The Role of Noradrenaline in Energy Homeostasis

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Contents

Abbreviations

α/β-MSH	α/β-melanocyte stimulating hormone
A	Adrenaline/adrenergic
AB	Antibody
AADC	Aromatic amino acid decarboxylase
AC	Adenylate cyclase
AD	Alzheimer's disease
AgRP	Agouti-related protein
AMPK	Adenosine monophosphate-activated protein kinase
AP	Action potential
ArP	Area postrema
AR	Adrenergic receptor/adrenoceptor
ARC	Arcuate nucleus
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BBB	Blood-brain-barrier
BMI	Body mass index
CA	Catecholamine
cAMP	Cyclic adenosine monophosphate
CART	Cocaine-amphetamine regulated transcript
CEC	Chloro-ethyl-clonidine
CNQX	6-Cyano-7-nitroquinoxaline-2,3-dione (AMPA/kainate receptor antagonist)
CNS	Central nervous system
CPP	Conditioned place preference
DA	Dopamine/dopaminergic

DAG	Diacylglycerine
DAP-5	DL-2-amino-5-phosphopentanoic acid (NMDA receptor antagonist)
DBH	Dopamine β-hydroxylase
DMH	Dorsomedial hypothalamus
DMSO	Dimethyl sulfoxide
DMT2	Diabetes mellitus type 2
DMV	dorsal motor nucleus of the vagus
DNB	Dorsal noradrenergic bundle
FFA	Free fatty acids
GABA	γ-Aminobutyric acid
GE	Glucose-excited
GI	Glucose-inhibited
GIRK	G-Protein activated inwardly rectifying potassium channel
GK	Glucokinase
GLUT	Glucose transporter
GPCR	G-Protein coupled receptor
HFD	High-fat diet
icv.	intracerebroventricular
IP_3	Inositoltrisphosphate
IPSC	Inhibitory postsynaptic current
K_{ATP}	ATP-dependent potassium channel
LepR	Leptin receptor
LH	Lateral hypothalamus
LC	Locus Coeruleus
LC-NA	Locus Coeruleus noradrenergic
MC3/4R	Melanocortin receptor 3 and 4
NA	Noradrenaline/noradrenergic
NAP	Numerical aperture

NCD	Normal chow diet
NPY	Neuropeptide Y
NPY1R	Neuropeptide Y receptor type 1
NTS	Nucleus tractus solitarius
PD	Parkinson's disease
PI3K	Phosphatidylinositol-3-kinase
PIP	Phosphatidylinositolphosphate
PIP_2	Phosphatidylinositol-4,5-bisphosphate
PIP_3	phosphatidylinositol-3,4,5-bisphosphate
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PNMT	Phenylethanolamine-N-methyltransferase
PTX	Picrotoxin ($GABA_A$ receptor antagonist)
POMC	Proopiomelanocortin
PVH	Paraventricular nucleus
R_S	Series resistance
REM	Rapid eye movement
SF-1	Steroidogenic factor 1
SFA	Spike frequency adaptation
SNA	Sympathetic nerve activity
SNS	Sympathetic nervous system
TH	Tyrosine hydroxylase
VGCC	voltage gated Ca^{2+} conductance
VMH	Ventromedial hypothalamus
VNB	Ventral noradrenergic bundle
WAT	White adipose tissue
WD	Working distance

WHO World health organization

Abstract

Obesity is a condition that is associated with excessive weight gain and fat mass storage whose prevalence is increasing within western populations. A variety of co-morbidities are linked to obesity such as type 2 diabetes mellitus, cardiovascular diseases and neurodegenerative disorders, including Alzheimer's disease and Parkinson's disease. Together, this contributes to substantial costs of healthcare programs. In non-obese individuals, energy intake and energy expenditure is precisely matched over a long time period in order to maintain energy resources and fat mass. This mechanism, termed energy homeostasis is accomplished by regulatory neuronal networks in the central nervous system (CNS).

To better understand and counteract obesity and its co-morbidities, increasing efforts are being made to define the control mechanisms in the CNS, that regulate body weight and energy homeostasis. The focus of this study is the noradrenergic (noradrenaline; NA) modulation of energy homeostasis. Anti-obesity drugs, for example amphetamines, can exert strong anorexigenic effects on eating behaviour in humans. However, these drugs generally affect multiple transmitter and neuromodulator pathways, such as the dopaminergic and serotonergic system, leading to undesired side effects. Pharmacological studies indicate that the anorexigenic effect of amphetamine and related drugs are caused in part by modulation of the NA system. In order to devise strategies and develop specific drugs with minimized side effects in support of weight loss programs, it is critical to understand in detail the mechanisms in the CNS by which NA contributes to energy homeostasis.

Besides the well established role of the paraventricular nucleus of the hypothalamus in NA-mediated modulation of food intake, studies indicate that NA input on the homeostatic system in the arcuate nucleus of the hypothalamus (ARC) might also modulate eating behaviour. In the ARC, two key neuronal populations, pro-opiomelanocortin (POMC) and agouti-related peptide (AgRP) expressing neurons sense and integrate peripheral and nutritional signals. Once activated, POMC neurons promote satiety and

activation of AgRP neurons leads to food intake and decreased energy expenditure. Mechanisms that mediate the possible NA action in the ARC are unknown. In this study, the effect of NA on POMC and AgRP expressing neurons has been investigated. Application of NA inhibits POMC neurons, while AgRP neurons are excited. Pharmacological experiments revealed that these effects are mediated by α_{2A}- and α_{1A}-adrenergic receptors (AR). This suggests a potent NA modulation of food intake. With respect to these effects, afferent projections from NA nuclei and the conditions under which NA is released into the ARC are of greatest interest.

As a potential NA source, the locus coeruleus (LC) in the brainstem contains 50% of the NA neurons in CNS. Efferent projections from the LC to the ARC have been identified. Besides the contribution to autonomic functions in general, studies indicate that the LC is also involved in glucose metabolism and the control of brown adipose tissue (BAT). Moreover, BAT thermogenesis is dependent on NA and plasma glucose. Therefore, the effects of changes in extracellular glucose concentrations have been investigated. Around 40% of neurons in the LC responded with increasing spike frequency due to elevated glucose levels, identifying these neurons as glucose-excited. A small subpopulation responded with a moderate inhibition and is considered as glucose-inhibited. Expression of a mutant variant of the ATP dependent potassium channel in mice silenced a large number of LC neurons and abolished responses to glucose. Moreover, sympathetic nerve activity was reduced and led to a white-adipose-tissue-like morphology of BAT, alongside with impairment of thermogenesis. As a consequence of decreased energy expenditure, these mice developed obesity.

The modulation of POMC and AgRP neurons by NA indicates a critical role of the catecholamine in the control of energy homeostasis. Moreover, this study reveals that the LC contains glucose-sensing neurons and contributes to the control of glucose metabolism and the activity of BAT. Its projection patterns in the CNS identify the LC as a potential source for NA release into the ARC. These results lead to new insights and the expansion of the current role of NA in the control of energy homeostasis. Importantly, this may help to develop new strategies and drugs with minimized side effects in the treatment of obesity.

Zusammenfassung

Adipositas stellt ein stark zunehmendes Gesundheitsproblem in Industrieländern dar. Eine Reihe an Begleiterkrankungen, wie Typ 2 Diabetes Mellitus , Herzkreislaufkrankheiten und neurodegenerative Störungen, wie z.B. Alzheimer und Parkinson verursachen hohe Zusatzkosten für Gesundheitssysteme. Bei nicht-adipösen Individuen sind Energieaufnahme und Verbrauch sehr präzise aufeinander abgestimmt, um über längere Zeit Stabilität von Energiereserven und Fettgewebe herzustellen. Dies ist definiert als Energiehomöostase und wird vom zentralen Nervensystem (ZNS) kontrolliert.

Um Adipositas-assoziierte Erkrankungen zu vermeiden, werden große Anstrengungen unternommen, die Regulation der Energiehomöostase im ZNS besser zu verstehen und zu beeinflussen. Die vorliegende Arbeit beschäftigt sich mit der noradrenergen (Noradrenalin; NA) Modulation der Energiehomöostase. Amphetamine und verwandte Wirkstoffe können Hunger-unterdrückende Wirkung haben. Dabei werden in der Regel mehrere Transmitter- bzw. Neuromodulatorsysteme beeinflusst, welches unerwünschte Nebenwirkungen zur Folge haben kann. Pharmakologische Studien deuten darauf hin, dass Amphetamine und verwandte Substanzen in der Behandlung von Adipositas zum Teil auf das NA System wirken. Detailliertes Wissen, wie NA auf die Energiehomöostase wirkt, ist daher unabdingbar in der Entwicklung von Medikamenten, die Adipositas-Patienten bei ihrer Therapierung unterstützend begleiten sollen.

Neben der bekannten NA Modulation der Nahrungsaufnahme im paraventrikulären Hypothalamus, sprechen Untersuchungen zusätzlich dafür, dass NA auch direkt im Arcuate Nucleus des Hypothalamus (ARC) wirkt. Dieser gilt als Schlüsselregion in der Kontrolle von Nahrungsaufnahme und Energieverbrauch. Er enthält unter anderem zwei Neuronenpopulationen, proopio-melanocortin (POMC) exprimierende und Agouti-related peptide (AgRP) exprimierende Neurone, die Signale aus der Peripherie im Zusammenhang mit dem Nahrungsstatus eines Körpers aufnehmen und weiterverarbeiten können. Die Erregung von POMC Neuronen hat Sattheit zur Folge, während

die Erregung von AgRP Neuronen in Nahrungsaufnahme und gesteigertem Energieverbrauch resultiert. Die Wirkmechanismen von NA im ARC sind bisher nicht geklärt. Daher wurde der Effekt von NA auf POMC und AgRP Neurone untersucht. NA inhibiert konzentrationsabhängig POMC Neurone, während es AgRP Neurone anregt. Diese Effekte werden durch die Aktvierung von α_{2A} - und α_{1A}-adrenergen Rezeptoren vermittelt. Aufgrund dieser Effekte sind die NA Projektionen in den ARC und die Umstände in denen NA dort ausgeschüttet wird von größtem Interesse.

Als eine mögliche Quelle beherbergt der Locus Coeruleus (LC) etwa 50% aller NA Neurone im ZNS. Die Innervation des ARC mit Efferenzen aus dem LC ist bekannt. Neben der Rolle des LC im autonomen Nervensystem, deuten Studien an, dass dieser sowohl an der Glukosehomöostase, als auch an der glukose- und NA-abhänigen Kontrolle des braunen Fettgewebes (BAT) beteiligt ist. In Anbetracht dieser Daten wurde untersuch, ob Neurone im LC auf Änderungen in der extrazellulären Glukosekonzentration reagieren. Etwa 40% der untersuchten Neurone erhöhten ihre Aktivität aufgrund einer höheren Glukosekonzentration und konnten daher als glukose-angeregt identifiziert werden. Ein kleiner Teil der Neurone reagierte mit einer Inhibition und wurde daher als glukose-inhibiert identifiziert. Die Expression eines mutierten ATP-abhängigen Kaliumkanals führte zur verminderter elektrischer Aktivität von LC Neuronen und verhinderte die Anregung aufgrund erhöhter extrazellulärer Glukosekonzentrationen. Weiterhin sank die Aktivität im Nervus Sympathikus und beeinflusste die Thermogenese im BAT negativ. Als Folge reduzierten Energieverbauchs entwickelten, die Mäuse Adipositas.

Effekte auf POMC und AgRP Neurone im ARC deuten auf eine Rolle NAs in der Energiehomöostase hin. Der LC beherbergt Neurone, die auf unterschiedliche Glukosekonzentrationen reagieren und zur Regulation des Glukosestoffwechsels und der Aktivität von BAT beitragen. In diesem Zusammenhang, identifizieren die Projektionen in den ARC, den LC als eine mögliche Quelle der NA-Ausschüttung. Die Ergebnisse dieser Arbeit beschreiben neue Erkenntnisse in der NA Modulation der Energiehomöostase. Dies könnte helfen neue Strategien und Medikamente zu entwickeln, die weniger Nebenwirkungen in der Behandlung von Adipositas zur Folge haben.

1 Introduction

Obesity, defined as an abnormal or excessive accumulation of body fat mass that impairs health, has become a worldwide epidemic with humans categorized as overweight or obese nearly doubled in number since the 1980s (World Health Organization, WHO; Fact sheet N°311). The increasing prevalence for obesity in both developed and non-developed countries is a major health threat in near future. Obesity and overweight are defined by the body-mass-index (BMI), a simple weight-for-height measure where a value greater than or equal to 25 is classified as overweight and a value greater than or equal to 30 is considered as obese (WHO, 2007). In 2008, 1.4 billion people, with the age of 20 or older were classified as overweight, of which 200 million men and 300 million women were listed as obese. This alarming trend can also be observed in children. By the end of 2010, 40 million children aged under five years were categorized as overweight, worldwide (WHO, Fact sheet N°311).

Certain co-morbidities associated with obesity are a major health problem. For instance, type 2 diabetes mellitus (T2DM) is diagnosed in a growing number of people throughout all ages (Must *et al.* , 1999). Besides, the risk for cardiovascular diseases like hypertension, stroke and heart attack, musculosceletal disorders and even certain forms of cancer, is increasing with higher BMIs (Guffey *et al.* , 2013; Lehrer *et al.* , 2013; Osmond *et al.* , 2009). Obesity also affects the function of the central nervous system (CNS). Alterations in brain morphology and decrease in brain volume have been associated with overweight or obesity in young adults (Bruce-Keller *et al.* , 2009). In part, these effects are also observed during aging and obesity increases the risk of neurodegenerative disorders including Alzheimer's disease (AD) and Parkinson's disease (PD) (Luchsinger, 2010; O'Rahilly, 2009). In 2012, the WHOs Regional Office for Europe reports that obesity is already responsible for 2-8 percent of overall health costs and 10-13 percent of deaths. Also in the United States, the increasing prevalence of obesity is responsible for substantial costs of health care programs. In 2009, a study by Finkelstein *et al.* (2009) evaluated the expected costs to be $85.7 billion. This has recently been extended to an

overall cost for obesity and obesity-related diseases of $209.7 billion (Cawley & Meyerhoefer, 2012). Due to this "economic burdon generated by obesity, its treatment has become one of the most urgent issues in medicine today" (Li & Cheung, 2009). It is critical to devise specific pharmacological strategies to assist obese patients in starting and maintaining a program of weight loss with no or minimized side effects. For example, amphetamine and its variants can be strong modulators of eating behaviour in humans, however strong side effects are a common problem of such anti-obesity strategies (Adan, 2013; Derosa & Maffioli, 2012; Fantasia, 2013). Therefore, neuronal mechanisms which underlie the control of body weight are of greatest interest in order to develop specific therapies in the treatment of overweight or obese patients.

In order to gain further knowledge about the neurotransmitter systems which regulate energy homeostasis, this thesis focusses on the specific role of noradrenaline (noradrenergic; NA) in this mechanism. In the following sections, a short introduction into the CNS in the general control of energy homeostasis with the focus on the melanocortin system of the hypothalamus is presented. As an example of a nutrient signal, glucose sensing is described as this mechanism is apparent in nuclei in the hypothalamus as well as in NA nuclei in the brainstem. The main focus of this study is the NA modulation of eating behaviour and its effect on the arcuate nucleus of the hypothalamus (ARC), a key region in the control of energy homeostasis. The locus coeruelus (LC) is introduced as a potential source for hypothalamic NA release and finally its role in glucose metabolism and control of brown adipose tissue (BAT) is described.

1.1 The central nervous system in control of energy homeostasis

Several heritable factors such as genetic predispositions and environmental influences like untimely food habits, reduced physical activity and increased food consumption along with unlimited access to food promote the development of overweight and obesity (Power, 2012). It is not massive periodic overconsumption that leads to obesity rather than a small mismatch in intake and expenditure over a long time period. A caloric consumption of 0.3 percent over energy expenditure is already sufficient for weight gain (Rosenbaum *et al.* , 1997). Energy homeostasis can be defined as the phys-

iological process whereby energy intake is matched to energy expenditure over time to promote the stability of energy resources stored as adipose tissue (Hagan & Niswender, 2012). This regulation is an exceedingly complex biological mechanism, which involves a variety of different biological behaviors and substrates. The question concerning how this regulation of food intake and energy expenditure is achieved has been thoroughly investigated. Initially regarded as a mechanism which is controlled by the body's periphery, it became increasingly clear that several parts of the CNS are critically involved in the regulation of energy homeostasis (Brobeck, 1946; Brobeck *et al.* , 1943).

1.1.1 The melanocortin system of the hypothalamus

A number of landmark studies highlighted the hypothalamus in the control of food intake (Anand & Brobeck, 1951; Kennedy, 1950; Mayer & Thomas, 1967). These studies led to the proposal of a "dual center model" with the ventromedial hypothalamus (VMH) being the "satiety center" and the lateral hypothalamus (LH) the "hunger center", as lesioning of one of these either decreases or increases food intake. Soon, the question arose of how these areas gather information to precisely determine levels of energy intake and expenditure. Parabiosis studies on lesioned rats led to the conclusion of a peripheral signal in relation to animals' lipostatics (Hervey, 1959). A rat with a lesion in the VMH was surgically connected with a normal rat, which allowed humoral factors to pass from one animal to the other. The lesioned rat developed obesity whereas its partner became hypohagic and lost weight, suggesting that a signal in proportion to the amount of fat mass is highly potent to inhibit food intake. Additional parabiosis studies on genetically obese mice, *ob/ob* and *db/db*, led to the assumption that the first lack the signal, while the latter are insensitive to it (Coleman, 1973, 1978). The later identification of the *ob* and *db* gene, which encode the hormone leptin and the respective receptor confirmed these experiments (Zhang *et al.* , 1994). These key studies led then to the further identification of various genes involved in the process of energy homeostasis, encoding peptides, receptors and transcription factors and most importantly the identification of the "melanocortin system" (Gao & Horvath, 2008).

The arcuate nucleus of the hypothalamus

The "melanocortin system" represents the key neuronal system in the control of energy homeostasis targeted by a large number of metabolic signals such as leptin, insulin, ghrelin as well as nutritional signals like glucose and free fatty acids (FFA; Brüning *et al.* 2000; He *et al.* 2006; Ibrahim *et al.* 2003; Parton *et al.* 2007; Spanswick *et al.* 1997, 2000). It is located in the ARC, which has an anatomically unique position because of its close proximity to fenestrated capillaries at the very medialbasal part of the hypothalamus (Burdakov *et al.* , 2005a; Ganong, 2000). Here, the blood brain barrier is highly permeable and thus provides access to peripheral signals for neurons (Benoit *et al.* , 2000; Cone *et al.* , 2001).

Two neuronal populations have been identified leading to opposing effects on food intake (Cone *et al.* , 2001). The first population expresses pro-opiomelanocortin (POMC) and cocaine- and amphetamine regulated transcript (CART). Activation of POMC neurons mediates satiety (anorexigenic). POMC is further cleaved into α- and β-melanocyte stimulating hormone (α- and β-MSH), which upon activity-dependent release stimulate the melanocortin receptor types 3 and 4 (MC3R, MC4R) in target areas and leading to reduced food intake and satiety (Boston *et al.* , 1997; Cone, 2005; Ellacott & Cone, 2004).

In contrast, neuropeptide Y (NPY) containing neurons in the ARC, expressing the agouti-related peptide (AgRP) mediate orexigenic signals (Aponte *et al.* , 2011). Consequently, NPY release increases food intake with a concomitant decrease in energy expenditure (Ollmann *et al.* , 1997; Stanley & Leibowitz, 1984). AgRP is a potent inverse agonist on MC3R and MC4R, thus preventing activation by their ligand α-MSH (Smith *et al.* , 2007). Additionally, AgRP expressing neurons co-express the inhibitory transmitter γ-aminobutyric acid (GABA) and form unidirectional synapses on POMC neurons, thus simultaneously inhibiting these anorexigenic neurons (Cowley *et al.* , 2001). This interaction may be seen as a important evolutionary blue-print that favors hunger over satiety by tonic inhibition of anorexigenic signals and thus can also lead to overconsumption in times of higher food availability (Atasoy *et al.* , 2012; Bates & Myers, 2003). The identification of this network in the ARC led to the question of how signals are further transferred and integrated to elicit adaptive behavior.

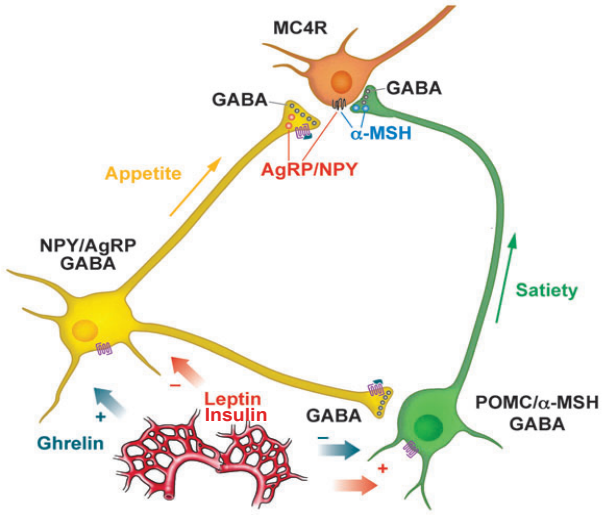


Figure 1.1: Melanocortin system in the ARC. Two opposing cell types in the ARC sense and process peripheral signals to second order neurons, which express MC4Rs. Insulin and leptin inhibit appetite signaling NPY/AgRP neurons and excite satiety signaling POMC neurons, leading to α-MSH release and the activation of second order neurons via MC4Rs. In contrast, ghrelin excites NPY/AgRP neurons, which form GABAergic synapses on POMC and second order neurons, leading to inhibition of satiety signals. NPY inhibits second-order neurons and AgRP potently antagonizes MC4Rs and activation by α-MSH. ARC, arcuate nucleus of the hypothalamus; α-MSH, α-melanocyte-stimulating hormone; AgRP, agouti-related peptide; POMC, proopiomelanocortin; MC4R, melanocyte receptor type 4; NPY, neuropeptide Y; GABA, γ-amino butoric acid. Modified from (Gao & Horvath, 2007).

Downstream targets of POMC and NPY/AgRP neurons

Various nuclei of the CNS exhibit dense innervation by POMC and NPY/AgRP projections, for example the paraventricular nucleus of the hypothalamus (PVH), the VMH, the dorsal medial hypothalamus (DMH) and the LH. MCR and/or NPY-receptor expression could be observed in all of the mentioned hypothalamic nuclei, thus identifying them as strong candidates for melanocortin signaling (Kishi *et al.* , 2003; Mountjoy *et al.* , 1994; Sahm *et al.* , 1994). An elegant study highlighted the PVH neurons as direct downstream targets for ARC POMC neurons and are therefore called second-order neurons in the melanocortin system (Balthasar *et al.* , 2005). The PVH has been studied extensively in the control of food intake and satiety. A study with lesioned PVH in rats, revealed that NPY and POMC signaling is still sufficient to regulate food intake (Dube *et al.* , 2006). However, Atasoy *et al.* (2012) provide data, in which a PVH subpopulation is necessary in processing the signals that are generated in first place in the ARC to other nuclei in

the CNS. Furthermore, a large body of literature suggests a role of the PVH in the NA control of energy homeostasis (Leibowitz, 1988; Wellman, 2000, 2005). Injections of NA and specific agonists and antagonists of adrenergic receptors (adrenoceptors; AR) into the PVH led to marked effects on feeding behaviour in rats.

In general, signals in relation to eating behaviour from hypothalamic nuclei are processed to nuclei in the brainstem, the Nucleus Tractus Solitarius (NTS) and the dorsal motor nucleus of the vagus (DMV) and are integrated with mechanosensory signals from the periphery (Suzuki *et al.* , 2010). One important signal is glucose, which modulates electrical activity of POMC and AgRP neurons in the ARC and brainstem nuclei (Diggs-Andrews *et al.* , 2010; Fioramonti *et al.* , 2007; Ibrahim *et al.* , 2003; Mizuno & Oomura, 1984; Parton *et al.* , 2007; Ritter *et al.* , 2011; Thorens, 2011; Wang *et al.* , 2008). A prerequisite to elicit adaptive behaviours in response to changes in glucose concentration is the perception of extracellular glucose levels, a mechanism termed "glucose sensing" (Levin *et al.* , 1999; Routh, 2002).

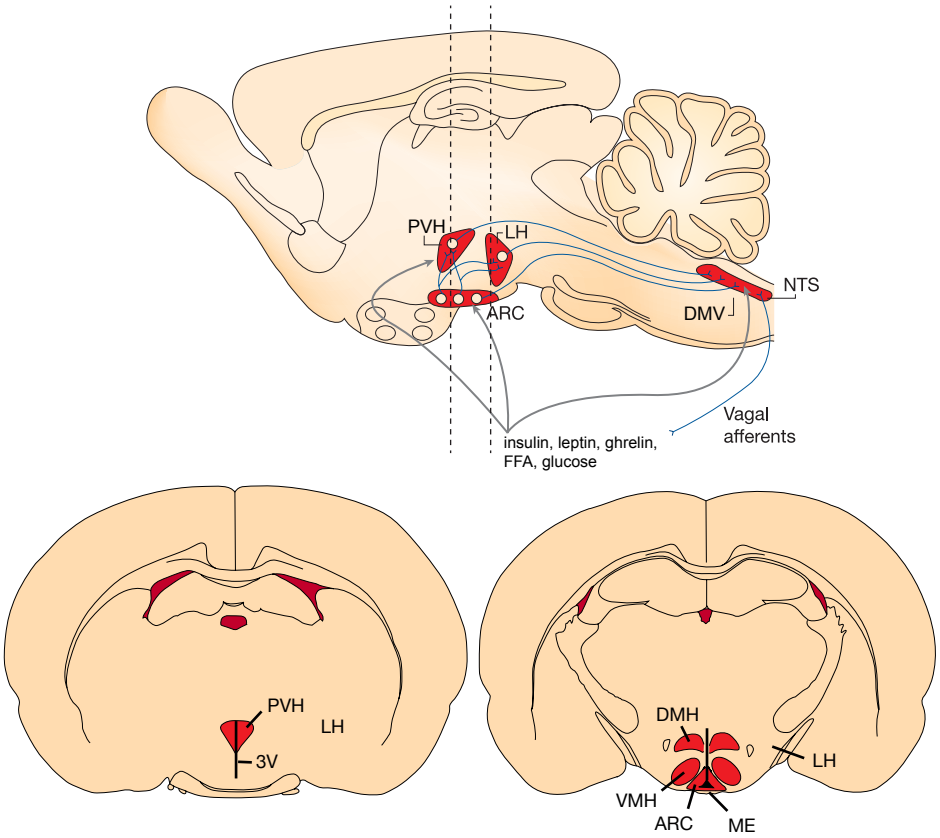


Figure 1.2: The melanocortin system and its downstream targets in the integration of peripheral signals. *Upper scheme* The peripheral signals ghrelin, insulin and leptin, FFA and glucose act on neurons in the brainstem and the hypothalamus. In the brainstem the NTS and DMV receive sensory information from vagal afferents and process signals to the hypothalamus. Here the respective information is integrated with the peripheral signals and processed back to the brainstem to elicit adequate behavior, i.e. food intake, satiety or energy expenditure. *Lower scheme* Coronal sections illustrated by dashed lines above show the positions of the relevant nuclei in the hypothalamus, which are involved in energy homeostasis. FFA, free fatty acids; NTS, nucleus tractus solitarius; DMV, dorsal motor nucleus of the vagus; PVH, paraventricular nucleus of the hypothalamus; LH, lateral hypothalamus; ARC, arcuate nucleus of the hypothalamus; 3V, 3rd ventricle; ME, median eminence; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamus. Modified from (Morton *et al.* , 2006; Schwartz *et al.* , 2000).

1.1.2 Glucose sensing

Glucose represents an important nutrient also mediating modulatory effects on neurons within the CNS (Marty *et al.* , 2007). In either high or low concentrations, glucose is able to elicit adaptive behaviours via afferent fibers from the periphery (Berthoud, 2008; Yuan & Yang, 2002). In the brainstem, this information is processed and integrated with signals from the CNS in terms of energy homeostasis (Watts & Donovan, 2010). Well studied examples include the cephalic phase of insulin secretion, initially elicited by activation of taste receptors in the oral cavity (Berthoud & Mogenson, 1977; Berthoud & Powley, 1990; Berthoud *et al.* , 1981) to control carbohydrate metabolism.

The CNS constitutes an organ with an specifically high demand in glucose, as it represents the sole energy store of the brain (Levin *et al.* , 2002). Thus, concentrations of CNS glucose must not fall under certain levels ($\sim$ 5 mM) and at critical times of lower concentrations, hepatic glucose production and adaptive behaviors, such as food intake or reduced energy expenditure are triggered (Marty *et al.* , 2007). These mechanisms postulate sites in the CNS that control food intake and energy expenditure in response to extracellular glucose concentrations and thus have the ability to sense extracellular glucose (Routh, 2002). First evidence that the CNS inherits sites of glucodetection was revealed in the 1950s (Mayer, 1953). Pioneering electrophysiological experiments in the 1960s suggested the existence of certain neuronal populations, which could change their firing in response to changes in extracellular glucose concentrations (Anand *et al.* , 1964). Further experiments could reveal two distinctly different populations of glucose-sensitive neurons, which either increase or decrease firing frequencies in response to elevation in extracellular glucose concentrations. Accordingly, they were termed "glucose-excited" (GE) and "glucose-inhibited" (GI) neurons (Belgardt *et al.* , 2009; Burdakov *et al.* , 2005b; Thorens, 2011). Until today, this classification has been expanded by the electrophysiological identification of neurons, responding to either excessively high or low concentrations of glucose high glucose-excited (HGE) and high glucose-inhibited (HGI; Fioramonti *et al.* 2007).

In favor of the identified CNS nuclei containing glucose-responsive neurons, great efforts have been made to unravel the mechanisms that couple extracellular glucose con-

centrations to certain intracellular membrane properties, that increase or decrease action potential (AP) frequencies. Mechanisms involving the adenosine-triphosphate (ATP)-dependent potassium channel (K_{ATP}) along with the expression of glucose-transporters (GLUTs) and glucokinase (GK) have been first identified in pancreatic β-cells and are also expressed in the CNS (Ashcroft *et al.* , 1984; Levin *et al.* , 1999). Briefly, extracellular glucose enters the cell via low affinity GLUTs. Subsequently, glucose is phosphorylated by GK and enters glucolysis leading to increased ATP concentrations. As a result, ATP closes K_{ATP} channels resulting in membrane potential depolarization and concomitant increase in firing frequencies (Ashford *et al.* , 1990; Dallaporta *et al.* , 2000; Lee *et al.* , 1999; Miki *et al.* , 2001; van den Top *et al.* , 2007). In contrast, glucose-inhibited neurons control their firing by activating an ATP driven Na^+/K^+- exchanger in response to elevated intracellular ATP concentrations. However, a second mechanism suggests the involvement of adenosine monophosphate-activated protein kinase (AMPK) pathways, which results in the opening of Cl^- channels and subsequent hyperpolarization of the neuron (Oomura *et al.* , 1974; Silver & Erecińska, 1998; Song & Routh, 2005; Song *et al.* , 2001). An overview of the proposed underlying mechansims is given in Figure 2 and reviewed by Jordan *et al.* (2010).

Various sites containing glucose-sensing populations have been identified including all key regulatory nuclei of energy homeostasis, such as the ARC, the PVH, VMH and LH (Burdakov *et al.* , 2005a). Further glucose sensing sites have been detected in the brainstem including, the NTS, the area postrema (ArP), the DMV and NA neurons in the basolateral medulla (Adachi *et al.* , 1995; Burdakov *et al.* , 2005a; Ritter *et al.* , 2011; Routh, 2002). A large body of work focused on NA neurons in the brainstem projecting to hypothalamic sites, which are involved in glucoprivic feeding responses (Ritter *et al.* , 2011). Further studies mark the main NA nucleus in the CNS, the LC, as a potential source for the NA modulation of energy homeostasis (Wellman, 2000). Therefore, NA and its role in the control of energy homeostasis is described in the following sections.

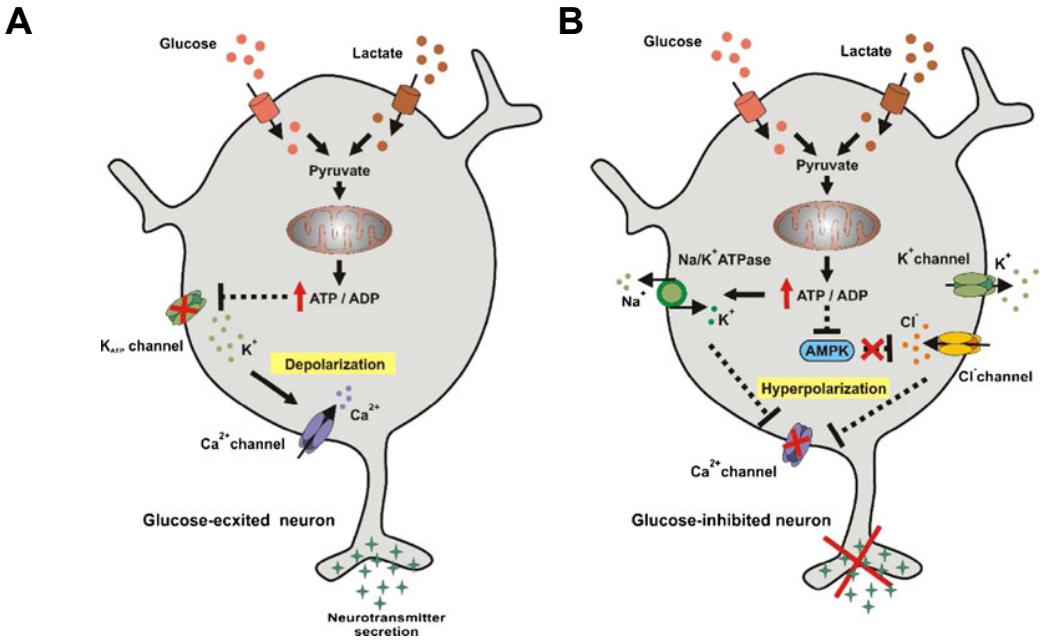


Figure 1.3: Neuronal glucose-sensing mechanisms. ***(A)*** In GE neurons, extracellular glucose enters the cell and is phosphorylated to Pyruvate by the kinase glucokinase (GK). Increasing intracellular ATP concentrations lead to the closure of K_{ATP} channels, thus increasing excitability and transmitter release. ***(A)*** In the proposed model for GI neurons, ATP activates a Na^+/K^+-ATPase decreasing membrane potential and transmitter release. Other models implicate AMPK mediated opening of Cl^- channels, other mechanisms involve K_{ATP} channel opening and decrease in Ca^{2+} conductance. GE, glucose-excited; GI, glucose-inhibited, K_{ATP}, ATP-dependent potassium channel; AMPK, adenosine monophosphate activated protein kinase; GK, glucokinase. Adopted and modified from Jordan *et al.* (2010).

1.2 The catecholamine noradrenaline in the control of energy homeostasis and food intake

Several drugs in the treatment of obesity target catecholaminergic (catecholamine; CA) neurotransmitter systems, among them the NA system (Hainer *et al.* , 2006a; Rosmond, 2004). Strong side effects of these drugs are a general problem. Amphetamines, which also target the NA system have anorexigenic effects but exhibit strong addictive potentials (Di Dalmazi *et al.* , 2013). In order to develop specific drugs with no or minimized side effects, a detailed understanding of these neurotransmitter systems in the modulation of eating behaviour is necessary. The present study focuses on the NA system in the control of energy homeostasis.

1.2.1 Noradrenaline

NA, adrenaline (adrenergic; A) and dopamine (dopaminergic; DA) belong to the CA class of monoamines with NA and DA representing the two primary CAs in the mammalian brain (Bloom, 2010). Almost five decades ago, these substances were identified by formaldehyde histofluorescence and soon matched to distinct neuronal populations(Carlsson *et al.* , 1962; Dahlström & Fuxe, 1964; Vogt, 1954).

NA can act either as a circulating hormone or neurotransmitter dependent on the site of biosynthesis and release. In the periphery, the medullae of the adrenal glands release NA and A into the blood, a process which is mainly associated with an adaptive behavior to stress, often referred to as "fight or flight" response (Jansen *et al.* , 1995). However, the classical view of NA release in the CNS underlies the postganglionic sympathetic neurons controlling cardiovascular responses to maintain blood pressure and a variety of other responses with the interplay of A (Esler *et al.* , 1985).

It became clear that NA as well as A are also released by various neurons serving as a classical synaptic neurotransmitter in the CNS (Fuxe, 1965). Various studies indicate, that projections of NA neurons can also be non-synaptic, thus releasing it nonspecifically within areas of brain-tissues where it rather acts in a hormone-like manner (Smeets & González, 2000). Release of NA in general is known to contribute to a variety of functions such as long-term synaptic plasticity, pain modulation, motor control, local blood flow, sleep wake cycles, arousal, task performance optimization and energy homeostasis (Aston-Jones & Cohen, 2005; Benarroch, 2009; Samuels & Szabadi, 2008a,b).

All CAs are synthesized by an specific enzymatic machinery. The neurotransmitter NA is synthesized in three steps starting with the amino acid tyrosine, which in a first step is converted to L-3,4-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine-hydroxylase (TH). L-DOPA represents the direct precursor for DA and DA is converted into NA by the enzyme dopamine-β-hydroxylase (DBH). Methylation by phenylethanolamine-N-methyltransferase (PNMT) finally converts NA to A, a process which is predominantly taking place in the medullae of the adrenal glands as A neuron groups are rather small compared to NA neuron groups (see figure 1.4 A; Smeets & González 2000).

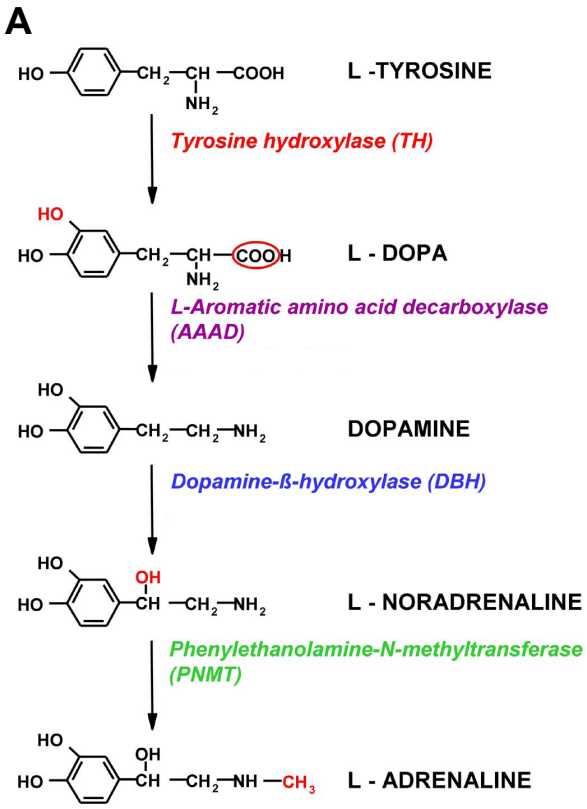


Figure 1.4: Catecholaminergic biosynthesis. ***(A)***Pathway for catecholamine biosynthesis and its enzymatic steps. The steps of conversion from L-tyrosine to L-noradrenaline are typical for sympathetic and some brain neurons, and the conversion of L-noradrenaline to L-adrenaline is typical for the adrenal medullary cells and some peripheral and central neurons. Modified from Kvetnansky *et al.* (2009).

The functional organization of the NA system consists of a peripheral (sympathetic ganglia) and a central part. Both share the common feature that their cell bodies are clustered in a small number of nuclei in the lower brainstem (Moore & Bloom, 1979).

First described in rats, seven NA nuclei have been identified in the brainstem and most of them have also been identified in primates and humans. Subdivided into three groups, the caudal (or medullary), central (medullo-pontine) and rostral (pontine) group, NA nuclei have been labeled from A1 to A7. However, 50% of all noradrenergic neurons are located in the A6 cell group, the LC.

Projections of the NA system are divided into two major groups: the caudal group (A1,A2,A5,A7) forms the ventral noradrenergic bundle (VNB) and central group (LC) gives rise to the dorsal noradrenergic bundle (DNB). Together, both bundles innervate almost the entire CNS. These wide projection pattern of the NA system reflects the

diverse functional role of NA neurons. The anatomy of the NA system is reviewed in Szabadi (2013).

The Locus Coeruleus - noradrenergic (LC-NA) system, is the main source of NA in the CNS. By innervating most structures, NA release from the LC modulates a large variety of systems (Berridge & Waterhouse, 2003). The control of sleep-wake cycles, promoting wakefulness, arousal and modulating task performance, are regarded as the major functions of the LC in the CNS. These contributions have been studied extensively *in vitro* and *in vivo*. The activation patterns of neurons in the LC have been matched to different states of wakefulness and arousal. During rapid eye movement (REM) sleep, neurons in the LC remain silent. They become tonically active during times of waking and increase firing with increasing arousal (Berridge *et al.* , 2012). In times of very high arousal (even stress and fear related) demanding optimal task performance, LC neurons are rhythmically active. An elegant study, using optogenetics, revealed the necessity of LC firing in maintaining wakefulness and also shows that LC activity is finely tuned in the control of attentional behavior (Carter *et al.* , 2010). Besides these roles, it is also known, that the LC contributes to autonomic function via the sympathetic nervous system (SNS). By innervating preganglionic sympathetic neurons in the spinal cord, the LC controls blood pressure and sweat glands and may also be involved in mediating iris reflexes in response to light stimuli (Samuels & Szabadi, 2008b). Additionally, retrograde labeling using pseudorabies viruses injected into brown adipose tissue (BAT) of different species has allowed the identification of the LC, implicated in the regulation of BAT sympathetic nerve activity (SNA) (Bamshad *et al.* , 1999; Cano *et al.* , 2003; Oldfield *et al.* , 2002). Upon cold exposure it was observed that LC neurons show increased spike activity, concomitant with higher thermogenesis in BAT (Kiyohara *et al.* , 1995; Miyata *et al.* , 1995). This clearly points towards a role of the LC in the control BAT activity.

NA exerts multiple potent effects on target neurons including the modulation of membrane potential, neuronal excitability, intracellular cascades and synaptic plasticity. *In vitro* studies indicate that these effects are rather complex and may critically rely on synaptic concentration as well as on the availability and affinity of certain receptor subtypes in any specific region (Hein, 2006; Philipp & Hein, 2004).

1.2.2 Noradrenergic receptors

The effect of NA and A is mediated via signaling of three major classes of receptors, α_1-, α_2- and β-adrenergic receptors (Adrenoceptors; ARs). These receptors are widely distributed in the body and the CNS (Young & Kuhar, 1980). Each of the three major types are further divided into three different subtypes (Docherty, 1998). Based on pharmacological characteristics, adrenoceptors were originally divided into α- and β-adrenergic receptors (Ruffolo, 1985; Ruffolo & Hieble, 1994). α-ARs were initially subdivided into α_1- and α_2-ARs based on the assumption that the first is expressed postsynaptically whereas the latter is expressed only presynaptically to inhibit transmitter release (Langer, 1974). However, this classification soon became obsolete. It was shown that α_2-ARs can be expressed pre - as well as postsynaptically (Rogawski & Aghajanian, 1982; Wellman *et al.* , 1993). Thus, pharmacological characterizations were used and led to the present classification scheme. An overview of the current nomenclature is given in figure 1.5.

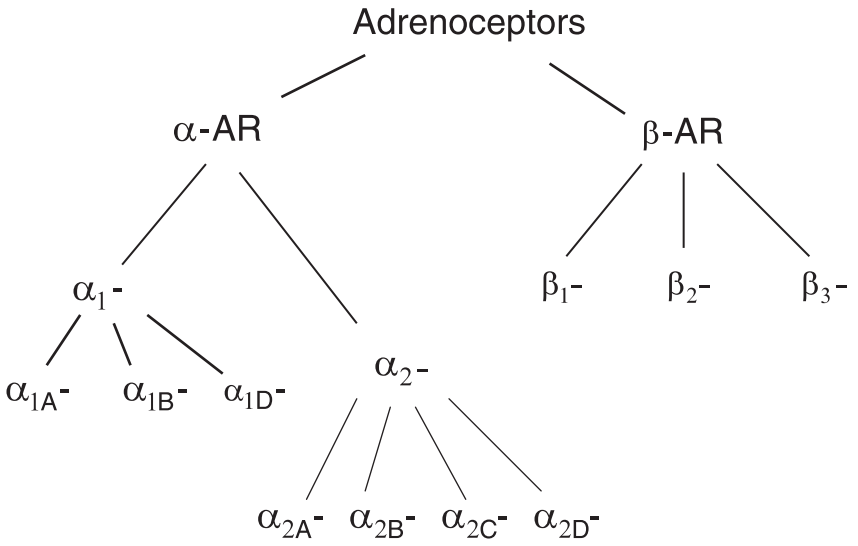


Figure 1.5: Current nomenclature of adrenoceptors based on pharamcological properties. ARs are divided in two major classes α-ARs and β-ARs. Modified from Woodcock (2007).

Several studies have revealed multiple actions of NA on intrinsic ionic currents and cellular properties (Hein, 2006). All of the three described major classes of AR belong to the class of G-protein coupled receptors (GPCRs) (Bloom, 1979; Insel, 1989; Ramos & Arnsten, 2007).

α_1-adrenergic receptors

In general, the class of α_1-ARs exerts its excitatory effect via coupling to G_q proteins, thereby activating phospholipase C (PLC) and phosphotidyl-inositol (PI) intracellular signaling (Macrez-Leprêtre *et al.* , 1997). This leads to activation of protein kinase C (PKC) and the release of intracellular Ca^{2+} via inositol-1,4,5-triphosphate (IP_3) (Benarroch, 2009; Birnbaum *et al.* , 2004). Further mechanisms have been implicated which also lead to excitatory effects on neurons. An elegant study by Pan *et al.* (1994) demonstrated the contribution of decreased K^+ conductances as well as increased voltage gated Ca^{2+} conductances (VGCC) to neuronal excitation. Dodt *et al.* (1991) could show that NA inhibits Ca^{2+}-activated K^+-currents via activation of α_1-ARs and thus leading to increased firing in response to excitatory stimuli. Mice carrying deletions for each of the α_1-AR subtype could reveal different systems they contribute to in the CNS. Various phenotypes were reported as most of the α_1-ARs are abundantly expressed (Tanoue *et al.* , 2002). The analyzed mice suggest that the subtypes of α_1-ARs are involved in the control of locomotion, cognition, control of motor activity as well as contribute to memory consolidation and fear-motivated exploratory activity. Consequently, patophysiology in relation to α_1-ARs exhibit various impairments and neurodegenerative phenotypes (Zuscik *et al.* , 2000). However, the detailed mechanisms by which α_1-ARs contribute to the different CNS functions remain largely unknown.

α_2-adrenergic receptors

The α_2-ARs, which exhibit the highest affinity to NA, are coupled to G_i proteins thus having inhibitory effects on target neurons. Activation of the G_i protein reduced the activity of the enzyme adenylcyclase (AC), which decreases the intracellular concentration of the second messenger cyclic adenosine monophosphate (cAMP; Bünemann *et al.* 2001; Hein 2006). Protein kinase A (PKA), a kinase regulating the activity of several cellular proteins including L-type Ca^{2+} channels can be activated by cAMP (Rosenbaum *et al.* , 2009). Additionally, activation of G protein coupled inwardly rectifying potassium channels (GIRKs) is a primary response to activation of G_i coupled receptors, which leads to potent inhibition of target neurons (Limbird, 1988; Lüscher & Slesinger, 2010). α_2-ARs

can be expressed either pre - or postsynaptically, where they serve different functions while sharing the same intracellular mechanism. Presynaptically expressed α_2-ARs are commonly autoreceptors (Callado & Stamford, 2000). Here, the synaptic availability of NA is controlled by auto-inhibition via the α_2-AR itself. In addition to their function as inhibitory autoreceptors, α_2-ARs can also regulate a number of other neurotransmitters in the central and peripheral nervous system (Richter *et al.* , 2012). In the brain, α_{2A}- and α_{2C}-ARs inhibit DA release in basal ganglia and serotonin (5-HT) secretion in mouse hippocampus and brain cortex (Bücheler *et al.* , 2002; Scheibner *et al.* , 2001). In the LC, postsynaptically expressed α_2-ARs have been shown to inhibit baseline activity and increase responsiveness to novel stimuli (Sara, 2009).

Further functional contributions of α_2-ARs are shown for pain perception, procession of sensory information, control of blood pressure and body temperature and also neuroprotectional effects are described (reviewed in Hein (2006); Rommelfanger & Weinshenker (2007); Weinshenker (2008). To mention a few, sensorimotor gating deficits, such as schizophrenia, attention deficit disorder and post-traumatic stress disorder are consequences of α_2-AR impairment (Brede *et al.* , 2004).

β-adrenergic receptors

β-ARs activate G_s proteins, which opposingly to α_2-ARs lead to an increase in cytosolic cAMP concentration, thus leading to excitatory effects (Benovic *et al.* , 1988). β-ARs are mostly known for their role in the regulation of cardiovascular, airway, uterine, and peripheral metabolic functions. Presynaptically expressed β-ARs on some peripheral and central nerve endings have been show to facilitate stimulation evoked neurotransmitter release. However, their major physiological significance is not known. Despite the wide expression of β_1- and β_2-ARs within the CNS, specific knockouts did not lead to any significant deficits in CNS function (Nicholas *et al.* , 1996).

The present study aims to gain further knowledge about the NA modulation of energy homeostasis. Therefore, general effects of NA and underlying receptor subtypes have been described. The following section provides an overview of NA modulation of

energy homeostasis at two sites, the ARC and the LC, both of which are in focus of this thesis.

1.2.3 The hypothalamus in the noradrenergic control of energy homeostasis

In the 1960s, first evidence appeared that NA directly affects food intake. Exogenous NA and A injected into various sites of the rat's forebrain was able to elicit feeding and drinking responses dependent on the injection site (Booth, 1967; Grossman, 1960). An important mapping study by Leibowitz (1978b) showed that infusions of NA into the medial hypothalamus, specifically into the PVH elicits remarkable bouts of food intake and that these responses were diminished by injecting NA anteriorly, laterally and dorsally to the PVH. Further studies revealed the presence of α_1- and α_2-ARs in the hypothalamus (Leibowitz *et al.* , 1982; Young & Kuhar, 1980). Pharmacological experiments showed that these receptors mediate the effect of exogenous NA. Clonidine, a specific agonist of α_2-ARs reliably mimicked the effect of NA injection. Consistently, this effect could be abolished by the administration of the specific α_2-AR antagonists Rauwolscine and Yohimbine (Goldman *et al.* , 1985; Leibowitz, 1988). In contrast, a variety of agonists of α_1-ARs led to reductions in food intake (for review see Wellman *et al.* 1993). The specific α_1-AR agonist SKF-89748 was able to reduce food intake in rats dose dependently (Morien *et al.* , 1993). Administration of benoxathian, a specific α_2-AR antagonist, prevented the reduction of food intake after the systemic administration of the α_2-AR agonist phenylpropanolamine (Wellman & Davies, 1991). In order to confirm these results, electrophysiological studies revealed a subpopulation of neurons in the PVH, which were either inhibited or excited by bath application of NA and/or specific agonists and antagonists (Inenaga *et al.* , 1986; Kow & Pfaff, 1989). Importantly, blocking synaptic transmission could demonstrate that these effects are due to cell intrinsic expression of ARs. Taken together, neurons in the PVH are differentially regulated by α_1- and α_2-AR subtypes, with the first leading to the suppression and the latter to the stimulation of food intake.

Anatomical studies aimed to reveal the specific sources in the brainstem which release NA in terms of energy homeostasis (Ritter *et al.* , 2000; Wellman, 2000, 2005).

Various NA nuclei show dense innervation of hypothalamic sites (Calaresu & Ciriello, 1980; Kataoka *et al.* , 1975; Loughlin *et al.* , 1986). As described earlier, the NA system innervates most parts of the CNS via the DNB, which carries the efferent projections of the LC, and the VNB. The identification of these fiber systems led to further pioneering experiments investigating the specific functions of the NA system with respect to feeding behavior. Almost 40 years ago, Ahlskog & Hoebel (1973) showed that chemical and electrolytic lesions of the VNB results in overeating and obesity. In contrast the interruption of the DNB, originating from the LC resulted in lowered body weight (Hoebel *et al.* , 1989). Genetic knockout (KO) of NA can be accomplished by the deletion of DBH. Consistently with the contribution of NA to feeding behavior, mice exhibited a smaller phenotype concomitant with reduced food intake. However, these mice could still increase feeding in response to overnight fasting (Cannon & Palmiter, 2003). Prior to this, a similar outcome has been noted by Rossi *et al.* (1982), where inhibition of DBH by the drug FLA-63 led to reduced eating behavior.

The discovery of the melanocortin system in the hypothalamus also led to experiments focusing on the effect of NA in the ARC or the medial hypothalamus in general. In this context, the ARC is regarded as the first order relay, containing neurons which adapt their activity to peripheral signals such as insulin, leptin, ghrelin, glucose and FFA and innervate downstream targets, especially the PVH, to either suppress or induce food intake (Gao & Horvath, 2007). A large body of literature suggests a role of NA in modulation of the action of these peripheral signals or vice versa (Brunetti *et al.* , 1999; Date *et al.* , 2006; Francis *et al.* , 2004; Levin *et al.* , 1998). While leptin inhibits NA release into the hypothalamus, insulin selectively downregulates the expression of α_2-AR specifically in the ARC (Brunetti *et al.* , 1999; Kawakami *et al.* , 2008; Levin *et al.* , 1998). Additionally, NA might also affect neurons that are located presynaptically to the PVH thereby changing excitatory and inhibitory synaptic input on neurons in the PVH (Han *et al.* , 2002). The ARC is a strong candidate in exerting these effects on PVH neurons and NA has been shown to activate ARs in the ARC (Kang *et al.* , 2000). However, these experiments lacked the identification of the respective neurons expressing the ARs.

Taken together, the role of NA signaling in the PVH with respect to food intake has been thoroughly investigated. Various studies also indicate direct or indirect modulatory effects of NA in the ARC and NA dependent postsynaptic currents have been measured in PVH neurons. POMC and AgRP neurons are located presynaptically to the PVH and are thus potent targets for NA in the regulation of energy homeostasis. The present study aims to analyze the effect of NA on these neurons. Moreover, this leads to the question which NA nuclei project and release NA into the ARC. The LC contains 50% of the NA neurons and therefore is regarded as a potential source for NA release into the ARC. Therefore the putative role of the LC in energy metabolism is a further subject of this thesis.

1.2.4 The Locus Coeruleus in the control of energy homeostasis and glucose metabolism

The LC NA system innervates BAT via the SNS and thus may contribute to the control of BAT activity. Besides its prominent role in thermoregulation, recent work has revealed that BAT is also involved in the control of glucose and lipid metabolism in rodents and may thus contribute to energy homeostasis (Bartelt *et al.* , 2011; Nedergaard *et al.* , 2011; Waldén *et al.* , 2012). BAT is a tissue with excessively high glucose uptake, a feature which accidentally led to its identification in humans while screening for tumors (Hany *et al.* , 2002). Importantly, NA release into BAT stimulates the expression of GLUT genes resulting in the uptake of glucose from blood vessels. Subsequently, glucose is pyruvated and finally oxidized in the mitochondria (Bartelt *et al.* , 2011). Given the sympathetic efferents of the LC into BAT and the effect of NA on glucose uptake, it is important to mention that studies support the hypothesis that the LC may also contribute to glucose metabolism (REF).

In order to maintain glucose homeostasis, the brainstem integrates information of the hypothalamus and visceral afferents arising in the periphery. A large body of evidence suggests that NA is the neurotransmitter which triggers food intake as response to glucoprivation (Emanuel & Ritter, 2010; Fraley & Ritter, 2003; Hudson & Ritter, 2004; Levin *et al.* , 1999; Ritter *et al.* , 2000, 2001, 2006). In this context, NA is suggested to

be mostly released by the A1 and A2 NA cell groups (Li *et al.* , 2009; Rinaman, 2011). However, about 50% of NA somata are located in the LC, which gives rise to the DNB. As aforementioned, lesioning of DNB fibers leads to phenotypes, which suggest a role in energy homeostasis. Identification of the melanocortin system and glucose-sensing neurons led to studies trying to uncover certain areas, that may sense extracellular glucose and may be involved in energy homeostasis. In order to find these areas, in-situ hybridizations for mRNA encoding proteins involved in the glucose-sensing machinery have been conducted. The expression of K_{ATP} channels and glucokinase has been anatomically matched with the LC, suggesting the existence of glucose-responsive neurons (Dunn-Meynell *et al.* , 1998; Finta *et al.* , 1993; Lynch *et al.* , 2000). Importantly, two studies in the 1990s by Murai *et al.* (1997a) and Illes *et al.* (1994) provides evidence of glucose-sensing behavior of LC neurons in the rat. In both studies, glucose free medium induced outward currents in a subset of LC neurons, which in the latter study could be blocked by application of the K_{ATP} channel blocker tolbutamide. The same authors could also show that metabolic inhibition due to hypoxia/anoxia induces outward currents, also sensitive to tolbutamide. In this context, hypoxia is thought to exert this effect via depletion of intracellular ATP, thus opening K_{ATP} channels (Grigg & Anderson, 1989). These results indicate the existence of GE neurons in the LC. In contrast, injection of 2-desoxy-glucose (2-DG) , a glucose variant which can't enter glycolysis mimicking conditions of glucoprivation, induced c-fos expression in the LC, suggesting the existence of GI neurons (Ritter *et al.* , 1998). However, this is not contradictory as c-fos expression due to 2-DG fails to label GE neurons, which should decrease electrical activity.

Taken together, a large body of literature provides evidence for a role of the NA system in the control of energy homeostasis. The NA modulation of neurons in the PVH has been well established. In the hypothalamus, the main center in the control of energy homeostasis is the ARC and a series of studies supports evidence for an action of NA in the ARC. However, a detailed analysis of the effect of NA, especially on POMC and NPY/AgRP neurons remains elusive.

The brainstem contains the majority of NA neurons in the CNS. Various nuclei exhibit dense efferent innervation of hypothalamic sites. The LC contains 50% of NA

somata and efferent projections to the ARC have been identified. This points towards a potential source for NA release into the ARC and a contribution to the control of energy homeostasis. A role of the LC in glucose metabolism via autonomic function has been proposed and LC neurons express a number of proteins, which identify these neurons as putative glucose-sensors. However, detailed electrophysiological data has not been described in this context.

1.3 Thesis objectives

The present study aims to expand the knowledge of NA modulation of energy homeostasis. Because various drugs in the treatment of obesity, at least in part, target the NA system, it is important to understand in detail the targets in the CNS and the mechanisms, by which NA modulates eating behaviour and energy expenditure. The presented experiments help to define the model of NA control of energy homeostasis, which is critical to develop specific drugs in the treatment of obesity with minimized side effects. Two systems are in the focus of this study:

1st: The Arcuate nucleus of the hypothalamus

1. Basic electrophysiological characteristics of POMC and AgRP neurons in the ARC have been analyzed
2. The effect of different concentrations of NA on POMC and NPY/AgRP neurons in the ARC has been investigated
3. The specific underlying receptors, expressed by POMC and NPY/AgRP neurons have been identified by pharmacological tools
4. Age- and diet-dependent effects on NA signaling in the ARC have been investigated for POMC neurons

2nd: The Locus Coeruleus

1. Basic electrophysiological characterization aimed to create a baseline for future experiments in our labaratory

2. Responses to changes in extracellular glucose have been investigated in LC neurons, as a potential source for NA release into the ARC

2 Materials and Methods

2.1 Animal care

Care of all animals was within institutional animal care committee guidelines. All animal procedures were approved by local government authorities (Bezirksregierung Köln, Cologne, Germany) and were in accordance with NIH guidelines. Mice were housed in groups of 3 – 5 animals at a temperature of 22 – 24°C with a 12 h light/12 h dark cycle. After weaning (P21), mice were either fed regular chow food (NCD; Teklad Global Rodent 2918; Harlan) containing 53.5 % carbohydrates, 18.5 % protein, and 5.5 % fat (12 % of calories from fat) or a high-fat diet (HFD; C1057; Altromin) containing 32.7 % carbohydrates, 20 % protein, and 35.5 % fat (55.2 % of calories from fat). All animals had access to water and chow *ad libitum*. The different mouse strains used for this study were kindly provided by Tim Klöckener, Sulay Tovar and Linda Verhagen of the Brüning group.

2.2 Brain slice preparation

The animals were anesthetized with halothane (B4388; Sigma-Aldrich, Taufkirchen, Germany) and subsequently decapitated. The brain was rapidly removed and a block of tissue containing the hypothalamus or brainstem was immediately cut out. Coronal slices (250 – 300 μm) were cut with a vibration microtome (HM-650 V; Thermo Scientific, Walldorf, Germany) under cold (4 °C), carbogenated (95% O2 and 5% CO2), glycerol-based modified artificial cerebrospinal fluid (GaCSF; Ye *et al.* 2006) to enhance the viability of neurons. GaCSF contained (in mM): 250 Glycerol, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1.2 NaH_2PO_4, 10 HEPES, 21 $NaHCO_3$, 5 Glucose and was adjusted to pH 7.2 with NaOH resulting in an osmolarity of ∼310 mOsm. Brain slices were transferred into carbogenated artificial cerebrospinal fluid (aCSF). First, they were kept for 20 min. in a 35 °C 'recovery bath' and then stored at room temperature (24 °C) for at least 30 min

prior to recording. For the recordings, slices were transferred to a Sylgard-coated (Dow Corning Corp., Midland, MI, USA) recording chamber (∼3 ml volume) and, if not mentioned otherwise, continuously perfused with carbogenated aCSF at a flow rate of ∼2 $ml \cdot min^{-1}$. aCSF contained (in mM): 125 NaCl, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1.2 NaH_2PO_4, 21 $NaHCO_3$, 10 HEPES, and 5 Glucose and was adjusted to pH 7.2 with NaOH resulting in an osmolarity of ∼310 mOsm.

2.3 Patch-clamp recordings

Current-clamp recordings in neurons of the hypothalamus and the brainstem were performed in the perforated patch-clamp configuration. In the hypothalamus, neurons which express POMC or NPY/AgRP were investigated. In the brainstem, NA neurons in the LC were investigated.

Neurons were visualized with a fixed stage upright microscope (BX51WI, Olympus, Hamburg, Germany) using 40× and 60× water-immersion objectives (LUMplan FL/N 40×, 0.8 numerical aperture, 2 mm working distance; LUMplan FL/N 60×, 1.0 numerical aperture, 2 mm working distance, Olympus) with infrared differential interference contrast optics (Dodt & Zieglgänsberger, 1990) and fluorescence optics.

POMC and NPY/AgRP neurons were identified by their anatomical location in the ARC and by their GFP fluorescence that was visualized with an X-Cite 120 illumination system (EXFO Photonic Solutions, Ontario, Canada) in combination with a Chroma 41001 filter set (EX: HQ480/40×, BS: Q505LP, EM: HQ535/50m, Chroma, Rockingham, VT, USA). Putative NA neurons were identified by their location ventrolateral to the 4th ventricle and/or by their GFP expression. Electrophysiological properties were analyzed to confirm the identity (i.e. slow and regular firing).

Electrodes with tip resistances between 4 and 6 MΩ were fashioned from borosilicate glass (0.86 mm inner diameter; 1.5 mm outer diameter; GB150-8P; Science Products) with a vertical pipette puller (PP-830; Narishige, London, UK).

Recordings in the ARC were made at room temperature. Recordings of LC NA neurons were made at ∼30-32 °C using an inline solution heater (SH27B; Warner Instruments, Hamden, CT, USA) operated by a temperature controller (TC-324B; Warner

Instruments). All recordings were performed with an EPC10 patch-clamp amplifier (HEKA, Lambrecht, Germany) controlled by the program PatchMaster (version 2.32; HEKA) running under Windows. Data were sampled at intervals of 100 μs (10 kHz) and low-pass filtered at 2 kHz with a four-pole Bessel filter. Cell capacitance was determined by using the capacitance compensation (C-slow) of the EPC10. Cell input resistances (R_M) were calculated from voltage responses to hyperpolarizing current pulses. The calculated liquid junction potential of 14.6 mV between intracellular and extracellular solution was compensated or subtracted offline (calculated with Patcher's Power Tools plug-in from `http://www.mpibpc.mpg.de/groups/neher/index.php?page=software` for IGOR Pro 6 [Wavemetrics, Lake Oswego, OR, USA]).

2.3.1 Perforated-patch clamp recordings

Perforated-patch experiments were conducted using protocols modified from Horn & Marty (1988) and Akaike & Harata (1994). Recordings were performed with ATP and GTP free pipette solution containing (in mM): 128 K-gluconate, 10 KCl, 10 HEPES, 0.1 EGTA, 2 $MgCl_2$ adjusted to pH 7.3 with KOH resulting in an osmolarity of $\sim$300 mOsm. ATP and GTP were omitted from the intracellular solution to prevent uncontrolled permeabilization of the cell membrane (Lindau & Fernandez, 1986). The patch pipette was tip filled with internal solution and back filled with 0.02% tetraethylrhodamine-dextran (D3308, Invitrogen, Eugene, OR, USA) added to the internal solution containing the ionophore to achieve perforated patch recordings.

Amphotericin B (A4888; Sigma) and Gramicidin (G5002; Sigma) were dissolved in dimethyl sulfoxide (DMSO; D8418, Sigma) following the protocols of Rae *et al.* (1991)and Kyrozis & Reichling (1995). The used DMSO concentration (0.1 – 0.3 %) had no obvious effect on the investigated neurons. All ionophores were added to the modified pipette solution shortly before use. The final concentration of nystatin and amphotericin B was $\sim$200 $\mu g \cdot ml^{-1}$, the final concentration of gramicidin was $\sim$10 – 75 $\mu g \cdot ml^{-1}$.

2.3.2 Single cell labeling

To label single cells, 1% biocytin (B4261; Sigma-Aldrich) was added to the pipette solution. Upon completion of the electrophysiological experiments, perforated-patch recordings were converted to the whole cell configuration and biocytin was allowed to diffuse into the cell for at least 5 min. The brain slices were fixed in Roti-Histofix (P0873; Carl Roth, Karlsruhe, Germany) overnight at 4°C and rinsed in 0.1 M Tris-HCl-buffered solution (pH 7.2; three times for 20 min each time; RT; TBS). Afterwards, the slices were incubated in TBS containing 1% Triton X-100 (39795.01, Serva, Heidelberg, Germany) and 10% normal goat serum (30 min; RT; S-1000; Vector Labs, Burlingame, CA, USA). Brain slices were washed in TBS (three times for 10 min each time) and subsequently incubated in Alexa Fluor 633 (Alexa 633)-conjugated streptavidin (1:600; 2 hours; RT; S21375; Invitrogen, Karlsruhe, Germany) that was dissolved in TBS containing 10% normal goat serum. Brain slices were rinsed in TBS (five times for 10 min each time), dehydrated, and then cleared and mounted in Permount (SP15B-500; Fisher Scientific, Nepean, Ontario, Canada).

2.3.3 Immunohistochemistry

Fixed slices containing single-cell labeled neurons (see section: 2.3.2; Single cell labeling) were incubated in TBS containing 2% Triton X-100 (39795.01, Serva, Heidelberg, Germany) and 10% normal goat serum (30 min; room temperature; S-1000; Vector Labs, Burlingame, CA, USA). Subsequently, slices were incubated in TBS containing 1% Triton x-100, 10% normal goat serum and the respective primary antibodies overight at RT (1:500; chicken anti-GFP polyclonal; ab13970; Abcam, Cambridge, UK; 1:250 rabbit anti-dopamine-β-hydroxylase polycolonal; ab43868; Abcam, Cambrige, UK). Afterwards, slices were rinsed in TBS (three times for 10 min each time) and incubated with secondary ABs (1:200 goat anti-chicken Alexa Fluor 488; ab150173; Abcam, Cambrige, UK; 1:100; goat anti-rabbit Alexa Fluor 488; ab96887; Abcam, Cambridge, UK). Finally slices were washed 5 times for 10 min each time, dehydrated and then cleared and mounted in Permount (SP15B-500; Fisher Scientific, Nepean, Ontario, Canada).

2.3.4 Image processing

Overview images of the preparations were taken with an LSM 510 Meta confocal laser scanning system (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) mounted on a fixed stage inverse microscope (Zeiss Axiovert 100M equipped with 10x Plan-Apochromat 0.45 NA, 20x Plan-Apochromat 0.75 NA, 40x oil-immersion Plan-Neofluar 1.30 NA, 63x oil-immersion DIC Plan-Apochromat 1.4 NA and 100x oil-immersion Plan-Neofluar 1.3 NA objectives).

Confocal images were captured using the multi track mode of the LSM 510 software. *Alexa 633* and *Alexa 488* were imaged with 633 nm and 488 nm excitation, respectively. Emission of *Alexa 633* and *Alexa 488* was collected through a 650 nm long pass and 505-530 nm band pass filter respectively. Confocal images were adjusted for contrast and brightness and overlaid in ImageJ (version 1.42q). For overview pictures overlapping imaging stacks (10x) were merged in Photoshop CS5 (Adobe Systems Incorporated, San Jose, CA).

2.3.5 Drugs

Noradrenaline-bitartrate(10nM-200 μM; I9278, Sigma), the specific α_{2A}-AR antagonist BRL 44408 (10 μM, C5776, Sigma), the specific α_{2B}-AR antagonist ARC 239, the specific α_{1A}-AR antagonist WB 4101 and the specific $\alpha_{1C,\,D}$-AR antagonist CEC (1 – 1000 nM, Q102, Sigma) were added to the normal aCSF. The K_{ATP} channel blocker tolbutamide (200 μM, T0891, Sigma) was dissolved in dimethyl-sulfoxide (DMSO, D8418, Sigma) and added to the normal aCSF with a final DMSO concentration of 0.1 – 0.25%. TTX (1μM,)and Cd^{2+} (500 μM,) to analyze pacemaking in LC neurons were also added to normal aCSF.

To isolate neurons from intact networks in acute brain slices, D-AP5 (50 μM), CNQX (10 μM) and PTX (100 μM) were dissolved in DMSO and added to the normal aCSF with a final DMSO concentration of 0.04%. All drugs were bath-applied in the given concentrations at a flow rate of $\sim$2-3 $\mathrm{ml \cdot min^{-1}}$.

The DMSO concentrations used to dissolve aforementioned compounds had no obvious effect on the investigated neurons.

2.3.6 Noradrenalin experiments

Concentrations of 10 nM to 100 μM were bath applied and perfused either for $\sim$5 minutes after NA reached the recording dish or until steady-state effects were visible. For analysis of membrane potentials and AP frequencies at least 30s at the end of each concentration have been used to calculate mean values. For conductance densities, three sets of hyperpolarizing current injections were delivered and mean input resistances were calculated, converted into conductance densities and normalized to respective cell capacitances, which were determined at the end of each experiment. The obtained values were plot as concentration-response expressed as normalization to the maximal response.

$$y = bottom + \frac{(top - bottom)}{1 + 10^{(LogEC50 - X) * Hillslope}}$$

Values versus agonist concentration data were then entered into Prism 5 (GraphPad Software, San Diego, CA) and concentration-response curves were constructed using a nonlinear least-squares curve fitting method. Each curve was fit with a standard variable slope between bottom (=0) and top (=1). The calculated EC50 value was used as a measurement of agonist potency.

2.3.7 Glucose sensing experiments

To study glucose sensing we used modified protocols from (Parton *et al.* , 2007) and varied bath glucose concentrations between 3 and 8 mM. in normal aCSF or aCSF containing 10^{-4} M PTX, 5 x 10^{-5} M D-AP5, and 10^{-5} M CNQX to reduce synaptic input. Since we found no difference in relative effects of external glucose changes by blocking synaptic input, the recorded cells were pooled for both saline. At the end of each experiment tolbutamide (200 μM) was applied to probe for K_{ATP} channels. We found that the basic

firing properties of TH positive LC neurons and their sensitivity to glucose were not homogenous. Therefore we used the '3 times standard deviation' criterion (Dhillon *et al.* , 2006; Kloppenburg *et al.* , 2007) and considered a neuron glucose responsive when the change in firing frequency between different glucose concentrations was 3 times larger than the standard deviation. The neurons were exposed to each glucose concentration for at least 10-15 min. For each neuron, the firing rate averaged from 30s intervals was taken as one data point. To determine the mean firing rate and standard deviation 10 data points at stable firing rates were averaged. The means for glucose responses were calculated from periods of peak hyperpolarizations or depolarizations, respectively

2.3.8 Data analysis

Data analysis was performed with Spike2 (version 6; Cambridge Electronic Design Ltd., Cambridge, UK), Igor Pro 6 (Wavemetrics, Portland, OR, USA) and Graphpad Prism (version 5.0b; Graphpad Software Inc., La Jolla, CA, USA). Coefficients of variation (CVs) were obtained according to (Wolfart *et al.* , 2001).

Spike frequency adaptation (SFA)

SFA ratios were calculated with modified protocols according to Vandecasteele *et al.* (2011) for each cell using the formula:

$$SFAratio = \frac{F_{initial}}{F_{final}}$$

where F_{init} is the initial instantaneous spike frequency (1/first interspike interval (ISI)) and F_{final} is the instantaneous frequency calculated from the last ISI. A neuron exhibiting no SFA (F_{init}/F_{final}) and one showing adaptation would have an SFA ratio of $\sim$1 and >1, respectively (Venance & Glowinski, 2003). Weak adaptation was categorized with values from 1-6 and strong adaptation was considered >6. For each neuron, the adaptation ratio was estimated for a depolarizing stimulation of which certain initial instantaneous frequencies to avoid differences due to large variation in input resistances.

Weak adaptation was categorized with values from 1-6 and strong adaptation was considered >6.

Pacemaking of LC neurons

in order to analyze pacemaking, 450 ISI have been plotted as frequency distribution. Bin size was automatically determined by Graphpad Prism. Distributions were fit to Gaussian model:

$$y = Amplitude * exp(-0.5 * ((X - Mean)/SD)^2)$$

Corresponding means and SDs have been used to calculate coefficients of variance:

$$coeff = 100 * \frac{SD}{mean}$$

Statistics

To determine differences in means of basic electrophysiological properties between the two different genotypes, unpaired *t* tests were used. To determine differences between treated and untreated states paired *t* tests or one-way ANOVA was performed; post hoc pairwise comparisons were performed using *t* tests with the Newman-Keuls method for p value adjustment.

A significance level of 0.05 was accepted for all tests. The '+' signs in the box plots show the mean, the horizontal line the median of the data. The whiskers were calculated according to the 'Tukey' method.

3 Results

CAs like DA and NA have been shown to modulate pathways that are involved in maintaining energy homeostasis (Könner *et al.* , 2011; Wellman, 2005). Various studies indicate effects of NA on the melanocortin signaling pathways in the hypothalamus and specifically the ARC (Brunetti *et al.* , 1999, 2004; Leibowitz *et al.* , 1988; Levin *et al.* , 1998). However, the detailed action of NA in the ARC remains elusive.

In order to investigate the specific role of NA in the ARC, different concentrations of NA have been bath applied combined with single cell patch-clamp recordings of POMC and NPY/AgRP neurons. To rule out unspecific cross-talk between CA systems, concentration-response curves were generated for both neuron types (Guiard *et al.* , 2008b). Synaptic isolation of neurons in acute brain slices was used to confirm that effects of NA on POMC and NPY/AgRP neurons are cell intrinsic. The effects of NA are mediated by certain subtypes of ARs. Specific agonists and antagonists are used to define the underlying AR subtypes. In this context, it is further of greatest interest where NA projections to the ARC arise and under which conditions NA is released.

The LC contains ∼50% of NA neurons (Szabadi, 2013) in the CNS. Efferent projections arising in the LC innervate almost the entire brain representing the main source of NA (Berridge, 2008; Sara, 1988; Szabadi, 2013). Besides the prominent role of the LC in sleep-wake cycles, arousal and attention a large body literature provides evidence of a function in energy metabolism in rats and monkeys (Ahlskog & Hoebel, 1973; Ammar *et al.* , 2001; Redmond *et al.* , 1977). The LC is also involved in the control of autonomic function and innervates BAT, thus serves a role in thermogenesis and energy expenditure (Samuels & Szabadi, 2008a,b). These mechanisms are known to be modulated by NA and glucose (Madden, 2012; Nedergaard *et al.* , 2011) and the expression of proteins, which are associated with glucose sensing has been matched with neurons in the LC (Dunn-Meynell *et al.* , 1998, 2002; Lynch *et al.* , 2000). In order to define the role of LC neurons in glucose metabolism and energy homeostasis, neurons are investigated for their ability to respond to changes in extracellular glucose concentrations. This aims

to further expand the knowledge of functions of the LC NA system and unravel NA modulation of energy homeostasis.

In detail, the experiments in the first part of this study aim to:

1. characterize basic electrophysiological properties of POMC neurons in the ARC by perforated patch-clamp recordings and if possible identify subpopulations
2. analyze the effect of NA on POMC neurons in the ARC and specify underlying receptor subtypes
3. characterize basic electrophysiological properties of NPY/AgRP neurons in the ARC by perforated patch-clamp recordings and if possible identify subpopulations

The LC exhibits wide efferent projections in the CNS and also innervates the hypothalamus including the ARC. It is thus a potential source for NA release on POMC and NPY/AgRP neurons.

In the second part, experiments in the LC aim to:

1. create an electrophysiological characterization of LC neurons in the perforated patch configuration, which serves as a baseline for future experiments in our labaratory
2. test for responses of LC neurons to changes in extracellular glucose concentrations as a fuel related signal

The identification of NA effects on the melanocortin system in the ARC and its effect on eating behavior in general aims to expand the model of NA's role in energy homeostasis. The LC represents a potential source for NA release into the ARC. It is important to identify conditions under which NA is released because anorexigenic drugs in the treatment of obesity often target CA neurotransmitter systems (Di Dalmazi *et al.* , 2013). In order to to develop specific pharmacological tools with minimized side effects in treatment of obesity, it is critical to understand in detail the mechanisms of the CA modulation of energy homeostasis.

3.1 Properties of POMC and NPY/AgRP neurons in the arcuate nucleus

In order to define specific subpopulations of the POMC and NPY/AgRP neurons, their basic electrophysiological properties were analyzed. Since previous descriptions are conducted in the whole cell patch-clamp configuration, these experiments aim to create a baseline for perforated patch-clamp experiments (Ernst *et al.* , 2009; Roepke *et al.* , 2012; Sohn *et al.* , 2011; van den Top *et al.* , 2007, 2004; Williams *et al.* , 2010; Yang *et al.* , 2012; Zhan *et al.* , 2013). All perforated patch-clamp recordings have been conducted in acute mouse brain slices containing the ARC and POMC or NPY/AgRP neurons were identified by their specific GFP expression.

3.1.1 Properties of POMC neurons

Basic membrane properties

POMC neurons in the ARC have been shown to comprise a heterogenous neuron cluster. Peripheral signals such as insulin and leptin are targeting a rather small amount of POMC neurons (Sohn & Williams, 2012; Williams *et al.* , 2010). In an elegant study, the same has been shown for the modulation by 5-HT (Sohn *et al.* , 2011). Consistently, electrophysiological properties of POMC neurons reflect this heterogeneity (see figure 3.1 A). Analysis of spontaneous activity of POMC neurons revealed large variability of frequencies resulting in a mean of 0.9 $\pm$ 0.4 Hz (see figure 3.1 B; n=15). Almost 50 % of POMC neurons did not exhibit any generation of spontaneous action potentials (AP). POMC membrane potentials resulted in a mean of -67.3 $\pm$ 2.1 mV (see figure 3.1 B; n=15). Input resistances also varied strongly covering a range from 1 to 4 GΩ(n=15). Measurement of cell capacitances at the end of each experiment resulted in a mean of 14.1 $\pm$ 2.1 pF (see figure 3.1 B; n=15).

SFA

Analysis of POMC neuron responses to long lasting depolarizing current injections of 10 seconds revealed three different types of SFA. This mechanism is closely related to

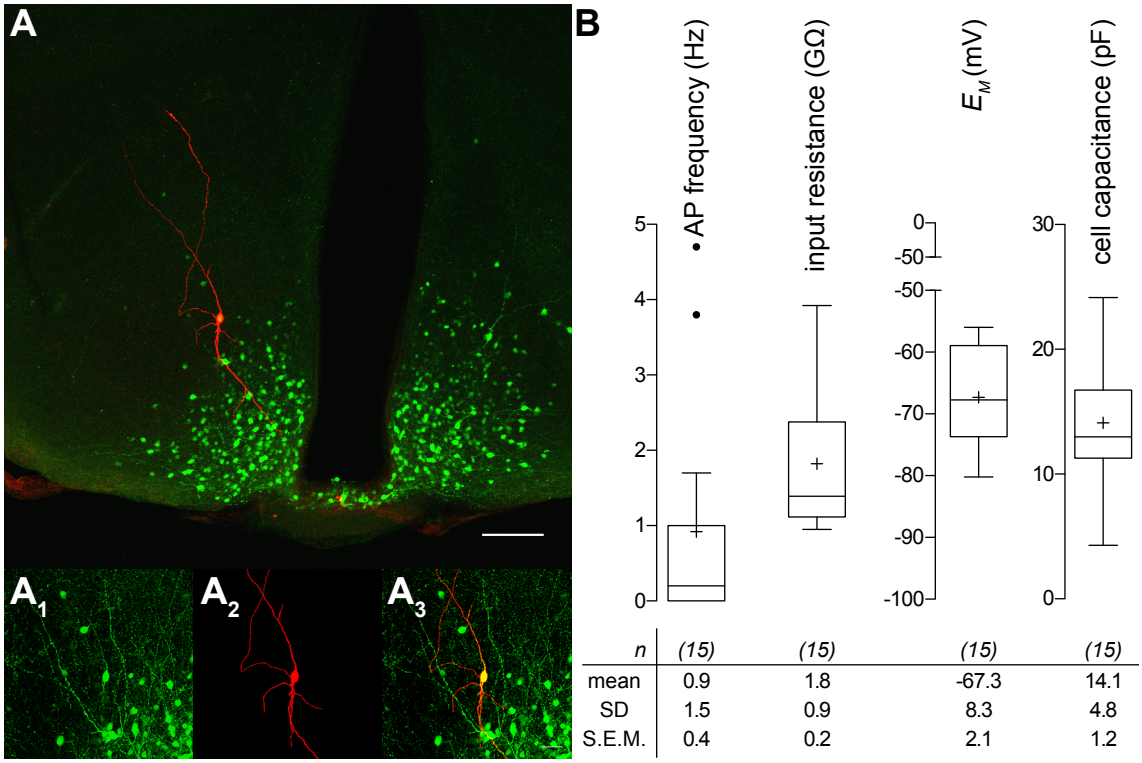


Figure 3.1: Properties of POMC neurons in the ARC. ***(A)*** *upper graphic* Localization of recorded neuron by post-hoc immunohistochemistry. ***(A_{1-3})*** *left* anti-GFP stain against POMC neurons. *mid* biocytin backfill of recorded neuron labeled by streptavidin-Alexa633 Fluor conjugate. *right* Overlay confirms doublelabelling of recorded neuron (yellow) and thus identifies the recorded cell as POMC neuron. ***(B)*** Basic electrophysiological properties of POMC neurons recorded in the arcuate nucleus and the respective means. Data are given as mean ± SEM and SD, scalebars in A: 100 μm; A_{1-3}): 20 μm. For details on boxplots see section Statistics in Materials and Methods.

the opening of voltage gated Ca^{2+} channels (VGCC) and subsequent activation of Ca^{2+} activated K^+ channels, thereby lowering instantaneous spike frequencies in response to a sustained stimulus. A tool to describe SFA is the "SFA ratio". It is defined as the fraction of the initial instantaneous frequency and the final instantaneous frequency (see section Materials and Methods on 29). POMC neurons exhibited strong (SFA ratio >6; n=1), weak (SFA Ratio 2-6; n=4) and no SFA (SFA ratio ∼1; n=1). The majority of POMC neurons was categorized as weak adapting and showing SFA ratios of approximately 4-5 (n=4; for single data points see figure 3.2 A *(mid trace)*, B *(blue)*, C *(blue)*). One neuron revealing strong SFA reduced AP frequency over time almost 13 fold (see figure 3.2 A *(upper trace)*, B *(black)*, C *(black)*, D). In contrast, the third type of SFA only resulted in a ratio of ∼ 1 (see figure 3.2 A *(lower trace)*, B *(red)*, C *(red)*, D). Instantaneous frequencies over time were fit to a mono-exponential decaying equation to analyze the time depen-

dent kinetics of SFA . Time constants varied from 159 ms to 1420 ms, again reflecting large variability (see figure 3.2 D; n=6). To summarize, the analysis of basic properties of POMC neurons revealed a heterogenous population covering a wide range of values. The analysis of SFA revealed three subtypes, regarding the SFA ratios. This is the first subclassification of POMC neurons, based on electrophysiological properties. None of the three investigated types could be assigned to other properties of POMC neurons, clearly pointing towards a large variety of POMC of neurons.

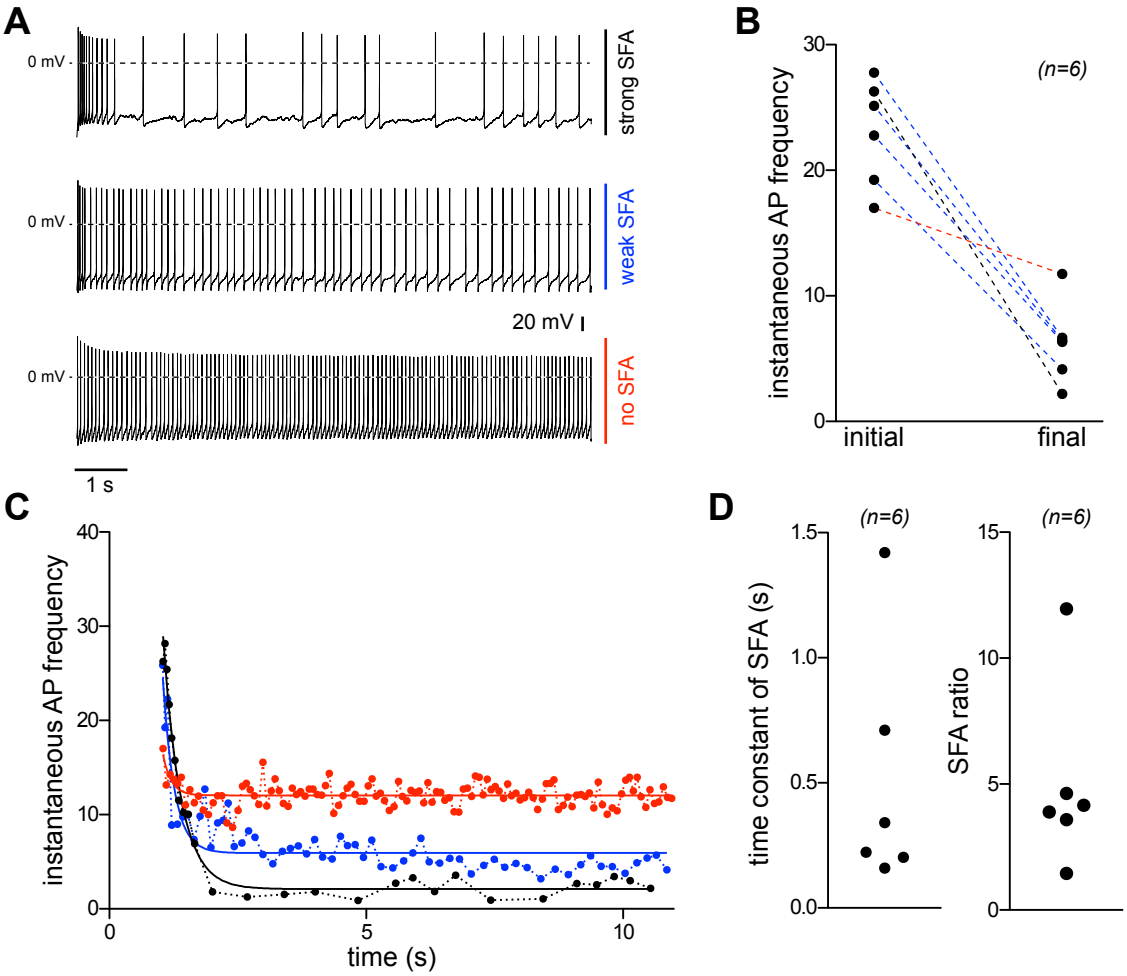


Figure 3.2: SFA properties of POMC neurons in the ARC. ***(A)*** Three different responses to long lasting current injections of 10 s reveal three types of SFA in POMC neurons; strong SFA *upper trace (black)*, weak SFA *mid trace (blue)*, and no SFA *lower trace (red)*. ***(B)*** Initial and final instantaneous frequencies of all single experiments, clearly showing the three distinct SFA types *(colorcode given in* ***(A)***). ***(C)*** Development of instantaneous frequencies of single experiment representing each type of SFA and the respective monoexponential fits to obtain time constants of inactivation.***(D)*** Time constants of inactivation obtained by monoexponential fits as illustrated in **(C)**) and SFA ratios as calculated by the instantaneous frequencies seen in **(B)**).

3.1.2 Properties of NPY neurons

Basic membrane properties

NPY/AgRP expressing neurons in the ARC have been reported to be a rather homogenous neuronal population compared to the large variation in electrophysiological properties of POMC neurons. However, there is no detailed description of GFP labeled NPY/AgRP neurons in the literature. Therefore, basic electrophysiological properties have been analyzed in a subset of mice that were used within this study. This aims to create a baseline, specifically for GFP expressing NPY/AgRP neurons for further experiments in our laboratory. A subset of neurons has been anterogradly labeled via with biocytin to analyze wether electrophysiological properties correlate with distinct localization of somata and morphology of NPY/AgRP neurons. In contrast to variability in morphology regarding dendritic projections, electrophysiological properties were homogenous. Almost all NPY/AgRP neurons exhibited generation of spontaneous action potentials ranging from 0.5 to 7 Hz (see figure 3.3 B; n=15). Respective membrane potentials showed a mean value of -54.4 $\pm$ 1.2 mV (see figure 3.3 B; n=15) . Cell capacitances of 9.7 $\pm$ 0.5 were slightly lower compared to POMC neurons (see figure 3.3 B; n=15). Control input resistances showed a larger variation and resulted in a mean of 2.3 $\pm$ 0.3 GΩ, slightly higher than input resistances of POMC neurons (see figure 3.3 B; n=14).

Spike frequency adaptation

Consistent with homogenous distribution of basic electrophysiological properties, analysis of responses to depolarizing current injections only revealed a single type of SFA (see figure 3.4). According to Ohm's law, large variations of input resistances led to large variations in voltage reflections upon current injection of 5 pA increments. Therefore, to compare SFA properties, current injections have been used which resulted in an initial instantaneous spike frequency of 30-40 Hz. Initial frequencies and final frequencies were used to obtain SFA ratios (see section Materials and Methods on page 29). Instantaneous frequencies over time were fit to a mono-exponential decaying equation to analyze the time dependent kinetics of SFA (see figure 3.4 B; n=15). In NPY/AgRP

neurons, SFA led to a 6-fold decrease in instantaneous spike frequency from a mean of 35.2 to 7.3 Hz resulting in an SFA ratio of 5.6 ± 2.3 (see figure 3.4 A, C, D; n=15).

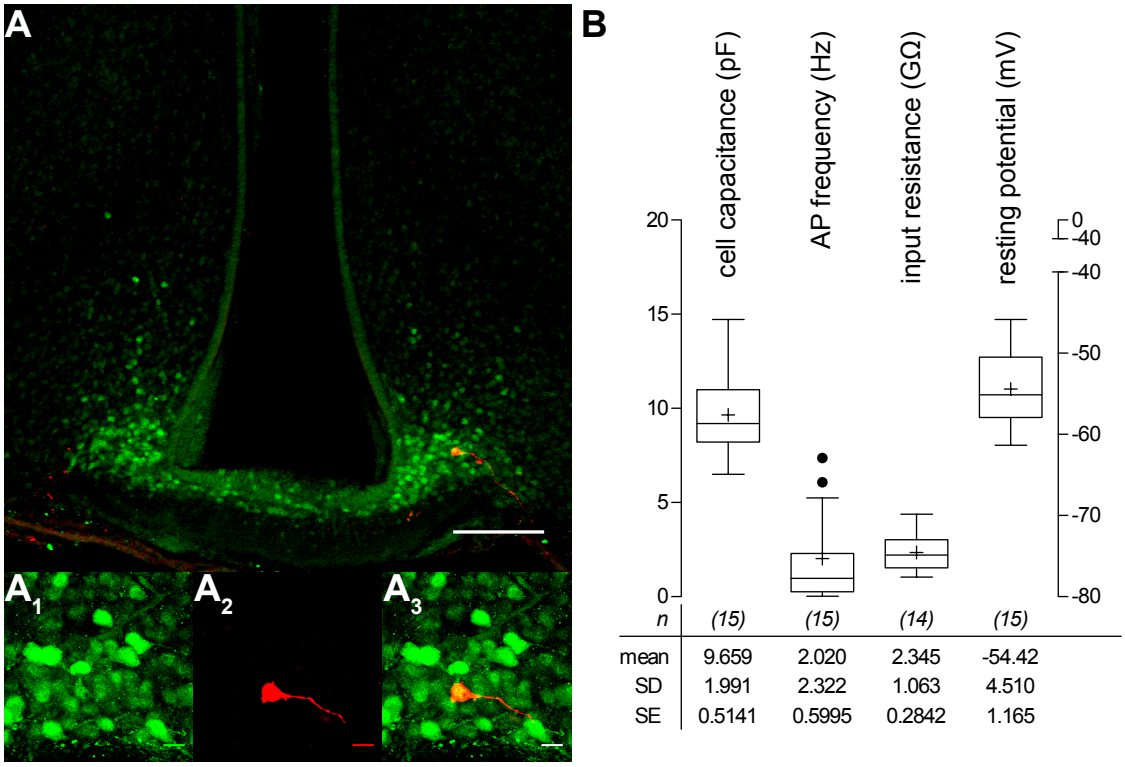


Figure 3.3: Properties of NPY/AgRP neurons of the ARC. ***(A)*** *upper graphic* Localization of recorded neuron by post-hoc immunohistochemistry. ***(A_{1-3})*** *left* anti-GFP staining of NPY/AgRP neurons. *mid* biocytin backfill of recorded neuron labeled by streptavidin-Alexa633-conjugate. *right* Overlay confirms co-labelling of recorded neuron (yellow) and thus identifies the recorded cell as NPY/AgRP neuron. ***(B)*** Basic electrophysiological properties of NPY/AgRP neurons recorded in the arcuate nucleus and the respective means. Data are given as mean ± SEM and SD, scalebars in A: 100 μm; A_{1-3}: 20 μm. For details on boxplots see section Statistics in Materials and Methods.

Taken together, basic properties of NPY/AgRP neurons resulted in a wide range of values, but did not lead to a subclassification. However, the properties of SFA were very uniform showing comparable values among all tested neurons, regardless of differences in other basic properties.

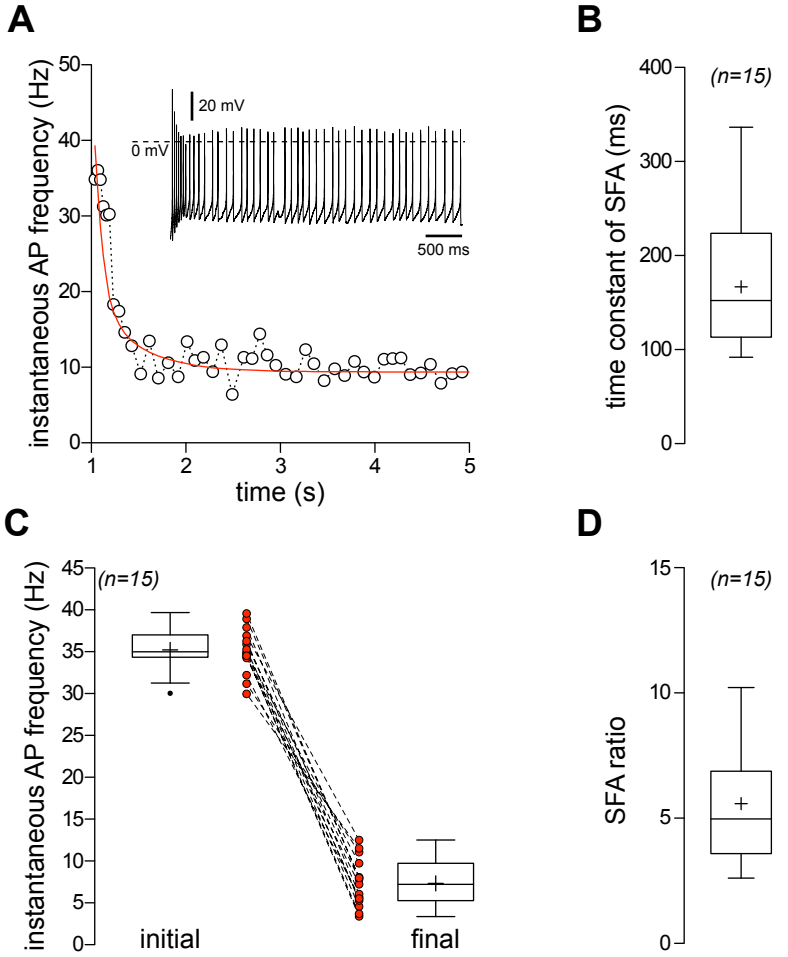


Figure 3.4: SFA properties of NPY/AgRP neurons in ARC. ***(A)*** Extract of a single experiment showing instantaneous spike frequency over time and the respective fit to a monoexponential equation. *inset* Respective voltage response to the depolarizing current injection. ***(B)*** Time constant of SFA as obtained by the monoexponential fits. ***(C)*** Initial and final instantaneous spike frequencies. Red circles and black dotted lines mark the single experiments and their respective reduction of frequency during SFA.***(D)*** SFA ratios as calculated by the frequencies seen in (C). For details on boxplots see section Statistics in Materials and Methods.

3.2 The effect of noradrenaline on POMC neurons of the melanocortin system in the arcuate nucleus

A substantial body of literature suggest NA effects in the ARC, mediated by the presence of ARs and NA synaptic endings (Brunetti *et al.* , 1999, 2002; Kang *et al.* , 2000; Levin *et al.* , 1999). These studies provide evidence, that leptin and insulin modulate NA effects in the hypothalamus and the ARC. Extracellular recordings revealed neurons in the ARC responding to application of NA, however these recordings lacked the identification of the respective neurons (Kang *et al.* , 2000). In order to analyze potential effects of NA on POMC neurons, different concentrations of NA have been bath applied and responses were tested for their dependence on the applied concentrations. Further experiments aimed to test wether the effect of NA is cell intrinsic and which are the underlying AR subtypes. These experiments aim to identify further contributions of the NA system to energy homeostasis and expand the present model. A detailed description of NA function in energy homeostasis is necessary to develop strategies and drugs that support the treatment of obesity with no or minimized side effects.

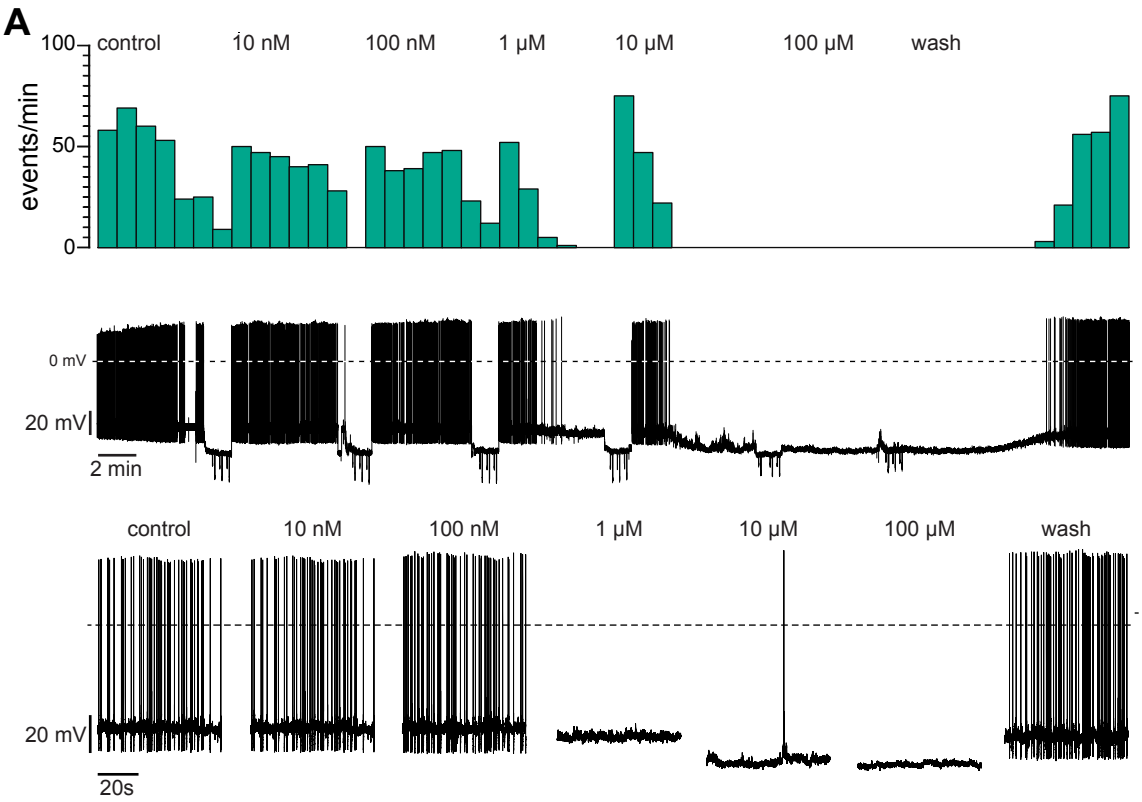
3.2.1 Noradrenaline inhibits POMC neurons dependent on concentration

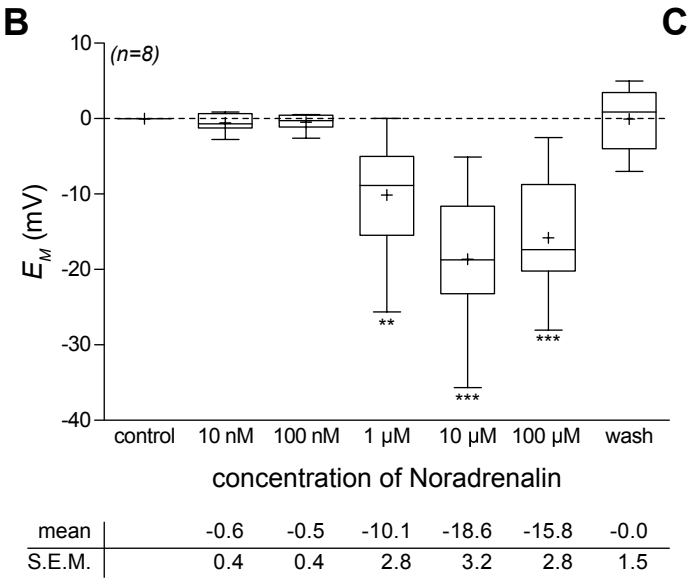
NA was bath applied in concentrations ranging from 100 nM to 100 μM to elucidate wether the CA changes membrane properties of POMC GFP neurons in the ARC. NA inhibited all recorded POMC neurons and decreased AP frequency in neurons which showed spontaneous activity (see figure 3.5 A). Not all tested POMC neurons exhibited the generation of spontaneous AP. Thus, the membrane potential was used to quantify the effect on POMC neurons. At a concentration of 10 and 100 nM, NA slightly hyperpolarized the membrane potential of POMC neurons of about -0.6 $\pm$ 0.4 mV for 10 nM and -0.5 $\pm$ 0.4 mV for 100 nM, respectively (see figure 3.5 A, B; n=8; *n.s.* $p \geq 0.05$).

Increasing concentrations of NA led to significant hyperpolarizations of the membrane potential reaching a peak effect at 10 μM with -18.6 $\pm$ 3.2 mV (see figure 3.5 A, B; n=8; ***$p < 0.001$). Further increasing the concentration to 100 μM was not able to increase the effect, rather leading to a slow desensitization. This was reflected by a weaker hyperpolarization of -15.8 $\pm$ 2.8 mV compared to the peak hyperpolarization

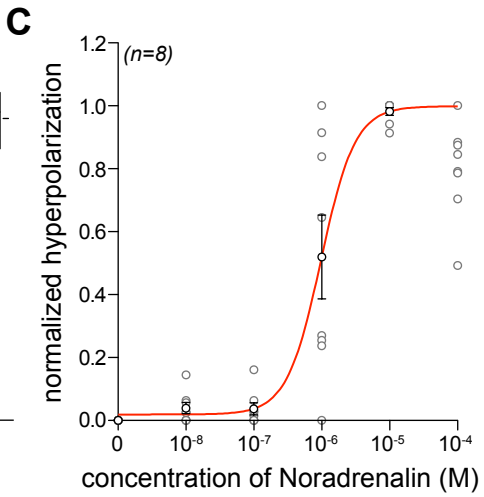
at 10 μM (see figure 3.5 B; n=8; *n.s.* $p \geq 0.05$). All effects were reversible at the tested concentrations (see figure 3.5 B for means and respective SEM of each applied concentration). After changing to control solutions the slow inhibition was followed by a slow and relatively long lasting (10 minutes) rebound excitation (see figure 3.5 A). The hyperpolarization clearly increased upon higher concentrations of NA. By fitting the values to a Hill equation, parameters for receptor activation were obtained and resulted in an EC_{50} of 975 nM and a respective slope of 1.74 (n=8).

Figure 3.5 ***(following page)*:** The effect of increasing concentrations of NA on the membrane potential of POMC neurons. ***(A)*** *upper panel* Rate histogram and overview of a representative recording *(mid panel)* of a POMC neuron and responses to increasing concentrations of NA ranging from 10 nM to 100 μM. The effect is clearly reversible at the end. *lower panel* Magnifications of the respective responses to each concentration of NA of the recording shown above for each concentration. The responses show a clear concentration dependency. Note the slow rebound excitation following the washout of NA. ***(B)*** Hyperpolarization of the membrane potential of POMC neurons after subtraction of the control membrane potential. Peak hyperpolarization was obtained at a concentration of 10 μM and resulted in a mean of -18.6 $\pm$ 3.2 mV. Application of higher concentrations led to slow desensitization of the response. ***(C)*** Mean normalized hyperpolarization is fit to a Hill equation to visualize concentration dependency and obtain kinetics for the responses to NA. Grey circles mark the single values. The fit results in a EC_{50} of 975.0 nM with a respective Hill slope of 1.74. Data are given as mean $\pm$ SEM., * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.





mean	-0.6	-0.5	-10.1	-18.6	-15.8	-0.0
S.E.M.	0.4	0.4	2.8	3.2	2.8	1.5



Hyperpolarization of membrane potential in POMC neurons was accompanied by decreases in input resistances (see figure 3.6 A; n=8), which were calculated to conductance densities (see figure 3.6 C) by normalizing to the cell capacitances of 12.7 $\pm$ 1.6 pF. Capacitances were measured at the end of each experiment (see figure 3.6 B; n=8). All changes in response to the application of NA were significant compared to measurements in control conditions. The response reached peak levels upon the application of 10 μM see figure 3.6). The NA induced mean peak conductance density resulted in 68.9 $\pm$ 10.1 S/F (n=8; ***$p < 0.001$; see figure 3.6 A, B, C for means and respective SEM of each applied concentration). The induced conductance densities were concentration dependent (see figure 3.5 B,C; ; n=8 3.6 C,D; n=8). By normalizing to the peak responses of each conductance density the effects of NA could be fit to a Hill equation to obtain parameters of receptor activation. The concentration of half maximal activation of the receptor (EC_{50}) was 1309 nM with a respective Hill slope of 2.1(see figure 3.6 D; n=8) .

Taken together, the effect of NA was concentration dependent with a maximal response at 10 μM, suggesting the expression of an inhibitory AR subtype. Increasing the applied concentration led to a desensitizing effect on the membrane properties. At the application of high concentrations, the wash out of NA resulted in a slow and long lasting (10 min) rebound excitation. The effective concentrations and receptor activation properties revealed by the fits to Hill equations point to a specific activation of the receptor by NA, rather than crosstalk mediated by high concentrations of the CA. These findings reveal the first evidence for noradrenergic modulation of the melanocortin system at the site of POMC neurons. This identifies the ARC as a target for NA release from brainstem nuclei and expands the established role of NA in the regulation of energy homeostasis and metabolism.

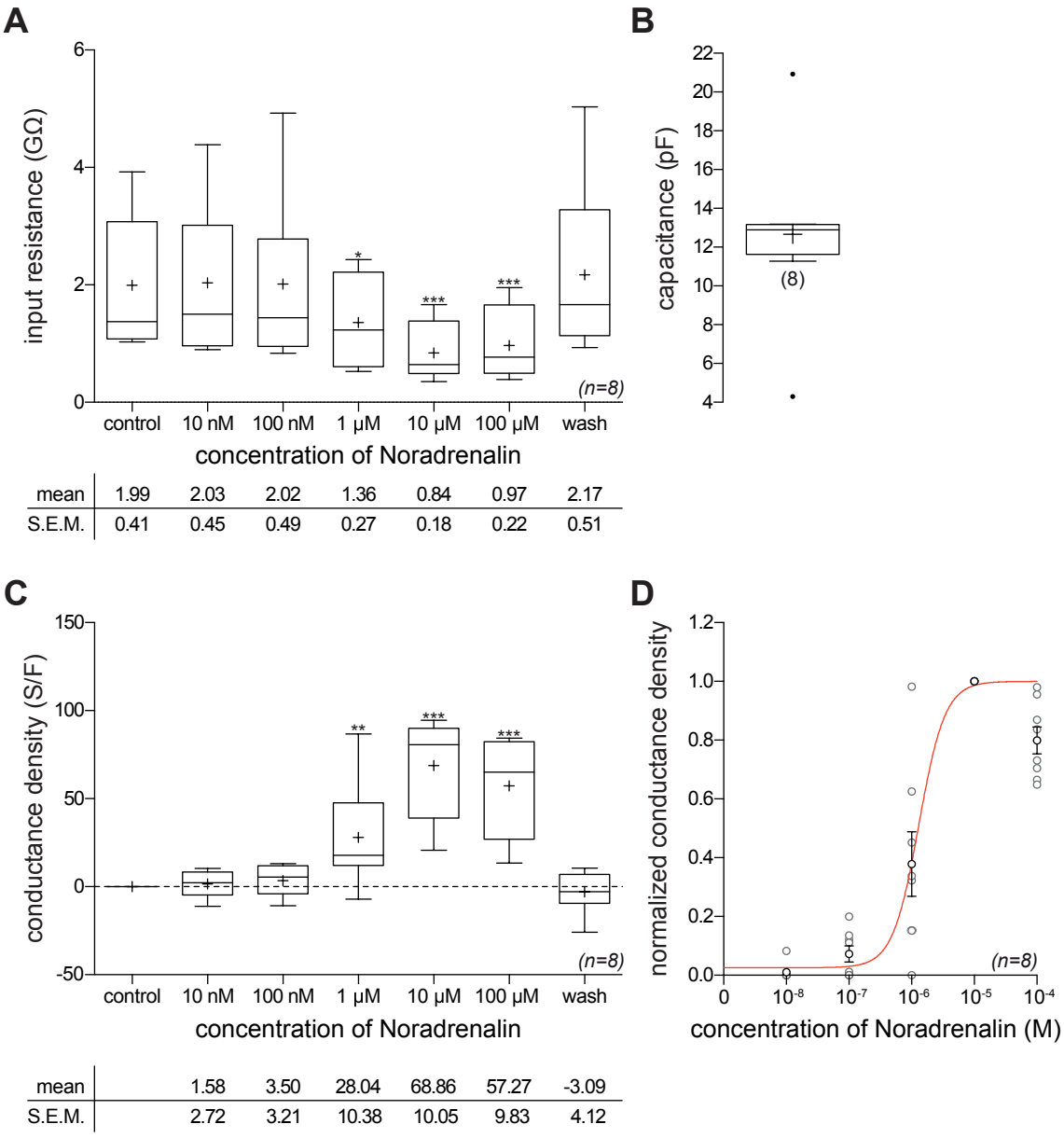


Figure 3.6: The effect of increasing concentrations of NA on the input resistance and conductance density of POMC neurons. ***(A)*** Increasing concentrations of NA from 10 nM to 100 μM lead to decreasing input resistances as measured by hyperpolarizing current injections. ***(B)*** Respective cell capacitance measured at the end of each experiment to obtain conductance densities. ***(C)*** Induced net conductance densities upon application of increasing concentrations of NA. ***(D)*** Mean normalized conductance density is fit to a Hill equation to visualize concentration dependency and obtain kinetics for the responses to NA. Grey circles mark the single values. The fit results in a EC50 of 1309 nM with a respective Hill slope of 2.1. Data are given as mean ± SEM, * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

3.2.2 The effect of noradrenaline on POMC neurons is cell intrinsic

To analyze wether the effect of the different concentrations of NA on the membrane properties of POMC neurons is pre - or postsynaptic, the response to a single concentration of NA has been tested in the presence of synaptic blockers (D-AP5 (50 μM), CNQX (10 μM) and PTX (100 μM)). First it was tested if the respective neurons respond to 10 μM NA in control solution, in which cells are not isolated from synaptic input. NA consistently inhibited POMC neurons (see figure 3.7 A *upper panel*) and increased conductance density to 118.2 $\pm$ 3.8 S/F compared to control levels of 52.8 $\pm$ 12.1 S/F (see figure 3.7 B; n=4; ***p<*0.001*). Application of the synaptic blockers did not lead to significant changes of conductance density, indicating low synaptic input to POMC neurons under control conditions (see figure 3.7 B; n=4; *n.s.* $p \geq$ *0.05*). After 15 minutes of perfusion with synaptic blockers, the response to 10 μM Noradrenalin was tested again. In the presence of synaptic blockers all tested neurons responded to NA and membrane potentials (see figure 3.7 A *lower panel*) as well as input resistances were significantly reduced resulting in an induced conductance density of 99.0 $\pm$ 5.8 S/F (see figure 3.7 B; n=4; ***p<*0.001*). The responses in the presence of the synaptic blockers were slightly decreased compared to those in the absence of synaptic blockers suggesting a small presynaptic contribution by NA responsive neurons, which project on POMC neurons (see figure 3.7 B; n=4; **p < *0.01*).

Taken together, the effects of NA on membrane properties of POMC neurons were reversible and present in all tested neurons. These findings show the expression of an inhibitory AR subtype intrinsically in POMC neurons. A small presynaptic contribution could be observed, indicating the modulation of neurons that form synapses on POMC neurons in the ARC. The hyperpolarization due to NA leads to the question which AR subtype mediates these effects.

3.2.3 Noradrenaline inhibits POMC neurons via the activation of α_{2A}-adrenergic receptors

The robust hyperpolarization of POMC neurons by NA raises the question which type of AR mediates the inhibitory responses upon the application of NA. A vast number of

subtypes have been classified by the help of pharmacological tools (Ruffolo & Hieble, 1994). Since α_2-ARs mediate inhibition and are expressed throughout the CNS, specific pharmacological tools were used to identify these receptors on POMC neurons (Hein, 2006). Experiments described in the previous chapter resulted in a weak contribution of presynaptic effects to the inhibition of POMC neurons by NA (see figure 3.7 B; n=4). Therefore all of the following experiments have been conducted in the presence of synaptic blockers.

The specific α_{2A}-AR antagonist BRL 44408 was bath applied and responses of 5 μM NA were compared to those in the presence of 10 μM BRL 44408. In control conditions, NA induced an increase in conductance density of 48.7 $\pm$ 9.1 S/F (see figure 3.8 A, B; n=5; ***$p < 0.001$). Upon application of 10 μM BRL 44408 alone, no significant increase in conductance density could be observed, suggesting no baseline activity of α_{2A}-ARs (see

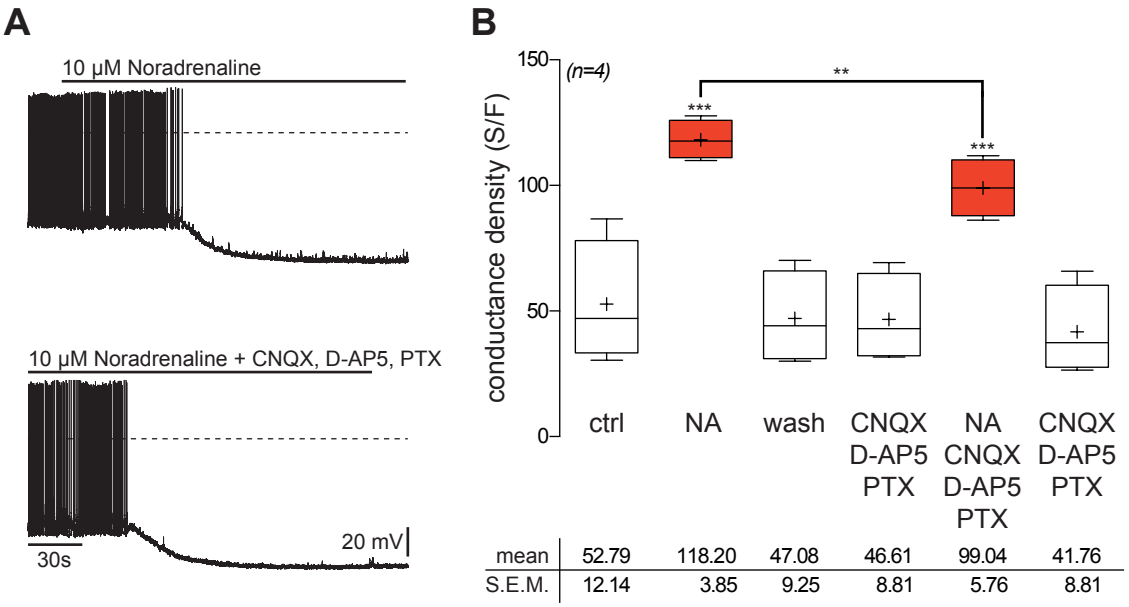


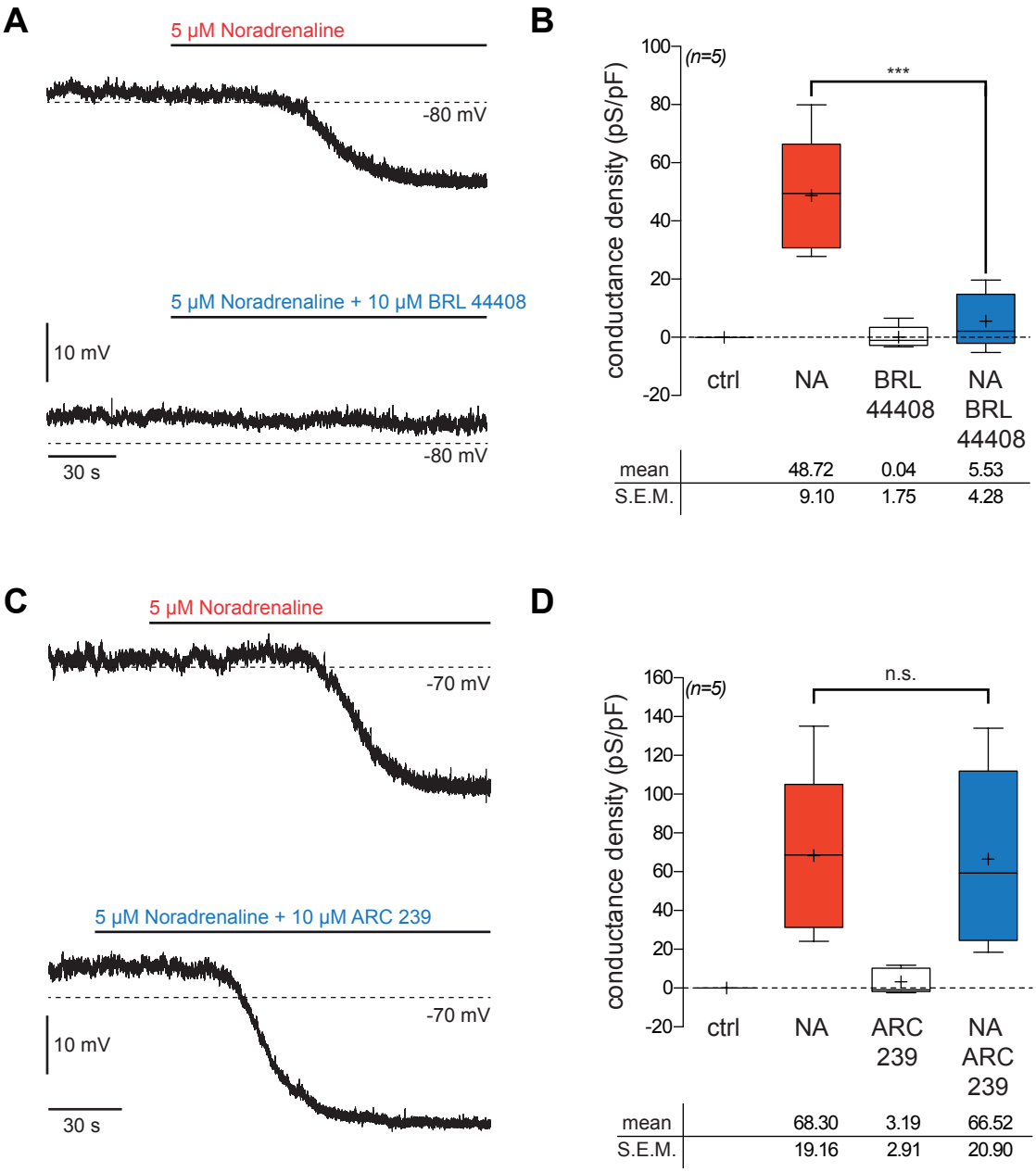
Figure 3.7: Response of synaptically isolated POMC neurons to NA application. ***(A)*** *upper panel* Representative response of a POMC neuron to 10 μM NA. Membrane potential is strongly hyperpolarized. Note the presence of small EPSPs. *lower panel* The response to 10 μM NA of the same neuron in the presence of the synaptic blockers CNQX, D-AP5 and PTX. Again NA strongly hyperpolarizes the membrane potential suggesting a postsynaptic effect on POMC neurons. Note the absence of EPSPs. ***(B)*** NA (10 μM) induces a strong increase in conductance density compared to control levels either in the presence or absence of synaptic blockers. Note the slightly reduced conductance density in presence of the synaptic blockers, suggesting a small presynaptic contribution to the conductance density induced by NA. Data are given as mean $\pm$ SEM, * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

figure 3.8 B; n=5; *n.s.* $p \geq 0.05$). The effect of 5 μM NA was almost completely abolished in the presence of BRL 44408 leading to a small increase of 5.5 $\pm$ 4.3 S/F (see figure 3.8 A, B; n=5; *n.s.* $p \geq 0.05$).

The compound BRL 44408 has been shown to specifically act on α_{2A}-ARs (Hopwood & Stamford, 2001; Owesson *et al.* , 2003; Pauwels & Colpaert, 2000). The almost complete blockade of the NA inhibition by BRL 44408 suggests that this effect is mediated by the activation of the α_{2A}-AR subtype. In order to further confirm this results a specific α_{2B}-AR antagonist was tested. In the presence of 1 μM ARC 239 the effect of NA on the membrane properties of POMC neurons remained unaltered (see figure 3.8 C, D; n=5; *n.s.* $p \geq 0.05$), suggesting that the α_{2B}-AR subtype does not contribute to the observed inhibition of NA on POMC neurons (see figure 3.8 B, D for means and respective SEM for each of the applied antagonists; n=5; ***$p<0.001$).

In line with the results obtained by Kang *et al.* (2000), NA exhibited effects on neurons in the ARC. Importantly, this could be demonstrated on POMC neurons, identified by the specific expression of GFP. This effect is the first described modulation of identified neurons in the ARC on a single cell level. POMC neurons regulate feeding behaviour in a network with AgRP neurons in the ARC. Therefore, it is critical to also test for NA modulation of AgRP neurons.

Figure 3.8 ***(following page)*:** NA inhibits POMC neurons via the activation of α_{2A}-ARs. ***(A)*** *upper panel* NA strongly hyperpolarizes POMC membrane potential in the presence of synaptic blockers. *lower panel* In the same neuron, the specific α_{2A}-AR antagonist BRL 44408 almost completely blocks the effect of NA. ***(B)*** Induced increase of conductance density by NA is significantly reduced by the application 10 μM BRL 44408. Upon application of BRL 44408 no significant increase in conductance density could be observed. ***(C)*** *upper panel*; NA strongly hyperpolarizes POMC membrane potential in the presence of synaptic blockers. *lower panel*; The response to NA in the same neuron remains unaltered in the presence of the α_{2B}-AR specific antagonist ARC 239. Data are given as mean $\pm$ SEM, * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.


A
5 µM Noradrenaline
-80 mV
5 µM Noradrenaline + 10 µM BRL 44408
10 mV
-80 mV
30 s
B
(n=5)
***
conductance density (pS/pF)
100
80
60
40
20
0
-20
ctrl
NA
BRL 44408
NA BRL 44408
mean 48.72 0.04 5.53
S.E.M. 9.10 1.75 4.28
C
5 µM Noradrenaline
-70 mV
5 µM Noradrenaline + 10 µM ARC 239
-70 mV
10 mV
30 s
D
(n=5)
n.s.
conductance density (pS/pF)
160
140
120
100
80
60
40
20
0
-20
ctrl
NA
ARC 239
NA ARC 239
mean 68.30 3.19 66.52
S.E.M. 19.16 2.91 20.90

3.3 The effect of noradrenaline on NPY/AgRP neurons

The consistent and concentration-dependent inhibition of POMC neurons by NA leads to the question if and how the membrane properties of NPY/AgRP neurons are modulated upon application of increasing concentrations of NA. In order to verify this hypothesis, the effect of different concentrations of NA on NPY/AgRP neurons has been tested.

3.3.1 Noradrenaline excites NPY/AgRP neurons dependent on concentration

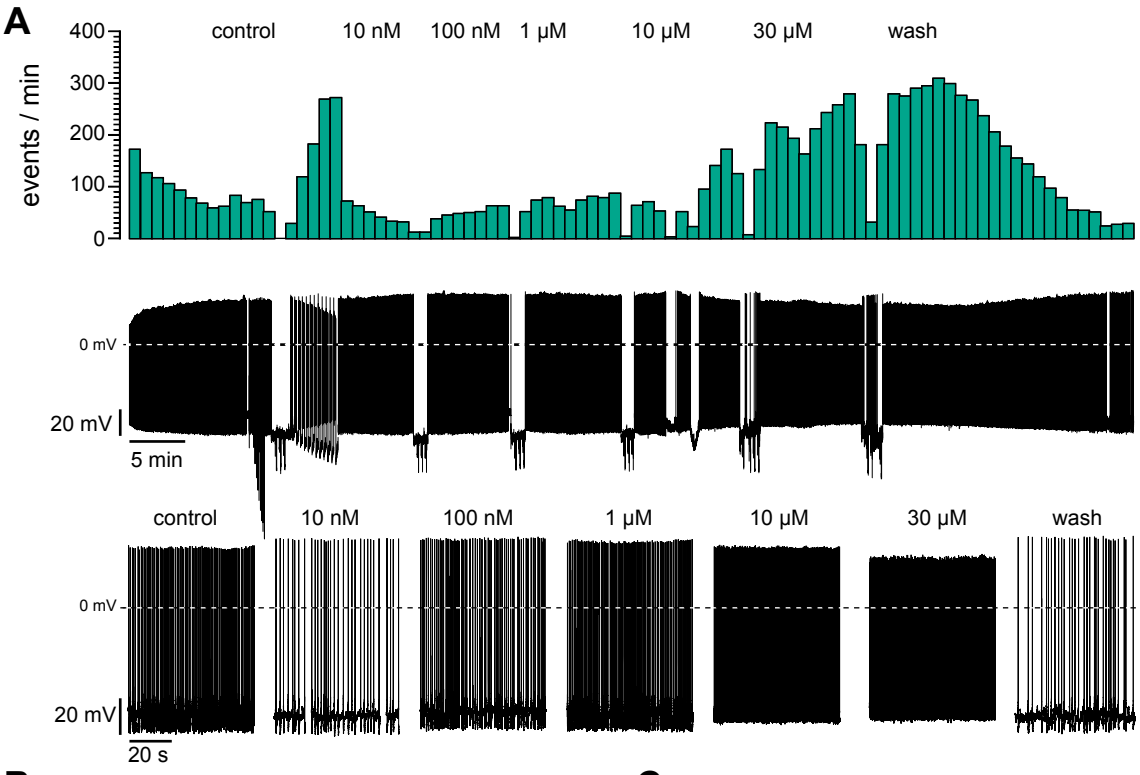
NPY/AgRP neurons were identified by their specific expression of GFP. Concentrations ranging from 10 nM to 100 μM were bath applied and responses were monitored in current clamp mode (see figure 3.9 A). At a concentration of 10 μM, NA excited AgRP neurons by depolarizing the membrane potential about 4.7 $\pm$ 0.8 mV (n=10; ***$p<0.001$) and thus increasing firing rates from 1.9 $\pm$ 0.7 Hz in control conditions to 4.2 $\pm$ 0.8 Hz (see figure 3.9 B, D for means and respective SEM; n=9; ***$p<0.001$). Membrane potential as well as firing frequencies could be fit to a Hill equation when normalized to their maximum effects of each concentration. The fit to Hill equations resulted in an EC50 of 1995 nM (n=9) for the AP frequency and 2046 nM (n=10) for the membrane potential (see figure 3.9 C, E). Respective Hill slopes resulted in values of 1.3 and 1.3.

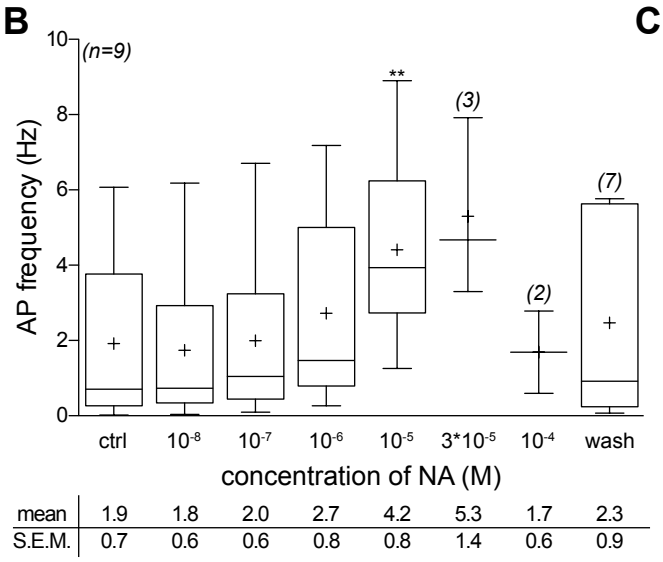
Opposite to POMC neurons, a concomitant increase in input resistance could be measured. This increase in input resistance and thus decrease in conductance density did not clearly follow a Hill relationship and thus was not fit to a Hill equation (data not shown). Together, this data suggests that NPY/AgRP neurons express ARs leading to an excitation of these neurons. Previous work on the melanocortin system has revealed differential regulation of POMC and NPY/AgRP neurons by various stimuli (Blouet & Schwartz, 2010). The present results show, that NA differentially regulates POMC and NPY/AgRP electrical activity by inhibition of POMC and excitation of NPY/AgRP neurons.

3.3.2 High concentrations of noradrenaline elicit bursting in NPY/AgRP neurons

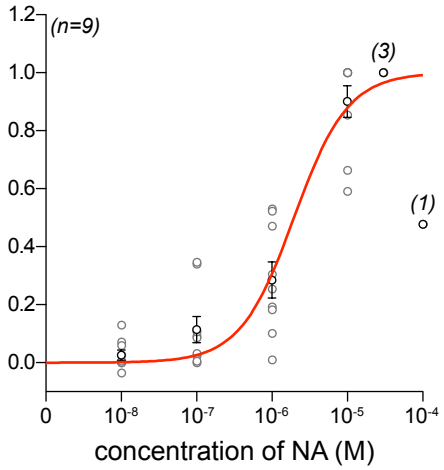
The application of 10 μM NA or higher elicited bursting firing patterns in six of sixteen recorded NPY/AgRP neurons (see figure 3.10). In the beginning, instantaneous frequencies were higher (see figure 3.10A-2, B-2) and with ongoing application decreased (see figure 3.10A). In contrast, burst duration increased and interburst intervals (IBI) decreased, leading to higher mean spike frequencies (see figure 3.10B).

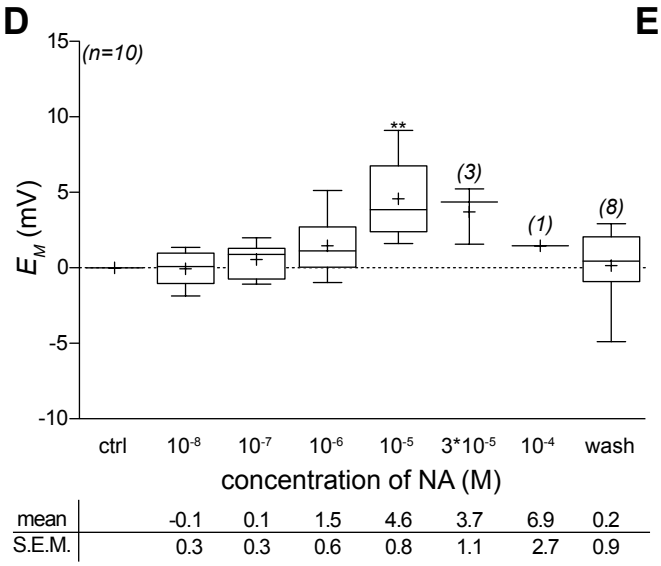
Figure 3.9 ***(following page)*:** NA excites NPY/AgRP neurons dependent on concentration. ***(A)*** *lower panel* Rate histogram and respective recording *(mid panel)* of an NPY/AgRP neuron to application of NA from 10 nM to 30μM. NA strongly excites NPY/AgRP dependent on the applied concentration. *lower panel* Magnifications for each of the applied concentrations of NA. ***(B)*** Increasing concentrations lead to higher AP frequencies with a peak response at 10 - 30 μM. ***(C)*** The normalized peak AP frequencies can be fit to a Hill equation obtaining parameters of receptor activation. ***(D)*** In line with increasing AP frequencies, NA depolarizes membrane potentials of NPY/AgRP neurons upon application of increasing concentrations. ***(E)*** The normalized peak depolarization can be fit to a Hill equation obtaining parameters of receptor activation. In ***(B-E)*** additional numbers in brackets over boxes indicate numbers of experiments for respective concentrations. Data are given as mean $\pm$ SEM, * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.



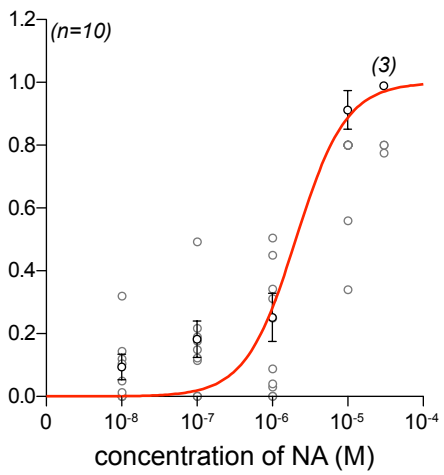


	ctrl	10^{-8}	10^{-7}	10^{-6}	10^{-5}	$3*10^{-5}$	10^{-4}	wash
mean	1.9	1.8	2.0	2.7	4.2	5.3	1.7	2.3
S.E.M.	0.7	0.6	0.6	0.8	0.8	1.4	0.6	0.9





	ctrl	10^{-8}	10^{-7}	10^{-6}	10^{-5}	$3*10^{-5}$	10^{-4}	wash
mean		-0.1	0.1	1.5	4.6	3.7	6.9	0.2
S.E.M.		0.3	0.3	0.6	0.8	1.1	2.7	0.9



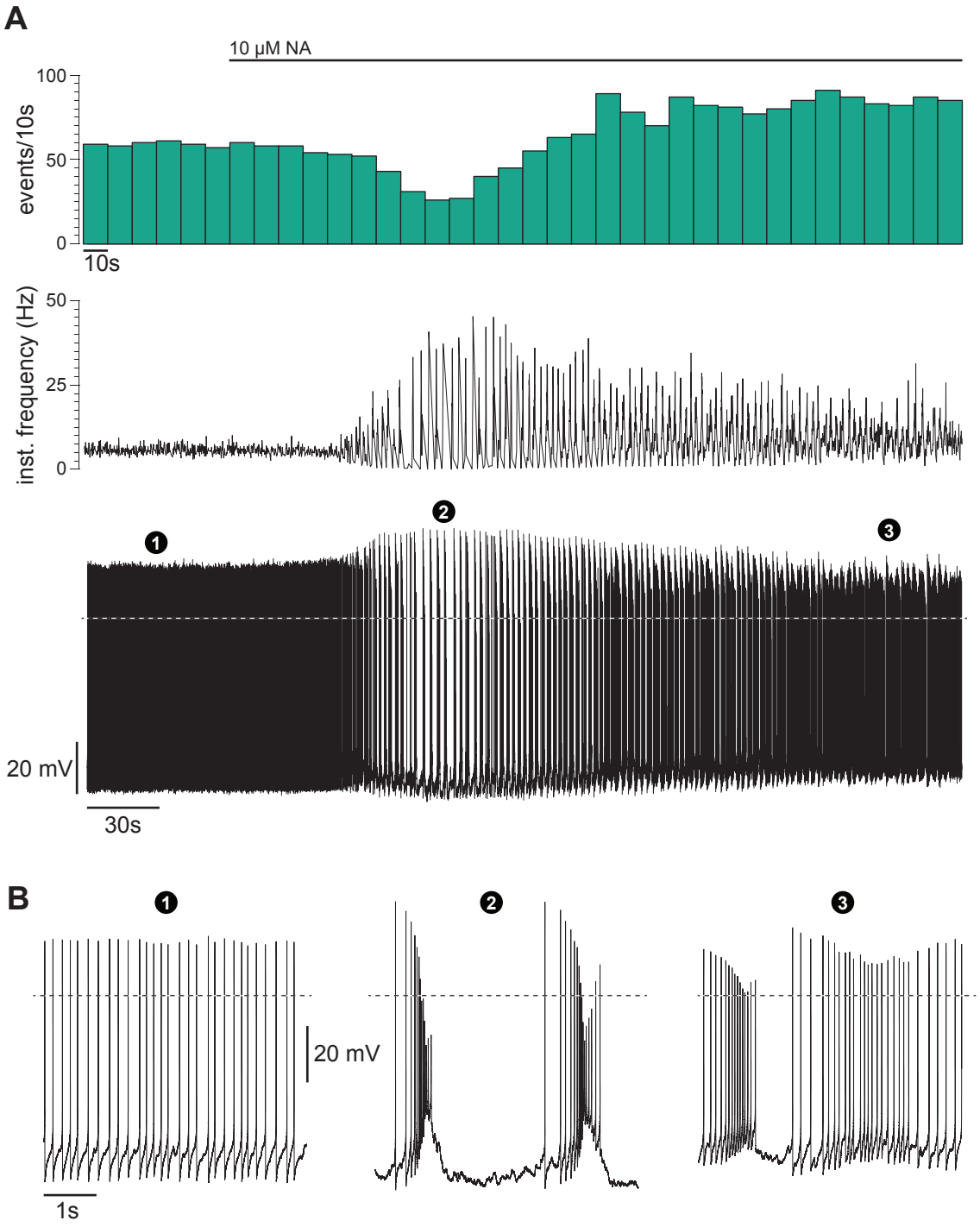


Figure 3.10: High concentrations of NA elicit bursting in NPY/AgRP neurons. ***(A)*** *T*he application of 10 μM NA elicits bursting in a subset of NPY/AgRP expressing neurons (6 of 16 neurons). upper panel Rate histogram of illustrating the overall increase in frequency due to the application of NA. mid panel Instantaneous spike frequency illustrates the oscillatory bursting behavior of NPY/AgRP neurons in response to NA. lower panel Respective recording corresponding to the rate histogram and instantaneous frequencies. Numbers indicate the relative time points of respective magnifications given in *B*. ***(B)*** Magnifications of the raw trace in *A, lower panel* at three different time points, control (1), early bursting (2) and late bursting (3). 10 μM NA elicits regular bursting of an NPY/AgRP neuron. Burst duration increases and IBIs decrease, thus increasing the overall mean frequency as seen in the rate histogram development in *A, upper panel*.

3.3.3 The effect of noradrenaline on NPY/AgRP neurons is cell intrinsic

The effect of NA on NPY/AgRP neurons was further investigated by applying NA in the presence of synaptic blockers. In line with previous results for POMC neurons in the ARC, synaptically isolated NPY neurons responded to the application of NA. Depolarized membrane potentials and increased firing frequencies of NPY/AgRP neurons were observed in all tested neurons (see figure 3.11; n=4; ***$p<0.001$). A concentration of 10 μM NA increased the AP frequency almost three times to 6.4 $\pm$ 1.7 Hz in the absence synaptic blockers (see figure 3.11 B; n=4; ***$p<0.001$). In the presence of synaptic blockers AP frequency was increased to 6.5 $\pm$ 1.7 Hz (see figure 3.11 B for means and respective S.E.M.; n=4; ***$p<0.001$). These experiments suggest that the excitation by NA is due to the cell intrinsic expression of ARs. In contrast to POMC neurons, no presynaptic contribution to the effect of NA could be observed (see figure 3.11 B; n=4; *n.s.* $p \geq 0.05$).

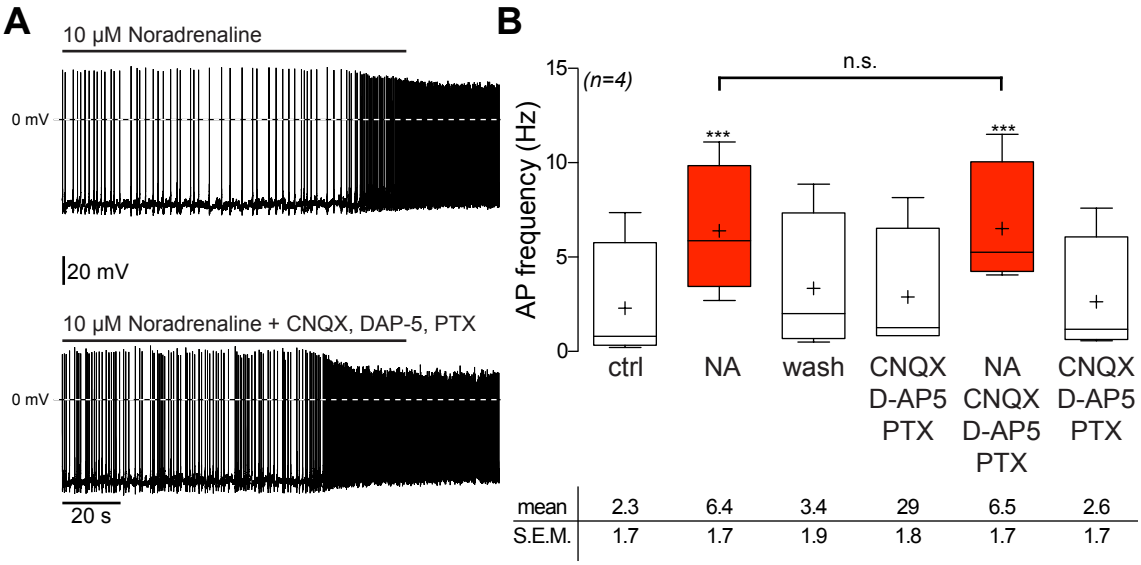


Figure 3.11: Response of synaptically isolated NPY/AgRP neurons to NA. ***(A)*** *upper trace* Representative response of a NPY/AgRP neuron to 10 μM NA. Membrane potential is strongly depolarized and increases AP frequency. *lower trace* The response to 10 μM NA of the same neuron in the presence of the synaptic blockers CNQX, D-AP5 and PTX. Again NA strongly depolarizes membrane potential suggesting a postsynaptic effect of NA on NPY/AgRP neurons. ***(B)*** Application of 10 μM NA induces a strong increase in AP frequency compared to control levels either in or without the presence of synaptic blockers. Synaptic isolation alone has no significant effect on NPY/AgRP neurons. Data are given as mean $\pm$ SEM, * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

3.3.4 Noradrenaline excites NPY/AgRP neurons via the activation of α_{1A}-adrenergic receptors

The fact that NPY/AgRP neurons are consistently excited upon the application of NA leads to question which receptor subtype mediates the excitatory effect on these neurons. It is reasonable to analyze wether the excitation of NPY/AgRP neurons is mediated by α_1-ARs, which have been shown to increase Ca^{2+} and decrease K^+ conductances and thus lead to excitation of neurons expressing these receptors. A variety of pharmacological tools is available to identify specific receptor subtypes of the α_1-ARs. Accordingly to the pharmacological experiments on POMC neurons, specific antagonists have been bath applied in the presence of synaptic blockers.

The effect of NA on all recorded NPY/AgRP neurons in control conditions showed an excitation of membrane properties that were significantly different to properties in the absence of NA (see figure 3.12 A, *upper trace*). At a concentration of 5 μM NA increased the firing frequency from 2.1 $\pm$ 0.6 Hz to 5.2 $\pm$ 0.9 Hz (see figure 3.12 B; n=4; ***$p<0.001$). The application of 100 nM WB 4101 alone did not change any properties to significant levels, suggesting no baseline activation of α_{1A}-ARs (see figure 3.12 B; n=4; *n.s.* $p\geq 0.05$). A second application of NA on the same neuron did not lead to any effects comparable to the control response of 5 μM NA (see figure 3.12 A, *lower trace*). AP frequency in the presence of WB 4101 stayed at values of 2.0 $\pm$ 0.6 Hz and thus did not reach any significance compared to control frequencies (see figure 3.12 B; for means and respective S.E.M.; n=4; *n.s.* $p\geq 0.05$). These experiments suggest the activation of α_{1A}-ARs by NA mediating the excitatory effect on NPY/AgRP neurons. To rule out further contribution of other subtypes of α_1-ARs, a second antagonist was tested specifically antagonizing the effect of α_{1B}- and α_{1C}-ARs. The application of 100 nM chloro-ethyl-clonidine (CEC) did not alter the responses to 5 μM NA (see figure 3.12 C;D n=4; *n.s.* $p\geq 0.05$). Under control conditions NA increased the AP frequency to 3.6 $\pm$ 0.2 Hz (n=4; ***$p<0.001$), which was not significantly different from the frequency of 3.5 $\pm$ 0.4 Hz in the presence of CEC (see figure 3.12 D; for means and respective SEM; n=4; *n.s.* $p\geq 0.05$). Taken together, these experiments demonstrate, that NA excites NPY/AgRP neurons by the activation of α_{1A}-ARs.

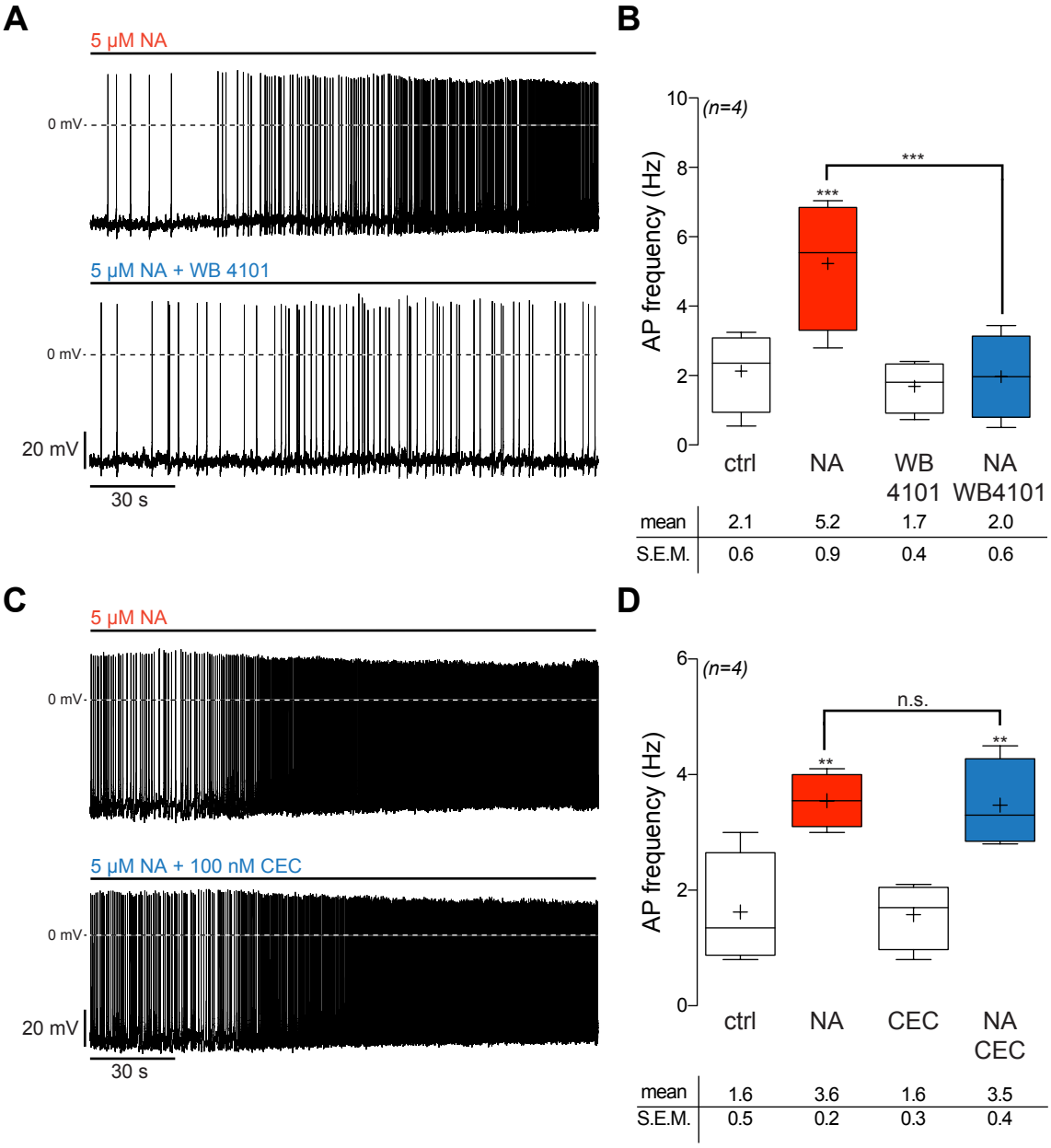


Figure 3.12: NA excites NPY/AgRP neurons via the activation of α_{1A}-ARs. ***(A)*** *upper trace* NA strongly depolarizes NPY/AgRP membrane potential and increases AP frequency in the presence of synaptic blockers. *lower trace* In the same neuron, the specific α_{1A}-AR antagonist WB 4101 blocks the effect of NA. ***(B)*** Increase of AP frequency by NA is almost completely blocked by WB 4104. Upon application of WB 4101 no significant effects could be observed. ***(C)*** *upper trace* NA strongly depolarizes NPY/AgRP membrane potential and increases AP frequency in the presence of synaptic blockers. *lower trace* The response to NA in the same neuron remains unaltered in the presence of the $\alpha_{1B,C}$-AR specific antagonist CEC. Data are given as mean ± SEM, * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

3.4 Effects of dietary changes and aging on responses of POMC neurons to noradrenaline

Aging as well as dietary changes have been shown to affect the melanocortin system in the ARC (Kim & Horvath, 2012; Newton *et al.* , 2013; Yang *et al.* , 2012). In order to investigate putative changes of aging and dietary changes, responses of POMC neurons to application of 10 μM NA have been recorded. Responses in mice aged 8 to 12 weeks were compared to respective responses in mice from 20 to 25 weeks. Induced conductance densities upon NA application decreased in mice at the age of 20 to 25 weeks (n=9) compared to younger mice (see figure 3.13; n=7; $^{**}p < 0.01$). In contrast, mice which had access to ad libitum HFD showed significantly increased conductance densities at the age of 20 to 25 weeks and older (see figure 3.13; n=12; $^{*}p < 0.05$). Taken together, these results indicate a significant age- and diet-dependent effect on NA modulation of POMC neurons in the ARC.

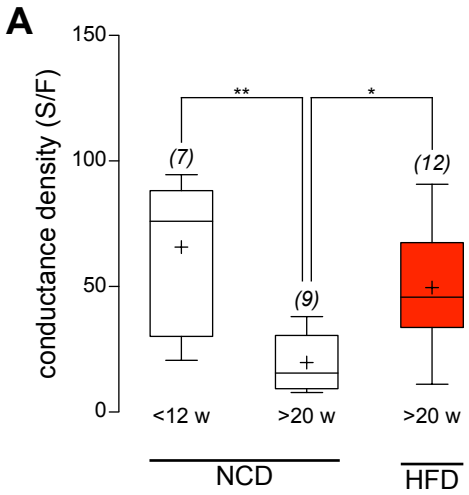


Figure 3.13: Aging and diet dependent changes on NA induced conductance density of POMC neurons in the ARC ***(A)*** Aging reduces induced conductance density upon NA application in mice at the age of 20 to 25 weeks compared to mice at the age of 8 to 12 weeks. Mice with ad libitum access to HFD from 3 weeks of age show increased conductance densities compared to NCD control animals at the age of 20 to 25 weeks. $^{*}p < 0.05$,$^{**}p < 0.01$, $^{***}p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

3.5 The effect of noradrenaline on POMC and NPY/AgRP neurons unifies both heterogenous populations

Within the last decades, the effect of several compounds has been tested for responses of anorexigenic POMC and orexigenic AgRP neurons in the ARC. The variety of stimuli included periphery-born hormones such as insulin, leptin and ghrelin, all of which have been shown to effectively modulate food intake when applied either intracerebroventricular (i.c.v.) or intraperitoenal (IP) (Brüning *et al.* , 2000; Cowley *et al.* , 2001; Ghamari-Langroudi, 2012; Spanswick *et al.* , 2000; Williams *et al.* , 2010). Other studies included extracellular glucose concentrations as well as neurotransmitters and biogenic amines, like Acteylcholine (ACh) and 5-HT (Fioramonti *et al.* , 2004; Mineur *et al.* , 2011; Parton *et al.* , 2007; Sohn *et al.* , 2011). However, the common feature of almost all tested stimuli was that it never affected the whole population of either POMC or NPY/AgRP neurons rather than effecting a subset of these populations (see figure 3.14). In line with the large variety of electrophysiological properties as well as different morphologies, these studies clearly show the heterogeneity of ARC neuronal populations. Strikingly, NA as well as A, was able to effect all tested POMC and NPY/AgRP neurons emphasizing the importance of NA modulation in the ARC.

The effects of NA on neurons in the ARC raises the question under which conditions it is released. The identification of distinct NA nuclei, which provide the NA release into the ARC could contribute to answer this question. NA nuclei reside in the brainstem. The LC represents the largest NA nucleus and innervates the whole CNS, including the ARC. As a potential source for NA, the next part of this thesis focusses on the LC and its putative contribution to energy homeostasis.

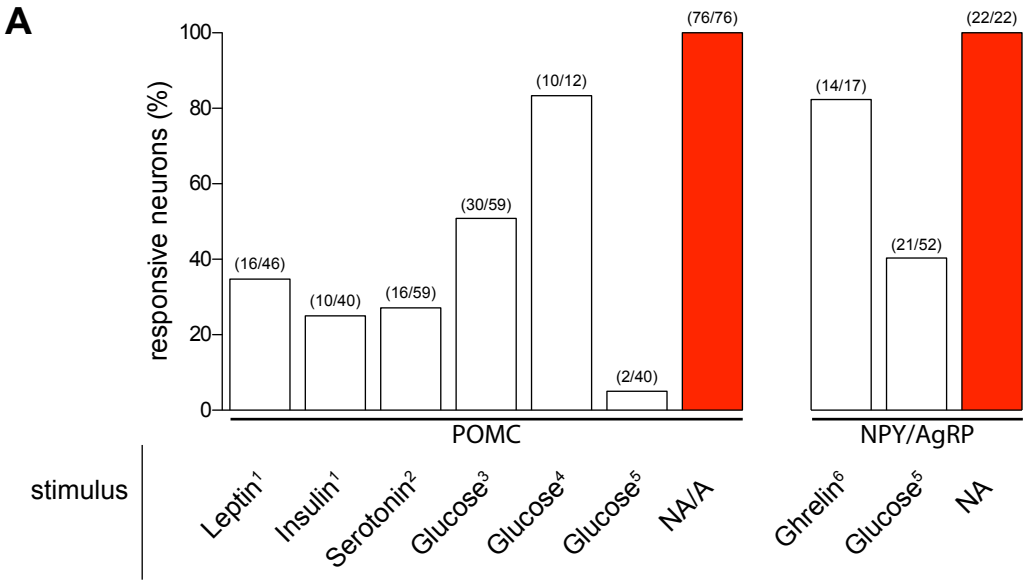


Figure 3.14: Responses of POMC and NPY/AgRP neurons to various stimuli ***(A)*** Bars showing the percentage of responding neurons to different stimuli. Numbers in brackets are total numbers of responding neurons in relation to overall recorded cells. The stimulus NA clearly sticks out among all tested in either POMC or NPY/AgRP population. [1]Williams *et al.* 2010; [2]Sohn *et al.* 2011; [3]Parton *et al.* 2007; [4]Ibrahim *et al.* 2003; [5]Fioramonti *et al.* 2007; [6]van den Top *et al.* 2007.

3.6 The role of the locus coeruleus in the control of energy homeostasis

The LC represents the main source of NA within the CNS. Specific efferent innervation from the LC to the ARC has been identified. This suggests that the LC is a potential source for NA release into the ARC and may thus exert modulatory effects on POMC and NPY/AgRP neurons.

Further widespread afferent and efferent projections reflects the contribution to a variety of different functions, such as attention, memory formation, sleep-wake-cycle, arousal as well as homeostatic mechanisms such as blood flow, chemosensitivity and energy homeostasis. Consequently, impairment of the LC has been shown to be involved of a vast number of diseases and syndroms like AD, PD, attention-deficity syndrome, hyperactivity, anxiety, disturbance in sleep wake cycle, tourett and depression. Various studies also indicate a role of the LC in mechanisms related to energy homeostasis, such as thermogenisis, the control of BAT and glucose metabolism. Since the LC has been shown to express K_{ATP} channels and GK, both of which have been shown to be expressed in glucose-sensitive neurons, the role of the LC in energy homeostasis and glucose metabolism was investigated by expressing a mutant variant of the K_{ATP} channel specifically in TH positive neurons. Expression of the mutant Kir6.2 variant resulted in silencing of the majority of neurons in the LC compared to their control littermates.

In order to identify and analyze LC neurons in C57BL/6-mice, an electrophysiological profile of LC neurons was first established. A small number of studies investigated the basic properties of LC neurons in acute slice preparations on a single cell level. However, these studies have been conducted by patch-clamp recordings in whole-cell configuration and various ages of mice, both of which may change properties of neurons (Cui *et al.* , 2011; de Oliveira *et al.* , 2010, 2011, 2012; Jin *et al.* , 2013; van den Pol *et al.* , 2002). LC neurons have been investigated in mice at the age of 10-15 weeks. This may serve as a baseline characterization for further experiments on modulation of LC neurons. Importantly, properties were identified that enable the specific identification of LC neurons by electrophysiological protocols.

3.6.1 Properties of noradrenergic neurons in the locus coeruleus

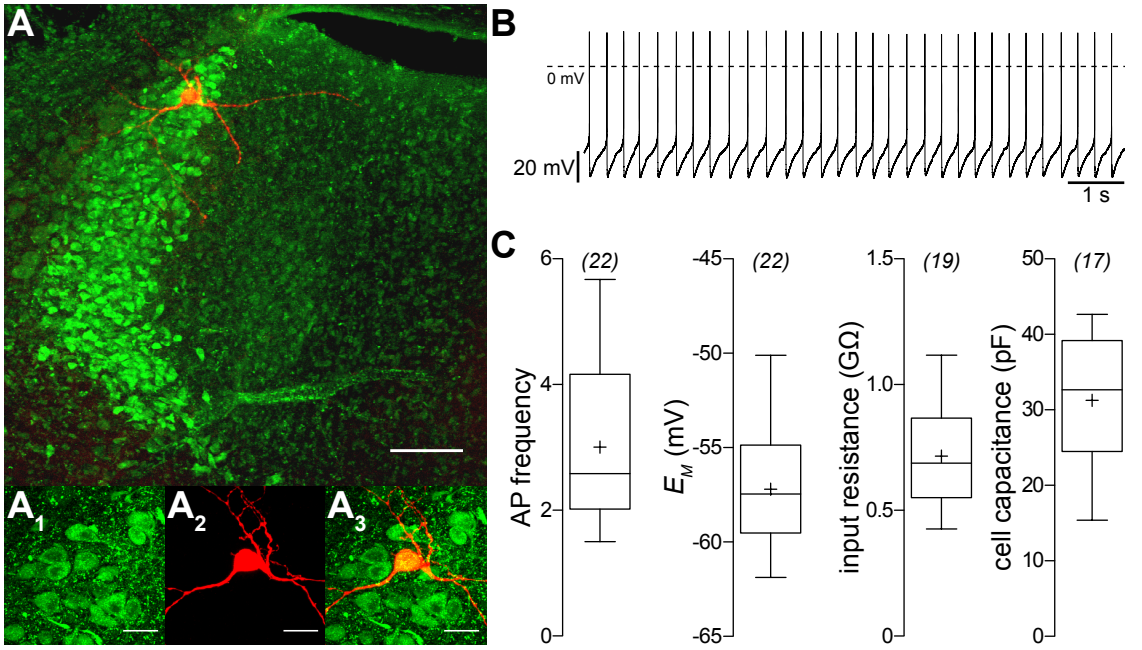
In the following, basic properties of LC neurons from C57BL/6-mice are described at the age of 10-15 weeks. Further characteristics, which are unique to LC neurons in this part of the brainstem are described. This aims to create a baseline for future experiments in our laboratory and to identify LC neurons by specific electrophysiological protocols.

Basic membrane properties

Consistent with previous work, basic properties of LC neurons resulted in a homogenous neuronal population. Streptavidin-stainings of biocytin-backfills of most of the recorded neurons revealed localization within the cluster of the LC and co-labeling with DBH-immunostaining (see figure 3.15 A). LC neurons generated spontaneous action potentials ranging from 1.5 Hz to almost 5.7 Hz corresponding to membrane potentials from $\sim$ -62 mV to -50 mV (see figure 3.15 B, C). The mean input resistance resulted in 716.2 $\pm$ 50.3 MΩ(see figure 3.15 C). Respective cell capacitances, measured at the end of each experiment were 32.0 $\pm$ 2.2 pF (see figure 3.15 C for details of basic properties including SD and SEM). The analyzed properties followed a gaussian distribution suggesting the homogeneity of this neuronal population.

Pacemaking in LC neurons

In vivo electrophysiological recordings of the LC in behaving rats and mice elaborated different activity patterns of these neurons including rhythmic bursting activity as well as continuous pacemaking or silent states (Berridge & Waterhouse, 2003). Consistent with work on dopaminergic neurons on the midbrain, however, in *in vitro* brain slice preparations the vast majority of LC neurons exhibited spontaneous generation of APs in a pacemaking fashion (see figure 3.16 A). A marker of pacemaking neurons is a gaussian distribution of ISIs when plotted as a histogram. Plotting 450 ISIs of each LC neuron revealed a gaussian distribution in each plotted histogram, suggesting a precise intrinsic pacemaking activity (see figure 3.16 B). With the parameters obtained by the gaussian fits coefficients of variances were calculated resulting in only $\sim$ 10 percent variation among ISIs of these neurons (see figure 3.16 C). To further investigate



mean	3.005	-57.21	0.7162	32.04
SD	1.247	3.010	0.2192	8.271
S.E.M.	0.2659	0.6418	0.05029	2.210
min	1.500	-61.87	0.4258	15.88
max	5.670	-50.10	1.117	42.67

Figure 3.15: Basic properties of DBH-positive neurons of the LC ***(A)*** anti-DBH-immunostaining reveals a typical banana shaped population lying ventrolateral to the 4th ventricle comprising the Locus Coeruleus. ***(A*** $_1 - 3$***)*** co-labeling of the recorded neuron by biocytin-backfill and subsequent staining with a streptavidin conjugated dye shows localization in the LC. ***(B)*** Basic membrane properties of LC neurons. For details see table underneath. Scalebars in A: 100 μm; $A_{1\text{-}3}$: 25 μm. For details on boxplots see section Statistics in Materials and Methods. DBH, dopamine-β-hydroxylase.

underlying mechanisms generating pacemaking, TTX was applied to abolish Na^+ driven action potentials. TTX decreased the amplitude of APs and decreased AP frequency but was not able to abolish pacemaking in LC neurons (see figure 3.16 D, E *(mid trace)*). Small spikes still appeared in a precise timing and ISIs could still be fit to a Gaussian equation, even if the distribution of ISIs was widened (see figure 3.16 F). The application of Cd^{2+} abolished any potentials, suggesting that in LC neurons, precise pacemaking is driven by Ca^{2+} conductances (see figure 3.16 D, E *(lower trace)*).

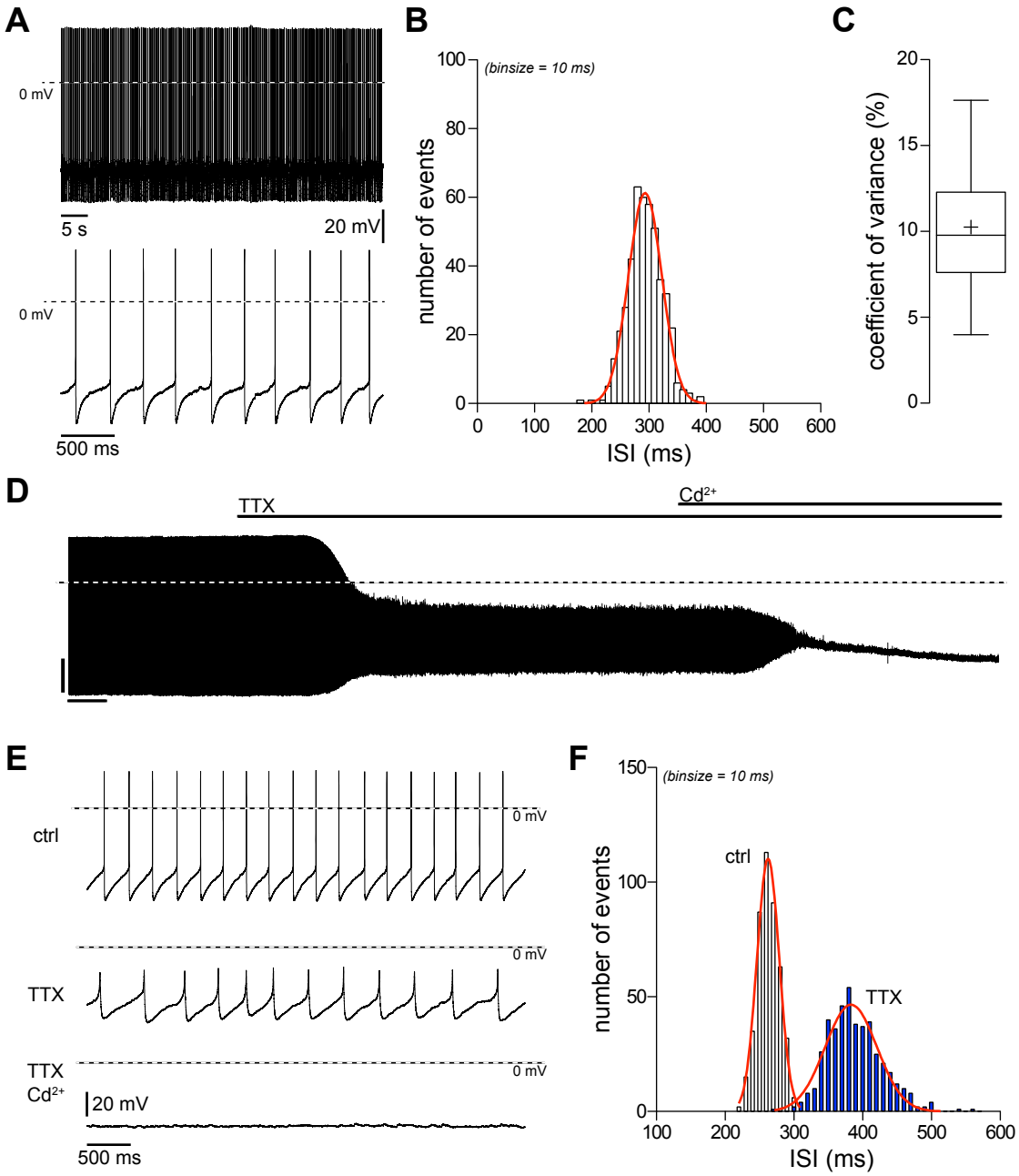


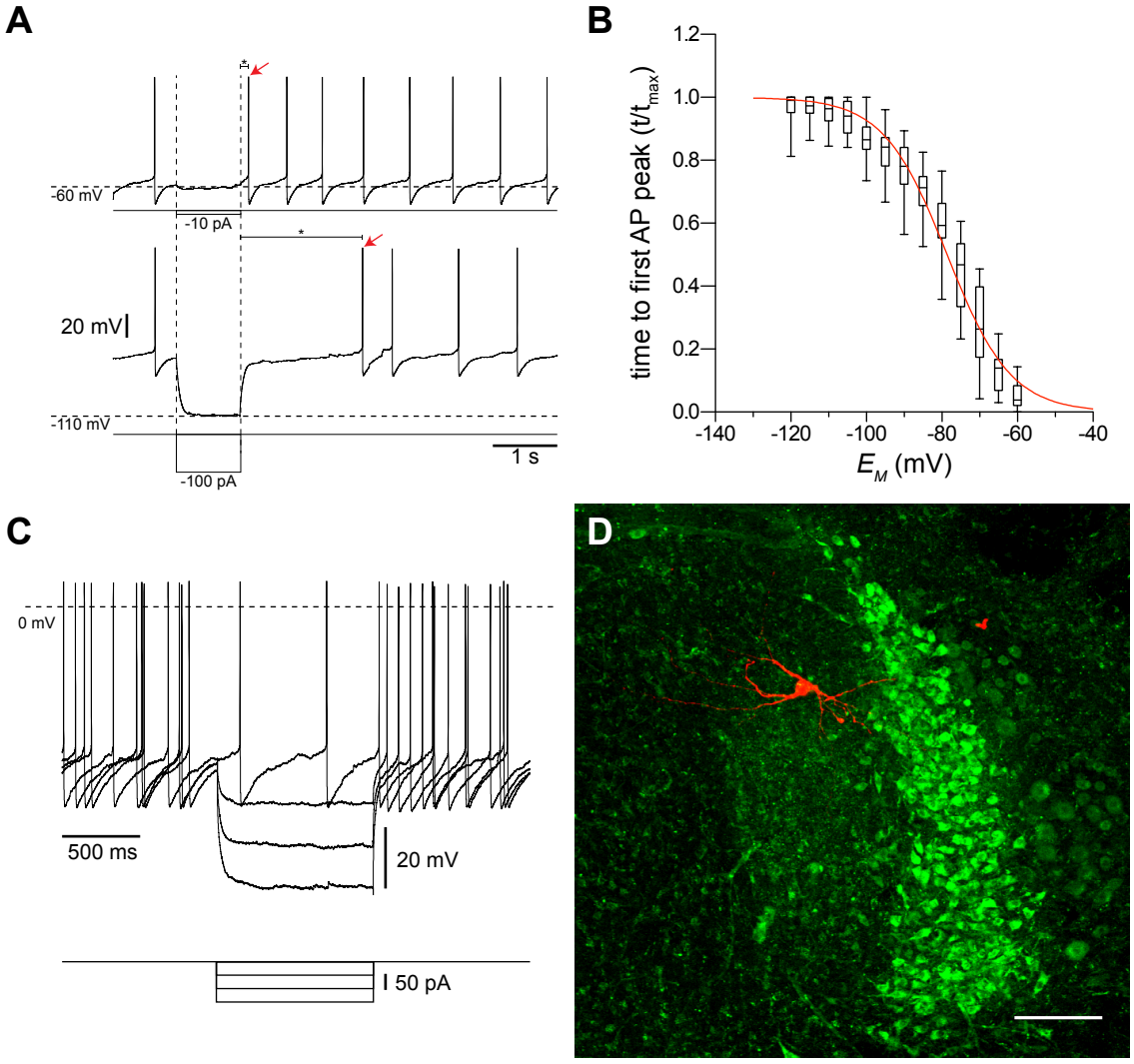
Figure 3.16: Pacemaking of LC neurons ***(A)*** Representative 5 minute section of a recording in the LC showing precise pacemaking activity *upper trace* and magnification emphasizing precesion of ISIs *lower trace*. ***(B)*** Respective ISI histogram of the recording in *A*. Distribution can be fit to a gaussian equation which serves to calculate coefficients of variance as a measure of pacemaking. ***(C)*** Coefficients of variance obtained by fits to gaussian equation. ***(D)*** Overview of a recording with application of 1 μM TTX and subsequent application of 500 μM of Cd^{2+} to further investigate mechanisms underlying the pacemaker activity in LC neurons. ***(E)*** Detailed extracts of the recording seen in *D*. *upper trace* Control shows characteristic pacemaking. *mid trace* Spike amplitude is drastically decreased by the application of TTX, however pacemaking activity is still present. *lower trace* Application of Cd^{2+} abolishes any potentials, thus showing pacemaking to be Ca^{2+}-dependent. ***(F)*** ISI histograms of traces seen in *E* in the presence of TTX *white bars* and TTX and Cd^{2+} *blue bars*. For details on boxplots see section Statistics in Materials and Methods. TTX, tetrodotoxin.

Excitation delay

In line with the aforementioned studies on electrophysiological properties of LC neurons, all recorded cells responded with a delay of excitation upon hyperpolarizing current injections (see figure 3.17 A). Plotting the time to the peak of the first spike following the offset of negative current injections to the respective hyperpolarized membrane potential revealed a voltage dependent mechanism (see figure 3.17 B). Delays increased with increasing current injections leading to more hyperpolarized membrane potentials (see figure 3.17 A). Normalized peak delays followed a Boltzmann equation suggesting this mechanism to underly the voltage dependency of voltage gated ion channels (see figure 3.17 B). The fit resulted in a halfmaximal delay at -78.6 mV and a slope of -5.1.

Interestingly, co-labeling of an DBH-immunostaining and an biocytin-backfill revealed a neuron without excitation delay lying outside of the LC, suggesting the delay to be a specific electrophysiological marker for neurons of the LC in this distinct area of the brainstem (see figure 3.17 C, D).

Figure 3.17 ***(following page)*****:** Excitation delay in neurons of the LC ***(A)*** Examples of responses to hyperpolarizing current injections of -10 pA and -100 pA, respectively. Note the prolonged delay to the peak of the first AP when the membrane potential is hyperpolarized to -110 mV compared to a hyperpolarization to -60 mV. Capped lines with asterisks indicate time from offset of current injection to the peak of the first AP, indicated by the red arrows. ***(B)*** Peaks of first APs were normalized to the maximal delay and fit to a single-exponential Boltzmann equation, obtaining parameters of voltage-dependency. ***(C)*** Neuron exhibiting no excitation delay in response to hyperpolarizing current injections of -50 pA, -100 pA and -150 pA. ***(D)*** Immunostaining of DBH and streptavidin-staining of biocytin-backfill reveals localization next to the LC for the respective neuron with the responses shown in *C*. For details on boxplots see section Statistics in Materials and Methods.


A
-60 mV
-10 pA
20 mV
-110 mV
-100 pA
1 s
B
time to first AP peak (t/t_max)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-140
-120
-100
-80
-60
-40
E_M (mV)
C
0 mV
500 ms
20 mV
50 pA
D

Spike frequency adaptation

The LC neurons showed weak spike frequency adaptation in responses to long lasting (3s) depolarizing stimuli (see figure 3.18 A). Current injections from 10 to 100 pA barely exhibited SFA ratio with values larger than 2 (see figure 3.18 A). With increasing current injections SFA ratios usually decreased to weaker SFA. Only a single of 15 analyzed neurons resulted in a SFA adaptation with SFA ratios consistently larger than two over the whole current injection increments to 100 pA (see figure 3.18 B). In line with the aforementioned properties, SFA also revealed a homogenous neuronal population in the LC.

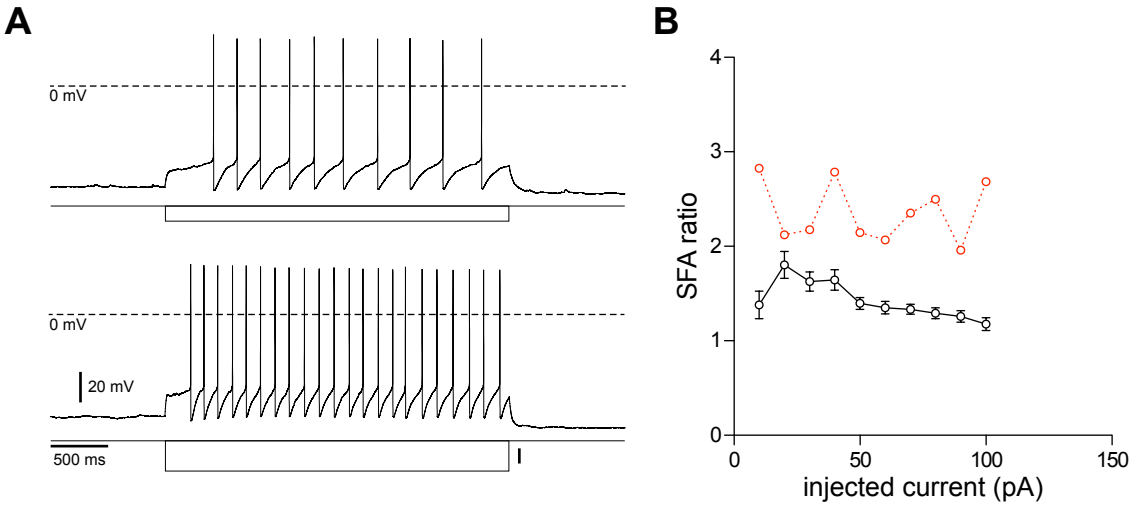


Figure 3.18: SFA in neurons of the LC ***(A)*** Examples of responses to depolarizing current injections of 50 *(upper trace)* and 100 pA *(lower trace)*, respectively. ***(B)*** SFA ratios plotted over respective depolarizing current injections (red trace marks single experiment with consistent SFA larger than 2).

Taken together, all analyzed basic properties of LC neurons lead to the assumption that the LC comprises a homogenous neuronal population. Pacemaking activity is suggested to underlie Ca^{2+} - and Ca^{2+}- dependent conductances, presumably. In line with previous work in mice, all LC neurons exhibited a delay of excitation upon increasing hyperpolarizing current injections which serves as a specific tool to identify LC neurons in the brainstem (Zhang *et al.* , 2010). The characterization of the aforementioned properties serves as a viable baseline for further investigation of the LC and its contribution to a vast number of mechanisms and functions.

3.6.2 The locus coeruleus: role in energy homeostasis, control of brown adipose tissue and glucose responsiveness

CA like DA and NA have been shown to be involved in the mechanism of energy homeostasis. The LC as the main source for NA in the CNS has been implicated in a vast number of homeostatic functions (Ammar *et al.* , 2001; AnselmoFranci *et al.* , 1997; Berridge & Waterhouse, 2003; Berridge *et al.* , 2012; Samuels & Szabadi, 2008a,b; Sara, 2009). In the brainstem, NA cell groups have been shown to be involved in adaptive responses to states of glucoprivation. In their review, Levin et al. cite the LC as a glucose sensing site referring to the expression of K_{ATP}, as well as GK in rats, both of which have been shown to be expressed in glucose-sensing neuronal populations and thus regarded as specific markers of gluco-responsive neurons (Dunn-Meynell *et al.* , 1998, 2002; Levin, 2001; Lynch *et al.* , 2000). To investigate the role of the LC in energy homeostasis, a mutant variant of the K_{ATP} channel Kir6.2 was expressed specifically under the control of the TH-promotor to target cells in the LC. Mice were phenotyped and compared to their wildtype littermates. To test for the ability of adapting electrical activity to changes in external glucose concentrations in LC neurons, perforated patch-clamp recordings were performed in acute mouse brain slices perfused with extracellular saline containing different concentrations of glucose.

3.6.3 Expression of the mutant variant Kir6.2 in catecholaminergic cells leads to obesity and altered brown adipose tissue morphology

***Data and textpassages described in this section is obtained and kindly provided by Sulay Tovar and Donald A. Morgan (Tovar *et al.* , 2013).**

From 3 weeks of age, body weight of control and Kir6.2^{THCre}-mice was monitored both under NCD and after exposure to a HFD. This analysis revealed a slight increase in body weight of the Kir6.2^{THCre}-mice compared to controls upon exposure to NCD (see figure 3.19 A). Importantly, it was previously demonstrated that THCre-mice on the same C57BL/6 genetic background did not exhibit alterations in body weight or energy homeostasis (Konner et al., 2011). Interestingly, the difference in body weight was more apparent when the animals were exposed to HFD (see figure 3.19 B). In fact, by the age

of 20 weeks, Kir6.2^{THCre}-mice on HFD gained 30% more weight than littermate controls on the same diet (see figure 3.19 B). Obesity in Kir6.2^{THCre}-mice was further confirmed by the relative increase in fat mass both under NCD and HFD conditions (see figure 3.19 C). Moreover, direct assessment of epididymal fat pad weight revealed a significant increase in Kir6.2^{THCre}-mice both on NCD and HFD (see figure 3.19 D). Obesity development in these animals was further reflected by the significant hyperleptinemia in Kir6.2^{THCre}-mice on HFD (see figure 3.19 E). and hyperglycemia. Finally, morphological analysis of white adipose tissue revealed significant hyperplasia of adipocytes in the Kir6.2^{THCre}-mice (see figure 3.19 F).

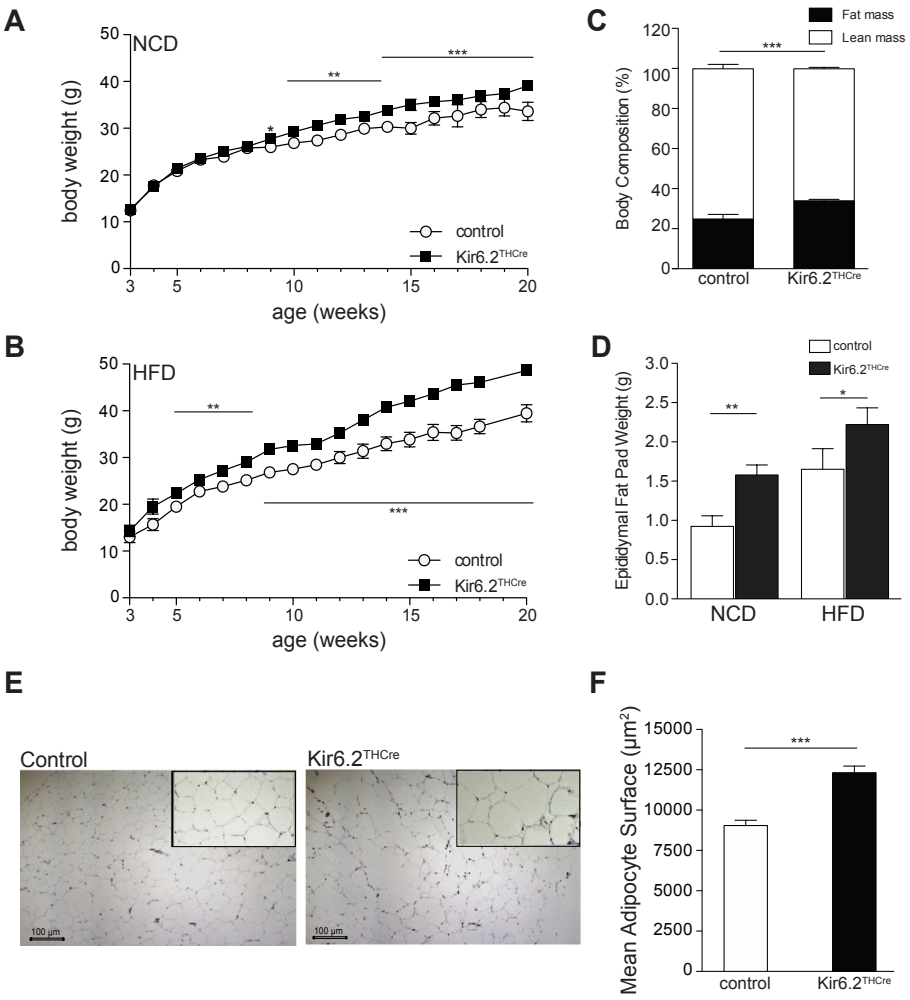


Figure 3.19: Kir6.2^{THCre}-mice develop obesity ***(A)*** Development of body weight in mice fed NCD over the timeframe of 20 weeks. Mice expressing the mutant Kir6.2 variant show elevated weight gain compared to their control littermates ***(B)*** Mice fed an ad libitum HFD exhibit exaggerated body weight gain and earlier onset of significantly higher weight gain. Data are given as mean $\pm$ SEM, * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. **Data and graphs were kindly provided by Sulay Tovar (Tovar *et al.* , 2013).**

Further analysis revealed no significant change in food intake of Kir6.2^{THCre}-mice on HFD compared to controls receiving the same diet, but significantly reduced energy expenditure. On the other hand, energy content in feces and food efficiency remained unaltered.

In the presence of reduced energy expenditure, the morphology of BAT was assessed. While BAT of control mice exhibited the normal appearance of multi-vacuolar brown adipocytes, the histomorphological characteristics of brown adipocytes in Kir6.2^{THCre}-mice had a rather macro-vacuolar, white-adipocyte-like phenotype (see figure 3.20 A). Moreover, BAT of Kir6.2^{THCre}-mice exhibited slightly reduced mRNA-expression of the brown adipocyte differentiation marker CIDEA, PGC-1 and UCP-1 as key regulators of mitochondrial biogenesis and uncoupling. Protein expression of UCP-1 in BAT of Kir6.2^{THCre}-mice compared to controls was similarly reduced (see figure 3.20 B). Since BAT function is tightly regulated by the SNS and since sympathetic denervation of BAT has been shown to result in similar histomorphological changes as observed in Kir6.2^{THCre}-mice BAT SNA was directly recorded in control and Kir6.2^{THCre}-mice(see figure 3.20 C; Minokoshi *et al.* 1986). This analysis revealed a significant reduction in BAT SNA in Kir6.2^{THCre}-mice compared to controls. In line, increase of SNA in response to systemic glucose injections was only observed in control animals (see figure 3.20 D). Impaired BAT-SNA led to reduced rectal temperature of animals exposed to cold (4C; see figure 3.20 E).

Collectively, these experiments indicate that decreased energy expenditure rather than increased food intake may account for the exacerbated obesity in Kir6.2^{THCre}-mice. Moreover, analysis of BAT revealed a WAT-like morphology and gene expression and reduced SNA into BAT alongside impaired glucose responsiveness and thermogenesis upon cold exposure.

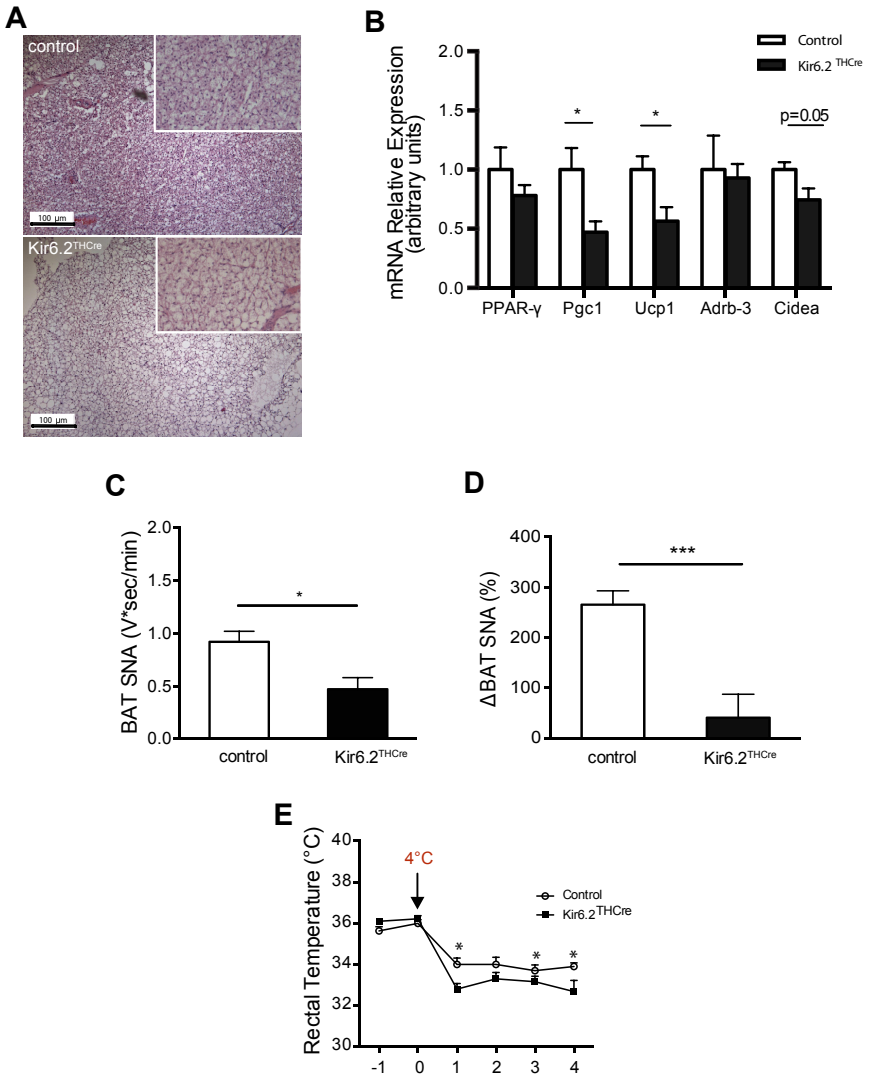


Figure 3.20: Impaired morphology and activity of BAT in Kir6.2^{THCre}-mice. ***(A)*** Representative HE staining of brown BAT of a 20-week-old male control *(upper panel)* and Kir6.2^{THCre}-mice *(upper panel)* on HFD (10x magnification in small square). ***(B)*** Relative expression of PPAR-γ, Pgc-1, Ucp1, β3-AR and Cidea in BAT extracts from 20-week-old control (n=6) and Kir6.2^{THCre}-mice (n=6) on a HFD. Expression of indicated mRNAs was normalized to that of HPRT and the resultant value for each group was normalized to expression of the target gene in control mice. ***(C)*** Quantification of BAT SNA of 15-week-old control (n=5) and Kir6.2^{THCre}-mice (n=5) on a HFD. ***(D)*** Comparison of BAT SNA responses induced by icv glucose (average of last hour of recording) between control and Kir6.2^{THCre}-mice. ***(E)*** Rectal temperature of 15-week-old control (n=6) and Kir6.2^{THCre}-mice (n=6) on a HFD upon cold exposure for 4 hours. Data are given as mean ± SEM, * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. PPAR-γ, peroxisome proliferator-activated receptor; Pgc-1, peroxisome proliferator-activated receptor-coactivator-1; Ucp-1, uncoupling protein 1; Cidea cell death-inducing DFF45-like effector protein a; icv, intracerebro-ventricular. **Data and graphs were kindly provided by Sulay Tovar and Donald A. Morgan. Modified from (Tovar *et al.* , 2013)**

3.6.4 A subpopulation of neurons in the locus coeruleus adapt their firing to changes in extracellular glucose in glucose-excited manner

The effect of changes in extracellular glucose concentrations has been investigated in acute mouse brain slices of Kir6.2^{THCre}-mice, their WT littermates and C57BL/6 mice. GFP expression and/or the shape of the 4th ventricle as a landmark has been used to identify LC neurons. In a first set of experiments, no differences could be observed between C57BL/6 mice and Kir6.2$^{-/-}$-mice , thus mice were pooled and in the following referred to as control or WT mice. (see figure 3.21 A; n=23, n=32; *n.s.* $p \geq 0.05$). However, as it was previously shown, the expression of the mutant Kir6.2 variant drastically reduces AP frequency. Almost 70 % of LC neurons did not generate any APs, which has neither been observed in C57BL/6 mice, nor in THCre control mice (see figure 3.21 A; n=24; ***$p<0.001$). This data demonstrates the successful expression of the mutant K_{ATP} channel under the control of the TH promotor and that this leads to silencing of the majority of LC neurons.

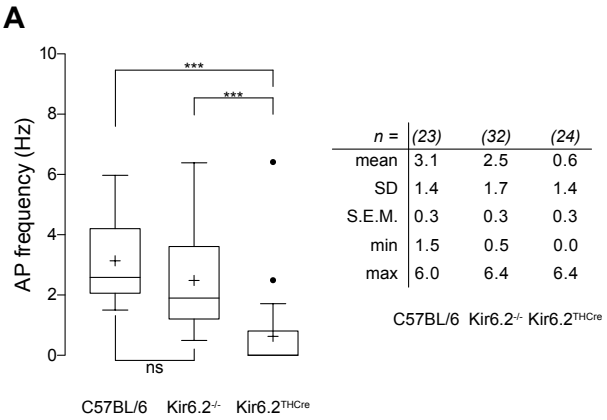


Figure 3.21: AP frequency is reduced in Kir6.2^{THCre}-mice ***(A)*** *left panel*, AP frequencies of C57BL/6 mice, THCre control mice and Kir6.2^{THCre}-mice. While AP frequency remains unaltered in THCre control mice compared to C57BL/6 mice, AP frequency is reduced in Kir6.2^{THCre}-mice. * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

In $\sim$ 43.8 % (7 of 16) of LC neurons in WT mice, decreasing extracellular glucose concentration from 5 mM to 3 mM significantly reduced spontaneous AP frequency (see figure 3.22 and figure 3.23 D; n=7; ; **$p < 0.01$). A response that could be reversed via blocking K_{ATP} channels through application of 200 μM tolbutamide (see figure 3.22;

n=7; ; $^{**}p < 0.01$). In the remaining neurons, changes in frequency did not reach significance. All together, a reduction from 5 to 3 mM of extracellular glucose reduced the firing in responding neurons from 3.4 $\pm$ 0.5 Hz to 2.8 $\pm$ 0.5 Hz, which corresponds to a reduction of 19.1 $\pm$ 6 % and the specific K_{ATP} channel blocker reversed the effect to $\sim$ 104%, suggesting a very small but present baseline conductance through K_{ATP} channels (see figure 3.22 C, D; n=7; $^{*}p < 0.05$).

Consistently with the role of K_{ATP} channels in glucose-mediated regulation of LC neurons, glucose responsiveness of these cells was abolished in Kir6.2^{THCre}-mice (see figure 3.23). Due to the expression of the mutant Kir6.2 variant, tested neurons did not exhibit any or very low spontaneous AP frequency (see figure 3.23). However, application of tolbutamide lead to robust depolarizations along with the appearance of spontaneous firing significantly different to control levels (see figure 3.23; n=10; $^{***}p<0.001$). Taken together, 43.8 % of LC neurons in control mice responded in a glucose-excited fashion, while in 100 % of Kir6.2^{THCre}-mice glucose responsiveness is abolished.

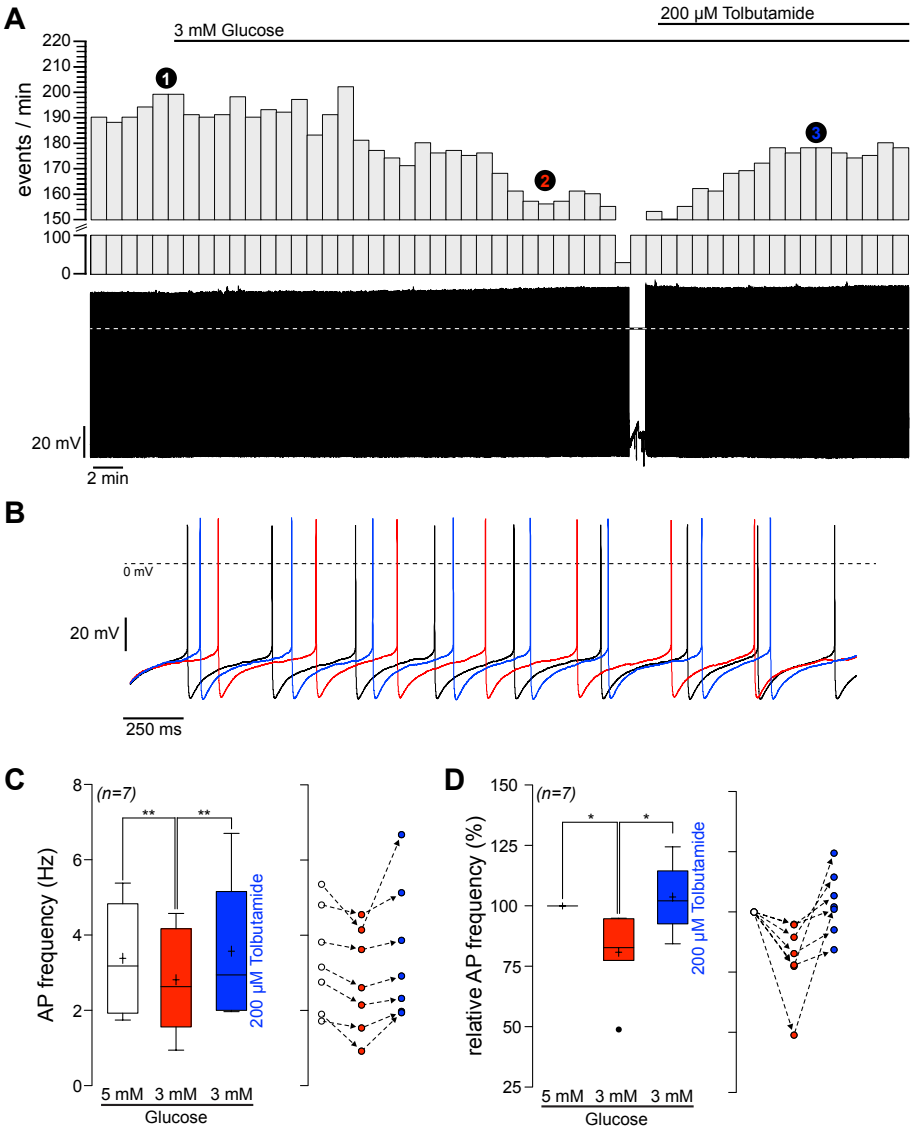


Figure 3.22: LC neurons adapt AP frequency in response to reductions of extracellular glucose concentration ***(A)*** Rate histogram and original corresponding raw data showing the effect of reducing extracellular glucose from 5 to 3 mM and following application of 200 μM tolbutamide. ***(B)*** Extract of APs normalized to the peak of the first AP visualizing the moderate decrease in AP frequency upon reduction of extracellular glucose to 3 mM (*red trace*) compared to control (*black trace*) and increase by the application of 200 μM tolbutamide (*blue trace*). ***(C)*** *left panel* Absolut change in frequency in response to glucose reduction and tolbutamide application. *right panel* Courses of single experiments. ***(D)*** *left panel* Relative change in frequency in response to glucose reduction and tolbutamide application. *right panel* Courses of single experiments. * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

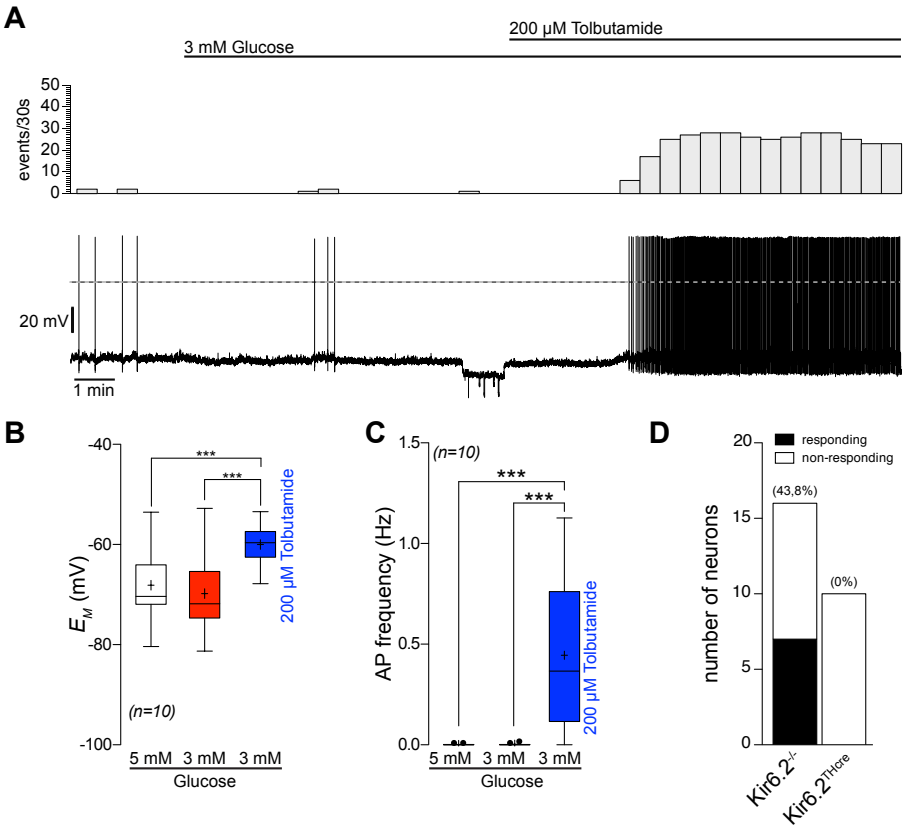


Figure 3.23: Responses to decreased extracellular glucose concentration are abolished in Kir6.2^{THCre}-mice. ***(A)*** Rate histogram and original corresponding raw data showing the effect of reducing extracellular glucose from 5 to 3 mM and following application of 200 μM tolbutamide. While the response to changes in glucose concentrations is absent, application of tolbutamide leads to depolarization and concomitant spontaneous firing ***(B)*** Membrane potential and firing ***(B)*** remains unaltered upon reduction of extracellular glucose concentration. Application of tolbutamide significantly depolarizes membrane potential and leads to generation of spontaneous APs. ***(D)*** 43.8 % of control mice were glucose responsive, while glucose responsiveness in Kir6.2^{THCre}-mice was completely abolished. * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

Conversely, increasing glucose concentrations from 5 mM to 8 mM increased firing of a subset (6 of 14; 42,9 %) of LC neurons in control mice (see figure 3.24 A, B, C; n=6; $*p < 0.05$); an effect that was absent in LC neurons of Kir6.2^{THCre}-mice (see figure 3.25 A, B, C; n=8).

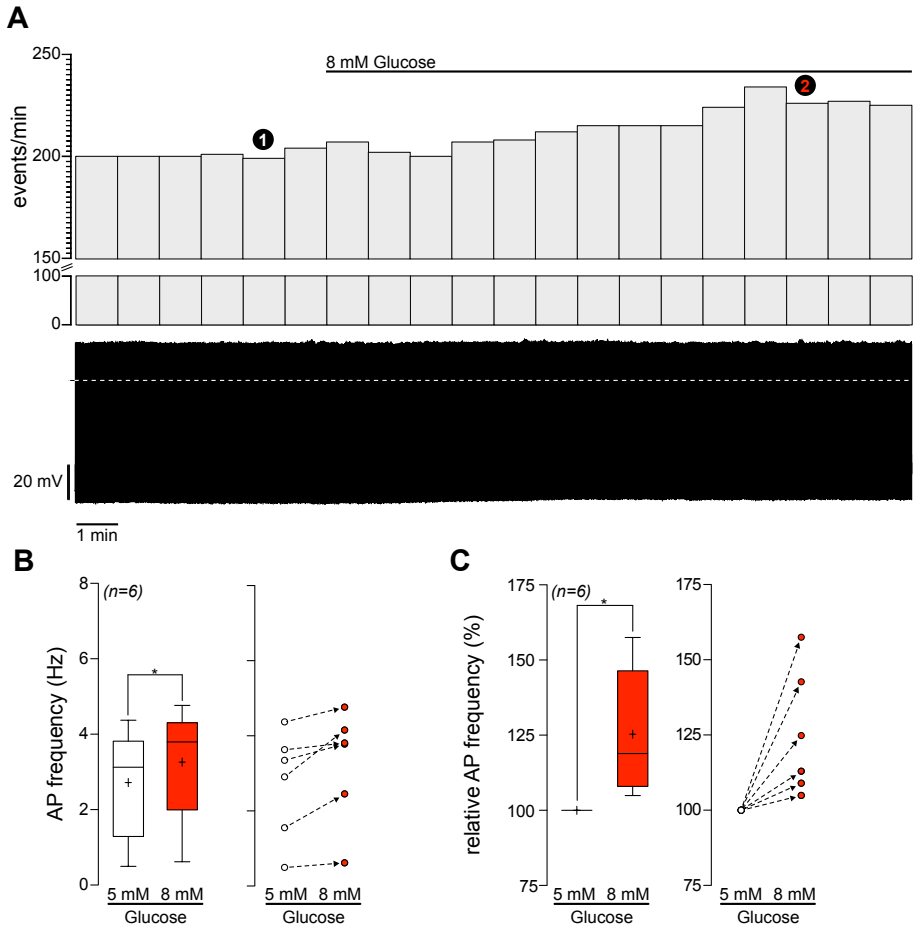


Figure 3.24: Kir6.2^{THCre}-mice develop obesity ***(A)*** Rate histogram and original corresponding raw data showing the effect of increasing extracellular glucose from 5 to 8 mM. Frequency is significantly increased. ***(B)*** *left panel*, Absolut change in frequency in response to glucose increase. *right panel*, Courses of single experiments. ***(C)*** *left panel*, Relative change in frequency in response to glucose increase. *right panel*, Courses of single experiments. $* p < 0.05$,$** p < 0.01$, $***p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

Consistent with the aforementioned experiments, application of tolbutamide in Kir6.2^{THCre}-mice led to depolarization and concomitant appearance of firing (see figure 3.25A, B, C, n=8; $***p<0.001$). These experiments reveal that a subpopulation of TH-positive neurons in the LC responds to alterations in extracellular glucose concentrations with concomitant changes in firing properties. The effects exhibit a K_{ATP} channel dependent manner and thus these cells can be considered as GE neurons.

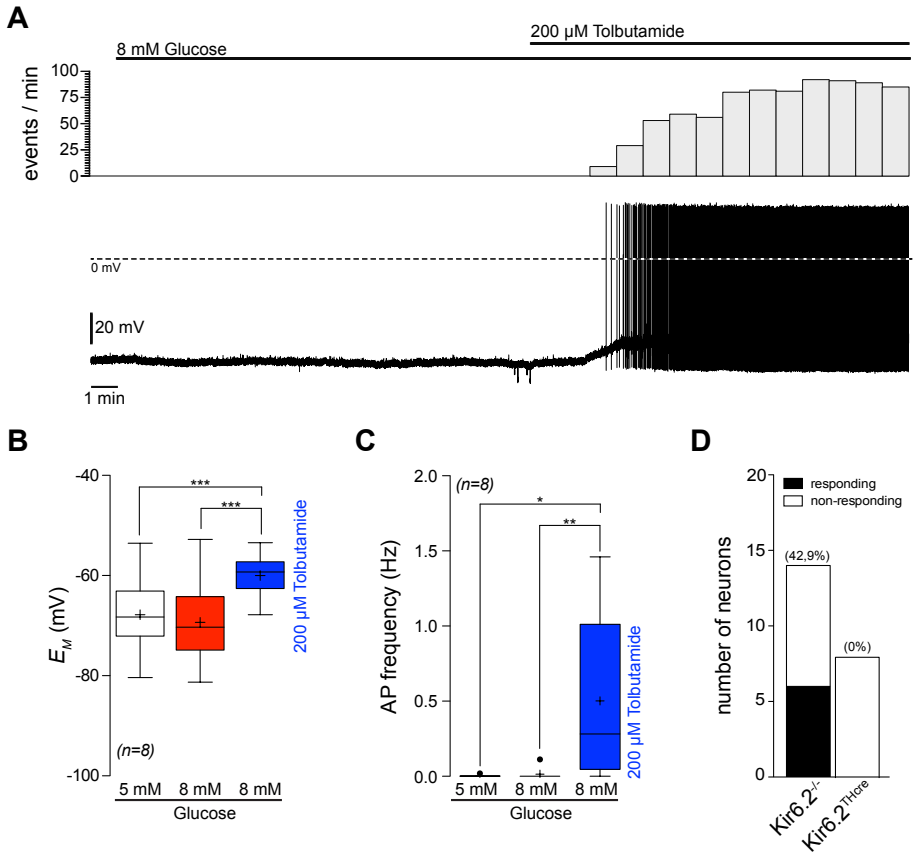


Figure 3.25: Glucose responsiveness to 8 mM is abolished in Kir6.2^{THCre}-mice ***(A)*** Rate histogram and original corresponding raw data showing the effect of increasing extracellular glucose from 5 to 8 mM and following application of 200 μM tolbutamide. While the response to changes in glucose concentration is absent, application of tolbutamide leads to depolarization and concomitant spontaneous firing ***(B)*** Membrane potential and firing ***(C)*** remains unaltered upon reduction of extracellular glucose concentration. Application of tolbutamide significantly depolarizes membrane potential and leads to generation of spontaneous APs. ***(D)*** 42.9 % of control mice were glucose responsive, while glucose responsiveness in Kir6.2^{THCre}-mice was completely abolished. * $p < 0.05$,** $p < 0.01$, ***$p < 0.001$. For details on boxplots see section Statistics in Materials and Methods.

Taken together, the aforementioned data indicate a role of LC NA neurons in energy homeostasis. Around 43 % of LC neurons showed characterizations compared to GE excited neurons. The application of the specific K_{ATP} channel blocker tolbutamide reversed the inhibiting effect of reducing extracellular glucose concentrations from 5 to 3 mM, suggesting a contribution of these channels in mediating the responses. A slightly increased firing compared to control levels points towards a weak baseline conductance through K_{ATP} channels. Conversely, increasing glucose concentrations to 8 mM resulted in an increase in firing. Around 57 % of the recorded neurons did not respond to alterations in glucose concentrations and thus can be considered as non glu-

cose responsive. 70% of LC neurons in Kir6.2^{THCre}-mice do not generate spontaneous APs and responses to either alteration of extracellular glucose concentration in all tested neurons is abolished. This data demonstrates that the LC is contributing to the analyzed obese phenotype in Kir6.2^{THCre}-mice and that this phenotype is at least in part due to silencing the majority of LC NA neurons.

3.6.5 A small subpopulation of neurons in the locus coeruelus adapt their firing to changes in extracellular glucose in glucose-inhibited manner

Interestingly, a small subset of LC neurons responded in the opposite manner to reduction of extracellular glucose (see figure 3.26 A, B). Two neurons increased their AP frequency of $\sim$ 8% and $\sim$ 15%, respectively (see figure 3.26 C). Therefore, these neurons are considered GI neurons and represent a third subpopulation in the LC with regard to glucose sensing.

The obtained data in the course of this study on LC neurons suggests that the LC is involved in the control of energy homeostasis. Glucose-dependent activity of GE neurons in the LC contributes to the control of thermogenesis in BAT and energy expenditure. This is clearly reflected by the reduced SNA and the impaired increase of SNA in response to elevated glucose levels in Kir6.2^{THCre}-mice. Due to the reduced energy expenditure, Kir6.2^{THCre}-mice develop mild obesity, which is exaggerated under HFD conditions. Exposure to cold of Kir6.2^{THCre}-mice reveals impaired thermogenesis in BAT. In control animals elevated glucose levels increase BAT-SNA activity. Impaired glucose-sensing in LC neurons reduces this response and contributes to the obese phenotype. A small proportion of LC neurons were identified to be GI. The aforementioned phenotype suggests that these neurons serve a different function. However, the exact contribution of these neurons to energy homeostasis remains unknown. Putative contributions will be discussed in the next sections.

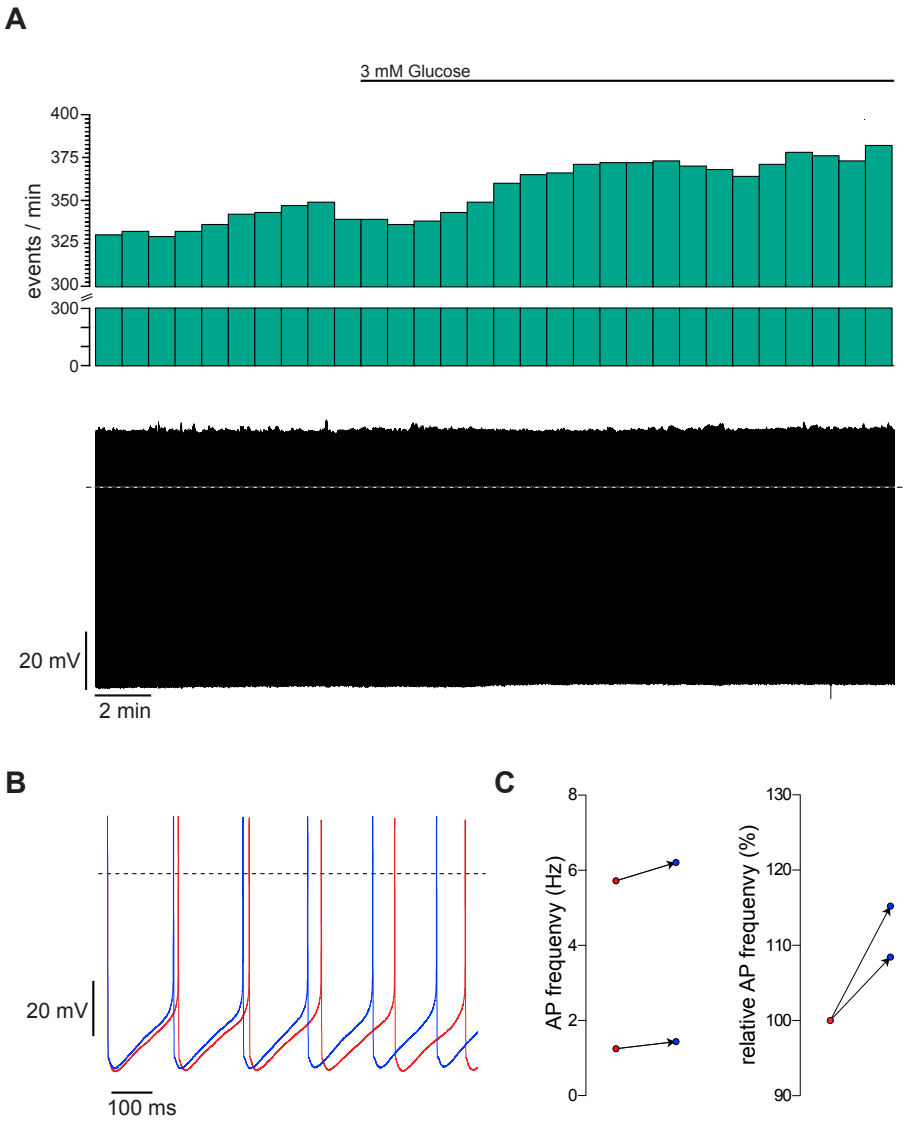


Figure 3.26: GI neurons in the LC ***(A)*** An LC neuron increases AP frequency in response to a reduction of extracellular glucose from 5 mM to 3 mM, thus identifying. *upper scheme* Rate histogram and respective recording *lower scheme*. ***(B)*** Spiketrains normalized to the peak of the first action potential, indicating higher frequency in 3 mM glucose *(blue)* compared to 5 mM glucose *(red)*. ***(C)*** Two neurons responded in GI manner, increasing their frequency to ∼108% and ∼115%, respectively. (GI, glucose inhibited.

4 Discussion

This study provides evidence for a role of NA in the regulation of energy homeostasis by modulating intrinsic membrane properties of POMC and NPY/AgRP neurons. Furthermore, experiments suggest a role of the LC in the control of glucose and energy metabolism. In the first part of this study, POMC and NPY/AgRP neurons have been analyzed with respect to NA modulation of their activity. The key results of the first part are:

1. POMC neurons of the ARC exhibit a large heterogeneity and show three different patterns of SFA
2. NA inhibits POMC neurons by the activation of α_{2A}-ARs
3. NPY/AgRP exhibit a large variation in their electrophysiological properties but do not reveal further subtypes
4. NA excites NPY/AgRP neurons by the activation of α_{1A}-ARs
5. High concentrations of NA elicit rhythmic bursting in NPY/AgRP neurons
6. Aging and chronic HFD feeding alter responses to NA in POMC neurons

In the second part, the LC has been investigated as a potential source for the release of NA into the ARC. Here, the key results are:

1. The LC comprises a homogenous neuronal population with specific electrophysiological properties, which help to identify LC-NA neurons in C57BL/6 mice.
2. Intrinsic pacemaking in acute mouse brain slices underlies Ca^{2+}-dependent mechanisms.
3. The LC contributes to the control of BAT activity via the SNS (Results and text passages obtained and kindly provided by Tovar *et al.* (2013))

4. A subpopulation of LC neurons was identified as glucose-sensing. LC neurons responded either as GE or GI neurons or did not respond to changes in extracellular glucose levels.

In the following sections, the described results are discussed and models of NA action on energy homeostasis are proposed (see figure 4.1 and 4.3).

4.1 Properties of POMC neurons

POMC neurons in the ARC have been of substantial interest, as these neurons are critical in mediating satiety by integrating signals, which are directly related to the energy state of the body such as insulin, leptin, ghrelin, glucose and FFAs (Gao & Horvath, 2007). In this context, a large amount of work has also focused on electrophysiological responses to these stimuli (Ghamari-Langroudi, 2012). However, a detailed description of intrinsic electrophysiological properties of these neurons is still lacking. Nonetheless, in a few studies values for basic properties of POMC neurons are given, which were determined as control values in experiments dealing with either responses to various neurotransmitters, hormones and fuel-sensing signals or unraveling the effect of dietary changes and aging (Kim & Horvath, 2012; Newton *et al.* , 2013; Roepke *et al.* , 2012; Yang *et al.* , 2012; Zhan *et al.* , 2013).

Basic properties

POMC neurons have been shown to exhibit spontaneous action potential generation in brain slices (Belgardt *et al.* , 2008; Claret *et al.* , 2007; Cowley *et al.* , 2001; Diano & Horvath, 2012; Ernst *et al.* , 2009; Klöckener *et al.* , 2011; Mineur *et al.* , 2011; Parton *et al.* , 2007; Plum *et al.* , 2006). However, recent studies also revealed POMC neurons, which exhibit hyperpolarized membrane potentials and lack spontaneous firing (Roepke *et al.* , 2012; Yang *et al.* , 2012; Zhan *et al.* , 2013). Results obtained from mice at different ages may change basic properties of POMC neurons (Kim & Horvath 2012; Newton *et al.* 2013; Yang *et al.* 2012, Pippow et. al, in preparation). A study pubslihed by Roepke *et al.* (2012) revealed properties of POMC neurons in adult mice at the age of 10-12 weeks. This overlaps with the age of mice used in this thesis. Values for membrane

potentials are well in line with their published data. Further publications revealed a large variability in spontaneous AP frequencies (Claret *et al.* , 2007; Cowley *et al.* , 2001; Diano *et al.* , 2011; Parton *et al.* , 2007; Plum *et al.* , 2007; Sohn *et al.* , 2011). Interestingly, input resistances revealed in the present thesis are slightly higher than reported for POMC neurons (Dicken *et al.* , 2012). Comparing the perforated patch-clamp to the conventional whole-cell patch-clamp method, shows that rupturing of the membrane leads to the dilution of intracellular molecules, in some cases leading to changes in intrinsic membrane properties also reflected in decreased input resistances (Oleson *et al.* 1993; Ueno *et al.* 1992; Simon Hess, PhD thesis, 2011).

Spike frequency adaptation

The issue of POMC heterogeneity has recently been in the focus of an elegant study by Williams *et al.* (2010) and reviewed by Sohn & Williams (2012). Electrophysiological experiments revealed three different types of POMC neurons concerning their ability to respond to the peripheral hormones insulin and leptin. Responses were clearly segregated in neurons only responding to leptin, to insulin or not responding to any of the applied stimuli. The authors mapped the different responding neurons to certain areas within the medial basal hypothalamus. Depending on characterization of SFA in the present thesis, experiments revealed three different types of POMC neurons. SFA was either weak, strong or was not apparent. Here, the question arises if any electrophysiological properties can be assigned to POMC neurons that respond to the different aforementioned stimuli, thus identifying cells as either leptin - / insulin - or non-responsive.

The identification of different types of SFA points towards the expression of different types of ion channels, which has been shown for invertebrates and vertebrates (Lewis *et al.* , 1986; Powers *et al.* , 1999)(Stocker, 2004). SFA has been observed in POMC neurons before but no further analysis to subdivide the population has been carried out (Acuna-Goycolea & van den Pol, 2005). First identified in motoneurons, the underlying ionic mechanisms of SFA has been investigated in detail (Sah & Davies, 2000; Wilanowski & Piotrkiewicz, 2012). In general, it is considered as a mechanism that reduces excitability to sustained stimuli (Peron & Gabbiani, 2009). For example, it contributes to various

functions within the CNS including long-term plasticity by optimized spike timing dependency and "forward masking", where first stimuli trigger full responses whereas later stimuli are masked by decreased excitability (Liu & Wang, 2001). Depending on the neuronal localization of the respective ion channels, SFA also contributes to transmitter release by changing the neuron's intrinsic excitability (Miles *et al.* , 2005).

Synaptic plasticity in the melanocortin system has been in focus of a recent review by Zeltser *et al.* (2012). An elegant study by Pinto *et al.* (2004) could reveal a first evidence for stimuli-dependent synaptic plasticity in the ARC. Leptin-deficient *(ob/ob)* mice exhibited differences in synaptic connections on POMC neurons, which was rapidly reversed by the administration of exogenous leptin. In this context, it also has to be taken into account that learning and experience might also exhibit substantial effects on the plasticity of the melanocortin system as "it would be beneficial, from an evolutionary standpoint, for animals in a nutrient-poor environment or those with intermittent access to food to respond more vigorously to food than animals with easy or abundant access to food" (Zeltser *et al.* , 2012). It has been argued if long-term synaptic plasticity changes SFA properties in neurons (Cohen-Matsliah *et al.* , 2010; Sun, 2009). Regarding the need for plasticity in the melanocortin system the question arises wether different types of SFA reflect different subpopulations of POMC neurons or different states of plasticity.

4.2 Properties of NPY/AgRP neurons

NPY/AgRP expressing neurons in the ARC have been identified as the opponent neuronal population to POMC neurons and have been in focus of a vast number of studies (Varela & Horvath, 2012). Numerous peripheral hormonal and fuel-sensing signals have been investigated for their modulatory effect on NPY/AgRP neurons (Belgardt *et al.* , 2009). In this thesis, electrophysiological properties of NPY/AgRP neurons have been investigated in acute mouse brain slices containing the ARC from 10-12 week old adult mice. In order to identify NPY expressing neurons a NPY^{GFP} mouseline was used (van den Pol *et al.* , 2009). It has been shown that 94 to 99% of NPY expressing neurons in the ARC also express AgRP mRNA. Hence, recordings of NPY^{GFP} can be considered as NPY/AgRP neurons (Hahn *et al.* , 1998).

Basic properties

Electrophysiological data revealed a more homogenous distribution of properties compared to POMC neurons. In spite of the variation of properties in NPY/AgRP neurons, no differences leading to further classification could be observed. This clearly points towards a rather homogenous neuronal population within the ARC. Accordingly, no segregation concerning their responses to peripheral stimuli has been observed until today but differences in electrophysiological properties like input resistances and baseline firing may influence the extent to which these neurons respond to different stimuli and consequently might lead to versatile outputs (Niimi *et al.* , 2012).

From an evolutionary point of view it is conceivable that hunger usually is favored over satiety, which serves to survive times of food deprivation by overeating at times of excessive food availability. A strong unilateral input on POMC neurons, that potently inhibits satiety signals is a prerequisite for the respective behavior (Bates & Myers, 2003; Horvath *et al.* , 1992, 2009). Here, the rather homogenous NPY/AgRP population with less variation in responses to peripheral signals could provide adequate input to the heterogenous POMC population. The effectiveness of NPY/AgRP neurons is further supported by *in vivo* experiments by Aponte *et al.* (2011) who show that activation of AgRP neurons leads to subsequent food intake, even if POMC neurons are stimulated simultaneously. Additionally, Atasoy *et al.* (2012) could show that activation of AgRP neurons lead to an direct onset of food intake, whereas POMC activation led to satiety and offset of food intake in a larger time frame, indicating the potency of the NPY/AgRP population.

4.3 Noradrenaline differentially modulates POMC and NPY/AgRP neurons

The effect of NA has been studied by the bath application of different concentrations after establishing perforated patch-clamp recordings. Neurons were identified by the expression of GFP under the control of either the POMC or the NPY promotor (Cowley *et al.* , 2001; van den Pol *et al.* , 2009). These experiments provide the first data

of NA modulation of the melanocortin system in the ARC on identified POMC and NPY/AgRP neurons and expand the model of the NA system in the control of energy homeostasis. The detailed understanding of NA modulation may help to develop strategies that specifically target certain transmitter systems in the treatment of obesity. These systems may interfere with each other and serve a variety of functions, which, upon modulation, can lead to undesired side effects (Guiard *et al.* , 2008a). Therefore it is critical to understand under which conditions and at which sites in the CNS these transmitter systems affect eating behavior and energy expenditure.

Concentration dependent effects of noradrenaline on POMC and NPY/AgRP neurons

NA exerts strong differential effects in POMC and NPY neurons. NA was able to inhibit POMC neurons, while NPY/AgRP were excited. In context of the melanocortin system in the ARC, comparable differential modulation has been shown for insulin, leptin and glucose (Ghamari-Langroudi, 2012). In the present study, concentrations from 10 nM to 100 μM hyperpolarized POMC neurons and depolarized NPY/AgRP neurons dependent on the applied concentration. The revealed EC_{50} for α_2-ARs, which mediate the inhibition of POMC neurons, are in line with values obtained from brain slices of rats (Jurgens *et al.* , 2007). In other studies on α_2-ARs in mouse and rat brain slices, EC_{50} values were 5 - to 15 fold higher (Alberto *et al.* , 2011; Li & van den Pol, 2005). However, the range of effective and tested concentrations is consistent with studies and differences in EC_{50} values can be due to differences in slice thickness, perfusion speed, application type and rely on receptor density as well as on the expression of different AR subtypes. Absence or presence of synaptic blockers may also shift concentration-response curves.

Since ARs do not discriminate between NA and A, it is important that A also inhibited POMC neurons dependent on concentration (Stephan Bremser, batchelor thesis, 2011). In line with the literature, the affinity of the receptor for A was 10 fold higher compared to the affinity for NA (Stephan Bremser, batchelor thesis, 2011; Jurgens *et al.* 2007). The strong decrease in input resistance upon NA application suggests, that the

inhibition is mediated by GIRKs, which are the targets of the G_i protein (Chen *et al.* , 2011).

The depolarization of NPY/AgRP neurons upon application of NA exhibits an EC_{50} slightly shifted to higher concentrations. This reflects the lower sensitivity of α_1-ARs to its endogenous ligand NA (Ruffolo *et al.* , 1991). Moreover, high concentrations (>10 μM) of NA induced rhythmic bursting activity in ∼40% of NPY/AgRP neurons. This effect has been also observed in sympathetic preganglionic neurons in rats and guinea pigs and here it underlies VGCCs (Carette, 1999; Yoshimura *et al.* , 1987). Neuropeptide expressing neurons need high spike frequencies to release their respective peptides. Bursting activity has been shown to potently lead to neuropeptide release (Tallent, 2008; van den Pol, 2012). An increase in VGCC has been shown as a target for α_1-ARs and also intracellular release of Ca^{2+} via IP3 is also possible, both of which could contribute to this bursting firing pattern (García-Sáinz *et al.* , 1999, 2000; Macrez-Leprêtre *et al.* , 1997). Moreover, the concomitant increase in input resistance suggest the closure of GIRKs, which has been shown for α_1-ARs via activation of the G_q protein.

It was further investigated if the effects of NA on POMC and NPY/AgRP neurons are post - or presynaptic. Therefore, a set of synaptic blockers have been added to the extracellular aCSF. The results in this thesis clearly demonstrate, that the recorded effects are postsynaptic and due to cell intrinsic expression of ARs. In contrast to the NPY/AgRP neurons, a small contribution of presynaptic modulation could be observed in POMC neurons, suggesting the innervation by neurons expressing ARs. It has been shown that NPY/AgRP neurons form unidirectional GABAergic synapses on POMC neurons. In slice preparations without synaptic blockade, the excitation of NPY/AgRP neurons leads to inhibitory postsynaptic currents (IPSCs) in POMC neurons due to GABA release. Moreover, NPY inhibits POMC neurons through the NPY receptor type 1(Y1R)-mediated activation of GIRK channels (Roseberry *et al.* , 2004). Excitation of NPY/AgRP neurons in the ARC by NA most likely mimics these effects. Further support is provided by the fact that the application of synaptic blockers alone has no effect on membrane properties of POMC neurons. However, the possibility of further synaptic endings of NA responsive cells cannot be excluded but baseline activity of these synapses could not

be observed. Neurons in the VMH for example have been shown to innervate POMC neurons and NA has been shown to exert effects on food intake when injected into the VMH (Klöckener *et al.* , 2011; Wellman, 2000). Albeit, there is no data which matches NA responses in the VMH with projections to POMC neurons, it cannot be ruled out that this contributes to the presynaptic effect observed in POMC neurons.

Identification of adrenergic receptors on POMC and NPY neurons

In support of the present results, expression of α_1- and α_2-ARs has been detected in the ARC (Acosta-Martinez *et al.* , 1999; Kang *et al.* , 2000; Young & Kuhar, 1979, 1980). In order to reveal the specific subtypes of ARs mediating the effects on POMC and NPY/AgRP neurons, specific pharmacological antagonists were used. Despite discrepancies concerning effective concentrations, BRL 44408 has been shown to specifically block the action of α_{2A}-ARs (Callado & Stamford, 2000; Gyires *et al.* , 2009; Hopwood & Stamford, 2001; Owesson *et al.* , 2003; Ruffolo, 1985). In contrast to the α_{2B}-AR specific antagonist ARC 239, BRL 44408 abolished $\sim$ 95% of NAs effect on POMC neurons. In this context, it is important to mention that BRL 44408 has also been shown to modify serotonergic 5-HT1_A-receptors. Although, serotonergic responses in POMC neurons have been characterized, these effects are suggested to underlie the activation of 5-HT2_C-receptors (Sohn *et al.* , 2011). Thus, there is no supporting evidence of crosstalk with other receptors on POMC neurons by BRL 44408.

In NPY/AgRP neurons the specific α_{1A}-AR antagonist WB 4101 potently blocks $\sim$ 95% of NA's effect. CEC, an selective antagonist for α_{1B}- and α_{1C}-ARs, has no effect on the NA mediated excitation. Both antagonists have been proven to specifically act on the mentioned subtypes suggesting that α_{1A}-ARs are responsible for the excitatory effect (Pan *et al.* , 1994; Zimnik *et al.* , 2012). For the first time on a single cell level, this study provides strong evidence for the expression of α_{2A}-ARs in POMC neurons and α_{1A}-ARs in NPY/AgRP neurons, which mediate the either inhibitory or excitatory effect, respectively.

Physiology of noradrenaline with relevance to the melanocortin system

Effects of NA on neuronal populations in the ARC have been investigated about a decade ago. A study by Kang *et al.* (2000) revealed that NA is able to either excite or inhibit neurons residing in the ARC. However, the experiments lacked the identification of the extracellularly recorded neurons. Studies also have revealed that the ARC is innervated by NA cell groups of the NTS as well as the main source of NA, the LC (Grill & Hayes, 2009). Yet, mostly the PVH has been in focus of NAs role in energy homeostasis (Wellman, 2000, 2005). This view has now to be expanded towards the melanocortin system in the ARC, which lies presynaptically to the PVH (Atasoy *et al.* , 2012).

In *in vivo* experiments, exogenous NA delivered by IP and ICV injections has been shown to potently induce feeding in rodents (Booth, 1967; Leibowitz, 1988; Wellman *et al.* , 1993). A proposed model by Wellman *et al.* (1993) suggests that induced food intake is due to inhibition of inhibitory neurons in the PVH by α_2-ARs. POMC and AgRP neurons in the ARC exert their potent effects on food intake via activation or inhibition of second order neurons, for example in the PVH (Gao & Horvath, 2008). Here, POMC neurons release α-MSH, which in turn excites neurons in the PVH by the activation of MC4Rs. In contrast, NPY and AgRP both are able to reduce firing of PVH neurons. These differential effects lead to the onset or offset of feeding (Ghamari-Langroudi *et al.* , 2011). The obtained data in this thesis indicates, that NA potently inhibits the melanocortin signaling at both sites, in the ARC and the PVH. At the level of the ARC, POMC neurons are inhibited which in turn leads to decreased release of α-MSH on second-order neurons. Simultaneously, NPY/AgRP neurons are excited, in turn leading to the GABAergic inhibition of POMC neurons. The release of NPY and AgRP on second order neurons in the PVH further decreases excitability in these neurons. Together, the orchestrated effect of NA on first and second order neurons suggest a potent pathway to elicit feeding via the shutdown of melanocortin signaling at both critical sites, the PVH and most importantly the ARC (see figure 4.1).

A body of evidence also supports the hypothesis on NA's modulatory effect on the melanocortin system in the ARC (Guy & Pelletier, 1988; Harfstrand *et al.* , 1986, 1987; Wellman, 2000). In this context, correlations between NA and other transmitters and

modulators have been suggested. Leptin for example, a hormone which is well known to exert its primary effect on the melanocortin system, has been shown to modulate NA release of catecholaminergic synapses in the ARC (Brunetti *et al.* , 1999; Francis *et al.* , 2004). In addition, insulin downregulates the expression of α_2-ARs specifically in ARC. Both effects would lead to an decrease in food intake due to the disinhibition of POMC neurons by the orchestrated effect of less receptor density and decreased ligand concentration. This suggests that both peripheral hormones exert their effects on food intake, at least in part, by the modulation of NA signaling in the ARC. Further experiments revealed an regulatory effect of NPY on NA release. NPY injections into the preoptic area, localized in the close vicinity of the ARC, increases NA release and food intake in rats (Myers *et al.* , 1996). NPY inhibits second order neurons in the PVH to induce feeding. The accompanying release of NA could lead to hyperpolarization of POMC neurons and depolarization of AgRP neurons in the ARC, thus augmenting release of NPY in the PVH.

Potential sources for the noradrenaline release

Given the consistent effects of exogenous NA on the ARC and the PVH the question remains under which physiological conditions endogenous NA is released and where exactly NA releasing projections to these sites arise. The first evidence of feeding related endogenous NA release was presented by (Martin & Myers, 1975). In their study, they preloaded hypothalamic sites with an radioactive variant of NA and examined the changes of NA concentrations during feeding. Changes were observed especially from midline structures of the hypothalamus at the level of the VMH. In contrast, nutrient infusions into the duodenum suppressed NA release in medial hypothalamic structures along with an increase in NA release into the lateral hypothalamus (Myers & McCaleb, 1980). In support of NAs action of increasing food intake, extracellular concentrations and NA release peak during the onset of the dark phase, a period that is critically related to feeding in rats (Morien *et al.* , 1995; Stanley *et al.* , 1989). Until now, most studies focused on NA's action on the PVH, which is conceivable as here endogenous and exogenous NA exhibit strong effects and can well be correlated with concomitant

behavioral patterns of animals. (Leibowitz, 1988; Wellman, 2000, 2005; Wellman *et al.* , 1993; **?**).

Mostly, the aforementioned physiological implications of NA release may rely on specific efferent projections from NA cell groups residing in the brainstem. Here the A1 (ventrolateral medulla; VLM) and the A2 (NTS) NA cell groups have been shown to specifically innervate the PVH (Goddard *et al.* , 2010). Additionally, the LC also innervates various sites of the hypothalamus, including the PVH as well as the ARC (Grzanna & Molliver, 1980; Samuels & Szabadi, 2008a). Interestingly, NA synapses on NPY neurons in the ARC have been shown, but the authors did not focus on the specific sources of these synaptic endings (Guy & Pelletier, 1988; Harfstrand, 1986, 1987; Harfstrand *et al.* , 1986). Thus, NA release on NPY/AgRP neurons is likely due to specific NA synapses. However, in the past two decades the development of the "volume transmission concept" expanded the functions of neuromodulators (Fuxe *et al.* , 2013; Zoli *et al.* , 1998). In contrast to the "wiring transmission", representing the classical synapse-mediated effect of neurotransmitters, the "volume transmission" allows neuromodulators to exert their effects at sites outside the synaptic cleft by 3-dimensional diffusion. With respect to this broad spatio-temporal effect, NA has been shown to be released at non-synaptic sites, thus mediating rather paracrine effects (Beaudet & Descarries, 1978; Callado & Stamford, 2000; Milusheva *et al.* , 2003; Séguéla *et al.* , 1990). Although it is likely that distinct synaptic projections of NA cell groups to specific hypothalamic regions contribute to the effects of NA on feeding behavior, synaptic connections thus are not a prerequisite. Previous work in the Horvath group and others has detected large expression of the uncoupling protein 2 (UCP2) in hypothalamic sites, which leads to heat production in mitochondria (Horvath *et al.* , 1999; Richard *et al.* , 1998). Further work revealed, that UCP2 and UCP3 are also expressed in a large number of NA and DA neurons and the concept of volume transmission is generally accepted for CA systems (Agnati *et al.* , 2005; Rivera *et al.* , 2006). Moreover, volume transmission may rely on temperature gradients (Agnati *et al.* , 1994). In this context, (Rivera *et al.* , 2006) argue, that UCP2/3 expression may produce temperature gradients in CA volume transmission, that lead

to "convective fluid movements" and thus "enhance migration of neurotransmitters". Together, the hypothesis is raised for a role of NA in the ARC by volume transmission.

In the following a hypothetic model is presented illustrating the NA effect on the ARC (see figure 4.1).

The effects of aging and chronic HFD feeding on noradrenergic responses in POMC neurons

Aging has been shown to result in changes in properties of POMC neurons in the ARC (Kim & Horvath, 2012; Newton *et al.* , 2013; Yang *et al.* , 2012). NA neurons in rats have been unaffected as no change in number could be detected during aging (**?**). However, on the level of ARs, numerous studies report a decreased receptor density with ongoing age (**?**). Decrease of α_2-AR expression has been shown in the prefrontal cortex in the context of AD (**?**). The responses recorded in this study on aged mice (20-25w) reveal a decreased conductance density upon application of 10 μM NA. This raises the possibility that α_2-AR expression is also decreased in POMC neurons during aging. However, the question if this effect is really due to reduced receptor density remains unanswered in these experiments.

In POMC neurons of mice fed a HFD from the age of 3 weeks on, this effect was reversed. Responses were not significantly different to that of POMC neurons in mice aged 8-12 weeks. In *ob/ob* mice, elevated NA levels have been detected in hypothalamic sites, however elevations in the ARC did not reach significance (Oltmans, 1983). Largest elevations were detected in the PVH. In line with the experiments in mice fed a HFD in this study, increased responses to injections of NA into hypothalamic structures have been shown in rodents (Kraszewski & Cincotta, 2000). Interestingly, insulin has been shown to exert effects on α_2-ARs specifically in the ARC (Levin *et al.* , 1998). In this thesis, the expression of α_2A-ARs has been shown in POMC neurons. Obesity leads to insulin resistance (Brüning *et al.* , 2000; Röhl *et al.* , 2004; Schubert *et al.* , 2004). Thus the possibility arises, that insulin resistance leads to impairment of intracellular cascades triggered by insulin receptor activation and following reduced expression of α_2A-ARs.

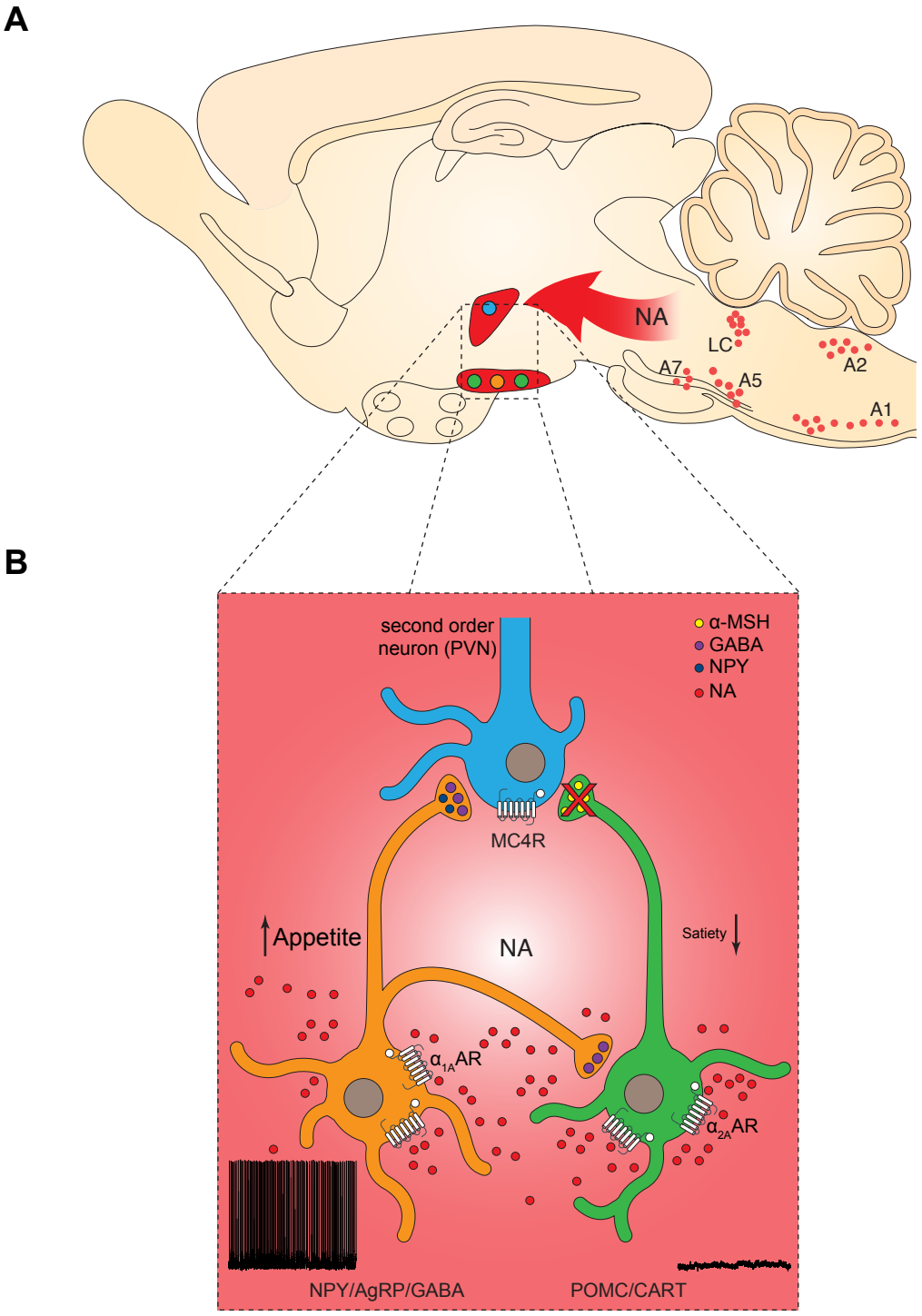


Figure 4.1: Modulation of the ARC melanocortin system by NA. ***(A)*** Schematic illutration of the mouse brain. NA cell groups residing in the brainstem project and release NA to hypothalamic sites controlling food intake, like the PVH and the ARC. ***(B)*** Upon release of NA into the ARC, NPY/AgRP/GABA neurons show increased firing due to the activation of excitatory α_{1A}-ARs. In contrast, NA inhibits POMC/CART expressing neurons in the ARC nucleus via the activation of inhibitory α_{2A}-ARs. POMC neurons thus remain silent. Further inhibition is mediated by unidirectional release of GABA from NPY/AgRP/GABA neurons on POMC/CART neurons. Consequently, α-MSH release on second order neurons is inhibited and NPY release on second order neurons antagonizes the effect of MC4R. Thus, satiety signals are depleted and appetite and feeding is the consequence. Modified from Dietrich & Horvath (2013); Sara (2009).

4.4 Properties of noradrenergic neurons in the locus coeruleus

Properties of NA neurons in the LC have been investigated using perforated patch-clamp techniques in acute brain slices of C57BL/6 at the age of 10-15 weeks. Neurons were identified by their anatomical location, ventrolaterally to the fourth ventricle. In most cases, after completion of electrophysiological protocols, recorded cells were labeled anterogradely with biocytin and slices were subsequently processed for AB-stainings. Immunohistochemical protocols against DBH alongside with streptavidin-biocytin-staining of the recorded cell, confirmed the neurons to be noradrenergic and located within the LC. Although, the enzyme DBH is less concentrated in NA cells compared to TH, stainings clearly silhouetted from background and typical shapes of the LC were revealed (see figure 3.15 A). Despite a few number of studies investigated basic properties of LC neurons in wildtype mice, none of these studies took advantage of the perforated patch-clamp technique, thus leaving intracellular solutions unchanged except for monovalent ions. Since the whole-cell patch-clamp technique has been shown to change properties of neurons, LC-NA neurons were analyzed again in order to create a sufficient baseline for future experiments in our laboratory (Simon Hess, PhD thesis 2011). For the first time, this study provides a detailed electrophysiological characterization of LC neurons analyzed by perforated patch-clamp recordings.

In the living rats, neurons of the LC display different types of activity. LC NA neurons are silent during phases of REM sleep. At times of waking, LC neurons increase tonic discharge and phasic patterns of action potentials can be observed during task performances and arousal stimuli as well as in states of high performance (Aston-Jones & Cohen, 2005; Berridge & Waterhouse, 2003; Sara, 2009). However, in acute slice preparations only tonic firing has been observed (de Oliveira *et al.* , 2010, 2011; Taneja *et al.* , 2009; van den Pol *et al.* , 2002; Williams *et al.* , 1985; Zhang *et al.* , 2010). This phenomenon is also described for the DA system and is arguably due to loss of afferent projections that contribute to the different firing patterns (Surmeier *et al.* , 2011).

LC neurons in slice preparations of C57BL/6 mice fired tonically with frequencies from 0.5 to 6 Hz, respectively. To further unravel mechanisms that underlie tonic pacemaking, Na^+ dependent spikes have been blocked by TTX. This revealed Cd^{2+}-

sensitive potentials that showed broader gaussian ISI distribution compared to TTX-sensitive spikes. These results suggest that pacemaking of LC neurons underlies a Ca^{2+}-dependent mechanism. However, the specific channels which are involved and a detailed analysis of underlying currents still remain elusive for the LC-NA neurons. In part, previous experiments support these findings as TTX application was not sufficient to block pacemaking in a subset of LC neurons. However, a second subset of neurons did not exhibit any pacemaking by the blockade of voltage gated Na^{+} currents (de Oliveira *et al.* , 2010). Here, differences may originate from different experimental procedures, as well as using different ages of mice. In a later study, the same authors investigated pacemaking again in 8-12 week old mice and found a subset of neurons, that did not show any spontaneous APs at all. Respective brain slices were reported to have a thickness of 140 μM and thus differ intensively from brain slices of 250 - 300 μM used in this thesis (de Oliveira *et al.* , 2011).

Basic electrophysiological properties have been investigated before in a study by Zhang *et al.* (2010). The authors prepared mouse brain slices in a comparable fashion and basic properties revealed values in line with this thesis, except for input resistances, which resulted in higher values in this study. The perforated patch-clamp technique can be responsible for the slight differences in input resistances (Falke *et al.* 1989; Oleson *et al.* 1993, Simon Hess, 2011).

Besides the pacemaking activity, the most prominent feature of the LC NA neurons is the excitation delay. In line with Zhang *et al.* (2010) the recorded neurons showed increasing times to the peak of the first AP in response to increasing hyperpolarizing current injections. This property could not be observed in a neuron which was located outside the LC (see figure 3.17 B). Thus, the excitation delay in response to hyperpolarizing current injections is unique to LC neurons and can be used as an electrophysiological tool to identify LC neurons in brain slices from C57BL/6 mice.

4.5 The locus coeruleus in the control of energy homeostasis and glucose metabolism

The LC is a potential source for NA release in the ARC (AnselmoFranci *et al.* , 1997; Grzanna & Molliver, 1980). Efferent projections have been identified and previous studies provide evidence for a role of the LC in the CNS-mediated control of energy homeostasis (Ammar *et al.* , 2001; Redmond *et al.* , 1977). Data provided by a recent collaboration reveals evidence for a role of the LC in glucose metabolism and control of BAT SNA. Glucose elevations lead to increased SNA and activation of BAT. In this context, the LC has been shown to innervate BAT via the SNS (Cano *et al.* , 2003). Therefore, glucose-sensing experiments have been conducted by singe cell perforated patch-clamp recordings in acute mouse brain slices. For the first time, GE and GI neurons have been detected in the LC of brain slice preparations and glucose-sensing in the LC was shown to contribute to BAT SNA. Thermogenesis and energy expenditure was reduced when LC neurons were silent and glucose-sensing was abolished. This leads to a further expansion of the NA system in control of energy homeostasis.

4.5.1 Kir6.2^{THCre}-mice develop obesity and impaired brown adipose tissue morphology and function

In the present section, data obtained and kindly provided by Sulay Tovar and Donald A. Morgan are discussed. Text passages are also provided by Tovar *et al.* (2013).

Kir6.2^{THCre}-mice developed obesity when fed NCD, an observation which was largely exaggerated in mice fed ad libitum HFD. Consistently, the surface of adipocytes increased in WAT and Kir6.2^{THCre}-mice exhibited impaired insulin sensitivity. Obesity has been further confirmed by elevated plasma leptin levels. Conversely, this obese phenotype could not be matched with increased food intake or decreased locomotor activity, pointing towards metabolic impairments at different levels of energy homeostasis. In line with this result, the analysis of BAT revealed severe morphological changes as well as differences in BAT specific gene expression, both of which yielding to an "white-adipocyte-like phenotype" of BAT. The specific function of BAT is thermogenesis by directly converting energy obtained by feeding into heat (Cannon & Nedergaard,

2004). Exposing animals to cold (4°C) could clearly show an impairment in maintaining rectal body temperature. Since this mechanism is controlled by NA fibers via the SNS, SNA activity was reduced in Kir6.2^{THCre}-mice compared to control mice (Bartness *et al.* , 2010).

BAT is further activated upon food intake and at times of high thermogenesis, the tissue is responsible for an characteristically large uptake of lipids and glucose (Collins *et al.* , 2001). When humans are exposed to cold it is reported that glucose uptake increases about 10 -15 fold compared to normal temperatures (Virtanen & Nuutila, 2011). Centrally applied glucose increases SNA activity in control mice, an effect which is abolished in Kir6.2^{THCre}-mice. The ability of centrally applied glucose to stimulate BAT SNA has been intensely studied, revealing that manipulating responses to glucose by either injecting 2-desoxy glucose in defined neuronal regions or lesioning attenuated the ability of glucose to activate BAT SNA (Egawa *et al.* , 1989a,b; Holt & York, 1989a,b; Madden, 2012). The attenuated ability of centrally applied glucose to activate BAT SNA may stem from direct impairment of glucose sensing in the sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM), Raphe Pallidus (RPa) or the intermediolateral nucleus (IML). At least Kir6.2-dependent glucose-mediated neuronal activation in the RVLM appears unlikely, since it has been demonstrated that RVLM-neurons are activated by glucoprivation and that this in turn activates GABAergic innervation of the RPa to inhibit BAT SNA (Madden, 2012). Nevertheless, it cannot be ruled out that abrogation of Kir6.2-mediated control of RPa, IML or other CA neurons may contribute to the obese phenotype of Kir6.2^{THCre}-mice. Apart from the aforementioned neuronal populations in control of BAT SNA, the LC has also been identified by retrograde tracing from BAT (Cano *et al.* , 2003). C-Fos immunoreactivity in the LC is activated within three to 24 hours of cold-exposure and bilateral lesions of the LC causes obesity in monkeys (Miyata *et al.* , 1995; Redmond *et al.* , 1977). Additionally, cold exposure leads to an increase of TH mRNA expression up to 200% in the LC, suggesting increased release of NA and increased thermogenesis as a response (Richard *et al.* , 1988). Taken together, these experiments point towards the possibility that neurons in the LC modulate BAT SNA.

4.5.2 Glucose sensing, brown adipose tissue and the locus coeruleus

Since glucose metabolism and BAT thermogenesis are related and the LC innervates and controls BAT via the SNS, glucose sensing was examined in Kir6.2^{THCre}-mice, their WT littermates and C57BL/6 mice (Bamshad *et al.* , 1999; Cano *et al.* , 2003; Mounien *et al.* , 2010; Oldfield *et al.* , 2002; Tews & Wabitsch, 2011). Comparable to LC NA neurons of C57BL/6 mice, THCre^{GFP} LC neurons were spontaneously active and exhibited a tonic pacemaking-like activity between 0.3 and 6 Hz. Importantly, this suggests that genomic modifications and GFP expression did not significantly alter properties of LC NA neurons.

To this day, support for several mechanisms of the reception of extracellular glucose levels exists and neurons are generally categorized as either glucose-excited (GE) or glucose-inhibited (GI), referring to their response to elevated glucose levels (Thorens, 2011). First discovered in pancreatic β-cells, the most prominent model of glucose-sensing includes the expression of two keyproteins, glucokinase and K_{ATP} (Ashcroft *et al.* , 1984; Ashcroft & Rorsman, 2004). Expression of both proteins has been detected in the LC (Dunn-Meynell *et al.* , 1998; Koyama *et al.* , 1999; Lynch *et al.* , 2000; Nieber *et al.* , 1995). In this particular context, the LC has already been cited as a glucose sensing site in a review by Levin (2001).

The present experiments reveal for the first time glucose-sensing neurons in the LC in acute mouse brain slices. Three different populations of LC neurons could be identified. Neurons were either GE, GI or were non-responsive.

Glucose sensing

Concentrations of extracellular glucose were altered from 5 mM to 8 mM and 5 mM to 3 mM, respectively. These concentrations have been shown to be effective for glucose sensing populations, for example POMC expressing cells in the ARC which have been subject of various studies concerning their ability of adapting firing to changes in extracellular glucose concetrations (Burdakov *et al.* , 2005a; Parton *et al.* , 2007). The experiments in this thesis led to the identification of a subpopulation of LC neurons, that respond in the manner of GE neurons. Here, increasing or decreasing extracellular

glucose concentration led to moderate excitation or inhibition of these neurons, respectively (see figure 3.22 and 3.24). Around 40% of LC neurons responded with a slight decrease of AP frequency to a reduction of extracellular glucose, a response that could be reversed by the K_{ATP} channel specific blocker tolbutamide. This suggests, that the response is mediated by the opening of K_{ATP} channels due to the consequent decrease in ATP/ADP ratio. On the other hand, the same proportion of LC neurons moderately increased AP frequency upon application of 8 mM extracellular glucose, suggesting the ATP dependent closure of K_{ATP} channels.

In support of these experiments, numerous studies reveal the activation of potassium currents due to reduction of extracellular glucose and induced hypoxia, which were sensitive to K_{ATP} channel blockers (Koyama *et al.* , 1999; Kuwahata, 2004; Murai *et al.* , 1997b; Nieber *et al.* , 1995)

The small number of neurons of the LC population responding as GI neurons, suggest a differential regulation of the LC population by changes in extracellular glucose. Opposing effects of glucose on neuronal populations have been revealed in the VMH, were neurons are either GE or GI (**?**). A few studies also suggest that the LC contains GI neurons (Ritter *et al.* , 1998; Sara, 1988). C-fos expression in response to 2-DG injections has been detected in the LC in rats (Ritter *et al.* , 1998). Importantly, this is not contradictory to the identification of GE neurons, as 2-DG induced c-fos expression only labels GI neurons. Glucoprivation in these studies led to increase in extracellular LC activity in rats and in living cats (Sara, 1988). The aforementioned studies support the results revealed in this thesis and point towards a differential regulation of glucose in the LC and a complex function of the LC in glucose metabolism and energy homeostasis. Importantly, differential regulation by glucose has been shown in the brainstem before. GI and GE neurons among a single cell population have been detected in the NTS (Mizuno & Oomura, 1984).

It is important to mention that used extracellular glucose concentrations largely vary among studies (Burdakov *et al.* , 2005b; Mizuno & Oomura, 1984; Parton *et al.* , 2007). In the CSF, the physiological range of glucose concentrations is still unclear. Additionally, concentrations may differ dependent on the location of the neuronal population

(Burdakov *et al.* , 2005a). It is certain, that increase in plasma glucose also rapidly leads to higher glucose concentrations in the brain (Silver & Erecińska, 1994). Here, concentrations are thought to vary around 10-30% to the corresponding plasma glucose levels. Given a range of plasma glucose from 5 to 8 mM, concentrations are expected to vary from 1 to 2.5 mM (Routh, 2002). Neuronal populations that lie in close proximity to structures known as circumventricular organs (CVO) might be exposed to much higher concentrations comparable to plasma glucose levels. CVOs are located around the third and fourth ventricle and are characterized by high permeability of the BBB. As an example, the median eminence, a prominent CVO thus enables the neighboring ARC to be exposed to higher glucose concentrations compared to other areas of the CNS (Fioramonti *et al.* , 2004; Ganong, 2000). An equivalent structure is also located in the brainstem. Here, the area postrema (ArP) around the fourth ventricle is highly permeable, and structures located in the vicinity may be exposed to glucose levels in the range of plasma glucose levels. In this context, a number of brainstem NA cell groups such as the NTS have been shown to respond to 2-DG, a glucose variant (Ritter *et al.* , 2011). Injections of 2-DG reliably lead to glucoprivic feeding (Ritter *et al.* , 2000). Additionally, electrophysiological data indicate glucose sensing in the DMV and NTS (Balfour *et al.* , 2006; Dallaporta *et al.* , 2000; Mizuno & Oomura, 1984).

Importantly, the effect of extracellular changes of glucose concentration has also been clarified in mice, which expressed the mutant Kir6.2 variant specifically in the LC (Tovar *et al.* , 2013).

Kir6.2^{THCre}-mice, glucose-sensing and physiology

Consistent with the reduced SNA activity, the Cre mediated expression of the mutant variant Kir6.2, which represents an constitutively open K^+ channel, resulted in severe inhibition of tonic activity in most LC neurons, most of which remained silent. In line with these results, the Cre mediated expression of the mutant K_{ATP} channel in LC neurons depletes electrical responses to changes in extracellular glucose levels by silencing these neurons. Application of tolbutamide significantly increased membrane potentials of LC neurons and concomitant appearance of APs was observed in most of the experi-

ments. This clearly shows that silencing of LC neurons is due to the activation of K_{ATP} channels. The presence of other described mechanisms of glucose sensing cannot be ruled out in LC neurons (Thorens, 2011). However, no significant changes in membrane potentials could be detected in Kir6.2^{THCre}-mice.

The obtained data of this study shows that a subpopulation of the LC responds to extracellular changes in glucose concentrations with moderate adaptation of AP frequency. The additional innervation of BAT by the LC and the well established role of NA in the control of BAT activity suggests a contribution of the LC to these mechanisms (Bamshad *et al.* , 1999; Bartness *et al.* , 2010; Cano *et al.* , 2003; Oldfield *et al.* , 2002). To support the hypothesis that the LC controls BAT SNA, the injection of an adeno-associated virus (AAV) into the LC led to site specific expression of the mutant K_{ATP} channel, thus minimizing the possibility of other TH expressing cells to influence glucose responses. Two weeks after the AAV-injections, mice started to develop obesity and phenotyping resulted in no difference compared to Kir6.2^{THCre}-mice. Consistently, extracellular recordings of sympathetic nerve activity, by which the LC controls brown adipose tissue, was reduced and revealed the depletion of responses to glucose injections. After glucose stimulation in control animals, SNA activity only increased in vehicle-injected mice. These experiments further support the role of the LC in control of BAT via SNA.

Given the role of NA in control of BAT it is reasonable to hypothesize that under conditions of lower glucose levels and thus decreased tonic activity of the LC, activity of BAT SNA is also decreased. This relation is also supported in a recent study by Shi *et al.* (2013). Fasting induced NPY signaling from the ARC to the PVH results in decreased expression of TH mRNA in the PVH and brainstem including the LC. They also observed a concomitant decrease in BAT activity. NA release is strongly correlated with the expression of TH mRNA, thus decreased BAT activity may underlie decreased NA release (Mitchell *et al.* , 1993). In summary, in times of food deprivation and hunger, energy expenditure via BAT is decreased due to reduced TH mRNA expression and NA release in the LC. The same conditions also lead to decreased glucose levels, which in

turn might amplify this effect via glucose-sensing of GE neurons in the LC innervating BAT, reflected by decreased SNA.

The identification of a second glucose-responsive subpopulation in the LC raises the question how these neurons contribute to energy homeostasis. While the data of citetTovar:1 along with the data obtained in this thesis clearly suggest, that GE neurons control SNA BAT, the role of GI neurons remains unknown. There is evidence, that lesions of DNB, which carries afferent projections of the LC, lead to hypophagia and reduced bodyweight (Hoebel *et al.* , 1989) and lesions of the LC attenuate oral intake (Ammar *et al.* , 2001). With respect to the obtained effect of NA in the ARC (and the PVH) and the identified efferent projections of the LC to the ARC (Grzanna & Molliver, 1980), reduced NA release would lead to decreased food intake and increased energy expenditure. Interestingly, a subpopulation of neurons in the LC co-express NPY (Smiałowska, 1988) in rats and NPY in the ARC leads to inhibition of POMC neurons and increased food intake when released into the PVH. GI neurons in the LC could release NA and/or NPY into the ARC and PVH to potently induce food intake. However, innervation of both regions by NPY-LC neurons has not been identified (Holets *et al.* , 1988).

Taken together, a substantial body of literature supports the results obtained in this study. In the LC, evidence for GE and GI neurons have been reported. It is therefore of great interest how the different subpopulations contribute to the functions of the LC. Here, GE and GI neurons could exhibit differences in there efferent projections to serve different roles in the response to changes in extracellular glucose levels. In this context, differential efferent projections has been shown in the LC before.

4.6 The role of noradrenalin in the pharmacotherapy of obesity

Drugs that aim to reduce food intake primarily act on neurotransmitters in the CNS (Ioannides-Demos *et al.* , 2006). In this context, the most frequently targeted transmitters include 5-HT and the CA DA and NA (Adan, 2013). However, how the class of monoamines is controlling energy homeostasis and food intake is only partly understood and the impact of these transmitters on other systems in the CNS is tremendous (Bloom, 2010; Guiard *et al.* , 2008a; Smeets & González, 2000; Wellman, 2005). Thus it is

not surprising, that in the history of anti-obesity drugs a large number of compounds that aim to modulate the monoaminergic systems exhibited a variety of side effects which consequently led to the withdrawal of respective drugs (Ryan & Bray, 2013). The anorexigenic effect of amphetamine has been of interest since decades but the addictive action of this drug, presumably due to unspecific effects also on the dopaminergic system does not recommend a long term use in the treatment of obesity (Jones & Caul, 1992; Pandit *et al.* , 2011; Wellman, 2005; White *et al.* , 2010). Recently, the drug sibutramine, which resulted in constant weight loss in patients over a longtime period, was withdrawn due to the increased risk for nonfatal myocardial infarction and nonfatal stroke in high-risk cardiac patients (James *et al.* , 2010). Sibutramine potently led to increased NA and 5-HT concentrations by blocking the reuptake of both biogenic amines (Powell *et al.* , 2011).

When focussing on the treatment of obesity by targeting specifically the NA system it is mandatory to mention that NA may have opposing effects on food intake and energy homeostasis (Wellman, 2005). As previously mentioned, NA projections in the CNS arise in the brainstem and travel by two distinct fiber systems and impairment of either of these fiber systems led to hyper - or hypophagia, respectively (Wellman, 2000). This further emphasizes the difficulty of anti-obesity treatment. Developing drugs which are specific for a particular system reduces the side effects but still it remains difficult to make the compounds effective at specific sites within the CNS. Additionally, NA, serotonergic and Dopaminergic systems may influence one another. For example, DA neurons in the midbrain also express ARs, thus manipulating the NA systems indirectly may influence the DA systems and hence might lead to side effects (Bonci *et al.* , 2003).

Within the last years, serotonergic and NA reuptake inhibitors have been more and more in focus as drugs for the treatment of obesity. Here, several compounds have been tested. However, some having severe effects on the emotional state of a human being. The role of 5-HT and NA in depression has been studied for years. Interestingly, a large community supports the hypothesis that depression and obesity may be linked diseases (Hainer *et al.* , 2006b; Rosmond, 2004). Some drugs are only indicated for short term use in treatment of obesity. Until today, sibutramine, a specific serotonin

and noradrenalin reuptake inhibitor represents the only indicated drug for a long term treatment of obesity. However, it also leads to various side effects and is specifically increasing the risk of nonfatal myocardial infarction and nonfatal stroke in patients with preexisting cardiovascular diseases (James *et al.* , 2010)

Together, these examples illustrate the difficulties in the long term treatment of obesity. It seems to be of specific importance in the development of anti-obesity drugs to unravel the single factors controlling energy homeostasis and food intake and evaluate the systems these factors interfere with.

"Nonetheless, obesity remains a disease mainly caused by an excess of caloric intake in relation to energy expenditure and on that basis, its treatment should be a healthy diet and physical activity. When these options alone are not sufficient, then additional pharmacotherapy with an acceptable efficacy and safety profile could provide a useful option" (Gouni-Berthold *et al.* , 2013).

4.7 Outlook and preliminary data

More experiments should be conducted to further define the impact of the NA system on the ARC in detail. Proposals for further experiments and respective preliminary datasets are are given in the following sections.

4.7.1 Energy homeostasis and noradrenergic signaling in the hypothalamus

The potent effects of NA on POMC and NPY/AgRP expressing cells in the ARC raise the question of how NA modulates behavior in the living animal and how this is physiologically relevant. Initially, intracerebral injections led to the identification of various sites in the hypothalamus that respond to the injection of NA with food intake and thus provided first evidence for NAs role in energy homeostasis (Booth, 1967; Booth & Jarman, 1976; Leibowitz, 1978a). Later, the same method revealed NA cell groups in the brainstem responding to the injection of 2-DG (Ritter *et al.* , 1998).

Since pharmacological experiments revealed that the inhibitory effect of NA on POMC neurons is mediated by α_2-ARs, the specific antagonist SKF 86466 has been used for IC injections. However, the unilateral injection into the ARC failed to significantly decrease

refeeding in fasted mice (see figure 4.2). Nevertheless, the obtained data showed a tendency towards decreased food intake. In the context of these experiments, two problems arise. First, it has been described that for POMC neurons unilateral IC injections fail to induce significant effects. These were only obtained by bilateral injections (Atasoy *et al.* , 2012). Second, the evolutionary advantage for hunger favors the NPY/AgRP drive and thus leads to more reliable responses on a short term range even when drugs are unilaterally injected (Aponte *et al.* , 2011; Atasoy *et al.* , 2012; Zhan *et al.* , 2013). Hence, in the future it is conceivable to rather focus on the NPY/AgRP neurons and since it is known that the excitatory effect of NA on these neurons is mediated by α_{1A}-ARs, there is also a large pharmacological toolbox to affect these neurons.

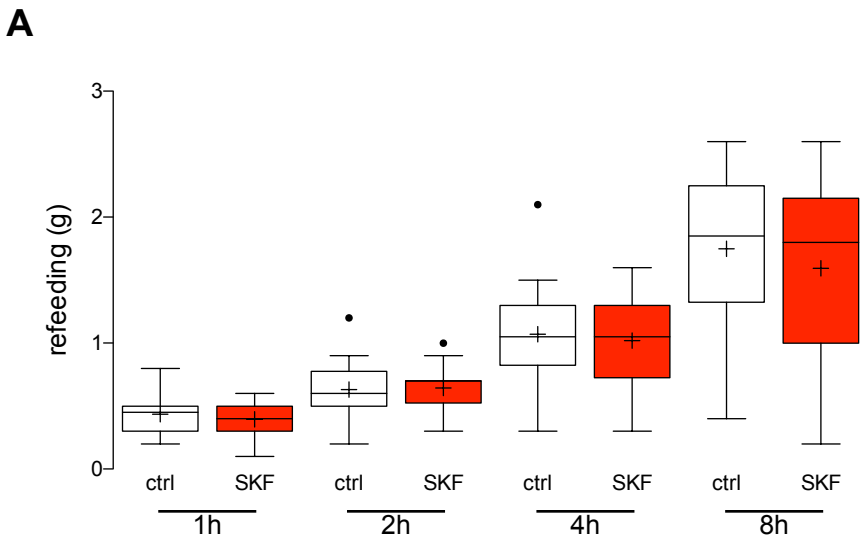


Figure 4.2: IC injections of the α_2-AR antagonist and the effect on refeeding in fasted mice ***(A)*** Unilateral IC injections of 10 μM of the specific α_2-AR antagonist SKF 86466 fails to reduce refeeding in fasted mice. Food intake has been analyzed at 1, 2, 4 and 8 hours after IC injections. At each timepoint there is s small tendency towards decreased food intake. Experiments were done and data was kindly provided by **Tim Klöckener**.

4.7.2 Anatomy of noradrenergic signaling to the hypothalamus

Despite the various anatomical works on the NA system and the known projections from NA cell groups to the medial basal hypothalamus and specifically the ARC, it is still unclear, which exact cell groups project on either or both POMC and NPY/AgRP neurons. TH-immunoreactive synaptic endings have been shown on NPY neurons of

the ARC in rats, however these findings lack the specific source of these projections. In this context, in a first step it should be addressed wether NA release into the ARC is realized only by "wired" or "volume transmission". In order to show direct synaptic projections from NA cell groups of the brainstem, DBH^{Cre} mice have been crossed with wheatgerm-agglutinin (WGA) expressing mice (Braz *et al.* , 2002). The use of the anterograde tracer WGA in combination with the DBH^{Cre} mouse enables to analyze specific synaptic projections from NA cell groups only. Interestingly, the success of this genetical tracing experiment has been published recently. However, the authors focused on a different area within the CNS and did not describe if cell bodies in the ARC were stained (Walling *et al.* , 2012).

4.7.3 Effect of insulin, leptin and ghrelin on the the noradrenergic modulation of POMC and NPY/AgRP neurons in the arcuate nucleus

As aforementioned, studies indicate the peripheral hormones insulin, leptin and ghrelin to have effects on NA release in the ARC as well as on the expression of α_2-ARs. In context of decreased expression of α_2-ARs upon insulin stimulation, recording of induced conductance densities upon NA application in either normal aCSF and slices pre-incubated with Insulin containing aCSF could provide first evidence if this is visible on a single cell level. In the case of leptin, responses should be compared in the presence and the absence of specific antagonists for either α_{1A}- or α_{2A}-ARs. Given that NA projections are still working in acute brain slice preparations, the effect of leptin should be decreased in the presence of the antagonists since leptin has been shown to reduce the release of NA into the ARC (Brunetti *et al.* , 1999; Francis *et al.* , 2004; Kawakami *et al.* , 2008). The following figure illustrates a proposed model of insulin and leptin action on the NA system modulating POMC and NPY/AgRP expressing neurons in the ARC.

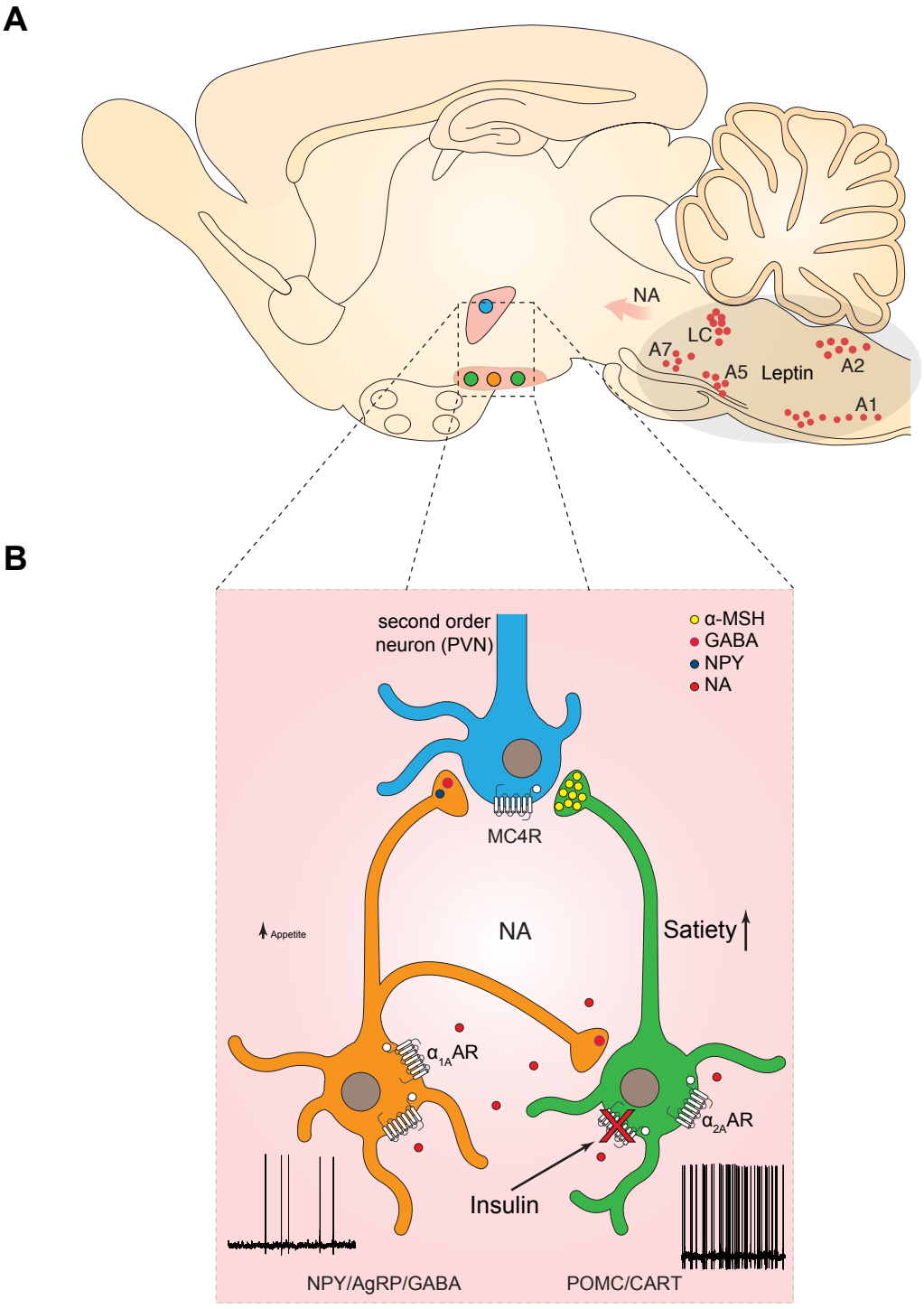


Figure 4.3: Proposed model of insulin and leptins action on the NA system modulation the ARC ***(A)*** Schematic illustration of the CNS with NA cell groups in the brainstem and the melanocortin system of the hypothalamus with the ARC and PVH. Leptin inhibits NA release into the hypothalamus, presumably by acting on NA neurons residing in the brainstem with efferent projections to the ARC. ***(A)*** Decreased NA concentration due to inhibited NA release by leptin into the ARC leads to decreased excitation of NPY/AgRP neurons and decreased inhibition of POMC/CART neurons, respectively. Thus, α-MSH release from POMC neurons on second order neurons is increased and satiety signals prevail. Additionally, GABA and NPY release from NPY/AgRP synaptic terminals is decreased and targeted neurons are less inhibited. Insulin leads to decreased α_{2A}-AR expression in the ARC and consequently higher α-MSH on second order neurons. Also compare to 4.1. Modified from Dietrich & Horvath (2013); Sara (2009).

4.7.4 Effects of dietary changes and aging

Noradrenalin content in the brain changes during aging as it has been shown for rats at different ages (Míguez *et al.* , 1999; Smith *et al.* , n.d.). Moreover, NA cell groups also are effected by aging. Expecially the LC is affected with ongoing age and loss of LC neurons is implicated in age-associated diseases like PD and AD. In the ARC, induced conductance densities upon NA application decrease in mice at the age of 20 weeks compared to younger mice. These results suggest less affinity of NA to α_2-ARs, however it is not clear wether this is reflected by decreased receptor expression or alterations in downstream targets of the receptors.

4.7.5 The role of the locus coeruleus in energy homeostasis

Mentioned before, lesioning of the DNB, which contains fibers arising from the LC and innervate the hypothalamus including the arcuate nucleus, leads to leanness and hypophagia in rats, suggesting the contribution of the LC-NA system to energy homeostasis and food intake. In this context, DBH KO mice consistently develop hypophagia and are smaller than their wildtype littermates. However, the effect on metabolism and the development of obesity in Kir6.2$^{\mathrm{THCre}}$-mice could not be assigned to a change in food intake or locomotive behavior. Obesity in these mice is more likely to be based on decreased energy expenditure due to the whitening of the BAT. In this context it is unclear to which extent glucose exerts its physiological effects via the LC or which further physiological effects it underlies.

Various neuronal populations that are involved in energy homeostasis are glucose-sensitive which leads to the question if and how the LC further contributes to the CNS-mediated control of energy homeostasis. In this regard, the question arises if the LC also is able to respond to other fuel sensing signals. Interestingly, leptin receptor expression has been detected in the rat LC and leptin modulates NA signaling (Grill *et al.* , 2002a; Hay-Schmidt *et al.* , 2001). Additionally, leptin delivery into the fourth ventricle reduces food intake in rats (Grill *et al.* , 2002b) but yet exact pathways remain elusive. In an elegant study Elmquist *et al.* (1997) evaluated Leptins effect on the CNS including the by c-fos expression, however this method fails when neurons decrease their electrical

activity in response to certain stimuli (Elmquist *et al.* , 1999; Hoffman *et al.* , 1993; Hyman *et al.* , 1993). In very recent experiments in our lab, we have evidence that leptin may have rather inhibitory effects on neurons in the LC. Consistently, Elmquist *et al.* (1997) did not report any activation in the LC but in other nuclei of the brainstem.

To this date, there is no evidence for any effects of Insulin on electrical activity on LC neurons, however studies revealed effects in gene expression as shown by in-situ hybridization for NET mRNA and TH mRNA upon Insulin administration of (Figlewicz *et al.* , 1993; Rusnák *et al.* , 1998).

Since the effects of glucose on the LC have been shown in Kir6.2^{THCre}-mice and mice injection with an AAV, specificity of the effects could be matched with the LC. However, since this method is time-consuming and invasive it would be of great interest to develop markers for different NA cell groups in the brainstem. A very recent study by Robertson *et al.* (2013) reveal these markers and thus enable to specifically target the LC and other NA cell groups with the advantage of neurogenetics. In favor of these important study, new insights of the LC-NA system in contribution to energy homeostasis can be revealed in the future.

List of Figures

Bibliography

Acosta-Martinez, M, Fiber, J M, Brown, R D, & Etgen, A M. 1999. Localization of alpha1B-adrenergic receptor in female rat brain regions involved in stress and neuroendocrine function. *Neurochemistry international*, **35**(5), 383–391.

Acuna-Goycolea, Claudio, & van den Pol, Anthony N. 2005. Peptide YY(3-36) inhibits both anorexigenic proopiomelanocortin and orexigenic neuropeptide Y neurons: implications for hypothalamic regulation of energy homeostasis. *The journal of neuroscience : the official journal of the society for neuroscience*, **25**(45), 10510–10519.

Adachi, A, Kobashi, M, & Funahashi, M. 1995. Glucose-responsive neurons in the brainstem. *Obesity research*, **3 Suppl 5**(Dec.), 735S–740S.

Adan, Roger A H. 2013. Mechanisms underlying current and future anti-obesity drugs. *Trends in neurosciences*, **36**(2), 133–140.

Agnati, L F, Cortelli, P, Biagini, G, Bjelke, B, & Fuxe, K. 1994. Different classes of volume transmission signals exist in the central nervous system and are affected by metabolic signals, temperature gradients and pressure waves. *Neuroreport*, **6**(1), 9–12.

Agnati, L F, Genedani, S, Lenzi, P L, Leo, G, Mora, F, Ferré, S, & Fuxe, K. 2005. Energy gradients for the homeostatic control of brain ECF composition and for VT signal migration: introduction of the tide hypothesis. *Journal of neural transmission (vienna, austria : 1996)*, **112**(1), 45–63.

Ahlskog, J E, & Hoebel, B G. 1973. Overeating and obesity from damage to a noradrenergic system in the brain. *Science (new york, ny)*, **182**(4108), 166–169.

Akaike, N, & Harata, N. 1994. Nystatin perforated patch recording and its applications to analyses of intracellular mechanisms. *The japanese journal of physiology*, **44**(5), 433–473.

Alberto, Christian O, Trask, Robert B, & Hirasawa, Michiru. 2011. Dopamine acts as a partial agonist for α2A adrenoceptor in melanin-concentrating hormone neurons. *The journal of neuroscience : the official journal of the society for neuroscience*, **31**(29), 10671–10676.

Ammar, A A, Södersten, P, & Johnson, A E. 2001. Locus coeruleus noradrenergic lesions attenuate intraoral intake. *Neuroreport*, **12**(14), 3095–3099.

Anand, B K, & Brobeck, J R. 1951. Localization of a "feeding center" in the hypothalamus of the rat. *Proceedings of the society for experimental biology and medicine. society for experimental biology and medicine (new york, n.y.)*, **77**(2), 323–324.

Anand, B K, Chhina, G S, Sharma, K N, Dua, S, & Singh, B. 1964. Activity of single neurons in the hypothalamic feeding centers: effect of glucose. *The american journal of physiology*, **207**(Nov.), 1146–1154.

AnselmoFranci, JA, Franci, CR, Krulich, L, AntunesRodrigues, J, & McCann, SM. 1997. Locus coeruleus lesions decrease norepinephrine input into the medial preoptic area and medial basal hypothalamus and block the LH, FSH and prolactin preovulatory surge. *Brain research*, **767**(2), 289–296.

Aponte, Yexica, Atasoy, Deniz, & Sternson, Scott M. 2011. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nature neuroscience*, **14**(3), 351–355.

Ashcroft, F M, Harrison, D E, & Ashcroft, S J. 1984. Glucose induces closure of single potassium channels in isolated rat pancreatic beta-cells. *Nature*, **312**(5993), 446–448.

Ashcroft, Frances, & Rorsman, Patrik. 2004. Type 2 diabetes mellitus: not quite exciting enough? *Human molecular genetics*, **13 Spec No 1**(Apr.), R21–31.

Ashford, M L, Boden, P R, & Treherne, J M. 1990. Tolbutamide excites rat glucoreceptive ventromedial hypothalamic neurones by indirect inhibition of ATP-K+ channels. *British journal of pharmacology*, **101**(3), 531–540.

Aston-Jones, Gary, & Cohen, Jonathan D. 2005. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. *Annual review of neuroscience*, **28**, 403–450.

Atasoy, Deniz, Betley, J Nicholas, Su, Helen H, & Sternson, Scott M. 2012. Deconstruction of a neural circuit for hunger. *Nature*, **488**(7410), 172–177.

Balfour, Robert H, Hansen, Ann Maria Kruse, & Trapp, Stefan. 2006. Neuronal responses to transient hypoglycaemia in the dorsal vagal complex of the rat brainstem. *The journal of physiology*, **570**(Pt 3), 469–484.

Balthasar, Nina, Dalgaard, Louise T, Lee, Charlotte E, Yu, Jia, Funahashi, Hisayuki, Williams, Todd, Ferreira, Manuel, Tang, Vinsee, McGovern, Robert A, Kenny, Christopher D, Christiansen, Lauryn M, Edelstein, Elizabeth, Choi, Brian, Boss, Olivier, Aschkenasi, Carl, Zhang, Chen-yu, Mountjoy, Kathleen, Kishi, Toshiro, Elmquist, Joel K, & Lowell, Bradford B. 2005. Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell*, **123**(3), 493–505.

Bamshad, M, Song, C K, & Bartness, T J. 1999. CNS origins of the sympathetic nervous system outflow to brown adipose tissue. *The american journal of physiology*, **276**(6 Pt 2), R1569–78.

Bartelt, Alexander, Bruns, Oliver T, Reimer, Rudolph, Hohenberg, Heinz, Ittrich, Harald, Peldschus, Kersten, Kaul, Michael G, Tromsdorf, Ulrich I,

Weller, Horst, Waurisch, Christian, Eychmüller, Alexander, Gordts, Philip L S M, Rinninger, Franz, Bruegelmann, Karoline, Freund, Barbara, Nielsen, Peter, Merkel, Martin, & Heeren, Joerg. 2011. Brown adipose tissue activity controls triglyceride clearance. *Nature medicine*, **17**(2), 200–205.

Bartness, T J, Vaughan, C H, & Song, C K. 2010. Sympathetic and sensory innervation of brown adipose tissue. *International journal of obesity and related metabolic disorders : journal of the international association for the study of obesity*, **34 Suppl 1**(Oct.), S36–42.

Bates, Sarah H, & Myers, Martin G. 2003. The role of leptin receptor signaling in feeding and neuroendocrine function. *Trends in endocrinology and metabolism: Tem*, **14**(10), 447–452.

Beaudet, A, & Descarries, L. 1978. The monoamine innervation of rat cerebral cortex: synaptic and nonsynaptic axon terminals. *Neuroscience*, **3**(10), 851–860.

Belgardt, Bengt F, Husch, Andreas, Rother, Eva, Ernst, Marianne B, Wunderlich, F Thomas, Hampel, Brigitte, Klöckener, Tim, Alessi, Dario, Kloppenburg, Peter, & Brüning, Jens C. 2008. PDK1 deficiency in POMC-expressing cells reveals FOXO1-dependent and -independent pathways in control of energy homeostasis and stress response. *Cell metabolism*, **7**(4), 291–301.

Belgardt, Bengt F, Okamura, Tomoo, & Brüning, Jens C. 2009. Hormone and glucose signalling in POMC and AgRP neurons. *The journal of physiology*, **587**(Pt 22), 5305–5314.

Benarroch, Eduardo E. 2009. The locus ceruleus norepinephrine system: functional organization and potential clinical significance. *Neurology*, **73**(20), 1699–1704.

Benoit, S, Schwartz, M, Baskin, D, Woods, S C, & Seeley, R J. 2000. CNS melanocortin system involvement in the regulation of food intake. *Hormones and behavior*, **37**(4), 299–305.

Benovic, J L, Bouvier, M, Caron, M G, & Lefkowitz, R J. 1988. Regulation of adenylyl cyclase-coupled beta-adrenergic receptors. *Annual review of cell biology*, **4**, 405–428.

Berridge, Craig W. 2008. Noradrenergic modulation of arousal. *Brain research reviews*, **58**(1), 1–17.

Berridge, Craig W, & Waterhouse, Barry D. 2003. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain research. brain research reviews*, **42**(1), 33–84.

Berridge, Craig W, Schmeichel, Brooke E, & España, Rodrigo A. 2012. Noradrenergic modulation of wakefulness/arousal. *Sleep medicine reviews*, **16**(2), 187–197.

Berthoud, H-R, & Mogenson, G J. 1977. Ingestive behavior after intracerebral and intracerebroventricular infusions of glucose and 2-deoxy-D-glucose. *The american journal of physiology*, **233**(3), R127–33.

Berthoud, H-R, & Powley, T L. 1990. Identification of vagal preganglionics that mediate cephalic phase insulin response. *The american journal of physiology*, **258**(2 Pt 2), R523–30.

Berthoud, H-R, Bereiter, D A, Trimble, E R, Siegel, E G, & Jeanrenaud, B. 1981. Cephalic phase, reflex insulin secretion. Neuroanatomical and physiological characterization. *Diabetologia*, **20 Suppl**(Mar.), 393–401.

Berthoud, Hans-Rudolf. 2008. The vagus nerve, food intake and obesity. *Regulatory peptides*, **149**(1-3), 15–25.

Birnbaum, S G, Yuan, P X, Wang, M, Vijayraghavan, S, Bloom, A K, Davis, D J, Gobeske, K T, Sweatt, J D, Manji, H K, & Arnsten, A F T. 2004. Protein kinase C overactivity impairs prefrontal cortical regulation of working memory. *Science (new york, ny)*, **306**(5697), 882–884.

Bloom, F E. 1979. Chemically coded transmitter systems. *Progress in brain research*, **51**, 125–131.

Bloom, Floyd E. 2010. The catecholamine neuron: Historical and future perspectives. *Progress in neurobiology*, **90**(2), 75–81.

Blouet, Clémence, & Schwartz, Gary J. 2010. Hypothalamic nutrient sensing in the control of energy homeostasis. *Behavioural brain research*, **209**(1), 1–12.

Bonci, Antonello, Bernardi, Giorgio, Grillner, Pernilla, & Mercuri, Nicola B. 2003. The dopamine-containing neuron: maestro or simple musician in the orchestra of addiction? *Trends in pharmacological sciences*, **24**(4), 172–177.

Booth, DA. 1967. Localization of adrenergic feeding system in rat diencephalon. *Science (new york, ny)*, **158**(3800), 515–&.

Booth, DA, & Jarman, SP. 1976. Inhibition of food-intake in rat following complete absorption of glucose delivered into stomach, intestine or liver. *Journal of physiology-london*, **259**(2), 501–522.

Boston, B A, Blaydon, K M, Varnerin, J, & Cone, R D. 1997. Independent and additive effects of central POMC and leptin pathways on murine obesity. *Science (new york, ny)*, **278**(5343), 1641–1644.

Braz, Joao M, Rico, Beatriz, & Basbaum, Allan I. 2002. Transneuronal tracing of diverse CNS circuits by Cre-mediated induction of wheat germ agglutinin in transgenic mice. *Proceedings of the national academy of sciences of the united states of america*, **99**(23), 15148–15153.

Brede, Marc, Philipp, Melanie, Knaus, Anne, Muthig, Verena, & Hein, Lutz. 2004. alpha2-adrenergic receptor subtypes - novel functions uncovered in gene-targeted mouse models. *Biology of the cell / under the auspices of the european cell biology organization*, **96**(5), 343–348.

Brobeck, J R. 1946. Mechanism of the development of obesity in animals with hypothalamic lesions. *Physiological reviews*, **26**(4), 541–559.

Brobeck, J R, Tepperman, J, & Long, C N. 1943. Experimental Hypothalamic Hyperphagia in the Albino Rat. *The yale journal of biology and medicine*, **15**(6), 831–853.

Bruce-Keller, Annadora J, Keller, Jeffrey N, & Morrison, Christopher D. 2009. Obesity and vulnerability of the CNS. *Biochimica et biophysica acta*, **1792**(5), 395–400.

Brunetti, L, Michelotto, B, Orlando, G, & Vacca, M. 1999. Leptin inhibits norepinephrine and dopamine release from rat hypothalamic neuronal endings. *European journal of pharmacology*, **372**(3), 237–240.

Brunetti, L, Recinella, L, Orlando, G, Michelotto, B, Di Nisio, C, & Vacca, M. 2002. Effects of ghrelin and amylin on dopamine, norepinephrine and serotonin release in the hypothalamus. *European journal of pharmacology*, **454**(2-3), 189–192.

Brunetti, L, Orlando, G, Recinella, L, Michelotto, B, Ferrante, C, & Vacca, M. 2004. Resistin, but not adiponectin, inhibits dopamine and norepinephrine release in the hypothalamus. *European journal of pharmacology*, **493**(1-3), 41–44.

Brüning, J C, Gautam, D, Burks, D J, Gillette, J, Schubert, M, Orban, P C, Klein, R, Krone, W, Müller-Wieland, D, & Kahn, C R. 2000. Role of brain insulin receptor in control of body weight and reproduction. *Science (new york, ny)*, **289**(5487), 2122–2125.

Bücheler, M M, Hadamek, K, & Hein, L. 2002. Two alpha(2)-adrenergic receptor subtypes, alpha(2A) and alpha(2C), inhibit transmitter release in the brain of gene-targeted mice. *Neuroscience*, **109**(4), 819–826.

Bünemann, M, Bücheler, M M, Philipp, M, Lohse, M J, & Hein, L. 2001. Activation and deactivation kinetics of alpha 2A- and alpha 2C-adrenergic receptor-activated G protein-activated inwardly rectifying K+ channel currents. *The journal of biological chemistry*, **276**(50), 47512–47517.

Burdakov, Denis, Luckman, Simon M, & Verkhratsky, Alexei. 2005a. Glucose-sensing neurons of the hypothalamus. *Philosophical transactions of the royal society of london. series b, biological sciences*, **360**(1464), 2227–2235.

Burdakov, Denis, Gerasimenko, Oleg, & Verkhratsky, Alexei. 2005b. Physiological changes in glucose differentially modulate the excitability of hypothalamic melanin-concentrating hormone and orexin neurons in situ. *The journal of neuroscience : the official journal of the society for neuroscience*, **25**(9), 2429–2433.

Calaresu, F R, & Ciriello, J. 1980. Projections to the hypothalamus from buffer nerves and nucleus tractus solitarius in the cat. *The american journal of physiology*, **239**(1), R130–6.

Callado, L F, & Stamford, J A. 2000. Spatiotemporal interaction of alpha(2) autoreceptors and noradrenaline transporters in the rat locus coeruleus: implications for volume transmission. *Journal of neurochemistry*, **74**(6), 2350–2358.

Cannon, Barbara, & Nedergaard, Jan. 2004. Brown adipose tissue: function and physiological significance. *Physiological reviews*, **84**(1), 277–359.

Cannon, C Matson, & Palmiter, R D. 2003. Peptides that regulate food intake: norepinephrine is not required for reduction of feeding induced by cholecystokinin. *American journal of physiology-regulatory integrative and comparative physiology*, **284**(6), R1384–8.

Cano, Georgina, Passerin, Alicia M, Schiltz, Jennifer C, Card, J Patrick, Morrison, Shaun F, & Sved, Alan F. 2003. Anatomical substrates for the central control of sympathetic outflow to interscapular adipose tissue during cold exposure. *The journal of comparative neurology*, **460**(3), 303–326.

Carette, B. 1999. Noradrenergic responses of neurones in the mediolateral part of the lateral septum: alpha1-adrenergic depolarization and rhythmic bursting activities, and alpha2-adrenergic hyperpolarization from guinea pig brain slices. *Brain research bulletin*, **48**(3), 263–276.

Carlsson, A, Falck, B, & Hillarp, N A. 1962. Cellular localization of brain monoamines. *Acta physiologica scandinavica. supplementum*, **56**(196), 1–28.

Carter, Matthew E, Yizhar, Ofer, Chikahisa, Sachiko, Nguyen, Hieu, Adamantidis, Antoine, Nishino, Seiji, Deisseroth, Karl, & de Lecea, Luis. 2010. Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nature neuroscience*, **13**(12), 1526–1533.

Cawley, John, & Meyerhoefer, Chad. 2012. The medical care costs of obesity: an instrumental variables approach. *Journal of health economics*, **31**(1), 219–230.

Chen, Shao-Rui, Chen, Hong, Yuan, Wei-Xiu, & Pan, Hui-Lin. 2011. Increased presynaptic and postsynaptic α2-adrenoceptor activity in the spinal dorsal horn in painful diabetic neuropathy. *The journal of pharmacology and experimental therapeutics*, **337**(1), 285–292.

Claret, Marc, Smith, Mark A, Batterham, Rachel L, Selman, Colin, Choudhury, Agharul I, Fryer, Lee G D, Clements, Melanie, Al-Qassab, Hind, Heffron, Helen, Xu, Allison W, Speakman, John R, Barsh, Gregory S, Viollet, Benoit, Vaulont, Sophie, Ashford, Michael L J, Carling, David, & Withers, Dominic J. 2007. AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. *Journal of clinical investigation*, **117**(8), 2325–2336.

Cohen-Matsliah, Sivan Ida, Motanis, Helen, Rosenblum, Kobi, & Barkai, Edi. 2010. A novel role for protein synthesis in long-term neuronal plasticity: maintaining re-

duced postburst afterhyperpolarization. *The journal of neuroscience : the official journal of the society for neuroscience*, **30**(12), 4338–4342.

Coleman, D L. 1973. Effects of parabiosis of obese with diabetes and normal mice. *Diabetologia*, **9**(4), 294–298.

Coleman, D L. 1978. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia*, **14**(3), 141–148.

Collins, S, Cao, WH, Daniel, KW, Dixon, TM, Medvedev, AV, Onuma, H, & Surwit, R. 2001. Adrenoceptors, uncoupling proteins, and energy expenditure. *Experimental biology and medicine*, **226**(11), 982–990.

Cone, R D, Cowley, M A, Butler, A A, Fan, W, Marks, D L, & Low, M J. 2001. The arcuate nucleus as a conduit for diverse signals relevant to energy homeostasis. *International journal of obesity and related metabolic disorders : journal of the international association for the study of obesity*, **25 Suppl 5**(Dec.), S63–7.

Cone, Roger D. 2005. Anatomy and regulation of the central melanocortin system. *Nature neuroscience*, **8**(5), 571–578.

Cowley, M A, Smart, J L, Rubinstein, M, Cerdán, M G, Diano, S, Horvath, T L, Cone, R D, & Low, M J. 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*, **411**(6836), 480–484.

Cui, Ningren, Zhang, Xiaoli, Tadepalli, Jyothirmayee S, Yu, Lei, Gai, Hongyu, Petit, James, Pamulapati, Ravi T, Jin, Xin, & Jiang, Chun. 2011. Involvement of TRP channels in the CO2 chemosensitivity of locus coeruleus neurons. *Journal of neurophysiology*, **105**(6), 2791–2801.

Dahlström, A, & Fuxe, K. 1964. Localization of monoamines in the lower brain stem. *Experientia*, **20**(7), 398–399.

Dallaporta, M, Perrin, J, & Orsini, J C. 2000. Involvement of adenosine triphosphate-sensitive K+ channels in glucose-sensing in the rat solitary tract nucleus. *Neuroscience letters*, **278**(1-2), 77–80.

Date, Yukari, Shimbara, Takuya, Koda, Shuichi, Toshinai, Koji, Ida, Takanori, Murakami, Noboru, Miyazato, Mikiya, Kokame, Koichi, Ishizuka, Yuta, Ishida, Yasushi, Kageyama, Haruaki, Shioda, Seiji, Kangawa, Kenji, & Nakazato, Masamitsu. 2006. Peripheral ghrelin transmits orexigenic signals through the noradrenergic pathway from the hindbrain to the hypothalamus. *Cell metabolism*, **4**(4), 323–331.

de Oliveira, R B, Howlett, M C H, Gravina, F S, Imtiaz, M S, Callister, R J, Brichta, A M, & van Helden, D F. 2010. Pacemaker currents in mouse locus coeruleus neurons. *Neuroscience*, **170**(1), 166–177.

de Oliveira, Ramatis B, Gravina, Fernanda S, Lim, Rebecca, Brichta, Alan M, Callister, Robert J, & van Helden, Dirk F. 2011. Developmental changes in pacemaker currents in mouse locus coeruleus neurons. *Brain research*, **1425**(Nov.), 27–36.

de Oliveira, Ramatis B, Gravina, Fernanda S, Lim, Rebecca, Brichta, Alan M, Callister, Robert J, & van Helden, Dirk F. 2012. Heterogeneous responses to antioxidants in noradrenergic neurons of the Locus coeruleus indicate differing susceptibility to free radical content. *Oxidative medicine and cellular longevity*, **2012**, 820285.

Derosa, Giuseppe, & Maffioli, Pamela. 2012. Anti-obesity drugs: a review about their effects and their safety. *Expert opinion on drug safety*, **11**(3), 459–471.

Dhillon, Harveen, Zigman, Jeffrey M, Ye, Chianping, Lee, Charlotte E, McGovern, Robert A, Tang, Vinsee, Kenny, Christopher D, Christiansen, Lauryn M, White, Ryan D, Edelstein, Elisabeth A, Coppari, Roberto, Balthasar, Nina, Cowley, Michael A, Chua, Streamson, Elmquist, Joel K, & Lowell, Bradford B. 2006. Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron*, **49**(2), 191–203.

Di Dalmazi, Guido, Vicennati, Valentina, Pasquali, Renato, & Pagotto, Uberto. 2013. The unrelenting fall of the pharmacological treatment of obesity. *Endocrine*, May.

Diano, Sabrina, & Horvath, L. 2012 (Sept.). *Peroxisome proliferation-associated control of reactive oxygen species sets melanocortin tone and feeding in diet-induced obesity : Nature Medicine : Nature Publishing Group*.

Diano, Sabrina, Liu, Zhong-Wu, Jeong, Jin Kwon, Dietrich, Marcelo O, Ruan, Hai-Bin, Kim, Esther, Suyama, Shigetomo, Kelly, Kaitlin, Gyengesi, Erika, Arbiser, Jack L, Belsham, Denise D, Sarruf, David A, Schwartz, Michael W, Bennett, Anton M, Shanabrough, Marya, Mobbs, Charles V, Yang, Xiaoyong, Gao, Xiao-Bing, & Horvath, Tamas L. 2011. Peroxisome proliferation-associated control of reactive oxygen species sets melanocortin tone and feeding in diet-induced obesity. *Nature medicine*, **17**(9), 1121–1127.

Dicken, Matthew S, Tooker, Ryan E, & Hentges, Shane T. 2012. Regulation of GABA and glutamate release from proopiomelanocortin neuron terminals in intact hypothalamic networks. *The journal of neuroscience : the official journal of the society for neuroscience*, **32**(12), 4042–4048.

Dietrich, Marcelo O, & Horvath, Tamas L. 2013. Hypothalamic control of energy balance: insights into the role of synaptic plasticity. *Trends in neurosciences*, **36**(2), 65–73.

Diggs-Andrews, Kelly A, Zhang, Xuezhao, Song, Zhentao, Daphna-Iken, Dorit, Routh, Vanessa H, & Fisher, Simon J. 2010. Brain insulin action regulates hypothalamic glucose sensing and the counterregulatory response to hypoglycemia. *Diabetes*, **59**(9), 2271–2280.

Docherty, JR. 1998. Subtypes of functional alpha(1)- and alpha(2)-adrenoceptors. *European journal of pharmacology*, **361**(1), 1–15.

Dodt, H U, & Zieglgänsberger, W. 1990. Visualizing unstained neurons in living brain slices by infrared DIC-videomicroscopy. *Brain research*, **537**(1-2), 333–336.

Dodt, H U, Pawelzik, H, & Zieglgänsberger, W. 1991. Actions of noradrenaline on neocortical neurons in vitro. *Brain research*, **545**(1-2), 307–311.

Dube, Michael G, Kalra, Satya P, & Kalra, Pushpa S. 2006. The hypothalamic paraventricular nucleus is not essential for orexigenic NPY or anorexigenic melanocortin action. *Peptides*, **27**(9), 2239–2248.

Dunn-Meynell, A A, Rawson, N E, & Levin, B E. 1998. Distribution and phenotype of neurons containing the ATP-sensitive K+ channel in rat brain. *Brain research*, **814**(1-2), 41–54.

Dunn-Meynell, AA, Routh, VH, Kang, L, Gaspers, L, & Levin, BE. 2002. Glucokinase is the likely mediator of glucosensing in both glucose-excited and glucose-inhibited central neurons. *Diabetes*, **51**(7), 2056–2065.

Egawa, M, Yoshimatsu, H, & Bray, G A. 1989a. Effects of 2-deoxy-D-glucose on sympathetic nerve activity to interscapular brown adipose tissue. *The american journal of physiology*, **257**(6 Pt 2), R1377–85.

Egawa, M, Yoshimatsu, H, & Bray, G A. 1989b. Lateral hypothalamic injection of 2-deoxy-D-glucose suppresses sympathetic activity. *The american journal of physiology*, **257**(6 Pt 2), R1386–92.

Ellacott, Kate L J, & Cone, Roger D. 2004. The central melanocortin system and the integration of short- and long-term regulators of energy homeostasis. *Recent progress in hormone research*, **59**, 395–408.

Elmquist, J K, Ahima, R S, Maratos-Flier, E, Flier, J S, & Saper, C B. 1997. Leptin activates neurons in ventrobasal hypothalamus and brainstem. *Endocrinology*, **138**(2), 839–842.

Elmquist, J K, Elias, C F, & Saper, C B. 1999. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron*, **22**(2), 221–232.

Emanuel, Alan J, & Ritter, Sue. 2010. Hindbrain catecholamine neurons modulate the growth hormone but not the feeding response to ghrelin. *Endocrinology*, **151**(7), 3237–3246.

Ernst, Marianne B, Wunderlich, Claudia M, Hess, Simon, Paehler, Moritz, Mesaros, Andrea, Koralov, Sergei B, Kleinridders, André, Husch, Andreas, Münzberg, Heike, Hampel, Brigitte, Alber, Jens, Kloppenburg, Peter, Brüning, Jens C, & Wunderlich, F Thomas. 2009. Enhanced Stat3 activation in POMC neurons

provokes negative feedback inhibition of leptin and insulin signaling in obesity. *The journal of neuroscience : the official journal of the society for neuroscience*, **29**(37), 11582–11593.

Esler, M D, Hasking, G J, Willett, I R, Leonard, P W, & Jennings, G L. 1985. Noradrenaline release and sympathetic nervous system activity. *Journal of hypertension*, **3**(2), 117–129.

Falke, L C, Gillis, K D, Pressel, D M, & Misler, S. 1989. 'Perforated patch recording' allows long-term monitoring of metabolite-induced electrical activity and voltage-dependent Ca2+ currents in pancreatic islet B cells. *Febs letters*, **251**(1-2), 167–172.

Fantasia, Heidi Collins. 2013. New developments in the pharmacologic treatment of obesity. *Nursing for women's health*, **17**(1), 53–62.

Figlewicz, D P, Szot, P, Israel, P A, Payne, C, & Dorsa, D M. 1993. Insulin reduces norepinephrine transporter mRNA in vivo in rat locus coeruleus. *Brain research*, **602**(1), 161–164.

Finkelstein, Eric A, Trogdon, Justin G, Cohen, Joel W, & Dietz, William. 2009. Annual medical spending attributable to obesity: payer-and service-specific estimates. *Health affairs (project hope)*, **28**(5), w822–31.

Finta, E P, Harms, L, Sevcik, J, Fischer, H D, & Illes, P. 1993. Effects of potassium channel openers and their antagonists on rat locus coeruleus neurones. *British journal of pharmacology*, **109**(2), 308–315.

Fioramonti, Xavier, Lorsignol, Anne, Taupignon, Anne, & Penicaud, Luc. 2004. A new ATP-sensitive K+ channel-independent mechanism is involved in glucose-excited neurons of mouse arcuate nucleus. *Diabetes*, **53**(11), 2767–2775.

Fioramonti, Xavier, Contie, Sylvain, Song, Zhentao, Routh, Vanessa H, Lorsignol, Anne, & Penicaud, Luc. 2007. Characterization of glucosensing neuron subpopulations in the arcuate nucleus: integration in neuropeptide Y and pro-opio melanocortin networks? *Diabetes*, **56**(5), 1219–1227.

Fraley, GS, & Ritter, S. 2003. Immunolesion of norepinephrine and epinephrine afferents to medial hypothalamus alters basal and 2-deoxy-D-glucose-induced neuropeptide Y and agouti gene-related protein messenger ribonucleic acid expression in the arcuate nucleus. *Endocrinology*, **144**(1), 75–83.

Francis, J, MohanKumar, SMJ, & MohanKumar, PS. 2004. Leptin inhibits norepinephrine efflux from the hypothalamus in vitro: role of gamma aminobutyric acid. *Brain research*, **1021**(2), 286–291.

Fuxe, K. 1965. Evidence for the existence of monoamine neurons in the central nervous system. 3. the monoamine nerve terminal. *Zeitschrift für zellforschung und mikroskopische anatomie (vienna, austria : 1948)*, **65**(Feb.), 573–596.

Fuxe, Kjell, Borroto-Escuela, Dasiel O, Romero-Fernandez, Wilber, Zhang, Wei-Bo, & Agnati, Luigi F. 2013. Volume transmission and its different forms in the central nervous system. *Chinese journal of integrative medicine*, **19**(5), 323–329.

Ganong, W F. 2000. Circumventricular organs: definition and role in the regulation of endocrine and autonomic function. *Clinical and experimental pharmacology and physiology*, **27**(5-6), 422–427.

Gao, Qian, & Horvath, Tamas L. 2007. Neurobiology of feeding and energy expenditure. *Annual review of neuroscience*, **30**, 367–398.

Gao, Qian, & Horvath, Tamas L. 2008. Neuronal control of energy homeostasis. *Febs letters*, **582**(1), 132–141.

García-Sáinz, J A, Vázquez-Prado, J, & Villalobos-Molina, R. 1999. Alpha 1-adrenoceptors: subtypes, signaling, and roles in health and disease. *Archives of medical research*, **30**(6), 449–458.

García-Sáinz, J A, Vázquez-Prado, J, & del Carmen Medina, L. 2000. Alpha 1-adrenoceptors: function and phosphorylation. *European journal of pharmacology*, **389**(1), 1–12.

Ghamari-Langroudi, Masoud. 2012. Electrophysiological Analysis of Circuits Controlling Energy Homeostasis. *Molecular neurobiology*, Feb.

Ghamari-Langroudi, Masoud, Srisai, Dollada, & Cone, Roger D. 2011. Multinodal regulation of the arcuate/paraventricular nucleus circuit by leptin. *Proceedings of the national academy of sciences of the united states of america*, **108**(1), 355–360.

Goddard, Andrew W, Ball, Susan G, Martinez, James, Robinson, Michael J, Yang, Charles R, russell, James M, & Shekhar, Anantha. 2010. Current perspectives of the roles of the central norepinephrine system in anxiety and depression. *Depression and anxiety*, **27**(4), 339–350.

Goldman, C K, Marino, L, & Leibowitz, S F. 1985. Postsynaptic alpha 2-noradrenergic receptors mediate feeding induced by paraventricular nucleus injection of norepinephrine and clonidine. *European journal of pharmacology*, **115**(1), 11–19.

Gouni-Berthold, Ioanna, Brüning, Jens C, & Berthold, Heiner K. 2013. Novel approaches to the pharmacotherapy of obesity. *Current pharmaceutical design*, **19**(27), 4938–4952.

Grigg, J J, & Anderson, E G. 1989. Glucose and sulfonylureas modify different phases of the membrane potential change during hypoxia in rat hippocampal slices. *Brain research*, **489**(2), 302–310.

Grill, H J, & Hayes, M R. 2009. The nucleus tractus solitarius: a portal for visceral afferent signal processing, energy status assessment and integration of their combined

effects on food intake. *International journal of obesity and related metabolic disorders : journal of the international association for the study of obesity*, **33**(Apr.), S11–S15.

Grill, Harvey J, Schwartz, Michael W, Kaplan, Joel M, Foxhall, James S, Breininger, John, & Baskin, Denis G. 2002a. Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. *Endocrinology*, **143**(1), 239–246.

Grill, HJ, Schwartz, MW, Kaplan, JM, Foxhall, JS, Breininger, J, & Baskin, DG. 2002b. Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. *Endocrinology*, **143**(1), 239–246.

Grossman, S P. 1960. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of hypothalamus. *Science (new york, ny)*, **132**(3422), 301–302.

Grzanna, R, & Molliver, M E. 1980. The locus coeruleus in the rat: an immunohistochemical delineation. *Neuroscience*, **5**(1), 21–40.

Guffey, Catherine R, Fan, Daping, Singh, Udai P, & Murphy, E Angela. 2013. Linking obesity to colorectal cancer: recent insights into plausible biological mechanisms. *Current opinion in clinical nutrition and metabolic care*, **16**(5), 595–600.

Guiard, B P, El Mansari, M, & Blier, P. 2008a. Cross-Talk between Dopaminergic and Noradrenergic Systems in the Rat Ventral Tegmental Area, Locus Ceruleus, and Dorsal Hippocampus. *Molecular pharmacology*, **74**(5), 1463–1475.

Guiard, Bruno P, El Mansari, Mostafa, & Blier, Pierre. 2008b. Cross-Talk between Dopaminergic and Noradrenergic Systems in the Rat Ventral Tegmental Area, Locus Ceruleus, and Dorsal Hippocampus. *Molecular pharmacology*, **74**(5), 1463–1475.

Guy, J, & Pelletier, G. 1988. Neuronal interactions between neuropeptide-y (npy) and catecholaminergic systems in the rat arcuate nucleus as shown by dual immunocytochemistry. *Peptides*, **9**(3), 567–570.

Gyires, Klara, Zadori, Zoltan S, Torok, Tamas, & Matyus, Peter. 2009. alpha(2)-Adrenoceptor subtypes-mediated physiological, pharmacological actions. *Neurochemistry international*, **55**(7), 447–453.

Hagan, Scott, & Niswender, Kevin D. 2012. Neuroendocrine regulation of food intake. *Pediatric blood & cancer*, **58**(1), 149–153.

Hahn, T M, Breininger, J F, Baskin, D G, & Schwartz, M W. 1998. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nature neuroscience*, **1**(4), 271–272.

Hainer, V, Kabrnova, K, Aldhoon, B, Kunesova, M, & Wagenknecht, M. 2006a. Serotonin and Norepinephrine Reuptake Inhibition and Eating Behavior. *Molecular and functional diversity of ion channels and receptors*, **1083**(1), 252–269.

Hainer, Vojtech, Kabrnova, Karolina, Aldhoon, Bashar, Kunesova, Marie, & Wagenknecht, Martin. 2006b. Serotonin and norepinephrine reuptake inhibition and eating behavior. *Molecular and functional diversity of ion channels and receptors*, **1083**(Nov.), 252–269.

Han, Seong Kyu, Chong, Wonee, Li, Long Hua, Lee, In Se, Murase, Kazuyuki, & Ryu, Pan Dong. 2002. Noradrenaline excites and inhibits GABAergic transmission in parvocellular neurons of rat hypothalamic paraventricular nucleus. *Journal of neurophysiology*, **87**(5), 2287–2296.

Hany, Thomas F, Gharehpapagh, Esmaiel, Kamel, Ehab M, Buck, Alfred, Himms-Hagen, Jean, & von Schulthess, Gustav K. 2002. Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *European journal of nuclear medicine and molecular imaging*, **29**(10), 1393–1398.

Harfstrand, A. 1986. Intraventricular administration of neuropeptide Y (NPY) induces hypotension, bradycardia and bradypnoea in the awake unrestrained male rat. Counteraction by NPY-induced feeding behaviour. *Acta physiologica scandinavica*, **128**(1), 121–123.

Harfstrand, A. 1987. Brain neuropeptide Y mechanisms. Basic aspects and involvement in cardiovascular and neuroendocrine regulation. *Acta physiologica scandinavica. supplementum*, **565**, 1–83.

Harfstrand, A, FUXE, K, Agnati, L F, Eneroth, P, Zini, I, Zoli, M, Andersson, K, von Euler, G, Terenius, L, Mutt, V, & Goldstein, M. 1986. Studies on neuropeptide Y-catecholamine interactions in the hypothalamus and in the forebrain of the male rat. Relationship to neuroendocrine function. *Neurochemistry international*, **8**(3), 355–376.

Harfstrand, A, Eneroth, P, Agnati, L, & Fuxe, K. 1987. Further-studies on the effects of central administration of neuropeptide-y on neuroendocrine function in the male-rat - relationship to hypothalamic catecholamines. *Regulatory peptides*, **17**(3), 167–179.

Hay-Schmidt, A, Helboe, L, & Larsen, P J. 2001. Leptin receptor immunoreactivity is present in ascending serotonergic and catecholaminergic neurons of the rat. *Neuroendocrinology*, **73**(4), 215–226.

He, Wu, Lam, Tony K T, Obici, Silvana, & Rossetti, Luciano. 2006. Molecular disruption of hypothalamic nutrient sensing induces obesity. *Nature neuroscience*, **9**(2), 227–233.

Hein, Lutz. 2006. Adrenoceptors and signal transduction in neurons. *Cell and tissue research*, **326**(2), 541–551.

Hervey, G R. 1959. The effects of lesions in the hypothalamus in parabiotic rats. *The journal of physiology*, **145**(2), 336–352.

Hoebel, B G, Hernandez, L, Schwartz, D H, Mark, G P, & Hunter, G A. 1989. Microdialysis studies of brain norepinephrine, serotonin, and dopamine release during ingestive behavior. Theoretical and clinical implications. *Molecular and functional diversity of ion channels and receptors*, **575**, 171–91– discussion 192–3.

Hoffman, G E, Lee, W S, Smith, M S, Abbud, R, Roberts, M M, Robinson, A G, & Verbalis, J G. 1993. c-Fos and Fos-related antigens as markers for neuronal activity: perspectives from neuroendocrine systems. *Nida research monograph*, **125**, 117–133.

Holets, V R, Hokfelt, T, Rökaeus, Å, Terenius, L, & Goldstein, M. 1988. Locus coeruleus neurons in the rat containing neuropeptide Y, tyrosine hydroxylase or galanin and their efferent projections to the spinal cord, cerebral cortex and hypothalamus. *Neuroscience*, **24**(3), 893–906.

Holt, S J, & York, D A. 1989a. Interaction of intracerebroventricular insulin and glucose in the regulation of the activity of sympathetic efferent nerves to brown adipose tissue in lean and obese Zucker rats. *Brain research*, **500**(1-2), 384–388.

Holt, S J, & York, D A. 1989b. Studies on the sympathetic efferent nerves of brown adipose tissue of lean and obese Zucker rats. *Brain research*, **481**(1), 106–112.

Hopwood, S E, & Stamford, J A. 2001. Noradrenergic modulation of serotonin release in rat dorsal and median raphé nuclei via alpha(1) and alpha(2A) adrenoceptors. *Neuropharmacology*, **41**(4), 433–442.

Horn, R, & Marty, A. 1988. Muscarinic activation of ionic currents measured by a new whole-cell recording method. *The journal of general physiology*, **92**(2), 145–159.

Horvath, T L, Naftolin, F, Kalra, S P, & Leranth, C. 1992. Neuropeptide-Y innervation of beta-endorphin-containing cells in the rat mediobasal hypothalamus: a light and electron microscopic double immunostaining analysis. *Endocrinology*, **131**(5), 2461–2467.

Horvath, T L, Warden, C H, Hajos, M, Lombardi, A, Goglia, F, & Diano, S. 1999. Brain uncoupling protein 2: uncoupled neuronal mitochondria predict thermal synapses in homeostatic centers. *The journal of neuroscience : the official journal of the society for neuroscience*, **19**(23), 10417–10427.

Horvath, Tamas L, Andrews, Zane B, & Diano, Sabrina. 2009. Fuel utilization by hypothalamic neurons: roles for ROS. *Trends in endocrinology and metabolism: Tem*, **20**(2), 78–87.

Hudson, Bryan, & Ritter, Sue. 2004. Hindbrain catecholamine neurons mediate consummatory responses to glucoprivation. *Physiology & behavior*, **82**(2-3), 241–250.

Hyman, S E, Kosofsky, B E, Nguyen, T V, Cohen, B M, & Comb, M J. 1993. Everything activates c-fos–how can it matter? *Nida research monograph*, **125**, 25–38.

Ibrahim, Nurhadi, Bosch, Martha A, Smart, James L, Qiu, Jian, Rubinstein, Marcelo, Rønnekleiv, Oline K, Low, Malcolm J, & Kelly, Martin J. 2003. Hypothalamic proopiomelanocortin neurons are glucose responsive and express K(ATP) channels. *Endocrinology*, **144**(4), 1331–1340.

Illes, Peter, Sevcik, Jan, Finta, Ervin P, Fröhlich, Rainer, Nieber, Karen, & Nörenberg, Wolfgang. 1994. Modulation of locus coeruleus neurons by extra- and intracellular adenosine 5'-triphosphate. *Brain research bulletin*, **35**(5-6), 513–519.

Inenaga, K, Dyball, R E, Okuya, S, & Yamashita, H. 1986. Characterization of hypothalamic noradrenaline receptors in the supraoptic nucleus and periventricular region of the paraventricular nucleus of mice in vitro. *Brain research*, **369**(1-2), 37–47.

Insel, P. 1989. Structure and Function of Alpha-Adrenergic Receptors. *The american journal of medicine*, **87**(2), S12–S18.

Ioannides-Demos, Lisa L, Proietto, Joseph, Tonkin, Andrew M, & McNeil, John J. 2006. Safety of drug therapies used for weight loss and treatment of obesity. *Drug safety : an international journal of medical toxicology and drug experience*, **29**(4), 277–302.

James, W Philip T, Caterson, Ian D, Coutinho, Walmir, Finer, Nick, Van Gaal, Luc F, Maggioni, Aldo P, Torp-Pedersen, Christian, Sharma, Arya M, Shepherd, Gillian M, Rode, Richard A, & Renz, Cheryl L. 2010. Effect of Sibutramine on Cardiovascular Outcomes in Overweight and Obese Subjects. *The new england journal of medicine*, **363**(10), 905–917.

Jansen, A S, Nguyen, X V, Karpitskiy, V, Mettenleiter, T C, & Loewy, A D. 1995. Central command neurons of the sympathetic nervous system: basis of the fight-or-flight response. *Science (new york, ny)*, **270**(5236), 644–646.

Jin, Xin, Cui, Ningren, Zhong, Weiwei, Jin, Xiao-Tao, & Jiang, Chun. 2013. GABAergic synaptic inputs of locus coeruleus neurons in wild-type and Mecp2-null mice. *American journal of physiology-cell physiology*, Feb.

Jones, J R, & Caul, W F. 1992. Effects of amphetamine on food intake and weight: timing of injections and food access. *Physiology & behavior*, **52**(3), 515–520.

Jordan, Sabine D, Könner, A Christine, & Brüning, Jens C. 2010. Sensing the fuels: glucose and lipid signaling in the CNS controlling energy homeostasis. *Cellular and molecular life sciences : Cmls*, **67**(19), 3255–3273.

Jurgens, Chris W D, Hammad, Hana M, Lichter, Jessica A, Boese, Sarah J, Nelson, Brian W, Goldenstein, Brianna L, Davis, Kylie L, Xu, Ke, Hillman, Kristin L, Porter, James E, & Doze, Van A. 2007. alpha(2A) adrenergic receptor activation inhibits epileptiform activity in the rat hippocampal CA3 region. *Molecular pharmacology*, **71**(6), 1572–1581.

Kang, Y M, Ouyang, W, Chen, J Y, Qiao, J T, & Dafny, N. 2000. Norepinephrine modulates single hypothalamic arcuate neurons via alpha(1)and beta adrenergic receptors. *Brain research*, **869**(1-2), 146–157.

Kataoka, K, Sorimachi, M, Okuno, S, & Mizuno, N. 1975. Innervation of hypothalamic and limbic areas by the cholinergic, the GABA-ergic and the catecholaminergic nerve fibers; a quantitative analysis. *Pharmacology biochemistry and behavior*, **3**(1 Suppl), 61–73.

Kawakami, Akio, Kawakami, Akio, Okada, Nobukazu, Rokkaku, Kumiko, Honda, Kazufumi, Ishibashi, Shun, & Onaka, Tatsushi. 2008. Leptin inhibits and ghrelin augments hypothalamic noradrenaline release after stress. *Stress: The international journal on the biology of stress*, **11**(5), 363–369.

Kennedy, G C. 1950. The Hypothalamic Control of Food Intake in Rats. *Proceedings of the royal society b: Biological sciences*, **137**(889), 535–549.

Kim, Jae Geun, & Horvath, Tamas L. 2012. mTOR Signaling Fades POMC Neurons during Aging. *Neuron*, **75**(3), 356–357.

Kishi, Toshiro, Aschkenasi, Carl J, Lee, Charlotte E, Mountjoy, Kathleen G, Saper, Clifford B, & Elmquist, Joel K. 2003. Expression of melanocortin 4 receptor mRNA in the central nervous system of the rat. *The journal of comparative neurology*, **457**(3), 213–235.

Kiyohara, T, Miyata, S, Nakamura, T, Shido, O, Nakashima, T, & Shibata, M. 1995. Differences in Fos expression in the rat brains between cold and warm ambient exposures. *Brain research bulletin*, **38**(2), 193–201.

Klöckener, Tim, Hess, Simon, Belgardt, Bengt F, Paeger, Lars, Verhagen, Linda A W, Husch, Andreas, Sohn, Jong-Woo, Hampel, Brigitte, Dhillon, Harveen, Zigman, Jeffrey M, Lowell, Bradford B, Williams, Kevin W, Elmquist, Joel K, Horvath, Tamas L, Kloppenburg, Peter, & Brüning, Jens C. 2011. High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. *Nature neuroscience*, **14**(7), 911–918.

Kloppenburg, Peter, Zipfel, Warren R, Webb, Watt W, & Harris-Warrick, Ronald M. 2007. Heterogeneous effects of dopamine on highly localized, voltage-induced Ca2+ accumulation in identified motoneurons. *Journal of neurophysiology*, **98**(5), 2910–2917.

Könner, A Christine, Hess, Simon, Tovar, Sulay, Mesaros, Andrea, Sánchez-Lasheras, Carmen, Evers, Nadine, Verhagen, Linda A W, Brönneke, Hella S, Kleinridders, André, Hampel, Brigitte, Kloppenburg, Peter, & Brüning, Jens C. 2011. Role for insulin signaling in catecholaminergic neurons in control of energy homeostasis. *Cell metabolism*, **13**(6), 720–728.

Kow, L M, & Pfaff, D W. 1989. Responses of hypothalamic paraventricular neurons in vitro to norepinephrine and other feeding-relevant agents. *Physiology & behavior*, **46**(2), 265–271.

Koyama, S, Jin, Y H, & Akaike, N. 1999. ATP-sensitive and Ca2+-activated K+ channel activities in the rat locus coeruleus neurons during metabolic inhibition. *Brain research*, **828**(1-2), 189–192.

Kraszewski, KZ, & Cincotta, AH. 2000. Increased responsiveness of ventromedial hypothalamic neurons to norepinephrine in obese versus lean mice: Relation to the metabolic syndrome. *International journal of molecular medicine*, **5**(4), 349–355.

Kuwahata, Takashi. 2004. Effects of adenosine and ATP on the membrane potential and synaptic transmission in neurons of the rat locus coeruleus. *The kurume medical journal*, **51**(2), 109–123.

Kvetnansky, Richard, Sabban, Esther L, & Palkovits, Miklos. 2009. Catecholaminergic Systems in Stress: Structural and Molecular Genetic Approaches. *Physiological reviews*, **89**(2), 535–606.

Kyrozis, A, & Reichling, D B. 1995. Perforated-patch recording with gramicidin avoids artifactual changes in intracellular chloride concentration. *Journal of neuroscience methods*, **57**(1), 27–35.

Langer, S Z. 1974. Presynaptic regulation of catecholamine release. *Biochemical pharmacology*, **23**(13), 1793–1800.

Lee, K, Dixon, A K, Richardson, P J, & Pinnock, R D. 1999. Glucose-receptive neurones in the rat ventromedial hypothalamus express KATP channels composed of Kir6.1 and SUR1 subunits. *The journal of physiology*, **515 (Pt 2)**(Mar.), 439–452.

Lehrer, Steven, Green, Sheryl, Ramanathan, Lakshmi, & Rosenzweig, Kenneth E. 2013. Obesity and deranged sleep are independently associated with increased cancer mortality in 50 US states and the District of Columbia. *Sleep & breathing = schlaf & atmung*, **17**(3), 1117–1118.

Leibowitz, S F. 1988. Hypothalamic paraventricular nucleus: interaction between alpha 2-noradrenergic system and circulating hormones and nutrients in relation to energy balance. *Neuroscience and biobehavioral reviews*, **12**(2), 101–109.

Leibowitz, S F, Jhanwar-Uniyal, M, DVORKIN, B, & Makman, M H. 1982. Distribution of alpha-adrenergic, beta-adrenergic and dopaminergic receptors in discrete hypothalamic areas of rat. *Brain research*, **233**(1), 97–114.

Leibowitz, S F, Sladek, C, Spencer, L, & Tempel, D. 1988. Neuropeptide Y, epinephrine and norepinephrine in the paraventricular nucleus: stimulation of feeding and the release of corticosterone, vasopressin and glucose. *Brain research bulletin*, **21**(6), 905–912.

Leibowitz, SF. 1978a. Hypothalamic catecholamines, hormones and feeding-behavior. *International journal of obesity*, **2**(3), 363–364.

Leibowitz, SF. 1978b. Paraventricular nucleus - primary site mediating adrenergic-stimulation of feeding and drinking. *Pharmacology biochemistry and behavior*, **8**(2), 163–175.

Levin, B E. 2001. Glucosensing neurons do more than just sense glucose. *International journal of obesity and related metabolic disorders : journal of the international association for the study of obesity*, **25 Suppl 5**(Dec.), S68–72.

Levin, B E, Dunn-Meynell, A A, & Routh, V H. 1999. Brain glucose sensing and body energy homeostasis: role in obesity and diabetes. *The american journal of physiology*, **276**(5 Pt 2), R1223–31.

Levin, Barry E, Dunn-Meynell, Ambrose A, & Routh, Vanessa H. 2002. CNS sensing and regulation of peripheral glucose levels. *Pages 219–258 of: International review of neurobiology*. Elsevier.

Levin, BE, Israel, P, & Lattemann, DPF. 1998. Insulin selectively downregulates alpha(2)-adrenoceptors in the arcuate and dorsomedial nucleus. *Brain research bulletin*, **45**(2), 179–181.

Lewis, D V, Huguenard, J R, Anderson, W W, & Wilson, W A. 1986. Membrane currents underlying bursting pacemaker activity and spike frequency adaptation in invertebrates. *Advances in neurology*, **44**, 235–261.

Li, Ai-Jun, Wang, Qing, Dinh, Thu T, & Ritter, Sue. 2009. Simultaneous silencing of Npy and Dbh expression in hindbrain A1/C1 catecholamine cells suppresses glucoprivic feeding. *The journal of neuroscience : the official journal of the society for neuroscience*, **29**(1), 280–287.

Li, Mingfang, & Cheung, Bernard M Y. 2009. Pharmacotherapy for obesity. *British journal of clinical pharmacology*, **68**(6), 804–810.

Li, Ying, & van den Pol, Anthony N. 2005. Direct and indirect inhibition by catecholamines of hypocretin/orexin neurons. *The journal of neuroscience : the official journal of the society for neuroscience*, **25**(1), 173–183.

Limbird, L E. 1988. Receptors linked to inhibition of adenylate cyclase: additional signaling mechanisms. *Faseb journal : official publication of the federation of american societies for experimental biology*, **2**(11), 2686–2695.

Lindau, M, & Fernandez, J M. 1986. A patch-clamp study of histamine-secreting cells. *The journal of general physiology*, **88**(3), 349–368.

Liu, Y H, & Wang, X J. 2001. Spike-frequency adaptation of a generalized leaky integrate-and-fire model neuron. *Journal of computational neuroscience*, **10**(1), 25–45.

Loughlin, SE, Foote, SL, & Bloom, FE. 1986. Efferent projections of nucleus locus-coeruleus - topographic organization of cells of origin demonstrated by 3-dimensional reconstruction. *Neuroscience*, **18**(2), 291–306.

Luchsinger, José A. 2010. Diabetes, related conditions, and dementia. *Journal of the neurological sciences*, **299**(1-2), 35–38.

Lüscher, Christian, & Slesinger, Paul A. 2010. Emerging roles for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease. *Nature reviews neuroscience*, **11**(5), 301–315.

Lynch, R M, Tompkins, L S, Brooks, H L, Dunn-Meynell, A A, & Levin, B E. 2000. Localization of glucokinase gene expression in the rat brain. *Diabetes*, **49**(5), 693–700.

Macrez-Leprêtre, N, Kalkbrenner, F, Schultz, G, & Mironneau, J. 1997. Distinct functions of Gq and G11 proteins in coupling alpha1-adrenoreceptors to Ca2+ release and Ca2+ entry in rat portal vein myocytes. *The journal of biological chemistry*, **272**(8), 5261–5268.

Madden, C J. 2012. Glucoprivation in the ventrolateral medulla decreases brown adipose tissue sympathetic nerve activity by decreasing the activity of neurons in raphe pallidus. *American journal of physiology-regulatory integrative and comparative physiology*, **302**(2), R224–32.

Martin, G E, & Myers, R D. 1975. Evoked release of [14C]norepinephrine from the rat hypothalamus during feeding. *The american journal of physiology*, **229**(6), 1547–1555.

Marty, Nell, Dallaporta, Michel, & Thorens, Bernard. 2007. Brain glucose sensing, counterregulation, and energy homeostasis. *Physiology (bethesda, md.)*, **22**(Aug.), 241–251.

Mayer, J. 1953. Glucostatic mechanism of regulation of food intake. *The new england journal of medicine*, **249**(1), 13–16.

Mayer, J, & Thomas, D W. 1967. Regulation of food intake and obesity. *Science (new york, ny)*, **156**(3773), 328–337.

Míguez, J M, Aldegunde, M, Paz-Valiñas, L, Recio, J, & Sánchez-Barceló, E. 1999. Selective changes in the contents of noradrenaline, dopamine and serotonin in rat brain areas during aging. *Journal of neural transmission (vienna, austria : 1996)*, **106**(11-12), 1089–1098.

Miki, T, Liss, B, Minami, K, Shiuchi, T, Saraya, A, Kashima, Y, Horiuchi, M, Ashcroft, F, Minokoshi, Y, Roeper, J, & Seino, S. 2001. ATP-sensitive K+ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. *Nature neuroscience*, **4**(5), 507–512.

Miles, G B, Dai, Y, & Brownstone, R M. 2005. Mechanisms underlying the early phase of spike frequency adaptation in mouse spinal motoneurones. *The journal of physiology*, **566**(2), 519–532.

Milusheva, E, Sperlágh, B, Shikova, L, Baranyi, M, Tretter, L, Adám-Vizi, V, & Vizi, E S. 2003. Non-synaptic release of [3H]noradrenaline in response to oxidative stress combined with mitochondrial dysfunction in rat hippocampal slices. *Neuroscience*, **120**(3), 771–781.

Mineur, Yann S, Abizaid, Alfonso, Rao, Yan, Salas, Ramiro, DiLeone, Ralph J, Gündisch, Daniela, Diano, Sabrina, De Biasi, Mariella, Horvath, Tamas L, Gao, Xiao-Bing, & Picciotto, Marina R. 2011. Nicotine decreases food intake through activation of POMC neurons. *Science (new york, ny)*, **332**(6035), 1330–1332.

Minokoshi, Y, Saito, M, & Shimazu, T. 1986. Metabolic and morphological alterations of brown adipose tissue after sympathetic denervation in rats. *Journal of the autonomic nervous system*, **15**(3), 197–204.

Mitchell, S N, Smith, K M, Joseph, M H, & Gray, J A. 1993. Increases in tyrosine hydroxylase messenger RNA in the locus coeruleus after a single dose of nicotine are followed by time-dependent increases in enzyme activity and noradrenaline release. *Neuroscience*, **56**(4), 989–997.

Miyata, S, Ishiyama, M, Shido, O, Nakashima, T, Shibata, M, & Kiyohara, T. 1995. Central mechanism of neural activation with cold acclimation of rats using Fos immunohistochemistry. *Neuroscience research*, **22**(2), 209–218.

Mizuno, Y, & Oomura, Y. 1984. Glucose responding neurons in the nucleus tractus solitarius of the rat: in vitro study. *Brain research*, **307**(1-2), 109–116.

Moore, R Y, & Bloom, F E. 1979. Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annual review of neuroscience*, **2**, 113–168.

Morien, A, MCMAHON, L, & Wellman, P J. 1993. Effects on food and water intake of the alpha 1-adrenoceptor agonists amidephrine and SK&F-89748. *Life sciences*, **53**(2), 169–174.

Morien, A, Wellman, P J, & Fojt, J. 1995. Diurnal rhythms of paraventricular hypothalamic norepinephrine and food intake in rats. *Pharmacology biochemistry and behavior*, **52**(1), 169–174.

Morton, G J, Cummings, D E, Baskin, D G, Barsh, G S, & Schwartz, M W. 2006. Central nervous system control of food intake and body weight. *Nature*, **443**(7109), 289–295.

Mounien, Lourdes, Marty, Nell, Tarussio, David, Metref, Salima, Genoux, David, Preitner, Frederic, Foretz, Marc, & Thorens, Bernard. 2010. Glut2-dependent

glucose-sensing controls thermoregulation by enhancing the leptin sensitivity of NPY and POMC neurons. *Faseb journal*, **24**(6), 1747–1758.

Mountjoy, K G, Mortrud, M T, Low, M J, Simerly, R B, & Cone, R D. 1994. Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Molecular endocrinology*, **8**(10), 1298–1308.

Murai, Y, Ishibashi, H, Koyama, S, & Akaike, N. 1997a. Ca2+-activated K+ currents in rat locus coeruleus neurons induced by experimental ischemia, anoxia, and hypoglycemia. *Journal of neurophysiology*, **78**(5), 2674–2681.

Murai, Y, Ishibashi, H, Koyama, S, & Akaike, N. 1997b. Ca2+-activated K+ currents in rat locus coeruleus neurons induced by experimental ischemia, anoxia, and hypoglycemia. *Journal of neurophysiology*, **78**(5), 2674–2681.

Must, A, Spadano, J, Coakley, E H, Field, A E, Colditz, G, & Dietz, W H. 1999. The disease burden associated with overweight and obesity. *Jama : the journal of the american medical association*, **282**(16), 1523–1529.

Myers, R D, & McCaleb, M L. 1980. Feeding: satiety signal from intestine triggers brain's noradrenergic mechanism. *Science (new york, ny)*, **209**(4460), 1035–1037.

Myers, R D, Lankford, M F, & Roscoe, A K. 1996. Neuropeptide Y perfused in the preoptic area of rats shifts extracellular efflux of dopamine, norepinephrine, and serotonin during hypothermia and feeding. *Neurochemical research*, **21**(6), 637–648.

Nedergaard, JAN, Bengtsson, Tore, & CANNON, BARBARA. 2011. New powers of brown fat: fighting the metabolic syndrome. *Cell metabolism*, **13**(3), 238–240.

Newton, A Jamila, Hess, Simon, Paeger, Lars, Vogt, Merly C, Fleming Lascano, Jenifer, Nillni, Eduardo A, Brüning, Jens C, Kloppenburg, Peter, & Xu, Allison W. 2013. AgRP innervation onto POMC neurons increases with age and is accelerated with chronic high-fat feeding in male mice. *Endocrinology*, **154**(1), 172–183.

Nicholas, AP, Hokfelt, T, & Pieribone, VA. 1996. The distribution and significance of CNS adrenoceptors examined with in situ hybridization. *Trends in pharmacological sciences*, **17**(7), 245–255.

Nieber, K, Sevcik, J, & Illes, P. 1995. Hypoxic changes in rat locus coeruleus neurons in vitro. *The journal of physiology*, **486 (Pt 1)**(July), 33–46.

Niimi, Keita, Horie, Shinichiro, Yokosuka, Makoto, Kawakami-Mori, Fumiko, Tanaka, Koichi, Fukayama, Haruhisa, & Sahara, Yoshinori. 2012. Heterogeneous electrophysiological and morphological properties of neurons in the mouse medial amygdala in vitro. *Brain research*, **1480**(Oct.), 41–52.

Oldfield, B J, Giles, M E, Watson, A, Anderson, C, Colvill, L M, & McKinley, M J. 2002. The neurochemical characterisation of hypothalamic pathways projecting polysynaptically to brown adipose tissue in the rat. *Neuroscience*, **110**(3), 515–526.

Oleson, DorothyR, DeFelice, LouisJ, & Donahoe, RobertM. 1993. A comparison of K+ channel characteristics in human T cells: Perforated-patch versus whole-cell recording techniques. *The journal of membrane biology*, **132**(3), 229–241.

Ollmann, M M, Wilson, B D, Yang, Y K, Kerns, J A, Chen, Y, Gantz, I, & Barsh, G S. 1997. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science (new york, ny)*, **278**(5335), 135–138.

Oltmans, G A. 1983. Norepinephrine and dopamine levels in hypothalamic nuclei of the genetically obese mouse (ob/ob). *Brain research*, **273**(2), 369–373.

Oomura, Y, Ooyama, H, Sugimori, M, Nakamura, T, & Yamada, Y. 1974. Glucose inhibition of the glucose-sensitive neurone in the rat lateral hypothalamus. *Nature*, **247**(439), 284–286.

O'Rahilly, Stephen. 2009. Human genetics illuminates the paths to metabolic disease. *Nature*, **462**(7271), 307–314.

Osmond, Jessica M, Mintz, James D, Dalton, Brian, & Stepp, David W. 2009. Obesity increases blood pressure, cerebral vascular remodeling, and severity of stroke in the Zucker rat. *Hypertension*, **53**(2), 381–386.

Owesson, Catarina A, Seif, Isabelle, McLaughlin, Daniel P, & Stamford, Jonathan A. 2003. Different alpha(2) adrenoceptor subtypes control noradrenaline release and cell firing in the locus coeruleus of wildtype and monoamine oxidase-A knockout mice. *The european journal of neuroscience*, **18**(1), 34–42.

Pan, Z Z, Grudt, T J, & Williams, J T. 1994. Alpha 1-adrenoceptors in rat dorsal raphe neurons: regulation of two potassium conductances. *The journal of physiology*, **478 Pt 3**(Aug.), 437–447.

Pandit, Rahul, de Jong, Johannes W, Vanderschuren, Louk J M J, & Adan, Roger A H. 2011. Neurobiology of overeating and obesity: the role of melanocortins and beyond. *European journal of pharmacology*, **660**(1), 28–42.

Parton, Laura E, Ye, Chian Ping, Coppari, Roberto, Enriori, Pablo J, Choi, Brian, Zhang, Chen-yu, Xu, Chun, Vianna, Claudia R, Balthasar, Nina, Lee, Charlotte E, Elmquist, Joel K, Cowley, Michael A, & Lowell, Bradford B. 2007. Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. *Nature*, **449**(7159), 228–232.

Pauwels, P J, & Colpaert, F C. 2000. Heterogeneous ligand-mediated Ca(++) responses at wt and mutant alpha(2A)-adrenoceptors suggest multiple ligand activation binding sites at the alpha(2A)-adrenoceptor. *Neuropharmacology*, **39**(11), 2101–2111.

Peron, Simon Peter, & Gabbiani, Fabrizio. 2009. Role of spike-frequency adaptation in shaping neuronal response to dynamic stimuli. *Biological cybernetics*, **100**(6), 505–520.

Philipp, Melanie, & Hein, Lutz. 2004. Adrenergic receptor knockout mice: distinct functions of 9 receptor subtypes. *Pharmacology & therapeutics*, **101**(1), 65–74.

Pinto, Shirly, Roseberry, Aaron G, Liu, Hongyan, Diano, Sabrina, Shanabrough, Marya, Cai, Xiaoli, Friedman, Jeffrey M, & Horvath, Tamas L. 2004. Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science (new york, ny)*, **304**(5667), 110–115.

Plum, Leona, Ma, Xiaosong, Hampel, Brigitte, Balthasar, Nina, Coppari, Roberto, Münzberg, Heike, Shanabrough, Marya, Burdakov, Denis, Rother, Eva, Janoschek, Ruth, Alber, Jens, Belgardt, Bengt F, Koch, Linda, Seibler, Jost, Schwenk, Frieder, Fekete, Csaba, Suzuki, Akira, Mak, Tak W, Krone, Wilhelm, Horvath, Tamas L, Ashcroft, Frances M, & Brüning, Jens C. 2006. Enhanced PIP3 signaling in POMC neurons causes KATP channel activation and leads to diet-sensitive obesity. *Journal of clinical investigation*, **116**(7), 1886–1901.

Plum, Leona, Rother, Eva, Muenzberg, Heike, Wunderlich, F Thomas, Morgan, Donald A, Hampel, Brigitte, Shanabrough, Marya, Janoschek, Ruth, Koenner, A Christine, Alber, Jens, Suzuki, Akira, Krone, Wilhelm, Horvath, Tamas L, Rahmouni, Kamal, & Bruening, Jens C. 2007. Enhanced leptin-stimulated Pi3k activation in the CNS promotes white adipose tissue transdifferentiation. *Cell metabolism*, **6**(6), 431–445.

Powell, A G, Apovian, C M, & Aronne, L J. 2011. New drug targets for the treatment of obesity. *Clinical pharmacology and therapeutics*, **90**(1), 40–51.

Power, Michael L. 2012. The human obesity epidemic, the mismatch paradigm, and our modern "captive" environment. *American journal of human biology : the official journal of the human biology council*, **24**(2), 116–122.

Powers, R K, Sawczuk, A, Musick, J R, & Binder, M D. 1999. Multiple mechanisms of spike-frequency adaptation in motoneurones. *Journal of physiology, paris*, **93**(1-2), 101–114.

Rae, J, Cooper, K, Gates, P, & Watsky, M. 1991. Low access resistance perforated patch recordings using amphotericin B. *Journal of neuroscience methods*, **37**(1), 15–26.

Ramos, Brian P, & Arnsten, Amy F T. 2007. Adrenergic pharmacology and cognition: focus on the prefrontal cortex. *Pharmacology & therapeutics*, **113**(3), 523–536.

Redmond, D E, Huang, Y H, Snyder, D R, Maas, J W, & Baulu, J. 1977. Hyperphagia and hyperdipsia after locus coeruleus lesions in the stumptailed monkey. *Life sciences*, **20**(9), 1619–1628.

Richard, D, Rivest, R, Huang, Q, Bouillaud, F, Sanchis, D, Champigny, O, & Ricquier, D. 1998. Distribution of the uncoupling protein 2 mRNA in the mouse brain. *The journal of comparative neurology*, **397**(4), 549–560.

Richard, F, Faucon-Biguet, N, Labatut, R, Rollet, D, Mallet, J, & Buda, M. 1988. Modulation of tyrosine hydroxylase gene expression in rat brain and adrenals by exposure to cold. *Journal of neuroscience research*, **20**(1), 32–37.

Richter, Hardy, Teixeira, Filipe M, Ferreira, Samira G, Kittel, Agnes, Köfalvi, Attila, & Sperlágh, Beáta. 2012. Presynaptic α(2)-adrenoceptors control the inhibitory action of presynaptic CB(1) cannabinoid receptors on prefrontocortical norepinephrine release in the rat. *Neuropharmacology*, **63**(5), 784–797.

Rinaman, Linda. 2011. Hindbrain noradrenergic A2 neurons: diverse roles in autonomic, endocrine, cognitive, and behavioral functions. *American journal of physiology-regulatory integrative and comparative physiology*, **300**(2), R222–35.

Ritter, S, Llewellyn-Smith, I, & Dinh, TT. 1998. Subgroups of hindbrain catecholamine neurons are selectively activated by 2-deoxy-D-glucose induced metabolic challenge. *Brain research*, **805**(1-2), 41–54.

Ritter, S, Dinh, TT, & Zhang, YB. 2000. Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain research*, **856**(1-2), 37–47.

Ritter, S, Bugarith, K, & Dinh, TT. 2001. Immunotoxic destruction of distinct catecholamine subgroups produces selective impairment of glucoregulatory responses and neuronal activation. *The journal of comparative neurology*, **432**(2), 197–216.

Ritter, Sue, Dinh, Thu T, & Li, Ai-Jun. 2006. Hindbrain catecholamine neurons control multiple glucoregulatory responses. *Physiology & behavior*, **89**(4), 490–500.

Ritter, Sue, Li, Ai-Jun, Wang, Qing, & Dinh, Thu T. 2011. Minireview: the value of looking backward: the essential role of the hindbrain in counterregulatory responses to glucose deficit. *Endocrinology*, **152**(11), 4019–4032.

Rivera, A, Agnati, L F, Horvath, T L, Valderrama, J J, de La Calle, A, & Fuxe, K. 2006. Uncoupling protein 2/3 immunoreactivity and the ascending dopaminergic and noradrenergic neuronal systems: relevance for volume transmission. *Neuroscience*, **137**(4), 1447–1461.

Robertson, Sabrina D, Plummer, Nicholas W, de Marchena, Jacqueline, & Jensen, Patricia. 2013. Developmental origins of central norepinephrine neuron diversity. *Nature neuroscience*, July, 1–10.

Roepke, T A, Smith, A W, Rønnekleiv, O K, & Kelly, M J. 2012. Serotonin 5-HT2C receptor-mediated inhibition of the M-current in hypothalamic POMC neurons. *American journal of physiology endocrinology and metabolism*, **302**(11), E1399–406.

Rogawski, M A, & Aghajanian, G K. 1982. Activation of lateral geniculate neurons by locus coeruleus or dorsal noradrenergic bundle stimulation: selective blockade by the alpha 1-adrenoceptor antagonist prazosin. *Brain research*, **250**(1), 31–39.

Röhl, Mathias, Pasparakis, Manolis, Baudler, Stephanie, Baumgartl, Julia, Gautam, Dinesh, Huth, Marion, De Lorenzi, Rossana, Krone, Wilhelm, Rajewsky, Klaus, & Brüning, Jens C. 2004. Conditional disruption of IkappaB kinase 2 fails to prevent obesity-induced insulin resistance. *Journal of clinical investigation*, **113**(3), 474–481.

Rommelfanger, K S, & Weinshenker, D. 2007. Norepinephrine: The redheaded stepchild of Parkinson's disease. *Biochemical pharmacology*, **74**(2), 177–190.

Roseberry, Aaron G, Liu, Hongyan, Jackson, Alexander C, Cai, Xiaoli, & Friedman, Jeffrey M. 2004. Neuropeptide Y-mediated inhibition of proopiomelanocortin neurons in the arcuate nucleus shows enhanced desensitization in ob/ob mice. *Neuron*, **41**(5), 711–722.

Rosenbaum, Daniel M, Rasmussen, Søren G F, & Kobilka, Brian K. 2009. The structure and function of G-protein-coupled receptors. *Nature*, **459**(7245), 356–363.

Rosenbaum, M, Leibel, R L, & Hirsch, J. 1997. Obesity. *The new england journal of medicine*, **337**(6), 396–407.

Rosmond, Roland. 2004. Obesity and depression: same disease, different names? *Medical hypotheses*, **62**(6), 976–979.

Rossi, J, Zolovick, A J, Davies, R F, & Panksepp, J. 1982. The role of norepinephrine in feeding behavior. *Neuroscience and biobehavioral reviews*, **6**(2), 195–204.

Routh, VH. 2002. Glucose-sensing neurons: Are they physiologically relevant? *Physiology & behavior*, **76**(3), 403–413.

Ruffolo, RR. 1985. Pharmacology of adrenoceptors. *Trends in pharmacological sciences*, **6**(1), 5–8.

Ruffolo, RR, & Hieble, JP. 1994. Alpha-adrenoceptors. *Pharmacology & therapeutics*, **61**(1-2), 1–64.

Ruffolo, RR, Nichols, AJ, Stadel, JM, & Hieble, JP. 1991. Structure and function of alpha-adrenoceptors. *Pharmacological reviews*, **43**(4), 475–505.

Rusnák, M, Jeloková, J, Vietor, I, Sabban, E L, & Kvetnanský, R. 1998. Different effects of insulin and 2-deoxy-D-glucose administration on tyrosine hydroxylase gene expression in the locus coeruleus and the adrenal medulla in rats. *Brain research bulletin*, **46**(5), 447–452.

Ryan, Donna H, & Bray, George A. 2013. Pharmacologic treatment options for obesity: what is old is new again. *Current hypertension reports*, **15**(3), 182–189.

Sah, P, & Davies, P. 2000. Calcium-activated potassium currents in mammalian neurons. *Clinical and experimental pharmacology and physiology*, **27**(9), 657–663.

Sahm, U G, Qarawi, M A, Olivier, G W, Ahmed, A R, Branch, S K, Moss, S H, & Pouton, C W. 1994. The melanocortin (MC3) receptor from rat hypothalamus: photoaffinity labelling and binding of alanine-substituted alpha-MSH analogues. *Febs letters*, **350**(1), 29–32.

Samuels, E R, & Szabadi, E. 2008a. Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part I: principles of functional organisation. *Current neuropharmacology*, **6**(3), 235–253.

Samuels, E R, & Szabadi, E. 2008b. Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part II: physiological and pharmacological manipulations and pathological alterations of locus coeruleus activity in humans. *Current neuropharmacology*, **6**(3), 254–285.

Sara, S J. 1988. Glucose effects on firing rate of neurons of the locus coeruleus: another attempt to put memory back in the brain. *Neurobiology of aging*, **9**(5-6), 730–732.

Sara, Susan J. 2009. The locus coeruleus and noradrenergic modulation of cognition. *Nature reviews neuroscience*, **10**(3), 211–223.

Scheibner, J, Trendelenburg, A U, Hein, L, & Starke, K. 2001. Alpha2-adrenoceptors modulating neuronal serotonin release: a study in alpha2-adrenoceptor subtype-deficient mice. *British journal of pharmacology*, **132**(4), 925–933.

Schubert, Markus, Gautam, Dinesh, Surjo, David, Ueki, Kojihiko, Baudler, Stephanie, Schubert, Dominic, Kondo, Tatsuya, Alber, Jens, Galldiks, Norbert, Küstermann, Eckehardt, Arndt, Saskia, Jacobs, Andreas H, Krone, Wilhelm, Kahn, C Ronald, & Brüning, Jens C. 2004. Role for neuronal insulin resistance in neurodegenerative diseases. *Proceedings of the national academy of sciences of the united states of america*, **101**(9), 3100–3105.

Schwartz, M W, Woods, S C, Porte, D, Seeley, R J, & Baskin, D G. 2000. Central nervous system control of food intake. *Nature*, **404**(6778), 661–671.

Séguéla, P, Watkins, K C, Geffard, M, & Descarries, L. 1990. Noradrenaline axon terminals in adult rat neocortex: an immunocytochemical analysis in serial thin sections. *Neuroscience*, **35**(2), 249–264.

Shi, Yan-Chuan, Lau, Jackie, Lin, Zhou, Zhang, Hui, Zhai, Lei, Sperk, Guenther, Heilbronn, Regine, Mietzsch, Mario, Weger, Stefan, Huang, Xu-Feng, Enriquez, Ronaldo F, Castillo, Lesley, Baldock, Paul A, Zhang, Lei, Sainsbury, Amanda, Herzog, Herbert, & Lin, Shu. 2013. Arcuate NPY Controls Sympathetic Output and BAT Function via a Relay of Tyrosine Hydroxylase Neurons in the PVN. *Cmet*, **17**(2), 236–248.

Silver, I A, & Erecińska, M. 1994. Extracellular glucose concentration in mammalian brain: continuous monitoring of changes during increased neuronal activity and upon

limitation in oxygen supply in normo-, hypo-, and hyperglycemic animals. *The journal of neuroscience : the official journal of the society for neuroscience*, **14**(8), 5068–5076.

Silver, I A, & Erecińska, M. 1998. Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons. *Journal of neurophysiology*, **79**(4), 1733–1745.

Smeets, W J, & González, A. 2000. Catecholamine systems in the brain of vertebrates: new perspectives through a comparative approach. *Brain research. brain research reviews*, **33**(2-3), 308–379.

Smiałowska, M. 1988. Neuropeptide Y immunoreactivity in the locus coeruleus of the rat brain. *Neuroscience*, **25**(1), 123–131.

Smith, Mark A, Hisadome, Kazunari, Al-Qassab, Hind, Heffron, Helen, Withers, Dominic J, & Ashford, Michael L J. 2007. Melanocortins and agouti-related protein modulate the excitability of two arcuate nucleus neuron populations by alteration of resting potassium conductances. *The journal of physiology*, **578**(Pt 2), 425–438.

Smith, Roy G, Betancourt, Lorena, & Sun, Yuxiang. Molecular Endocrinology and Physiology of the Aging Central Nervous System. *edrv.endojournals.org*.

Sohn, Jong-Woo, & Williams, Kevin W. 2012. Functional Heterogeneity of Arcuate Nucleus Pro-Opiomelanocortin Neurons: Implications for Diverging Melanocortin Pathways. *Molecular neurobiology*, Feb.

Sohn, Jong-Woo, Xu, Yong, Jones, Juli E, Wickman, Kevin, Williams, Kevin W, & Elmquist, Joel K. 2011. Serotonin 2C receptor activates a distinct population of arcuate pro-opiomelanocortin neurons via TRPC channels. *Neuron*, **71**(3), 488–497.

Song, Z, & Routh, V H. 2005. Differential effects of glucose and lactate on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes*, **54**(1), 15–22.

Song, Z, Levin, B E, McArdle, J J, Bakhos, N, & Routh, V H. 2001. Convergence of pre- and postsynaptic influences on glucosensing neurons in the ventromedial hypothalamic nucleus. *Diabetes*, **50**(12), 2673–2681.

Spanswick, D, Smith, M A, Groppi, V E, Logan, S D, & Ashford, M L. 1997. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature*, **390**(6659), 521–525.

Spanswick, D, Smith, M A, Mirshamsi, S, Routh, V H, & Ashford, M L. 2000. Insulin activates ATP-sensitive K+ channels in hypothalamic neurons of lean, but not obese rats. *Nature neuroscience*, **3**(8), 757–758.

Stanley, B G, & Leibowitz, S F. 1984. Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. *Life sciences*, **35**(26), 2635–2642.

Stanley, B G, Schwartz, D H, Hernandez, L, Hoebel, B G, & Leibowitz, S F. 1989. Patterns of extracellular norepinephrine in the paraventricular hypothalamus: relationship to circadian rhythm and deprivation-induced eating behavior. *Life sciences*, **45**(4), 275–282.

Stocker, Martin. 2004. Ca(2+)-activated K+ channels: molecular determinants and function of the SK family. *Nature reviews neuroscience*, **5**(10), 758–770.

Sun, Qian-Quan. 2009. Experience-dependent intrinsic plasticity in interneurons of barrel cortex layer IV. *Journal of neurophysiology*, **102**(5), 2955–2973.

Surmeier, D J, Guzman, J N, Sanchez-Padilla, J, & Schumacker, P T. 2011. The role of calcium and mitochondrial oxidant stress in the loss of substantia nigra pars compacta dopaminergic neurons in Parkinson's disease. *Neuroscience*, Aug.

Suzuki, Keisuke, Simpson, Katherine A, Minnion, James S, Shillito, Joyceline C, & Bloom, Stephen R. 2010. The role of gut hormones and the hypothalamus in appetite regulation. *Endocrine journal*, **57**(5), 359–372.

Szabadi, Elemer. 2013. Functional neuroanatomy of the central noradrenergic system. *Journal of psychopharmacology (oxford, england)*, June.

Tallent, Melanie K. 2008. Presynaptic inhibition of glutamate release by neuropeptides: use-dependent synaptic modification. *Results and problems in cell differentiation*, **44**, 177–200.

Taneja, Praveen, Ogier, Michael, Brooks-Harris, Gabriel, Schmid, Danielle A, Katz, David M, & Nelson, Sacha B. 2009. Pathophysiology of Locus Ceruleus Neurons in a Mouse Model of Rett Syndrome. *The journal of neuroscience : the official journal of the society for neuroscience*, **29**(39), 12187–12195.

Tanoue, Akito, Koshimizu, Taka-aki, & Tsujimoto, Gozoh. 2002. Transgenic studies of alpha(1)-adrenergic receptor subtype function. *Life sciences*, **71**(19), 2207–2215.

Tews, D, & Wabitsch, M. 2011. Renaissance of Brown Adipose Tissue. *Hormone research in paediatrics*, **75**(4), 231–239.

Thorens, B. 2011. Brain glucose sensing and neural regulation of insulin and glucagon secretion. *Diabetes, obesity & metabolism*, **13 Suppl 1**(Oct.), 82–88.

Tovar, S, Paeger, L, Hess, S, Morgan, DA, Hausen, A C, Broenneke, H S, Hampel, B, Ackermann, J, Evers, N, Buening, H, Wunderlich, F T, Rhamouni, K, Kloppenburg, P, & Bruening, J C. 2013. KATP-Channel-dependent Glucose Sensing in the Locus Coeruleus controls Energy Homeostasis via Regulation of Sympathetic Innervation of Brown Adipose Tissue. *Cell metabolism*, **18**(4), 1–11.

Ueno, S, Ishibashi, H, & Akaike, N. 1992. Perforated-patch method reveals extracellular ATP-induced K+ conductance in dissociated rat nucleus solitarii neurons. *Brain research*, **597**(1), 176–179.

van den Pol, AN, Ghosh, PK, Liu, RJ, Li, Y, Aghajanian, GK, & Gao, XB. 2002. Hypocretin (orexin) enhances neuron activity and cell synchrony in developing mouse GFP-expressing locus coeruleus. *Journal of physiology-london*, **541**(1), 169–185.

van den Pol, Anthony N. 2012. Neuropeptide transmission in brain circuits. *Neuron*, **76**(1), 98–115.

van den Pol, Anthony N, Yao, Yang, Fu, Li-Ying, Foo, Kylie, Huang, Hao, Coppari, Roberto, Lowell, Bradford B, & Broberger, Christian. 2009. Neuromedin B and gastrin-releasing peptide excite arcuate nucleus neuropeptide Y neurons in a novel transgenic mouse expressing strong Renilla green fluorescent protein in NPY neurons. *The journal of neuroscience : the official journal of the society for neuroscience*, **29**(14), 4622–4639.

van den Top, M, Lyons, D J, Lee, K, Coderre, E, Renaud, L P, & Spanswick, D. 2007. Pharmacological and molecular characterization of ATP-sensitive K(+) conductances in CART and NPY/AgRP expressing neurons of the hypothalamic arcuate nucleus. *Neuroscience*, **144**(3), 815–824.

van den Top, Marco, Lee, Kevin, Whyment, Andrew D, Blanks, Andrew M, & Spanswick, David. 2004. Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nature neuroscience*, **7**(5), 493–494.

Vandecasteele, M, Deniau, J-M, & Venance, L. 2011. Spike frequency adaptation is developmentally regulated in substantia nigra pars compacta dopaminergic neurons. *Neuroscience*, **192**(Sept.), 1–10.

Varela, Luis, & Horvath, Tamas L. 2012. Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis. *Embo reports*, **13**(12), 1079–1086.

Venance, L, & Glowinski, J. 2003. Heterogeneity of spike frequency adaptation among medium spiny neurones from the rat striatum. *Neuroscience*, **122**(1), 77–92.

Virtanen, Kirsi A, & Nuutila, Pirjo. 2011. Brown adipose tissue in humans. *Current opinion in lipidology*, **22**(1), 49–54.

Vogt, M. 1954. The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *The journal of physiology*, **123**(3), 451–481.

Waldén, Tomas B, Hansen, Ida R, Timmons, James A, Cannon, Barbara, & Nedergaard, Jan. 2012. Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. *American journal of physiology endocrinology and metabolism*, **302**(1), E19–31.

Walling, Susan G, Brown, Robert A, Miyasaka, Nobuhiko, Yoshihara, Yoshihiro, & Harley, Carolyn W. 2012. Selective wheat germ agglutinin (WGA) uptake in the

hippocampus from the locus coeruleus of dopamine-β-hydroxylase-WGA transgenic mice. *Frontiers in behavioral neuroscience*, **6**, 23.

Wang, Wei-Guang, Chen, Xi, Jiang, Hong, & Jiang, Zheng-Yao. 2008. Effects of ghrelin on glucose-sensing and gastric distension sensitive neurons in rat dorsal vagal complex. *Regulatory peptides*, **146**(1-3), 169–175.

Watts, Alan G, & Donovan, Casey M. 2010. Sweet talk in the brain: glucosensing, neural networks, and hypoglycemic counterregulation. *Frontiers in neuroendocrinology*, **31**(1), 32–43.

Weinshenker, David. 2008. Functional consequences of locus coeruleus degeneration in Alzheimer's disease. *Current alzheimer research*, **5**(3), 342–345.

Wellman, P J, & Davies, B T. 1991. Reversal of phenylpropanolamine anorexia in rats by the alpha-1 receptor antagonist benoxathian. *Pharmacology biochemistry and behavior*, **38**(4), 905–908.

Wellman, PJ. 2000. Norepinephrine and the control of food intake. *Nutrition*, **16**(10), 837–842.

Wellman, PJ. 2005. Modulation of eating by central catecholamine systems. *Current drug targets*, **6**(2), 191–199.

Wellman, PJ, Davies, BT, Morien, A, & McMahon, L. 1993. Modulation of feeding by hypothalamic paraventricular nucleus alpha-1-adrenergic and alpha-2-adrenergic receptors. *Life sciences*, **53**(9), 669–679.

White, Wesley, Hundley, Marcus B, & White, Ilsun M. 2010. The effects of dose and repeated administration on the longer-term hypophagia produced by amphetamine in rats. *Pharmacology biochemistry and behavior*, **97**(2), 384–391.

Wilanowski, Grzegorz, & Piotrkiewicz, Maria. 2012. Is spike frequency adaptation an artefact? Insight from human studies. *Frontiers in cellular neuroscience*, **6**, 50.

Williams, JT, Henderson, G, & North, RA. 1985. Characterization of alpha-2-adrenoceptors which increase potassium conductance in rat locus coeruleus neurons. *Neuroscience*, **14**(1), 95–101.

Williams, Kevin W, Margatho, Lisandra O, Lee, Charlotte E, Choi, Michelle, Lee, Syann, Scott, Michael M, Elias, Carol F, & Elmquist, Joel K. 2010. Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *The journal of neuroscience : the official journal of the society for neuroscience*, **30**(7), 2472–2479.

Wolfart, J, Neuhoff, H, Franz, O, & Roeper, J. 2001. Differential expression of the small-conductance, calcium-activated potassium channel SK3 is critical for pacemaker control in dopaminergic midbrain neurons. *The journal of neuroscience : the official journal of the society for neuroscience*, **21**(10), 3443–3456.

Woodcock, Elizabeth A. 2007. Roles of alpha1A- and alpha1B-adrenoceptors in heart: insights from studies of genetically modified mice. *Clinical and experimental pharmacology and physiology*, **34**(9), 884–888.

Yang, Shi-Bing, Tien, An-Chi, Boddupalli, Gayatri, Xu, Allison W, Jan, Yuh Nung, & Jan, Lily Yeh. 2012. Rapamycin Ameliorates Age-Dependent Obesity Associated with Increased mTOR Signaling in Hypothalamic POMC Neurons. *Neuron*, **75**(3), 425–436.

Ye, Jiang Hong, Zhang, Jingli, Xiao, Cheng, & Kong, Jian-Qiang. 2006. Patch-clamp studies in the CNS illustrate a simple new method for obtaining viable neurons in rat brain slices: glycerol replacement of NaCl protects CNS neurons. *Journal of neuroscience methods*, **158**(2), 251–259.

Yoshimura, M, Polosa, C, & Nishi, S. 1987. Noradrenaline induces rhythmic bursting in sympathetic preganglionic neurons. *Brain research*, **420**(1), 147–151.

Young, W S, & Kuhar, M J. 1979. Noradrenergic alpha 1 and alpha 2 receptors: autoradiographic visualization. *European journal of pharmacology*, **59**(3-4), 317–319.

Young, W S, & Kuhar, M J. 1980. Noradrenergic alpha 1 and alpha 2 receptors: light microscopic autoradiographic localization. *Proceedings of the national academy of sciences of the united states of america*, **77**(3), 1696–1700.

Yuan, Pu-Qing, & Yang, Hong. 2002. Neuronal activation of brain vagal-regulatory pathways and upper gut enteric plexuses by insulin hypoglycemia. *American journal of physiology endocrinology and metabolism*, **283**(3), E436–48.

Zeltser, Lori M, Seeley, Randy J, & Tschöp, Matthias H. 2012. Synaptic plasticity in neuronal circuits regulating energy balance. *Nature neuroscience*, **15**(10), 1336–1342.

Zhan, Cheng, Zhou, Jingfeng, Feng, Qiru, Zhang, Ju-En, Lin, Shuailiang, Bao, Junhong, Wu, Ping, & Luo, Minmin. 2013. Acute and long-term suppression of feeding behavior by POMC neurons in the brainstem and hypothalamus, respectively. *The journal of neuroscience : the official journal of the society for neuroscience*, **33**(8), 3624–3632.

Zhang, Xiaoli, Cui, Ningren, Wu, Zhongying, Su, Junda, Tadepalli, Jyothirmayee S, Sekizar, Sowmya, & Jiang, Chun. 2010. Intrinsic membrane properties of locus coeruleus neurons in Mecp2-null mice. *American journal of physiology-cell physiology*, **298**(3), C635–46.

Zhang, Y, Proenca, R, Maffei, M, Barone, M, Leopold, L, & Friedman, J M. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature*, **372**(6505), 425–432.

Zimnik, Nathan C, Treadway, Tyler, Smith, Richard S, & Araneda, Ricardo C. 2012. Alpha1A-Adrenergic Regulation of Inhibition in the Olfactory Bulb. *The journal of physiology*, Dec.

Zoli, M, Torri, C, Ferrari, R, Jansson, A, Zini, I, Fuxe, K, & Agnati, L F. 1998. The emergence of the volume transmission concept. *Brain research. brain research reviews*, **26**(2-3), 136–147.

Zuscik, M J, Sands, S, Ross, S A, Waugh, D J, Gaivin, R J, Morilak, D, & Perez, D M. 2000. Overexpression of the alpha1B-adrenergic receptor causes apoptotic neurodegeneration: multiple system atrophy. *Nature medicine*, **6**(12), 1388–1394.

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DANKE

In loving memory of my father
1950 - 2010

** The truth is rarely pure and never simple **

Oscar Wilde
1854 - 1900

Erklärung

Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit – einschließlich Tabellen, Karten und Abbildungen –, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von unten angegebenen Teilpublikationen – noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.

Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Peter Kloppenburg betreut worden.

Köln, den 26.08.2013

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(Lars Paeger)

Teilpublikationen

Artikel

Husch, A, Paehler, M, Fusca, D, Paeger,L, Kloppenburg, P. 2009. Calcium current diversity in physiologically different local interneuron types of the antennal lobe. *J. Neurosci.*, **29**(3), 716–726.

Husch, A, Paehler, M, Fusca, D, Paeger,L, Kloppenburg, P. 2009. Distinct electrophysiological properties in subtypes of nonspiking olfactory local interneurons correlate with their cell type-specific Ca2+ current profiles. *J. Neurophysiol.*, **102**(5), 2834–2845.

Klöckener, T, Hess, S, Belgardt, B F, Paeger, L, Verhagen, L A, Husch, A, Sohn, J W, Hampel, B, Dhillon, H, Zigman, J, Lowell, B B, Williams, K W, Elmquist, J K, Horvath, T L, Kloppenburg, P, Brüning, J C. 2011. High-fat Feeding Promotes Obesity via Insulin Receptor/PI3k-Dependent Inhibition of SF-1 VMH Neurons. *Nat. Neurosci.*, **14**(7), 911–18.

Almajan,E R, Richter, R, Paeger, L, Martinelli, P, Barth, E, Decker, T, Larsson, N G, Kloppenburg, P, Langer, T, Rugarli, E I. 2011. AFG3L2 supports mitochondrial protein synthesis and Purkinje cell survival. *J. Clin. Invest.*, **122**(11), 4048–4058.

Newton,A J, Hess,S, Paeger, L, Vogt, M C, Lascano, J F, Nillni, E A, Brüning, J C, Kloppenburg, P, Xu, A W. 2011. AgRP innervation onto POMC neurons increases with age and is accelerated with chronic high-fat feeding in male mice. *Endocrinology*, **154**(1), 172–183.

Tovar, S, Paeger, L, Hess, S, Morgan, D A, Hausen, A C, Brönneke, H S, Hampel, B, Ackermann, J, Evers, N, Büning, H, Wunderlich, F T, Rhamouni, K, Kloppenburg, P, Brüning, J C. 2013. K_{ATP}-Channel-dependent Glucose Sensing in the Locus Coeruleus controls Energy Homeostasis via Regulation of Sympathetic Innervation of Brown Adipose Tissue. *Cell Metab.* (accepted).

Vogt, M C, Paeger, L, Hess, S, Steculorum, S M, Hampel, B, Neupert, S, Nicholls, H C, Hausen, A C, Mauer, J, Predel, R, Kloppenburg, P, Horvath, T L, Brüning, J C. 2013. Neonatal insulin action in POMC neurons impairs hypothalamic neurocircuit formation in response to maternal high fat feeding. *Cell* (submitted).

Pippow, A, Paehler, M, Hess, S, Paeger, L, Joucla, S, Klöckener, T, Pouzat, C, Brüning, J C, Kloppenburg, P. 2013. High fat diet decreases neural activity in anorexigenic POMC neurons of the nucleus arcuatus by altering Ca^{2+} handling properties. (in preparation).

Poster

Paeger, L, Kloppenburg, P. 2009. Characterization of Transient Potassium Currents in Identified Olfactory Interneurons of *Periplaneta Americana*. *Proceedings of the 32th Göttingen Neurobiology Conference and the 8th Meeting of the German Neuroscience Society.*

Pippow, A, Paehler, M, Paeger, L, Hess, S, Klöckener, T, Vogt, M, Pouzat, C, Brüning, J C, Kloppenburg, P. 2010. High fat induced obesity impairs intrinsic properties of anorexigenic POMC neurons in the hypothalamus. *CECAD Meeting 2010*

Schleicher, S, Rotte, C, Paeger, L, Kloppenburg, P. 2011. Characterization of Transient Potassium Currents in Identified Olfactory Interneurons of *Periplaneta Americana*. *Proceedings of the 33th Göttingen Neurobiology Conference and the 9th Meeting of the German Neuroscience Society.*

Pippow, A, Paehler, M, Paeger, L, Hess, S, Klöckener, T, Vogt, M, Pouzat, C, Brüning, J C, Kloppenburg, P. 2011. High fat induced obesity impairs intrinsic properties of anorexigenic POMC neurons in the hypothalamus. *Proceedings of the 33th Göttingen Neurobiology Conference and the 9th Meeting of the German Neuroscience Society.*

Pippow, A, Demmer, H, Fusca, D, Hess, S, Husch, A, Paehler, M, Wratil, H, Pouzat,C, Kloppenburg, P. 2011. Distinct calcium handling properties of identified insect olfactory interneurons. *Annual Meeting of the Society for Neuroscience (SfN). Abstract, Washington, DC*

Paeger, L , Pippow, A, Hess, S, Paehler, M, Vogt, M C, Klöckener, T, Pouzat, C, Brüning, J C, Kloppenburg, P 2011. Ca2+- activated K+ currents contribute to high fat diet induced inhibition of POMC neurons of the mouse nucleus arcuatus. *41st Annual Meeting of the Society for Neuroscience (SfN). Abstract, Washington, DC*

Tovar, S, Paeger, L, Hess, S, Morgan, D A, Hausen, A C, Brönneke, H S, Hampel, B, Ackermann, J, Evers, N, Büning, H, Wunderlich, F T, Rhamouni, K, Brüning, J C, Kloppenburg, P 2012. K_{ATP}-channel-dependent glucose Ssensing in the Locus Coeruleus controls brown adipose tissue and sympathetic traffic and energy homeostasis. *Joint FEPS and Spanish Physiological Society Scientific Congress 2012. Santiago de Compostela, Spain*

Paeger, L, Tovar, S, Hess, S, Morgan, D A, Hausen, A C, Brönneke, H S, Hampel, B, Ackermann, J, Evers, N, Büning, H, Wunderlich, F T, Rhamouni, K, Brüning, J C, Kloppenburg, P 2013. A glucose responsive subpopulation of the Locus Coeruleus contributes to brown adipose tissue sympathetic traffic and energy homeostasis. *Proceedings of the 34^{th} Göttingen Neurobiology Conference and the 10^{th} Meeting of the German Neuroscience Society.*

Paeger, L, Tovar, S, Hess, S, Morgan, D A, Hausen, A C, Brönneke, H S, Hampel, B, Ackermann, J, Evers, N, Büning, H, Wunderlich, F T, Rhamouni, K, Brüning, J C, Kloppenburg, P 2013. Electrophysiological properties of neurons of the Locus Coeruleus and evidence for their role in energy homeostasis. *43rd Annual Meeting of the Society for Neuroscience (SfN). Abstract, San Diego, CA*

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