BLOOD PRESSURE COMPLEXITY IN PRIMATE PREGNANCY

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The one thing all maps do is provide readers with orientation. Mapmaking is not simply stacking data on top of data. There is an art and a science to how cartographers prioritise and integrate the data. The interaction between the layers is the story of the map. It is the narrative structure of what we see when we read a map. The layers tell us the hierarchy of what is the most important.

Kirk Goldsberry

STATEMENT OF ORIGINALITY

This thesis is the result of original research conducted by the candidate whilst enrolled for the degree of Doctor of Philosophy with Sydney Medical School, Faculty of Medicine and Health at The University of Sydney. It has not been previously submitted, in part or whole, to any university or institution for any degree, diploma, or other qualification.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

AUTHORSHIP ATTRIBUTION STATEMENT

This thesis contains material I have previously published in collaboration with my supervisors, Professor Annemarie Hennessy and Professor Angela Makris. Section 2.3 of this thesis is published as Pears S, Makris A, Hennessy A. The chronobiology of blood pressure in pregnancy. Pregnancy Hypertens. 2018 Apr; 12:104-109. I reviewed the literature and wrote the drafts of the review article.

As a supervisor for the candidature upon which this thesis is based, I can confirm that the authorship attribution statement above is correct.

ABSTRACT

This thesis explores the effect of antihypertensive drugs commonly used in pregnancy (labetalol, methyldopa, hydralazine) on blood pressure (BP) response in an experimental model of preeclampsia (EPE) whilst awake, during sleep, during the early morning BP surge (EMS) and in the context of stressors including general anaesthesia (ketamine, propofol, sevoflurane), noise, eating and during enclosure cleaning.

The EPE was established in *Papio hamadryas* (n = 9) by reducing uteroplacental blood flow. The EPE was confirmed by the presence of proteinuria (urine protein creatinine ratio), elevated BP (telemeter) and raised soluble fms-like tyrosine kinase receptor-1 (sFLT-1) (ELISA). Continuous BP readings were collected via telemetry before and after antihypertensive treatment was established. Equipotent doses of labetalol, methyldopa and hydralazine equivalent to starting dose rates in humans were given. The BP response to these antihypertensive agents was established whilst awake, during sleep, during EMS and in the context of stressors including general anaesthesia (ketamine, propofol, sevoflurane), noise, eating and enclosure cleaning. The BP response was evaluated using a multidimensional approach involving assessment of BP average, BP load, short- and long-term BP variability (defined by SD1 and SD2 respectively and calculated using Poincaré plot geometry) and a published algorithm (The Chaos Decision Tree Algorithm) classifying BP data as stochastic, periodic or chaotic.

All animals developed EPE: rise in systolic BP 7mmHg ($p < 0.05$), diastolic BP 8mmHg, ($p < 0.05$). The average rise in proteinuria was 19.6 mg/mmol ($p = 0.2$). sFlt-1 levels increased significantly two weeks following induction of EPE, increasing from 2670 +/- 531 pg/ml prior to EPE and increasing to 10563 +/- 12324 pg/ml ($p < 0.05$).

Average BP determined that there was no significant difference between the average systolic and diastolic awake or sleep BP before and after antihypertensive treatment was established.

However, BP load showed that systolic and diastolic BP load increased significantly whilst awake on labetalol treatment ($p < 0.001$). Diastolic BP load increased significantly on methyldopa and hydralazine treatment (both $p < 0.001$) however there was no significant

change to systolic BP load. Systolic and diastolic BP load during sleep changed significantly (overall increase) on labetalol, methyldopa and hydralazine treatment (all $p < 0.05$).

Variability analysis found a significant reduction in short-term variability/SD1 of diastolic BP during sleep on labetalol ($p < 0.01$) and methyldopa ($p < 0.001$) treatment. There was a significant reduction in long-term variability/SD2 of systolic BP on hydralazine treatment ($p = 0.02$).

The Chaos Decision Tree Algorithm largely classified the awake and sleep BP data as periodic on antihypertensive treatment, except in the case of diastolic BP on labetalol (awake and sleep) and diastolic BP on methyldopa (awake), which were classified as stochastic.

Average systolic BP significantly increased during the EMS period on labetalol, methyldopa or hydralazine treatment (all $p < 0.001$). Average diastolic BP also increased significantly on labetalol and hydralazine treatment ($p < 0.001$) and remained unchanged on methyldopa treatment.

BP load also significantly increased during the EMS period on labetalol, methyldopa or hydralazine treatment (all $p < 0.001$) except in there was no change in diastolic BP load on labetalol treatment.

Despite BP average and BP load showing an increase on treatment, variability analysis showed that short-term systolic and diastolic BP variability/SD1 significantly decreased on labetalol, methyldopa and hydralazine (all $p < 0.05$). There was no significant change in long-term BP variability/SD2. The Chaos Decision Tree Algorithm classified all the EMS BP data as stochastic.

Average systolic BP during general anaesthesia with ketamine and sevoflurane was significantly different ($p = 0.003$). The average systolic BP before/on antihypertensive drug treatment (labetalol, methyldopa, hydralazine combined) during ketamine anaesthesia was 136/137 mmHg, whereas this was 112/104 mmHg during sevoflurane.

BP load during anaesthesia (ketamine, propofol or sevoflurane) before and on labetalol, methyldopa or hydralazine treatment was calculated. Systolic and diastolic BP load reduced significantly on methyldopa treatment during propofol anaesthesia ($p < 0.01$) and increased on hydralazine treatment ($p = 0.02$). Systolic and diastolic BP also increased significantly on labetalol treatment during sevoflurane anaesthesia (both $p < 0.05$) whereas systolic and diastolic BP load decreased significantly on methyldopa and hydralazine treatment during sevoflurane anaesthesia (all $p < 0.01$).

Variability analysis identified a significant decrease in long-term variability/SD2 on labetalol treatment during propofol anaesthesia ($p = 0.02$) and a significant increase on methyldopa treatment during ketamine anaesthesia ($p = 0.002$). There were no other significant differences between the different antihypertensive drug treatments under the different anaesthetics.

The Chaos Decision Tree Algorithm mostly classified the BP data from the propofol group as either chaotic or periodic and the BP data from the ketamine and sevoflurane groups were mostly classified as stochastic.

Average BP response to noise stressors on antihypertensive treatment was varied and increased significantly on labetalol, methyldopa or hydralazine during everyday, familiar sounds ($p = 0.001$) whereas during classical music, average systolic BP decreased on labetalol ($p < 0.001$). When exposed to the sound of lions roaring, average systolic BP decreased on labetalol treatment whereas systolic BP effectively remained the same before and on methyldopa and hydralazine treatment. When exposed to sounds of other baboons at rest, average systolic BP increased significantly on treatment with labetalol, methyldopa or hydralazine (all $p < 0.05$).

Systolic and diastolic BP load during everyday, familiar sounds also increased significantly on labetalol, methyldopa or hydralazine (all $p < 0.01$), which was consistent with the BP average for this data set. During classical music systolic BP load increased significantly on labetalol and methyldopa treatment (both $p < 0.01$). The BP load in this instance shows a different pattern than identified by BP average; average BP during classical music

decreased on labetalol whereas systolic BP load during classical music increased on labetalol. When exposed to the sound of lions roaring, diastolic BP load changed significantly on labetalol treatment ($p = 0.04$), consistent with the average BP result. When exposed to sounds of other baboons at rest, systolic BP load increased significantly on labetalol, methyldopa or hydralazine treatment (all $p < 0.01$); this was also consistent with average BP.

Variability analysis of the noise stressors showed a significant reduction in short-term variability/SD1 during sounds of lions roaring when on labetalol or methyldopa treatment (both $p = 0.02$). BP average and load showed labetalol as having the only significant effect and the variability findings are in line with this, however, variability analysis also identified methyldopa as reducing short-term variability. Labetalol, methyldopa and hydralazine all significantly reduced long-term variability of systolic and diastolic BP during the noise stressor of lions roaring (all $p < 0.05$). Labetalol and methyldopa also significantly reduced long-term variability of systolic BP during sounds of other baboons at rest (both $p < 0.05$). The Chaos Decision Tree Algorithm classified all BP data collected during exposure to controlled stressors as stochastic.

Average systolic and diastolic BP increased significantly on methyldopa whilst eating ($p = 0.016$). Systolic BP load decreased on labetalol, methyldopa and hydralazine treatment (all $p < 0.01$) and only on labetalol did diastolic BP load change significantly ($p < 0.001$). Variability analysis revealed no significant changes to short- or long-term variability of systolic or diastolic BP whilst eating. The Chaos Decision Tree Algorithm classified the BP data sets collected whilst animals were eating as stochastic.

Average systolic and diastolic BP increased significantly on labetalol, methyldopa and hydralazine whilst eating (all $p < 0.001$). This finding was consistent with the BP load results which increased significantly on labetalol, methyldopa and hydralazine whilst eating (all $p < 0.01$).

Variability analysis found a significant increase in short-term variability of diastolic BP on labetalol ($p = 0.03$). The Chaos Decision Tree Algorithm classified the BP data collected during cleaning of the animal's enclosure as stochastic.

The key findings were that the combination of sevoflurane and labetalol, or sevoflurane and hydralazine created the most preferable BP dynamics during anaesthesia. Further, labetalol was the most effective overall at controlling BP when exposed to the range of stressors. Labetalol, methyldopa or hydralazine created similar BP dynamics during the EMS period.

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ABPM	Ambulatory blood pressure monitoring
ACOG	American College of Obstetricians and Gynaecologists
AEC	Animal ethics committee
ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANBC	Australian National Baboon Colony
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BCA	Bicinchoninic acid assay
BID	bis in die/twice a day
BP	Blood pressure
BPH	Blood Pressure High
BPV	Blood pressure variability
CHIPS	Chronic Hypertension in Pregnancy Study trial
CI	Confidence interval
CVS	Chorionic villous sample
EF	Silence/low level of everyday, familiar sounds
ELISA	Enzyme linked immunosorbent assay
EMS	early morning surge
EPE	Experimental preeclampsia model
ESC	European Society of Cardiology
ESH	European Society of Hypertension
GGT	Gamma glutamyl-transferase

HELLP	Haemolysis, elevated liver enzymes and low platelet syndrome
HR	Heart rate
HYPITAT	Hypertension and Preeclampsia Intervention Trial At Term study
IM	Intramuscular
ISSHP	International Society for the Study of Hypertension in Pregnancy
IV	Intravenous
K	Mathematical constant that is the result of the 0–1 chaos test; approaches 1 for chaotic systems and approaches 0 for periodic systems
mmHg	Millimetres of mercury
MP	Music with long-term periodicity (MP), specifically, the Sonata in D major for Two Pianos, K. 448 by Wolfgang Amadeus Mozart
NATA	National Association of Testing Authorities
NICE	United Kingdom's National Institute for Health and Care Excellence guidelines
NP	Non-pregnant
PlGF	Placental growth factor
PT	Lion's roaring - a potential threat to baboons
SC same	Calm vocal sounds made by conspecifics/baboons – i.e., animals of the species
SEM	Standard error of the mean
SC	Subcutaneous
SD1	Standard deviation of a Poincaré plot perpendicular to the line-of-identity and corresponds to the width of a plot. In the context of this thesis SD1 represents short-term blood pressure variability.
SD2	Standard deviation of a Poincaré plot along the line-of-identity and corresponds to the length of a plot. In the context of this thesis SD2 represents long-term blood pressure variability (i.e., over the course of a defined data collection period)
sFlt-1	Soluble FMS-like tyrosine kinase-1

SHHT	Spontaneous hypertension and heart failure
SOMANZ	Society of Obstetric Medicine of Australia and New Zealand
uACR	Urine albumin to creatinine ratio
UPCR	Urine total protein to creatinine ratio UPCR
UPI	Uteroplacental ischemia
VEGF	Vascular endothelial growth factor
VEGF R1	Vascular endothelial growth factor receptor 1
WHO	World Health Organisation

1 BACKGROUND AND LITERATURE REVIEW

1.1 Preeclampsia

1.1.1 Definition and diagnostic criteria

Preeclampsia is classified as a hypertensive disorder of pregnancy and is diagnosed by the presence of new onset hypertension (≥140 mmHg/and or 90 mmHg) after 20 weeks gestation accompanied by evidence of end organ dysfunction e.g. proteinuria, maternal acute kidney injury, liver dysfunction, neurological features, haemolysis or thrombocytopenia, seizures, placental abruption and adverse pregnancy outcomes including fetal growth restriction.[1] This range of potential diagnostic features indicates the complex and multi-organ system nature of the disease. There has been debate about the definition of preeclampsia, and the International Society for the Study of Hypertension in Pregnancy (ISSHP) released a consensus statement in 2018 in an attempt to resolve this,[2] acknowledging the need for a broad definition of preeclampsia in a clinical setting to minimise the risk of false negative diagnoses, whilst having a more precise definition when recruiting patients into clinical research studies. The 2021 ISSHP guidelines identify a remaining challenge centred on using the term 'suspected preeclampsia' for a broad range of women.[1] The guidelines point out that women with 'suspected preeclampsia' have been studied where preeclampsia was defined only by gestational hypertension and proteinuria and as such, would have already satisfied the current ISSHP broad definition of preeclampsia.[1] Subsequently, the guidelines advise using a diagnosis of 'suspected preeclampsia' for no longer than 24 hours.[1] This thesis seeks to further

investigate features of blood pressure (BP) in pregnancy in an experimental model of preeclampsia.

Preeclampsia has a significant global heath impact; it is responsible for over 500,000 fetal and neonatal deaths and over 70,000 maternal deaths worldwide each year.[1-211] Further adding to this impact is the fact that affected women have an increased risk of developing cardiovascular disease later in life.[3-7] The principal underlying abnormality of preeclampsia, endothelial dysfunction, may persist in women that have had preeclampsia although most acute markers of endothelial dysfunction (proteinuria and acute hypertension) do reverse in the immediate term (up to 3 months). It appears that endothelial damage could contribute to the increased risk of developing cardiovascular disease later in life.[3,6,8,9] The current 2021 ISSHP guidelines recommend that women receive annual medical review for 5 - 10 years following a hypertensive pregnancy, as this is the window where cardiovascular risk factors and disease appear most frequently; calculating the lifetime (not 10-year) cardiovascular risk scores to estimate cardiovascular risk in these women is also an updated recommendation.[1]

1.1.2 Pathogenesis

The pathophysiology of preeclampsia involves both maternal, fetal and placental factors as presented in the two-stage model of preeclampsia.[10-12] This model proposes that the establishment of a poorly perfused placenta in early pregnancy (stage 1) interacts with maternal risk factors leading to clinical maternal endothelial disease (stage 2).[13] In an uncomplicated pregnancy, the vasculature at the placental implantation site remodels so that the terminal part of the spiral arterioles are wide open, creating a high-capacity, low-resistance system ideal for maternal-fetal exchange of nutrients and oxygen.[14] In preeclampsia, however, shallow placentation and failure of the spiral arteries to remodel early in pregnancy, (weeks to months before development of clinical manifestations of the disease) leads to less than ideal blood flow between the placenta and uterus and comparatively hypoxic trophoblast tissue.[15,16] A state of stress then develops in the placenta, which in turn adversely affects villous angiogenesis.[17] As pregnancy progresses, the placenta increasingly secretes antiangiogenic factors including soluble *fms-like* tyrosine kinase-1 (sFlt-1) and endoglin (amongst other factors) into the maternal circulation.[18] The sFlt-1 and endoglin bind vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), which leads to systemic maternal vascular inflammation, endothelial dysfunction and vascular injury, resulting in the clinical manifestations of preeclampsia including (but not limited to) hypertension and proteinuria.[18,19]

This is one of the many pathophysiological pathways that have been investigated, and has allowed pre-clinical testing in women at risk. Other intersecting pathways that have been examined include the role of oxidative stress in the dysfunction of placental endothelial nitric oxide synthase,[20] alterations to the hypothalamic-pituitary-adrenal axis[21] and abnormal circulating levels of relaxin to name just a few.[22] It should also be noted that the syncytiotrophoblast stress in cases of late-onset PE are mostly related to chronic cardiometabolic and inflammatory processes.

1.1.3 Diagnostic challenges: hypertension in preeclampsia

As described above, preeclampsia is diagnosed by the presence of new onset hypertension ($\geq$140 mmHg systolic BP or $\geq$90 mmHg diastolic BP) after 20 weeks gestation.[1] However, women with preeclampsia may not meet the diagnostic criteria for hypertension at the time of assessment if their blood pressure fluctuates. The degree of maternal hypertension and proteinuria as well as the presence of other clinical manifestations of preeclampsia can be highly variable.[14] Almost all women with preeclampsia have hypertension eventually, but a small proportion of those with HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome and rare cases of eclampsia do not meet current diagnostic criteria for the hypertension component of the disorder.[14] It is possible that in these women, BP is increased above their personal baselines, but their BP reading does not meet the diagnostic criteria for hypertension in pregnancy.[14,23] Another possibility is that the variability is not captured by the classical clinical definitions.[24] Dr Chesley made the point that a rise in blood pressure is more significant than a random blood pressure reading, and that on the blood pressure spectrum, some people will just have higher levels than others.[25] He proposes that dividing abnormal BP from normal according to a conventional dividing line is inaccurate and arbitrary whilst acknowledges the usefulness of doing so in a clinical setting.[25]

Stroke leading to death or disability is one of the most serious complications of preeclampsia[26] with most strokes being preceded by severe, fluctuating blood pressure levels.[27,28] Lowering blood pressure can reduce this risk.[14] An enquiry into 22 preeclampsia-related maternal deaths in the United Kingdom found that inadequate treatment of systolic hypertension followed by stroke was the major failing in clinical care leading to the deaths.[29] The findings from this enquiry draw attention to the risks associated with systolic hypertension in pregnancy and the importance of treating it as a medical emergency.[29]

It is universally agreed that severe hypertension in women with preeclampsia should be lowered immediately to prevent severe complications.[2,30] There has been debate, however, as to what constitutes severe hypertension and advice in treatment guidelines from around the world has differed.[7] The Society of Obstetric Medicine of Australia and New Zealand (SOMANZ) older Guidelines for the Management of Hypertensive Disorders of Pregnancy in 2014 defined severe hypertension requiring urgent treatment as a systolic blood pressure greater than or equal to 170 mmHg with or without diastolic blood pressure greater than or equal to 110 mmHg.[30] This was seen as representing a level of blood pressure above which the risk of maternal morbidity and mortality is increased.[29,31] The goal of BP treatment is to stabilise the maternal condition either peripartum or for expectant management. There is mounting evidence that cerebral perfusion pressure is altered in pregnant women which makes them more susceptible to cerebral haemorrhage, posterior reversible encephalopathy syndrome and hypertensive encephalopathy.[32,33] The SOMANZ guidelines (currently under review) now stipulate that treatment for severe hypertension should commence at systolic blood pressure greater than or equal to 160 mmHg/110 mmHg along with American College of Obstetricians and Gynaecologists (ACOG).[30,34] The ISSHP revised its recommendations in 2021 and now advises that treatment for hypertension should commence at 140/90 mmHg[1,2] as does the United Kingdom's National Institute for Health and Care Excellence (NICE)[35] and Canadian guidelines.[36] This differs from the guidelines from Brazil,[37] Germany,[38] Ireland[39] and European Society of Cardiology (ESC)/European Society of Hypertension (ESH)[40] which advise treatment should commence at systolic pressure greater than or equal to 150/90 mmHg (95 mmHg for ESC/ESH). There is significant and increasing evidence of poor outcomes linked to BP exceeding 160/110 mmHg however, poor outcomes can and do occur at BPs less than these thresholds.[31] The 2021 ISSHP guidelines identify that although there is disagreement as to the definition of severe preeclampsia, consensus is building.[1] Clinicians most likely have limited or no immediate treatment options for many of the risk factors for preeclampsia e.g. nulliparity, obesity, prior preeclampsia, multifetal pregnancy and chronic kidney disease. However, given that blood pressure treatment is readily available in an accessible form to the clinician, hypertension is a readily modifiable risk factor. These changing guidelines highlight the need to understand in more detail the etiology of these variations in BP to facilitate management. Currently, clinical guidance is defined in terms of threshold, however when there is blood pressure instability, as is often seen in women with preeclampsia, a clinical threshold may be of limited use. An aspect of these BP variations, the influence of various stressors on BP (anaesthesia, noise, environmental stressors), was explored in this thesis.

Research about the impact of specific environmental and noise stressors on BP reactivity in women with preeclampsia, as well as what treatment choices might best manage this, is limited.

It is becoming increasingly well documented that prenatal maternal stress is associated with poor maternal and newborn outcomes.[41] Stress in this type of physiological context refers to the internal and external demands a person faces, with stress becoming negative when coping mechanisms fail.[41] Prenatal stress can range from severe (e.g. trauma) to moderate (e.g. life event changes) to mild (e.g. experience of daily hassles)[41] and the ability of a pregnant woman to cope with these stressors is highly individual and dependant on the balance of her allostatic load and resilience.[42] The Optimal Pregnancy Environment Risk Assessment program was developed to identify and mitigate risk associated with poor maternal and newborn outcomes due to stressors.[43] The prenatal maternal stress literature notes that the response to stressors is highly individualised and related to many factors, particularly whether a pregnant woman feels able to cope.[42] Acute prenatal maternal circulatory reactivity has been used as an index of maternal neuroendocrine function, with BP increases being associated with increases in corticotropin and catecholamines and used as a surrogate indicator of stress.[44] Ice-water studies have shown that the stress response in pregnant women is blunted however in women with preeclampsia, this is not so.[45] The clinical stress response is not referred to by clinical preeclampsia guidelines and is not currently used in clinical management. Women with preeclampsia have blood pressure that can suddenly fluctuate significantly, making clinical management challenging, inciting investigation in this thesis of the influence of various sounds and aspects of daily routine on blood pressure reactivity in the EPE model and the associated effect of labetalol, methyldopa and hydralazine.

In a hypertensive pregnancy as compared to hypertension in the non-pregnant (NP) population, the relationship between blood pressure and outcome is accelerated in that the vasculature has less time to adapt, and is responding in a setting of normal physiological vasodilation and hypotension.[46] Further, in preeclampsia there is impaired cerebral autoregulation that is evident regardless of the BP level.[46-48] Also in pregnancy, systolic hypertension (particularly when BP is very high) is important in determining stroke risk regardless of age, and even after very short periods of raised BP, intracerebral haemorrhage is more common relative to the NP population.[46] Therefore, it is possible that rapid rises and falls in BP might be associated with adverse outcomes with more of an effect than in the NP population.[46] It is important to note that

women with an unexceptional mean BP but increased BP variability may form a newly defined and at-risk group, and identifying this group may help guide treatment choices.[46]

1.2 Blood Pressure

Following is a table of terms used to describe BP and their definitions (Table 1-1). These terms will be used throughout the thesis.

Term	Definition
Blood pressure (BP)	Blood pressure is the pressure, measured in millimetres of mercury (mmHg), within the major arterial systems of the body. It is conventionally separated into systolic and diastolic determinations. Systolic pressure is the maximum blood pressure during contraction of the heart's ventricles; diastolic pressure is the minimum pressure recorded just prior to the next contraction.[49] Only systemic (not pulmonary) will feature in this thesis.
Blood pressure variability (BPV)	The term BPV encompasses a wide range of BP variations, occurring over seconds or minutes (very short-term BPV), over 24 hours (short-term BPV, usually assessed by ambulatory BP monitoring), and between days (medium-term or day-to-day BPV, assessed with home BP monitoring or repeat clincial observations). [50,51] Long-term BPV has also been described, including seasonal BP variations and changes between clinic visits over months or years (visit-to-visit BPV).[50,51]
Usual blood pressure	Usual blood pressure is used to describe the concept of the theoretical 'true' underlying level of blood pressure.[52]
Labile hypertension	The term labile hypertension generally refers to sudden rises of BP and is considered a clinical impression without defined criteria or treatment guidance.[53] There is no globally accepted definition of labile hypertension.[51] This term is not usually applied to sudden hypotension.
Paroxysmal hypertension (pseudo-pheochromocytoma)	Paroxysmal hypertension refers to dramatic episodes of abrupt and severe blood pressure elevation; the episodes occur suddenly, are recurrent and occur independently of current stress or perceived emotional distress.[54]
Blood pressure instability	Blood pressure instability describes transient fluctuations in blood pressure, usually in response to a specific stimulus, such as change in posture, emotional stress, or pain.[52]
White-coat hypertension	White-coat hypertension refers to elevated clinic (≥140/90 mmHg) blood pressure, but normal blood pressure measured at

	home or work (< 135/85 mmHg).[2] It conveys an increased risk for preeclampsia.[2]
Masked hypertension	Masked hypertension is another form of hypertension, is difficult to diagnose and is characterised by blood pressure that is normal at a clinic visit but elevated at other times.[2] Masked hypertension has been shown as a strong predictor of cardiovascular risk.[55]

Table 1-1 Blood pressure terms and definitions

1.2.1 Blood pressure as a continuous variable

Blood pressure is a dynamic variable, and is characterised by continuous short-term fluctuations within a 24 hour period and long-term fluctuations occurring over more-prolonged periods of time.[52,56] Blood pressure changes in response to many things and reflects endogenous internal rhythms, which have links to variation between heartbeats, respiratory cycle variation, immediate activity variation, diurnal variation (light-linked) and stages of life variation including pregnancy. Blood pressure has been considered in the past as a definitive, constant, physiological characteristic[57] however it is now well recognised that blood pressure is a continuous variable, impossible to characterise accurately except by multiple or continuous readings under various conditions.[58,59] From a perspective of data classification, continuous variables can take on an unlimited number of values between the lowest and highest points of measurement whereas discrete data are associated with a limited number of possible values.[60] Binary data are a type of discrete data that encompass information that is confined to two possible options.[60] Blood pressure has been treated as binary: either it meets a clinically defined threshold that categorises it as 'high' or it doesn't. Reduction to a binary threshold limits the possibility to predict its effects and outcomes.

1.2.2 Blood Pressure Variability

Blood pressure functions to ensure constantly adequate organ perfusion and BP levels adjust in response to changing demands (e.g., BP increases when facing physical or emotional stress and BP is lower during sleep).[50] The size and patterns characterising these BP variations define BPV.[61] Blood pressure variability represents a dynamic and characteristic physiologic feature of the cardiovascular system, with the BPV range being different among individuals in response to their daily activities and challenges, and also influenced by the unique reactivity of their

cardiovascular control mechanisms.[62] On the other hand, from a clinical perspective, BPV has been described as a source of noise that makes it difficult to assess an individual's 'true' or 'usual' BP level.[52] There is however, significant and increasing evidence showing that BPV is an independent predictor of cardiovascular risk in the NP population.[61,63] While the actual level of blood pressure is very important, it is also clear that variability in BP has an independent effect on cardiovascular risk.[64-66] Increased BP variation is thought to reflect pathophysiological imbalanced cardiovascular regulation in response to internal and external haemodynamic stressors; it may also reflect changes in renal regulatory mechanisms, cardiovascular damage, or other underlying pathological conditions associated with autonomic dysfunction.[61,67] Variation in BP has been shown to be reproducible rather than a random occurrence that hinders the ascertainment of 'usual' BP measurements.[52,68,69] There is, however, no clear definition or standard criteria to distinguish between normal and abnormal blood pressure fluctuations.[70] There is also minimal guidance available for treatment of blood pressure behaving in such a way.[53]

A secondary analysis from the Chronic Hypertension in Pregnancy Study (CHIPS) trial demonstrated relationships with adverse pregnancy outcomes for both mean BP level and BP variability.[46] Higher BP level was associated with more adverse maternal and perinatal outcomes, and the observed association extended down to below 140/90 mmHg for many maternal (severe hypertension, preeclampsia) and perinatal outcomes (low birth weight and premature delivery).[46] Higher BP variability was associated with increased risk of severe hypertension and preeclampsia, but interestingly not perinatal outcomes. In an additional sensitivity analysis, there was evidence that increased diastolic BP variability (as measured by standard deviation and average real variability) was associated with lower perinatal risk.[46]

Occurrence of BP peaks, whether assessed in a clinic or at home, provides independent prognostic information beyond average BP.[71] This is supported by findings describing the association between the morning surge in BP and the incidence of cardiovascular events, an association that increases when the morning BP surge is particularly pronounced.[71,72] It has been shown that whatever the time of day a BP surge occurs, whether in the morning after night time sleep or in the afternoon after a siesta, a parallel surge in stroke events also occurs.[73]

The issue of BP variability is not new. In 1987, Parati *et al*. showed that for nearly any level of mean 24 hour BP assessed intra-arterially, subjects with high BP variability had more severe target organ damage, an observation confirmed by long term follow-up in the same patients.[74] The issue reported by Rothwell *et al*.[75] showing that different antihypertensive drugs might have different effects on BP variability, (which may in turn affect the degree of cardiovascular protection provided), needs further research and, if confirmed, should lead to translation and action into clinical practice.[71] Comparable work in any pregnant population is limited.

'Usual' blood pressure is a term used to describe the theoretical 'true' underlying level of blood pressure, which cannot be measured with total precision, but is widely considered to be the most important component of blood pressure and if this usual BP is exceeded, this is what determines the adverse effects associated with BP and accounts for the benefits of antihypertensive drugs.[52] Rothwell *et. al.* describes the common acceptance that underlying 'usual blood pressure' can account for all blood-pressure-related risk of vascular events as well as for the benefits of antihypertensive drugs. They suggests that other potentially informative measures including variability in clinic blood pressure have been overlooked, and the effects of antihypertensive drugs on this variable is mostly unknown.[69]

Labile hypertension is generally used to refer to sudden rises of BP. Historically, the term was used for the first time in 1969 and intended to describe BP levels that varied above 140/90 mmHg.[51] It was thought to represent the initial stage in development of fixed essential hypertension. However, the concept of labile hypertension is not well studied and there is not a globally accepted definition. Therefore, it is considered more of a clinical impression in lieu of a specific diagnosis and the clinical impact of labile hypertension is debated.[51]

Blood pressure instability contributes to overall BP variability and will often have similar clinical associations, such as arterial stiffness and baroreceptor dysfunction. However, instability differs from variability in that it refers specifically to sudden changes in blood pressure, the consequences of which might differ from more gradual fluctuations.[52] This term appears to be used interchangeably with 'labile hypertension'.

Paroxysmal hypertension presents as dramatic episodes of abrupt and severe blood pressure elevation. Patients with paroxysmal hypertension experience recurrent, sudden, unprovoked,

symptomatic, and severe elevations of blood pressure that occur independently of current stress or perceived emotional distress. Causes of paroxysmal hypertension include phaeochromocytoma and may also be physiological or psychosomatic in nature.[54] Although the terms 'labile hypertension' and 'paroxysmal hypertension' are used interchangeably, their cause, clinical presentation and management differ substantially.[54]

White coat hypertension is now recognised as a condition that is not entirely benign.[2,76] The situation of being in a clinical environment often leads a patient to experience an alerting reaction and a transient increase in BP. This represents a major problem associated with BP measurement in clinical practice, as it prevents BP measures obtained in the clinic from accurately reflecting the patient's 'usual' blood pressure value.[77] The current definition of white coat hypertension based on the clinic-ambulatory BP difference is intended to help clinicians with decision making, i.e., whether or not to start a patient on antihypertensive treatment.[77] The reported incidence of white coat hypertension in pregnancy varies, ranging between 4% and 30%.[78-81] White coat hypertension can be diagnosed through the use of Ambulatory Blood Pressure Monitoring (ABPM) however diagnosing masked hypertension presents more of a challenge as women with masked hypertension have by definition, BP that is normal at a clinic visit and elevated at other times.[82,83] Much less is known about masked hypertension compared with white coat hypertension. It is typically diagnosed by home BP monitoring or ABPM when there is evidence of hypertensive target organ damage such as unexplained chronic kidney disease, left ventricular cardiac hypertrophy or uteroplacental dysfunction in the absence of hypertension in the clinic.[1] The prevalence of masked hypertension is unknown in uncomplicated pregnancies however it has been reported that 30% of high risk pregnancies have masked hypertension.[2,55,83]

1.2.3 Methods of blood pressure measurement and associated limitations

Office BP measurement using auscultatory, oscillometric semiautomatic or automatic sphygmomanometers is described by hypertension guidelines as the preferred method for clinical BP measurement.[40,84] Intra-arterial versus non-invasive cuff-based methods provides direct assessment of BP levels and is the gold standard for measuring BP levels and short-term BPV.[85] The ISSHP recommendation for treatment of hypertension in pregnancy is based on clinic or home measurements and states that home BP monitoring and/or ABPM can be a useful adjunct to office measurements when investigating hypertension in pregnancy.[2] The most recent ESH clinical practice guidelines provide thresholds for hypertension that are diagnosed with the use of an average calculation of the second and third office BP readings, an average of

24 hour ambulatory BP monitoring readings or an average of home BP monitoring readings taken for multiple days (at least 3 days with at least 12 readings).[86] These approaches have the effect of evening out the fluctuations in BP levels.[85] It is acknowledged that there are no absolute definitions of 'normal' ABPM and as such 24 hour means are not appropriate for the diagnosis of hypertension in individual patients as this measure may conceal periods of daytime hypertension or if means from smaller blocks of time are used, outlying values may significantly alter the mean values.[87] The median 24 hour BP has been proposed as an alternative, and has often been used in the assessment of antihypertensive drug therapy in the NP population, however neither the mean or median allow evaluation of BP responsiveness or control. The area under the BP curve is another way to measure BP load, with the option of using various thresholds to calculate this. The entire area under the curve could be calculated, however this value is similar to mean 24 hour BP. The area on a curve under 140/90 mmHg during waking hours and under 120/80 mmHg during sleep have been shown in the NP population to be more clinically valuable than mean ambulatory BP. When presented this way on a graph, efficacy of an antihypertensive drug can be visualised, and the nuance of the data is not lost. The rate or frequency of significant change in BP is not represented with this approach however, and it is acknowledged that rate and frequency patterns of BP behaviour are clinically relevant.[87] Although the previously mentioned guidelines acknowledge the evidence supporting the prognostic value of BPV, this knowledge has not yet been translated into routine clinical practice.[85]

1.2.4 Indices currently used to measure blood pressure variability

There are currently no standardised methods, or protocols for measuring BP variation and assessing its prognostic value[85] however there are various active research projects investigating BP changes throughout pregnancy.[68 88] As described earlier, BPV is classified and measured in different BP ranges: very short-term (beat-to-beat), short-term (within 24 hour, minute-to-minute, hour-to-hour and day-to-night), mid-term (day-to-day) and long-term (visit-to-visit over weeks, months and years).[61] These classifications represent complex and continuous interactions between intrinsic (neurohormonal, cardiovascular) and extrinsic (environmental, behavioural) factors.[85] Blood pressure variation may correspond to adaptations aimed at maintaining BP homeostasis, or conversely, it may indicate underlying pathology.[85] The dynamic nature of BP has led to challenges in accurate assessment and classification. BPV indices aim to quantify overall variability by describing BP dimensions, namely range, dispersion, order and BP irregularity as well as describing specific BP patterns such as

nocturnal BP decline, morning BP surge and in pregnancy, the second trimester dip.[85,89] Blood pressure load was proposed as a clinically useful parameter based on the hypothesis that it might indicate the haemodynamic burden on the cardiovascular system and augment the information carried by average BP.[90]

Term	Definition
Standard deviation	Standard deviation measures the dispersion of the BP dataset relative to its mean and is reliant on the mean.[85]
Coefficient of variation	Coefficient of variation is the ratio of the standard deviation to the mean. This measure is not dependent on the mean, and as such datasets with different averages can be compared.
Average real variability	Average real variability assesses the sequence of BP readings and is the average of the differences between consecutive BP measurements in a dataset.
BP load	Blood pressure load is the proportion of times (expressed as a percentage) that the BP exceeds normal values of the total number of recorded BP within a dataset/defined time period.[90]
Cumulative BP load	Cumulative BP load refers to an output of ambulatory BP monitoring data. Cumulative BP load is defined as the area between the fluctuating ambulatory BP curve and the time axis and aims to measure the 'dose' of BP over time.[85,91]
Variability independent of the mean	The variability independent of the mean is a transformation of standard deviation that is defined to be uncorrelated with mean levels. It is impractical for use at an individual level because it relies on previous derivation of equation coefficients for a given population.[52]
Time rate of BP variation	The time rate of BP variation measures the speed and steepness of BP change over time. This index considers the sequence and the incline of BP changes and is useful whenever multiple changes in BP trends occur between measurements.[92]

Table 1-2 Indices used to measure blood pressure variability.

The aim of the indices listed in Table 1-2 is to describe and quantify overall BP variability in terms of distribution and order. Where BPV is assessed, commonly used approaches include standard deviation and the coefficient of variation. However standard deviation is dependent on the average BP value and neither take into consideration the temporality of the measurements.[61,93] Average real variability and time rate of BP variation do take sequence and temporality into consideration however are used exclusively in research.[61,71,85,92,93] Many of the measures of BP variability do not take into consideration the temporal structure of a sequence of measurements. For example, the following two sequences, A = (1,2, 3, 2,1,2, 3,2,1,2, 3,2,1) and B = (1, 1,1,1,2,2,2,2,2,2,3,3,3) have the same variability, as measured by amplitude of range, mean and standard deviation but completely different structures.[88] Measures that are sensitive to the temporal organisation of a signal are important in characterising and discriminating different dynamic systems.[88] It is worth noting when discussing approaches to assessing BPV that BP is a nonlinear physiological signal as this determines what indices and types of data analysis tools are appropriate for BP data sets.[88] Cardiovascular regulation has long been accepted as highly complex and nonlinear.[94] The baroreflex mechanism regulates BP in the short term and other paracrine and hormonal mechanisms, including nitric oxide and angiotensin II related pathways, are also involved in the maintenance of BP.[94] The interrelationships of these regulatory mechanisms and the inherent nonlinearities of each individual mechanism, result in the complex and nonlinear behaviour of the BP controlling system. This type of system is more effectively analysed using nonlinear techniques, most of which are adopted from chaos theory. [95]

1.3 The chronobiology of blood pressure in pregnancy

This section, 1.3, is derived from a review published in Pregnancy Hypertension, 12: 104-109, 2018. As outlined in the review, blood pressure reflects endogenous internal rhythms, which have links to variation between heartbeats, respiratory cycle variation, immediate activity variation, diurnal variation (light-linked) and stages of life variation including pregnancy. Even in pregnancy, blood pressure has been considered in the past as a definitive, constant, physiological characteristic.[57] It is now well recognised that blood pressure is a continuous variable, impossible to characterise accurately except by multiple or continuous readings under various conditions.[58] Blood pressure is influenced by level of activity, exercise or rest, degree of wakefulness or sleep; environmental factors such as temperature, mood and a multitude of other emotional and psychological factors that reflect a person's response to the internal and external milieu[96,97] many of which are related to circadian rhythms. This review focused on the chronobiology of blood pressure in pregnancy, and more specifically, in the common disease state of high blood pressure in pregnancy or preeclampsia.

1.3.1 Blood pressure in normal pregnancy and preeclampsia

In normal pregnancy, the physiological changes to the cardiovascular system are significant. By eight weeks' gestation, the cardiac output has already increased by 20%.[98] Peripheral vasodilation is increased and is mediated by factors including nitric oxide synthesis, oestradiol upregulation and vasodilatory prostaglandins.[98] This peripheral vasodilation leads to a 25–30% fall in systemic vascular resistance, and to compensate for this, cardiac output increases by around 40% during the entire pregnancy, which is reflected in an increase in stroke volume and heart rate.[98] Stroke volume declines towards term and the increase in maternal heart rate of between 10–20 beats per minute is maintained, sustaining the increased cardiac output.[98] Arterial under-filling in pregnancy leads to the stimulation of arterial baroreceptors, activating the renin-angiotensin-aldosterone and the sympathetic nervous systems stimulating a release of antidiuretic hormone.[99] These changes lead to sodium and water retention and create the hypervolaemic, hypo-osmolar state characteristic of pregnancy.[99] Extracellular volume increases by 30–50% and plasma volume by 30–40% which plays a critical role in maintaining circulating blood volume, blood pressure and uteroplacental perfusion during pregnancy.[100]

Given the nature of blood pressure in pregnancy, identifying blood pressure changes that are unsustained or episodic high blood pressure can present a challenge, particularly in the context of office/one-off measurements over a short period of time. The ISSHP recommend a minimum of two blood pressure measurements to establish a diagnosis of hypertension and preferably, for accuracy in diagnosing, that the blood pressure remain elevated after overnight rest in hospital or in a day (time) assessment unit.[101]

Hermida *et al.* found that in a study of 289 pregnant women (189 of these were uncomplicated pregnancies, 71 women went on to develop gestational hypertension and 29 developed preeclampsia), both systolic and diastolic blood pressure in uncomplicated pregnancies steadily decreased up to the 20th week of pregnancy and then increased until the day of delivery, with the final blood pressure values being similar to what was found in early pregnancy for the same women.[102] In contrast to this, the women that went on to develop gestational hypertension and preeclampsia had blood pressure values that demonstrated significant linear increases throughout their pregnancies, with an even more pronounced elevation in the second half of pregnancy when compared to the women with uncomplicated pregnancies.[102] The authors point out the importance of noting that blood pressure measurements at the beginning of gestation in this group of women were similar for the complicated and uncomplicated pregnancies. By the 14th week of gestation (i.e., end of the first trimester), the blood pressure values for women that developed gestational hypertension and preeclampsia reached 115/68 mmHg, whereas the women that went on to have uncomplicated, normotensive pregnancies had an average blood pressure of 102/59 mmHg at the end of the first trimester. Further, these results show that differences in blood pressure between complicated and uncomplicated pregnancies can be observed long before the blood pressure exceeds the defined threshold to allow an actual clinical diagnosis of gestation hypertension or preeclampsia to be made.[102]

Additionally, Hermida *et al*. found increased 24 hour BP mean in pregnancies with gestational hypertension or preeclampsia when compared with normotensive pregnancies.[103] The trend of blood pressure increasing as gestation progresses during the second half of pregnancy has been found to be more significant for women who develop preeclampsia when compared to those who developed gestational hypertension.[103] More specifically, this study documented first trimester differences of approximately 12 mmHg in the systolic blood pressure 24 hour mean and approximately 7 mmHg in diastolic blood pressure 24 hour mean in women that were diagnosed with gestational hypertension or preeclampsia later in the pregnancy as compared

with normotensive pregnancies. In the second trimester, this study found differences between healthy and complicated pregnancies of approximately 13 mmHg in systolic blood pressure 24 hour mean and 7 mmHg in diastolic blood pressure 24 hour mean. The documented differences in the BP 24 hour mean of between women with healthy and complicated pregnancies undergoing sampling during the third trimester are approximately 15 mmHg for systolic blood pressure and 9 mmHg for diastolic blood pressure.[103]

1.3.2 The blood pressure cycles and circadian rhythms of normal pregnancy and preeclampsia

Continuous BP monitoring around the clock has demonstrated that the blood pressure values collected over a 24 hour period form a characteristic circadian pattern. The 24 hour diurnal circadian rhythm of blood pressure in pregnancy is similar to the non-pregnant state,[104] with a nocturnal decrease, especially during sleep, of 10–20% followed by an increase early in the morning.[105] In women with normal pregnancies, significant BP decreases occur overnight, with BP increasing during the waking hours and reaching a maximum by late evening.[106-109] Brown *et al.* (1997) found that in a study of 276 women with uncomplicated pregnancies, the average upper limits of normal were 130/77, 132/79, 133/81, and 135/86 mmHg. These measurements were taken between week 9-17, 18-22, 26-30, and >30 weeks' gestation respectively; the systolic blood pressure fell by 12% to 14% and diastolic blood pressure by 18% to 19% during sleep at all stages of pregnancy. Awake ambulatory blood pressure monitoring systolic measurements were 11 to 12 mmHg higher than clinic measurements and diastolic measurements were 5 to 11 mmHg higher throughout pregnancy. Maximum BPV ranged from 8 to 13 mmHg.[104]

Ambulatory, continuous blood pressure monitoring has also shown a circadian BPV over the course of pregnancy that characterises clinically healthy pregnant women as well as women who go on to develop gestational hypertension or preeclampsia.[103,110-112] Maternal cardiovascular adaptations to pregnancy involve considerable change throughout the trimesters as described earlier. In healthy pregnant women, the systolic and diastolic blood pressures begin to fall during the 1st trimester, continuing to decrease until halfway through pregnancy (an average of 5 to 10 mmHg for systolic blood pressure and 10 to 15 mmHg for diastolic blood pressure) and then the gradually return to pre-pregnancy baseline levels by the end of the 9th month.[111,113-116]

One study of forty-eight normal pregnancies showed lowest blood pressure noted during the 1st trimester, a minimal increase noted during the 2nd trimester, and an incremental rise during the last trimester.[117] Blood pressures were found to rise during the day and fall at night for each trimester of pregnancy with a recorded mean nocturnal decrease in arterial pressure averaging 15% was noted in all participants during each trimester.[117] In addition, a decrease in systolic blood pressure of at least 10% was noted in all participants during each trimester.[117]

Similar pregnancy circadian rhythms are seen in animals. Pregnancy in baboons is characterised by lower blood pressure than in the non-pregnant state.[118] As baboon pregnancy advances, the diastolic and mean BP increases but systolic BP stays low.[118] Starka *et al*. found evidence that blood pressure of healthy pregnant baboons demonstrated diurnal variation, similar to the 24 hour circadian periodicity to humans.[119] Haemodynamic adaptation in early rat pregnancy is characterised by a fall in blood pressure and total peripheral resistance, together with a rise in cardiac output and stroke volume – the same pattern as seen in human pregnancy.[120]

Melatonin influences rhythmic changes in blood pressure by affecting the body's master clock in the hypothalamus and consequently sympathetic and parasympathetic tone.[121,122] The physiological nocturnal fall in BP is associated with the nocturnal decrease in adrenergic activity and the increase in parasympathetic activity.[123] In addition, several investigators have shown that the nocturnal surge of endogenous melatonin may contribute directly to arterial vasodilation that leads in turn to nocturnal blood pressure fall in normotensives and dippers with essential hypertension.[124-126]

Furthermore, Brown *et al*. (2014) found that within a cohort of two hundred and forty-one pregnant women diagnosed with essential hypertension in early pregnancy, 32% of these women in fact had white-coat hypertension, supporting the notion that white-coat hypertension is a common phenomenon in pregnant women that appear to have essential hypertension according to routine blood pressure measurement in early pregnancy. [127]

1.3.3 Allostatic load and circadian rhythm

Allostasis is the ability to maintain stability and homeostasis during external changes. Allostatic load, which refers to the bodily compensations made in the pursuit of allostasis, causes physiological and pathophysiological changes.[128] Major allostatic responses involve the sympathetic nerve system and the hypothalamus–pituitary–adrenal axis and when these systems are activated, catecholamines are released from the adrenal medulla and from the sympathetic nerves.[128] This response normally ceases as soon as the stress has passed, with plasma catecholamine and cortisol levels returning to baseline.[128] Overexposure to allostatic load can occur either because of hyperresponsiveness or delayed recovery, inducing a hyperactivity of the hypothalamus-pituitary axis and sympathetic nerves, and ultimately leading to cardiovascular disease on chronic phase.[128]

The link between allostatic load and circadian rhythm changes are demonstrated by associations between exposure to earthquakes (an allostatic model) and cardiovascular disease (for example, coronary artery disease). Such associations have been reported in Thessaloniki in 1978, Newcastle in 1991, Northridge in 1994 and in Hanshin-Awaji in 1995.[129] Chen *et al*. showed that participants in the study following the Wenchuan earthquake demonstrated a non-dipper diurnal blood pressure change.[130] This finding is relevant because it means that overexposure to an allostatic load caused a significantly abnormal circadian rhythm.[128]

Further, a study by Higgins *et al*. found that the women who work had higher mean daytime BP as well as higher 24 hour systolic pressures compared with those not working. Their study also found that the rate of subsequent development of preeclampsia was significantly higher among those at work compared with those not working.[131] The explanation for the association between work and preeclampsia is unknown, but it has been suggested that work is associated with an increased catecholamine release and sympathetic response over the course of a working day that increases blood pressure.[132] However other studies have found no association between work and hypertensive disorders of pregnancy.[133]

Blood pressure assessment in pregnant women generally relies on a few office measurements that would be affected by sleep/wake schedule, physical activity, diet, emotional state, among many other factors.[89,134-136] These casual, time-unspecified measurements are not sufficient in

predicting serious hypertensive episodes nor are they sufficient to inform decisions around dose refinement or timing of antihypertensive medications.[136]

Preeclampsia is associated with increased maternal morbidity and mortality and there is increasing evidence that the negative health effects of preeclampsia may not be restricted to pregnancy.[137] Women diagnosed with preeclampsia are also at increased risk of cardiovascular events in the future (Figure 1-1).[137] Charlton *et al*. demonstrated that the principal underlying abnormality, endothelial dysfunction, persists in women who have had preeclampsia and that it is this damage that increases the risk of developing cardiovascular disease in later life.[3] The contributions of hypertension and dyslipidaemia before and during the pregnancy are also important and contribute to future risk.[3]

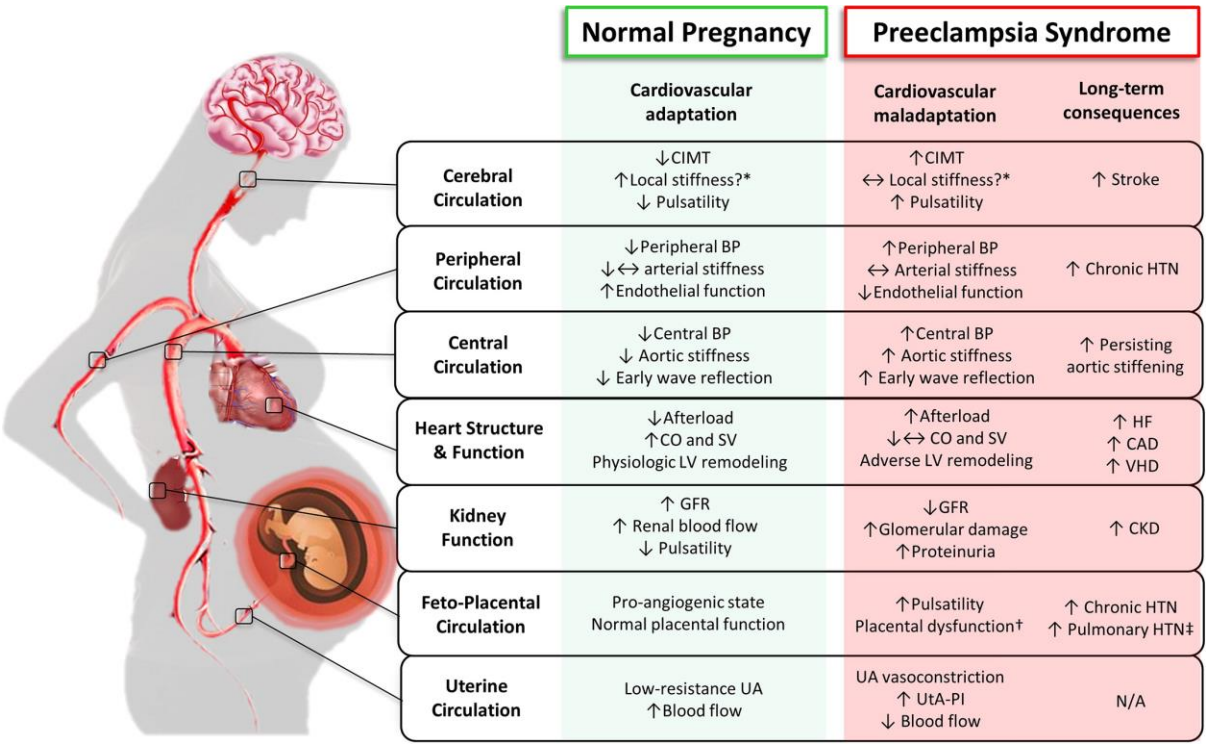

Figure 1-1 Comparison between cardiovascular adaptation in normal pregnancy and cardiovascular maladaptation in preeclampsia and associated long-term cardiovascular consequences.[138]

1.3.4 How do blood pressure cycles change in preeclampsia?

Preeclampsia appears to interfere with the normal circadian decrease in blood pressure at night, sometimes resulting in no decrease or an increase. This phenomenon of nocturnal hypertension in preeclampsia has been observed in several studies. Redman *et al*. describe the nocturnal blood pressure trends in a group of pregnant women, including nine normotensive women, nine women with untreated chronic hypertension and nine women with severe preeclampsia.[139] In this group of women, the mean nocturnal fall for the diastolic pressures of the normotensive and chronic hypertensive women at 28 weeks of gestation were similar (-4.8 mmHg and -6.0 mmHg, respectively). The systolic blood pressure profiles of the women with chronic hypertension, however, revealed a significantly greater fall, -16.3 mmHg compared with -11.7 mmHg in the normotensive women.[140]

Halligan *et al*. (1996) studied 48 pregnant women (24 normotensive and 24 with preeclampsia), demonstrated that the blood pressure fall at night was greater in the normotensive women and smaller in the women with preeclampsia.[141] The mean day-night difference for diastolic blood pressure was found to be greater for normotensive women (17.2 mmHg) than for women with preeclampsia (9.0 mmHg).[141] The mean day-night difference for systolic blood pressure showed a similar pattern, with a greater difference for normotensive women (15.7 mmHg) than for women with preeclampsia (9.4 mmHg).[141]

Another study had similar findings whereby the decrease in blood pressure during sleep is significantly less in women with preeclampsia and significantly greater in normotensive patients.[142] The group with chronic hypertension showed a markedly different nocturnal blood pressure pattern.

In a study by Sawyer et al. (1981) three groups of 15 women in their third trimester were studied: normotensive, chronically hypertensive, and those with preeclampsia (blood pressure at or above 160/110 mmHg with proteinuria and/or oedema). Compared with the overnight systolic blood pressure fall in normotensive women, the fall in the women with chronic hypertension was significantly greater during sleep and significantly less in women with preeclampsia. Although the diastolic pressure fell during the night as well, no significant difference was found among the three groups. The circadian rhythm was abolished in the two participants with severe preeclampsia with these women experiencing no decrease or increase in blood pressure during sleep.[108]

The clinical relevance of establishing the presence of an abnormal blood pressure pattern lies in its proven association in the NP population with more severe hypertensive target organ damage and increased cardiovascular risk.[143-145] Left ventricular hypertrophy, carotid intima-media thickening, microalbuminuria and cerebrovascular disease are more prevalent in non-dippers than in dippers.[146,147]

Definitions of dippers and non-dippers vary among authors, and a general description of a dipper is someone who shows a reduction in BP of 10% or 10/5 mmHg during sleep.[148] Those who have less than a 10% reduction in BP during sleep are defined as non-dippers.[149] The time period measured showing the nocturnal blood pressure decrease shows some variation amongst authors, depending on the definition of night and day.[149] A commonly used definition of daytime is the period from 6am to 10pm, with night time being defined as when patients go to sleep, or as 10pm to 6am.[148]

As discussed above, patients with preeclampsia are frequently characterised by altered diurnal circadian blood pressure rhythms. There is also evidence that the nocturnal hypertension often seen in preeclampsia is related to elevated levels of molecules that cause endothelial damage.[150] In the general population, the non-dipping blood pressure profile is considered a risk factor for cardiovascular events and target organ damage.[151] The underlying mechanisms have not been fully clarified, but there is evidence that endothelial activation, dysfunction or damage could play a pivotal role.[152] Markers of endothelial dysfunction-activation (molecules such as von Willebrand Factor and soluble Vascular Cell Adhesion Molecule-1) are elevated in women with preeclampsia.[150] Impaired endothelial function and assumed accompanying procoagulant changes are long known to be involved in the pathogenesis of atherosclerosis.[150] In addition, a history of preeclampsia is associated with an increased risk for cardiovascular disease.[150]

One study also found that non-dippers and dippers with preeclampsia differ not only in their haemodynamic response during night, but also in their physiological response to darkness, as shown by the blunted nocturnal melatonin secretion in non-dippers.[122]

Interestingly, animal studies have shown that fetal diurnal rhythms are to a large extent driven by the mother.[153] Kennaway *et al*. demonstrated that infants born after intrauterine growth restriction and preeclampsia have delayed appearance of melatonin rhythmicity and a marked reduced day–night difference in melatonin production between 46 and 55 weeks' post-conception as compared with full-term infants.[154]

During pregnancy the pineal gland is not the exclusive source of melatonin, it is also secreted from the placenta.[122] According to Lanoix *et al*., preeclampsia leads to depressed melatonin levels in the placenta.[155] Reduced melatonin levels in women with preeclampsia, as showed in this study, might be in part due to reduced placental secretion of melatonin.[122] Studies in animal models of hypertension in pregnancy suggest that shallow placentation and reduction in uteroplacental perfusion during pregnancy triggers the release of biologically active factors which affect the sympathetic tone and lead to generalised vasoconstriction, hypertension and disturbance of the circadian blood pressure rhythm.[156] Dysfunction of the autonomic nervous system is suspected to be a major contributor to the increase in peripheral vascular resistance in preeclampsia.[122] This overactivity has been suggested as a contributing factor in the loss of nocturnal BP dip.[151] Impaired nocturnal melatonin secretion, shown in the study conducted by Bouchlariotou *et al*., might be an additional contributor to autonomic nervous system dysfunction and thus indirectly of nocturnal hypertension in preeclampsia.[122] Preliminary clinical trial evidence for a potential role of melatonin therapy in the management of preeclampsia is promising and clinical evaluation is warranted.[157]

1.3.5 What implications does blood pressure variability have for treatment?

Rather than representing 'background noise', these BP variations have consistently been shown to be the result of complex interactions between extrinsic environmental and behavioural factors and intrinsic cardiovascular regulatory mechanisms.[61] The adverse cardiovascular impacts of hypertension have been shown to be associated with absolute blood pressure values, however evidence from a number of studies point toward these outcomes also being dependent on the degree of BPV.[61] Increased short-term and long-term BPV are associated with the development, progression, and severity of cardiac, vascular, and renal damage and with an increased risk of cardiovascular events and mortality.[61] One-off BP measurements are relatively easy to report and quantify however it is unclear which variability index correctly identifies and quantifies true BPV.[158] Parati *et al*. draws attention to the findings from post-hoc analyses of large intervention trials in hypertension that show within-patient visit-to-visit BPV is a strong prognostic indicator for cardiovascular morbidity and mortality.[61] This result has prompted

discussion on whether antihypertensive treatment in the NP population should be targeted not only towards reducing mean blood pressure levels, but also to stabilising BPV with the aim of achieving consistent blood pressure control over time, which might favour cardiovascular protection[61] with some evidence to suggest that amlodipine is effective for minimising BPV in NP hypertensive individuals.[159] Particularly in the case of preeclampsia, many of the most serious adverse outcomes result from acute episodes of BPV, as occurs with cerebral haemorrhage. Cerebral haemorrhage is the main cause of maternal mortality in preeclampsia. [160]

The Control of Hypertension in Pregnancy Study (CHIPS) based eligibility on BP measurements falling within the eligible range twice, i.e. based on evidence of sustained hypertension.[161] In the event of a patient having labile or episodic hypertension, they may have been excluded from the trial if their second BP reading fell below the required range. The Hypertension and Preeclampsia Intervention Trial At Term (HYPITAT) study also excluded patients that did not have sustained BP within a defined range over the course of two readings taken at least six hours apart.[162]

Approximately 15% of women with preeclampsia have severe, episodic hypertension and can't be treated; highly variable, episodic hypertension is a limit to treatment.[163] There is minimal literature describing this phenomenon in pregnant women, however the body of research around episodic hypertension in the NP population is growing. The only area where there is substantial literature describing episodic hypertension in women with preeclampsia relates to blunting the hypertensive response associated with intubation, as this hypertensive response has been identified as a direct cause of maternal mortality. There are no published guidelines for the clinical management of this response. [164,165]

Chronopharmacology, the study of how biologic rhythms interact with medications, has led to new understandings and concepts about the behaviour of therapeutic agents.[166] Chronotherapeutics is a relatively new concept in medicine, and aims to increase the positive effects of medications and manage their adverse effects.[166] It is indicated for medical conditions that show predictable time variation in symptoms and risk for life-threatening events.[166]

While the guidelines for treating hypertension in pregnancy use absolute measures to start treatment, they do not consider the important rhythms of hypertension including night and daytime readings, let alone at times of different treatment. These variations are likely to have strong impacts on pregnancy outcomes, risk, and long-term hypertension risk.

1.4 Anaesthetic considerations in the management of preeclampsia

Management of preeclampsia may require intrapartum general anaesthesia and challenges may include stabilising the blood pressure, preventing seizures and pulmonary oedema. Causes of death in women with preeclampsia are intracranial haemorrhage and cerebral infarction, acute pulmonary oedema, respiratory failure and hepatic failure or rupture.[29,167] Severe maternal complications include antepartum haemorrhage due to placental abruption, eclampsia, cerebrovascular accidents, organ failure and disseminated intravascular coagulation.[29,167] These complications as well as delivery itself often require surgical intervention, therefore anaesthetic considerations are of the utmost importance in the multi-disciplinary management of critically ill women with preeclampsia.[168] Additional risks associated with anaesthesia in women with preeclampsia include greater haemodynamic instability, especially at induction and intubation and again at extubation.[169,170] These are a few reasons amongst many others, why generally, neuraxial anaesthesia is preferred where possible.

Cesarean delivery may be indicated in the management of women with preeclampsia. When a neuraxial anaesthetic is not used for cesarean delivery, general anaesthesia presents clinical risks. At the time of intubation, a major concern is the acute increase in blood pressure that can precipitate intracranial haemorrhage associated with direct laryngoscopy.[171] The hypertensive response to intubation has been identified as a direct cause of maternal mortality. [29,167] Many strategies for controlling the haemodynamic response to laryngoscopy and intubation have been investigated in the general surgical population, with conflicting results.[171] Few studies specifically address women with preeclampsia, and clinical practice among anaesthetists varies widely.[172]

Due to the risk of haemorrhagic stroke in the presence of systolic hypertension, preeclampsia treatment guidelines advise lowering blood pressure to a target systolic level of 140 - 150mmHg and a diastolic of 90 - 100 mmHg using oral labetalol or other options.[31,168,173] As previously mentioned, there is some variation between guidelines. Systolic blood pressure > 160 mmHg is defined as a hypertensive crisis and a medical emergency requiring immediate treatment.[29] If left untreated, this degree of hypertension is associated with an increased risk of intracerebral haemorrhage.[167] By reducing levels of severe hypertension, the risk of mortality is decreased.[168,174] Sudden, rapid falls in blood pressure should also be avoided, which may induce maternal or fetal complications relating to critical hypoperfusion thresholds.[168] Elevated blood pressure is recommended to be lowered to levels of systolic 140 – 150 mmHg and diastolic 80 – 100 mmHg at a rate of 10 – 20 mmHg every 10 – 20 min although this recommendation is based

on weak data as there is not a randomised controlled trial investigating this area specifically.[168] The purpose of stabilising the blood pressure is to reduce the risk of stroke and cerebral haemorrhage whilst maintaining appropriate organ and fetal perfusion. Because of the disruption of cerebral autoregulation in preeclampsia, women are at a higher risk for intracranial bleeding at much lower blood pressures (> 155 mmHg).[31] [47] The duration and periodicity of these elevations with respect to this risk have never been defined, especially those created events involved in general anaesthesia.

In obstetric anaesthesia, historically thiopental and currently propofol are the most commonly used drugs.[171] Ketamine and etomidate are used occasionally for induction of anaesthesia for cesarean delivery, but their haemodynamic profiles make them less appealing for prevention of hypertension during laryngoscopy and tracheal intubation.[171] Ketamine is a nonbarbiturate dissociative anaesthetic and a cyclohexanone derivative that is rapidly acting and produces profound anaesthesia and analgesia.[175] Ketamine's onset of action, when given IV, is within seconds and when administered IM, the onset of action is approximately 4 minutes, with a duration of action from 15 to 30 minutes and elimination half-life of approximately 45 minutes.[175] Propofol is a general anaesthetic with unknown mechanism of action, possibly due to γ-aminobutyric acid receptor agonism or glutamate receptor antagonism. Onset time is 30 to 60 seconds, the duration of effect is 3 to 10 minutes and the elimination half-life is 40 minutes. The most serious maternal side effect of propofol is a decrease in systemic vascular resistance, causing hypotension.[171]

Safety studies comparing thiopental with propofol in healthy women undergoing cesarean delivery showed lower blood pressures during and after tracheal intubation with propofol 2 to 2.5 mg/kg compared with thiopental 4 to 5 mg/kg.[176-178] No expanded investigation into thiopental or propofol dose variations has been done in healthy pregnant women or women with preeclampsia. It is possible that both the timing and dose of induction drug significantly affect the haemodynamic response to laryngoscopy and tracheal intubation. There is limited literature regarding the optimal choice and clinical effect of pharmacologic medications for control of arterial blood pressure during the rapid-sequence induction of general anaesthesia in women with preeclampsia.[171] There are no trials available that compare the medications used in current practice, in particular with propofol as the induction drug, and there is insufficient evidence to support one particular drug as first line therapy. It may be that a combination of

drugs from different classes, along with a patient-specific dose of induction drug, leads to optimal haemodynamic stability.[171]

1.5 Experimental Models of Preeclampsia

Currently preeclampsia treatment is based on expectant management as there is no cure, apart from delivery. Experimental animal models of preeclampsia allow investigation of the disease and the associated physiological interrelationships in a whole body system, as well as potential treatments, especially where BP management is concerned.[179] There have been developments in the prevention of preeclampsia with regard to daily low-dose aspirin before week 16 of pregnancy reducing the risk of early onset preeclampsia. Aspirin however does not reduce the risk of late-onset preeclampsia, which accounts for a significant percentage of cases.[180-182] Additionally, calcium supplementation may reduce risk of preeclampsia in women with low dietary intake however neither aspirin or calcium prevent development of preeclampsia in all women.[179] Lack of effective therapies for preeclampsia create the need for preclinical animal models of preeclampsia to identify causes, develop new therapies, and to assess safety and efficacy of interventions before evaluation in clinical trials in pregnant women.[179] Experimental models of preeclampsia are also needed in order to determine the whole body and pregnancy impacts of therapies. Preclinical animal models have consistent environmental and clinical conditions, with less genetic variation than in human studies and facilitate direct assessment of the underlying mechanisms for responses to interventions using *in vivo, in vitro* and molecular approaches where whole body responses and pregnancy outcomes need to be assessed.[179]

Preeclampsia is generally seen as a human disease with few reports in other species.[183-185] One report describes a suspected case in a chimpanzee at Dallas Zoo in 1971. Clinical signs noted over the course of this chimpanzee's pregnancy included coma, seizures, mild oedema of extremities and mild albuminuria. Preeclampsia was suspected given these signs and the chimpanzee responded to treatment that included calcium supplementation, corticosteroids, dietary management and there was an apparently spontaneous recovery following delivery. [183]

Spontaneous preeclampsia has been described in several other non-human primate species, including gorillas[186] and patas monkeys.[187] Conclusive evidence of preeclampsia is often lacking in these cases as access to diagnostic information and samples, including blood pressure data and renal tissue histopathology, is often limited. Spontaneous preeclampsia in a baboon (*Papio hamadryas*) pregnant with twins has been documented, with the features of preeclampsia resolving postpartum.[188] There has been a report of adult African green monkeys (*Chlorocebus aethiops sabaeus*) developing hypertension spontaneously.[189] Other preclinical models in which preeclampsia develops spontaneously include the Blood Pressure High (BPH)/5 mouse

model,[190] the SHHT (spontaneous hypertension and heart failure) rat[191] and Dahl salt-sensitive rat as examples.[192] These strains of rodent are hypertensive before pregnancy. The placental structure of non-human primates most closely resembles human placental structure.[193] As such, it is possible to do proof-of-principle studies in non-human primates.[188] Baboons (*Papio anubis* and *Papio hamadryas*) and rhesus monkeys (*Macaca mulatta*) have been used to model preeclampsia, predominantly by initiating uteroplacental ischemia via surgical ligation of placental blood supply.[194,195] The advantages of the baboon model relates to reproductive and physiological similarity to humans including early vasodilation of pregnancy, haemomonochorial placentation, singleton pregnancies, upright posture and renal histopathological correlation.[196]

Models of preeclampsia are characterised by the development of hypertension during pregnancy in combination with proteinuria and renal pathology. Most preclinical models are induced by interventions that act at specific points on pathways thought to lead to preeclampsia in humans, which may be downstream of the initiating factors (Stage 1), and so do not reflect the complete phenotype of human preeclampsia. [179]

Late-onset preeclampsia in humans is not, however, associated with impaired spiral artery remodelling and, therefore, likely results from defects in other pathways.[197] Studies in animal models show that a preeclampsia-like phenotype can be induced through surgical restriction of placental perfusion, hypoxia, inflammatory challenges such as lipopolysaccharide, prothrombotic challenges such as phosphatidylserine/phosphatidylcholine microvesicles, and in genetic models that over activate the renin-angiotensin system, reduce adenosine 5-monophosphate-activated protein kinase activation, or increase circulating antiangiogenic factors.[179] These animal models demonstrate that multiple factors, including restricted uterine blood flow, hypoxia, elevated maternal inflammatory cytokines, overactivation of the renin-angiotensin system, and either decreased angiogenic factors such as VEGF, PlGF or increased antiangiogenic factors (sFlt-1 or soluble endoglin) in the maternal circulation, are sufficient to induce a phenotype consistent with the human definition of preeclampsia.[194,198,199] The wide range of factors that can induce preeclampsia-like features adds support to the hypothesis that preeclampsia is a syndrome that can result from disturbances in different pathogenic pathways.[179]

Directly measuring BP through arterial cannulation is also possible in animals[200] in ways not safe, feasible or acceptable in humans. Implanted telemeter devices allow continuous BP measurement in a non-invasive way, to measure the sorts of variability that contribute to the risks in humans described above. They also allow for blood pressure monitoring without the stress artefact that may be created by manually measuring BP in animals[201] with a tail or limb cuff.[202] The impact of anaesthesia and surgical procedures, the impact of the environment (e.g. noise stressors) and even the influences of the day-night cycle and associated BP surge changes can be explored in a model of a preeclampsia phenotype of sufficient duration to provide meaningful data.

1.6 Aims and Hypotheses

Managing hypertension in women with preeclampsia with antihypertensives such as labetalol, methyldopa or hydralazine reduces the risk of serious complications. Antihypertensive therapy may also achieve better overall control of the variations in blood pressure induced by events such as anaesthesia, noise exposure and circadian rhythms. There may be differences in the effect on variability of different antihypertensive treatments in response to events.

This work aimed to investigate:

1. The variability of blood pressure in an experimental *Papio hamadryas* (baboon) model of preeclampsia (EPE) and the potential ways to control blood pressure using three antihypertensive agents commonly used in the clinical setting.

2. The blood pressure response to general anaesthetic agents commonly used in women with preeclampsia in an experimental model of preeclampsia, and the effect of the different antihypertensives on blood pressure control during anaesthesia.

3. The blood pressure response to controlled stressors (sounds, eating, and enclosure cleaning) in an experimental model of preeclampsia, and the effect of the different antihypertensives on blood pressure during exposure to controlled stressors.

2 MATERIALS AND METHODS

2.1 Overview of Experiments

The experiment involved establishing an experimental model of preeclampsia in nine pregnant baboons. The study was approved and overseen by the Sydney Local Health District Animal Ethics Committee (AEC) and abided by the Australian code for the care and use of animals for scientific purposes (The Code)[1] and the Principles and guidelines for the care and use of non-human primates for scientific purposes.[203]

There were six individual studies undertaken within each single pregnancy:

Study 1: Blood pressure whilst awake at rest and during sleep
Study 2: Early morning blood pressure surge
Study 3: Blood pressure under anaesthesia (ketamine, propofol, sevoflurane)
Study 4: Exposure to controlled stressors (sounds)
Study 5: Blood pressure whilst eating
Study 6: Blood pressure during cleaning of enclosure

Each study occurred collectively during a seven-week period during each animals' pregnancy (table 3-1). Animals were spontaneously pregnant within family groups at the Australian

National Baboon Colony (ANBC) where breeding was planned based on normal colony requirement reproduction rates. The colony has water *ad libitum*, and an established and stable vegetarian and commercial pellet diet. At 4-5 days prior to 130 days of gestation, animals were transported by climate controlled utility vehicle (as per the ANBC's AEC approved Standard Transport Operating Procedure) with companions from the ANBC to the Royal Prince Alfred Animal House (Sydney). After completion of the seven week study, animals returned to their family group at the ANBC prior to their delivery date. The gestation period for *Papio hamadryas* is typically 182 days.

Each animal had a telemeter surgically implanted at approximately 130 days gestation, and surgery or angiography procedure to establish the experimental model of preeclampsia (uteroplacental ischemia) approximately one week later. Continuous blood pressure data was collected from this point onwards (Figure 2-4 – Timeline of procedures). These established techniques have been published elsewhere.[204]

The animals were then assigned to receive antihypertensive drugs in a particular sequence (see table 2-1). The drugs selected for this study are those commonly used to treat women with preeclampsia in a clinical setting, labetalol (40 mg BID), methyldopa (10 mg/kg BID) and hydralazine (15 mg BID) and doses were adjusted for female weight.

Whilst receiving antihypertensive drug treatment, the animals were also allocated to receive a ketamine, propofol or sevoflurane anaesthetic before and after antihypertensive drug treatment was established (see table 2-2). The anaesthetic protocols selected for this study are either used routinely in baboons at the ANBC when required for veterinary care purposes or commonly used in women with preeclampsia when required in a clinical setting. Of the nine animals in the study, three received the ketamine anaesthetic, three received the propofol anaesthetic and three received the sevoflurane anaesthetic.

The animals were anaesthetised before and whilst on drug treatment. Whilst under anaesthesia blood pressure was continuously monitored via the implanted telemetry system and the animals underwent physical examination, fetal ultrasound, chorionic villous sampling as well as blood and urine collection. Anaesthetising animals before and after establishment of drug

treatment allowed investigation of the effect of drug and the absence of drug on BPV under three different types of anaesthesia.

The effect of various environmental factors on BPV whilst before treatment and whilst receiving treatment was also assessed. Blood pressure data was continuously collected from animals while they were awake at rest, during sleep, while they were eating, while their enclosure was being cleaned and whilst a series of potentially stressor or relaxing sounds was played to the animals including Mozart Music, lions roaring, and baboons barking and grunting (Figure 2-4 – Timeline of procedures).

2.2 Animal Selection, acclimatisation, training, and environmental enrichment

Baboons were selected for the experiment if they were considered healthy and met the following criteria:

- between 5 and 20 years of age (life span up to 27 years)
- regular menstrual cycling (usual cycle 35 days)
- not used in any other research studies within the past 12 months
- not previously used in uteroplacental ischemia (UPI) studies
- no abnormalities in the most recent routine health screen (which includes screening for tuberculosis, full blood count, biochemistry and urinalysis)

Once selected, female baboons were then housed with breeding male companions. Baboons that were screened to be potentially pregnant based on > 35 days amenorrhoea, had an abdominal-pelvic ultrasound to confirm pregnancy. Every animal studied has been published in this thesis.

Once selected, baboons were transported to the animal house at least 3 days prior to telemeter insertion surgery, to allow time to adjust to the new environment. Baboons were always transported and housed with at least two other familiar female baboons for companionship. Baboons were transported to an animal house by temperature-controlled utility vehicle in transport crates, and by experienced and familiar staff. Once at the animal house, a training program commenced to familiarise the animals with the carers as well as the enclosure. A

positive reinforcement approach was used to train the animals to move between the cages with food treats as rewards.

The housing facilities comprised a room with four cages: individual data collection cage 1, individual data collection cage 2, the restraint cage and a group cage (Figure 2-1). These areas were interlinked by steel mesh fully enclosed walkways to allow the animals to move between the cages. Individual data collection cages 1 and 2 were data collection cages (1 m x 1.2 m x 1.6 m) fitted with telemetry receivers for collecting transmitted data from the telemeters implanted in the animals. These cages were also fitted with an elevated perch to allow for the normal resting and sleeping position preferred by baboons. Between these two cages was a restraint cage (0.8 m x 0.8 m) to allow for brief restraint for injections to be administered without sedation or anaesthesia. This restraint cage is similar in principle to a 'cattle crush' or 'livestock stall' that allows for holding animals safely while they are given treatment. The animals are trained to move in and out of the restraint cage using positive reinforcement. The group cage was a large corral-type enclosure (3.7 m x 2.6 m x 2.0 m) and fitted with various perches, ladders and ropes to allow for social interaction, stimulation and exercise during the day. The floor of the group cage was covered in a wood shaving substrate to provide opportunity for normal foraging behaviour. A range of nuts and seeds was scattered in this substrate daily.

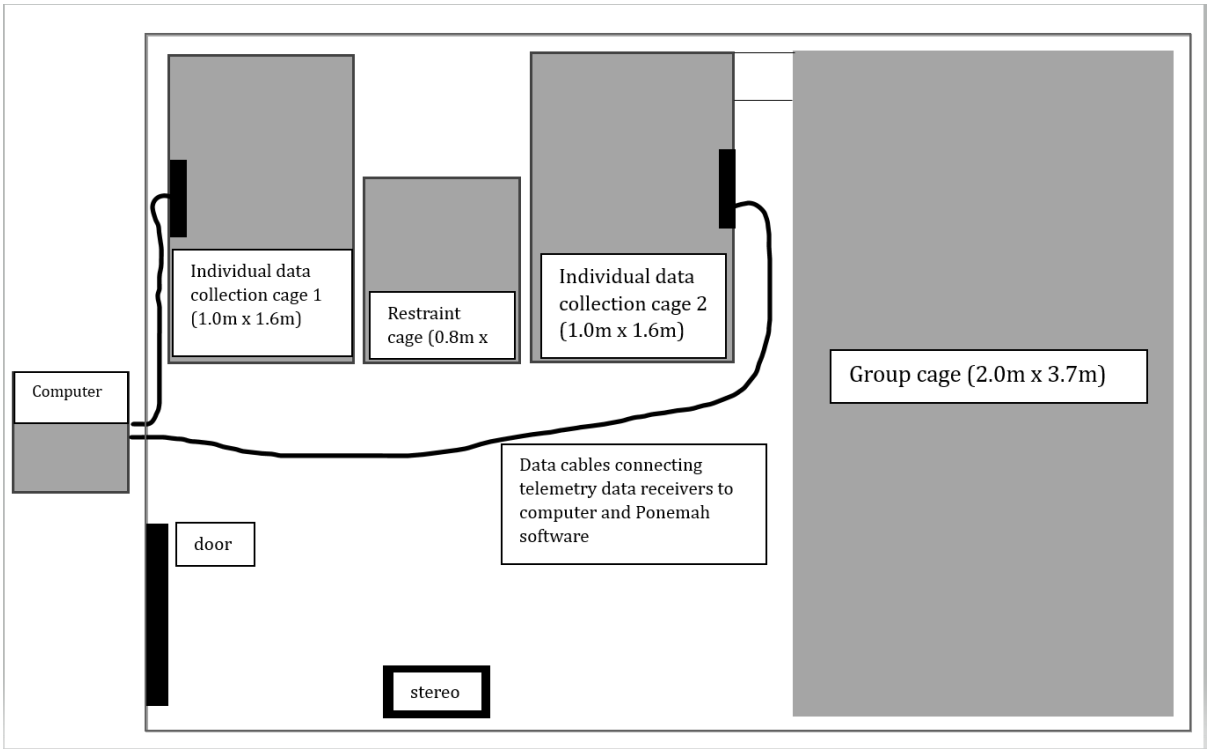

Figure 2-1 Experimental room set up for remote BP monitoring via telemeters of pregnant baboons before and after the experimental model of preeclampsia is established. Telemetry data was collected via receivers in Individual data collection cage 1 and Individual data collection cage 2. The restraint cage was used to administer sedation. The animals socialised in the Group cage during the day, outside of the defined data collection periods.

A daily routine for the animals was established. A semi-artificial day-night cycle was created using timed lighting in addition to the presence of a skylight in the experimental room. The daytime light cycle was set from 6am to 6pm and the night-time light cycle set to 6pm to 6am.

Environmental enrichment for the animals was primarily aimed at providing opportunities to engage in the behaviours they would in the wild as much as possible. Baboons in the wild will typically spend much of their day grooming each other (which maintains relationships and their social organisation), as well as foraging and exploring their environment. While the animals were in the group cage during the day, they spent much of the time grooming each other. The

group cage was furnished with perches, ladders, swings, tyres, baffle balls full of nuts/seeds. Water was freely available in all the cages: a trough and sipper in the group cage and sippers in the individual data collection cages. Added environmental enrichment was provided in the individual data collection cages in the form of seed tubes (a type of food toy), baffle balls as well as scattered food and nuts. The animals were moved into the individual data collection cages at night.

2.3 Experimental Design and Antihypertensive treatment

The experimental design was a cross-over design, where each animal received antihypertensive drug treatment (labetalol, methyldopa or hydralazine) with a wash-out period between each drug. The order in which the treatment was given to the animals was randomised using a Latin-square design[205] to account for potential temporal effects on attenuation of the model (Table 2-1). This type of design was feasible as the half-life of all the antihypertensive drugs used is relatively short ($t_{1/2}$ labetalol = 6 hours, $t_{1/2}$ methyldopa = 10 hours, $t_{1/2}$ hydralazine= 4 hours). The wash-out period provided an opportunity for the animals to return to their baseline blood pressure levels before receiving the next treatment. A potential disadvantage of this design is a risk of carryover effects confounding the estimated treatment effect. The advantage of the cross-over design is that each animal formed its own control group, thereby increasing the statistical power of the data.

Individual Animal Identifier	Drug given first	Drug given second	Drug given third
P04	labetalol	methyldopa	hydralazine
U20	methyldopa	hydralazine	labetalol
U30	hydralazine	labetalol	methyldopa
Y60	methyldopa	hydralazine	labetalol
F21	labetalol	methyldopa	hydralazine
B38	hydralazine	labetalol	methyldopa
Y46	hydralazine	labetalol	methyldopa
L50	labetalol	methyldopa	hydralazine
T40	methyldopa	hydralazine	labetalol

Table 2-1 Latin square design representing the order in which each animal (1-9) received labetalol, methyldopa and hydralazine. The codes PO4-T40 are unique animal identifiers.

The drugs were crushed and placed inside of preferred foods. These drugs maintain their potency after crushing and this is a viable method of preparing the drugs for human ingestion. The amount of drug ingested by the baboons at the time of each dosing was estimated by observing the animals consuming the food that the tablet was embedded in and checking the amount of leftover food and estimating how much of the tablet likely wasn't consumed. If the food containing the tablet was rejected entirely, a different type of food was selected, and the medication placed inside. Observation to ensure ingestion was for as long as necessary, generally between 5 and 60 minutes. The sample collected after a washout period was also the 'before treatment' sample for the next drug treatment.

Each drug (labetalol, methyldopa or hydralazine) was given to each animal twice daily for four days followed by a three-day washout period where no drug was given. Following the washout period, the second drug treatment would commence. Drugs were given orally, hidden in a piece

of banana, chocolate or peanut butter. Depending on the individual animals' preference, sometimes 1-2 squares of chocolate were melted, the crushed tablet mixed in, and some nuts and dried fruit were sprinkled on top then this was placed in the fridge to set. Sometimes the animals preferred large pieces of dried fruit (pawpaw, pineapple) so the crushed tablet was sprinkled inside of a sliced piece of dried fruit then the pieces were squeezed back together and given to the animal.

Labetalol (labetalol hydrochloride, Alphapharm Pty. Ltd., 100 mg tablets) was administered at a dose of 40 mg per day divided into two doses, given for a period of four days. This dose was selected as it is equivalent to a low starting dose used in a clinical setting. The 40 mg dose of tablet was measured by cutting the 100 mg tablet and weighing a 40 mg portion in a Square Plastic Anti-Static Weighing Dish using a laboratory scale (Sartorius analytical laboratory scale, model 1702). Labetalol lowers blood pressure by blocking peripheral alpha- and beta-adrenoceptors subsequently reducing peripheral vascular resistance.[206]

Methyldopa (anhydrous methyldopa, Alphapharm Pty. Ltd., 250 mg tablets) was administered at a dose rate of 10 mg/kg divided into two doses per day, given for a period of four days. The dose of tablet for each individual animal was measured by cutting the 250 mg tablet and weighing the portion in a weighing dish using a laboratory scale. Methyldopa was not administered in banana as it has been reported that there is a significant drug-food interaction between the polyphenol oxidase found in bananas and methyldopa, that causes the methyldopa to rapidly degrade.[207] Methyldopa binds to alpha-2 adrenergic receptors leading to a reduction in vasoconstrictor signals, subsequently reducing total peripheral resistance.[208]

Hydralazine (hydralazine hydrochloride, Alphapharm Pty. Ltd., 25 mg tablets) was administered at a dose rate of 15 mg per day divided into two doses, given for a period of four days. This dose was selected as it is equivalent to a weight-adjusted low starting dose used in a clinical setting. The 15 mg dose of tablet was measured by cutting the 25mg tablet and weighing a 15 mg portion in a weighing dish using a laboratory scale. Hydralazine alters cellular calcium metabolism which has the effect of relaxing and dilating vascular smooth muscle.[209]

The preferred food was selected and the weighing dish containing the weighed, crushed tablet was mixed into the selected food. When banana was used, an end of the banana was sliced off,

still in the skin, and some of the seeded middle part of the banana was removed, the crushed tablet placed in, and the middle part placed back on top like a plug. When chocolate was used, it was Cadbury's Dairy Milk chocolate, and this was softened in the microwave for 15 seconds in a weighing dish. The crushed tablet was mixed with the softened chocolate. Nuts including almonds, hazelnuts, cashews, walnuts, and Brazil nuts were added to the top of the chocolate. This chocolate + nut + tablet mix was placed into the refrigerator for approximately 3 minutes to harden, then given to the animals. Baboons are often suspicious of new types of food, so prior to the course of tablets, animals were given extra treats including extra bananas, dried fruit, chocolate, chocolate covered sultanas etc. This had the added function of acclimatising the animals to being handed treat foods at certain times of day.

2.4 Anaesthesia for surgical procedures and sample collections

In addition to being allocated to an antihypertensive drug treatment group, the nine animals in this study were also allocated to either the ketamine, propofol or sevoflurane anaesthetic protocol groups as outlined below in Table 2-2. The group of three animals receiving each anaesthetic were each receiving a different sequence of antihypertensive medication (described in Table 2-1).

Individual Animal Identifier	**Anaesthetic protocol**
P04	ketamine
U20	ketamine
B38	ketamine
F21	propofol
Y60	propofol
U30	propofol
T40	sevoflurane
Y46	sevoflurane
L50	sevoflurane

Table 2-2 Anaesthetic protocol allocation. Routine procedures were conducted under each of the anaesthetic protocols.

The anaesthetic protocols selected for this study were either the standard ANBC anaesthetic protocol or anaesthetic protocols commonly used in women with preeclampsia in a clinical setting,[210] and the purpose of the anaesthetic procedures undertaken were to allow for physical exam, fetal ultrasound as well as blood and urine collection.

The animals were anaesthetised before and whilst on each drug treatment. General anaesthesia was administered to achieve Stage 3 of surgical anaesthesia (Guedel's classification). Whilst under general anaesthesia, heart rate and blood pressure were continuously monitored via

telemetry and vital signs monitored via pulse oximetry. The animals allocated to the propofol and sevoflurane protocols were intubated and the animals allocated to the ketamine protocol received oxygen via face mask. Whilst anaesthetised, animals had a physical exam and fetal ultrasound, blood, urine and chorionic villous samples (CVS) were collected. Anaesthetising animals before and whilst on drug treatment allowed investigation of the effect of each antihypertensive drug on blood pressure complexity under the three different types of anaesthesia.

2.4.1 Anaesthetic Protocol: Ketamine

Anaesthetic induction was achieved using intramuscular ketamine (Ketamil®, Troy Laboratories, NSW, Australia) at a dose rate of 5-10 mg/kg. Atropine (0.04 mg/kg; Pfizer Australia, NSW, Australia) and metoclopramide (0.25 mg/kg; Pfizer Aust, NSW, Australia) were given intramuscularly following induction to prevent hypersalivation and vomiting. Oxygen was administered via face mask at a rate of 10 l/min. An intravenous cannula (21 gauge peripheral intravenous cannula, Becton Dickinson Pty. Ltd, Australia) was then placed into the cephalic vein to administer a constant rate infusion of ketamine (0.2 mg/kg/min) in saline. Clonazepam (0.01 mg/kg; Rivotril®, Roche Products, NSW, Australia) was administered intravenously to prevent any seizure activity that the ketamine infusion may cause.

2.4.2 Anaesthetic Protocol: Propofol

Anaesthetic induction was achieved using intramuscular ketamine (Ketamil®, Troy Laboratories, NSW, Australia) at a dose rate of 5-10 mg/kg. Atropine (0.04 mg/kg; Pfizer Australia, NSW, Australia) and metoclopramide (0.25 mg/kg; Pfizer Aust, NSW, Australia) were given intramuscularly following induction to prevent hypersalivation and vomiting. Oxygen supplementation via face mask at a rate of 10 l/min commenced. An intravenous cannula was then placed into the cephalic vein to administer an additional induction dose of propofol (2-4 mg/kg to effect; Provive®, Claris Lifesciences Ltd., Gujarat, India) to allow intubation. Animals were then intubated in the supine position using an appropriately sized cuffed endotracheal tube (sizes ranged from 4.5 to 6.5) and laryngoscope (Miller style straight blade, size 3). Oxygen was supplied through the endotracheal tube at a flow rate of 30 ml/kg/min via rebreathing system on a continuous flow Boyle's anaesthetic machine. Anaesthesia was maintained by a constant rate intravenous infusion of propofol (0.4–0.6 mg/kg/min).

2.4.3 Anaesthetic Protocol: Sevoflurane

Anaesthetic induction was achieved using intramuscular ketamine (Ketamil®, Troy Laboratories, NSW, Australia) at a dose rate of 5-10 mg/kg. Atropine (0.04 mg/kg; Pfizer Australia, NSW, Australia) and metoclopramide (0.25 mg/kg; Pfizer Aust, NSW, Australia) were given intramuscularly following induction to prevent hypersalivation and vomiting. Oxygen supplementation via face mask commenced. An intravenous cannula was then placed into the cephalic vein to administer an additional induction dose of propofol (2-4 mg/kg to effect; Provive®, Claris Lifesciences Ltd., Gujarat, India) to allow for rapid sequence intubation. This was followed immediately by a dose of intravenous suxamethonium (1 mg/kg; Astra Zeneca 100 mg/2 ml) to facilitate tracheal intubation. Suxamethonium stops spontaneous breathing for the duration of its half-life (1-2 minutes). Animals were then intubated in rapid sequence in the supine position using an appropriately sized cuffed endotracheal tube (sizes ranged from 4.5 to 6.5) and laryngoscope (Miller style straight blade, size 3). Animals were ventilated manually at a rate of 10-12 breaths per minute with intermittent positive pressure using a one litre non-latex anaesthetic breathing bag (GE Healthcare) until they recommenced spontaneous breathing. Anaesthesia was maintained by sevoflurane (inhalational) to effect delivered via rebreathing system on a continuous-flow anaesthetic machine. Oxygen was supplied at a flow rate of 30 ml/kg/min.

2.5 Uteroplacental ischemia induction (UPI)

Animals were anaesthetised for the induction of UPI. The animal was laid supine with both legs extended and secured. Two techniques to establish UPI were used.

2.5.1 UPI - Surgical ligation model

Animals were positioned semi-laterally to limit possible supine hypotension and to tilt the uterus away from the uterine artery. Prior to surgery, the uterine artery was located using colour Doppler ultrasonography (GE Healthcare Voluson E8 Expert ultrasound machine). Based on the relative blood velocities, the uterine artery thought to be the smaller was identified. Following sterile preparation of the surgical field using 7% povidone-iodine (Betadine Antiseptic Liquid, Alpha Medical Solutions, Australia), suprapubic transperitoneal incision was made followed by a small incision in the broad ligament. The uterine artery was identified, dissected and irrigated with 1% lignocaine solution (lignocaine hydrochloride, Pfizer, Australia) to reduce arterial spasm. The vessel was then ligated with 4.0 silk sutures. The peritoneum, muscle, and skin were closed in layers.[194]

2.5.2 UPI - Angiography

The femoral artery contralateral to the telemeter insertion site was used. Following sterile preparation of the surgical field a 0.3cm dermal incision was made to allow insertion of the needle and guidewire into the femoral artery. A micro puncture kit was used to gain access to the artery and a 5 Fr Glide-sheath Slender® introducer sheath (Terumo Interventional Systems, New Jersey, USA) used to access the femoral artery for the procedure.

A percutaneous aortogram was performed first. A catheter (0.49mm PROGREAT™, Terumo Interventional Systems, New Jersey, USA) was inserted into the aorta, using image intensification (Siemens Healthineers Cios Select Mobile C-arm machine), at the level of the renal arteries. Radio-opaque contrast (Omnipaque ® 300 mg/ml, GE Healthcare, Illinois, USA) was injected at 2 ml/sec for 6 seconds. This allowed the visualisation of the left and right uterine arteries and the distribution of vascularisation within each placenta. The catheter was inserted into the right uterine artery and contrast injected at 1 ml/sec for approximately 5 seconds (selective right uterine artery angiogram). This second procedure allowed visualisation only of the areas of the placenta supplied by the right uterine artery. Each spiral vessel

generated a well-defined 'blush' of contrast, which corresponded to dye entering the intervillous space of each cotyledon. The target uterine artery was the non-dominant, to allow adequate perfusion for the pregnancy to continue.

Reduction of uterine blood flow was achieved by inserting particles (Contour™ PVA Embolisation particles, Boston Scientific, USA) reconstituted with 10 ml of normal saline; 1-2 ml of the 10 ml particle-saline solution was injected along with coils (1.8mm figure 8, Boston Scientific, Massachusetts, USA). After the catheter was placed in the selected uterine artery, coils were positioned in the artery until there was no visible flow into the artery. To prevent clotting in the surrounding vessels because of the instrumentation, an ACDA-A Citrate (Baxter Healthcare, Australia) infusion (12.5mg/min) and calcium gluconate (Phebra, NSW) infusion (31mg/min) were administered for the duration of the angiogram.

Prophylactic intravenous antibiotics were administered at the end of the procedures. Antibiotics administered were benzyl penicillin (300 mg, Crown Scientific Limited, Victoria, Australia) and tobramycin (20 mg, David Bull Laboratories, Victoria, Australia). For all surgical procedures, pre-operative and post-operative buprenorphine (0.02 mg/kg, Temgesic®, Reckitt Benckiser Pty. Ltd., Australia) was administered to provide analgesia. Animals were closely monitored post-operatively and additional analgesia was administered where required.

The UPI model was established surgically in the first eight animals. The ninth animal in the protocol had UPI induced via angiography. Surgical induction of the UPI became increasingly difficult and less feasible, due to variability of the anatomy and identifying an appropriate uterine artery using doppler to target to induce a reliable phenotype. Subsequently, the approach to establishing the model was reviewed and adjusted with angiography being used to assist in visualising the arteries and providing the access to use the interventional radiology techniques of particle and coil placement to achieve the reduction in uterine blood flow in place of surgical ligation of the uterine artery. Angiography provides clear radiographic visualisation of the arteries which enabled accurate identification of the appropriate artery to ligate.

2.6 Telemeter insertion

Blood pressure data was collected by telemeters (PA-D70, Figure 2-2 and M10-F1, Figure 2-3; Data Sciences International, St. Paul, MN, USA). The telemeters were surgically implanted to measure intra-arterial blood pressure. The PA-D70 implants were used in the first seven animals of the protocol and the M10-F1 implants were used in the last two animals of the protocol as the manufacturer upgraded the implant and the PA-D70 implants were discontinued. The primary difference between these two implants is that the updated, M10-F1 series can collect data over a greater range and the battery life is longer with no difference in the sensitivity or algorithm for data collection.

The devices are designed for use in large animals and record pressures ranging between -20 and 300 mmHg using a pressure transmission catheter. The transmitter bodies are made from silicone elastomer with the PA-D70 implant weighing 37 grams (50 x 50mm) and the M10-F1 implant weighing 13.7 grams (40 x 26 x 14 mm). The implants are magnetically activated on and off allowing storage without loss of battery life. As the devices are surgically implanted into animals, they are sterilised by the manufacturer and double wrapped in sterile packaging for transportation.

Figure 2-2 Picture of PA-D70 implant, a telemeter for data collection.

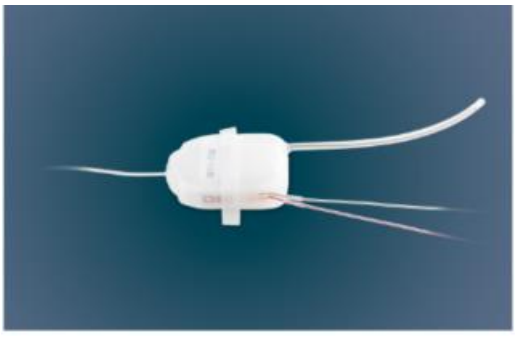

Figure 2-3 Picture of M series implant, an upgraded telemeter for data collection.[211]

Surgical implantation of the devices involved a 3-5cm inguinal incision to access the profunda femoris artery via blunt dissection. The catheter component of the telemeter was inserted into the profunda femoris and placed a calculated distance into the mid-abdominal aorta. The catheter is secured to the blood vessel with insoluble sutures (6.0 silk, US Surgical Corporation, Norwalk, CT, USA). The muscle above the vessel is closed in layers using a simple continuous suture pattern. The body of the telemeter is placed in a subcutaneous pocket over the abdominal musculature. Both skin incisions are closed with dissolvable buried sutures. Prophylactic intravenous antibiotics were administered at the end of each procedure: benzyl penicillin (300 mg, Crown Scientific Limited, Victoria, Australia) and tobramycin (20 mg, David Bull Laboratories, Victoria, Australia). Pre-operative and post-operative buprenorphine (0.02 mg/kg, Temgesic®, Reckitt Benckiser Pty. Ltd., Australia) was administered to provide analgesia.

2.7 Blood pressure data collection

The BP data collected by the telemeters included systolic and diastolic blood pressure, heart rate, pulse pressure, transmitter signal strength, temperature and animal activity. This data was transmitted from the telemeter to a remote receiver (RMC-1 receiver; Data Sciences International) and then transferred via cable to a data exchange matrix (Data Sciences International), which was connected to a computer that recorded blood pressure readings every 20s (Ponemah ™ software).

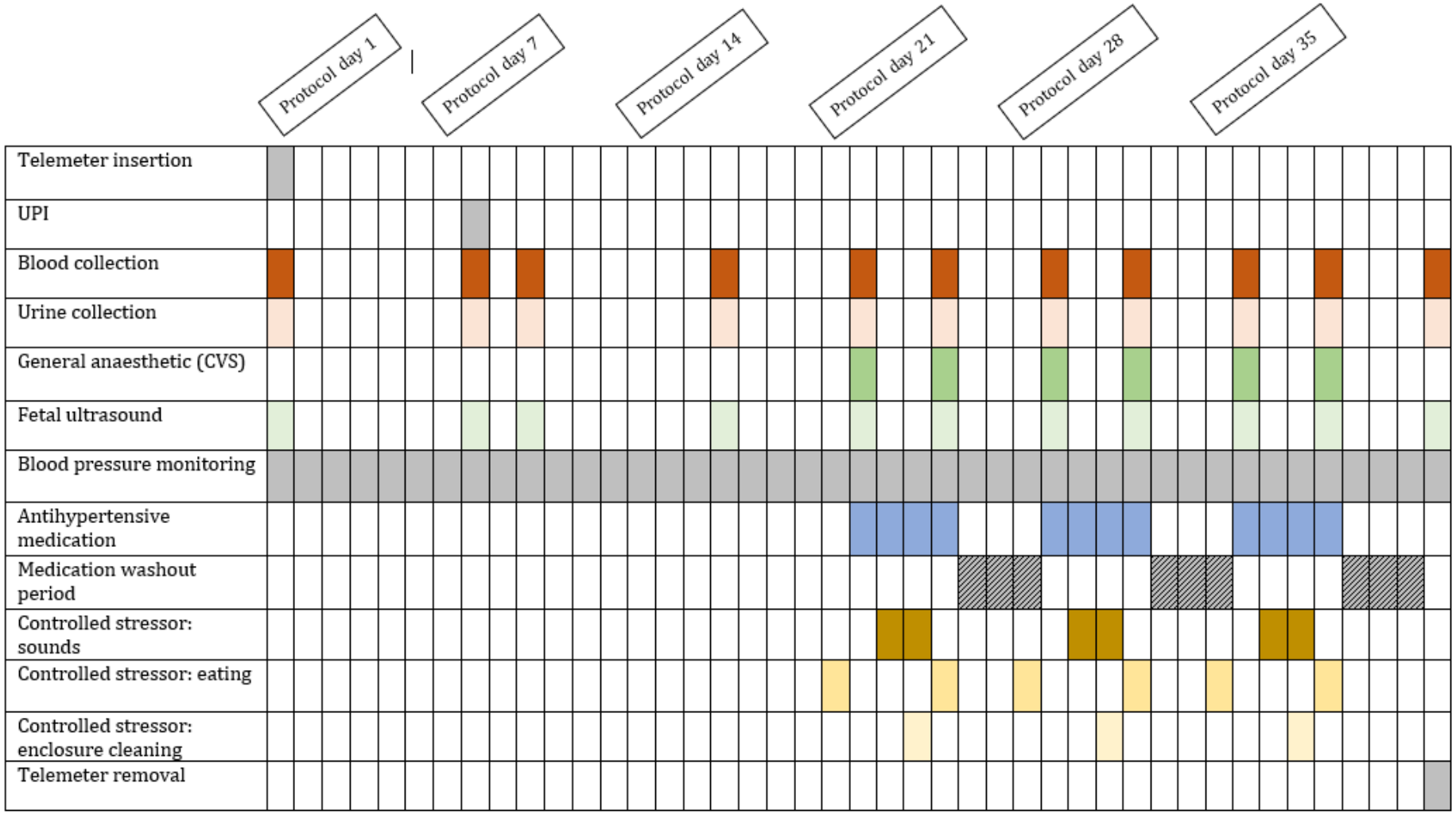

Figure 2-4 Timeline of procedures and sample collections.

2.8 Blood pressure data collection parameters

2.8.1 Confirming the UPI model

The induction of UPI resulted in the development of the experimental model of preeclampsia in the study animals as described in the literature.[194,212] This was demonstrated by an increase in blood pressure, development of proteinuria, and increase in circulating sFlt-1.[194,212]

Blood pressure data was collected two days before the UPI procedure whilst the animals were awake (between 4pm – 8pm) and asleep (11pm – 3am). This was defined as the baseline BP. Blood pressure data 14 days after the UPI procedure was used as the time point to confirm establishment of the UPI model; the literature reports that after 14 days of reduced placental blood flow the cardinal features of the model are present namely renal histological changes identical to human preeclampsia and increased sFlt-1 expression.[194]

2.8.2 Study 1: Blood pressure whilst awake at rest and during sleep

Blood pressure data whilst animals were awake and at rest was collected between 4-8pm each day of the experimental protocol. The data was refined using activity counts, a measure generated by the telemetry software. Activity is a relative measurement in this context and used to determine if the animal is moving (active) or not (inactive). Activity counts are not directly generated by the implant, but instead are generated by the Data Exchange Matrix. As the animals move about in their enclosure, the telemetry signal transmitted to the receiver varies in strength. This variation is typically due to position or distance of the animal relative to the receiver. When the signal strength changes by a certain amount, the Data Exchange Matrix creates an 'activity count'. The number of counts generated is dependent on both distance and speed of movement. Blood pressure data collected that corresponded to a period of activity (such as moving around the enclosure, jumping on or off a perch) was excluded from the awake at rest data set. The same algorithm was used for all animals.

Blood pressure data was collected during sleep between the hours of 11pm and 3am. This data was also refined using the activity count as described above. Given baboons are diurnal, activity overnight was uncommon.

2.8.3 Study 2: Early morning blood pressure surge

The early morning surge (EMS) period for the purposes of this study was defined as the period 30 minutes from the time the lights went on in the enclosure in the morning, as determined by a preset light timer. This definition was established for pragmatic reasons due to the difficulty of accurately determining when the animals woke up without creating a disturbance.

2.8.4 Study 3: Blood pressure during anaesthesia (ketamine, propofol or sevoflurane) before and on antihypertensive treatment (labetalol, methyldopa or hydralazine)

Blood pressure data was collected from the time of anaesthetic induction to the time of recovery, before and after antihypertensive treatment was established. The average duration of all anaesthetics was 46 minutes and the variation between anaesthetic time (Table 2-3) was associated with the additional time required to intubate and administer suxamethonium (see section 2.4).

Anaesthetic protocol	Average anaesthetic duration - minutes (mean ± SEM)
ketamine	34 ± 2.3
propofol	49 ± 2.2
sevoflurane	56 ± 2.3

Table 2-3 Average duration (minutes) for ketamine, propofol and sevoflurane anaesthetic protocols.

2.8.5 Study 4: Exposure to controlled stressors

The BP data was collected during exposure to controlled stressors in the form of exposure to various sounds and music, before and after antihypertensive drug treatment was established. The stereo was set up in the room with the enclosure and set to a volume of approximately 60 decibels. All the sounds and periods of silence were on one recording so it was only necessary to be in the room once to set the stereo up and press play. Following

set up of the stereo, there was a period of 15 minutes of silence to allow for BP to return to baseline. Between each sound, a period of 15 minutes of silence/everyday familiar sounds were allowed to permit BP to return to baseline (Figure 2-5 and 2-6).

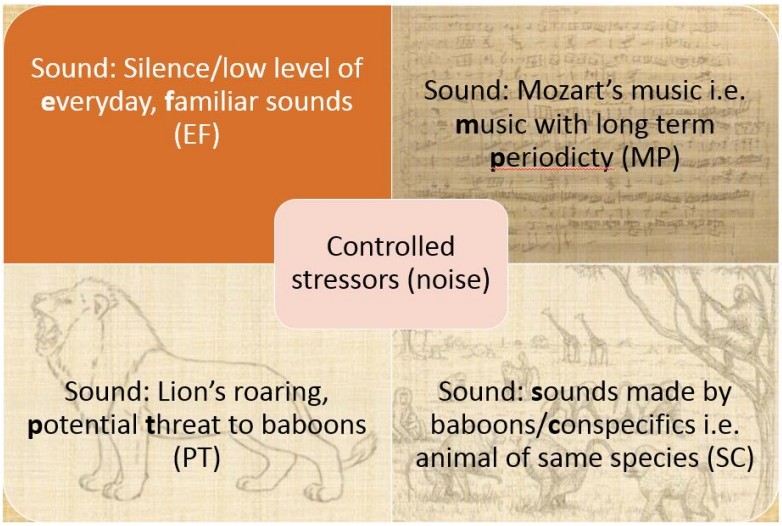

Figure 2-5 Pictogram of the four controlled stressor sounds.

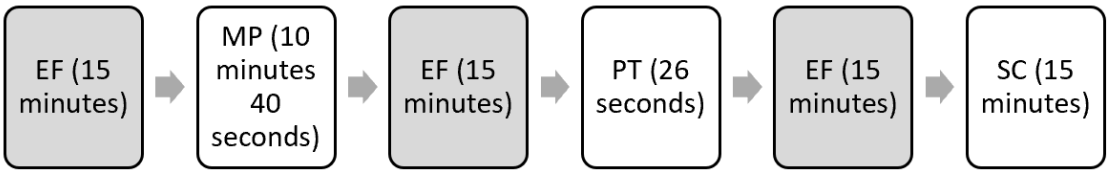

Figure 2-6 The sequence and duration of the controlled stressor sounds. This sequence was played to the animals twice per data collection session.

2.8.6 Study 5: Blood pressure whilst eating

Data was collected during the afternoon feeding time. Feeding time was a positive event; the animals routinely called out in anticipation when the food is being prepared and move towards the food as soon as it's scattered. The period of feeding was defined as 30 minutes from time food was given. Most of the food was consumed during the 30 minutes following the start of feeding.

2.8.7 Study 6: Blood pressure during cleaning of enclosure

Blood pressure data was collected during the weekly group cage clean. The group cage clean was considered a stressor for the animals. During this time the animal care staff moved the animals into the individual data collection cages and cleaned the group cage. This involved removing the enclosure substrate of wood chips/shavings, hosing the floors, walls, perches and water trough and relining the enclosure with fresh bedding which would take approximately two hours.

2.8.8 Duration of data collection periods

Data collection occurred during each week of antihypertensive treatment with labetalol, methyldopa or hydralazine and the duration of these data collection periods are outlined in Table 2-4 below. See Figure 2-4 for timeline of procedures outlining frequency of data collections during each week of antihypertensive treatment and section 2.9 for full description of data collection including how data was refined.

BP data collection parameter	**Duration of data collection period**
BP whilst awake (at rest)	4 hours per day
BP during sleep	4 hours per night
BP during EMS period	30 minutes
BP during general anaesthesia	46 minutes (average)
BP during exposure to controlled stressor EF	4 hours
BP during exposure to controlled stressor MP	40 minutes
BP during exposure to controlled stressor PT	1 minute 20 seconds
BP during exposure to controlled stressor SC	1 hour
BP whilst eating	30 minutes
BP during cleaning of enclosure	2 hours

Table 2-4 Duration of data collection periods for Study 1-6. EMS – Early Morning Surge, EF – Everyday familiar sounds, MP – Music with long-term periodicity, PT - Lion's roaring - a potential threat to baboons, SC - Calm vocal sounds made by conspecifics/baboons – i.e., animals of the same species.

2.9 Sample collection and processing

2.9.1 Routine Biochemistry, Haematology and Urinalysis

Routine biochemistry and haematology were used to monitor animal welfare, and especially the complications of experimental preeclampsia. The biochemistry, haematology and urinalysis were performed by commercial, National Association of Testing Authorities (NATA) accredited laboratory testing conducted at the nearby pathology service, on automated and approved machines.

Blood samples (5 ml) were collected from the cephalic vein via a 21 gauge sterile needle (Becton Dickinson Pty. Ltd., Australia) at baseline (before treatment) and after a 3-day course of either labetalol, methyldopa or hydralazine. Blood samples were also collected at the time of telemeter insertion, UPI and telemeter removal. The blood was immediately transferred to the appropriate processing tubes following collection: 500 ul was transferred to a paediatric K2EDTA tube (Becton Dickinson Pty. Ltd., Australia) for haematology and 500 ul was transferred to a paediatric Plasma Separator Tube (Becton Dickinson Pty. Ltd., Australia) for biochemistry. The blood collected for use in ELISAs (4 ml) was transferred to a K2EDTA tube (Greiner Bio-One International) then centrifuged at 3000 rpm for 10 minutes to separate the plasma. Aliquots of 250 ul were then transferred to 2 ml microcentrifuge tubes (Eppendorf, Hamburg, Germany) and snap frozen in liquid nitrogen before being stored in a -80°C freezer.

Albumin and liver function tests (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and g-glutamyl transpeptidase) were measured as an indicator of hepatic function. Acute changes in plasma creatinine and urea concentration were used as an indicator of renal function. Serum electrolytes (sodium, potassium, chlorine, bicarbonate, calcium, phosphate and magnesium) were monitored as an indicator of hydration status throughout the pregnancy. Haemoglobin and haematocrit were monitored to identify anaemia should it occur.

Platelets were also monitored to ensure adequate clotting as there was a theoretical potential for thrombocytopenia because of the UPI. White cell counts were monitored to assess for any infection.

The concentration of albumin, protein and creatinine in the urine were measured and a microalbumin/creatinine and protein/creatinine ratio were calculated (mg/mmol). Clinically significant proteinuria in the experimental model was based on the current clinical criteria for preeclampsia in humans i.e., a protein excretion rate of greater than 30 mg/mmol. These parameters were calculated using the Bicinchoninic acid (BCA) assay

which has the benefit of being able to detect proteins at concentrations as low as 5 ug/ml. Urine samples were collected at the same time points as the blood collections, namely at baseline (before treatment) and after a 3-day course of either labetalol, methyldopa or hydralazine and at the time of telemeter insertion, UPI and telemeter removal. Sterile graduated infant feeding tubes (5 Fr, Livingstone, NSW, Australia) were used in lieu of urinary catheters as they were appropriately malleable and sized for the procedure. These were inserted into the urethra under clean conditions using sterile lubrication jelly. Urine was collected from the tube into sterile urine collecting specimen containers, with the first 1-2 ml being discarded to minimise any risk of sample contamination.

2.9.2 Ultrasound

An ultrasound machine (Voluson Women's Health Ultrasound, GE Healthcare) was used to assess fetal health, fetal heart rate and monitor growth measurements (including biparietal diameter, head circumference and femur length), fetal morphology as well as facilitate collection of the chorionic villous sample.

2.9.3 Chorionic Villous Sampling

The CVS were collected transabdominally via a 19 gauge 1.5 inch needle and 10 mL syringe containing 2 ml of sterile Hams F10 nutrient media (Gibco, NSW, Australia) inserted into the placenta using ultrasound guidance to ensure the needle did not puncture the basal plate. The sample obtained was inoculated into a Petri dish containing Hams F10 media. The fronds of the placental tissue were then immediately pipetted into a 2 ml microcentrifuge tube (Eppendorf, Hamburg, Germany) and centrifuged at 1200 repetitions per minute for 3 minutes. The sample was separated from the excess Hams solution and snap frozen in liquid nitrogen following addition of an RNA stabilisation agent (RNALater, Qiagen, Australia). Following this procedure, fetal heartbeat was detected, and heartrate calculated to ensure the fetus was not compromised during the procedure.

2.9.4 ELISA for sFlt-1

Enzyme linked immunosorbent assay (ELISA) was performed using commercial kits for sFlt-1, (human VEGF R1/Flt-1 kit, catalogue number DVR100C, R&D Systems, MN, USA). The principle of the assay is based on the quantitative sandwich enzyme immunoassay technique whereby a monoclonal antibody specific for VEGF R1 is pre-coated on a microplate and any VEGF R1 present in the samples and standards pipetted into the wells are bound by the immobilised antibody. The unbound substances are washed away, then an enzyme-linked polyclonal antibody specific for VEGF R1 is added to the wells and by

adding the substrate/colour solution, colour develops in proportion to the amount of VEGF R1 bound in the initial step, and the intensity of the colour development is quantified using the optical density unit.

All reagents, standards and samples were prepared as follows:

1. Wash buffer was brought to room temperature and 20 ml of buffer was diluted into deionised water to prepare 500 ml of wash buffer.
2. Substrate solution – colour reagents A (stabilised hydrogen peroxide) and B (stabilised tetramethylbenzidine) were mixed in equal volumes within 5 minutes of use and protected from light.
3. VEGF R1 standard (lyophilised recombinant human VEGF R1) was reconstituted using 1 ml of deionised water to produce a stock solution of 20,000 pg/ml and manually agitated gently and allowed to sit for 15 minutes prior to making dilutions.
4. Dilutions were made up by pipetting 900ul of Calibrator Diluent RD6-10 into a test tube (2000 pg/ml) and 500 ul of RD6-10 into another six test tubes. The VEGF R1 20,000 pg/ml stock solution was used to create a dilution series of the following concentrations: 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml and 31.2 pg/ml. The 2000 pg/ml standard served as the high standard and Calibrator Diluent RD6-10 served as the zero standard (0 pg/ml).
5. Baboon plasma samples brought to room temperature from frozen (stored in – 80 °C freezer) immediately prior to assay and diluted serially 10-fold up to 100-fold where required using Calibrator Diluent RD6-10.

2.9.4.1 Assay Procedure

Calibrator Diluent RD6-10 (100ul) was added to each well of the pre-coated 96-well ELISA plate. Plasma sample (100ul) was added to each well. The wells were then covered with adhesive plastic film and incubated for 2 hours at room temperature on an orbital shaker (Platform Shaker, 200 rpm, orbit diameter 32 mm Ratek, Boronia, VIC, Australia) set to approximately 500 rpm.

The plate was then washed manually (using a pipette) four times with wash buffer ensuring that any remaining wash buffer was removed during the final wash by

inverting the plate upside down against clean paper towels. VEGF R1 conjugate (polyclonal antibody against VEGF R1 conjugated to horseradish peroxidase with preservatives), 200ul, was added to each well and the plate was again covered with adhesive plastic film and incubated for 2 hours at room temperature on the shaker. After incubation, the plate was again washed four times as described above, then 200ul of substrate solution was added to each well followed by another 30-minute incubation period in the cupboard at room temperature to protect from light. Stop solution was provided in the kit ready to use (2 N sulfuric acid), 50ul, was then added to each well and within 30 minutes, the optical density of each well was determined using a microplate reader (Flexstation 3, Molecular Devices, San Jose, CA, USA) set to 450 nm with wavelength correction set to 540 nm.

2.10 Methods of blood pressure analysis

2.10.1 Blood pressure averaging calculations

Blood pressure measurements collected for studies 1-6 (as outlined in sections 3.9.1 – 3.9.7) were exported from the Ponemah telemetry software into SPSS Statistics 25.0.0 (IBM Corporation, USA) for analysis.

2.10.2 Blood pressure load calculations

Blood pressure load is defined as the percentage of abnormally elevated BP readings, and it is usually calculated based on ambulatory BP monitoring. The equivalent of this was achieved in the baboon model using remote telemetry. Generally, BP load is a concept developed to evaluate how often BP remains above a particular threshold, and it has been suggested as a clinically useful parameter complementing the quantification of corresponding average ambulatory BP levels.[91,213] To provide an interpretation on the BP data presented in this thesis that is consistent with the concept of BP load and applicable to baboons, all sets of BP data collected during the defined periods described above were analysed by creating a Microsoft Excel Macro (Microsoft Office 16). The macro was designed to categorise data sets from the six studies into three categories: within one standard deviation above the mean BP, between one and two standard deviations of the mean BP and greater than two standard deviations of the mean BP. This approach was used in an effort to define BP burden in the animals in an equivalent, but more species-applicable way, than if the approach used to describe human BP load were to be directly applied to the baboons' BP data. Using the mean BP for each animal as the baseline, then categorising the BP values according to how many standard deviations from the mean the values were, allowed in some way individualisation of the data categorisation, and as such made the categories more species appropriate. Allocating the three categories a colour, mirroring the traffic light approach used when analysing human BP load, allowed the BP load data to be presented in a similar way to how a 24 hour BP profile can be presented in a clinical ambulatory BP monitoring report. This provided a comparative way to view the animal and human data. Again, the categories are defined, and colour coded as follows:

- Readings < 1 standard deviation above baseline
- Readings > 1 and < 2 standard deviations above baseline
- Readings > 2 standard deviations above baseline

2.10.3 Calculating blood pressure variability using SD1 and SD2 values generated by Poincaré Plots

In this study, the Poincaré plot was the method used to geometrically represent the non-linear components and trends of BP within the defined experimental periods. The Poincaré plot is a scatter graph that visualises the correlation between two consecutive data points in a time-series (i.e., x-axis: N versus y-axis: N+1).[214] In the past, Poincaré plots have been used in the analysis of physiological fluctuations (e.g., heart rate variability analysis) and have enabled researchers to measure short- and long-term variability separately.[215] The width of the plots (SD1) represent short-term BPV (represented in red in Figure 2-6 below) and the length of the plots (SD2) represent long-term BPV (represented in green on Figure 2-6 below). Poincaré plots have been used for their visual representations because their shape changes according to the nature of the variability of the data, and quantitatively by analysing the geometric components, i.e., width and length of the plot. In this study, the quantitative outputs (SD1/SD2) of the plots have been used due to the large volume of data.

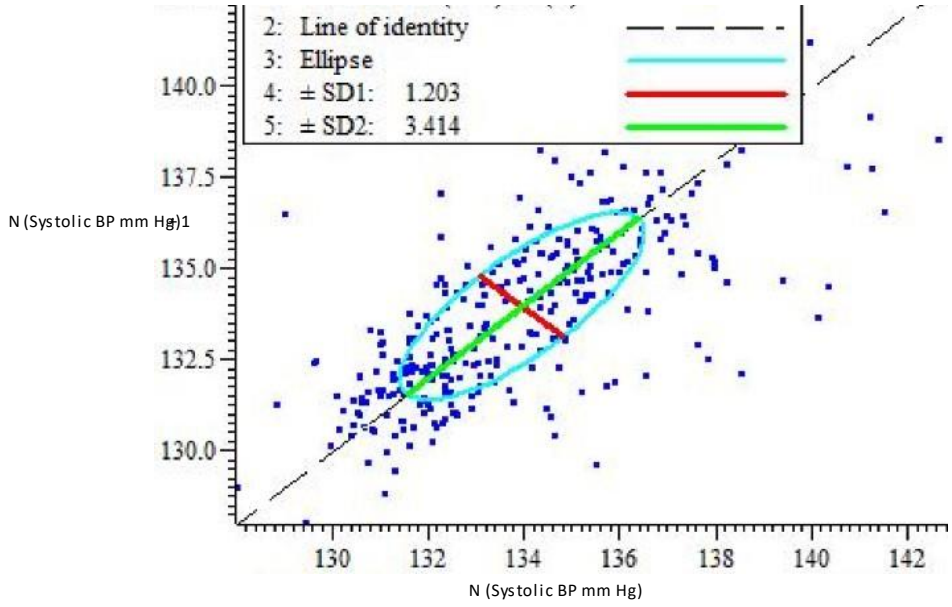

Figure 2-7 Example of Poincaré plot of systolic BP data showing the orientation of the axes and SD1/SD2 values.

The script creating the plot and ellipse was written by Geoff Horseman for Spike2 v8.06 x64 Unicode, copyright © Cambridge Electronic Design 1988-2015.

Specifically, the script generates a joint interval plot, also known as a Poincaré plot in which each blood pressure reading is plotted against the subsequent blood pressure reading to create a scatter plot. An ellipse is fitted to the data (similar to a 'line of best fit') and the standard deviation of the points on two axes is calculated. SD1 is perpendicular to the line of identity and SD2 runs along the line of identity. The script also calculates the standard deviation of blood pressure readings and the root mean square of differences between successive blood pressure readings. The Poincaré plot is a technique that is most commonly used in a clinical context to assess the dynamics of heart rate variability.[216]

2.10.4 Classifying blood pressure data as either stochastic, periodic, or chaotic

The publication: 'A simple method for detecting chaos in nature'[217] provided the code for the Chaos Decision Tree Algorithm via the online open access repository, Figshare. The Chaos Decision Tree Algorithm[217] was downloaded from this source, imported into MATLAB R2022B and applied to the BP data from each study (1-6). The output of this algorithm is a descriptor, or a classification that describes the dynamic of the BP data set as stochastic, periodic or chaotic.

The Chaos Decision Tree Algorithm, as published, consists of four steps, including determining if the data are stochastic using permutation entropy and a combination of Amplitude Adjusted Fourier transform surrogates and Cyclic Phase Permutation surrogates.[217] The data is then denoised using the Schreiber de-noising algorithm, corrected for possible signal oversampling, and then tested for chaos using a modified 0–1 test for chaos (Figure 2-8). The modified 0–1 test provides a single statistic, K, which approaches 1 for chaotic systems and approaches 0 for periodic systems. [217]

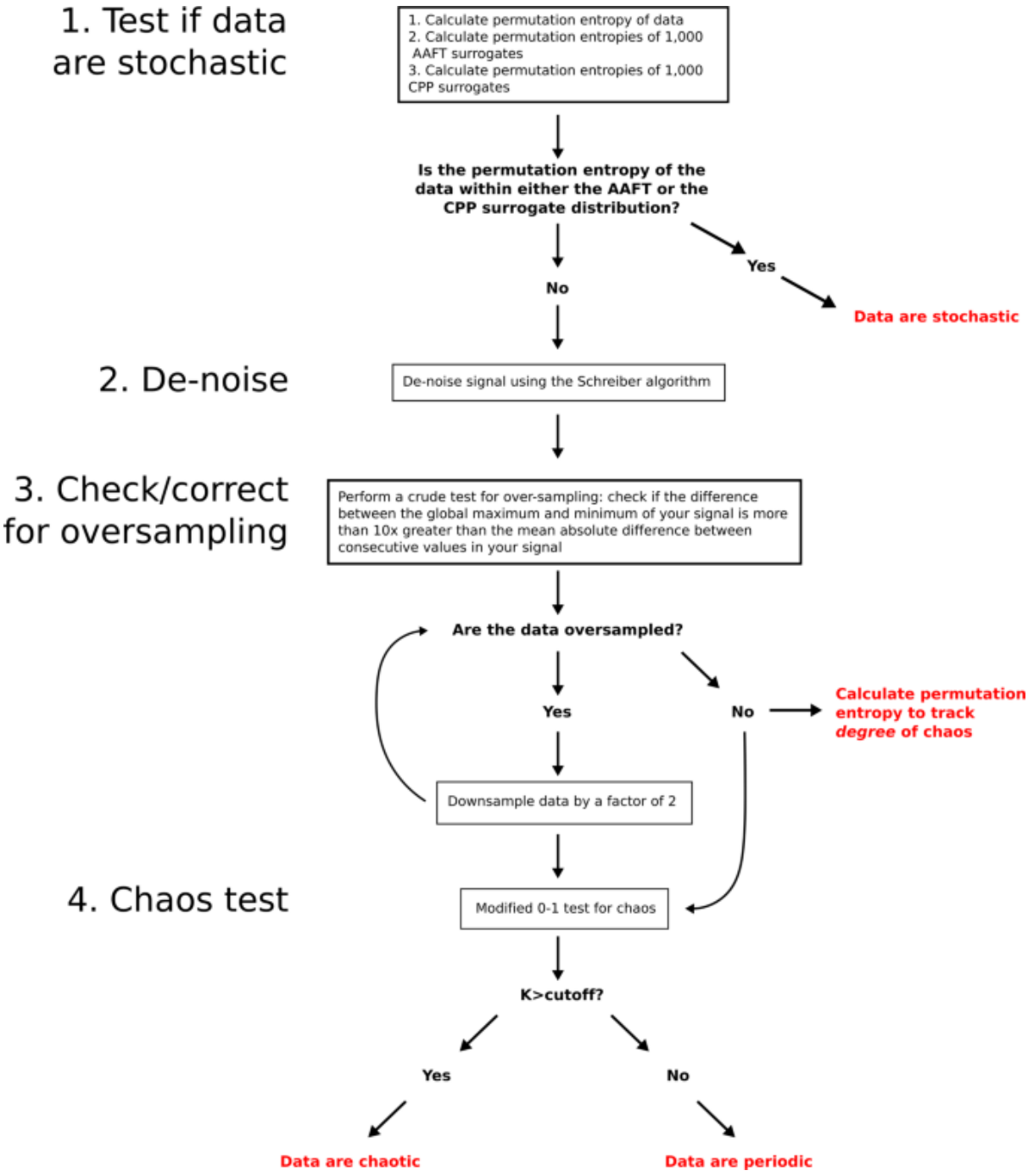

Figure 2-8 The Chaos Decision Tree Algorithm[217]

2.11 Statistical analysis

2.11.1 Confirming the UPI model

SPSS Statistics version 25.0.0 (IBM Corporation, USA) was used to perform the statistical analyses. Analysis of the data used to assess establishment of the UPI model was done using a Wilcoxon Signed Ranks Test with significance set at $p \leq 0.05$.

2.11.2 Average blood pressure

SPSS Statistics version 25.0.0 (IBM Corporation, USA) was used to perform the statistical analyses. Overall significance was determined using a two-level repeated measures ANOVA. Post-hoc pairwise comparisons were made using a Bonferroni adjustment for multiple comparisons. Significance was set at $p < 0.05$.

2.11.3 Blood pressure load

Statistical analysis of blood pressure load was calculated using a Pearson's chi-square test (Social Science Statistics Calculator) with significance set at $p < 0.05$. The BP data, a continuous variable, was transformed into categorical data for the purpose of BP load analysis. The BP data was categorised into three categories in a similar way to how a 24 hour BP profile presented in a clinical ambulatory BP monitoring report. As described in Section 2.11.2 the categories were defined, and colour coded as follows:

Readings < 1 standard deviation above baseline

Readings > 1 and < 2 standard deviations above baseline

Readings > 2 standard deviations above baseline

2.11.4 Blood pressure variability

SPSS Statistics version 25.0.0 (IBM Corporation, USA) was used to perform the statistical analyses. Overall significance was determined using a two-level repeated measures ANOVA. Post-hoc pairwise comparisons were made using a Bonferroni adjustment for multiple comparisons. Significance was set at $p < 0.05$.

3 RESULTS

3.1 Animal Clinical Characteristics

Data was collected from nine, healthy, pregnant *Papio hamadryas* (baboons). The age range of the animals at the time of the study was 5 – 13 years of age, the weight range was 10.9 – 17.5 kilograms at the time study commenced, and number of previous pregnancies was between 0-1 (Table 3-1). The study was approved and overseen by the Sydney Local Health District Animal Ethics Committee and abided by the Australian code for the care and use of animals for scientific purposes[1] and the Principles and guidelines for the care and use of non-human primates for scientific purposes.[203] Data from every animal studied has been published in this thesis. The animals returned to their family group at the ANBC at the conclusion of the seven week study.

Animal Clinical Characteristics									
Individual Animal Identifier	P04	U20	B38	F21	Y60	U30	T40	Y46	L50
Age at time of study (years)	6	7	8	7	5	7	7	13	8
Weight at start of study (kg)	12.2	12.4	16	13	15.4	17.5	12	10.9	11.4
Previous pregnancies	0	0	0	1	0	0	0	1	0

Table 3-1 Animal Clinical Characteristics. Age (years), weight (kilograms) and number of previous pregnancies.

The results will be presented in the following order:

i. Evaluation of data demonstrating that the UPI model was established in the cohort of the nine animals in the study (section 4.2).

ii. Study 1-6 in each animal (n=9) while on each of the 3 antihypertensives:

Study 1: Blood pressure whilst awake at rest and during sleep

Study 2: Blood pressure during anaesthesia (ketamine n=3, propofol n=3, sevoflurane n=3)

Study 3: Exposure to controlled stressors

Study 4: Early morning blood pressure surge

Study 5: Blood pressure whilst eating

Study 6: Blood pressure during cleaning of enclosure

iii. Each of the six studies above have been analysed in four ways, with calculations undertaken to ascertain:

Average blood pressure

Blood pressure load

Blood pressure variability using SD1 and SD2 values generated by Poincaré Plots

Classification of blood pressure data as stochastic, periodic or chaotic

3.2 Confirming the UPI model

3.2.1 Blood pressure before and after UPI

There was an increase in mean systolic BP (awake) after the UPI procedure as compared to baseline (p = 0.03) (Figure 3-1). Baseline BP data was collected between 4-8pm two days before the UPI procedure and compared to BP data collected during 4-8pm 14 days after the UPI procedure, i.e. after allowing time for the UPI syndrome to develop.[194] The mean systolic BP at baseline before UPI was 130 +/- 5.9 mmHg and after the UPI procedure was 137 +/- 4.6 mmHg.

There was also a significant increase in mean diastolic BP (awake) after the UPI procedure compared to baseline (p = 0.011) (Figure 4-1). The mean diastolic BP at baseline before UPI was 87 +/- 5.5 mmHg and after the UPI procedure 95 +/- 4.5 mmHg.

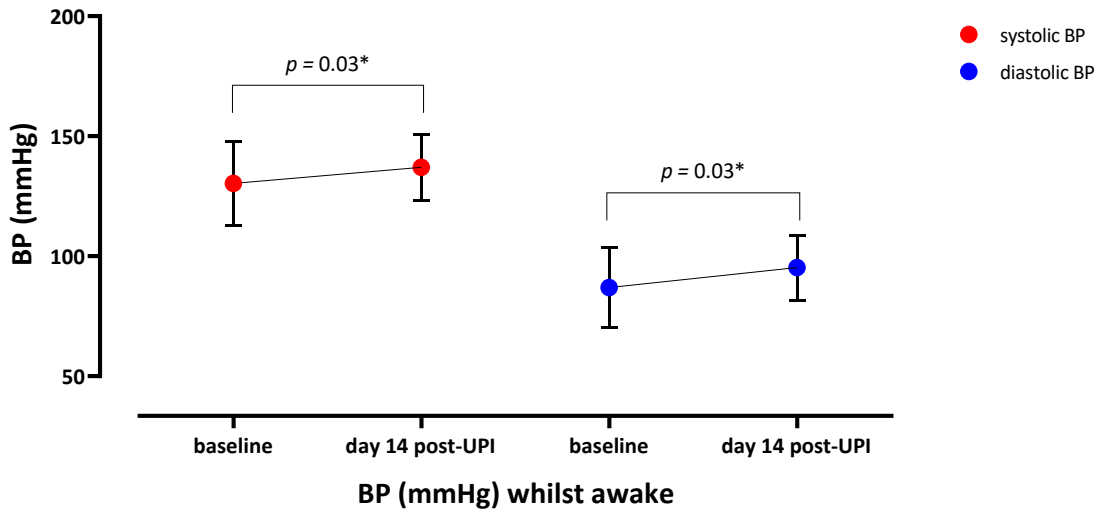

Figure 3-1 Average BP (awake) at baseline and 14 days after UPI (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$)

The increase in mean systolic BP during sleep after the UPI procedure compared to baseline although not statistically significant (p = 0.058) was elevated, (Figure 3-2). The mean systolic BP at baseline before UPI was 128 +/- 7.2 mmHg and after the UPI procedure was 136 +/- 4.2 mmHg.

There was a significant increase in mean sleep diastolic BP compared to baseline after the UPI procedure (p = 0.03). The mean diastolic BP at baseline before UPI was 83 +/- 6.6 mmHg and after the UPI procedure 94 +/- 3.9 mmHg.

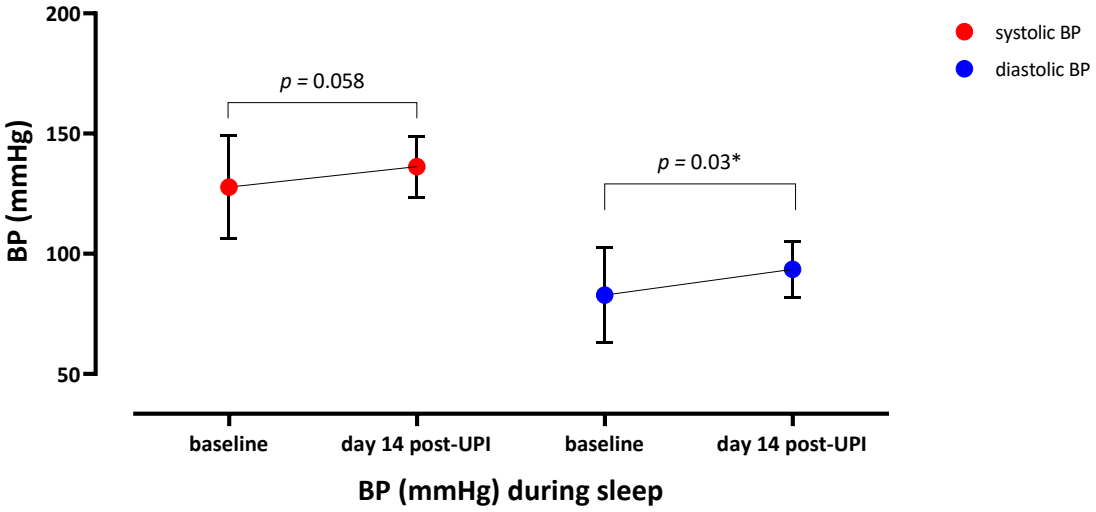

Figure 3-2 Average BP (sleep) systolic and diastolic BP at baseline and 14 days after UPI (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$)

3.2.2 Laboratory markers of the UPI model

3.2.2.1 sFlt-1 concentration

There was a significant increase in sFlt-1 concentration after the UPI procedure (p = 0.008). The mean concentration before UPI was 2670 +/- 531 pg/ml and after the UPI procedure mean concentration was 10563 +/- 12324 pg/ml (Figure 3-3).

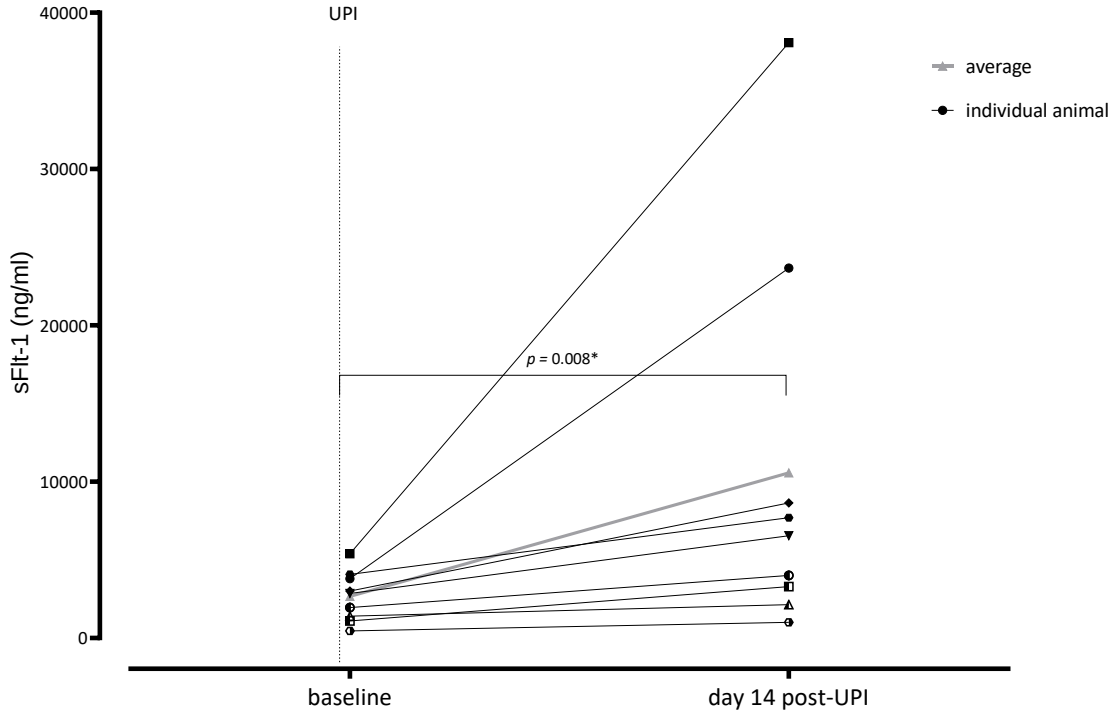

Figure 3-3 sFlt-1 concentration (ng/ml) at baseline and 14 days after UPI (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$). sFlt-1 – soluble fms-like tyrosine kinase 1, ng – nanograms, ml – millilitres.

3.2.2.2 Biochemistry and Haematology

After UPI, average serum albumin decreased (27.0 +/-2.1 g/l before UPI and 22.7 +/- 2.1 g/l after UPI; p = 0.01) which is an expected acute phase response. Other parameters including platelet count, urea, creatinine concentration, liver function tests (ALP, ALT, AST, GGT) did not differ significantly before and after UPI. This is in keeping with the mild phenotype of this model.

3.2.2.3 Urinalysis

The concentration of albumin, protein and creatinine in the urine were measured and a microalbumin/creatinine and protein/creatinine ratio (UPCR) were calculated (mg/mmol). Average urine protein to creatinine ratio was 10.5 mg/mmol before (*SD* = 18.4) and 30.1 mg/mmol (*SD* = 22.6) after the UPI procedure, however this was not statistically significant ($p = 0.2$), (Figure 3-4). Average urine microalbumin to creatinine ratio was 5.4 mg/mmol before (*SD* = 9.6) and 24.8 mg/mmol (*SD* = 52.8) after the UPI procedure; this change was also not statistically significant ($p = 0.4$) (Figure 3-5).

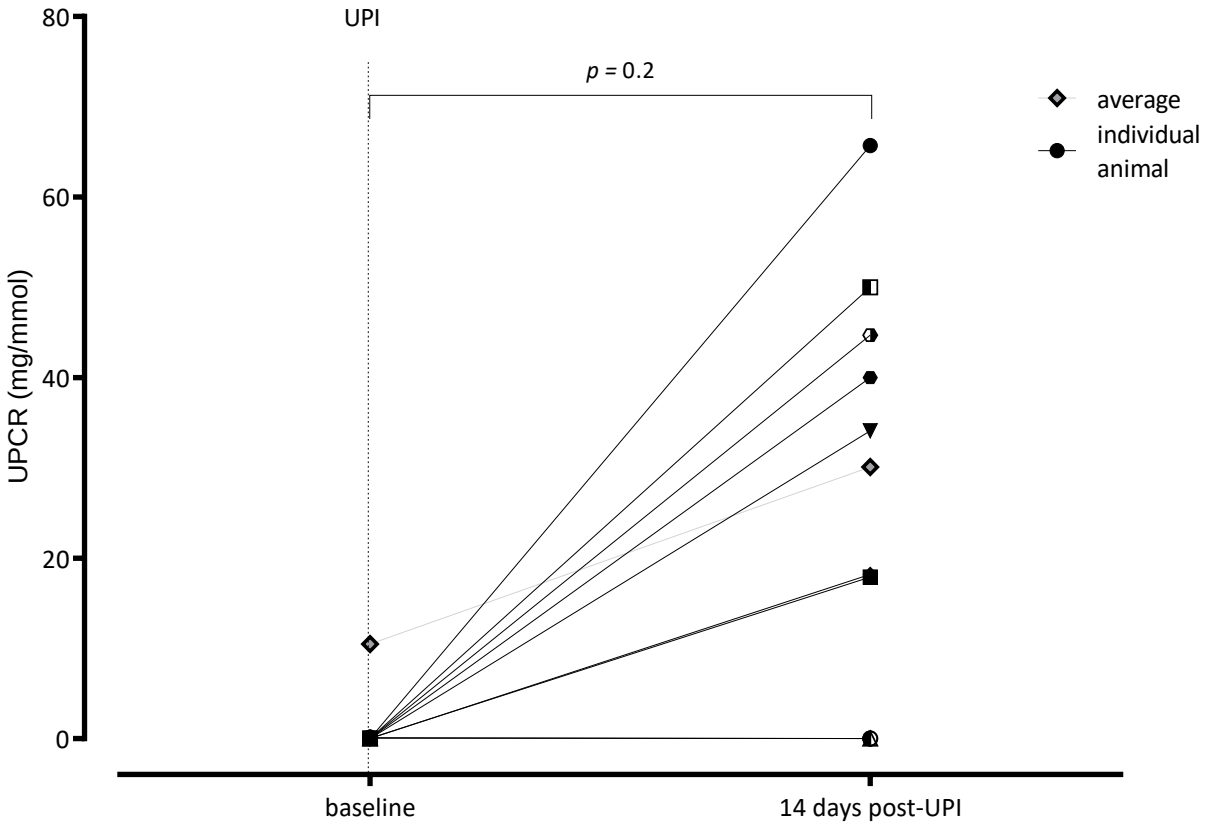

Figure 3-4 Urine total protein to creatinine ratio at baseline and 14 days after UPI (n = 9). Statistical significance defined as $p \leq 0.05$. UPCR – urine total protein to creatinine ratio, mg – milligrams, mmol – millimoles.

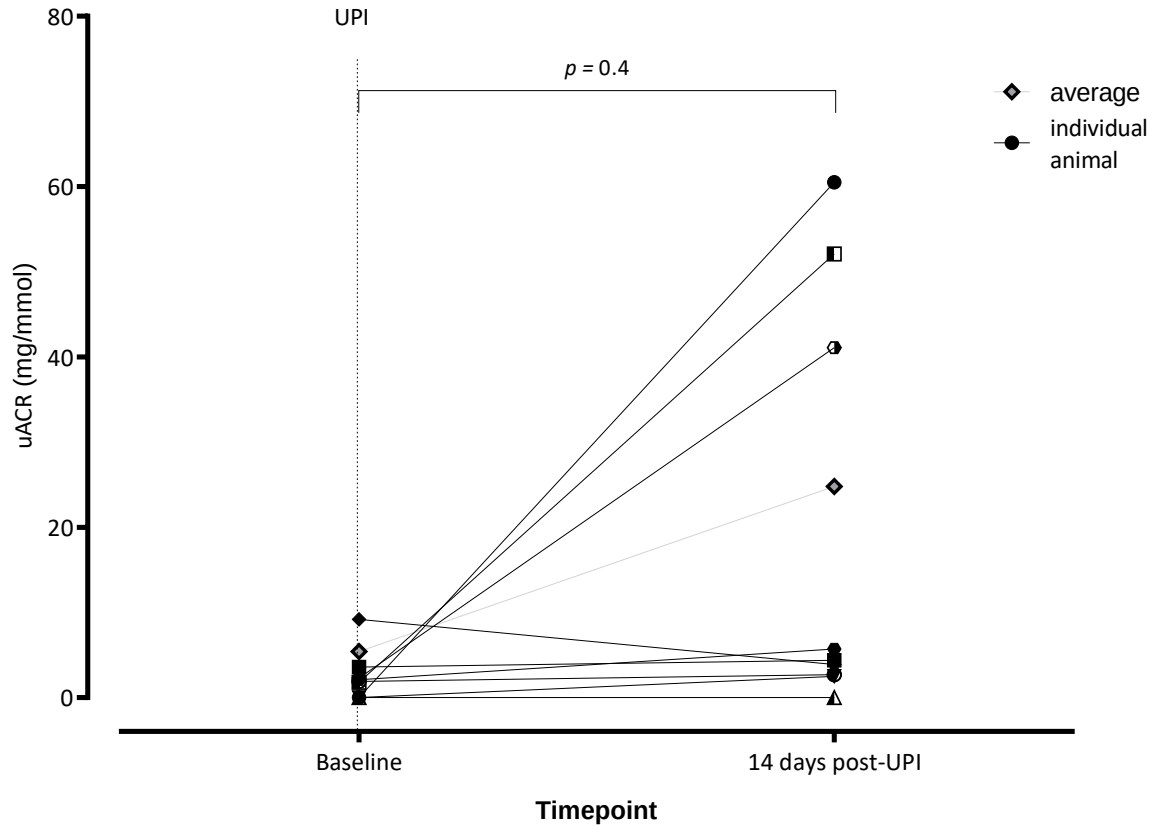

Figure 3-5 Urine albumin to creatinine ratio at baseline and 14 days after UPI (n = 9). Statistical significance defined as $p \leq 0.05$. uACR – Urine albumin to creatinine ratio, mg – milligrams, mmol – millimoles.

Thus, given the increase in the BP and sFlt-1 successful UPI was obtained. Although the increase in other parameters was mild, this is representative of the mild nature of the model's phenotype.

3.3 Blood Pressure

As described in the Methods chapter (section 2.9.2 – 2.9.7), blood pressure data was collected under six different conditions:

Study 1: Blood pressure whilst awake at rest and during sleep
Study 2: Early morning blood pressure surge
Study 3: Blood pressure under anaesthesia (ketamine, propofol, sevoflurane)
Study 4: Exposure to controlled stressors (sounds)
Study 5: Blood pressure whilst eating
Study 6: Blood pressure during cleaning of enclosure

Antihypertensive treatment (labetalol, methyldopa, hydralazine) was established for at least 3 days during each of these studies (section 3.3). Blood pressure data from each study was analysed using a multifaceted approach to determine average BP, BP load, BP variability using SD1 and SD2 values generated by Poincaré Plots and classification of BP data as stochastic, periodic or chaotic (Figure 3-6).

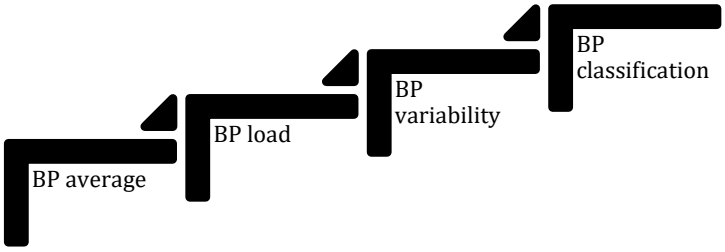

Figure 3-6 Ideogram representing layers of analysis investigating BP complexity.

3.3.1 Study 1: Blood pressure whilst awake at rest and during sleep

3.3.1.1 Average blood pressure: awake (rest) and during sleep

3.3.1.1.1 Awake

There was no significant difference between the average systolic (Figure 3-7) and diastolic (Figure 3-8) awake BP before and after antihypertensive treatment was established; labetalol, methyldopa and hydralazine combined (systolic $p = 0.6$, diastolic $p = 0.4$). The systolic/diastolic BP mean before treatment was 138.5 / 96.6 mmHg, (95% CI: 133 – 144 / 91 – 102), and after treatment established was 139.3 / 98.1 mmHg, (95% CI: 133 – 146 / 91 – 105).

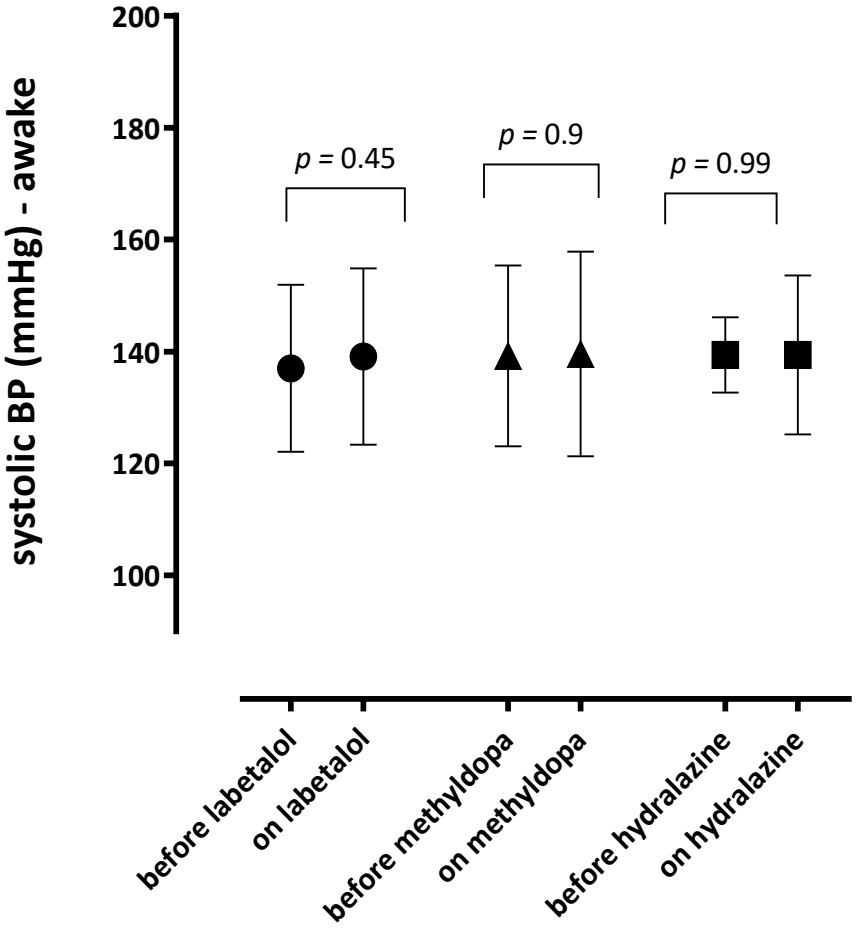

Figure 3-7 Average systolic BP (awake) – before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). There was no significant difference in the systolic BP (awake) before and after treatment was established with each of the antihypertensives (n = 9 animals). Statistical significance defined as $p \leq 0.05$.

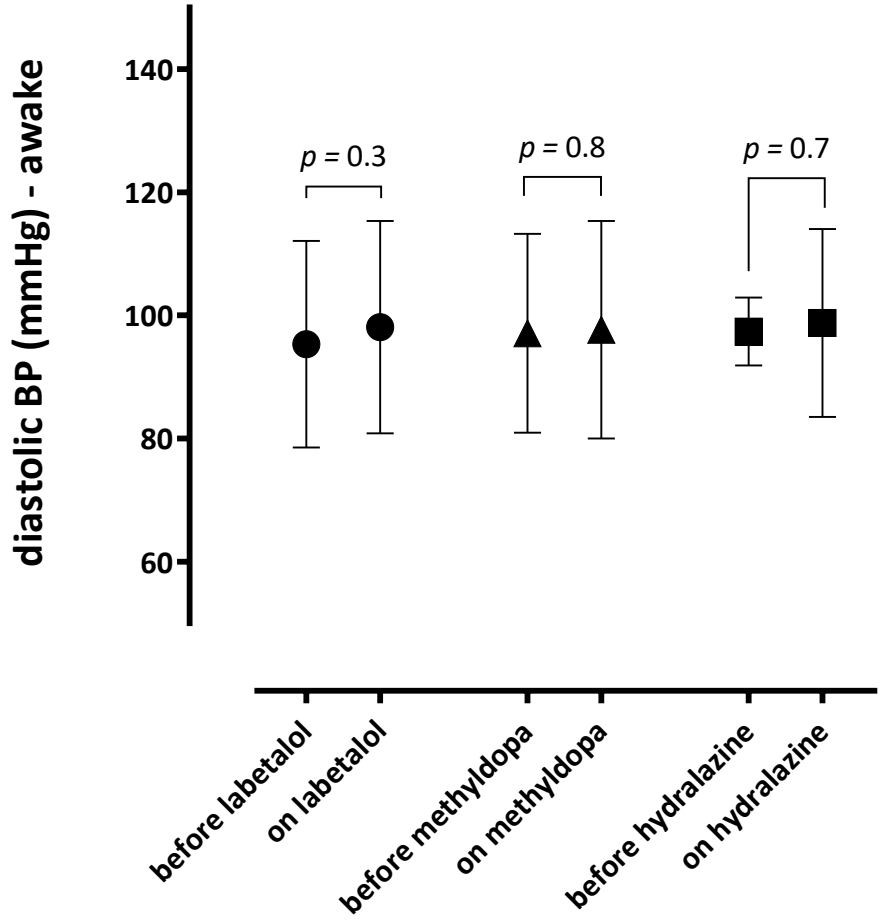

Figure 3-8 Average diastolic BP (awake) – before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). There was no significant difference in the diastolic BP (awake) before and after treatment with each of the antihypertensives was established. Statistical significance defined as $p \leq 0.05$.

3.3.1.1.2 Sleep

There was also no significant difference between the average systolic (Figure 3-9) and diastolic (Figure 3-10) sleep BP before and after antihypertensive treatment with labetalol, methyldopa and hydralazine (systolic p = 0.5, diastolic p = 0.9). The systolic/diastolic BP mean before treatment was 137.8 / 96.6 mmHg, (95% CI: 132 – 143 / 89 – 104), and on drug treatment mean was 138.8 / 96.7 mmHg, (95% CI: 133 – 145 / 90 – 103).

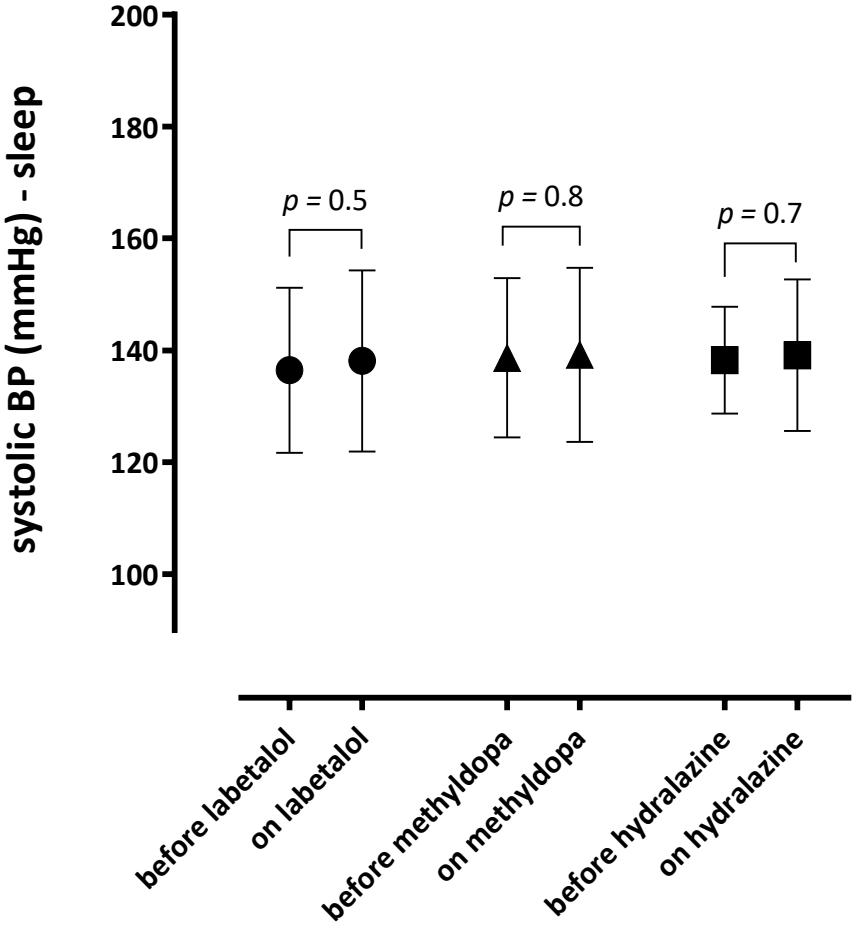

Figure 3-9 Average systolic BP (sleep) – before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). There was no significant difference in the systolic BP during sleep before and on treatment with each of the antihypertensives (n = 9). Statistical significance defined as $p \leq 0.05$.

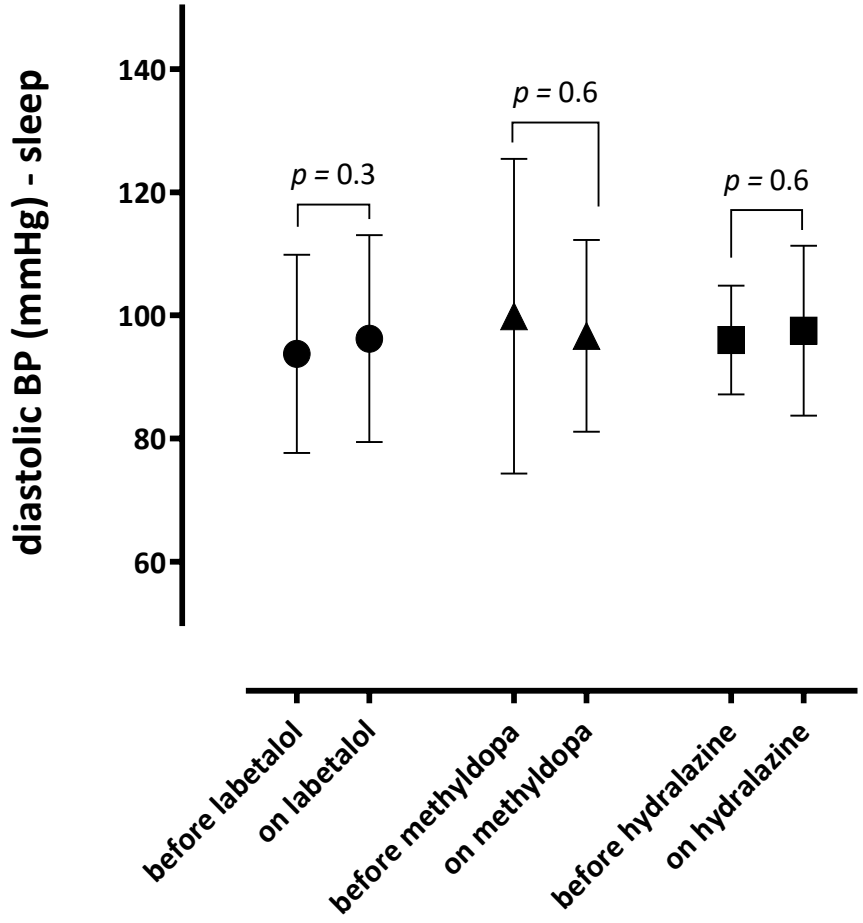

Figure 3-10 Average diastolic BP (sleep) – before and on antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). There was no significant difference in the diastolic BP during sleep before and on treatment with each of the antihypertensives. Statistical significance defined as $p \leq 0.05$.

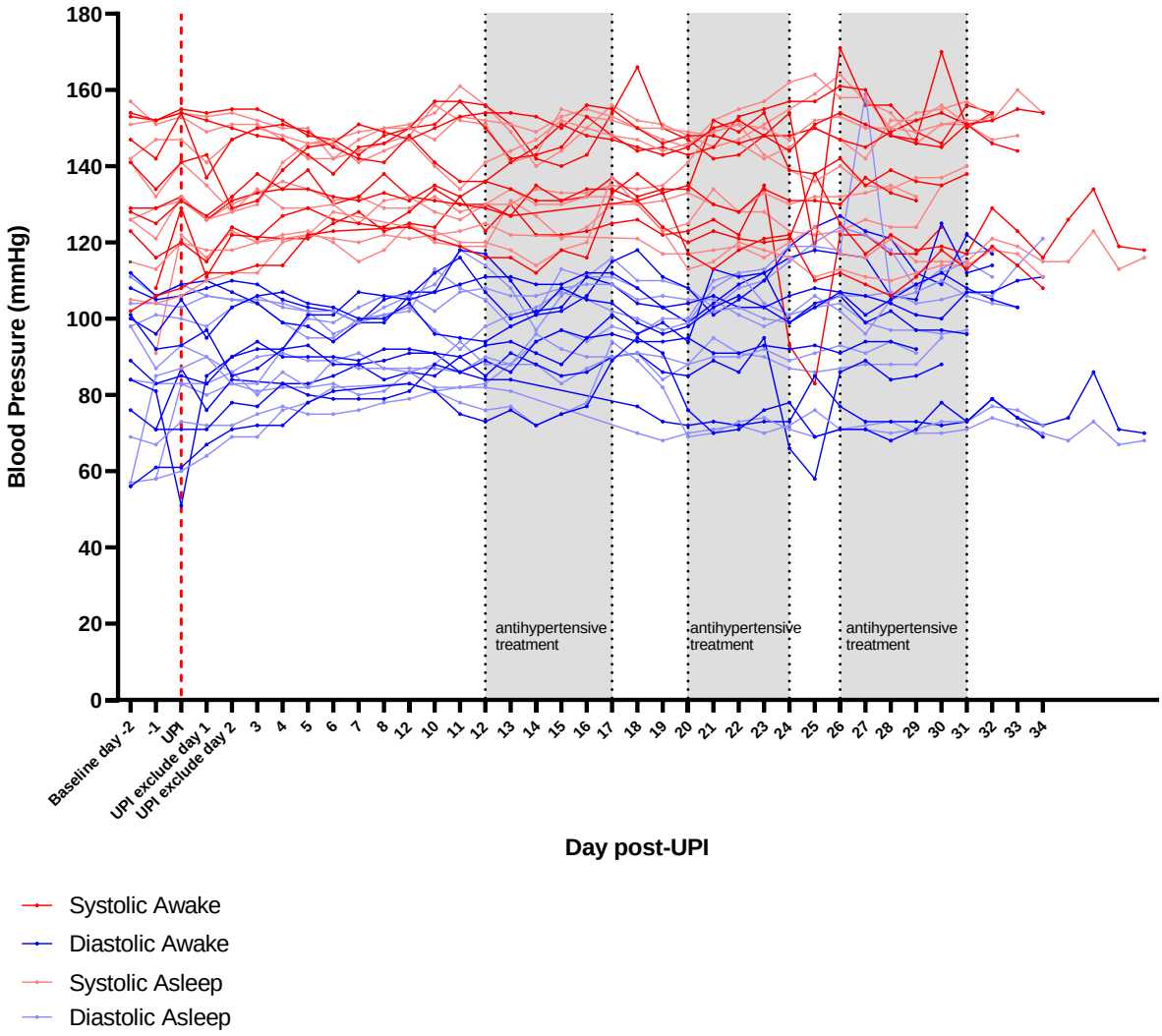

Figure 3-11 Average daily awake and sleep BP – individual animals. This graph describes the average BP whilst awake and during sleep for each of the nine individual animals over the course of the experimental period. Baseline BP was defined as BP data collected between 4-8pm two days before the UPI procedure. Each animal received the antihypertensive treatment in sequence according to the (Table 2-1) Latin Square Design.

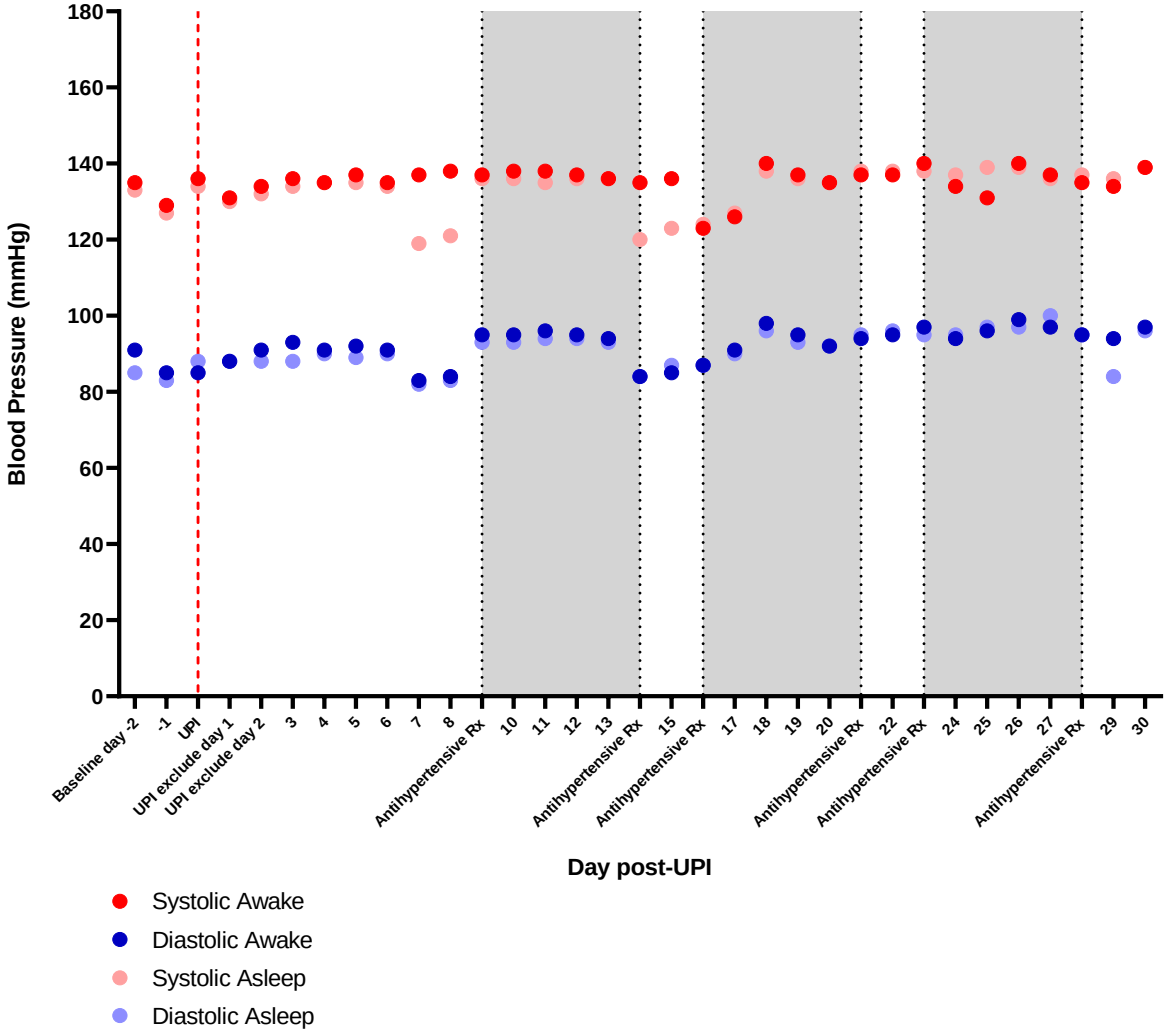

Figure 3-12 Average daily awake and sleep BP – average of all nine animals. Each point on this graph describes the average BP of all nine animals whilst awake and during sleep on each day throughout the course of the experimental period. Baseline BP was defined as BP data collected between 4-8pm two days before the UPI procedure. Each animal received the antihypertensive treatment in sequence according to the (Table 2-1) Latin Square Design.

3.3.1.2 Blood pressure load: awake (rest) and during sleep

As described in the Methods Chapter, BP load was calculated by categorising data according to what percentage of BP readings were within one standard deviation of the baseline BP level (optimal BP), what percentage of BP readings were between one and two standard deviations from the baseline BP level and what percentage of BP readings were greater than two standard deviations above the baseline BP level. Baseline BP level was defined as the group average the day before each new drug treatment commenced.

3.3.1.2.1 Awake

Systolic BP load increased significantly on labetalol treatment as determined by chi-square test: $\chi 2$ (4, N = 9708) = 287.07, $p < 0.001$. Diastolic BP load also increased significantly: $\chi 2$ (4, N = 8774) = 747.85, $p < 0.001$, i.e., BP load was generally higher on labetalol treatment (Figure 3-13).

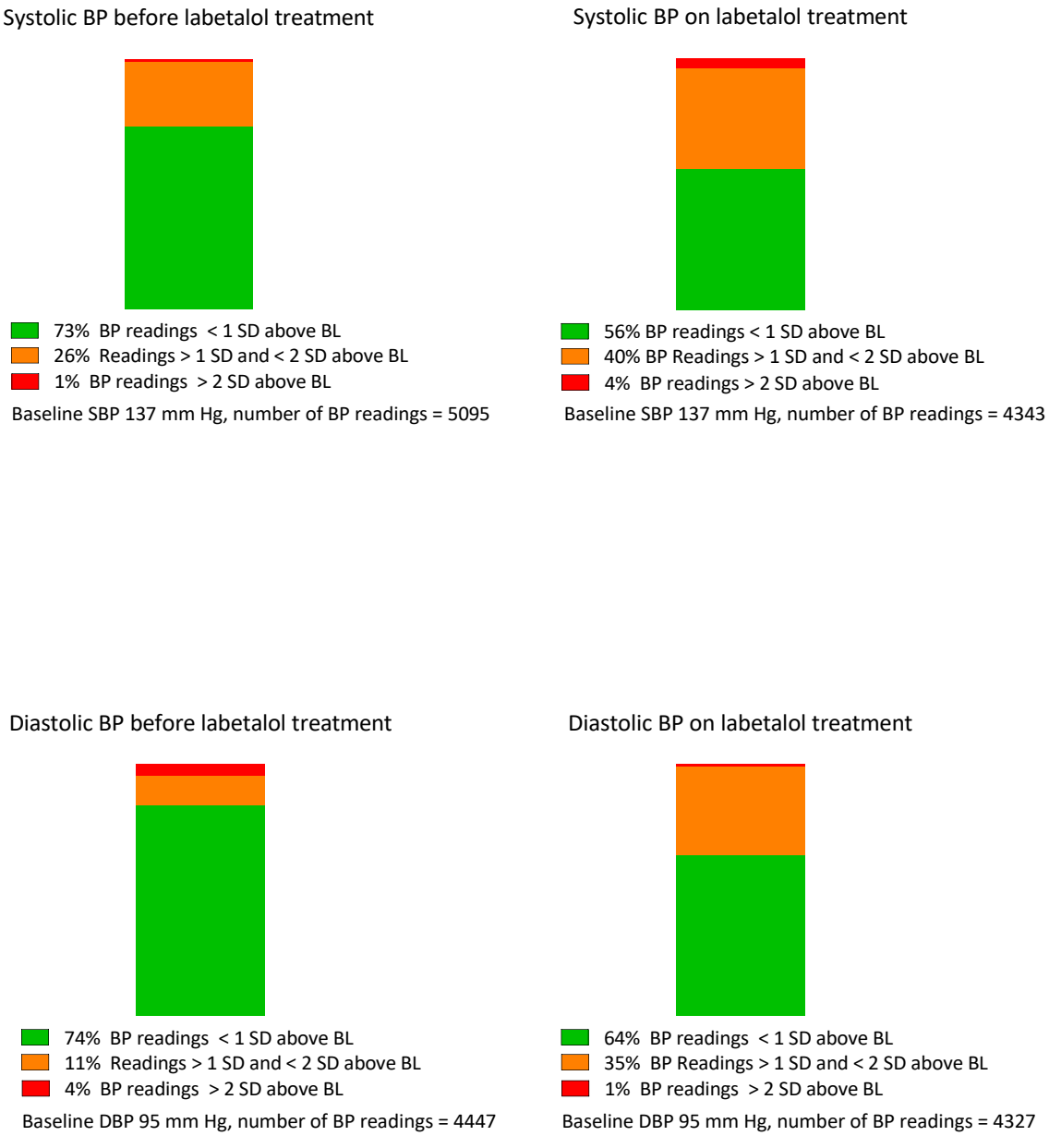

Figure 3-13 Systolic and diastolic BP load (awake) before and after labetalol treatment was established (n = 9). Baseline BP was defined as the average BP of all nine animals before labetalol treatment commenced.

Systolic BP load did not change significantly on methyldopa treatment as determined by chi-square test: $\chi 2$ (4, N = 9708) = 3.41, p = 0.18. Diastolic BP load increased significantly: $\chi 2$ (4, N = 8658) = 204.43, $p < 0.001$ (Figure 3-14).

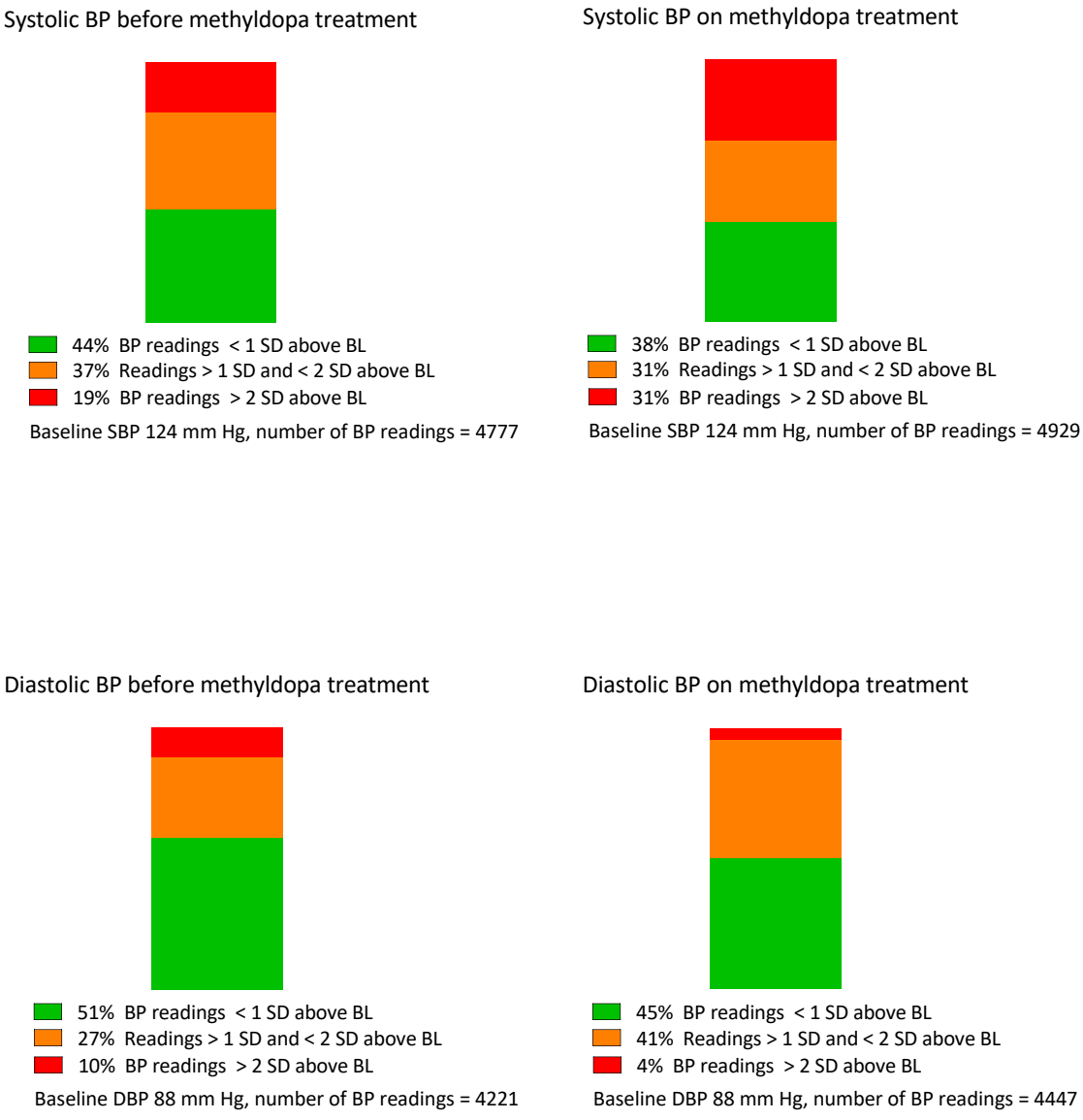

Figure 3-14 Systolic and diastolic BP load (awake) before and on methyldopa treatment (n = 9). Baseline BP was defined as the average BP of all nine animals before methyldopa treatment commenced.

Systolic BP load decreased significantly on hydralazine treatment as determined by chi-square test: $\chi 2$ (4, N = 9233) = 156.1, p < 0.001. Diastolic BP load also decreased significantly: $\chi 2$ (4, N = 7465) = 46.3, p < 0.001 (Figure 3-15).

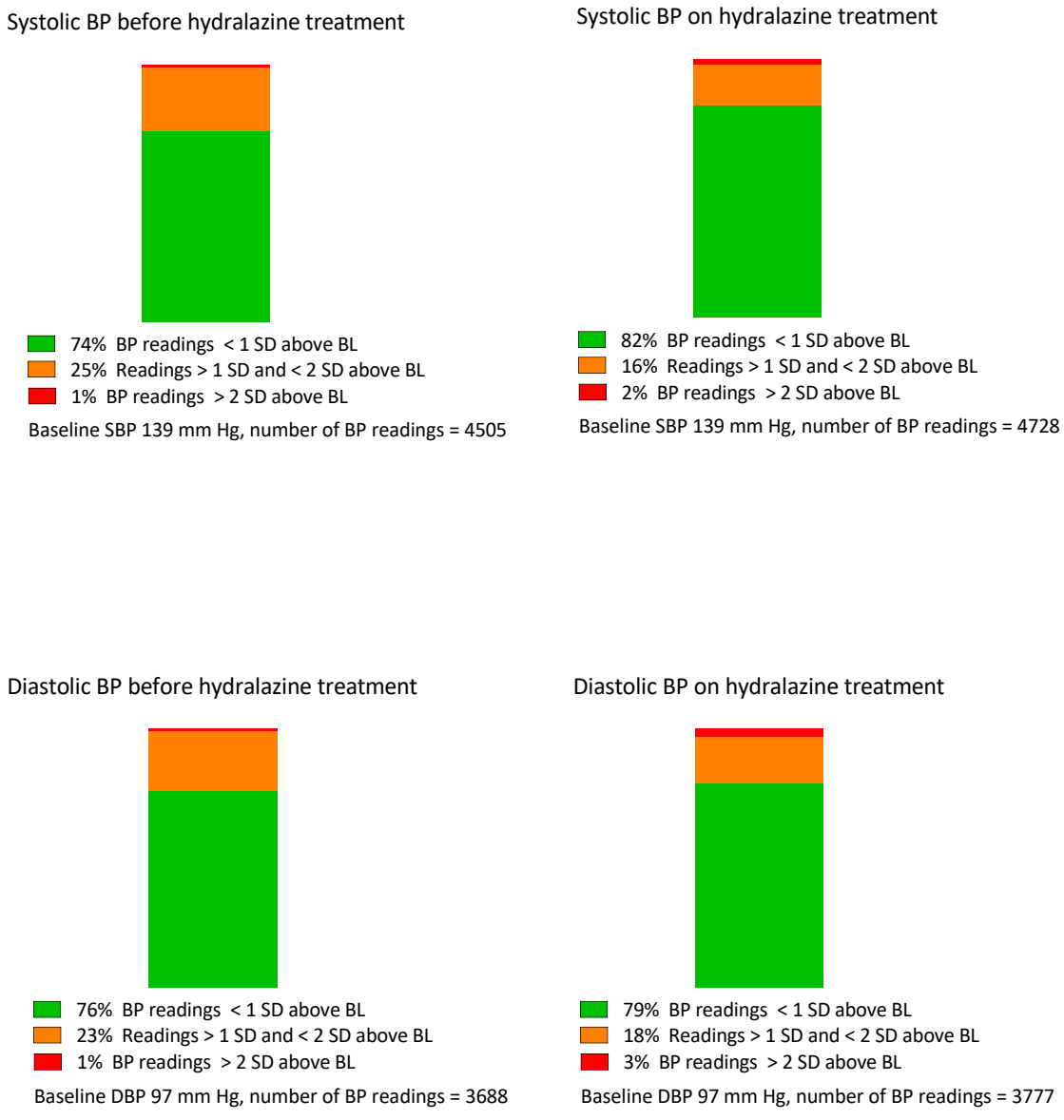

Figure 3-15 Systolic and diastolic BP load (awake) before and after hydralazine treatment was established (n = 9). Baseline BP was defined as the average BP of all nine animals before hydralazine treatment commenced.

3.3.1.2.2 Sleep

Systolic BP load during sleep significantly increased on labetalol treatment as determined by chi-square test: $\chi 2\,(4,\ N = 12593) = 1438.44,\ p < 0.001$. Diastolic BP load also significantly increased: $\chi 2\,(4, N = 12751) = 1494.25, p < 0.001$ (Figure 3-16).

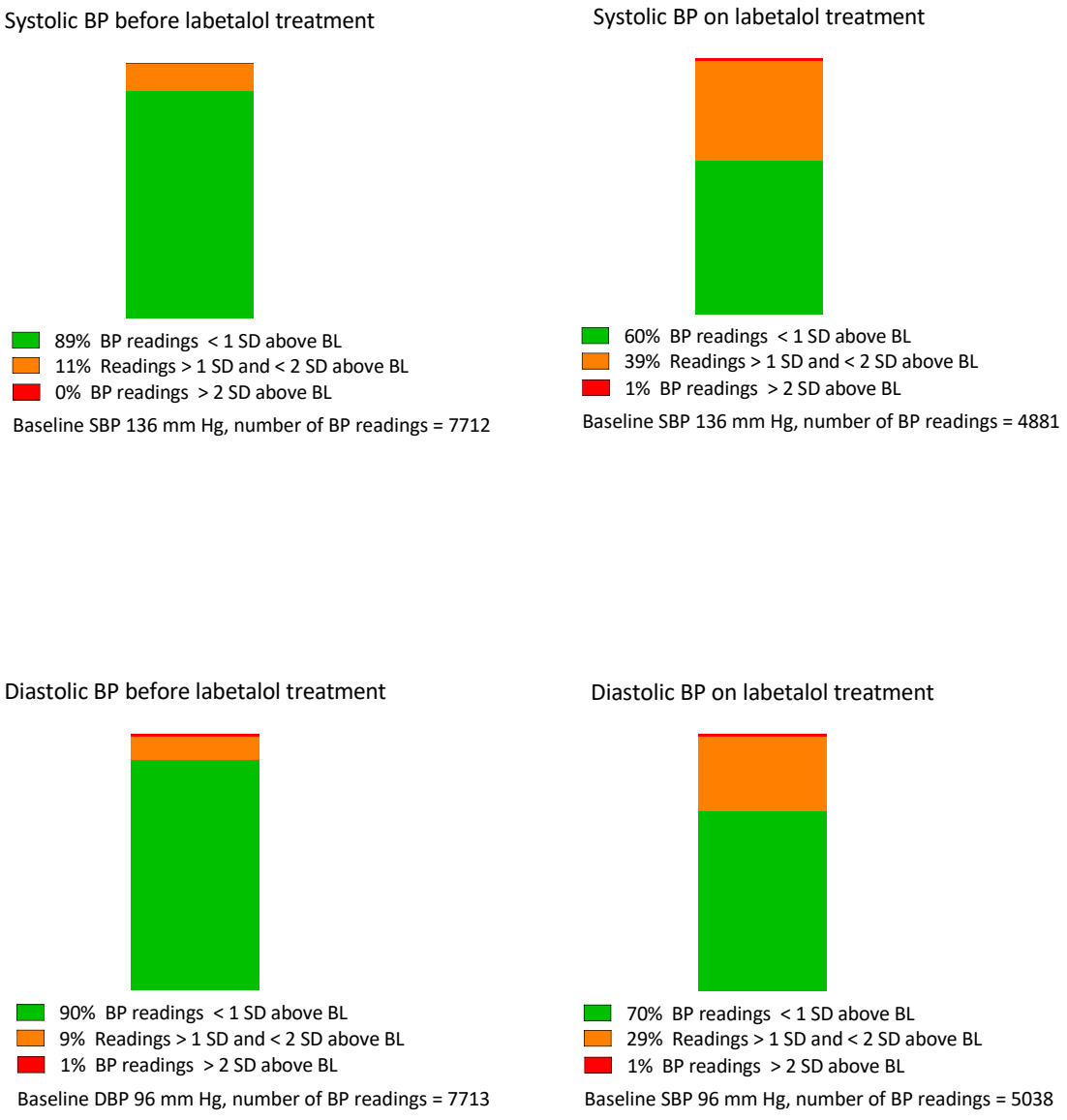

Figure 3-16 Systolic and diastolic BP load (sleep) before and after labetalol treatment was established (n = 9). Baseline BP was defined as the average BP of all nine animals before labetalol treatment commenced.

Systolic BP load significantly increased on methyldopa treatment as determined by chi-square test: $\chi 2$ (4, N = 8658) = 204.43, $p < 0.001$. Diastolic BP load also increased significantly: $\chi 2$ (4, N = 9420) = 93.64, $p < 0.001$ (Figure 3-17).

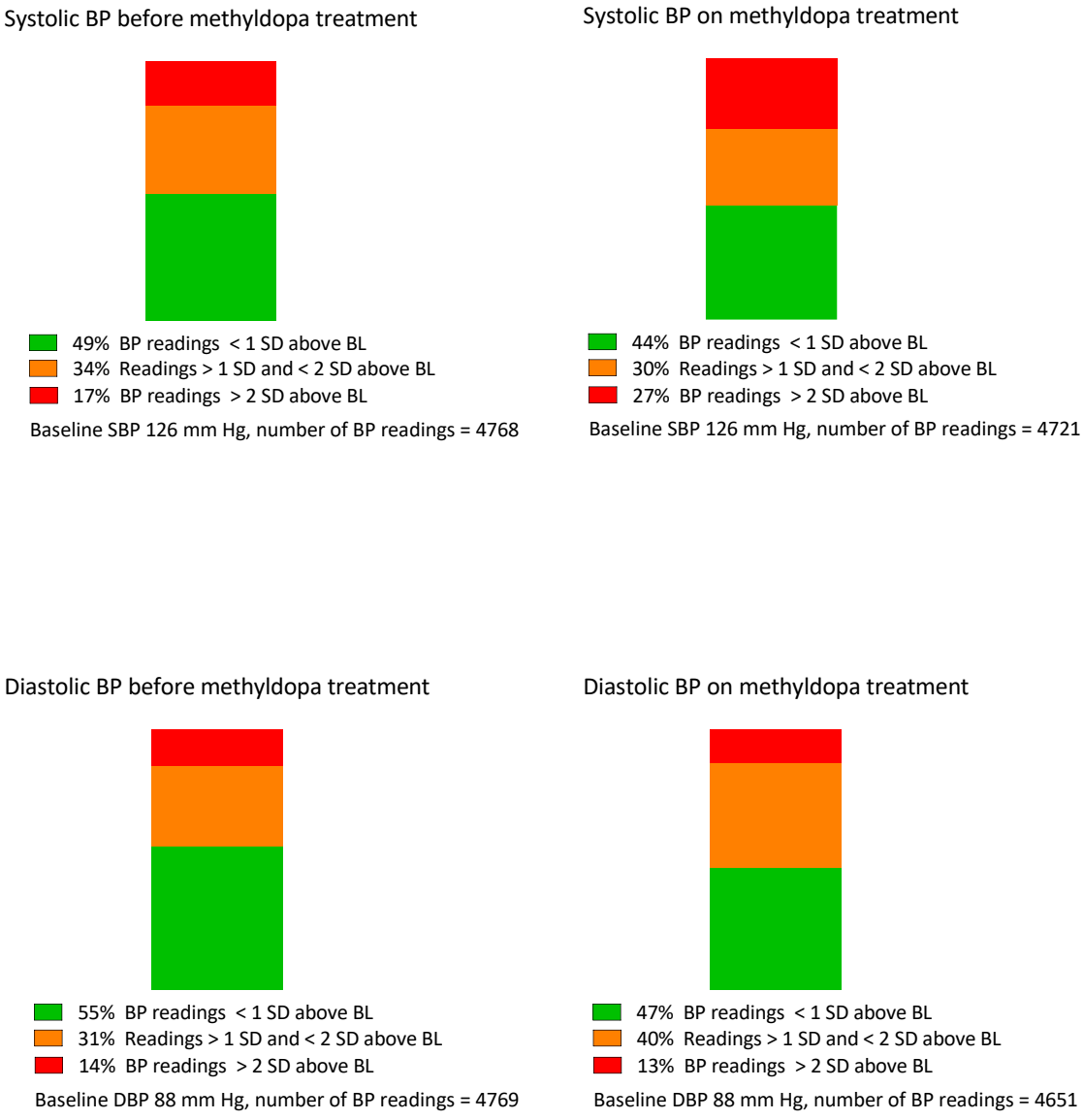

Figure 3-17 Systolic and diastolic BP load (sleep) before and after methyldopa treatment was established (n = 9). Baseline BP was defined as the average BP of all nine animals before methyldopa treatment commenced.

Systolic BP load decreased significantly on hydralazine treatment as determined by chi-square test: $\chi 2$ (4, N = 9489) = 152.11, p < 0.001. Diastolic BP load also increased significantly: $\chi 2$ (4, N = 8334) = 7.79, p = 0.02 (Figure 3-18).

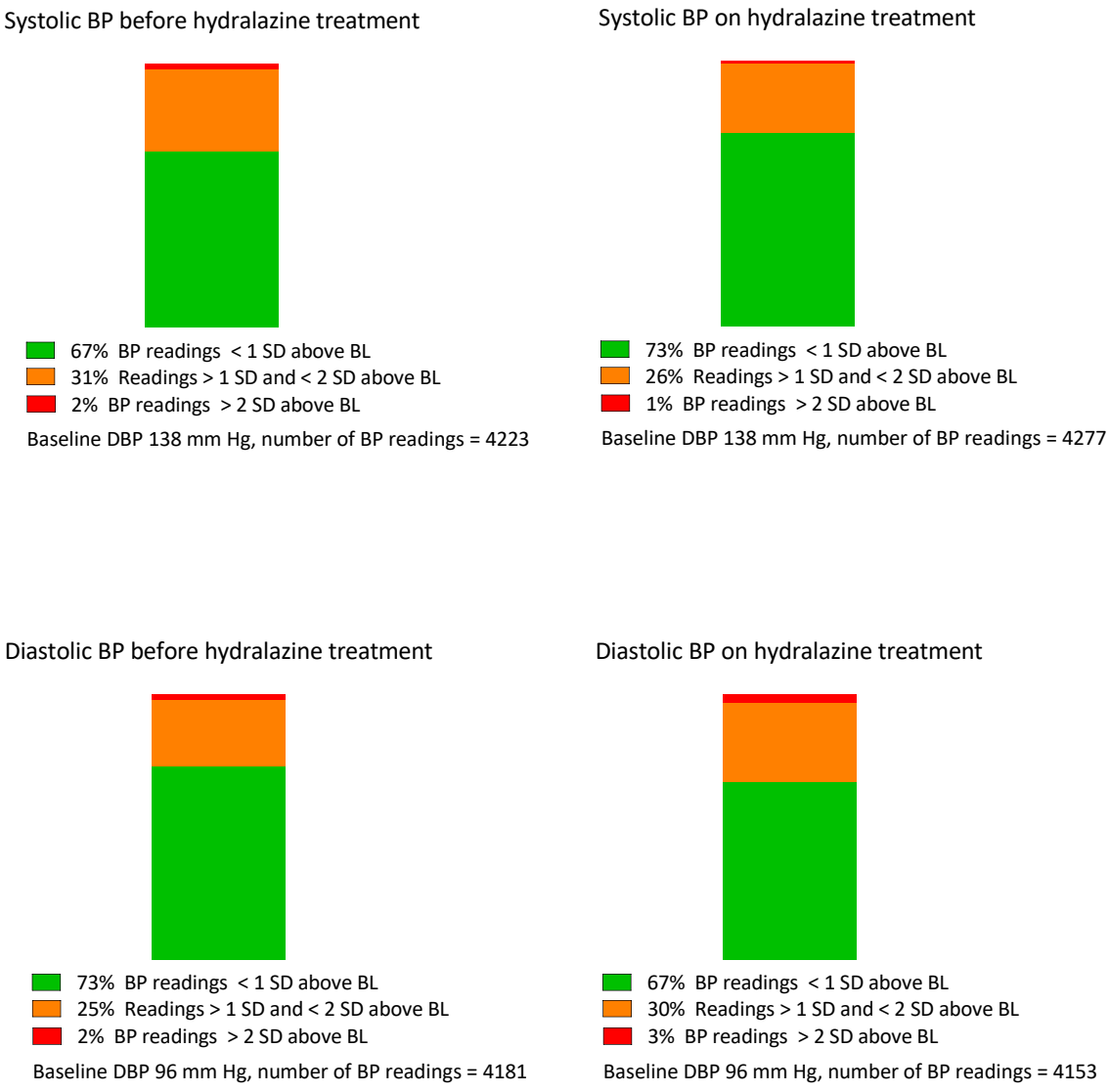

Figure 3-18 Systolic and diastolic BP load (sleep) before and after hydralazine treatment was established (n = 9). Baseline BP was defined as the average BP of all nine animals before hydralazine treatment commenced.

Overall, in terms of BP load whilst awake at rest and during sleep, there was a statistically significant increase in BP load with labetalol and methyldopa treatment and a decreased BP load associated with hydralazine treatment.

3.3.1.3 Blood pressure variability using SD1 and SD2 values generated by Poincaré Plots: awake (rest) and during sleep

As described in the Methods Chapter, BPV is defined by SD1 and SD2 values which are measures of short-term variability (i.e., change from one data point to the next) and long-term variability (variability over the course of the data collection period), respectively. Values were generated using Poincaré plots (as described in Section 2.11.3).

3.3.1.3.1 Awake

There was no significant difference between the SD1/SD2 systolic or diastolic awake before and after antihypertensive treatment was established (Figure 3-19 – Figure 3-22).

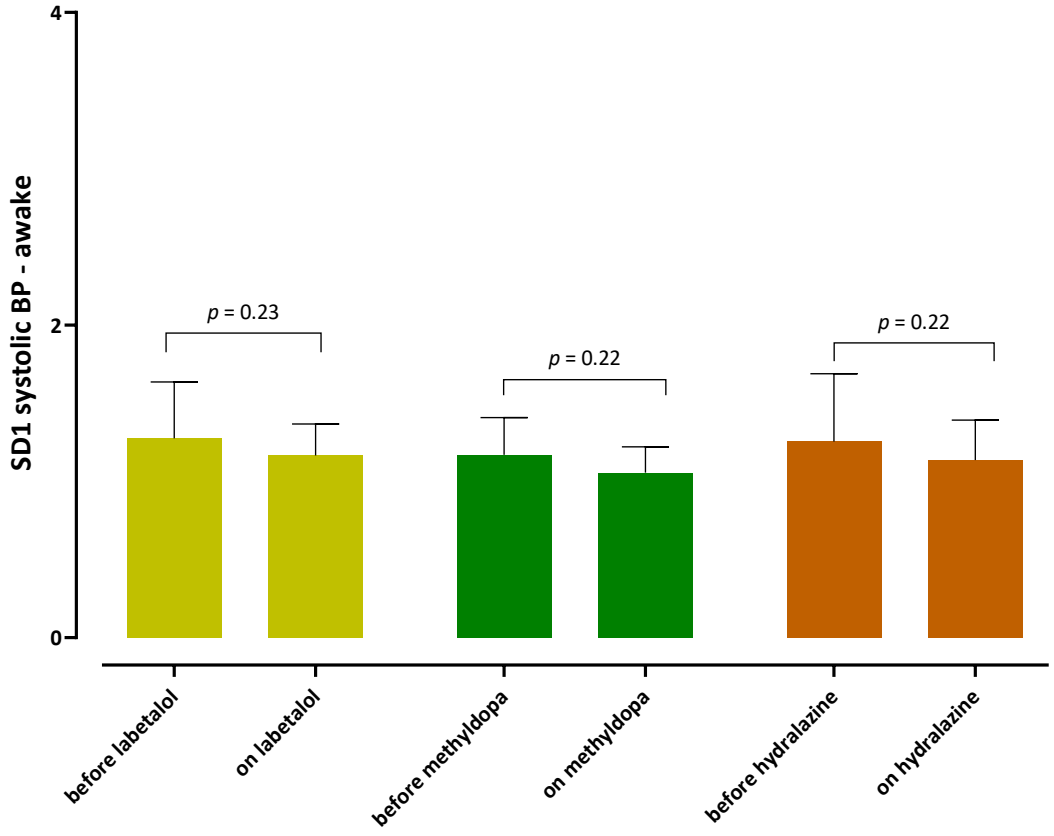

Figure 3-19 Short term variability (SD1) of systolic BP (awake) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Statistical significance defined as $p \leq 0.05$.

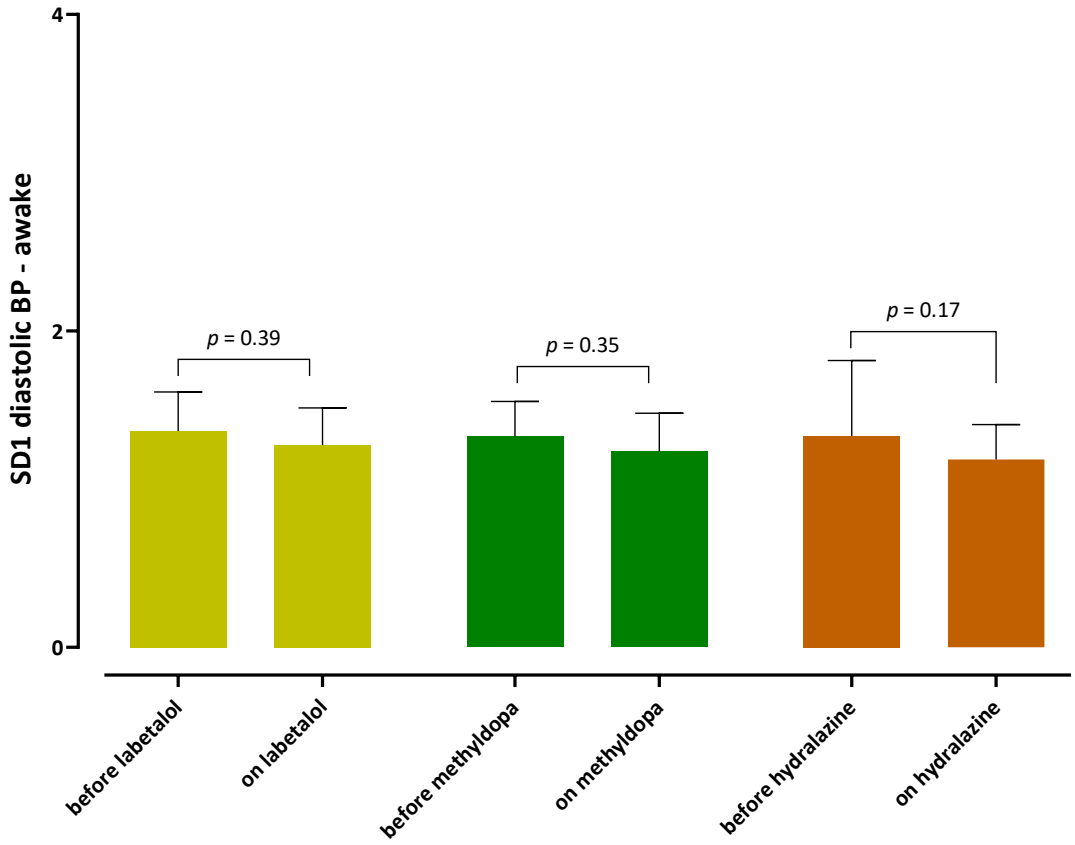

Figure 3-20 Short term variability/SD1 of diastolic BP (awake) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Statistical significance defined as $p \leq 0.05$.

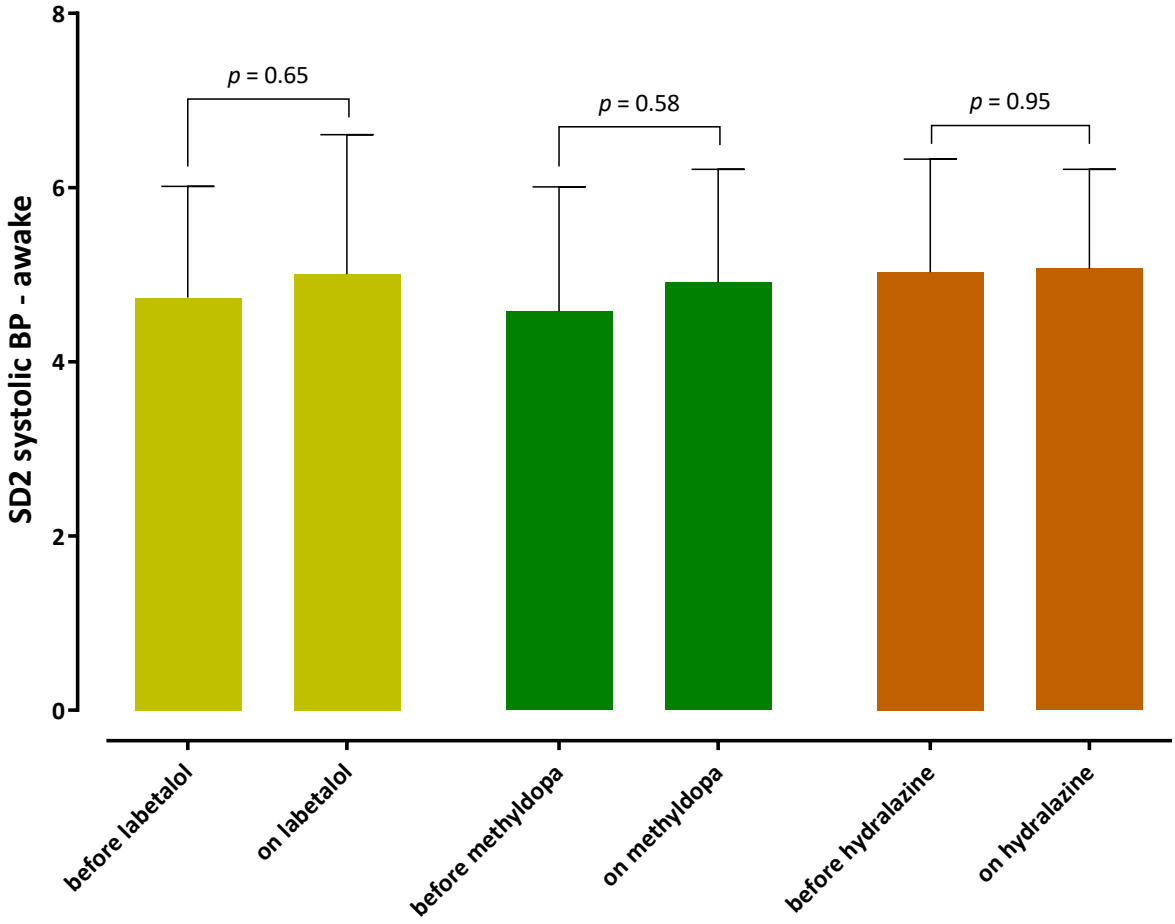

Figure 3-21 Long term variability (SD2) of systolic BP (awake) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Statistical significance defined as $p \leq 0.05$.

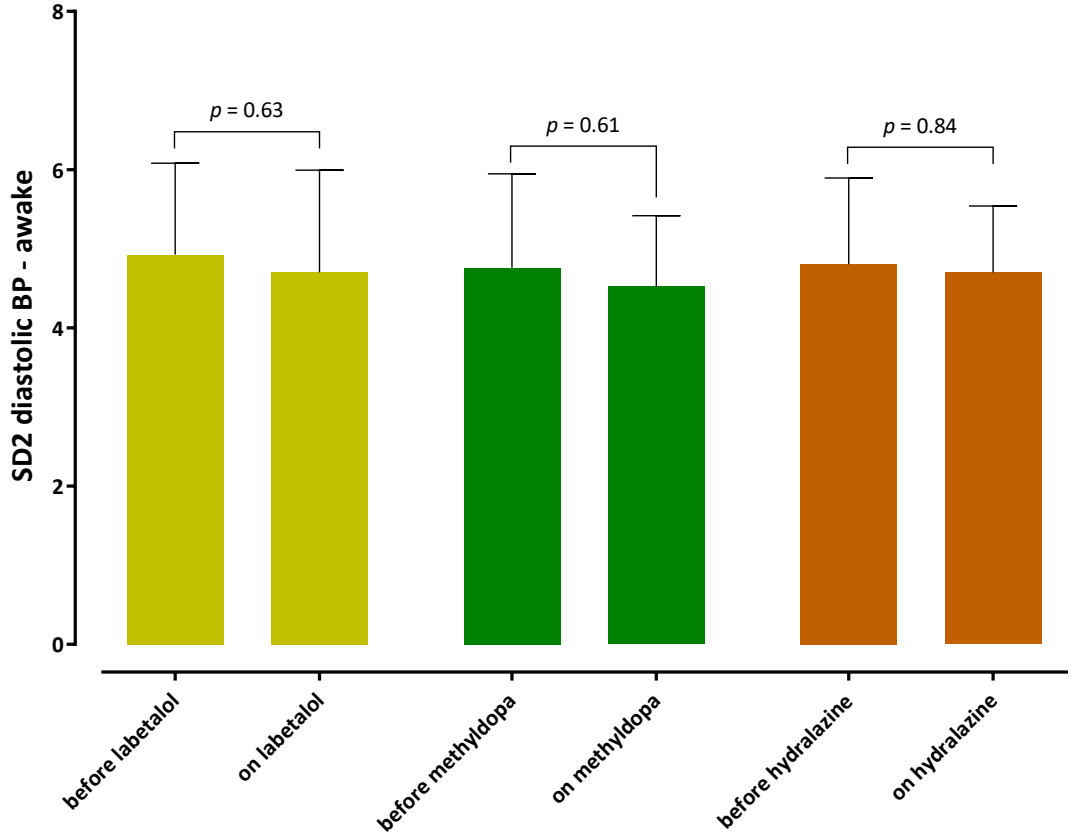

Figure 3-22 Long term variability (SD2) of diastolic BP (awake) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Statistical significance defined as $p \leq 0.05$.

Overall, short and long term variability as represented by SD1 and SD2 respectively, did not change significantly with any of the antihypertensive treatments.

3.3.1.3.2 Sleep

Variability analysis found a significant reduction in SD1 of diastolic BP during sleep on labetalol and methyldopa treatment (Figure 3-24). There was a significant reduction in SD2 of systolic BP on hydralazine treatment (Figure 3-25). There was no significant difference in SD1 of systolic BP (Figure 3-23) or SD2 of diastolic BP (Figure 3-26) during sleep on antihypertensive treatment.

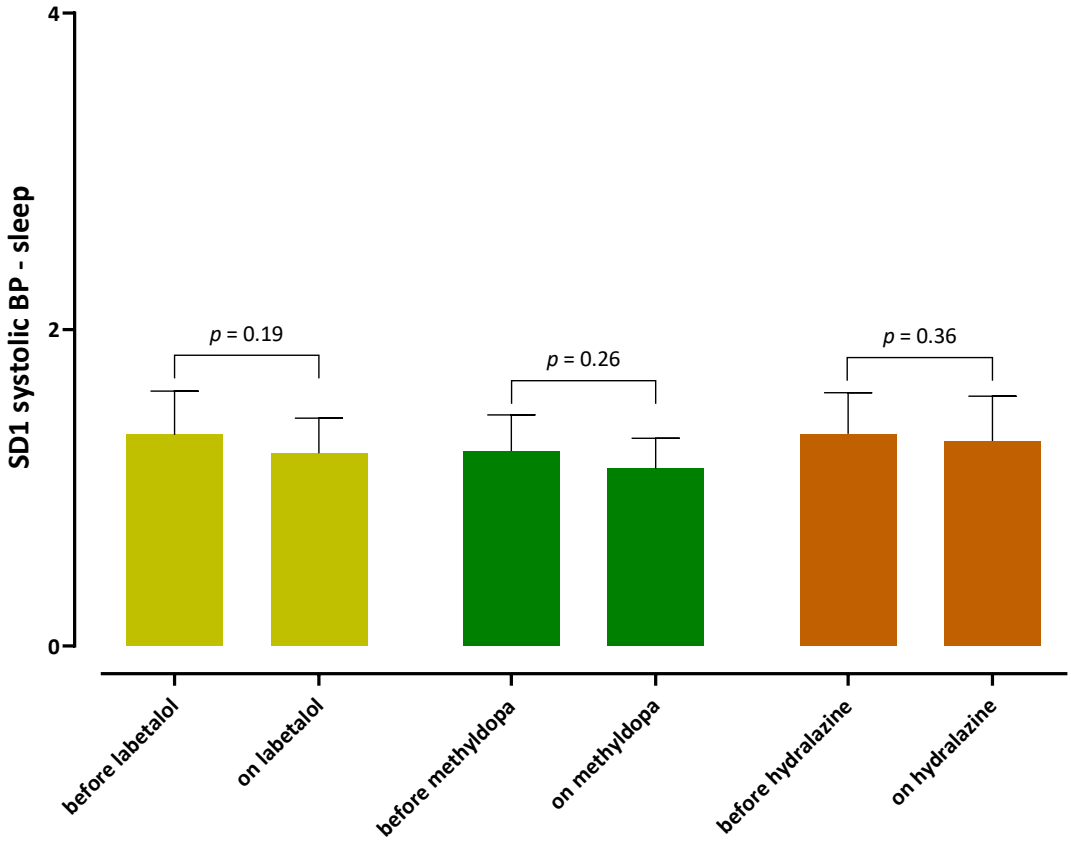

Figure 3-23 Short term variability (SD1) of systolic BP (during sleep) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Statistical significance defined as $p \leq 0.05$.

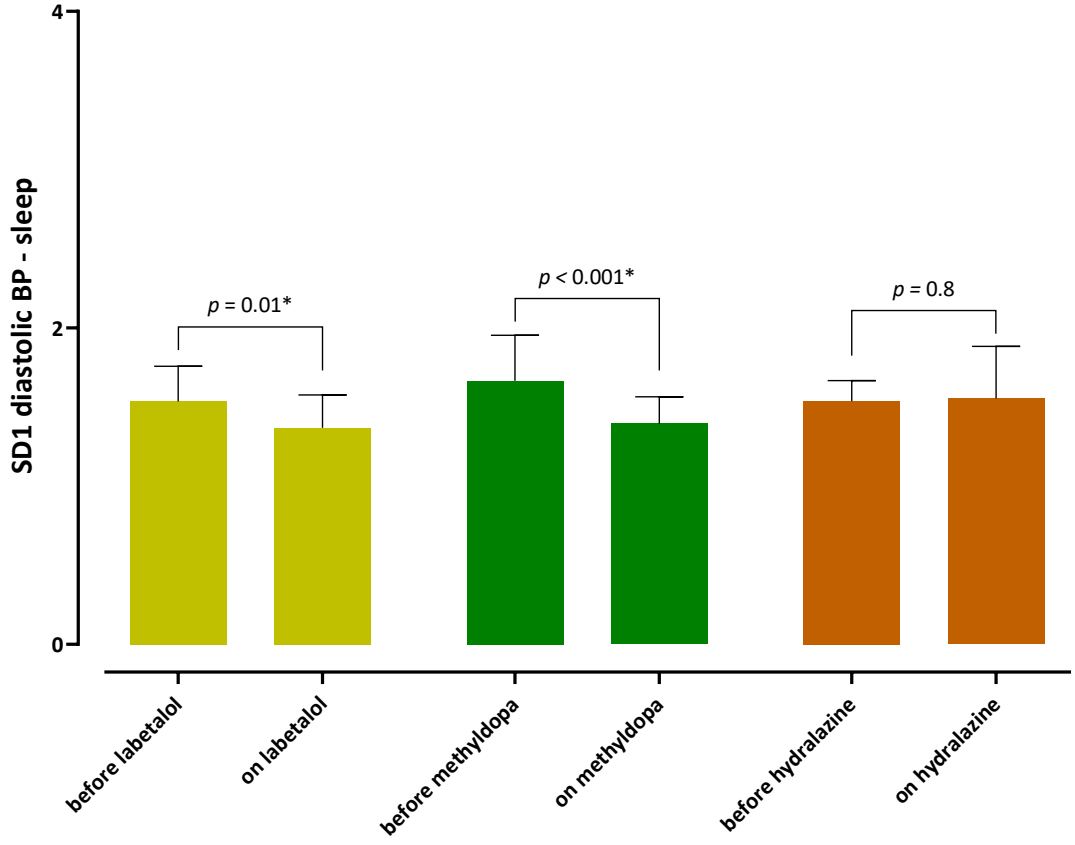

Figure 3-24 Short term variability (SD1) of diastolic BP (during sleep) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$)

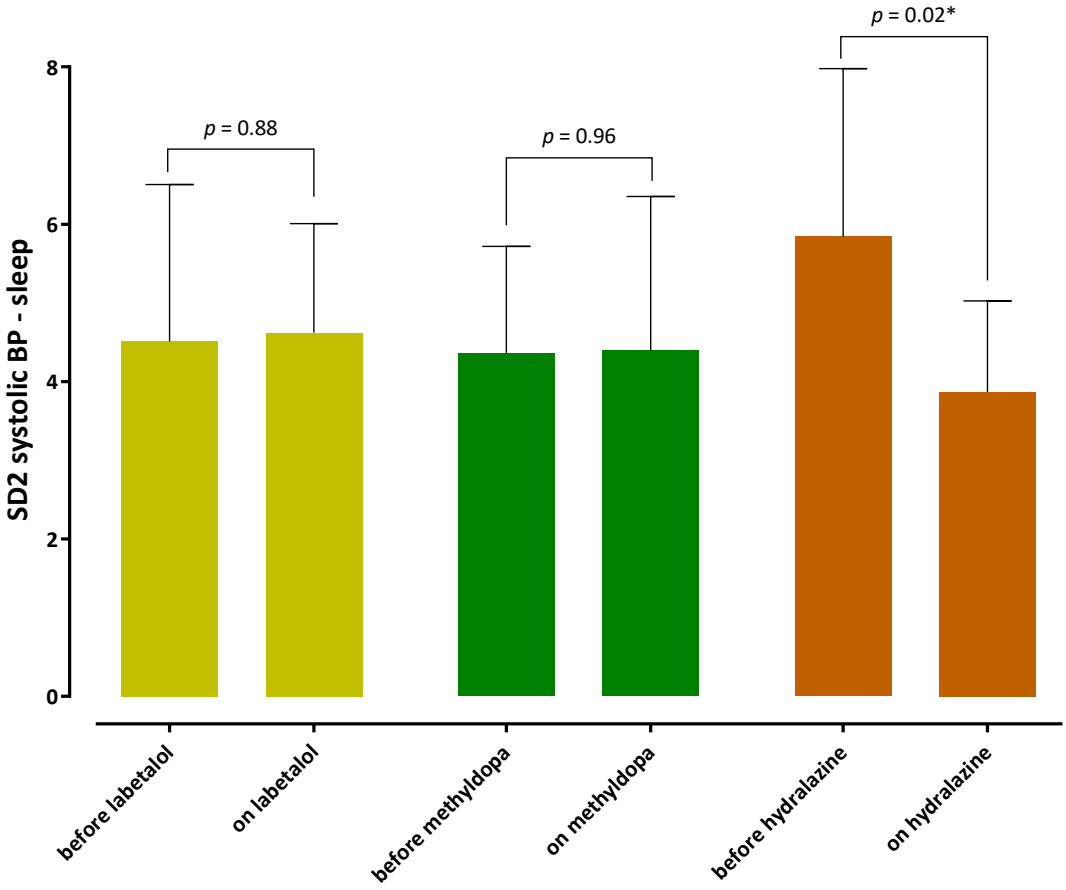

Figure 3-25 Long term variability (SD2) of systolic BP (during sleep) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$)

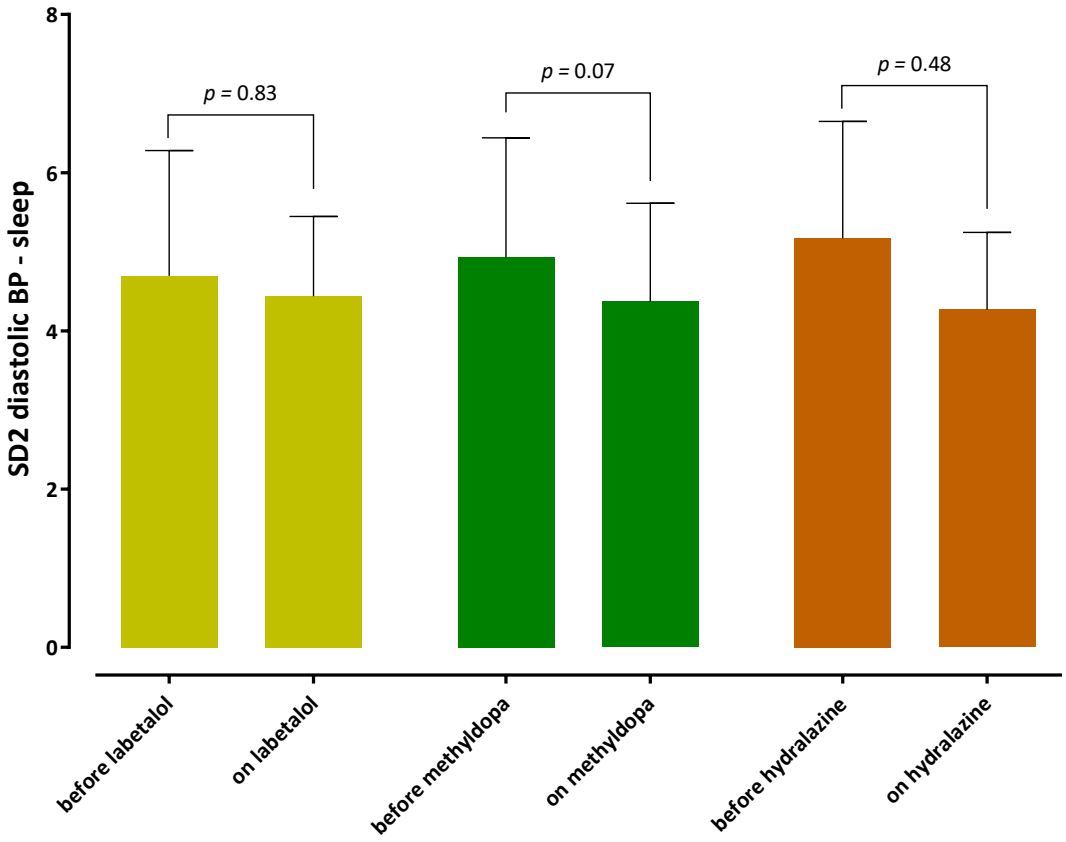

Figure 3-26 Long term variability (SD2) of diastolic BP (during sleep) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Statistical significance defined as $p \leq 0.05$.

Overall, the short-term variability (SD1) of diastolic BP during sleep decreased significantly with labetalol and methyldopa treatment, and the long-term variability (SD2) of systolic BP during sleep decreased significantly with hydralazine treatment.

3.3.1.4 Classification of blood pressure data as stochastic, periodic or chaotic: awake (rest) and during sleep

The awake and sleep BP data sets were processed using the Chaos Decision Tree Algorithm[217] and the data sets were classified as either stochastic, periodic or chaotic (Table 3-3). The Chaos Decision Tree Algorithm consists of four steps, including determining if the data are stochastic using permutation entropy and a combination of Amplitude Adjusted Fourier transform surrogates and Cyclic Phase Permutation surrogates.[217] The data is then denoised using the Schreiber de-noising algorithm, corrected for possible signal oversampling, and then tested for chaos using a modified 0–1 test for chaos. The modified 0–1 test provides a single statistic, K, which approaches 1 for chaotic systems and approaches 0 for periodic systems. [217]

Stochastic	Periodic	Chaotic
diastolic BP awake and during sleep (labetalol) systolic BP awake (before labetalol) diastolic BP awake (before and on methyldopa) systolic and diastolic BP sleep (before hydralazine)	systolic BP awake (labetalol, K: 0.5172) systolic BP awake (before and on methyldopa, K:0.4817, K: 0.5601) systolic BP awake (before and on hydralazine, K: 0.4884, K: 0.5533) diastolic BP awake (before labetalol, K: 0.4783) diastolic BP awake (before and on hydralazine, K: 0.4083, K: 0.6745) systolic BP sleep (before and on labetalol, K: 0.3909, K: 0.5220) systolic BP sleep (before and on methyldopa, K: 0.3698, K: 0.4188) systolic BP sleep (hydralazine, K: 0.5823) diastolic BP sleep (labetalol; K: 0.2523) diastolic BP sleep (before and on methyldopa, K: 0.5167, K: 0.4709) diastolic BP sleep (hydralazine, K: 0.5872)	Nil

Table 3-2 Classification of systolic and diastolic BP awake and sleep data sets as stochastic, periodic or chaotic as defined by the Chaos Decision Tree Algorithm. K - the Chaos Decision Tree Algorithm performs the 0–1 chaos test on the BP data and this test provides a single statistic, K, which approaches 1 for chaotic systems and approaches 0 for periodic systems.[217]

3.3.2 Study 2: Early morning blood pressure surge

3.3.2.1 Average blood pressure: EMS

There was a significant increase in the average EMS systolic BP before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established ($p < 0.001$) as shown in Table 3-4 and Figure 3-27 below.

	Systolic BP average before treatment	**Systolic BP average after treatment was established**	***p*-value**
Labetalol	132 mmHg (CI 95%: 131-133)	143 mmHg (CI 95%: 142-144)	< 0.001*
Methyldopa	140 mmHg (CI 95%: 140-141)	143 mmHg (CI 95%: 142-144)	< 0.001*
Hydralazine	128 mmHg (CI 95%: 127-128)	144 mmHg (CI 95%: 143-145)	< 0.001*

Table 3-3 Mean systolic BP (mmHg) during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$). CI – 95% confidence interval.

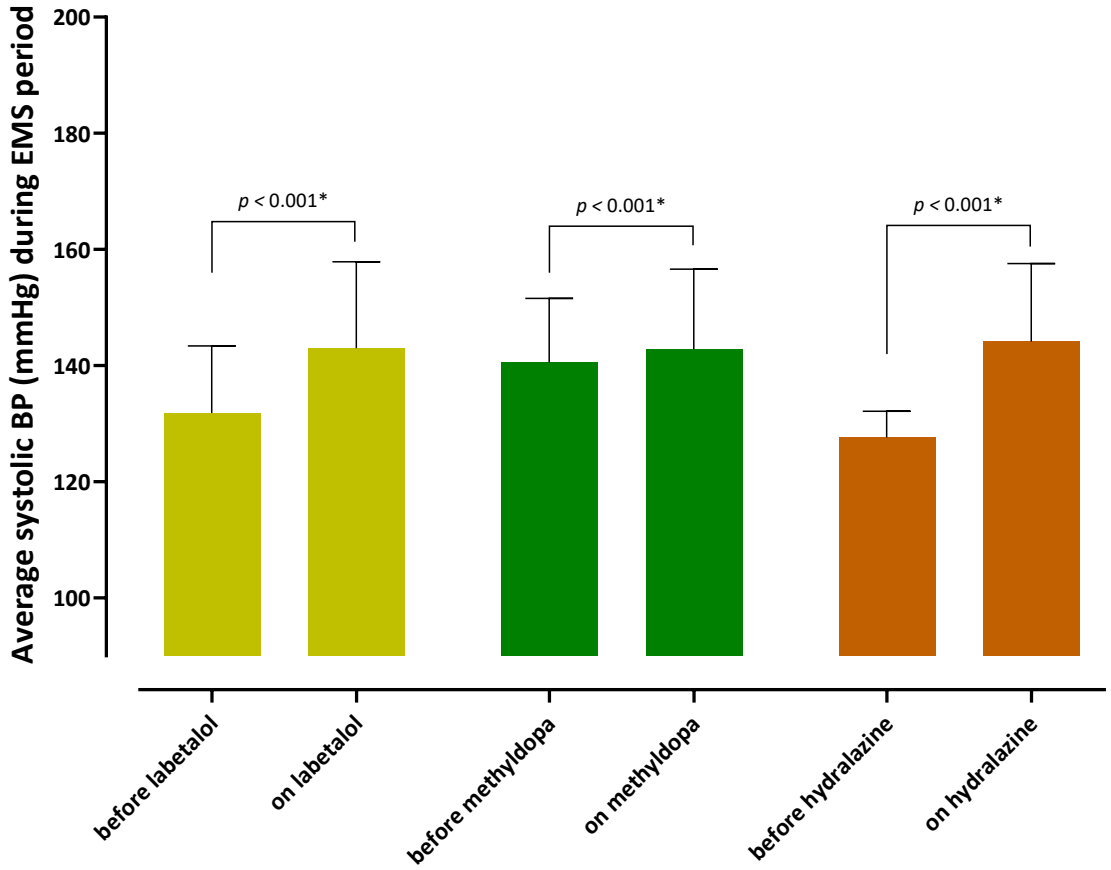

Figure 3-27 Mean systolic BP during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). There was a statistically significant increase in systolic BP after treatment for each drug. * indicates statistically significant difference between groups ($p \leq 0.05$)

There was a significant increase between the average EMS diastolic BP before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established ($p < 0.001$) as shown in Table 3-5 and Figure 3-28 below.

	Diastolic BP average before treatment	**Diastolic BP average on treatment**	***p*-value**
Labetalol	88 mmHg (CI 95%: 88-89)	97 mmHg (CI 95%: 96-98)	< 0.001*
Methyldopa	96 mmHg (CI 95%: 95-97)	96 mmHg (CI 95%: 95-97)	0.69
Hydralazine	85 mmHg (CI 95%: 84-86)	99 mmHg (CI 95%: 98-100)	< 0.001*

Table 3-4 Mean diastolic BP during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$). CI – 95% confidence interval.

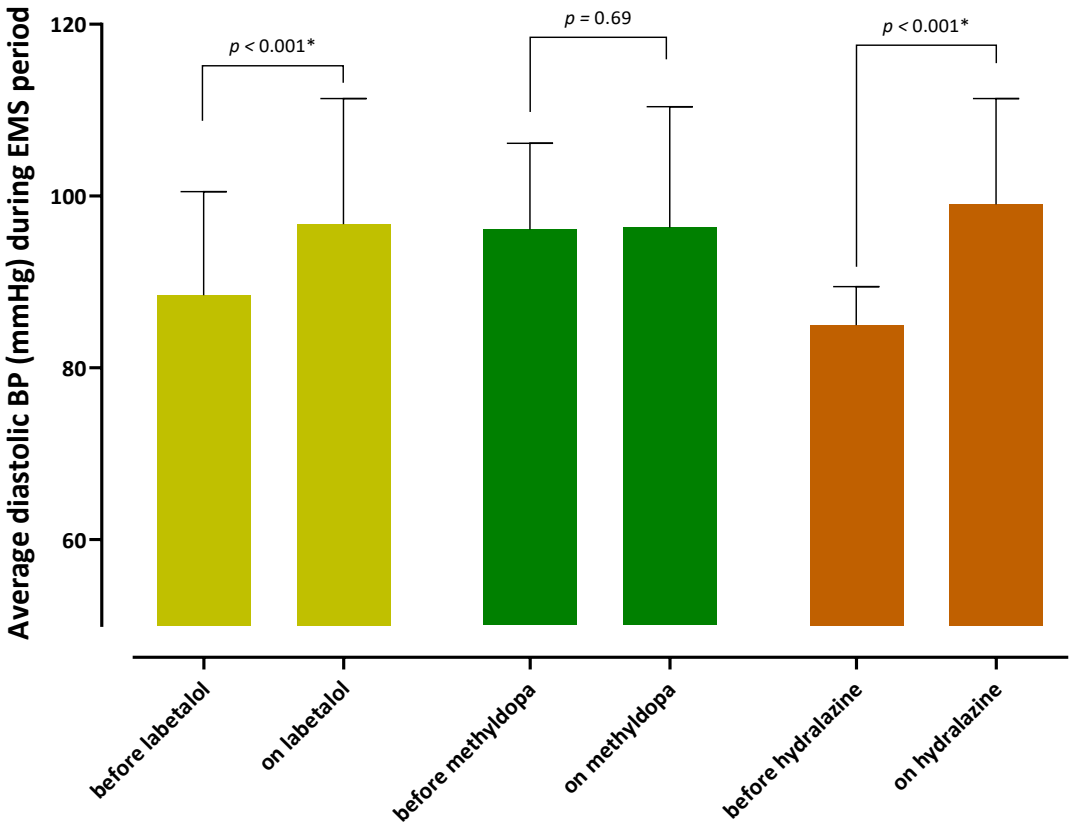

Figure 3-28 Mean diastolic BP during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). There was a statistically significant increase in diastolic BP on treatment with labetalol or hydralazine. * - statistically significant, i.e., $p \leq 0.05$.

3.3.2.2 Blood pressure load: EMS

The BP load during before and on antihypertensive treatment was calculated. Baseline BP was defined as the average BP of all nine animals before each new antihypertensive treatment commenced. Systolic BP load increased significantly after labetalol, methyldopa or hydralazine treatment was established during the EMS period as determined by chi-square test $\chi 2$ (4, N = 4878) = 19.45, $p < 0.01$, $\chi 2$ (4, N = 4848) = 49.50, $p < 0.01$, $\chi 2$ (4, N = 4754) = 74.64, $p < 0.01$ respectively (Figure 3-29).

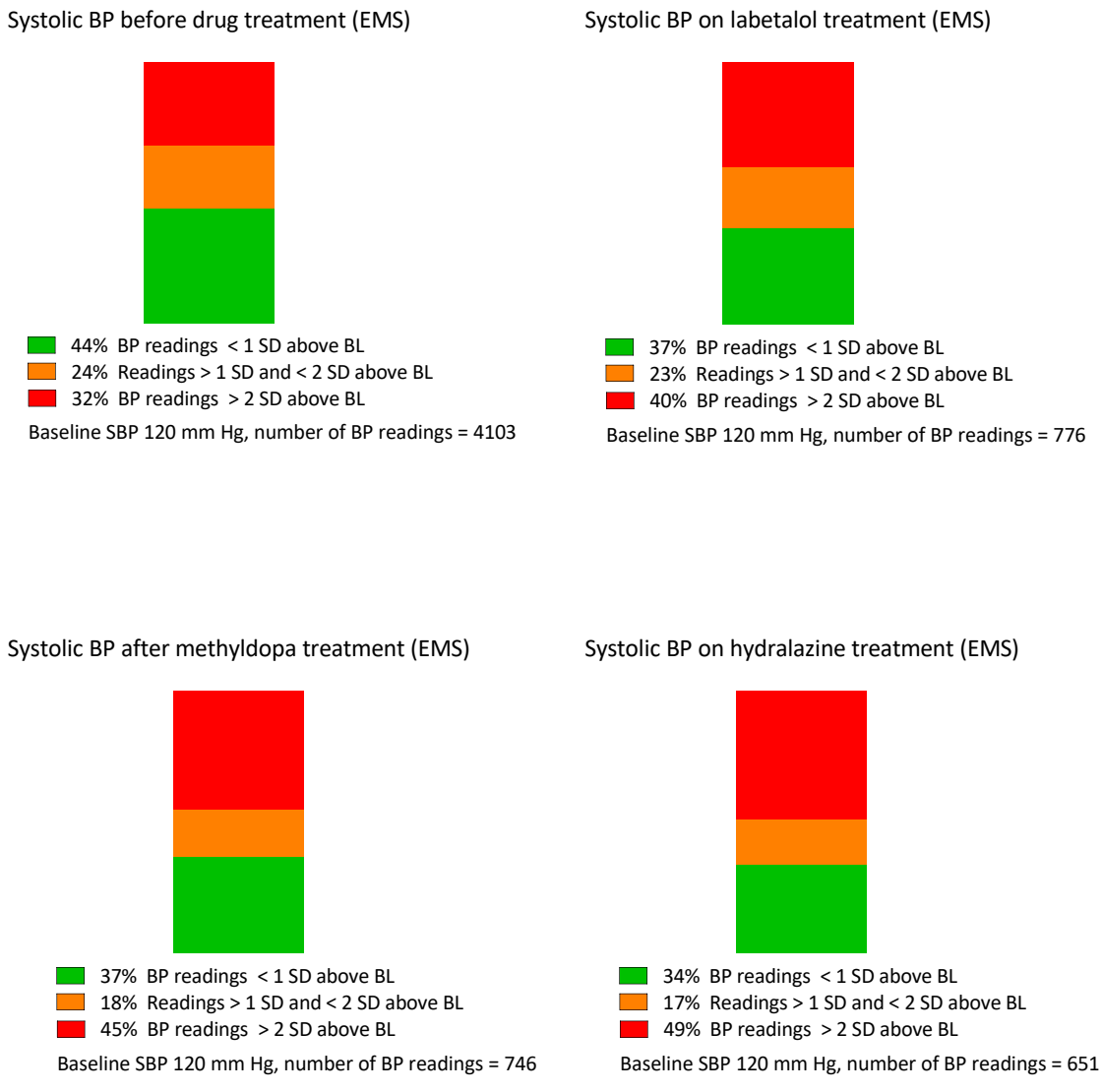

Figure 3-29 Systolic BP load during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9).

Diastolic BP load increased significantly on methyldopa and hydralazine treatment during the EMS period as determined by chi-square test $\chi 2$ (4, N = 4848) = 24.35, $p < 0.01$, $\chi 2$ (4, N = 4754) = 53.85, $p < 0.01$ respectively. Diastolic BP load did not change significantly on labetalol treatment: $\chi 2$ (4, N = 4879) = 2.15, $p = 0.34$ (Figure 3-30).

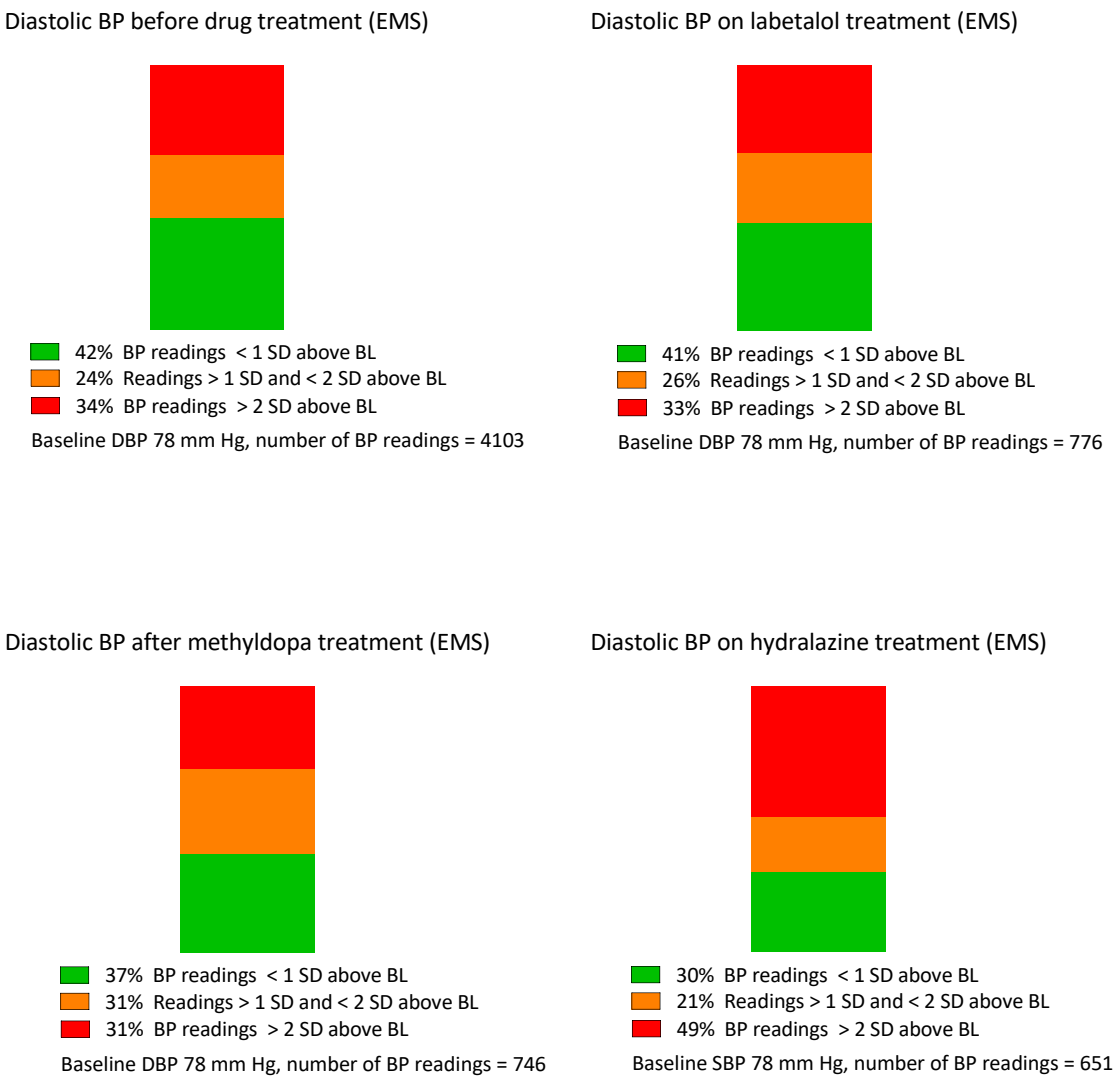

Figure 3-30 Diastolic BP load during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9).

BP load during the EMS period mostly increased with antihypertensive treatment (except in the case of diastolic BP after labetalol treatment, where there was no significant difference).

3.3.2.3 Blood pressure variability using SD1 and SD2 values generated by Poincaré Plots: EMS

There was a significant difference between the short-term variability as measured by SD1 of EMS systolic BP (Figure 3-31) and diastolic BP (Figure 3-32) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (both $p < 0.001$). There was no significant difference between the antihypertensive types.

There was no significant difference between the long-term variability as measured by SD2 of EMS systolic BP (Figure 3-33) or diastolic BP (Figure 3-34) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established.

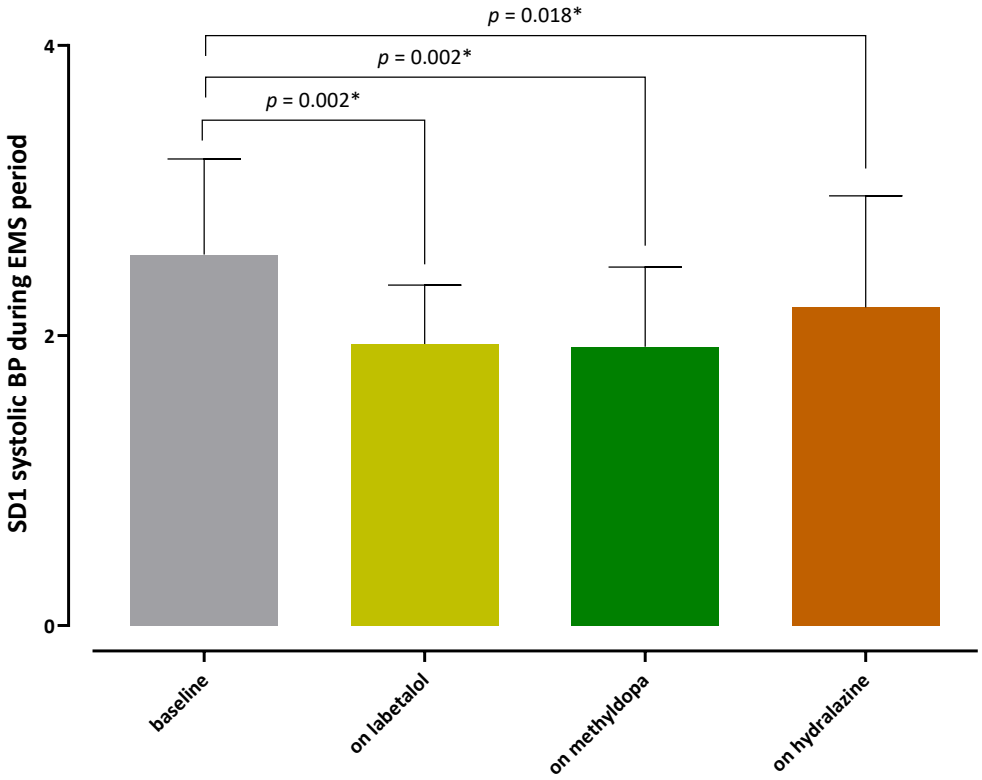

Figure 3-31 Mean short-term variability (SD1) of systolic BP during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Baseline BP was defined as the group average before any antihypertensive treatment commenced. * indicates statistically significant difference between groups (p ≤ 0.05).

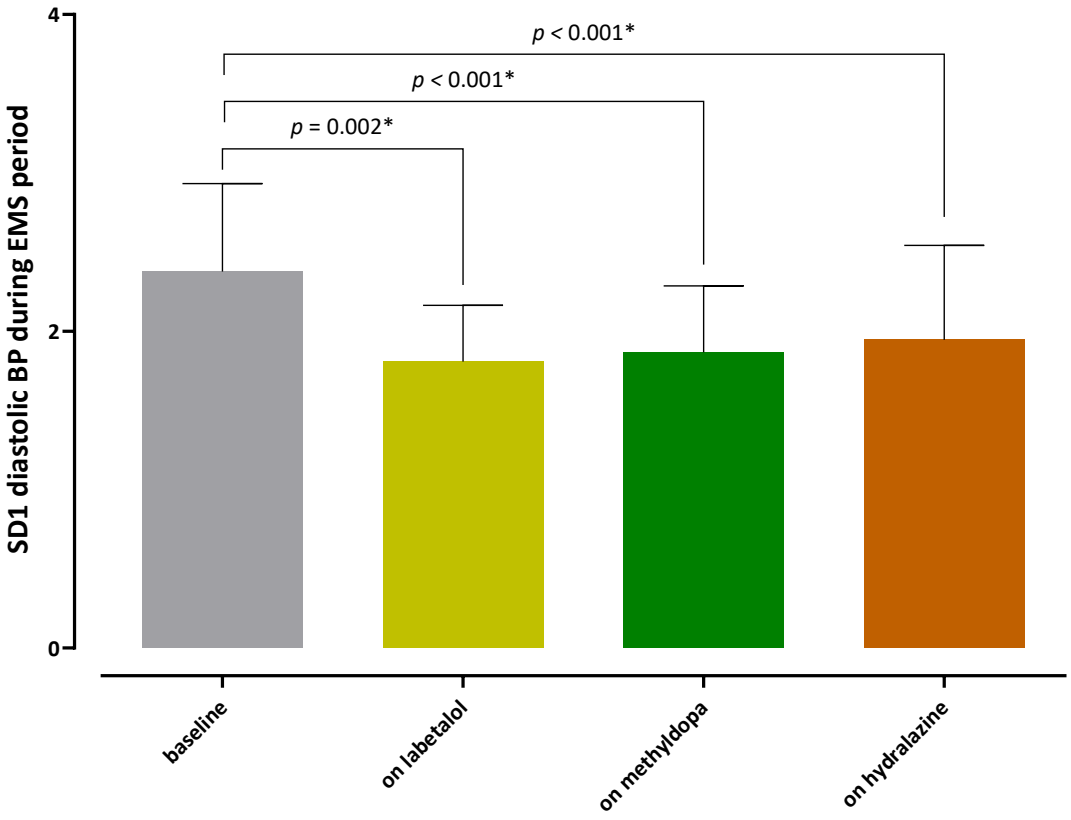

Figure 3-32 Mean short-term variability (SD1) of diastolic BP during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Baseline BP was defined as the group average before any antihypertensive treatment commenced. * indicates statistically significant difference between groups ($p \leq 0.05$).

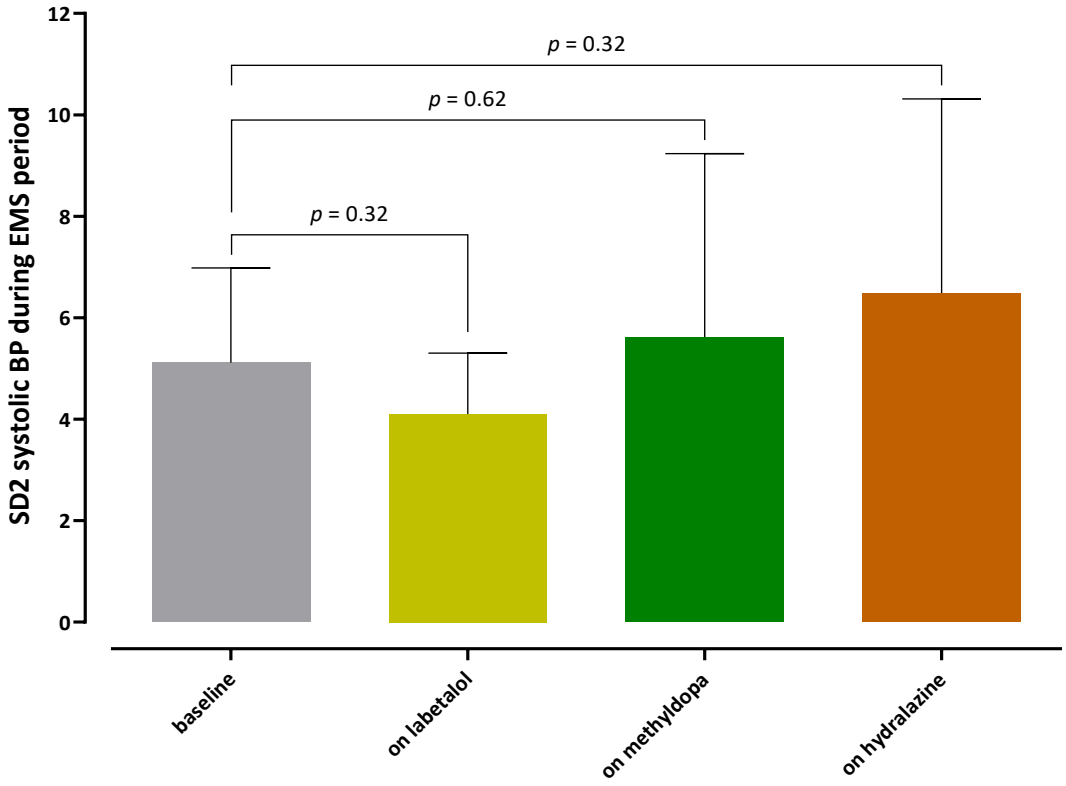

Figure 3-33 Mean long-term variability (SD2) of systolic BP during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Baseline BP was defined as the group average before any antihypertensive treatment commenced. Statistical significance defined as $p \leq 0.05$.

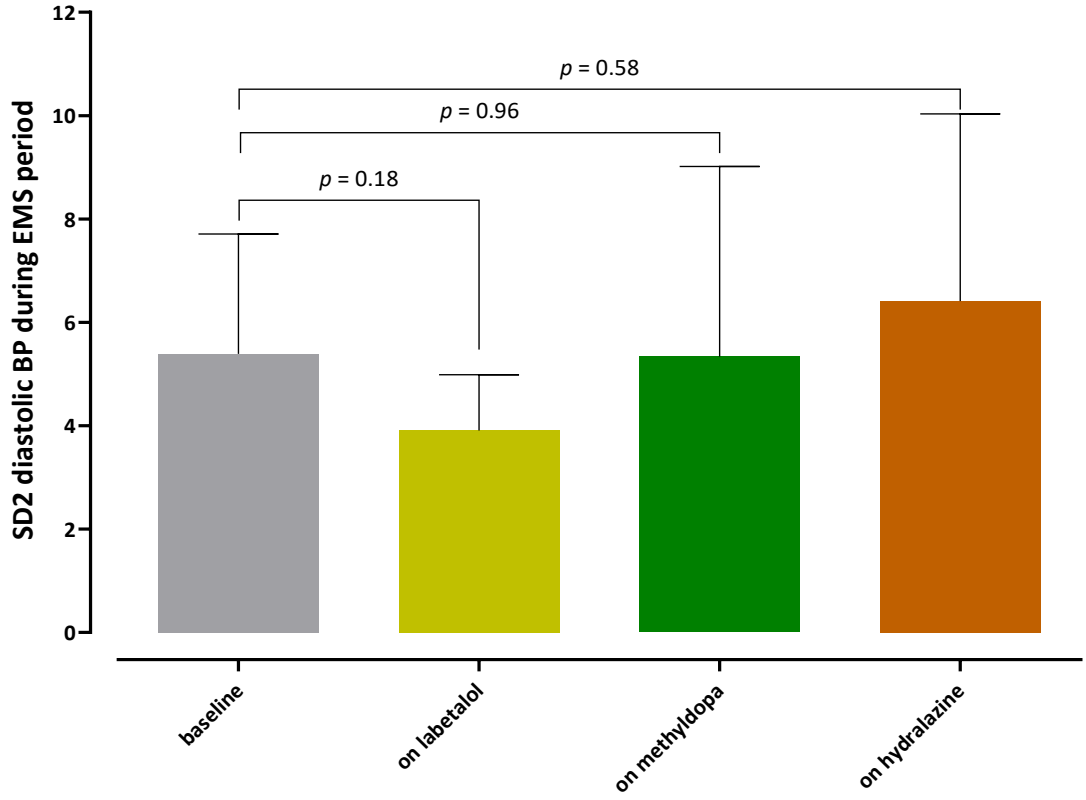

Figure 3-34 Mean long-term variability (SD2) of diastolic BP during the EMS period before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). Baseline BP was defined as the group average before any antihypertensive treatment commenced. Statistical significance defined as $p \leq 0.05$.

3.3.2.4 Classification of blood pressure data as stochastic, periodic or chaotic: EMS

The Chaos Decision Tree Algorithm classified all the EMS BP data as stochastic.

3.3.3 Study 3: Blood pressure during anaesthesia (ketamine, propofol, sevoflurane) before and on antihypertensive treatment (labetalol, methyldopa or hydralazine)

Of the nine animals in this study, three were allocated to the ketamine anaesthetic protocol, three were allocated to the propofol anaesthetic protocol and three were allocated to the sevoflurane anaesthetic protocol propofol as outlined in Table 2-2. The group of three animals receiving each anaesthetic were concurrently receiving a different sequence of antihypertensive medication (described in Table 2-1).

3.3.3.1 Average blood pressure: anaesthesia

There was a significant difference between the average systolic BP during general anaesthesia with ketamine and sevoflurane (p = 0.003). The average systolic before/after drug treatment established (labetalol, methyldopa, hydralazine combined) under ketamine anaesthesia was 136/137 mmHg, whereas this was 112/104 mmHg under sevoflurane (Table 3-7). There was no significant difference between the different antihypertensive drug treatments under the different anaesthetics.

Mean systolic BP	before labetalol treatment (mmHg)	on labetalol treatment (mmHg)	before methyldopa treatment (mmHg)	on methyldopa treatment (mmHg)	before hydralazine treatment (mmHg)	on hydralazine treatment (mmHg)
ketamine	133 (CI: 115-152)	139 (CI: 118-160)	137 (CI: 119-156)	136 (CI: 115-158)	141 (CI: 122-159)	137 (CI: 116-159)
propofol	119 (CI: 100-137)	117 (CI: 95-138)	137 (CI: 119-156)	116 (CI: 95-138)	119 (CI: 101-138)	122 (CI: 100-143)
sevoflurane	109 (CI: 91-128)	100 (CI: 78-121)	118 (CI: 100-137)	109 (CI: 87-130)	109 (CI: 86-132)	105 (CI: 79-131)

Table 3-5 Mean systolic BP before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during ketamine, propofol and sevoflurane anaesthesia (n = 9). CI – 95% confidence interval.

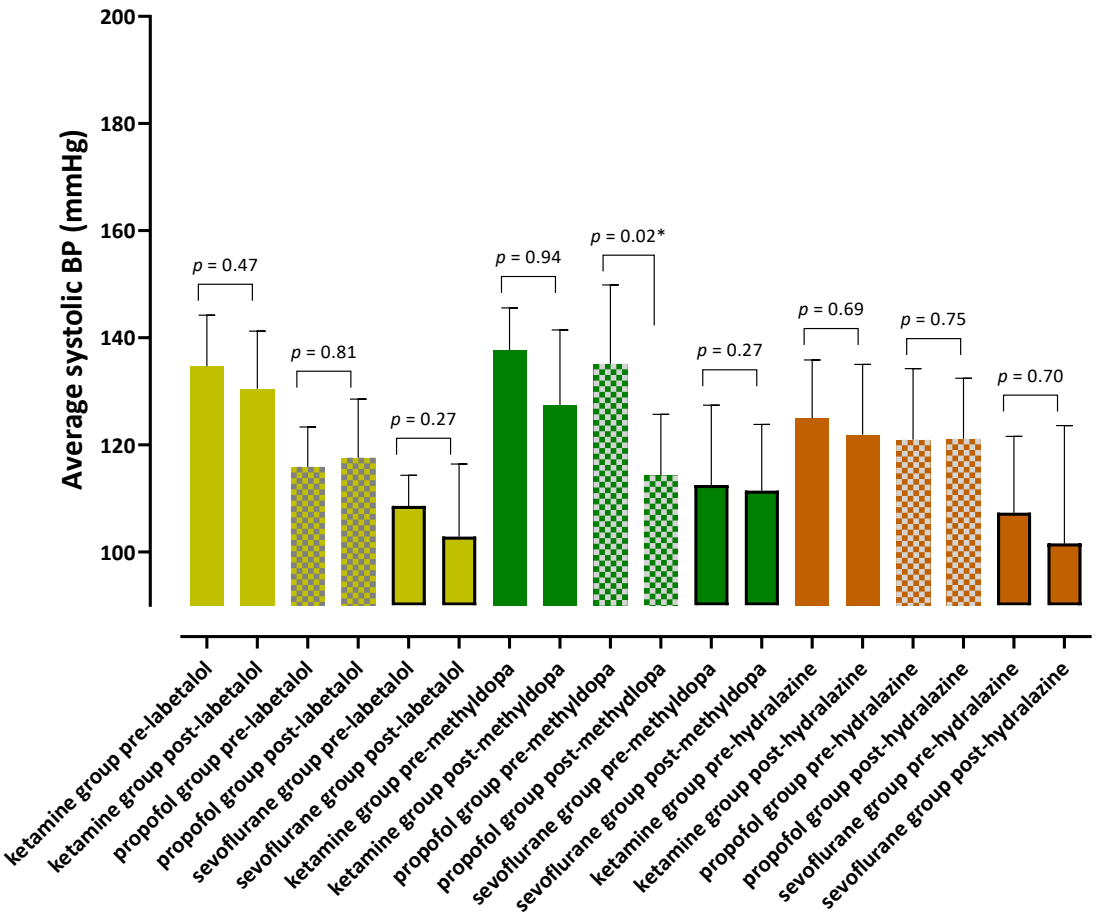

Figure 3-35 Average systolic BP before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during ketamine (n = 3), propofol (n = 3) or sevoflurane (n = 3) anaesthesia. There was a statistically significant difference between average systolic BP before and on methyldopa treatment during propofol anaesthesia (*p* = 0.02). There was no other significant difference between the different antihypertensive drug treatments under the different anaesthetics. There was a significant difference between the average systolic BP during anaesthesia with ketamine compared with sevoflurane (*p* = 0.003). * indicates statistically significant difference between groups (p ≤ 0.05). NB: The sevoflurane pre- and post-hydralazine group uses data from two animals only as one animal (T50) from this group delivered early. * - statistical significance, i.e., *p* = 0.05.

There was a significant difference between the average diastolic BP during general anaesthesia with ketamine and sevoflurane on drug treatment (p = 0.02). The average diastolic BP before/on drug treatment (all combined) under ketamine anaesthesia was 89/90 mmHg, whereas this was 75/66 mmHg under sevoflurane. There was no significant difference between the different antihypertensive drug treatments under the different anaesthetics.

Anaesthetic type	**Mean diastolic BP – before labetalol (mmHg)**	**Mean diastolic BP – on labetalol (mmHg)**	**Mean diastolic BP – before methyldopa (mmHg)**	**Mean diastolic BP – on methyldopa (mmHg)**	**Mean diastolic BP – before hydralazine (mmHg)**	**Mean diastolic BP – on hydralazine (mmHg)**
ketamine	83 (CI: 68-98)	93 (CI: 75-111)	93 (CI: 78-108)	88 (CI: 70-106)	93 (CI: 78-108)	90 (CI: 72-108)
propofol	81 (CI: 66-96)	79 (CI: 61-97)	98 (CI: 83-113)	78 (CI: 60-96)	78 (CI: 63-93)	83 (CI: 65-101)
sevoflurane	68 (CI: 53-83)	64 (CI: 46-82)	78 (CI: 63-93)	69 (CI: 51-87)	78 (CI: 60-96)	66 (CI: 44-88)

Table 3-6 Mean diastolic BP (mmHg) before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during ketamine (n = 3), propofol (n = 3) or sevoflurane anaesthesia (n = 3). CI – 95% confidence interval.

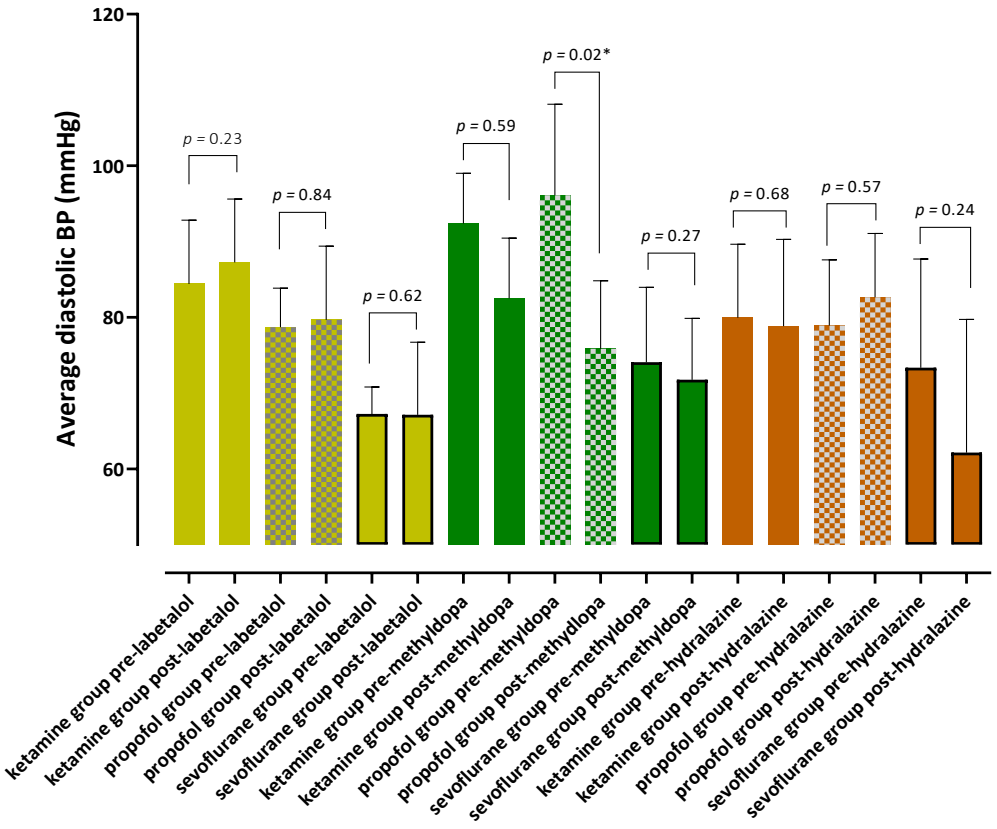

Figure 3-36 Average diastolic BP before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during ketamine (n = 3), propofol (n = 3) or sevoflurane (n = 3) anaesthesia. There was no significant difference between the different antihypertensive drug treatments under the different anaesthetics. There was a significant difference between the average diastolic BP during anaesthesia with ketamine compared with sevoflurane (p = 0.02). NB: The sevoflurane pre- and post-hydralazine group uses data from two animals only as one animal from this group delivered early. * - statistical significance, i.e., p = 0.05.

3.3.3.2 Blood pressure load: anaesthesia

The BP load during ketamine anaesthesia before and on labetalol treatment was calculated. Baseline BP was defined as the average BP of all nine animals during ketamine anaesthesia before labetalol treatment commenced. Systolic BP load did not change significantly on labetalol treatment during ketamine anaesthesia as determined by chi-square test: $\chi 2$ (4, N = 445) = 0.33, p = 0.8. Diastolic BP load did not change significantly: $\chi 2$ (4, N = 445) = 1.52, p = 0.47 (Figure 3-37).

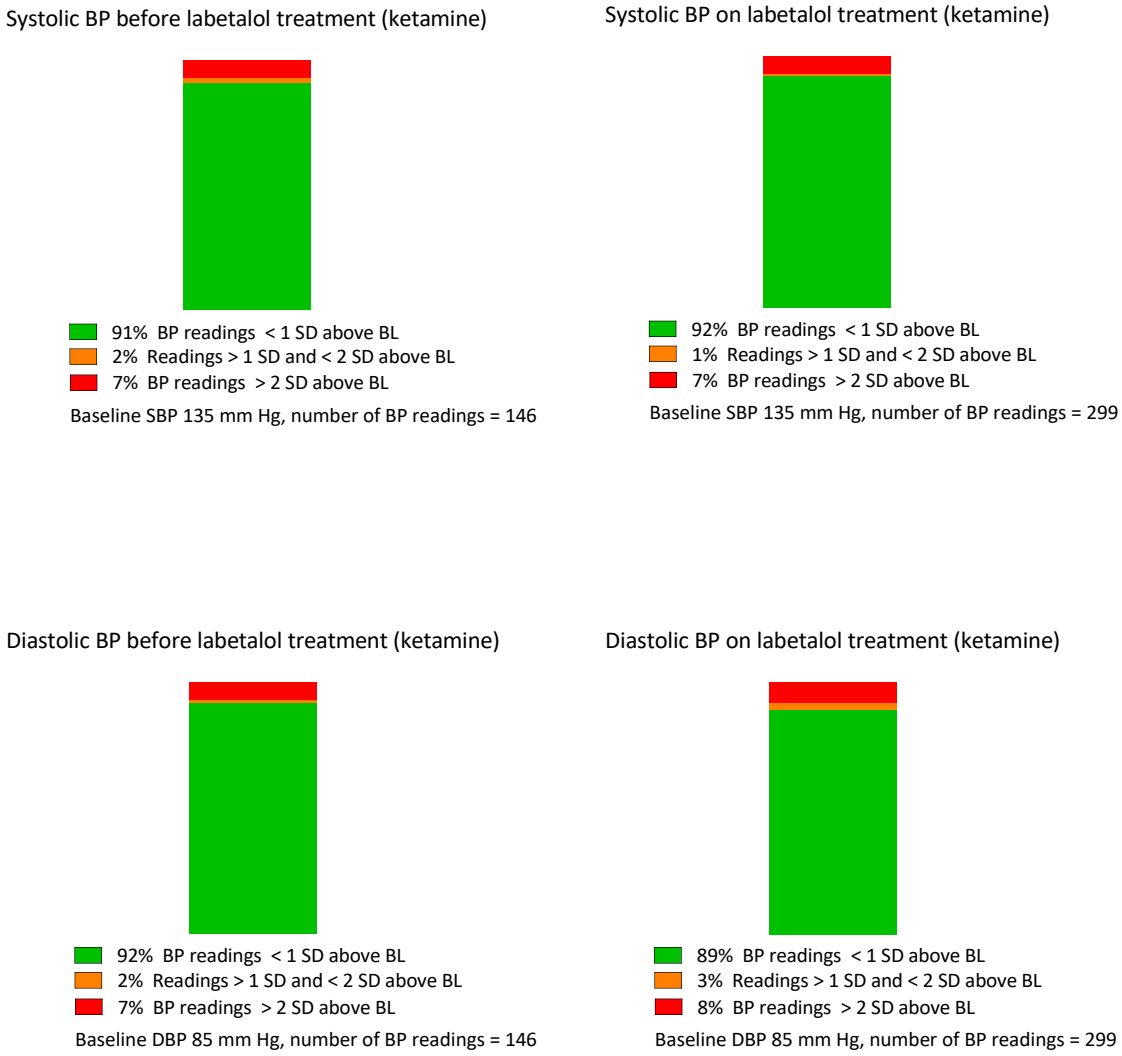

Figure 3-37 Systolic and diastolic BP load during ketamine anaesthesia before and on labetalol treatment (n = 3).

The BP load during ketamine anaesthesia before and on methyldopa treatment was calculated. Baseline BP was defined as the average BP of all nine animals during ketamine anaesthesia before methyldopa treatment commenced. Systolic BP load did not change significantly on methyldopa treatment during ketamine anaesthesia as determined by chi-square test: $\chi 2$ (4, N = 381) = 0.52, p = 0.78. Diastolic BP load did not change significantly: $\chi 2$ (4, N = 381) = 1.03, p = 0.60 (Figure 3-38).

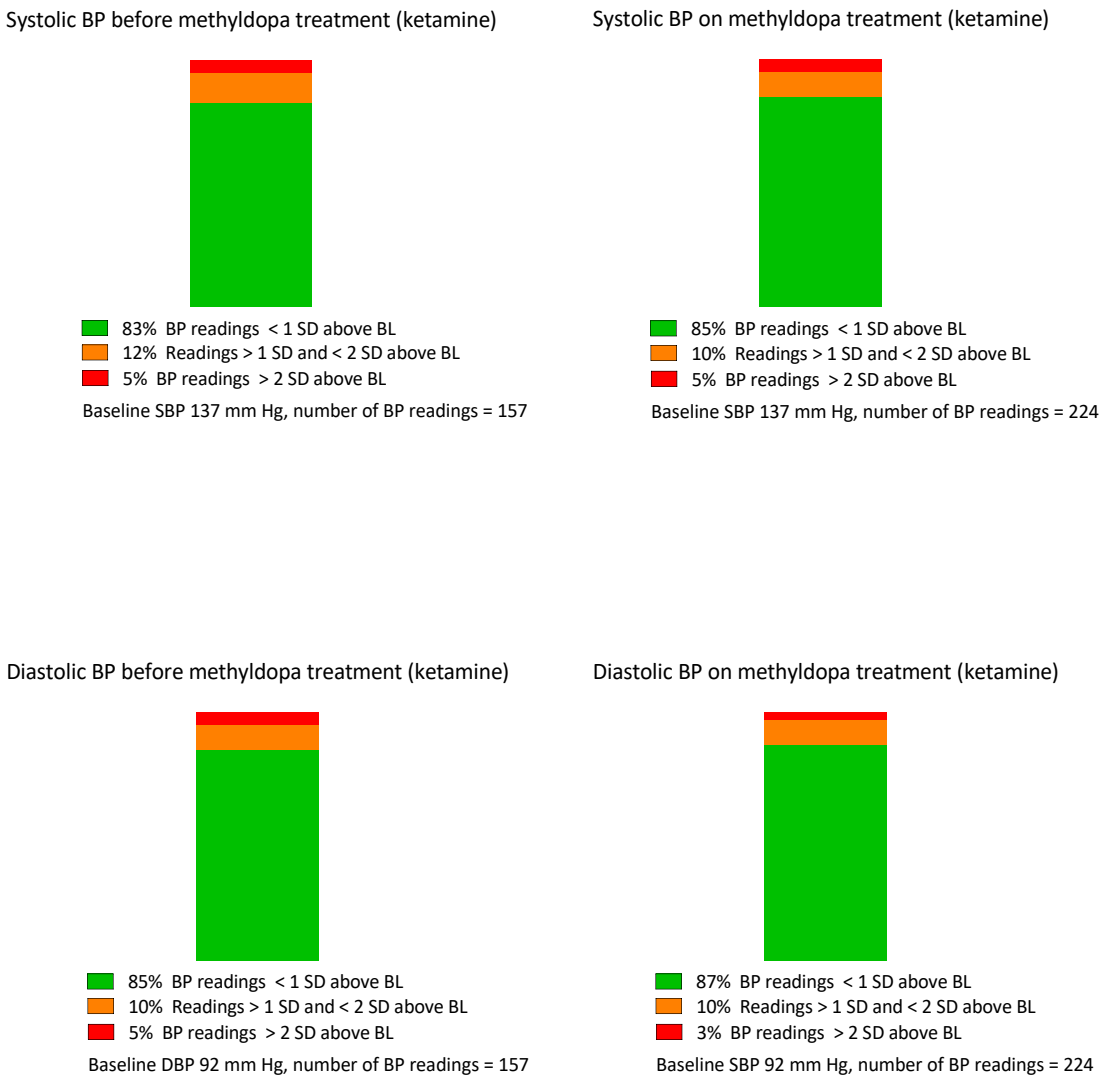

Figure 3-38 Systolic and diastolic BP load during ketamine anaesthesia before and on methyldopa treatment (n = 3).

The BP load during ketamine anaesthesia before and on hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals during ketamine anaesthesia before hydralazine treatment commenced. Systolic BP load did not change significantly on hydralazine treatment during ketamine anaesthesia as determined by chi-square test: $\chi 2$ (4, N = 334) = 0.21, p = 0.9. Diastolic BP load did not change significantly: $\chi 2$ (4, N = 334) = 0.03, p = 0.99 (Figure 3-39).

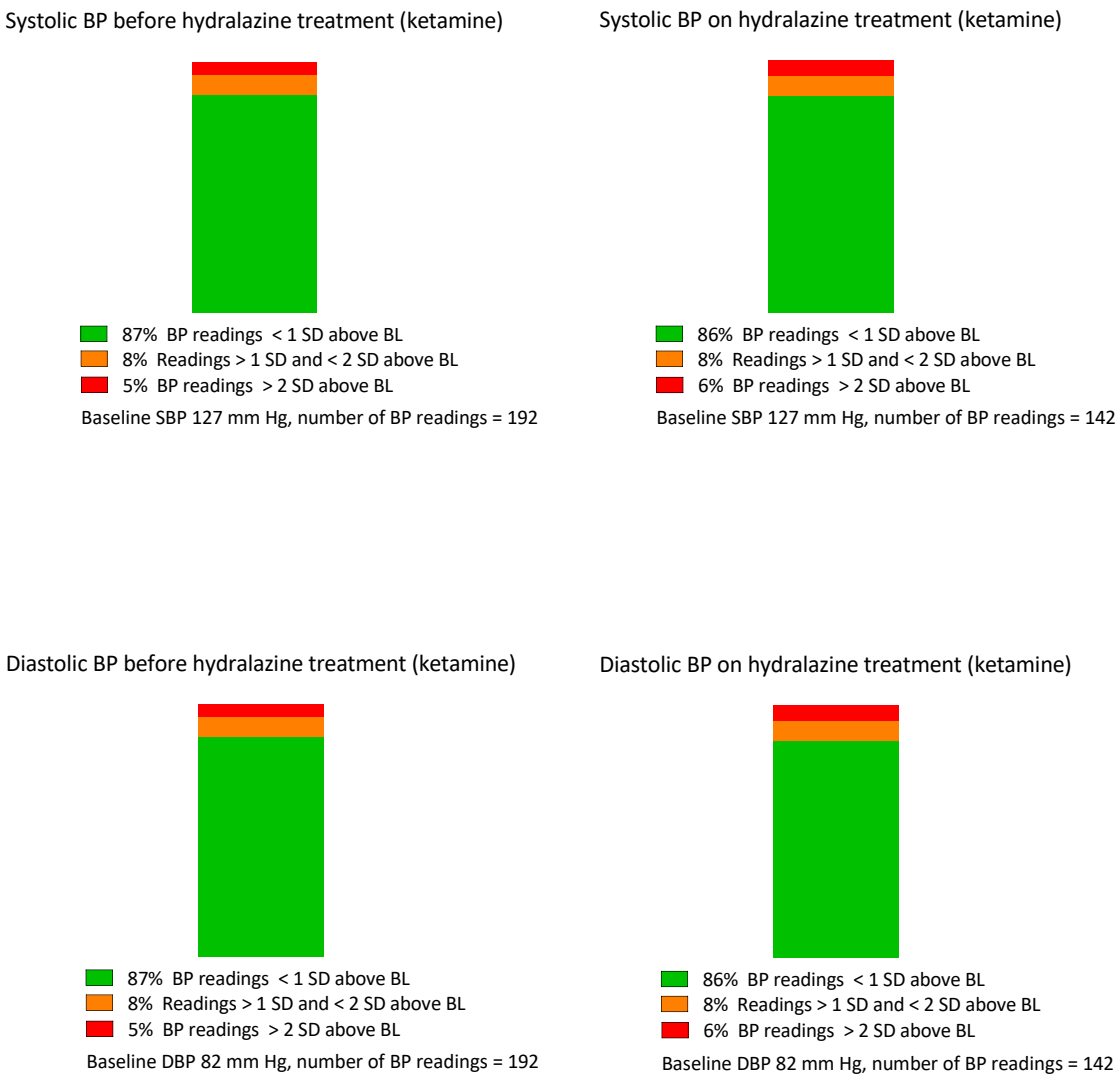

Figure 3-39 Systolic and diastolic BP load during ketamine anaesthesia before and on hydralazine treatment (n = 3).

The BP load during propofol anaesthesia before and on labetalol treatment was calculated. Baseline BP was defined as the average BP of all nine animals during propofol anaesthesia before labetalol treatment commenced. Systolic BP load decreased significantly on labetalol treatment during propofol anaesthesia as determined by chi-square test: $\chi 2$ (4, N = 647) = 17.33, $p < 0.01$. Diastolic BP load decreased significantly: $\chi 2$ (4, N = 647) = 12.80, $p = 0.002$ (Figure 3-40).

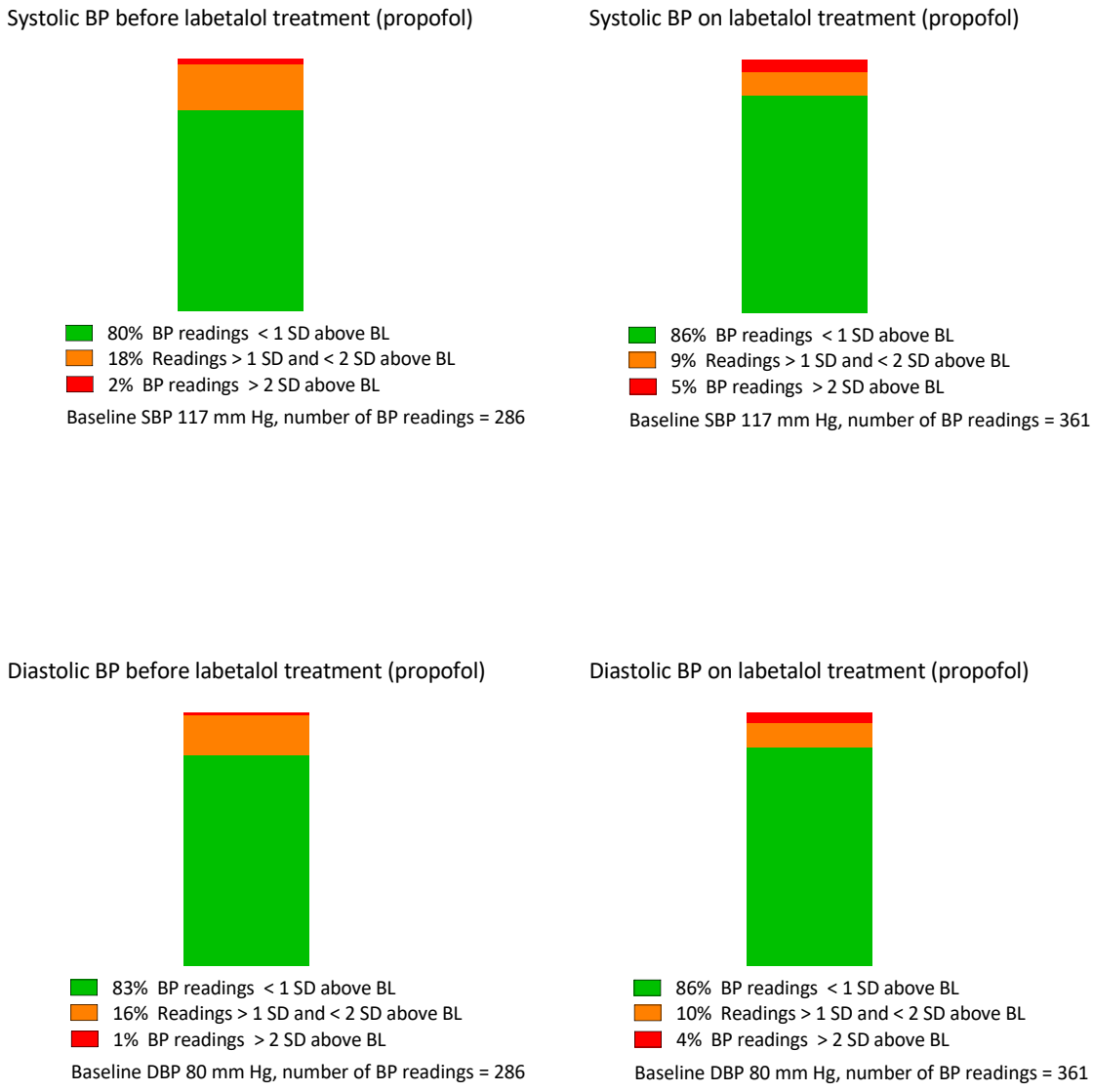

Figure 3-40 Systolic and diastolic BP load during propofol anaesthesia before and on labetalol treatment (n = 3).

The BP load during propofol anaesthesia before and on methyldopa treatment was calculated. Baseline BP was defined as the average BP of all nine animals during propofol anaesthesia before methyldopa treatment commenced. Systolic BP load decreased significantly on methyldopa treatment during propofol anaesthesia as determined by chi-square test: $\chi 2$ (4, N = 488) = 29.20, $p < 0.01$. Diastolic BP load decreased significantly: $\chi 2$ (4, N = 488) = 38.6, $p < 0.001$ (Figure 3-41).

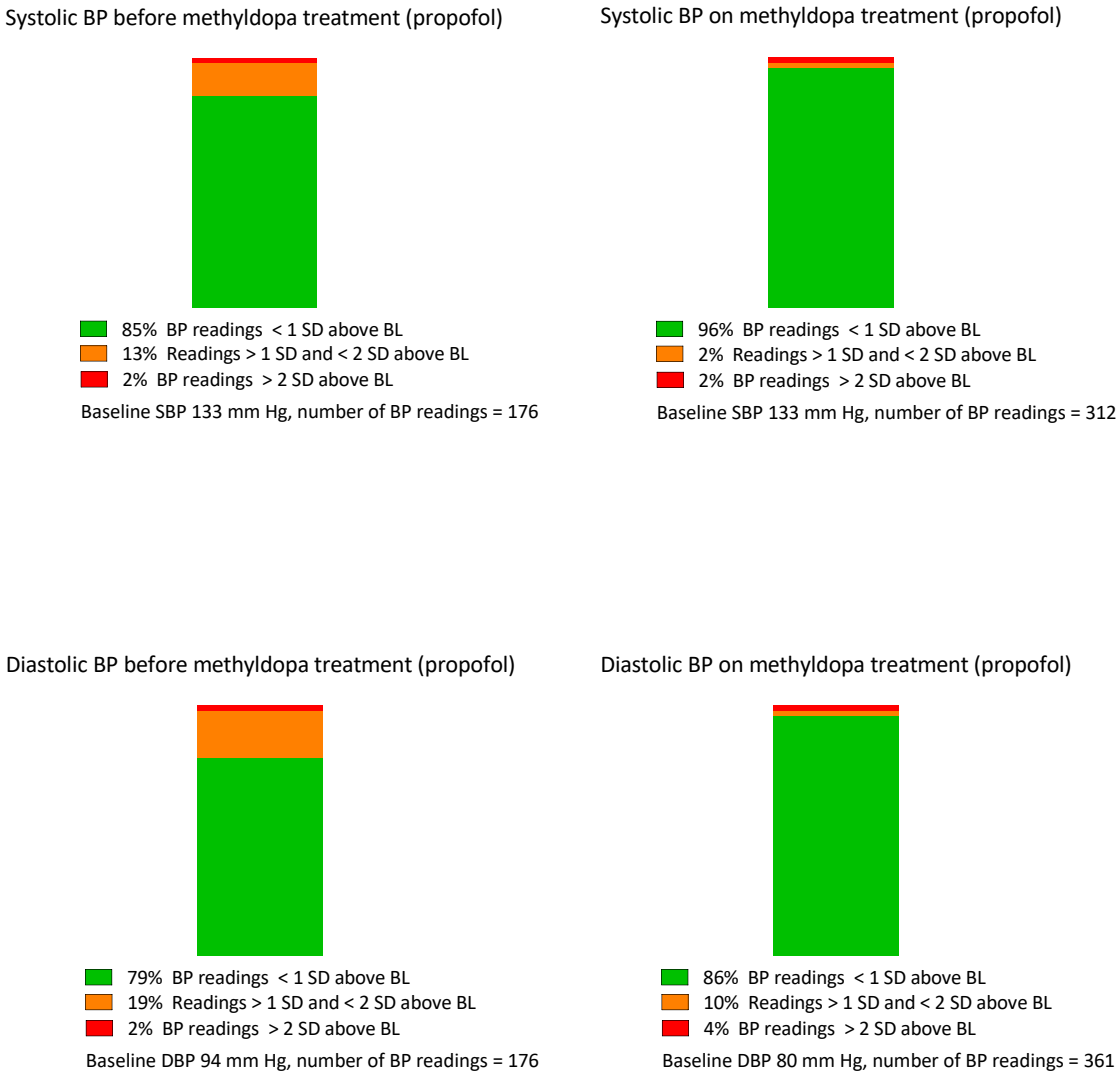

Figure 3-41 Systolic and diastolic BP load during propofol anaesthesia before and on methyldopa treatment (n = 3).

The BP load during propofol anaesthesia before and on hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals during propofol anaesthesia before hydralazine treatment commenced. Systolic BP load did change significantly on hydralazine treatment during propofol anaesthesia as determined by chi-square test: $\chi 2$ (4, N = 624) = 8.03, p = 0.019. Diastolic BP load did not change significantly: $\chi 2$ (4, N = 624) = 4.42, p = 0.12 (Figure 3-42).

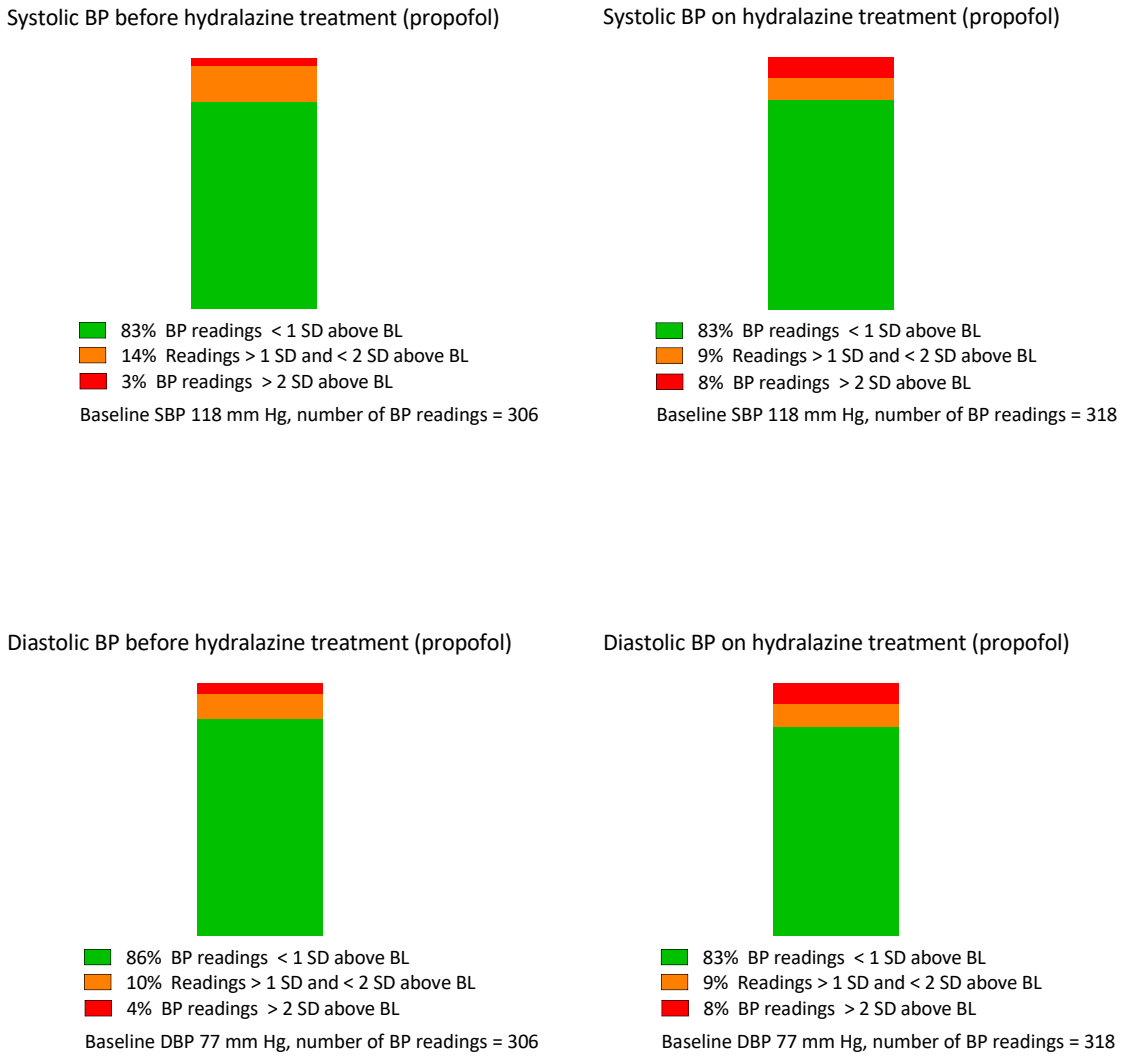

Figure 3-42 Systolic and diastolic BP load during propofol anaesthesia before and on hydralazine treatment (n = 3).

The BP load during sevoflurane anaesthesia before and on labetalol treatment was calculated. Baseline BP was defined as the average BP of all nine animals during sevoflurane anaesthesia before labetalol treatment commenced. Systolic BP load increased significantly on labetalol treatment during sevoflurane anaesthesia as determined by chi-square test: $\chi 2$ (4, N = 790) = 25.22, $p < 0.01$. Diastolic BP load also increased significantly: $\chi 2$ (4, N = 790) = 5.99, $p = 0.049$ (Figure 3-43).

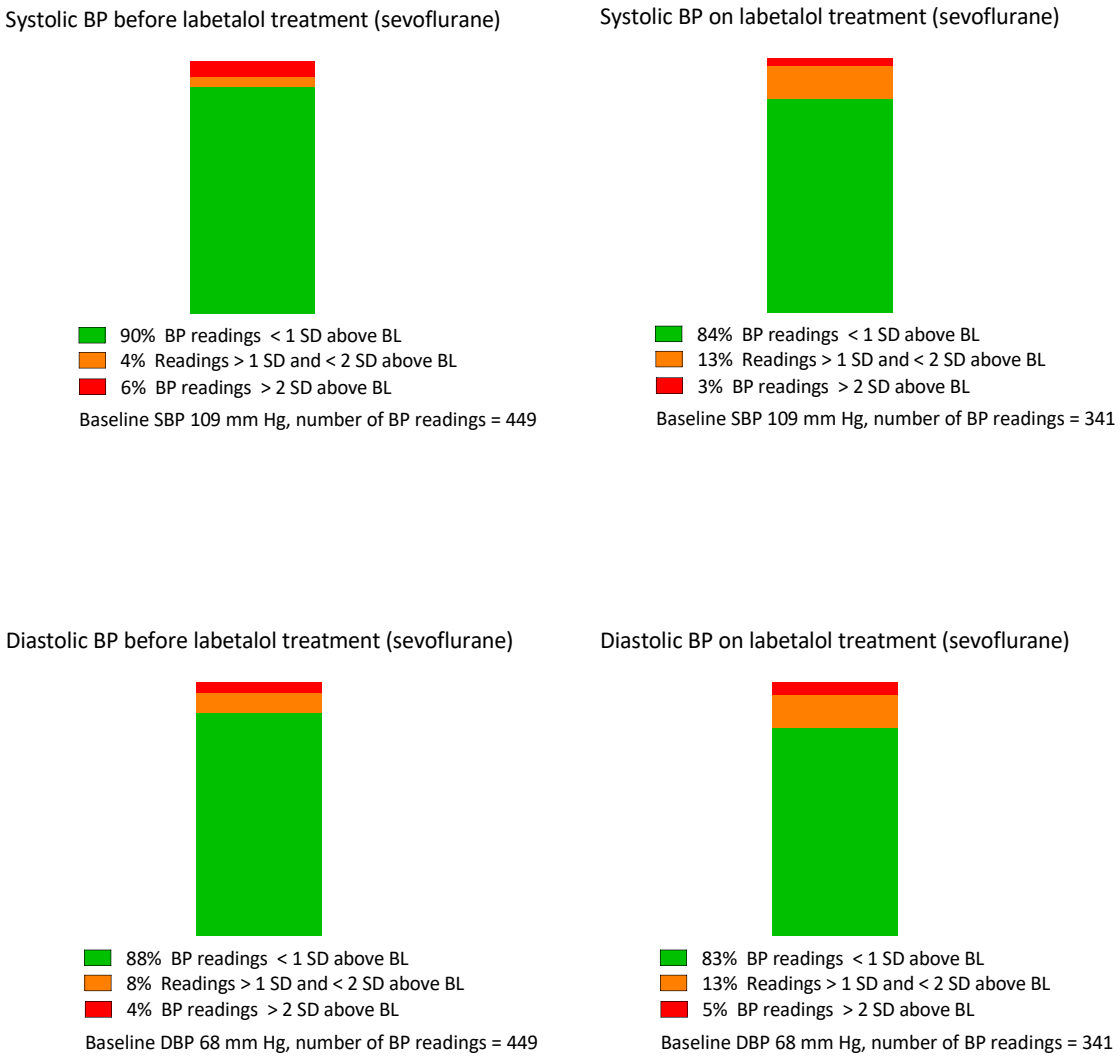

Figure 3-43 Systolic and diastolic BP load during sevoflurane anaesthesia before and on labetalol treatment (n = 3).

The BP load during sevoflurane anaesthesia before and on methyldopa treatment was calculated. Baseline BP was defined as the average BP of all nine animals during sevoflurane anaesthesia before methyldopa treatment commenced. Systolic BP load decreased significantly on methyldopa treatment during sevoflurane anaesthesia as determined by chi-square test: $\chi 2$ (4, N = 873) = 19.42, p < 0.01. Diastolic BP also decreased significantly: $\chi 2$ (4, N = 873) = 22.43, p < 0.001 (Figure 3-44).

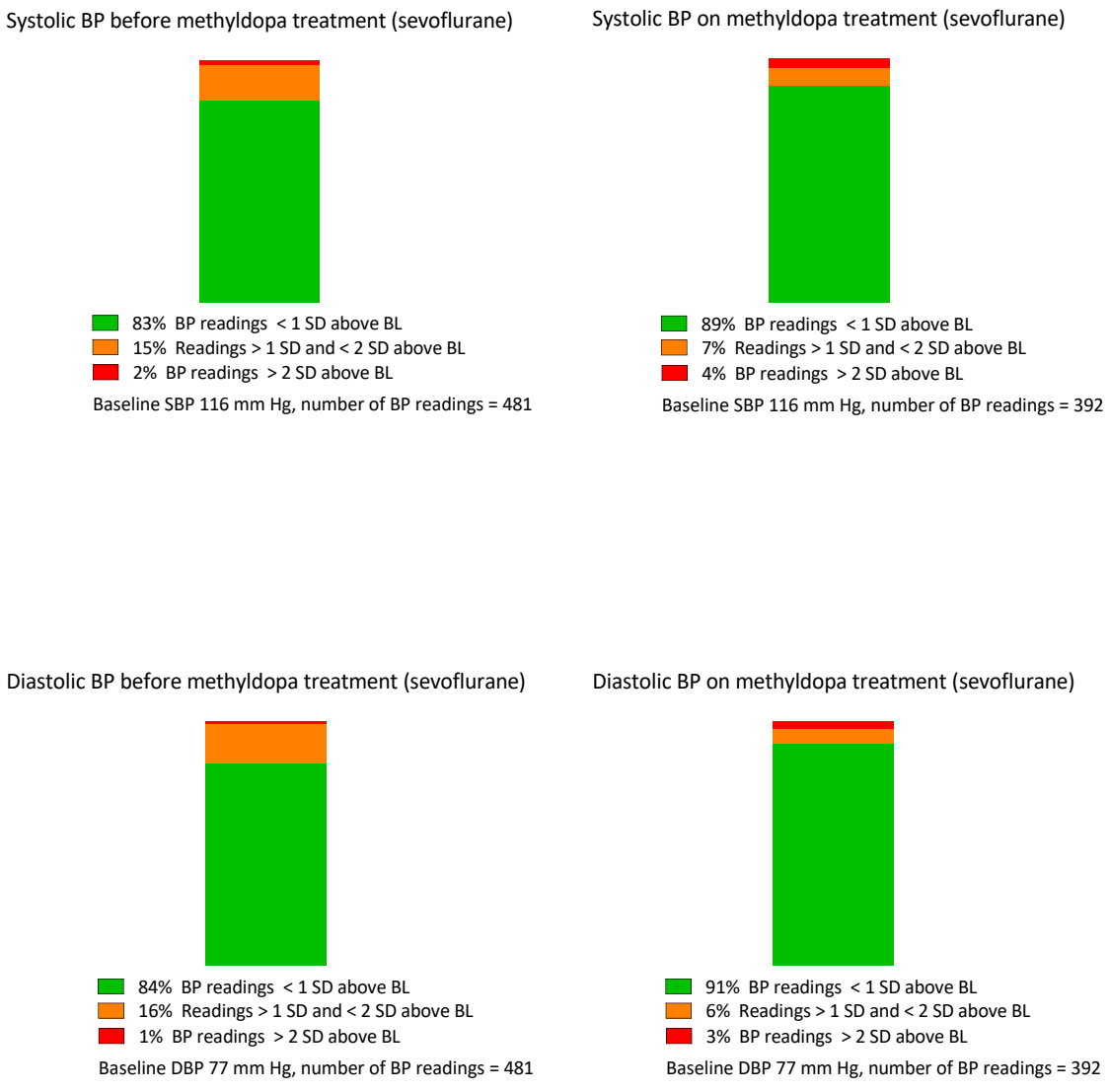

Figure 3-44 Systolic and diastolic BP load during sevoflurane anaesthesia before and on methyldopa treatment (n = 3).

The BP load during sevoflurane anaesthesia before and on hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals during sevoflurane anaesthesia before hydralazine treatment commenced. Systolic BP load decreased significantly on hydralazine treatment during sevoflurane anaesthesia as determined by chi-square test: $\chi 2$ (4, N = 444) = 9.63, $p < 0.01$. Diastolic BP load also decreased significantly: $\chi 2$ (4, N = 444) = 13.82, $p < 0.01$ (Figure 3-45).

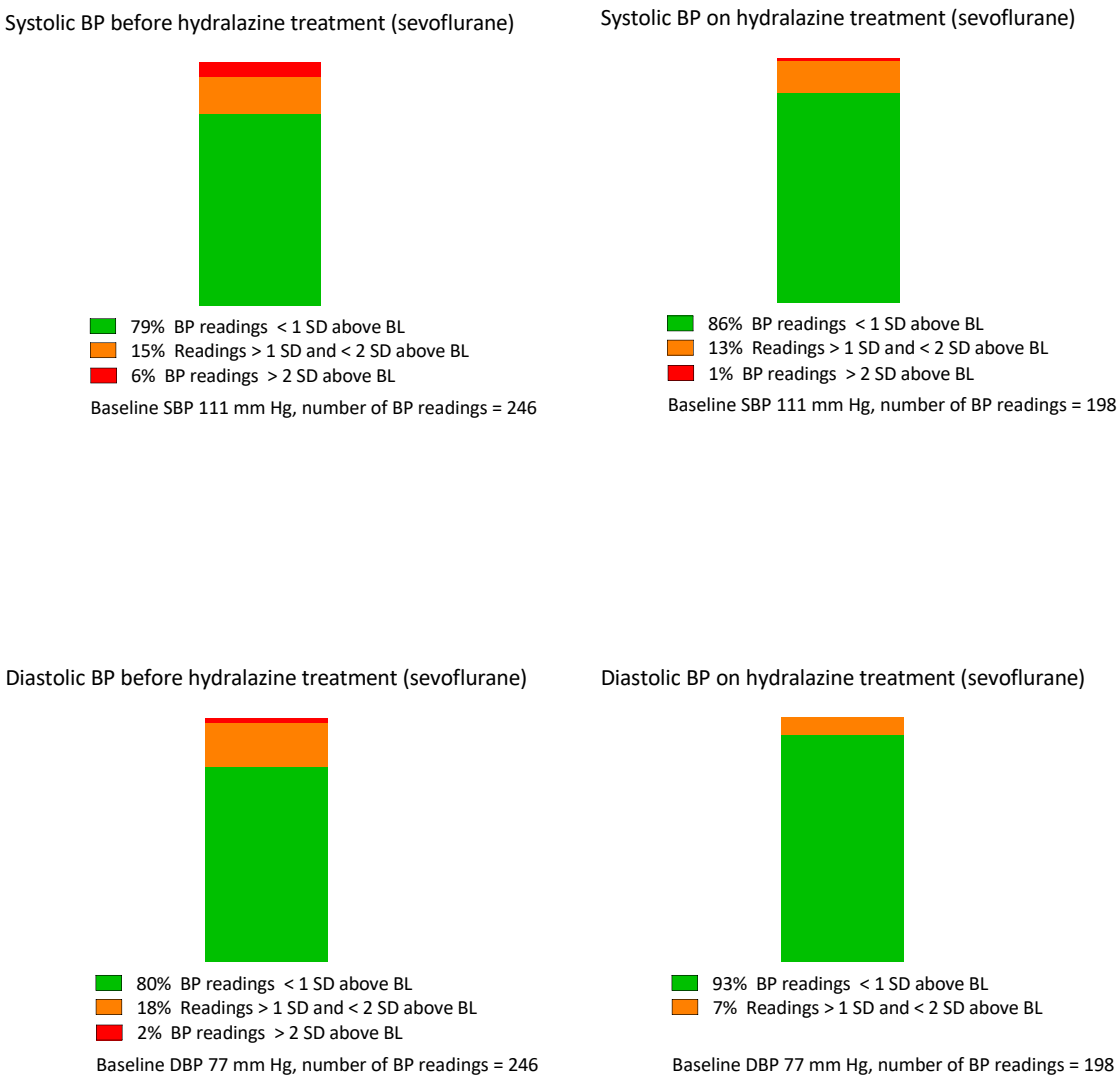

Figure 3-45 Systolic and diastolic BP load during sevoflurane anaesthesia before and on hydralazine treatment (n = 3).

Overall, the anaesthetic-antihypertensive combination that had the greatest proportion of readings in the 'green' zone (within one standard deviation of the baseline BP) and lowest proportion in the 'red' zone (greater than two standard deviations of the baseline BP) was sevoflurane and hydralazine.

3.3.3.3 Blood pressure variability using SD1 and SD2 values generated by Poincaré Plots: anaesthesia

There was a significant difference between the diastolic long-term variability as measured by SD2 under ketamine and sevoflurane anaesthesia after treatment with methyldopa was established (73.6 vs. 16.6 +/- 13.9, CI: 8 – 106, $p < 0.05$). There was no significant difference in diastolic long-term variability between the other anaesthetic and antihypertensive conditions.

There was no significant difference between the systolic and diastolic short-term variability and systolic long-term variability under the different anaesthetics: ketamine, propofol or sevoflurane before and after treatment with labetalol, methyldopa or hydralazine was established.

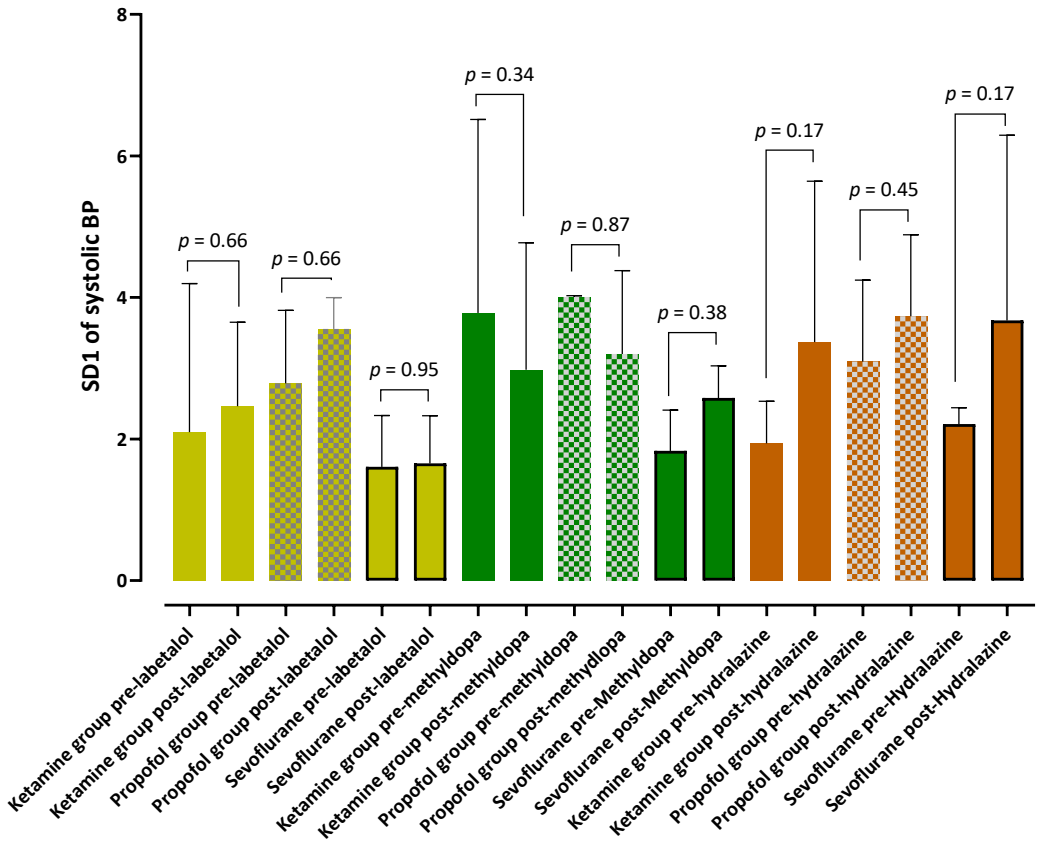

Figure 3-46 Short-term variability, as represented by SD1, of systolic BP before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during ketamine (n = 3), propofol (n = 3) or sevoflurane (n = 3) anaesthesia. There was no significant difference between the different antihypertensive drug treatments under the different anaesthetics. Statistical significance defined as $p \leq 0.05$. NB: The sevoflurane pre- and post-hydralazine group uses data from two animals only as one animal from this group delivered early.

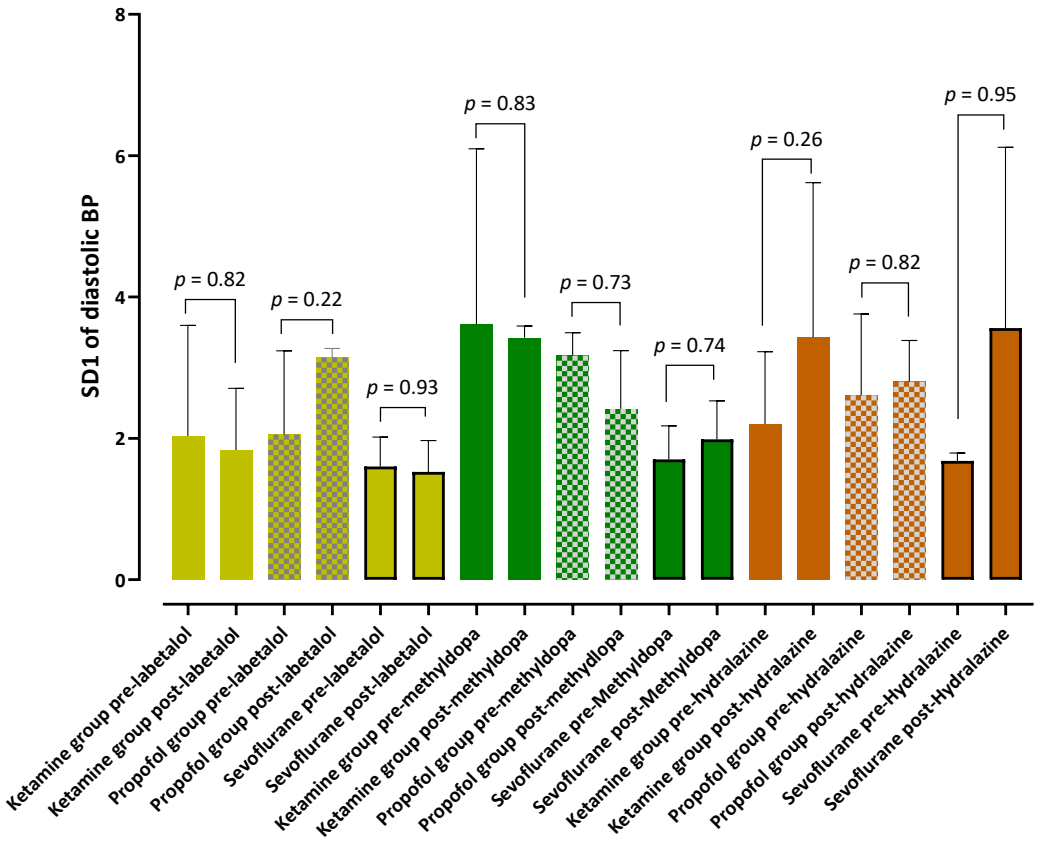

Figure 3-47 Short-term variability, as represented by SD1, of diastolic BP before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during ketamine (n = 3), propofol (n = 3) or sevoflurane (n = 3) anaesthesia. There was no significant difference between the different antihypertensive drug treatments under the different anaesthetics. Statistical significance defined as $p \leq 0.05$. NB: The sevoflurane pre- and post-hydralazine group uses data from two animals only as one animal from this group delivered early.

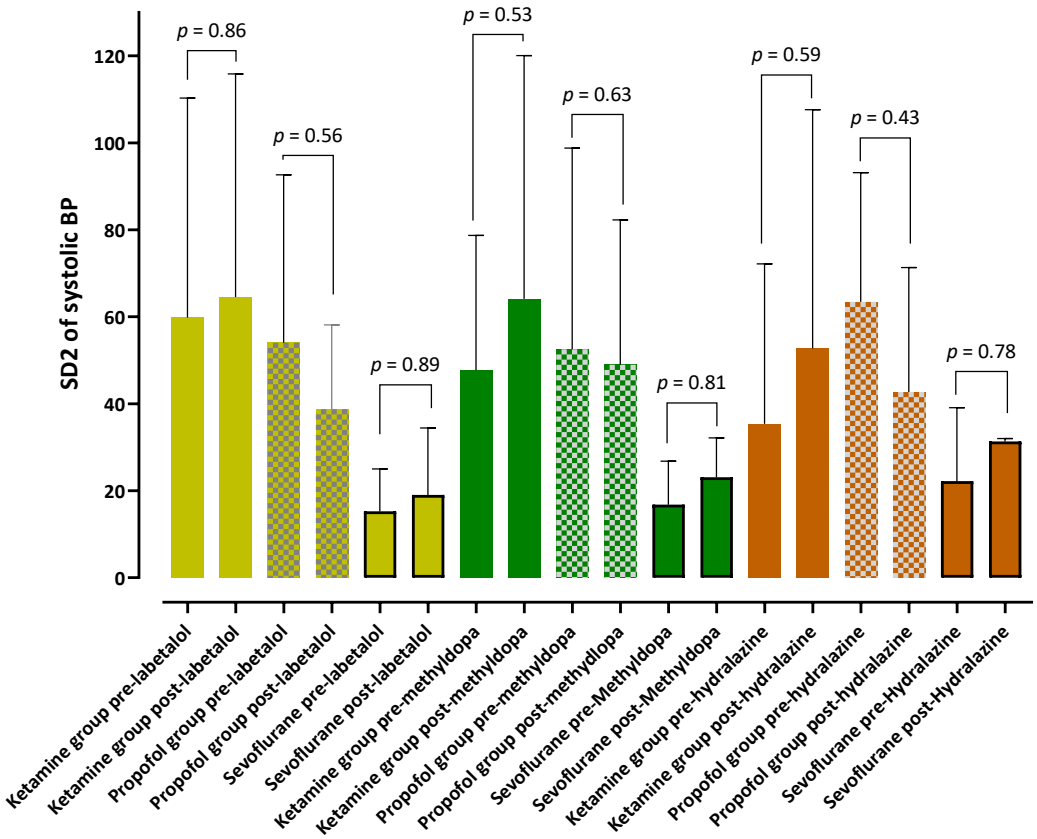

Figure 3-48 Long-term variability, as represented by SD2, of systolic BP before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during ketamine (n = 3), propofol (n = 3) or sevoflurane (n = 3) anaesthesia. There was no significant difference between the different antihypertensive drug treatments under the different anaesthetics. Statistical significance defined as $p \leq 0.05$. NB: The sevoflurane pre- and post-hydralazine group uses data from two animals only as one animal from this group delivered early.

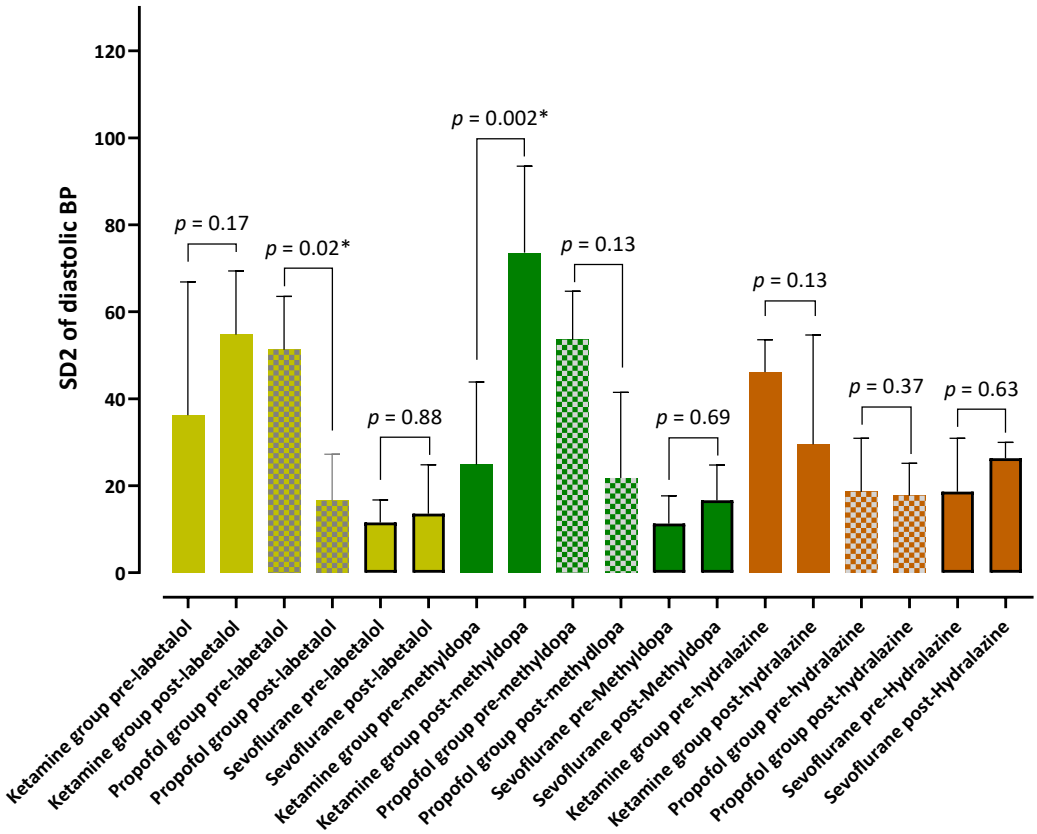

Figure 3-49 Long-term variability, as represented by SD2, of diastolic BP before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during ketamine (n = 3), propofol (n = 3) or sevoflurane (n = 3) anaesthesia. There was a significant decrease in SD2 on labetalol treatment during propofol anaesthesia and a significant increase on methyldopa treatment during ketamine anaesthesia. There were no other significant differences between the different antihypertensive drug treatments under the different anaesthetics. NB: The sevoflurane pre- and post-hydralazine group uses data from two animals only as one animal from this group delivered early. * - statistical significance, i.e., $p \leq 0.05$.

3.3.3.4 Classification of blood pressure data as stochastic, periodic or chaotic: anaesthesia

The BP data sets collected during anaesthesia were processed using the Chaos Decision Tree Algorithm and the following results (Table 3-9) were returned:

Stochastic	**Periodic**	**Chaotic**
All ketamine and sevoflurane data sets (systolic BP and diastolic BP) before and after all antihypertesives (except sevoflurane systolic BP on hydralazine – see periodic column). Propofol group (systolic BP and diastolic BP) before labetalol, diastolic BP on labetalol, and before methyldopa (diastolic BP)	Propofol group (systolic BP) on labetalol (K: 0.0939) Propofol group (systolic BP) before hydralazine (K: 0.7105) Sevoflurane group (systolic BP) on hydralazine (K: 0.5469)	Propofol group (systolic BP and diastolic BP) on methyldopa (K: 0.9567, K: 0.8920) Propofol group (systolic BP) on hydralazine (0.9626) and diastolic BP before and on hydralazine (K: 0.7595, K: 0.9275)

Table 3-7 Classification of systolic and diastolic BP data sets before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during ketamine (n = 3), propofol (n = 3) and sevoflurane (n = 3) anaesthesia as stochastic, periodic or chaotic as defined by the Chaos Decision Tree Algorithm. K - the Chaos Decision Tree Algorithm performs the 0–1 chaos test on the BP data and this test provides a single statistic, K, which approaches 1 for chaotic systems and approaches 0 for periodic systems.[217]

3.3.4 Study 4: Blood pressure during exposure to controlled stressors

As described, the nine baboons were given antihypertensive treatment (labetalol, methyldopa or hydralazine). On the different treatments, the baboons were then exposed to controlled stressors EF, MP, PT and SC (Figure 2-5).

3.3.4.1 Average blood pressure: noise stressors

During the everyday, familiar sounds (EF), average systolic BP increased significantly after labetalol, methyldopa or hydralazine treatment was established ($p < 0.001$ for all). The difference between systolic BP on labetalol and hydralazine two drugs was significant ($p = 0.001$), as shown in Figure 3-50.

Whilst Mozart's sonata (MP) was playing, average systolic BP decreased on labetalol treatment ($p < 0.001$) and increased on methyldopa and hydralazine treatment (ns). The difference between systolic BP on labetalol treatment when compared with methyldopa and hydralazine treatment was significant ($p < 0.001$), as shown in Figure 3-50.

Whilst a recording of lions roaring was being played, i.e., a potential threat (PT) to baboons, average systolic BP decreased on labetalol treatment ($p < 0.001$) and was significantly different from the average systolic BP on methyldopa and hydralazine treatment (both $p < 0.001$). Systolic BP effectively remained the same before and on methyldopa and hydralazine treatment (Figure 3-50).

During a recording of baboon noises, i.e., sounds of animals from the same species (SC) average systolic BP increased on treatment with each of the three drugs (labetalol, methyldopa or hydralazine). There was a significant difference in systolic BP before and on labetalol treatment ($p < 0.05$) as well as methyldopa or hydralazine treatment (both $p < 0.001$), as shown in Figure 3-50. The average systolic BP values before and on antihypertensives in the presence of the EF, MP, PT and SC controlled noise stressors is also outlined in Table 3-10.

Sound	Mean systolic BP – no drug (mmHg)	Mean systolic BP – labetalol (mmHg)	Mean systolic BP – methyldopa (mmHg)	Mean systolic BP – hydralazine (mmHg)
EF	151 (95%CI: 149-152)	156 (95%CI: 155-157)	157 (95%CI: 155-159)	159 (95%CI: 158-161)
MP	154 (95%CI: 152-156)	149 (95%CI: 148-152)	157 (95%CI: 155-159)	154 (95%CI: 152-157)
PT	142 (95%CI: 137-146)	129 (95%CI: 126-132)	141 (95%CI: 136-145)	142 (95%CI: 137-147)
SC	152 (95%CI: 150-153)	154 (95%CI: 153-155)	157 (95%CI: 156-158)	155 (95%CI: 154-157)

Table 3-8 Mean systolic BP (mmHg) before and on antihypertensive treatment with labetalol, methyldopa or hydralazine during exposure to controlled stressors (n = 9). EF – everyday, familiar sounds, MP – music with long-term periodicity i.e., Mozart's music, PT – potential threat i.e., sounds of lion's roaring, SC – sounds of other baboons/conspecifics, CI – 95% confidence interval.

During EF, diastolic BP increased after treatment with each of the three drugs was established. There was a significant difference seen during EF before and on treatment with labetalol and hydralazine (both $p < 0.001$), 7- and 5-mmHg respectively. Further, this difference in diastolic BP between these two drugs was significant ($p < 0.05$). The diastolic BP was also significantly different on methyldopa treatment when compared to labetalol ($p < 0.001$) and hydralazine ($p < 0.05$), as shown in Figure 3-51.

There was no statistically significant difference between the diastolic BP before and on drug treatment during MP (Figure 3-51).

During PT, diastolic BP decreased on labetalol treatment ($p < 0.001$), remained the same on methyldopa and increased on hydralazine treatment (ns).

During SC, diastolic BP increased significantly on treatment with all three drugs ($p < 0.001$), as shown in Figure 3-51. The average diastolic BP values before and on antihypertensives in the presence of the EF, MP, PT and SC controlled noise stressors is also outlined in Table 3-11.

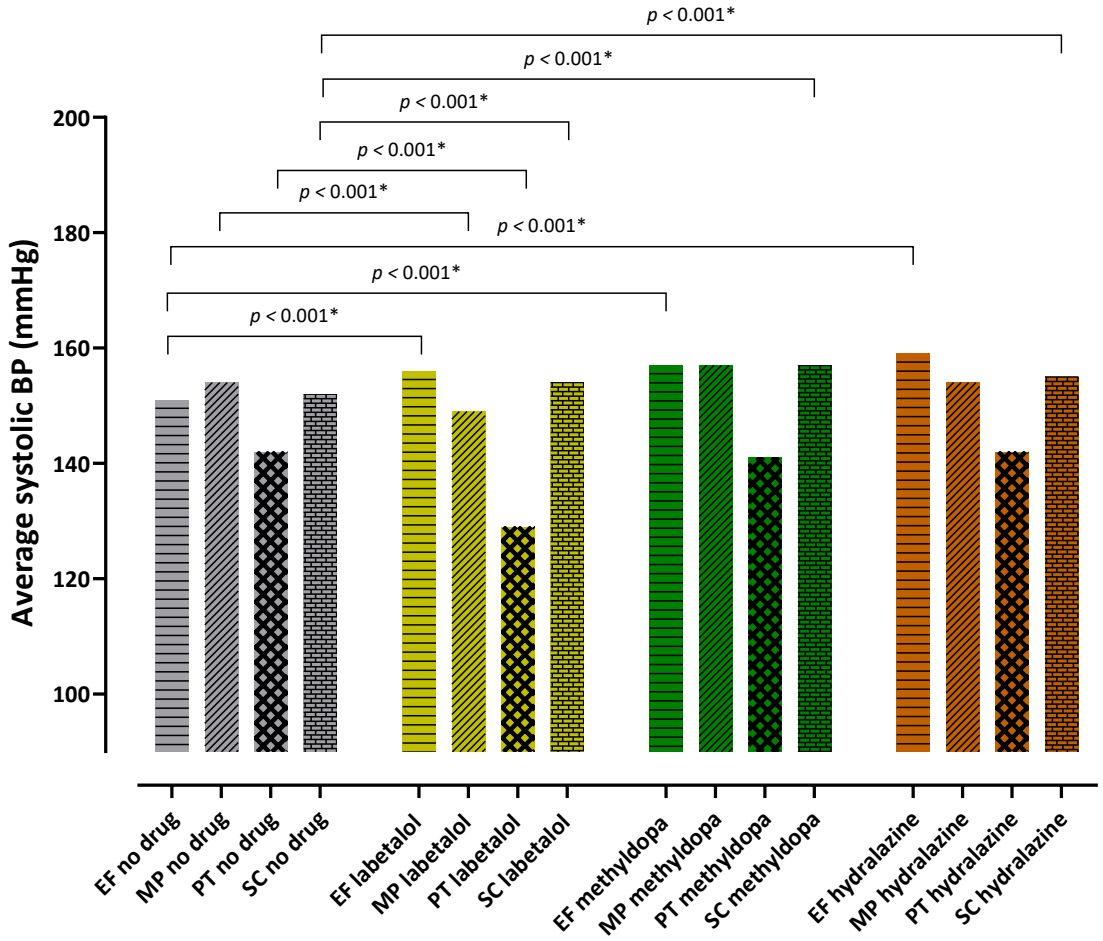

Figure 3-50 Average systolic BP before and on antihypertensive treatment was established with labetalol, methyldopa or hydralazine during exposure to controlled stressors (n = 9). Statistical significance defined as $p \leq 0.05$. EF – everyday, familiar sounds, MP – music with long-term periodicity i.e., Mozart's music, PT – potential threat i.e., sounds of lion's roaring, SC – sounds of other baboons/conspecifics.

*** indicates statistically significant difference between groups ($p \leq 0.05$)**

Sound	Mean diastolic BP – no drug (mmHg)	Mean diastolic BP – labetalol (mmHg)	Mean diastolic BP – methyldopa (mmHg)	Mean diastolic BP – hydralazine (mmHg)
EF	101 (CI: 100-103)	108 (CI: 107-109)	103 (CI: 102-104)	106 (CI: 104-107)
MP	104 (CI: 103-106)	104 (CI: 103-105)	106 (CI: 104-108)	103 (CI: 100-105)
PT	94 (CI: 90-98)	83 (CI: 81-86)	94 (CI: 90-98)	97 (CI: 93-101)
SC	103 (CI: 102-104)	109 (CI: 108 – 109)	105 (CI: 104-106)	107 (CI: 105-108)

Table 3-9 Mean diastolic BP before and on antihypertensive treatment was established with labetalol, methyldopa or hydralazine during exposure to controlled stressors (n = 9). EF – everyday, familiar sounds, MP – music with long-term periodicity i.e., Mozart's music, PT – potential threat i.e., sounds of lion's roaring, SC – sounds of other baboons/conspecifics, CI – 95% confidence interval.

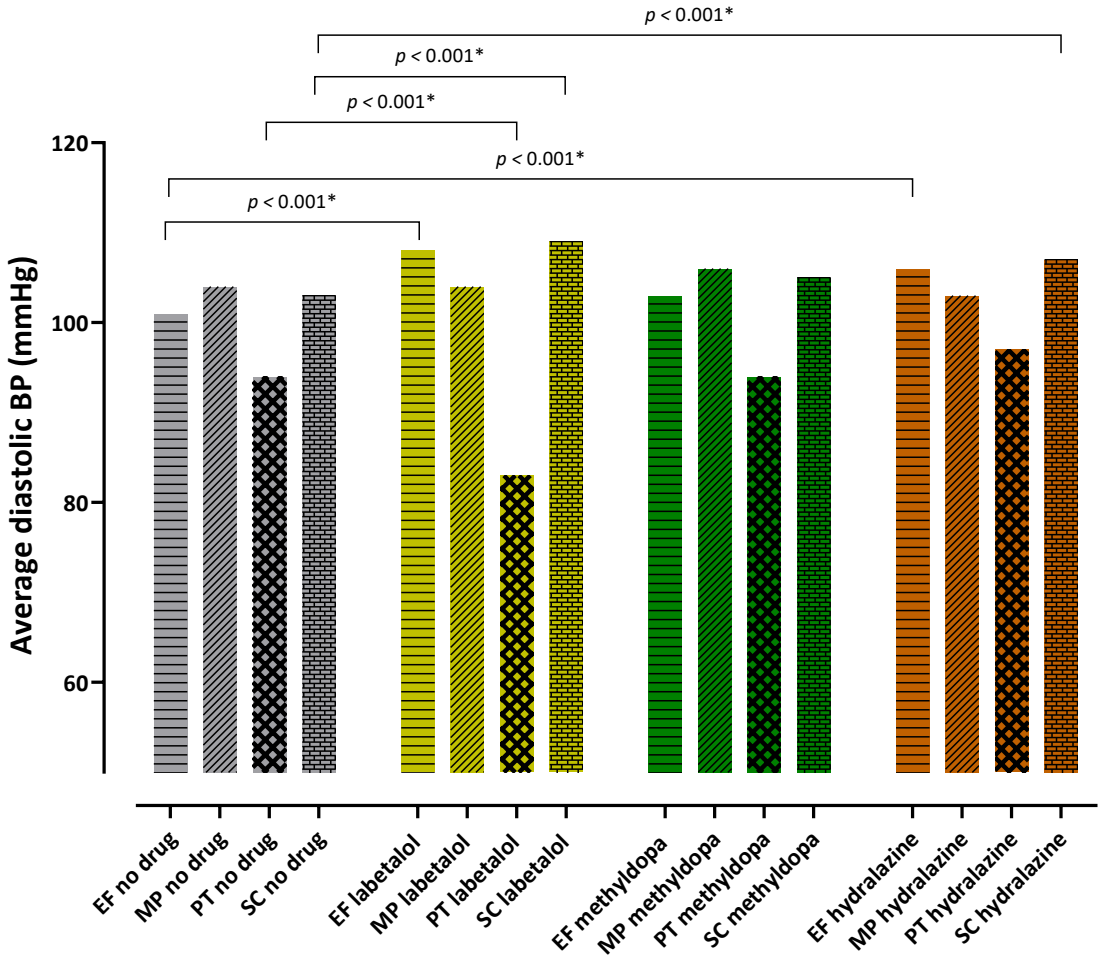

Figure 3-51 Mean diastolic BP before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established during exposure to controlled stressors (n = 9). Statistical significance defined as $p \leq 0.05$. EF – everyday, familiar sounds, MP – music with long-term periodicity i.e., Mozart's music, PT – potential threat i.e., sounds of lion's roaring, SC – sounds of other baboons/conspecifics.

*** indicates statistically significant difference between groups ($p \leq 0.05$)**

3.3.4.2 Blood pressure load: noise stressors

The systolic BP load during exposure to EF sounds before and on labetalol, methyldopa or hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Systolic BP load increased significantly after labetalol, methyldopa or hydralazine treatment was established during exposure to everyday, familiar sounds as determined by chi-square test: $\chi 2$ (4, N = 85) = 13.18, $p < 0.01$, $\chi 2$ (4, N = 88) = 76.69, $p < 0.01$, $\chi 2$ (4, N = 85) = 44.9, $p < 0.01$ respectively (Figure 3-52).

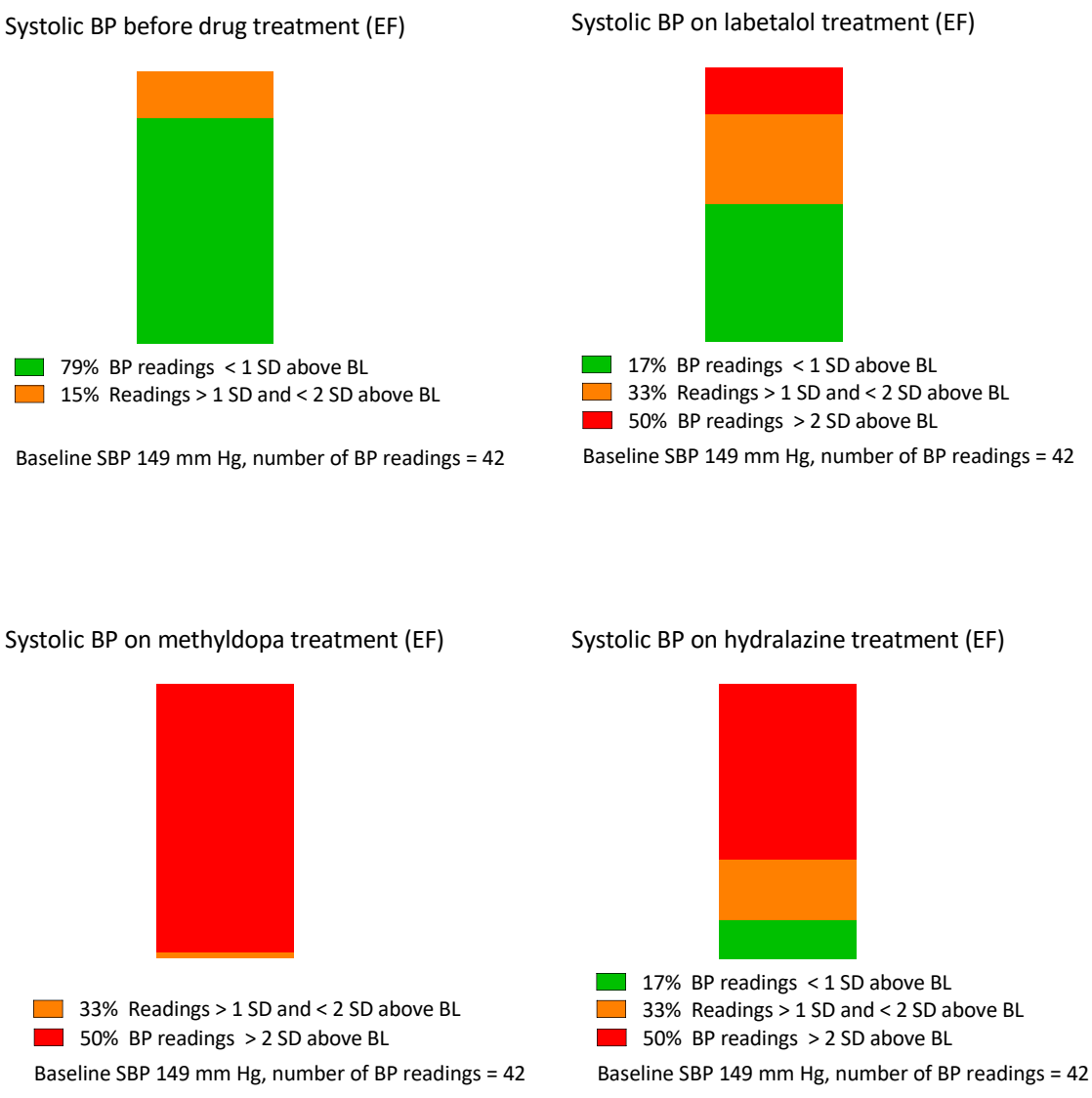

Figure 3-52 Systolic BP load during everyday, familiar (EF) sounds before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9).

The diastolic BP load during exposure to EF sounds before and on labetalol, methyldopa or hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Diastolic BP load increased significantly after labetalol, methyldopa or hydralazine treatment was established during exposure to everyday, familiar sounds as determined by chi-square test: $\chi 2$ (4, N = 85) = 39.55, $p < 0.01$, $\chi 2$ (4, N = 85) = 47.98, $p < 0.01$, $\chi 2$ (4, N = 85) = 21.04, $p < 0.01$ respectively (Figure 3-53).

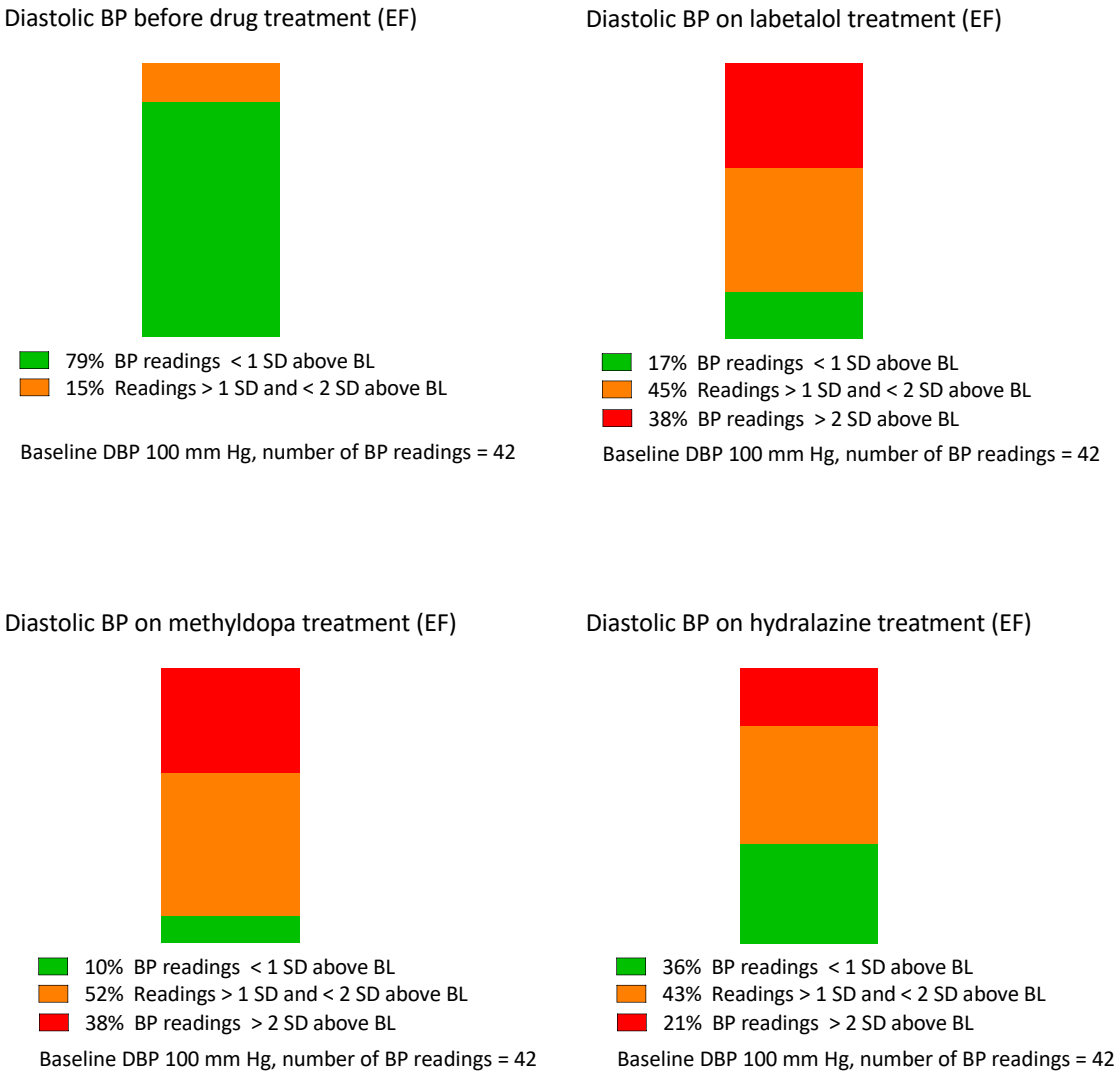

Figure 3-53 Diastolic BP load during everyday, familiar (EF) sounds before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9).

The systolic BP load during exposure to MP sounds before and on labetalol, methyldopa or hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Systolic BP load increased significantly after labetalol and methyldopa treatment during exposure to MP sounds as determined by chi-square test: $\chi 2$ (4, N = 90) = 24.52, $p < 0.01$, $\chi 2$ (4, N = 84) = 9.84, $p = 0.007$ respectively. Systolic BP load did not change significantly after hydralazine treatment: $\chi 2$ (4, N = 85) = 0.38, $p = 0.83$ (Figure 3-54).

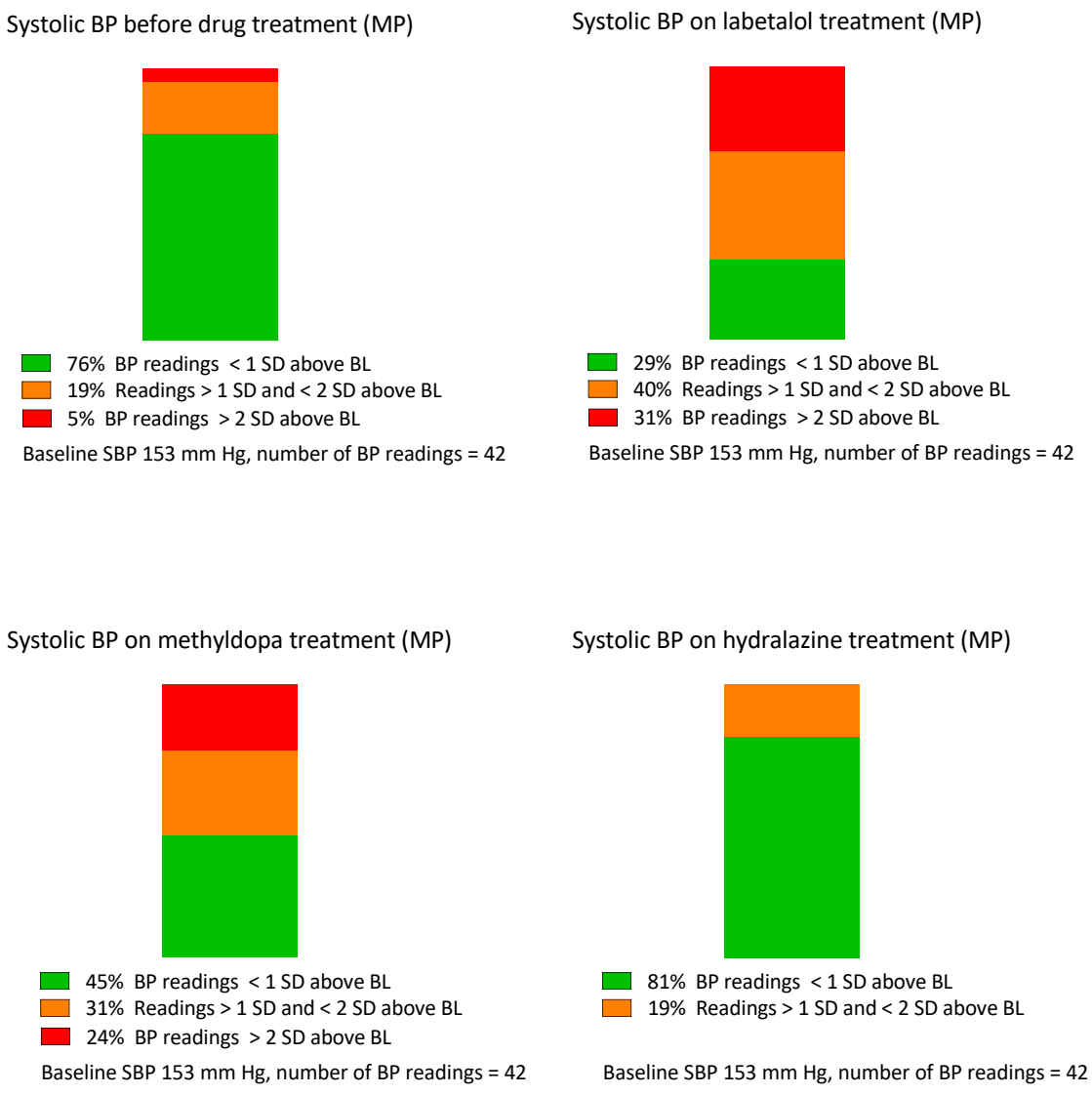

Figure 3-54 Systolic BP load during exposure to Mozart's music with long-term periodicity (MP) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9).

The diastolic BP load during exposure to MP sounds before and on labetalol, methyldopa or hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Diastolic BP load increased significantly after labetalol treatment during exposure to MP sounds as determined by chi-square test: $\chi 2$ (4, N = 84) = 47.9, $p < 0.01$. Diastolic BP load did not change significantly after methyldopa or hydralazine treatment: $\chi 2$ (4, N = 84) = 2.85, p = 0.24, $\chi 2$ (4, N = 85) = 1.49, p = 0.47 respectively (Figure 3-55).

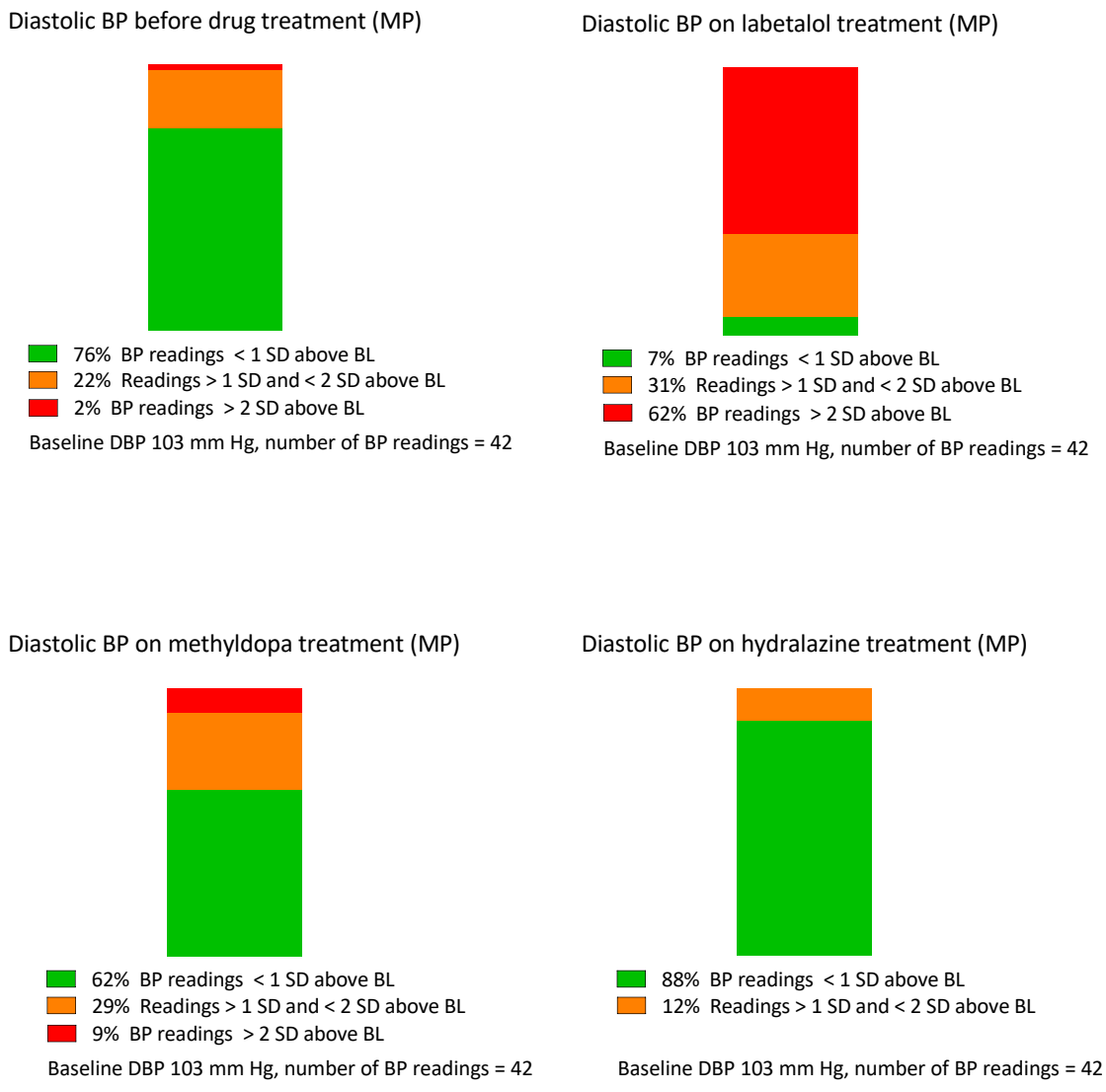

Figure 3-55 Diastolic BP load during exposure to Mozart's music with long-term periodicity (MP) before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9).

The systolic BP load during exposure to PT sounds before and on labetalol, methyldopa or hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Systolic BP load did not change significantly after labetalol, methyldopa or hydralazine treatment was established during exposure to PT sounds as determined by chi-square test: $\chi 2$ (4, N = 86) = 4.97, p = 0.08., $\chi 2$ (4, N = 85) = 0.07, $\chi 2$ (4, N = 86) = 0.08, p = 0.96 respectively (Figure 3-56).

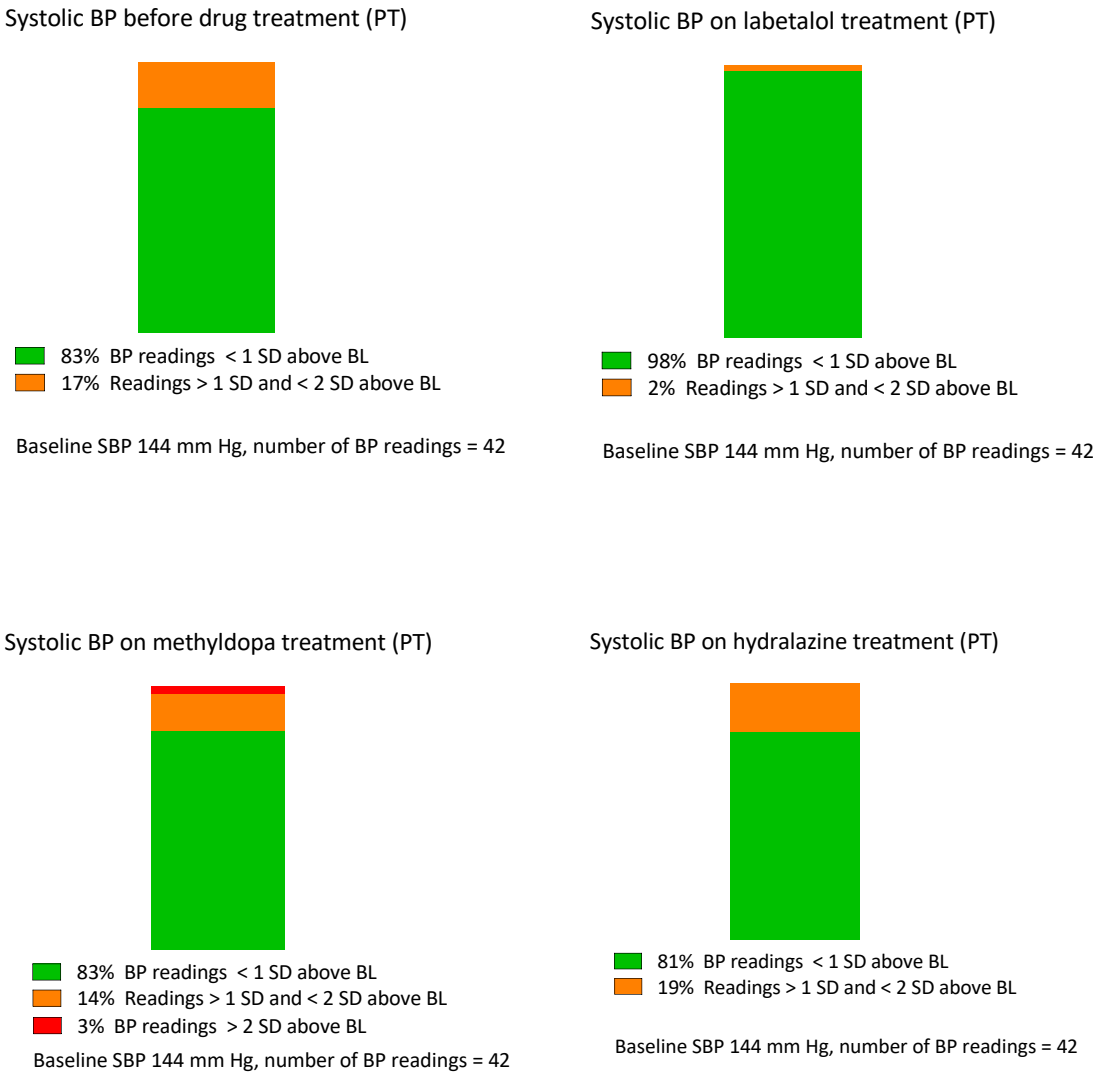

Figure 3-56 Systolic BP load during exposure to sounds of a potential threat (PT) i.e., lion's roaring before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9).

The diastolic BP load during exposure to PT sounds before and on labetalol, methyldopa or hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Diastolic BP load decreased significantly after labetalol treatment was established during exposure to PT sounds as determined by chi-square test: $\chi 2$ (4, N = 87) = 6.28, p = 0.04. Diastolic BP load did not change significantly after methyldopa and hydralazine treatment was established $\chi 2$ (4, N = 86) = 0.82, p = 0.66, $\chi 2$ (4, N = 86) = 0.08, p = 0.96 respectively (Figure 3-57).

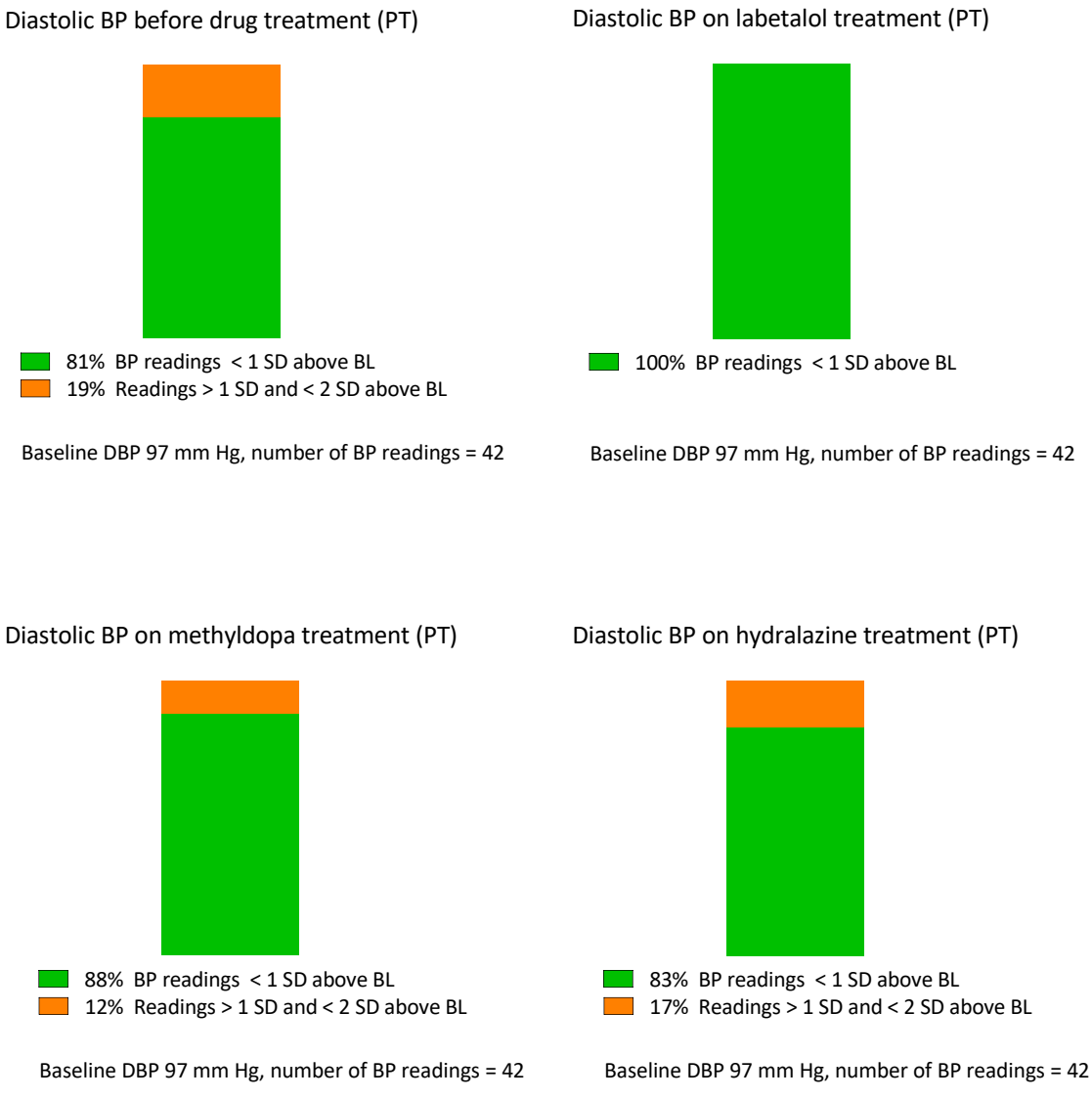

Figure 3-57 Diastolic BP load during exposure to sounds of a potential threat (PT) i.e., lion's roaring before and after antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9).

The systolic BP load during exposure to SC sounds before and on labetalol, methyldopa or hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Systolic BP load increased significantly on labetalol, methyldopa and hydralazine treatment during exposure to SC sounds as determined by chi-square test: $\chi 2$ (4, N = 85) = 45.86, $p < 0.01$, $\chi 2$ (4, N = 85) = 61.53, $p < 0.01$, $\chi 2$ (4, N = 85) = 13.91, $p < 0.01$ respectively (Figure 3-58).

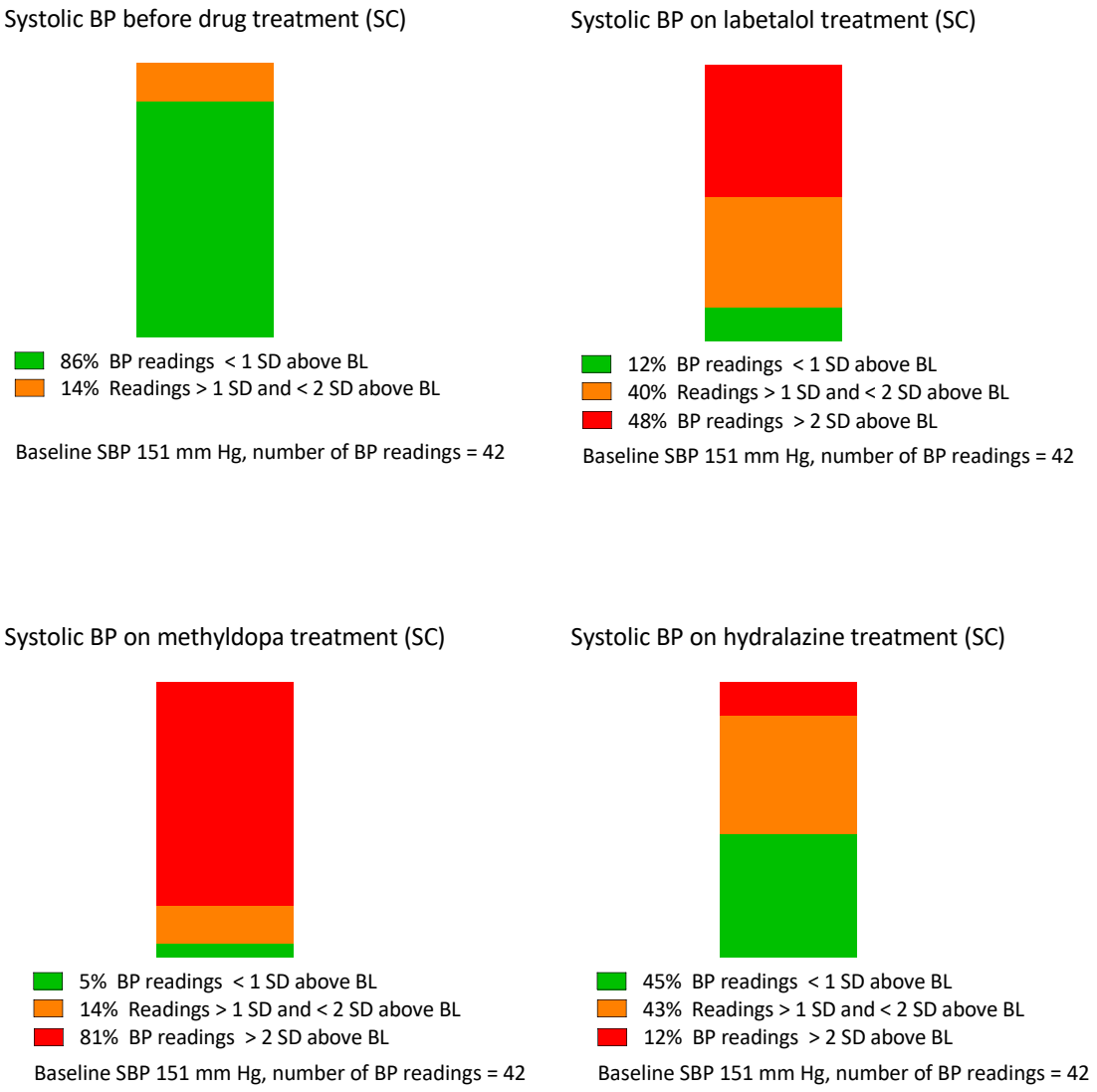

Figure 3-58 Systolic BP load during exposure to sounds of other baboons/conspecifics (SC) before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9).

The diastolic BP load during exposure to SC sounds before and on labetalol, methyldopa or hydralazine treatment was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Diastolic BP load increased significantly on labetalol and hydralazine treatment during exposure to SC sounds as determined by chi-square test: $\chi 2$ (4, N = 85) = 30.61, $p < 0.01$, $\chi 2$ (4, N = 85) = 9.35 respectively. Diastolic BP load did not change significantly on methyldopa treatment: $\chi 2$ (4, N = 85) = 5.06, $p = 0.08$ (Figure 3-59).

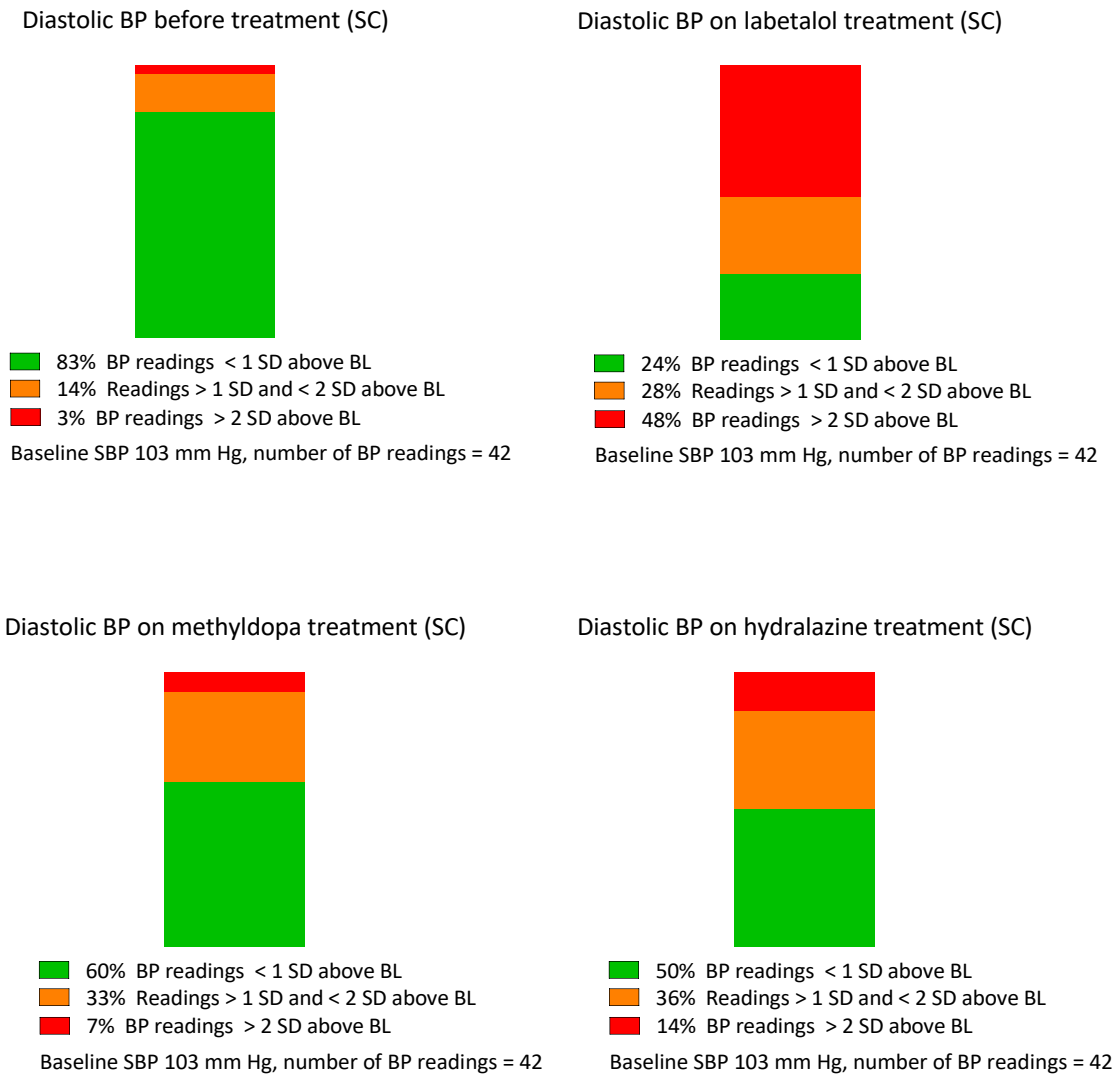

Figure 3-59 Diastolic BP load during exposure to sounds of other baboons/conspecifics (SC) before and on antihypertensive treatment with labetalol, methyldopa and hydralazine (n = 9).

3.3.4.3 Blood pressure variability using SD1 and SD2 values generated by Poincaré Plots: noise stressors

The short-term variability, as represented by SD1, and long-term variability, as represented by SD2, of systolic BP did not differ significantly in response to the different antihypertensive drugs when exposed to the different sounds. There was a significant difference between SD1 of systolic BP on labetalol or methyldopa treatment during exposure to PT sounds (Figure 3-60). There was also significant difference between SD2 of systolic BP on labetalol, methyldopa or hydralazine treatment during exposure to PT sounds as well as on labetalol or methyldopa during exposure to SC sounds (Figure 3-62).

Short-term variability of diastolic BP did not differ significantly in response to the different antihypertensive drugs when exposed to the different sounds (Figure 3-61).

Long-term diastolic BPV differed significantly in response to the different antihypertensive drugs when exposed to the different sounds (p = 0.013) (Figure 3-63).

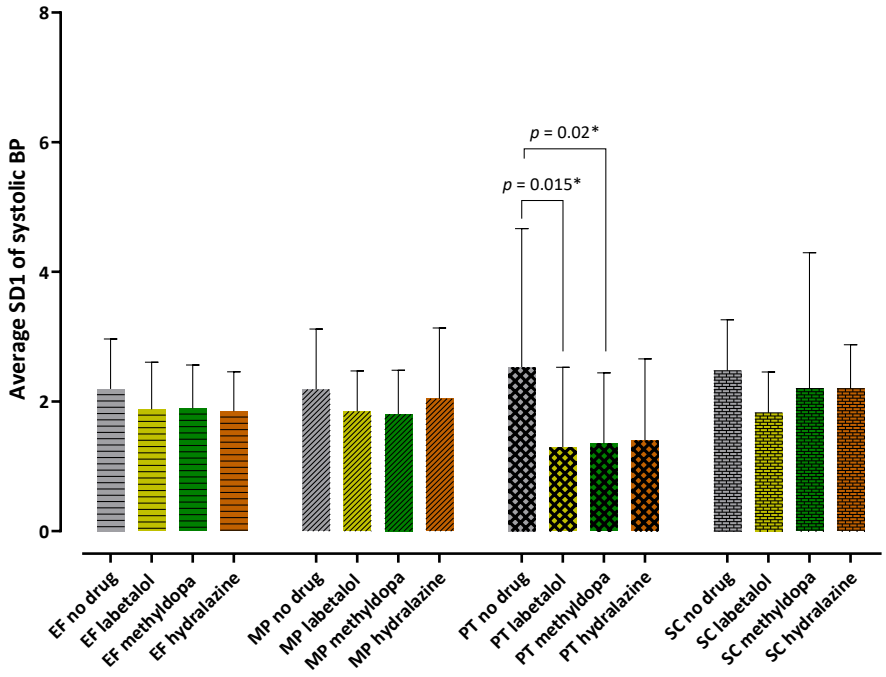

Figure 3-60 Average systolic short-term variability, as represented by SD1, during exposure to controlled stressors (EF, MP, PT and SC) before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$). EF – everyday, familiar sounds, MP – music with long-term periodicity i.e., Mozart's music, PT – potential threat i.e., sounds of lion's roaring, SC – sounds of other baboons/conspecifics.

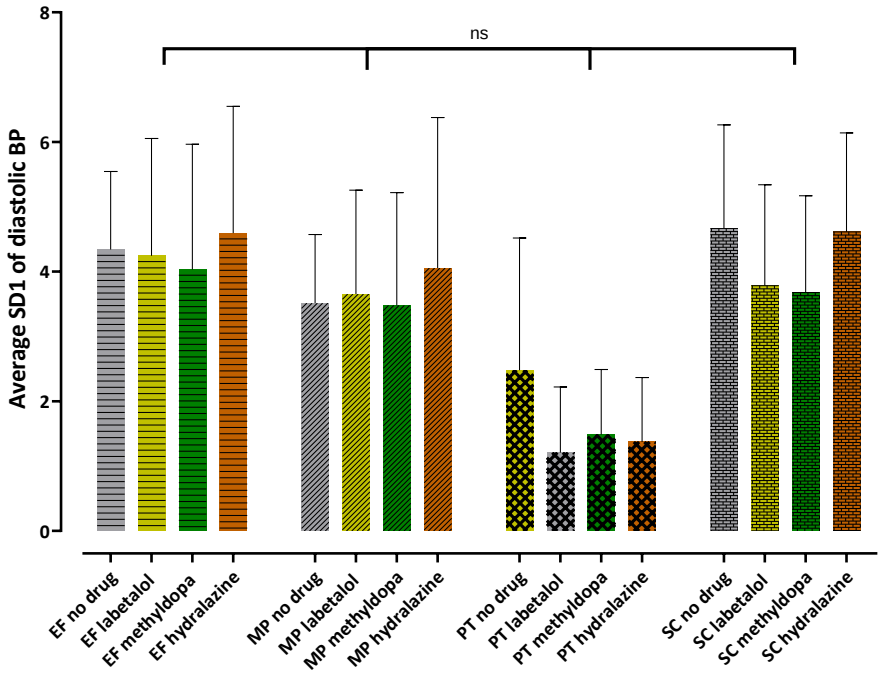

Figure 3-61 Average diastolic short-term variability, as represented by SD1, during exposure to controlled stressors (EF, MP, PT and SC) before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). There was no significant difference between SD1 of the different antihypertensive drugs when exposed to the different sounds. Statistical significance defined as $p \leq 0.05$. EF – everyday, familiar sounds, MP – music with long-term periodicity i.e., Mozart's music, PT – potential threat i.e., sounds of lion's roaring, SC – sounds of other baboons/conspecifics.

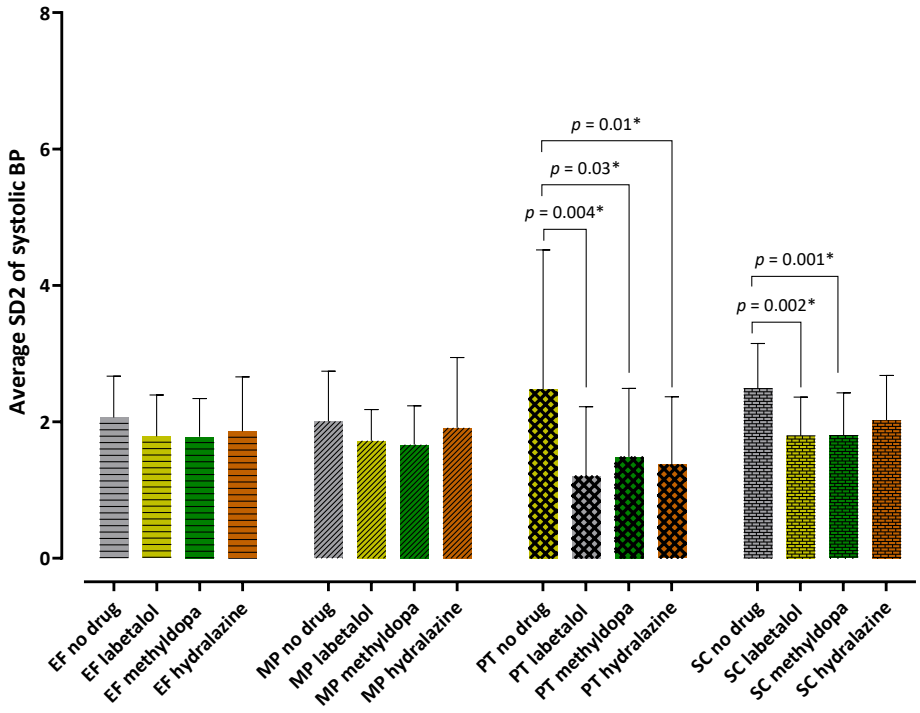

Figure 3-62 Average systolic long-term variability, as represented by SD2, during exposure to controlled stressors (EF, MP, PT and SC) before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$). EF – everyday, familiar sounds, MP – music with long-term periodicity i.e., Mozart's music, PT – potential threat i.e., sounds of lion's roaring, SC – sounds of other baboons/conspecifics.

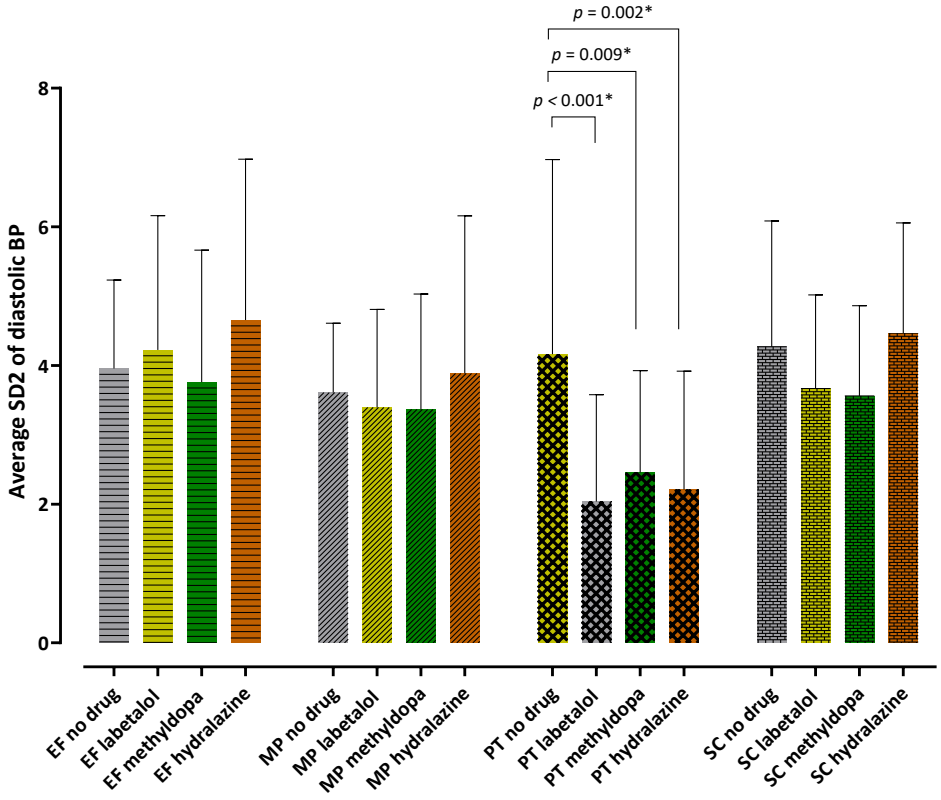

Figure 3-63 Average diastolic long-term variability, as represented by SD2, during exposure to controlled stressors (EF, MP, PT and SC) before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). * indicates statistically significant difference between groups ($p \leq 0.05$). EF – everyday, familiar sounds, MP – music with long-term periodicity i.e., Mozart's music, PT – potential threat i.e., sounds of lion's roaring, SC – sounds of other baboons/conspecifics.

3.3.4.4 Classification of blood pressure data as stochastic, periodic or chaotic: noise stressors

The Chaos Decision Tree Algorithm classified all BP data collected during exposure to controlled stressors (EF, MP, PT, SC) as stochastic.

3.3.5 Study 5: Blood pressure whilst eating

3.3.5.1 Average blood pressure: eating

There was no significant difference between the mean systolic BP whilst eating before and on treatment (labetalol, methyldopa or hydralazine data combined). There was a significant difference between the mean diastolic BP before and on drug treatment (labetalol, methyldopa or hydralazine), with no significant difference between the drugs (Table 3-13).

Average BP whilst eating before and on drug treatment			
	Before drug treatment mean (mmHg)	On drug treatment mean (mmHg)	*p*-value
Systolic BP	149.0 (95% CI: 148-150)	149.1 (95% CI: 148-150)	0.89
Diastolic BP	102.8 (95% CI: 102-103)	103.5 (95% CI: 103-104)	0.04*

Table 3-10 Mean systolic and diastolic BP whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). The BP data was collected during the afternoon feeding time. The feeding period was defined as the period 30 minutes following the time food was given. Most of the food was consumed during the 30 minutes following the start of feeding. CI – 95% confidence interval. * - statistically significant, $p < 0.05$.

There was no significant difference between the mean systolic BP whilst eating before and on treatment with labetalol or hydralazine. There was a significant difference increase in mean systolic BP before and on treatment with methyldopa (Table 3-14 and Figure 3-64).

Average systolic BP whilst eating before and on treatment with labetalol, methyldopa and hydralazine			
	Before treatment (mmHg)	On treatment (mmHg)	*p*-value
Labetalol	149.4 (CI 95%: 148-150)	148.2 (CI 95%: 147-149)	0.07
Methyldopa	148.9 (CI 95%: 148-150)	150.5 (CI 95%: 149-152)	0.016*
Hydralazine	148.9 (CI 95%: 148-150)	148.6 (CI 95%: 147-150)	0.69

Table 3-11 Mean systolic BP whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). CI – 95% confidence interval. * - statistically significant, p < 0.05.

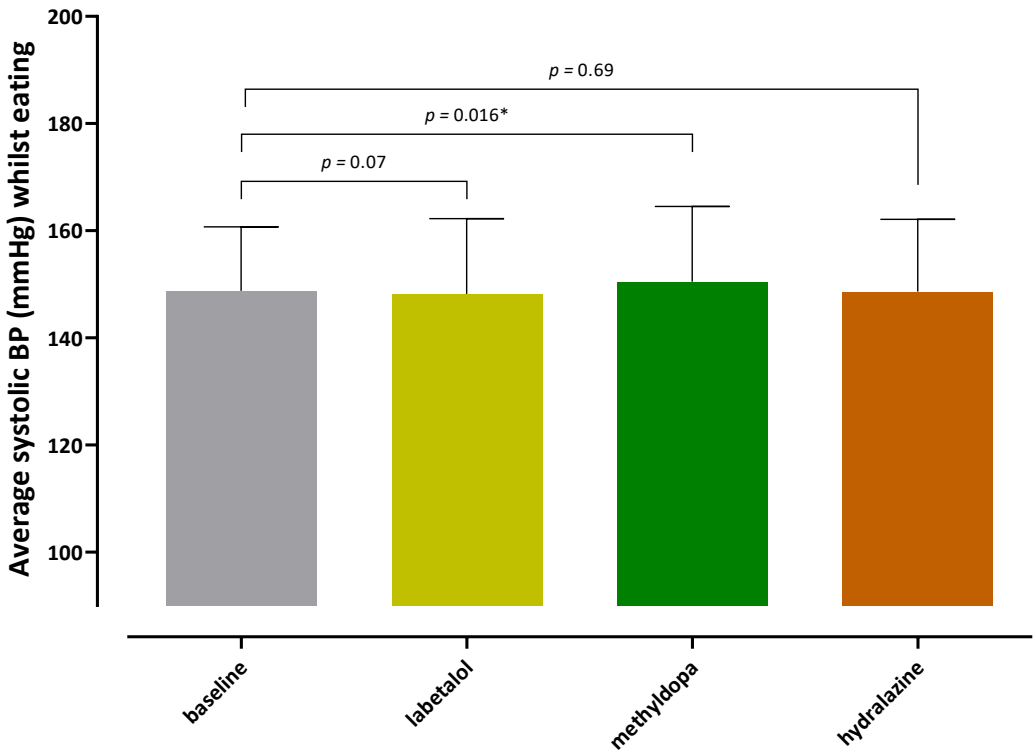

Figure 3-64 Mean systolic BP whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). Baseline BP was defined as the average BP of all nine animals before any antihypertensive treatment commenced. There was a statistically significant difference between the average systolic BP before and on methyldopa treatment only (p = 0.016), and not with the labetalol or hydralazine. * indicates statistically significant difference between groups ($p \leq 0.05$).

There was no significant difference between the mean diastolic BP whilst eating before and on treatment with labetalol and hydralazine. There was a significant difference between the mean diastolic BP before and on treatment with methyldopa (Table 3-15 and Figure 3-65).

Average diastolic BP whilst eating before and on treatment with labetalol, methyldopa and hydralazine			
	Before treatment (mmHg)	On treatment (mmHg)	*p*-value
Labetalol	103.1 (CI 95%: 102-104)	103.0 (CI 95%: 102-104)	0.85
Methyldopa	102.6 (CI 95%: 102-104)	104.3 (CI 95%: 103-106)	0.008*
Hydralazine	102.6 (CI 95%: 102-104)	103.2 (CI 95%: 102-104)	0.26

Table 3-12 Mean diastolic BP whilst eating before and on antihypertensive treatment with labetalol, methyldopa and hydralazine (n = 9). CI – 95% confidence interval. * - statistically significant, p < 0.05.

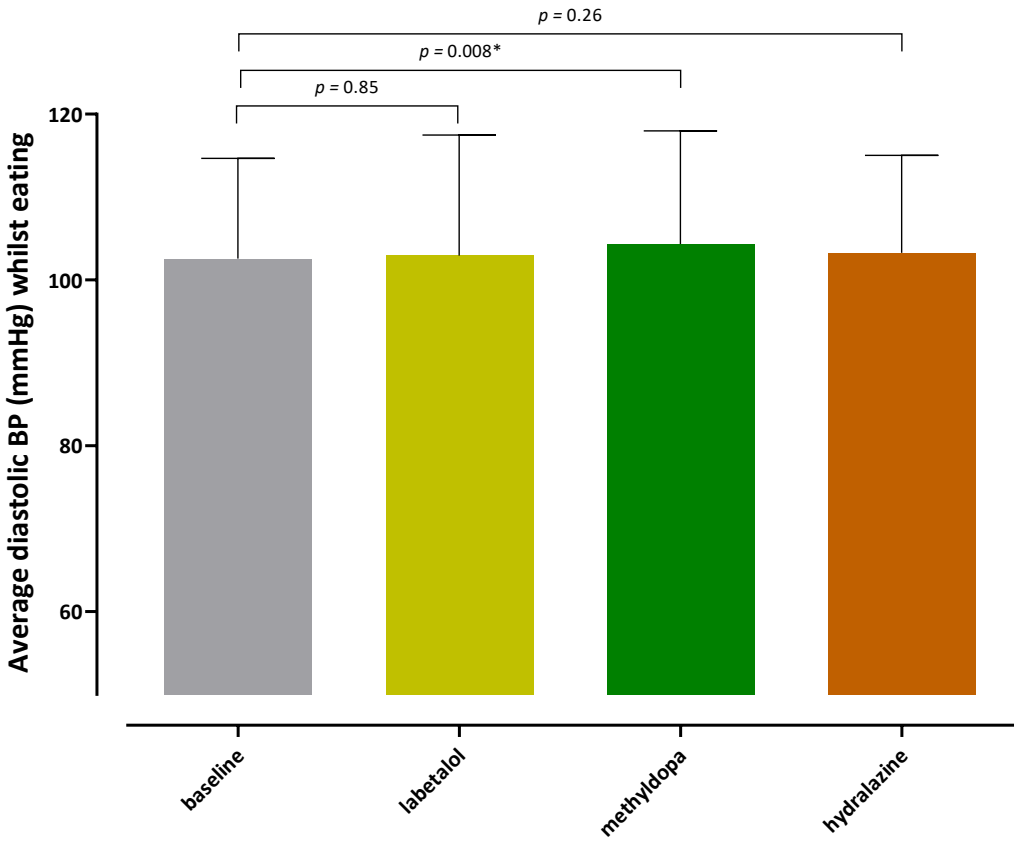

Figure 3-65 Mean diastolic BP whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). Baseline BP was defined as the average BP of all nine animals before any antihypertensive treatment commenced. There was a statistically significant difference between the average diastolic BP before and on drug treatment. * indicates statistically significant difference between groups ($p \leq 0.05$).

3.3.5.2 Blood pressure load: eating

The BP load whilst animals were eating before and on antihypertensive treatment was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive treatment commenced. Systolic BP load decreased significantly after labetalol, methyldopa or hydralazine treatment whilst animals were eating as determined by chi-square test: $\chi 2$ (4, N = 1008) = 62.27, $p < 0.01$, $\chi 2$ (4, N = 994) = 8.63, $p = 0.01$, $\chi 2$ (4, N = 1008) = 23.98, $p < 0.01$ respectively (Figure 3-66).

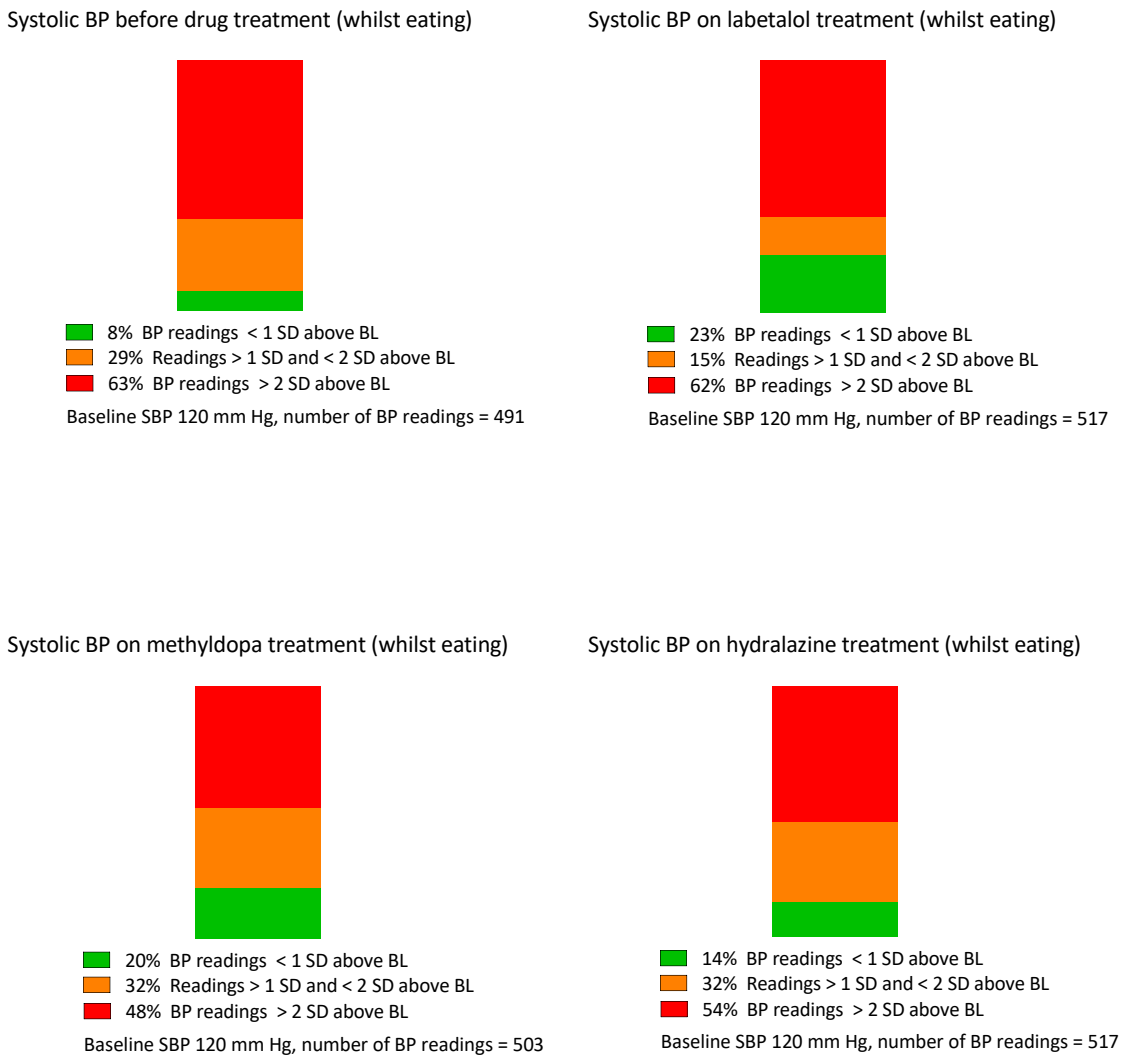

Figure 3-66 Systolic BP load whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9).

Diastolic BP load increased significantly on labetalol treatment whilst animals were eating as determined by chi-square test $\chi 2$ (4, N = 200) = 15.05, $p < 0.001$. Diastolic BP load did not change significantly on methyldopa or hydralazine treatment whilst animals were eating as determined by chi-square test $\chi 2$ (4, N = 994) = 3.02, $p = 0.22$, $\chi 2$ (4, N = 1008) = 1.11, p = 0.57 respectively (Figure 3-67).

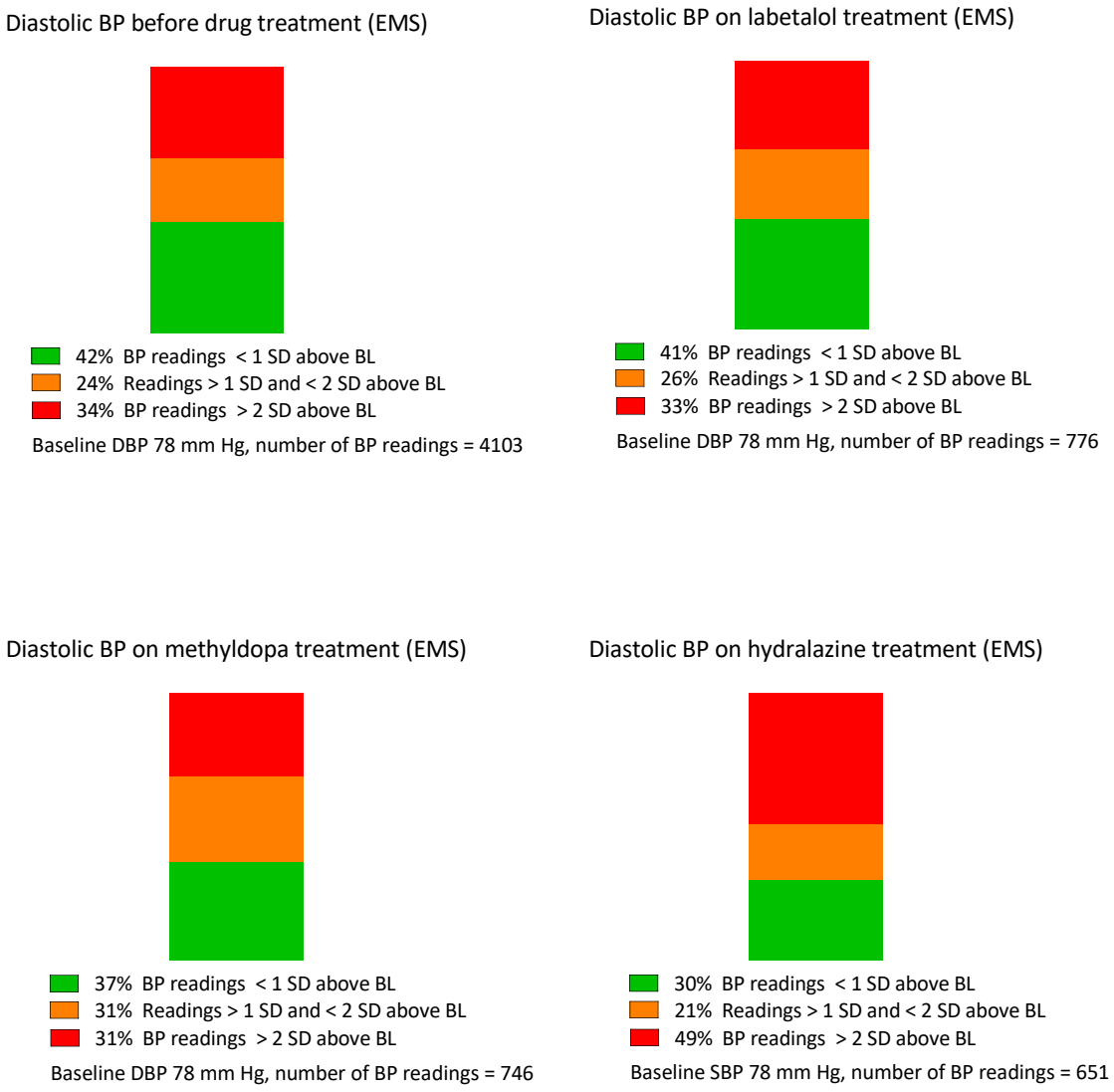

Figure 3-67 Diastolic BP load whilst eating before and on antihypertensive treatment with labetalol, methyldopa and hydralazine (n = 9).

3.3.5.3 Blood pressure variability using SD1 and SD2 values generated by Poincaré Plots: eating

There was no significant difference between the short-term systolic or diastolic BP variability as measured by SD1 (Figure 3-68 and Figure 3-69), long-term systolic or diastolic BP variability as measured by SD2 (Figure 3-70 and Figure 3-71) whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine.

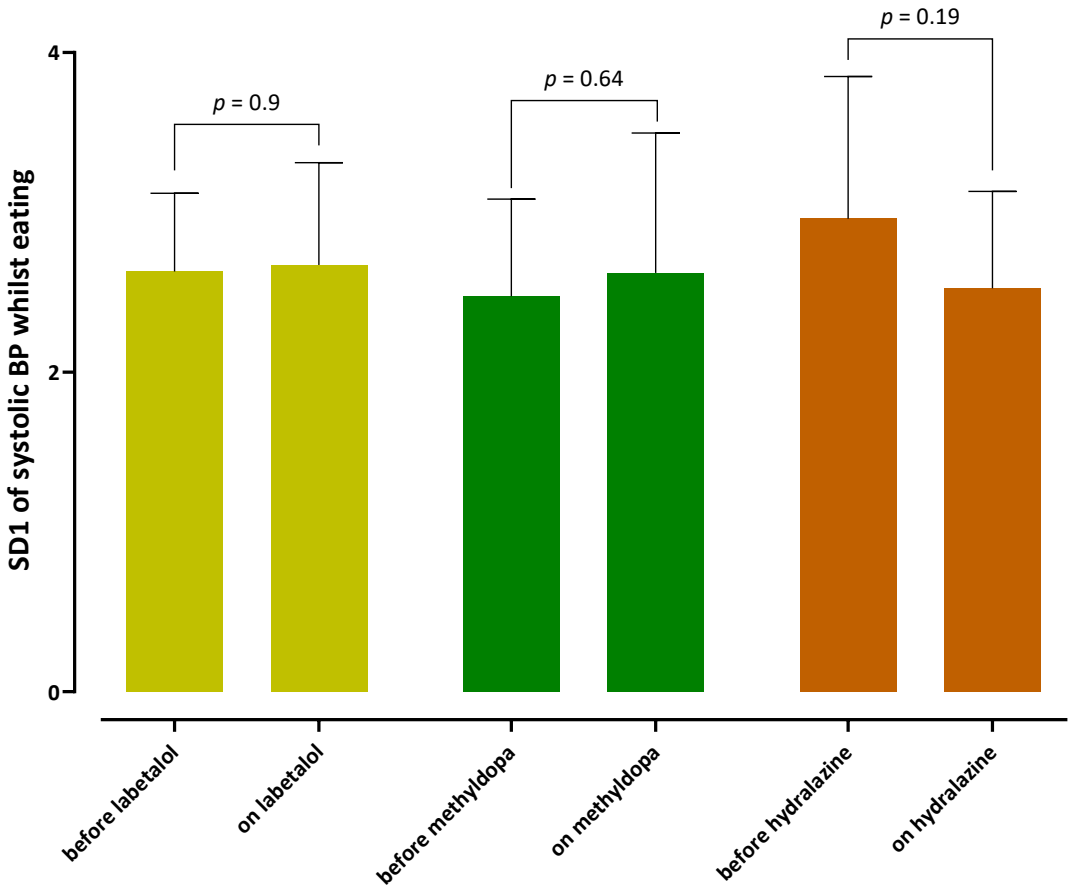

Figure 3-68 Mean short-term variability (SD1) of systolic BP whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). There was no significant difference in mean SD1 with any of the antihypertensive treatments. Statistical significance defined as $p \leq 0.05$.

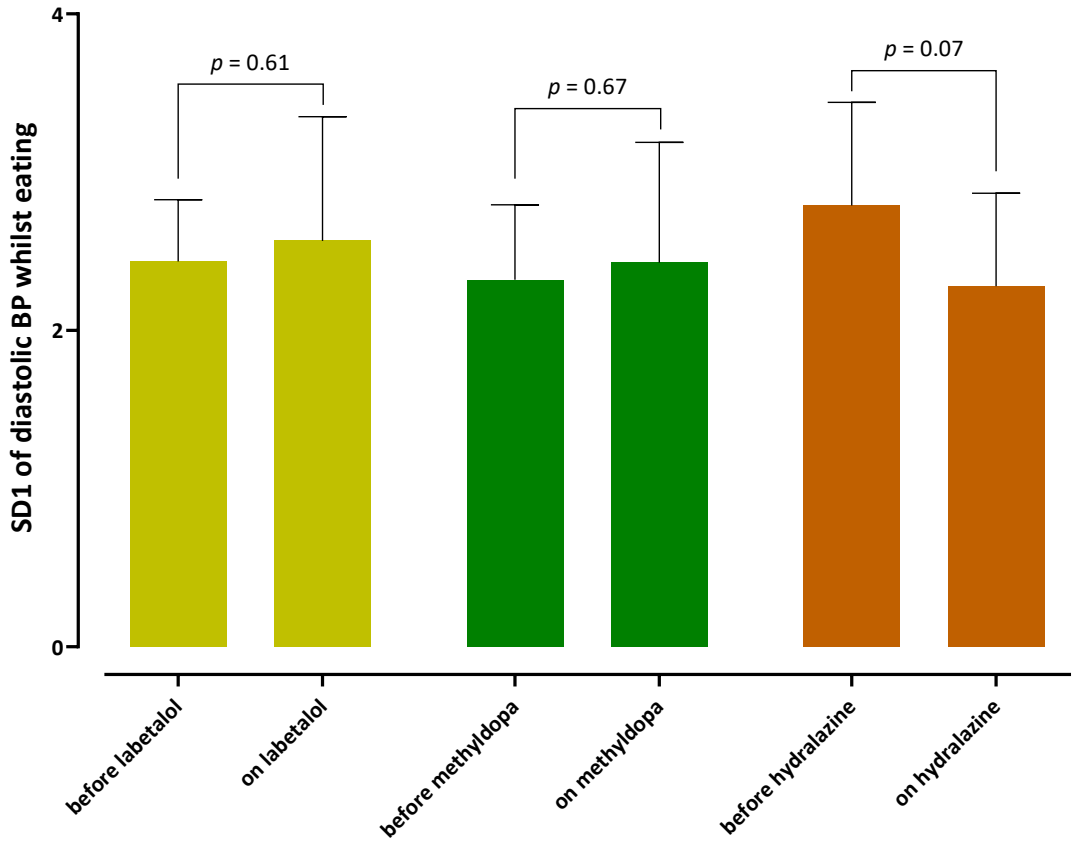

Figure 3-69 Mean short-term variability (SD1) of diastolic BP whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). There was no significant difference in mean SD1 with any of the antihypertensive treatments. Statistical significance defined as $p \leq 0.05$.

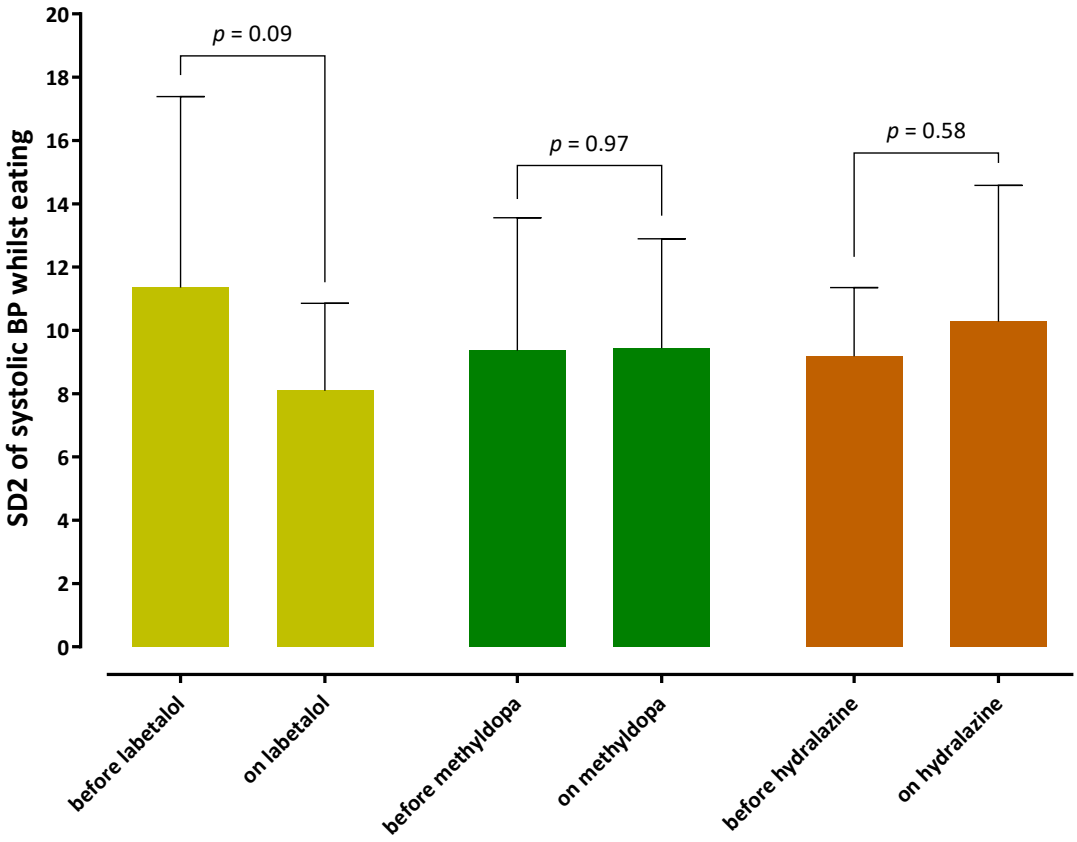

Figure 3-70 Mean long-term variability (SD2) of systolic BP whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine was established (n = 9). There was no significant difference in mean SD2 with any of the antihypertensive treatments. Statistical significance defined as $p \leq 0.05$.

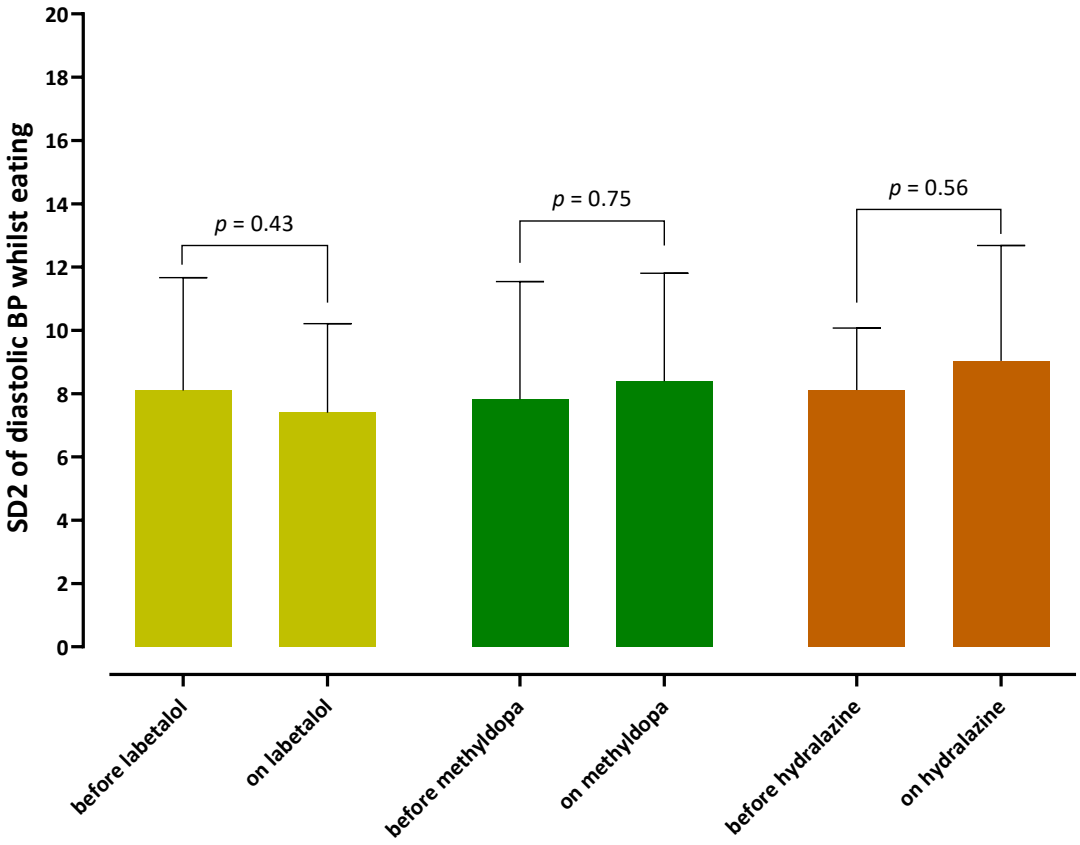

Figure 3-71 Mean long-term variability (SD2) of diastolic BP whilst eating before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). There was no significant difference in mean diastolic SD2 before and on antihypertensive treatment whilst eating. Statistical significance defined as $p \leq 0.05$.

3.3.5.4 Classification of blood pressure data as stochastic, periodic or chaotic: eating

The Chaos Decision Tree Algorithm classified the BP data sets collected whilst animals were eating was classified as stochastic.

3.3.6 Study 6: Blood pressure during cleaning of enclosure

3.3.6.1 Average blood pressure: cleaning

There was a significant difference between mean systolic during enclosure cleaning before and on drug treatment (labetalol, methyldopa or hydralazine combined); the average BP increased after drug treatment (Figure 3-72).

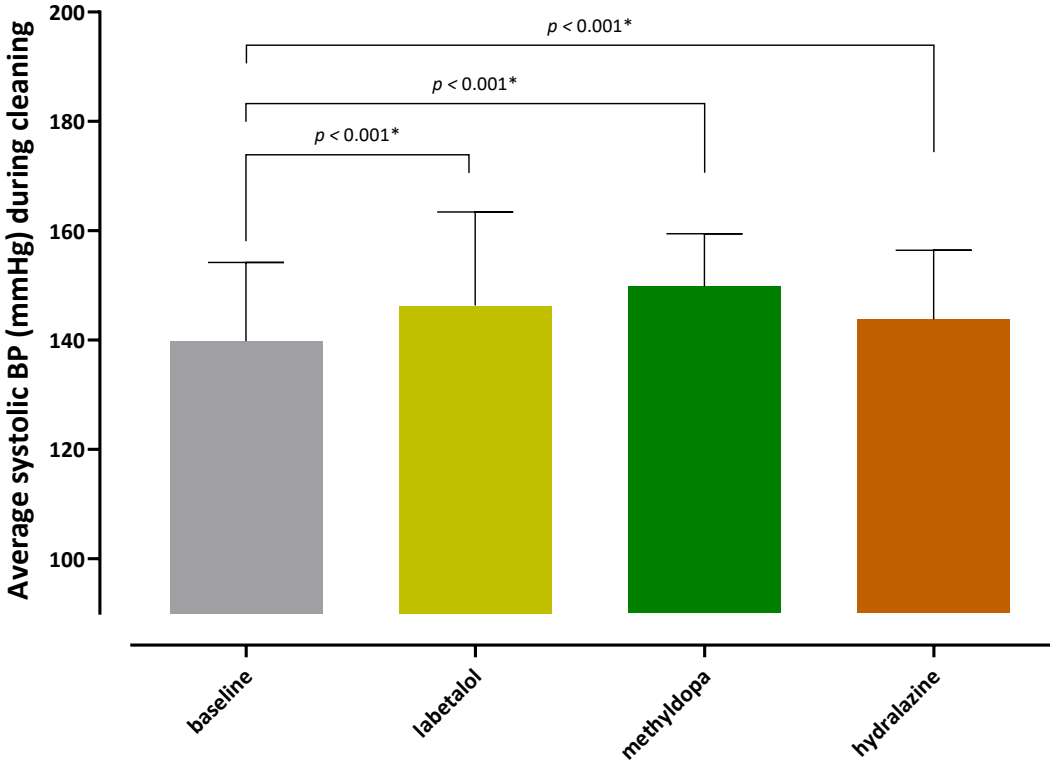

Figure 3-72 Mean systolic BP whilst enclosure was being cleaned before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. There was a statistically significant difference (p < 0.001) between average systolic BP before and on treatment; average systolic BP increased on treatment. * indicates statistically significant difference between groups ($p \leq 0.05$).

There was a significant difference between mean diastolic during enclosure cleaning before and on drug treatment (labetalol, methyldopa or hydralazine results combined); the average BP increased after drug treatment (Figure 3-73).

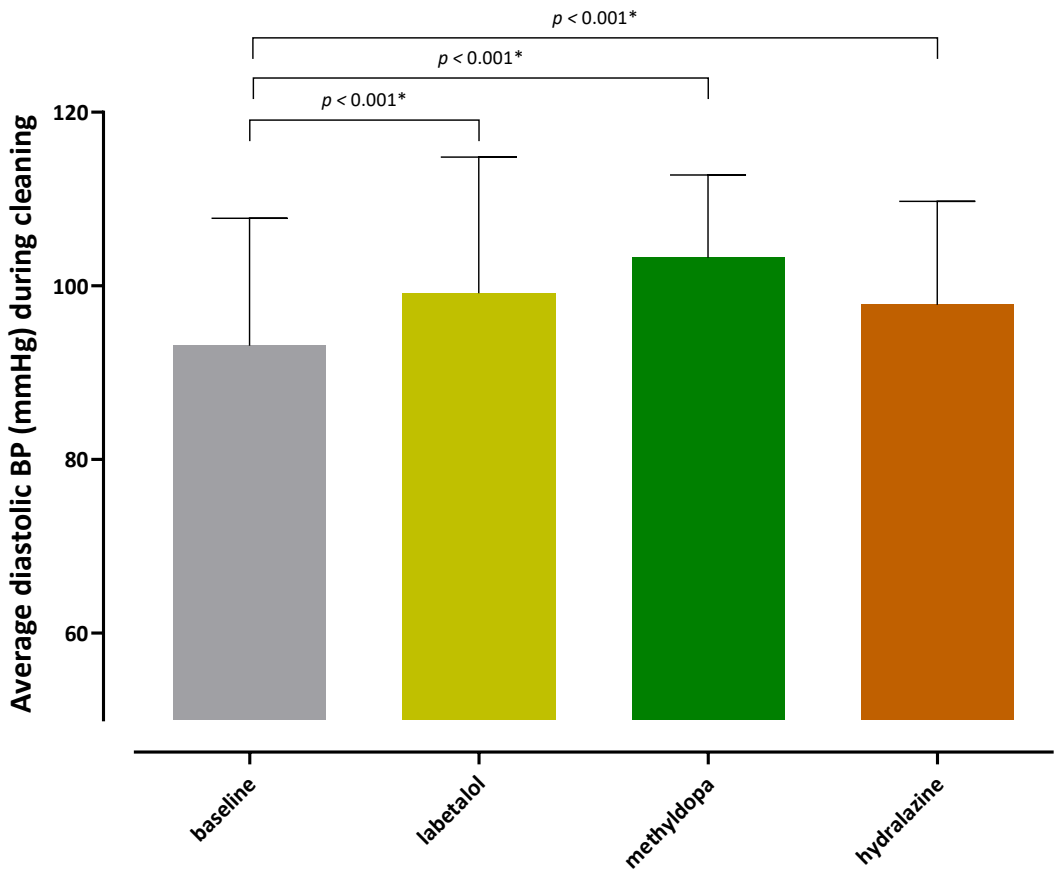

Figure 3-73 Mean diastolic BP whilst enclosure was being cleaned before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. There was a statistically significant difference (p < 0.001) between average diastolic BP before and on treatment; average systolic BP and diastolic BP increased on treatment. * indicates statistically significant difference between groups ($p \leq 0.05$)

3.3.6.2 Blood pressure load: cleaning

The systolic BP load during cleaning of the enclosure was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Systolic BP load increased significantly after labetalol, methyldopa or hydralazine treatment whilst the animals' enclosure was being cleaned as determined by chi-square test: $\chi 2$ (4, N = 3728) = 61.49, $p < 0.01$, $\chi 2$ (4, N = 3649) = 413.09, $p < 0.01$, $\chi 2$ (4, N = 4466) = 51.1, $p < 0.01$ respectively (Figure 3-74).

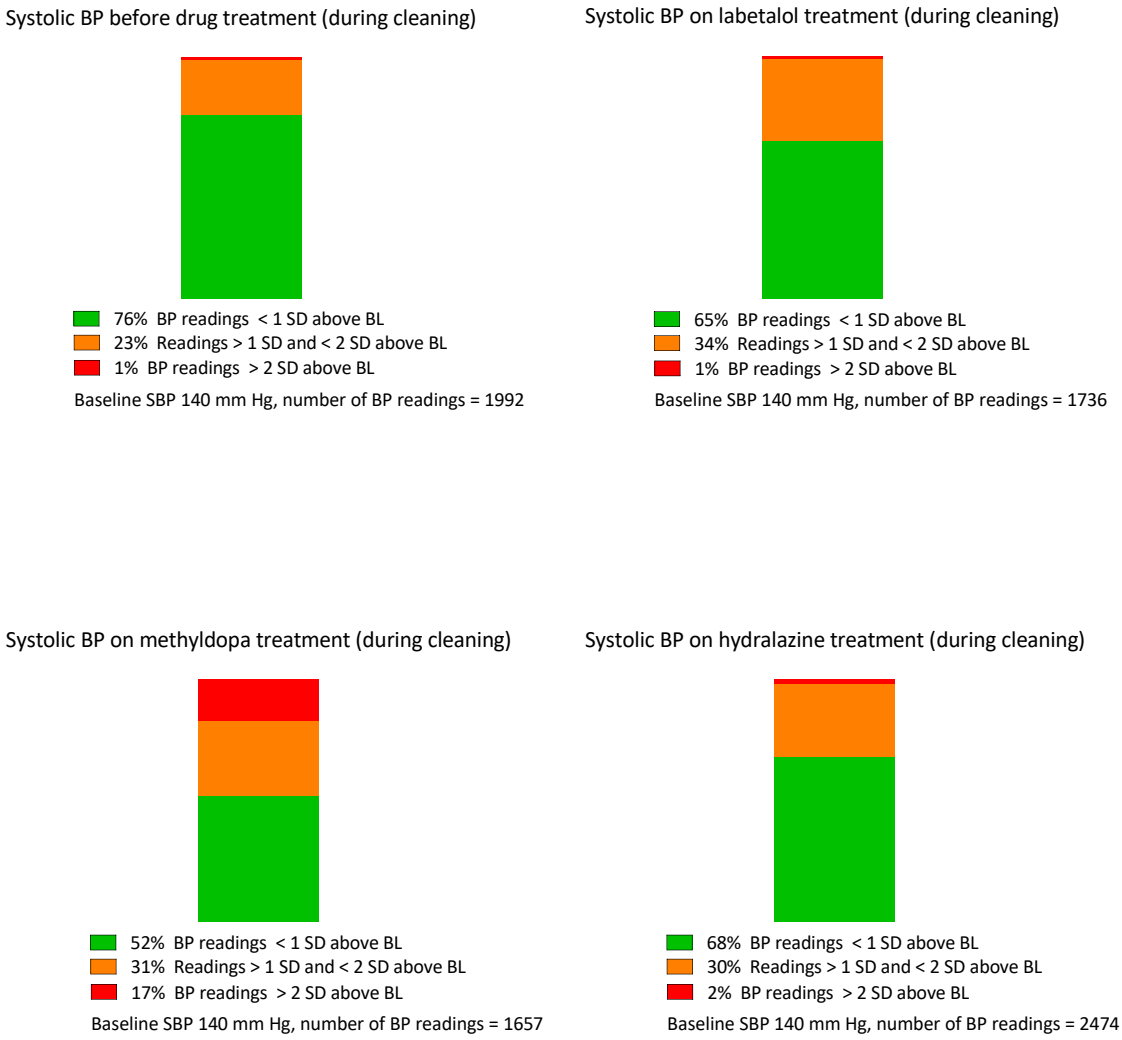

Figure 3-74 Systolic BP load during enclosure cleaning before and after antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9).

The diastolic BP load during cleaning of the enclosure was calculated. Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. Diastolic BP load increased significantly after labetalol, methyldopa or hydralazine treatment whilst the animals' enclosure was being cleaned as determined by chi-square test: $\chi 2$ (4, N = 3727) = 69.74, $p < 0.01$, $\chi 2$ (4, N = 3648) = 533.69, $p < 0.01$, $\chi 2$ (4, N = 4465) = 94.78, $p < 0.01$ respectively (Figure 3-75).

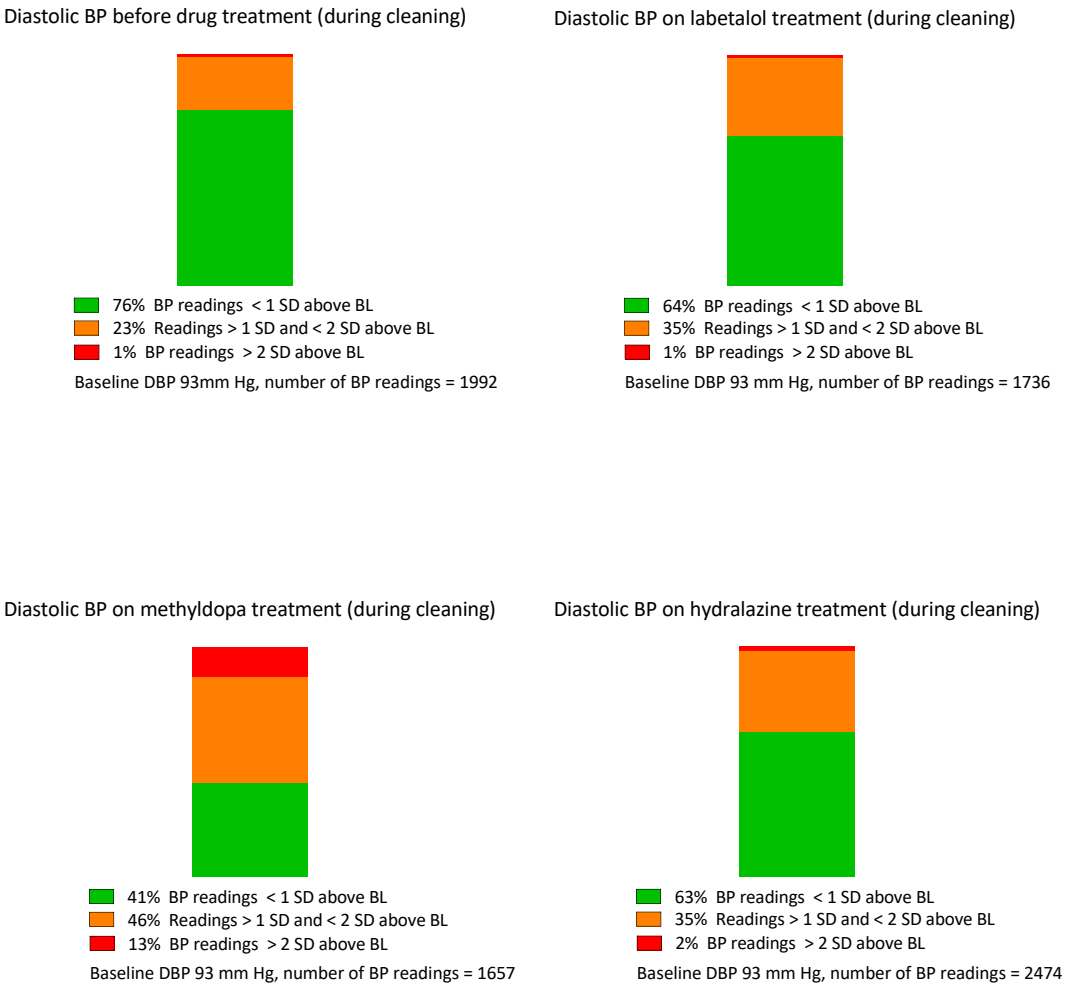

Figure 3-75 Diastolic BP load during enclosure cleaning before and after antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9).

3.3.6.3 Blood pressure variability using SD1 and SD2 values generated by Poincaré Plots: cleaning

There was no significant difference between SD1 of systolic BP during enclosure cleaning before and on drug treatment (labetalol, methyldopa or hydralazine combined), as shown in Figure 3-76. There was a significant increase in SD1 of diastolic BP on labetalol treatment (Figure 3-77). There was no significant difference in SD2 of systolic or diastolic BP before and on antihypertensive treatment (Figure 3-78 and Figure 3-79).

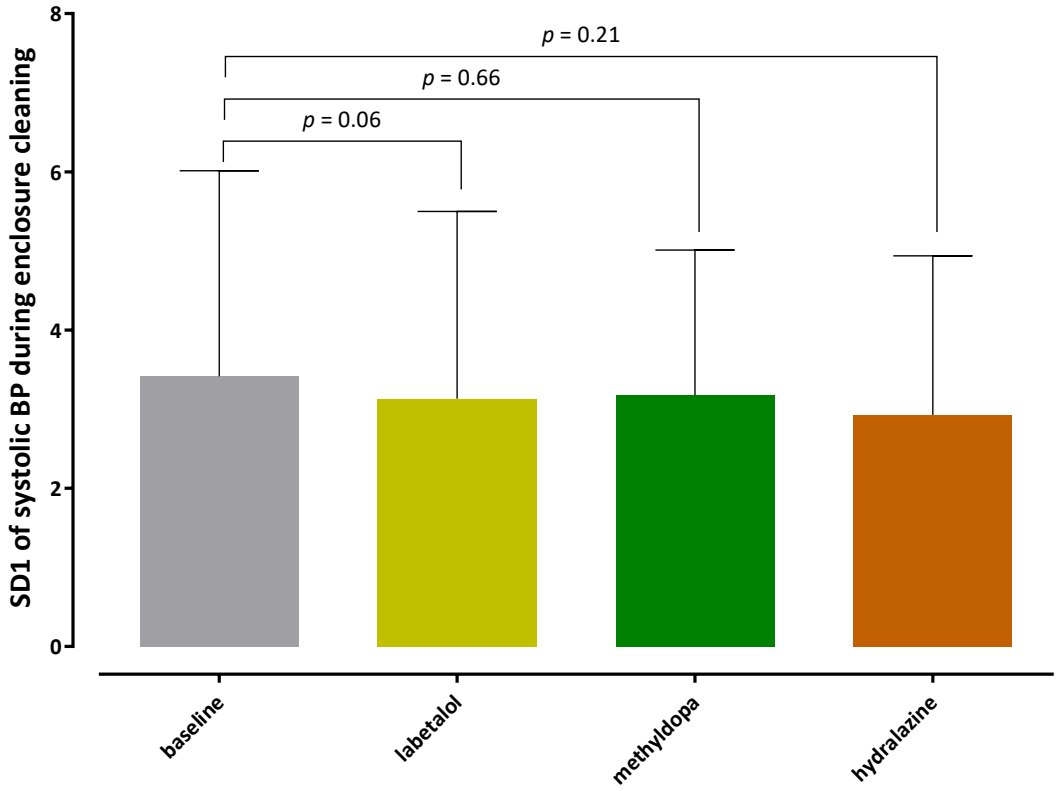

Figure 3-76 Mean short-term variability of systolic BP, as represented by SD1, during enclosure cleaning before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. There was no significant difference in mean systolic SD1 before and on antihypertensive treatment. Statistical significance defined as $p \leq 0.05$.

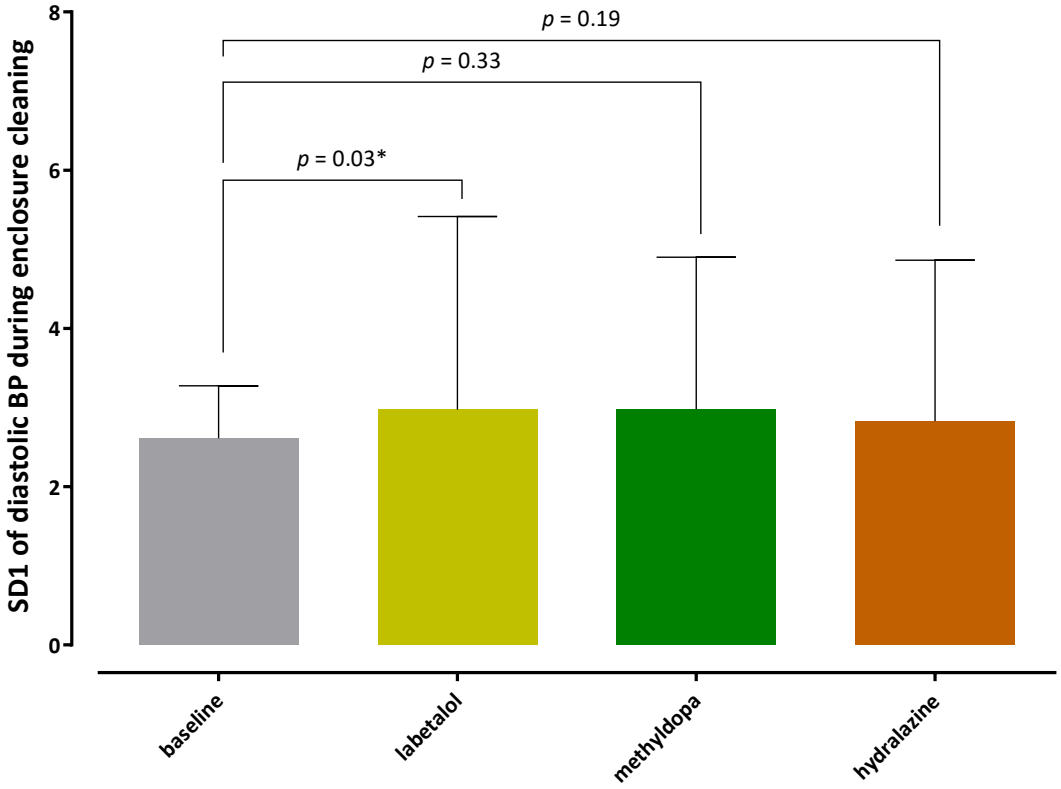

Figure 3-77 Mean short-term variability of diastolic BP, as represented by SD1, during enclosure cleaning before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. There was a significant difference between average diastolic SD1 on labetalol treatment only. *indicates statistically significant difference between groups ($p \leq 0.05$).

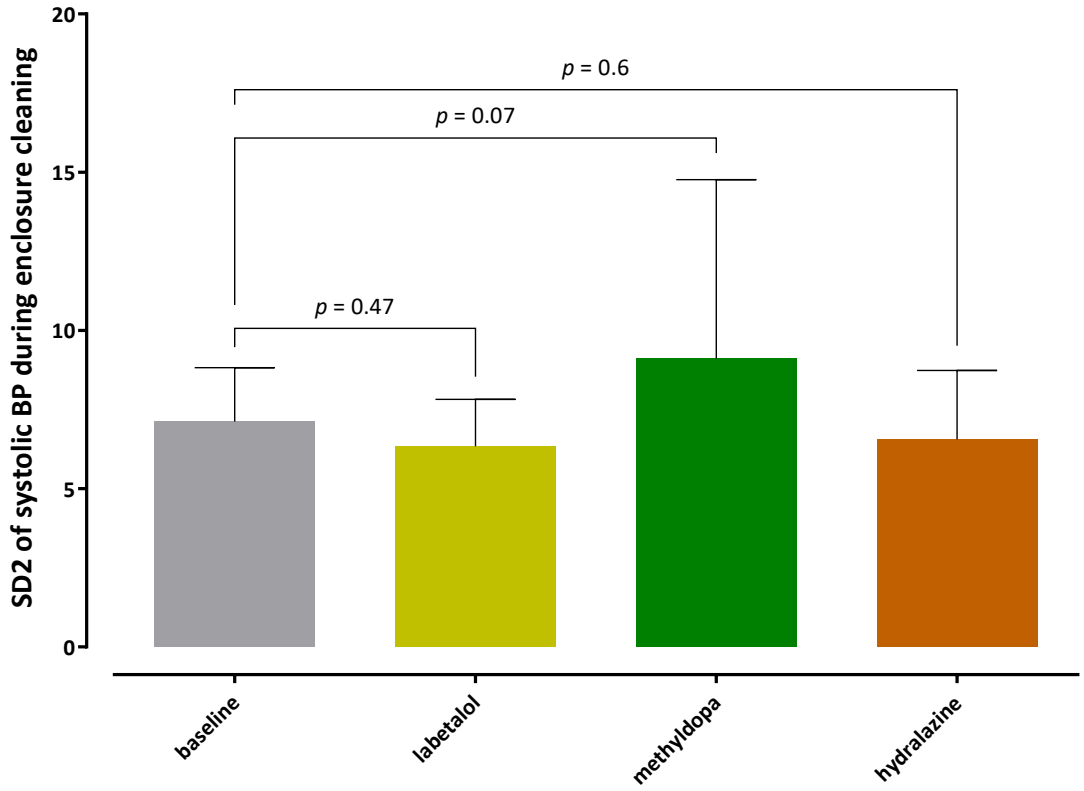

Figure 3-78 Mean long-term variability (SD2) of systolic BP during enclosure cleaning before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. There was no significant difference in mean systolic SD2 before and on antihypertensive treatment. Statistical significance defined as $p \leq 0.05$.

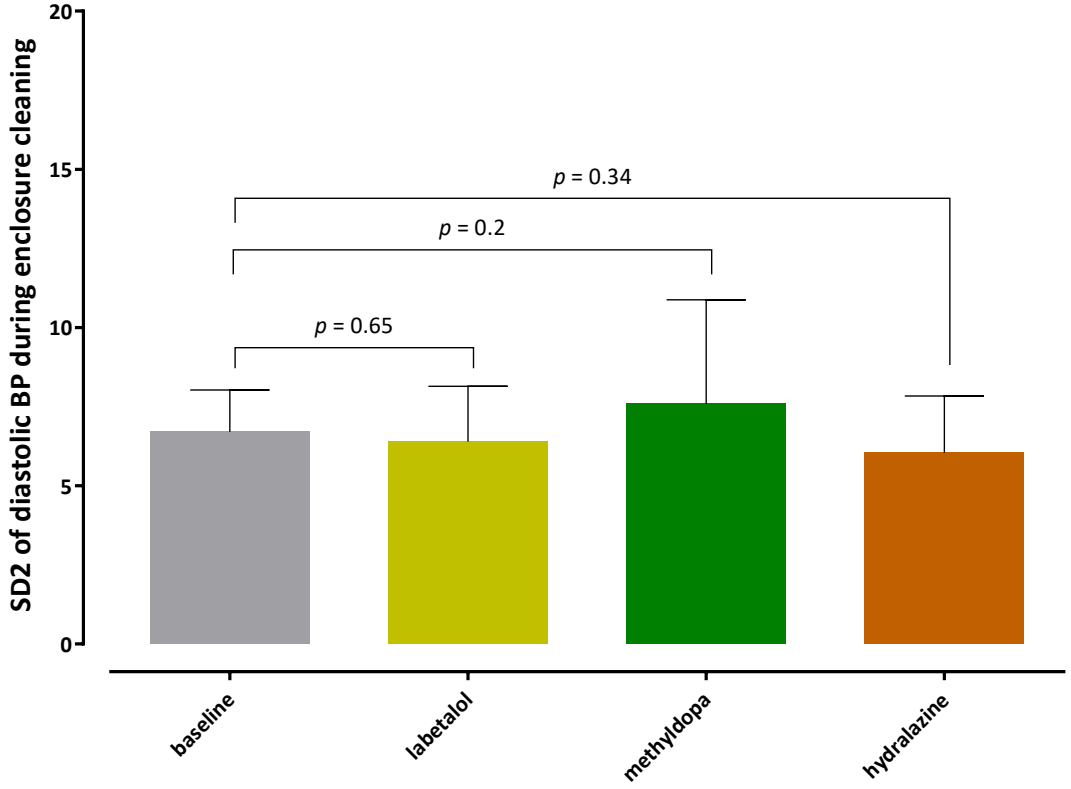

Figure 3-79 Mean long-term variability (SD2) of diastolic BP during enclosure cleaning before and on antihypertensive treatment with labetalol, methyldopa or hydralazine (n = 9). Baseline BP was defined as the average BP of all nine animals before any antihypertensive drug treatment commenced. There was no significant difference in mean diastolic SD2 before and on antihypertensive treatment. Statistical significance defined as $p \leq 0.05$.

3.3.6.4 Classification of blood pressure data as stochastic, periodic or chaotic: cleaning

The Chaos Decision Tree Algorithm classified the BP data collected during cleaning of the animal's enclosure set as stochastic.

4 DISCUSSION

The focus of this thesis is the clinical complexity of BP readings in an experimental model of preeclampsia and the effect of the oral antihypertensives labetalol, methyldopa and hydralazine on BP in response to different stressors. There are limited human studies exploring the clinical complexity of oral antihypertensives in women with preeclampsia on BP load and BP variability.

The induction of UPI in this cohort resulted in a condition clinically analogous to human preeclampsia, as described by Makris *et al*.[194] This was illustrated by the overall increase in blood pressure, development of proteinuria, and elevation in circulating sFlt-1 (due to syncytiotrophoblast stress) by precedent set in BP measurement.

Systolic and diastolic mean BP (awake) increased significantly after the UPI procedure as compared to baseline, with respective increases of 7 and 8 mmHg respectively (Figure 4-1). The increase in mean systolic BP during sleep after the UPI procedure compared to baseline increased, however was not statistically significant. There was however a significant increase in mean sleep diastolic BP of 11 mmHg compared to baseline after the UPI procedure.

In terms of BP averages, there was no statistically significant change in the BP in the experimental model of preeclampsia before and on treatment with labetalol, methyldopa or hydralazine whilst awake at rest and during sleep. The average increase in systolic and

diastolic (awake) BP following UPI was statistically significant, as was the increase in sFlt-1. The usefulness of angiogenic markers to mark uteroplacental dysfunction is becoming increasingly recognised, with the ISSHP guidelines introducing recommendations to assess angiogenic markers where available, stating that with angiogenic imbalance, the diagnosis of preeclampsia is strengthened.[1] The amount of drug ingested by the baboons at the time of each dosing was estimated by observing the animals consuming the food that the tablet was embedded in and checking the amount of leftover food and estimating how much of the tablet likely wasn't consumed. If the food containing the tablet was rejected entirely, a different type of food was selected, and the medication placed inside. Observation to ensure ingestion was for as long as necessary, generally between 5 and 60 minutes. An LC-MS method to detect labetalol, methyldopa and hydralazine in baboon plasma was developed, and a possible future direction of this research includes further evaluating samples using this method. The preliminary results from this work did show qualitative evidence that labetalol, methyldopa and hydralazine was absorbed.

In terms of BP during anaesthesia, the significant change in BP found was decreased average BP with methyldopa treatment during propofol anaesthesia. Typical BP range for baboons is similar to human ranges i.e., 110-120/80 mmHg. In the experimental model of preeclampsia, BP rises 5-10 mmHg. The average BP before methyldopa treatment in the group receiving propofol anaesthesia was 137/98 mmHg, which is considered high for this species. After methyldopa treatment the average decreased to 116/78 mmHg, which would be considered within the optimal range. There was no significant difference in average BP before and on treatment in the ketamine and sevoflurane groups. Average BP during ketamine anaesthesia was the highest of the three anaesthetics, with BP remaining high before and on treatment with each antihypertensive drug. Average BP during sevoflurane anaesthesia was clinically low before treatment and even lower after antihypertensive treatment although the average change was not statistically significant. The BP results from this analysis showed the propofol/methyldopa combination placed average BP in the optimal range.

In terms of change in average BP on treatment in the presence of controlled noise stressors (EF, MP, PT and SC), there was somewhat of a counterintuitive result: the average BP mostly increased on treatment compared with pre-treatment averages in the presence of the everyday/familiar sounds (EF) and whilst listening to the sounds of conspecifics (SC). Average systolic BP did decrease significantly in the presence of the

sound of a potential threat (PT) and classical music (MP) after labetalol treatment however there was no statistically significant change at all during PT and MP sounds after methyldopa or hydralazine treatment. These results show labetalol was more effective in reducing average systolic BP during PT and MP noise exposure when compared to methyldopa or hydralazine. The PT and MP sounds could be considered novel as the baboons are captive bread and not encountering the sounds of wild lions or Mozart's sonatas; exposure to those sounds brought about no change in BP reactivity. The everyday, familiar sounds of quiet, their companions and animal care staff working (EF) and a recorded sound of other baboons (SC) are sounds they are accustomed to; it's possible that the animals are conditioned to these sounds and as such they carry implications to the animals in the context of their social interactions, hierarchies and daily routine and that this, combined with the act of administering the medication led to increased blood pressure that was not controlled by the treatments.

The continuous BP data provided by the telemetry provided the opportunity to investigate the BP reactivity to events that would occur in the daily routine (alongside the anaesthesia and other studies) such as feeding, enclosure cleaning and exposure to everyday sounds associated with the other companion animals and routine running of the facility. The addition of the recording of classical music with long-term periodicity (MP) was to investigate the association between classical music and stress reduction in the experimental model of preeclampsia; in the NP literature listening to classical music is associated with stress reduction as measured by physiological parameters such as HR and BP.[218-221]

Group average systolic BP decreased significantly after labetalol treatment during exposure to classical music (MP) when compared with pre-treatment values. The findings in the literature regarding physiological response to music with long term periodicity demonstrate a range of responses. Temporal-spatial reasoning has been shown to improve in groups for 15 minutes after listening to Mozart's sonatas.[222,223] Other findings have shown that listening to slow classical music significantly decreases HR and BP in the NP population.[219] One proposed theory is that music affects the cardiovascular system via the autonomic system; the vagus nerve may respond to vibrations of the eardrum that reflect the tempo of the music, triggering a relaxation response.[219] The concept of hypertension in the NP population caused by chronic noise exposure is generally accepted, with some studies demonstrating that noise exposure causes an increase in BP whereas findings are

contradicted in other studies.[224-226] The effect of sudden, short-term noises/sounds on BP reactivity in the NP has been shown to cause an increase in BP associated with peripheral vasoconstroction.[227] It's clear from the sound therapy literature that various sounds can have a stress reducing effect on physiology as measured by BP and HR.[228,229] Interestingly, music or sound preference matters; studies have shown that patient selected music in a clinical setting shows more beneficial effects than music chosen by someone else.[230] The baboons' responses to the sounds may perhaps be explained as a response based on previous conditioning/sound exposure or lack thereof.

Average BP increased significantly whilst eating with methyldopa treatment and remained largely unchanged with labetalol or hydralazine treatment. For baboons, eating and obtaining food could be considered a positive experience, and a competitive one. The anticipation of such routine husbandry events can lead to stress, irrespective of whether the events themselves are positive or aversive.[231] Hamadryas baboons have a distinctive, multi-level social system where the social hierarchy is apparent, but not necessarily rigid.[232] This hierarchy amongst groups of females specifically would usually be negotiated by submissive behaviour rather than access to food, although in an all-female group such as the groups studied, non-dominant animals have been observed to leave a preferred food type e.g. bananas, allowing the dominant female to come over and take the bananas. All considered, feeding behaviours in this context reflect complex social dynamics. It is likely that these dynamics change day-to-day/feed-to-feed which may be in part the reason for the increase in BP on treatment with the act of handfeeding a tablet-laced treat likely played a role as well. The animals were however acclimatised to the process of being hand fed treats by animal care staff in the lead up to being given food containing antihypertensive agents to mitigate this confounding variable as much as possible. Further, the results were group averages and by taking the average, nuance of the data is lost. This same data is analysed in terms of BP load, BP variability and the chaos algorithm below.

Average BP increased significantly with all treatments (labetalol, methyldopa, hydralazine) during enclosure cleaning. These results show that the blood pressure response to the enclosure cleaning stressor was not controlled by the treatments. The animal care staff considers enclosure cleaning to be a negative stressor for the animals and this is supported by the literature in a range of group-housed species.[233-235] Hoses and water are used, which the animals retreat from. *Papio hamadryas* are observed within the

colony environment to move out of the rain and seek cover in their night house or under outdoor shelters and move away from any water being sprayed from a high-pressure hose or other. The juvenile animals are observed to regularly jump into and play in pools and ponds in their enclosure. Overall, the presence of care staff hosing and scrubbing enclosures is considered a negative stressor and the high average BP results are consistent with this presumption. The even higher average BP on treatment was not expected, however this is thought to be due to the combined impact of the presence of care staff feeding food treats containing treatment as well as the cleaning process itself. It is likely that animal care staff feeding treats containing medication, and returning to assess how much had been ingested, was a negative stressor for the animals. The experimental design included acclimatisation of the animals to the animal care staff feeding treats prior to induction of UPI, however this acclimatisation did not include repeated checks to ensure animals had ingested the treatment-laced treat. In terms of real-life application of this finding, this data suggests that exposure to a negative stressor increases BP, despite antihypertensive therapy with labetalol, methyldopa or hydralazine in an experimental model of preeclampsia. There is evidence that pregnancy is protective of stress related BP increases and that in cases of preeclampsia that protection is not apparent. This is consistent with the findings from this study. The cardiovascular and neurohumoral response to physiological stress is reduced in healthy pregnant women as compared to nonpregnant women,[236] while in women with preeclampsia this response is increased.[45] Further, acral skin cooling/vasoconstriction as a stressor has been shown to raise blood pressure in pregnancy, with the greatest BP increases seen in women with preeclampsia.[237] Interestingly, vasoconstriction of arteriovenous anastomoses also occurs in response to auditory or other centrally mediated stimuli, including the startle response, single deep breath, pain and mental arithmetic, which Hartgill and Perhonen[237] propose may explain the labile blood pressures observed in women with preeclampsia.

With regards to the outcomes of average EMS BP data the average BP largely increased on treatment with labetalol, methyldopa or hydralazine. Average BP has not been a sensitive enough indicator to detect change in most of the data sets in comparison to other analyses below. One of the disadvantages of averaging BP data is that the average value is affected by the highest and lowest values, and the average value may not actually coincide with any of the recorded values. Current hypertension guidelines advise taking three BP measurements at 1 minute intervals and using the average of the last two measurements as a basis for clinical decision making.[86,238] The advantage of average calculations is that it facilitates clinical decision making by providing evidence to classify BP and treat, however

the disadvantage is that BP averages statistically disregard the dynamic fluctuations in BP levels and associated prognostic significance.[52,56,71,85] As reflected in figure 3-11, average daily awake and sleep BP of the individual animals, some animals' BP increased with antihypertensive treatment and some decreased, with the net result being no statistically significant change overall. In women with preeclampsia, a decrease in the slope of the morning blood pressure surge when compared with normotensive pregnancies has been described. In women with normotensive pregnancies, the slope has been found to increase from the first to the second trimester and then significantly decrease during the third trimester, however in women who developed preeclampsia, the slope was found to decrease with gestational age, mainly for diastolic BP.[239]

Blood pressure load is defined as the percentage of abnormally elevated BP readings over a defined period,[240] usually 24 hours, and it is usually calculated based on ambulatory BP monitoring. BP load was a concept developed based on the hypothesis that it might be an index of hemodynamic burden on the cardiovascular system.[90] Some studies in the NP population of patients with untreated hypertension show that BP load is a better determinant of poor cardiovascular outcomes than either casual office or mean ambulatory BP.[241] However this is debated, with other studies showing that repeatability of BP impacts predictive power of BP load, and in individuals with outlying BP values BP load is less accurate as a predictor.[242] There are limited studies of the effect of different antihypertensive treatment on BP load in women with preeclampsia with BP load studies relating to hypertension in pregnancy focussing largely on preeclampsia risk and predicting the development of preeclampsia.[240,243]

In terms of BP load whilst awake at rest and during sleep, there was overall a statistically significant increase in BP load with labetalol and methyldopa treatment and a decreased BP load associated with hydralazine treatment. This contrasts with the average BP finding whereby there was no statistically significant change in the average BP whatsoever with labetalol, methyldopa or hydralazine treatment whilst awake at rest and during sleep. The average BP results from this data set suggest that antihypertensive choice does not significantly affect the BP in any way however the BP load results suggest that hydralazine reduced the BP load whereas labetalol and methyldopa increase the BP load. This data show that labetalol, methyldopa or hydralazine affect BP load differently in this experimental model of preeclampsia, differences not detected by BP average.

A 2019 trial[244] found no studies that directly compared three commonly used oral antihypertensives: labetalol, methyldopa, and nifedipine for management of severe hypertension in pregnancy, with this study's review of evidence finding insufficient data to recommend a specific antihypertensive drug and recommendations that the choice of antihypertensive should depend on clinicians' experience and familiarity with the drug, known adverse effects, and women's experiences. This trial found that nifedipine, labetalol and methyldopa reduced blood pressure to the reference range in most women and as single drugs, and nifedipine resulted in a greater frequency of primary outcome attainment than labetalol or methyldopa use. This study also found that methyldopa, nifedipine, and labetalol are viable initial options for treating severe hypertension in low-resource settings. While the World Health Organisation (WHO) recommends the use of antihypertensives for treatment of severe hypertension in pregnancy, it does not recommend a specific first line of treatment oral drug. The WHO also decided not to issue a recommendation on the treatment of mild/moderate hypertension until further evidence becomes available.[245] The NICE guidelines advise offering labetalol to treat hypertension in pregnant women with preeclampsia in the first instance and to offer nifedipine then methyldopa if labetalol is not suitable and base the choice on any pre-existing treatment, side-effect profiles, risks (including fetal effects) and the woman's preference.[35] ISSHP guidelines list the acceptable first-line anti-hypertensives as: labetalol, oxprenolol, methyldopa, nifedipine, diltiazem; with prazosin and hydralazine usually used as 2nd or 3rd line agents.[2] These guidelines are largely based on studies using office BP measurements as per current recommendations. The results of this thesis found that the hydralazine treated groups had the least amount of BP load whist awake at rest and during sleep i.e., the greatest proportion of BP readings within one standard deviation of the mean. To contextualise this BP load finding, BP data was categorised according to how many standard deviations from the mean it was i.e., within one standard deviation above the mean BP, between one and two standard deviations of the mean BP and greater than two standard deviations of the mean BP. This approach was used in an effort to define BP burden in the animals in an equivalent, but more species-applicable way, than if the approach used to describe human BP load were to be directly applied to the baboons' BP data. Using the mean BP for each animal as the baseline, then categorising the BP values according to how many standard deviations from the mean the values were, allowed individualisation of the data categorisation, and as such made the categories more species appropriate. Allocating the three categories a colour, mirroring the traffic light approach used when analysing human BP load, allowed the BP load data to be presented in a similar way to how a 24 hour BP profile can be presented in a clinical ambulatory BP monitoring report.

With the significant change in average BP during anaesthesia being a reduction in average BP with methyldopa treatment during propofol anaesthesia, BP load results mirrored this finding and showed that BP load was also significantly reduced with methyldopa or hydralazine treatment during sevoflurane anaesthesia when compared with pre-treatment values during sevoflurane anaesthesia. Where there was no significant change in average BP with labetalol treatment during anaesthesia of any type, BP load showed an increase with labetalol treatment during propofol and sevoflurane anaesthesia. This dynamic was not detected by the BP averaging. The average shows that BP goes down under propofol and sevoflurane anaesthesia on labetalol treatment. If BP load results from this study were to be a guide to antihypertensive and anaesthetic choices, methyldopa coupled with propofol or sevoflurane, or hydralazine and sevoflurane would be preferable to labetalol with either propofol or sevoflurane.

The change in BP load in the presence of controlled sound stressors was generally consistent with average BP; BP load for the most part increased on treatment with labetalol, methyldopa or hydralazine in the presence of EF, MP and SC however there was mostly no significant change in BP load when in the presence of sounds of a PT, in fact there was a significant decrease in diastolic BP load after labetalol treatment when the animals' were listening to sounds of a PT. The BP load analysis of controlled sounds stressors suggests labetalol as being most effective out of the three antihypertensive drugs at controlling BP in this context when compared to methyldopa or hydralazine.

The BP load findings during the EMS period, whilst eating and during cleaning showed an overall increase in BP load on treatment with labetalol, methyldopa or hydralazine, i.e., mostly similar to the corresponding average BP outcomes. This could be interpreted as the medications did not have a BP lowering effect on the EMS and when exposed to stressors, which could be relevant for women with preeclampsia and their treating team.

BP load increased significantly on treatment with all three antihypertensive drugs at feeding time. In comparison to average BP, where average BP increased significantly whilst eating with methyldopa treatment and remained statistically unchanged with labetalol or hydralazine treatment, BP load showed that significant increases occurred with all three antihypertensive drugs. As expected, BP load was a more sensitive indicator

of BP changes than average BP in this data set. Interestingly, BP load during feeding time was the highest overall of all the studies, both before and on treatment. This is most likely related to positive/excitement associated with eating as well as competition/social dynamics and the physical act of exerting themselves to get to the food before their companions.

BP load increased significantly after labetalol, methyldopa or hydralazine treatment whilst the animals' enclosure was being cleaned, which is consistent with average BP results. As expected, the BP load results showed changes in BP not detected by average alone. In terms of BP load graphs, the area under the 24 hour BP curve with respect to time, more specifically the area of the curve under 140/90 mmHg curve during waking hours and under 120/80 mmHg during sleep, have been shown in the NP population to be more clinically valuable that mean ambulatory BP.[87] The rate or frequency of significant change in BP is not represented with this approach to analysis however, and it is acknowledged that this pattern of BP behaviour is clinically relevant.[87]

Blood pressure variability analysis revealed more subtle changes whilst awake at rest and during sleep before and on treatment with labetalol, methyldopa or hydralazine. Short-term diastolic BP variability during sleep was significantly lower with labetalol and methyldopa treatment when compared to pre-treatment values. Long-term systolic BP variability was significantly lower with hydralazine treatment when compared to pre-treatment values. The short and long term variability in this study was calculated using SD1 and SD2 respectively, as generated by Poincaré plots. Here, each blood pressure reading was plotted against the subsequent blood pressure reading. An ellipse was then fitted to the data (similar to a 'line of best fit') and the standard deviation of the points on the two axes was calculated. SD1 is perpendicular to the 'line of best fit' and SD2 runs along this line. In the context of this study, the width of the plots (SD1) represents short-term BPV and the length of the plots (SD2) represent long-term BPV. By plotting each data point against the preceding data point the temporal aspect of the data is incorporated into the outputs. This shows whether a physiological system holds association between events separated by time. [214] One of the limitations of a number of described measures of BPV is that temporality is not accounted for, such as with standard deviation and the coefficient of variation. Average real variability and time rate of BP variation do take sequence and temporality into consideration however are used exclusively in research.[61,71,85,92,93] Poincaré plots have been used in the analysis of physiological fluctuations (e.g., heart rate

variability analysis) in research[214,246,247] and clinical[216,248,249] contexts and have enabled measurement of short- and long-term variability in various contexts separately.[215]

The variability results were mostly consistent with the BP averages for this awake/sleep data set in that there was slight change overall to the awake/sleep BP before and on treatment. When interpreting these results alongside the BP load findings, there was an overall increase in load with labetalol and methyldopa treatment but reduced short-term diastolic BP variability during sleep with labetalol and methyldopa treatment and reduced long-term systolic BP variability with hydralazine treatment. So, whilst the overall BP values as measured by BP load increased with labetalol and methyldopa treatment, the short-term variability decreased. SD1 and SD2 are in this context a measure of short- and long-term variability over the course of a data collection period. While BP load in this study grouped BP data according to how far away from the mean each value was, the Poincaré plot analysis described the short and long term variability of the data sets. When BP load and Poincaré plot analysis is applied to the same data, different patterns of BP behaviour are revealed.

Regarding the EMS data, there was a uniform decrease in short-term variability on treatment with labetalol, methyldopa or hydralazine however the long-term variability remained unchanged on treatment i.e., there was less change between successive BP data points on treatment however the change over the course of the EMS data collection period was not significantly different on treatment. The EMS BP average and BP load increased as mentioned previously; so, while the BP average and load mostly increased on treatment the short-term variability decreased with the three treatments. This data set shows that labetalol, methyldopa or hydralazine treatment decreased short-term variability and had no significant impact on long-term variability in the EMS period. Again, in this data set the variability analysis detects different patterns of BP behaviour than BP average and load. BP variability has prognostic implications, demonstrated in the NP population and increasingly studied in the pregnant population. This data shows that in an experimental model of preeclampsia, labetalol, methyldopa or hydralazine treatment decreased short-term variability in the early morning surge period compared with pre-treatment values. There was no significant difference between the antihypertensive types. In terms of reducing short-term variability associated with the EMS, this data shows that labetalol, methyldopa or hydralazine were equally effective.

Labetalol, methyldopa or hydralazine treatment had little impact on overall BP variability during anaesthesia, with the significant results being a reduction in long-term diastolic BPV after labetalol treatment during propofol anaesthesia and a significant increase in long-term diastolic BPV after methyldopa treatment during ketamine anaesthesia when compared with pre-treatment values.

In terms of clinically relevant BPV findings, this data suggests that labetalol would reduce diastolic BPV over the course of a propofol general anaesthetic, i.e., reduced BPV over the course of the anaesthetic period. This data also suggest that methyldopa treatment would increase long-term diastolic BPV during ketamine anaesthesia when compared with pre-treatment values, so these results suggest that the combination of ketamine anaesthesia with methyldopa is not an ideal combination for minimising long-term diastolic BPV.

Outcomes of the variability analysis of the BP data from the controlled stressor studies (noise exposure, eating and enclosure cleaning) showed the significant changes in variability on treatment related to the sound of a potential threat; short-term variability of systolic BP decreased with labetalol and methyldopa treatment and long-term variability of diastolic BP decreased on treatment with all three antihypertensives (labetalol, methyldopa, hydralazine). There was also a reduction in long-term systolic BP variability with labetalol and methyldopa treatment in the presence of the sounds of conspecifics. This data suggests that labetalol and methyldopa are effective at reducing systolic short and long-term variability when exposed to controlled sound stressors PT and SC. Long-term variability of diastolic BP also decreased significantly after labetalol treatment during enclosure cleaning. BPV did not change on treatment with labetalol, methyldopa or hydralazine whilst eating when compared with pre-treatment values. Overall, labetalol was the most effective in the most controlled stressor contexts in reducing BP variability.

There were patterns of BP behaviour identified by the variability analysis not identified by averaging the BP or calculating BP load. The Poincaré plot described the correlation pattern of the BP time series signal and this approach may identify differences that are not easily detectable with traditional time and frequency domain approaches to BP measurement.[248] The plots are able to identify cycles between the data-points and patterns in the data.[249,250] As BP variability is associated with prognosis, there is clinical relevance in identifying changes in BP variability.[52,69,251] An obstacle to doing so in clinical

practice has been practical limitation associated with describing and classifying BPV in the pregnant population as well as data regarding which type of antihypertensive agents manage BPV most effectively in the pregnant population. Another consideration when evaluating the outcomes of this variability analysis is that the experimental preeclampsia model is typically a clinically mild syndrome with increases in BP of between 5-10 mmHg and higher degrees of variability using this approach to analysis may be seen in clinically unwell patients with preeclampsia.

In this thesis the BP data from sets 1-6 was also described using the Chaos Decision Tree Algorithm.[217] This algorithm classifies data as stochastic, periodic or chaotic. The majority of the data were classified as stochastic. There is limited literature regarding classifications of BP data using this approach. There is however a growing body of literature describing heart rate variability using mathematical chaos.[252] A recent study hypothesised that beat-to-beat BPV has a similar physiological basis to heart rate variability.[253] The heart rate variability literature suggests that stochastic, or random behaviour regarding heart rate variability is a healthy state for a heart to be in with reduced variability being associated with poorer cardiovascular health overall.[254] This predominately is thought to mean that a heart is adapting to increased or decreased physiological demands appropriately.[252] Exactly how much variability is healthy and what is pathological is not clearly defined. The animals in this study were considered healthy pregnant animals with an induced model of experimental preeclampsia. Findings of primarily stochastic BP is consistent with this.

BP was periodic during the day whilst animals were awake (at rest) and during sleep on treatment with labetalol, methyldopa or hydralazine. A defining feature of healthy function is adaptability, or the ability to respond to unpredictable stimuli.[255] It has been proposed that fractal physiology may inhibit the emergence of very periodic behaviours that greatly narrow system responsiveness.[255] This hypothesis is supported by findings from life-threatening conditions such as chronic heart failure, where the breakdown of fractal correlations is often accompanied by the emergence of a dominant mode, e.g., the Cheyne-Stokes frequency. Abrupt transitions to strongly periodic dynamics are observed in many other pathologies, including at high altitudes[256] and with obstructive sleep apnoea[257] sudden cardiac death,[258] epilepsy, and fetal distress syndromes.[259] The results from this thesis showing that when the animals are awake (at rest), and during sleep while they are receiving labetalol, methyldopa or hydralazine, their blood pressure is periodic suggests

that the antihypertensive treatment is causing this. Although periodic, metronomic physiologic behaviour may have a narrowed system responsiveness, in the case where BP may rise dramatically potentially resulting in catastrophic strokes, cerebral haemorrhage and end organ damage, periodic BP behaviour induced by antihypertensives is likely preferable.

Notably, the only data that met the criteria for a chaotic data set was that collected during propofol anaesthesia after methyldopa or hydralazine treatment. Given that chaotic systems can destabilise in response to a small change in conditions, and that a number of studies have associated chaotic dynamics with pathological rather than normal cardiac behaviour[260] it's likely that chaotic BP during general anaesthesia is not ideal. Interpreting the results from this study using this principle, propofol anaesthesia combined with methyldopa or hydralazine create chaotic BP dynamics, whereas BP remains stochastic when propofol is combined with labetalol. As such, if using propofol anaesthesia, labetalol would be the preferable antihypertensive. This chaos algorithm supports sevoflurane as the anaesthetic of choice, as BP dynamics in combination with labetalol, methyldopa or hydralazine are largely stochastic, although systolic BP with hydralazine treatment was periodic. Blood pressure during ketamine anaesthesia was stochastic with all the antihypertensive treatments. If results from the chaos algorithm, were to be a guide to antihypertensive and anaesthetic choices, sevoflurane combined with labetalol or methyldopa would be preferable. Although BP dynamics during ketamine anaesthesia were stochastic, analysis of BP using average BP and BP load reveal BP during ketamine anaesthesia was the highest of the three anaesthetics.

The cardiovascular system is regulated via many feedback mechanisms occurring with different time delays and can be considered nonlinear and complex,[261] [262] resulting in conditions in which very small variation of a given input, such as cardiac output or the concentration of a neurotransmitter, might result in a dramatic change in an output.[263] The baroreflex mechanism is responsible for the regulation of BP in the short term through the control of the action of the heart and vessels.[94,264]. Other paracrine and hormonal mechanisms, including those that involve nitric oxide and angiotensin II are also involved in the maintenance of BP.[264] The relationship of these regulatory mechanisms and the inherent nonlinearities of each individual mechanism, result in the complex and nonlinear behaviour of the BP controlling system.[94] Chaos theory is a recognised way to

describe non-linear systems.[95,255,259] Most published investigations of non-linear dynamics relating to the circulatory system focus on the NP population.

Circulatory disorders can show a pattern of decreased chaos such as in the case of congestive heart failure or an increase in chaos in the case of fibrillation.[265] Vasomotion plays a large role in the control of BP and studies have established that vasomotion is chaotic and that this dynamic allows a high degree of flexibility to the control of microcirculatory blood flow.[265-268] Vasomotion may contribute to the non-linear dynamics observed in systemic blood pressure.[265] It has been suggested that the sensitivity of chaotic systems to initial conditions allows large, efficient changes in state[269] and may be an advantage for control of such a system.[270] However with such a system, small changes can easily destabilise periodic or steady-state behaviour.[265,271] Clinicians recognise that there is a cohort of women with preeclampsia that have blood pressure that suddenly and unexpectedly rises, and this occurrence often precipitates a worsening of their condition. These women may not meet the clinical criteria for hypertension at the time their BP is measured, or they may have BP readings that are considered low-normal shortly followed by BP readings considered high (or as representing severe hypertension).[24] It's possible that describing the BP dynamics of women with preeclampsia using non-linear approaches may be useful in identifying BP that may change suddenly; chaotic systems are known to shift abruptly from a regular behaviour because of the critical change of a given parameter. Whilst this thesis focussed on describing BP in the experimental model of preeclampsia before and on antihypertensive treatment using non-linear approaches including Poincaré plots and the chaos algorithm, a future direction of research could be applying non-linear analysis to BP data from women with preeclampsia with the aim of identifying BP that may destabilise suddenly in a clinically relevant way. Another question is: does the placenta function as a chaotic attractor in women with preeclampsia?

Blood pressure is complex, dynamic and regulated by many different systems in the body, and it is understood by more than just a single BP reading or average BP. Blood pressure load, BP variability and the chaos algorithm have each spotlighted different patterns in the BP data than was detected by average BP alone. Faced with the complex clinical case of a woman with preeclampsia with unstable BP, how could the results of this thesis potentially be applied?

Whilst average BP showed that the propofol/methyldopa combination achieved BP in the optimal range as compared to ketamine and sevoflurane, BP variability (SD1/SD2) showed

reduced long-term diastolic BP variability on labetalol treatment during propofol anaesthesia. The chaos algorithm also showed the BP during propofol anaesthesia remained either stochastic or periodic on labetalol treatment compared to BP being classified as chaotic during propofol anaesthesia on methyldopa and hydralazine treatment. Whilst the significance of chaotic BP is yet to be determined, stochasticity in the cardiovascular system is typically associated with a healthy state, whereas a loss of complexity, in the case of periodic or chaotic behaviours, has usually been associated with a pathological state. Following this line of practical reasoning, propofol anaesthesia may not prove to be the ideal choice for women with preeclampsia, as in this study, it routinely generated chaotic BP dynamics. From this standpoint, sevoflurane, combined with labetalol or methyldopa, may be preferable.

In terms of exposure to controlled noise stressors, where there was any effect, labetalol was the most effective at managing BP. The effect was seen with average BP and BP variability analysis, with no change seen with BP load analysis. BP remained stochastic during exposure to controlled stressors. Whilst eating, average BP increased significantly on methyldopa treatment whilst remained largely unchanged with labetalol or hydralazine treatment. In this context, labetalol or hydralazine would be preferable. Given labetalol's favourable effects during anaesthesia and during exposure to noise stressors, labetalol could be considered preferable over hydralazine. None of the antihypertensives showed any more effect over the other during enclosure cleaning. BP uniformly increased with all three of the antihypertensive drug treatments.

5 LIMITATIONS

The main limitation of this study was the small size of the cohort (n = 9) resulting in a somewhat low statistical power for detecting differences between groups. Working with primates using this model of experimental preeclampsia is highly complex, and as such, large cohorts aren't feasible. This limitation was somewhat mitigated by the experimental cross-over design. The advantage of the cross-over design is that each animal formed its own control group, thereby increasing the statistical power of the data. A potential disadvantage of this design is a risk of carryover effects confounding the estimated antihypertensive treatment effect.

The model of experimental preeclampsia that the UPI establishes is generally considered a mild, controlled syndrome and as such, increases in parameters associated with HELLP syndrome is generally not seen. Typically, increases in BP of between 5-10 mmHg develop in the model. In the studies where there were no or minimal changes in BP, it may be due to the mild nature of the syndrome and BP increases. Perhaps higher degrees of variability using SD1 and SD2 as markers of BPV may be seen in clinically unwell patients with preeclampsia.

Insufficient ingestion of antihypertensive medication was a possible reason there was no change in average BP whilst the animals were awake and at rest, or during sleep. The animals were observed at the time of dosing to ensure that they ate all the food containing the tablet, as described in section 2.3. If the food containing the tablet was rejected entirely, a different type of food was selected, and a new dose of the medication was prepared and placed inside. It was estimated that all the animals ingested greater than

90% of all the doses. It's possible that the medication may have interacted with the food stuffs, however this was unlikely. Bananas do affect methyldopa absorption[207] and subsequently bananas were not fed during the time of methyldopa therapy.

Insufficient absorption of antihypertensive medication is another possible factor that may have affected outcomes. An LC-MS method to detect labetalol, methyldopa and hydralazine in baboon plasma was developed, and a possible future direction of this research includes further evaluating samples using this method. The preliminary results from this work did show qualitative evidence that labetalol, methyldopa and hydralazine was absorbed.

The thesis primarily focussed on clinical BP complexity in the experimental preeclampsia model and investigations into the role of the autonomic nervous system and management of hypertensive emergencies was not explored.

6 CONCLUSIONS

Blood pressure is complex and dynamic, and even more so in the context of preeclampsia. This complexity is reflected in the changing classifications of BP in the ISSHP and other guidelines. This thesis aimed to investigate the blood pressure response in an experimental model of preeclampsia whilst awake at rest and during sleep, during exposure to several different stressors including the early morning surge period, general anaesthesia, noise, eating and enclosure cleaning and the potential ways to control BP using three antihypertensive agents commonly used in the clinical setting: labetalol, methyldopa or hydralazine. The BP response was analysed using four different metrics to assess BP dynamics: BP average, BP load, BP variability and the chaos algorithm.

Dramatically fluctuating BP in a woman with preeclampsia is a high-risk clinical situation. Managing these sudden, severe fluctuations of BP is the clinical aim in such a situation. This thesis explored the clinical complexity of antihypertensive treatment, anaesthesia and stressor exposure in an experimental model of preeclampsia and found that not all the antihypertensive medications and anaesthetic combinations behaved in equivalent ways. These findings are relevant because they show different antihypertensive medications are more effective in different contexts, and there are different BP dynamics during anaesthesia with ketamine, propofol or sevoflurane during treatment with labetalol, methyldopa or hydralazine that may have implications for anaesthetic and other outcomes.

Average BP is currently used in clinical practice as is BP load to varying degrees. Most commonly, one-off BP measurements and average BPs from these one-off point of care measurements are used. The additional types of blood pressure analysis (blood pressure variability analysis and chaos constant) may be of use in future clinical practice to describe and identify BP dynamics that are not detected by the current BP analysis tools (i.e. one-off BP measurements, BP averages and BP load). These additional types of analysis may be of particular use in identifying the subset of women with preeclampsia at risk of complications associated with highly variable BP. The additional types of analysis are not necessarily invasive (dependant on BP monitoring device used) but may provide additional clinical insights. Future directions of this work could include applying these methods of BP analysis to BP measurements from women with varying presentations of preeclampsia, on a variety of different treatments.

The key findings were that the combination of sevoflurane and labetalol or hydralazine created the most preferable BP dynamics during anaesthesia. Further, labetalol was the most effective overall at controlling BP when exposed to the range of stressors. Labetalol, methyldopa or hydralazine created similar BP dynamics during the early morning surge period. In terms of translating this finding to clinical practice, future directions could include investigating the efficacy of labetalol in managing variable BP in women with preeclampsia whilst concurrently using analytics such as BP load, BP variability analysis and the chaos algorithm that describe BP dynamics not captured by one-off office BP measurements or average BP. The prognostication value of these BP tools could also be investigated in women with preeclampsia not yet on BP treatment.

Another future direction of research may be applying non-linear analysis to BP data from women with preeclampsia with the aim of identifying BP that may destabilise suddenly using the chaos algorithm. Further research may also involve investigating whether the placenta functions as a chaotic attractor in women with preeclampsia, and whether the K constant could function as an ‘instability constant’ that flags a BP dynamic at risk of destabilising in a clinically relevant way to support point of care decision making.

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