlers and Kupfer, 1997; Neubauer, 1999). The results of the present study showed that there was no evidence of a statistically significant reduction in SWS with age, but that the age effect on REM sleep approached significance with REM sleep decreasing with increasing age. This reduction in REM sleep could be seen in both the healthy volunteers and the diabetic patients.

It is unclear why there was no evidence of a loss of SWS with increasing age in this present study however it may possibly be due to the narrow age range of patients and volunteers investigated. As diabetic patients were required to have a history of painful diabetic peripheral neuropathy a complication which normally appears many years after diabetic diagnosis, the majority of patients and aged matched healthy volunteers were in the narrow age range of 55–75 years.

With regards to gender differences and sleep, SWS has also previously been reported to differ between males and females, with females having longer duration SWS compared with age-matched males (Ehlers and Kupfer, 1997). Indeed, the results from the current showed that gender did indeed have a significant effect on SWS with females having longer duration SWS compared with men. The gender effect was strongest with regard to stage 4 sleep with females having almost twice as much stage 4 sleep as their male counterpart. This gender difference could be seen across both the diabetic patient group and healthy volunteer group, however it was most prominent in the diabetic group with diabetic males having far less SWS
and stage 4 sleep compared to not only diabetic and healthy females but also compared with healthy males. This would suggest that the diabetic condition has a more detrimental effect on SWS in men compared with females as diabetic females and healthy females had similar amount of SWS when compared and it was only between the males that a difference was seen. There was no significant effect of gender on any other sleep stages or on any variables of sleep quality and continuity.

In conclusion, both the healthy volunteer group and the diabetic patient group displayed expected changes with respect to age and gender. However, age and gender could not explain the difference in sleep seen between the two groups as the analysis was based on age and gender matched groups. Overall, diabetic patients with painful DPN appear to have worse sleep quality, less total sleep time, a lower amount of REM sleep and reduced stage 4 sleep. The loss of REM and SWS may be of importance as REM sleep is thought to be involved in memory consolidation whilst SWS is thought to be the restorative part of sleep (Kamel and Gammack, 2006; Najib, 2006; Tasali et al., 2007; Vgontzas and Kales, 1999). Whilst one can establish such differences, it is still difficult to determine the cause. It appears from the analysis that changes in BMI cannot explain the differences seen as BMI was used as a covariate in the analysis and did not change the differences seen in the results. However, the poor sleep in this diabetic population may result from a combination of factors such as possible sleep disorders like sleep apnoea, periodic limb movements, glucose control and/or neuropathic pain itself. These factors will be investigated further in chapter five.

Poor sleep can have a profound effect on everyday functioning and quality of life with sleep loss and poor sleep quality known to be associated with cognitive decline and changes in mood (Benbow et al., 1998; Davies et al., 2006; Galer et al., 2000). All of these negative effects of poor sleep can add further to the burden of living with DM and it is therefore
important to take sleep problems into consideration when looking at different treatment options for DM patients with painful DPN. This will be investigated and discussed further in chapter 4.

There are limited PSG data published looking at sleep in diabetic patients with painful DPN compared with healthy subjects. This exploratory analysis shows that there are objective differences in sleep between these two groups. In order to more fully elucidate the impact of diabetes and pain on sleep future studies should include a patient group with diabetes but without painful DPN as well as a planned healthy control group matched for age and gender. This would enable the effect of neuropathic pain, glucose control and other factors associated with the diabetic condition to be explored and modelled in order to establish the main causes of sleep disturbance in both diabetic patients and diabetic patients with further complications.
CHAPTER 4 - RESULTS
CHAPTER 4 RESULTS

4. The effects of pregabalin, duloxetine and amitriptyline on sleep architecture, sleep quality and pain in type 1 and type 2 diabetic patients with painful DPN.

4.1 Introduction

Chronic painful diabetic peripheral neuropathy (DPN) is often very difficult to treat, with treatment regimes often inadequate at controlling pain and limited by side-effects and drug tolerance (Barbano et al., 2004; Quattrini and Tesfaye, 2003; Vinik and Mehrabyan, 2004). The aim when treating chronic painful DPN is to reduce pain and improve a patient’s quality of life (QOL).

Various treatments, both pharmacological and non-pharmacological are available. One non-pharmacological treatment that has been reported to reverse symptoms of DPN is the Anodyne Therapy System. A study by Leonard et al showed that treatment with this near-infrared medical device improved sensation in feet, improved balance and also reduced pain in subjects with DPN (Leonard et al., 2004). Pharmacological treatments however are more common and include lidocaine patches, opioid analgesics, tricyclic antidepressants and antiepileptics such as gabapentin, and more recently pregabalin.

Of the pharmacological agents most commonly used to treat chronic pain in DPN we have focused on three treatments: amitriptyline (AMI), duloxetine (DUL) and pregabalin (PGB) and have investigated the effects of the treatments on pain, sleep architecture and sleep

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quality in type 1 and 2 DM patients with painful DPN. More detailed information on mechanism of action, efficacy and safety of each of these drugs is given below.

4.1.1 Amitriptyline

Tricyclic antidepressants (TCA) are known to have an analgesic effect on neuropathic pain and have been considered as the first-line therapy in painful DPN (Joss, 1999, McQuay et al., 1996). Amitriptyline is known to be the most effective TCA in the treatment of neuropathic pain and its good efficacy has been shown in several clinical trials, however, it is not licensed for neuropathic pain (Dalocchio et al., 2000; Max et al., 1988; Max et al., 1987; Morello et al., 1999). Side effects are known to be many and the drug is therefore not well tolerated and these are limiting factors for the usage of AMI (Dalocchio et al., 2000; Morello et al., 1999).

4.1.1.1 Pharmacology and mechanism of action

Amitriptyline inhibits the re-uptake of noradrenaline and serotonin initially potentiating the effect of these neurotransmitters but longer term inducing changes in post- and pre-synaptic receptors which is thought to underlie the principal mechanism of action of the drug. Amitriptyline is not highly selective and acts on a number of receptors including muscarinic acetylcholine receptors, H1 histamine receptors and α1-adrenergic receptors. Amitriptyline effects on both the muscarinic acetylcholine and histaminergic receptors have the potential to impact sleep. Muscarinic receptors are thought to be involved in the regulation of REM sleep with muscarinic antagonists lengthening REM latency and reducing REM activity and density, and histamine H1 receptors are involved in wake regulation with antagonism of these receptors linked to promotion of sleep (Haas and Panula, 2003). However, the full mechanism of action of amitriptyline in humans is not known (Bryson and Wilde, 1996; Gupta et al., 1999; Haas and Panula, 2003).
4.1.1.2 Pharmacokinetics

Amitriptyline is rapidly absorbed with peak plasma concentration usually occurring between 4 and 8 hours after administration and an elimination half-life of between 10-28 hours, average 15 hours. It is metabolized in the liver and the active metabolite nortriptyline has a half-life of 16 to 80 hours. It is excreted in the urine and small amounts are excreted in the bile (Bowden et al., 1985; U’Prichard et al., 1978).

4.1.1.3 Dose range and efficacy in Painful DPN

Amitriptyline is administered orally in 25 mg tablets containing amitriptyline hydrochloride. It is normally administered 75mg/day in either divided doses or as a single dose at night time. The dose can then be increased up to 150mg/day if required.

Amitriptyline has been shown to be effective in the treatment of painful DPN even at very low doses (McQuay et al., 1992). The mechanism of action of the analgesic effect of amitriptyline is not fully understood but it is suggested that it activates the opioid endogenous system through an elevation in noradrenaline levels possibly caused through the antagonism of α2-adrenoceptors. In addition, AMI has been shown to block both Na+ channels and Ca2+ channels. The inhibition of serotonin re-uptake and also blockade of muscarinic receptors may also play a role. These mechanisms combined are thought to contribute to the analgesic effects seen with amitriptyline (Ashina et al., 2004; Mico et al. 2006).

4.1.1.4 Adverse events

Adverse events from AMI are common and this is as mentioned earlier a limiting factor in treatment regimes. Several studies have shown that night time dosing with AMI leads to 'residual effects' the morning after, with increased drowsiness, increased subjective rating of
sedation and difficulty waking as well as worsened subjective alertness and increased
daytime somnolence when compared with placebo (Hindmarch et al., 2000; Rosenzweig et
al., 1998; Versiani et al., 1999). Other common side effects include weight gain, nausea,
dizziness, headache, increase sweating, irritability, dry mouth and constipation. Some of
these side effects are thought to result from the antimuscarinic, antihistaminic and α-
adrenergic activites of AMI, and are present even at low dosages (Bryson and Wilde, 1996;
Dallocchio et al., 2000; Gupta et al. 1999).

4.1.1.5 Effect on sleep and quality of life

Amitriptyline has been shown to have an effect on sleep with several studies demonstrating
that it causes REM suppression (Kerkhofs et al., 1990; Mertz et al., 1998; Staner et al., 1995).
A study by Mertz et al (1998) showed that 50 mg amitriptyline per night not only reduced
REM sleep, but also resulted in poorer sleep efficiency and an increased number of arousals
when compared with placebo. A study by Casper et al (1994) however, contradicted the latter
and suggested that sleep efficiency and total sleep time were actually increased with
amitriptyline (100-150 mg). This contradiction in findings between the two studies may be
due to the difference in dose administered.

As mentioned earlier, residual effects and cognitive impairment are common with
amitriptyline treatment. Subjects may experience next day sedation, trouble waking up from
sleep, worsening in alertness and in behaviour upon waking compared with placebo. This can
impair patients' cognitive function and also have a negative effect on patients' QOL
(Hindmarch et al., 2000; Rosenzweig et al., 1998).
4.1.2 Duloxetine

Duloxetine was initially developed as an antidepressant drug, however pain and depression often co-exist and clinical trials also showed an improvement in pain (Detke et al., 2002; Goldstein et al., 2005; Goldstein, 2007). Duloxetine was the first drug to get regulatory approval (in 2004) for the treatment of painful DPN in the US by Food and Drug Administration (Raskin et al., 2005; Smith, 2006). It was licensed in the UK for the treatment of major depressive episodes in early 2005 and was later in 2005 licensed for the treatment of painful DPN (UK Medicine information, NHS).

4.1.2.1 Pharmacology and mechanism of action

Duloxetine is a selective serotonin and noradrenaline re-uptake inhibitor and differs from the tricyclic antidepressant amitriptyline by being more selective in its action with no other significant channel or receptor activities (Goldstein et al., 2005; Katoh et al., 1995; Smith, 2006). It is a balanced serotonin and noradrenaline re-uptake inhibitor which means its affinity for both serotonin and noradrenaline reuptake inhibition is relatively equal (Goldstein, 2007). However, duloxetine's exact mechanism of action in humans is still unknown (Goldstein et al., 2005; Raskin et al., 2005).

4.1.2.2 Pharmacokinetics

Duloxetine is rapidly absorbed after oral administration and have a peak plasma concentration of around 6 hours post dose. The elimination half-life of duloxetine is about 12 hours with a range of 8 to 17 hours. Duloxetine is eliminated through hepatic metabolism involving cytochrome P450 enzymes, CYP2D6 and CYP1A2. Most of the metabolites are found in the urine and around 20% is excreted in the faeces (Data from medication guide,
duloxetine hydrochloride, delayed-release capsules, Eli Lilly & Co, 2004; Westanmo et al., 2005).

4.1.2.3 Dose range and efficacy in painful DPN

Duloxetine is administered orally and is given as capsules containing 20, 30 or 60 mg of duloxetine. In the treatment of diabetic neuropathic pain, it is recommended that duloxetine is given at a dose of 60 mg/day, once a day, however, a lower starting dose may be considered for some patients.

Several studies have evaluated the efficacy of duloxetine for the management of neuropathic pain from DPN and duloxetine has been reported to be safe and effective in the treatment of neuropathic pain in diabetes patients. In a study by Raskin et al (2005) it was reported that both duloxetine 60mg once a day and 60 mg (twice a day) was safe, well tolerated and effective as a treatment of diabetic peripheral neuropathic pain. These results were supported in another study by Goldstein et al (2005). The results of this 12 week double-blind, placebo controlled study showed that the treatments of duloxetine 60mg/day or 120mg/day significantly improved pain severity scores compared with placebo, while 20mg/day dose was not significantly more effective than placebo. It was also considered safe and well tolerated with less than 20% discontinuation due to adverse events (Goldstein et al., 2005). Clinical trial data suggested that there was no significant improvement in analgesic action with the higher, 120 mg/day, dose and therefore 60mg/day is the current recommended dose. The mechanism of action of the analgesic effect of duloxetine is not fully understood but it is thought that the increase of neurotransmitter noradrenaline due to the re-uptake inhibition is involved in the drug's effectiveness against neuropathic pain.
4.1.2.4 Adverse events

The most common adverse events seen in duloxetine-treated DPN patients are nausea, somnolence, dizziness, dry mouth, decreased appetite, constipation and hyperhidrosis (excessive sweating), asthenia (feeling of weakness) (American Society of Health-System Pharmacists 2009; Data from medication guide, duloxetine hydrochloride, delayed-release capsules, Eli Lilly & Co, 2004; Westanmo et al., 2005).

4.1.2.5 Effect on sleep and quality of life

A study by Chalon et al (2005) showed that duloxetine had a similar effect on sleep as other antidepressant drugs including REM suppression and increased REM latency. It was also found that sleep continuity was significantly reduced with duloxetine 60 mg twice a day compared with placebo, although there was some evidence that 80 mg once a day acted to improve sleep continuity (Chalon et al., 2005). Furthermore, data from medication guide (Eli Lilly & Co) showed an increase in prevalence of insomnia in patients with painful DPN when treated with duloxetine 20 mg once a day and 60 mg once and twice a day compared with placebo, with studies showing that the incidence of insomnia was greater with the highest doses of duloxetine 60 mg twice a day (Data from medication guide, duloxetine hydrochloride, delayed-release capsules, Eli Lilly & Co, 2004).

4.1.3 Pregabalin

Pregabalin is a relatively new compound, derived from gabapentin. It was initially launched as a adjunctive therapy for treatment of partial seizures in adults with epilepsy. It is now licensed for the treatment of peripheral neuropathic pain in adults (Sills, 2006).
4.1.3.1 Pharmacology and mechanism of action

Pregabalin is an analog of the neurotransmitter gamma-aminobutyric acid (GABA) but does not appear to interact with the GABA receptors or alter GABA uptake. It has anxiolytic, anticonvulsant and analgesic effects and is thought to have a greater analgesic activity than its sister compound gabapentin. Pregabalin’s full mechanism of action is still unclear but it’s thought that when it binds to the α2-δ subunit of voltage-gated calcium channels in the central nervous system it causes a reduction in calcium influx which decreases the release of excitatory neurotransmitters such as glutamate. It is this modulation of neurotransmitter release that is thought to contribute to pregabalin’s analgesic effect (Ben-Menachem, 2004; Frampton and Foster, 2005; Frampton and Scott, 2004; Richter et al., 2005; Rosenstock et al., 2004; Tassone et al., 2007).

4.1.3.2 Pharmacokinetics

The pharmacokinetics of pregabalin has been studied in healthy volunteers as well as in patients with chronic pain. Pregabalin is rapidly absorbed after oral administration with the time taken to peak plasma concentration being around 1.5 hours. Hepatic metabolism involving cytochrome P450 enzymes is minimal and 98% of pregabalin is eliminated as unchanged drug. Pregabalin (on single dose of 300mg in healthy volunteers) has an elimination half-life of between 4.6-6.8 hours following a single dose and was appears to be independent of the dosage. Furthermore, elimination of pregabalin occurs primarily by renal excretion (Frampton and Foster, 2005; Frampton and Scott, 2004).

4.1.3.3 Dose range and efficacy in Painful DPN

Pregabalin is administered orally in a hard capsule each containing either 25, 50, 75, 100, 150, 200 or 300mg of pregabalin. Pregabalin is usually taken two or three times a day and the
recommended dosage in neuropathic pain is 150-600mg/day. Normally treatment is started at 75mg twice a day with doubling of the dose if necessary at weekly intervals (150mg and 300mg twice a day, respectively) (Frampton and Foster, 2005; Frampton and Scott, 2004).

The efficacy of pregabalin in neuropathic pain has been evaluated in several studies. A study by Rosenstock et al (2004) showed that pregabalin 300 mg/day was safe, well tolerated and effective in reducing pain associated with painful DPN. This study had a 1 week baseline phase, followed by a 8 weeks fixed-dose treatment phase. 146 diabetic patients with painful DPN were randomized and received either pregabalin 300mg/day or placebo for the whole duration of the 8 weeks treatment period. Pain was significantly reduced compared with placebo and pregabalin was well tolerated and safe as most adverse events were mild to moderate and did not result in withdrawal. Another study by Tölle et al (2008) showed that pregabalin doses ranging from 150-600mg/day were all well tolerated with generally mild to moderate adverse events reported. The study was a 12 week trial with 395 diabetic patients with painful DPN randomized to placebo, 150, 300 or 600 mg/day pregabalin. In regards to pain reduction, pregabalin 600mg/day significantly improved pain scores compared with placebo, while 150 and 300mg/day did not change from placebo. This was supported by another study by Richter et al (2005) were 246 patients with painful DPN were randomized to placebo or pregabalin (150 or 600mg/day) for 6 weeks. The results showed that pregabalin 600mg/day significantly reduced pain compared with placebo controls, while pregabalin 150mg/day did not differ from placebo. Furthermore, in a study by Freynhagen et al (2005), it was found that both a flexible schedule of 150, 300, 450 and 600 mg pregabalin per day, with a weekly dose escalation and a fixed schedule of 300mg/day for a week followed by 600mg/day for 11 weeks, significant relieved pain compared with placebo controls.
4.1.3.4 Adverse events

Adverse events reported during clinical trials with pregabalin are usually mild. Most common adverse events reported are dizziness and somnolence. Other adverse events include, dry mouth, increased appetite and weight gain, peripheral oedema, memory impairment, confusion, vertigo, disturbance in attention, blurred vision, vomiting, and fatigue (Frampton and Foster, 2005; Frampton and Scott, 2004; Richter et al., 2005; Rosenstock et al., 2004).

4.1.3.5 Effect on sleep and quality of life

Several studies have suggested that pregabalin has a positive effect on sleep by improving pain-related sleep disturbance (Freynhagen et al., 2005; Lesser et al., 2004). Better night time sleep may result in improved mood and there is evidence that patients may also feel less fatigued during the day and that this together with pain relief can improve patients’ quality of life (QOL) (Rosenstock et al., 2004). Lesser et al (2004) found that pregabalin 300 and 600 mg/day had a beneficial effect on sleep in patients with diabetic DPN and a randomized, double-blind, placebo controlled study by Rosenstock et al (2004) showed that pregabalin 300mg/day improved mood, sleep disturbance and QOL. A study by Freynhagen et al (2005) reported that both a fixed dose regime of 300 mg/day, increasing to 600 mg/day after one week and flexible dose regime of 150-600 mg/day significantly improved pain-related sleep interference. Similar results were seen in studies done by Tolle et al (2008) and Richter et al (2005) were pregabalin 600mg/day reduced pain-related sleep interference. Furthermore, Hindmarch et al (2005) found that pregabalin also had an effect on sleep in healthy volunteers with 450mg/day improving sleep efficiency, increasing the proportion of slow wave sleep (SWS) and reducing sleep onset latency compared with placebo.
4.1.4 Pain and sleep

All above medications are commonly used in the treatment of neuropathic pain with good documentation of efficacy and safety as mentioned earlier. However, the full mechanisms by which the treatments affect sleep are not fully understood. The effects on sleep may be through a direct pharmacological action or via indirect routes such as pain relief. It is known for example, that sleep disturbance is very common in patients with pain and between 50-70% of people seeking help for chronic pain have reported sleep impairment (Gore et al., 2005). In a study by Galer and associates it was shown that painful DPN had a high impact on sleep and more than half of the patients experienced worsening of the pain at night time (Galer et al., 2000). This was also supported in a study by Benbow et al. where the diabetic study group had problems with their sleep as their neuropathic pain got worse at night (Benbow et al., 1998).

4.1.5 Study Aim

The results of the analysis in chapter 3 suggest that DM patients with painful DPN have differences in their sleep architecture when compared with healthy aged and gender matched controls. Patients with painful DPN appear to have reduced sleep quality and a higher level of daytime sleepiness compared with healthy subjects of the same age and gender. This may be due to neuropathic pain and other common complications seen in diabetes. Pregabalin, duloxetine and amitriptyline are all used in treatment of neuropathic pain and one would therefore like to investigate the effect of these three drugs on pain, sleep architecture and sleep quality in type 1 and 2 DM patients with painful DPN. There are currently no published reports comparing pregabalin, duloxetine and amitriptyline in the same clinical trial. Furthermore, most studies with regards to pregabalin’s effect on sleep in patients with painful DPN have only reported subjective data (Richter et al., 2005; Rosenstock et al., 2005; Tölle
et al., 2008). In fact, a literature search showed that objective polysomnography assessment of the effect of pregabalin on sleep in patients with painful DPN is limited and published data only found in healthy subjects or in patients with epilepsy (de Haas et al., 2007; Hindmarch et al., 2005) as well as one ongoing study with patients with fibromyalgia (clinicaltrials.gov). The aim of the current study was to assess the effects of all three treatments on sleep and pain in patients with painful DPN and to determine, if possible, whether improvement of sleep was related to reduced pain or directly as a result of the treatment regime. As both pain and poor quality of sleep can have a negative effect on a patient’s health and quality of life it is important to establish the treatment effect on both of these variables. The ideal treatment would be one that reduced pain and improved sleep without evidence of daytime sedative effects.

4.2 Methods

4.2.1 Subjects

A total of 83 type 1 and 2 DM patients were randomised (26 females and 57 males) onto the trial. Subjects had to be over 18 years old and have been diagnosed with DM for at least a year with neuropathic pain of diabetic origin and a score above 12 on the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS). For more detailed description inclusion and exclusion criteria see general methods and materials section 2.1.

4.2.2 PSG overnight recording and Sleep staging

PSG overnight recordings in order to assess the effects of each drug on sleep architecture were carried out on visit 1 = night 7 (baseline with placebo), visit 2 = night 21 (chronic low dose), and visit 3 = night 35 (chronic high dose). ‘Low dose’ was defined as pregabalin 150mg (twice a day), duloxetine 60mg (every morning), or amitriptyline 25mg (twice a day)
and 'high dose' was defined as pregabalin 300 mg (twice a day), duloxetine 60mg (twice a day), or amitriptyline 25mg (every morning); 50mg (every evening). See section 2.2 of general methods and materials for more detailed description of the study, and an overview of the study schedule can be seen in appendix 1. All PSG recordings were carried out in sleep labs at the Surrey Clinical Research Centre as stated in general methods section 2.3 using the Compumedics Siesta System® (Compumedics Ltd., Australia). Time in bed was 8 hours (2300-0700). All subjects had a habituation night before each study night in order to get use to the environment (equal to night 6, 20 and 34). The PSG nights were staged by a single sleep stager according to the Rechtschaffen and Kales criteria as stated in general methods and materials section 2.4.

4.2.3 Karolinska sleepiness Scale

The Karolinska Sleepiness Scale (KSS) was used to assess levels of daytime sleepiness and was carried out at 0800h, 1000h, 1300h and 1600h on day 8 for placebo (visit 1), day 22 for low dose (visit 2) and day 36 for high dose (visit 3). A brief description of the KSS can be found under general methods section 2.5.3.

4.2.4 Leeds Sleep Evaluation Questionnaire

The Leeds Sleep Evaluation Questionnaire (LSEQ) was carried out at 0800h on day 8 for placebo (visit 1), day 22 for low dose (visit 2) and day 36 for high dose (visit 3), and was used to assess subjective ratings of sleep quality and early morning behaviour (Hindmarch and Gudgeon, 1980; Parrot and Hindmarch, 1980; Zisapel and Laudon, 2003). A description of the LSEQ can be found in general materials and methods section 2.5.1.
4.2.5 Brief Pain Inventory

The brief pain inventory (BPI) was used to assess patient’s pain and was filled in on a daily basis throughout the study. A description of BPI can be found in general materials and method section 2.5.4. Patients was divided into mild, moderate and severe pain group defined as BPI worse pain score of 0-3 for mild, 4-6 for moderate and 7 -10 for severe.

4.2.6 Visual Analogue Scale

The Visual Analogue Scale (VAS) was used for rating subjective pain and was carried out at 0800h, 1000h, 1300h and 1600h on day 8 for placebo (visit 1), day 22 for low dose (visit 2) and day 36 for high dose (visit 3). The VAS is a common tool to assess the analgesic properties of various drugs (Langley and Sheppeard, 1985). A description of VAS can be found in general materials and methods section 2.5.5.

4.2.7 Statistical methods

Data set was analysed in a linear mixed model using SAS® PROC MIXED 9.1. Observations under treatment were analysed in a linear mixed model using SAS® PROC MIXED 9.1. The observations were the dependent variable and fixed effect was treatment with visits being a repeated measure. Subjects were added as a random effect. Additional independent covariates included body mass index (BMI) and age. Statistical significance level was set to 5% (0.05).

The sample size of the study groups were decided based on power calculations for the BPI pain variables. Decision on concentration of study drugs were as mentioned earlier based on previously documented effective analgesic dosages.
4.3 Results

4.3.1 Demographics and Screening results

A total of 83 patients (57 males and 26 females) were randomised onto the trial and 65 subjects completed all 3 treatment visits. 27 patients were randomised to pregabalin, 28 to duloxetine and 28 to amitriptyline (see table 4.1 for all demographics). 11 patients had type 1 DM and 72 patients had type 2 DM. 48 patients were receiving insulin treatment and 35 patients were not receiving insulin treatment.

<table>
<thead>
<tr>
<th></th>
<th>Pregabalin</th>
<th>Duloxetine</th>
<th>Amitriptyline</th>
<th>All</th>
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<tr>
<td></td>
<td>N = 27</td>
<td>N = 28</td>
<td>N = 28</td>
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<td>Age (years)</td>
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<tr>
<td>Mean (SD)</td>
<td>66.3 (7.5)</td>
<td>65.0 (9.6)</td>
<td>64.2 (9.6)</td>
<td>65.1 (8.9)</td>
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<tr>
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<tr>
<td>Max</td>
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</table>

Table 4.1: Demographic characteristics of study population.

4.3.2 Subjective Pain measures

4.3.2.1 Visual analogue scale

Subjective pain was significantly reduced over time in all three treatment groups with subjects having less pain at visit 2 (low dose of treatment) compared with placebo at visit 1 (pregabalin P < 0.05, duloxetine and amitriptyline P < 0.01). Furthermore, subjects in the duloxetine group had significantly reduced pain at visit 3 compared with the placebo visit (P < 0.001), table 4.3. There were large differences between treatment groups at baseline so in
order to correct for this baseline was added as a co-variant. However, there was still no significant difference in treatment effect between the three treatments (figure 4.1).

Figure 4.1: Treatment effect on visual analogue scale (VAS) pain score. VAS showed that pain was reduced in all three treatment groups; a high VAS score indicates worse pain. There was no significant difference in treatment effect between the drugs, pregabalin (●), duloxetine (■), amitriptyline (▲). Symbols show mean score ± standard error of mean (SEM).
4.3.2.2 Brief pain inventory

Pain severity and pain interference on sleep was significantly reduced in all treatment groups, (table 4.3). Subjects self-rated pain severity score decreased at visit 2 compared with placebo visit 1 (pregabalin and amitriptyline $P < 0.05$, duloxetine $P < 0.01$). Pain interference on sleep was significantly reduced at visit 2 and 3 compared with placebo (Pregabalin; $P < 0.01$ and 0.05 for visit 2 and 3, respectively; amitriptyline; $P < 0.01$ for both visits and duloxetine; $P < 0.05$ for visit 2 and $P < 0.01$ for visit 3).

There was a difference between the treatment groups as baseline and baseline was therefore added as a co-variant. However, there was still no significant difference of treatment effect on pain when comparing the three treatments (figure 4.2).

![Figure 4.2](image_url)

**Figure 4.2** Treatment effect on pain: **A. BPI - Pain Severity Score.** BPI pain severity was significantly reduced from visit 1 to visit 2 in all three treatment groups (a high BPI pain score indicates worse pain). There was no significant difference in treatment effect between the drugs. **B. BPI - Pain Interference on Sleep.** BPI pain interference on sleep was significantly reduced from visit 1 to visit 2 and 3 in all three treatment groups (a higher pain interference score indicates a higher level of pain interfering with sleep). There was no significant difference in treatment effect between the drugs. pregabalin(●), duloxetine (■), amitriptyline (▲). Symbols show mean score ± standard error of mean (SEM)
Table 4.2: Patient disposition.

Not randomized (n = 21, 20%)

Randomized (n = 83, 80%)

Pregabalin (n = 27)
- Completed study (n = 19)
  - Withdrawn (n = 8, 30%)
    - Adverse event (n = 6)
    - Lack of compliance (n = 1)
    - Protocol deviation (n = 1)
- Completed study (n = 23)
- Withdrawn (n = 5, 18%)
  - Adverse event (n = 3)
  - Withdrawal of consent (n = 1)
  - Protocol deviation (n = 1)

Duloxetine (n = 28)
- Completed study (n = 23)
- Withdrawn (n = 5, 18%)
  - Adverse event (n = 1)
  - Withdrawal of consent (n = 1)
  - Protocol deviation (n = 2)

Amitriptyline (n = 28)
- Completed study (n = 23)
- Withdrawn (n = 5, 18%)
  - Adverse event (n = 2)
  - Withdrawal of consent (n = 1)
  - Protocol deviation (n = 1)
Table 4.3: Change in subjective variables from baseline (placebo) to each treatment visit.

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<th>Amitriptyline</th>
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<tr>
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<td>49.3 (2.09)</td>
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<td>46.0 (3.23)</td>
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<td>52.7 (2.47)</td>
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<td>49.8 (2.14)</td>
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<td>LSEQ Behaviour Following Sleep</td>
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<td>pic v high</td>
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<td>49.0 (2.40)</td>
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</tbody>
</table>

BPI = Brief Pain Inventory, VAS = Visual Analogue Scale, KSS = Karolinska Sleepiness Scale, LSEQ = Leeds Sleep Evaluation Questionnaire
Plc = Placebo, Low = lower dose of study medication, high = higher dose of study medication
NS = Not significant
### Table 4.4: Change in objective sleep variables from baseline (placebo) to each treatment visits

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<tr>
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<th>Pregabalin</th>
<th>Duloxetine</th>
<th>Amtriptyline</th>
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<td></td>
<td>Mean (SE)</td>
<td>Time contrast</td>
<td>Mean (SE)</td>
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<td>Low dose</td>
<td>High dose</td>
<td>Cohen's d</td>
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<tr>
<td>N</td>
<td></td>
<td></td>
<td>p-value</td>
</tr>
<tr>
<td>TST (min)</td>
<td>371.6 (11.84)</td>
<td>380.6 (9.14)</td>
<td>410.3 (10.24)</td>
</tr>
<tr>
<td>SE (%)</td>
<td>77.3 (2.46)</td>
<td>79.2 (1.90)</td>
<td>85.4 (2.13)</td>
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<tr>
<td>SOL (min)</td>
<td>22.3 (2.47)</td>
<td>25.1 (4.72)</td>
<td>18.8 (2.38)</td>
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<tr>
<td>LPS (min)</td>
<td>29.0 (4.25)</td>
<td>26.7 (5.41)</td>
<td>14.1 (1.77)</td>
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<tr>
<td>WASO (min)</td>
<td>90.9 (11.77)</td>
<td>81.8 (8.83)</td>
<td>57.2 (10.30)</td>
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<tr>
<td>Duration of Wake</td>
<td>108.9 (11.84)</td>
<td>99.9 (9.14)</td>
<td>70.2 (10.24)</td>
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<tr>
<td>Duration of stage 1 sleep</td>
<td>67.2 (8.81)</td>
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<td>Duration of stage 2 sleep</td>
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<td>Duration of stage 4 sleep</td>
<td>25.8 (5.31)</td>
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<td>Duration of SWS (min)</td>
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<td>Duration of NREM (min)</td>
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<td>319.0 (8.89)</td>
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<td>Duration of REM (min)</td>
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<td>REM Latency (min)</td>
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<td>99.0 (11.65)</td>
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NS = Not significant, pic = placebo, low = lower dose of study medication, high = higher dose of study medication, N = number of subjects

TST = total sleep time, SE = sleep efficiency, SOL = sleep onset latency, LPS = latency to persistent sleep, REM = rapid eye movement, NREM = non rapid eye movement, SWS = slow wave sleep, NAW = number of awakenings
**Table 4.5: Treatment by visit effect.**

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<td>SE (%)</td>
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<td>SWS Latency (min)</td>
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</table>

*When gender is a covariant = significant treatment by visit effect, p < 0.05, NS = Not significant

PGB = pregabalin, DUL = duloxetine, AMI = amitriptyline, TST = total sleep time, SE = sleep efficiency, SOL = sleep onset latency, LPS = latency to persistent sleep, REM = rapid eye movement sleep, NREM = non rapid eye movement sleep, SWS = slow wave sleep, NAW = number of nocturnal awakenings.
4.3.3 Polysomnography (PSG)

PSG variables analysed included; latency to sleep onset (SOL, min), latency to persistent sleep (LPS, min), wake after sleep onset (WASO, min), number of awakenings (NAW), and duration of time awake (stage 0, min) throughout the night. Total duration (min) and percentage of stage 1, 2, 3, 4, rapid eye movement (REM) sleep, non rapid eye movement (NREM) sleep and slow wave sleep (SWS) (stages 3 and 4 combined) were also analysed together with variables such as sleep efficiency (SE) and total sleep time (TST). See appendix 6 for more detailed definition of sleep variables.

4.3.3.1 Sleep continuity and quality of sleep

SE, TST, WASO, duration of wake and NAW all reflect sleep continuity and quality of sleep. Comparison of the three treatments showed that there was a significant treatment by visit effect on SE, TST, wake and WASO (P<0.001 for SE, TST and duration of wake, P<0.01 for WASO, table 4.5). Duloxetine significantly reduced TST, SE and increased the duration of wake when compared with pregabalin and amitriptyline (low dose; duloxetine v pregabalin, P< 0.001 and duloxetine v amitriptyline P< 0.001, high dose; duloxetine v pregabalin, P< 0.001 and duloxetine v amitriptyline P< 0.01), (figure 4.3 for TST and SE). Duloxetine significantly increased WASO compared with pregabalin and amitriptyline (duloxetine v pregabalin P<0.01 for both low and high dose, duloxetine v amitriptyline P<0.001 and P<0.05 for low and high dose respectively), (figure 4.3C). There was no significant treatment by visit effect on NAW when comparing the three drugs, (figure 4.3D).

Post hoc analysis showed that low and high dose duloxetine had a medium to strong treatment effect with a significant reduction in SE and TST compared with placebo (SE LS mean for placebo 79.4 %, reduced to 70.4% with low dose and to 74.2% with high dose, P<0.0001 and P<0.05, respectively, TST LS mean was 381.4 min reduced to 338.1 min for
low dose and to 356.6 min for high dose, P<0.0001 and P< 0.05, respectively), while WASO was significantly increased with low dose (LS mean 85.6 min for placebo, 120.2 min low dose and 100.5 min high dose, P<0.01 for low dose). In contrast, pregabalin significantly increased SE and TST and reduced WASO at visit 3 (high dose) compared with placebo at visit 1 (LS mean for SE was 77.3% at placebo increased to 85.4 % with high dose and TST was 371.6 min for placebo and increased to 410.3 min with high dose and WASO was 90.9 min for placebo and 57.2 min for high dose, P<0.01 for all. Amitritptyline had no significant treatment effect on TST or SE but did significantly reduce WASO compared with placebo (LS mean 91.0 min for placebo and 66.6 min for high dose, P<0.05, table 4.4).

NAW was the only one of above variables that did not change significantly with any of the three treatments, (figure 4.3D).
Figure 4.3: Treatment effect on sleep continuity and sleep quality. There was an overall significant treatment by visit effect on SE and TST (P<0.001 for both) and WASO (P<0.01), but no significant effect on NAW. Comparison between treatments; duloxetine v pregabalin = *, duloxetine v amitriptyline = †. Level of significance †/ = P<0.05, ††/** = P<0.01 and †††/** = P<0.001. Plots shows mean, ± standard error of mean (SEM). Amitriptyline (▲), pregabalin (●), duloxetine (■) A. Sleep efficiency (SE) and B. Total sleep time (TST). There was a significant difference between duloxetine compared with pregabalin and amitriptyline, with duloxetine significantly reducing SE and TST, while pregabalin increased SE and TST. Amitriptyline showed no changed compared with placebo. C. Wake after sleep onset. There was a significant difference between duloxetine compared with pregabalin and amitriptyline, with duloxetine significantly increasing WASO, while pregabalin and amitriptyline significantly decreased WASO compared with placebo. D. Number of nocturnal awakenings (NAW). There was no significant difference in NAW between the three treatments or compared with placebo.
4.3.3.2 Latency to sleep

Both SOL and LPS are standard PSG variables used to assess how long it takes to fall asleep and both variables were significantly affected by the three treatments (treatment by visit effect = P<0.01 for both), table 4.5. Pregabalin significantly decreased SOL and reduced LPS compared with duloxetine and amitriptyline (SOL; pregabalin v duloxetine P<0.05 for low dose and P<0.001 for high dose and pregabalin v amitriptyline P<0.01 for high dose, LPS; pregabalin v duloxetine P<0.001, figure 4.4A and B).

When looking at the effect of dose, pregabalin significantly reduced LPS at the higher dose (LS mean 29.0 min for placebo compared with 14.1 min for high dose, P<0.05) and decreased SOL, even if the latter was not statistically significant when compared with placebo (LS mean 22.3 min for placebo to 18.8 min with high dose). In contrast, duloxetine high dose significantly increased LPS and SOL compared with placebo (LPS; LS mean 16.1 min for placebo compared with 33.6 min for high dose, P<0.01, SOL; LS mean 17.5 min with placebo to 30.9 min with high dose, P<0.001). Amitriptyline treatment did not change LPS or SOL significantly when compared with placebo, (table 4.4).
Figure 4.4: Treatment effect on sleep onset latency SOL and LPS. There was an overall significant treatment by visit effect on SOL and LPS (P<0.01 for both). Comparison between treatments; duloxetine vs pregabalin = *, amitriptyline vs pregabalin = x and duloxetine vs amitriptyline = t. Level of significance t/* = P<0.05, xx = P<0.01 and *** = P<0.001. Plot shows mean, ± standard error of mean (SEM). Amitriptyline (▲), pregabalin (●), duloxetine (▲). 

A. Sleep Onset Latency. There was a significant difference between pregabalin when compared with amitriptyline and duloxetine. Pregabalin decreased SOL and duloxetine increased SOL compared with placebo, while amitriptyline did not change significantly compared with placebo.

B. Latency to Persistent Sleep. There was a significant difference between the treatments with duloxetine significantly increasing LPS compared with pregabalin and amitriptyline. Pregabalin decreased LPS and duloxetine increased LPS compared with placebo, while amitriptyline did not change significantly from placebo.
4.3.3.3 Sleep architecture NREM and REM

When looking at patients' sleep architecture there was no significant treatment by visit effect on stage 1, 2 and 3 sleep and there was therefore no significant differences between amitriptyline, duloxetine or pregabalin treatments with regards to the amount of stage 1, 2 and 3 sleep (figure 4.5). There was a significant treatment by visit effect (P<0.05) for stage 4 sleep with a difference between duloxetine and amitriptyline at visit 2, (P<0.05, figure 4.5).

Post hoc analysis showed that there was a significant visit effect, likely to be due to dosage. Duration of stage 1 increased across all treatment groups, and all treatments showed a moderate to strong treatment effect (table 4.4). Pregabalin: LS mean 67.2 min with placebo at visit 1 to 82.3 min low dose and 85.5 min high dose (P<0.05 and P = 0.0513, respectively). Duloxetine: LS mean 65.8 min placebo to 85.4 min low dose and 93.7 min high dose, (P<0.01 for both), and amitriptyline: LS mean 59.3 min placebo to 82.5 min low dose and 79.5 min high dose, (P<0.01 for both). In addition, patients in the pregabalin and amitriptyline treatment groups had a significant increase in duration of stage 2 sleep on visit 2 and 3 compared with placebo at visit 1, with high dose pregabalin and low and high dose amitriptyline increasing stage 2 sleep, while there was no significant difference in the duration of stage 2 sleep with duloxetine treatment compared with placebo (table 4.4). Pregabalin: LS mean 170.3 min for placebo to 212.2 min for high dose, P<0.01 and amitriptyline: LS mean 178.7 min for placebo compared with 197.1 min for low dose and 215.4 min for high dose, (P<0.05 and P<0.01, respectively). There was no significant change in duration of stage 3 sleep in any of the treatment groups compared with placebo, and duration of stage 4 sleep only showed significant difference in duloxetine group with decrease in stage 4 sleep at visit 2 compared with placebo at visit 1(P<0.01).

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Figure 4.5: Treatment effect on sleep architecture. There was no significant treatment by visit effect on stage 1, 2 and 3 sleep, while there was a significant treatment by visit effect on stage 4 sleep (P<0.05). Comparison between treatments; duloxetine v amitriptyline = t. Level of significance ⋆ = P<0.05. Plots shows mean, ± standard error of mean (SEM). Amitriptyline (▲), pregabalin (○), duloxetine (■) A. Stage 1 sleep duration. There was no difference between treatments. All treatments increased the duration of stage 1 sleep compared with placebo. B. Stage 2 sleep duration. There was no difference between treatments. Amitriptyline and pregabalin significantly increased the amount of stage 2 sleep compared with placebo, while duloxetine did not change significantly compared with placebo. C. Stage 3 sleep duration. There was no significant difference between treatments or when compared with placebo. D. Stage 4 sleep duration. There was a significant difference between treatments with duloxetine having less stage 4 sleep compared with amitriptyline at visit 2. Duloxetine low dose also significantly reduced stage 4 sleep compared with placebo, while stage 4 sleep during treatment with pregabalin and amitriptyline did not change significantly.
When stage 3 and 4 sleep was combined (Slow Wave Sleep, SWS) there was no significant difference in the duration of SWS between the three treatment groups (table 4.4 and figure 4.6). A similar result was seen when looking at the thirds of night as there was no significant difference between the three treatments in SWS on the 1st, 2nd or 3rd third of the night. However, there was a significant difference between visits (P<0.05). On the 1st third of the night high dose pregabalin significantly increased the amount of SWS compared with placebo (LS mean 27 min with placebo to 41 min with high dose, P<0.01), while amitriptyline and duloxetine had no significant effect on the amount of SWS in the 1st third of the night compared with placebo (DUL; 28 min placebo and 29 min high dose; AMI; 29.5 min placebo and 31 min high dose).

When looking at the latency to SWS there was a significant difference between the treatments (P<0.01), with pregabalin significantly decreasing SWS latency compared with amitriptyline and duloxetine (table 4.5 and figure 4.6). Pregabalin decreased SWS latency compared with placebo (LS mean 63.2 min at placebo compared with 44.8 min for low dose and 38.8 min for high dose, P<0.01 for both), while duloxetine increased SWS latency (LS mean 55.8 min at placebo compared with 62.8 min for low dose and 88.6 min for high dose, P<0.05 for high dose). There was no significant change with amitriptyline when compared with placebo (table 4.4).
Figure 4.6: Treatment effect on duration SWS and latency to SWS. There was no significant treatment by visit effect on duration of SWS, while there was a significant treatment by visit effect on SWS latency ($P < 0.01$). Comparison between treatments; pregabalin v duloxetine = * and pregabalin v amitriptyline = x Level of significance *= $P < 0.05$, **=$P < 0.01$, ***=$P < 0.001$. Plots shows mean, ± standard error of mean (SEM). Amitriptyline (▲), pregabalin (•), duloxetine (■) A. SWS duration. There was no significant difference between the treatments or compared with placebo. B. SWS latency. There was a significant difference between treatments, with pregabalin decreasing latency to SWS and duloxetine increasing latency to SWS compared with placebo. There was no significant effect of amitriptyline on SWS latency when compared with placebo.
When looking at stage 1, 2, 3 and 4 sleep combined (NREM sleep), the overall treatment by visit effect was approaching significance (P=0.0526). Analysis showed a significant treatment by visit effect with the duloxetine group having significantly less NREM sleep compared with amitriptyline at visit 2 (low dose) and compared with pregabalin at visit 3, high dose (P<0.05 for both, table, 4.5, figure 4.7). There was evidence of a significant visit effect with NREM sleep increasing in all three treatments groups. Pregabalin increased NREM duration compared with placebo (LS mean 291.5 min compared with 319.0 min for low dose and 348.3 for high dose, P<0.01 and P<0.0001, respectively). Amitriptyline showed similar results (LS mean 291.6 min for placebo, increasing to 328.6 min for low dose and further increase to 343.6 min for high dose, P<0.0001 for both). Duloxetine was the only treatment that did not significantly increase NREM duration at the lower dose, however there was a significant increase at the high dose (LS mean 298 min for placebo compared with 326.7 min for high dose, P<0.05, table 4.4).

For REM sleep duration there was a significant treatment by visit effect when comparing the three treatments with each other (P<0.01, table, 4.5). Low dose of duloxetine significantly decreased REM sleep duration compared with pregabalin and amitriptyline (P<0.001 and 0.05, respectively) and the same could be seen with high dose duloxetine (P<0.001 and 0.01, respectively, figure 4.7).

There was also a significant visit effect (P<0.0001) with a significant reduction in REM sleep duration across all three treatment groups, (table 4.4). Pregabalin decreased REM sleep with a LS mean of 80.1 min for placebo compared with 61.6 min for low dose and 62 min for high dose (P<0.01 for both), duloxetine had an even stronger treatment effect and reduced REM sleep from a LS mean of 83.4 min for placebo to 34.8 min low dose and 29.9 min high dose.
and amitriptyline had a LS mean of 77.0 min for placebo compared with 49.7 min for low dose and 50.2 min for high dose (P<0.0001 for both). 

Figure 4.7: Treatment effect on duration of NREM/REM sleep. There was a significant treatment by visit effect on the duration of REM sleep (P<0.01), while the duration of NREM sleep approached significance (P=0.0526); Comparison between treatments; pregabalin v duloxetine = * and duloxetine v amitriptyline = †. Level of significance †/* = P<0.05, *** = P<0.001. Plots shows mean, ± standard error of mean (SEM). Amitriptyline (△), pregabalin (○), duloxetine (□) A. NREM sleep duration. All treatments significantly increased the duration of NREM sleep compared with placebo. There was also a difference between the treatments with duloxetine having significantly less NREM sleep compared with amitriptyline at low dose and pregabalin at high dose. B. REM sleep duration. All treatments significantly reduced the duration of REM sleep compared with placebo. There was still a significant difference between treatments with duloxetine significantly decreasing the duration of REM sleep compared with pregabalin and amitriptyline.
Furthermore, REM latency and the number of REM periods during the night were also significantly affected by treatment, (treatment by visit effect, \( P<0.01 \) and \( P<0.0001 \), respectively) when comparing the three drugs (table 4.5). REM latency was significantly increased by low dose duloxetine compared with low dose pregabalin and amitriptyline (\( P<0.05 \) for both) and the same effect was observed at high dose (\( P<0.001 \) and \( P<0.05 \), respectively, figure 4.8). Post hoc analysis showed that there was also a significant change with visit (table 4.4). Both duloxetine and amitriptyline significantly increased REM latency compared with placebo. Duloxetine (LS mean of 79.9 min at placebo compared with 166.8 min with low dose and 226.7 min with high dose, \( P<0.0001 \) for both) and amitriptyline (LS mean 65.2 min for placebo compared with 148.0 min at low dose and 148.8 min at high dose, \( P<0.0001 \) for both). Pregabalin low and high dose had no significant effect on REM latency compared with placebo.

For the number of REM periods duloxetine significantly reduced the number of REM periods when compared with pregabalin, both at the low dose and high dose (\( P<0.0001 \) for both). In addition, low dose duloxetine reduced the number of REM periods compared with amitriptyline (\( P<0.001 \)). There was also a significant reduction in the number of REM periods following high dose amitriptyline when compared with pregabalin (\( P<0.01 \), figure 4.8). With regards to changes with visits (Table 4.4), duloxetine and amitriptyline significantly reduced the number of REM periods compared with placebo. Duloxetine had a LS mean of 3.8 REM periods for placebo compared with 1.5 at low dose and 1.8 at high dose (\( P<0.0001 \) for both) and amitriptyline had LS mean 3.7 for placebo compared with 2.7 low dose and 2.3 high dose (\( P<0.001 \) and \( P<0.0001 \), respectively). Pregabalin demonstrated a weaker treatment effect with a LS mean of 3.3 REM periods for placebo, 2.8 at low dose and 3.1 at high dose) with only the low dose, visit 2 being significantly different compared with placebo (\( P<0.05 \)).
Figure 4.8: Treatment effect on REM latency and number of REM periods. There was a significant treatment by visit effect on both REM latency and the number of REM periods $P < 0.01$ and $P < 0.0001$, respectively. Comparison between treatments; pregabalin vs duloxetine $= *$ pregabalin vs amitriptyline $= \times$ and duloxetine vs amitriptyline $= \dagger$. Level of significance $*/* = P < 0.05$, $** = P < 0.01$, $\dagger\dagger\dagger/***/*** = P < 0.001$. Plots show mean, ± standard error of mean (SEM). Amitriptyline (▲), pregabalin (●), duloxetine (■). A. REM latency. There was a significant difference between treatments with pregabalin shortening REM latency compared with amitriptyline and duloxetine. The latter two significantly increased REM latency when compared with placebo, while pregabalin did not significantly change from placebo. B. Number of REM periods. There was a significant difference between the treatments with duloxetine significantly reducing REM periods compared with amitriptyline and pregabalin. Duloxetine and amitriptyline decreased number of REM periods compared with placebo.
4.3.3.4 Sleep stages as a percentage of TST

When looking at stage 1, 2, 3 and 4 sleep as a percentage of TST there was no significant treatment by visit effect. There was no difference between the treatments and no difference when comparing the treatments with placebo. However, when looking at REM sleep there was a significant treatment by visit effect (P<0.05) with the high dose of duloxetine reducing % of REM sleep significantly more compared with pregabalin and amitriptyline (P< 0.001 and P<0.01, respectively). Post hoc analysis showed that all three treatments reduced the % of REM sleep when compared with placebo and this reduction was significant both at the low and high doses (P<0.0001 for all, figure 4.9).

As a result of the significant change in % REM sleep, there was also an overall significant treatment by visit effect (P<0.05) for % NREM sleep, with high dose of duloxetine increasing % of NREM sleep of TST significantly more when compared with pregabalin and amitriptyline (P< 0.001 and P<0.01, respectively). All treatments showed an increase in the % NREM sleep compared with placebo and this was significant in both low and high doses (P<0.0001 for all, figure 4.10).
Figure 4.9: Treatment effect on % REM sleep of TST. There was a significant treatment by visit effect on %REM sleep of TST when comparing the three treatments (P<0.05). Comparison between treatments: duloxetine v amitriptyline = † Level of significance: †† = P<0.01, ††† = P<0.001. All three treatment reduced % REM sleep compared with placebo (*** = P<0.001 for all). Bars show mean, ± standard error of mean (SEM).

Figure 4.10: Treatment effect on % NREM sleep of TST. There was a significant treatment by visit effect on %NREM sleep of TST when comparing the three treatments (P<0.05). Comparison between treatments: duloxetine v amitriptyline = † Level of significance: †† = P<0.01, ††† = P<0.001. All three treatment increased % NREM sleep compared with placebo (*** = P<0.001 for all). Bars show mean, ± standard error of mean (SEM).
4.3.4 Correlation between pain and sleep

Pearson correlations were done to assess possible relationship between pain and sleep. Pain variables included pain severity score, pain interference score and pain interference on sleep score all from the BPI. Sleep variables included NA W, SE, TST, WASO, and LPS. There were no significant correlations between pain and any of above sleep variables at baseline (visit 1) and there were no significant correlations when looking at the change from placebo at baseline to visit 3. This dissociation between pain and sleep was still present when looking at correlations within each drug treatment.

Patients were also divided into groups of mild, moderate and severe pain but again results showed no significant correlations between pain and sleep in the moderate or severe pain patient groups when compared at baseline. The only group that showed significant correlations was the mild pain patient group at baseline. Increased pain severity score and pain interference score correlated with an increase in LPS (P<0.05 for both). Correlation with pain severity score and SE and TST approached significance with an increase in the pain severity score correlating with a decrease in SE and TST (P<0.065 for both). BPI pain interference on sleep showed a significant correlation with TST and WASO. Increased pain interference on sleep correlated with a decrease in TST and an increase in WASO (P<0.05 for both, Table 4.6).

There were no significant correlations when comparing change from baseline to visit 3 in any of the pain groups (mild; N= 18, moderate; N=22 and severe; N=11) suggesting that any improvement in pain did not lead to significant improvements in sleep quality or continuity.
Table 4.6: Pearson’s correlations between pain and sleep at baseline.

<table>
<thead>
<tr>
<th></th>
<th>Pain severity score</th>
<th>Pain interference score</th>
<th>Pain interference on sleep score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (N=67)</td>
<td>Mild (N=22)</td>
<td>Moderate (N=29)</td>
</tr>
<tr>
<td>LPS (min)</td>
<td>NS</td>
<td>P&lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>NAW</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>SE (%)</td>
<td>NS</td>
<td>P=0.065</td>
<td>NS</td>
</tr>
<tr>
<td>TST (min)</td>
<td>NS</td>
<td>P=0.065</td>
<td>NS</td>
</tr>
<tr>
<td>WASO (min)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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LPS = latency to persistent sleep, NAW = number of nocturnal awakenings, SE = sleep efficiency, TST = total sleep time, WASO = wake after sleep onset,
N = number of subjects, NS = not statistically significant


4.3.5 Subjective sleep measures and daytime sleepiness

4.3.5.1 Karolinska sleepiness scale

There was no evidence of a significant treatment by visit effect with no significant difference in treatments when all three drugs were compared with each other, table 4.5 and figure 4.11. There was also no significant difference in daytime sleepiness in the pregabalin and duloxetine treatment groups when compared with placebo. However, subjects in the amitriptyline treatment group showed a significant increase in daytime sleepiness compared with placebo with a KSS LS mean of 4.1 for visit 1 (placebo) compared with 4.6 for visit 2 (low dose) and 4.3 for visit 3 (high dose), (P<0.05 for both, table 4.3)

Figure 4.11: Treatment effect on Karolinska Sleepiness Scale (KSS). There was no significant treatment by visit effect. Plot shows mean ± standard error of mean (SEM) amitriptyline (▲), pregabalin (●), duloxetine (■)
4.3.5.2 Leeds sleep evaluation questionnaire

There was no significant treatment by visit effect on any of the LSEQ variables (Getting to Sleep, GTS; Quality of Sleep, QOS; Awakening From Sleep, AFS; Behaviour Following Awakening, BFW) when comparing the three drugs with each other, (table 4.5 and figure 4.12).

With respect to each visit, subjects in the pregabalin treatment group found it significantly easier to get to sleep and had significantly better subjective quality of sleep at visit 3 compared with placebo at visit 1 (P<0.01 for both, table 4.3). There was no significant difference in the ease of getting to sleep, nor subjective quality of sleep when looking at the duloxetine or amitriptyline treatment groups compared with placebo. There was no significant difference in awakening from sleep and behaviour following sleep with any of the treatment groups when compared with placebo, (table 4.3).
Figure 4.12: Treatment effect on Leeds Sleep Evaluation Questionnaire (LSEQ). There was no significant treatment by visit effect on any of the LSEQ questions when comparing the three drugs. Plots show mean, ± standard error of mean (SEM). Amitriptyline (▲), pregabalin (●), duloxetine (■). The black line indicates no change in the subjective feeling of ease of getting to sleep compared to normal (before treatment began).

A. LSEQ-Getting to sleep. There was no significant difference between the treatments. Pregabalin significantly improved getting to sleep compared with placebo while amitriptyline and duloxetine did not change significantly compared with placebo. B. LSEQ-Quality of sleep. There was no significant difference between the treatments. Pregabalin significantly improved quality of sleep compared with placebo while amitriptyline and duloxetine did not change significantly compared with placebo. C. LSEQ-awakening following sleep. There was no significant difference when comparing the three treatments or when compared with placebo. D. LSEQ-behaviour following sleep. There was no significant difference when comparing the three treatments or when compared with placebo.
4.3.6 Safety data

Safety data showed that 83 patients were randomized, 75 completed visit 1, 69 completed visit 2 and 65 patients completed all 3 visits, (table 4.2). 4.82% (n=4) were excluded due to protocol deviation and 1.20% (n=1) were excluded due to non-compliance and 3.61% (n=3), withdrew consent. 12% (n=10) withdrew from the study due to adverse events. Adverse events included; panic attack (n=1), hepatitis (n=1), salmonella enteritis (n=1), weakness (n=1), diarrhoea (n=1), chest pain (n=1), balance disorder (n=1), flulike symptoms (n=1), infection in left foot (n=1), and ruptured abdominal aortic aneurysm (n=1). None of the above adverse events were classified, by the Principal Investigator, as likely to be due to any of the three treatments. Other reported adverse events were mild to moderate and did not result in withdrawal from the study. Protocol deviations and non-compliance resulting in withdrawal were for example feeling of claustrophobia when in the sleep laboratory, ingestion of analgesic medication not permitted by the protocol and non compliance in regards to study procedures.
4.4 Discussion

Pregabalin, duloxetine and amitriptyline are prescribed in the treatment of neuropathic pain in patients with painful DPN, and they all have good documentation of efficacy and safety (Frampton and Foster, 2004; Goldstein et al., 2005; McQuay et al., 1992; Raskin et al., 2005; Rosenstock et al., 2004). The results in this study supported this as all doses were safe and well tolerated with generally mild to moderate adverse events. Only 12% (n=10) of patients withdrew from the study and no withdrawals were classified as being likely to be due to treatment. Furthermore, all three medications significantly reduced pain compared with placebo. Although subjective pain was reduced in all treatment groups during the study one has to be careful when saying that this pain reduction was a therapeutic effect of the given medical intervention as this study did not have a placebo arm in order to confirm this. The main reason for not including a placebo arm was that it was not considered ethically correct to have a placebo arm for the whole duration of the study (total of 5 weeks) since the safety and efficacy of all three treatments had previously been reported (Goldstein et al., 2005; McQuay et al., 1992; Raskin et al., 2005; Richter et al, 2005; Rosenstock et al., 2004). Therefore, the pain reduction seen in the current study may be either a drug effect or an effect of time or a combination of both, however it is more likely that it is due directly to the treatment. There was no significant difference between any of the treatment groups with respect to pain relief and therefore the conclusion was that no drug was superior with regards to pain reduction in this patient group.

Pain is thought to impair sleep (Benbow et al., 1998; Galer et al., 2005) and it has been suggested that patients with painful DPN have worse sleep compared with a healthy population (see chapter 3). It is already known that pregabalin, duloxetine and amitriptyline are effective at reducing pain (Goldstein et al., 2005; McQuay et al., 1992, Raskin et al., 2005; Richter et al, 2005; Rosenstock et al., 2004).
2005; Richter et al., 2005 and Rosenstock et al., 2004), but this is the first time all three drugs have been compared with each other in the same clinical trial and the first time it has been shown that there is no significant difference in pain relief when the three drugs are compared together.

It is not fully known how these medications affect sleep, and no studies have compared the three drugs and their effect on sleep in painful DPN patients. The results from this study showed that sleep in patients with painful DPN was significantly affected by all three medications and analysis showed that there was a significant difference between the treatment groups. TST and SE, both good indicators of quality of sleep, were significantly reduced by duloxetine while pregabalin significantly increased TST (min) and SE (%) when compared with placebo. SE was for example increased from a mean of 77 % (± SEM 2.46) to a mean of 85% (± SEM 2.13) in patients on the pregabalin treatment regime. A SE of 85% is similar to that seen in a healthy population of the same age and gender (see results chapter 3). WASO was also significantly affected with high dose of pregabalin reducing WASO while low dose duloxetine increased WASO. The reduction in sleep continuity with increased WASO and reduced SE has been seen previously with duloxetine in healthy subject (Chalon et al., 2005). In the present study it appeared that the negative effect on sleep was particularly prominent at low dose duloxetine. WASO was only significantly increased at the low dose and there was also a greater reduction in TST and SE with low dose duloxetine compared with high dose (although the high dose still significantly reduced TST and SE compared with placebo).

SOL and LPS were also greatly affected by the drug treatments with duloxetine increasing both SOL and LPS, meaning that it took longer for patients to fall asleep when on duloxetine treatment compared with placebo. In fact duloxetine at the high dose (120mg/day) had a
strong negative treatment effect, resulting in a SOL and LPS almost twice as long on high dose as compared with placebo. These results indicate similarities with previously published data. Data published by Eli Lilly and Co (2004) showed an increased prevalence of insomnia (characterized as difficulties initiating and/or maintaining sleep and commonly associated with impairments of functioning while awake) in patients with painful DPN and the prevalence was highest with the dose of 120mg/day. In contrast, pregabalin reduced SOL and LPS with the latter significantly shorter compared with placebo. Unlike pregabalin and duloxetine, amitriptyline had little effect on objective sleep. Amitriptyline had no significant effect on, TST, SE, LPS, SOL or duration of wake when compared with placebo and only WASO was significantly reduced at the high dose of amitriptyline.

Although objective measures of sleep quality (TST and SE) and ease of getting to sleep (SOL and LPS) were improved by pregabalin and worsened on duloxetine, subjectively there was no significant difference between the three treatments. It was however possible to see improvements in subjective sleep variables in the pregabalin treatment group, with the pregabalin group by visit 3 showing improved subjective quality of sleep and ease to get to sleep when compared with placebo.

The positive effect on sleep seen with pregabalin was only present at the high dose of 600mg/day with the lower dose of 300 mg/day showing little difference compared with placebo. The effects of pregabalin on sleep have been previously reported although the majority of studies have investigated subjective and not objective sleep variables. Studies done by Tölle et al (2008) and Richter et al (2005) showed that 600mg/day pregabalin significantly lowered sleep interference score compared with placebo, while a study by Rosenstock et al (2004) showed significant reduction in sleep interference score with 300mg/day pregabalin when compared with placebo. There are limited data published with
regards to objective PSG variables. One PSG study by Hindmarch et al (2005) found that pregabalin 450/mg day improved SE and reduced SOL compared with placebo when assessed in healthy volunteers. Another PSG study in patients with epilepsy showed that pregabalin 300mg/day significantly improved SE and reduced number of awakenings compared with baseline, and WASO approached significant difference (de Haas et al., 2007). The result of the present study shows a similar improvement in sleep in patients with painful DPN (on pregabalin equivalent to 600mg/day). Any improvement in sleep in the patients with DPN may have been thought to be due to pain relief, however as a similar level of improvement has also been seen in healthy volunteers, this suggests that pregabalin improves sleep through another distinct mechanism other than that of reducing neuropathic pain.

It was hypothesised that an improvement in sleep quality or continuity might enhance the analgesic efficacy of pregabalin when compared to other treatments (e.g. duloxetine) that did not improve but instead disrupted sleep. This however was not shown as there was no significant difference in the three treatments on analgesic efficacy.

On closer examination of the sleep data one could also see a significant change in sleep architecture with the three treatments. There was a strong and significant treatment by visit effect with regards to REM sleep. REM sleep was significantly suppressed by the low and high doses of all three treatments when compared with placebo. With amitriptyline this was expected as classic antidepressants (tricyclic antidepressants, TCAs) are known for their REM suppression (Mertz et al., 1998; Staner et al., 1995). The REM suppression seen with duloxetine however, was significantly greater compared with that of pregabalin and amitriptyline. Closer inspection suggested that the first REM period was lost following duloxetine treatment as the REM latency increased from 79 min to almost 227 min and the number of REM periods was reduced from approximately 4 to 2. These results are in
agreement with previous published data where duloxetine has been shown to cause REM suppression and increased REM latency in healthy subjects (Chalon et al., 2005). Another PSG study in patients with major depression showed that there was a significant increase in REM latency from 58.5 min at baseline to 193.6 min with duloxetine (60mg/day) and REM sleep duration was also significantly reduced (Kluge et al., 2007). The REM suppression seen with SSNRIIs such as duloxetine is similar to that seen with other antidepressants such as TCAs and SSRIs. Studies for the past 30 years have showed a suppression of REM sleep and also a marked increase in REM latency with these types of drugs (Gillin et al.1978; Kerkhofs et al., 1990; Mertz et al., 1998; Staner et al., 1995). This data would suggest that the first REM period in sleep is fragile and is easily disrupted accounting for the increase in REM latency seen in patients treated with SSNRIIs, SSRIs and TCAs. The long term effect of REM suppression is not fully known, some suggest that REM sleep has an important role in memory consolidation but evidence for this is found to be weak (Siegel, 2001).

There was no significant difference in any of the other stages of sleep when comparing the three treatments. All three drugs increased the amount of stage 1 compared with placebo and stage 2 sleep was increased with pregabalin and amitriptyline. Stage 3 and 4 and SWS (stage 3 and 4 combined) remained unchanged. However, there was a slight reduction in stage 4 sleep and SWS with all three medications even if this was not statistically significant. This reduction was unexpected, especially with pregabalin which is known to enhance stage 4 sleep and SWS (Hindmarch et al., 2005). The reduction may be due to the high dosages used in this study as one need higher dosage of, for example pregabalin, in order to achieve good analgesic efficacy and this may therefore have a negative impact on SWS. However, when looking at SWS as thirds of the night it was shown that high dose pregabalin significantly increased / consolidated the amount of SWS in the 1st third of the night to 41min of SWS compared with 27 min for placebo, while SWS in the 1st third of night on amitriptyline and
duloxetine remained unchanged. Pregabalin also reduced the latency to SWS compared with amitriptyline and duloxetine. The reduction in SWS latency on pregabalin from 63 min to 39 min brought the DPN patients in line with an age and gender matched healthy volunteer historical control group who had a mean SWS latency of 30 min (see chapter 3). This reduction, together with the increase of SWS in the 1st third of the night suggested that even if the amount of SWS overall was not significantly increased with any of the treatments, it was shifted to the earlier part of the night in the patient group on pregabalin.

When looking at the percentage of NREM and REM of TST there was also a significant difference both between the treatments as well as compared with placebo. During the placebo treatment all groups could be considered to have a normal percentage of REM/NREM sleep with around 20-22% spent in REM sleep and 78-80% spent in NREM (Kryger et al., 2005). However, during active treatment the REM suppression seen with all of the treatments altered the REM/NREM percentage and the percentage of REM was significantly reduced to 8.4% for duloxetine to 12.7% with amitriptyline and 15% with pregabalin and NREM percentage increased.

With regards to day time sleepiness, as assessed by KSS, there was no significant difference between the three treatments and there was no difference between pregabalin and duloxetine compared with placebo, while amitriptyline slightly increased daytime sleepiness compared with the placebo visit. In addition, there was no significant difference on awakening following sleep and behaviour following sleep (LSEQ) between the three treatments or when compared with placebo. Finally there was no significant difference between any of the treatments or compared with placebo on next day performance. This was assessed using a psychometric test battery including memory tasks and reaction tasks (internal communication, Miss Laura Gribble, University of Surrey). It was of interest that there was
no evidence of significant objective daytime performance impairment with amitriptyline and only slightly increased daytime sleepiness as daytime sedation, excessive sleepiness and impairment the next day has been reported in previous studies with amitriptyline and therefore might have been expected (Hindmarch et al., 2000; Rosenzweig et al., 1998).

In conclusion, the results suggest that all three treatments are effective in reducing neuropathic pain of diabetic origin and it has for the first time been shown that none of the drugs have a superior analgesic effect. Duloxetine disrupted sleep and showed a strong negative treatment effect on sleep with reduced SE and TST, increased wake time and an increase in the time taken to fall asleep which may be due to an alerting effect of the drug. Selective serotonin re-uptake inhibitors have for example showed increase in wakefulness and a general alerting effect (Hicks et al., 2002; Wilson et al., 2000). Despite the negative effect on sleep with duloxetine there was no evidence of excessive daytime sleepiness or a subjective perception of worse sleep. However this may also be due to the alerting effect of the drug giving cognitive enhancement and a general feeling of improved wake performance. Whether any direct alerting effect could be maintained longer term is unknown, and the disruption of sleep continuity and sleep quality may have a detrimental effect long term, but future studies are needed to establish whether this is the case. In contrast, pregabalin improved sleep in patients with painful DPN and had a positive treatment effect on both subjective and objective sleep and no significant daytime sleepiness or sedative effects the next day. Amitriptyline did not have any effect on subjective or objective sleep, no significant sedation effect or impairment the next day, but showed some evidence of excessive daytime sleepiness.

The lack of correlations between pain and sleep suggest that although pregabalin improved sleep quality this improvement in sleep did not lead to subsequent improvements in pain.
relief or perception of pain interference on sleep. The reason for this is unclear, however pain improvements may have become more apparent with a longer treatment regime and it may not have been possible to see such effects over a short period. Another possible reason why there did not seem to be a significant interplay between improvement in quality of sleep and further improvement of pain may be because there was a distribution of patients classified as suffering from mild, moderate and severe pain (as defined by BPI worst pain question) with the majority of patients in the mild-moderate grouping (40 patients) and only 11 patients in the severe pain group. This was also particularly apparent in the pregabalin treatment group with the pregabalin group having the lowest mean pain severity score at the baseline, placebo visit. Future research may be to include patients reporting only moderate or severe pain rather than mild as one may expect a greater treatment effect and a possible difference in analgesic efficacy between the different treatments.
CHAPTER 5 – RESULTS
5. Clinical sleep characteristics of type 1 and 2 diabetic patients with painful DPN and possible treatment effects.

5.1 Introduction

One of the previous chapters (see chapter 3) showed that diabetic patients with painful DPN indeed had worse sleep when compared with a healthy historically created control group which was matched for age and gender. The diabetic patient group took longer to fall asleep, had worse sleep quality (defined by reduced sleep efficiency and total sleep time, and more time awake), and they also felt sleepier during the day compared with the healthy volunteer group.

The reduced sleep quality seen may have been due, at least in part, to the observation that diabetic patients with painful DPN often find that their pain has a negative effect on their sleep and it is well documented that one of the most common reported complaints of chronic pain is sleep disturbance (Benbow et al., 1998; Galer et al., 2000; Gore et al., 2005; Moldofsky, 2001) however, the results in chapter 4 showed little correlation between pain and objective sleep. Other factors such as periodic limb movement (PLMS), sleep disordered breathing and nocturnal hyperglycaemia/hypoglycaemia may also be contributing to the worsening in sleep quality and the increase in wake time seen in this patient group as all of these features are commonly associated with diabetes (Chansens, 2007; Foley et al., 2004; Resnick et al., 2003; Vigg 2003).

PLMS are often associated with arousals which can lead to difficulties in staying asleep, resulting in sleep fragmentation and excessive daytime sleepiness (Ancoli-Israel and Ayalon,
Sleep apnoeas are often associated with oxygen desaturation which can lead to nocturnal hypoxemia and also cause arousals and awakenings (Alsaeedi and Alshammari, 2005; Neubauer, 1999; Pallayova et al., 2006; Paulson, 2000). Furthermore, it is thought that sleep apnoea, severe oxygen desaturations and sleep fragmentation are risk factors for altered glucose metabolism and are associated with nocturnal hyperglycaemia (Pallayova et al., 2006; Punjabi and Polotsky, 2005), with several studies showing a link between obstructive sleep apnoea, sleep loss and insulin resistance (Pallayova et al. 2006; Punjabi et al., 2002 Punjabi et al., 2004, Spiegel et al., 2005).

Nocturnal glucose levels alone may also contribute to worse sleep. Hypoglycaemic episodes are for example thought to reduce slow wave sleep and increase in awakenings, although the effect on sleep is not fully known (Bendtson et al., 1992).

**5.1.1 Study Aim**

Several studies have looked in isolation at factors that may affect the sleep of diabetic patients with painful DPN (Alsaeedi and Alshammari, 2005; Paulson, 2000; Resnick et al., 2003; Rijsman et al., 2004; West et al., 2006). In this study one aim was to explore the relationship between pain, clinical sleep characteristics and nocturnal glucose and also to ascertain what the key factors in this population group were. One hypothesis to be tested was that an increase in the apnoea/hypopnoea index (AHI) was correlated with increased oxygen desaturations and hypoxemia leading to nocturnal hyperglycaemia. As hyperglycaemia is also thought to be a risk factor for worsening of peripheral neuropathy, correlations between nocturnal glucose levels and pain severity were also to be explored in this patient group.

Another aim of the study was to establish whether PLMS and/or obstructive sleep apnoea correlated with the poor sleep seen.
Finally, the effects of duloxetine, amitriptyline and pregabalin, on clinical sleep parameters and glucose levels were assessed in order to gain a full profile of the treatments.

5.2 Methods

5.2.1 Subjects

A total of 83 type 1 and 2 DM patients were included in the analysis. Subjects had to be over 18 years old and have been diagnosed with DM for at least a year and have neuropathic pain of diabetic origin with a score above 12 on the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS). For more detailed description of recruitment and inclusion and exclusion criteria see general methods and materials section 2.1

5.2.2 PSG overnight recording

PSG overnight recordings were carried out on visit 1 = night 7 (baseline with placebo), visit 2 = night 21 (chronic low dose), and visit 3 = night 35 (chronic high dose). The low dose visit corresponded to pregabalin 150mg (twice a day), duloxetine 60mg (every morning), or amitriptyline 25mg (twice a day) and high dose corresponded to pregabalin 300 mg (twice a day), duloxetine 60mg (twice a day), or amitriptyline 25mg (every morning); 50 mg (every evening). See section 2.2 of general methods and materials for more detailed description of the study, and an overview of the study schedule can be seen in appendix 1. All PSG recordings were carried out in sleep labs at the Surrey Clinical Research Centre as stated in general method section 2.3 using the Compumedics Siesta System® (Compumedics Ltd., Australia). Time in bed was 8 hours (2300-0700). The PSG nights were staged according to the Rechtschaffen and Kales criteria as stated in general methods and materials section 2.4.
5.2.3 Clinical Sleep Assessment

Clinical sleep variables analysed included arousals, PLMS and sleep apnoeas/hypopnoeas. Arousals were measured using the EEG recording with an arousal defined as awakening of least 3 secs in duration and less than 15 secs (see section 2.4.2.1 Micro Arousals for more information). PLMS and sleep apnoeas were assessed from a full body PSG assessment, including leg EMG, respiratory belt, oximeter and thermistor. Arousals, PLMS and the apnoea/hypopnoea index (AHI) were all scored according to the criteria of The AASM Manual for the Scoring of Sleep and Associated Events, 2007. For statistical analysis, the arousal index was used and defined as number of arousals per hour of sleep, the PLMS index was defined as the number of PLMS per hour of sleep and the AHI index was defined as number of apnoeas and hypopnoea per hour of sleep. For more detailed description of how apnoeas/hypopnoeas and PLMS were scored and defined, see general method and material chapter 2 section 2.4.2.2 Sleep Apnoea and Hypopnoea and section 2.4.2.3 Periodic Limb Movement of Sleep (PLMS).

5.2.4 Continuous Glucose Monitoring

The interstitial glucose levels were monitored overnight using the CGMS® System Gold™ Continuous Glucose Monitoring (Medtronic MiniMed, Inc., CA U.S.A). This system was designed to provide continuous measurements of interstitial glucose levels over the range 2.2-22.0 m.mol/l. See general method and material chapter 2 in section 2.6 Continuous Glucose Monitoring for more detailed description of the CGMS.
5.2.5 Statistical methods

Pearson correlations were used to analyse possible relationship between different variables of the baseline data at visit 1. Low correlation was defined 0.2 to 0.4, moderate 0.4 to 0.7 and strong 0.7 to 0.9. Statistical significance level was set to 5% (P<0.05).

Observations under treatment were analysed in a linear mixed model using SAS® PROC MIXED 9.1. The observations were the dependent variable and fixed effect was treatment with visits being a repeated measure. Additional independent covariates included body mass index (BMI) and age. Statistical significance level was set to 5% (P<0.05).

5.3 Results

5.3.1 Demographics and Screening results

A total of 83 patients (57 males and 26 females) were randomised onto the trial, 27 patients were randomised to pregabalin, 28 to duloxetine and 28 to amitriptyline and 65 subjects completed all visits (Table 4.2 Patient disposition in chapter 4 result section for more detailed description). Mean age of all patients was 65.1 ± 8.9 years and mean BMI was 32 ± 5.4 kg/m² (Table 4.1 Demographic characteristics of study population in chapter 4 result section for more detailed description). 72 patients had type 2 DM. 48 patients were receiving insulin treatment and 35 patients were not receiving insulin treatment. Mean HbA1c at screening was 7.93 ± 1.47% and at the end of the study 7.83 ± 1.29 %.
5.3.2 Baseline characteristics

5.3.2.1 Clinical Sleep Variables

5.3.2.1.1 Periodic Limb Movements in Sleep

The results showed that the diabetic patients (n = 70) had a mean PLMS index (PLM/hour of sleep) of 17 ± 20.7 with an index ranging from 0-85. 59% of patients had a PLMS index of greater than 5 and 39% had a PLMS index greater than 15. PLMS are known to increase with increasing age, however during this study there was no significant correlation between age and PLMS index (r = 0.11, figure 5.1).

Figure 5.1: Correlation between PLMS index and age. There was no correlation between increase in PLMS Index and increasing age (r = 0.11), n = 70 diabetic patients.
5.3.2.1.2 Sleep Apnoeas

The results showed that 36% of the diabetic patients in this study population had mild sleep apnoea, defined as AHI > 5. Only 3% of the patients had moderate sleep apnoea defined as AHI 15-30 and no patient had severe sleep apnoea defined as AHI > 30. The mean AHI in this study group was 4.3 ± 5.2 with a range from 0-27. Sleep apnoea is commonly associated with BMI, with a higher AHI with increasing BMI. There was a significant correlation between these two variables in this study population, (r = 0.30, P<0.05, figure 5.2).

![Graph showing correlation between AHI and BMI](image)

**Figure 5.2: Correlation between AHI and BMI.** There was a correlation between AHI and BMI in this study group (r = 0.30, P<0.05). n = 66 diabetic patients.

Nocturnal oxygen (O₂) levels (% of O₂ saturated haemoglobin = SPO₂), and number of O₂ desaturations (O₂ desats) during the night were also measured. Mean nocturnal SPO₂ in this diabetic study group was 92 ± 3 % (range from 79-98%) and the mean number of O₂ desaturations was 3.3 ± 3.8 per hour of sleep (range from 0-19).
There was no significant correlation between mean SPO$_2$ and AHI, ($r = 0.045$, figure 5.3). However, there was a very strong correlation between AHI and number of O$_2$ desaturations during the night ($r = 0.91$), and this correlation was significant ($P<0.0001$, figure 5.4).

![Figure 5.3: Correlation between AHI and mean nocturnal SPO$_2$. There was no correlation between the AHI and SPO$_2$ in this study group ($r = 0.045$ and $r^2 = 0.002$). $n = 66$ diabetic patients. SPO$_2$ = % of oxygen saturated haemoglobin.]

![Figure 5.4: Correlation between AHI and number of O$_2$ desaturations (O$_2$desat). There was a strong correlation between an increase in the number of apnoeas and hypopnoea and an increase in the number of O$_2$ desaturations ($r = 0.91$, $P<0.0001$). $n = 66$ diabetic patients.]

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5.3.2.1.3 Arousals

The number of nocturnal arousals was also analysed. Arousals were divided into spontaneous arousals, arousals associated with PLMS, and arousals associated with apnoea and hypopnoea. For spontaneous arousals patients had a mean of $4.0 \pm 2.9$ arousals/hour (range 0.8-15), while for arousals associated with PLMS and arousals associated with apnoea/hypopnoea patients had a mean of $1.6 \pm 2.0$ (range 0-12) and $0.7 \pm 1.5$ (range 0-8.2) arousals/per hour of sleep, respectively.

5.3.2.2 Glycosylated haemoglobin and glucose levels

5.3.2.2.1 Glycosylated haemoglobin (HbA1c)

HbA1c measurements were used to assess long term glycaemic control. The International Diabetes Federation recommends HbA1c values below 6.5% for diabetic patients, while the American Diabetes Association recommends a value below 7%. The mean HbA1c for this study group was $7.93 \pm 1.47\%$ and the result showed that only 13% of all diabetic patients in this study had HbA1c values below the recommended 6.5% (figure 5.5).
5.3.2.2 Glucose levels

Patients’ glucose levels were analysed by using a CGMS. The mean glucose levels for the entire placebo visit (~ 48 hours) was $9.4 \pm 2.3$ mmol/L, range 6-16 mmol/L ($n=66$). The mean nocturnal glucose for night 2 visit 1 (baseline) was $7.8 \pm 2.1$ mmol/L, range 3-15 mmol/L ($n=71$).

Nocturnal hypoglycaemic events (defined as glucose levels < 3.3 mmol/L) was rare in this patient group, analysis showed that only 4% of patients ($n=3$) had a hypoglycaemic event during the night. Nocturnal hyperglycaemia was far more common with 79% of patients ($n=56$) having hyperglycaemic events during the night (defined as glucose levels > 7.8 mmol/L), and a quarter of these patients spent the entire night with glucose levels >7.8 mmol/L. If looking at hyperglycaemic events > 11.0 mmol/L the result showed that 31%
(n=22) of patients had nocturnal hyperglycaemic events > 11.0 mmol/L and 4% (n=3) had events >15 mmol/L. Figure 5.6 demonstrates the differences in nocturnal glucose between patients. (A) A patient who is classified as having nocturnal hyperglycaemia with a mean glucose of >7.8mmol/L for the entire night. (B) A patient with a stable glucose curve and no hyperglycaemic/hypoglycaemic events during the night. (C) A patient with hypoglycaemic events and nocturnal glucose levels <3.3 mmol/L.

Figure 5.6: Nocturnal glucose curve. Graph shows representative traces of nocturnal glucose levels for 3 different patients on night 7, visit 1. Patient A had nocturnal hyperglycaemia with a mean glucose of >7.8mmol/L for the whole night, patient B showed a stable glucose curve with no hyperglycaemic/hypoglycaemic events during the night, while C shows an example of patient with hypoglycaemic events and glucose levels <3.3 mmol/L.
5.3.2.3 Correlations between AHI, oxygen saturations, sleep, glucose and pain at baseline

Results in section 5.3.2.1.2 Sleep Apnoeas, showed a significant correlation between AHI and the number of oxygen desaturations (P<0.0001). Further correlations showed that both AHI and number of oxygen desaturations correlated with number of awakenings (NAW) (r = 0.35, P<0.05 and r = 0.37, P<0.01, respectively, figure 5.7). The increase in apnoeas, number of oxygen desaturations and NAW did not correlate with nocturnal glucose levels (mean nocturnal glucose or glucose >7.8mmol/L). And there was no correlation between nocturnal glucose levels and pain severity or pain interference.

Figure 5.7: Correlation between AHI, number of O2 desaturations and number of awakenings (n =58). A. Correlation between AHI and number of awakenings. Increase in AHI correlated with increase in number of awakenings (r = 0.35 and P<0.05) n = 57. B. Correlation between number of O2 desaturations and number of awakenings. Increase in number of O2 desaturations correlated with increase in number of awakenings (r = 0.37 and P<0.01).
When looking at AHI prevalence at different sleep stages the result showed that AHI was most commonly associated with stage 1 sleep with increased stage 1 sleep duration associated with an increase in the AHI ($P<0.01$, figure 5.8). In contrast, an increase in both stage 3 and 4 sleep resulted in a decrease in the AHI and this was statistically significant for stage 4 sleep ($P<0.05$) and approached significance for stage 3 sleep ($P=0.0507$). There was no correlation between stage 2 or REM sleep duration and AHI (figure 5.8).

Similar results could be seen with NAW, where an increase in NAW was associated with an increase in stage 1 sleep ($P<0.0001$) whereas when stage 3 and 4 sleep increased this was associated with a decrease in NAW ($P<0.001$ and $P<0.05$, respectively). There was no correlation between NAW and REM sleep.
Figure 5.8: Correlation between AHI and duration of sleep stages. (n = 57) A. Correlation between AHI and duration of stage 1 sleep. Increase in AHI correlated significantly with increase in stage 1 sleep (r = 0.36 and P < 0.01). B. Correlation between AHI and duration of stage 2 sleep. There was no significant correlation between AHI and duration of stage 2 sleep (r = 0.17). C. Correlation of AHI and duration of stage 4 sleep. Increase in AHI correlated significantly with an increase in stage 4 sleep (r = 0.28 and P < 0.05). D. Correlation between AHI and duration of REM sleep. There was no significant correlation between amount of REM sleep and AHI (r = 0.14).
5.3.3 Treatment effects on clinical variables

The following section describes the effect of the three treatments (pregabalin, amitriptyline and duloxetine) on clinical variables. For clinical sleep variables (PLMS, AHI and arousals) only the visit 1 (placebo baseline) and visit 3 (chronic high dose) were included in the analysis. The reason for inclusion of visit 3 data alone was that in the time available it was not possible to perform complete analysis of visit 2 (chronic low dose) data and that if a drug effect was seen then it would be expected to be most apparent in the highest dose. Future work could be to score and analyse the low dose records in order to see if a dose response exists. For HbA1c levels assessment was made at medical screening before the clinical start of the study and then again at the follow up visit after treatment had been terminated. The analysis of glucose levels however included data from all three visits: visit 1 (placebo baseline), visit 2 (chronic low dose) and visit 3 (chronic high dose).

5.3.3.1 Clinical Sleep

5.3.3.1.1 Periodic Limb Movements in Sleep

There was a significant treatment by visit effect (P<0.001) on PLMS Index with a significantly lower PLMS Index with high dose pregabalin treatment compared with duloxetine and amitriptyline (P<0.001 for both, table 5.1 and figure 5.9).

Post hoc analysis showed that pregabalin significantly decreased PLMS compared with placebo (LS mean 19.7 for placebo to 12 with high dose Cohen’s $d = 0.68$ and $P<0.01$), while duloxetine and amitriptyline significantly increased PLMS compared with placebo. Duloxetine had a LS mean of 16.2 for placebo compared to 24.4 with high dose (Cohen’s $d = 0.66$ and $P<0.05$) and amitriptyline had a LS mean of 16.2 for placebo compared with 19.9 with high dose (Cohen’s $d = 0.74$ and $P<0.01$, figure 5.9).
Figure 5.9: Treatment effect on PLMS index. There was a significant treatment by visit effect (P<0.001). Pregabalin significantly lowered the PLMS index compared with pregabalin and duloxetine. Comparison between treatments = †. Pregabalin significantly decreased PLMS index while duloxetine and amitriptyline significantly increased PLMS index compared with placebo at baseline. Comparison with placebo = *. Level of significance: ††† = P<0.001, ** = P<0.01, * = P<0.05). Bars show mean, ± standard error of mean (SEM) PGB = pregabalin, DUL = duloxetine, AMI = amitriptyline.
Table 5.1: Treatment by visit effect.

<table>
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<tr>
<th></th>
<th>Lower dose</th>
<th>Higher dose</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Effect p-value</td>
<td>Treatment</td>
</tr>
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<td>PLMS Index (PLM/hours of sleep)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>PGB v AMI</td>
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<tr>
<td></td>
<td></td>
<td>DUL v AMI</td>
</tr>
<tr>
<td>AHI (Apnoea-Hypopnoea/hour of sleep)</td>
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<tr>
<td></td>
<td></td>
<td>PGB v AMI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DUL v AMI</td>
</tr>
<tr>
<td>Spontaneous arousals</td>
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<tr>
<td></td>
<td></td>
<td>PGB v AMI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DUL v AMI</td>
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<tr>
<td>Respiratory arousals</td>
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<tr>
<td></td>
<td></td>
<td>PGB v AMI</td>
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<td></td>
<td></td>
<td>DUL v AMI</td>
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<tr>
<td>Arousal associated with PLMS</td>
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<tr>
<td></td>
<td></td>
<td>PGB v AMI</td>
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<td></td>
<td>DUL v AMI</td>
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<tr>
<td>Mean number of nocturnal O2 desaturations</td>
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<tr>
<td></td>
<td></td>
<td>PGB v AMI</td>
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<td></td>
<td></td>
<td>DUL v AMI</td>
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<tr>
<td>Mean nocturnal O2 saturation (%)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>PGB v AMI</td>
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<td></td>
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<td>DUL v AMI</td>
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<tr>
<td>Mean nocturnal glucose levels (mmol/L)</td>
<td>0.01</td>
<td>PGB v DUL</td>
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<td></td>
<td></td>
<td>PGB v AMI</td>
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<tr>
<td></td>
<td></td>
<td>DUL v AMI</td>
</tr>
<tr>
<td>Mean glucose levels for whole visit (mmol/L)</td>
<td>NS</td>
<td>PGB v DUL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PGB v AMI</td>
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<td></td>
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<td>DUL v AMI</td>
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<tr>
<td>Mean % of nocturnal glucose levels &lt; 3.3 mmol/L</td>
<td>NS</td>
<td>PGB v DUL</td>
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<td></td>
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<td>PGB v AMI</td>
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<td></td>
<td></td>
<td>DUL v AMI</td>
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<tr>
<td>Mean % of nocturnal glucose levels &gt; 7.8 mmol/L</td>
<td>NS</td>
<td>PGB v DUL</td>
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<tr>
<td></td>
<td></td>
<td>PGB v AMI</td>
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<td>DUL v AMI</td>
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NS = Not significant

PGB = pregabalin, DUL = duloxetine, AMI = amitriptyline,
PLMS = periodic limb movement in sleep, AHI = apnoea-hypopnoea Index
5.3.3.1.2 Sleep Apnoeas

There was a significant treatment by visit effect on AHI (P<0.0001) with a significant increase in apnoeas and hypopnoeas in the pregabalin treatment group compared with duloxetine and amitriptyline (P<0.0001 and P<0.001, respectively, table 5.1, figure 5.10).

Pregabalin significantly increased the AHI compared with placebo at visit 1 (LS mean 5.8 apnoea/hypopnoea per hour of sleep at placebo to 11.9 apnoea/hypopnoea per hour of sleep with high dose at visit 3, Cohen’s $d = 1.26$ and P<0.0001), while duloxetine and amitriptyline did not change significantly the AHI compared with placebo at baseline (figure 5.10).

Figure 5.10: Treatment effect on the apnoea-hypopnoea index (AHI). There was a significant treatment by visit effect (P<0.0001). Pregabalin significantly increased AHI compared with duloxetine and amitriptyline. Comparison between treatments = †. Pregabalin significantly increased AHI compared with placebo while duloxetine and amitriptyline had no significant effect on AHI compared with placebo at baseline. Comparison with placebo = *. Level of significance: +++/*** = P<0.001. Bars show mean, ± standard error of mean (SEM). PGB = pregabalin, DUL = duloxetine, AMI = amitriptyline.
The number of $O_2$ desaturations/hour of sleep also demonstrated a significant treatment by visit effect ($P<0.0001$), with the pregabalin treatment group having a significantly higher number of $O_2$ desaturations / hour compared with both the duloxetine and amitriptyline treatment groups ($P<0.001$ for both, table 5.1 and figure 5.11). Further analysis showed that high dose pregabalin increased number of $O_2$ desaturations compared with placebo (LS mean 3.9 at placebo to 10.2 with high dose, Cohen’s $d = 1.31$ and $P<0.0001$) while amitriptyline and duloxetine had no significant effect on $O_2$ desaturations compared with placebo at baseline, (figure 5.11).

Figure 5.11: Treatment effect on number $O_2$ desats/hour of sleep. There was a significant treatment by visit effect ($P<0.0001$). Pregabalin significantly increased number of $O_2$ desaturations compared with duloxetine and amitriptyline. Comparison between treatments = †. Pregabalin significantly increase number $O_2$ desaturations compared with placebo, while with duloxetine and amitriptyline there was no significant change compared with placebo at baseline. Comparison with placebo = *. Level of significance: †††/*** = $P<0.001$. Bars show mean, ± standard error of mean (SEM). PGB = pregabalin, DUL = duloxetine, AMI = amitriptyline.
There was no significant treatment by visit effect on mean nocturnal $O_2$ saturation with all treatment groups having similar mean $O_2$ saturation when compared with each other as well as compared with placebo (pregabalin; 92.4% for placebo and 93.3% for high dose, duloxetine; 93.2% for placebo and 93.9% for high dose, amitriptyline; 93.7% for both placebo and high dose, figure 5.12).

**Figure 5.12: Treatment effect on mean $O_2$ saturation.** There was no significant treatment by visit effect. Bars show mean, ± standard error of mean (SEM) PGB = pregabalin, DUL = duloxetine, AMI = amitriptyline.
5.3.3.1.3 Arousals

There was no significant treatment by visit effect on the number of spontaneous arousals, no significant difference between the treatments or when compared with placebo (table 5.1 and figure 5.13).

![Graph showing mean number of spontaneous arousals/hour for various treatments](image)

**Figure 5.13: Treatment effect on number of spontaneous arousals.** There was no significant treatment by visit effect and no significant difference when compared with placebo. Bars show mean, ± standard error of mean (SEM). PGB = pregabalin, DUL = duloxetine, AMI = amitriptyline

Although there was a significant treatment effect on number of PLMS, there was no such treatment effect on the arousals associated with the PLMS. Pregabalin significantly decreased PLMS while duloxetine and amitriptyline both increased number of PLMS, however, the arousals associated with PLMS remained unchanged and similar between treatment and to that seen at the placebo baseline (table 5.1 and figure 5.14).
Figure 5.14: Treatment effect on number of arousals associated with PLMS. There was no significant treatment by visit effect and no significant difference compared with placebo. Bars show mean, ± standard error of mean (SEM). PGB = pregabalin, DUL = duloxetine, AMI = amitriptyline.

For respiratory arousals the treatment by visit effect approached significance, P=0.055 with respiratory arousals mirroring the changes in the AHI. There was a significant increase in the number of respiratory arousals in the pregabalin group compared with amitriptyline and duloxetine (P<0.05, for both). Post hoc analysis showed that pregabalin significantly increased the number of respiratory arousals compared with the placebo at baseline (LS mean 1.4 with placebo to 3.4 with high dose, Cohen’s d = 0.70 and P<0.01), while duloxetine and amitriptyline had no significant effect on respiratory arousals (table 5.1 and figure 5.15).
**Figure 5.15: Treatment effect on number of respiratory arousals.** The treatment by visit effect approached significance, $P = 0.055$. Pregabalin significantly increased the number of respiratory arousals compared with duloxetine and amitriptyline. Comparison between treatments = †. Pregabalin significantly increased the number of respiratory arousals compared with placebo, while there was no significant change with duloxetine and amitriptyline compared with placebo at baseline. Comparison with placebo = *. Level of significance: † = $P < 0.05$, ** = $P < 0.01$. Bars show mean, ± standard error of mean (SEM). PGB = pregabalin, DUL = duloxetine, AMI = amitriptyline.

### 5.3.3.2 Glycosylated haemoglobin and glucose levels

#### 5.3.3.2.1 Glycosylated haemoglobin (HbA$_1c$)

There was no significant difference in HbA$_1c$ measurements post treatment compared with before treatment had started ($7.93 \pm 1.47\%$ before treatment and $7.83 \pm 1.29\%$ post treatment) and no significant difference between the treatments (Table 5.2). Even though a slight improvement was observed the glycosylated haemoglobin levels were still above the International Diabetes Federation recommended HbA$_1c$ value of below 6.5%.
Table 5.2: HbA1c levels pre treatment and post treatment. There was no significant difference between HbA1c levels at pre treatment compared with post treatment and there was no significant difference between the treatments.

5.3.3.2.2 Glucose levels

Glucose levels were measured and analysed for all 3 visits. There was a significant treatment effect on mean nocturnal glucose levels (P<0.01) with the pregabalin treatment group having significantly higher nocturnal glucose levels compared with the duloxetine and amitriptyline treatment groups (low dose; pregabalin v duloxetine P<0.05, high dose; pregabalin v duloxetine P<0.001, pregabalin v amitriptyline P<0.05, table 5.1 and figure 5.16).

Post hoc analysis showed that pregabalin significantly increased glucose levels at visit 3 compared with placebo with LS mean of 7.1 mmol/L for placebo and 8.7 mmol/L for high dose (Cohen’s $d = 0.68$ and $P<0.01$). In contrast, duloxetine significantly decreased nocturnal glucose levels compared with placebo with LS mean 8.1 mmol/L for placebo to 6.8 mmol/L low dose and 7.0 mmol/L high dose (Cohen’s $d = 0.78$ and $P<0.01$ for low dose and Cohen’s $d = 0.60$ and $P<0.05$ for high dose) while amitriptyline had no significant effect on nocturnal glucose levels, (figure 5.16).
Figure 5.16: Treatment effect on nocturnal glucose levels. The overall treatment by visit effect was significant (P < 0.01). Comparison between treatments; pregabalin v duloxetine = * and pregabalin v amitriptyline = x. Both low and high dose duloxetine decreased glucose levels and high dose pregabalin increased glucose levels when compared with placebo, while amitriptyline did not change glucose levels significantly compared with placebo at baseline. Level of significance * / x = P < 0.05, *** = P < 0.001. Plot shows mean, ± standard error of mean (SEM). Amitriptyline (▲), pregabalin (●), duloxetine (■).

When looking at hypoglycaemia (defined as glucose levels < 3.3 mmol/L) during the night results showed that there was no significant difference between any of the three treatments or compared with placebo for percentage of nocturnal glucose < 3.3 mmol/L (table 5.1). A similar result was seen when looking at hyperglycaemia (defined as glucose levels > 7.8 mmol/L). Again there was no significant difference between any of the three treatments or change when compared with placebo (table 5.1).
When looking at glucose levels for the whole visit (~ 48 hours) there was no significant treatment by visit effect. There was no significant difference in overall mean glucose levels either between the three treatments or when compared with the placebo baseline (table 5.1 and figure 5.17).

Figure 5.17: Treatment effect on glucose levels (whole visit). The overall treatment by visit effect was not significant and there was no significant difference between the three treatments compared with placebo. Duloxetine ( ), pregabalin ( ), amitriptyline ( ). Plot shows mean, ± standard error of mean (SEM).
5.4 Discussion

This study was the first one to investigate the inter-relationship of several factors that may affect sleep in diabetic patients with painful DPN and to analyse if pregabalin, duloxetine and amitriptyline could affect these factors and therefore possibly improve pain and sleep in this patient group. The aim was to explore the relationship between pain, clinical sleep characteristics such as PLMS and sleep apnoea, nocturnal oxygen desaturations and nocturnal glucose to ascertain what were the key factors in this population group.

In previous chapters (chapter 3 and 4) it was shown that diabetic patients with painful DPN had worse sleep compared with healthy subjects of the same age and gender, however results indicated that there was little correlation between pain and worsening in sleep quality and sleep continuity. Therefore, other factors mentioned above were thought to be contributing to the disruption in sleep.

The results in this chapter showed that the diabetic patients had a mean PLMS index (PLM/hour of sleep) of 17, SD 20.7 with an index ranging from 0-85 PLM/hour of sleep. 59% of patients had a PLMS index of greater than 5 and 39 % had a PLMS index greater than 15. Between 30 - 45% of the older adult population have been estimated to have PLMS (Ancoli-Israel and Ayalon, 2009; Kaplan and Fischer, 2005). PLMS may cause arousals which can lead to difficulties in staying asleep, and result in sleep fragmentation (Ancoli-Israel and Ayalon, 2009). However, only 1.6 of the 17 PLM/hour were associated with nocturnal arousals and there was no correlation between an increase in PLMS and any of the sleep variables TST, SE, WASO, LPS, and NAW, suggesting that the high incidence of PLMS in this patient group did not have a negative effect on sleep.
The prevalence of sleep apnoea in this diabetic patient group was perhaps not as high as expected. The results showed that 36% of the diabetic patients had mild sleep apnoea, defined as AHI > 5 and 3% of the patients had moderate sleep apnoea defined as AHI 15-30 with no patients being classified as having severe sleep apnoea defined as AHI > 30. There was a significant correlation between BMI and sleep apnoea, with a higher BMI correlating with an increase in the AHI. This was expected as it is known that obesity is a common risk factor in the development of obstructive sleep apnoea (Resnick et al., 2003). Clinical diagnosis of sleep apnoea is normally made at an AHI ≥ 10-15 and is more common in older adults. The results from the Sleep Healthy Heart Study showed that prevalence increased with aged and 20% of those aged 60 years or older had an AHI ≥ 15 (Ancoli-Isreal and Ayalon, 2006). Sleep apnoea can be associated with oxygen desaturation which can lead to nocturnal hypoxemia and this can also cause arousals and awakenings (Alsaeedi and Alshammari, 2005; Neubauer, 1999; Pallayova et al., 2006; Paulson, 2000). The results of the current study showed a strong correlation between AHI and number of awakenings and oxygen desaturations. Increase in AHI correlated significantly with an increase in oxygen desaturations ≥ 4% and with an increase in number of awakenings. Mean nocturnal oxygen level was 92% (range 79-98%), but did not correlate with AHI.

The prevalence of apnoeas and hypopnoeas also correlated with distinct sleep stages. An increase in AHI and number of awakenings correlated significantly with an increased duration of lighter, stage 1, sleep. This might suggest that apnoeas and hypopnoeas cause awakenings and lightening of sleep resulting in patients having difficulty entering and maintaining stage 2 sleep. In contrast, patients with a longer duration of stage 3 and 4 sleep (SWS) show a reduced AHI and reduced number of awakenings. In particular, there was a significant correlation between the increase in stage 4 sleep and the decrease in number of awakenings and AHI, while this correlation approached significance with regards to stage 3
sleep. There was no evidence of a correlation between stage 2 and REM sleep and the AHI or number of awakenings. A reduced number of awakenings with increases in slow wave sleep but not with REM sleep have previously been shown in healthy subjects (Dijk et al., 2006). This indicates that an increase in slow wave sleep reflects less fragmented sleep with fewer numbers of awakenings during the night.

Furthermore, it is thought that sleep apnoea and severe oxygen desaturations are associated with altered glucose metabolism resulting in nocturnal hyperglycaemia and several studies have shown a link between obstructive sleep apnoea, sleep loss and insulin resistance (Pallayova et al. 2006; Punjabi et al., 2002 Punjabi et al., 2004, Spiegel et al., 2005). Nocturnal glucose levels may also contribute to worse sleep (Bendtson et al., 1992). One hypothesis was that an increase in AHI would correlate with an increase in oxygen desaturations resulting in an increase in the duration of lighter stage 1 sleep and nocturnal awakenings and this could indeed be seen in the analysis. Furthermore, sleep fragmentation and hypoxemia are reported to alter glucose metabolism (Punjabi and Polotsky, 2005) and therefore it was expected that with an increase in the AHI there would be increased nocturnal glucose levels (hyperglycaemia) and a worsening of the neuropathic pain. The results however, showed that there was no correlation between hypoxemia (number of desaturations and mean nocturnal oxygen levels), and glucose levels (mean nocturnal glucose levels and % above 7.8mmol/L). Furthermore, there was no correlation between NAW or WASO and nocturnal glucose levels suggesting that sleep fragmentation did not result in hyperglycaemia. The reversed effect has also been reported with nocturnal hypoglycaemia and/or hyperglycaemia affecting sleep in a negative way (Bendtson et al., 1992). There was however no correlation between hyperglycaemic or hypoglycaemic events and sleep quality or sleep continuity in this patient group. The lack of correlation between nocturnal hypoglycaemic
events (defined as glucose levels < 3.3 mmol/L) and sleep may be due to that these events were rare in this patient group and analysis showed that only 4% of patients had a hypoglycaemic event during the night during the residential visits. Nocturnal hyperglycaemia was far more common with 79% of patients having hyperglycaemic events during the night (defined as glucose levels > 7.8 mmol/L), and a quarter of these patients spent the entire night with glucose levels > 7.8 mmol/L, but there was still no correlation between hyperglycaemia and sleep.

However, even if there was no evidence that nocturnal glucose levels had a negative effect on sleep one should always aim to lower glucose levels. The results showed that the patient population group had a high degree of nocturnal hyperglycaemia, with a mean HbA1c of 7.93 ± 1.47% and only 13% of patients having a value equal to or below the recommended 6.5%, indicating poor glycaemic control in this patient group (International Diabetes Federation). HbA1c is a measure of long term glucose control and a high value is known to be a risk factor in developing microvascular complications such as peripheral neuropathy. Hyperglycaemia is also associated with a worsening of peripheral neuropathy and an increased risk of cardiovascular complications (Gore et al., 2005; Haffner and Cassells, 2003; Idris et al., 2006; Laakso, 1999; Tesfaye and Kempler, 2005; Vinik and Flemmer, 2002). A worsening in peripheral neuropathy could lead to increase in neuropatic pain, but there was no correlation between hyperglycaemia and pain severity or pain interference in this patient group.

The incidence and severity of obstructive sleep apnoea was also affected by treatment with a significant increase in apnoeas and hypopnoeas in the pregabalin treatment group compared with duloxetine and amitriptyline and compared with placebo. This finding support previous data which have indicated that pregabalin increases snoring index compared with placebo (Saletu-Zyhlarz et al. 2006). Furthermore, it was expected that both duloxetine and
amitriptyline might have a beneficial effect on the prevalence of apnoeas and hypopnoeas as it has previously been reported that TCAs and SSRIs might enhance ventilation and provide some benefit in the treatment of OSA (Hanzel et al., 1991) although the exact mechanism for this is unclear. The result of this study showed however no significant difference between amitriptyline and duloxetine when compared with placebo.

Finally the study investigated the effects of pregabalin, duloxetine and amitriptyline on glucose levels and HbA1c to see if any of the treatments would have a protective / beneficial effect on the metabolic condition itself. There are very little data published in regards to the three treatments possible effect on glucose levels. An analysis done by Hardy et al. (2007) collecting data from three different clinical trials with duloxetine showed that duloxetine treatment (60mg/day or 120mg/day) increased fasting plasma glucose levels in both short and long-term studies. However, this increase was modest (0.5 and 0.67 mmol/L, respectively). The results of the present study showed no significant difference in long term glucose as measured by HbA1c with none of the treatments causing a significant difference post treatment compared with before treatment had started (7.93 ± 1.47% before treatment and 7.83 ± 1.29% post treatment). Even though a slight improvement was observed, the glycosylated haemoglobin levels were still above the International Diabetes Federation recommended HbA1c value of below 6.5%. T. However, HbA1c is a measure of long term glucose control of the past 2-3 months (Özmen and Boyvada, 2003) so longer treatment may be needed to see possible full effect if any.

There was evidence that nocturnal glucose levels were affected by treatment with results suggesting that the pregabalin treatment group had significantly higher nocturnal glucose levels compared with the duloxetine and amitriptyline treatment groups and a significant increase in nocturnal glucose levels at the high dose pregabalin visit compared with placebo.
In contrast, duloxetine significantly decreased nocturnal glucose levels compared with placebo with while amitriptyline had no significant effect on nocturnal glucose levels. Although the results showed a statistically significant difference between treatments these results must be read with caution as the overall increase in nocturnal blood glucose with pregabalin was only 1.6 mmol/L and the decrease in nocturnal glucose with duloxetine was only 1.3 mmol/L (with mean glucose levels ranging from 6.8 mmol/L to 8.7 mmol/L across treatment groups and visits). It is unlikely that these changes would represent a clinically significant change. In addition, there was no significant difference between the treatments or in comparison with placebo with regards to nocturnal hyperglycaemic or hypoglycaemic events.

In conclusion, the results of this study suggest that diabetic patients with painful DPN have a higher prevalence of PLMS compared with general population but the PLMS index overall was considered mild (Ancoli-Israel and Ayalon, 2009; Kaplan and Fischer, 2005) and was not associated with many arousals and did not disturb sleep in these patients. In contrast although OSA in these patients was considered to be predominantly mild, sleep apnoeas and hypopnoeas appeared to disturb sleep with an increased number of awakenings, an increase in stage 1 sleep and a reduction in the deeper, SWS (stage 3 and 4). There was some evidence that pregabalin increased the AHI significantly. However, whether pregabalin led to the development or worsening of apnoeas or whether the patients in this treatment group had a higher prevalence of apnoeas and were more unstable needs to be investigated further. However the study highlights the importance of comprehensive assessment of drug effects as the results presented here would suggest that pregabalin should be prescribed with caution in patients with a history of sleep apnoea.
Finally, it can be established that patients with painful DPN in this study have overall a poor glucose control with high HbA$_{1c}$ and an increased prevalence of hyperglycaemia. The treatments appeared to have no effect on HbA$_{1c}$ or hyperglycaemic/hypoglycaemic episodes within a 2-4 week period of treatment. Although poor glucose control did not appear to affect sleep directly it may contribute to a worsening of peripheral neuropathy and increase the risk of other complications such as cardiovascular disease.
CHAPTER 6 - GENERAL DISCUSSION
6.1 General Discussion

Diabetes is a very complex metabolic disorder affecting millions of people of all ages all over the world. The prevalence is estimated to be over 220 million with 150 million having type 2 diabetes. Type 2 diabetes is increasing in epidemic proportions with the prevalence predicted to double by 2025 (International Diabetes Federation; King et al., 1998). A large amount of research is currently being undertaken in this field to try to prevent and treat the disease but more research is needed.

Living with diabetes is often difficult with a risk of a number of debilitating complications and as a result reduced quality of life. One common complication is painful DPN. The mechanisms of painful DPN are poorly understood and drug treatment often has a poor response rate and poor patient tolerance long term. The aim of our study was to try to identify which of the most commonly used drug treatments might be the most effective in the treatment of painful DPN; reducing pain, improving sleep and daytime functioning and as a result hopefully improving quality of life.

The compounds investigated in this study were duloxetine, pregabalin and amitriptyline and this was the first study to compare the three drugs in the same clinical trial. Eighty-three type 1 and 2 diabetic patients with painful DPN were recruited and randomized onto the trial. Forty of these patients were randomly selected to be used in a sub analysis comparing sleep in this patient group with that of a healthy population. A historically healthy control group was created from the large Surrey Sleep Research Centre (SSRC) database. Polysomnography was used to assess sleep in both diabetes group and healthy control group. It could be established that diabetic patients with painful DPN had poor sleep in comparison...
with the healthy volunteers (table 6.1). Several studies have previously shown that diabetic patients have poor sleep using subjective sleep questionnaires but few studies have assessed sleep objectively using polysomnography and, as far as the author is aware, no previous study has compared objective sleep in this patient group with that of healthy controls.

The main analysis of the study included all 83 randomized patients of which 65 completed the whole study (all three visits). The study consisted of a single blind placebo run in of one week, a 2 week double-blind dosing period of a lower dosage of study medication and a 2 week double-blind dosing period of a higher dose of study medication. During the dosing periods patients were admitted to the Surrey CRC for a comprehensive (48 hr) assessment at baseline placebo (day 6-8), chronic low dose (day 20-22) and chronic high dose (day 34-36).

Baseline analysis at visit 1 suggested that the poor sleep in the diabetic study group was not associated with pain or nocturnal glucose levels with hyperglycaemic/hypoglycaemic events during the night. Prevalence of periodic limb movement was high in this patient group but the number of events was considered mild and not associated with any arousals or awakenings. Sleep apnoea did however correlate with number of awakenings and there was a correlation between an increase in lighter, stage 1 sleep, and an increase in the apnoea index.

Analysis of the low and high dose of treatments at visits 2 and 3 respectively, showed some very interesting results (table 6.2). For the first time one could compare the analgesic efficacy of these three drugs directly with each other and the results showed that all three treatments improved subjective pain and reduced pain interference on sleep in patients with painful DPN. As sleep is now one of the most common complaints in patients with diabetes one would hope that by reducing pain the treatments would also improve the subjective quality of sleep as well as objectively measured sleep parameters. The data showed that pregabalin indeed improved both subjective and objective sleep. Pregabalin improved sleep when
compared with placebo but also significantly improved sleep compared with duloxetine and
to some extent with amitriptyline. In contrast, duloxetine significantly worsened sleep with
patients spending more time awake, taking a longer time to fall asleep, and consequently
having reduced SE and TST. Amitriptyline appeared to have little effect on sleep when
compared with placebo (table 6.2).

Although pregabalin improved sleep and duloxetine disrupted sleep there was no evidence
that any of the treatments affected (improved or impaired) levels of daytime sleepiness when
compared with placebo. In addition, a parallel analysis of the same study looking at the next
day performance was carried out by Miss Laura Gribble (University of Surrey). A
psychometric test battery was used to assess cognitive function and this demonstrated that
there was no significant effect of treatment on cognitive tasks such as memory and attention
but that there was some evidence that pregabalin impaired divided attention (tracking
deviation) and that duloxetine exhibited an alerting effect with improved critical flicker
fusion performance and reduced reaction time (personal communication with Miss Laura
Gribble, University of Surrey). Whether this alerting affect of the duloxetine could be
sustained longer term is unknown as the poor sleep seen with duloxetine might eventually
result in a higher level of daytime sleepiness and reduced alertness.

Deciding on the ideal treatment drug for painful DPN remains difficult, especially when there
is no clear differentiation on pain outcome. However, duloxetine has a strong negative
treatment effect on sleep and this may affect the patients long term, and one should take this
into consideration when prescribing duloxetine, especially if patients have previously
complained about sleep problems. Pregabalin on the other hand has a strong positive effect
on sleep and the true benefits of this in regards to reduced pain and improved quality of life
may not be seen in such a short duration as with this trial. However, the data from this study
suggest that pregabalin should be prescribed with caution in patients with a history of sleep apnoea as the prevalence of sleep apnoea was increased in the pregabalin treatment group. This study highlights the importance of comprehensive assessment of drug effects looking at all aspects with regards to possible benefits and also potential side effects. This study makes a substantial scientific contribution which will help diabetic consultants and GPs to make informed treatment choices for their patients.

6.1.1 Future Work

Future research would be to more fully understand the effect of diabetes on sleep. Studies should compare sleep in diabetic patients with painful DPN with diabetic patients without painful DPN and a real time healthy volunteer group, all matched for age, gender and BMI. The study should aim to control for clinical features such as PLMS and apnoeas/hypopnoes by possibly excluding people with a high prevalence of PLMS and AHI. This would allow the impact of diabetes and neuropathic pain on sleep to be more fully elucidated. The study should also consider using habitual bedtimes rather than a strict study regime of sleep at 2300-0700 in order to get a good picture of sleep efficiency and latency to sleep across all groups and patients. Reason for this is that the patients on the current study were mainly older adults with a mean age of 65 years and therefore a bedtime of 23:00 was possibly a bit late and not necessarily aligned with their circadian phase as older adults tend to go to bed earlier. Habitual bedtimes were collected for the duration of the study and varied greatly between subjects, with some subjects having a mean habitual bedtime as early as 21:00 and some subjects having a mean bedtime as late as 01:00. This could possibly have had an effect on for example sleep onset latency and REM sleep when subject had restricted bedtime of 23:00 during study days.
Further studies are also required to more fully investigate the effect of pregabalin on sleep apnoea. A range of patients should be included from those with no evidence of sleep apnoea, mild, moderate and severe apnoea in order to assess whether pregabalin worsens existing sleep apnoea and/or if pregabalin induces sleep apnoea in non apnoea patients.
Table 6.1: Summary of results of the comparison between DM patients and healthy subjects

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<th>Healthy subjects</th>
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<tr>
<td>Mean (SE)</td>
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<tr>
<td>TST (min)</td>
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<td>412 (5.1)</td>
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<td>SE (%)</td>
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<td>95 (4.2)</td>
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<td>KSS</td>
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<td>2.6 (0.2)</td>
<td>&lt;0.001</td>
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DM = Diabetes Mellitus, TST = total sleep time, SE = sleep efficiency, LPS = latency to persistent sleep, WASO = wake after sleep onset, DUR = duration in minutes per sleep stage, KSS = karolinska sleepiness scale
<table>
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<th>Treatment by visit</th>
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<th>Amitriptyline</th>
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<td>PRC LS Q</td>
<td>PRC LS Q</td>
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<td>13.2 (1.72)</td>
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<td>4.2 (0.22)</td>
<td>4.7 (0.25)</td>
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<tr>
<td>LSEQ-AFS</td>
<td>46.5 (3.19)</td>
<td>49.4 (5.07)</td>
<td>49.2 (4.27)</td>
</tr>
<tr>
<td>LSEQ-BFS</td>
<td>49.3 (4.02)</td>
<td>53.3 (4.06)</td>
<td>50.6 (4.06)</td>
</tr>
<tr>
<td>TST (min)</td>
<td>371.6 (11.84)</td>
<td>280.6 (9.14)</td>
<td>410.3 (12.10)</td>
</tr>
<tr>
<td>SE (%)</td>
<td>77.3 (2.46)</td>
<td>79.2 (1.90)</td>
<td>85.4 (2.13)</td>
</tr>
<tr>
<td>SOL (min)</td>
<td>22.3 (2.47)</td>
<td>25.1 (4.72)</td>
<td>18.8 (4.27)</td>
</tr>
<tr>
<td>LPS (min)</td>
<td>29.0 (4.25)</td>
<td>26.7 (5.41)</td>
<td>14.1 (1.77)</td>
</tr>
<tr>
<td>WASO (min)</td>
<td>90.9 (11.77)</td>
<td>81.8 (8.63)</td>
<td>57.2 (10.30)</td>
</tr>
<tr>
<td>Duration of Wake (min)</td>
<td>108.9 (11.84)</td>
<td>99.9 (9.14)</td>
<td>70.2 (10.24)</td>
</tr>
<tr>
<td>Duration of SWS (min)</td>
<td>54.0 (7.88)</td>
<td>45.5 (4.29)</td>
<td>50.6 (7.39)</td>
</tr>
<tr>
<td>Duration of NREM (min)</td>
<td>291.5 (10.57)</td>
<td>319.0 (8.89)</td>
<td>348.3 (10.07)</td>
</tr>
<tr>
<td>Duration of REM (min)</td>
<td>80.1 (5.97)</td>
<td>61.6 (6.85)</td>
<td>62.0 (6.85)</td>
</tr>
</tbody>
</table>

**NS** = Not significant, **plc** = placebo, **low** = lower dose of study medication, **high** = higher dose of study medication, **N** = number of subjects, **KSS** = Karolinska sleepiness scale, **GTS** = getting to sleep, **QOS** = quality of sleep, **BFS** = behaviour following sleep, **TST** = total sleep time, **SE** = sleep efficiency, **SOL** = sleep onset latency, **LPS** = latency to persistent sleep, **REM** = rapid eye movement, **NREM** = non rapid eye movement, **SW** = slow wave sleep, **WASO** = wake after sleep onset.
REFERENCES
REFERENCES


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APPENDICES
### APPENDIX 1: Overview of study schedule

<table>
<thead>
<tr>
<th>STUDY PROCEDURES</th>
<th>Pre-study</th>
<th>TREATMENT PERIOD</th>
<th>Post-study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Screening</td>
<td>1 D1-D8</td>
<td>2 D9-D22</td>
</tr>
<tr>
<td>Informed Consent</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leeds Assessment of Neuropathic Symptoms and Signs (LANSS)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mini Mental State Exam (MMSE)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short-Form 36 (SF-36)</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Physical examination</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrocardiogram (ECG)</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory tests</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical history/medications</td>
<td>X</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Alcohol Breathalyser</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Polysomnographic (PSG) recording</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Continuous Glucose Monitoring System (CGMS)</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Psychometric assessment</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Wrist Actigraphy</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Overview of study schedule.** Alcohol breathalyser was used on D6, D20 and D34 before transported to the Surrey CRC. CGMS was carried out while the patients were in the Surrey CRC from D6-D8 in treatment period 1, D20-D22 in period 2 and D34-36 in period 3. PSG recording was carried out night 6, 7, 20, 21 and 34, 35. Psychometric testing was carried out on D7 and D8 for treatment period 1, D21 and D22 for period 2 and D35 and D36 for period 3. Wrist actigraphy was attached at screening and used for the whole study period.
APPENDIX 2:

LEEDS SLEEP EVALUATION QUESTIONNAIRE

Subj. Init.: Datum Subj. No: 
Visit No: Datum Date: 

- How would you compare getting to sleep last night with getting to sleep normally, i.e. without study medication?

<table>
<thead>
<tr>
<th>Easier than usual</th>
<th>Harder than usual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quicker than usual</td>
<td>Slower than usual</td>
</tr>
<tr>
<td>Felt more drowsy than usual</td>
<td>Felt less drowsy than usual</td>
</tr>
</tbody>
</table>

- How would you compare the quality of last night’s sleep with your usual sleep?

<table>
<thead>
<tr>
<th>More restful than usual</th>
<th>Less restful than usual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fewer periods of wakefulness than usual</td>
<td>More periods of wakefulness than usual</td>
</tr>
</tbody>
</table>

- How did your awakening today compare with your usual pattern of awakening?

<table>
<thead>
<tr>
<th>Easier than usual</th>
<th>Harder than usual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Took shorter than usual</td>
<td>Took longer than usual</td>
</tr>
</tbody>
</table>

- How did you feel on waking?

<table>
<thead>
<tr>
<th>Alert</th>
<th>Tired</th>
</tr>
</thead>
</table>

- How was your sense of balance and co-ordination upon getting up?

| Less clumsy than usual | More clumsy than usual |
APPENDIX 3:  
Karolinska Sleepiness Scale

Please indicate your level of sleepiness for the previous 5 minutes using the scale below.

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very alert</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Alert – normal level</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Neither alert nor sleepy</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Sleepy, but no effort to keep awake</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td>Very sleepy, great effort to keep awake</td>
<td>9</td>
</tr>
</tbody>
</table>
APPENDIX 4:

Brief Pain Inventory (Short Form)

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these everyday kinds of pain today?

☐ Yes  ☐ No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.

3. Please rate your pain by marking the box beside the number that best describes your pain at its worst in the last 24 hours.

☐ 0  ☐ 1  ☐ 2  ☐ 3  ☐ 4  ☐ 5  ☐ 6  ☐ 7  ☐ 8  ☐ 9  ☐ 10

No Pain

Pain As Bad As You Can Imagine

4. Please rate your pain by marking the box beside the number that best describes your pain at its least in the last 24 hours.

☐ 0  ☐ 1  ☐ 2  ☐ 3  ☐ 4  ☐ 5  ☐ 6  ☐ 7  ☐ 8  ☐ 9  ☐ 10

No Pain

Pain As Bad As You Can Imagine

5. Please rate your pain by marking the box beside the number that best describes your pain on the average.

☐ 0  ☐ 1  ☐ 2  ☐ 3  ☐ 4  ☐ 5  ☐ 6  ☐ 7  ☐ 8  ☐ 9  ☐ 10

No Pain

Pain As Bad As You Can Imagine

6. Please rate your pain by marking the box beside the number that tells how much pain you have right now:

☐ 0  ☐ 1  ☐ 2  ☐ 3  ☐ 4  ☐ 5  ☐ 6  ☐ 7  ☐ 8  ☐ 9  ☐ 10

No Pain

Pain As Bad As You Can Imagine

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Pain Research Group
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7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please mark the box below the percentage that most shows how much relief you have received.

<table>
<thead>
<tr>
<th>0%</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
<th>70%</th>
<th>80%</th>
<th>90%</th>
<th>100%</th>
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</thead>
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<tr>
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</tbody>
</table>

No Relief

9. Mark the box beside the number that describes how, during the past 24 hours, pain has interfered with your:

A. General Activity

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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</table>

Does Not Interfere

B. Mood

<table>
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<tr>
<th>0</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</table>

Does Not Interfere

C. Walking ability

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<tr>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</table>

Does Not Interfere

D. Normal Work (includes both work outside the home and housework)

<table>
<thead>
<tr>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<th>7</th>
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</table>

Does Not Interfere

E. Relations with other people

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<tr>
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<th>3</th>
<th>4</th>
<th>5</th>
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Does Not Interfere

F. Sleep

<table>
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Does Not Interfere

G. Enjoyment of life

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<th>3</th>
<th>4</th>
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</tbody>
</table>

Does Not Interfere

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Pain Research Group
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APPENDIX 5: LINE ANALOGUE RATING SCALE

Remember, the marks you make are to show how you feel now compared to your usual feelings.

<table>
<thead>
<tr>
<th>More Competent</th>
<th>Less Competent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Less Anxious</th>
<th>More Anxious</th>
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<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Less Alert</th>
<th>More Alert</th>
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<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Less Drowsy</th>
<th>More Drowsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

<table>
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<tr>
<th>Less Relaxed</th>
<th>More Relaxed</th>
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<tbody>
<tr>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Less Clumsy</th>
<th>More Clumsy</th>
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<tr>
<td></td>
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<table>
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<tr>
<th>More Sad</th>
<th>Less Sad</th>
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<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>More Tired</th>
<th>Less Tired</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Less Dizzy</th>
<th>More Dizzy</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
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<table>
<thead>
<tr>
<th>Less Energetic</th>
<th>More Energetic</th>
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<table>
<thead>
<tr>
<th>More Depressed</th>
<th>Less Depressed</th>
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<td></td>
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<table>
<thead>
<tr>
<th>More Slow-witted</th>
<th>Less Slow-witted</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>More Light-headed</th>
<th>Less Light-headed</th>
</tr>
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<tr>
<td></td>
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</table>
## Polysomnographic assessments and definitions

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LIGHTOFF</strong></td>
<td>Lights off time Defined as the time (hh:mm:ss) when laboratory lights are switched off. Corresponds with the start of the analysis period</td>
</tr>
<tr>
<td><strong>LIGHTON</strong></td>
<td>Lights on time Defined as the time (hh:mm:ss) when laboratory lights are switched on. Corresponds with the end of the analysis period</td>
</tr>
<tr>
<td><strong>SOL2</strong></td>
<td>Sleep onset Latency (min) Defined as the time in minutes occurring from lights off to the first epoch of stage 2, 3, 4 or REM</td>
</tr>
<tr>
<td><strong>LPS</strong></td>
<td>Latency to persistent sleep (min) Defined as the time in minutes from lights off to the first consecutive 20 epochs of NREM or REM</td>
</tr>
<tr>
<td><strong>FINALAWK</strong></td>
<td>Final awakening Defined as the first epoch of wake which is not followed by any epoch of NREM or REM, or lights on whichever comes first</td>
</tr>
<tr>
<td><strong>TIB</strong></td>
<td>Time in bed (mins) Defined as the time in minutes from lights out to lights on</td>
</tr>
<tr>
<td><strong>TST</strong></td>
<td>Total sleep time (min) Defined as the time in minutes scored as NREM or REM but excluding epochs of MVT and Unscored within the period between lights off and lights on</td>
</tr>
<tr>
<td><strong>SPT</strong></td>
<td>Sleep Period Time (min) Defined as the total time in minutes scored as NREM, REM, WAKE or MVT occurring from SOL2 until lights on or the FINALAWK</td>
</tr>
<tr>
<td><strong>DUR0</strong></td>
<td>Stage 0 duration (min) Defined as the time in minutes scored as WAKE from lights off to lights on</td>
</tr>
<tr>
<td><strong>DUR1</strong></td>
<td>Stage 1 duration (min) Defined as the time in minutes scored as 1 from lights off to lights on</td>
</tr>
<tr>
<td><strong>PSPT1</strong></td>
<td>Percent of SPT for Stage 1 Defined as the percentage of epochs scored as 1 within the Sleep Period Time</td>
</tr>
<tr>
<td><strong>DUR2</strong></td>
<td>Stage 2 duration (min) Defined as the time in minutes scored as 2 from lights off to lights on</td>
</tr>
<tr>
<td><strong>PSPT2</strong></td>
<td>Percent of SPT for Stage 2 Defined as the percentage of epochs scored as 2 within the Sleep Period Time</td>
</tr>
<tr>
<td><strong>DUR3</strong></td>
<td>Stage 3 duration (min) Defined as the time in minutes scored as 3 from lights off to lights on</td>
</tr>
<tr>
<td><strong>PSPT3</strong></td>
<td>Percent of SPT for Stage 3 Defined as the percentage of epochs scored as 3 within the Sleep Period Time</td>
</tr>
<tr>
<td><strong>DUR4</strong></td>
<td>Stage 4 duration (min) Defined as the time in minutes scored as 4 from lights off to lights on</td>
</tr>
<tr>
<td><strong>PSPT4</strong></td>
<td>Percent of SPT for Stage 4 Defined as the percentage of epochs scored as 4 within the Sleep Period Time</td>
</tr>
<tr>
<td><strong>SWS</strong></td>
<td>Duration of Stage 3 + 4 sleep (min) Defined as the time in minutes scored as 3 or 4 from lights off to lights on</td>
</tr>
<tr>
<td><strong>PSPTSW</strong></td>
<td>Percent of SPT for Stage 3 + 4 sleep Defined as the percentage of epochs scored as 3 or 4 within the Sleep Period Time</td>
</tr>
<tr>
<td><strong>DUR5</strong></td>
<td>Duration of REM sleep (min) Defined as the time in minutes scored as REM from lights off to lights on</td>
</tr>
<tr>
<td><strong>PSPTREM</strong></td>
<td>Percent of SPT for REM sleep Defined as the percentage of epochs scored as REM within the Sleep Period Time</td>
</tr>
<tr>
<td><strong>DURNREM</strong></td>
<td>Duration of NREM sleep Defined as the time in minutes scored as NREM from lights off to lights on</td>
</tr>
<tr>
<td><strong>DURMVT</strong></td>
<td>Duration of MVT Defined as the time in minutes scored as MVT from lights off to lights on</td>
</tr>
<tr>
<td><strong>SEFF</strong></td>
<td>Sleep Efficiency Defined as the percentage of TST against (TST + total wake + total MVT)</td>
</tr>
<tr>
<td><strong>STAGEC</strong></td>
<td>Number of stage changes Defined as the number of stage transitions from SOL2 until lights on or FINALAWK</td>
</tr>
<tr>
<td><strong>TAWAKE</strong></td>
<td>Total Time Awake from SOL (min) Defined as the time in minutes scored as wake from SOL2 to lights on or FINALAWK</td>
</tr>
<tr>
<td><strong>NAW</strong></td>
<td>Number of night awakenings after LPS until lights on Defined as the number of blocks of consecutive epochs of wake from LPS until lights on</td>
</tr>
<tr>
<td><strong>NAWSP</strong></td>
<td>Number of awakenings after LPS until final Defined as the number of blocks of consecutive epochs of wake from LPS until FINALAWK</td>
</tr>
<tr>
<td><strong>Term</strong></td>
<td><strong>Definition</strong></td>
</tr>
<tr>
<td>----------</td>
<td>----------------</td>
</tr>
<tr>
<td>WASO</td>
<td>Wake after sleep onset (min) Defined as the time in minutes of epochs scored as wake from SOL2 until lights on</td>
</tr>
<tr>
<td>WASOSP</td>
<td>Total duration of awakenings after sleep onset until the final awakening Defined as the time in minutes of epochs scored as wake from SOL2 until FINALAWK</td>
</tr>
<tr>
<td>WAS</td>
<td>Wake after sleep Defined as the time in minutes from final awakening to lights on</td>
</tr>
<tr>
<td>S2LAT</td>
<td>Latency to stage 2 Defined as the time in minutes from lights out to the first epoch of 2</td>
</tr>
<tr>
<td>SWSLAT</td>
<td>Latency to SWS (min) Defined as the time in minutes from SOL2 to the first epoch of 3 or 4</td>
</tr>
<tr>
<td>SWS1</td>
<td>Duration of Stage 3 + 4 sleep during 1st 3rd of night (min) Defined as the time in minutes scored as 3 or 4 occurring within the 1st 3rd of the period from lights off to lights on</td>
</tr>
<tr>
<td>SWS2</td>
<td>Duration of Stage 3 + 4 sleep during 2nd 3rd of night (min) Defined as the time in minutes scored as 3 or 4 occurring within the 2nd 3rd of the period from lights off to lights on</td>
</tr>
<tr>
<td>SWS3</td>
<td>Duration of Stage 3 + 4 sleep during the final 3rd of night (min) Defined as the time in minutes scored as 3 or 4 occurring within the 2nd 3rd of the period from lights off to lights on</td>
</tr>
<tr>
<td>RLAT</td>
<td>REM Sleep Latency Defined as the time in minutes from SOL2 to the first epoch of REM</td>
</tr>
<tr>
<td>NCYCLES</td>
<td>Number of REM/non-REM cycles Defined as the number of REM cycles</td>
</tr>
<tr>
<td>NPERIODS</td>
<td>Number of REM periods Defined as the number of REM periods</td>
</tr>
<tr>
<td>DURCYC</td>
<td>Duration of REM/non-REM cycles Defined as the average time in minutes, of all of the REM cycles in the sleep period.</td>
</tr>
<tr>
<td>REMRATIO</td>
<td>REM/non-REM ratio Defined as the total duration of REM divided by the total duration of NREM from SOL2 to the FINALAWK or lights on</td>
</tr>
<tr>
<td>RLAT1,2,3,4,5,6</td>
<td>Latency to each REM cycle Defined as the time in minutes from SOL2 to the first epoch of each REM cycle</td>
</tr>
<tr>
<td>REM1</td>
<td>Duration of REM sleep during 1st 3rd of night (min) Defined as the time in minutes scored as REM occurring within the 1st 3rd of the period from lights off to lights on</td>
</tr>
<tr>
<td>REM2</td>
<td>Duration of REM sleep during 2nd 3rd of night (min) Defined as the time in minutes scored as REM occurring within the 2nd 3rd of the period from lights off to lights on</td>
</tr>
<tr>
<td>REM3</td>
<td>Duration of REM sleep during the final 3rd of night (min) Defined as the time in minutes scored as REM occurring within the final 3rd of the period from lights off to lights on</td>
</tr>
</tbody>
</table>