Gp130 Signaling In POMC Neurons Is Required For CNTF-Induced Anorexia

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4. Abbreviations

°C	degrees Celsius
3’	three prime end of DNA sequences
5’	five prime end of DNA sequences
A	adenosine
a.m.	ante meridiem
ACTH	adrenocorticotrophin
AgRP	agouti-related peptide
AKT	proteinkinase B
ALS	amyotrophic lateral sclerosis
AMPK	AMP activated protein kinase
ARC	arcuate nucleus
BAT	brown adipose tissue
BBB	blood brain barrier
BMI	body mass index
bp	base pairs
C	cytosine
c	DNA concentration
$CaCl_2$	calcium chloride
cAMP	cyclic adenosine monophosphate
cDNA	complementary DNA
CLAMS	Comprehensive Laboratory Animal Monitoring System
cm	centimeter
CNS	central nervous system
CNTF	ciliary neurotrophic factor
$CNTF_{Ax15}$	Axokine, recombinant human variant of CNTF
Cre	site specific recombinase from phage P1
CRH	corticotrophin-releasing hormone
CT-1	cordiotrophin-1
CVO	circumventricular organs
d	deci-

Da	Dalton
DAPI	4',6-diamidino-2-phenylindole
db/db	mice homozygous for a point mutation in the leptin receptor gene
ddH_2O	double destilled water
DMH	dorsomedial hypothalamic nucleus
DMSO	dimethylsulfoxide
DNA	desoxyribonucleic acid
DNase	desoxyribonuclease
dNTP	desoxyribonucleotide-triphosphate
DTT	1,4-Dithio-DL-threitol
e.g.	exempli gratia
ECL	enhanced chemiluminescence
EDTA	ethylendiamine tetraacetate
eGFP	enhanced green fluorescent protein
ELISA	enzyme-linked immunosorbent assay
Erk	extracellular signal-regulated kinase
EtBr	ethidium bromide
EtOH	ethanol
floxed / lox	loxP flanked
FOXO1	forkhead-O transcription factor 1
g	gram
G	guanine
G6Pase α	glucose-6-phosphatase α
Gab	growth factor receptor binding protein associated binder
GABA	γ-aminobutyric acid
GFP	green fluorescent protein
GLP-1	glucagon-like peptide 1
GLUT-4	glucose transporter 4
gp130	glycoprotein 130
Grb2	growth factor receptor binding protein 2
GTT	glucose tolerance test
Gusb	glucuronidase beta
h	hour

H&E	hematoxylin/eosin
H_2O_2	hydrogen peroxide
HCl	hydrochloric acid
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethansulfonic acid
HFD	high fat diet
HGP	hepatic glucose production
HPA	hypothalamic-pituitary-adrenal
Hprt-1	hypoxanthine guanine phosphoribosyl transferase-1
i.e.	id est
ip.	intraperitoneal
icv	intracerebroventricular
IGF-1	insulin-like growth factor-1
IKK	inhibitor of kappaB kinase
IL-11	interleukin-11
IL-6	interleukin-6
IL-6R	interleukin-6 receptor
IR	insulin receptor
IRES	internal ribosome entry site
IRS	insulin receptor substrate
ITT	insulin tolerance test
JAK	Janus kinase
JNK	c-Jun N-terminal kinase;
k	kilo
kb	kilobase pairs
KCl	potassium chloride
kDa	kilodalton
KOH	potassium hydroxide
L	liter
LacZ	gene encoding the enzyme ß-galactosidase
Lepr	leptin receptor
LH	lateral hypothalamic area
LIF	leukaemia inhibitory factor
loxP	recognition sequence for Cre (locus of x-ing over phage P1)

m	milli
M	molar
MAPK	mitogen-activated protein kinase
MCR	melanocortin receptor
ME	median eminence
MEK	MAPK/Erk Kinase
$MgCl_2$	magnesium chloride
min	minute
mm	milimeter
mRNA	messenger RNA
MSH	melanocyte-stimulating hormone
n	nano-
n	counts
Na_2HPO_4	disodium hydrogen phosphate
Na_3O_4V	sodium orthovanadate
NAc	nucleus accumbens
NaCl	sodium chloride
NaF	sodium fluoride
NaH_2PO_4	monosodium phosphate
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
NIRKO	neuron-restricted insulin receptor knockout mice
NMR	nuclear magnetic resonance
NPY	neuropeptide Y
ob/ob	mice homozygous for mutation in the leptin gene
ObRb	long isoform of the leptin receptor
OD	optical density
OSM	oncostatin M
p	pico-
P	phospho
PAGE	polyacrylamid gel electrophoresis
PB	phosphate buffer
PBS	phosphate buffered saline

PCR	polymerase chain reaction
PDK1	phosphoinositide-dependent protein kinase 1
PFA	paraformaldehyde
PH	pleckstrin homology
PI3	kinase phosphatidylinositol 3 kinase
PIP2	phosphatidylinositol (4,5) bisphosphate
PIP3	phosphatidylinositol (3,4,5) trisphosphate
POMC	pro-opiomelanocortin
PTB	phosphotyrosine binding
PTEN	phosphatase and tensin homolog
PVN	paraventricular nucleus
Raf	proto-oncogene serine/threonine protein kinase
Ras	Ras small GTPase
rhCNTF	recombinant human CNTF
RNA	ribonucleic acid
RNAi	RNA interference
RNase	ribonuclease
rpm	rounds per minute
RT	room temperature
RT-PCR	real-time polymerase chain reaction
s	second
s.c.	subcutanous
SCD-1	stearoyl-CoA desaturase-1
SDS	sodiumdodecylsulfate
sec	second
SEM	standard error of the mean
SH	src homology
Shp-2	tyrosine phosphatase-2
SIM-1	single minded homolog-1
SN	substantia nigra
SOCS	suppressor of cytokine signaling
SOS	son of sevenless
ß-ga	ß-galactosidase

ß-me	ß-mercaptoethanol
STAT	signal transducer and activator of transcription
TAE	Tris-acetic acid-EDTA buffer
TBP	TATA-box binding protein
TBS	Tris buffered saline
TE	Tris-EDTA buffer
Th	tyrosine hydroxylase
TNF-α	tumor necrosis factor-α
Tris	2-amino-2-(hydroxymethyl-)1,3-propandiole
Trp	tryptophan
TWEEN	polyoxethylene-sorbitan-monolaureate
Tyr	tyrosine
U	units
UV	ultraviolet
V	Volt
v/v	volume per volume
VMH	ventromedial nucleus of the hypothalamus
VO2	volume of consumed oxygen
VTA	ventral tegmental area
w/v	weight per volume
WAT	white adipose tissue
WHO	World Health Organization
$ZnSO_4$	zinc sulfate
µ	micro-

5. Introduction

5.1. Obesity

The prevalence of adult obesity and overweight among children has increased dramatically during the last decades. The World Health Organization (WHO) released that in 2005 approximately 1.6 billion adults were overweight and at least 400 million adults were obese. The WHO further projects that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese. Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health (1), either leading to serious health consequences. Risk increases progressively as Body Mass Index (BMI) increases. Raised BMI is a major risk factor for chronic diseases including cardiovascular diseases (e.g. hypertension, heart diseases and stroke), diabetes mellitus type 2, sleep apnea and certain forms of cancer (2-4).

The BMI is a simple index of weight-for-height that is commonly used in classifying overweight and obesity in adult populations and individuals. It is defined as the weight in kilograms divided by the square of the height in meters (kg/m2). The World Health Organization defines "overweight" as a BMI equal to or more than 25, and "obesity" as a BMI equal to or more than 30 (1).

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed on one hand, and calories expended on the other hand. The rapid increase in obese people results from a combination of genetic predisposition, enhanced availability of high-energy foods, and decreased physical activity in modern society. Lifestyle modifications and obesity drugs are the current efforts to diminish the growing epidemic, but so far, none of them is capable of ensuring a consistent and effective weight loss. As a result, new therapeutic approaches are required to treat the obesity epidemic.

5.2. Energy homeostasis

Obesity is the result of a long-term positive body energy balance namely, energy intake is greater than energy expenditure thus, excess calories are stored in the form of fat. On the other hand, negative energy balance leads to a decreased body mass. Thus, understanding the regulation of energy balance is crucial in the development of therapeutically strategies in the treatment of obesity (5, 6).

Energy homeostasis is primarily regulated by the central nervous system (CNS) (5). Neuronal systems involved in regulation of energy intake, energy expenditure and endogenous glucose production sense and integrate input from peripheral hormones and nutrient-related signals that transmit information about body energy stores and energy availability to the brain (5). Under circumstances of sufficient energy stores and energy availability, the CNS receives afferent signals that lead to the activation of anabolic pathways (inhibition of energy intake and endogenous glucose production, increase of energy expenditure and mobilization of fat stores). Conversely, in the state of low energy stores and food deficiency, catabolic responses are activated to promote positive energy balance (increased energy intake and glucose production, decreased energy expenditure).

The afferent signals that are crucial for the regulation of energy homeostasis include the pancreatic β-cell hormone insulin as well as the adipocyte-derived hormone leptin.

5.3. Insulin and the insulin receptor

The anabolic peptide-hormone insulin is produced by the β-cells of the pancreatic islets of Langerhans. Insulin was discovered in 1921 by F. Banting, C. Best, J. Macleod and J. Collip., two years later, F. Banting and J. Macleod were awarded the Nobel Prize in Medicine for their discovery. In 1953, the primary sequence of insulin was decoded (7), followed by the crystal structure of the active form in 1966 (8). One year later, Steiner *et al*. identified the inactive precursor proinsulin, which is cleaved to insulin by different endopeptidases (9, 10).

Insulin secretion from the pancreatic ß-cells is triggered predominantly in response to increased blood glucose levels (11, 12). Additionally, plasma insulin levels are also directly linked to the degree of body adiposity (13, 14). Insulin acts on peripheral tissues, particularly on muscle cells and adipocytes via translocation of the glucose transporter to remove the glucose from the blood and store it in form of glycogen (11, 15). Moreover, insulin promotes anabolic processes such as stimulation of amino acid uptake and protein synthesis in muscle, glycogen synthesis in liver and muscle as well as lipogenesis in adipose tissue. Catabolic processes, on the other hand, such as glycogenolysis, lipolysis and proteolysis are inhibited by insulin (16).

In addition to its peripheral action, insulin circulates in proportion to the amount of body fat, crosses the blood-brain-barrier via a saturable transporter and acts on the CNS as an adiposity signal (17-20).

The heterotetrameric insulin receptor is ubiquitously expressed and belongs to the family of ligand-activated receptor tyrosine kinases. Binding of insulin leads to autophosphorylation of the receptor on three intracellular tyrosine residues resulting in complete activation of the intrinsic tyrosine activity of the receptor.
Receptor autophosphorylation results in recruitment and binding of intracellular proteins via their phosphotyrosine binding domains. Signaling molecules for the insulin receptor include the insulin receptor substrate (IRS) proteins 1-4 which are phosphorylated upon binding to the receptor, and thereby serve as a docking platform for other proteins, leading to the action of different downstream signaling pathways.
There are two major pathways activated by the IRS proteins, the Ras/Raf Mitogen activated protein (MAP) kinase and the PI3 (phosphatidyl-inositol 3) kinase pathway (21).

5.4. Leptin

Leptin is a 16-kDa protein encoded by the *ob* (obese) gene with a structure similar to the 4-α-helical bundle cytokines (22). It is a circulating hormone secreted by the adipose tissue. First described in 1994 (23), leptin has proven to be a key metabolic protein that has actions throughout the body. Mice with mutations of the gene encoding leptin (*ob/ob* mice) as well as mice with mutations compromising the leptin receptor (*db/db* mice) become pathologically obese, infertile, hyperphagic, hypothermic, and diabetic (24). Analogous to insulin, plasma leptin levels are correlated directly with adipose tissue mass, and circulating leptin is transported into the brain via a saturable process (25, 26). A neuron-specific deletion of the leptin receptor bears analogy to the phenotype of the *db/db* mouse, indicating that the major role of leptin action to control energy homeostasis is accounted for by its signaling in the CNS (27).

5.4.1. The leptin receptor

Several leptin receptor isoforms (LEPR-A to F) are encoded by a single leptin receptor gene, each containing an identical ligand binding domain, but varying by the presence or absence of a transmembrane domain or a complete cytosolic domain (28). Among the different isoforms, all arising from alternative mRNA splicing and/or proteolytic processing of the single *Lepr* gene (29, 30), only the long form of the leptin receptor (Ob-Rb) has been shown to activate intracellular signaling (31). Many effects of leptin on energy homeostasis are assignable to its action in the CNS, especially in the mediobasal hypothalamus, where the Ob-Rb is highest expressed (32). Ob-Rb is highly conserved among species and is essential for leptin action as observed in *db/db* mice, which actually lack only the Ob-Rb (31, 33).

The leptin receptor is a single membrane-spanning receptor, belongs to the class I cytokine receptor family and shows the highest similarity to gp130 (glycoprotein 130) which is the common signal transducer for the interleukin 6 (IL-6) family of cytokines (34). Hormone binding to the Ob-Rb results in dimerization of the receptor and activation of the janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathway (35). Activation of the constitutively associated JAK2 leads to autophosphorylation on three different tyrosine residues of the receptor, whereas each phosphorylated tyrosine residue results in the activation of a different downstream signaling pathway (in figure 1 exemplified with STAT3) (36, 37). Phosphorylated STAT molecules dimerize and translocate to the nucleus to modulate transcription of multiple target genes via STAT-responsive elements (36). Here. STAT3 activates transcription of suppressor of cytokine signaling (SOCS) 3 (38), which is a negative regulator of proximal leptin signaling and directly binds to JAK2 in a leptin-dependent manner (39). SOCS3 attenuates leptin receptor signaling by inhibiting JAK-induced tyrosine phosphorylation of the receptor.

Binding of leptin to the leptin receptor also mediates tyrosine phosphorylation of IRS and thereby activation of the PI3-kinase signaling pathway (40).

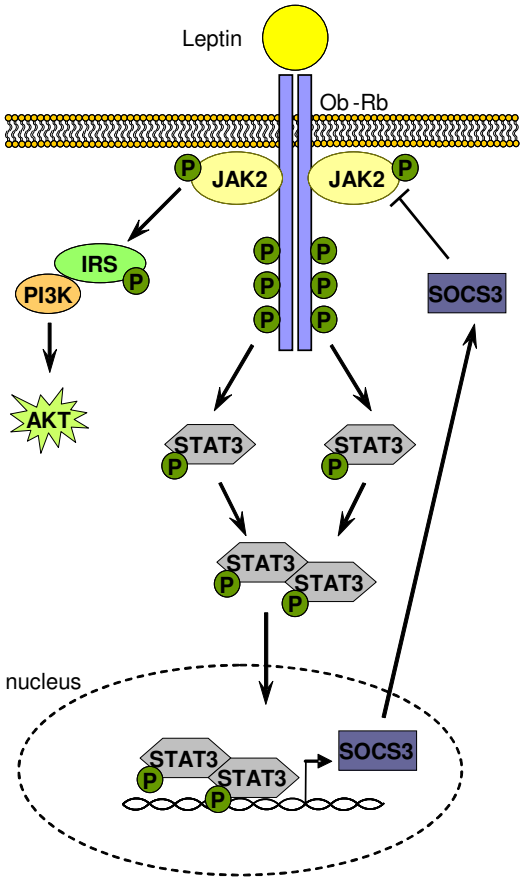

Figure 1.1: Leptin receptor signaling.

Binding of leptin to Ob-Rb leads to activation of JAK2 and subsequently to JAK2-mediated phosphorylation of three different intracellular tyrosine residues of the receptor, resulting in the activation of different downstream signaling pathways. Phosphorylation and activation of STAT3 molecules lead to a dimerization of STAT3. The dimers translocate to the nucleus and activate target genes including SOCS3, resulting in inhibition of leptin signaling via negative feedback loop. Additionally, phosphorylated JAK2 directly activates the IRS/PI3K and the Shp2/Erk (not illustrated) signaling pathway. Abbreviations: AKT, protein kinase B; IRS, insulin receptor substrate; JAK, janus kinase; Ob-Rb, long form of the leptin receptor; PI3K, phosphatidyl inositol 3 kinase; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcript.

5.5. Central regulation of energy homeostasis

5.5.1. The hypothalamus

During the last decades, the research on central regulation of energy balance has focused on the hypothalamus. In vertebrates, the hypothalamus is a part of the diencephalon located below the thalamus, building up the floor of the third cerebral ventricle. Conventional histological techniques revealed nuclei as clusters of neurons within the hypothalamus. The mammalian hypothalamus consists of more than 40 histologically distinct nuclei areas, which can often be further divided into subnuclei (41). Classical experiments utilizing lesions or electrical stimulation indicated that some of these nuclei act as discrete feeding and satiety centers (42). Here, the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the ventromedial nucleus of the hypothalamus (VMH), the dorsomedial hypothalamic nucleus (DMH) and the lateral hypothalamus (LH) are the nuclei, which have been shown to be involved in regulation of feeding behavior (figure 1.4). Importantly, the hypothalamus has been shown to be involved in the regulation of many other body functions, e.g. the cardiovascular system, thermoregulatory- and stress responses, the regulation of drinking behavior and the autonomous nervous system (43). In addition, the hypothalamus plays an essential role in assuring the survival of the species by controlling the expression of sexual and maternal behaviors. Of fundamental importance to the function of the hypothalamus is its close connection with the pituitary gland and the now clearly established pathways for neural control of endocrine secretion patterns (44, 45).

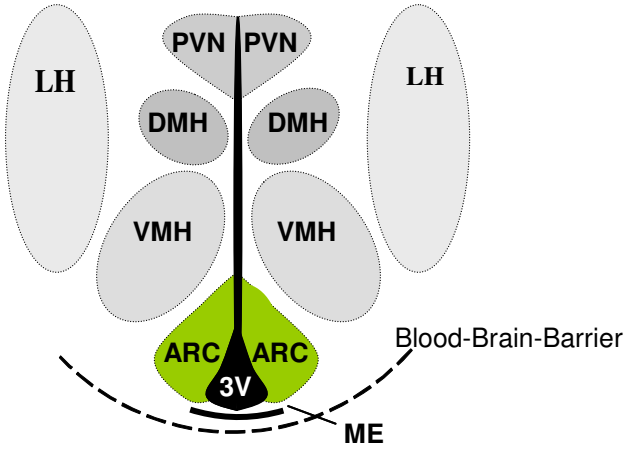

Figure 1.4: Schematic anatomical structure of the hypothalamus.
Diagram of the mediobasal hypothalamus, showing major hypothalamic regions implicated in regulation of food intake and energy expenditure. Abbreviations: 3V, third ventricle; ARC, arcuate nucleus, DMH, dorsomedial hypothalamic nucleus; LH, lateral hypothalamus; ME, median eminence; VMH, ventromedial nucleus of the hypothalamus; PVN, paraventricular nucleus.

The ARC is located in the mediobasal hypothalamus flanking the base of the third cerebral ventricle right above the median eminence (figure 1.4), which is part of the inferior boundary of the hypothalamus. In the CNS, there are discrete areas localized in the ventricular walls, known as circumventricular organs (CVO) (46), in most of which the blood-brain-barrier (BBB) displays special characteristics. The median eminence is one of the brain windows which allows peptides and proteins secreted by the neural tissue to reach the blood stream, and enables neural cells to sense circulating plasma molecules, including insulin and leptin (47-49). The PVN is located at the dorsal end of the third ventricle and represents the hypothalamic part where numerous neuronal pathways involved in the regulation of energy balance converge, including projections from the ARC (41). Lesions of the PVN as well as the VMH, which is located directly above the ARC, results in severe hyperphagia and obesity (50, 51). The DMH is located dorsal of the VMH and was suggested to integrate information from the ARC, the PVN, and the LH (52). The LH, a very large and heterogenous area, is the most extensively interconnected area of the hypothalamus, thereby allowing it to modulate different functions, including cognitive and autonomic functions (43).

5.5.2. POMC, the melanocortin system and the regulation of energy homeostasis

The melanocortin model is of great importance explaining the neuronal control of energy balance (5, 53). In this model, the ARC is considered a critical region for diverse reasons. Two functionally opposing neuronal populations have been studied in great detail: the orexigenic agouti related peptide / neuropeptide Y (AgRP/NPY)-expressing and the anorexigenic pro-opiomelanocortin (POMC)-expressing neurons (figure 1.5). AgRP and NPY are anabolic neuropeptides that stimulate food intake and reduce energy expenditure (54-57), while the catabolic neuropeptide POMC suppresses food intake and increases energy expenditure (58).

The orexigenic neuropeptide NPY, a 36 amino acid peptide, is one of the most abundant neurotransmitters (59, 60). NPY is widely expressed throughout the brain with highest concentration in the ARC (61). It is the most potent endogenous orexigenic signal, stimulates food intake, reduces energy expenditure (55, 56, 62), and centrally administered, NPY promotes positive energy balance resulting in obesity (56).

The orexigenic neurotransmitter AgRP consists of 132-amino acids. It is co-expressed with NPY in a distinct neuronal population of the ARC and functions as a melanocortin receptor 3/4 (MC3R/MC4R) antagonist. The adrenal gland is the only peripheral tissue with detectable AgRP expression (63). AgRP mRNA levels in the ARC are increased upon fasting (64), and reduction of hypothalamic AgRP mRNA results in increased metabolic rate and reduction of body weight without effecting food intake (65). Transgenic mice with ubiquitous overexpression of AgRP are obese (63). Central injection of AgRP increases food intake and is also capable of blocking intracerebroventricular (icv) α-melanocyte stimulating hormone (MSH) stimulated food intake (54, 66, 67).

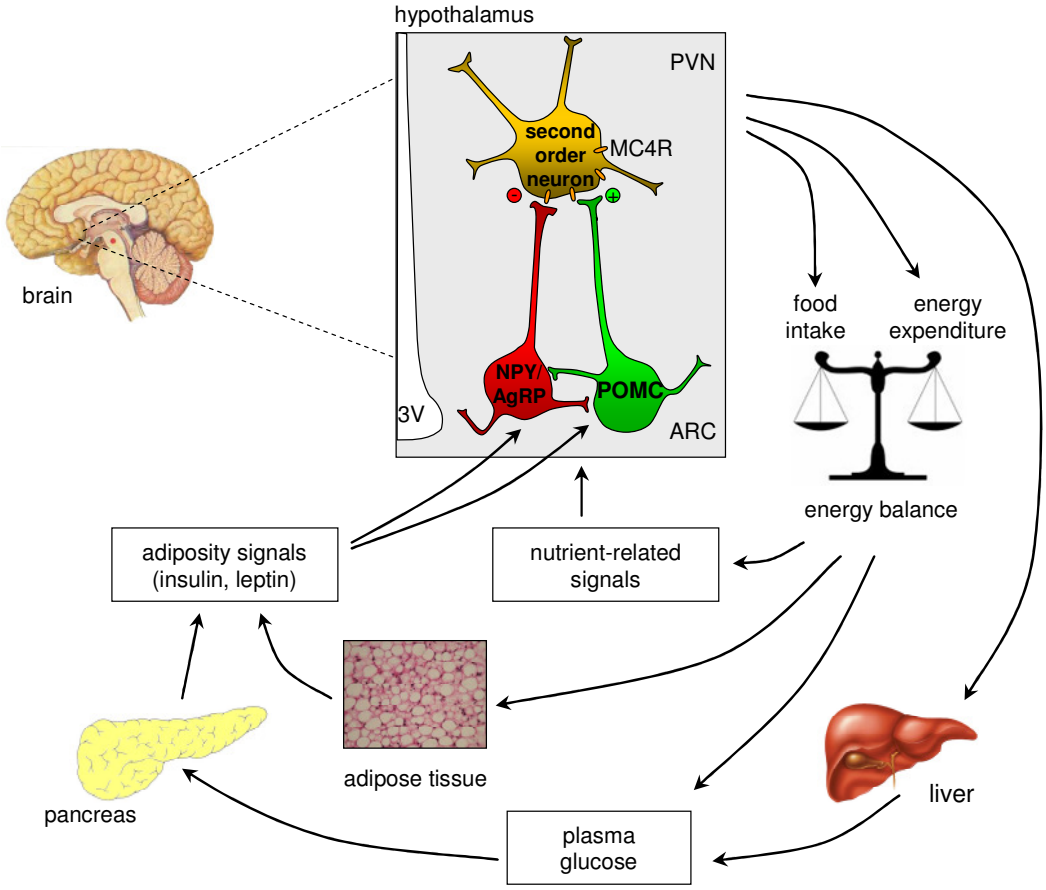

Figure 1.5: Central regulation of energy homeostasis.

The ARC contains both NPY/AgRP and POMC neurons, which are located close to the BBB, where they have preferential access to peripheral humoral signals such as the pancreas-derived hormone insulin and the adipocyte-secreted hormone leptin as well as nutritional signals such as glucose and free fatty acids. Both neuronal populations exert potent effects on energy balance mediated by their characteristic neuropeptides, which allow modulation of second order neurons. POMC neurons project predominantly to second order neurons in the PVN of the hypothalamus, where the POMC cleavage product α-MSH acts on MC4R to suppress food intake. AgRP serves as an inverse agonist for the MC4 receptor, thus counteracting the function of α-MSH. Abbreviations: α-MSH, α-melanocyte-stimulating hormone; AgRP, agouti-related peptide; ARC, arcuate nucleus; MC4R, melanocortin receptor 4; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PVN, paraventricular nucleus. [Adapted from Schwartz MW, Porte D (68)]

While neonatal ablation of AgRP/NPY neurons has negligible impact on feeding, selective ablation of AgRP/NPY neurons in adult mice results in acute reduction of food intake (69, 70).

The anorexigenic 32 kDa prohormone, POMC, is expressed in the pituitary gland, skin and hypothalamus (71-73). In a tissue-specific manner it is processed by prohormone convertases (PC) to different bioactive products including adreno-corticotropin (ACTH), β-endorphin and α-, β-, and γ-MSH (74, 75). The energy status of the body is reflected by POMC mRNA levels, as POMC gene expression is reduced in negative energy balance and increased in positive energy balance (64, 76, 77). The brain expresses two types of MCR, MC3R and MC4R (78, 79). Both receptor types are highly expressed in regions of the brain established to be important in the control of food intake and body weight (79, 80). MC4R knockout-mice develop a maturity-onset obesity syndrome associated with increased food intake, hyperinsulinemia, hyperglycemia, and an increase in linear growth (81). Whereas ACTH is the predominant POMC product secreted by corticotroph cells in the pituitary, the principal identified agonist to MCRs expressed in the brain is α-MSH (82). Icv injection of α-MSH reduces food intake and increases energy expenditure (58), and mice lacking the POMC-derived peptides are obese (83).

Both, AgRP/NPY and POMC neurons express insulin and leptin receptors and are targeted by the respective hormones (84, 85), and large body of evidence has revealed an important role for leptin-activated STAT3 signaling in the CNS and POMC/AgRP neurons specifically in control of energy homeostasis. Neuron-specific disruption of STAT3 results in hyperphagia, obesity, diabetes and infertility (86). Along this line, mice with disruption of STAT3 specifically in leptin receptor-expressing neurons develop profound obesity (87). Lack of leptin receptor signaling or inactivation of STAT3 specifically in POMC neurons results in obesity and other elements of the metabolic syndrome in rodents (88, 89), indicating a role for STAT3 signaling in POMC/AgRP neurons for maintaining normal energy homeostasis Deletion of STAT3 in AgRP/NPY neurons results in modest weight gain accompanied by mild hyperphagia (90). Interestingly, mice with a constitutive activation of STAT3 signaling specifically in AgRP neurons are lean due to increased locomotor activity (185), assigning AgRP neurons and specifically STAT3 signaling a role in control of locomotor activity. Administration of leptin to the ARC stimulates expression of POMC (85, 91), and inhibits expression of AgRP and NPY (92, 93). Moreover,

administration of a MC4R antagonist attenuates the anorexigenic response to leptin (94).

A major target site for leptin signaling is the ARC of the hypothalamus. Aside from the DMH and the VMH, the highest levels of Ob-Rb expression are found in the ARC (32). Both AgRP/NPY and POMC neurons within the ARC express the long form of the leptin receptor (95, 96) and are directly regulated by leptin. While the orexigenic AgRP/NPY-producing neurons are inhibited by leptin (97, 98), the anorexigenic POMC neurons are activated (97, 99). Thus, leptin stimulates the production and secretion of anorexigenic neuropeptides and reciprocally suppresses levels of orexigenic peptides. Consistently, ob/ob and db/db mice exhibit elevated levels of AgRP and NPY mRNA and reduced levels of POMC mRNA (76, 100, 101). Administration of leptin stimulates expression of POMC (85, 91) and inhibits expression of AgRP and NPY (92, 93). Moreover, administration of a MC4R antagonist attenuates the anorexigenic response of leptin (94). It has been shown that leptin increases the frequency of action potentials in POMC neurons by depolarization through a non-specific cation channel and reduced inhibition by local NPY neurons (99).

Both AgRP/NPY and POMC neurons coexpress the insulin- and the leptin receptor (102, 103). Insulin signals to the ARC of the hypothalamus to inhibit orexigenic AgRP/NPY neurons and to stimulate anorexigenic POMC neurons (5, 102, 104, 105). Central administration of insulin results in increased POMC and decreased NPY expression, without affecting the expression of AgRP (92) Recently, it was shown that insulin signaling in AgRP neurons is required for insulin's ability to suppress hepatic glucose production (106).

Besides the ability of the CNS to integrate information about the body energy status derived from hormones like insulin and leptin, the hypothalamus can also respond directly to circulating concentrations of nutrients, in particular of glucose and free fatty acids. Central administration of glucose decreases blood glucose levels (107) and central administration of oleic acid inhibits food intake by inhibiting NPY gene expression (108).

5.6. The hypothalamic-pituitary-adrenal axis

The Hypothalamic-Pituitary-Adrenal (HPA) axis is complex system of direct influences and feedback interactions among the PVN of the hypothalamus, the anterior lobe of the pituitary gland and the adrenal glands. It is a major part of the neuroendocrine system that controls reactions to stress, and regulates multiple body processes including the immune system, energy storage and expenditure and sexuality. Corticotrophin-releasing hormone (CRH) synthesized and released by the PVN is the principal regulator of anterior pituitary corticotroph ACTH secretion (109). Interleukin-2, interferons, and the gp130 cytokine family participate in ACTH regulation and mediate the immuno-neuroendocrine interface. Pituitary corticotroph POMC expression is regulated by ;CRH as well as the gp130 receptor cytokine family (110, 111). Gp130 cytokines activate the HPA axis even in the absence of CRH (112-114). ACTH stimulates adrenal glucocorticoid release that in turn acts as a negative feedback on CRH and ACTH production.

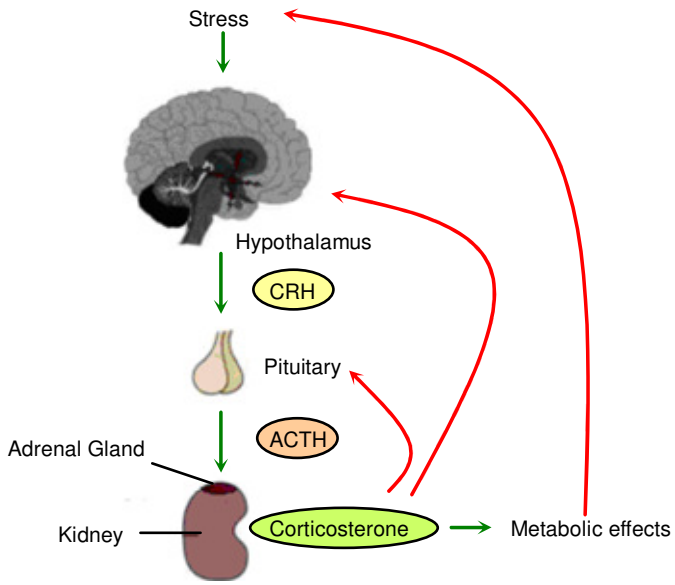

Figure 1.6: The HPA-axis

During inflammation and stressors that induce systemic cytokine production, CRH production by the PVN of the hypothalamus leads to POMC expression and ACTH release in the pituitary gland. ACTH stimulates adrenal corticosterone production, leading to a negative feedback cycle [red arrows = negative regulation, green arrows = positive regulation]. Abbreviations: CRH; corticotrophin releasing hormone, ACTH; adrenocorticotropin.

5.7. Gp130 and cytokine signaling

5.7.1. Cytokines

Pleiotropy and redundancy in biological activities are characteristic features of most cytokines (115, 116). As soluble mediators acting at low concentrations (nano- to pico-molar) they play an important role in the communication between cells. Unlike hormones, cytokines are not stored in glands, but are rapidly synthesized and secreted by different cells predominantly after stimulation. They exert their biological functions through specific cell surface receptors on their target cells. Cytokines have been classified on the basis of molecular cloning and structural analysis (117, 118) Furthermore, molecular and structural analysis of the related receptors accounted for the classification of different cytokine families (119).

5.7.2. The interleukin-6-type cytokine family

Originally, IL-6 was identified as a B cell differentiation factor, inducing B cell maturation to antibody forming plasma cells (120). However, cloning of IL-6 revealed that IL-6 is a typical multifunctional cytokine that affects not only immune response but also the hematopoietic, endocrine, hepatic and even the neural system (116, 121-124). IL-6 is closely related to a group of other cytokines, such as interleukin-11 (IL-11), oncostatin M (OSM), leukaemia inhibitory factor (LIF), cardiotrophin (CT)-1 and ciliary neurotrophic factor (CNTF), all of them exhibit a similar helical structure characterized by four antiparallel α-helices (119).

The so called “IL-6-type cytokines” reveal not only a redundancy in biological activities but furthermore, all members of this family signal via receptors with common structural features, comprising the class I cytokine receptor family (125-130).

5.7.3. Class I cytokine receptors and gP130

The receptors involved in IL-6-type cytokine signaling are type I membrane proteins (extracellular N-terminus, one transmembrane domain), with the exception of the CNTF receptor (CNTFR), which is linked to the plasma membrane by a glycosyl-phosphatidyl inositol anchor (128). All class I cytokine receptors are defined by the presence of at least one cytokine-binding molecule consisting of two fibronectin–

type-III-like domains of which the N-terminal domain contains a set of four conserved cysteine residues and the C-terminal domain a WSXWS (tryptophan-serine-X-tryptophan-serine) motif (119).

Molecular cloning of the IL-6 receptor (IL-6R) revealed that the IL-6R complex consists of two components. First, an IL-6 binding molecule termed IL-6Rα, a relatively short cytoplasmatic region containing only 82 amino acids, and second the signal transducing 130-kDa membrane gp130 which has no IL-6-binding capacity by itself but plays a critical role in the formation of high-affinity IL-6-binding sites (131, 132). Other receptors for IL-11, OSM, LIF, CT-1 and CNTF were molecularly cloned and revealed a structure similar to the IL-6Rα (figure 1.2) (119, 133).

The functional redundancy of the IL-6 family of cytokines can be explained by the molecular biology of the cytokine receptor system. All class-I-receptor complexes share gp130 as a common signal transducer, but each cytokine requires a private ligand-specific receptor. IL-6, IL-11, and CNTF first bind specifically to their α receptor subunits (IL-6Rα, IL-11Rα, and CNTFRα, respectively) before the signaling receptor subunits are engaged. Also, an α-subunit for CT-1 has been postulated, but since it has not been cloned yet, its existence is uncertain (134). The complexes between IL-6 and IL-6Rα, and between IL-11 and IL-11Rα signal through gp130 homodimers; the complex of CNTF and CNTFRα signals through a heterodimer of gp130 and the LIFR, another signal-transducing receptor subunit. The cytokines LIF and OSM do not require binding to non-signaling α receptors; they engage the signaling receptor subunits directly. LIF uses a heterodimer of gp130 and LIFR to transduce signals. Human OSM has the exceptional capability to recruit two different receptor complexes it forms both, LIFR/gp130 and OSM-specific receptor component (OSMR)/gp130 heterodimers (135) (figure 1.2).

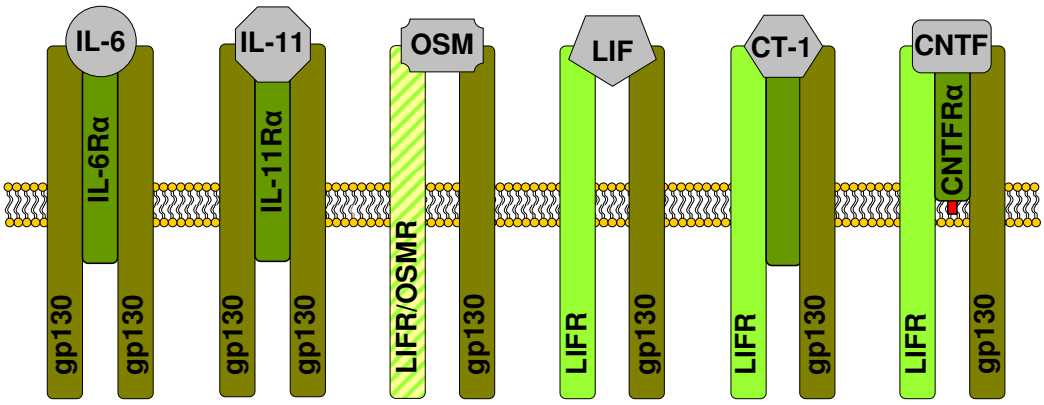

Figure 1.2: Class I cytokine receptors.

Schematic models of the receptors for IL-6, IL-11, OSM, LIF, CT-1 and CNTF. IL-6 and IL-11 first bind specifically to their α-receptor subunits, IL-6Rα and IL-11Rα, only the complex of cytokine and α-receptor efficiently recruits gp130 for homodimerization. OSM utilizes two different heterodimers, either LIF/gp130 or OSM/gp130. LIF binds to the LIFR, which then becomes heterodimerized with gp130. The LIFR/gp130 heterodimer is further utilized for CT-1 binding and signaling. Like IL-6 and IL-11, CNTF first binds to its α-receptor subunit, CNTFRα, which is structural closely related to the extracellular region of IL-6R and is anchored to the membrane via a glycosyl-phosphatidyl-inositol linkage. Abbreviations: CNTF, ciliary neurotrophic factor; CT-1, corticotrophin-1; gp130, glycoprotein 130; IL-6, interleukin-6; IL-11, interleukin-11; LIF, leukaemia inhibitory factor; OSM, oncostatin M; R, receptor.

5.7.4. Cytokine signaling

IL-6 type cytokines utilize tyrosine kinases of the JAK family and transcription factors of the STAT family as major mediators of signal transduction. Additionally, the MAPK cascade or a signaling cascade involving PI3K/AKT can be activated by IL-6 type cytokines (135, 136).

The first event in IL-6-type cytokine signaling is the ligand-induced homo-or hetero-dimerization of signal-transducing receptor subunits resulting in activation of the associated JAKs, which phosphorylate themselves and the receptor. These phosphorylation sites serve as docking sites for the SH2 domain containing STATs, such as STAT3, and for SH2-containing proteins and adaptors that link the receptor to MAPK or PI3K/AKT.

Receptor-bound STATs, phosphorylated by JAKs, dimerize and translocate into the nucleus to regulate target gene transcription. Members of the SOCS protein family reduce receptor signaling via homologous or heterologous feedback regulation (analogous to leptin signaling, see figure 1.1).

In the event of the MAPK pathway, the activated receptor binds to the Grb2-SOS complex via its docking protein SHP2. The SOS protein then activates Ras, which in turn phosphorylates a series of MAP kinases (Raf, MEK, MAPK). MAPK enters the nucleus and subsequently phosphorylates and activates transcription factors.

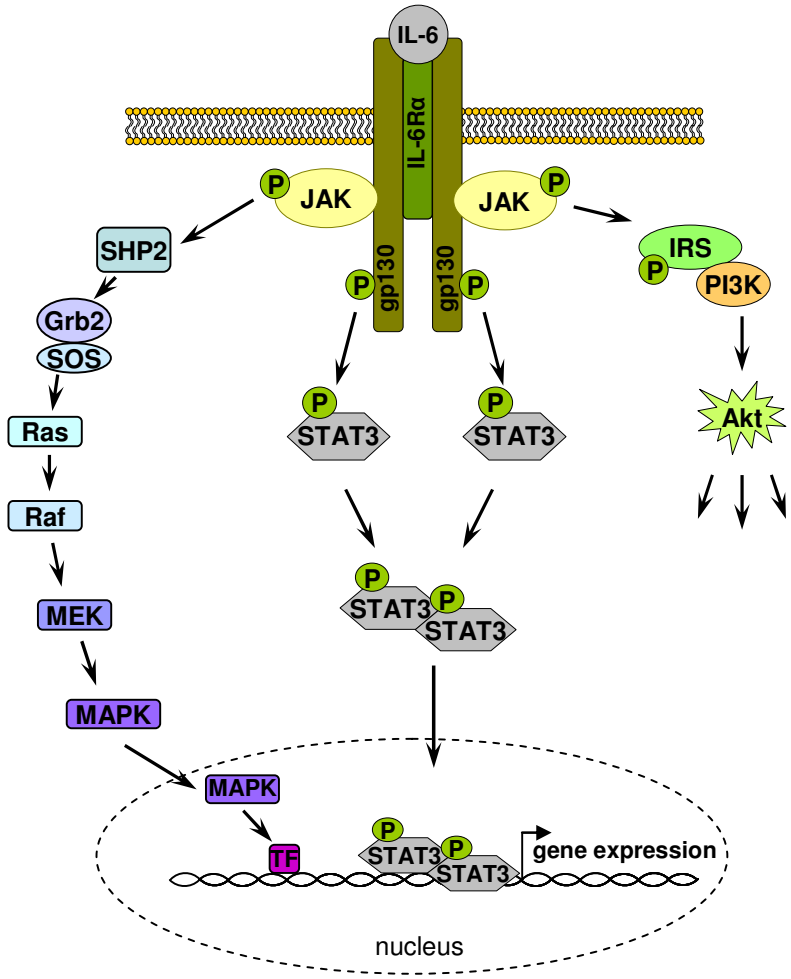

Figure 1.3: Cytokine signaling.

The figure illustrates the principles of cytokine signaling exemplified with IL-6. IL-6 binding to IL-6Rα leads to activation of JAK and subsequently to JAK-mediated phosphorylation of intracellular tyrosine residues of the receptor, resulting in the activation of different downstream signaling pathways. Phosphorylation and activation of STAT 3 molecules lead to a dimerization of STAT3 molecules. The dimers translocate to the nucleus and activate target genes. Additionally, phosphorylated JAK leads to the activation of the MAPK cascade and can also trigger the PI3K signaling pathway. Abbreviations: AKT, protein kinase B; gp130, glycoprotein 130; Grb2, growth factor receptor-bound protein 2; IL-6, interleukin-6; MAPK, mitogen-activated protein kinase; MEK, MAPK/Erk kinase; PI3K, phosphatidyl inositol 3 kinase; R, receptor; Raf, proto-oncogene serine/threonine protein kinase; Ras, small GTPase; SOS, son of sevenless; SHP2, SH2-containing phosphatase 2; TF, transcription factor.

5.8. CNTF

In 1979, CNTF, a 24 kDa protein, was first identified and partially purified as a factor which promoted survival of chick ciliary ganglion neurons (137). Subsequently, it was shown that CNTF is also present in large amounts in sciatic nerves of adult rats and rabbits, which led to its final purification and cloning (138, 139). Further studies revealed that CNTF is expressed in glial cells within the central and peripheral nervous systems. It stimulates gene expression, cell survival or differentiation in a variety of neuronal cell types such as sensory, sympathetic, ciliary and motor neurons. In addition, non-neuronal cells, such as oligodendrocytes, microglial cells, liver cells, and skeletal muscle cells, respond to exogenously administered CNTF, both *in vitro* and *in vivo* (140).

CNTF mediates its effects by binding to the CNTF-receptor-α (CNTFRα) (141). As described above, ligand binding leads to heterodimerization of gp130 and the LIFR, activating the JAK/STAT signaling pathway (128). Interestingly, the IL-6R is able to serve as an α-receptor for CNTF (142), both, CNTF and IL-6 possess a specific combination of three binding epitopes, which leads to different ligand-receptor interactions (143). CNTFRα expression is most abundant in the CNS; however, it is expressed in numerous peripheral tissues including skeletal muscle (128, 141).

CNTF was first considered as an antiobesogenic pharmaceutical, when amyotrophic lateral sclerosis (ALS) patients were treated with recombinant human CNTF (rhCNTF), in attempt to slow disease progression. The endpoint of the study, the slope of decline of isometric muscle strength in treated versus placebo patients, showed no statistically significant difference between rHCNTF and placebo-treated patients, remarkably, the side effects of rhCNTF included anorexia and weight loss (144). Since this study, numerous rodent studies have further substantiated the antiobesogenic properties of CNTF and its second-generation analog, Axokine (Axokine) (145-150).

In peripheral tissues such as skeletal muscle, CNTF upregulates AMPK activation, thereby increasing fatty acid oxidation (151). Furthermore, CNTF acts to decrease steatosis in the liver and lipid build up in skeletal muscle (147, 152). The promotion of fatty acid oxidation and lowered lipid accumulation in liver and skeletal muscle decrease the activation of serine threonine kinases (JNK and IKK) and the transcription of stearoyl-CoA desaturase (SCD)-1 in liver to improve lipid-induced insulin resistance.

5.8.1. CNTF and the CNS

In situ hybridization with cRNA probes for murine Ob-Rb and CNTFRα, revealed that both receptors are co-localized in the arcuate nucleus and the paraventricular nuclei, hypothalamic regions involved in the regulation of energy balance (148, 153). CNTF treatment of *ob/ob* mice was found to reduce adiposity, hyperphagia, and hyperinsulinemia associated with leptin deficiency. Unlike leptin, CNTF also reduced obesity-related phenotypes in *db/db* mice, and in mice with diet-induced obesity, which are partially resistant to the actions of leptin (148). Furthermore, recombinant CNTF was able to reverse the obese phenotype in leptin resistant rodent models and to prevent rebound weight gain after the end of treatment (150). Pharmacological experiments indicated that CNTF, similar to leptin is capable of suppressing NPY mRNA levels, and NPY application counteracts the weight reducing effects of CNTF (154). However, CNTF and leptin initiate differential patterns of gene expression in the ARC and CNTF possesses inflammatory properties distinct from leptin (155, 156), indicating diverging mechanisms of action and potentially differential target sites of both hormones.

5.8.2. Axokine

Axokine ($CNTF_{Ax15}$), a second-generation neurotrophic factor that is related to CNTF, was developed by Regeneron Pharmaceuticals for the potential treatment of obesity and associated complications such as diabetes mellitus type 2. Axokine is the designation for recombinant human CNTF with the following modifications: substitutions of alanine for cystein at position 17 and arginine for glutamine at position 63, and deletion of the 15 C-terminal amino acids. Axokine is four to five times more potent than the CNTF parent molecule as measured by *in vitro* neuronal survival assays and *in vivo* studies and has improved stability properties over CNTF (157-160).

5.9. Objectives

Obesity and type 2 diabetes mellitus are intimately connected diseases and their incidences are steadily increasing worldwide. Therefore, there is an urgent need for the development of therapeutically approaches to treat the obesity epidemic. CNTF exerts anorectic effects by overcoming leptin resistance via activation of hypothalamic neurons. However, the exact site of CNTF action in the hypothalamus has not been identified yet. Using Cre-loxP-mediated recombination *in vivo*, the common gp130 signal-transducing subunit, which is required for functional CNTF signaling, was ablated selectively in POMC-expressing neurons (*gp130*$^{\Delta POMC}$ mice). Characterization of *gp130*$^{\Delta POMC}$ mice elucidated the physiological role of gp130 signaling in POMC neurons, particularly on the regulation of energy homeostasis and neuropeptide expression. Additionally, the effect of exogenous CNTF on food intake and body weight was analyzed by central CNTF administration.

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6. Material and Methods

6.1. Chemicals

Table 1: Chemicals

Chemicals	Supplier
Acrylamide (Rotiphorese Gel 30®)	Roth, Karlsruhe, Germany
Actrapid® (Insulin)	Novo Nordisk, Bagsværd, Denmark
Agarose	Invitrogen, Karlsruhe, Germany
Aprotinin	Sigma-Aldrich, Seelze, Germany
APS	Sigma-Aldrich, Seelze, Germany
Avertin (2,2,2-tribromoethanol)	Sigma-Aldrich, Seelze, Germany
Benzamidin	Sigma-Aldrich, Seelze, Germany
Bromphenol Blue	Merck, Darmstadt, Germany
CNTF	R&D Systems,
DEPC	Applichem, Darmstadt, Germany
Dimethylsulfoxide (DMSO)	Merck, Darmstadt, Germany
Dithiothreitol DTT	Applichem, Darmstadt, Germany
Enhanced Chemiluminscence (ECL) Kit	Perbio Science, Bonn, Germany
Ethanol	Applichem, Darmstadt, Germany
Ethidium Bromide	Sigma-Aldrich, Seelze, Germany
Ethylendiamine tetraacetate (EDTA)	Applichem, Darmstadt, Germany
Forene® (Isoflurane)	Abbott, Wiesbaden, Germany
Glucose, 20%	DeltaSelect, Pfullingen, Germany
Glycerine	Merck, Darmstadt, Germany
Glycin	Applichem, Darmstadt, Germany
Hepes	Applichem, Darmstadt, Germany
Isopropanol	Roth, Karlsruhe, Germany
Leptin	Sigma-Aldrich, Seelze, Germany
LPS	Sigma-Aldrich, Seelze, Germany
Methanol	Roth, Karlsruhe, Germany
NaF	Applichem, Darmstadt, Germany
Paraformaldehyde (PFA)	Applichem, Darmstadt, Germany
Phenylmethylsulfonylfluoride (PMSF)	Sigma-Aldrich, Seelze, Germany
Phosphate buffered NaCl (PBS)	Gibco BRL, Eggenstein, Germany

Proteinase K	Roche, Mannheim, Germany
Sodium Azide	Merck, Darmstadt, Germany
Sodium Chloride, 0,9%	DeltaSelect, Pfullingen, Germany
Sodium Dodecyl Sulfate (SDS)	Applichem, Darmstadt, Germany
Sodium fluoride	Merck, Darmstadt, Germany
Sodium orthovanadate	Sigma-Aldrich, Seelze, Germany
Sucrose	
Trishydroxymethylaminomethane (Tris)	Applichem, Darmstadt, Germany
Triton X-100	Applichem, Darmstadt, Germany
Tween 20	Applichem, Darmstadt, Germany
Western Blocking Reagent	Roche, Karlsruhe, Germany
β-Mercaptoethanol (β-ME)	Applichem, Darmstadt, Germany

6.2. Animals

All animal procedures were conducted in compliance with protocols approved by local government authorities (Bezirksregierung Köln) and were in accordance with National Institutes of Health guidelines. Mice were housed in groups of 3-5 at 22-24°C in a 12 : 12 h light/dark cycle with lights on at 6 a.m. Animals were either fed regular chow food (Teklad Global Rodent # T.2018.R12; Harlan, Germany) containing 53.5% of carbohydrates, 18.5% of protein, and 5.5% of fat (12% of calories from fat) or a high fat containing diet (HFD) (# C1057; Altromin, Germany) containing 32.7%, 20% and 35.5% of carbohydrates, protein and fat (59.36% of calories from fat), respectively. Water was available *ad libitum* and food was only withdrawn if required for an experiment. Body weight was measured once a week. Genomic DNA was isolated from tail tips, genotyping was performed by PCR. At the end of the study period, the animals were killed in isoflurane anaesthesia.

6.2.1. Generation of PomcCre-gp130lox/lox mice

PomcCre mice (88) were mated with $gp130^{lox/lox}$ mice (161), and a breeding colony was maintained by mating $gp130^{lox/lox}$ with PomcCre-$gp130^{lox/lox}$ mice. $Gp130^{lox}$ mice had been backcrossed for at least 5 generations on a C57BL/6 background, and PomcCre mice (initially established on a FVB background) had been backcrossed for two generations on a C57BL/6 before intercrossing them with $gp130^{lox}$ mice. Only animals from the same mixed background strain generation were compared to each other. PomcCre mice were genotyped by PCR as previously described (88), $gp130^{lox}$ mice were genotyped by PCR with primers flanking the first loxP site (5gp130, 3gp130). Germline deletion was excluded using a third primer, binding ~300 bp downstream the second loxP site. For visualization of Cre-mediated recombination, mice were crossed with either the indicator strain RosaArte1 (*LacZ* reporter) (162) or the Z/EG reporter strain (163).

6.2.2. Restraint stress and LPS treatment

Mice were adapted to gentle handling for approximately 8 weeks prior to the experiment. To determine basal serum corticosterone and IL-6 levels, blood was drawn from the tail vein during the first 3 hours (h) of the light phase. The following

day, mice were subjected to 1h of restraint stress at the same time of the light phase. Restraint stress was achieved by enclosing the animals in a plastic tube with a diameter of 3 cm and openings at both ends for tail and nose. The length of the tube was adjusted to the size of the animal to ensure complete immobilization. At the end of the experiment, blood samples were again drawn from the tail vein.

Seven days later the animals were injected intraperitoneal (ip.) with 0.9%NaCl during the first 3 h of the light phase, blood was collected from the tail vein 1 h after injection. After five days of recovery, each mouse received an ip. injection of 300µg LPS (L 2630 Sigma, Germany), 1 h after injection blood samples were taken. Two days after LPS injection the animals were sacrificed in isoflurane anaesthesia.

6.2.3. Glucose and insulin tolerance tests

Glucose tolerance tests were performed with 16h fasted animals. After determination of fasted blood glucose levels, each animal received an ip. injection of 20%Glucose (10ml/kg). Blood Glucose levels were detected after 15, 30, 60 and 120 minutes (min).

Insulin tolerance tests were performed with mice fed *ad libitum*. After determination of basal blood glucose levels, each animal obtained an ip. injection of insulin, 0,75U/kg (Actrapid®). Additional blood glucose values were collected 15, 30 and 60 min after insulin injection.

6.2.4. Indirect calorimetry and food intake

All measurements were performed with a Comprehensive Laboratory Animal Monitoring System (CLAMS, Oxymax Windows 3.0.3; Columbus Instruments, OH, USA). Mice were placed at room temperature (22°-24°C) in 3.0-liter chambers of the CLAMS open circuit calorimetry. Settling time was set at 150 seconds (sec) and measuring time at 60 sec with room air as reference. Food and water were *ad libitum* provided in the appropriate devices. Mice were allowed to acclimatize in the chambers for 24 h. Parameters of indirect calorimetry were measured for at least the following 72 h. Food intake was measured continuously in the CLAMS during the experiment. Presented data are average values obtained in these recordings.

6.2.5. Icv cannula implantation

12 week old mice were anesthetized by ip. injection of Avertin® (240mg/kg) and placed in a stereotactic device. A sterile osmotic pump connector cannula (Bilany Consultants GmbH, Germany) was implanted into the lateral brain ventricle (0.2 mm posterior and 1.0 mm lateral relative to bregma and 2.3 mm below the surface of the skull). The support plate of the catheter was attached to the skull with superglue. The catheter was prefilled with sodium chloride (NaCl) and connected to a sealed micro-renathane catheter (MRE-025; Braintree Scientific, Inc., MA, USA).

6.2.6. Icv injections

After 5 days of recovery, the sealed micro-renathane catheter was removed. Over 1 min, 5µl NaCl was injected, to avoid a backflow, the catheter was sealed with hot clamps. The following day, 4µl recombinant Rat CNTF, 0.25 mg/ml (557-NT/CF, R&D Systems) were injected followed by 1µl of NaCl to assure complete drug absorption to the lateral ventricle. Injections were performed in isoflurane anaesthesia 1h before the onset of the dark cycle.

Food intake and body weight were measured 4h and 24 h (female mice) and 6h/24h (male mice) after the injection. Correct position of the icv cannula was verified in each animal by injection of methyl blue after sacrificing the mice. Only data from animals successfully injected with NaCl and CNTF was analyzed

6.2.7. Axokine treatment

Axokine was purchased from Regeneron Pharmaceuticals, Terrytown, NY. Placebo was freshly prepared every 3-4 days containing 10mM sodium phosphate, 0, 05% Tween®80, 3% PEG 3350, and 20% sucrose in ddH_2O.

After weaning, mice were fed with high fat containing diet. At the age of 10 weeks, only mice with sufficient weight gain were accustomed to daily subcutaneous (sc.) injection 1 hour before onset of the dark period with placebo for 5 days. The body weight of all groups at the beginning of the experiment averaged out between 33 and 34 g. Subsequent treatment with either Placebo or Axokine (0.1µg/kg/day) for 7 days was followed by 16 days of continuous monitoring.

6.2.8. Molecular biology

Standard methods of molecular biology were performed according to Sambrook and Russell, unless stated otherwise.

6.2.9. Isolation of genomic DNA

Mouse tail biopsies were incubated 1 hour (h) in lysis buffer (100 mM Tris-HCl (pH 8.5), 5 mM EDTA, 0.2% (w/v) SDS, 0.2 M NaCl and 500 mg/ml proteinase K) in a thermomixer (Eppendorf, Hamburg, Germany) at 56°C. DNA was then precipitated from the solution by adding an equivalent of isopropanol. After centrifugation, the DNA pellet was dried at 60°C for 10 min and resuspended in doubly distilled water (ddH2O).

6.2.10. Polymerase chain reaction (PCR)

The PCR method was used to genotype mice for the presence of floxed alleles or transgenes with customized primers listed in table 3. Reactions were performed in a Thermocycler iCycler PCR machine (Bio-Rad, München, Germany) or in a Peltier Thermal Cycler PTC-200 (MJ Research, Waltham, USA). All amplifications were performed in a total reaction volume of 25µl, containing a minimum of 50ng template DNA, 25pmol of each primer, 25µM dNTP Mix, 10 x RedTaq reaction buffer and 1 unit of RedTaq DNA Polymerase. PomcCre PCR program started with 5 min denaturation at 95°C, followed by 35 cycles consisting of denaturation at 94°C for 60 sec, annealing at 55°C for 30 sec and elongation at 72°C for 90 sec and a final elongation step at 72°C for 7 min. gp130-deletion/gp130-flox PCR program started with 4 min denaturation at 94°C, followed by 35 cycles consisting of denaturation at 94°C for 30 sec, annealing 54°C for 30 sec and elongation at 72°C for 45 sec and a final elongation step at 72°C for 7 min.

Table 2: Oligonucleotides for genotyping

Primer	Sequence (5'-3')
PomcCre AA03	GAG ATA TCT TTA ACC CTG ATC
PomcCre N57R	CAC ATA AGC TGC ATC GTT AAG
PomcCre N16R	TGG CTC AAT GTC CTT CCT GG
5gp130	GGT GGC TGA TTC ACC TGC A
3gp130	TAC GCT GGG CAG CGT CCT
gp130Δ	GAA ACA CTC ATG CTG AAA CC

All primers were purchased from Eurogentec, Cologne, Germany. PCR-amplified DNA fragments were applied to 1% - 3% (w/v) agarose gels (1 x TAE, 0.5mg/ml ethidium bromide) and electrophoresed at 120V.

6.2.11. Hypothalamic neuropeptide expression

Neuropeptide mRNA was analyzed using quantitative real-time (RT)-PCR. RNA was isolated from hypothalamic blocks respectively pituitaries using the Qiagen RNeasy Kit (Qiagen, Germany). The RNA was reversely transcribed with EuroScript Reverse Transcriptase (Eurogentec, Belgium) and amplified using TaqManR Universal PCR-Master Mix, NO AmpErase UNG with TaqManR Assay on demand kits (Applied Biosystems, CA, USA) with the exception of the detection of POMC mRNA. Customized primers were used with POMC sense 5'-GACACGTGGAAGATGCCGAG-3'; anti-sense, 5'-CAGCGAGAGGTCGAGTTTGC-3'; probe sequence, 5'-FAM-CAACCTGCTGGCTTGCATCCGG-TAMRA-3'. Relative expression of neuropeptide mRNA was determined using standard curves based on hypothalamic copy (c) DNA and samples were adjusted for total RNA content by TATA-box binding protein (TBP) RNA quantitative PCR. Calculations were performed by a comparative method (2-ddCT). Quantitative PCR was performed on an ABI-PRISM 7700 Sequence Detector (Applied Biosystems, Germany). Assays were linear over 4 orders of magnitude.

6.3. Analytical procedures

6.3.1. ELISA and RIA

Blood glucose levels were determined from whole venous blood using an automatic glucose monitor (GlucoMen® GlycÓ; A. Menarini Diagnostics, Italy). Insulin, leptin and Il-6 levels in serum were measured by ELISA using mouse standards according to manufacturer's guidelines (Mouse Leptin ELISA, Mouse/Rat Insulin ELISA; CrystalChem, IL, USA / BD OptEIA™Mouse Il-6 ELISA Kit; BD Biosciences, Pharmingen CA). Corticosterone serum levels were determined by RIA using mouse standards according to manufacturer's guidelines (ImmuChem™Double Antibody Corticosterone ^{125}I RIA Kit; INC Biomedicals, Inc., CA, USA).

6.3.2. Protein extraction

Snap-frozen tissues were thawed and homogenized in lysis buffer (50mM HEPES (pH 7.4), 1% Triton X-100, 0.1M Sodiumfluoride, 10mM EDTA, 50mM Sodiumchloride, 10mM Sodiumorthovanadate, 0,1% SDS, 10µg/ml Aprotinin, 2mM Benzamidine, 2mM PMSF) using a polytron homogenizer (IKA Werke, Staufen, Germany). Particulate matter was removed by centrifugation for 1h at 4°C. The supernatant was transferred to a fresh vial and protein concentrations were determined using a Bradford assay. Protein extracts were diluted to 1mg/ml with lysis buffer and 4 x SDS sample buffer (125mM Tris-HCl (pH 6.8), 5% SDS, 43.5% glycerine, 100mM DTT, and 0.02% bromophenol blue), incubated at 95°C over 5min and stored at -80°C.

6.3.3. Western blot analysis

Frozen protein extracts were thawed at 95°C for 5min, then separated on 8-10% (v/v) SDS polyacrylamide gels and blotted onto PVDF membranes (Bio-Rad, München, Germany). Membranes were then incubated with 1% blocking reagent (Roche, Mannheim, Germany) for 1h at RT or over night at 4°C. Subsequently, primary antibodies (gp130 (# sc-656, Santa Cruz Biotechnology, Inc., CA, USA) and Pten (# MS-1250-P; NeoMarkers, CA, USA) as loading control) diluted in 0.5% blocking solution were applied for 1h at RT or over night at 4°C. PVDF membranes

were then washed twice for 10min with 1 x TBS/Tween and incubated twice for 10min with 0.5% blocking solution. After 1h incubation at RT with the respective secondary antibodies, membranes were washed 4 times for 5min with 1 x TBS/Tween, incubated for 1min in Pierce ECL Western Blotting Substrate (Perbio Science, Bonn, Germany), sealed in a plastic bag and exposed to chemiluminescence film (Amersham, Braunschweig, Germany) at -80°C. Films were developed in an automatic developer.

6.4. Immunohistochemistry

6.4.1. Intraveneous stimulation and tissue preparation

Control and PomcCre-GP130$^{lox/lox}$ mice were mated with RosaArte1 reporter mice (162). At the age of 10 weeks, *ad libitum* fed mice received one intravenous injection of 5g recombinant rat CNTF, 20µg Leptin (L-3772, Sigma) or NaCl into the tail vein. 30min after injection the mice were perfused with 0.9% NaCl followed by 4% paraformaldehyde (in PBS), the brains were extracted, kept in 4% PFA over night at 4°C and stored in 20% sucrose at 4°C.

For one series, we performed double-labeling of p-Stat3 and β-galactosidase (indicating POMC-expressing neurons). Therefore, free-floating tissue sections were extensively washed to remove cryoprotectant, then pre-treated with 100% methanol pre-cooled to 20°C and incubated for 20min at room temperature in 0.3% glycine in PBS for 10min. and 0.03% SDS in PBS for 10min including PBS washes during the steps. After that, sections were blocked for 1h (3% normal donkey serum in PBS/0.4% Triton X-100/ 0.2% sodium-azide) followed by incubation of the primary antibodies rabbit anti-p-Stat3 (1:3000 diluted in blocking solution; Cell Signalling, MA, USA) and goat anti-β -galactosidase (1:3000 diluted in blocking solution; Biogenesis, UK) for 48h at 4°C. Sections were extensively washed in PBS, incubated protected from light for 2h at room temperature in anti-rabbit-Alexa488 (Molecular Probes; OR, USA) for labelling of p-Stat3 antibodies and biotinylated anti-goat antibody (West Grove, PA, USA) for detection of β-galactosidase antibodies (both at 1:200 dilution in blocking solution without sodium-azide). The biotinylated anti-goat antibody was then further labelled with Alexa568-conjugated streptavidin (1:200; Molecular Probes) for 1h in the dark at room temperature. Sections were mounted onto gelatine coated

slides, dried and coverslipped using mounting media (ProLong® Gold; Molecular Probes). Fluorescence signals were detected under a fluorescent microscope (BX-51; Olympus, NY, USA) and representative pictures of the ARC were taken with a digital colour camera (DP70; Olympus).

6.4.2. Tissue preparation without stimulation

Animals were perfused with PBS followed by 4%PFA for 5min. Brain and pituitary were separated and post-fixed in 4%PFA at 4°C over night. The following day the tissue was transferred to 20% Sucrose/PBS, after 6h the brains/pituitaries were washed in PBS, dabbed and frozen in tissue freezing medium. After cutting into 25µm sections the sections were stored in PBS-azide at 4°C until further use.

Day1: Brain sections were rinsed with PBS 2x for 10min, washed in freshly prepared 0.3%H_2O_2 for 30min and rinsed in PBS 3x for 10min. After blocking in PBT-azide w/3% donkey serum for 1h, the sections were transferred into primary antibody (Anti-GFP rabbit serum, Molecular Probes #A-6455, Invitrogen, Germany) and incubated on a shaker ~50rpm over night (prim. antibody, 1:10000, + 3% donkey serum + PBT-Azide).

Day2: Sections were washed with PBS 5x for 10min and transferred into secondary antibody (anti-rabbit IgG, # 711-065-125, Jackson ImmunoResearch, PA, USA) on a shaker ~50rpm for 1h (2nd antibody, 1:500,+ 3% donkey serum + PBT, no azide).

After incubation, the sections were rinsed with PBS, 3x for 10min, incubated in ABC (Avidin Biotin Complex-Vectastain Elite) for 1h, again washed with PBS, 2x for 10min and incubated in 0,04% DAB and 0,01% H_2O_2 ~4 min. Subsequently the sections were rinsed with PBS 2x for 10min, dried, mounted onto gelatine-coated slides and covered with glycerine.

Pituitary was stained with the same antibodies, using Tyramide Signal Amplification kit (TSA™ Fluorescence Systems, Perkin Elmer, MA, USA).

6.4.3. POMC cell counting

For quantification of β-galactosidase positive POMC cells, tissues were processed as described for pStat3/β-galactosidase double immunohistochemistry and stained for β-galactosidase. Pictures from every 4th section throughout the ARC (generally 12-13 adjacent sections bregma -1.1mm to -2.7mm) were taken and all sections were

allocated in a rostral to caudal manner to visualize the distribution of POMC neurones throughout the ARC. Using Adobe Photoshop software β-galactosidase positive neurones were counted and marked digitally to prevent multiple counts. Cell counts were performed on 3 animals per group and cell numbers represents every 4th section throughout the ARC.

The percentage of double positive cells compared to the total amount of β-galactosidase positive POMC cells after CNTF stimulation was also determined by counting.

6.4.4. Immunostainig for c-Fos

As described above, catheter implanted animals received 1h before onset of the dark phase an injection of either NaCl or recombinant rat CNTF. 6h later, the mice were anesthetised by Avertin® and perfused with NaCl followed by Somogyi-Takagi-Fixative. The brains were dissected, kept in glutaraldehyde-free fixative at 4°C for at least 2h and were stored in 0.1M phosphate buffer at 4°C until further preparation. Immunostaining for c-Fos was carried out as previously described (164).

6.5. Statistical methods

Data was analyzed for statistical significance using a two-tailed unpaired student's T-Test.

7. Results

7.1. POMC-restricted inactivation of gp130.

To investigate the role of gp130 signaling in POMC neurons, mice carrying the loxP-flanked (flox) gp130 allele (161) were crossed with mice expressing the Cre recombinase under control of the *Pomc* promoter (figure 3.1) (88).

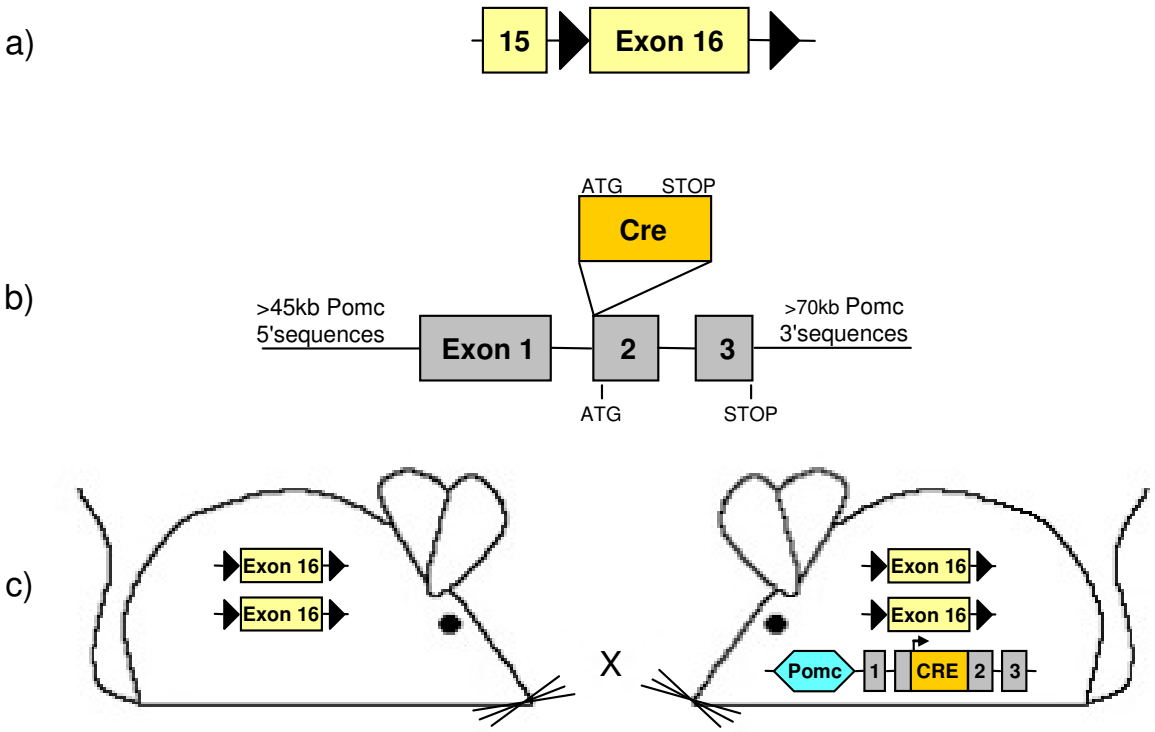
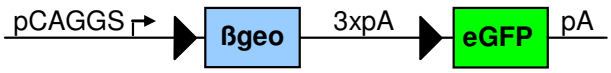


Figure 3.1: POMC-specific inactivation of the *gp130* gene.
(a) Map of the loxP-flanked *gp130* gene exon 16. Cre mediated recombination of this allele ($gp130^{lox}$) leads to removal of the transmembrane exon (exon 16) and a resulting frame shift, thereby leading to generation of $gp130^{\Delta}$. **(b)** Map of the *PomcCre* transgene. Mice expressing Cre recombinase (Cre) under the control of the *Pomc* promoter were generated by engineering a POMC bacterial artificial chromosome. The Cre translational initiation site (ATG) was inserted to the POMC ATG and deleted the first 30bp of the *Pomc* gene. **(c)** Breeding strategy for $gp130^{\Delta}$ mice: Mice, homozygous for the loxP flanked exon 16 of the gp130 allele ($gp130^{lox/lox}$) were crossed with $gp130^{lox/lox}$ mice expressing the Cre protein under the control of the *Pomc* promoter.

Cre-mediated recombination was visualized by crossing PomcCre mice to a reporter mouse strain *(Z/EG*) which expresses the enhanced green fluorescent protein (eGFP) after Cre-mediated deletion of a lox-P flanked *lacZ* gene (figure 3.2a) (163). These mice showed a pattern of eGFP immunoreactivity in the ARC of the hypothalamus similar to POMC (figure 3.2b).

a)



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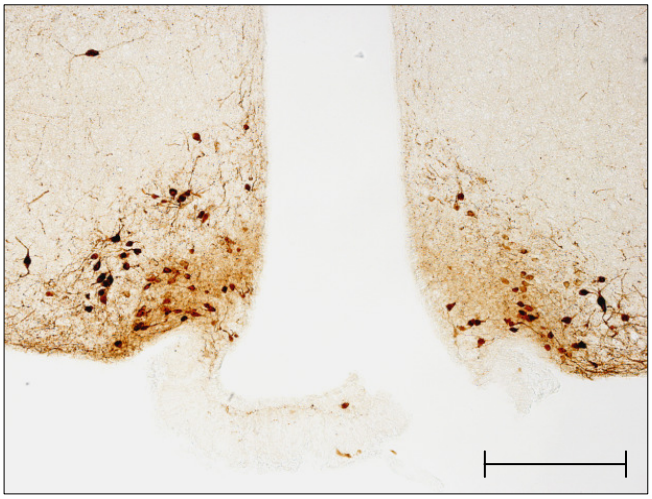

Figure 3.2: eGFP immunoreactivity pattern in the ARC of gp130$^{\Delta POMC}$ mice.

(a) Map of the Z/EG expression construct. The Z/EG transgene consists of the strong *pCAGGS* (chicken ß actin promoter with upstream CMV enhancer) promoter, directing expression of a loxP-flanked *ßgeo* (*lacZ*/neomycin-resistance) fusion gene and three SV40 polyadenylation sequences (3xpA). Followed by the coding sequence of the enhanced green fluorescence protein (eGFP) and a rabbit ß globin polyadenylation sequence (pA). In this configuration, *ßgeo* is expressed before Cre excision, and eGFP is expressed after Cre excision.

(b) Mice carrying the Z/EG reporter construct were mated with *gp130*$^{\Delta POMC}$ and immunohistochemistry for eGFP was performed in double transgenic mice (scale bar 100µm).

To confirm the specificity of gp130 inactivation, Western blot analyses were performed. Consistent with restricted inactivation in POMC neurons, Western blot analysis revealed no alterations in overall brain and hypothalamic gp130 protein expression (figure 3.3). Accordingly, gp130 expression in peripheral tissues remained unchanged in *gp130*$^{\Delta POMC}$ mice (figure 3.3).

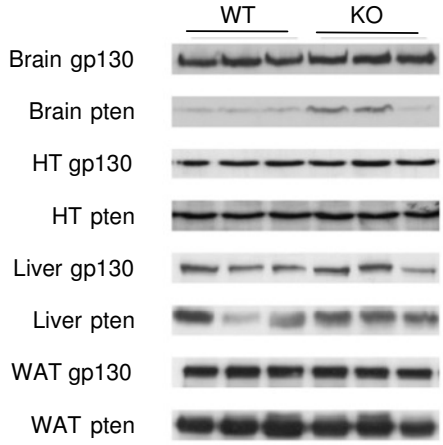

Figure 3.3: Western blot analysis of gp130 expression.

Western Blot analysis of gp130 and pten (loading control) in brain, hypothalamus (HT), liver and white adipose tissue (WAT) in wildtype (WT) and *gp130*$^{\Delta POMC}$ (KO) mice.

7.2. Abolished CNTF-stimulated STAT3 tyrosine phosphorylation in POMC cells of *gp130*$^{\Delta POMC}$ mice.

To determine the effects of a POMC-restricted gp130 deficiency on CNTF`s ability to induce STAT3 phosphorylation, wildtype and *gp130*$^{\Delta POMC}$ mice were mated to a second reporter mouse strain. In this strain transcription of the *ß-galactosidase* gene (*LacZ*) under control of the ubiquitously expressed *Rosa26* promotor is prevented by a floxed *hygromycin* resistance gene (*LacZ* reporter mice), thus leading to ß-galactosidase (ß-gal) expression only in cells expressing the Cre recombinase (figure 3.4a) (162). In wildtype reporter mice, injection of recombinant CNTF intravenously resulted in profound activation of STAT3 phosphorylation in both POMC- and non-POMC- expressing neurons of the ARC (figure 3.4b). In contrast, in *gp130*$^{\Delta POMC}$ reporter mice, CNTF treatment resulted in tyrosine phosphorylation of STAT3 in non-POMC-cells, but it failed to activate STAT3 phosphorylation in POMC neurons (figure 3.4b and c).

To determine the specificity of gp130 ablation in POMC neurons for gp130-dependent cytokine signaling, both wildtype and *gp130*$^{\Delta POMC}$ mice were treated with intravenous leptin injection.

Leptin treatment evoked a profound stimulation of STAT3 tyrosine phosphorylation in both POMC- and non-POMC- cells of wildtype and *gp130*$^{\Delta POMC}$ mice (figure 3.4b).

These data indicate, that POMC-restricted inactivation of gp130 selectively inhibits signaling via gp130-coupled cytokine receptors such as the CNTF receptor, without abolishing the gp130 independent cytokine receptor signaling such as that mediated by the leptin receptor

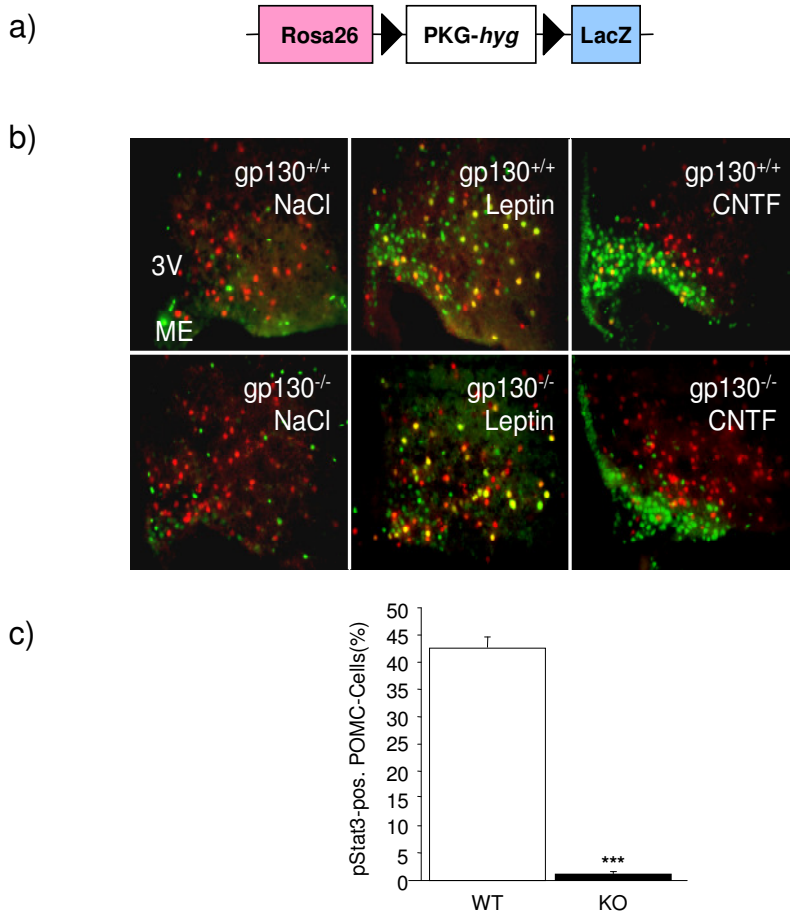

Figure 3.4: Specific ablation of gp130 signaling in POMC neurons.

(a) Map of the *LacZ* transgene for Cre-mediated expression from the *Rosa26* locus. pGK-hyg: *hygromycin resistance* gene driven by the *pGK* (*phosphoglycerate kinase*) promoter. **(b)** Double immunohistochemistry for p-STAT3 (green) and ß-gal (red) in POMC neurons of wildtype (gp130$^{+/+}$) and *gp130*$^{\Delta POMC}$ (gp130$^{-/-}$) mice 30 minutes after intravenous stimulation with NaCl, leptin or recombinant CNTF. (ME = median eminence; 3V = third ventricle) **(c)** Quantification of p-STAT3-positive POMC cells after CNTF stimulation in wildtype (WT) and *gp130*$^{\Delta POMC}$ (KO) mice (mean ± SEM of three animals in each group) ($P < 0.001$).

7.3. Unaltered POMC cell numbers and stress response in *gp130*$^{\Delta POMC}$ mice.

Gp130 has been demonstrated to play a critical role in neurogenesis (165). Therefore, it was determined whether lack in functional gp130 signaling alters the number or distribution of hypothalamic POMC cells.

This analysis revealed equal numbers and distribution of POMC cells in both wildtype and *gp130*$^{\Delta POMC}$ mice (figure 3.5). These data indicate that disrupted gp130 signaling did not affect neurogenesis and cell survival of POMC cells.

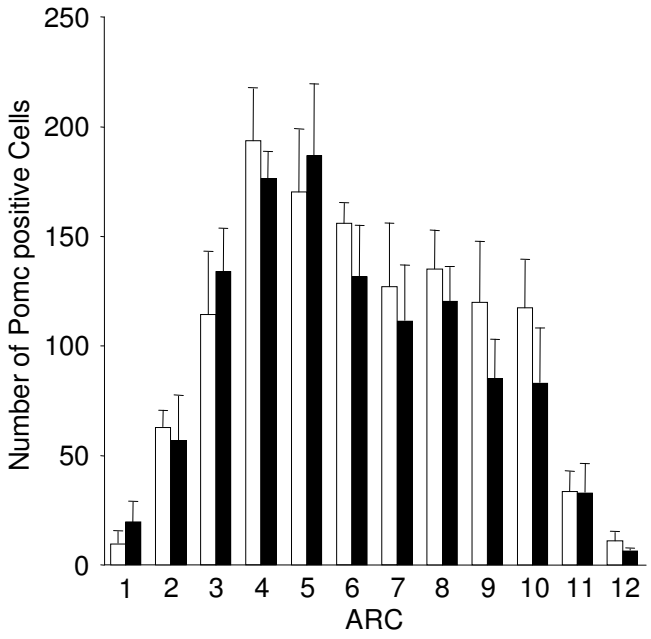

Figure 3.5: Unaltered POMC cell numbers and distribution.

Quantitative and spatial analysis of POMC cells in wildtype (open bars) and *gp130*$^{\Delta POMC}$ (filled bars) mice (n = 3 of each genotype).

Consistent with the expression pattern of endogenously expressed POMC, the only non-hypothalamic site of recombination detectable in *PomcCre*-transgenic mice was the pituitary (figure 3.6a).

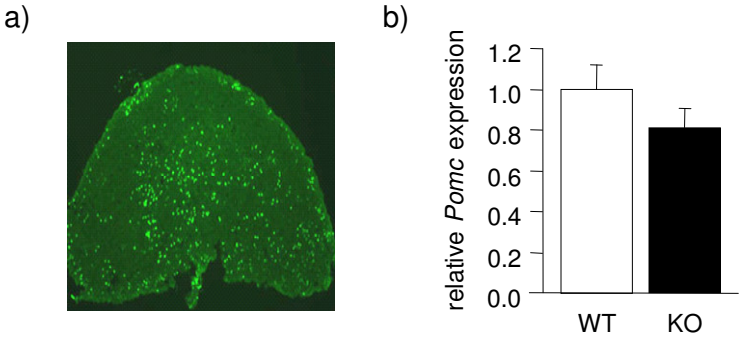

Figure 3.6: Pituitary POMC expression.

(a) Immunohistochemistry for eGFP performed in the pituitary of *LacZ* reporter mice. **(b)** Relative expression of POMC mRNA in pituitaries of wildtype (WT) and *gp130*$^{\Delta POMC}$ (KO) mice (n = 7-8).

Because cytokines that signal through gp130-dependent receptors such as leukaemia inhibiting factor (LIF) and IL-6 have been demonstrated to regulate adrenocorticotropin expression and release in cultured pituitary cells (166), we next characterized the functional stress response of *gp130*$^{\Delta POMC}$ mice.

POMC mRNA expression in the pituitary of wildtype and *gp130*$^{\Delta POMC}$ mice was comparable (figure 3.6b). To determine the stress response in wildtype and *gp130*$^{\Delta POMC}$ mice, we used two paradigms activating the corticotropic response via distinct mechanisms, i.e. restraint stress and LPS injection. Both stimuli dramatically increased plasma corticosterone concentrations to a comparable extent in wildtype and *gp130*$^{\Delta POMC}$ mice (figure 3.7a). As expected, LPS injection resulted in a profound increase of circulating IL-6 concentrations (figure 3.7b)(167). This increase was indistinguishable between wildtype and *gp130*$^{\Delta POMC}$ mice.

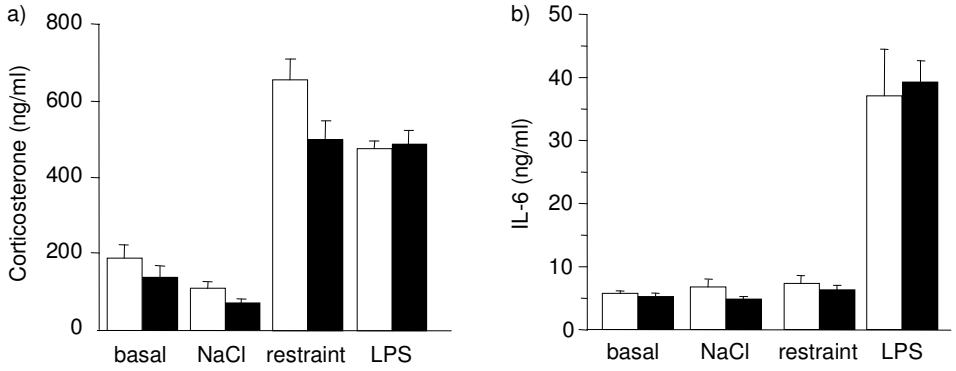

Figure 3.7: Unaltered stress response in *gp130$^{\Delta POMC}$* mice.

(a) Plasma corticosterone concentrations: basal and 1h after NaCl injection, restraint stress, or LPS treatment in wildtype (open bars; n = 6-10) and *gp130$^{\Delta POMC}$* (filled bars; n= 7-12) mice. **(b)** IL-6 plasma concentrations: basal and 1h after NaCl injection, restraint stress, or LPS treatment in wildtype (open bars; n = 6) and *gp130$^{\Delta POMC}$* (filled bars; n= 7) mice.

Taken together, these data implicate that inactivation of gp130 signaling in POMC cells of the hypothalamus and pituitary does not impair responses to restraint and LPS-induced stress in these animals.

7.4. Normal energy and glucose homeostasis in *gp130$^{\Delta POMC}$* mice.

To determine the importance of endogenous gp130-dependent signaling in POMC cells on the regulation of energy homeostasis, the body weight of wildtype and *gp130$^{\Delta POMC}$* mice from weaning until 6 month of age was monitored. This analysis revealed no change in body weight in the presence of POMC-restricted gp130 deficiency both under standard and high fat diets (figure 3.9a). White adipose tissue mass and circulating plasma leptin concentrations were indistinguishable between wildtype and *gp130$^{\Delta POMC}$* mice (figure 3.8b and c). In wildtype and *gp130$^{\Delta POMC}$* mice, there was an increase in adipose tissue mass and plasma leptin concentrations upon exposure to high–fat diet (figure 3.8b and c). Food intake and basal metabolic rate were also indistinguishable between wildtype and *gp130$^{\Delta POMC}$* mice (figure 3.8d and e). Furthermore, the expression of anorexigenic neuropeptides such as POMC and cocaine- and amphetamine-related transcript (CART) was unaltered in wildtype and *gp130$^{\Delta POMC}$* mice (figure 3.8f). Moreover, body length was indistinguishable between wildtype and *gp130$^{\Delta POMC}$* mice, a further indication of intact function of the melanocortin pathway in the absence of gp130 signaling in POMC neurons (figure 3.8g).

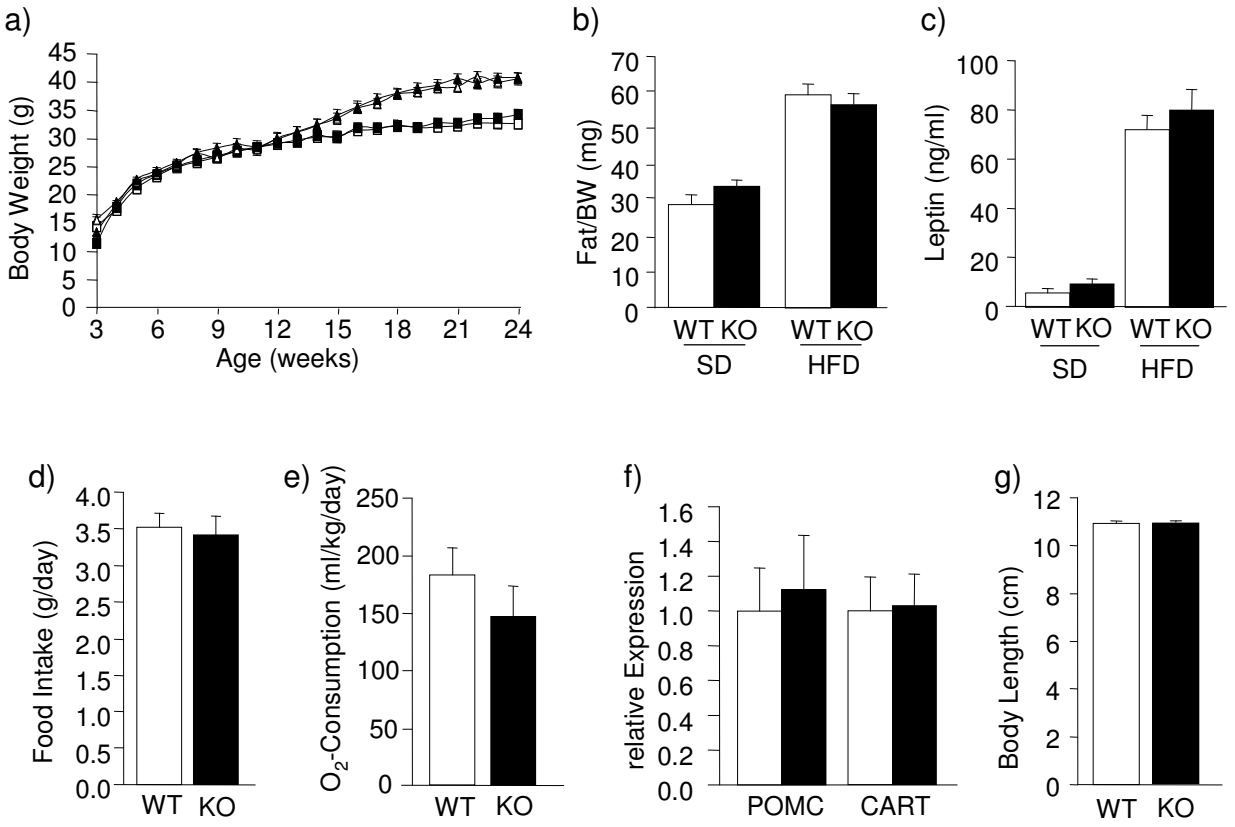

Figure 3.8: Normal energy homeostasis in *gp130$^{\Delta POMC}$* mice.

(a) Body-weight curve of wildtype (open squares and open triangles) and *gp130$^{\Delta POMC}$* (filled squares and filled triangles) mice on standard diet (SD) (squares; n = 12-18) and high fat-diet (HFD) (triangles; n = 12-31). **(b)** Epigonadal fat pads were dissected and weighed. Data represent the mean ± SEM of 14-31 mice in each group. **(c)** Serum leptin levels of wildtype (n = 10-16) and *gp130$^{\Delta POMC}$* (n = 10-16) mice on SD and HFD at the age of 24 weeks. **(d)** Daily food intake of wildtype (n = 6) and *gp130$^{\Delta POMC}$* (n = 9) mice at the age of 11 weeks. **(e)** Basal metabolic rate (BMR) of wildtype (n = 6) and *gp130$^{\Delta POMC}$* (n =9) mice at the age of 11 weeks. **(f)** Relative expression of POMC and CART on wildtype (open bars; n = 7-8)) and *gp130$^{\Delta POMC}$* (filled bars; n = 6-7) mice. **(g)** Body length of wildtype (n = 13) and *gp130$^{\Delta POMC}$* (n = 13) mice.

Consistent with unaltered energy homeostasis in *gp130$^{\Delta POMC}$* mice, these animals exhibit normal glucose metabolism as assessed by glucose tolerance tests, insulin tolerance tests, and blood glucose and plasma insulin concentrations (figure 3.9a, c, e and f). Exposure to high fat diet resulted in impaired glucose tolerance, increased blood glucose concentrations, and elevated plasma insulin concentrations as an indirect measure of insulin resistance in a similar manner in wildtype and *gp130$^{\Delta POMC}$* mice (figure 3.9b and d-f).

Taken together, these data indicate that gp130 signaling in POMC neurons is dispensable for normal wildtype of body weight, food intake, energy expenditure, and glucose metabolism.

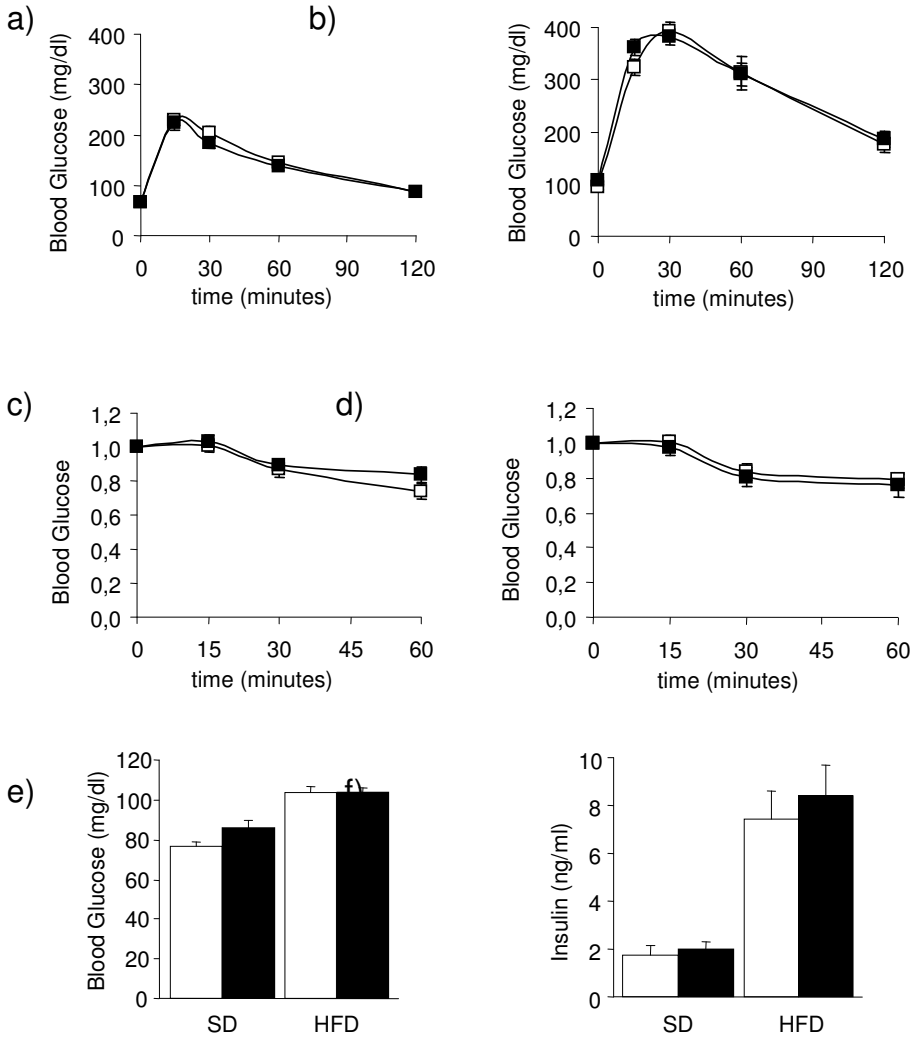

Figure 3.9: Unaltered glucose metabolism in *gp130*$^{\Delta POMC}$ mice.

(a) GTT of wildtype (□, n=10) and *gp130*$^{\Delta POMC}$ (■, n=10) mice at the age of 17 weeks on standard diet.

(b) GTT of wildtype (□, n=13) and *gp130*$^{\Delta POMC}$ (■, n=17) mice at the age of 17 weeks on high fat diet.

(c) ITT of wildtype (□, n=10) and *gp130*$^{\Delta POMC}$ (■, n=10) mice at the age of 22 weeks on standard diet.

(d) ITT of wildtype (□n=13) and *gp130*$^{\Delta POMC}$ (■, n=19) mice at the age of 22 weeks on high fat diet.

(e) Basal blood glucose levels of wildtype (white bars, n=20) and *gp130*$^{\Delta POMC}$ (black bars, n=30-32) mice on standard diet (SD) and on high fat diet (HFD) at the age of 17-22 weeks.

(f) Serum insulin levels of wildtype (white bars) and *gp130*$^{\Delta POMC}$ (black bars) mice on standard diet and on high fat diet at the age of 20 weeks (*n* = 10-13 for each group and genotype)

7.5. Blunted anorectic response to centrally injected CNTF in *gp130*$^{\Delta POMC}$ mice.

To analyze the effect of exogenous CNTF on food intake, cannulae were implanted into the lateral ventricle of wildtype and *gp130*$^{\Delta POMC}$ mice and after a few days of recovery, central injection of NaCl or CNTF was conducted.

Notably, female wildtype mice exhibited a highly significant reduction in food intake, 4h (-83%) and 24h (-43%) after central CNTF injection, compared to NaCl injection, respectively (figure 3.10a). Consequently, a delayed but highly significant reduction of body weight followed. 4h after CNTF injection, wildtype females had lost 4,6% of body weight, after 24h the loss added up to 10,2%, compared to weight loss after NaCl stimulation, respectively (figure 3.10b). Female *gp130*$^{\Delta POMC}$ mice (figure 3.10b) also exhibited a significant reduction in food intake 4h after CNTF injection, but in contrast to the more than 80% reduction in food intake in wildtype mice, food intake was only reduced by 35% in *gp130*$^{\Delta POMC}$ mice. Strikingly, 24h after CNTF injection, no significant difference in food intake after NaCl- and CNTF- injection was detectable in female *gp130*$^{\Delta POMC}$ mice (figure 3.10a). Again as a consequence of reduction of food intake, *gp130*$^{\Delta POMC}$ females exhibited a weight loss of 2,8% 4h after CNTF injection. 24h after CNTF injection they hat lost 4,4% compared to weight loss after NaCl injection (figure 3.10b). Although body weight was significantly reduced in female *gp130*$^{\Delta POMC}$ mice, the effect of centrally administered CNTF was more potent in wildtype mice.

Strikingly, however, when male wildtype and *gp130*$^{\Delta POMC}$ mice were acutely treated with CNTF: 6-h food intake after single central injection was significantly reduced by 60% in wildtype animals, whereas male *gp130*$^{\Delta POMC}$ mice failed to exhibit a significant reduction of food intake (figure 3.10c). Body weight significantly decreased by 4% in CNTF-treated wildtypes, whereas it remained unchanged in male *gp130*$^{\Delta POMC}$ mice (figure 3.10d). Taken together, CNTF rapidly and severely impaired food intake over a 24-h period in wildtype females and males. Notably, milder effects were observed in female *gp130*$^{\Delta POMC}$ mice, whereas in male *gp130*$^{\Delta POMC}$ mice the acute anorectic effect of POMC- mediated CNTF signaling was almost completely blunted.

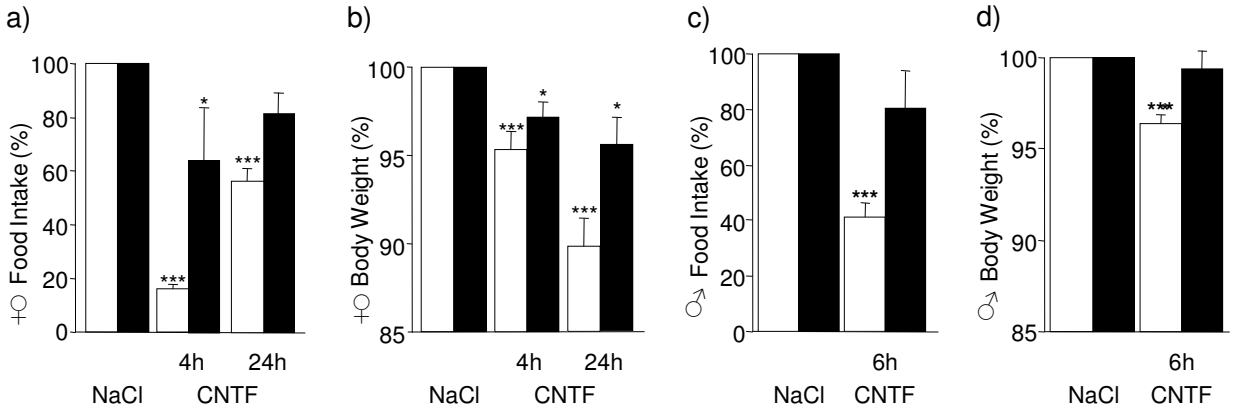

Figure 3.10: Blunted anorectic response to centrally injected CNTF in *gp130*$^{\Delta POMC}$ mice.
Central injection of either 0.9% NaCl or 1µg of recombinant rat CNTF into the lateral ventricle of 13- to 15-week-old mice. **(a)** Food intake of female wildtype (open bars) and *gp130*$^{\Delta POMC}$ (filled bars) mice, 0.9% NaCl-treated, 4 and 24h after icv CNTF injection (mean ± SEM of 9-10 animals in each group) ($P < 0.05$, $P < 0.01$). **(b)** Body weight of female wildtype (open bars) and *gp130*$^{\Delta POMC}$ (filled bars) mice, 0.9% NaCl-treated, 4 and 24h after icv. CNTF injection (mean ± SEM of 9-10 animals in each group) ($P < 0.05$). **(c)** Food intake of male wildtype (open bars) and *gp130*$^{\Delta POMC}$ (filled bars) mice, 0.9% NaCl-treated, 6h after icv CNTF injection (mean ± SEM of 6-16 animals in each group) ($P < 0.01$). **(d)** Body weight of male wildtype (open bars) and *gp130*$^{\Delta POMC}$ (filled bars) mice, 0.9% NaCl-treated, 6h after icv CNTF injection (mean ± SEM of 6-16 animals in each group) (∗: $p \leq 0{,}05$; ∗∗:$p \leq 0{,}01$; ∗∗∗:$p \leq 0.001$).

7.6. CNTF fails to induce c-Fos immunoreactivity in the PVN of g*p130*$^{\Delta POMC}$ mice.

The PVN of the hypothalamus is a major site of action of POMC efferents in the regulation of feeding (168). Therefore, the activation of PVN neurons in response to acute CNTF treatment in wildtype and *gp130*$^{\Delta POMC}$ mice was analyzed. In wildtype animals, CNTF evoked a strong induction of c-Fos immunoreactivity in the PVN (figure 3.11). Strikingly, however, CNTF treatment completely failed to enhance c-Fos immunoreactivity in the PVN of male *gp130*$^{\Delta POMC}$ mice (figure 3.11).

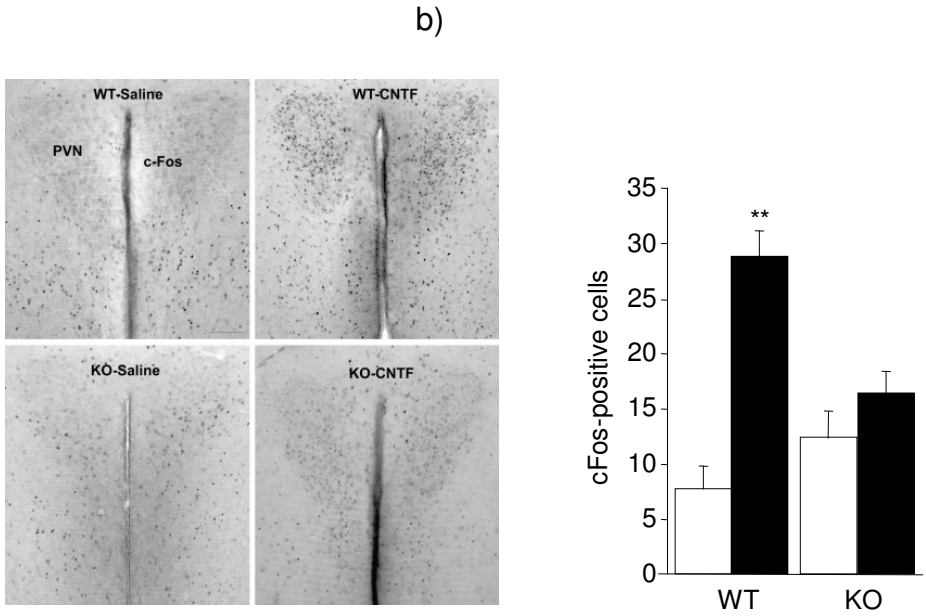

Figure 3.11: *Gp130*$^{\Delta POMC}$ mice are resistant to the acute anorectic effect of centrally injected CNTF.

(a) c-Fos expression in the PVN of wildtype and *gp130*$^{\Delta POMC}$ mice 6h after icv injection of 0.9% NaCl or CNTF. **(b)** Quantification of c-Fos positive cells in the PVN of wildtype and *gp130*$^{\Delta POMC}$ mice 6h after icv. injection of 0.9% NaCl or CNTF

Thus, CNTF application acutely and strongly activates neurons in the PVN that are characterized as important mediators of energy homeostasis regulation (168). The absence of PVN neuronal activation in response to CNTF application in *gp130*$^{\Delta POMC}$ mice clearly indicates that PVN activation critically depends on functional CNTF signaling in POMC neurons.

7.7. Reduced weight loss in *gp130*$^{\Delta POMC}$ mice after chronic Axokine treatment.

Chronic treatment with the human recombinant variant of CNTF, Axokine, reduces body weight, increases metabolic rate and improves diabetic parameters (146, 147). With the objection of extending the prior short-term experiments, a seven-day period of daily Axokine injections in wildtype and *gp130*$^{\Delta POMC}$ mice was performed, followed by 16 days of monitoring. During the injection period, additional groups of wildtype and *gp130*$^{\Delta POMC}$ mice were substituted with placebo.

As expected, the placebo group remained unaffected and showed a normal weight gain after the injection period (figure 3.12).

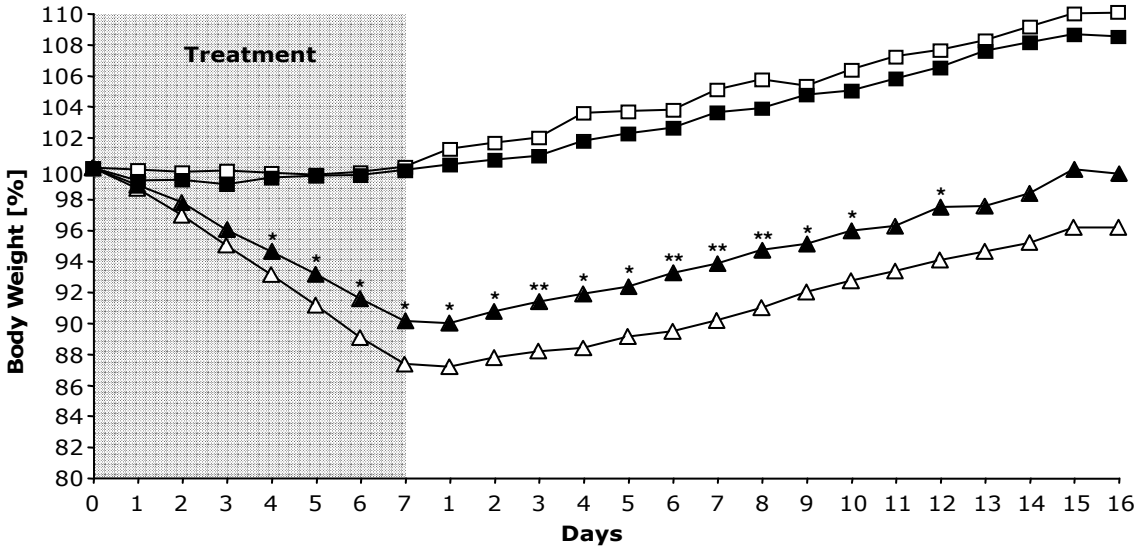

Figure 3.12:. The effect of chronic Axokine treatment is impaired in *gp130*$^{\Delta POMC}$ mice

Relative body weight changes of wildtype (open symbols) and *gp130*$^{\Delta POMC}$ mice (filled symbols).Seven days of daily Axokine injections are followed by a 16 day follow-up care. Body weight was measured daily, and is shown as the percentage difference of the initial body weight.

Placebo injected mice (squares) maintained their original body weight during the treatment and exhibited a constant weight gain afterwards. Axokine treated mice (0.1µg/kg/day, triangles) revealed a drastic weight loss during the treatment, however, *gp130*$^{\Delta POMC}$ mice lost significantly less weight compared to wildtype mice. Analog to placebo treated mice, in both, wildtype and *gp130*$^{\Delta POMC}$ mice, a subsequent weight gain followed. The body weight difference between placebo and Axokine treated mice remained constant during the follow-up care period. (□: placebo treated wildtype mice, n=16; ■: placebo treated *gp130*$^{\Delta POMC}$ mice, n=13; ∆: Axokine treated wildtype mice, n=16; ▲: Axokine treated *gp130*$^{\Delta POMC}$ mice, n=15; ∗: $p \leq 0,05$; ∗∗:$p \leq 0,01$).

In contrast, Axokine treated wildtype mice sustained a clear weight loss up to 13% during the injection period (figure 3.12). The Axokine dependent weight loss of *gp130*$^{\Delta POMC}$ mice was significantly attenuated compared to Axokine-treated wildtype mice. *Gp130*$^{\Delta POMC}$ mice only lost up to 10 % body mass. The weight difference between Axokine-treated wildtype and *gp130*$^{\Delta POMC}$ mice persisted during the 16 day follow-up care period but the relative body weight regain was comparable to the placebo treated wildtype groups.

In summary, the anorectic effect of chronic Axokine treatment is significantly diminished in animals with POMC-specific ablation of gp130 signaling.

8. Discussion

8.1. CNTF: a potential therapeutic tool for the treatment of obesity.

Obesity and type 2 diabetes are the most prevalent metabolic diseases in the western world (1). Many disorders directly correlate with obesity, including glucose intolerance, dyslipidemia and insulin resistance. Several pharmacological substances are in use to treat these disorders, unfortunately the current therapeutic strategies exhibit disadvantages and limitations, therefore the aim of research is the development of an omnipotent drug. The discovery of leptin and the leptin receptor presented a completely new idea in the therapeutic control of obesity, because it established a link between a circulating molecule and modification of central feeding behavior. After years of research, it became evident, that obesity is characterized by a dysfunctional leptin signaling leading to insulin resistance (169). Hence, the idea of leptin as an obesity therapeutic received less consideration for years and the focus changed to subjects more promising. However, recent studies demonstrate profound anti-obesity effects of combining leptin with other substances, e.g. rimonabant, a cannabinoid type 1 receptor antagonist (170), amylin a hormone co-secreted with insulin (171), or the combination of amylin and cholecystokinin a peptide involved in the control of appetite (172).

Formerly, in 1979, CNTF was identified as a factor which promoted survival of chick ciliary ganglion neurons (173, 174). In the 1990s, CNTF was considered as a promising treatment for ALS and several studies were conducted, first in animal models (175, 176) and subsequent in clinical trials (144, 177). The observation that anorexia and weight loss are side effects of CNTF treatment, lead to further rodent studies, investigating the anti-obesogenic properties of CNTF and recombinant human CNTF (Axokine). CNTF exerts anorectic effects by engaging CNS sites distinct from leptin (155) and has provided an attractive therapeutic tool for the treatment of obesity (146, 148, 150, 178).

It`s clinical use so far has been limited due to the development of neutralizing antibodies in patients treated with the recombinant variant of CNTF, although ongoing research aims to overcome this immunity (146).

Despite intense research over the years, the exact site of CNTF action in mediating its anorectic effect has yet not been identified. Expression of the CNTF receptor is

most abundant in the CNS (141), furthermore it is present in numerous peripheral tissues (128).

POMC neurons are an important primary site of leptin action in the brain and critical for energy homeostasis (76, 179, 180). Considering previous studies describing co-localization of the CNTFRα and the Ob-Rb in brain regions involved in the regulation of energy balance (85, 148), the question was raised whether POMC neurons contribute to the anorectic effects of CNTF. The CNTF signaling cascade depends on gp130 signaling molecules (136), accordingly, neuron-specific deletion of gp130 represents a functional approach for exploring CNTF`s mode of action.

8.2. POMC-specific deletion of gp130.

In order to explore the role of POMC cells in mediating CNTF`s biological effects on feeding, a POMC cell-specific deletion of gp130 (*gp130*$^{\Delta POMC}$) was generated. Therefore, mice carrying the loxP-flanked gp130 allele (161) were crossed with mice expressing the Cre recombinase under control of the *Pomc* promoter (88).

In the present study, Western Blot analysis showed no alterations in overall brain, hypothalamic and peripheral tissue expression of gp130. However, immunohistochemistry revealed an abolished STAT3 tyrosine phosphorylation in POMC cells of *gp130*$^{\Delta POMC}$ mice, providing a functional confirmation of the gp130 signaling disruption in these neurons. Nevertheless, in *gp130*$^{\Delta POMC}$ mice the pattern of STAT3 phosphorylation within the hypothalamus differed between leptin and CNTF stimulated mice. This is consistent with previous studies reporting a robust phospho (p)-STAT3 staining in the ventromedial part of the ARC after CNTF stimulation, whereas leptin-stimulated STAT3 phosphorylation was more prominent in the lateral ARC and the ventromedial hypothalamus (160). However, even though this points to a differential expression of CNTF and leptin receptors within different neuronal populations of the ARC, the functional results of this study reveal that CNTF signaling plays a critical role in POMC cells. Thus, both CNTF and leptin target at least this cell type.

Gp130 has been demonstrated to play a critical role in neurogenesis (181). In addition, Shimazaki et al. suggest, that CNTF/LIFR/gp130-mediated signaling supports the maintenance of forebrain neuronal stem cells (182). Therefore, in the present study arcuate POMC cell number in *gp130*$^{\Delta POMC}$ mice was assessed. Quantitative and spatial analysis revealed an unaltered number of POMC cells in the

arcuate nucleus, indicating that disrupted gp130 signaling in *gp130*$^{\Delta POMC}$ mice does not affect neurogenesis and cell survival of POMC neurons.

Despite from the CNS, POMC is expressed in pituitary corticotrophs, for this reason POMC expression in *gp130*$^{\Delta POMC}$ pituitary cells was visualized by immunohistochemistry, and a normal expression pattern was visible. Additionally, mRNA expression was unaltered in *gp130*$^{\Delta POMC}$ mice. Given that pituitary corticotroph POMC gene expression is amongst others regulated by members of the gp130 cytokine family (183) and gp130 cytokines activate the HPA axis even in the absence of CRH (112), systemic stress tests were conducted to evaluate the effects of the POMC specific gp130 knockout on the HPA axis. Neither endotoxic nor restraint stress revealed a significant difference between wildtype and *gp130*$^{\Delta POMC}$ mice, implicating that POMC cell dependent gp130 signaling in the pituitary is negligible in respect of the systemic stress response.

Since leptin receptor signaling in POMC neurons is required for normal body weight homeostasis (88), and administration of CNTF activates leptin-like pathways and results in reduced feeding, body weight and insulin levels in leptin-resistant mouse models (150) it is likely, that the lack of gp130 mediated CNTF signaling in POMC neurons leads to an imbalance of energy regulation. However, extensive phenotyping exhibited that the POMC-specific deficiency of gp130 has no impact on body weight, food intake, energy expenditure and glucose metabolism, both in mice fed with standard diet and mice on high fat diet, indicating that neither endogenous CNTF nor any other endogenous gp130 cytokine do influence energy homeostasis via POMC neurons.

In summary, *gp130*$^{\Delta POMC}$ mice represent a functional POMC specific gp130 knockout without effecting vital functions or energy metabolism.

8.3. Impaired anorectic response to acute central CNTF treatment and reduced body weight loss during chronic systemic Axokine treatment in *gp130*$^{\Delta POMC}$ mice.

The anorectic and weight reducing effects of CNTF previously described by multiple studies result from pharmacological doses of CNTF (146, 150, 154, 155, 160, 178, 184). The majority of these studies performed subcutaneous or intraperitoneal injections of Axokine, a recombinant variant of CNTF (150, 152, 178, 185). In these experiments, Axokine administration activates gp130 signaling in both, peripheral

organs and the CNS (150, 185). Considering the neuronal specificity of the gp130 knockout, central application of CNTF represents a more specific and direct approach. Therefore, in the present study the effect of acute central CNTF treatment was investigated in wildtype and *gp130*$^{\Delta POMC}$ mice. As expected, wildtype mice exhibited a highly significant reduction in food intake, subsequently followed by a profound decrease in body weight. Strikingly, pharmacological doses of centrally injected CNTF, resulted in a blunted anorectic response in male *gp130*$^{\Delta POMC}$ mice. There were no significant changes in food intake and body weight after central administration of CNTF, indicating that POMC-mediated gp130 signaling plays a critical role in mediating CNTF`s acute anorectic effect. Surprisingly, female *gp130*$^{\Delta POMC}$ mice revealed a significant reduction in food intake 4h after CNTF injection. Notably, this effect was less evident compared to wildtype mice and 24h after CNTF injection, no significant difference was detectable in CNTF treated animals compared to NaCl injection. Body weight reduction occurred delayed but according to food intake reduction, therefore, female *gp130*$^{\Delta POMC}$ mice exhibited a significant reduction in body weight over the 24-h period but it is worth mentioning, that the body weight reducing effect in wildtype mice was more pronounced compared to *gp130*$^{\Delta POMC}$ mice.

Even though the underlying mechanism for this sexually dimorphic phenotype remains unsolved, our data are in line with other studies using genetic mouse models of obesity (84, 186, 187). Other studies, analyzing the effect of centrally administered agents such as insulin and leptin on energy homeostasis (188) also report different phenotypes and pharmacological responses depending on gender.

A recent study reported, that estrogen-induced decrease in body weight was dependent on STAT3 activation in the brain (189), since chronic estrogen administration in a mouse model of brain STAT3 knockout had no effect on body weight. In contrast, estrogen centrally injected into the third ventricle of rats significantly reduced food intake 4-14h after treatment (189).

Furthermore, the authors demonstrated an increased number of c-Fos expressing POMC neurons in the ARC upon estrogen treatment in rats, indicating that estrogen might be able to elevate POMC tone in a leptin like manner (189, 190). Bearing in mind that estrogen exerts multiple effects on the hypothalamus including influencing energy balance (191), one could speculate, that estrogen action in some extent bypasses the absent POMC-mediated CNTF signaling in female *gp130*$^{\Delta POMC}$ mice.

Thus, CNTF signaling in arcuate cell populations other than POMC expressing cells remained unaffected in female *gp130*$^{\Delta POMC}$ mice and, central stimulation with pharmacological doses of CNTF triggers an increase in STAT3 expression. This, in turn might lead to an estrogen-mediated reduction in food intake, thereby inducing the observed weight loss. Given that male mice have lower levels of estrogen, the consequences of disrupted POMC-mediated CNTF signaling might be more pronounced. Unfortunately, the interactions between estrogen and hypothalamic cytokine signaling remain to be investigated. Therefore, further studies are necessary to elucidate the sexually dimorphic phenotypes in energy homeostasis

The PVN of the hypothalamus is a major site of action of POMC efferents in the regulation of feeding (168). Stereotaxic microinjection of either α-MSH or AgRP into the PVN alters food intake and energy expenditure (192, 193). Furthermore, animals with PVN lesions exhibit distinct hyperphagia (194). Therefore, in the present study the activation of PVN neurons in response to acute CNTF treatment was analyzed. Strikingly, however, CNTF application completely failed to enhance c-Fos immunoreactivity in the PVN of *gp130*$^{\Delta POMC}$ mice. According to this, the present study revealed that CNTF treatment acutely and strongly activates neurons in the PVN that are characterized as key mediators of energy homeostasis regulation (168). Thus, the current experiments define a functional neuronal circuit in which CNTF signaling in POMC neurons is essential for CNTF's acute inhibitory effect on food intake and the activation of PVN neurons. These results again are in line with the fact that CNTF engages similar intracellular signaling mechanisms to those activated by leptin stimulation (150, 155).

Remarkably, one unique feature of CNTF is protection from weight rebound upon termination of treatment (150, 178). Long-term observations in diet-induced obese mice revealed a persisting effect on body weight even after termination of Axokine treatment (178). Furthermore, they report that Axokine alters energy homeostasis and improves metabolic control in diet-induced obese mice (178). Therefore, it is crucial to determine whether this long-term protection also depends on functional gp130 signaling in POMC neurons. Consequently, Axokine treatment was performed over a 7-dy period in diet-obese wildtype and *gp130*$^{\Delta POMC}$ mice in anology to previous studies (147, 150, 160, 178). As expected, subcutaneously applied Axokine significantly reduced body weight in wildtype animals. In contrast, the body weight reducing effect of Axokine was impaired in g*p130*$^{\Delta POMC}$ mice. While Axokine-treated

wildtype mice sustained a clear weight loss up to 13% during the injection period, *gp130*$^{\Delta POMC}$ mice only lost up to 10 % body mass during Axokine treatment. These observations confirm the results of centrally applied CNTF, indicating that both, systemic and central administration of CNTF lead to comparable short-term effects and that peripheral action of CNTF does not further increase the overall anorectic effect of CNTF. Although a persisting weight difference between Axokine-treated wildtype and *gp130*$^{\Delta POMC}$ mice during the 16-day follow-up care period was detectable, further experiments are necessary to sufficiently assess the contribution of POMC-mediated cytokine signaling to the observed long-term protection capacities of CNTF.

A previous study described the utilization of a pair-fed group to differentiate the effects of reduced food intake versus increased energy expenditure. Interestingly, significant differences in body weight gain between the pair-fed- and the Axokine treated group indicated an effect of Axokine to increase energy expenditure (178).

Thus, to investigate whether Axokine action on POMC neurons accounts for such changes in energy expenditure and hence exerts long-term protection from weight rebound, food intake in wildtype and *gp130*$^{\Delta POMC}$ mice has to be determined, and a pair-fed group is reasonable for a precise evaluation. Furthermore, analysis in metabolic chambers might be a supplementary procedure to substantiate the achieved results.

8.4. Hypothalamic and peripheral effects of CNTF

Additional, intriguing mechanisms for CNTF action have been described. CNTF has been demonstrated to induce neurogenesis in the ARC of the adult brain (181). Importantly, this effect appeared to be necessary for protection against the above-mentioned weight rebound effects. Kokoeva *et al*. provided evidence that newly proliferated neurons exhibit either NPY or POMC expression (181). Therefore, one could speculate that CNTF's effect on neurogenesis requires gp130 signaling in adult neuronal stem cells before the newborn neurons acquire either a POMC or an NPY phenotype. Moreover, Kokoeva *et al.* demonstrated that mitotic blockade of CNTF-stimulated cell proliferation in the ARC does not affect the acute CNTF-dependent weight loss, but abrogates the long-term effect on body weight regulation. The authors suggest that the long-term effects on body weight of CNTF-treated mice

require functional neurogenesis in the ARC, whereas short-term effects of CNTF result from acute signaling in existing neurons. According to this model, the protective effect against weight rebound would be retained in *gp130*$^{\Delta POMC}$ mice, and long term-studies should be extended regarding neurogenesis and cell fate.

Additionally, another recent finding underlines the important role of hypothalamic gp130 signaling in mediating the anorectic response to CNTF. Belsham *et al*. identified GLP-1-receptor signaling as an important mechanism downstream of CNTF's central action (195). Primary neuronal cultures from the hypothalamus of GLP1-receptor-knockout mice completely lacked CNTF-mediated neurogenesis, therefore connecting the induction of CNTF-dependent neurogenesis in the hypothalamus to GLP-1 receptor signaling (195).

Furthermore, GLP-1 has been shown to be a potent activator of firing of POMC cells in the ARC (196), thus, CNTF action in neuronal populations other than POMC expressing cells could be important for an additional anorectic effect mediated by GLP1-signaling in POMC neurons.

Steinberg *et al*. recently demonstrated that an acute reduction in hypothalamic AMP-dependent kinases (AMPK) signaling by Axokine represents a potentially important mechanism in mediating Axokine effects on food intake (185). Injections of Axokine increased arcuate nucleus STAT3 activation and reduced AMPK signaling, but did not alter STAT3 phosphorylation and/or AMPK activity in the PVN, the posterior hypothalamus or cortex (185).

Furthermore, it has become evident that CNTF increases fatty-acid oxidation and reduce insulin resistance in skeletal muscle by activating AMPK (197). Intraperitoneal administration of CNTF enhanced activation of STAT3 and AMPK in skeletal muscle whereas central injection of CNTF failed to have any effect on skeletal muscle cells, indicating that CNTF can act independently of central mechanisms (197). Therefore, *gp130*$^{\Delta POMC}$ mice as well as mice with targeted disruption of gp130 signaling in other neuronal populations of the ARC and in peripheral organs will allow to define the contribution of organ-specific gp130 signaling to the improvement of glucose metabolism and sustained weight loss upon chronic CNTF treatment.

8.5. Disruption of gp130 provides insight to several signaling pathways

Gp130$^{\Delta POMC}$ mice provide an excellent tool to dissect the primary site of other cytokines acting in the CNS to regulate energy homeostasis and signaling via gp130-dependent receptor complexes.

Recently, Grossberg *et al.* also used POMC specific gp130 knockout mice to examine the contribution of POMC neurons to the anorectic effects of the proinflammatory cytokine LIF (198). Interestingly, they observed, that mice lacking gp130 specifically in POMC neurons failed to respond to centrally administered LIF. Additionally, transgenic hypothalamic explants did not release α-MSH upon LIF stimulation, indicating that POMC neurons also mediate the acute anorectic effects of centrally injected LIF, providing a mechanistic link between inflammation and food intake (198).

Furthermore, the immune-regulating and acute phase-inducing cytokine IL-6 exerts anti-obesity effects in rodents when administered centrally, possibly by increasing energy expenditure (199). Although, expression of the IL-6R has been described in the human ARC (200) and in the hypothalamus of rats (201, 202), currently, there is no evidence for arcuate IL-6R expression in mice (198). Nevertheless, to define the neuronal population mediating the anti-obesity effects of IL-6, it will be crucial to analyze the effect of centrally administered IL-6 in mice with targeted disruption of gp130 in neuronal populations expressing the IL-6R.

Overall, it is necessary to keep in mind, that the knockout of gp130 signaling bears a certain risk of misinterpretation. Thus, instead disrupting the signaling pathway of one specific cytokine for example by knocking out one particular receptor, in the current experimental approach the signaling of a whole family of cytokines is impaired. In that line of thought, it is surprising that ablation of gp130 from POMC cells has no effect on steady-state regulation of energy homeostasis, which might either indicate that signaling via gp130 (and thus signaling of this cytokine family) is not necessary for the regulation of body weight and fuel metabolism. Alternatively, since gp130 signaling is ablated upon POMC expression, which takes place around embryonic day 17 in mice, it may be the case, that compensatory mechanisms play a role here. Notably, the developing/neonatal hypothalamus still has the capacity to compensate for an anorectic tone in order to promote survival of the organism. This is in contrast to the adult hypothalamus, since ablation of POMC or AgRP neurons during adult life

leads to uncontrolled hyperphagia or hypophagia, respectively (69, 70). Thus, generation of an inducible PomcCre line might help to rule out potential compensatory mechanisms that mask the real effect.

8.6. Perspectives

The targeted disruption of gp130 signaling provides the opportunity to investigate cytokine signaling in a variety of cell populations throughout the CNS, not only in the ARC, but also in the PVN, the DMH and other hypothalamic nuclear regions, allowing for the dissection of neuronal networks targeted by different cytokines to regulate energy homeostasis and metabolism.

Furthermore, generation of an inducible PomcCre line might contribute to the differentiation of compensatory mechanisms and real effects evoked from the disrupted cytokine signaling.

Additionally, the Axokine experiment has to be extended. It is necessary to determine food intake and to include a pair-fed group. Furthermore, metabolic chambers for the evaluation of energy expenditure, and assessment of glucose metabolism before, during and after Axokine treatment will help to identify the mechanisms underlying Axokine`s weight reducing effect.

Last but not least, given that gp130 is ubiquitously expressed, not only the CNS but also peripheral organs provide interesting targets for analyzing the effect of disrupted cytokine signaling.

9. Summary

Ciliary neurotrophic factor (CNTF) exerts anorectic effects by overcoming leptin resistance via activation of hypothalamic neurons. However, the exact site of CNTF action in the hypothalamus has not yet been identified. Using Cre-loxP-mediated recombination in vivo, the common cytokine signaling chain gp130, which is required for functional CNTF signaling, was selectively inactivated in proopiomelanocortin (POMC)-expressing neurons. POMC-specific gp130 knockout mice exhibit unaltered numbers of POMC cells and normal energy homeostasis under standard and high fat diet. Endotoxin (LPS) and stress-induced anorexia and adrenocorticotropin regulation were unaffected in these animals. Strikingly, the anorectic effect of centrally administered CNTF was abolished in POMC-specific gp130 knockout mice. Correspondingly, in these animals, CNTF failed to activate STAT3 phosphorylation in POMC neurons and to induce c-Fos expression in the paraventricular nucleus. Supplemental long-term studies revealed an impaired body weight reduction in POMC-specific gp130 knockout mice. These data reveal POMC neurons as a critical site of CNTF action in mediating its anorectic and body weight reducing effect.

10. Zusammenfassung

Der ciliare neurotrophe Faktor (CNTF) übt seine anorexigene Wirkung über die Aktivierung hypothalamischer Neurone aus und ist damit sogar in der Lage, eine bestehende Leptin-Resistenz zu überwinden. Die genaue Wirkweise von CNTF an Neuronen des Hypothalamus ist bislang jedoch unklar. Um den Effekt der CNTF-Wirkung an Pro-opiomelanocortin- (POMC-) ausprägenden Neuronen des Hypothalamus zu untersuchen, wurde mithilfe des Cre-loxP-Systems in der Maus das für die CNTF-Signalkaskade notwendige Glykoprotein 130 (GP130) selektiv in POMC ausprägenden Neuronen inaktiviert. Die so entstandenen POMC-spezifischen GP130-Knockout-Mäuse wiesen sowohl unter Standardernährung als auch unter fettreicher Ernährung eine reguläre Anzahl an POMC-Zellen und normale Parameter der Energie-Homöostase auf. Die durch Endotoxin (LPS) und Stress induzierte Anorexie war in diesen Tieren ebenso wenig verändert, wie die Regulation von Adrenokortikotropin. Bemerkenswert war jedoch, dass POMC-spezifische GP130-Knockout-Mäuse nach zentraler CNTF-Gabe eine deutlich abgeschwächte Reduktion der Nahrungsaufnahme zeigten. Ebenso war CNTF in diesen Tieren nicht in der Lage, eine STAT3-Phosphorylierung zu bewirken und c-Fos Expression im paraventrikulären Nukleus hervorzurufen. Zusätzliche Langzeitstudien zeigten, dass POMC-spezifische GP130-Knockout-Tiere im Verlauf einer systemischen CNTF-Behandlung deutlich weniger Körpergewicht verlieren, als Wildtyp-Mäuse. Diese Daten verdeutlichen, dass POMC Neurone eine entscheidende Rolle in der Vermittlung der anorexigenen und Körpergewichts-reduzierenden Wirkung von CNTF spielen.

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13. Erklärung

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