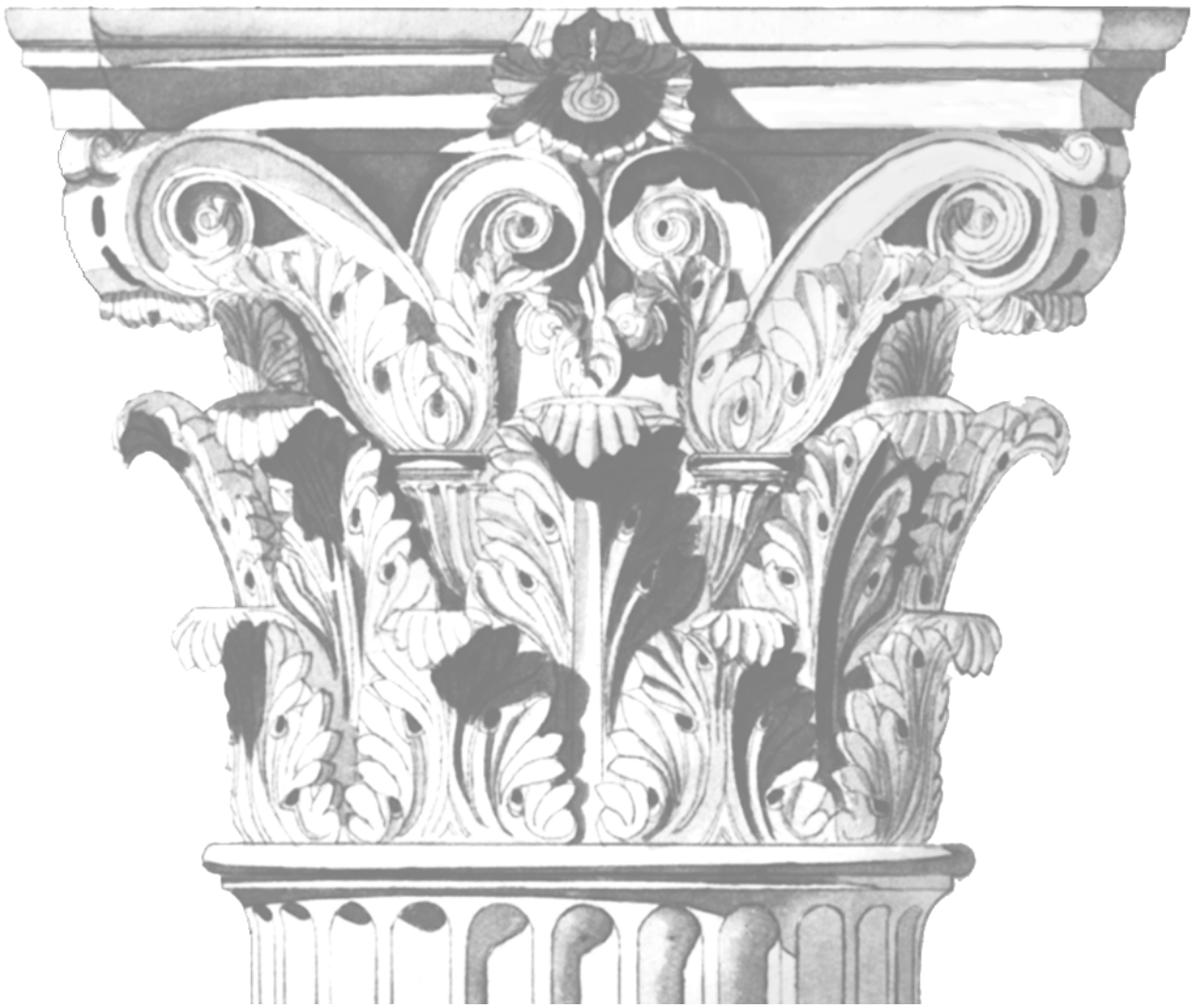
CARDIOVASCULAR AUTONOMIC DYSFUNCTION IN PARKINSONIAN SYNDROMES

MIKA KALLIO

Department of Clinical Neurophysiology,
University of Oulu
Department of Neurology,
University of Oulu

OULU 2001



MIKA KALLIO

CARDIOVASCULAR AUTONOMIC DYSFUNCTION IN PARKINSONIAN SYNDROMES

Academic Dissertation to be presented with the assent of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium 8 of the University Hospital of Oulu, on August 17th, 2001, at 12 noon.

OULUN YLIOPISTO, OULU 2001

Manuscript received 13 July 2001
Manuscript accepted 24 July 2001

Communicated by
Docent Joel Hasan
Professor Esko Länsimies

ISBN 951-42-6448-7 (URL: http://herkules.oulu.fi/isbn9514264487/)

ALSO AVAILABLE IN PRINTED FORMAT
ISBN 951-42-6447-9
ISSN 0355-3221 (URL: http://herkules.oulu.fi/issn03553221/)

OULU UNIVERSITY PRESS
OULU 2001

Kallio, Mika, Cardiovascular autonomic dysfunction in Parkinsonian syndromes
Department of Clinical Neurophysiology, University of Oulu, P.O.Box 5000, FIN-90014 University of Oulu, Finland, Department of Neurology, University of Oulu, P.O.Box 5000, FIN-90014 University of Oulu, Finland
2001
Oulu, Finland
(Manuscript received 13 July 2001)

Abstract

Autonomic nervous system (ANS) disturbances are common in Parkinson's disease (PD), but also in other Parkinsonian syndromes, especially in multiple system atrophy (MSA). The differentiation between various Parkinsonian syndromes may be difficult, but it is important for prognostic and therapeutic purposes. The aim of this study was to determine the ability of different analysis methods to reveal cardiovascular regulation disturbances in PD and to evaluate the diagnostic capacity of autonomic tests to differentiate between various Parkinsonian syndromes. Furthermore, this study aimed to evaluate the relationships between ANS disturbances and the clinical characteristics of PD. In addition, the cardiac autonomic function was evaluated during various sleep stages for the first time in untreated PD patients by using spectral heart rate variability (HRV) measures to determine possible sleep stage specific cardiovascular regulation disturbances.

Cardiovascular autonomic reflexes were evaluated in 62 untreated and newly diagnosed PD patients, 34 PD patients under antiparkinsonian medication, 47 MSA patients and 15 patients with progressive supranuclear palsy (PSP). The usefulness of different analysis methods was evaluated in a subgroup of 32 untreated PD patients. A further 21 untreated PD patients underwent one-night polysomnography for nocturnal heart rate variability analysis.

PD patients with hypokinesia/rigidity as their initial onset sign had a significantly lower max-min ratio in the deep breathing test than those patients with tremor as the initial sign. MSA patients showed significant reductions in both HRV and blood pressure responses during orthostatic provocation, whereas PSP patients had normal results. Absolute spectral measures yielded the clearest indicators separating the PD patients from the controls, while the cardiovascular reflexes proved more useful than the normalised spectral HRV measures in revealing the differences between the two groups. HRV was abnormally decreased during non-REM sleep in PD patients but not during REM sleep or the S1 sleep stage. The normalized high frequency power was significantly decreased in PD patients during sleep stages S2-4, while the standard deviation of the R-R intervals was increased during the same sleep stages, possibly corresponding to the increased motility of PD patients during these sleep stages.

The clinical characteristics of PD deserve particular attention in connection with ANS disturbances, since autonomic failure seems to be more pronounced in PD patients with hypokinesia/rigidity as their initial sign. The evaluation of the autonomic function may also be helpful in the differential diagnosis of Parkinsonian syndromes. Spectral analysis methods should be implemented in the evaluation of ANS dysfunction to achieve the best possible efficacy in the differentiation of pathological responses from normal ones. Nocturnal analysis of cardiovascular regulation revealed new and interesting features of pathologic HRV in PD patients, thus when HRV is evaluated, the different sleep stages should be analysed separately.

Keywords: heart rate variability, autonomic nervous system, Parkinsonian syndromes, sleep

To my Family

Acknowledgements

This work was carried out at the Department of Clinical Neurophysiology, Universities of Oulu and Göteborg, during the years 1995-2000.

I wish to express my deepest gratitude to Docent Uolevi Tolonen, M.D., Head of the Department of Clinical Neurophysiology at the beginning of this study, who kindly suggested the subject of this study to me, and who has been my teacher and supervisor in scientific and clinical work. I thank him for his excellent and tireless guidance, encouragement and patience throughout the years of the study.

I am deeply indebted to Professor Vilho Myllylä, M.D., Vice Rector of Oulu University and Docent Kyösti Sotaniemi, M.D., for their excellent instructive guidance and valuable criticism that was essential for the accomplishment of this thesis.

I am also deeply grateful to Professor Gunnar Wallin, M.D., Head of the Department of Clinical Neurophysiology at the University of Göteborg, for his support and co-operation during my visits in Göteborg. He kindly gave me the opportunity to work with him in his world-renowned laboratory, taught me the secrets of microneurography and guided me during my visits. I also wish to express my warmest gratitude to Associate Professor Mikael Elam, M.D., for providing me the possibility to participate in the ongoing autonomic nervous system research study.

I am very grateful to Kalervo Suominen, M.Sc., for his irreplaceable and invaluable technical and developmental guidance and help during these years.

I also wish to thank my co-workers Anna Bianchi, Ph.D., Vesa-Pekka Heikkilä, Ms.C., and Timo Mäkikallio, M.D., for providing me the specific heart rate variability analysis tools and my co-writers Tarja Haapaniemi, M.D. and Björn Holmberg, M.D., Jukka Turkka, M.D., and Sergei Astafiev, Ph.D. for their interest on our mutual work and for fruitful conversations.

I am also greatly indebted to Docent Joel Hasan, M.D., and Professor Esko Länsimies, M.D., the official reviewers of my thesis. Their scientific experience and constructive criticism was invaluable during the process of reviewing the thesis. I also wish to thank Ian Morris-Wilson, M.A., Ph.D., for his excellent revising and valuable advice regarding the contents of the text, which I truly appreciate.

I am also grateful to Professor Matti Hillbom, M.D., for providing some of the facilities for the study. Docent Ville Jäntti, M.D., the present Head of the Department of Clinical Neurophysiology is valued for his scientific enthusiasm.

I wish to thank the whole staff of the Department of Clinical Neurophysiology, especially Marja-Riitta Kauppila, R.N. and Raija Remes, R.N., who performed all the sleep polygraphy recordings either with or without me. I also wish to thank the whole staff of the Department of Neurology and all the patients and control subjects who made this thesis possible.

I also wish to thank my nearest colleagues Usko Huuskonen, M.D. and Seppo Rytky, M.D., for being my friend and creating a pleasant working atmosphere. I also thank my family and all my friends for their support during all these years.

Finally, I wish to express my warmest thanks to my wife Marjo, for understanding a workaholic husband during all the long evenings that I have been working. I also thank her for giving birth to our almost 10 months old son Niilo, the joy of my life.

This study was supported by the Finnish Neurological Foundation and Oulu University Foundation.

Oulu, July 2001 Mika Kallio

Abbreviations

ANS	autonomic nervous system
AR	autoregressive model
BM	body movement
BP	blood pressure
CAP	cyclic alternating pattern
CBD	corticobasal degeneration
CNS	central nervous system
ECG	electrocardiogram
EEG	electroencephalogram
EOG	electro-oculogram
EMG	electromyogram
FD	fractal dimension
FFT	fast Fourier transform
HF	high frequency
HR	heart rate
HRV	heart rate variability
LF	low frequency
MSA	multiple system atrophy
MSNA	muscle sympathetic nerve activity
NN50	interval differences of successive R-R intervals greater than 50 ms
PD	Parkinson’s disease
PSD	power spectral density
PSP	progressive supranuclear palsy
REM	rapid eye movement
RMSSD	square root of the mean squared differences of successive R-R intervals
RSA	respiratory sinus arrhythmia
SDNN	standard deviation of R-R intervals
SSNA	skin sympathetic nerve activity
SSR	sympathetic skin response
VLF	very low frequency

List of original publications

This thesis is based on the following publications, which are referred to in the text by their Roman numerals:

I Kallio M, Haapaniemi T, Turkka J, Suominen K, Tolonen U, Sotaniemi K, Heikkilä V-P, Myllylä V (2000) Heart rate variability in patients with untreated Parkinson's disease. Eur J Neurol 7: 667-72.

II Holmberg B, Kallio M, Johnels B, Elam M (2001) Cardiovascular reflex testing contributes to clinical evaluation and differential diagnosis of Parkinsonian syndromes. Mov Disord 16: 217-25.

III Kallio M, Suominen K, Bianchi AM, Mäkikallio T, Haapaniemi T, Astafiev S, Sotaniemi KA, Myllylä VV, Tolonen U. Comparison of heart rate variability analysis methods. A controlled study in patients with untreated Parkinson's disease. Submitted.

IV Kallio M, Suominen K, Haapaniemi T, Sotaniemi K, Myllylä V, Astafiev S, Tolonen U. Nocturnal cardiac autonomic regulation in Parkinson's disease. Submitted.

Contents

1 Introduction

Disorders of the autonomic nervous system (ANS) can be seen either as secondary to the diseases of the peripheral nervous system and to those of the central nervous system (CNS), or as a primary, selective disease involving only the ANS. In Parkinsonian syndromes, which include Parkinson's disease (PD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), the involvement of ANS failure has remained somewhat obscure even though it has significant implications for diagnosis, prognosis and management (Mathias 1997). In MSA the ANS failure has been reported to be a characteristic and more usual finding than in PD (Wenning *et al.* 1994, Klein *et al.* 1997), whereas in PSP cases both with and without the involvement of ANS failure have been seen (Van Dijk *et al.* 1991, Gutrecht 1992, Kimber *et al.* 2000). Despite the use of uniform clinical criteria (Quinn 1989, Hughes *et al.* 1992, 1993, Daniel & Lees 1993, Golbe & Davis 1993, Litvan *et al.* 1996, Gilman *et al.* 1999) the diagnosis is still unreliable, especially in early and atypical Parkinsonian patients (Litvan *et al.* 1997), where several neurological features may overlap (Lees 1987).

For the evaluation of cardiovascular autonomic control the current bedside golden standard is the study of cardiovascular reflexes (Ravits 1997, Myllylä *et al.* 2000), and it has been suggested that these reflexes will even reveal ANS dysfunction in untreated patients with early PD (Awerbuch & Sandyk 1992, Turkka *et al.* 1997). However, it is still unclear whether the disease itself or its treatment is the cause of ANS failure. The clinically most profound ANS disturbances have assumed to be linked with bilateral rigidity in PD patients (Marttila 1974); but this has not been confirmed by objective laboratory tests. The integrity of the ANS during sleep has scarcely been studied, mostly because of the demanding methodology involved. On the other hand the study of heart rate reactions to spontaneous body movements during sleep has confirmed that both parasympathetically and sympathetically mediated ANS failure is present in PD (Laihinen *et al.* 1987, Smirne *et al.* 1990, Ferini-Strambi *et al.* 1992, Ferini-Strambi & Smirne1997). However, spectral analysis of heart rate variability has not been utilised in nocturnal studies.

Further knowledge about cardiovascular autonomic dysfunction in Parkinsonian syndromes and its relations to clinical features and different physiological conditions such as sleep stages may further improve the diagnostic and therapeutic accuracy in individual cases of Parkinsonian syndromes.

2 Review of the literature

2.1 General aspects of the autonomic nervous system

An English physiologist Langley was the first to introduce the term autonomic nervous system in 1898 when he discovered the nerves now known as the parasympathetic division of the ANS. Later the ANS has been divided anatomically and functionally into two main branches that are the parasympathetic and the sympathetic divisions. The main role of this involuntary system is to maintain bodily homeostasis and to adapt the essential visceral functions to the changing endogenous or environmental circumstances.

The parasympathetic and sympathetic divisions of the ANS constantly cooperate, either facilitating or inhibiting various organ functions including almost exclusively the cardiovascular, gastrointestinal, urinary, sexual and thermal functions, and partially also the respiratory, renal and endocrine functions. The parasympathetic and sympathetic divisions are further divided into the pre- and postganglionic tracts. The parasympathetic preganglionic fibres originate in the brainstem and in the second, third and fourth sacral segments of the spinal cord, leave the CNS via the cranial and sacral spinal nerves, and synapse with the postganglionic neurons in the parasympathetic ganglia that are situated near the end organ. The most widespread cranial parasympathetic output is carried through the vagus nerve, which originates from the dorsal vagal nucleus and the nucleus ambiguus in the medulla (Loewy & Spyer 1990). The sympathetic preganglionic fibres originate in the intermediolateral columns of the thoracolumbar spinal cord and project to the paravertebral sympathetic ganglia where they synapse with the postganglionic neurons. The chemical transmitter in all preganglionic and postganglionic parasympathetic neurons is acetylcholine. Sympathetic postganglionic fibres are noradrenergic, except those cholinergic fibers that innervate sweat glands and some cholinergic vasodilator fibers that innervate muscles (Willis 1993).

Emotional situations such as passion, hate and fear are accompanied by alterations of the heart rate and blood pressure, and negative emotional states like anger and chronic stress have been associated with the occurrence of cardiac diseases such as sudden death, arrhythmias, and myocardial infarctions. Hence, it is evident that the CNS controls

autonomic functions, and CNS damage may affect the function of the ANS. There are three main CNS structures that control the ANS functions: the frontal lobe cortex, the limbic lobe and the amygdala, and the hypothalamus (Benarroch 1993, 1997). The frontal lobe cortex appears to be the highest level of integration of ANS functions while the limbic system, which is critical for emotional and affective expressions, controls a large variety of visceral functions. The hypothalamus is the most important regulator of autonomic functions and its primary role is to serve as an integrating and modifying mechanism between the cortical autonomic structures and the preganglionic autonomic neurons. Although the hypothalamus exerts major overall control of the ANS, many autonomic functions do not require continuous monitoring, and, for instance, transection of the brainstem above the pons leaves regulation of the cardiovascular and respiratory functions virtually intact.

2.1.1 Cardiovascular autonomic regulation

Fluctuations in heart rate (HR) and blood pressure (BP) reflect the dynamic response of the cardiovascular control system to physiological changes. The HR is controlled by membrane processes of the sinoatrial node, which is modulated by innervations from both the parasympathetic and sympathetic divisions of the ANS. The heart receives extrinsic efferent (sympathetic and parasympathetic) and afferent innervations, and possesses intracardiac nerve supplies (Benarroch 1997, Crick *et al.* 2000). This intracardiac intrinsic nervous system interacts with efferent nerve fibres in a complex manner to help maintain adequate cardiac output. Intrinsic neurones also receive afferent inputs from mechanosensory and chemosensory nerve endings located in cardiovascular and pulmonary tissues, and afferent fibers from centrally located neurons (Crick *et al.* 2000). Noradrenaline and acetylcholine are the predominant neurotransmitters utilized by the heart, although many other types of neurotransmitters or neuromodulators have been localised in cardiac nerves (Burnstock 1986).

The main periodic fluctuations are respiratory sinus arrhythmia (RSA), baroreflex-related and thermoregulation related variability of the HR. Perhaps the most conspicuous fluctuation is the effect of respiration on HR which is the RSA, seen as oscillations in the R-R intervals in the frequency range of 0.15-0.4 Hz, which are generally believed to be mediated predominantly by fluctuations of vagal-cardiac nerve traffic and thus reflect vagal activity (Wheeler & Watkins 1973, Katona & Jih 1975, Eckberg 1983). The HR accelerates during inspiration and decelerates during expiration, and the magnitude of this response depends on the rate and the depth of the respiration (Hirsch & Bishop 1981). The inspiratory inhibition is evoked primarily by central impulses from the medullary respiratory center to the cardiovascular center (Akselrod *et al.* 1985, Richter & Spyer 1990). Peripheral reflexes due to hemodynamic changes and thoracic stretch receptors contribute to RSA (Akselrod *et al.* 1985). Although RSA can be abolished by atropine or vagotomy (Akselrod *et al.* 1985, McCabe *et al.* 1985), RSA has been shown to be a somewhat imperfect index of vagal activity and the sympatho-vagal balance in HR variability (HRV) analysis can also be questioned (Kollai & Mizsei 1990, Eckberg 1997).

Furthermore, it has been shown that arterial BP variations significantly influence the amplitude of HR variations in the deep breathing test (Diehl *et al.* 1997)

Slower fluctuations in the HR than in the RSA occur at around 0.1 Hz, and this fluctuation in the HR originates from self-oscillation in the vasomotor part of the baroreflex loop as a result of negative feedback in the baroreflex (Madwed *et al.* 1989) and is accompanied by synchronous fluctuations in the blood pressure known as Meyer waves. This fluctuation decreases with both parasympathetic and sympathetic blockade (Akselrod *et al.* 1985, Pomeranz *et al.* 1985). Yet slower fluctuations are thought to arise from thermoregulatory peripheral blood flow adjustments and are mediated by the sympathetic nervous system (Rosenbaum & Face 1968, Lindqvist *et al.* 1989).

The neural regulation of the circulatory function operates mainly through the interplay of the sympathetic and vagal outflows. In most physiological conditions, the activation of either one of these outflows is accompanied by the inhibition of the other. The sympatho-vagal balance is tonically and phasically modulated by the interaction of at least three major factors: central neural integration, peripheral inhibitory reflex mechanisms (with negative feedback characteristics), and peripheral excitatory reflex mechanisms (with positive feedback characteristics).

The insular and medial prefrontal cortical areas and the extended amygdala (the central nucleus of the amygdala and the nucleus of the stria terminalis) are involved in high-order processing of autonomic control (Benarroch 1993). Reciprocal interactions between all components of the central autonomic system allow continuous feedback interactions and integration of autonomic responses, and central autonomic control depends on the activity of several parallel pathways. Transmission of information within the central autonomic network involves several neurotransmitters, including amino acids, acetylcholine, monoamines, and neuropeptides. Amino acids mediate rapid, point-to-point communication through ion channel receptors. Acetylcholine, monoamines and neuropeptides mediate slower modulatory influences mainly through G-protein (guanosine triphosphate-binding protein) coupled receptors (Benarroch 1993, 1997).

Afferent fibers from mechanosensitive and/or chemosensitive endings respond to a variety of physical and/or chemical changes, respectively, via a number of different cardiac reflexes (Smith & Thames 1994). Viscerosensory inputs from baroreceptors, cardiac receptors, pulmonary receptors and carotid-aortic chemoreceptors are carried by the glossopharyngeal and vagus nerves and relay to the nucleus tractus solitarius at the brainstem (Loewy 1990). Humoral inputs may be either direct or through specialized receptor structures terminating in the circumventricular organs (Ferguson 1992).

The best-known negative feedback mechanism is the baroreceptor reflex. The baroreceptors, which are mainly located in the carotid sinuses, are sensitive to pressure and are referred to as high-pressure receptors. Afferent fibres join the vagus and glossopharyngeal nerves and connect the cardiovascular centers of the medulla oblongata. Increased arterial pressure increases the firing frequency of the afferent impulses to the CNS, causing a tonic suppression of the cardiovascular sympathetic drive and an increase in the parasympathetic vagal drive, which results in a decrease in the HR and in peripheral resistance. An initial decrease in blood pressure has opposite effects.

2.1.2 Cardiovascular autonomic regulation during sleep

Tonic, reflex, and integrative control of autonomic functions depends on the physiological and behavioural state of the person. Thus it can be affected by respiration, the sleep wake cycle, emotional state, attention, and several other factors. During sleep dramatic changes occur in the autonomic function although not so long ago, sleep was generally viewed as a passive process. Recognition of a new phase of sleep, i.e. rapid eye movement sleep, or REM sleep, gave rise in the 1950's (Aserinsky & Kleitman, 1953) to the concept of sleep's dynamic state of consciousness.

Overall, sleep is considered a condition in which vagal activity is high and sympathetic activity is relatively peaceful (Parmeggiani 1994). During non-REM sleep, parasympathetic activity and autonomic stability are increased, whereas during REM sleep, the tonic decrease in sympathetic activity is interrupted by irregular episodes of sympathetic activation and parasympathetic deactivation (Mancia 1993, Parmeggiani 1994).

During the four stages of non-REM sleep (S1-4), which can be distinguished by the appearance of sleep spindles, K-complexes and by the amount of delta activity in electroencephalogram (EEG), respiration and the HR are markedly regular when compared to other states of vigilance, and this regularity increases as the stage of the non-REM sleep deepens. The spectral high frequency (HF) component of the HRV increases as the low frequency (LF) and the LF:HF-ratio decreases towards the deepest stages of non-REM sleep (Lisenby *et al.* 1976, Baharav *et al.* 1995, Vaughn *et al.* 1995, Bonnet & Arand 1997, Scholz *et al.* 1997). However, during the last 15 minutes of non-REM sleep, prior to the onset of REM sleep, an increase in the LF:HF-ratio can be seen even before any other sign of the incipient sleep stage change (Scholz *et al.* 1997). Spontaneous sympathetic skin responses (SSR) are at their highest during non-REM sleep, but the evoked SSR can only be induced during wakefulness or arousal from light sleep (Liguori *et al.* 2000). The muscle sympathetic nerve activity (MSNA) decreases with increasing depth of non-REM sleep, but K-complexes are associated with short-lasting increases of MSNA (Hornyak *et al.* 1991) and the burst latency shortens (Xie *et al.* 1999). These results suggest that frequent arousals, which can be caused by e.g. periodic leg movements or disordered breathing, may augment the magnitude of arterial pressure perturbations during non-REM sleep.

REM sleep is characterised by paralysis of skeletal muscles, rapid eye movements, irregular respiration and heart rate, impaired homeostatic regulation and activation of the limbic structures (Braun *et al.* 1997). The EEG becomes desynchronised and resembles that of early drowsiness or S1. The changes seen in the HRV are opposite to those of non-REM sleep, i.e. the HF component decreases and the LF increases with the LF:HF-ratio. Spontaneous SSR decline markedly during REM sleep, and evoking of the SSR is unsatisfactory (Liguori *et al.* 2000). During REM sleep a clear increase of MSNA can be seen and this increase occurs mainly in short irregular periods which are often but not always related to rapid eye movements. An inverse relationship between the duration of REM sleep and the increase of total MSNA can also be found, which may, at least in part, be related to the finding that the longer the REM sleep period the shorter the relative duration of rapid eye movements (Hornyak *et al.* 1991). Skin sympathetic nerve activity

(SSNA) is also increased during REM sleep, but not during non-REM stages (Noll *et al.* 1994). The simultaneous recording of MSNA and SSNA during non-REM sleep has shown that both are centrally suppressed during light sleep. MSNA and/or SSNA frequently accompany spontaneous and arousal stimuli-induced K-complexes, and burst properties of MSNA become similar to those of SSNA during light sleep, thus suggesting that MSNA and SSNA may share a common origin in the central nervous system (Takeuchi *et al.* 1994).

The cyclic alternating pattern (CAP) consists of periodic or cyclic alterations in two different EEG activity levels during sleep, which is related to a fluctuation of vigilance between these two levels and represents different morphology and responsiveness to stimulation (Terzano *et al.* 1985). Recently it has been shown that CAP events corresponding to transient arousals are related to significant increases in LF power and in the LF:HF-ratio (Ferini-Strambi *et al.* 2000), thus shifting the balance of cardiac autonomic regulation towards a sympathetic predominance. This CAP may bias studies on nocturnal HRV in which the microstructure of sleep has not been considered.

2.2 General aspects of Parkinsonian syndromes

James Parkinson (1817) was the first to describe six patients with a disease characterised by tremor and slowness of movements, or the shaking palsy, today known as Parkinson's disease. His description of the disease was very thorough, and the diagnosis of PD still rests on clinical findings. He noted that the clinical manifestations of PD are not restricted to the classic triad of akinesia, rigidity and tremor, but also include ANS disorders. Of these, the most common are sialorrhea, seborrhea, hyperhidrosis, constipation, sphincter disturbances, dysphagia, postural hypotension and other vasomotor abnormalities, heat intolerance and impotence (Appenzeller & Goss 1971, Gross *et al.* 1972, Turkka 1986).

After the role of dopamine as a neural transmitter with important motor functions had been described (Carlsson *et al.* 1957), and once it was observed that neuronal damage in the basal ganglia leads to abnormal low concentrations of dopamine (Ehringer & Hornykiewitz 1960), oral levodopa treatment could be developed to help PD patients (Cotzias *et al.* 1967). It was soon noted that some of the patients responded well to levodopa treatment while others did not. Patients developing additional symptoms not usually seen in PD were soon recognised a special group and called Parkinson-plus syndromes, and now includes multiple system atrophy, progressive supranuclear palsy and corticobasal degeneration. These patients have a poor response to levodopa treatment and develop a more widespread degeneration of the nervous system (Gibb & Lees 1988), and present symptoms such as pyramidal signs, early cerebellar symptoms, dementia or early dysautonomic features that are not normally seen in PD. The differential diagnosis between these various diseases may be difficult, especially in the early course of the diseases and in patients with atypical signs (Hughes *et al.* 1992, Litvan *et al.* 1996).

2.2.1 Parkinson's disease

PD comprises about 75% of all Parkinsonian syndromes (Jellinger 1987), and the definition of PD rests on the typical clinical picture combined with a clear pathology. Tremor is the most common initial symptom in PD; bradykinesia and rigidity are also early clinical presentations albeit less specifically. A good and consistent response to levodopa therapy is crucial for the diagnosis; however, typical fluctuations in the clinical state usually develop as the disease progresses.

Neuronal loss in the substantia nigra and neuronal inclusion bodies (Lewy bodies) are typical neuropathological findings, but neuronal damage and/or Lewy bodies are also seen in the cortex, in the hypothalamus and in other brainstem cells as well as in the paravertebral sympathetic ganglia (Jellinger 1991). In addition to the degeneration of the dopaminergic system, PD involves the noradrenergic, serotonergic and cholinergic systems and also affects the central autonomic network and peripheral parts of the ANS (Jellinger 1991, Lowe *et al.* 1997).

2.2.2 Multiple system atrophy

MSA is defined as a sporadic progressive adult onset disorder characterised by autonomic dysfunction, parkinsonism and ataxia in any combination (Gilman *et al.* 1999). It comprises about 10% of Parkinsonian syndromes and it is the most common differential diagnosis for PD. Clinically the differential diagnosis between MSA and PD can be difficult, but there are certain features such as marked orthostatic hypotension, levodopa unresponsiveness, and pyramidal and/or cerebellar signs that should make the diagnosis of PD dubious. Cold hands may also raise the suspicion of MSA.

Typical neuropathological findings are specific gliocytoplasmatic bodies (Papp & Lantos 1994), gliosis and nerve cell loss in the putamen, substantia nigra, basis pontis, inferior olives, cerebellar folia, in the intermediolateral column of the spinal cord and in Onuf's nucleus. The locus coeruleus, dorsal vagal nucleus, pyramidal tract and anterior horn cells are also affected by the disease (Daniel 1999, Wenning *et al.* 1997).

2.2.3 Progressive supranuclear palsy

It has been proposed that about 4% of all patients presenting Parkinsonism have PSP. PSP should be suspected when the patient has supranuclear gaze palsy and postural instability with early falling (Golbe *et al.* 1993, Litvan *et al.* 1996). The bradykinesia is bilateral and the benefit of levodopa is usually small and declines gradually further. Men are more affected and tend to have a worse prognosis. Although PSP is usually considered to be a sporadic disorder, it has been suggested that its cause may be hereditary (Yebenes *et al.* 1995, Bennett *et al.* 1998).

The characteristic microscopic pathology has been reported as consisting of neurofibrillatory tangles, neurophil threads and neuronal loss in the globus pallidus, the

subthalamic nucleus, the substantia nigra, the superior colliculus, the periaquaductal grey, the pretectal areas, the brainstem and the medulla (Hauw *et al.* 1994, Lantos 1994). Presynaptic damage to the dopaminergic and cholinergic systems has been reported (Young 1985, Brooks 1993, Ruberg *et al.* 1993).

2.3 Autonomic dysfunction in Parkinsonian syndromes

Autonomic dysfunction in PD is not rare, and the prevalence is especially high in patients with bilateral severe bradykinesia and rigidity (Spiegel *et al.* 1969, Marttila 1974, Haapaniemi *et al.* 2000). However, clinical autonomic failure as an early or severe sign in patients with Parkinsonism is usually an indication for a different diagnosis than PD, namely MSA (Wenning *et al.* 2000). On the other hand, excessive sweating, constipation, bladder and sexual dysfunction, postural dizziness, sleep and breathing disturbances are common in PD patients (Turkka *et al.* 1987, Niimi *et al.* 1999, Haapaniemi *et al.* 2000).

Using standard cardiovascular reflex tests parasympathetically mediated but also sympathetically mediated autonomic dysfunction has been reported in PD. HR responses to normal and controlled deep breathing, the Valsalva manoeuvre and tilting have been reported as being diminished in PD patients (Appenzeller & Goss 1971, Sachs *et al.* 1985, Camerlingo *et al.* 1986, Goetz *et al.* 1986, Turkka *et al.* 1987, 1997, Piha *et al.* 1988, van Dijk *et al.* 1993, Haapaniemi *et al.* 2000). The BP responses to tilting and isometric work have also been reported as being pathological (Gross *et al.* 1972, Turkka *et al.* 1987, Van Dijk *et al.* 1991, Turkka *et al.* 1997). However, some authors (Ludin *et al.* 1987) have not been able to replicate pathological cardiovascular autonomic reflexes, and it has been proposed that age could explain most instances of HRV (van Dijk *et al.* 1993).

The recent use of 24-hour ECG recordings has revealed diminished spectral power values (Mastrocola *et al.* 1999). In this study the daytime and the nighttime periods were evaluated separately and the HF absolute spectral power was diminished only during the night. However, the HRV was not studied separately in relation to sleep stages. Other studies on HR reactions caused by spontaneous sleep movements have revealed disturbed HR reactions in PD patients both during non-REM and REM sleep, reflecting both parasympathetically and sympathetically mediated defective cardiac autonomic modulation (Laihinen *et al.* 1987, Smirne *et al.* 1990, Ferini-Strambi *et al.* 1992, Ferini-Strambi & Smirne1997).

The levels of serum noradrenalin and its main metabolite in the spinal fluid have been found to be low in PD patients when measured in responses to standing up (Turkka 1986, Turkka *et al.* 1987, Koike & Takahashi 1997). The use of meta-[(123) I] iodobenzylguanidine (MIBG) myocardial scintigraphy has demonstrated a reduced MIBG uptake in the majority of PD patients, and even in the early stage of the disease, indicating cardiac sympathetic nerve disturbances (Hakusui *et al.* 1994, Orimo *et al.* 1999). Parkinsonian syndromes other than PD do not demonstrate a significant reduction in MIBG uptake in any organs, except for the lower legs in MSA (Taki *et al.* 2000).

Autonomic dysfunction is a characteristic and early feature in MSA. It may be present as e.g. breathing disturbances, both during the day as involuntary gasps and at night as

sleep apnoeas, or as bladder and sexual dysfunctions (Wenning *et al.* 1994, Bannister & Mathias 1999). HR responses to the cardiovascular reflex test have been reported as being more diminished in MSA than in idiopathic PD (Bordet *et al.* 1996, Kimber *et al.* 2000). In a recent study cardiovascular autonomic dysfunction has been proposed as an exclusionary feature in the diagnosis of PSP (Kimber *et al.* 2000).

2.4 Methods for assessing autonomic functions

Various methods of analysing HR and BP variability have been increasingly used for the evaluation of the autonomic function during the last few years (Ravits 1997). The parasympathetic and sympathetic neural mechanisms that control the cardiovascular system generate different rhythms in HRV and arterial BP (Omboni *et al.* 1996) and the measurement of HRV has become the most widely used indirect measure of vagal nerve function (Ravits 1997). Microneurography is widely accepted as a golden standard in measuring muscle and skin sympathetic nerve traffic (Wallin & Elam 1997), but this laborious technique is not easily applicable for clinical studies. The bedside golden standard for assessing the cardiovascular autonomic function today is the use of cardiovascular reflex tests (Ravits 1997, Myllylä *et al.* 2000). Other tests are the thermoregulatory sweating test, the quantitative sudomotor axon reflex test, the SSR, and the laser Doppler flowmetry of skin vasomotor reflexes. Recently the evaluation of cardiovascular autonomic control has been assessed during different sleep stages by utilising either short-term HR changes to body movements or HRV analysis (Zemaityte *et al.* 1984, Franceschi *et al.* 1986, Scholz *et al.* 1993).

2.4.1 Cardiovascular autonomic reflex tests

Standard cardiovascular autonomic reflex tests rely on HR and BP changes to certain stimuli. The usual test package comprises evaluation both of respiratory sinus arrhythmia during normal and paced breathing, during the Valsalva manoeuvre and tilting, and of the BP changes to tilting and isometric work under standardized environmental conditions (Piha 1988, 1993, Ewing 1992, Wieling & Karemaker 1999). HR reactions are commonly accepted as indicating predominantly parasympathetically mediated regulation, whereas BP changes are thought to reflect sympathetically mediated regulation. However, these boundaries are not watertight since e.g. it has been shown that both parasympathetic and sympathetic hypofunction can alter HR variation in the deep breathing test and the Valsalva manoeuvre (Diehl *et al.* 1997, Freeman 1997).

HR reactions have been shown to be age and baseline HR dependent, and the BP response to isometric work depends on gender (Vargas & Lye 1980, Vita *et al.* 1986, Piha 1988). Therefore, HR responses must be adjusted for mean the age and mean baseline HR, and the BP values must be analysed separately for both genders in isometric work test.

It has been proposed that only the presence of two or more abnormal test results (i.e. outside the 95% percentile of the control subjects) indicates definite ANS involvement

(Ewing 1992). This “Ewing protocol” has lately been criticized (Wieling & Karemaker 1999) and e.g. a marked orthostatic hypotonia alone is now accepted as fulfilling the criteria for dysautonomia (Consensus statement 1996).

Cardiovascular reflex tests have proven useful in detecting ANS regulation failure in numerous pathological conditions such as peripheral neuropathies due to e.g. diabetes (Bennet *et al.* 1977, Shahani *et al.* 1990, Ewing 1992), alcoholism (Johnson & Robinson 1988), uraemia (Solders *et al.* 1985, Wang *et al.* 1994) and Guillain-Barré syndrome (Tuck & McLeod 1981). Abnormal cardiovascular reflexes have also been found in neurological diseases such as MSA and pure autonomic failure (Cohen *et al.* 1987, Ravits *et al.* 1995), PD (Sachs *et al.* 1985, Turkka *et al.* 1987, Piha *et al.* 1988, Van Dijk *et al.* 1993, Turkka *et al.* 1997), amyotrophic lateral sclerosis (Pisano *et al.* 1995, Linden *et al.* 1998), migraine (Havanka-Kanniainen *et al.* 1988), stroke (Korpelainen *et al.* 1994) and epilepsy (Isojärvi *et al.* 1998).

2.4.2 Heart rate variability analysis methods

Variable phenomena can be described not only as a function of time, but as the sum of elementary oscillatory components, defined by their frequency and amplitude. Stationary and sufficient amount of data are requirements for spectral analysis, especially for nonparametric methods such as fast Fourier transform (FFT), in order to achieve reliable results. For non-stationary situations, such as HR and other physiological phenomena, various methods are available for characterising the signal and its dynamics (Bianchi *et al.* 1990, Huikuri 1995, Task Force 1996, Berntson *et al.* 1997, Hoyer *et al.* 1998). Usual HRV analysis measures are based on time domain, frequency domain and non-linear methods.

HRV reflects fluctuations rather than absolute levels of autonomic tone. The respiratory rhythm of HRV is considered to reflect vagal modulation. Absolute but not normalised (i.e. as percentages of the total power minus very low frequency power component) respiratory frequency spectral power correlates significantly with the R-R interval shortening after a large dose of atropine, the current gold standard for vagal tone (Hayano *et al.* 1991). The rhythm corresponding to vasomotor waves and present in HR and arterial pressure variability is considered to be a marker of both sympathetic and vagal modulation. However, neither absolute nor normalised spectral power near 0.1 Hz correlates significantly with MSNA (Saul *et al.* 1990) or with cardiac noradrenalin spill-over (Kingwell *et al.* 1994). A reciprocal relation exists between these two rhythms that is considered similar to that characterising the sympathovagal balance.

The time window for HRV analysis may be either short (some minutes) or long (several hours) and both time windows have their advantages and disadvantages. Short-term recordings may fail to detect some very low frequency oscillations, while long-term recordings are affected by alternating environmental conditions that may confound the results of HRV analysis.

Time and frequency domain HRV analysis methods have proved useful in evaluating tonic autonomic effects in both health and disease such as diabetes, coronary artery disease and stroke (Bianchi *et al.* 1990, Huikuri 1995, Korpelainen *et al.* 1996, 1999).

2.4.2.1 Time domain measures of HRV

Time domain measures of HRV are based on either statistical or geometrical analyses of the HR or the intervals between successive normal complexes (Task Force 1996). The square root of variance, or the standard deviation of R-R intervals (SDNN), is the simplest variable for calculating the statistical time domain measure. The SDNN reflects all the cyclic components responsible for variability in the period of recording, and the durations of the recordings used to determine the SDNN should be standardised, as the SDNN depends on the period length. The square root of the mean squared differences of successive R-R intervals (RMSSD), and the NN50, i.e. the number of interval differences of successive R-R intervals greater than 50 ms (Ewing *et al.* 1984), and the proportion of it (pNN50), are the most commonly used measures derived from R-R interval differences, and are estimates of HF variations of the HR (Task Force 1996, Berntson *et al.* 1997).

Geometrical methods present R-R intervals in geometrical patterns and various methods exist to derive HRV measures from these patterns. One approach is to quantitatively analyse the Poincaré plots (Huikuri *et al.* 1996, Tulppo *et al.* 1996), which are scattergrams plotting each R-R interval as a function of the previous R-R interval. Utilising this method the instantaneous beat-to-beat and continuous long-term variability of R-R intervals can be determined. The shape of the plot can also be classified into several pattern-based categories, i.e. the qualitative analysis of Poincaré plots (Woo *et al.* 1994). Another geometrical method is e.g. the triangular index, where the geometric pattern is interpolated by a mathematically defined shape (Malik *et al.* 1989, Farrell *et al.* 1991).

2.4.2.2 Frequency domain measures of HRV

Various spectral methods have been used in the analysis of the HRV. Power spectral density (PSD) analysis provides the basic information about how variance distributes as a function of frequency, though only an estimate can be obtained by proper mathematical algorithms (Task Force 1996). The methods for the calculation of the PSD are generally classified as nonparametric (e.g. FFT) and parametric (e.g. the autoregressive model approach (AR)). Nonparametric methods have the advantages of having simple algorithms and a high processing speed, whereas the advantages of parametric methods are smoother spectral components, easy post-processing and an accurate estimation of the PSD even on a small number of samples. The basic disadvantage of the parametric method is the need for verification of the suitability of the chosen model, and variations of this model order within or between subjects may make quantitative comparisons of results invalid.

The power spectrum is commonly divided into three or four frequency bands which are: ultra low frequency (ULF) <0.0033 Hz, very low frequency (VLF) 0.0033-0.04 Hz, LF 0.04-0.15 Hz and HF 0.15-0.40 Hz (Task Force 1996). The power of the different components along with the total power is expressed as absolute units (ms^2). LF and HF components can also be expressed as normalised units and the LF:HF-ratio is also commonly used in order to present the controlled and balanced behaviour of the two

branches of the ANS. Both normalised and absolute units should be presented in order to describe the distribution of power in spectral components completely (Task Force 1996).

2.4.2.3 Non-linear measures of HRV

Non-linear methods related to chaos tend to deal with autonomous systems, i.e. systems where there is no input or where the input has a very simple form (Hoyer *et al.* 1998). It has been suggested that a healthy heart rhythm is chaotic and shows a fractal form that may be broken down by a disease (Goldberger *et al.* 1990, Goldberger 1996). However, it has been reported that the most essential parts of the deterministic-chaotic properties of HRV may be vagally mediated (Zwiener *et al.* 1996). The present non-linear methods for evaluating HRV are far less developed and more complex than the linear methods and there are many problems in their theoretical foundations and their practical applications. Most importantly, the non-linear parameters describe long-term regulation mechanisms, and a reliable estimation of non-linear measures requires long data sets, longer than the few minutes necessary for the time or frequency domain measures.

The parameters that have been used to measure non-linear properties of HRV include, to name a few, the fractal dimension, 1/f scaling of power spectra, detrended fluctuation analysis, Kolmogorov entropy and approximate entropy. These methods have detected abnormal HRV in various cardiovascular conditions such as coronary artery disease with or without previous myocardial infarction (Bigger *et al.* 1996, Mäkikallio *et al.* 1996, 1997, 1998). 1/f power scaling has been shown to be a more powerful predictor of survival than traditional markers both in healthy elderly and in patients with impaired left ventricular dysfunction (Brouwer *et al.* 1996, Huikuri *et al.* 1998). In the future more advanced approaches, such as isofunctional modelling and surrogate data analysis as an example of bivariate data analyses, as well as bicoherence, may reveal new insights into HRV analysis (Hoyer *et al.* 1998).

2.4.3 Assessment of nocturnal autonomic functions

Polysomnographic studies have demonstrated that sleep is a complex highly organised physiological state which is composed of two distinct states: REM and non-REM sleep. These two states are further divided into tonic and phasic REM stages, and S1-S4 non-REM sleep stages. The simultaneous and continuous recording of the EEG, electro-oculogram (EOG) and electromyogram (EMG) is needed to distinguish the different sleep stages from each other (Rechtschaffen & Kales 1968).

Several approaches for assessing nocturnal autonomic functions in relation to different sleep stages have been presented. HR, BP, respiration, pupillometry, temperature, electrodermal changes and microneurographical measurements among others have been utilised in many species to investigate the autonomic control in relation to the sleep stages (Parmeggiani 1994). In man, the most common methods used are the measurement of the HR and the measurement of HRV.

2.4.3.1 HR reactions to nocturnal spontaneous body movements

The normal motor behaviour during sleep consists of many kinds of movements: rapid eye movements are often accompanied by phasic muscle twitches, typically involving peripheral muscles. Muscle activity is also seen during non-REM sleep, and consists of e.g. sudden jerks often associated with falling asleep, arousals and K-komplexes, or larger movements with sleep transitions (Oswald 1959, Muzet *et al.* 1972).

Sleep movements are often associated with a small increase or decrease in the HR (Townsend *et al.* 1975, Alihanka 1982). The onset of body movement is followed by HR acceleration, and at the end of the movement the HR decelerates, often remaining decreased for 10-60 s (Alihanka 1982). The measure need to describe the HR reaction to body movements is the difference between the preceding mean R-R interval and the minimum R-R interval during body movement (Alihanka 1982).

An alternative measure was introduced by Franceshi *et al.* (1986). They evaluated tonic and phasic HR changes in relation to spontaneous body movements occurring independently from apnoea or nocturnal myoclonus by calculating both the ratio of the mean R-R interval before the body movement to the mean R-R interval during wakefulness (the sleep/wakefulness ratio Rsw), and the ratio of the longest R-R interval before the body movement to the shortest one after the body movement (the body movement ratio, Rbm). The abnormal HR reactions to body movements have been reported in patients with Alzheimer's disease (Franceschi *et al.* 1986), PD (Laihinen *et al.* 1987, Ferini-Strambi *et al.* 1992), multiple sclerosis (Ferini-Strambi *et al.* 1995) and panic disorder (Ferini-Strambi & Smirne 1997).

2.4.3.2 HRV during sleep

Of the various spectral analysis methods available usually FFT or AR have been used to evaluate nocturnal HRV. The results have shown increases in the HF components and decreases in the LF components of HRV across non-REM stages and opposite changes in REM sleep and wakefulness in healthy man (Zemaityte *et al.* 1984, Scholz *et al.* 1993, Baharav *et al.* 1995, Bonnet & Arand 1997, Scholz *et al.* 1997).

In pathological conditions only limited data exist on HRV in relation to sleep stages. Using 24-h ECG recordings Mastrocola *et al.* (1999) reported diminished variability of standard R-R intervals and spectral measures of HRV in PD patients under medication. In this study the daytime and nighttime periods were evaluated separately and the HF absolute spectral power was diminished only during the night. However, the HRV was not evaluated separately during the different sleep stages. Vanoli *et al.* (1995) demonstrated pathological HRV during both non-REM and REM sleep in patients after myocardial infarction.

2.4.3.3 Sympathetic nerve activity during sleep

Using microneurography, both muscle and skin sympathetic nerve activity can be recorded from the C-fibres of the peripheral nerve (Hagbart & Vallbo 1968, Hagbart *et al.* 1972). The features of MSNA differ from those of SSNA, MSNA showing a rhythmic and pulse synchronous discharge pattern while SSNA has an irregular firing pattern and high response to arousal stimuli (Wallin & Elam 1997). In muscle nerves the sympathetic activity is dominated by vasoconstrictor impulses, whereas skin nerves the sympathetic activity consists of vasoconstrictor, vasodilatator and sudomotor impulses.

Intraneural recordings are made with a tungsten microelectrode having a shaft diameter of 0.2 mm and an uninsulated tip of a few micrometers. Most sympathetic recordings are made in the peroneal, tibial, median or radial nerves but sometimes small cutaneous nerves are used (Nordin 1990). Manual handling of the microelectrode is preferable when searching for an appropriate intraneural recording site, and clear criteria exist for the identification of skin or muscle nerve fascicles (Vallbo *et al.* 1979).

It has been shown that during non-REM sleep MSNA decreases and during REM it increases (Hornyak *et al.* 1991, Okada *et al.* 1991, Somers *et al.* 1993). High amplitude K-complexes are accompanied by brief increases of MSNA (Hornyak *et al.* 1991) and a shortening of burst latency (Xie *et al.* 1999). SSNA has been found to be increased during REM sleep, but not during non-REM stages (Noll *et al.* 1994).

It is also possible to record MSNA and SSNA simultaneously. In one study in which this was carried out Takeuchi *et al.* (1994) showed that there are similarities in the burst properties of MSNA and in those of SSNA during S2, thus suggesting that MSNA and SSNA may share a common origin in the CNS. So far no data exists on nocturnal MSNA or SSNA in pathological conditions.

3 The aims of the study

The purpose of this study was to evaluate the cardiovascular ANS regulation in patients with PD, MSA and PSP. More precisely defined this study aimed:

1. To evaluate cardiovascular responses as a marker of ANS failure in newly diagnosed untreated patients with PD and to assess the relationship between them and the clinical characteristics of PD.
2. To investigate autonomic function in patients fulfilling strict clinical diagnostic criteria for PD, MSA and PSP, and to evaluate the diagnostic potential of laboratory autonomic tests.
3. To determine the efficacy of and the relationships between various time domain, frequency domain, non-linear and geometrical analysis methods in evaluating the cardiovascular regulation in untreated PD patients and controls.
4. To evaluate the cardiac autonomic function in relation to sleep stages both in newly diagnosed PD patients and in healthy controls by studying HRV with various methods, and by analysing HR changes associated with sleep movements.

4 Subjects and methods

4.1 Subjects

This study was carried out in the departments of Clinical Neurophysiology and Neurology at Oulu University Hospital, Finland (I, III and IV) and at Sahlgrenska University Hospital, Gothenburg, Sweden (II). Patients participating in the study had been referred to the Departments of Neurology in these two hospitals. The study was approved by the local Ethics Committees of the corresponding medical faculties, and was carried out according to the principles of the Declaration of Helsinki. All patients and control subjects gave their informed consent before their inclusion in the study.

In Study I, 50 consecutive untreated patients with idiopathic PD fulfilling the Parkinson's Disease Society Brain Bank clinical criteria (Daniel & Lees 1993), referred to the Department of Neurology, Oulu University Hospital because of extrapyramidal symptoms were included in the study. The diagnosis of PD was made on clinical grounds and was confirmed with a follow-up extending for up to 3 years. Patients with manifestations of other central or peripheral nervous system disorders or with any other disease or medication known to affect the ANS were excluded. For the analysis of the clinical subgroups, the patients were divided according to the age of the patients, the duration of the symptoms and the clinical features at the onset of PD (dominant sign: tremor, hypokinesia/rigidity, combined tremor and hypokinesia/rigidity, right or left side onset).

In Study II, the inclusion criteria for PD were the British Brain Bank research criteria for clinically definite PD (Hughes *et al.* 1992), whereas the inclusion criteria for MSA were the criteria for probable MSA presented by Quinn (1989), and for PSP the criteria proposed by Golbe & Davis (1993), later affirmed by new and more specific clinical criteria for PSP (Litvan *et al.* 1996) and MSA (Gilman *et al.* 1999). Thirty-four PD patients, 47 MSA patients and 15 PSP patients were included in the study along with 18 age-matched controls.

In Study III a further 12 untreated PD patients were evaluated along with 20 patients from Study I, for the comparison of different HRV analysis methods, the patient group thus comprising 32 PD patients.

In Study IV 21 untreated newly diagnosed PD patients underwent one-night polysomnography for nocturnal HRV analysis. One patient was excluded from the study due to scanty sleep and one further patient was excluded due to periodic sleep apnoeas.

Table 1 presents the characteristics of the patients and control subjects in the individual studies. All the controls were healthy volunteers without any disease or medication known to affect the ANS. The control subjects were recruited from relatives and acquaintances of the patients and hospital staff.

Table 1. Characteristics of the subjects in individual studies.

Study	No. of subjects		Age, mean years (SD)	Duration of disease, mean years (SD)	Hoehn and Yahr stage, median	UPDRS total score Mean (SD)
	women	men				
Study I						
PD patients	22	28	60(9)	2.2(1.8)	1.7	-
Control subjects	28	27	56(12)			
Study II						
PD patients	15	19	61(10)	11.7(6.9)	2.8	-
MSA patients	20	27	63(9)	5.2(3.2)	3.5	-
PSP patients	6	9	68(5)	4.7(2.6)	3.5	-
Control subjects	9	9	63(6)			
Study III						
PD patients	14	18	58(12)	1.8(1.8)	1.9	34.1(13.3)
Control subjects	14	10	54(13)			
Study IV						
PD patients	7	14	58(12)	1.8(2.0)	1.5	25.8(11.7)
Control subjects	12	10	56(13)			

UPDRS = Unified Parkinson's Disease Rating Scale.

4.2 Methods

4.2.1 Clinical examination

In Study I patients were clinically examined by either Jukka Turkka, M.D., Ph.D., or by Tarja Haapaniemi, M.D., and in Study II by Björn Holmberg, M.D., Ph.D. In Studies III and IV neurologist Tarja Haapaniemi, M.D. examined the patients. The clinical disability caused by PD was graded using the Hoehn and Yahr staging (Studies I and II, Hoehn & Yahr 1967) and the unified Parkinson's disease rating scale (UPDRS, Fahn *et al.* 1987,

Studies III and IV). The clinical severity of the autonomic failure was graded in Studies I, III and IV by using a rating scale from 0-2 (0=absent, 1=mild, 2=severe) for different modalities of the ANS measures, which are postural dizziness and disturbances of sweating, urinary function, bowel function, sexual function, HR regulation, salivation and breathing and signs of peripheral circulation disturbances, sluggish pupillary reactions and seborrhoea (Turkka 1986, Turkka *et al.* 1987).

4.2.2 Cardiovascular autonomic reflex tests (I, II)

Cardiovascular autonomic function tests based on HR and BP responses at rest and after various forms of stimulation were performed under standardized environmental conditions. The next test was not begun until the HR and BP had returned to the rest level after the previous test.

In Study I the ECG and nasal thermistore signals were conveyed through a 12 bit A/D converter to a PC with a sampling frequency of 320 Hz. The recorded signals in deep breathing, the Valsalva manoeuvre and the isometric handgrip tests were checked off-line and only technically adequate performances were accepted for analyses (Suominen 1997).

The investigations proceeded as follows: first, with the patient in a supine position on the tilt table, the maximum contraction power (handgrip) of the patient's dominant hand was measured three times with a dynamometer for the isometric work test. During a 30-minute resting period, the BP was measured three times using an automatic arm sphygmomanometer. Thereafter, the following five tests were performed: normal breathing, paced breathing at six breaths per minute, the Valsalva manoeuvre, upright tilting and the isometric handgrip test (Turkka *et al.* 1997).

The following HR responses were calculated:

1. In the normal breathing test the consecutive R-R intervals for all patients were measured from the ECG for a period of 1 minute, and the standard deviation (SD) and the square root of the mean squared differences of successive R-R intervals (RMSSD) were used as the variables.
2. In the deep breathing test the median of 5 consecutive ratios of the longest (expiration at 5 sec) to the shortest (inspiration at 5 sec) R-R interval was selected. The test was performed twice and the higher R-R interval ratio was used as the "max-min ratio".
3. In the Valsalva manoeuvre the ratio of the longest R-R interval after blowing (40 mmHg or at least over 50 % of maximum blowing capacity for 15 seconds) and the shortest interval during blowing were calculated. The highest ratio of three manoeuvres was used as the "Valsalva ratio".
4. In the tilt table test the ratio of the longest R-R interval around beat 30 (20-40) to the shortest interval around beat 15 (10-20) after quick upright tilting (2 seconds, 90^{o}) was used as the "30:15 ratio".

The following BP responses were used:

1. The BP response to upright tilt was quantified as the largest drop (or lowest increase) in systolic and diastolic pressure (mmHg) immediately after, and at 1 minute and 2 minutes after tilting.
2. In the isometric work test the largest increase in the systolic and diastolic pressure during a 4-minute period of sustained handgrip with a dynamometer at 30% of the subject's maximum voluntary force was recorded.

In Study I, in a subgroup of 20 PD patients and 24 controls, HRV was also evaluated by means of spectral analysis, fractal dimension (FD) and NN50 during a 10-minute recording with the subject in a stationary supine position at rest.

In Study II subjects were placed and secured on a tilting table, and recordings of heart rate and BP where initiated (Deuschl & Eisen 1999). Both a continuous non-invasive finger BP measurement according to the volume-clamp principle (Finapres^{R}, Ohmeda Monitoring Systems, Englewood, Colorado, USA) and an automatic sphygomanometric method with the cuff placed on the left upper arm were used. Heart rate was calculated with a computer program developed in the laboratory of Sahlgrenska University Hospital, triggered by the pulse signal from the Finapres. Sinus arrhythmia was evaluated during 60 seconds periods, at rest and during controlled breathing (6 respiratory cycles per period), and calculated according to the equation

$$100 \cdot (HRmax - HRmin)/HRmean$$

Orthostatic tests were performed subsequently, with the time used for raising the tilt table from the horizontal position to 75^{0} head-up tilt limited to two seconds. Quantitative HR and BP changes during eight-minute periods of tilt were based on sphygomanometric data.

4.2.3 Heart rate variability analysis methods (III)

4.2.3.1 Time domain analyses

The time domain parameters of the HRV that were used were the standard deviation of R-R intervals (SDNN) and pNN50, i.e. the percentage number in the consecutive R-R interval change greater than 50 ms (Ewing *et al.* 1984), measured during the 10-minute resting phase.

The moving polynomial method allows derivation of components of HRV within specified frequency bands (Porges 1985). This method is mainly a time domain method, but like spectral techniques it describes the HRV within specified frequency bands. The details of this method have been described previously (Donchin *et al.* 1985). The natural logarithm of the variance of HRV within the high frequency band (0.15-0.40 Hz) was calculated and presented as the vagal tone index (VTI) (ln/msec2) with a PC-based software package (Mxedit2.01, Delta-Biometrics, Bethesda, USA).

4.2.3.2 Frequency domain analyses

Spectral analysis of 512 consecutive R-R intervals of patients and controls was performed within the 10-minute rest period, during normal breathing, using FFT and AR (Berger *et al.* 1986, Task Force 1996).

The R-R intervals of the 10-minute rest period were converted to a smoothed instantaneous HR time series at 4 Hz, and an exact Hamming window was applied before FFT. An area spectrum was applied to calculate the power spectral distribution with FFT.

The power in three frequency bands was determined by integrating the power spectrum: 0.15-0.40 Hz for the HF; 0.04-0.15 Hz for the LF; and 0.0033-0.04 Hz for the VLF. The values of the spectral power are presented as absolute units and normalized units i.e. as percentages of the total power minus the VLF component.

The LF and HF components were also determined visually for each subject from the Fourier series, and the relative and absolute values were quantified by measuring the area of the frequency bands.

The power spectral distribution was also calculated using AR. The parameters of the model and the variance of the driving white noise were estimated by means of the recursive Levinson Durbin algorithm and the optimal order was chosen in accordance with the Akaike information criterion (Kay & Marple 1981). The whiteness of the prediction error was verified in order to match the model hypothesis (Box & Jenkins 1976, Task Force 1996). The PSD was divided into a sum of spectral components by means of a spectral decomposition method based on the residual integration (Zetterberg 1969, Baselli *et al.* 1987). It was then possible to evaluate the peak frequency and the power of the most relevant spectral components, the spectral components whose central frequencies are within the previously defined LF and HF bands. The automatic detection was visually checked and modified if necessary. Both the absolute and normalized power values, according to Task Force (1996), were calculated, together with the LF:HF ratio.

4.2.3.3 Non-linear analyses

Non-linear HR dynamics were measured by a custom-made analysis program (Hearts 5 software package, Heart Signal Co., Oulu, Finland). Detailed descriptions of these methods have been published elsewhere (Huikuri *et al.* 1998, Pincus 1991, Saul *et al.* 1987, Pincus & Goldberger 1994, Peng *et al.* 1995, Bigger *et al.* 1996, Ho *et al.* 1997).

To compute the FD we used a customized Mandelbrot-ε-blanket method described by Mandelbrot and further developed by Peleg (Mandelbrot 1982, Peleg *et al.* 1984). In this method the length of the HRV curve is determined a maximum of 20 times with different scales. The FD can be computed by plotting the lengths versus the scale on a log-log scale from the slope of the best fitting straight line.

Approximate entropy (ApEn) is a measure that quantifies regularity and complexity of mathematical data and has been developed for time series (Pincus 1991). ApEn values were computed from R-R interval segments and averaged to obtain a mean value of ApEn characterising the whole recording.

Detrended fluctuation analysis (DFA) is a modified root-mean-square analysis that quantifies the presence or absence of fractal-like correlation properties and has been validated for time series (Peng *et al.* 1995). DFA HR correlations were defined for both short-term (• 11 beats, DFA1) and long-term (> 11 beats, DFA2) fluctuations in the R-R interval time series. The cutoff point of 11 beats was chosen based on the previous findings of a "crossover point" on the log-log plot (Peng *et al.* 1995). Scaling exponents were computed from consecutive R-R intervals and averaged to obtain a mean value.

4.2.3.4 Geometrical HRV analysis

Poincaré plots, scattergrams in which each R-R interval of a tachogram were plotted as a function of the previous R-R interval, were analysed quantitatively. The standard deviations of the instantaneous beat-to-beat variability (SD1) and long-term R-R interval variability (SD2) were determined. The details of this method have been described earlier (Huikuri *et al.* 1996, Tulppo *et al.* 1996).

4.2.4 Methods for assessing nocturnal autonomic functions (IV)

All subjects underwent polysomnography for one night including an EEG with C3/A2, C4/A1, O1/A2 and O2/A1 tracings, a submental EMG, EOG, ECG, airflow with nasal thermistore, movement recording with a static charge sensitive bed SCSB (Alihanka and Vaahtoranta, 1979) and oxygen saturation recordings. Sleep stages (non-REM stages S1-4 and SREM) were scored off-line using the standardized criteria with 30 s epochs (Rechtschaffen and Kales, 1968). R-R intervals were checked manually after automatic detection of the R-spikes from the ECG and all R-R intervals were included in the analysis. However, in some occasional cases where no R-spikes could be detected or an ectopic beat occurred, the erroneous or missing R-spike was substituted with a point dividing the R-R interval equally.

4.2.4.1 Sleep movement related HR changes

Phasic and tonic HRV time domain measures were evaluated in relation to spontaneous body movements during sleep according to a previously described method (Franceschi *et al.*, 1986). The Rbm represents an index of phasic heart rate increase induced by body movement, i.e. the ratio of the mean R-R interval before body movement to the shortest one after body movement. The sleep-wakefulness ratio (Rs/w) was considered as an index of tonic heart rate decrease induced by sleep, i.e. the ratio of the mean R-R interval before body movement to the mean R-R interval during wakefulness. We also included movements lasting for over 2 seconds instead of using only movements lasting for more than 6 seconds (Franceschi *et al.*, 1986).

4.2.4.2 HRV in various sleep stages

Instead of using only short segments of R-R intervals considered as representative of each sleep stage, all data with the same sleep stage including all corresponding R-R intervals were pooled into one segment before analysis, thus representing the given sleep stage.

Three parameters were calculated as time domain measures of the HRV: the SDNN, the RMSSD, and the pNN50. For the frequency domain measures of HRV during the different sleep stages both FFT and AR were used. The FD was used as the non-linear measure. These parameters were calculated as described in chapters *4.2.3.2* and *4.2.3.3*.

4.3 Statistics

Before statistical analyses were conducted, the values of both BP responses and the logarithmically transformed HR measures were adjusted for both the mean age and the mean baseline of the R-R intervals using multiple regression (I,III,IV). For the isometric work responses the genders were analysed separately (I). For Study II a non-parametric one-way analysis of variance was used to compare age, disease duration, calculated HRV at rest and controlled breathing between the diagnostic groups. If the result of the test was significant, post hoc tests were performed, using the Mann-Whitney U test with Bonferroni corrections for multiple comparisons. The significance levels for the comparisons between the patients and control subjects were obtained by using analysis of covariance (ANCOVA), the χ^2 –test, Student's t-test and the Mann-Whitney U test. In Study III correlation and covariance coefficients were calculated to evaluate the relationship between the parameters and forward stepwise logistic regression analysis was performed to find the strongest independent predictor to separate the PD group from the healthy controls using SPSS for Windows Release 9.0. The level of statistical significance was set at the p value of 0.05.

5 Results

5.1 Cardiovascular autonomic reflexes

Standard cardiovascular reflex tests were used to evaluate cardiovascular autonomic regulation in PD patients (Studies I and II), and in MSA and PSP patients (Study II). In Study I, the results of the standard cardiovascular reflex tests showed diminished responses in untreated newly diagnosed PD patients when compared to the controls. The max-min ratio of the deep breathing test and the 30:15 ratio of the tilting test were lower in patients, and the systolic BP fall in the tilting test was more pronounced in patients (Table 2).

When the clinical characteristics of PD were taken into consideration, patients with hypokinesia/rigidity or tremor as their initial sign had diminished HR responses to the deep breathing test and in the tilting test. When the subgroups were compared to each other, patients with a hypokinesia/rigidity onset sign had a lower max-min ratio than those with tremor as initial sign (Table 3). The severity of clinical autonomic dysfunction did not correlate with cardiovascular disturbances.

In Study I, with subgroups of 20 PD patients and 24 controls, HRV was also evaluated by means of spectral analysis, fractal dimension and pNN50 during 10-minute recordings with the subjects in a stationary supine position at rest. The HF of both FFT and AR derived spectral analyses were diminished in PD patients, along with the AR derived LF and LF:HF ratio.

In Study II, the max-min ratio of the deep breathing test was significantly lower in the MSA patient group than in the controls, whereas in the PD and PSP patient groups no significant difference was seen (Figure 1). No significant difference between the groups was found for HRV during the normal breathing test. Sixty percent of the MSA patients, 39% of the PD and 29% of the PSP patients had a result outside the 95th percentile for HRV during controlled breathing of the control group. In the tilting test both MSA and PD patients had pathological BP responses, while PSP patients had preserved BP responses compared with control subjects (Figure 2). PD patients showed pathological BP responses in the late course of the disease and with increasing age, whereas MSA patients

had pathological responses at all ages and durations of the disease (Figure 3). Pathological results in both the max-min ratio and the tilting test were found in 40% of the MSA patients, 30% of the PD patients and 7% of the PSP patients, whereas normal results in both tests were found in 18%, 40% and 64%, respectively.

Table 2. HR responses of PD patients and control subjects to normal breathing [standard deviation (SDNN) square root of the mean squared differences of successive R-R intervals (RMSSD)], deep breathing (Max-min ratio), the Valsalva ratio and the tilting test (30:15 ratio). Blood pressure changes (mmHg) to the tilting test and the isometric handgrip test are also presented. The statistical significance between the groups was obtained using ANCOVA. (I)

Variable	Patients (n=50)	SD	Controls (n=55)	SD	p
SDNN	25.3	2.00	29.0	1.51	ns
RMSSD	28.6	2.54	39.3	13.4	ns
Max-min ratio	1.17	0.01	1.25	0.02	0.000
Valsalva ratio	1.64	0.05	1.74	0.06	ns
30:15 ratio	1.06	0.01	1.15	0.01	0.000
Isometric handgrip					
systolic BP (females)	28.7	4.9	28.7	3.8	ns
diastolic BP (females)	21.1	3.9	17.8	2.7	ns
systolic BP (males)	35.4	3.0	34.9	3.6	ns
diastolic BP (males)	20.9	2.0	22.3	2.1	ns
Tilting test 2 min					
systolic BP	-5.7	1.9	0.0	1.9	0.041
diastolic BP	6.2	1.4	4.9	1.1	ns

ns= non significant

Table 3. HR responses in PD patients with hypokinesia/rigidity or tremor as initial sign. The statistical significance was obtained using ANCOVA. (I)

Variable	Hypokinesia/ rigidity	SD	Tremor	SD	p
SDNN	21.6	3.1	25.1	2.7	ns
Max-min ratio	1.12	0.02	1.18	0.02	0.037
Valsalva ratio	1.60	0.12	1.60	0.06	ns
30:15 ratio	1.06	0.02	1.05	0.01	ns

For abbreviations see Table 2.

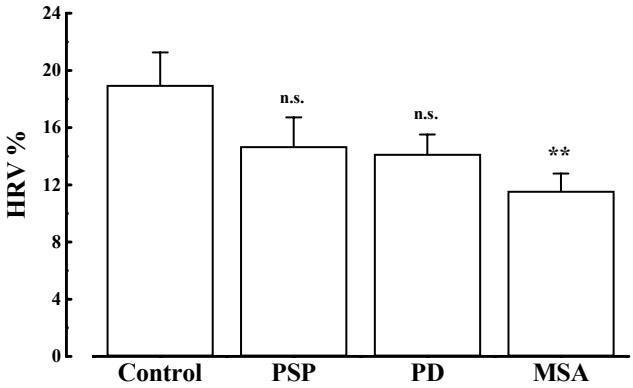


Fig. 1. HRV during deep breathing for healthy controls, PSP, PD and MSA patients. Mean levels and standard errors of means are shown. **p<0.01 using Mann-Whitney U test. (II)

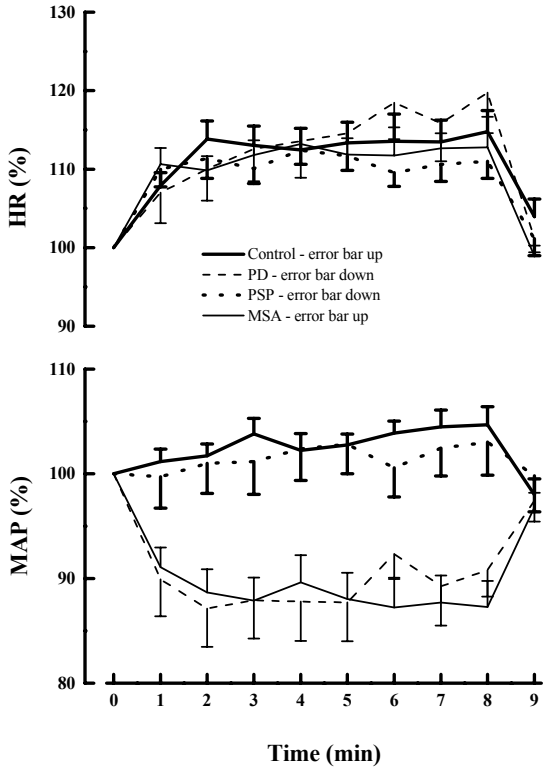


Fig. 2. HR and BP responses in control subjects, PD, PSP and MSA patients during the tilting test from baseline (0 min) to standing (1-8 min) and resumed horizontal position (9 min). The relative mean arterial pressure (MAP%) was significantly decreased in PD (p<0.01) and MSA patients (p<0.001) during 2-8 min of tilting. The significance of the difference was obtained using Student's t-test. (II)

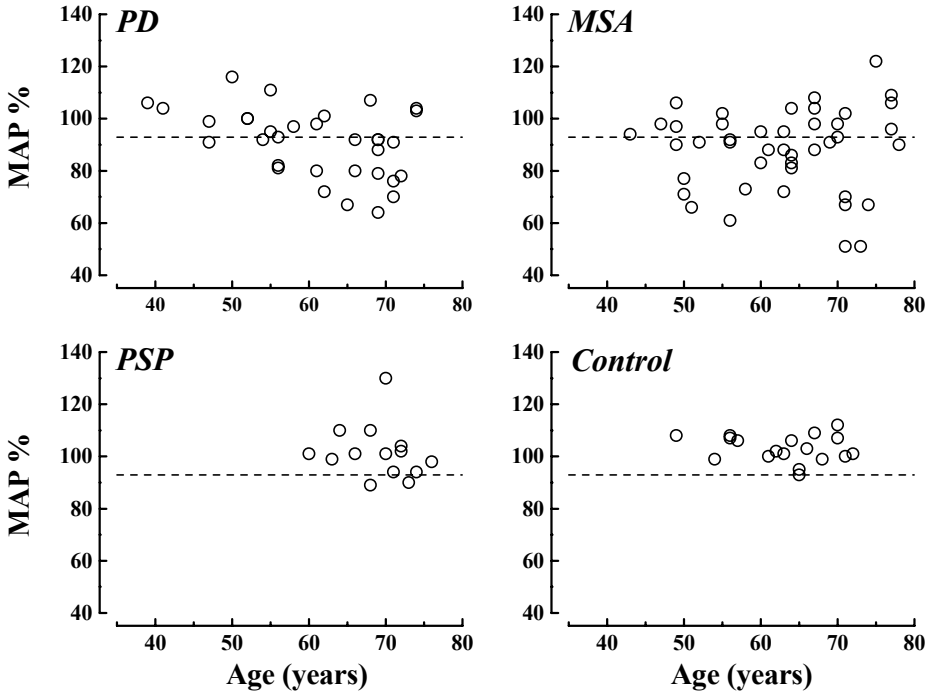


Fig. 3. Relative mean arterial blood pressure (MAP%) during the tilting test for individual PD, MSA and PSP patients and control subjects. The dotted lines indicate the limit for a pathological BP response (relative MAP –2SD for the control groups). The BP fall can be seen in the MSA group at all ages and durations of the disease, whereas in the PD group with increasing age and disease duration. (II)

5.2 Comparison of various analysis methods

In Study III, the efficacy and mutual relationships of different analysis methods in revealing abnormal HRV was evaluated in PD patients and control subjects. Both time and frequency domain measures revealed attenuated cardiovascular autonomic regulation in the PD patients compared to the controls (Table 4) whereas non-linear or geometrical methods did not reveal any statistically significant differences between the groups.

Using both absolute and normalised spectral measures, both the HF and LF absolute spectral powers were the most effective measures, in that order, for distinguishing the two groups (Table 5). When only normalised spectral units were included in the comparison, the two best single parameters for separating the groups were the 30:15 and the max-min ratio. When estimating the correlations between the different measures, the time domain measures, the fractal dimension and the absolute spectral measures correlated with each other, but other non-linear estimates did not associate with either time or the frequency domain measures.

Table 4. Cardiovascular reflex test and HRV measures by time and frequency domain in PD patients and control subjects. (III)

Variable (units)	Patients (n=32)	SD	Controls (n=24)	SD	p
Cardiovascular reflex test measures					
max-min ratio	1.17	0.02	1.27	0.04	0.020
Valsalva ratio	1.76	0.07	1.60	0.07	ns
30:15 ratio	1.07	0.01	1.15	0.03	0.012
Time domain measures					
SDNN (ms)	30.7	2.4	31.5	3.2	ns
pNN50 (%)	0.64	0.24	2.12	0.84	0.013
VTI (ln(ms^2))	4.26	0.28	5.23	0.22	0.016
Frequency domain measures					
Autoregressive model					
Absolute units					
Total power (ms^2)	698	139	1134	256	0.000
VLF (ms^2)	326	103	619	123	ns
LF (ms^2)	117	41	141	69	ns
HF (ms^2)	77	21	223	86	0.010
Normalized units					
LF(n)	42.9	7.1	31.7	6.9	ns
HF(n)	28.3	5.2	50	7.9	0.019
LF:HF ratio	1.5	0.5	0.7	0.3	ns
Fast Fourier transform					
Absolute units					
Total power (ms^2)	290	27	410	36	0.008
VLF (ms^2)	74	6	101	9	0.008
LF (ms^2)	101	11	143	14	0.017
HF (ms^2)	51	6	92	11	0.001
0.04-0.15 Hz (ms^2)	95	11	127	13	ns
0.15-0.40 Hz (ms^2)	93	11	143	17	0.012
Normalized units					
LF(n)	42.5	2.4	39.5	1.4	ns
HF(n)	30.6	2.0	36.5	1.9	0.044
0.04-0.15 Hz (n)	43.5	2.0	41.6	1.5	ns
0.15-0.40 Hz (n)	42.8	1.8	46.6	1.8	ns
LF:HF-ratio	1.40	0.2	1.10	0.1	ns

pNN50= changes in the R-R interval greater than 50 ms; VTI= vagal tone index; VLF= very low frequency power; LF= low frequency power; HF = high frequency power. For other abbreviations see Table 2. The statistical significance levels between the groups were obtained using ANCOVA.

Table 5. Forward stepwise logistic regression classification table for the calculated parameters when A) both absolute (a) and normalized (n) units or B) only normalized units are included in analysis. (III)

Variable	Score	p	r
A) absolute and normalized units			
HF (FFT)	21.677	0.000	0.537
LF (FFT)	14.161	0.000	0.422
LF (AR)	8.347	0.004	0.305
30:15 ratio	7.755	0.005	0.290
max-min ratio	7.468	0.006	0.283
B) normalized units			
30:15 ratio	7.755	0.005	0.290
max-min ratio	7.468	0.006	0.283
VTI	6.473	0.011	0.256
HF(n) (AR)	6.448	0.011	0.255
pNN50	5.922	0.015	0.240

Score= ranking score; r= regression coefficient. For abbreviations see Tables 2 and 3.

5.3 Nocturnal cardiac autonomic regulation in PD

In Study IV, HRV was evaluated separately for each sleep stage by using both time and frequency domain methods along with non-linear ones, and by analysing HR changes associated with spontaneous sleep movements.

The characteristics of the sleep in the PD patients and the controls are presented in Table 6 and no differences were found between the two groups. However, the patients moved more than the controls during sleep: during S3 there were 11.8±9.7 movements per hour in the PD patients vs. 4.6±5.9 movements per hour in the controls (p=0.003, Student's t-test), during S4 12.9±7.6 vs. 4.9±4.5 (p=0.0001) and during SREM 15.1±9.6 vs. 8.9±6.7 (p=0.008). One PD patient had periodic limb movement syndrome (PLMS) with 36 movements per hour, and three patients had REM sleep behaviour disorder (RBD) without violence. Five patients had 2.4-3.5 episodes of hypopnoea per hour and two of the controls had 1.7-4.2 hypopnoea episodes per hour during sleep (p=0.2680, ^{2}-test).

The body movement ratio was decreased in PD patients during non-REM (1.32±0.01 in patients vs. 1.38±0.01 in controls, p=0.0002) and REM (1.25±0.01 vs. 1.34±0.02, p=0.0001) sleep stages but the sleep-wakefulness ratio did not differ between the groups. The SDNN was significantly increased in PD patients during slow wave sleep (S3 + S4) (45.3±5.1 vs. 32.2±3.6, p=0.031). Other time domain measures and the FD did not differ significantly between the patients and the controls.

The normalized HF power was diminished and the LF:HF ratio was increased in awake PD patients (Table 7). During non-REM sleep the normalized HF power was also diminished while during S1 the normalized LF was low in PD patients. AR revealed that the absolute VLF and LF powers were increased during S3 (VLF power: 180±59 in patients vs. 88±21 in controls, p=0.048; LF power respectively: 121±47 vs. 54±14, p=0.047 during S3 sleep stage). However, absolute FFT measures revealed no differences (Table 8).

Table 6. The characteristics of sleep in PD patients and control subjects. (IV)

Variable	Patients n=19	Range	Controls n=20	Range	p
Time in bed, min.	488.3	413-584.7	510.9	309.7-569	ns
Total sleep time, min.	345.1	209.9-519	391.8	185.4-535	ns
Sleep efficiency %	77.8	46.9-95.4	81.8	49.1-95.1	ns
Wake after sleep onset, min	38.0	7.2-86.8	25.1	1.4-89.9	ns
Sleep latency, min.	19.3	12.8-53.8	20.9	16.4-68.7	ns
S2 latency, min.	33.2	6.1-76.4	32.1	11.3-85.6	ns
REM latency, min.	110.4	63.8-278.2	134.8	56.1-229.5	ns
%S1	19.1	8.2-39.9	14.5	7.6-32.8	ns
%S2	50.3	34.5-59.6	52.2	46.5-67.1	ns
%S3	6.5	2.8-13.2	6.6	1.9-12.0	ns
%S4	8.2	0.9-13.0	7.0	1.8-16.1	ns
%REM	14.7	5.9-32.6	17.1	4.6-24.8	ns

Values are median.
ns = non significant.
The significance levels between the groups were obtained using the Mann-Whitney U-test.

Table 7. FFT spectral measures expressed as normalized units during various sleep stages in PD patients and control subjects. (IV)

Variable	Sleep stage	Patients n=19	SD	Controls n=20	SD	p
LF(n)	Awake	41.1	1.4	41.3	1.6	ns
	REM	44.8	1.5	43.0	1.5	ns
	S1	42.1	1.3	45.3	1.0	0.049
	S2	44.2	2.1	40.5	2.2	ns
	S3	42.1	2.2	40.3	2.4	ns
	S3+4	41.3	1.7	39.2	2.6	ns
	S4	41.0	1.6	39.0	2.4	ns
HF(n)	Awake	18.3	1.0	23.5	1.2	0.002
	REM	20.0	1.5	21.6	1.2	ns
	S1	19.6	1.4	21.0	1.1	ns
	S2	26.9	1.7	33.0	2.2	0.031
	S3	30.0	1.5	35.1	1.9	0.044
	S3+4	29.4	1.9	36.8	2.4	0.019
	S4	30.3	2.3	38.4	2.8	0.029
LF:HF ratio	Awake	2.2	0.2	1.8	0.1	0.032
	REM	2.2	0.2	2.0	0.1	ns
	S1	2.2	0.2	2.2	0.1	ns
	S2	1.6	0.2	1.2	0.1	ns
	S3	1.4	0.1	1.2	0.1	ns
	S3+4	1.4	0.1	1.1	0.1	ns
	S4	1.4	0.1	1.1	0.1	ns

The statistical significance levels between the groups were obtained using ANCOVA.

Table 8. FFT spectral measures expressed as absolute units (ms^2) during various sleep stages in PD patients and control subjects. (IV)

Variable	Sleep stage	Patients n=19	SD	Controls n=20	SD	p
total power	Awake	264	39	269	26	ns
	REM	295	47	238	28	ns
	S1	323	32	363	28	ns
	S2	246	31	211	25	ns
	S3	428	37	364	38	ns
	S3+4	271	27	215	20	ns
	S4	342	42	276	26	ns
VLF	Awake	65	10	58	6	ns
	REM	61	10	48	6	ns
	S1	80	8	88	9	0.05
	S2	50	6	38	4	ns
	S3	75	9	60	7	ns
	S3+4	50	8	35	4	ns
	S4	62	10	44	5	ns
LF	Awake	81	15	87	9	ns
	REM	105	17	82	11	ns
	S1	102	11	123	10	ns
	S2	86	11	70	8	ns
	S3	146	13	122	16	ns
	S3+4	90	11	71	9	ns
	S4	114	14	90	12	ns
HF	Awake	36	6	49	6	ns
	REM	47	9	41	5	ns
	S1	47	7	57	6	ns
	S2	67	7	67	10	ns
	S3	133	12	128	15	ns
	S3+4	83	9	77	8	ns
	S4	108	12	101	11	ns

The statistical significance levels between the groups were obtained using ANCOVA.

6 Discussion

6.1 General aspects

The purpose of the present study was to assess the usefulness of various methods for evaluating cardiovascular autonomic dysfunction in PD, MSA and PSP patients. It also aimed to elucidate whether any particular clinical characteristics of PD are associated with autonomic disturbances. To evaluate the diagnostic potential of cardiovascular autonomic tests autonomic functions were investigated in patients fulfilling strict clinical criteria for PD, MSA and PSP. Furthermore, nocturnal autonomic regulation in PD was studied in order to reveal sleep stage specific HRV.

From the methodological point of view the measurement of standard cardiovascular reflexes has achieved a gold standard in the clinical testing of autonomic functions (Myllylä *et al.* 2000). However, the main problem is the considerable physiological variation between the subjects as also seen in our controls (Ravits 1997). Moreover, the cardiovascular reflexes are not direct measures of autonomic nervous tract function, but they are responses to complex neural, humoral and hormonal interactions at multiple levels of neural axis (Piha 1988). Muscle and skin sympathetic activity can be measured directly in humans by using microneurographic recording technique (Wallin & Elam 1997). This method provides direct information about sympathetic physiology and pathophysiology but is not applicable for clinical diagnostic work. A consensus statement including suggestions for useful parameters exists for studying HRV (Task Force 1996) and these recommendations were taken into consideration in this study. However, the validity of these proposals in neurological diseases has not been examined until now.

ANS symptoms are common in PD; 80-90 % of PD patients have some symptoms when all cases are included (Turkka 1986). The most commonly reported disturbances of ANS function in PD are cardiovascular symptoms, i.e. orthostatic hypotension and postural dizziness (Turkka 1986). A variety of other symptoms such as increased perspiration and salivation, oily skin, bladder disturbance, decreased potency and libido, lachrymation, hypersecretion of the nasal glands and gastrointestinal disturbances also exist. Even though the methods of the present study aimed at examining cardiovascular

integrity in PD, in Studies I, III and IV the ANS symptoms of the patients were collected using a structural questionnaire (Turkka 1986) including all main ANS symptoms described in PD. Cardiovascular signs are clinically objectively difficult to verify and so in the present study the cardiovascular reflexes and the HR reactions during sleep were used and tested as indicators for ANS disturbance. However, no significant correlation between clinical ANS symptoms and cardiovascular reflex disturbances were found. This may be due to that in early PD ANS symptoms are not prominent and/or the tests are not sensitive enough.

In Parkinsonian syndromes, which include PD, MSA, PSP and CBD, especially in early and atypical Parkinsonian patients, diagnosis may be unreliable (Litvan *et al.* 1997). However, ANS failure may have significant implications for the diagnosis of Parkinsonian syndromes (Mathias 1996). Hence, the present Study II aimed to evaluate the diagnostic potential of cardiovascular autonomic tests in Parkinsonian syndromes. Indeed, especially the diagnoses of MSA and PSP may be defined with the aid of ANS findings. In Studies I, III and IV the idiopathic PD diagnosis was based on the internationally accepted criteria of the Parkinson's Disease Society Bank (Daniel & Lees 1993), and the PD diagnoses in these studies were further confirmed during the clinical follow-up, which in most cases lasted several years.

The mean age of the patients in Studies I and III was higher than that of the controls, but the age-dependence of the ANS measures was the same in the patients and controls, and in the statistical analyses the HR was adjusted for both the mean age and mean baseline R-R intervals by using multiple regression. Therefore, the significant ANS parameter differences between the patients and controls were most probably not due to an age-related difference but to the disease itself, as was shown in Studies II and IV with age-matched groups.

Even though sleep was considered for a long time to be a as static state, it has come to be recognised as an active period in the ANS function, the HR and BP variation differing in various sleep stages in some parameters even divergently (Scholz *et al.* 1997). In Study IV HRV in PD patients was evaluated for the first time with respect to different sleep stages. In addition to traditional time and frequency domain methods based on linear fluctuation of HR, a non-linear fractal dimension analysis method was also used to quantify the complexity of HR dynamics. Sleep stage dependent differences in HRV were obtained when using both time and frequency domain methods, but not when using FD. This result may be due to the fact that HR oscillations become more stable during non-REM sleep (Zemaityte *et al.* 1984, Scholz *et al.* 1993, Baharav *et al.* 1995, Bonnet & Arand 1997, Scholz *et al.* 1997). Upcoming methods for evaluating non-linear HR oscillations may further enlighten the dynamics of HRV during sleep (Hoyer *et al.* 1998).

6.2 Cardiovascular autonomic regulation in PD

In Study I the presence of disturbed cardiovascular regulation in untreated patients with early PD was seen, and hypokinesia/rigidity as the initial manifestation of PD was associated with the most pronounced involvement of the cardiovascular responses. This finding was confirmed later by another study undertaken by our research group

(Haapaniemi *et al.* 2001). Patients with more advanced disease according to higher Hoehn-Yahr stage scores did not have a more significant cardiovascular ANS involvement, which is in contradiction to an earlier finding (van Dijk *et al.* 1993). In that study, some of the patients had antiparkinsonian medication, the disease was more severe and the duration was longer than in patients of Study I. Although evidence exists for the increasing prevalence of Lewy bodies with aging (Fearnley & Lees 1991), clinical and pathological features of the PD process do not seem to differ in young- and old-onset patients (Gibb & Lees 1988). The present results confirmed this, showing no statistically significant difference between young- and old-onset patients when age-related physiological changes were considered. Some studies have also demonstrated that autonomic failure may be different for left and right hemisphere lesions in patients with stroke (Korpelainen *et al.* 1993, Naver 1996), but in the present Study I the ANS dysfunction was similar in patients with right and left onset of PD symptoms.

The reason for the more advanced cardiovascular ANS dysfunction in patients with hypokinesia/rigidity onset found in the present study is a matter of interest. This difference might be due to more advanced neuronal damage in hypokinesia/rigidity onset PD, the damage affecting more autonomic centers or peripheral ANS structures, and indeed there is some evidence in different subpopulations for pigmentation and differential cell death of the substantia nigra in PD (Gibb & Lees 1991). Some studies have suggested that autonomic symptoms are more common in PD patients with severe bilateral bradykinesia and rigidity (Spiegel *et al.* 1969, Marttila 1974). Our results indicate that this similar degrees of autonomic dysfunction are also found in unilateral onset cases.

In addition to traditional time domain cardiovascular reflex tests, a significant deficit was also found in HR spectral parameters including both the HF-component (related to respiration) and the LF-component (related to Meyer wave sinus arrhythmia). A healthy heartbeat is a temporal fractal, normal subjects showing a high degree of HR fluctuation that is associated with more than just respiration. However, the fractal dimension, a parameter that does not have a characteristic scale of time but rather has self-similar fluctuations on multiple scales of time, was not significantly low among our patients. A larger number of patients and a longer recording time for the FD calculation might have segregated the patients with PD from the controls better.

Is the ANS failure in PD parasympathetic or sympathetic in origin? In our study the most pronounced changes were seen in the HRV measures, and the orthostatic BP reaction was also diminished. In our spectral analyses of the HRV both the HF-component (mainly parasympathetically mediated) and also mildly the LF-component (sympathetically mediated) were decreased in the patient group. Both parasympathetic and sympathetic hypofunction can alter pulse variation in the deep breathing test and the Valsalva manoeuvre (Diehl *et al.* 1997; Freeman 1997). Goldstein *et al.* (1997) have shown an impaired myocardial uptake of the marker on PET favoring a loss of myocardial sympathetic nerve terminals in the PD patients with autonomic failure. Furthermore, Hakusui *et al.* (1994) and Orimo *et al.* (1999), using meta-[(123)I] iodobenzylguanidine (MIBG) myocardial scintigraphy, have found a reduced MIBG uptake in the majority of patients with PD and even in the early stages of the disease, indicating cardiac sympathetic nerve disturbances. So, evidently both parasympathetic and sympathetic deficits may occur in PD, even in the early stages.

6.3 The clinical efficacy of autonomic tests

In patients presenting Parkinsonian features, autonomic dysfunction has significant implications for diagnosis, prognosis and management (Mathias 1997). Several proposed methods for differentiating Parkinsonian disorders exist based on pathophysiological differences. Significantly higher levels of neurofilament protein in the cerebrospinal fluid have been found in MSA and PSP patients compared to PD patients (Holmberg *et al.* 1998). A levodopa test can also discriminate PD from MSA as well as from PSP (Johnels *et al.* 1993). When these methods are used in combination, their differential diagnostic precision is further improved. However, other methods are needed to distinguish MSA patients from PSP patients. Urethral sphincter-EMG has been proposed as a useful tool for the identification of MSA (Eardley *et al.* 1989, Pramstaller *et al.* 1995, Palace *et al.* 1997) but the test's reproducibility and ability to exclude PSP patients has been questioned (Schwarz *et al.* 1997, Vallderiola *et al.* 1995). In this situation, autonomic cardiovascular tests are valuable complements and have the advantage of being non-invasive and easily reproduced. Moreover, post-mortem validated studies have shown clinical signs of dysautonomia to be the best clinical features for differentiation of these disorders (Litvan *et al.* 1996, Wenning *et al.* 1999, 2000).

In Study II laboratory autonomic function tests were limited to two commonly used procedures, and this could have reduced the sensitivity to detecting all expressions of autonomic failure but beneficially increased the statistical power for the comparisons between the diagnostic groups. In spite of the limited number of tests, the sensitivity to recognising autonomic failure was high; 24 patients without obvious clinical autonomic dysfunction had laboratory impairment of cardiovascular reflexes. Furthermore, 13 MSA patients had urinary incontinence with abnormal BP tilt, and/or HRV responses in 11 cases.

The MSA group showed significantly reduced HRV for deep breathing, indicating vagal dysfunction, while HRV in the PD and PSP groups was unchanged compared to the controls. In Studies I and III, however, the HRV during deep breathing was significantly diminished in PD patients, since in these studies the absolute max-min ratio were used instead of relative max-min ratio in Study II. However, the aim of Study II was different. Patients with pathological HRV were more frequent in the MSA group than in the PD and PSP groups. The autonomic abnormalities in MSA patients have been described as involving the supraspinal cardiovascular centres, including the dorsal vagal nuclei, and loss of preganglionic sympathetic neurons in the intermediolateral columns of the spinal cord (Wenning *et al.* 1997, Benarroch 1997, Daniel 1999). In the present study impairment of HRV was found in 12 PD (37%) and four PSP (27%) patients, and tended to appear more late in the disease, but this finding was made in MSA regardless of disease duration. The study raised an interesting possibility for differentiating young MSA patients in the early stages of the disease from those patients with PD, but this could not be further evaluated as the PD and MSA groups were insufficiently matched for disease duration.

Twenty-eight patients without obvious symptoms of orthostatic failure had pathological results in the orthostatic provocation test. In PD, a significant impairment of BP control was frequently seen and the relative mean arterial pressure was negatively

related to age as well as to disease duration. Consequently, the severe cases of symptomatic orthostatic failure with pronounced BP falls were seen in PD patients aged over 60 and with disease duration exceeding 10 years. Compared to PD patients in Studies I and III, the PD patients in Study II had longer disease duration and had also had antiparkinsonian medication, which all contributes to more pronounced pathological responses. The pathological BP responses were most frequent and severe in MSA, and were found regardless of age or disease duration. The BP fall was rapid in both PD and MSA and a significant change was found after two minutes standing in both groups, and hence autonomic failure can obviously be a distinctive finding in both syndromes. Only two PSP patients showed a limited BP fall (less than 11%) and the PSP group did not differ significantly from the controls. Consequently, the test was useful for the differentiation of PSP patients from those with MSA and, when used in combination with analysis of cerebrospinal fluid neurofilament light protein concentrations, also from those with PD.

Clinically diagnosed groups of PD, MSA and PSP always include a selection bias due to statements in the clinical criteria. Autonomic failure is mandatory for a clinically probable MSA diagnosis according to Gilman *et al.* (1999), whereas the criteria for MSA proposed by Quinn (1989), that were used in this study, do not include autonomic failure as mandatory for the diagnosis, although postural hypotension could be one of the features required for a diagnosis. The frequent finding of a BP drop and HRV loss in the MSA group may therefore represent a selection bias, but 28 patients without postural hypotension were regarded as having MSA in our study and included either as a result of urological disturbance or without any clinical autonomic dysfunction. The frequencies of abnormal BP tilt and/or HRV responses among these MSA patients were clearly higher (67%) than in the PSP group (33%).

Several limitations of this clinical study resulting from concomitant pharmacological treatment need to be stressed. The anti-Parkinson treatment in the clinical study groups had been optimised by the clinicians before hospitalisation and was not altered because of the autonomic testing. These drugs could have affected either the HRV or BP responses, as they are known to lower BP due to central hypotensive effects (Calne *et al.* 1970). Selegiline may also have negative effects on cardiovascular reflexes (Turkka *et al.* 1997). However, a moderate improvement of orthostatic hypotension induced by levodopa and dopamine agonists has been reported in MSA (Aminoff *et al.* 1973). Tests both with and without pharmacological treatment would have to be done to further analyse its effect on cardiovascular reflexes but they are difficult to perform due to the risk of severe adverse effects in disabled patients. Furthermore, tricyclic antidepressants, anticholinergics and clozapine should be considered when a pathological result is reported as they decrease HRV. In this study, the group results of the HRV and orthostatic provocation test did not change by excluding patients treated with these drugs, and the frequency of pathological HRV findings was still highest in the MSA group. Drugs likely to increase BP levels such as fludrocortisone or various sympathomimetic substances were given to the most disabled PD and MSA patients. These drugs probably counteracted the BP falls. For three MSA patients, concomitant treatment with beta-blockers could have increased the BP fall for the orthostatic provocation test and decreased the HR response, but the results of the group comparisons did not change by excluding them.

6.4 Comparison of different HRV methods

In Study III various HRV analysis methods in a short-term 10-min stationary recording at rest and in cardiovascular reflex test measurements, as has previously been documented by time domain measures revealed attenuated cardiovascular regulation in untreated PD patients (Awerbuch & Sandyk 1992, Turkka *et al.* 1997). The highest efficacy in segregating the PD patients from the controls was obtained by absolute power spectra, highlighting the importance of HR spectral analysis in the evaluation of cardiovascular autonomic integrity. It is noteworthy that when a short-term HRV recording is used the non-linear or geometrical measures do not separate the patients from the controls. Each of the examined parameters provides a different kind of information concerning HRV and their concomitant use enlightens different aspects of the disturbances in cardiovascular autonomic regulation in PD.

To our knowledge this study is the first to compare different HRV analysis methods using cardiovascular reflex tests with stationary HRV recordings, and to reveal cardiovascular modulation differences between patients with a central nervous system disease and controls. This kind of a study was considered appropriate since several different approaches in HRV analysis are used at present, and the defining of the most efficient setting of methods is important for clinical applications. ANS disorders may be subjectively uncomfortable for the patient in many ways. Moreover, in many diseases the cumulative risk of e.g. sudden cardiac death is high in patients with associated autonomic dysfunction (Kleiger *et al.* 1987, Ewing 1988, Brouwer *et al.* 1996, Huikuri *et al.* 1996, Huikuri *et al.* 1998).

In healthy subjects the correlations between cardiovascular reflex test measures and spectral analysis have been determined (Hayano *et al.* 1991, Šega *et al.* 1993, Linden & Diehl 1996). However, in pathological conditions they have scarcely been studied in short-term recordings (Bianchi *et al.* 1990, Freeman *et al.* 1991). Time domain ratios of cardiovascular reflex tests are measures of the sequential heart rate response to specific physiological perturbations, and they do not measure beat-to-beat HRV. Therefore direct comparison between time and frequency domain methods may not be relevant. However, it has been shown that the time domain indexes of deep breathing and the 30:15 ratio to standing correlate strongly with parasympathetically mediated absolute spectral parameters in diabetic patients and also in healthy subjects (Freeman *et al.* 1991, Linden & Diehl 1996). In PD patients in the present study these time domain measures correlated not only with parasympathetically but also with sympathetically mediated absolute spectral measures. In healthy subjects the Valsalva ratio correlated only with the sympathetic absolute spectral measure (Linden & Diehl 1996) whereas in diabetic patients the Valsalva ratio had a correlation only with the parasympathetic absolute spectral power (Freeman *et al.* 1991). In PD patients the Valsalva ratio was associated with both HF and LF absolute spectral estimates. Hayano *et al.* (1991) showed, which our results confirm, that in normal subjects absolute but not normalized power shows a significant correlation with cardiac vagal tone in the 10-min registration of HRV. In 24-h ECG recordings absolute FFT powers also appear to be the most effective predictors of risk of sudden cardiac death (Myers *et al.* 1986). Absolute spectral units are strictly

correlated to the total power and hence to the variance of the signal, and thus they are especially indicated when variations in the total variability are investigated.

The correlation between various estimates of HRV exists because of both mathematical and physiological relationships. For example, the mathematical counterparts the SD, the square root of variance, and the total spectral power, equal to variance, correlated strongly with each other in PD patients. Both physiological ANS divisions generally affect the time domain parameters and the normalized spectral units. Indeed, in the present study significant correlations were found between the various parasympathetically and sympathetically mediated estimates of HRV.

The present results also point out that in short time recordings (5-10 minutes) the spectral peaks should be selected manually rather than using predetermined bands. Manual selection of e.g. the actual HF band allows precise adjustment to the corresponding breathing frequency.

Non-linear methods related to chaos tend to deal with autonomous systems, i.e. systems where there is no input or where the input has a very simple form (Hoyer *et al.* 1998). It has been suggested that a healthy heart rhythm is chaotic and shows a fractal form which may be broken down by a disease (Goldberger 1996). It has also been reported that the most essential parts of deterministic-chaotic properties of HRV may be vagally mediated (Zwiener *et al.* 1996). In Study III the highest correlation of the FD were with the max-min ratio of deep breathing, the Valsalva ratio and the VTI (Table 5). However, the non-linear methods were not as effective as expected in the separation of the patients from the controls. This may be due to the fact that present methods are far less developed than linear methods and there are many possible pitfalls in their approach. Most importantly, non-linear parameters describe long-term regulation mechanisms, and reliable estimation requires long data sets, longer than the few minutes necessary for the spectral analysis. In future more advanced approaches, such as isofunctional modelling and surrogate data analysis as an example of bivariate data analyses as well as bicoherence, may reveal new insights into HRV analysis (Hoyer *et al.* 1998).

ApEn is a measure quantifying the regularity and complexity of time series (Pincus & Goldberger 1994). Lower values of ApEn indicate a regular (less complex) signal while higher values indicate irregularity (greater complexity). In Study III there was no difference in ApEn between the PD patients and controls. This is in accordance with our finding that ApEn does not correlate with traditional time domain or frequency domain parameters (Table 2). It has earlier been shown that ApEn does not correlate with cardiovascular risk factors in contrast to time domain and frequency domain measures (Pikkujämsä 1999). Both these findings suggest that at least in these situations the ApEn of HR time series is not a clinically applicable measure of ANS integrity.

In the present study, in order to reduce the vast number of data obtained by several HRV analysis techniques and parameters, forward stepwise logistic regression analysis was used. The absolute spectral powers were the most useful three parameters distinguishing the patients from the controls. The usefulness of spectral analysis for evaluation of HR changes is further supported by the fact that spectral estimates have also been shown to be useful for examination of dynamic HR responses e.g. after tilting (Linden & Diehl 1996). However, it was interesting that when only normalized spectral powers were used in the analysis, the two best predictors of cardiovascular regulation

dysfunction in PD were the more traditional 30:15 ratio and the max-min ratio of deep breathing in that order.

6.5 Autonomic control during sleep

Study IV revealed disturbed nocturnal cardiac autonomic control in newly diagnosed PD patients during sleep and waking. HR reactions were decreased both during sleep and waking and this finding corresponded to the attenuated HRV as reported in earlier papers with daytime cardiovascular reflex tests. A new finding was the increased variance of HR during non-REM sleep.

Study IV was the first to measure HRV using spectral analysis in relation to different sleep stages in PD patients, or indeed in any neurological disease. In a recent study with 24-hour ECG recording HRV was analysed separately for day and night, revealing a decreased variance in HR with SDNN and absolute LF power both during the day and night but with absolute HF power only during the night (Mastrocola *et al.*, 1999). The increased variance in HR during non-REM sleep of Study IV disagrees with this finding. However, these two studies have several differences: the patients of the other study had more advanced PD according to the Hoehn and Yahr staging, and they also took antiparkinsonian drugs that most likely may, as recently reported, alter the HRV values (Camerlingo *et al.*, 1990; Turkka *et al.*, 1997). An additional difference may be caused by the need to exclude ECG periods with abundant artefacts in 24-hour ECG recordings. In Study IV every R-spike in recordings was manually checked and used for the analysis and therefore all events in the HRV were included. The sleep stage scoring and the comprehensive analysis pattern, including e.g. both normalized and absolute spectral measures as recommended by Task Force (1996), also enabled the examination of HRV in more detail than earlier reports.

The increased variance of HR during non-REM sleep seems to be associated with the increased motility of the PD patients during sleep, a phenomenon that has been reported earlier (Trenkwalder, 1998). Furthermore, the hypopnoea events during the S2 sleep of five patients and two of the controls and the PLMS of one patient may also contribute to the increased variance of the HR due to the cardiac arousal (Sforza *et al.*, 2000). Thus in the light of Study IV it seems that in the early phase of PD the cardiovascular system is still able to adapt its essential reactions to the changing internal environmental circumstances.

The normalized HRV spectral measures, which describe the relative distribution of power within the spectral components, are decreased in untreated PD patients as revealed by the diminished standardized daytime cardiovascular reflex measurements (Awerbuch & Sandyk 1992). Similarly, in the present study transient/phasic HRV time domain measure reactions were also decreased in the PD patients during both the non-REM and REM stages, agreeing with an earlier report (Ferini-Strambi *et al.*, 1992). The observations of Study IV also confirm the results of another study with a different methodological approach that revealed decreased heart rate reaction to spontaneous body movements during sleep (Laihinen *et al.*, 1987). All these results show that HRV is decreased in PD.

HRV dynamics may also break down in several pathological conditions, as shown in long-term ECG recordings (Mäkikallio *et al.*, 1996; Korpelainen *et al.*, 1999). Using representative stationary segments of sleep stages, Scholz *et al.* (1997) have demonstrated changes in the balance of cardiovascular autonomic regulation in normal subjects in respect to different sleep stages; the more synchronised sleep is, the more the LF:HF ratio is decreased, whereas the LF:HF is significantly increased during REM sleep. In our study without segmentation this physiological phenomenon was seen in the control subjects but not in the PD patients, indicating disturbed ultradian cardiac autonomic regulation even in the early phase of PD.

It should be remembered that the conditions in which long-term recordings of HRV are performed most likely influence the results. It is well known, for example, that sympathetic activity is increased during wakefulness and physical activity, whereas parasympathetic activity dominates at rest (Hornyak *et al.*, 1991; Karemaker, 1997). There are also clear differences in LF and HF spectral powers and in their ratios during different sleep stages (Vaughn *et al.*, 1995; Scholz *et al.*, 1997). Transient physiological phenomena, such as body movements and K-complexes during sleep, also seem to result in altered HRV (Scholz *et al.*, 1997), as was also found in Study IV. Other internal phenomena also vary with sleep stages; for example, Liguori *et al.* (2000) have shown that spontaneous sympathetic skin responses are most abundant during S4 sleep and lowest during REM sleep.

In addition to physiological conditions clinical features of a disease may also contribute to HRV, as was demonstrated in Study IV by the increased variance of HR in association with nocturnal movements. If only mean HRV values of long-term ECG recordings are calculated, the results represent dominant recording periods, as in 24-hour ECG recordings while awake, and valuable information concerning shorter periods may be lost. Thus, when long-term HRV is evaluated, the recording conditions should be standardized and HRV should be studied separately while the subjects are awake and different sleep stages, as done in Study IV.

7 Conclusions

1. Untreated patients with early PD have cardiovascular ANS regulation failure, although at this early stage of PD the observed changes do not reach significance at the individual level. This failure is pronounced in patients with hypokinesia/rigidity as their initial sign, and thus PD subgroup differentiation may deserve particular attention when the treatment for PD and concomitant diseases is considered.
2. Analysis of HRV during forced respiration and BP response during tilt recognizes abnormal cardiovascular reflexes in a further 44% of Parkinsonian patients compared with clinical evaluation. A simultaneous impairment of HRV and BP control during tilt, as well as orthostatic BP falls >15% below supine level, should question a proposed diagnosis of PSP. Impairment of cardiovascular reflexes was found in the MSA group at all ages and even in the early stages of the disease, whereas in PD patients at only high age and after a long disease duration.
3. Absolute spectral units could reveal impaired cardiovascular autonomic regulation in untreated PD patients most efficiently. However, the traditional time domain measures separated the patients from the healthy controls most efficiently when only normalized spectral units were taken into account. The results emphasize the importance of using both time and frequency domain HRV estimates as a measure of cardiac control.
4. ANS dysfunction was found during sleep in untreated PD patients. The normalized HRV spectral measures were decreased during S4 sleep stage, as earlier shown in the standardized daytime cardiovascular reflexes. Respectively, transient/phasic HR reactions to spontaneous body movements were also decreased during sleep. However, the evaluation of HRV during various sleep stages revealed also new features of cardiovascular regulation. During non-REM sleep, an increase of HR variance in association with increased number of sleep movements was found indicating that in spite of ANS dysfunction in this early stage of PD, the cardiovascular regulation system was still able to react to internal environmental circumstance changes in a suitable manner.

8 Future perspectives

1. Verify peripheral sympathetic autonomic dysfunction with microneurography registrations.
2. Investigate the relationships of the slow oscillations in the EEG, BP, respiration and HRV.
3. Investigate whether patients with hypokinesia as the initial clinical sign have more progressive dysautonomia than patients with tremor as their initial sign.
4. Investigate the effects of medication and disease progression on nocturnal autonomic cardiovascular regulation.

9 References

Akselrod S, Gordon D, Madwed JB, Snidman NC, Shannon DC, Cohen RJ (1985) Hemodynamic regulation: investigation by spectral analysis. Am J Physiol 249: H867-875.

Alihanka J, Vaahtoranta K (1979) A static charge sensitive bed. A new method for recording body movements during sleep. Electroenceph clin Neurophysiol 46: 731-734.

Alihanka J (1982) Sleep movements and associated autonomic nervous activities in young male adults. Academic dissertation. Acta Physiol Scand 511 (Suppl).

Aminoff MJ, Wilcox CS, Woakes MM, Kremer M (1973) Levodopa therapy for Parkinsonism in the Shy-Drager syndrome. J Neurol Neurosurg Psychiatry 36: 350-353.

Appenzeller O, Goss JE (1971) Autonomic deficits in Parkinson's syndrome. Arch Neurol 24: 50-57.

Aserinsky E, Kleitman N (1953) Regularly occurring periods of eye motility, and concomitant phenomena during sleep. Science 118: 273-274.

Awerbuch GI, Sandyk R (1992) Autonomic functions in the early stages of Parkinson's disease. Int J Neurosci 64: 7-14.

Baharav A, Kotagal S, Gibbons V, Rubin BK, Pratt G, Karin J, Akselrod S (1995) Fluctuations in autonomic nervous activity during sleep displayed by power spectrum analysis of heart rate variability. Neurology 45: 1183-1187.

Bannister R, Mathias CJ (1999) Clinical features and evaluation of the primary chronic autonomic failure syndromes. In: Bannister R, Mathias CJ (eds) Autonomic Failure: a textbook of clinical disorders of the autonomic nervous system, 4th edition. Oxford University Press, NY, p 307-316.

Baselli G, Cerutti S, Civardi S, Lombardi F, Malliani A, Merri M, Pagani M, Rizzo G (1987) Heart rate variability signal processing: a quantitative approach as an aid to diagnosis in cardiovascular pathologies. Int J Bio Med Comput 20: 51-70.

Benarroch EE (1993) The Central Autonomic Network: Functional Organization, Dysfunction, and Perspective. Mayo Clin Proc 68: 988-1001.

Benarroch EE (1997) Overview of the organization of the central autonomic network. In: Benarroch EE (ed) Central autonomic network: Functional organization and clinical correlations. Futura Publishing Company, Inc., Armonk, NY, p 3-28.

Bennet T, Femtem PH, Fitton D, Hampton JR, Hoskins DJ, Riggot PA (1977) Assessment of vagal control of the heart in diabetes: measures of R-R interval variation under different conditions. Br Heart J 39: 25-28.

Bennett P, Bonifati V, Bonuccelli U, Colosimo C, De Mari M, Fabbrini G, Marconi R, Meco G, Nicholl DJ, Stocchi F, Vanacore N, Vieregge P, Williams AC (1998) Direct genetic evidence for involvement of tau in progressive supranuclear palsy. European Study Group on Atypical Parkinsonism Consortium. Neurology 51: 982-985.

Berger RD, Akselrod S, Gordon D, Cohen R (1986) An efficiant algorithm for spectral analysis of heart rate variability. IEEE Trans Biomed Eng 33: 900-904.

Berntson GG, Bigger JT, Eckberg DL, Grossman P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH, van der Molen MW (1997) Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology 34: 623-648.

Bianchi AM, Bontempi B, Cerutti S, Gianoglio P, Comi G Natali Sora MG (1990) Spectral analysis of heart rate variability signal and respiration in diabetic subjects. Med Biol Eng Comput 28: 205-211.

Bigger JT, Steinman RC, Rolnitzky LM, Fleiss JL, Albrecht P, Cohen RJ (1996) Power law behaviour of R-R-interval variability in healthy middle-aged persons, patients with recent acute myocardial infarction, and patients with heart transplants. Circulation 93: 2142-2151.

Bonnet MH, Arand DL (1997) Heart rate variability: sleep stage, time of night, and arousal influences. Electroenceph Clin Neurophysiol 102: 390-396.

Bordet R, Benhadjali J, Destee A, Hurtevent JF, Bourriez JL, Gieu JD (1996) Sympathetic skin response and R-R interval variability in multiple system atrophy and idiopathic Parkinson's disease. Mov Disord 11: 268-272.

Box GEP, Jenkins GM (1976) Time series analysis: forecasting and control. Holden-Day, San Francisco.

Braun AR, Balkin TJ, Wesenstein NJ, Carson RE, Varga M, Baldwin P, Selbie S, Belenky G, Herscovitch P (1997) Regional cerebral blood flow throughout the sleep-wake cycle. An H2 15O PET study. Brain 120: 1173-1197.

Brooks DJ (1993) PET studies on the early and differential diagnosis of Parkinson's disease. Neurology 43: 6-16.

Brouwer J, van Veldhuisen DJ, Man int Veld AJ, Haaksma J, Dijk WA, Visser KR, Boomsma F, Dunselman PHJM, Lie KI (1996) Prognostic value of heart rate variability during long-term follow-up in patients with mild to moderate heart failure. J Am Coll Cardiol 28: 1183-1189.

Burnstock G (1986) Autonomic neuroefector junctions: current developments and future directions. J Anat 146: 1-30.

Calne DB, Brennan J, Spiers AS, Stern GM (1970) Hypotension caused by L-dopa. Br Med J 21: 474-475.

Camerlingo M, Aillon C, Bottachi E, Gambaro P, D'Alessandro G, Franceschi M, Devoti G, Mamoli A (1986) Parasympathetic assessment in Parkinson's disease. Adv Neurol 45: 267-269.

Camerlingo M, Ferraro B, Gazzaniga GC, Casto L, Cesana BM, Mamoli A (1990) Cardiovascular reflexes in Parkinson's disease: long-term effects of levodopa treatment on de novo patients. Acta Neurol Scand 81: 346-348.

Carlsson A, Lindqvist M, Magnusson T (1957) 3,4-Dihydroxyphenylalanine and 5-hydroxytyramine as reserpine antagonists. Nature 180: 1200-1202.

Cohen J, Low P, Fealey RD (1987) Somatic and autonomic function in progressive autonomic failure and multiple system atrophy. Ann Neurol 22: 692-699.

Cotzias CG, Van Woert MH, Schiffer LM (1967) Aromatic amino acids and modification of Parkinsonism. N Engl J Med 276: 374-379.

Consensus statement on the definition of orthostatic hypotension, pure autonomic failure, and multiple system atrophy (1996) Neurology 46: 1470.

Crick SJ, Sheppard MN, Anderson RH (2000) Neural supply of the heart. In: Horst TJ (ed) The nervous system and the heart. Human Press Inc. Totowa, New Jersey, p 3-54.

Daniel SE, Lees AJ (1993) Parkinson's Disease Society Brain Bank, London: overview and research. J Neural Transm. 39(suppl): S165-172.

Daniel SE (1999) The neuropathology and neurochemistry of multiple system atrophy. In: Mathias CJ, Bannister R (eds) Autonomic failure: a textbook of the autonomic nervous system. 4th edition, Oxford University Press, NY, p 321-328.

Deuschl G, Eisen A (eds) (1999) Recommendations for the Practice of Clinical Neurophysiology: Guidelines of the International Federation of Clinical Neurophysiology. Elsevier, Amsterdam.

Diehl RR, Linden D, Berlit P (1997) Determinants of heart rate variability during deep breathing: basic findings and clinical applications. Clin Auton Res 7: 131-135.

Donchin Y, Feld JM, Porges, SW (1985) Respiratory sinus arrhythmia during recovery from isoflurane-nitrous oxide anesthesia. Anesthesia and Analgesia 64:811-815.

Eardley I, Quinn NP, Fowler CJ, Kirby RS, Parkouse HF, Marsden CD, Bannister R (1989) The value of urethral sphincter electromyography in the differential diagnosis of Parkinsonism. Br J Urol 64: 360-362.

Eckberg DL (1983) Human sinus arrhythmia as an index of vagal cardiac outflow. J Appl Physiol 54: 961-966.

Eckberg DL (1997) Sympathovagal balance: a critical appraisal. Circulation 96: 3224-3232.

Ehringer H, Hornykiewitz O (1960) Verteilung von Noradrenaline und Dopamin (3-hydroxytyramin) im Gehirn des Menchen und ihr Verhalten bei Erkrankerung des extrapyramidalen Systems. Klin Wochenschr 38: 1236-1239.

Ewing DJ (1992) Analysis of heart rate variability and other non-invasive tests with special reference to diabetic mellitus. In: Bannister R, Mathias CJ, (eds) Autonomic failure. A textbook of clinical disorders of the autonomic nervous system. 3rd edition. Oxford University Press, NY, p 312-333.

Ewing DJ, Neilson JMM, Travis P (1984) New method for assessing cardiac parasympathetic activity using 24 hour electrocardiograms. Brit Heart J 52: 396-402.

Ewing DJ (1988) Recent advances in the non-invasive investigation of diabetic autonomic neuropathy. In: Bannister R (ed) Autonomic failure. A textbook of clinical disorders of the autonomic nervous system. Oxford University Press, NY, p 667-689.

Fahn S, Elton RL, and the members of the UPDRS Development Committee (1987) UPDRS: unified Parkinson's Disease Rating Scale in recent development in Parkinson's disease. In: Fahn S, Marsden CD, Calne DB, Goldstein M (eds) Macmillan Health Care Information. Florham Park, NJ, p 153-164.

Farrell TG, Paul V, Cripps TR, Malik M, Bennett ED, Ward D, Camm AJ (1991) Baroreflex sensitivity and electrophysiological correlates in patients after acute myocardial infarction. Circulation 83: 945-952.

Fearnley JM, Lees AJ (1991) Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 114: 2283-2301.

Ferguson AV (1992) Neurophysiological analysis of mechanisms for subfornical organ and area postrema involvement in autonomic control. Prog Brain Res 91: 413-421.

Ferini-Strambi L, Franceschi M, Pinto P, Zucconi M. Smirne S (1992) Respiration and heart rate variability during sleep in untreated Parkinson patients. Gerontology 38: 92-98.

Ferini-Strambi L, Rovaris M, Oldani A, Martinelli V, Filippi M, Smirne S, Zucconi M, Comi G (1995) Cardiac autonomic function during sleep and wakefulness in multiple sclerosis. J Neurol 242: 639-643.

Ferini-Strambi L, Smirne S (1997) Cardiac autonomic function during sleep in several neuropsychiatric disorders. J Neurol 244(Suppl 1): S29-36.

Ferini-Strambi L, Bianchi A, Zucconi M, Oldani A, Castronovo V, Smirne S (2000) The impact of cyclic alternating pattern on heart rate variability during sleep in healthy young adults. Clin Neurophysiol 111: 99-101.

Franceschi M, Ferini-Strambi L, Minicucci F, Sferrazza-Papa A, Smirne S (1986) Signs of cardiac autonomic dysfunction during sleep in patients with Alzheimer's disease. Gerontology 32: 327-334.

Freeman R (1997) Noninvasive evaluation of heart rate variability. The time domain. In: Low PA, (ed) Clinical Autonomic Disorders, 2nd edition. Lippincott-Raven Publishers, Philadelphia, p 297-307.

Freeman R, Saul JP, Roberts MS, Berger RD (1991) Spectral analysis of heart rate in diabetic autonomic neuropathy. A comparison with standard tests of autonomic function. Arch Neurol 48: 185-190.

Gibb WR, Lees AJ (1988) A comparison of clinical and pathological features of young- and old-onset Parkinson's disease. Neurology 38: 1402-1406.

Gibb WR, Lees AJ (1991) Anatomy, pigmentation, ventral and dorsal subpopulations of the substantia nigra, and differential cell death in Parkinson's disease. J Neurol Neurosurg Psychiatry 54: 388-396.

Gilman S, Low PA, Quinn N, Albanese A, Ben-Shlomo Y, Fowler CJ, Kaufmann H, Klockgether T, Lang AE, Lantos PL, Litvan I, Mathias CJ, Oliver E, Robertson D, Schatz I, Wenning GK (1999) Consensus statement on the diagnosis of multiple system atrophy. J Neurol Sci 163: 94-98.

Goetz CG, Lutge W, Tanner CM (1986) Autonomic dysfunction in Parkinson's disease. Neurology 36: 73-75.

Golbe L, Davis P (1993) Progressive Supranuclear Palsy. In: Parkinson's Disease and Mov Disord. Williams and Wilkins, p 145-161.

Goldberger AL, Rigney DR, West BJ (1990) Chaos and fractals in human physiology. Sci Am 262: 42-49.

Goldberger AL (1996) Non-linear dynamics for clinicians: chaos theory, fractals, and the complexity at the bedside. Lancet 347: 1312-1314.

Goldstein DS, Holmes C, Cannon III RO, Eisenhofer G, Kopin IJ (1997) Sympathetic cardioneuropathy in dysautonomias. New Engl J Med 336: 696-702.

Gross M, Bannister R, Godwin-Austen R (1972) Orthostatic hypotension in Parkinson's disease. Lancet 1: 174-176.

Gutrecht JA (1992) Autonomic cardiovascular reflexes in progressive supranuclear palsy. J Auton Nerv Syst 39: 29-35.

Haapaniemi TH, Kallio MA, Korpelainen JT, Suominen K, Tolonen U, Sotaniemi KA, Myllyla VV (2000) Levodopa, bromocriptine and selegiline modify cardiovascular responses in Parkinson's disease. J Neurol 247: 868-874.

Haapaniemi TH, Pursiainen V, Korpelainen JT, Huikuri HV, Sotaniemi KA, Myllylä VV (2001) Ambulatory ECG and analysis of heart rate variability in Parkinson's disease. J Neurol Neurosurg Psychiatry 70: 305-310.

Hagbart KE, Vallbo ÅB (1968) Pulse and respiratory grouping of sympathetic impulses in human peripheral nerves. Acta Physiol Scand 74: 96-108.

Hagbart KE, Hallin RG, Hongell A, Torebjörk HE, Wallin BG (1972) General characteristics of sympathetic activity in human skin nerves. Acta Physiol Scand 84: 164-176.

Havanka-Kanniainen H, Tolonen U, Myllylä VV (1988) Autonomic dysfunction in migraine: a survey of 188 patients. Headache 28: 465-470.

Hauw JJ, Daniel SE, Dickson D, Horoupian DS, Jellinger K, Lantos PL, McKee A, Tabaton M, Litvan I (1994) Preliminary NINDS neuropathology criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). Neurology 44: 2015-2019.

Hakusui S, Yasuda T, Yanagi T, Takahashi A, Hasegawa Y, Inoue M (1994) A radiological analysis of heart sympathetic function with meta-[(123)I] iodobenzylguanidine in neurological patients with autonomic failure. Auton Nerv Syst 32: 384-390.

Hayano J, Sakakibara Y, Yamada A, Yamada M, Mukai S, Fuhinami T, Fujinami T, Yokoyama K, Watanabe Y, Takata K (1991) Accuracy of assessment of cardiac heart rate variability in normal subjects. Am J Cardiol 67: 199-204.

Hirsch JA, Bishop B (1981) Respiratory sinus arrhythmia in humans: how breathing pattern modulates heart rate. Am J Physiol 241: H620-629.

Ho KKL, Moody GB, Peng C-K, Mietus JE, Larson MG, Levy D, Goldberger AL (1997) Predicting survival in heart failure case and control subjects by use of fully automated methods for deriving nonlinear and conventional indices of heart rate dynamics. Circulation 96: 842-848.

Hoehn MM, Yahr MD (1967) Parkinsonism: onset, progression, and mortality. Neurology 17: 427-442.

Holmberg B, Rosengren L, Karlsson JE, Johnels B (1998) Increased cerebrospinal fluid levels of neurofilament protein in progressive supranuclear palsy and multiple system atrophy compared to Parkinson's disease. Mov Disord 13: 70-77.

Hornyak M, Cejnar M, Elam M, Matousek M, Wallin BG (1991) Sympathetic muscle nerve activity during sleep in man. Brain 114: 1281-1295.

Hoyer D, Kaplan D, Palus M, Pompe B, Seidel H (1998) New systems-analytical approaches to non-linear coordination. IEEE Eng Med Biol 17: 58-61.

Hughes AJ, Daniel SE, Kilford L, Lees AJ (1992) Accuracy of clinical diagnosis of idiopathic Parkinsonism - a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 55: 181-184.

Hughes AJ, Daniel SE, Kilford L, Lees AJ (1993) A clinicopathologic study of 100 cases of Parkinson's disease. Arch Neurol 50: 140-148.

Huikuri HV, Mäkikallio TH, Airaksinen KEJ, Seppänen T, Puukka P, Räihä IJ, Sourander LB (1998) Power-law relationship of heart rate variability as a predictor of mortality in the elderly. Circulation 97: 2031-2036.

Huikuri HV, Seppänen T, Koistinen MJ, Airaksinen KEJ, Ikäheimo MJ, Castellanos A, Myerburg RJ (1996) Abnormalities in beat-to-beat dynamics of heart rate before the spontaneous onset of life-threatening ventricular tachyarrhytmias in patients with prior myocardial infarction. Circulation 93: 1836-1844.

Huikuri HV (1995) Heart rate variability in coronary artery disease. J Int Med 237: 349-357.

Isojärvi JI, Ansakorpi H, Suominen K, Tolonen U, Repo M, Myllylä VV (1998) Interictal cardiovascular autonomic responses in patients with epilepsy. Epilepsia 39: 420-426.

Jellinger K (1987) The pathology of parkinsonism. In: Marsden CD, Fahn S (eds), Movement disorder 2. Butterworths, London, p 124-165.

Jellinger KA (1991) Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. Mol Chem Neuropathol 14: 153-197.

Johnels B, Ingvarsson PE, Holmberg B, Matousek M, Steg G (1993) Single dose l-dopa response in early Parkinson's disease: measurements with optoelectronic recording technique. Mov Disord 8: 56-62.

Johnson RH & Robinson BJ (1988) Mortality in alcoholics with autonomic neuropathy. J Neurol Neurosurg Psychiatry 51: 476-480.

Karemaker JM (1997) Analysis of blood pressure and heart rate variability. Theoretical considerations. In: Low PA, (ed) Clinical Autonomic Disorders, 2nd edition. Lippincott-Raven Publishers, Philadelphia, p 309-322.

Katona PG & Jih F (1975) Respiratory sinus arrhythmia: non-invasive measure of parasympathetic cardiac control. J Appl Physiol 39: 801-805.

Kay SM, Marple SL (1981) Spectrum analysis: a modern perspective. Proc IEEE 69: 1380-1418.

Kimber J, Mathias CJ, Lees AJ, Bleasdale-Barr K, Chang HS, Churchyard A, Watson L (2000) Physiological, pharmacological and neurohormonal assessment of autonomic function in progressive supranuclear palsy. Brain 123: 1422-1430.

Kingwell BA, Thompson JM, Kave DM, McPherson GA, Jennings GL, Esler MD (1994) Heart rate spectral analysis, cardiac norepinephrine spillover, and muscle sympathetic nerve activity during human sympathetic nervous activation and failure. Circulation 90: 234-240.

Kleiger RE, Miller JP, Bigger JT Jr, Moss AJ (1987) Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 59: 256-262.

Klein C, Brown R, Wenning G, Quinn N (1997) The "cold hands sign" in multiple system atrophy. Mov Disord 12: 514-518.

Koike Y, Takahashi A (1997) Autonomic dysfunction in Parkinson's disease. Eur Neurol 38 (Suppl.2): S8-12.

Kollai M & Mizsei G (1990) Respiratory sinus arrhythmia is a limited measure of cardiac parasympathetic control in man. J Physiol (Lond) 424: 329-342.

Korpelainen JT, Sotaniemi KA, Myllylä VV (1993) Asymmetric sweating in stroke: a prospective quantitative study of patients with hemispheral brain infarction. Neurology 43: 1211-1214.

Korpelainen JT, Sotaniemi KA, Suominen K, Tolonen U, Myllylä VV (1994) Cardiovascular autonomic reflexes in brain infarction. Stroke 25: 787-792.

Korpelainen JT, Sotaniemi KA, Huikuri HV, Myllylä VV (1996) Abnormal heart rate variability as a manifestation of autonomic dysfunction in hemispheric brain infarction. Stroke 27: 2059-2063.

Korpelainen JT, Sotaniemi KA, Mäkikallio A, Huikuri HV, Myllylä VV (1999) Dynamic behaviour of heart rate in ischemic stroke. Stroke 30: 1008-1013.

Laihinen A, Alihanka J, Raitasuo S, Rinne UK (1987) Sleep movements and associated autonomic nervous activities in patients with Parkinson's disease. Acta Neurol Scand 76: 64-68.

Lantos PL (1994) The neuropathology of progressive supranuclear palsy. J Neural Transm Suppl 42: 137-152.

Lees AJ (1987) The Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). In: Marsden CD, Fahn S (eds) Movement disorders 2. Butterworths, London, p 272-287.

Liguori R, Donadio V, Foschini E, Di Stasi V, Plazzi G, Lugaresi E, Montagna P (2000) Sleep stage-related changes in sympathetic sudomotor and vasomotor skin responses in man. Clin Neurophysiol 111: 434-439.

Linden D, Diehl RR (1996) Comparison of standard autonomic tests and power spectral analysis in normal adults. Muscle Nerve 19: 556-562.

Linden D, Diehl RR, Berlit P (1998) Reduced baroreflex sensitivity and cardiorespiratory transfer in amyotrophic lateral sclerosis. Electroencephalogr Clin Neurophysiol 109: 387-390.

Lindqvist A, Parviainen P, Kolari P, Tuominen P, Tuominen J, Välimäki I, Antila K, Laitinen LA (1989) A non-invasive method for testing neural circulatory control in man. Cardiovasc Res 23: 262-272.

Lisenby MJ, Richardson PC, Welch AJ (1976) Detection of cyclic sleep phenomena using instantaneous heart rate. Electroenceph Clin Neurophysiol 40: 169-177.

Litvan I, Agid Y, Calne D, Campbell G, Dubois B, Duvoisin RC, Goetz CG, Golbe LI, Grafman J, Growdon JH, Hallett M, Jankovic J, Quinn NP, Tolosa E, Zee DS (1996) Clinical Research criteria for the diagnosis of progressive supranuclear palsy (Steele -Richardson- Olszewski syndrome): Report of the NINDS-SPSP International Workshop. Neurology 47: 1-9.

Litvan I, Campbell G, Mangone CA, Verny M, McKee A, Chaudhuri KR, Jellinger K, Pearce RK, D'Olhaberriague L (1997) Which clinical features differentiate progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome) from related disorders? A clinicopathological study. Brain 120: 65-74.

Loewy AD, Spyer KM (1990) Vagal preganglionic neurons. In: Loewy AD, Spyer KM (eds) Central regulation of autonomic functions. Oxford University Press, NY, p 68-85.

Loewy AD (1990) Central autonomic pathways. In: Loewy AD, Spyer KM (eds) Central regulation of autonomic functions. Oxford University Press, NY, p 88-103.

Lowe J, Lennox G, Leugh PN (1997) Disorders of movement and system degenerations. In: Graham DI & Lantos P (eds) Greenfields neuropathology 6th edition. E Arnold, London, p 280-366.

Ludin SM, Steiger UH, Ludin HP (1987) Autonomic disturbances and cardiovascular reflexes in idiopathic Parkinson's disease. J Neurol 235: 10-15.

Madwed JB, Albrecht P, Mark RG, Cohen RJ (1989) Low-frequency oscillations in arterial pressure and heart rate: a simple computer model. Am J Physiol 256: H1573-1579.

Malik M, Farrell T, Cripps TR, Camm AJ (1989) Heart rate variability in relation to prognosis after myocardial infarction: selection of optimal processing techniques. Eur Heart J 10: 1060-1074.

Mancia G (1993) Autonomic modulation of the cardiovascular system during sleep. N Engl J Med 328: 347-349.

Mandelbrot BB (1982) The fractal geometry of nature. W.H. Freeman and Company, New York.

Marttila R (1974) Epidemiological, clinical, and virus-serological studies of Parkinson's disease. Academic dissertation. University of Turku, Finland.

Mastrocola C, Vanacore N, Giovani A, Locuratolo N, Vella C, Alessandri A, Baratta L, Tubani L, Meco G (1999) Twenty-four-hour heart rate variability to assess autonomic function in Parkinson's disease. Acta Neurol Scand 99: 245-247.

Mathias CJ (1996) Disorders affecting autonomic function in Parkinsonian patients. Adv Neurol 69: 383-391.

Mathias CJ (1997) Autonomic disorders and their recognition (editorial). N Engl J Med 336: 721-724.

McCabe PM, Yongue BG, Ackles PK, Porges SW (1985) Respiratory modulation of baroreceptor and chemoreceptor reflexes affecting heart rate and cardiac vagal efferent nerve activity. Psychophysiology 22: 195-203.

Mäkikallio TH, Seppänen T, Niemelä M, Airaksinen KEJ, Tulppo M, Huikuri HV (1996) Abnormalities in beat to beat complexity of heart rate dynamics in patients with previous myocardial infarction. J Am Coll Cardiol 28: 1005-1011.

Mäkikallio TH, Seppänen T, Airaksinen KE, Koistinen J, Tulppo MP, Peng CK, Goldberger AL, Huikuri HV (1997) Dynamic analysis of heart rate may predict subsequent ventricular tachycardia after myocardial infarction. Am J Cardiol; 80: 779-783.

Mäkikallio TH, Ristimäe T, Airaksinen KE, Peng CK, Goldberger AL, Huikuri HV (1998) Heart rate dynamics in patients with stable angina pectoris and utility of fractal and complexity measures. Am J Cardiol; 81: 27-31.

Myers GA, Martin GJ, Magid MN, Barnett PS, Schaad JW, Weiss JS, Lesch M, Singer DH (1986) Power spectral analysis of heart rate variability in sudden death: comparison to other methods. IEEE T Bio Med Eng 33: 1149-1156.

Muzet A, Naitoh, P, Townsend BE, Johnson LC (1972) Body movements during sleep as a predictor of stage change. Psycho Sci 29:7-10.

Myllylä VV, Korpelainen JT, Tolonen U, Havanka H, Saari A (2000) Neuropathology and cardiovascular regulation. In: GJ Ter Horst (ed) The nervous system and the heart. Human Press Inc. Totowa, NJ, p 181-237.

Naver HK (1996) Dysfunction of the autonomic nervous system after stroke. Academic dissertation, Göteborg University, Sweden.

Niimi Y, Ieda T, Hirayama M, Koike Y, Sobue G, Hasegawa Y, Takahashi A (1999) Clinical and physiological characteristics of autonomic failure with Parkinson's disease. Clin Auton Res 9: 139-144.

Noll G, Elam M, Kunimoto M, Karlsson T, Wallin BG (1994) Skin sympathetic nerve activity and effector function during sleep in humans. Acta Physiol Scand 151: 319-329.

Nordin M (1990) Sympathetic discharges in the human supraorbital nerve and their relation to sudo- and vasomotor responses. J Physiol 423: 241-255.

Okada H, Iwase S, Mano T, Sugiyama Y, Watanabe T (1991) Changes in muscle sympathetic nerve activity during sleep in humans. Neurology 41: 1961-1966.

Omboni S, Parati G, Di Rienzo M, Wieling W, Mancia G (1996) Blood pressure and heart rate variability in autonomic disorders: a critical review. Clin Auton Res 6: 171-182.

Orimo S, Ozawa E, Nakade S, Sugimoto T, Mizusawa H (1999) (123)I-metaiodobenzylguanidine myocardial scintigraphy in Parkinson's disease. J Neurol Neurosurg Psychiatry 67: 189-194.

Oswald I (1959) Sudden bodily jerks on falling asleep. Brain 82: 92-103.

Palace J, Chandiramani VA, Fowler CJ (1997) Value of sphincter electromyography in the diagnosis of multiple system atrophy. Muscle Nerve 20: 1396-1403.

Papp MI, Lantos PL (1994) The distribution of oligodendroglial inclusions in multiple system atrophy and its relevance to clinical symptomatology. Brain 117: 235-243.

Parkinson James (1817) An essay on the shaking palsy. Sherwood, Neely and Jones, London.

Parmeggiani PL (1994) The autonomic nervous system in sleep. In: Kryger MH, Roth T, Dement WC (eds) Principles and practice of sleep medicine. WB Saunders, Philadelphia, p 194-203.

Peleg S, Naor J, Hartley R, Avnir D (1984). Multiple resolution texture and classification. IEEE Trans Pattern Anal Machine Intell 6: 518-523.

Peng CK, Havlin S, Stanley HE, Goldberger AL (1995) Quantification of scaling exponents and crossover phenomenon in nonstationary heart beat series. Chaos 5: 82-87.

Piha SJ (1988) Cardiovascular autonomic function tests. Responses in healthy subjects and determination of the age-related reference values. Academic dissertation. Publications of the social insurance institution, ML:85, Finland.

Piha SJ, Rinne JO, Rinne UK, Seppänen A (1988) Autonomic dysfunction in recent onset and advanced Parkinson's disease. Clin Neurol Neurosurg 90: 221-226.

Piha SJ (1993) Cardiovascular responses to various autonomic tests in males and females. Clin Auton Res 3: 15-20.

Pikkujämsä S (1999) Heart rate variability and baroreflex sensitivity in subjects without heart disease. Effects of age, sex and cardiovascular risk factors. Academic dissertation. Acta Univ Oul. D 520. University of Oulu, Finland.

Pincus SM, Goldberger AL (1994) Physiologic time-series analysis: what does regularity quantify? Am J Physiol 226: H1643-1656.

Pincus SM (1991) Approximate entropy as a measure of system complexity. Proc Natl Acad Sci USA 88: 2297-2301.

Pisano F, Miscio G, Mazzureo G, Lantranchi P, Colombo R, Pinelli P (1995) Decreased heart rate variability in amyotrophic lateral sclerosis. Muscle Nerve 18: 1225-1231.

Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ et al. (1985) Assessment of autonomic function in humans by heart rate spectral analysis. Am J Physiol 248: H151-153.

Porges SW (1985) Method and apparatus for evaluating rhytmic oscillations in aperiodic physiological response systems. United States Patent No. 4,510,9444. April 16.

Pramstaller PP, Wenning GK, Smith SJ, Beck RO, Quinn NP, Fowler CJ (1995) Nerve conduction studies, skeletal muscle EMG, and sphincter EMG in multiple system atrophy. J Neurol, Neurosurg Psychiatry 58: 618-621.

Quinn NP (1989) Multiple System Atrophy-the nature of the beast. J Neurol Neurosurg Psychiatry Special Supplement: 78-89.

Ravits J, Hallet M, Nilsson J, Polinsky R, Dambrosia J (1995) Electrophysiological tests of autonomic function in patients with idiopathic autonomic failure syndromes. Muscle Nerve 19: 758-763.

Ravits JM (1997) Autonomic nervous system testing. Muscle Nerve 20: 919-937.

Rechtschaffen A, Kales A (1968) A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Washington, DC: Public Health Service, US Government Printing Office.

Richter DW, Spyer KM (1990) Cardiorespiratory control. In: Loewy AD & Spyer KM (eds) Central regulation of autonomic functions. Oxford University Press, Oxford, p 189-207.

Rosenbaum M, Race D (1968) Frequency-response characteristics of vascular resistance vessels. Am J Physiol 215: 1397-1402.

Ruberg M, Hirsch E, Javoy-Agid F (1993) Neurochemistry. In: Litvan I, Agid Y (eds) Progressive Supranuclear Palsy. Clinical and research approaches. Oxford University Press, Oxford, p 89-109.

Sachs C, Berglund B, Kaijser L (1985) Autonomic cardiovascular responses in parkinsonism: effect of levodopa with dopa-decarboxylase inhibition. Acta Neurol Scand 71: 37-42.

Saul JP, Albrecht P, Berger RD, Cohen RJ (1987) Analysis of long-term heart rate variability: methods, 1/f scaling and implications. In: Computers in Cardiology. Silver Spring. Md: IEEE Computer Society Press, p 419-422.

Saul JP, Rea RF, Eckberg DL, Berger RD, Cohen RJ (1990) Heart rate and muscle sympathetic nerve variability during reflex changes of autonomic activity. Am J Physiol 258: H713-721.

Scholz UJ, Bianchi AM, Cerutti S, Kubicki S (1993) Monitoring of the vegetative backround of sleep by means of spectral analysis of heart rate variability. Proc IEEE Conf Comp in Cardiol, London, p 321-324.

Scholz UJ, Bianchi AM, Cerutti S, Kubicki S (1997) Vegetative background of sleep: spectral analysis of the heart rate variability. Physiol Behav 62: 1037-1043.

Schwarz J, Kornhuber M, Bischoff C, Straube A (1997) Electromyography of the external anal sphincter in patients with Parkinson's disease and multiple system atrophy: frequency of abnormal spontaneous activity and polyphasic motor unit potentials. Muscle Nerve 20: 1167-1172.

Šega S, Jager F, Kiauta T (1993) A comparison of cardiovascular reflex tests and spectral analysis of heart rate variability in healthy subjects. Clin Auton Res 3: 175-182.

Sforza E, Jouny C, Ibanez V (2000) Cardiac activation during arousal in humans: further evidence for hierarchy in the arousal response. Clin Neurophysiol 111: 1611-1619.

Shahani BT, Day TJ, Cros D, Khaill N, Kneebone CS (1990) RR interval variation and the sympathetic skin response in the assessment of autonomic function in peripheral neuropathy. Arch Neurol 47: 659-664.

Smirne S, Ferini-Strambi L, Zucconi M, Pinto P, Franceschi M (1990) Cardiac autonomic dysfunction during sleep in some neurological diseases. Neurophysiol Clin 20: 131-136.

Smith ML, Thames MD (1994) Cardiac receptors: discharge characteristics and reflex effects. In: Armour JA, Ardell JL (eds) Neurocardiology, Oxford University Press, NY, p 19-52.
Solders G, Persson A, Gutierrez A (1985) Autonomic dysfunction in non-diabetic terminal uraemia. Acta Neurol Scand 71: 321-327.
Somers VK, Dyken ME, Mark AL, Abboud FM (1993) Sympathetic-nerve activity during sleep in normal subjects. N Engl J Med 328: 303-307.
Spiegel EA, Wycis HT, Schor S, Schwartz HA, Fabioni FR (1969) The incidence of vegetative symptoms in Parkinson patients with and without bradykinesia. In: Gillingham FJ, Donaldson TMC (eds) Third symposium on Parkinson's disease. Livingstone, Edinburgh, p 200.
Suominen K (1997) Design and implementation of a PC-based data acquisition system for measuring ECG and respiratory signals. Int J Clin Monit Com 14: 225-230.
Taki J, Nakajima K, Hwang EH, Matsunari I, Komai K, Yoshita M, Sakajiri K, Tonami N (2000) Peripheral sympathetic dysfunction in patients with Parkinson's disease without autonomic failure is heart selective and disease specific. Eur J Nucl Med 27: 566-573.
Takeuchi S, Iwase S, Mano T, Okada H, Sugiyama Y, Watanabe T (1994) Sleep-related changes in human muscle and skin sympathetic nerve activities. J Auton Nerv System 47: 121-129.
Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996) Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Circulation 93: 1043-1065.
Terzano MG, Mancia D, Salati MR, Costani G, Decembrino A, Parrino L (1985) The cyclic alternating pattern as a physiological component of normal NREM sleep. Sleep 8: 137-145.
Townsend RE, Johnson LC, Naitoh P, Muzet AG (1975) Heart rate preceding motility in sleep. Psychophysiology 12: 217-219.
Trenkwalder C (1998) Sleep dysfunction in Parkinson’s disease. Clin Neurosci 5: 107-114.
Tuck RR & McLeod JG (1981) Autonomic dysfunction in Guillain-Barré syndrome. J Neurol Neurosurg Psychiatry 44: 983-990.
Tulppo MJ, Mäkikallio TH, Takala TES, Seppänen T, Huikuri HV (1996) Quantitative beat-to-beat analysis of heart rate dynamics during exercise. Am J Physiol 271: H244-252.
Turkka J, Suominen K, Tolonen U, Sotaniemi K and Myllylä V (1997) Selegiline diminishes cardiovascular autonomic responses in Parkinson’s disease. Neurology 48: 662-667.
Turkka J (1986) Autonomic dysfunction in Parkinson’s disease. Academic dissertation, Acta Universitatis Ouluensis, Series D Medica No. 142, Oulu, Finland.
Turkka JT (1987) Correlation of autonomic dysfunction to CSF concentrations of Noradrenaline and 3-methoxy-4hydroxyphenylglycol in Parkinson’s disease. Eur Neurol 26: 29-34.
Turkka JT, Tolonen U, Myllylä VV (1987) Cardiovascular reflexes in Parkinson’s disease. Eur Neurol 26: 104-112.
Vallbo Å, Hagbart K-E, Torebjörk HE, Wallin BG (1979) Somatosensory, proprioceptive and sympathetic activity in human peripheral nerves. Physiol Rev 59: 919-957.
Valldeoriola F, Valls-Sole J, Tolosa ES, Marti MJ (1995) Striated anal sphincter denervation in patients with progressive supranuclear palsy. Mov Disord 10: 550-555.
Van Dijk JG, Haan J, Koenderik M, Roos RA (1991) Autonomic nervous function in progressive supranuclear palsy. Arch Neurol 48: 1083-1084.
Van Dijk JG, Haan J, Zwinderman K, Kremer B, van Hilten BJ, Roos RAC (1993) Autonomic nervous system dysfunction in Parkinson’s disease: Relationship with age, medication, duration, and severity. J Neurol Neurosurg Psychiatry 56: 1090-1095.
Vanoli E, Adamson PB, Ba-Lin, Pinna GD, Lazzara R, Orr WC (1995) Heart rate variability during specific sleep stages. A comparison of healthy subjects with patients after myocardial infarction. Circulation 91: 1918-1922.
Vargas E & Lye M (1980) The assessment of autonomic function in the elderly. Age Ageing 9: 210-214.
Vaughn BV, Quint SR, Messenheimer JA, Robertson KR (1995) Heart period variability in sleep. Electroenceph Clin Neurophysiol 94: 155-162.
Vita G, Princi P, Calabro R, Toscana A, Manna L, Messina C (1986) Cardiovascular reflex tests. Assessment of age-adjusted normal range. J Neurol Sci 75: 263-274.

Wallin BG, Elam M (1997) Microneurography and Autonomic Dysfunction. In: Low PA (ed) Clinical Autonomic Disorders, Evaluation and Management. 2nd edition, Lippincort-Raven, Philadelphia p 243-252.

Wang SJ, Liao KK, Liou HH, Lee SS, Tsai CP, Link P, Kao KP, Wu ZA (1994) Sympathetic skin response and R-R interval variation in chronic uremic patients. Muscle Nerve 17: 411-418.

Wenning GK, Quinn N, Magalhaes C, Mathias C, Daniel SE (1994) "Minimal change" Multiple System Atrophy. Mov Disord 9: 161-166.

Wenning GK, Tison F, Ben Shlomo, Daniel SE, Quinn NP (1997) Multiple system atrophy: A review of 203 pathologically proven cases. Mov Disord 12: 133-147.

Wenning GK, Scherfler C, Granata R, Bosch S, Verny M, Chaudhuri KR, Jellinger K, Poewe W, Litvan I (1999) Time course of symptomatic orthostatic hypotension and urinary incontinence in patients with postmortem confirmed parkinsonian syndromes: a clinicopathological study. J Neurol Neurosurg Psychiatry 67: 620-623.

Wenning GK, Ben Shlomo Y, Hughes A, Daniel SE, Lees A, Quinn NP (2000) What clinical features are most useful to distinguish definite multiple system atrophy from Parkinson's disease? J Neurol Neurosurg Psychiatry 68: 434-440.

Wheeler T, Watkins PH (1973) Cardiac denervation in diabetes. BMJ 4: 584-586.

Wieling W, Karemaker JM (1999) Measurement of heart rate and blood pressure to evaluate disturbances in cardiovascular control. In: Mathias CJ, Bannister R (eds) Autonomic failure. A textbook of clinical disorders of the autonomic nervous system. 4th edition. Oxford University Press, NY, p 196-210.

Willis W D Jr (1993) The Autonomic Nervous System and its Central Control. In: Berne RM, Levy MN (eds) Physiology, 3rd edition. Mosby-Year Book, St Louis, p 244-259.

Woo MA, Stevenson WG, Moser DK, Middlekauff HR (1994) Complex heart rate variability and serum norepinephrine levels in patients with advanced heart failure. J Am Coll Cardiol 1; 23: 565-569.

Xie A, Skatrud JB, Puelo DS, Morgan BJ (1999) Arousal from sleep shortens sympathetic burst latency in humans. J Physiol 515: 616-628.

Yebenes J Sarasa J, Daniel SE, Lees A (1995) Familiar progressive supranuclear palsy – description of pedigree and review of literature. Brain 118: 1095-1103.

Young A (1985) Progressive supranuclear palsy: postmortem chemical analysis. Ann Neurol 18: 521-522.

Zemaityte D, Varoneckas G, Sokolov E (1984) Heart rhythm control during sleep. Psychophysiol 21: 279-289.

Zetterberg LH (1969) Estimation parameters for a linear difference equation with application to EEG analysis. Math Biosci 5: 227-275.

Zwiener U, Hoyer D, Lüthke B, Schmidt K, Bauer R (1996) Relations between parameters of spectral power densities and deterministic chaos of heart-rate variability. J Autonom Nerv Syst 57: 132-135.