



UvA-DARE (Digital Academic Repository)

The circadian system

A regulatory feedback network of periphery and brain

Buijs, F.N.

Publication date

2019

Document Version

Other version

License

Other

[Link to publication](#)

Citation for published version (APA):

Buijs, F. N. (2019). *The circadian system: A regulatory feedback network of periphery and brain*. [Thesis, externally prepared, Universiteit van Amsterdam].

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.

Appendices

Summary

Samenvatting

Author Affiliations

Publications

Portfolio

Acknowledgments

Summary

In this thesis we investigate and discuss the role of the suprachiasmatic nucleus (SCN) as part of a circadian network, integrating peripheral feedback in order to effectively regulate day-night fluctuations in the physiological functions of the body.

The earth revolves around the sun, bringing us seasons, while it rotates on its axis once every 24 hours giving all organisms on earth exposure to alternating cycles of light and darkness and environmental changes. In mammals, a central autonomous pacemaker, located in the hypothalamic suprachiasmatic nucleus (SCN), drives rhythmic behavioral, neuroendocrine and autonomic output, providing a daily organization of physiology. The endogenous nature of the neuronal activity generated in the SCN ensures that behavioral and physiological rhythms are maintained even in constant dark conditions (DD). The SCN is located above the optic chiasm (i.e., *suprachiasmatic*) through which it receives photic (light) information, which synchronizes the ~24-hour (i.e., *circadian*) rhythm generated by the molecular SCN clock to the exact 24-hour of the environment. This circadian timing mechanism allows organisms to anticipate day-night changes in the environment, best preparing the physiology for upcoming challenges.

In **chapter one** we introduce the molecular organization of SCN activity and explain how clock genes, found in almost all cell types in brain and body, are involved in maintaining the autonomous rhythm of the SCN and the rhythmicity of our organs. SCN neurotransmitters are essential in coordinating this rhythmicity among different cells within the SCN but also to relay circadian cues to different nuclei in the hypothalamus, and thus to the brain and periphery (the body). We discuss numerous conducted experiments illustrating the importance of the SCN in organizing circadian rhythmicity in organisms and the importance of the SCN as an integrator of peripheral feedback essential for regulating and adapting the daily oscillations in physiology. Furthermore, light is not the sole input or only synchronizer of the SCN as it also receives feedback from hormones, other brain nuclei and behavioral activity amongst others. In this thesis we investigated the possibility whether the SCN could be part of a large network of oscillators all functioning within a series of feedback loops maintaining the organism in synchrony with its environment.

In **chapter two** we investigated how the SCN is incorporated in such a neuronal feedback circuit arising from the nucleus tractus solitarius (NTS), modulating cardiovascular reactivity. The NTS is known to be the medullar integration center of visceral information including blood pressure (BP). We provide evidence of the importance of the SCN as an integration site in physiological circuits regulating BP. In Wistar rats we showed that the SCN receives cardiovascular feedback via the NTS. Glutamate is one of the neurotransmitters involved in the NTS-SCN neural pathway, with projections terminating in the ventrolateral

part of the SCN, where light information also enters the SCN. We demonstrated, using the immediate early gene *c-Fos* as an activity marker, that the activity of the SCN is changed directly following blood pressure changes in the periphery. This provided evidence for a direct neuronal feedback pathway from the NTS to the SCN, transmitting blood pressure information. We also showed that there is an active involvement of the SCN in short-term blood pressure regulation. As such, we provided evidence that the SCN is incorporated in a neuronal feedback circuit arising from the NTS, modulating cardiovascular reactivity.

In **chapter three**, we investigated blood pressure regulation under the influence of antipsychotic drugs that induce broad cardiometabolic side effects. Second-generation antipsychotics (SGA) are associated with adverse cardiometabolic side effects contributing to premature mortality in patients and non-compliance. While mechanisms mediating these cardiometabolic side effects remain poorly understood, three independent studies recently demonstrated that melatonin was protective against cardio-metabolic risk in SGA-treated patients. We showed Olanzapine induces *c-Fos* immunoreactivity in the SCN with consecutive activation of the paraventricular nucleus (PVN) and dorsal motor nucleus of the vagus (DMV), indicating a potent induction of parasympathetic tone. Through cholera toxin B subunit (CtB) tracing we proved the existence of an SCN-parasympathetic neuronal pathway further illustrated by demonstrating a direct olanzapine-induced decrease in blood pressure and heart rate. Bilateral lesions of the SCN prevented the effects of olanzapine on parasympathetic activity. Likewise, melatonin abolished this olanzapine-induced SCN-parasympathetic pathway activation as well as its cardiovascular effects while brain areas associated with the beneficial effects of olanzapine, including the striatum, ventral tegmental area, and nucleus accumbens, remained activated. This is important as increased parasympathetic activity induced by olanzapine favors the appearance of cardiometabolic adverse effects like obesity, as well as lipid, insulin and glucose disturbances. In conclusion these results demonstrated the SCN is a key nucleus mediating the early side effects of olanzapine on cardiovascular function and showed melatonin has an opposing and potentially protective effect in olanzapine use.

Chapter two and three therefore illustrate that in addition to receiving information on BP excursions through its direct connections with the NTS, the SCN is also able to modify BP changes, altering its output dependent on the peripheral feedback it receives; as we showed e.g. by melatonin.

From cardiovascular regulation we shifted our focus to the importance of metabolic feedback. Seeing the influence of cardiometabolic changes on the SCN and considering early reports that metabolic changes influence neuropeptide Y (NPY) concentrations in the SCN we investigated the intergeniculate leaflet (IGL) which is classically known as the area of the thalamic lateral geniculate complex providing the SCN with non-photoc information via its NPY innervation. In **chapter four** we therefore investigated whether

this non-photoc input might also be related to the metabolic state of the animal. Using male Wistar rats in different metabolic states (refed, fasted, ad libitum), including a fasted monosodium glutamate-arcuate-lesioned and an IGL lesioned group we showed that it is the IGL, and not the ARC, that is at the origin of most NPY projections to the SCN and that the IGL responds to metabolic conditions. Fasting induces important changes in the NPY expression in the IGL, coinciding with similar changes of NPY/gamma-aminobutyric acid (GABA) projections of the IGL to the SCN. Consequently we demonstrated the SCN does not depend exclusively on the ARC or NTS to obtain information about the metabolic status of the body, but that the IGL can also transmit metabolic information that it receives from NTS and the Nucleus Gracilis. This integration of metabolic information in the SCN may serve to adapt its output to the periphery in order to regulate the physiology not only according to the day-night cycle but also to the energy status of the body.

Further investigating metabolic feedback to the SCN and the position of the SCN in the hypothalamic circadian network we investigated the function of SCN-ARC interconnectivity in **chapter five**. The ARC is known as *the* hypothalamic structure receiving metabolic information from the periphery and transmits information on e.g. food intake, temperature and reproductive changes to different hypothalamic centers. We showed the involvement of the SCN in this feedback circuit by demonstrating that fasting alters SCN activity. The importance of the ARC was demonstrated by placing knife cuts—between the SCN and ARC, so disrupting their connectivity—preventing this activity change in the SCN and thus illustrating the importance of ARC metabolic feedback to the SCN. Surprisingly, this interruption of SCN-ARC communication also resulted in a loss of rhythm in locomotor activity, temperature and corticosterone secretion in constant dark conditions. It did not affect SCN clock gene rhythmicity but caused the ARC to desynchronize its activity from the rhythm of the SCN. Moreover, when placing unilateral SCN lesions and contralateral knife cuts this resulted in the same arrhythmicity of physiology, indicating that it is indeed the reciprocal connections synchronizing the ARC with the SCN and that interaction between SCN and ARC is essential for the expression of circadian physiological rhythms. Moreover, following glucose administration in fasted animals, a decreased SCN c-Fos staining was observed in control animals, while the knife cut prevented all changes in SCN activity, demonstrating the importance of the ARC as metabolic modulator of SCN neuronal activity. This confirms that the SCN functions as part of a larger circadian network of tightly linked oscillatory feedback circuits whose integral function is essential for regulating physiological and behavioral functions.

Finally, to further illustrate the functioning of the SCN inside a larger tightly coupled circadian network, we investigated the organization of reproductive feedback to the SCN in **chapter six**. There we investigated the role of different hypothalamic areas including the ARC, the anteroventral periventricular nucleus (AVPV) and SCN in the

complex regulation of the reproductive cycle. In this chapter we demonstrated neuronal feedback received by the SCN through Kisspeptin (Kiss) neurons, known as a stimulating factor for gonadotropin-releasing hormone (GnRH) release. We showed that Kiss neurons, mainly expressed in the AVPV and ARC, project to the ventral part of the SCN where they form close apposition with vasoactive intestinal peptide (VIP) neurons. Interestingly, projections from Arg-Phe-NH₂ related peptide-3 or RFamide-related peptide-3 (RFRP-3) neurons (an inhibitory factor in GnRH release) in the dorsomedial hypothalamic nucleus also form close apposition with VIP neurons in the SCN. We showed that: 1) Kiss feedback to the SCN originates from both the AVPV and ARC, 2) Kiss expresses a diurnal variation in the SCN and 3) Kiss terminates on VIP neurons in the ventral part of the SCN. Interestingly, VIP neurons receiving direct retinal input have been proven critical in maintaining SCN synchrony. This indicates a role for Kisspeptin and RFRP-3 signaling to the SCN, whereby their influence may provide a basis for the optimal conditions under which the LH surge and consequential ovulation can take place synchronizing behavior and the estrous cycle. Herein, we argue, the SCN may form part of a Kisspeptin feedback network to adequately time the reproductive cycle in accordance with seasonal, diurnal and metabolic environmental changes.

In **chapter seven** we draw several conclusions from the presented chapters and we examine possible implications of our findings for the clinic. We argue that long-term deleterious feedback to the SCN, e.g. by untimely food intake or activity, causes circadian desynchronization, which is associated with chronic diseases such as diabetes, hypertension, cancer, and psychiatric disorders. We examine evidence that these diseases might stem from the SCN, where desynchronized peripheral feedback disrupts/modifies its output.

We conclude that these multiple intertwined feedback loops of the circadian system make it robust and adaptable, capable of withstanding brief erroneous feedback. However, months or years of conflicting feedback, ill-timed behavior or chronic jetlag/shift work, will increase an individuals' susceptibility to pathology and disease.

Further investigation and insight in the complexity of the day-night organization of physiological functions and all the ramifications of the circadian system, will be necessary to better understand the functional changes taking place in adverse conditions and pathogenesis. A more broad application of circadian timing principles when developing new therapeutic strategies should also be made. This will likely maximize efficacy, reduce toxicity, and decrease the adverse effects patients experience from drugs during their treatment.

Nederlandse Samenvatting

In dit proefschrift onderzoeken en bespreken wij de rol van de suprachiasmatische kern (SCN) die, als onderdeel van een circadiaan netwerk, perifere feedback integreert om zo dag-nacht fluctuaties in de fysiologische functies van het lichaam effectief te reguleren.

De aarde draait om de zon, wat ons seizoenen brengt, terwijl hij tevens iedere 24 uur om zijn as draait. Hierdoor worden alle organismen op aarde blootgesteld aan wisselende cycli van licht en duisternis en de daarmee samenhangende veranderingen in de omgeving. Bij zoogdieren stimuleert een centrale autonome pacemaker, gelegen in de hypothalamische SCN, ritmisch gedrag met samenhangende neuro-endocrine en autonome output, en voorziet zo in een dagelijkse, 24-uurs organisatie van de fysiologie. De endogene aard van de neuronale activiteit die gegenereerd wordt in de SCN zorgt ervoor dat gedrags- en fysiologische ritmes worden gehandhaafd, zelfs in constant donker (DD). De SCN bevindt zich net boven het optisch chiasma (suprachiasmatisch) waardoor het direct fotonische (licht) informatie van de retina ontvangt. Dit omgevingslicht, wat een exact 24-uurs ritme heeft, synchroniseert de SCN die middels zijn moleculaire SCN-klok een ~24-uurs of circadiaan ritme aanhoudt met de buitenwereld. Dit circadiane timingmechanisme stelt organismen in staat om te anticiperen op dag-nachtveranderingen die optreden in de omgeving, waarbij de fysiologie wordt voorbereid op aankomende slaap of activiteits periode.

In **hoofdstuk één** introduceren wij de moleculaire organisatie van SCN-activiteit en leggen wij uit hoe klokgenen, die in bijna alle celtypen van de hersenen en het lichaam voorkomen, betrokken zijn bij het handhaven van het autonome ritme van de SCN en het ritme van onze organen. SCN-neurotransmitters zijn essentieel in het coördineren van het ritme tussen de verschillende cellen binnen de SCN, maar ook om circadiane signalen door te geven aan verschillende kernen in de hypothalamus en daarbij aan de hersenen en de periferie (het lichaam). Wij bespreken talrijke uitgevoerde experimenten die het belang illustreren van de SCN voor het organiseren van circadiane ritmes. Hierbij is feedback vanuit de periferie essentieel is voor het reguleren en aanpassen van dagelijkse oscillaties in de SCN en in de fysiologie. Licht is daarbij niet de enige input of synchroniserend signaal van de SCN maar de SCN ontvangt ook feedback van onder meer hormonen, andere hersenkernen en organen. In dit proefschrift onderzochten wij de mogelijkheid of de SCN deel uitmaakt van een groter netwerk van oscillatoren die allemaal functioneren binnen een reeks feedbackcircuits die het organisme met zijn omgeving synchroniseert.

In **hoofdstuk twee** hebben wij onderzocht hoe de SCN is ingebouwd in een dergelijk neuronaal feedbackcircuit dat middels de nucleus tractus solitarius (NTS) cardiovasculaire veranderingen reguleert. De NTS, gelokaliseerd in de medulla, staat bekend als het integratiecentrum van viscerale informatie waaronder bloeddruk (BP). Wij toonden

het belang aan van de SCN als een integratieplaats van fysiologische circuits die BP reguleren. In Wistar ratten lieten wij zien dat de SCN cardiovasculaire feedback ontvangt via de NTS. Glutamaat is een van de neurotransmitters die betrokken is bij deze NTS-SCN neuronale verbinding. De NTS projecties eindigen in het ventrolaterale deel van de SCN waar ook lichtinformatie de SCN binnenkomt. Met behulp van de transcriptiefactor voor snelle genexpressie c-Fos als een neuronale activiteit marker, toonden wij aan dat de activiteit van de SCN direct veranderd na bloeddrukveranderingen in de periferie. Dit leverde bewijs voor een directe neuronale feedbackroute van de NTS naar de SCN die bloeddruk-informatie verzendt. Bovendien lieten wij zien dat er actieve betrokkenheid is van de SCN bij de regulering van de korte termijn bloeddruk fluctuaties. Zodoende hebben wij bewijs geleverd dat de SCN is opgenomen in een neuronaal feedbackcircuit dat voortkomt uit de NTS en cardiovasculaire reactiviteit moduleert.

In **hoofdstuk drie** onderzochten wij bloeddrukregulatie onder invloed van antipsychotica die brede cardiometabole bijwerkingen veroorzaken. Tweede generatie antipsychotica (SGA) zijn geassocieerd met cardiometabole bijwerkingen die bijdragen aan vroegtijdige sterfte bij patiënten en therapieontrouw. Hoewel mechanismen die deze cardiometabole bijwerkingen mediëren nog steeds slecht worden begrepen, hebben drie onafhankelijke onderzoeken onlangs aangetoond dat melatonine beschermt tegen de cardiometabole bijwerkingen die zijn geassocieerd met SGA behandelde patiënten. Wij toonden aan dat olanzapine de immunoreactiviteit van c-Fos in de SCN induceert met daaropvolgende activatie van de paraventriculaire kern (PVN) en de dorsale motorische kern van de vagus (DMV), wat wijst op een verhoging van de parasymphatische tonus. Door middel van de cholera-toxine B (CtB) tracer hebben wij het bestaan aangetoond van een SCN-parasymphatisch neuronaal circuit. Het bestaan van dit circuit werd ondersteund door het aantonen van een directe door olanzapine geïnduceerde verlaging van de bloeddruk en hartslag. Bilaterale laesies van de SCN voorkwamen de effecten van olanzapine op de parasymphatische activiteit. Op gelijke wijze zorgde melatonine ervoor dat deze olanzapine geïnduceerde activatie van het SCN-parasymphatische neuronale circuit achterwege bleef. Opvallend genoeg bleven hersengebieden die geassocieerd zijn met de gunstige effecten van olanzapine, waaronder het striatum, het ventrale tegmentale gebied en de nucleus accumbens, geactiveerd. Dit is belangrijk omdat de door olanzapine geïnduceerde verhoogde parasymphatische activiteit geassocieerd is met ontstaan van cardiometabole pathologie zoals obesitas, alsook lipiden-, insuline- en glucosestoornissen. Samengevat toonden deze resultaten aan dat de SCN een kern is dat de vroege bijwerkingen van olanzapine op de cardiovasculaire functie medieert en dat melatonine een tegengesteld en potentieel beschermend effect heeft op het ontstaan van de bijwerkingen geassocieerd met het gebruik van olanzapine.

Hoofdstuk twee en drie illustreren niet alleen dat de SCN informatie over BP-excursies via zijn directe verbindingen met de NTS ontvangt, maar dat de SCN ook in staat is BP

veranderingen op te vangen door zijn output aan te passen afhankelijk van de perifere feedback.

Van cardiovasculaire regulatie verlegden wij ons focus naar het belang van het ontvangen van metabole feedback. Gezien de invloed van cardiometabole veranderingen op de SCN en rekening houdend met studies die hebben aangetoond dat metabole veranderingen de neuropeptide Y (NPY) concentraties in de SCN beïnvloeden, hebben wij de intergeniculate leaflet (IGL) onderzocht. De IGL staat erom bekend dat het de SCN van non-fotische informatie voorziet via zijn NPY projecties. In **hoofdstuk vier** hebben wij daarom onderzocht of deze niet-fotische input ook gerelateerd kan zijn aan de metabole status van het dier. Wij onderzochten mannelijke Wistar ratten in verschillende metabole condities (gevoed na vasten, nuchter, ad libitum) en een groep gevaste dieren met monosodiumglutamaat en IGL-laesies. Hierbij lieten wij zien dat het de IGL en niet de ARC is, die de oorsprong vormt van de meerderheid van de NPY projecties naar de SCN en dat de IGL reageert op veranderende metabole condities. Vasten induceert belangrijke veranderingen in de NPY-expressie in de IGL die samenvallen met vergelijkbare veranderingen van NPY en gamma-aminoboterzuur (GABA) projecties van de IGL naar de SCN. Daarbij hebben wij aangetoond dat de SCN niet uitsluitend afhankelijk is van de ARC of NTS voor het verkrijgen van informatie over de metabole staat van het lichaam, maar dat ook de IGL metabole informatie verzendt die deze informatie op zijn beurt van de NTS en Nucleus Gracilis ontvangt. Deze integratie van metabole informatie in de SCN kan dienen om de output van de SCN aan te passen aan veranderende omstandigheden. Dit om de fysiologie niet alleen te reguleren volgens een dag-nacht ritme, maar ook afhankelijk van de energiestatus van het lichaam.

Om de metabole feedback naar de SCN en de positie van de SCN in het hypothalame circadiane netwerk verder te onderzoeken, hebben wij in **hoofdstuk vijf** de functie van de SCN-ARC-interconnectiviteit onderzocht. De ARC staat bekend als de hypothalame structuur die metabole informatie ontvangt van de periferie en informatie doorstuurt naar verschillende hypothalame kernen over bijvoorbeeld voedselinname, temperatuur en reproductieve veranderingen. Wij lieten de betrokkenheid van de SCN zien in dit feedbackcircuit door aan te tonen dat vasten de SCN-activiteit verandert. Wij toonden vervolgens het belang van de ARC hierin aan door micro-messneden te plaatsen tussen de SCN en ARC. Dit verstoort de directe connectiviteit tussen de twee kernen waardoor de eerder aangetoonde veranderingen in SCN activiteit na vasten werden voorkomen. Verrassend resulteerde deze onderbreking van SCN-ARC-communicatie ook in een verlies van ritme in motorische activiteit, temperatuur en corticosteron secretie in constant donker omstandigheden. Het had echter geen invloed op de ritmiciteit van SCN-klokgenen maar zorgde ervoor dat de activiteit van de ARC zich desynchroniseerde met die van de SCN. Bovendien resulteerde het plaatsen van unilaterale SCN-laesies en contralaterale messneden in diezelfde aritmie van de fysiologie. Dit gaf aan dat het inderdaad de

reciproque SCN-ARC verbindingen zijn die de ARC synchroniseren met de SCN en dat die interactie tussen SCN en ARC essentieel is voor de expressie van circadiane fysiologische ritmes. Bovendien werd na glucosetoediening in nuchtere dieren een afgenomen SCN c-Fos-kleuring waargenomen in vergelijking met controledieren. Een messnede tussen SCN en ARC voorkwam deze verandering in SCN-activiteit, hetgeen het belang aantoont van de ARC als metabole modulator van SCN-neuronale activiteit. Dit bevestigt tevens dat de SCN functioneert als onderdeel van een groter circadiaans netwerk van nauw verbonden oscillerende feedbackcircuits waarvan de integrale functie essentieel is voor het reguleren van de fysiologie en gedrag.

Tenslotte, om de werking van de SCN binnen een groter, nauw gekoppeld, circadiaans netwerk verder te illustreren, hebben wij de organisatie van reproductieve feedback op de SCN in **hoofdstuk zes** onderzocht. We bekeken de rol van verschillende hypothalamische gebieden, waaronder de ARC, de anteroventrale periventriculaire nucleus (AVPV) en de SCN in de complexe regulatie van de reproductieve cyclus. Wij demonstreerden dat de SCN neuronale feedback ontvangt van Kisspeptin (Kiss) neuronen; bekend als een stimulerende factor voor de afgifte van gonadotropin-releasing hormone (GnRH). Wij lieten zien dat Kiss neuronen, die zich voornamelijk in de AVPV en ARC bevinden, naar het ventrale deel van de SCN projecteren waar ze contact maken met vasoactieve intestinale peptide (VIP) neuronen. Interessant is dat projecties van Arg-Phe-NH₂ related peptide-3 of Rfamide-related peptide-3 (RFRP-3) neuronen (een remmende factor in GnRH-afgifte) in de dorsomediale hypothalamische nucleus ook soortgelijke contacten vormen met VIP-neuronen in de SCN. Wij toonden aan dat: 1) Kiss feedback naar de SCN afkomstig is van zowel de AVPV als de ARC, 2) Kiss expressie een dagelijkse fluctuatie in de SCN laat zien en 3) Kiss eindigt op VIP-neuronen in het ventrale deel van de SCN. Interessant is dat VIP-neuronen die directe retinale input ontvangen, van cruciaal belang zijn gebleken om de SCN gesynchroniseerd te houden. Dit duidt er op dat de invloed van Kisspeptine en RFRP-3 op de SCN een basis kan vormen voor de specifieke omstandigheden waaronder de LH-piek en de daaruit voortvloeiende ovulatie kan plaatsvinden, gesynchroniseerd met gedrag. Hierin, zo stellen wij, kan de SCN deel uitmaken van een Kisspeptin-feedbacknetwerk om de reproductieve cyclus adequaat te timen in overeenstemming met seizoensgebonden, dagelijkse en metabole veranderingen van de omgeving.

In **hoofdstuk zeven** trekken wij een aantal conclusies uit de gevonden resultaten in de verschillende hoofdstukken en tonen wij aan hoe onze bevindingen mogelijke implicaties hebben voor de kliniek. Wij stellen dat schadelijke feedback naar de SCN op de lange termijn, bijvoorbeeld bij activiteit of inname van voedsel buiten de door de SCN aangegeven momenten, circadiane desynchronisatie veroorzaakt wat geassocieerd is met het ontstaan van chronische aandoeningen zoals diabetes, hypertensie, kanker en psychiatrische stoornissen. Dit leidt tot de hypothese dat deze ziekten veroorzaakt kunnen worden

door een disbalans tussen de signalen van de SCN, en de gedesynchroniseerde perifere feedback welke de output van de SCN verstoort/aanpast.

Wij concluderen dat onder normale omstandigheden deze in elkaar verweven feedbackcircuits het circadiane systeem robuust en aanpasbaar maakt, in staat om kortdurende verstoringen goed te weerstaan. Echter, maanden of jaren van tegenstrijdige feedback, slecht getimed gedrag of chronische jetlag / ploegendiensten zullen voor individuen de kans op het ontwikkelen van pathologie en ziekte vergroten.

Verder onderzoek en inzicht naar de complexiteit van de dag-nacht, waak-slaap, organisatie van fysiologische functies en alle vertakkingen van het circadiane systeem zullen noodzakelijk zijn om de functionele veranderingen die plaatsvinden gedurende ongunstige omstandigheden en tijdens pathogenese beter te begrijpen. Het is noodzakelijk dat er een bredere toepassing van circadiane timing bij het ontwikkelen van nieuwe therapeutische strategieën wordt gemaakt. Dit zal waarschijnlijk de werkzaamheid van geneesmiddelen kunnen maximaliseren en de toxiciteit en bijwerkingen kunnen verminderen die patiënten ervaren tijdens hun behandeling.

Author Affiliations

MariCarmen Basualdo

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Gloria Benítez-King

Laboratorio de Neurofarmacología, Subdirección de Investigaciones Clínicas, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico city, DF, Mexico

Frederik N Buijs

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico
Netherlands Institute for Neuroscience, an Institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, The Netherlands

Ruud M Buijs

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Fernando Cazarez-Márquez

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

David Centurion

Department Farmacobiología, CINVESTAV, Mexico city, DF, Mexico

Carolina Escobar

Departamento de Anatomía, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Nathalie Guerrero-Vargas

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico
Departamento de Anatomía, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Mara A Guzmán-Ruiz

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Departamento de Anatomía, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Gerhard Heinze

Departamento de Psiquiatría y Salud Mental, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Andries Kalsbeek

Netherlands Institute for Neuroscience, an Institute of the Royal Netherlands Academy of Arts and Sciences, Amsterdam, The Netherlands

Luis León-Mercado

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Mercedes Perusquía

Endocrinology of Reproduction Laboratory, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Francisco Romo-Nava

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Departamento de Psiquiatría y Salud Mental, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Department of Psychiatry and Behavioral Neuroscience, Division of Bipolar Disorder Research, University of Cincinnati, Cincinnati, Ohio

Nadia Saderi

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Laboratorio de Biología Celular y Fisiología, Facultad de Ciencias, Universidad Autónoma de San Luis Potosí, San Luis Potosí (SLP), Mexico

Roberto C Salgado-Delgado

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Laboratorio de Biología Celular y Fisiología, Facultad de Ciencias, Universidad Autónoma de San Luis Potosí, San Luis Potosí (SLP), Mexico

Frank AJL Scheer

Division of Sleep Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, United States

Eva Soto-Tinoco

Laboratory of Hypothalamic Integration Mechanism, Instituto de Investigaciones Biomedicas, Universidad Nacional Autónoma de México (UNAM), Mexico city, DF, Mexico

Marcela Valdés-Tovar

Laboratorio de Neurofarmacología, Subdirección de Investigaciones Clínicas, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico city, DF, Mexico

List of Publications

1. The NPY intergeniculate leaflet projections to the Suprachiasmatic nucleus transmit metabolic conditions. Saderi N, Cazarez-Márquez F, Buijs FN, Salgado-Delgado RC, Guzman-Ruiz MA, del Carmen Basualdo M, Escobar C, Buijs RM. *Neuroscience*. 29;246:291-300. (2013)
2. The Suprachiasmatic nucleus is part of a neural feedback circuit adapting blood pressure response. Buijs FN, Cazarez F, Basualdo MC, Scheer FA, Perusquía M, Centurion D, Buijs RM. *Neuroscience*. 25;266:197-207. (2014)
3. The circadian system: A regulatory feedback network of periphery and brain. Buijs FN, León-Mercado L, Guzmán-Ruiz M, Guerrero-Vargas NN, Romo- Nava F, Buijs RM. *Physiology (Bethesda)*. 31(3):170-81. (2016)
4. Suprachiasmatic nucleus interaction with the Arcuate nucleus; Essential for organizing physiological rhythms. Buijs FN, Guzmán-Ruiz M, León- Mercado L, Basualdo MC, Escobar C, Kalsbeek A, Buijs RM. *Eneuro* 4 (2). (2017)
5. Olanzapine-induced early cardiovascular effects are mediated by the biological clock and prevented by melatonin. Romo-Nava F, Buijs FN, Valdés-Tovar M, Benítez-King G, Basualdo M, Perusquía M, Heinze G, Escobar C, Buijs RM. *J Pineal Res*. 62(4). (2017)
6. The Suprachiasmatic nucleus is part of a Kisspeptin feedback network involving the anterior ventral part of the third ventricle and Arcuate nucleus. Buijs FN, Soto-Tinoco E, Basualdo MC, Kalsbeek A, Buijs RM. To be submitted

Outside thesis

7. A role for VGF in the hypothalamic Arcuate and Paraventricular nuclei in the control of energy homeostasis. Saderi N, Buijs FN, Salgado-Delgado R, Merkenstein M, Basualdo MC, Ferri GL, Escobar C, Buijs RM. *Neuroscience*. 8;265:184-95. (2014)
8. Neuropeptide changes in the Suprachiasmatic nucleus are associated with the development of hypertension. Yilmaz A, Buijs FN, Kalsbeek A, Buijs RM. Submitted

Portfolio

Frederik Nicolaas Buijs

PhD period: February 2013 - January 2019

Promotores: Prof. dr. A. Kalsbeek and Prof. dr. D. F. Swaab

Courses

2013 Animal Surgery, Facultad de Medicina, UNAM

2013 Introduction in experimental Sciences, Facultad de Medicina, UNAM

2013 Biostatistics, Instituto de Investigaciones Biomedicas, UNAM

2013 Introduction in Neurosciences, Facultad de Medicina, UNAM

2013 Chronobiology in mammals, Instituto de Investigaciones Biomedicas, UNAM

2014 Neurochemistry, Facultad de Medicina, UNAM

2014 Comparative Endocrinology, Instituto de Investigaciones Biomedicas, UNAM

2014 Neuroscience of the body, Instituto de Investigaciones Biomedicas, UNAM

Seminars

Weekly department seminars

Presentations

14-18 June 2014

Big Sky, Montana, United states of America

Poster presentation: Interaction between the arcuate nucleus and suprachiasmatic nucleus is essential for activity, temperature and corticosterone circadian rhythmicity

SRBR, Society for Research on Biological Rhythms conference

12-17 June 2011

Lucca, Italy

Poster presentation: The suprachiasmatic nucleus is involved in blood pressure regulation

Chronobiology, Gordon Research Conference

5-9 May 2011

Puebla, Mexico

Poster presentation: The suprachiasmatic nucleus is involved in blood pressure regulation

III World Congress of Chronobiology

Teaching

Student coaching/mentor scientific research projects – Medical/Psychology bachelor students

Acknowledgments

The last, but not least important part of my thesis. My acknowledgements to all that have made this thesis possible, as it is not a solitary endeavor. Without the support of many, I would only have achieved little.

I cannot start my acknowledgments without naming Inez Bausch. She has always been a joyful little ray of sunlight in our family and she was like a little big sister to me. Inez, you will always have a special place in my heart.

Pappa, I owe you all my thanks for your tireless support which enabled me to realize this thesis. First of all, you have been my scientific mentor. I greatly enjoyed our afternoon walks in the botanic garden where we had discussions on life but also many brainstorm sessions thinking of different hypothesis' for surprising experimental outcomes ever molding our vision on how the circadian system is organized. I have very much enjoyed building my thesis one experiment at a time; developing my scientific understanding, my analytical skills and learning to solve the most challenging puzzles. You were always there to guide me in the right direction.

Secondly you were there as my father. I feel lucky that I have been able to experience working side by side with my dad. Working closely together on something that, I see, brings you so much joy. It was an opportunity many do not get.

Without your flexibility, endless positivity and perseverance I might not have made it to the finish line. We came from far. We have had a difficult period where we spoke little. You initiated the opportunity for me to come to Mexico, for us to work together on this big project. It opened up the way for us to resolve our differences. Pap, thank you for this unique experience and great adventure that we completed together. I am happy I decided to take the leap across the ocean creating a warm memory I will forever cherish.

Mamma. Without you I would literally not have been here, nor where I find myself today. You have always been there for me, giving me words of wisdom, holding up the mirror so I could reflect, challenging my views but most of all, taking time to listen. Mam, I hold dear the special bond we have. The trips we have made, the museums and concerts we visit together. You helped view things in a different light and have always forced me see the other side of the coin. While this is not always easy, it has given me an open mind. Something I have come to depend on while maneuvering through life. But this open mind has also enabled me to maneuver through hypotheses', thinking of new experiments or trying to solve complicated puzzles on brain feedback circuits. Thank you for the endless support during the ups and down I experienced while making this thesis. We have been through a difficult time as a family, though in each other we found strength and support, and it brought us closer together. For this I am grateful.

Hannah and Dorthe, my two older sisters. What would I have done without you? I feel so blessed having two older sisters who I could always count on. My thesis has been a scientific journey but also an emotional journey for many reasons. You have laughed with me, cried with me, struggled with me and been angry with me. I was in Mexico but you were never far away. With you I never feel alone, no matter where I am I know there is always someone looking over my shoulder, someone who I can always call.. Hannah and Dorthe, thank you for your faith in a good ending, your positivity and your never-ending support for me and my work.

Many many thanks to my friends in Amsterdam whom I have neglected for two and a half years while living in Mexico. You welcomed me with open arms when I came back as if I had never left. That is true friendship.

Thijs, you are both the small devil on my shoulder and the angel watching over me. You were there to distract me when I was down and.....when I had to work. You supported me when times got tough and I spent nights/ weekends/ vacations working on my thesis. You never stopped reminding me of my strengths, when I was focusing on my weaknesses. Without you this project would have remained a life's work. Thank you for being there and being my friend, I hold it dear.

Ernst and Stephan, my paranymphs, my personal consultants, my best friends.

Stephan, you were there to coach me when things seemed insurmountable. From the strong friendship we have built up the past 15 years, you know me all too well. It is through this understanding that you could really help me. You highly required help with my time management, organizational skills and tireless pushing for me to finish my damn thesis already. I would play my part by repeating it was almost done. Thank you for those many last pushes and giving me the opportunity to now actually be able to thank you for helping me cross the finish line.

Ernst, you gave me great support and advice as an excellent planner. We talked so much about how I could better organize my work, I believe you just might have caused some permanent alterations to my hardwiring. You persuaded me back to work, pushed me when I needed pushing and never stopped believing in me. I missed a difficult moment in your life when I was in Mexico, it was not easy being so far away. You had to make an important choice in your career and you chose for family and friendship, something that has also helped us remain close. I value what we have built up... You and Stephan both made invaluable contributions to my work and without you two I could not have finished this once in a lifetime project. Thank you for being my best friends and my advisors.

Bart, I cherish our deep conversations and our superficial ones, our hilarious moments and our never to forget time at Europaplein. This is where we really got to know each other's inner child and respect one another as adult. You saw me embark on this wild adventure and have piloted me through the rough seas I encountered on the way. You have been there through the length of my endeavor, no matter how tough it got. Thank you for

being there as my best friend, without you I could not have pulled this off.

Tammo, roommate, friend for life. We have had quite a rollercoaster ride together in the past two years, but we pulled each other through the both fun and hard times. I enjoy our nights philosophizing about important issues and trivial subjects, women, work, sales tactics, writing books, women, friendship, sports, women.... Thank you for being there, your endless support and uplifting personality.

Jan, I love those moments where our worlds meet and amplify each other. Thank you for the great time I had with you at Roelof Hart. I truly value the friendship that has arisen from it.

Tommie, little Buddha, thank you for your wisdom and being there for me. Remember, I will always be there for the journey of your own as well.

Thanks to you, my friends I have not named, I have not forgotten you.

To my colleagues at the OLVG, UMC (Dane, thank you for the insightful nightly discussions) and AMC, thank you. I look back at great moments.

Hillechien, you have been blessed with seeing me finish this thesis but missing most of the struggles, frustration and late hours spent finishing this thing. You now bear the fruit of me having time, so we can embark on a brand-new adventure, together exploring every corner of the world. I cannot wait...

A great mention and thank you for all my friends in Mexico who have welcomed me with open arms when first arriving to Mexico. You made me feel right at home and have made my time in Mexico an unforgettable experience.

A special thanks to you MariCarmen. You have helped me tremendously with the technical aspects of my research. Your special gift and talent made it all seem easy, until I tried it myself. Without you I would not have had anything to write about. Muchas gracias por tu tiempo y tu paciencia. Likewise, many many thanks to my co-authors, colleagues and friends from the UNAM Biomedicas lab. My respect for your work ethic, always helping hand and openness in making Mexico City my second home. Eva, Nathalie, Mara and Luis, thank you. Fernando, amigo, hermano, mi tutor en Español/Chilango y science liaison. You taught me discipline and perseverance. Without you, getting up at 6 AM every morning, go running and head for the lab would not have been possible. A la chamba! Thank you for accompanying me during the weekend and evening lab sessions and for chela's when we needed them.

Daniel y Patrick, gracias por estar mis hermanos Mexicanos. You guys welcomed me in your family and made me feel I truly had two brothers on the other side of the ocean. Thank you for taking me in and showing me how great it is to be a Chilango.

Dear Prof. Swaab and Prof. Kalsbeek. Dick and Dries, thank you for being my promotores, my advisors, for leading me through the process of creating this thesis. And above all... for your patience. You have had to guide me while I was in Mexico, which was not always easy. After that, I took on a full-time position at the OLVG trying to juggle my job and my PhD at the same time. This delayed my progress considerably, but you did not lose hope. Through much patience, asking good critical questions and valuable advice you have allowed me to bring this thesis to a good ending. It has been a great journey and a valuable learning experience. Thank you for your trust and willingness to embark on this journey together.

Dear Prof. van Dijk, Prof. la Fleur, Prof. Fliers, Prof. Meijer, dr. van Montfrans and dr. Yi. Thank you for being willing to be part of my thesis committee. I hope you have enjoyed reading my thesis and will likewise enjoy my defense.

