DISSERTATIONES
NEUROSCIENTIAE
UNIVERSITATIS
TARTUENSIS
26

TRIIN TEKKO

Neurodevelopmental Approach in the Study of the Function of *Wfs1* and *Lsamp,* Potential Targets in the Regulation of Emotional Behaviour

DISSERTATIONES NEUROSCIENTIAE UNIVERSITATIS TARTUENSIS
26

DISSERTATIONES NEUROSCIENTIAE UNIVERSITATIS TARTUENSIS
26

TRIIN TEKKO

Neurodevelopmental Approach in the Study of the Function of *Wfs1* and *Lsamp*, Potential Targets in the Regulation of Emotional Behaviour

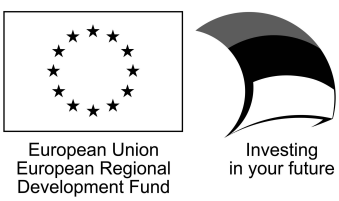
UNIVERSITY OF TARTU
Press

Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia

Dissertation was accepted for the commencement of the degree of Doctor of Philosophy (Neurosciences) on April 24th, 2018 by the Joint Council for the Curriculum of Neurosciences

Supervisors:	Kersti Lilleväli, PhD, Senior Research Fellow, Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
	Eero Vasar, MD, PhD, Professor, Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
	Mari-Anne Philips, PhD, Senior Research Fellow, Department of Physiology, Institute of Biomedicine and Translational Medicine, University of Tartu, Tartu, Estonia
Reviewers:	Tambet Tõnissoo, PhD, Associate Professor, Institute of Molecular and Cell Biology, University of Tartu, Estonia
	Kaia Palm, PhD, Associate Professor, Institute of Chemistry and Biotechnology, Tallinn University of Technology, Estonia
Opponent:	Luis Puelles, MD, PhD, Professor, Department of Human Anatomy and Psychobiology, University of Murcia, Spain
Commencement:	August 23rd, 2018

This research was supported by the European Union through the European Regional Development Fund (Project No. 2014-Q8 2020.4.01.15-0012), and by the grant from the Estonian Research Foundation (IUT 20-41).



ISSN 1736-2792
ISBN 978-9949-77-748-8 (print)
ISBN 978-9949-77-749-5 (pdf)

University of Tartu Press
www.tyk.ee

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I. **Tekko T**, Lilleväli K, Luuk H, Sütt S, Truu L, Örd T, Möls M, Vasar E. 2014. Initiation and developmental dynamics of *Wfs1* expression in the context of neural differentiation and ER stress in mouse forebrain. *Int J Dev Neurosci* 35:80–88.

II. **Tekko T**, Lakspere T, Allikalt A, End J, Kõlvart KR, Jagomäe T, Terasmaa A, Philips MA, Visnapuu T, Väärtnõu F, Gilbert SF, Rinken A, Vasar E, Lilleväli K. 2017. *Wfs1* is expressed in dopaminoceptive regions of the amniote brain and modulates levels of D1-like receptors. *PLoS One* 12(3):e0172825.

III. Philips MA, Lilleväli K, Heinla I, Luuk H, Hundahl CA, Kongi K, Vanaveski T, **Tekko T**, Innos J, Vasar E. 2015. *Lsamp* is implicated in the regulation of emotional and social behavior by use of alternative promoters in the brain. *Brain Struct Funct* 220(3):1381–1393.

The published papers used in this study have been reprinted with the permission of the copyright owners.
Contribution of the author:

I. The author participated in designing the study, performed most experiments and wrote the manuscript
II. The author participated in designing the study, performed most of the *in situ* hybridization and immunohistochemistry experiments and wrote most parts of the manuscript
III. The author performed the non-radioactive *in situ* hybridization experiments and contributed to writing the manuscript

ABBREVIATIONS

5-HT	5-hydroxytryptamine/serotonin
AC8	adenylyl cyclase 8
Acb	nucleus accumbens
ACo	core nucleus of the amygdala
aCPu	anterior caudate-putamen
AD	anterodorsal nucleus of the thalamus
ADAM a	disintegrine and metalloproteinase family of proteases
ADHD	attention deficit/hyperactivity disorder
ADo	dorsal region of the amygdala
APir	amygdalopiriform transition area
Atf4	activating transcription factor 4
Atf6	activating transcription factor 6
AV	anteroventral nucleus of the thalamus
Bdnf	brain-derived neurotrophic factor
BL	basolateral nucleus of the amygdala
BLP	basolateral nucleus of the amygdala, posterior part
BM	basomedial nucleus of the amygdala
BMP	basomedial nucleus of the amygdala, posterior part
BR	blocking reagent
BSA	bovine serum albumin
BST	bed nucleus of stria terminalis
CA	cornu ammonis region of the hippocampus
CA1	cornu ammonis 1 region of the hippocampus
cAMP	cyclic adenosine monophosphate
cDNA	complementary DNA
CeA	central nucleus of the amygdala
Chop	C/EBP-homologous protein
Cisd2	CDGSH Iron Sulphur Domain 2, same as Wfs2
CoA	cortical nucleus of the amygdala
CPu	caudate-putamen
CREB	cAMP responsive element binding protein
CRH	corticotropin-releasing hormone
D1	dopamine receptor 1
D2	dopamine receptor 2
DAT	dopamine transporter
DC	dorsal cortex
Disc1	disrupted in schizophrenia 1
DLG	dorsal lateral geniculate nucleus
DM	dorsomedial hypothalamus
Drd1a	dopamine receptor 1 a
Drd2	dopamine receptor 2
Drd3	dopamine receptor 3
Drd5	dopamine receptor 5

DVR	dorsal ventricular ridge
E	embryonic day
EA	extended amygdala
Ect	ectorhinal cortex
eIF2α	eukaryotic translation initiation factor 2, alpha subunit
Ent	entorhinal cortex
Epha2	ephrin type-A receptor 2
eQTL	expression quantitative trait locus
ER	endoplasmic reticulum
ERSE	endoplasmic reticulum stress response element
Fgfr1	fibroblast growth factor receptor 1
Fgfr2	fibroblast growth factor receptor 2
Fgfr3	fibroblast growth factor receptor 3
GABA	γ-aminobutyric acid
$GABA_A$	γ-aminobutyric acid receptor A
Gabra1	γ-aminobutyric acid receptor A, α1 subunit
Gabra2	γ-aminobutyric acid receptor A, α2 subunit
GAP43	growth associated protein 43
Glp-1	glucagon-like peptide 1
GPI	glycosylphosphatidylinositol
Grp78	78-kDa glucose regulated protein
Grp94	94-kDa glucose regulated protein
H^+ V-ATPase	vacuolar-type H^+-ATPase
Her2	human epidermal growth factor receptor 2
Her4	human epidermal growth factor receptor 4
Hprt1	hypoxanthine phosphor-ribosyl-transferase 1
Hrd1	HMG-CoA reductase degradation 1 homolog
Hsp70	70 kilodalton heat shock protein
HSP90	90 kilodalton heat shock protein
IA	intercalated amygdala
IgLON	protein family (immunoglobulin Lsamp, Obcam, neurotrimin)
InP	intrapeduncular nucleus
IP_3	inositol triphosphate
Ire1	inositol-requiring protein-1
KO	knockout
LacZ	β-galactosidase gene
LacZNeo	beta-galactosidase/neomycin fusion gene
LC	lateral cortex
LGE	lateral ganglionic eminence
LHb	lateral habenula
LP	lateral posterior thalamic nucleus
Lsamp	limbic system associated membrane protein
LSt	lateral striatum
MC	medial cortex
MeA	medial amygdala

MG	medial geniculate nucleus of the thalamus
MGE	medial ganglionic eminence
MHb	medial habenula
MSt	medial striatum
MYA	million years ago
Negr1	neuronal growth regulator 1
NF-κB	nuclear factor kappa B family of transcription factors
Ntm	neurotrimin
NTMT	0.1 M NaCl, 0.1 M Tris-HCl pH 9.5, 50 mM $MgCl_2$, 0.001% Tween-20
Opcml	opioid binding protein/cell adhesion molecule like
P	postnatal day
Pa	paraventricular nucleus of the hypothalamus
PB	sodium phosphate buffer
PBS	phosphate-buffered saline
pCPu	posterior caudate-putamen
PCR	polymerase chain reaction
PERK	protein kinase RNA (PKR)-like ER kinase
PFA	paraformaldehyde
PFC	prefrontal cortex
Pir	piriform cortex
PRh	perirhinal cortex
PT	pallial thickening
qRT-PCR	quantitative real-time PCR
RS	retrosplenial cortex
Rt	reticular nucleus of the thalamus
RyR	ryanodine receptor
SAP	stretch-attend posture
SC	superior colliculus
SDS	sodium dodecyl sulphate
SEM	standard error of the mean
Serca2	sarco/endoplasmic reticulum calcium transport ATPase 2
Smurf1 E3	ubiquitin ligase Smad ubiquitination regulatory factor 1
SNc	substantia nigra, pars compacta
SNr	substantia nigra, pars reticulata
SSC	saline sodium citrate buffer
StAm	strioamygdaloid transition area
StC	striatal capsule
StPal	striopallidal area
Syp1	synaptophysin
TBST	25 mM Tris-HCl pH 7.5; 140 mM NaCl; 2.7 mM KCl; 0.1% Tween-20
TRH	thyrotropin-releasing hormone
TTBS	25 mM Tris-HCl pH 7.5; 140 mM NaCl; 2.7 mM KCl; 0.25% Triton X-100

Tu	olfactory tubercle
TuSt	striatal part of the olfactory tubercle
TuStPal	striopallidal part of the olfactory tubercle
UPR	unfolded protein response
UTR	untranslated region
VMH	ventromedial hypothalamus
VPL	ventral posterolateral thalamic nucleus
VPM	ventral posteromedial thalamic nucleus
VTA	ventral tegmental area
Wfs1	Wolfram syndrome 1
Wfs2	Wolfram syndrome 2, same as Cisd2
WS	Wolfram syndrome
Xbp1	X-box binding protein 1
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
Zbtb20	zinc finger and BTB domain containing 20

INTRODUCTION

Emotions, cognition and memory are all higher-order brain functions with a largely shared neural substrate and sensory input. Their operation needs to be precisely orchestrated and adjusted through feedback loops in accordance to the changing environment. The formation of such feedback loops includes setting of the transcription activity of numerous genes and forming and remodelling synapses depending on the crosstalk with the environment. As the emotional circuits are among those to take the longest to develop and considering the importance of the dialogue between genetics and the environment in sculpting the emotional responsiveness of the brain, these neural networks prove the most vulnerable to detrimental factors. This is underlined by the existence of a wide range of psychiatric disorders targeting the emotional functioning of the brain, such as mood, anxiety, impulse control and substance abuse disorders as well as psychotic diseases.

The median age of onset of mental disorders falls into late childhood to early adulthood and shows a specificity across the diagnoses (Kessler et al., 2005) which indicates a strong developmental factor in the incidence of most psychiatric diseases. Adverse life events such as traumatic emotional experience or periods of sustained stress can result in the emergence of a genetically predisposed mental disease.

The distant origins of the present thesis are experiments done in early 2000s in the Institute of Physiology, University of Tartu. In these experiments, the rats were exposed to a cloth immersed in cat odour and gene expression changes were measured in the amygdala (Kõks et al., 2002; Kõks et al., 2004). To rodents, cat odour indicates the presence of a predator and initiates a fear response (File et al., 1993). In the aforementioned experiments, wolframin (*Wfs1*) and the limbic system associated membrane protein (*Lsamp*) were among those genes which had elevated expression levels in the amygdala of the cat odour-exposed rats (Kõks et al., 2002; Kõks et al., 2004). The amygdala is the central regulator of emotional behaviour and its different nuclei are involved in diverse aspects of the perception and expression of fear and anxiety (Roozendaal et al., 2009). At the time of the cat odour experiments, *Wfs1* had been identified as the causative gene of Wolfram syndrome, a rare condition characterized by the co-occurrence of diabetes mellitus, diabetes insipidus, sensorineural deafness and optic atrophy and often involving psychiatric symptoms; nevertheless, not much was known about its function. LSAMP had been identified as a member of the immunoglobulin superfamily that takes part in the circuit formation of the emotional brain. While the function in the development of the nervous system is established for LSAMP, which takes part in neurite outgrowth, axon targeting and synaptogenesis (Eagleson et al., 2003; Hashimoto et al., 2009; Keller et al., 1989; Mann et al., 1998; Pimenta et al., 1995; Zhukareva and Levitt, 1995), there are only a few studies pointing to the possible neurodevelopmental role of WFS1 (Ghirardello et al., 2014; Hershey et al., 2012). A linkage to mood and anxiety disorders, schizophrenia and suicidal behaviour has been established for

both *WFS1* and *LSAMP* (Chen et al., 2018; Furlong et al., 1999; Galfalvy et al., 2013; Koido et al., 2005; Koido et al, 2012; Koido et al., 2014; Must et al., 2009; Seifuddin et al., 2013; Sokolowski et al., 2016; Swift et al., 1991; Swift et al., 1998; Swift and Swift, 2005; Torres et al., 2001; Zalsman et al., 2009).

Since the roots of neuropsychiatric disorders often lie within brain development, the main goal of this thesis is to question during which developmental periods *Wfs1* and *Lsamp* are required and investigate the function of these genes in the developing and adult brain. First, we studied the initiation pattern of *Wfs1* expression in the mouse brain, viewing it in the setting of neural differentiation and developmental endoplasmic reticulum (ER) stress. In the mouse bain, the expression of *Wfs1* was first initiated at the embryonic day 15.5 (E15.5) and the initiation pattern roughly followed the synaptic maturation of brain structures. The widespread expression of *Wfs1* during the perinatal period was not related to the regulation of developmental ER stress. Secondly, the anatomical distribution of *Wfs1* expression in the brain was studied in a wider evolutionary context and the relation between WFS1 and D1-type dopamine receptors was investigated. The expression of *Wfs1* was evolutionarily conserved in the striatal and subpallial amygdaloid regions but showed greater variation in the pallial structures in mammalian, avian and chelonian brains. The expression pattern of *Wfs1* was notably overlapping with the expression of *Drd1a*, gene encoding the dopamine receptor D1. The ligand binding of D1-type dopamine receptors was increased in the hippocampi of *Wfs1* knockout mice. Thirdly, we studied the activity patterns of the two alternative promoters of the *Lsamp* gene in the developing and adult mouse brain and examined the behavioural outcomes related to the expression levels of the alternative transcripts. The two alternative promoters showed largely complementary activity patterns: 1a promoter was prevailing in the limbic-related structures, while the activity of 1b promoter delineated somatosensory pathways. The expression levels of 1a and 1b transcripts in different brain regions correlated with characteristics of anxiety-related and social behaviour of mice.

REVIEW OF LITERATURE

1. *Wfs1*

1.1 *Wfs1* gene and Wolfram syndrome

WFS1, or *Wolfram syndrome 1*, was named after a syndrome first described by Wolfram and Wagener in 1938 (Inoue et al., 1998; Strom et al., 1998; Wolfram and Wagener, 1938). Wolfram syndrome (WS) is an autosomal recessive disorder characterized by diabetes insipidus, juvenile-onset diabetes mellitus, optic atrophy and sensorineural deafness, hence, it is also known under acronym DIDMOAD (OMIM #222300). It is a progressive neurodegenerative disease and other symptoms often include disturbances in bladder function, ataxia, peripheral neuropathy, epilepsy, brain atrophy, cognitive impairment, and psychiatric manifestations (Chaussenot et al., 2011; Medlej et al., 2004; Rigoli et al., 2011; Urano 2016). The most common cause of death in WS patients is respiratory failure resulting from brainstem atrophy (Barrett et al., 1995). The prevalence of WS is 1/770 000 with the carrier frequency of 1/354 in the population of the United Kingdom (Barrett et al., 1995). Most of the cases of WS are caused by mutations in the *WFS1* gene, although there is another causative gene called *WFS2* or *CISD2*, mutations of which are related to a much rarer variant of WS (Amr et al., 2007; El-Shanti et al., 2000; Inoue et al., 1998; Urano, 2016). The promoter of *WFS1* gene, which is situated in 4p16.1 in the human genome (Rigoli et al., 2011), contains a sequence that resembles the endoplasmic reticulum (ER) stress response element (ERSE), a regulatory sequence for many ER-stress-activated genes (Kakiuchi et al., 2006). In compliance with this, the transcription of *Wfs1* is upregulated by ER stress (Fonseca et al., 2005; Ueda et al., 2005; Yamaguchi et al., 2004). Several ER stress pathway mediators can upregulate *Wfs1* transcription, including Xbp1, Ire1α, PERK, and ATF6β (Fonseca et al., 2005; Kakiuchi et al., 2006; Odisho et al., 2016). Importantly, a mood stabilizer valproate has been shown to induce the transcription of *WFS1* in neuronal cells (Kakiuchi et al., 2009).

A wide range of mutations and polymorphisms has been described in the *WFS1* gene (Cano et al., 2007; Chaussenot et al., 2011; Cryns et al., 2003b; Gomez-Zaera et al., 2001; Hardy et al., 1999; Khanim et al., 2001; Kytövuori et al., 2013; van den Ouweland et al., 2003; Qian et al., 2015; Rohayem et al., 2011; Yu et al., 2010). In addition to WS, some mutations of *WFS1* are shown to cause nonsyndromic autosomal dominant hearing impairment (Cano et al., 2007; De Franco et al., 2017; Young et al., 2001) and diabetes (Bonnycastle et al., 2013), and recently, three dominant mutations of *WFS1* causing co-occurrence of neonatal diabetes, sensorineural deafness, congenital cataracts and hypotonia were reported (De Franco et al., 2017). Heterozygous carriers of *WFS1* mutations are at risk to develop diabetes mellitus (Sandhu et al., 2007; Wasson and Permutt, 2008) and mental illness (Cryns et al., 2003b; Khanim et al., 2001; Koido et al., 2005; Kytövuori et al., 2013; Swift et al., 1998; Swift

and Swift, 2005). The most common psychiatric manifestations linked to polymorphisms in *WFS1* gene include mood disorders and suicidal behaviour (Furlong et al., 1999; Koido et al., 2005; Must et al., 2009; Sequeira et al., 2003; Zalsman et al., 2009). The pathologies arising from the mutations of WFS1 are probably caused by the reduced or diminished level of protein and not by accumulation of defective protein (Hofmann et al., 2003; Hofmann and Bauer, 2006). However, in case of dominant missense mutations, aggregation of mutant protein and resulting ER stress seems to lead to the disease symptoms (De Franco et al., 2017).

Wfs1 is expressed in a wide range of tissues, with the highest mRNA levels in heart, brain, spinal cord, placenta, intestine, lung and pancreas (Becker et al., 2008; Inoue et al., 1998; Strom et al., 1998). Antibody staining has revealed that the protein expression in the human tissues can vary notably depending on the developmental stage: in most tissues, strongest immunostaining of WFS1 is observable in late gestation, whereas in adult organism, WFS1 expression is reduced or diminished in many cell types (De Falco et al., 2012). Skin, respiratory system, myocardium, spleen and thymus show considerable developmental downregulation, while substantial immunostaining is found in striated muscles, smooth muscles of the gastrointestinal system, epithelia of the oesophagus and cervix, in liver, endocrine pancreas, thyroid gland, adrenal gland, kidney, and testis of adult human (De Falco et al., 2012). In mouse, high WFS1 protein levels are reported in heart, brain, pancreas, muscle and liver (Hofmann et al., 2003).

More meticulous studies on *Wfs1* expression are performed on the tissues and organ systems affected by WS: visual (Kawano et al., 2008; Schmidt-Kastner et al., 2009; Yamamoto et al., 2006) and auditory system (Cryns et al., 2003a; Suzuki et al., 2016), brain (Kawano et al., 2009; Luuk et al., 2008; Philbrook et al., 2005; Takeda et al., 2001) and pancreas (Ishihara et al., 2004; Philbrook et al., 2005). In the visual system, WFS1 protein expression is most prominent in retinal ganglion cells (Kawano et al., 2008; Schmidt-Kastner et al., 2009; Yamamoto et al., 2006). In the inner ear, WFS1 protein is expressed in different cell types bordering the scala media of the cochlea, in spiral ganglion, and in vestibular hair cells (Cryns et al., 2003a; Suzuki et al., 2016).

In rodent brain, the most distinct mRNA signal and protein expression is present in the CA1 region of the hippocampus, central nucleus of the amygdala, parasubiculum, nucleus accumbens, olfactory tubercle, piriform cortex, layer II/III of the neocortex, lateral bed nucleus of the stria terminalis, interstitial nucleus of the posterior limb of the anterior commissure, and posterior caudate putamen (Kawano et al., 2009; Luuk et al., 2008; Takeda et al., 2001). Notably, *Wfs1* mRNA and/or protein are found in numerous brainstem nuclei and in the cerebellum (Kawano et al., 2009; Luuk et al., 2008; Takeda et al., 2001), dysfunction of which might be related to the common symptoms in WS patients, e.g. sensorineural deafness, peripheral neuropathy, dysphagia, dysarthria, nystagmus, sleep anomalies, gait abnormalities and ataxia (Chaussenot et al., 2011; Medlej et al., 2004).

In this context, it is important to point out the major findings in the brains of human WS patients. Numerous studies show neurodegenerative changes that encompass brain stem, cerebellum, cerebral cortex, optic nerves, chiasm and tracts, lateral geniculate nucleus, hypothalamic paraventricular and supraoptic nuclei, and posterior pituitary (Chaussenot et al., 2011; Galluzzi et al., 1999; Genis et al., 1997; Hilson et al., 2009; Pakdemirli et al., 2005; Rando et al., 1992; Shannon et al., 1999). In addition to neurodegenerative processes, WS causes neurodevelopmental abnormalities: already in young patients, loss of myelination in main white matter tracts, reduced volume of brain stem and cerebellar white matter, and thinning of several cortical areas have been found (Hershey et al., 2012; Lugar et al., 2016).

1.2 The structure of WFS1 protein and its functions

WFS1 protein comprises of 890 amino acids that make up nine membrane-spanning segments with long hydrophilic termini (Fig. 1). It assembles into tetrameric complexes in the membrane of ER. The N-terminus is glycosylated and faces the cytosolic side of the membrane while C-terminus remains in the lumen (Hofmann et al., 2003). While it predominantly localizes in the ER membrane (Takeda et al., 2001), it has been shown to also reside in the membranes of secretory granules both in pancreatic beta cells and neuronal cells (Gharanei et al., 2013; Hatanaka et al., 2011).

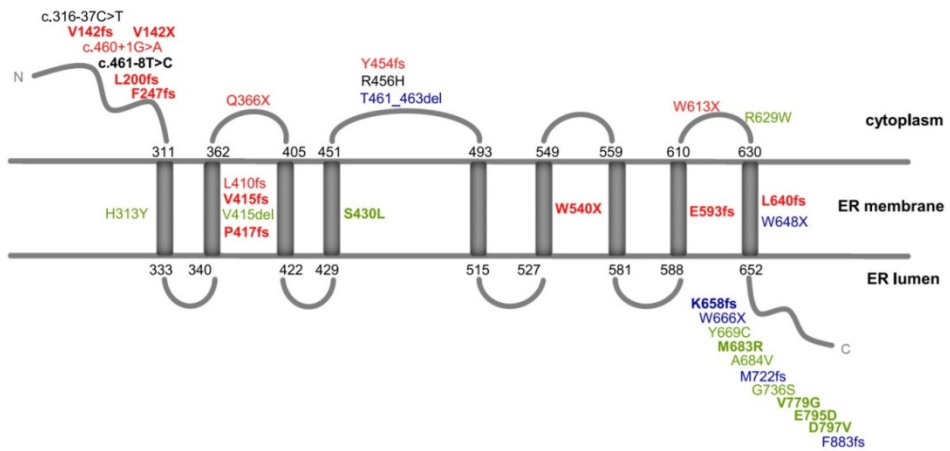


Figure 1. A scheme of human WFS1 protein with relative positions of WS-causing mutations within the polypeptide chain. Mutations are color-coded according to severity: red – complete loss of function; blue – partial loss of function; green – minor loss of function; black – unspecified. Adapted from Rohayem et al., 2011.

Several binding partners have been identified for WFS1 protein. The E3 ubiquitin ligase Smad ubiquitination regulatory factor 1 (SMURF1) interacts with WFS1 at the ER and promotes its ubiquitination and proteasomal degradation (Guo et al., 2011). Interaction between WFS1 and another E3 ubiquitin ligase HRD1 helps to stabilize the latter (Fonseca et al., 2010). GRP94, an ER resident member of the HSP90 family of molecular chaperones, is another binding partner of WFS1, and probably helps to keep WFS1 in an inactive state (Kakiuchi et al., 2009). Through its N-terminal domain, WFS1 can bind calmodulin in a calcium-dependent manner (Yurimoto et al., 2009).

In pancreatic beta cells, WFS1 downregulates unfolded protein response (UPR) by promoting the degradation of one of the key transcription factors of ER stress, ATF6α (Fonseca et al., 2010; see Fig. 2). Thus, in case of deficiency in functional WFS1, ER stress pathways are reinforced in pancreatic beta cells, which leads to apoptosis and diabetes mellitus (Ishihara et al., 2004; Riggs et al., 2005; Yamada et al., 2006). Increased UPR has also been reported in WFS1-depleted neuronal cell lines (Gharanei et al., 2013). WFS1 has been shown to interact with ß1-subunit of Na^+/K^+-ATPase and V_1A subunit of vacuolar-type H^+-ATPase (H^+ V-ATPase), probably helping to stabilize them while maturation in the ER, for the levels of both protein subunits are reduced by the depletion of WFS1 in various cell lines (Gharanei et al., 2013; Zatyka et al., 2008). Several studies show that lack of functional WFS1 is related to alterations in the activity or amount of prohormone convertases and peptide hormone processing, but the underlying mechanisms remain to be elaborated (Gabreels et al., 1998; Hatanaka et al., 2011; Tein et al., 2015).

WFS1 has been shown to modulate the filling state of ER Ca^{2+} store (Cagalinec et al., 2016; Hara et al., 2014; Takei et al., 2006) and cytoplasmic Ca^{2+} concentration (Cagalinec et al., 2016; Hara et al., 2014; Lu et al., 2014; Osman et al., 2003; Takei et al., 2006; Zatyka et al., 2015). WFS1 modulates the activity and turnover rate of sarco/endoplasmic reticulum calcium transport ATPase 2 (SERCA2), a protein serving the function of pumping Ca^{2+} ions from cytoplasm to ER (Zatyka et al., 2015). In addition, both ER membrane-resident Ca^{2+} channels – inositol 1,4,5-trisphosphate receptor (IP_3R; Cagalinec et al., 2016) and ryanodine receptor (RyR; Lu et al., 2014) might be involved in causing the elevated cytoplasmic Ca^{2+} concentration and blunted Ca^{2+} transients reported in various models of WFS1 depleted cells (Cagalinec et al., 2016; Ishihara et al., 2004; Zatyka et al., 2015). Importantly, elevated cytoplasmic Ca^{2+} concentration is a well-known cause of ER stress (Krebs et al., 2015). Thus, in addition to via reduced degradation of ATF6α (Fonseca et al., 2010), lack of functional WFS1 contributes to ER stress through the rise of cytoplasmic Ca^{2+} beyond tolerable levels. This, in turn, promotes apoptotic pathways via calpain hyperactivation (Lu et al., 2014).

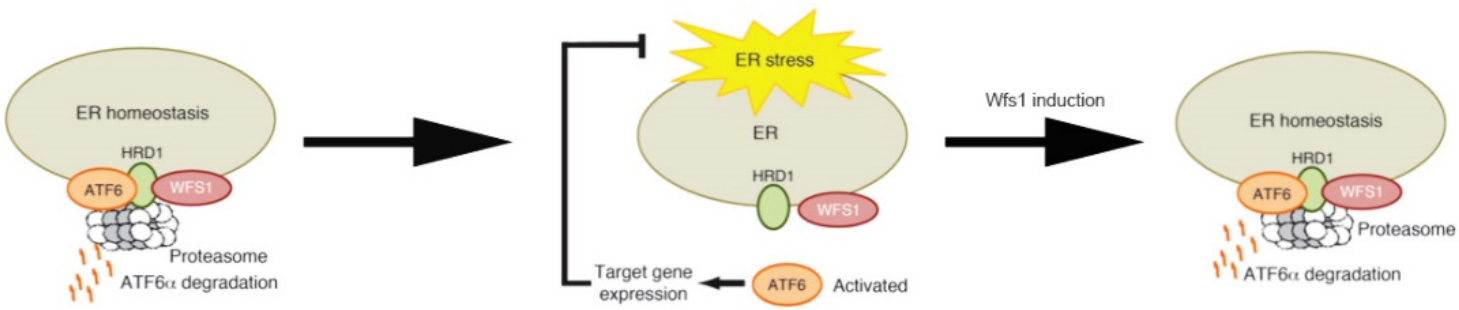


Figure 2. The role of WFS1 in regulating ER stress response. WFS1 recruits the transcription factor ATF6α to the E3 ubiquitin ligase HRD1. HRD1 marks ATF6α with ubiquitin for proteasomal degradation. Under ER stress, ATF6α dissociates from WFS1 and its soluble aminoportion, p60ATF6α, translocates to the nucleus, where it upregulates ER stress target genes. At a later time point, WFS1 is induced by ER stress, which causes the eventual degradation of ATF6α. Adapted from Fonseca et al., 2010.

Another important issue related to the ER/cytoplasmic calcium imbalance is the resulting disturbance in mitochondrial function, since the energy production in mitochondria, mitochondrial dynamics, and mitophagy are highly dependent on cellular calcium homeostasis and the ER/mitochondria dialogue through IP_3 receptor (Decuypere et al., 2011). Activation of ER stress pathways, alterations in ER-mitochondria association and dysregulation of calcium flow from ER to mitochondria have been implicated in the pathology of Parkinson`s disease (Bellucci et al., 2011; Calì et al., 2012), Alzheimer`s disease (Area-Gomez et al., 2012; Hoozemans et al., 2005), amyotrophic lateral sclerosis (Ito et al., 2009; Stoica et al., 2014) and Huntington`s disease (Lajoie and Snapp, 2011). In this aspect, WS pathology in neurons might share common mechanisms with these higher incidence diseases (for a review, see Delprat et al., 2018). Deviations from normal mitochondrial function and increased mitophagy have been shown to result from knockdown of WFS1 (Cagalinec et al., 2016) or lack of the protein encoded by the other WS-causative gene, *Wfs2*/*Cisd2* (Chen et al., 2009; Wiley et al., 2013). Due to the resemblance to mitochondrial diseases (deafness, optic atrophy and psychiatric findings which indicate neurodegeneration caused by the energy deficit), mitochondrial dysfunction has been long hypothesized to be the underlying cause of WS symptoms (Barrett et al., 2000; Kanki and Klionsky, 2009; Newman, 2005).

Considerable body of data on the functional importance of *Wfs1* has been obtained by using genetic invalidation. To date, four *Wfs1* knockout (KO) mouse models have been generated, two of which harbour the invalidated *Wfs1* gene in all cells (Ishihara et al., 2004; Kõks et al., 2009) and two are conditional knockouts lacking *Wfs1* in cerebral cortical derivatives (Shrestha et al., 2015) and pancreatic β cells (Riggs et al., 2005). Besides the mouse lines, three rat lines with disrupted *Wfs1* gene have been generated, where exon 5 is targeted by deletions (2/3), or an insertion (1/3; Plaas et al., 2017).

The *Wfs1* KO mice generated in our laboratory (Kõks et al., 2009; Luuk et al., 2008) exhibit impaired glucose tolerance and higher anxiety in novel and stressful situations, which is underlined by higher plasma corticosterone level upon stressful events when compared to wild-type (wt) mice, and increased sensitivity to the anxiolytic effect of diazepam and ethanol (Luuk et al., 2009; Raud et al., 2015). The behavioural deviations of these *Wfs1*$^{-/-}$ mice probably arise from alterations in their GABAergic, dopaminergic and serotonergic systems (Luuk et al., 2009; Raud et al., 2009; Visnapuu et al., 2013a; Visnapuu et al., 2013b). The expression levels of $GABA_A$ receptor subunits *Gabra1* and *Gabra2* are reduced in the frontal cortex and temporal lobe of the *Wfs1*$^{-/-}$ mice; similar reduction can be obtained in wt mice by exposing them to elevated plus maze (Raud et al., 2009). The dopaminergic transmission is changed both pre- and postsynaptically in the *Wfs1*$^{-/-}$ mice (Luuk et al., 2009; Visnapuu et al., 2013a). The maximal volume of dopamine output in the striatum is lower compared to wt (Matto et al., 2011), and in compliance with this, the expression of dopamine transporter is reduced in the midbrain of *Wfs1*$^{-/-}$ animals (Visnapuu et al., 2013a). The 5-HT transporter is reduced in the pons of the *Wfs1*$^{-/-}$ mice,

accompanied by the lack of increase in serotonin/5-hydroxytryptamine (5-HT) metabolites following a stressful challenge, indicating reduced serotonergic transmission in the *Wfs1*$^{-/-}$ mice generated in our laboratory (Visnapuu et al., 2013b).

The other *Wfs1* KO mouse generated by Ishihara and colleagues (Ishihara et al., 2004) develops glucose intolerance similarly to our mouse model (Reimets et al., 2016), but otherwise exhibits less severe phenotype, demonstrating only subtle deviations from normal behaviour indicating either longer reaction time or slightly increased behavioural despair (Kato et al., 2008). Different genetic background might contribute to the differences between the two *Wfs1* KO mouse lines. While the *Wfs1*$^{-/-}$ mouse studied by Kato and colleagues is bred in C57BL/6J background, the *Wfs1*$^{-/-}$ mouse line generated in our laboratory has C57BL/6 × 129S6/SvEvTac mixed background. Compared to C57BL/6, 129S6/SvEvTac mice are more anxious and display lower exploratory activity (Abramov et al., 2008), which might be related to the mutation of *Disc1* gene present in this mouse strain (Koike et al., 2006).

2. *Lsamp*

2.1 IgLON gene family

IgLON gene family is a small family of genes encoding neural adhesion molecules, which in turn belongs to the immunoglobulin (Ig) superfamily of cell adhesion molecules. Currently, five members of IgLON family are known: opioid binding protein/cell adhesion molecule like (*Opcml*; also known as *Obcam*, *Opcm* and *IgLON1*; Schofield et al., 1989), neurotrimin (*Ntm*; also known as *HNT*, *NTRI* and *IgLON2* in humans, the chick homologue is called *CEPU-1*; Struyk et al., 1995), limbic system-associated membrane protein (*Lsamp*; also known as *Lamp* and *IgLON3*; Keller et al., 1989; Levitt, 1984; Pimenta et al., 1995), neuronal growth regulator 1 (*Negr1*; also known as *Kilon*, *MGC46680*, *IgLON4*, and neurotractin; Funatsu et al., 1999), and *IgLON5* (from: IgLON family member 5; also known as *LOC402665*; Gray et al., 2015). All IgLON family proteins are extensively glycosylated, contain three immunoglobulin domains and are tethered to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor (Itoh et al., 2008). IgLONs can form homodimers and heterodimers, which are called dimeric IgLONs or DIgLONs (Akeel et al., 2011; Lodge et al., 2000). Forming homo- or heterophilic dimers is important for promoting or inhibiting neurite outgrowth (Akeel et al., 2011; Gil et al., 2002; Reed et al., 2004). In addition, IgLONs take part in synaptogenesis (Hashimoto et al., 2009) and axonal pathfinding and fasciculation (Chen et al., 2001; Keller et al., 1989; Mann et al., 1998; Schmidt et al., 2014; Singh et al., 2018). NEGR1, NTM and LSAMP can promote neurite outgrowth as soluble factors after being shed from the cell surface by ADAM family metalloproteinases (Pischedda and Piccoli, 2016; Sanz et al., 2015; Sanz et al., 2017). Several lines of evidence indicate that receptor tyrosine kinases are involved in the growth regulating effects of IgLONs. Soluble NEGR1 exerts its neurite

growth-promoting functions via the FGFR2 (fibroblast growth factor receptor 2) dependent intracellular pathway (Pischedda and Piccoli, 2016), and the interaction of OPCML with different receptor tyrosine kinases (including EPHA2, FGFR1, FGFR3, HER2, HER4) leads to the internalisation and degradation of the latter (McKie et al., 2012). OPCML regulates the growth and proliferation of astrocytes (Sugimoto et al., 2010) and acts as a tumour suppressor gene in several tissues (Cui et al., 2008; McKie et al., 2012; Reed et al., 2007; Sellar et al., 2003). Tumour suppressor role has also been suggested for LSAMP and NEGR1 (Barøy et al., 2014; Kim et al., 2014; Ntougkos et al., 2005). Allelic variants of *NEGR1* have been associated with the reduced myelination of neuronal pathways in the brain (Dennis et al., 2014), depression (Hyde et al., 2016), and obesity (Willer et al., 2008). Elevated level of NEGR1 has been associated with psychiatric disorders including major depression, bipolar disorder and schizophrenia (Maccarrone et al., 2013), and deletion of *NEGR1* causes language disabilities and learning deficiency (Genovese et al., 2015; Tassano et al., 2015; Veerappa et al., 2013). NTM is involved in axonal fasciculation and synaptogenesis in the central nervous system and promoting Schwann cell migration in the peripheral nervous system (Chen et al., 2001; Yu et al., 2012). *NTM* has been associated with intelligence (Pan et al., 2011) and with its close neighbour *OPCML* belongs to the risk locus for Alzheimer`s disease, ADHD, depression, schizophrenia and autism at 11q25 (Baye et al., 2008; Blacker et al., 2003; Liu et al., 2007; Magri et al., 2010; Ogdie et al., 2003; Schol-Gelok et al., 2010; Vorstman et al., 2006; Ye et al., 2012). Association to schizophrenia has been suggested to all IgLON genes besides the most recently discovered *IgLON5* (Chen et al., 2018; Koido et al., 2014; O'Donovan et al., 2008; Panichareon et al., 2012; Ripke et al., 2014; Wang et al., 2010). Increased mRNA expression levels of *NEGR1* and *NTM* 1b as well LSAMP and NEGR1 protein have been found in the dorsolateral prefrontal cortex of the patients of schizophrenia (Behan et al., 2009; Karis et al., 2018). *IgLON5* was brought under wider attention after identification as a protein targeted by autoantibodies in case of a tauopathic sleep disorder involving parasomnia and obstructive sleep apnoea and often accompanied by ataxia, dysphagia and hypoventilation (Gelpi et al., 2016; Sabater et al., 2014).

2.2 The structure of *Lsamp* gene and the function of LSAMP protein

LSAMP gene (also known as *Lamp* or *IgLON3*) is located at 3q13.31 in the human genome and it is composed of 11 exons distributed over 2.2 megabases (Mb; Pimenta and Levitt, 2004). *LSAMP* gene contains two alternative first exons with separate promoters, which encode distinct signal peptides, but yield an identical mature protein (see Fig. 3; Pimenta and Levitt, 2004). Additional variation becomes from alternative splicing near the 3′ end of the mRNA, which results in including or omitting an insertion of 23 amino acids (exons 7 and 8; Pimenta and Levitt, 2004). *Lsamp* gene, including its two-promoter structure, is highly conserved among vertebrates. The cDNA nucleotide sequence identity is 94% and amino acid sequence identity is 99% between rat and human (Pimenta et al., 1996a) and 91% between rat and chicken (Brümmendorf et al., 1997). Mature LSAMP is a 64–68 kDa protein (Pimenta et al., 1995). It contains three immunoglobulin domains and a GPI anchor in its C-terminal region, which binds it to the extracellular side of the plasma membrane of neurons of the limbic system (Levitt, 1984; Pimenta et al., 1995; Zacco et al., 1990).

Polymorphisms of *LSAMP* gene are associated with schizophrenia (Chen et al., 2018; Koido et al., 2014), depression (Koido et al., 2012), panic disorder (Koido et al., 2012), and suicide (Galfalvy et al., 2013). It is worth noting that besides *LSAMP*, 3q13.31 chromosomal locus contains several genes implicated in the neural development and psychiatric disease, including *DRD3*, *ZBTB20*, and *GAP43* (Blennow et al., 1999; Chen et al., 2018; Davies et al., 2014; Lowther et al., 2014; Nunokawa et al., 2010), which, residing in proximity to each other, could share common regulatory mechanisms. According to Fromer et al. (2016), the location of the expression quantitative trait locus (eQTL, *i.e.* site that influences the level of gene expression) (rs74731643) that is influencing the expression level of *LSAMP* gene is more than 2 Mb upstream of *LSAMP* gene (see also Karis et al., 2018). This provides evidence that the regulatory areas of *LSAMP* can span over a wider genomic area and *LSAMP* can be coregulated with other genes in the neighbourhood, contributing to the genetic risk for neurodevelopmental/neuropsychiatric conditions.

LSAMP specifically participates in regulating the formation of limbic circuits by promoting cell adhesion, synapse formation, neurite outgrowth and axon targeting in limbic structures (Eagleson et al., 2003; Hashimoto et al., 2009; Keller et al., 1989; Pimenta et al., 1995; Schmidt et al., 2014; Zukhareva and Levitt, 1995). While attracting axons from limbic-associated thalamic nuclei, it can mediate repulsion for non-limbic thalamocortical axons (Mann et al., 1998). The inhibition might be mediated by heterophilic binding with other IgLON family members, for the coexpression of LSAMP and NTM inhibits neurite outgrowth in several models (Akeel et al., 2011; Gil et al., 2002). Likewise, coexpression of LSAMP and NTM reduces the number of synapses on hippocampal neurons (Hashimoto et al., 2009). In dorsal root ganglions, shedding of LSAMP from the cell surface by metalloproteinase ADAM10 relieves the inhibition of neurite outgrowth (Sanz et al., 2017).

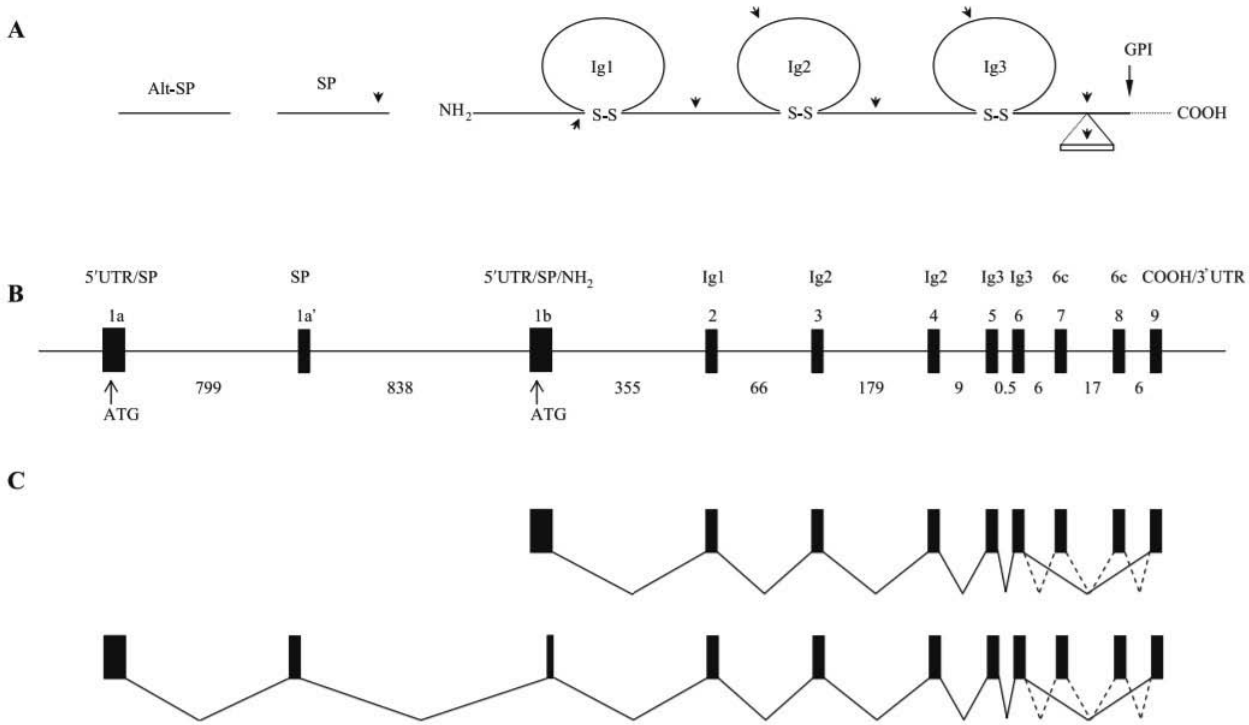


Figure 3. The structure of mouse LSAMP protein and *Lsamp* gene. A: Schematic representation of the LSAMP structure. From left to right: two alternative signal peptides, N-terminal domain, three Ig domains, putative site for the insertion of 23 amino acids (open bar), GPI anchor attachment site in the C-terminal domain. Splice sites defining the *Lsamp* exons are indicated by short arrows. B: Map of *Lsamp* gene. Exons are depicted as boxes. Transcription initiation sites are indicated by arrows. Sizes of introns are indicated in kilobases. C: Scheme of *Lsamp* cDNA forms with the alternative signal peptides and alternative splicing of exons 7 and 8. Adapted from Pimenta and Levitt, 2004.

Besides having role in the development and functioning of the nervous system, LSAMP also serves as a tumour suppressor. Reduced level of LSAMP resulting from deletions or methylation of the promoter region has been shown in patients of osteosarcoma (Barøy et al., 2014; Kresse et al., 2009; Pasic et al., 2010; Yen et al., 2009), acute myeloid leukaemia (Kühn et al., 2012), prostate cancer (Petrovics et al., 2015), clear cell renal sarcoma (Chen et al., 2003) and epithelial ovarian cancer (Ntougkos et al., 2005). One study associates a case of acute myeloid leukaemia with increased expression level of LSAMP (Coccaro et al., 2015). Polymorphism in *LSAMP* gene yielding lower transcription level has been associated with coronary artery disease (Dungan et al., 2016; Wang et al., 2008).

LSAMP was first described in the rat brain as a protein with specific expression in the limbic system – the network of cortical structures, subcortical nuclei and pathways involved in processing of emotional information in the brain (Horton and Levitt, 1988; Levitt, 1984). In the foetal rat brain, the mRNA of *Lsamp* is first detected at E13-E14 in the developing striatum, preoptic area, amygdala, diencephalon, midbrain and hindbrain (Pimenta et al., 1996b), and LSAMP protein is first found at E15 in the fibres coursing from diencephalon to the cortex through the internal capsule (Horton and Levitt, 1988). The *in situ* hybridization studies in the developing and adult rat brain confirmed the prominent expression of *Lsamp* in limbic structures, such as hippocampus, extended amygdala, ventral and dorsal striatum, and preoptic and hypothalamic areas, but additionally, *Lsamp* mRNA was found in several brain regions related to somatosensory and motor function, e.g. somatosensory cortical regions and thalamic nuclei, midbrain and hindbrain (Pimenta et al., 1996b; Reinoso et al., 1996). In addition to rat, the expression of LSAMP protein has been studied in the brain of cat (Chesselet et al., 1991), cynomolgus monkey (*Macaca fascicularis*; Côté et al., 1995; Côté et al., 1996), human (Prensa et al., 1999; Prensa et al., 2003; Uroz et al., 2004) and pigeon (Yamamoto and Reiner, 2005; Yamamoto et al., 2005), confirming its presence principally in the limbic structures in different evolutionary lineages. During the development, LSAMP is present along the growing axons, cell somata and both pre- and postsynaptically in the forming synapses, whereas in adult brain, the immunoreactivity only remains in the dendrites and cell body (Horton and Levitt, 1988; Zacco et al., 1990). In the chick embryo, Lsamp protein expression is found in the retinotectal pathway, axon-dense regions of the spinal cord, ventral root of the spinal nerve, and sympathetic and dorsal root ganglia (Brümmendorf et al., 1997).

AIMS OF THE STUDY

The overall aim of the present thesis is to give insight into the mechanisms by which two genes, *Wfs1* and *Lsamp*, are involved in the regulation of the development and functioning of the neural circuits underlying behaviour. Describing the neuroanatomical distribution and elucidating the roles of WFS1 and LSAMP in the brain development helps to better understand their functions related to the susceptibility to psychiatric diseases. The precise aims are as follows:

1. To describe the initiation pattern of *Wfs1* expression in relation to major developmental events in the mouse brain.
2. To study the role of WFS1 in the regulation of the developmental ER stress in the mouse brain.
3. To study the evolutionary conservation of the expression of *Wfs1* in amniote brain.
4. To explore the relation between *Wfs1* and dopaminergic system and target the role of WFS1 in D1-like receptor dependent signalling.
5. To describe the spatial activity pattern of the two alternative promoters of the *Lsamp* gene from the developmental aspect and to investigate their significance in anxiety-related and social behaviour in mice.

MATERIALS AND METHODS

1. Ref I

The expression of WFS1 had earlier been studied in our laboratory by Luuk and colleagues in the adult mouse brain by immunohistochemistry and X-gal staining of *Wfs1*-β-galactosidase knockin mouse brains (Luuk et al., 2008). Nothing was known about the initiation and embryonic expression of *Wfs1*, therefore, we decided to focus on these events, taking advantage of the non-radioactive *in situ* RNA hybridization method to see the earliest events and localize the cell bodies that express *Wfs1*. We also added several postnatal stages in our study, to provide a continuous series of coronal sections helping to understand the general changes in the expression pattern in each brain structure and to complement the *in situ* hybridization study published by Kawano et al. in 2009. Because one of the established functions of WFS1 is regulating the ER stress, we hypothesized that WFS1 might take part in the regulation of the developmental ER stress in neurons. To test our hypothesis, we applied *in situ* RNA hybridization of *Grp78* and *Grp94* genes and real-time quantitative polymerase chain reaction (qRT-PCR) on ER stress markers *Grp78*, *Grp94* and *CHOP* in wt and *Wfs1*$^{-/-}$ mouse brains.

2. Ref II

In the second paper, we took an evolutionary approach to study the function of WFS1 in the tetrapodean brain, using mouse, domestic chick/common quail and red-eared slider turtle as representatives of the different phylogenetic lineages. Regarding the distribution of WFS1 in the dopaminoceptive brain regions and the reported deviations in the dopaminergic system of *Wfs1* KO mice (Luuk et al., 2009; Visnapuu et al., 2013a), we took under closer investigation the relation between WFS1 and D1-type dopamine receptors. For this, we used *in situ* RNA hybridization, immunohistochemistry and radioactive ligand binding assay.

3. Ref III

Because *Lsamp* gene contains two conserved promoters that yield an mRNA with the different first exons, but identical protein, we aimed to study the functional significance of the dual promoter structure. First, we studied the pattern of the promoter activity in the developing and adult mouse brain by radioactive and non-radioactive *in situ* RNA hybridization and by X-gal staining of *Lsamp*-β-galactosidase knockin mouse brains. Secondly, since *Lsamp* KO mice as well as psychiatric patients show deviations in fear and anxiety related and social behaviour, we studied the correlation between the *Lsamp* promoter activity and measures of trait anxiety and social behaviour. Additionally, we measured the expression of *Lsamp* 1a and 1b transcripts upon acute fear response. In the first

part of behavioural testing, we used a battery of elevated plus-maze test, locomotor activity test, and social interaction test. Following the last test, the expression of *Lsamp* transcripts was measured in the ventral striatum, hippocampus and temporal lobe by qRT-PCR. In the second part of behavioural testing, fear conditioning was applied to study the effect of acute fear on the expression of *Lsamp* transcripts in the hippocampus and temporal lobe. The expressions of *Lsamp* transcripts and immediate early gene *c-Fos* were measured by qRT-PCR.

4. Animals

All the mice (*Mus musculus*) were housed under standard laboratory conditions (12-h light/dark cycle with free access to food and water) at the Laboratory Animal Centre of the Institute of Biomedicine and Translational Medicine, University of Tartu (accreditation number KL1210), and were killed by cervical dislocation or decapitation. In case of transcardial perfusion, animals were deeply anaesthetized by intraperitoneal injection of ketamine and xylazine (100 mg/kg and 10 mg/kg, respectively; paper II) or mixture of Hypnorm® (yielding 0.3 mg/kg of fentanyl citrate and 10.7 mg/kg fluanisone; Janssen Animal Health, Buckinghamshire, UK) and Dormicum® (yielding 1.3 mg/kg of midazolam; Roche, Mannheim, Germany; paper III, immunohistochemistry) or pentobarbital (paper III, radioactive *in situ* hybridization). Two lines of knockout mice were used in the present studies; the description of the generation of the knockout mouse lines is provided below. In paper I, wt C57BL/6 mice were used for *in situ* hybridization studies and wt and *Wfs1*$^{-/-}$ [(129S6/SvEvTac (Taconic) × C57BL/6) × (129S6/SvEvTac × C57BL/6)] mice were used in quantitative real-time PCR experiments. In paper II, wt C57BL/6 mice were used for *in situ* hybridization and immunohistochemistry and male wt and *Wfs1*$^{-/-}$ [(129S6/SvEvTac × C57BL/6) × (129S6/SvEvTac × C57BL/6)] were used for radioligand binding experiments. In paper III, C57BL/6 mice were used for *in situ* hybridization experiments, *Lsamp*$^{-/-}$ [(129S6/SvEvTac × C57BL/6) × (129S6/SvEvTac × C57BL/6)] mice were used for X-gal staining and male C57BL/6 mice were used for behavioural studies.

In paper II, in addition to mice, we used chicken (*Gallus gallus domesticus*), quails (*Coturnix coturnix*) and red-eared slider turtles (*Trachemys scripta*). Chicken were obtained from commercial poultry farm Tallegg and from the Science Centre AHHAA in Tartu, Estonia in cooperation with Tallegg. Quails were obtained from commercial quail farming company Järveotsa Vutifarm OÜ. Turtles were purchased commercially from the Kliebert Turtle and Alligator Farm (Hammond, Louisiana). Chicken and quails were killed by decapitation. Turtles were anaesthetized by intramuscular ketamine and xylazine injection (90 mg/kg and 6 mg/kg, respectively) combined with hypothermia induced by keeping the animal in ice before decapitation. The approximate number and other data of all the animals used for the preparation of papers I, II and III is shown in Table 1.

Table 1. Data of the animals used in Refs I, II and III.

Species	Geno-type	Background	Sex	Age	Number	Experiment	Ref
House mouse	wt	C57BL/6	nd	E15.5, E18.5, P0, P2, P4, P7, P20, P60	20	*in situ* hybridization	I
House mouse	wt	129S6/SvEvTac × C57BL/6	nd	E17, P0, P2, P4	28	real-time PCR	I
House mouse	*Wfs1*$^{-/-}$	129S6/SvEvTac × C57BL/6	nd	E17, P0, P2, P4	28	real-time PCR	I
House mouse	wt	129S6/SvEvTac × C57BL/6	male	3 months	40	radioligand binding	II
House mouse	*Wfs1*$^{-/-}$	129S6/SvEvTac × C57BL/6	male	3 months	42	radioligand binding	II
House mouse	wt	C57BL/6	nd	adult	2	*in situ* hybridization	II
House mouse	wt	C57BL/6	nd	adult	3	immuno-histochemistry	II
House mouse	wt	C57BL/6	nd	E13.5, E15.5, adult	10	*in situ* hybridization	III
House mouse	*Lsamp*$^{-/-}$	129S6/SvEvTac × C57BL/6	nd	E13.5, E15.5, adult	8	X-gal staining	III
House mouse	wt	C57BL/6	male	2 months	31	Behavioural experiments	III
House mouse	wt	C57BL/6	nd	adult	1	immuno-histochemistry	III
Domestic chick			nd	E10, E13, E15, E20, P0, adult	6	*in situ* hybridization	II
Common quail			nd	adult	2	immuno-histochemistry	II
Red-eared slider turtle			nd	adult	2	*in situ* hybridization	II

nd – not determined

4.1 *Wfs1* knockout mice

The knockout strategy for creating *Wfs1*$^{-/-}$ mouse line is published by Luuk et al., 2008 and Kõks et al., 2009. In brief, by using targeted homologous recombination, amino acids 360–890 of WFS1 protein were replaced by and an in-frame NLSLacZNeo cassette, resulting in fusion protein consisting of WFS1 residues 1–360 and β-galactosidase. *Wfs1*$^{-/-}$ mice (*Wfs1*-β-galactosidase knockin mice) with mixed genetic background [(129S6/SvEvTac × C57BL/6) × (129S6/SvEvTac × C57BL/6)] were used for quantitative real-time PCR experiments in Ref I and radioligand binding experiments in Ref II. Mixed genetic background was used to avoid robust background effects on gene expression.

4.2. *Lsamp* knockout mice

The knockout strategy for creating *Lsamp*$^{-/-}$ mouse line is described in detail in Innos et al., 2011. In brief, exon 1b of *Lsamp* gene was replaced by a NLSLacZNeo cassette by targeted homologous recombination. *Lsamp*$^{-/-}$ (*Lsamp*-β-galactosidase knockin) [(129S6/SvEvTac × C57BL/6) × (129S6/SvEvTac × C57BL/6)] mice were used for X-gal staining in Ref III.

5. Non-radioactive *in situ* RNA hybridization (papers I, II and III)

The used mouse *Wfs1* riboprobe template sequence is available at: GenBank ref |NM_011716.2| Range 1: 2210–2686. In case of *Lsamp*, the cDNA fragment specific for 1a promoter (400 bp) consisted of 1a specific 5′UTR, exon 1a and exon 1a′. Universal *Lsamp* probe (567 bp) was transcribed from a cDNA fragment consisting of exons 2–6. cDNA fragment sequences used as templates for Dig-labelled riboprobes for other genes were obtained using the following primers (containing restriction sites of HindIII and XhoI for *Syp1*, SalI and NotI for all others):

Mouse *Syp1* For	CCCA▾AGCT▴Tgggggtcagttccgggtggt
Mouse *Syp1* Rev	CCGC▾TCGA▴Gcttcacatcggacaggcctt
Mouse *Grp78* For	TTTG▾TCGA▴Ctgcagcaggacatcaagttc
Mouse *Grp78* Rev	TTTGC▾GGCC▴GCaatccttgcttgatgctga
Mouse *Grp94* For	TTTG▾TCGA▴Catggcacagtggaagaggac
Mouse *Grp94* Rev	TTTGC▾GGCC▴GCgtttcctcttgggtcagcaa
Mouse *Drd1a* For	TTTGC▾GGCC▴GCctctgctgcttttggacag
Mouse *Drd1a* Rev	TTTG▾TCGA▴Ctaggggcagagcattggtag
Mouse *Drd5* For	TTTGC▾GGCC▴GCgagaactgtgactccagcct
Mouse *Drd5* Rev	TTTG▾TCGA▴Cgacatgtgatcgaaaggccc
Chick *Drd1a* For	TTTGC▾GGCC▴GCatgacttggaacgacaccact
Chick *Drd1a* Rev	TTTG▾TCGA▴Cagttgctctcaggttgctgg
Chick *Wfs1* For	TTTGC▾GGCC▴GCgacagaagaggcatcacttctgagaa
Chick *Wfs1* Rev	TTTG▾TCGA▴Cctcatgtagcttgtcactgtgaagaa

The brains of mice, chicken and turtles were dissected and fixed at 4°C in 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline, pH 7.4 (PBS) for 1–5 days depending on the size of the brain. Subsequently, the brains were immersed overnight at 4°C in the cryoprotectant consisting of 20% sucrose in 2%PFA/PBS and kept frozen in -80°C until further processing. *in situ* RNA hybridization was carried out on free-floating 40 µm cryosections using digoxigenin-UTP (Roche) labelled sense and antisense RNA probes. At first, the sections were rinsed twice in PBS containing 0.25% Triton X-100 (PBST) and once in 5X SSC (pH 5). Next, the sections were prehybridized for 1 hour at 65°C in prehybridization mixture containing 50% formamide, 5X SSC (pH 5), 2% blocking reagent (BR; Roche), following overnight hybridization in the same conditions with hybridization mixture containing 50% formamide, 5X SSC, 1% BR and 1 µg/ml probe (heated at 80°C for 5 min and cooled on ice prior to adding to the hybridization mixture). To control the specificity of RNA probe binding, some sections were incubated with the sense probe. After the hybridization, the sections were washed for 30 min at 65°C in solution containing 50% formamide, 5X SSC and 1% SDS, following 2 x 30 min at 60°C in 50% formamide, 2X SSC. Next, the sections were rinsed three times in TBST (25 mM Tris-HCl pH 7.5; 140 mM NaCl; 2.7 mM KCl; 0.1% Tween20) and blocked against non-specific antibody binding for 1h in TBST containing 2% BR. Then, the sections were incubated overnight at 4°C in 1% BR/TBST with 1:2000 anti-digoxigenin antibody conjugated to alkaline phosphatase (Roche). On the third day, the sections were washed three times in TBST, following two washes in NTMT (0.1 M NaCl, 0.1 M Tris-HCl pH 9.5, 50 mM $MgCl_2$, 0.001% Tween20). Then, the sections were transferred into the alkaline phosphatase substrate BM Purple AP Substrate (Roche) and when sufficient staining had appeared, the reaction was terminated by transferring the sections into PBS. All washing and incubation steps were carried out under rocking. The stained sections were transferred onto slides in 0.5% gelatine, air dried and mounted with Pertex (Histolab).

In case of turtle, we used chick *Wfs1* and *Drd1a* probes and the hybridization was carried out at 60°C and post-hybridization washes at 60°C and 57°C. Using NCBI BLAST, we analysed the sequence identity between the chick cDNA sequences corresponding to the probes and the transcriptome of *Trachemys scripta* (accessible from Kaplinsky et al., 2013). The sequence identity was 88% (682 nucleotides of 773) in case of *Drd1a* and 89% (786 nucleotides of 887) in case of *Wfs1*.

In case of E13.5 mouse brains in paper III, whole-mount *in situ* hybridization was carried out. The dissected brains and/or embryos were fixed in 4% PFA/PBS at 4°C overnight, then dehydrated in increasing concentrations of methanol in 0.1% Tween-20/PBS (25%, 50%, 75%, 2 x 100%) and rehydrated with the same concentrations of methanol in 0.1% Tween-20/PBS in reversed order, following washing with 0.1% Tween-20/PBS. To degrade the proteins, the specimens were treated with 10 µg/ml proteinase K (Fermentas) in PBS for 10 min and the reaction was terminated by 2 mg/ml glycine in 0.1% Tween-

20/PBS. Then, following washing with 0.1% Tween-20/PBS, the specimens were refixated with 4% PFA/0.2% glutaraldehyde/PBS for 20 min. After washing from the fixative, the specimens were put into prehybridization mix containing 50% formamide and 5 x SSC (pH 5), where they were stored at -20°C until further processing. The prehybridization was carried out in 50% formamide/5 x SSC/1% SDS/1% BR at 70 °C for 1.5h under rocking and hybridization with 1 µg/ml heat-activated (see above) RNA probe was carried out overnight in the same conditions. The following steps were the same as described before for the 40 µm sections, with minor modifications. The first washing step after the hybridization with 50% formamide, 5X SSC and 1% SDS was carried out 2 x 30 min and after the incubation with anti-digoxigenin antibody (Roche), the specimens were washed with TBST for 6 times and left washing in TBST at 4 °C until the next day. The next day, the specimens were washed three times in NTMT and kept in BM Purple AP Substrate (Roche) until desired staining developed. For cutting 50-µm vibratome sections, the stained specimens were inserted into 1 ml of 0.5% gelatine/30% bovine serum albumin (BSA)/20% sucrose/PBS, wherein 140 µl of 25% glutaraldehyde was added immediately before insertion and incubated for 10 min. The sections were mounted on glass slides with 70% glycerol.

6. Radioactive *in situ* RNA hybridization

Mouse *Lsamp* sequence accessible from UCSC Genome Browser, genome.ucsc.edu (Accession No. uc007zfr.1) was used as a template sequence for ^{33}P-dUTP labelled antisense 40′mer DNA oligonucleotide probes:

Mouse *Lsamp*1a 5′-accccagcacccagacgctgtgcagccagtaggtcctcat-3′
Mouse *Lsamp*1b 5′-gaagaaggcagagcagtctcagtaggaccagcggcaactg-3′
Mouse *Lsamp*UNI 5′-agagcatggcgcttctccagctcaacccgagggtccagag-3′

In situ hybridization on free-floating sections was carried out as described in Hundahl et al. 2010.

7. Immunohistochemistry (papers II and III)

For non-fluorescent immunohistochemistry, the mice were transcardially perfused with 2% PFA/PBS under ketamine and xylazine anaesthesia. The brains were dissected and kept in 2% PFA/PBS for 1 h and cryoprotected overnight in 20% sucrose in 1% PFA/PBS at 4°C. Until further processing, the brains were kept frozen at -80°C. Immunohistochemical protein detection was carried out on free-floating 40 µm cryosections. First, the sections were washed two times in Tris-buffered saline containing 0.25% Triton X-100 (TTBS) and treated with 0.3% H_2O_2 in ddH_2O for 15 min to quench the endogenous peroxidase activity, following three washes in TTBS. Next, unspecific antibody binding was blocked with 5% horse serum (Vector Laboratories) in TTBS for 1h. The

incubation with primary antibody (diluted in 2% horse serum in TTBS) was carried out at room temperature for 1h, then at 4°C overnight. The antibodies were: rabbit polyclonal WFS1 (the same as in Luuk et al., 2008, diluted 1:1000), rabbit polyclonal D1 (#ADR-001, Alomone Labs, diluted 1:1000), and rabbit polyclonal D5 (#ADR-005, Alomone Labs, diluted 1:1000). The next day, the sections were washed three times in TTBS and incubated with the biotinylated anti-rabbit antibody (Vector Laboratories, dilution 1:400) in 1% horse serum in TTBS for 1h. The antibody binding was detected using the Vectastain Elite ABC Kit (Vector Laboratories) according to the protocol provided by the manufacturer. Briefly, the biotinylated secondary antibody was bound to streptavidin and horseradish peroxidase reaction with diaminobenzidine (DAB; Vector Laboratories) was used to visualize immunoreactivity. The stained sections were transferred onto slides in 0.5% gelatine, air dried and mounted with Pertex (Histolab). All washing steps and longer incubations were carried out under rocking. Specificity of the immunohistochemistry was determined by incubations without the primary antibody.

For fluorescent immunohistochemistry, quail and turtle brains were fixed for 4 h in 4% PFA/PBS, washed with PBS following impregnation with 30% sucrose in ddH_2O at 4°C, and were frozen and stored at -80°C. The brains were cut into 40 μm freely floating coronal cryosections. The sections were permeabilized with 0.3% TritonX-100/PBS over 30 min and blocked against non-specific antibody binding with 5% donkey serum (Jackson ImmunoResearch Laboratories Inc.) /1% BSA (Sigma)/PBS over 1 h. Next, the incubation with WFS1 antibody (1:400 in 1% BSA /0.1% Tween-20/PBS) was carried out at room temperature for 1h, then at 4°C overnight. After three washing steps with 0.3% TritonX-100/PBS, the sections were incubated in FITC conjugated goat anti-rabbit secondary antibody solution (1:1000, Jackson ImmunoResearch Laboratories Inc.) in 0.1% Tween-20/1% BSA/PBS for 2 h. Nuclei were counterstained with DAPI (4,6-diamidino-2-phenylindole, Sigma Aldrich) 1: 2000 dilution in secondary antibody buffer. Sections were further washed in PBS and mounted in Fluoromount (Sigma Aldrich) mounting medium. All washing steps and longer incubations were carried out under rocking. Specificity of the immunohistochemistry was determined by incubations without the WFS1 primary antibody.

To visualize neuronal nuclei, immunohistochemistry with NeuN antibody (Millipore, clone A60, cat. no. MAB377) was performed in Ref III. Briefly, 5% normal goat serum (Dako) was used for blocking, NeuN antibody was diluted to 1:1000 in 0.25% TritonX-100/PBS with 2% goat serum, and goat anti-mouse IgG antibody coupled to Alexa Fluor 488 fluorescent dye (cat. no. A11001, Molecular Probes, diluted 1:500 in 0.25% TritonX-100/PBS with 2% goat serum) was used as the secondary antibody. After staining, the sections were transferred to gelatinized glass slides and mounted in a 1:1 mix of glycerol and PBS.

8. X-gal staining (paper III)

To demonstrate the activity of *Lsamp* 1b promoter, the detection of β-galactosidase activity was performed by X-gal staining. The adult *Lsamp*-β-galactosidase knockin mice were transcardially perfused under combined fentanyl, fluanisone and midazolam anaesthesia with 2% PFA in 0.1 M sodium phosphate buffer (PB), pH 7.4. The brains were dissected, cut into halves and kept overnight in 20% sucrose/1% PFA in PB, frozen, and cut into 100 µm free-floating sections. The sections were stained overnight in 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 1 mg/ml X-gal, 0.125% Triton X-100 in PB at room temperature. After staining, the sections were incubated in 2% PFA solution in PB, transferred to glass slides and mounted with Pertex (Histolab). For X-gal staining, E13.5 (embryonic day 13.5) and E15.5 brains and/or embryos were fixed in 4% PFA/PBS at 4 °C for 30 min. For cutting 50-µm vibratome sections, the stained (E13.5 and E15.5) specimens were inserted into 1 ml of 0.5% gelatine/30% BSA/20% sucrose/PBS, with 140 µl of 25% glutaraldehyde as described above. The sections were mounted on glass slides with 70% glycerol.

9. Quantitative real-time PCR (papers I and III)

The mice were decapitated, and brains were dissected out. Whole forebrains (paper I) or temporal lobes, hippocampi and ventral striata (paper III) were frozen in liquid nitrogen. Taqman system (Applied Biosystems) was employed for measuring the expression levels of *Grp78*, *Grp94* (paper I), *Lsamp* 1a and 1b transcripts and *c-Fos* (paper III). The detailed description of the method used for measuring the expression levels of *Grp78* and *Grp94* is provided in Visnapuu et al., 2013b. For the description of the method used for measuring the expression levels of *Lsamp* transcripts and *c-Fos*, see Ref III. The expression analysis of *Chop* was performed using SYBR Green system as in Örd et al. (2012). In all cases *Hprt1* was used as the housekeeper gene, as it has consistently shown stable results as a reference gene for brain tissues in our laboratory.

The primer sequences and assays used for real-time PCR experiments were the following:

Mouse *Grp94* (*Hsp90B1*)	Taqman assay Mm00441927_m1
Mouse *Grp78* For	5’- cataaaccccgatgaggctgta -3’
Mouse *Grp78* Rev	5’-cacctgtatcctgatcaccagaga-3’
Mouse *Grp78* probe	5’-VIC-tggtgccgctgtccaggctg-MGB-3’
Mouse *CHOP* For	5’-ctgcctttcaccttggagac-3’
Mouse *CHOP* Rev	5’-cgtttcctggggatgagata-3’
Mouse *Hprt1* For	5’-gcagtacagccccaaaatgg-3’
Mouse *Hprt1* Rev	5’- aacaaagtctggcctgtatccaa-3’
Mouse *Hprt1* probe	5’-VIC-aagcttgctggtgaaaaggacctctcg-TAMRA-3’
Mouse *Lsamp* probe	5’-FAM-aaccgaggcacggacaac-MGB-3’
Mouse *c-Fos*	Taqman assay Mm00487425_m1

In paper I, the impact of genotype and mouse age on the expression levels of *Grp94*, *Grp78*, and *Chop* was estimated with Type III ANOVA realized in the SAS procedure GLM (SAS Inst Inc, 2008). The terms of the underlying GLM model were the main effects of gene and genotype, their interaction and the mouse stage subjected to gene and genotype. Comparison of the effects of selected factor combinations was estimated with the Estimate option provided by the GLM procedure. For each of the 24 combinations of gene × stage × genotype, seven animals were measured.

In paper III, all data were analysed using Statistica version 8.0 (StatSoft, Inc.). As the behavioural scores were not normally distributed, Spearman's rank-order method was used for the calculation of correlation coefficients. In the fear conditioning study, one-way ANOVA (conditioning type as grouping variable) was performed. Tukey HSD post hoc analysis was used when applicable after statistically significant ANOVA.

10. Radioligand [^{3}H]SCH23390 binding assay (paper II)

To better understand the relation between Wfs1 and D1-type dopamine receptors, we used radioligand binding assay to determine the amount of D1-type dopamine receptors in wt and *Wfs1*$^{-/-}$ mice. The detailed description of the radioligand binding assay is provided in paper II. Briefly, the mice were decapitated, the hippocampi were rapidly dissected, frozen in liquid nitrogen and stored at -80°C until further processing. The hippocampi were homogenized by ultrasound sonication, and membrane preparations were made by centrifugation at 30 000 g. In radioligand binding curve experiments, the hippocampal membranes of 6 mice from corresponding group (wt or *Wfs1*$^{-/-}$) were pooled and used at concentration of 20 mg tissue/ml. The membranes were incubated with different dilutions (0.06–8.2 nM) of a D1-like receptor radioligand [^{3}H]SCH23390 (81.9 Ci/mmol, PerkinElmer) in the absence (for total binding) or in the presence (for nonspecific binding) of 10 μM (+)-butaclamole (Sigma-Aldrich), a dopaminergic antagonist. Samples were incubated for 60 min at 25°C and the reactions were stopped by rapid filtration through thick GF/B glass fibre filtermats using FilterMate Harvester (both from PerkinElmer). Solid scintillant MeltiLexTM B/HS was then impregnated into the filter by using MeltiLexTM Heatsealer. The filter-bound radioactivity was counted using a Wallac MicroBeta TriLux 1450 LSC Luminescence Counter (all from PerkinElmer). The total concentrations of radioligand dilutions were determined in vials with 3 ml of liquid scintillation cocktail OptiPhase HiSafe (PerkinElmer).

The number of binding sites of D1-like receptors in wt and *Wfs1* KO mice hippocampi was estimated by determination of specific binding of 4 nM [^{3}H]SCH23390 as described above. The tissue concentration in these experiments was 6.7 mg/ml.

All the data were analysed using GraphPad Prism 5.0 (GraphPad Software Inc). Data are presented as mean ± SEM of at least three independent experi-

ments carried out at least in duplicates. Statistically significant differences were determined by the Student t test, where $p < 0.05$ was taken as the criterion of significance.

11. Behavioural experiments (paper III)

All behavioural studies were done with 2 months old male C57BL/6 mice. Male mice were used to avoid the possible impact of the oestrous cycle on the experimental results. In the first part of the behavioural studies, we investigated the correlations between general behavioural profile and the expression of *Lsamp* transcripts in the hippocampus, temporal lobe and ventral striatum. All mice (n = 15) were subjected to a battery of three behavioural tests: elevated plus-maze test (day 0), locomotor activity test (day 5), and social interaction test (day 10). The motility box test was carried out as described in Innos et al. 2013. Four behavioural parameters were recorded: (1) time spent moving (move, s); (2) distance travelled (distance, m); (3) time spent in the centre (time centre, s); and (4) time spent in the corners (time corner, s). The plus-maze experiment was performed as described in Philips et al. (2010) and eight behavioural parameters were recorded: (1) the number of closed arm entries; (2) the number of open arm entries; (3) the ratio between open and closed arm entries; (4) the latency to enter open arm (latency, s); (5) time spent on open arms; (6) the number of protected head-dips; (7) the number of unprotected head-dips; and (8) the number of stretch-attend postures (SAPs). The social interaction test was carried out as described previously (Innos et al. 2012), briefly: 14 mice were matched into 7 pairs of two unfamiliar mice according to the bodyweight. (1) The time spent sniffing the partner's anogenital area (anogenital sniffing, s) and (2) the time spent sniffing other body regions (sniffing other body parts, s) were recorded separately. These two measures were also summarized for (3) the time of total social sniffing for each animal (time of social sniffing, s). The animals were decapitated 10 days after the last experiment and the ventral striatum (including nucleus accumbens and olfactory tuberculi), hippocampus and temporal lobe (including temporal cortex and amygdala) were dissected from the brains.

In the second part of the behavioural studies, we investigated the influence of acute fear reaction on the expressional activity of the *Lsamp* gene. In order to induce acute fear reaction, fear conditioning was carried out by means of a computer-controlled Multi Conditioning System (TSE). Sixteen mice were divided into three groups: "naïve" (n = 5); "pre-conditioning" (n = 5) and "conditioned fear" (n = 6). The mice in "pre-conditioning" and "conditioned fear" groups underwent fear conditioning, which was performed in a dimly illuminated (15 lx) acrylic cage (30 × 30 × 30 cm) with stainless steel rod floor. Between subjects the cages were cleaned with isopropanol. On the first day after 150-s acclimation period animals received six trials with the following stimuli: 15 s tone (12 kHz; 70 dB) and bright light (pulsing at 200 ms) were

terminated by a 2-s electric shock (0.6 mA) during which the light was constant. Inter-trial interval was 120 s (±50%). After the last trial the animals were returned to their home cages. On the second day, animals in the "conditioned fear" group were placed into the conditioning cages and exposed to similar stimuli without an electric shock for about 45 min (20 trials). The animals were killed immediately afterwards. "Naïve" and "Pre-conditioning" groups received no treatment on the second day. The hippocampus and temporal lobe (including the temporal cortex and the lateral, basolateral, central and medial nuclei of the amygdala) were dissected from the brains.

The data were analysed using Statistica version 8.0 (StatSoft, Inc.). As the behavioural scores were not normally distributed, Spearman's rank-order method was used for the calculation of correlation coefficients. In the fear conditioning study, one-way ANOVA (conditioning type as grouping variable) was performed. Tukey HSD post hoc analysis was used when applicable after statistically significant ANOVA. Data are presented as mean ± SEM.

RESULTS AND DISCUSSION

1. *Wfs1* expression is initiated at the time of intense synaptogenesis and achieves mature pattern after puberty (Ref I, Ref II)

Neuroimaging studies in young WS patients have revealed defects in axon myelination and brain growth (Ghirardello et al., 2014; Hershey et al., 2012; Lugar et al., 2016), suggesting a role in the brain development for WFS1. Therefore, we addressed the initiation of *Wfs1* expression in the developing brain, by studying it in parallel with the marker of synaptic activity, synaptophysin (*Syp1*).

First, when comparing the initiation pattern of *Wfs1* in two evolutionarily distant lineages, mouse and domestic chick, it appears that *Wfs1* is first expressed in the phylogenetically older striatal and amygdaloid regions at the time when most neurons have migrated to their destination and begin to make contacts to build up the neural network. This finding suggests that *Wfs1* is not involved in neurogenesis, neuronal migration or neurite outgrowth, but more likely in later neurodevelopmental processes.

In mouse brain, the mRNA of *Wfs1* could be first detected at E15.5 in the developing striatum and central and medial amygdala (CeA and MeA, respectively; see Fig. 4E-H, same as Ref I, Fig. 1E-H). This falls in the time window of the beginning of synaptic functioning in the subcortical regions of the brain, as shown by the expression of *Syp1* (Fig. 4A-D, same as Ref I, Fig. 1A-D). In the domestic chick brain, the expression of *Wfs1* started between E10 – E13 (Ref II, S2 Fig. A-B). Like in the mouse, the first structures that expressed *Wfs1* in the chick brain were striatal and subpallial amygdaloid regions. Another similarity between the two species was that the most widespread expression of *Wfs1* occurred during the development, around the time of birth in the mouse (at P2; Ref I, Fig. 2A-D) and hatching in the chick (at E20; Ref II, S2 Fig. C-D). The early expression pattern of *Wfs1* in mouse and chick brain suggests that it follows the ontogenetic development of the brain structures, appearing first in the phylogenetically older regions and later in the phylogenetically newer pallial structures. It is possible that the expression of *Wfs1* is activated by the synaptic activation of neurons or by the high embryonic expression of ER stress pathway mediators, such as spliced XBP1 (Hayashi et al., 2007; Kakiuchi et al., 2006).

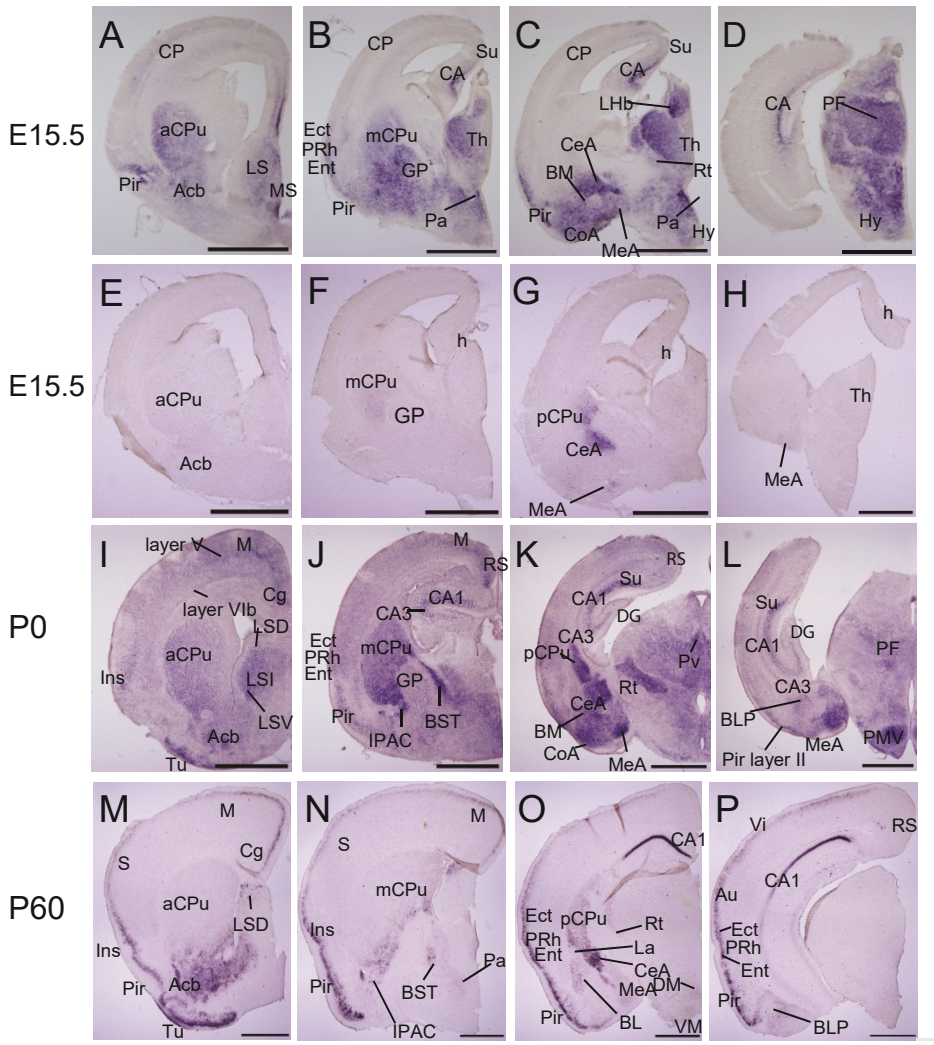


Figure 4. The initiation and developmental dynamics of *Wfs1* expression in the mouse brain. mRNA *in situ* hybridization on coronal brain sections. For comparison, *Syp1* expression as a marker of synaptic maturation is depicted in A-D. The expression of *Wfs1* is initiated at E15.5 (E-H), achieves widespread expression in most of the fore-brain and diencephalon by birth (I-L) and shows widespread downregulation and specific upregulation by adulthood (M-P). Abbreviations: Acb-nucleus accumbens, aCPu-anterior caudate-putamen, Au-auditory cortex, BL-basolateral nucleus of the amygdala, BLP-basolateral amygdala, posterior part, BM-basomedial nucleus of the amygdala, BST-bed nucleus of stria terminalis, CA-cornu ammonis, CA1-cornu ammonis 1, CA3-cornu ammonis 3, CeA-central nucleus of the amygdala, Cg-cingulate cortex, CoA-cortical nucleus of the amygdala, CP-cortical plate, DG-dentate gyrus, DM-dorsomedial hypothalamus, Ect-ectorhinal cortex, Ent-entorhinal cortex, GP-globus pallidus, h-hippocampus, Hy-hypothalamus, Ins-insular cortex, IPAC-interstitial nucleus of the posterior limb of the anterior commissure, La-lateral nucleus of the amygdala, LHb-lateral habenula, LS-lateral septal nucleus, LSD-lateral septal nucleus,

dorsal part, LSI-lateral septal nucleus, intermediate part, LSV, lateral septal nucleus, ventral part, M-motor cortex, mCPu-medial caudate-putamen, MeA-medial nucleus of the amygdala, MS-medial septal nucleus, pCPu-posterior caudate-putamen, Pa-paraventricular nucleus of the hypothalamus, PF-parafascicular nucleus of the thalamus, Pir-piriform cortex, PMV-ventral premammillary nucleus, PRh-perirhinal cortex, PV-paraventricular nucleus of the thalamus, RS-retrosplenial cortex, Rt-reticular thalamic nucleus, S-somatosensory cortex, Su-subiculum, Th-thalamus, Tu-olfactory tubercle, Vi-visual cortex, VM-ventromedial hypothalamus. Scale bar 1 mm. Adapted from Ref I, Fig. 1 and 2.

The shaping out of the expression pattern of *Wfs1* progressed gradually, involving numerous temporary events. In mouse, there were several brain regions that lost the expression of *Wfs1* entirely or almost entirely by adulthood. The early postnatal widespread expression in the diencephalon (Ref I, Fig. 1O-P, Fig. 2C-D) remained only weakly in the reticular nucleus of the thalamus (Rt) and paraventricular, dorsomedial and ventromedial hypothalamic nuclei (Ref I, Fig. 2N-O). In the septum, the expression of *Wfs1* became restricted to the dorsal part of the lateral septal nucleus (LSD) in the adult brain (Ref I, compare Fig. 2A *vs* Fig. 2M). In the anterior caudate-putamen (aCPu), *Wfs1* expression persisted only in the ventral and medial tiers (Ref I, compare Fig. 1M-N, Fig. 2A-B *vs* Fig. 2M-N). In the caudal aspects of the amygdala, including MeA, posterior basomedial (BMP) and posterior basolateral (BLP) nuclei, the remarkably strong perinatal signal was downregulated to weak level by the adulthood (Ref I, Fig. 1K-L, P, Fig. 2D, O-P).

Since the formation of the neural network underlying the emotional behaviour, including fear and anxiety, largely depends on environmental feedback, the shaping out of these circuits takes place late after birth (Bouwmeester et al., 2002; Casey et al., 2017; Cressman et al., 2010; Wolterink et al., 2001). The developmental dynamics of *Wfs1* in the mouse MeA, BMP and BLP could reflect the prolonged maturation of the fear-associated circuits. MeA receives prominent vomeronasal input and its function is involved in sensing the presence of a predator and generating a predator-induced fear response (Dielenberg et al., 2001; Martinez et al., 2011; Scalia and Winans, 1975). The activation of the basolateral amygdala promotes the expression of fear and is required for storing and modulating fear memories (Maren et al., 1996; Sierra-Mercado et al., 2011). Neural activity in the basomedial amygdala suppresses fear behaviour (Adhikari et al., 2015). The posterior division of basomedial amygdala is particularly interesting in the context of *Wfs1* expression, as it mostly projects to other *Wfs1*-expressing regions like pre- and infralimbic cortices, ecto- and perirhinal cortices, parts of lateral, central, medial and cortical amygdala, nucleus accumbens, bed nucleus of stria terminalis, ventromedial hypothalamus and posterior caudate-putamen (Petrovich et al., 1996). This probably indicates the requirement of WFS1 during the formation of certain interconnected fear-related neural pathways.

The developmental dynamics of the *Wfs1* expression pattern in the mouse septum correlates well with the maturation of the septal serotonergic, dopaminergic and enkephalinergic afferent connections and hippocampal input, which advances in medial to lateral manner and accomplishes after the second postnatal week (Sheehan et al., 2004). Likewise, in the chick, the maturation of *Wfs1* expression in the septum was prolonged, as it was present only in the medial septal nucleus of the adult bird, but not in any of the developmental stages considered (Ref I S3 Fig. A, C).

The developmentally transient expression of *Wfs1* in the anterior CPu of the mouse suggests that in the dorsal striatum, *Wfs1* expression is initiated during the time of synaptic activation and plays a role specifically during the development. CPu is a large structure primarily involved in the generation of voluntary movements, but it also participates in associative and motivational aspects of behaviour. As a matter of fact, the dorsomedial striatum is connected to the cortical and thalamic regions attributed to limbic functions, and takes part in goal-directed behaviour, *versus* habitual behaviour controlled by the dorsolateral striatum (Burton et al., 2015; Voorn et al., 2004). Consistent with this, the expression of *Wfs1* persists near the medial margin of the aCPu, reassuring its involvement in the emotional circuits of the brain. The other subregion of the CPu retaining the *Wfs1* expression in adulthood is the posterior CPu (pCPu), which is the site for the convergence of auditory and dopaminergic stimulation (Arnauld et al., 1996).

Of the cortical structures of the mouse, the expression of *Wfs1* first appeared at E18.5 in the regions that are phylogenetically older and showed established *Syp1* signal by E17.5 (see Ref I, Fig. 3C-D), namely in the piriform cortex (Pir), insular cortex, ecto-, ento- and perirhinal cortices (Ect, Ent and PRh, respectively), cortical amygdala (CoA) and hippocampus (Ref I, Fig. 1I-L). In these structures, the expression gained strength during the subsequent days and became prominently strong by P20 (Ref I, Fig. 1M-P, Fig. 2A-L). The expression in the hippocampus spread throughout the CA region and the developing dentate gyrus after birth (Ref I, Fig. 1N-P, Fig. 2C-D, F-H) and became restricted to the CA1 only after P7 (Ref I, Fig. 2K-L, Fig. 5M). The first entorhinal axons arborize in the CA region of the hippocampus at E17 (Supèr and Soriano, 1994), just before the beginning of *Wfs1* expression. Dentate gyrus, which is one of the main input gateways to the hippocampus in adulthood, is shaped out during the postnatal development (Altman and Bayer, 1990; Leuner and Gould, 2010; see also Ref I, Fig. 3C-H). Therefore, we propose that the initiation of *Wfs1* expression in the hippocampus is caused by the early synaptic activity and the refinement of the expression pattern occurs after the main synaptic input through the dentate gyrus becomes functional.

In the neocortex, which is the last structure to become anatomically and functionally mature, the *Wfs1* expression took long to initiate. In accordance with the inside-out order of the development of the cerebral cortex (Angevine and Sidman, 1961; Rakic, 1974), the deeper layers showed specific pattern of *Syp1* expression first (Ref I, Fig. 4B, D, F). The expression of *Wfs1* also

appeared first in the deeper layers and later in more superficial layers. Layer II/III, the *Wfs1*-expressing layer in the adult brain, started expressing *Wfs1* as late as at P4, beginning with auditory, motor, cingulate and retrosplenial cortical fields (Ref I, Fig. 2E-H). Layer V, which is the source of long subcortical projections, showed transient expression between birth and adulthood (Ref I, Fig. 1M-O, Fig. 2A-P, Fig. 4C, E, G, I, K, M). Likewise, layer VIb/subplate showed temporary expression of *Wfs1* between P0 and P4 (Ref I, Fig. 1M, Fig. 2A-C, E, Fig. 4E). Majority of the neurons in layer VIb are short-lived and they fulfil specific role during the development, establishing the first pioneering axons that guide the thalamocortical axons to their cortical target (Molnár et al., 1998a; Molnár et al., 1998b; Price et al., 1997). When reaching the developing cortex, the growing thalamocortical axons usually arrest for several days and make synaptic contacts with the neurons of layer VIb (Higashi et al., 2005; Molnár et al., 1998a; Molnár et al., 1998b). It is worth pointing out, that layer VIb resembles the reticular nucleus of the thalamus (Rt) in many ways: also, Rt loses its volume by adulthood and serves as a resting and rearranging compartment for the growing corticothalamic axons (Jacobs et al., 2007; Molnár and Cordery, 1999; Montiel et al., 2011). We first observed the expression of *Wfs1* in the Rt at E18.5 (Ref I, Fig. 1K), which is the time when the growing corticothalamic axons leave Rt and start to invade the dorsal thalamus (Jacobs et al., 2007). All in all, the pattern of the initiation of *Wfs1* in the neocortex and Rt favours the hypothesis that the expression of *Wfs1* is initiated by the beginning of synaptic activity and (largely) transient expression in layers V and VIb and in Rt might refer to the temporarily increased synaptic activity during the time of *Wfs1* expression. In general, our findings in the mouse were well in accordance with the study published by Kawano and co-workers in 2008, which focussed on the postnatal changes in the expression pattern of *Wfs1*. An overview of the developmental expression pattern of *Wfs1* in the mouse brain is in Supplementary Table 2a in Ref I.

Like in the mouse, there were some regions in the chick brain where the expression of *Wfs1* was downregulated by adulthood. Most notably, the relatively widespread expression in the pallial amygdala of the embryonic chick became only faintly detectable in the dorsal region of the amygdala (ADo) and amygdalopiriform transition area (APir) in the adult chick (Ref II, compare S2 Fig. C-D *vs* Fig. 5H). This suggests the common developmental dynamics of *Wfs1* expression in the posterior pallial amygdala in mammalian and avian brain, which is more thoroughly discussed in the third chapter of this section.

2. Lack of functional WFS1 does not result in the alteration of ER stress markers in the developing mouse brain (Ref I)

ER plays essential role in the post-translational modification, folding and trafficking of secretory and membrane-bound proteins, phospholipid biosynthesis and calcium storage. Perturbed functioning of the ER results in ER stress, which is manifested by the accumulation of unfolded proteins. The protein folding status in the ER is sensed by the luminal domains of three ER membrane-resident proteins: inositol-requiring protein-1 (Ire1), activating transcription factor-6 (Atf6) and protein kinase RNA (PKR)-like ER kinase (PERK). To alleviate the ER stress, these proteins initiate the intracellular signalling pathways collectively known as unfolded protein response (UPR) or ER stress response. UPR involves the general attenuation of protein synthesis, while upregulating the production of chaperones and degrading misfolded proteins. It also mediates apoptosis under severe ER stress (for a review, see Ron and Walter, 2007). While ER stress can result from numerous environmental challenges and pathological conditions (Zhao and Ackerman, 2006), activation of UPR signalling pathways is also essential during the normal development in different cell types (Rutkowski and Hedge, 2010). Most notably these include the cells possessing immense secretory capacity, such as antibody-producing B lymphocytes (Gass et al., 2002; Reimold et al., 2001; Zhang et al., 2005) and exocrine cells of the pancreas and salivary glands (Lee et al., 2005), but also cardiomyocytes (Masaki et al., 1999), hepatocytes (Reimold et al., 2000), osteoblasts (Pereira et al., 2004; Saito et al., 2011), dendritic cells (Iwakoshi et al., 2007), and placental cells (Iwawaki et al., 2009).

Physiological ER stress also occurs during the development of the nervous system. ER stress pathways are activated in the brain during the embryonic (Zhang et al., 2007) and postnatal development (Hayashi et al., 2007) and UPR signalling is required for neuronal differentiation (Cho et al., 2009) and neurite growth (Favero et al., 2013). The correct timing of attenuation of the developmental UPR is crucial for proper neural development, as shown in the mouse cerebral cortex (Laguesse et al., 2015).

The most well-known function of WFS1 is alleviating ER stress response/ UPR, which has been extensively studied in the pancreas (Fonseca et al., 2005; Fonseca et al., 2010; Shang et al., 2014; Ueda et al., 2005; Yamada et al., 2006). There is also evidence that depletion of WFS1 in neuronal cells upregulates ER stress response pathways and apoptosis (Cagalinec et al., 2016; Gharanei et al., 2013).

Regarding the remarkably widespread expression of *Wfs1* at the perinatal period, it is tempting to hypothesize that WFS1 is needed in many neurons specifically during the beginning of synaptic activity, which might be related to the developmental ER stress. Birth or hatching drastically exposes the animal to large number of new sensory stimuli that need to be processed in the brain. This

requires the acquisition of the full capacity of the protein synthesis machinery, which is challenging to the neurons. Widespread expression of *Wfs1* seen in the brain around the time of birth/hatching might reflect its role in coping with the strenuous intracellular environment. We addressed this question by measuring the levels of UPR genes in the brains of wild-type (wt) and *Wfs1*$^{-/-}$ mice during the perinatal period between E17.5 and P4. We confirmed the developmental downregulation of ER stress response genes *Grp78*, *Grp94* and *CHOP* (Fig. 5; Ref I, Fig. 5a, b, S Table 4). Each was highly expressed in the embryonic brain and underwent abrupt decrease by the time of birth. Interestingly, the expression level of *Grp78* temporarily rose again during the following postnatal days before obtaining its low adult level (Fig. 5; Ref I, Fig. 5a, b). We did not observe differences in the expression dynamics of *Grp78*, *Grp94* and *CHOP* between *Wfs1*$^{-/-}$ and wild-type (wt) mice, suggesting that the lack of Wfs1 does not alter the activity of UPR pathways in the developing brain between E17.5 and P4 (Fig. 5; Ref I, Fig. 5b, S Table 4). There are still some limitations to this result, for we did not investigate the expression of ER stress response related genes in specific brain structures, but in the whole forebrain. It might still be the case that in some structures where WFS1 is expressed, it could take part in regulating the ER stress response. Secondly, there are several other mediators in the ER stress pathways. Whereas the upregulation of *Grp*-s is general outcome of all ER stress pathways, upregulation of *CHOP* reflects a severe stress and results from the activation of PERK-eIF2α-ATF4 pathway (Kadowaki and Nishitoh, 2013). Also, different regions in the brain are vulnerable to excess ER stress at different times during the development. Wang and co-workers (2015) showed that the injection of ER stress inducer tunicamycin upregulated UPR pathways in the developing brain and caused caspase-3 activation in the layer II of parietal cortex, CA region of the hippocampus and external granule cell layer of the cerebellum at P4. At P12 the activation of caspase-3 had shifted to the dentate gyrus of the hippocampus, deep cerebellar nuclei, and pons, and no caspase-3 activation was detected in mice injected at P25 (Wang et al., 2015). Indeed, the lack of WFS1 contributes to the activation of IRE1-dependent ER stress pathway in the temporal lobe upon exposure to mild stress in adult mice (Altpere et al., 2017).

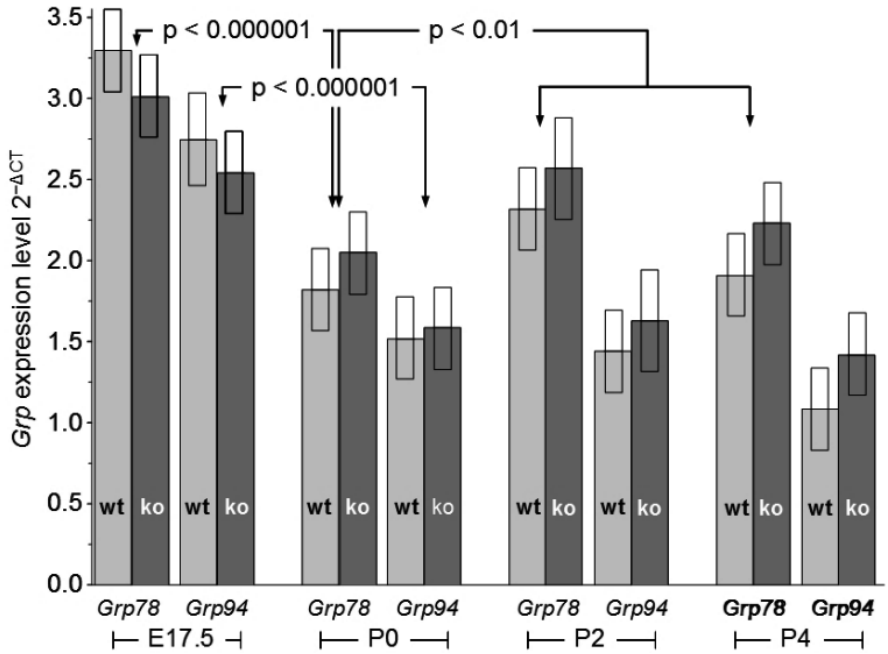


Figure 5. The expression of *Grp78* and *Grp94* in the brains of wt and *Wfs1* knockout (ko) mice during the perinatal period as revealed by quantitative real-time PCR analysis. Estimated expression levels with 95% confidence limits (indicated as rectangles); significantly differing expression levels are shown with arrows. Modified from Ref I, Fig. 5.

Taken together, we can conclude that the reason why the expression of *Wfs1* is initiated in a much wider variety of brain regions during the perinatal development is probably not the requirement for downregulating ER stress response. Instead, it might be necessary for the modelling of neural network. WFS1 has been shown to interact with the E3 ubiquitin ligases SMURF1 and HRD1, which are involved in the neurodevelopmental processes (Bryan et al., 2005; Kawada et al., 2014). In particular, WFS1 stabilizes the ER stress-inducible HRD1 (Fonseca et al., 2010) and is degraded by ER stress-sensitive SMURF1 (Guo et al., 2011). In the medial prefrontal cortex of adult mice, the restraint stress-induced production of WNT7A and neurotrophin3 (NTF3), two proteins involved in neural development and synaptic remodelling, is altered by the deletion of *Wfs1* (Shrestha et al., 2015). Thus, the widespread perinatal expression of *Wfs1* might be needed for the regulation of the developmental processes dependent on Wnt family proteins, neurotrophins or ubiquitin ligases (Ascano et al., 2012; Hall et al., 2000; Upadhyay et al., 2017).

3. The expression of *Wfs1* is conserved in subpallial regions, but more varied across lineages in the pallial derivatives of the amniote forebrain (Ref I, Ref II)

We decided to address the evolutionary conservation of the expression of *Wfs1* by comparatively studying it in rodent (mouse), avian (domestic chick) and chelonian (red-eared slider turtle) lineage. The divergence between the mammalian and sauropsid (including birds and reptiles) lineages occurred approximately 317 million years ago (MYA) and the split between birds and turtles occurred about 257 MYA (Shen et al., 2011). Studying the conservation of gene expression can bring us closer to understanding the function of our gene of interest, also, it might indicate how the functions of the homologous brain structures have diverged during the evolution. In addition, the developmental pattern of gene activation can provide valuable information about the ancestral situation and formation of brain structures and circuits.

As also shown by other authors (Kawano et al., 2009; Luuk et al., 2008), the highest *Wfs1* expressing regions in mouse were the CA1 region of the hippocampus, central extended amygdala, Pir, pCPu, and ventral striatum (Ref I, Fig. 2M-P). In the chick brain, the strongest expression was seen in the medial striatum (MSt), other regions with considerably strong signal were the caudal part of lateral striatum (LSt), striopallidal area (StPal), strioamygdaloid transition area (StAm), extended amygdala (EA), striatal part of the olfactory tubercle (TuSt), and striopallidal part of the olfactory tubercle (TuStPal; Ref II, Fig. 5A-C, G). In the red-eared slider turtle, *Wfs1* was expressed in striatal regions (including striatum (St), nucleus accumbens (Acb), olfactory tubercle (Tu)), striatoamygdalar area (StA), dorsal ventricular ridge (DVR), pallial thickening (PT), dorsal cortex (DC) and partially in the medial cortex (MC; Ref II, Fig. 7A-D).

Altogether, in mouse, chick and turtle, striatal structures and the subpallial nuclei of the amygdala were expressing *Wfs1* while the pallidal regions lacked the expression. This suggests that the expression in the subpallium shares a common developmental origin. The projection neurons of the striatal structures, including the dorsal and ventral striatum, as well as the central and intercalated nuclei of the amygdala are derived from the lateral ganglionic eminence (LGE) in the developing brain, while the medial ganglionic eminence (MGE) gives rise to the globus pallidus and ventral pallidum and the striatal and cortical interneurons (Deacon et al., 1994; Marin et al., 2000; Olsson et al., 1998). As the CPu, Acb, Tu and CeA of the mouse (Ref I, Fig. 2M-O, Ref II, Fig. 1A, G), MSt, LSt, intrapeduncular nucleus (InP), Acb, TuSt, TuStPal, StPal, StAm and EA of the chick (Ref II, Fig. 5A-C, G) and St, Acb, Tu and StA of the turtle (Ref II, Fig. 7A-C) were all expressing *Wfs1*, while globus pallidus and ventral pallidal territories were devoid of expression, a conclusion can be derived that the expression of *Wfs1* might be common for the LGE derivatives across the tetrapods.

The medial to lateral gradient of *Wfs1* expression across the avian striatum further supports the proposal that Wfs1 functions in emotional and motivational rather than purely somatomotor circuits in the striatum (see chapter 1 of Results and Discussion). The medial division of the avian MSt receives pallial input from the hippocampus and olfactory bulb (Veenman et al., 1995) and thalamic projections from the midline and intralaminar thalamic nuclei associated with viscerolimbic functions (Veenman et al., 1997), while the dopaminergic input preferentially arises from the ventral tegmental area (Mezey and Csillag, 2002). More lateral MSt receives pallial input from regions involved in somatosensory, visual, auditory and motor function (Veenman et al., 1995) and dopaminergic input from the substantia nigra (Mezey and Csillag, 2002). Also, LSt receives its input mainly from somatic pallial and thalamic regions (Veenman et al., 1995; Veenman et al., 1997) and might be comparable to the mammalian dorsolateral CPu (Kuenzel et al., 2011). Thus, Wfs1 might have a conserved role in the viscerolimbic functions of the striatum.

An interesting finding was that the mammalian intercalated amygdala (IA) and its putative avian homologue, striatal capsule (StC) were both expressing *Wfs1* (Ref II, Fig. 2A, C, E), supporting the homology proposed by Abellán and Medina, 2009, and suggesting that Wfs1 has a conserved function in this structure, probably related to the regulation of fear behaviour. The intercalated cell masses in the amygdala are providing feedforward inhibitory control over the basolateral and central nuclei, thus suppressing the fear behaviour (Ehrlich et al., 2009; Paré et al., 2003). Considering the behavioural outcomes of the ablation of *Wfs1* (Luuk et al., 2009), it would be reasonable to guess that the function of the intercalated amygdala might be affected in *Wfs1*$^{-/-}$ mice. As the intercalated cells are GABA-ergic, and changes in the GABA-ergic system have been shown to underscore the anxiety-related behaviour in *Wfs1*$^{-/-}$ mice (Raud et al., 2009; Raud et al., 2015), the role of WFS1 in the intercalated amygdala would deserve a more in-depth study.

In the bed nucleus of the stria terminalis (BST), the expression of *Wfs1* was present in both mouse and chick (Ref I, Fig. 1J, N, Fig. 2B, J, N; Ref II, Fig. 5C, G, S1Fig. B, G), but showed developmental downregulation in the latter (compare Ref II, Fig. 5G *vs* Ref II, S1Fig. G). BST belongs to the extended amygdala system, together with the central and medial nuclei of the amygdala. While the central nucleus of the amygdala mainly derives from the LGE, then BST and medial amygdala contain cell groups derived from the MGE, LGE, anterior peduncular area, commissural preoptic area, as well as ventral pallial and extratelencephalic progenitor domains (García-López et al., 2008). Similarly to mammals, the heterogenic developmental origin of the BST and medial amygdala has been established in the avian and reptilian brain (Abellán and Medina, 2009; Moreno et al., 2010; Puelles et al., 2000; Vicario et al., 2014). We did not distinguish the BST in the turtle brain, but the comparison with the detailed study of Moreno and colleagues, 2010 suggests that the BST is situated in the *Wfs1*-expressing region on the septal side of the ventral horn of the lateral ventricle, adjacent to the Acb (Ref II, Fig. 7A, B). In addition, the

Wfs1-expressing region designated as the circumventricular organ striopallidal organ (SPO) in our study (Ref II, Fig. 5B, C, S1Fig. A, B, F, G), based on Puelles et al., 2007, might in fact represent the dorsal and dorsomedial divisions of the lateral BST (Abellán and Medina, 2009; Vicario et al., 2014), but further study with region-specific markers would be needed to clarify this.

Despite the closer evolutionary relatedness, the expression pattern of *Wfs1* in the pallial brain regions was considerably different between turtle and chick. While the expression of *Wfs1* was largely lacking in the chick pallium, it was relatively widespread in the pallial structures of turtle and mouse. Notably, the derivatives of the dorsal and medial pallium almost entirely lacked the expression in the chick brain but showed conspicuously strong expression in mouse and turtle. In both, mouse and turtle, *Wfs1* was strongly expressed in the hippocampal homologue, in the CA1 region of the hippocampus in mouse and in the MC in the turtle (Ref I, Fig. 2 O-P; Ref II, Fig. 7A-C). In the turtle MC, *Wfs1* was expressed in the region proximal to the DC (Ref II, Fig. 7A-C). In the hippocampal formation of the chick, only subset of cells in the parahippocampal area were expressing *Wfs1* (Ref II, Fig. 5I). Since the avian parahippocampal area and reptilian dorsomedial cortex both correspond to the regions in mammalian hippocampus proper (Manns and Eichenbaum, 2009), the expression of *Wfs1* specifically in these regions might reflect the ancestral state in stem amniotes.

The distribution of the expression of *Wfs1* in the derivatives of the dorsal pallium, *i.e.* neocortex in the mouse, hyperpallium in the chick and dorsal cortex in the turtle, suggested independent gain in mouse and turtle, or alternatively, loss in the avian lineage. In mouse, *Wfs1* was expressed in the layer II/III of all neocortical fields except the retrosplenial cortex (RS), nevertheless, during the development, RS too showed the expression of *Wfs1* (Ref I, Fig. 2C, G, M-P). In the chick hyperpallium, there was no *Wfs1* expression at any developmental stage. In turtle, DC was the strongest *Wfs1* expression site, which probably reflects its importance in this brain structure.

We propose that the strong expression of *Wfs1* in the DC of the red-eared sliding turtle is a consequence of functional specification rather than indicator of evolutionary conservation. The cortical neurons of freshwater turtles are remarkably resistant to hypoxia and excitotoxicity (Wilson and Kriegestein, 1991; Doll et al., 1991). The resistance to neuronal damage includes reducing neuronal energy expenditure, downregulating excitatory amino acid release while increasing GABA release, inhibiting pro-apoptotic and activating pro-survival pathways (Kesaraju et al., 2009; Lutz and Milton, 2004; Nayak et al., 2016). In addition, the brains of freshwater turtles express innately higher levels of Hsp70 family heat shock proteins, stress-responsive transcription factor NF-κB, and harbour enhanced capacity to fight reactive oxygen species (Lutz and Milton, 2004; Prentice et al., 2004; Rice et al., 1995; Willmore and Storey, 1997). Interestingly, *Grp94*, a molecular partner of Wfs1, is among the genes that are strongly induced in the brain of red-eared slider turtle in response to anoxia (Kesaraju et al., 2009). Several studies show that ER stress and hypoxia

response are interconnected and UPR pathways can be activated in response to hypoxia (Koumenis et al., 2002; Pereira et al., 2014; Romero-Ramirez et al., 2004; see Wouters and Koritzinsky, 2008 for review). Based on this evidence, and the role of Wfs1 in regulating ER stress response (Fonseca et al., 2005; Fonseca et al., 2010; Yamada et al., 2006), we hypothesize that the strong expression in the DC of the red-eared slider turtle is related to the hypoxia tolerance of this species. Therefore, it would be interesting to compare the obtained results with the expression of *Wfs1* in a non-hypoxia resistant chelonian species.

The lateral pallial derivative pallial thickening showed strong *Wfs1* expression in the turtle (Ref II, Fig. 7A-C), its putative mammalian homologues claustrum and endopiriform nucleus (Bruce, 2009) did not express *Wfs1* at any developmental stage. Nevertheless, Puelles et al., 2016, suggest that the mammalian homologue for the pallial thickening could be the insular cortex, which showed strong expression of *Wfs1* in the mouse (Ref I Fig. 2M-N). The lateral cortex (LC) of the turtle did not express *Wfs1*, but its putative mammalian homologue Pir is one of the strongest *Wfs1*-positive regions in the mouse brain (Ref II Fig. 7A-C, Fig. 1A, G; Bruce, 2009). However, the homology between many pallial derivatives is still disputable. Martínez-García et al., 2009 favour considering the reptilian lateral cortex homologous to mammalian posterolateral cortical amygdala and amygdalopiriform transition area, which also express *Wfs1*, but less than Pir (Ref I Fig. 2; Ref II Table 1). The question about the evolution of the tetrapodean pallium has been most extensively brought under attention in studies on avian brain and it has given rise to fervent debates between scientists (Jarvis et al., 2005; Montiel and Molnár, 2013). Thus, the consensus in the homology between avian and mammalian pallial amygdala is still lacking these days. Whereas some authors consider the avian ADo and core nucleus of the amygdala (ACo) homologous to the mammalian basolateral (BL) and basomedial (BM) amygdaloid complex (Martínez-García et al., 2009), others argue that ADo and ACo correspond to the mammalian neocortical layers V and IV, respectively (Reiner et al., 2004; Reiner et al., 2005). The developmental dynamics of *Wfs1* in the aforementioned structures in the chick and mouse brain (see chapter 1 in this section) does not contradict with either proposal of homology. However, the role of the avian posterior arcopallium in the regulation of fear behaviour advocates for the homology to the mammalian pallial amygdala (Saint-Dizier et al., 2009). We did not perform the developmental gene expression study on the turtle brain, therefore, we cannot make conclusions about the developmental dynamics of the expression of *Wfs1* in the turtle caudal DVR, the counterpart of mammalian BM and BL according to Bruce et al., 2009, and Martínez-García et al., 2009. Nevertheless, the ventricular edge of the caudal DVR was one of the strongest *Wfs1* expression sites in the turtle brain (Ref II, Fig. 7D), which might reflect the evolutionary conservation of *Wfs1* expression in the ventral and lateral pallial derivatives.

In conclusion, our results suggest the common ancestral pattern of *Wfs1* expression in the subpallium. On the development of pallium, we favour the position that the expression of *Wfs1* is conserved in the pallial amygdaloid structures and perhaps in the hippocampal complex but gained independently in the derivatives of the dorsal pallium and subset of lateral pallial derivatives across the three evolutionary lineages studied.

On the development of pallium, we favour the position that the expression of *Wfs1* is conserved in the pallial amygdaloid structures and perhaps in the hippocampal complex but gained independently in the derivatives of the dorsal pallium and subset of lateral pallial derivatives across the three evolutionary lineages studied.

4. *Wfs1* is expressed in the brain regions that receive dopaminergic input and it influences the number of ligand binding sites of D1-like receptor (Ref II)

Since *Wfs1*$^{-/-}$ mice display alterations in their dopaminergic system (Luuk et al., 2009; Matto et al., 2011; Visnapuu et al., 2013a) and *Wfs1* is expressed in the brain regions that are known as targets of the dopaminergic pathways, such as ventral and dorsal striatum, hippocampus, central extended amygdala and cerebral cortex (Björklund and Dunnett, 2007; Wise, 2004), we aimed to take the relation of WFS1 and dopamine system under a closer investigation. We decided to study the relation between WFS1 and D1-type dopamine receptors, since the D1-type receptors are the most widespread dopamine receptors in the brain, being present in many *Wfs1*-expressing regions (Missale et al., 1998).

The mRNA expression of the two D1-type dopamine receptors, *Drd1a* (also known as *Drd1* in mammals) and *Drd5*, was rather evenly distributed throughout the mouse brain, with *Drd1a* showing more expression in the striatum and *Drd5* in the cortical structures and amygdala (Ref II, Fig. 1B, C, E, F, H, I, K, L, N, O). The immunohistochemical evaluation showed more specific confinement of both proteins to the well-known dopaminoceptive regions (Ref II, Fig. 3E-L). The expression pattern of D1-like dopamine receptors, especially that of DRD1A, was resembling the expression of WFS1 on the protein level (Ref II, Fig. 3, Fig. 4). In the chick brain, the mRNA expressions of *Wfs1* and *Drd1a* were almost completely overlapping (Ref II Fig. 5A-H, J, K). Likewise, in the turtle brain, the anatomical distributions of *Wfs1* and *Drd1a* mRNA were considerably similar (Ref II, Fig. 7).

Inspired by the largely overlapping expression pattern of *Wfs1* and *Drd1a*, we decided to examine the D1-like receptor specific dopaminergic transmission in wt and *Wfs1* KO mice. For this, we assayed the radioactive ligand binding of D1-like receptors in the hippocampi of wt and *Wfs1*$^{-/-}$ mice. We found that the ligand binding was increased in the *Wfs1*$^{-/-}$ hippocampi, indicating that *Wfs1* KO mice have more D1 receptor ligand binding sites than the wt mice (Fig. 6, same

as Ref II, Fig. 9). We propose that the increase in the D1-like receptor binding sites reflects a homeostatic change in response to pre- or postsynaptic variation of dopaminergic signalisation in the *Wfs1*$^{-/-}$ mice. It could be directly caused by the lack of WFS1 in the postsynaptic cells or alternatively reflect a compensatory change in response to the reduced dopamine output, as shown in the striatum of the *Wfs1*$^{-/-}$ mice (Matto et al., 2011). Since the intracellular environment is severely affected in WFS1 deficient neurons (Cagalinec et al., 2016), it could be the case that signal transduction pathways commencing from D1 receptor are deranged in *Wfs1* deficiency. Regarding that the D1-like receptors mediate changes in intracellular calcium levels (Lin et al., 1995; Liu et al., 1992; Surmeier et al., 1995; Swapna et al., 2016), lack of WFS1 is likely to interfere with these processes.

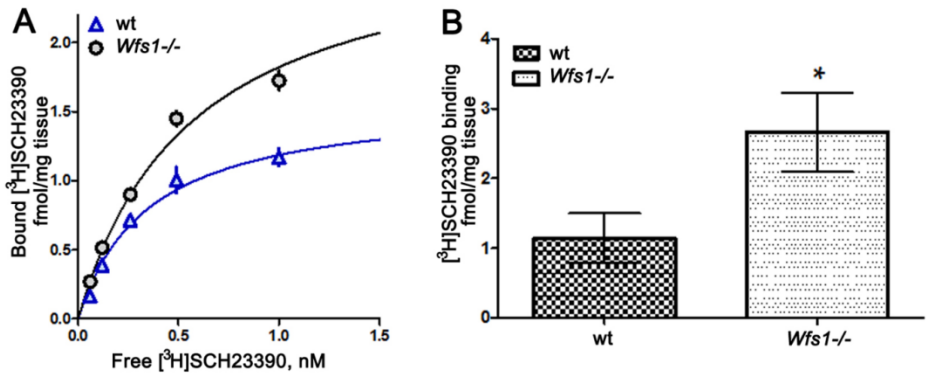


Figure 6. Binding of D1-type dopamine receptor specific ligand [^{3}H]SCH23390 to hippocampal membranes of wt and *Wfs1*$^{-/-}$ mice. (A) Binding curve of [^{3}H]SCH23390 binding to pooled hippocampal samples of wt (triangle) and *Wfs1*$^{-/-}$ (circle) mice. The membrane suspensions (3 mg/well) were incubated with different concentrations of [^{3}H]SCH23390 for 60 min and bound radioactivity was measured. Data are presented as mean ± SEM from experiments (n = 3) performed in duplicates. (B) The level of [^{3}H]SCH23390 binding sites of individual wt and *Wfs1*$^{-/-}$ mice determined in hippocampal membrane suspensions (6.7 mg/ml) incubated with 4 nM radioligand. Data are presented as mean ± SEM of all the mice tested. *$p < 0.05$. Same as Fig. 9 in Ref II.

The upregulation of D1-like receptors could explain the increased sensitivity of *Wfs1*$^{-/-}$ mice to the locomotor stimulatory effect of dopamine receptor agonist apomorphine (Luuk et al., 2009). Other significant changes in the functioning of the dopaminergic system of *Wfs1* KO mice include the reduced level of dopamine transporter (DAT) mRNA expression in the midbrain, reduced expression of dopamine receptor 2 (*Drd2*) in the dorsal striatum of the male individuals, and reduced sensitivity to the presynaptic dopamine agonist amphetamine (Luuk et al., 2009; Visnapuu et al., 2013a).

In our study, we did not evaluate the abundance or expression pattern of D2-like dopamine receptors. In contrast to D1-like receptors, D2-like receptors inhibit the adenylyl cyclase activity (Missale et al., 1998) and are predominantly expressed on different cells, at least in the dorsal and ventral striatum (Valjent et al., 2009). Therefore, it would be essential to find out if on the cellular level WFS1 colocalizes with D1- or D2-like receptors and/or if D2-receptor binding is affected in *Wfs1*$^{-/-}$ mice. In addition, the functioning of the intracellular pathways of both D1- and D2-like receptors should be studied in *Wfs1*$^{-/-}$ mice.

Curiously, the genetic deletion of calcium/calmodulin dependent adenylyl cyclases affects the same behavioural characteristics as the invalidation of *Wfs1* gene. Adenylyl cyclase 8 (*AC8*) KO mice show altered stress-induced anxiety (Bernabucci and Zhuo, 2016; Schaefer et al., 2000) and adenylyl cyclase 1/8 double KO mice display deficit in hippocampus-dependent long-term memory (Wong et al., 1999), which is also slightly impaired in *Wfs1*$^{-/-}$ mice (Luuk et al., 2009). In addition, adenylyl cyclase 1/8 double KO mice show dysregulated dopamine homeostasis in the ventral striatum and display reduced locomotor stimulation by methamphetamine, as well as attenuated behavioural sensitization upon repeated treatment with methamphetamine (Bosse et al., 2015). These findings resemble the phenotype of *Wfs1*$^{-/-}$ mice (Luuk et al., 2009; Visnapuu et al., 2013a). Furthermore, fear conditioning elevated the level of *Wfs1* expression in the hippocampus of wt mice but failed to do so in adenylyl cyclase 1/8 double KO mice (Wieczorek et al., 2010).

The hippocampal expression of *AC8* is restricted to the CA1 region similarly to *Wfs1* (Schaefer et al., 2000; Wei et al., 2002). *AC8* KO mice display lack of stress-induced CREB phosphorylation specifically in the CA1 region of the hippocampus, which probably underlies the deficiency in experience-dependent fear memory in these mice (Schaefer et al., 2000). To investigate the possible role of WFS1 in the D1 dependent adenylyl cyclase activation, measuring the intracellular cAMP and the activation of cAMP-dependent pathways in response to D1-receptor agonist in *Wfs1*-deficient neurons would be the next milestone in understanding the relation between WFS1 and D1-like dopamine receptors.

5. The differential expression of the two alternative transcripts of *Lsamp* gene correlates with the measures of trait anxiety and social interaction (Ref III)

Similarly to *Wfs1*, *Lsamp* is highly expressed in the brain regions involved in emotional circuits and its expression level in the temporal lobe changes in response to predator-related stimulus. After revising the genomic structure of *Lsamp* gene, two alternative promoters were described in 2004 by Pimenta and Levitt (Pimenta and Levitt, 2004). Although the protein expression pattern is

studied in several species (Brümmendorf et al., 1997; Chesselet et al., 1991; Côté et al., 1995; Côté et al., 1996; Levitt, 1984; Yamamoto and Reiner, 2005; Yamamoto et al., 2005) and mRNA expression is described in both foetal and adult rat brain (Pimenta et al., 1996b; Reinoso et al., 1996), the activity pattern of 1a and 1b promoters of the *Lsamp* gene had not been addressed. Therefore, we aimed to provide an anatomical overview of the alternative promoter activity in the adult and developing mouse brain. Although including or omitting exons 7 and 8 adds additional variation to the *Lsamp* transcripts (see Fig. 3), we distinguished the transcripts only based on the 5`end encoding the signal peptide. For simplicity, the transcripts obtained from the alternative promoters are called 1a and 1b transcript in this study. To better understand the importance of the *Lsamp* 1a and 1b transcripts, we examined the role of each transcript in relation to trait anxiety and social behaviour.

We found that the expression pattern of the two alternative transcripts was discernibly complementary, as *Lsamp* 1a transcript prevailed in the classical limbic structures (see Fig. 7) and 1b transcript was more abundant in the brain regions associated with sensory functions. Of the classical limbic structures (Heimer and Van Hoesen, 2006; Morgane et al., 2005), *Lsamp* 1a was strongly expressed in the hippocampus, ventral striatum, in limbic cortices including piriform, prelimbic, infralimbic, cingulate, insular and retrosplenial granular cortex, amygdala (nucleus of the lateral olfactory tract, lateral, basolateral, medial, posterolateral and posteromedial cortical nuclei), amygdalohippocampal transition area, dorsal lateral septum and medial preoptic area (Ref III, Fig. 1e-h, Fig. 2b, f, j, Fig. S1e, f, k). In general, the activity of *Lsamp* 1b promoter was much lower or not present in these structures. Of the limbic structures, considerable activity of *Lsamp* 1b promoter was present in the lateral septum, piriform cortex, and in some nuclei of the amygdala (central amygdala, posterolateral and posteromedial cortical amygdala; Ref III, Fig. 1i-l, Fig. 2c-d, g-h, k-l).

In the thalamus, *Lsamp* 1a promoter activity tended to localize into limbic-related nuclei, while 1b was active in several limbic-related nuclei and in numerous regions ascribed to participate in sensory communication. *Lsamp* 1a promoter activity was outstanding in the anterior thalamic nuclei, including anterodorsal (AD) and anteroventral (AV) nucleus (Ref III, Fig. S1g). In the AD, also 1b promoter was strongly activated (Ref III, Fig. S1h). The anterior thalamic nuclei are interconnected with the limbic structures such as hippo-campus, retrosplenial and entorhinal cortices, and mediate the formation of contextual fear memory (Marchand et al., 2014; Shibata, 1993; Wright et al., 2010). Paraventricular nucleus of the thalamus, which showed strong activity of both *Lsamp* 1a and 1b promoters (Ref III, Fig. S1g, h, k, l), receives extensive innervation by orexin, corticotropin-releasing hormone and monoamines and is reciprocally connected to numerous brain areas involved in emotion processing and motivation (Hsu et al., 2014). *Lsamp* 1a expression was strong in the parafascicular nucleus of the thalamus (PF), that mainly has connections to the brain regions involved in somatosensory and motor functions (Berendse and

Groenewegen, 1990; Cornwall and Phillipson, 1988). Together with the paracentral thalamic nucleus, where both *Lsamp* promoters were active, it belongs to the group of intralaminar thalamic nuclei, which are involved in behavioural flexibility and decision making (Bradfield et al., 2013; Brown et al., 2010) and nociceptive processing related to the affective and motivational aspects of pain (Sewards and Sewards, 2002; Sugiyo et al., 2006).

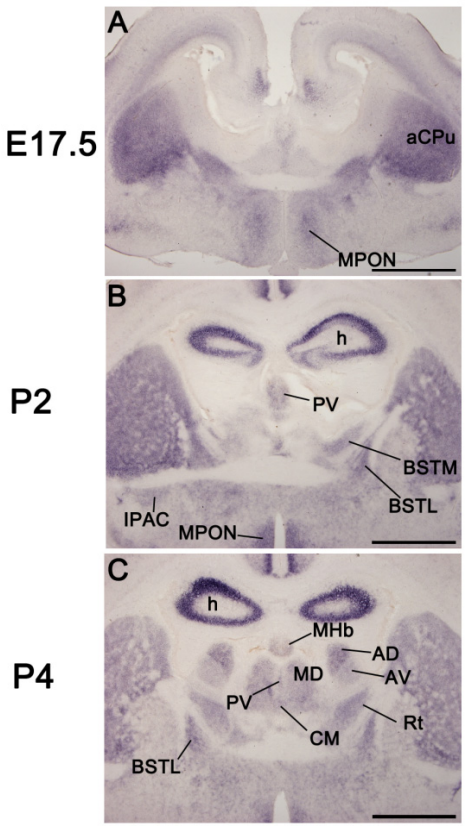


Figure 7. The expression of *Lsamp* 1a transcript during the development of emotional circuits. mRNA *in situ* hybridization. *Lsamp* 1a transcript is widely expressed in the limbic-related anterior, midline and intralaminar thalamic nuclei, medial preoptic area, parts of the extended amygdala (IPAC, BSTL, BSTM) and specifically during the development in the anterior CPu. Abbreviations: aCPu-anterior caudate-putamen, AD-anterodorsal thalamic nucleus, AV-anteroventral thalamic nucleus, BSTL-lateral bed nucleus of stria terminalis, BSTM-medial bed nucleus of stria terminalis, CM-central medial thalamic nucleus, h-hippocampus, IPAC- interstitial nucleus of the posterior limb of the anterior commissure, MD-mediodorsal thalamic nucleus, MHb-medial habenula, MPON-medial preoptic nucleus, PV-paraventricular thalamic nucleus, Rt- reticular thalamic nucleus. Scale bar 1 mm.

The activity of *Lsamp* 1b promoter well delineated auditory, visual and somatosensory pathways in the brain, being present in the brainstem sensory nuclei, thalamic relay nuclei and primary and secondary cortices of the respective perceptive fields. Along the auditory pathway, *Lsamp* 1b promoter was active in the dorsal and ventral cochlear nucleus, trapezoid body, superior olivary complex, lateral lemniscus, inferior colliculus, medial geniculate nucleus of the thalamus (MG), and auditory cortices (Ref III, Fig. 1j-k, Fig. S1n-p). Inferior colliculus functions as the integrator of auditory information to multi-modal sensory perception and passes the information on to the MG. MG represents the thalamic relay of the auditory pathway, passing information to

the auditory cortex, ibid sending projections to several subcortical areas involved in emotional behaviour and autonomic function (central and lateral amygdala; caudate-putamen; ventromedial hypothalamus; LeDoux et al., 1984).

In the visual system there was intensive *Lsamp* 1b transcript-specific staining in the superior colliculus (SC), dorsal lateral geniculate nucleus (DLG), lateral posterior thalamic nucleus (LP), and primary and secondary visual cortex (Ref III, Fig. S1n, Fig. 1j-l). DLG is the major thalamic relay receiving visual information from the retina and providing input to the primary visual cortex (Guillery and Sherman, 2002). LP integrates signals from the SC, cortex and retina and communicates with both higher visual cortical areas and subcortical sites, such as the amygdala, linking visual signals to emotional salience (Kaas and Lyon, 2007; Wei et al., 2015).

The brainstem nuclei of the somatosensory pathways, namely spinal trigeminal nucleus, solitary nucleus and cuneate nucleus were expressing *Lsamp* 1b transcript, as their thalamic targets ventral posteromedial nucleus (VPM), ventral posterolateral nucleus (VPL) and posterior thalamic nuclear group, and the somatosensory cortex (Ref III, Fig. 1i-k, Fig. S1s).

Both *Lsamp* 1a and 1b promoters were active in the brain regions associated with gustatory and olfactory perception, which is in accordance with the involvement of these brain regions (e.g. insular, piriform and entorhinal cortices, amygdala) in emotion processing. While the solitary nucleus and VPM, that are involved in mediating the taste signals, showed the activity of *Lsamp* 1b promoter (Ref III, Fig. 1i-k, Fig. S1s), the insular cortex, which is the cortical target of the gustatory pathway (Matsumoto, 2013), was exclusively expressing *Lsamp* 1a transcript (Ref III, Fig. 2b, f, Fig. S1e, f). In the olfactory system, the olfactory bulb, mediodorsal thalamic nucleus (MD; Ref III, Fig. 1i, Fig. 2k-l), and entorhinal cortex (Ref III, Fig. S1n) showed 1b specific X-gal staining, while the expression of 1a transcript was prevailing in the olfactory tubercle (Ref III, Fig. 2b, f) and piriform cortex (Ref III, Fig. 1e-h, Fig. S1k). In the nucleus of the lateral olfactory tract, which integrates olfactory and vomeronasal information (Pro-Sistiaga et al., 2007; Vaz et al., 2017), the expression of *Lsamp* 1a was distinctly strong, while no 1b activity was observed in this nucleus (Ref III, Fig. S1k).

In the sensory cortices, the promoter 1b was active in layers IV and VI (Ref III, Fig. S1d), underlining the distribution of 1b transcript in the brain regions involved in sensory processing. Layer IV is the major target for the thalamic afferents (Benshalom and White, 1986; Staiger et al., 1996) and layer VI is the main cortical layer that sends feedback to the thalamic nuclei (Lam and Sherman, 2010).

Both *Lsamp* 1a and 1b promoter were highly active in the medial habenula (MHb; Ref III, Fig. 1e, i, j), while the lateral habenula (LHb) showed moderate activity of 1b promoter (Ref III, Fig. 1i). Together with the bed nucleus of the anterior commissure, which also showed strong 1b promoter activity (Ref III, Fig. 2h), the MHb is part of septo-habenular pathway which is implicated in anxiety and fear responses (Yamaguchi et al., 2013). The neural pathway from

MHb to its main output target interpeduncular nucleus is involved in both addiction to multiple drugs of abuse and mood-associated conditions (McLaughlin et al., 2017). Neurons of the LHb participate in predicting negative rewards by sending inhibitory signals to the midbrain dopaminergic neurons upon cues associated with lack of reward (Matsumoto and Hikosaka, 2007).

Lsamp mRNA was rather widely distributed in the hypothalamus (Ref III, Fig. 1a-d, Fig. 2e, i), but the expression regions of the two alternative transcripts were generally segregated. Strong expression of 1a transcript was present in the dorsomedial and ventrolateral parts of the ventromedial hypothalamus (VMH; Ref III, Fig. 1e), which is involved in feeding, fear, aggression and sexual behaviour (King, 2006; Lin et al., 2011; Silva et al., 2013; Yang et al., 2017). *Lsamp* 1b-specific staining was present in the anterior part of the VMH (Ref III, Fig. 2k). Dorsomedial hypothalamus (DM), which functions as a thermoregulatory centre and integrator of autonomic, endocrine and behavioural responses to emotional stress (Fontes et al., 2011), showed strong 1a promoter activity (Ref III, Fig. 1f) as well as 1b specific X-gal staining in its compact subdivision (Ref III, Fig. 2l). *Lsamp* 1a transcript expression was remarkably strong in the medial preoptic area (Ref III, Fig. 2f), which provides important inhibitory input to the DM (Hunt et al., 2010) and is involved in thermoregulation, maternal behaviour, and sexual behaviour (Boulant, 2000; Stolzenberg and Numan, 2011). In the caudal hypothalamus, mammillary bodies, which belong to the spatial memory circuit joining ventral tegmentum and hippocampus with the anterior thalamic nuclei (Dillingham et al., 2015), originally proposed by Papez as the central circuit for emotion (Papez, 1937; Shah et al., 2012), were strongly expressing *Lsamp* 1a and 1b (Ref III, Fig. 1g-h). Moderate *Lsamp* 1a transcript expression was seen in the lateral hypothalamus (Ref III, Fig. 1f-g), which is involved in feeding, arousal and reward-seeking, and is the source of orexin/hypocretin input to various brain regions (Peyron et al., 1998; Stuber and Wise, 2016). Paraventricular hypothalamic nucleus (Pa) was one of the few hypothalamic regions, where the activity of both 1a and 1b promoters was strongly represented (Ref III, Fig. S1k-l). Pa contains different neurosecretory cells that produce various peptide hormones and neurotransmitters, including oxytocin, vasopressin, corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH), and it constitutes an important output station for the hypothalamo-pituitary-adrenal axis involved in stress regulation (Herman et al., 2003). The activity of *Lsamp* 1b promoter circumscribed the neuropeptide-producing nuclei of the hypothalamus: in addition to the Pa, 1b promoter was active in the periventricular (Ref III, Fig. 1i, Fig. S1i, j, l), anteroventral periventricular (Ref III, Fig. 2g, h), suprachiasmatic (Ref III, Fig. S1i, j, l), supraoptic (Ref III, Fig. S1l) and arcuate nucleus (Ref III, Fig. 1j, k).

Distinct pattern of *Lsamp* promoter activity was evident in the cerebellum. *Lsamp* 1b-specific staining was strong in the Purkinje cell layer and moderate in the molecular layer (Ref III, Fig. S1r), while the granule cell layer was weakly expressing 1a transcript (Ref III, Fig. S1q). The granule cell layer is the

principal glutamatergic cell layer in the cerebellum, which receives input from the cerebrum and sends projections to the Purkinje cells and to the molecular layer. The output of the cerebellum is the inhibitory signal from the Purkinje cells to the deep cerebellar nuclei. The Purkinje cells, in turn, are inhibited by the GABAergic cells of the molecular layer. This circuit is responsible for the motor coordination and a multitude of cognitive and emotional processing tasks (Saab and Willis, 2003).

In the developing brain, we detected the first signals for both promoters of the *Lsamp* gene at around E12.5. The expression of *Lsamp* 1a transcript first appeared in the outer layers of the neural tube (Ref III, Fig. 3a, b); conversely, the activation of 1b promoter marked the proliferative zone, lining the ventricular side of the developing striatum (Ref III, Fig. 3d, e). By E15.5, *Lsamp* 1b promoter activity was also present in the lining of the aqueduct and in the deep cortical layers near the corticostriatal junction (Ref III, Fig. 3f). The main expression regions of *Lsamp* 1a transcript in E15.5 brain were the piriform cortex, superficial part of the somatosensory cortical region and deeper zone of the remaining cortical plate, medial amygdala, anterior thalamus and the CPu (Ref III, Fig. 3c). Interestingly, *Lsamp* mRNA is almost lost from the CPu in the adult brain (Ref III, Fig. 2a-c, e-g), where weak 1a expression remains only in the anterior CPu (Ref III, Fig. 2b) and the caudal-most region shows activity of 1b promoter (Ref III, Fig. 1j). This suggests for the specific role of LSAMP during the development of the dorsal striatum. In the lateral habenula, LSAMP takes part in guiding the growing dopaminergic axons (Schmidt et al., 2014), which form a feedback loop between the ventral tegmental area (VTA) and LHb to signal the positive or negative rewarding value of the stimuli (Shen et al., 2012, Stamatakis et al., 2013). The CPu displays heterogenous LSAMP protein expression in its striosomal vs matrix compartments, suggesting for the segregation of limbic-related connections in relation to the nature of dopaminergic input (Chesselet et al., 1991). Therefore, the role for LSAMP in the correct dopaminergic innervation of the CPu can be hypothesized and needs further study.

Pimenta et al., 1996b report the first appearance of the 8.0 kb transcript of *Lsamp* gene (later referred to as 1a transcript) at E15.5 in the hindbrain of the rat, while 1.6 kb transcript (later referred to as 1b transcript) first appeared in the hindbrain of E12.5 rat. Presence of *Lsamp* expression at this early age precedes active neurite outgrowth and path-finding, suggesting that *Lsamp* is important already at the time of neuroepithelial patterning. Horton and Levitt, 1988 argue that LSAMP immunoreactivity appears 24 – 36 hours after the neurons undergo the final mitosis and predates the occurrence of synaptic transmission, probably reflecting the designation as a part of the limbic circuitry almost from the time of the initial differentiation. In the anterior-most end of the chick embryo, *Lsamp* mRNA is already present at the stage of three primary brain vesicles (Hamburger-Hamilton stage 10). By stage 14 (E2), *Lsamp* mRNA is expressed in the midbrain, neural crest cells and notochord, and by stage 25 (approximately E5), instead of notochord, the expression is found in the floor

plate and motoneuron columns (Kimura et al., 2001). The expression of the two alternative transcripts of the *Lsamp* mRNA undergoes developmental changes in the chick brain (Brümmendorf et al., 1997). Somewhat counter-intuitively, in the chick brain, *Lsamp* 1a transcript (referred to as g11/g19 form) is present at earlier stages compared to *Lsamp* 1b transcript (referred to as g9 form; Brümmendorf et al., 1997). Altogether, it is evident that the distinct developmental regulation of the two *Lsamp* transcripts in both rodent and chick brain underlines the significance of the alternative promoter activity in the development of the nervous system.

Comparing the present results with the protein expression in the embryonic rat brain described by Horton and Levitt, 1998 suggests that the LSAMP protein expression resembles more the distribution of *Lsamp* 1a transcript around E15. The distribution of LSAMP protein is also described in the adult rat (Levitt, 1984; Reinoso et al., 1996). For better overview, the comparison between protein expression described in the rat brain by Levitt et al., 1984 and Reinoso et al., 1996 and *Lsamp* 1a and 1b promoter activity studied in Ref III is presented in the Table 3 in the 6-th chapter of Results and Discussion.

In general, the expression of LSAMP protein is more similar to the regional distribution of *Lsamp* 1a transcript, as the limbic-specific identity suggests. Particularly, the protein expression studied by Levitt, 1984 mirrors the expression of 1a transcript. Nevertheless, several regions showing only *Lsamp* 1b promoter activity are expressing LSAMP protein, including entorhinal and somatosensory cortices, CPu, globus pallidus, sensorimotor thalamus (laterodorsal, mediodorsal, ventral posterolateral and posteromedial, ventrolateral, ventromedial, dorsal lateral and medial geniculate and posterior thalamic nuclei), lateral habenula, inferior and superior colliculi of the midbrain, sensory and motor nuclei of the brainstem, as well as molecular and Purkinje cell layer of the cerebellum (Levitt, 1984; Reinoso et al., 1996; Ref III, Fig. 1i-l, Fig. 2k, l, Fig. S1m-p, r, s, Supplementary table S2; Table 3). In addition, several neuropeptide-producing nuclei, including periventricular hypothalamic nucleus, anteroventral periventricular hypothalamic nucleus, supraoptic, suprachiasmatic and arcuate nucleus were among those where LSAMP protein expression was reduplicating the activity of *Lsamp* 1b promoter. Thus, from the comparison of *Lsamp* transcript and protein expression, two patterns emerge: the protein is made from solely 1b transcript in several sensory and motor regions and additionally, in most of the neurosecretory nuclei that express *Lsamp*.

As the somatomotor system is inevitably interconnected with the limbic system, the presence of LSAMP in the sensory and motor brain regions might be the witness of such association. In this context, special attention must be paid on the cerebellum as the powerful processing centre for cognitive, emotional and memory-related functions. Renowned for its role in motor coordination, the cerebellum is also involved in coordinating thought, affection, memory and language processes (Stoodley and Schmahmann, 2010). Thus, in the cerebellum, LSAMP could take part in the formation and maintenance of the emotion-related connectivity. In the dentate nucleus, which is one of the main

output stations of the cerebellum, the projections to and from different cortical regions are arranged in a topographic manner, forming a map of multiple closed-loop circuits (Dum and Strick, 2003). Considering the function of LSAMP and other IgLON family proteins in the formation of neural circuits, it is plausible that their expression could be required to establish such connectivity. It is important to note that most of the IgLON family genes are expressed in the cerebellum and in all dual promoter IgLON genes including *Lsamp*, *Opcml* and *Ntm*, 1b transcript is strongly prevailing in the cerebellum (Vanaveski et al., 2017).

Among the neurosecretory nuclei, the Pa, which is one of the most important neuroendocrine centres in the brain (Ferguson et al., 2008), outstands as an exception. In addition to strong *Lsamp* 1b activity, it moderately expressed 1a transcript (Ref III, Fig. S1k, l) and shows high LSAMP protein levels in its magnocellular part and low protein levels in the parvocellular part (Reinoso et al., 1996). Levitt, 1984 did not detect LSAMP protein expression in the Pa, the controversy might arise from the highly heterogenous structure of this nucleus (Swanson and Kuypers, 1980).

Another member of the IgLON family, OPCML/OBCAM is present in the neurosecretory granules in the vasopressin-secreting magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus (Miyata et al., 2003). Similarly to *Lsamp*, *Opcml* gene possesses two evolutionarily conserved alternative promoters leading to separate transcripts (1a and 1b) which are differentially expressed across the brain and yield an identical protein (Vanaveski et al., 2017). Concerning the pattern of *Lsamp* 1a and 1b promoter activity in comparison with the protein expression (see Table 3), it is intriguing to guess that in some neurosecretory nuclei (periventricular hypothalamic nucleus, supraoptic nucleus, suprachiasmatic nucleus and arcuate nucleus), where only *Lsamp* 1b promoter was active, LSAMP protein might be targeted to the neurosecretory granules. To elucidate the functional significance of the two alternative promoters, this question should be addressed in the future studies.

Regarding the ultrastructural distribution of LSAMP protein, it has been proposed that LSAMP fulfils different roles in the developing and adult brain (Zacco et al., 1990). During the development, LSAMP protein is extensively expressed on the surface of fasciculating axons (Horton and Levitt, 1988), whereas in the adult brain, only cell bodies and dendrites show LSAMP immunoreactivity (Zacco et al., 1990). While at P10, LSAMP is present in both pre- and postsynaptic membranes of the developing synapse in rat, it proves exclusively postsynaptic in adulthood (Zacco et al., 1990). Fimbria/fornix and corpus callosum, fibre tracts that transiently express LSAMP, lose the expression during the third postnatal week in the rat (Horton and Levitt, 1988; Zacco et al., 1990).

The developmentally transitory presence of LSAMP in the axons is in line with the participation in the axon growth and guidance (Eagleson et al., 2003; Keller et al., 1989; Mann et al., 1998). LSAMP has been shown to specifically promote the growth of limbic thalamic and cortical axons while inhibiting the

growth of nonlimbic thalamic axons (Mann et al., 1998) and facilitate the target invasion and collateralization of the septohippocampal axons (Keller at al., 1989). The neurite outgrowth- and adhesion-promoting activity of LSAMP (Pimenta et al., 1995; Zhukareva and Levitt, 1995) is mediated by its first Ig-domain, whereas interactions involving the second Ig domain mediate the inhibition of neurite outgrowth in heterophilic manner (Eagleson et al., 2003). One such heterophilic binding partner of LSAMP is Neurotrimin. Neurotrimin and LSAMP show rather complementary protein distribution in the rodent brain (in somatosensory and motor *vs* limbic brain regions; Gil et al., 2002), and their interaction inhibits neurite outgrowth, whereas both can promote neurite outgrowth in homophilic manner (Gil et al., 1998; Gil et al., 2002; Sanz et al., 2015). In the dorsal root ganglia, LSAMP inhibits neurite outgrowth and the attenuation of the inhibition occurs through LSAMP ectodomain shedding by metalloproteinase ADAM10 (Sanz et al., 2017).

In contrast to the rather wide evidence of the involvement of LSAMP in the neurodevelopmental processes, the role of LSAMP in the adult brain remains elusive. Considering its strong expression in the dendritic compartments of the limbic system neurons, one can hypothesize that it might take part in the activity-dependent remodelling of the neural network. Since LSAMP is present in the postsynaptic membrane in the adult rat (Zacco et al., 1990) and it can promote synaptogenesis in the hippocampus (Hashimoto et al., 2009), it is probable that it might take part in the plasticity or formation of synapses in the adult brain. In our laboratory, it has been shown that LSAMP is involved in the regulation of environmental enrichment-induced brain-derived neurotrophic factor (*Bdnf*) expression (Heinla et al., 2015). BDNF is a crucial regulator of proliferation, survival, differentiation, synaptogenesis and activity-dependent synaptic plasticity in neurons (reviewed in Binder and Scharfman, 2004; Gottmann et al., 2009). The lack of enrichment-induced elevation in *Bdnf* expression in the hippocampi of adult *Lsamp*$^{-/-}$ mice suggests that LSAMP could serve as a positive modulator of BDNF regulated neuronal pathways (Heinla et al., 2015). This assumption is further supported by the finding that the expression levels of *Lsamp* 1a and 1b transcripts positively correlate with the expression of *Bdnf* in the hippocampus and frontal cortex of mice (Heinla et al., 2015).

To elucidate the functional significance of the two alternative *Lsamp* promoters, we studied the expression level of *Lsamp* 1a and 1b in relation to the anxiety-related and social behavioural characteristics of mice. First, behavioural parameters in the motility box and elevated plus maze were compared to the expression level of *Lsamp* gene transcripts in the hippocampus, temporal lobe and ventral striatum. The results are presented in the Table 2 (same as Table 1 in Ref III). While there were no significant correlations between the *Lsamp* transcript expression and behavioural parameters in the motility box, the exposure to elevated plus maze revealed correlations between the anxiety-related behaviour and the expression of *Lsamp* transcripts. The expression level of *Lsamp* 1a transcript in the temporal lobe showed correlation with several behavioural measures in the plus maze: it was negatively correlated with the

number of open arm entries, time spent on open arms, ratio between open and closed arm entries, and number of unprotected head-dips, and positively correlated with the latency before entering the open arm. The time spent on open arms was also negatively correlated with the level of *Lsamp* 1b transcript expression in the temporal lobe.

Secondly, a social interaction test was carried out, where two unfamiliar mice were put in the same cage and the time spent sniffing the partner`s anogenital area as well as other body parts and total time of social sniffing was recorded. In the hippocampus, the expression of both *Lsamp* 1a and 1b transcript negatively correlated with the time spent sniffing other body parts besides the anogenital area. In the ventral striatum, the expression level of *Lsamp* 1a correlated positively with the time involved in anogenital sniffing.

Table 2. Correlation analysis between relative expression levels ($2^{-\Delta CT}$) of *Lsamp* 1a and 1b transcript in three brain areas and behavioural parameters in the motility box, elevated plus-maze and social interaction test. Table 1 in Ref III.

	Hippocampus		**Temporal lobe**		**Ventral striatum**	
	1a	**1b**	**1a**	**1b**	**1a**	**1b**
Motility box						
Move, s	−0.01	−0.06	0.11	0.06	0.12	−0.34
Distance, m	−0.06	−0.16	0.19	0.08	0.05	−0.30
Time centre, s	−0.40	−0.36	0.43	0.36	0.05	0.03
Time corner, s	0.21	0.33	−0.38	−0.10	−0.13	0.17
Elevated plus maze						
Closed arm entries	−0.20	−0.43	0.17	−0.02	0.08	−0.14
Open arm entries	0.16	0.16	−0.57*	−0.40	0.01	−0.34
Ratio open/closed arm entries	0.08	0.13	−0.63 *	−0.38	−0.11	−0.34
Latency, s	0.03	−0.01	0.53*	0.22	−0.15	0.45
Time on open arms, s	0.24	0.15	−0.65**	−0.66**	0.14	−0.21
Protected headdips	0.17	0.08	−0.06	−0.06	−0.12	−0.26
Unprotected headdips	0.10	0.12	−0.61*	−0.50	0.20	−0.40
SAPs	−0.05	0.10	0.24	0.23	0.11	0.17
Social interaction test						
Anogenital sniffing, s	−0.10	−0.22	−0.09	−0.26	0.54*	0.11
Sniffing other body parts, s	−0.67**	−0.65*	0.06	0.15	−0.19	−0.07
Time of social sniffing, s	−0.51	−0.60*	−0.01	−0.09	0.14	−0.06

The behavioural measures have been presented in either counts, seconds (s) or meters (m).
* $p < 0.05$, ** $p < 0.01$ (Spearman's rank-order correlation)

In the second part of the behavioural studies, we investigated the influence of acute fear reaction on the expressional activity of the *Lsamp* gene. Mice were divided into three groups: 1) experimentally naïve mice, 2) mice that underwent fear conditioning (15 s tone and pulsing bright light terminated by electric shock) but did not receive treatment on the day of tissue collection, and 3) mice that underwent fear conditioning and were exposed to the conditioned stimuli prior to tissue collection. We did not detect changes in the expression of *Lsamp* transcripts in the temporal lobe or hippocampus between the groups on the selected time point, 45 minutes after the onset of the exposure to the conditioned stimuli (Ref III, Fig. 4e). Nevertheless, the rise in neural activity in the temporal lobe and hippocampus was confirmed by measuring the expression level of immediate early gene *c-Fos*. In the 3rd group of mice that were exposed to the conditioned stimuli prior to tissue collection, *c-Fos* expression was upregulated in both, temporal lobe and hippocampus (Ref III, Fig. 4d). Stronger upregulation in the temporal lobe *vs* hippocampus reflected the specific activation of the amygdaloid area in response to conditioned fear.

Previously, changes in the expression level of *Lsamp* have been implicated in predator-associated fear and fear of pain. Exposure of rats to cat odour (Kõks et al., 2004) and electric foot shock (Lamprecht et al., 2009) increased the expression of *Lsamp* mRNA in the amygdala. In addition, *Lsamp* shows link to trait anxiety. Increased level of *Lsamp* mRNA in different brain regions involved in behavioural control (amygdala, periaqueductal grey, raphe nuclei, hippocampus and frontal cortex) has been associated with lower exploratory activity and higher level of anxiety (Alttoa et al., 2010; Nelovkov et al., 2006). However, the importance of the *Lsamp* 1a and 1b transcripts in fear and anxiety related behaviour had not been addressed before. Our results, which showed the elevation of *Lsamp* 1a and 1b transcripts in the temporal lobe in more anxious mice are well in accordance with the previous findings obtained on rats. The expression of *Lsamp* 1a transcript showed correlations with more measures of anxiety in the elevated plus maze than 1b transcript, probably reflecting its role in the shaping of anxiety-related neural circuits in the temporal lobe. Why we could not see differences in *Lsamp* expression in the acute fear test, could partly be explained by the findings of Lamprecht et al., 2009, who found that in the lateral amygdala, the timing of the elevation of *Lsamp* expression upon fear-generating stimulus (footshock) depends on whether the stimulus is paired to a conditioned stimulus (tone) or not. Thus, when the footshock was paired with the tone, the expression level of *Lsamp* showed elevation at the 5-h time point, and when the footshock was not paired with the tone, *Lsamp* expression was elevated at the 30-minute time point (Lamprecht et al., 2009). In our study design, we were not able to detect the elevation of *Lsamp* expression at our chosen time point, but this does not exclude the possibility that the rise in *Lsamp* expression could have occurred later.

In the social interaction test, significant correlations between the sniffing behaviour and the expression level of *Lsamp* transcripts occurred in the hippocampus and ventral striatum, but not in temporal lobe. The expression of *Lsamp*

1a transcript in the ventral striatum positively correlated with the sniffing of the anogenital area of the other male, which can be considered as an agonistic behaviour as it often precedes aggressive attacks (Innos et al., 2011). The *Lsamp*$^{-/-}$ mice show notable decrease in agonistic behaviour manifested by lack of whisker trimming and aggressive attacks as well as reduced anogenital sniffing between males (Innos et al., 2011). Ventral striatum is involved in reward-motivated behaviour, which includes, among others, reward related to social defeat of conspecifics (van Erp and Miczek, 2000; Ferrari et al., 2003). Therefore, the function of the ventral striatum might be altered in case of *Lsamp* deficiency.

Sniffing of other body parts negatively correlated with the expression level of both *Lsamp* 1a and 1b transcripts in the hippocampus. Notably, several hippocampal functions are affected following the genetic invalidation of *Lsamp*. *Lsamp*$^{-/-}$ mice display spatial memory deficit, reduction in the CA1 long term potentiation, and increased activity in novel environment (Catania et al., 2008; Innos et al., 2011; Qiu et al., 2010). Known for its principal role in spatial and contextual memory, the function of the hippocampus is also involved in anxiety-related and social behaviour (File et al., 2000; Moser and Moser, 1998). General social sniffing is not altered in *Lsamp*$^{-/-}$ mice (Innos et al., 2011; Innos et al., 2012); thus, less time spent involved in social sniffing might reflect the higher trait anxiety that correlates with the expression of *Lsamp* transcripts in the hippocampus. Less social interaction is a measure of higher anxiety, as shown by File and Hyde, 1978, who designed the test to evaluate the effect of anxiolytic drugs. *Lsamp*$^{-/-}$ mice are less anxious in novel and stressful environments (Catania et al., 2008; Innos et al., 2011; Innos et al., 2012). Both the *Lsamp* knockout studies and the present findings endorse the role of LSAMP in various hippocampal functions, including the regulation of anxiety.

The role of *Lsamp* in the regulation of behaviour is related to the functioning of major neurotransmitter systems in the brain. GABA-ergic, serotonergic and dopaminergic transmission is altered in the *Lsamp*$^{-/-}$ mice (Innos et al., 2011; Innos et al, 2013). In the temporal lobe of *Lsamp*$^{-/-}$ mice, the ratio between the transcripts of the α subunit of $GABA_A$ receptor genes *Gabra2* and *Gabra1* is shifted in favour of the *Gabra2* gene (Innos et al., 2011). This could be a possible reason for the reduced anxiety in *Lsamp* KO mice, since the α2 subunit is most relevant in mediating the anxiolytic effect of benzodiazepine drugs through the $GABA_A$ receptor (Löw et al., 2000; Möhler, 2002). In addition, *Lsamp* KO mice display increased serotonergic tone in the dorsal striatum and mesencephalon and lower sensitivity to amphetamine underlined by lower expression of dopamine transporter in the midbrain (Innos et al., 2013). The elevation in serotonergic transmission could be responsible for the reduced aggressiveness and social dominance, as decreased levels of 5-HT are widely shown to promote social aggression (Montoya et al., 2012).

In humans, polymorphisms in the *LSAMP* gene have been associated with predisposition to mood and anxiety disorders (Koido et al., 2012) and schizophrenia (Chen et al., 2018; Koido et al., 2014). In post-mortem patients of

schizophrenia, the protein level of LSAMP is increased in the dorsolateral prefrontal cortex (Behan et al., 2009) and orbitofrontal cortex (Velásquez et al., 2017), brain regions involved in memory, speech, and cognitive and executive functions. In the dorsolateral prefrontal cortex of the schizophrenia patients, abnormally high neuronal density has been reported, suggesting neuronal atrophy in this brain region (Selemon et al., 1995; Selemon et al., 1998; Selemon et al., 2003). Regarding the role of LSAMP in neural development and synaptogenesis, either directly or through the induction of BDNF, it is possible that LSAMP might participate in the compensatory mechanisms triggered by pathological changes in the neural connectivity of the dorsolateral prefrontal cortex of schizophrenia patients. As the two-promoter structure of *Lsamp* is strictly conserved across different species (Pimenta and Levitt, 2004), it is probable that the alternatively regulated expression is needed for the integrity of the functions performed by LSAMP protein. All the single nucleotide polymorphisms shown to associate with increased risk for depression and bipolar disorder reside in the first intron (Koido et al., 2012). Furthermore, *LSAMP* polymorphisms causing susceptibility for coronary artery disease also reside in the first intron and bring about reduced expression of *LSAMP* 1a transcript (Wang et al., 2008). These lines of evidence and the present study suggest for distinct roles of the 1a and 1b transcripts of *Lsamp* gene in the regulation of the development and functioning of neural networks. Further studies are needed to gain better understanding of the significance of the two-promoter structure of *Lsamp* in the context of subcellular localisation, neurite growth, synaptic remodelling, regulation of behaviour and psychiatric disease.

6. Table of *Lsamp* expression

Table 3. LSAMP protein expression and *Lsamp* 1a and 1b promoter activity in the rodent brain. The principal function of each brain structure is brought out in the right column. Protein expression data are from Levitt, 1984 and Reinoso et al., 1996.

Region	Protein Levitt 1984	Protein Reinoso 1996	1a	1b	Principal functions
Olfactory bulb//mitral cell layer	0	3	1	1	Olfactory
Cerebral cortex					
Orbital cortex/medial prefrontal cortex//prefrontal cortex	4	3	2	0	Decision-making, memory
Pre- and infralimbic cortex/medial prefrontal cortex//Pre- and infralimbic cortex	4	2	3	0	Control of fear behaviour
Dorsal peduncular cortex			0	3	Nociception, fear behaviour

Region	Protein Levitt 1984	Protein Reinoso 1996	1a	1b	Principal functions
Cingulate cortex/anterior and posterior cingulate cortex//anterior and posterior cingulate cortex	3 and 2	3 and 2	4	0	Emotions, motivation
Retrosplenial agranular cortex			0	2	Visually guided navigation, memory
Retrosplenial granular cortex			3	1	Navigation, memory
Granular insular cortex/insular cortex//insula	3	3	3	1	Emotions, autonomic control, gustatory
Agranular insular cortex/insular cortex//insula	3	3	3	0	Emotions, autonomic control
Ectorhinal cortex//perirhinal cortex		2	1	0	Object recognition, memory
Entorhinal cortex	4	2	0	1	Navigation, spatial memory
Claustrum			0	1	Consciousness
Dorsal endopiriform nucleus			0	2	Olfactory, gustatory
Piriform cortex	0	3	3	2	Olfactory
Temporal association cortex			2	1	Object recognition
Parietal association cortex			1	1	Attention
Primary auditory cortex/auditory cortex//auditory cortex	0	1	1	2	Auditory
Secondary auditory cortex/auditory cortex//auditory cortex	0	1	1	2	Auditory
Primary visual cortex/visual cortex//visual cortex	0	0	0	3	Visual
Secondary visual cortex/visual cortex//visual cortex	0	0	0	1	Visual
Primary somatosensory cortex/sensorimotor cortex//sensorimotor cortex	0	1	0	4	Sensory
Secondary somatosensory cortex/sensorimotor cortex//sensorimotor cortex	0	1	1	3	Sensory
Primary motor cortex/sensorimotor cortex//sensorimotor cortex	0	1	1	1	Motor
Secondary motor cortex/sensorimotor cortex//sensorimotor cortex	0	1	1	0	Motor
Septal and basal forebrain regions					
Medial septal nucleus/septum// medial septal nucleus	4	2	1	0	Generation of theta rhythm, spatial memory

Region	Protein Levitt 1984	Protein Reinoso 1996	1a	1b	Principal functions
Lateral septal nucleus, dorsal part/septum//lateral septal nucleus, dorsal part	4	2	2	3	Social and motivated behaviour, stress response
Lateral septal nucleus, ventral part/septum//lateral septal nucleus, ventral part	4	2	2	2	Social and motivated behaviour, stress response
Lateral septal nucleus, intermediate part/septum//lateral septal nucleus, intermediate part	4	1	0	1	Social and motivated behaviour, stress response
Septofimbrial nucleus			2	3	Unknown
Subfornical organ		2	1	2	Osmoregulation, cardiovascular regulation, feeding
Bed nucleus of the anterior commissure			0	4	Fear response
Lateral bed nucleus of stria terminalis/bed nucleus of stria terminalis//bed nucleus of stria terminalis	4	2	1	1	Anxiety, motivated behaviour
Medial bed nucleus of stria terminalis/bed nucleus of stria terminalis//bed nucleus of stria terminalis, encapsulated	4	3	3	1	Anxiety, reproductive behaviour, maternal behaviour
Basal ganglia					
Olfactory tubercle	3	3	2	0	Olfactory, motivated behaviour
Nucleus accumbens core/nucleus accumbens// nucleus accumbens	4	2	3	1	Motivated behaviour
Nucleus accumbens shell/nucleus accumbens// nucleus accumbens	4	2	3	1	Motivated behaviour
Caudate putamen	2	1	0	1	Motor, motivated behaviour
Globus pallidus		2	0	1	Motor
Lateral stripe of striatum			0	3	Unknown
Hippocampal formation					
CA1/hippocampus//CA1	4	3	4	1	Memory, navigation, stress
CA3/hippocampus//CA3	4	4	4	1	Memory, navigation, stress
Dentate gyrus/hippocampus// anterodorsal and caudoventral dentate gyrus	4	2 and 4	4	1	Memory, navigation, stress

Region	Protein Levitt 1984	Protein Reinoso 1996	1a	1b	Principal functions
Subiculum	4	3	2	0	Memory, navigation, stress
Amygdala					
Amygdalohippocampal area			4	0	Emotions
Nucleus of the lateral olfactory tract			3	0	Olfactory
Intercalated nuclei/ amygdala	4		0	1	Fear extinction
Central nucleus/amygdala// central nucleus	4	2	1	3	Fear response, motivated behaviour
Lateral nucleus/amygdala// lateral nucleus	4	2	3	1	Fear memory
Basolateral nucleus/ amygdala//basolateral nucleus	4	3	3	0	Fear response and memory
Basomedial nucleus/ amygdala	4		3	0	Anxiety
Medial nucleus/amygdala// medial nucleus	4	2	3	1	Social and reproductive behaviour, predator fear
Medial nucleus, posterodorsal/amygdala// medial nucleus, posterodorsal	4	4	2	1	Social and reproductive behaviour
Medial nucleus, posteroventral/amygdala	4		4	0	Predator fear
Posterolateral cortical nucleus/amygdala//cortical nucleus	4	3	3	1	Olfactory
Posteromedial cortical nucleus/amygdala//cortical nucleus	4	3	4	1	Reproductive behaviour
Hypothalamus					
Medial preoptic nucleus		3	4	0	Reproductive behaviour
Medial preoptic area/ preoptic area//medial preoptic area	4	3	2	0	Reproductive and parental behaviour, thermoregulation
Lateral preoptic area/ preoptic area//lateral preoptic area	4	2	2	0	Thirst, sleep
Periventricular hypothalamic nucleus	1	2	0	4	Production of hormones that regulate growth, metabolism and reproduction
Anteroventral periventricular nucleus		2	0	4	Reproductive behaviour
Lateral hypothalamus	4	2	2	0	Feeding, arousal, reward-related, neuroendocrine and autonomic functions

Region	Protein Levitt 1984	Protein Reinoso 1996	1a	1b	Principal functions
Lateroanterior hypothalamus//anterior hypothalamus		2	2	0	Aggression
Supraoptic nucleus	3	4	0	2	Osmoregulation, stress
Suprachiasmatic nucleus		2	0	4	Circadian rhythms
Paraventricular nucleus/ para-ventricular nucleus// magnocellular and parvocellular regions of the paraventricular nucleus	0	4 and 2	3	4	Energy homeostasis, neuroendocrine and autonomic functions, social and reproductive behaviour, stress
Medial tuberal nucleus			3	0	Aggression
Dorsomedial hypothalamus, compact			3	2	Emotional stress response, circadian rhythms, arousal
Dorsomedial hypothalamus	3	2	3	0	Emotional stress response, circadian rhythms, arousal
Ventromedial hypothalamic nucleus, anterior part/ ventromedial hypothalamic nucleus// ventromedial hypothalamic nucleus	4	3	0	2	Satiety
Ventromedial hypothalamic nucleus, ventrolateral part/ ventro-medial hypothalamic nucleus// ventromedial hypothalamic nucleus	4	3	3	0	Aggression, reproductive behaviour, satiety
Ventromedial hypothalamic nucleus, dorsomedial part/ ventro-medial hypothalamic nucleus// ventromedial hypothalamic nucleus	4	3	3	0	Fear behaviour
Arcuate nucleus	0	1	0	4	Regulation of feeding, growth and reproduction
Mammillary bodies/mammillary bodies// medial mammillary nucleus	3	2	4	4	Spatial and episodic memory
Ventral tuberomammillary nucleus			2	4	Sleep, arousal, energy homeostasis, memory
Ventromedial preoptic nucleus			3	0	Reproductive behaviour
Thalamus					
Anterodorsal thalamic nucleus/ anterior thalamic nuclei//antero-dorsal thalamic nucleus	4	4	3	4	Learning, spatial memory

Region	Protein Levitt 1984	Protein Reinoso 1996	1a	1b	Principal functions
Anteroventral thalamic nucleus/ anterior thalamic nuclei//antero-ventral thalamic nucleus	4	1	2	0	Learning, spatial memory
Anteromedial thalamic nucleus/ anterior thalamic nuclei//antero-medial thalamic nucleus	4	3	1	0	Learning, spatial memory
Paraventricular thalamic nucleus	4	4	2	3	Emotional arousal, anxiety, stress
Ventrolateral thalamic nucleus	0	1	0	1	Motor
Ventromedial thalamic nucleus		1	0	0	Motor
Ventral posteromedial thalamic nucleus/ ventrobasal complex// ventral posteromedial thalamic nucleus	0	2	0	3	Gustatory, somatosensory
Ventral posterolateral thalamic nucleus/ ventrobasal complex// ventral posterolateral thalamic nucleus	0	2	0	3	Somatosensory
Parataenial thalamic nucleus		4	1	4	Viscerolimbic
Mediodorsal thalamic nucleus	4	3	0	3	Olfactory processing, memory, cognition
Laterodorsal thalamic nucleus//laterodorsal thalamic nucleus//rostral and posterior laterodorsal thalamic nucleus	2	3 and 2	0	2	Spatial learning, somatosensory processing
Central medial thalamic nucleus		3	2	0	Cognitive, arousal
Paracentral thalamic nucleus			1	2	Cognitive, pain processing
Parafascicular thalamic nucleus		2	3	2	Behavioural flexibility, pain processing
Reuniens thalamic nucleus		2	0	1	Spatial navigation, fear memory, resilience to depressive states
Reticular thalamic nucleus		3	2	0	Attention, sensory gating
Xiphoid thalamic nucleus			1	2	Fear
Interanteromedial thalamic nucleus			0	2	Spatial navigation and memory
Medial habenula/ habenula//medial habenula	2	3	4	4	Anxiety, spatial memory, inhibitory control of behaviour
Lateral habenula/habenula// lateral habenula, medial and lateral part	2	2 and 1	0	2	Negative reward prediction
Posterior thalamic nuclear group		2	0	2	Somatosensory, auditory

Region	Protein Levitt 1984	Protein Reinoso 1996	1a	1b	Principal functions
Lateral posterior thalamic nucleus		1	0	2	Visual, sensory association
Dorsal lateral geniculate nucleus/ lateral geniculate nucleus//dorsal lateral geniculate nucleus	0	2	0	4	Visual
Ventral lateral geniculate nucleus/ lateral geniculate nucleus//ventral lateral geniculate nucleus	0	0	0	1	Visual, circadian rhythm
Medial geniculate nucleus	0	2	0	3	Auditory
Subthalamic nucleus		2	1	2	Control of movements, impulse control, cognition
Zona incerta		0	0	0	Viscerolimbic, pain processing, sensory gating
Brainstem					
Superior colliculus	4	2	0	2	Visual
Inferior colliculus, central nucleus/ inferior colliculus// inferior colliculus	0	2	0	3	Auditory
Substantia nigra, pars reticulata/ substantia nigra// substantia nigra, pars reticulata	3	1	1	2	Motor control, motivation
Substantia nigra, pars compacta/ substantia nigra// substantia nigra, pars compacta	3	2	1	2	Motor control and learning, regulation of sleep
Ventral tegmental area	3	2			Motivation, cognition, learning
Interpeduncular nucleus		2			Aversive states
Periaqueductal grey		2	1	1	Modulation of pain, fear behaviour, maternal behaviour
Mesencephalic raphe nuclei//dorsal raphe	2	2			Memory, reward-related behaviour, sleep-wake cycle
Red nucleus	0	1			Motor
III cranial nerve nucleus//oculo-motor nucleus	0	2			Motor
IV cranial nerve nucleus//trochlear nucleus	0	2			Motor
Dorsal tegmental nucleus	3	3			Arousal
Ventral tegmental nucleus	3	2			Memory, generation of theta rhythm

Region	Protein Levitt 1984	Protein Reinoso 1996	1a	1b	Principal functions
Reticular formation//lateral reticular nucleus	2	3			Arousal, consciousness, motor and visceral control
Nuclei of the lateral lemniscus		2	0	3	Auditory
Solitary nucleus	4	2	0	1	Multisensory
Superior olivary complex		2	0	1	Auditory
Nucleus of the trapezoid body		3	0	2	Auditory
Dorsal cochlear nucleus/ cochlear nuclei//dorsal cochlear nucleus	0	1	0	3	Auditory
Ventral cochlear nucleus/ cochlear nuclei//ventral cochlear nucleus	0	3	0	3	Auditory
Cuneate nucleus			0	2	Somatosensory
Gracile nucleus			0	2	Somatosensory
Spinal trigeminal nucleus/V cranial nerve nucleus// spinal trigeminal nucleus	0	2	0	2	Somatosensory
V cranial nerve nucleus//principal sensory trigeminal nucleus	0	2			Somatosensory
V cranial nerve nucleus//motor trigeminal nucleus	0	2			Motor
VI cranial nerve nucleus// abducens nucleus	0	2			Motor
VII cranial nerve nucleus// facial nucleus	0	3			Motor
VIII cranial nerve nucleus// superior, medial and lateral vestibular nucleus	0	1, 2, 1			Sensory
XII cranial nerve nucleus// hypo-glossal nucleus	0	2			Motor
Pontine and medullary raphe nuclei//nucleus raphe magnus	0	2			Breathing, digestion, regulation of cardiac function, modulation of pain
Locus coeruleus	2	3			Stress response, arousal, sleep, memory, emotions
Parabrachial nucleus	4				Gustatory, feeding, breathing, arousal, thermoregulation
Nucleus ambiguus		2			Somatomotor, visceromotor
Nucleus of dorsal motor vagus	4	3			Visceromotor

Region	Protein Levitt 1984	Protein Reinoso 1996	1a	1b	Principal functions
Cerebellum					Motor control, pain processing, learning, memory, emotions
Molecular layer	1	1	0	1	Inhibitory control of Purkinje cells
Granule cell layer	0	0	1	0	Processing of cerebral input, excitatory input to Purkinje cells and molecular layer
Purkinje cell layer	0	2	0	3	Inhibitory control of cerebellar output
Deep cerebellar nuclei// dentate nucleus	0	2			Cerebellar output, motor, cognitive, visuospatial
Deep cerebellar nuclei// interpositus nucleus	0	1			Cerebellar output, motor
Deep cerebellar nuclei// fastigial nucleus	0	0			Cerebellar output, motor
Spinal cord					
Dorsal horn/lamina II	3		2	2	Relaying somatosensory information from the body
Ventral horn/all other laminae	0		2	2	Motor
Intermediolateral cell column	2				Parasympathetic innervation of the body

Lsamp 1a promoter activity is studied by radioactive and non-radioactive RNA *in situ* hybridization, *Lsamp* 1b promoter activity is studied by radioactive RNA *in situ* hybridization and X-gal staining X-gal staining of *Lsamp*-β-galactosidase knockin mouse brains. Categories of the estimation of staining intensity: 0 – not detectable; 1 – barely detectable, 2 – low, 3 – moderate, 4 – high. The brain regions are named as follows: name according to Ref III (after Franklin and Paxinos, 1997)/name in Levitt, 1984//name in Reinoso et al., 1996.

SUGGESTIONS FOR FURTHER STUDIES

The development of the emotional brain begins during the embryonic stage and continues postnatally for an extended period and is adjusted by the crosstalk between the genes and environment. Predisposition to psychopathologies, such as mood and anxiety disorders and psychotic conditions is often genetically based, but the outbreak of the disease depends on environmental factors. Therefore, it is important to understand the genetic determinants of the development and how they interact with the intrinsic milieu of the organism and external environment. The results of the experiments included in this thesis have widened the knowledge of the function of WFS1 and LSAMP in the context of development and behavioural regulation, but also raised new questions and pointed towards further research directions.

In case of WFS1, it is essential to understand how the dysfunction of the protein leads to defects in myelination and how the developmental shortcoming is related to neurodegeneration widely reported in older WS patients. As several lines of evidence indicate a link between WFS1 and other proteins implicated in neurodevelopmental processes (Fonseca et al., 2010; Guo et al., 2011; Kawada et al., 2014; Shrestha et al., 2015), the mechanisms how WFS1 affects the developmental events through direct or indirect interaction with these proteins (namely, WNT7A, NTF3 and E3 ubiquitin ligases SMURF1 and HRD1) require further investigation.

The disturbances in the dopaminergic and serotonergic systems in case of the lack of functional WFS1 need further study. Specifically, the relation between WFS1 and the intracellular pathway of D1-type dopamine receptors requires investigating. The possible involvement of WFS1 in regulating the adenylyl cyclase activity, which is an intracellular effect of D1 receptor binding, is of particular interest. Also, the relation between WFS1 and D2-type dopamine receptors needs to be addressed.

In addition, it would be essential to verify in which cell types *Wfs1* is expressed and to study the connectivity between *Wfs1*-expressing cells to better understand the function of WFS1 on the circuit level. Here, the intercalated amygdala, being involved in the feedforward inhibition of the central amygdala activity and expression of fear extinction (Ehrlich et al., 2009; Likhtik et al., 2008), provides an interesting site for dissecting the function of WFS1 in the amygdala fear circuits. In conjunction with this, the role of WFS1 in the GABAergic system should be elaborated in further detail.

From the evolutionary perspective, studying the expression of *Wfs1* in the amphibian brain could give more information on the ancestral state of pallial and subpallial distribution of WFS1 and help to resolve the question of common developmental origin of the *Wfs1* expression pattern in the pallial amygdala. Also, an interesting topic that might shed light to the molecular function of WFS1 would be the expression of *Wfs1* in a non-hypoxia resistant chelonian species.

An interesting feature of the developmental expression of both *Lsamp* and *Wfs1* was their largely transient mRNA expression in the dorsal striatum. The role of such extensive developmental expression of both is unknown and further studies could provide important knowledge regarding the formation of neural circuits important for motivation, emotion and executive functioning.

In case of *Lsamp*, further studies are needed to understand the importance of the conserved dual promoter structure. It is essential to map the activity of the two alternative promoters in the developmental stages parting E15.5 and adulthood. The differential pattern of *Lsamp* 1a and 1b promoter activity in comparison with the protein expression points to two important aspects that need further study. First, the intracellular localization of the protein synthesized from each transcript should be thoroughly investigated. Secondly, the mechanisms how *Lsamp* 1a and 1b transcripts contribute to the formation of distinct neural circuits need to be elucidated.

The underlying mechanisms and causal relations between the *Lsamp* transcripts' expressions and measures of anxiety and social behaviour should be clarified. Also, measuring the level of LSAMP protein in different brain regions in relation to behavioural characteristics could add to the knowledge of the role of *Lsamp* in behaviour and psychiatric disease. In wider context, the regulatory mechanisms determining the expressional activity of *Lsamp* gene and influencing the translational activity, functionality and stability of LSAMP protein need to be studied.

CONCLUSIONS

The overall objective of the research presented in this dissertation was to bring us closer to understanding how *WFS1* and *LSAMP*, two genes implicated in fear and anxiety regulation and susceptibility to psychiatric diseases, are related the neurodevelopmental aspects of behaviour. The age of onset of common neuropsychiatric diseases, such as depression, bipolar disorder, anxiety disorders, and schizophrenia falls into late childhood to early adulthood, suggesting a developmental aetiology. Since both *WFS1* and *LSAMP* have been associated with psychiatric diseases, understanding their role in brain development can lead us closer to comprehending the causes of psychiatric diseases and elaborating better treatment. Both *Wfs1* and *Lsamp* are expressed in the embryonic brain, and the expression of both undergoes developmental changes which suggests a specific role during development. The knowledge obtained from the experiments included into this thesis helps to better understand the roles of *Wfs1* and *Lsamp* in the formation and functioning of different brain structures and points at possible directions for succeeding studies.

The main results are summarized as follows:

1. The expression of *Wfs1* is initiated at around E15.5 in the mouse brain. The initiation of *Wfs1* expression falls into the time window of the beginning of synaptic activity after the end of the peak of the neuronal migration. The expression of *Wfs1* is initiated in numerous regions of the brain and roughly follows the maturation of synaptic functionality. The first expression regions of *Wfs1* are the dorsal striatum and the amygdala; by the time of birth, the expression is widespread in all striatal and amygdaloid regions and diencephalon and emerging in the cortical structures, including neocortical and olfactory areas and hippocampus. After postnatal day 2, the widespread expression of *Wfs1* becomes gradually downregulated, achieving its relatively restricted adult pattern (mainly ventral striatum, caudal CPu, central extended amygdala, piriform cortex, layer II/III of the neocortex and sulcal cortical areas, CA1 region of the hippocampus, reticular thalamic nucleus) after puberty. Our findings extend the previous results that describe the expression of *Wfs1* in the postnatal and adult mouse brain.
2. During embryonic and early postnatal development, the dynamics of *Wfs1* expression shows opposing regulation compared to the markers of developmental endoplasmic reticulum stress. During the perinatal period, the expression of *Wfs1* is upregulated in a wide range of brain structures, whereas the expression of endoplasmic stress markers *Grp78*, *Grp94* and *CHOP* shows gradual downregulation. The expression levels do not differ between wt and *Wfs1*$^{-/-}$ pups. These results indicate that the transient widespread expression of *Wfs1* in the perinatal brain is not involved in the regulation of the developmental ER stress.
3. Across different evolutionary lineages, the expression of *Wfs1* is conserved in the striatal and subpallial amygdaloid structures and lacking in the pallidal regions. In pallial regions, the expression of *Wfs1* shows considerable

variation between the studied species: house mouse, domestic chick and red-eared slider turtle. The expression of *Wfs1* lacks in the hyperpallium of the avian brain, while being present in the neocortex of the mouse and dorsal cortex of the turtle. This suggests independent gain in the dorsal pallial derivatives in the mammalian and chelonian lineages or loss in the avian lineage. The presence of *Wfs1* expression in the hippocampal homologue and pallial amygdaloid structures of all lineages favours the hypothesis of common ancestral pattern.

4. Our expression analysis in the brain of mouse, domestic chick and red-eared slider turtle indicates that the relation between Wfs1 and D1-type dopamine receptors is evolutionarily conserved. Across different evolutionary lineages, the expression of *Wfs1* shows considerable overlapping with the expression of *Drd1a*, the gene encoding the most widespread D1-type dopamine receptor. In the hippocampus of *Wfs1*$^{-/-}$ mice, the ligand binding of D1-type dopamine receptors is increased. This result adds further evidence to the previous findings pointing to the altered dopaminergic system in *Wfs1*$^{-/-}$ mice and shows the specific involvement of D1-type dopamine receptors.
5. The two alternative promoters of the *Lsamp* gene show rather complementary patterns of activity in the mouse brain. While *Lsamp* 1a transcript is predominantly restricted to the limbic regions (e.g. hippocampus, amygdala, limbic-associated cortices, anterior thalamus, limbic-related hypothalamic regions), *Lsamp* 1b promoter shows strong activity in the sensory and motor pathways (visual, somatosensory and auditory pathways). The olfactory and gustatory pathways, which are largely integrated with the emotion-processing functions, show activity of both *Lsamp* 1a and 1b promoters. A higher expression of *Lsamp* 1a transcript in the temporal lobe correlates with higher trait anxiety in mice. A lower level of *Lsamp* 1a and 1b transcripts in the hippocampus correlates with a prolonged time of engagement in social sniffing between unfamiliar mice. Higher level of *Lsamp* 1a transcript in the ventral striatum positively correlates with the time involved in anogenital sniffing. As *Lsamp* 1a was predominantly expressed in the brain regions belonging to the emotion-processing circuits, the higher expression level indicating higher anxiety reveals its relevance for anxiety-related behavioural control. Correlations between the expression level of *Lsamp* gene transcripts and measures of social behaviour underline previous findings showing that loss of function of *Lsamp* reduces agonistic behaviour.

REFERENCES

Abellan A and Medina L. 2009. Subdivisions and derivatives of the chicken subpallium based on expression of LIM and other regulatory genes and markers of neuron subpopulations during development. *J Comp Neurol* 515:465–501.

Abramov U, Puussaar T, Raud S, Kurrikoff K, Vasar E. 2008. Behavioural differences between C57BL/6 and 129S6/SvEv strains are reinforced by environmental enrichment. *Neurosci Lett* 443(3):223–227.

Adhikari A, Lerner TN, Finkelstein J, Pak S, Jennings JH, Davidson TJ et al. 2015. Basomedial amygdala mediates top-down control of anxiety and fear. *Nature* 527(7577):179–185.

Akeel M, McNamee CJ, Youssef S, Moss D. 2011. DIgLONs inhibit initiation of neurite outgrowth from forebrain neurons via an IgLON-containing receptor complex. *Brain Res* 1374:27–35.

Altman J and Bayer SA. 1990. Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. J Comp Neurol. 301(3):365–381.

Altpere A, Raud S, Sütt S, Reimets R, Visnapuu T, Toots M et al. 2017. Mild stress induces brain region-specific alterations of selective ER stress markers' mRNA expression in Wfs1-deficient mice. *Behav Brain Res* pii: S0166-4328(17)30713-1.

Alttoa A, Kõiv K, Hinsley TA, Brass A, Harro J. 2010. Differential gene expression in a rat model of depression based on persistent differences in exploratory activity. *Eur Neuropsychopharmacol* 20(5):288–300.

Amr S, Heisey C, Zhang M, Xia XJ, Shows KH, Ajlouni K et al. 2007. A homozygous mutation in a novel zinc-finger protein, ERIS, is responsible for Wolfram syndrome 2. *Am J Hum Genet* 81(4):673–683.

Angevine JB and Sidman RL. 1961. Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nature* 192:766–768.

Area-Gomez E, Del Carmen Lara Castillo M, Tambini MD, Guardia-Laguarta C, de Groof AJ, Madra M et al. 2012. Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. *EMBO J* 31(21):4106–4123.

Arnauld E, Jeantet Y, Arsaut J, Demotes-Mainard J. 1996. Involvement of the caudal striatum in auditory processing: c-fos response to cortical application of picrotoxin and to auditory stimulation. *Brain Res Mol Brain Res* 41(1–2):27–35.

Ascano M, Bodmer D, Kuruvilla R. 2012. Endocytic trafficking of neurotrophins in neural development. *Trends in Cell Biology* 22(5):266–273.

Barøy T, Kresse SH, Skårn M, Stabell M, Castro R, Lauvrak S, et al. 2014. Reexpression of LSAMP inhibits tumor growth in a preclinical osteosarcoma model. *Mol Cancer* 13:93.

Barrett TG, Bundey SE, Macleod AF. 1995. Neurodegeneration and diabetes: UK nationwide study of Wolfram (DIDMOAD) syndrome. *Lancet* 346(8988):1458–1463.

Barrett TG, Scott-Brown M, Seller A, Bednarz A, Poulton K, Poulton J. 2000. The mitochondrial genome in Wolfram syndrome. *J Med Genet* 37:463–466.

Baye TM, Perry RT, Wiener HW, Chen Z, Harrell LE, Go RC. 2008. Candidate gene discovery procedure after follow-up confirmatory analyses of candidate regions of interests for Alzheimer's disease in the NIMH sibling dataset. *Dis Markers* 24(6): 293–309.

Becker AJJ, Befort YK, Blad C, Filliol D, Ghate A, Dembele D et al. 2008. Transcriptome analysis identifies genes with enriched expression in the mouse central extended amygdala. *Neuroscience* 156:950–965.

Behan AT, Byrne C, Dunn MJ, Cagney G, Cotter DR. 2009. Proteomic analysis of membrane microdomain-associated proteins in the dorsolateral prefrontal cortex in schizophrenia and bipolar disorder reveals alterations in LAMP, STXBP1 and BASP1 protein expression. *Mol Psychiatry* 14(6):601–613.

Bellucci A, Navarria L, Zaltieri M, Falarti E, Bodei S, Sigala S et al. 2011. Induction of the unfolded protein response by α-synuclein in experimental models of Parkinson's disease. *J Neurochem* 116(4):588–605.

Benshalom G and White EL. 1986. Quantification of thalamocortical synapses with spiny stellate neurons in layer IV of mouse somatosensory cortex. *J Comp Neurol* 253:303–314.

Berendse HW and Groenewegen HJ. 1990. Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. *J Comp Neurol* 299:187–228.

Bernabucci M and Zhuo M. 2016. Calcium activated adenylyl cyclase AC8 but not AC1 is required for prolonged behavioral anxiety. *Molecular Brain* 9:60.

Binder DK and Scharfman HE. 2004. Brain-derived neurotrophic factor. *Growth Factors* 22(3):123–131.

Björklund A and Dunnett SB. 2007. Dopamine neuron systems in the brain: an update. *Trends Neurosci* 30:194–202.

Blacker D, Bertram L, Saunders AJ, Moscarillo TJ, Albert MS, Wiener H et al. 2003. Results of a high-resolution genome screen of 437 Alzheimer's disease families. *Hum Mol Genet* 12:23–32.

Blennow K, Bogdanovic N, Gottfries CG, Davidsson P. 1999. The growth-associated protein GAP-43 is increased in the hippocampus and in the gyrus cinguli in schizophrenia. *J Mol Neurosci* 13(1–2):101–109.

Bonnycastle LL, Chines PS, Hara T, Huyghe JR, Swift AJ, Heikinheimo P et al. 2013. Autosomal dominant diabetes arising from a Wolfram syndrome 1 mutation. *Diabetes* 62(11):3943–3950.

Bosse KE, Charlton JL, Susick LL, Newman B, Eagle AL, Mathews TA et al. 2015. Deficits in behavioral sensitization and dopaminergic responses to methamphetamine in adenylyl cyclase 1/8-deficient mice. *J Neurochem* 135(6):1218–1231.

Boulant JA. 2000. Role of the preoptic-anterior hypothalamus in thermoregulation and fever. *Clin Infect Dis* 31 Suppl 5:S157–161.

Bouwmeester H, Wolterink G, van Ree JM. 2002. Neonatal development of projections from the basolateral amygdala to prefrontal, striatal, and thalamic structures in the rat. *J Comp Neurol* 442(3):239–249.

Bradfield LA, Hart G, Balleine BW. 2013. The role of the anterior, mediodorsal, and parafascicular thalamus in instrumental conditioning. *Front Syst Neurosci* 7:51.

Brown HD, Baker PM, Ragozzino ME. 2010. The parafascicular thalamic nucleus concomitantly influences behavioural flexibility and dorsomedial striatal acetylcholine output in rats. *J Neurosci* 30:14390–14398.

Bruce LL. Evolution of the nervous system in reptiles In: Kaas JH, editor. Evolutionary Neuroscience. Academic Press; 2009. pp. 233–264.

Brümmendorf T, Spaltmann F, Treubert U. 1997. Cloning and characterization of a neural cell recognition molecule on axons of the retinotectal system and spinal cord. *Eur J Neurosci* 9(6):1105–1116.

Bryan B, Cai Y, Wrighton K, Wu G, Feng XH, Liu M. 2005. Ubiquitination of RhoA by Smurf1 promotes neurite outgrowth. *FEBS Lett* 579(5):1015–1019.

Burton AC, Nakamura K, Roesch MR. 2015. From ventral-medial to dorsal-lateral striatum: Neural correlates of reward-guided decision-making. *Neurobiol Learn Mem* 0:51–59.

Cagalinec M, Liiv M, Hodurova Z, Hickey MA, Vaarmann A, Mandel M et al. 2016. Role of Mitochondrial Dynamics in Neuronal Development: Mechanism for Wolfram Syndrome. *PLoS Biol* 14(7):e1002511.

Calì T, Ottolini D, Negro A, Brini M. 2012. α-Synuclein controls mitochondrial calcium homeostasis by enhancing endoplasmic reticulum-mitochondria interactions. *J Biol Chem* 287(22):17914–17929.

Cano A, Rouzier C, Monnot S, Chabrol B, Conrath J, Lecomte P et al. 2007. Identification of novel mutations in WFS1 and genotype-phenotype correlation in Wolfram syndrome. *Am J Med Genet A* 143A(14):1605–1612.

Casey B, Heller AS, Gee DG, Cohen AO. 2017. Development of the emotional brain. *Neurosci Lett*, in press https://doi.org/10.1016/j.neulet.2017.11.055

Catania EH, Pimenta A, Levitt P. 2008. Genetic deletion of Lsamp causes exaggerated behavioral activation in novel environments. *Behav Brain Res* 188(2):380–390.

Chaussenot A, Bannwarth S, Rouzier C, Vialettes B, El Mkadem SA, Chabrol B et al. 2011. Neurologic features and genotype–phenotype correlation in Wolfram syndrome. *Ann Neurol* 69:501–508.

Chen S, Gil O, Ren YQ, Zanazzi G, Salzer LJ, Hillman ED. 2001. Ntm expression during cerebellar development suggests roles in axon fasciculation and synaptogenesis. *J Neurocytol* 30:927–937.

Chen J, Lui WO, Vos MD, Clark GJ, Takahashi M, Schoumans J et al. 2003. The t(1;3) breakpoint-spanning genes LSAMP and NORE1 are involved in clear cell renal cell carcinomas. *Cancer Cell* 4(5):405–413.

Chen YF, Kao CH, Chen YT, Wang CH, Wu CY, Tsai CY et al. 2009. Cisd2 deficiency drives premature aging and causes mitochondria-mediated defects in mice. *Genes Dev* 23:1183–1194.

Chen X, Long F, Cai B, Chen X, Chen G. 2018. A novel relationship for schizophrenia, bipolar and major depressive disorder Part 3: evidence from chromosome 3 high density association screen. *J Comp Neurol* 526(1):59–79.

Chesselet MF, Gonzales C, Levitt P. 1991. Heterogeneous distribution of the limbic system-associated membrane protein in the caudate nucleus and substantia nigra of the cat. *Neuroscience* 40(3):725–733.

Cho YM, Jang YS, Jang YM, Chung SM, Kim HS, Lee JH et al. 2009. Induction of unfolded protein response during neuronal induction of rat bone marrow stromal cells and mouse embryonic stem cells. *Exp Mol Med* 41:440–452.

Coccaro N, Zagaria A, Tota G, Anelli L, Orsini P, Casieri P et al. 2015. Overexpression of the LSAMP and TUSC7 genes in acute myeloid leukemia following microdeletion/duplication of chromosome 3. *Cancer Genet* 10:517–522.

Cornwall J and Phillipson OT. 1988. Afferent projections to the parafascicular thalamic nucleus of the rat, as shown by the retrograde transport of wheat germ agglutinin. *Brain Res Bull* 20(2):139–50.

Côté PY, Levitt P, Parent A. 1995. Distribution of limbic system-associated membrane protein immunoreactivity in primate basal ganglia. *Neuroscience* 69:71–81.

Côté PY, Levitt P, Parent A. 1996. Limbic system-associated membrane protein (LAMP) in primate amygdala and hippocampus. *Hippocampus* 6:483–494.

Cressman VL, Balaban J, Steinfeld S, Shemyakin A, Graham P, Parisot N et al. 2010. Prefrontal cortical inputs to the basal amygdala undergo pruning during late adolescence in the rat. *J Comp Neurol* 518:2693–2709.

a) Cryns K, Thys S, Van Laer L, Oka Y, Pfister M, Van Nassauw L et al. 2003. The WFS1 gene, responsible for low frequency sensorineural hearing loss and Wolfram syndrome, is expressed in a variety of inner ear cells. *Histochem Cell Biol* 119:247–256.

b) Cryns K, Sivakumaran TA, Van den Ouweland JM, Pennings RJ, Cremers CW, Flothmann K et al. 2003. Mutational spectrum of the WFS1 gene in Wolfram syndrome, nonsyndromic hearing impairment, diabetes mellitus, and psychiatric disease. *Hum Mutat* 22(4):275–827.

Cui Y, Ying Y, van Hasselt A, Ng KM, Yu J, Zhang Q et al. 2008. OPCML is broad tumor suppressor for multiple carcinomas and lymphomas with frequently epigenetic inactivation. *PLoS One* 3:e2990.

Davies MN, Krause L, Bell JT, Gao F, Ward KJ, Wu H et al. 2014. Hypermethylation in the ZBTB20 gene is associated with major depressive disorder. *Genome Biol* 15(4):R56.

De Falco M, Manente L, Lucariello A, Baldi G, Fiore P, Laforgia V et al. 2012. Localization and distribution of wolframin in human tissues. *Front Biosci (Elite Ed)* 4:1986–1998.

De Franco E, Flanagan SE, Yagi T, Abreu D, Mahadevan J, Johnson MB et al. 2017. Dominant ER Stress-Inducing WFS1 Mutations Underlie a Genetic Syndrome of Neonatal/Infancy-Onset Diabetes, Congenital Sensorineural Deafness, and Congenital Cataracts. *Diabetes* 66(7):2044–2053.

Deacon TW, Pakzaban P, Isacson O. 1994. The lateral ganglionic eminence is the origin of cells committed to striatal phenotypes: neural transplantation and developmental evidence. *Brain Res* 668(1–2):211–219.

Decuypere J-P, Monaco G, Missiaen L, De Smedt H, Parys JB, Bultynck G. 2011. IP3 Receptors, Mitochondria, and Ca2+ Signaling: Implications for Aging. *J Aging Res* 2011:920178.

Delprat B, Maurice T, Delettre C. 2018. Wolfram syndrome: MAMs' connection? *Cell Death Dis* 9(3):364.

Dennis EL, Jahanshad N, Braskie MN, Warstadt NM, Hibar DP, Kohannim O et al. 2014. Obesity gene NEGR1 associated with white matter integrity in healthy young adults. *Neuroimage* 102:548–557.

Dielenberg RA, Hunt GE, McGregor IS. 2001. “When a rat smells a cat”: the distribution of Fos immunoreactivity in rat brain following exposure to a predatory odor. *Neuroscience* 104:1085–1097.

Dillingham CM, Frizzati A, Nelson AJ, Vann SD. 2015. How do mammillary body inputs contribute to anterior thalamic function? *Neurosci Biobehav Rev* 54:108–119.

Doll CJ, Hochachka PW, Reiner PB. 1991. Effects of anoxia and metabolic arrest on turtle and rat cortical neurons. *Am J Physiol* 260:R747–R755.

Dum RP and Strick PL. 2003. An unfolded map of the cerebellar dentate nucleus and its projections to the cerebral cortex. *J Neurophysiol* 89(1):634–639.

Dungan JR, Qin X, Horne BD, Carlquist JF, Singh A, Hurdle M, et al. 2016. Case-only survival analysis reveals unique effects of genotype, sex, and coronary disease severity on survivorship. *PLoS One* 11:e0154856.

Eagleson KL, Pimenta AF, Burns MM, Fairfull LD, Cornuet PK, Zhang L, et al. 2003. Distinct domains of the limbic system-associated membrane protein (LAMP) mediate discrete effects on neurite outgrowth. *Mol Cell Neurosci* 24:725–740.

Ehrlich I, Humeau Y, Grenier F, Ciocchi S, Herry C, Lüthi A. 2009. Amygdala inhibitory circuits and the control of fear memory. *Neuron* 62(6):757–771.

El-Shanti H, Lidral AC, Jarrah N, Druhan L, Ajlouni K. 2000. Homozygosity mapping identifies an additional locus for Wolfram syndrome on chromosome 4q. *Am J Hum Genet* 66(4):1229–1236.

Favero CB, Henshaw RN, Grimsley-Myers CM, Shrestha A, Beier DR, Dwyer ND. 2013. Mutation of the BiP/GRP78 gene causes axon outgrowth and fasciculation defects in the thalamocortical connections of the mammalian forebrain. *J Comp Neurol* 521(3):677–696.

Ferguson AV, Latchford KJ, Samson WK. 2008. The paraventricular nucleus of the hypothalamus – a potential target for integrative treatment of autonomic dysfunction. *Expert Opin Ther Targets* 12(6):717–727.

Ferrari PF, van Erp AM, Tornatzky W, Miczek KA. 2003. Accumbal dopamine and serotonin in anticipation of the next aggressive episode in rats. *Eur J Neurosci* 17(2):371–378.

File SE and Hyde JR. 1978. Can social interaction be used to measure anxiety? *Br J Pharmacol* 62(1):19–24.

File SE, Zangrossi HJ, Sanders FL, Mabbutt PS. 1993. Dissociation between behavioral and corticosterone responses on repeated exposures to cat odor. *Physiol Behav* 54: 1109–1111.

File SE, Kenny PJ, Cheeta S. 2000. The role of the dorsal hippocampal serotonergic and cholinergic systems in the modulation of anxiety. *Pharmacol Biochem Behav* 66(1):65–72.

Fonseca SG, Fukuma M, Lipson KL, Nguyen LX, Allen JR, Oka Y et al. 2005. WFS1 is a novel component of the unfolded protein response and maintains homeostasis of the endoplasmic reticulum in pancreatic beta-cells. *J Biol Chem* 280(47):39609–39615.

Fonseca SG, Ishigaki S, Oslowski CM, Lu S, Lipson KL, Ghosh R et al. 2010. Wolfram syndrome 1 gene negatively regulates ER stress signaling in rodent and human cells. *J Clin Invest* 120(3):744–755.

Fontes MA, Xavier CH, de Menezes RC, Dimicco JA. 2011. The dorsomedial hypothalamus and the central pathways involved in the cardiovascular response to emotional stress. *Neuroscience* 184:64–74.

Franklin KBJ, Paxinos G. The mouse brain in stereotaxic coordinates. San Diego, London: Academic Press; 1997.

Fromer M, Roussos P, Sieberts SK, Johnson JS, Kavanagh DH, Perumal TM et al. 2016. Gene expression elucidates functional impact of polygenic risk for schizophrenia. *Nat Neurosci* 19:1442–1453.

Funatsu N, Miyata S, Kumanogoh H, Shigeta M, Hamada K, Endo Y et al. 1999. Characterization of a novel rat brain glycosylphosphatidylinositol-anchored protein (Kilon), a member of the IgLON cell adhesion molecule family. *J Biol Chem* 274(12):8224–8230.

Furlong RA, Ho LW, Rubinsztein JS, Michael A, Walsh C, Paykel ES et al. 1999. A rare coding variant within the wolframin gene in bipolar and unipolar affective disorder cases. *Neurosci Lett* 277(2):123–126.

Gabreels BA, Swaab DF, De Kleijn DP, Dean A, Seidah NG, Van De Loo JW et al. 1998. The vasopressin precursor is not processed in the hypothalamus of Wolfram syndrome patients with diabetes insipidus: evidence for the involvement of PC2 and 7B2. *J Clin Endocrinol Metab* 83:4026–4033.

Galfalvy H, Zalsman G, Huang YY, Murphy L, Rosoklija G, Dwork AJ et al. 2013. A pilot genome wide association and gene expression array study of suicide with and without major depression. *World J Biol Psychiatry* 14(8):574–582.

Galluzzi P, Filosomi G, Vallone IM, Bardelli AM, Venturi C. 1999. MRI of Wolfram syndrome (DIDMOAD) *Neuroradiology* 41:729–731.

García-López M, Abellán A, Legaz I, Rubenstein JLR, Puelles L, Medina L. 2008. Histogenetic Compartments of the Mouse Centromedial and Extended Amygdala Based on Gene Expression Patterns during Development. *J Comp Neurol* 506(1): 46–74.

Gass JN, Gifford NM, Brewer JW. 2002. Activation of an unfolded protein response during differentiation of antibody-secreting B cells. *J Biol Chem* 277:49047–49054.

Gelpi E, Höftberger R, Graus F, Ling H, Holton JL, Dawson T et al. 2016. Neuropathological criteria of anti-IgLON5-related tauopathy. *Acta Neuropathol* 132(4): 531–543.

Genís D, Dávalos A, Molins A, Ferrer I. 1997. Wolfram syndrome: a neuropathological study. *Acta Neuropathol* 93(4):426–429.

Genovese A, Cox DM, Butler MG. 2015. Partial Deletion of Chromosome 1p31.1 Including only the Neuronal Growth Regulator 1 Gene in Two Siblings. *J Pediatr Genet* 4(1):23–28.

Gharanei S, Zatyka M, Astuti D, Fenton J, Sik A, Nagy Z et al. 2013. Vacuolar-type H+-ATPase V1A subunit is a molecular partner of Wolfram syndrome 1 (WFS1) protein, which regulates its expression and stability. *Hum Mol Genet* 22(2):203–217.

Ghirardello S, Dusi E, Castiglione B, Fumagalli M, Mosca F. 2014. Congenital central diabetes insipidus and optic atrophy in a Wolfram newborn: is there a role for WFS1 gene in neurodevelopment? *Ital J Pediatr* 40:76.

Gil O. D., Zanazzi G., Struyk A. F., Salzer J. L. 1998. Neurotrimin mediates bifunctional effects on neurite outgrowth via homophilic and heterophilic interactions. *J. Neurosci* 18:9312–9325.

Gil OD, Zhang L, Chen S, Ren YQ, Pimenta A, Zanazzi G et al. 2002. Complementary expression and heterophilic interactions between IgLON family members Ntm and LAMP. *J Neurobiol* 51:190–204.

Gomez-Zaera M, Strom TM, Rodriguez B, Estivill X, Meitinger T, Nunes V. 2001. Presence of a major WFS1 mutation in Spanish Wolfram syndrome pedigrees. *Mol Genet Metab* 72:72–81.

Gottmann K, Mittmann T, Lessmann V. 2009. BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses. *Exp Brain Res* 199(3–4):203–234.

Gray KA, Yates B, Seal RL, Wright MW, Bruford EA. 2015. genenames.org: the HGNC resources in 2015. *Nucleic Acids Res* 43(Database issue):D1079–85.

Guillery RW and Sherman SM. 2002. Thalamic relay functions and their role in corticocortical communication: generalizations from the visual system. *Neuron* 33(2):163–175.

Guo X, Shen S, Song S, He S, Cui Y, Xing G et al. 2011. The E3 Ligase Smurf1 Regulates Wolfram Syndrome Protein Stability at the Endoplasmic Reticulum. *J Biol Chem* 286(20):18037–18047.

Hall AC, Lucas FR, Salinas PC. 2000. Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. *Cell* 100:525–535.
Hara T, Mahadevan J, Kanekura K, Hara M, Lu S, Urano F. 2014. Calcium efflux from the endoplasmic reticulum leads to β-cell death. *Endocrinology* 155(3):758–768.
Hardy C, Khanim F, Torres R, Scott-Brown M, Seller A, Poulton J et al. 1999. Clinical and molecular analysis of 19 Wolfram syndrome kindreds demonstrating a wide spectrum of mutations in WFS1. *Am J Hum Genet* 65:1279–1290.
Hashimoto T, Maekawa S, Miyata S. 2009. IgLON cell adhesion molecules regulate synaptogenesis in hippocampal neurons. *Cell Biochem Funct* 27:496–498.
Hatanaka M, Tanabe K, Yanai A, Ohta Y, Kondo M, Akiyama M et al. 2011. Wolfram syndrome 1 gene (WFS1) product localizes to secretory granules and determines granule acidification in pancreatic beta-cells. *Hum Mol Genet* 20(7):1274–1284.
Hayashi A, Kasahara T, Iwamoto K, Ishiwata M, Kametani M, Kakiuchi C et al. 2007. The role of brain-derived neurotrophic factor (BDNF)-induced XBP1 splicing during brain development. *J Biol Chem* 282(47):34525–34.
Heimer L and Van Hoesen GW. 2006. The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. *Neurosci Biobehav Rev* 30(2):126–147.
Heinla I, Leidmaa E, Kongi K, Pennert A, Innos J, Nurk K et al. 2015. Gene expression patterns and environmental enrichment-induced effects in the hippocampi of mice suggest importance of Lsamp in plasticity. *Front Neurosci* 9:205.
Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC et al. 2003. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol* (3):151–180.
Hershey T, Lugar HM, Shimony JS, Rutlin J, Koller JM, Perantie DC et al. 2012. Early brain vulnerability in Wolfram syndrome. *PLoS One* 7(7):e40604.
Higashi S1, Hioki K, Kurotani T, Kasim N, Molnár Z. 2005. Functional thalamocortical synapse reorganization from subplate to layer IV during postnatal development in the reeler-like mutant rat (shaking rat Kawasaki). *J Neurosci* 25(6):1395–406.
Hilson JB, Merchant SN, Adams JC, Joseph JT. 2009. Wolfram syndrome: a clinico-pathologic correlation. *Acta Neuropathol* 118(3):415–428.
Hofmann S, Philbrook C, Gerbitz KD, Bauer MF. 2003. Wolfram syndrome: structural and functional analyses of mutant and wild-type wolframin, the *WFS1* gene product. *Hum Mol Genet* 12(16):2003–2012.
Hofmann S and Bauer MF. 2006. Wolfram syndrome-associated mutations lead to instability and proteasomal degradation of wolframin. *FEBS Lett* 580:4000–4004.
Hoozemans JJ, Veerhuis R, Van Haastert ES, Rozemuller JM, Baas F, Eikelenboom P et al. 2005. The unfolded protein response is activated in Alzheimer's disease. *Acta Neuropathol* 110(2):165–172.
Horton HL and Levitt P. 1988. A unique membrane protein is expressed on early developing limbic system axons and cortical targets. *J Neurosci* 8:4653–4661.
Hsu DT, Kirouac GJ, Zubieta J-K, Bhatnagar S. 2014. Contributions of the paraventricular thalamic nucleus in the regulation of stress, motivation, and mood. *Front Behav Neurosci* 8:73.
Hundahl CA, Allen GC, Hannibal J, Kjaer K, Rehfeld JF, Dewilde S et al. 2010. Anatomical characterization of cytoglobin and neuroglobin mRNA and protein expression in the mouse brain. *Brain Res* 1331:58–73.

Hunt JL, Zaretsky DV, Sarkar S, DiMicco JA. 2010. Dorsomedial hypothalamus mediates autonomic, neuroendocrine, and locomotor responses evoked from the medial preoptic area. *Am J Physiol Regul Integr Comp Physiol* 298(1):R130-R140.

Hyde CL, Nagle MW, Tian C, Chen X, Paciga SA, Wendland JR et al. 2016. Identification of 15 genetic loci associated with risk of major depression in individuals of European descent. *Nat Genet* 48:1031–1036.

Innos J, Philips MA, Leidmaa E, Heinla I, Raud S, Reemann P et al. 2011. Lower anxiety and a decrease in agonistic behaviour in Lsamp-deficient mice. *Behav Brain Res* 217(1):21–31.

Innos J, Philips MA, Raud S, Lilleväli K, Kõks S, Vasar E. 2012. Deletion of the Lsamp gene lowers sensitivity to stressful environmental manipulations in mice. *Behav Brain Res* 228(1):74–81.

Innos J, Leidmaa E, Philips MA, Sütt S, Alttoa A, Harro J et al. 2013. Lsamp$^{-/-}$ mice display lower sensitivity to amphetamine and have elevated 5-HT turnover. *Biochem Biophys Res Commun* 430(1):413–418.

Inoue H, Tanizawa Y, Wasson J, Behn P, Kalidas K, Bernal-Mizrachi E et al. 1998. A gene encoding a transmembrane protein is mutated in patients with diabetes mellitus and optic atrophy (Wolfram syndrome). *Nat Genet* 20(2):143–148.

Ishihara H, Takeda S, Tamura A, Takahashi R, Yamaguchi S, Takei D et al. 2004. Disruption of the WFS1 gene in mice causes progressive beta-cell loss and impaired stimulus-secretion coupling in insulin secretion. *Hum Mol Genet* 13(11):1159–1170.

Ito Y, Yamada M, Tanaka H, Aida K, Tsuruma K, Shimazawa M et al. 2009. Involvement of CHOP, an ER-stress apoptotic mediator, in both human sporadic ALS and ALS model mice. *Neurobiol Dis* 36(3):470–476.

Itoh S, Hachisuka A, Kawasaki N, Hashii N, Teshima R, Hayakawa T et al. 2008. Glycosylation analysis of IgLON family proteins in rat brain by liquid chromatography and multiple-stage mass spectrometry. *Biochemistry* 47:10132–10154.

Iwakoshi NN, Pypaert M, Glimcher LH. 2007. The transcription factor XBP-1 is essential for the development and survival of dendritic cells. *J Exp Med* 204:2267–2275.

Iwawaki T., Akai R., Yamanaka S., Kohno K. 2009. Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability. *Proc Natl Acad Sci U S A* 106:16657–16662.

Jacobs EC, Campagnoni C, Kampf K, Reyes SD, Kalra V, Handley V et al. 2007. Visualization of corticofugal projections during early cortical development in a tau-GFP-transgenic mouse. *Eur J Neurosci* 25:17–30.

Jarvis ED, Güntürkün O, Bruce L, Csillag A, Karten H, Kuenzel W et al. 2005. Avian brains and a new understanding of vertebrate brain evolution. *Nat Rev Neurosci* 6(2):151–159.

Kaas JH and Lyon DC. 2007. Pulvinar contributions to the dorsal and ventral streams of visual processing in primates. *Brain Res Rev* 55:285–296.

Kadowaki H and Nishitoh H. 2013. Signaling Pathways from the Endoplasmic Reticulum and Their Roles in Disease. *Genes* 4(3):306–333.

Kakiuchi C, Ishiwata M, Hayashi A, Kato T. 2006. XBP1 induces WFS1 through an endoplasmic reticulum stress response element-like motif in SH-SY5Y cells. *J Neurochem* 97(2):545–555.

Kakiuchi C, Ishigaki S, Oslowski CM, Fonseca SG, Kato T, Urano F. 2009. Valproate, a Mood Stabilizer, Induces WFS1 Expression and Modulates Its Interaction with ER Stress Protein GRP94. *PLoS One* 4(1):e4134.

Kanki T and Klionsky DJ. 2009. Mitochondrial abnormalities drive cell death in Wolfram syndrome 2. *Cell Res* 19:922–923.
Kaplinsky NJ, Gilbert SF, Cebra-Thomas J, Lilleväli K, Saare M, Chang EY et al. 2013. The Embryonic Transcriptome of the Red-Eared Slider Turtle (Trachemys scripta). *PLoS One* 8(6):e66357.
Karis K, Eskla K-L, Kaare M, Täht K, Tuusov J, Visnapuu T et al. 2018. Altered Expression Profile of IgLON Family of Neural Cell Adhesion Molecules in the Dorsolateral Prefrontal Cortex of Schizophrenic Patients. *Front Mol Neurosci* 11:8.
Kato T, Ishiwata M, Yamada K, Kasahara T, Kakiuchi C, Iwamoto K et al. 2008. Behavioral and gene expression analyses of Wfs1 knockout mice as a possible animal model of mood disorder. *Neurosci Res* 61:143–158.
Kawada K, Iekumo T, Saito R, Kaneko M, Mimori S, Nomura Y et al. 2014. Aberrant neuronal differentiation and inhibition of dendrite outgrowth resulting from endoplasmic reticulum stress. *J Neurosci Res* 92(9):1122–1133.
Kawano J, Tanizawa Y, Shinoda K. 2008. Wolfram syndrome 1 (Wfs1) gene expression in the normal mouse visual system. *J Comp Neurol* 510:1–23.
Kawano J, Fujinaga R, Yamamoto-Hanada K, Oka Y, Tanizawa Y, Shinoda K. 2009. Wolfram syndrome 1 (Wfs1) mRNA expression in the normal mouse brain during postnatal development. *Neurosci Res* 64:213–230.
Keller F, Rimvall K, Barbe MF, Levitt P. 1989. A membrane glycoprotein associated with the limbic system mediates the formation of the septo-hippocampal pathway in vitro. *Neuron* 3:551–561.
Kesaraju S, Schmidt-Kastner R, Prentice HM, Milton SL. 2009. Modulation of stress proteins and apoptotic regulators in the anoxia tolerant turtle brain. J Neurochem 109(5):1413–1426.
Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE. 2005. Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 62(6):593–602.
Khanim F, Kirk J, Latif F, Barrett TG. 2001. *WFS1/wolframin* mutations, Wolfram syndrome, and associated diseases. *Hum Mutat* 17(5):357–367.
Kim H, Hwang JS, Lee B, Hong J, Lee S. 2014. Newly identified cancer-associated role of human Neuronal Growth Regulator 1 (NEGR1). *J Cancer* 5:598–608.
Kimura Y, Katoh A, Kaneko T, Takahama K, Tanaka H. 2001. Two members of the IgLON family are expressed in a restricted region of the developing chick brain and neural crest. *Develop Growth Differ* 43:257–263.
King BM. 2006. The rise, fall, and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. *Physiol Behav* 87(2):221–244.
Koido K, Kõks S, Nikopensius T, Maron E, Altmäe S, Heinaste E et al. 2005. Polymorphisms in wolframin (WFS1) gene are possibly related to increased risk for mood disorders. *Int J Neuropsychopharmacol* 8(2):235–244.
Koido K, Traks T, Balõtšev R, Eller T, Must A, Kõks S et al. 2012. Associations between LSAMP gene polymorphisms and major depressive disorder and panic disorder. *Transl Psychiatry* 2:e152.
Koido K, Janno S, Traks T, Parksepp M, Ljubajev Ü, Veiksaar P et al. 2014. Associations between polymorphisms of LSAMP gene and schizophrenia. *Psychiatry Res* 215:797–798.
Koike H, Arguello PA, Kvajo M, Karayiorgou M, Gogos JA. 2006. Disc1 is mutated in the 129S6/SvEv strain and modulates working memory in mice. *Proc Natl Acad Sci U S A* 103(10):3693–3697.

Kõks S, Planken A, Luuk H, Vasar E. 2002. Cat odour exposure increases the expression of wolframin gene in the amygdaloid area of rat. *Neurosci Lett* 322(2):116–120.
Kõks S, Luuk H, Nelovkov A, Areda T, Vasar E. 2004. A screen for genes induced in the amygdaloid area during cat odor exposure. *Genes Brain Behav* 3(2):80–89.
Kõks S, Soomets U, Paya-Cano JL, Fernandes C, Luuk H, Plaas M et al. 2009. Wfs1 gene deletion causes growth retardation in mice and interferes with the growth hormone pathway. *Physiol Genomics* 37:249–259.
Koumenis C, Naczki C, Koritzinsky M, Rastani S, Diehl A, Sonenberg N et al. 2002. Regulation of protein synthesis by hypoxia via activation of the endoplasmic reticulum kinase PERK and phosphorylation of the translation initiation factor eIF2alpha. *Mol Cell Biol* 22(21):7405–7416.
Krebs J, Agellon LB, Michalak M. 2015. Ca(2+) homeostasis and endoplasmic reticulum (ER) stress: An integrated view of calcium signaling. *Biochem Biophys Res Commun* 460(1):114–121.
Kresse SH, Ohnstad HO, Paulsen EB, Bjerkehagen B, Szuhai K, Serra M et al. 2009. LSAMP, a novel candidate tumor suppressor gene in human osteosarcomas, identified by array comparative genomic hybridization. *Genes Chromosomes Cancer* 48(8):679–93.
Kuenzel WJ, Medina L, Csillag A, Perkel DJ, Reiner A. 2011. The avian subpallium: new insights into structural and functional subdivisions occupying the lateral subpallial wall and their embryological origins. *Brain Res* 1424:67–101.
Kühn MW, Radtke I, Bullinger L, Goorha S, Cheng J, Edelmann J et al. 2012. High-resolution genomic profiling of adult and pediatric core-binding factor acute myeloid leukemia reveals new recurrent genomic alterations. *Blood* 119(10):e67–75.
Kytövuori L, Seppänen A, Martikainen MH, Moilanen JS, Kamppari S, Särkioja T et al. 2013. WFS1 variants in Finnish patients with diabetes mellitus, sensorineural hearing impairment or optic atrophy, and in suicide victims. *J Hum Genet* 58(8): 495–500.
Laguesse S, Creppe C, Nedialkova DD, Prévot PP, Borgs L, Huysseune S. 2015. A Dynamic Unfolded Protein Response Contributes to the Control of Cortical Neurogenesis. *Dev Cell* 35(5):553–567.
Lajoie P and Snapp EL. 2011. Changes in BiP availability reveal hypersensitivity to acute endoplasmic reticulum stress in cells expressing mutant huntingtin. *J Cell Sci* 124(Pt 19):3332–3343.
Lam YW and Sherman SM. 2010. Functional organization of the somatosensory cortical layer 6 feedback to the thalamus. *Cereb Cortex* 20(1):13–24.
Lamprecht R, Dracheva S, Assoun S, LeDoux JE. 2009. Fear conditioning induces distinct patterns of gene expression in lateral amygdala. *Genes Brain Behav* 8(8):735–743.
LeDoux JE, Sakaguchi A, Reis DJ. 1984. Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. *J Neurosci* 4(3):683–698.
Lee AH, Chu GC, Iwakoshi NN, Glimcher LH. 2005. XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. *EMBO J* 24(24):4368–4380.
Leuner B and Gould E. 2010. Structural plasticity and hippocampal function. *Annu Rev Psychol* 61:111–140, C1–3.
Levitt P. 1984. A monoclonal antibody to limbic system neurons. *Science* 223(4633): 299–301.

Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Paré D. 2008. Amygdala intercalated neurons are required for expression of fear extinction. *Nature* 454:642–645.
Lin CW, Miller TR, Witte DG, Bianchi BR, Stashko M, Manelli AM et al. 1995. Characterization of cloned human dopamine D1 receptor-mediated calcium release in 293 cells. *Mol Pharmacol* 47(1):131–139.
Lin D, Boyle MP, Dollar P, Lee H, Lein ES, Perona P et al. 2011. Functional identification of an aggression locus in the mouse hypothalamus. *Nature* 470(7333):221–226.
Liu YF, Civelli O, Zhou QY, Albert PR. 1992. Cholera toxin-sensitive 3',5'-cyclic adenosine monophosphate and calcium signals of the human dopamine-D1 receptor: selective potentiation by protein kinase A. *Mol Endocrinol* 6(11):1815–1824.
Liu F, Arias-Vasquez A, Sleegers K, Aulchenko YS, Kayser M, Sanchez-Juan P et al. 2007. A genomewide screen for late-onset Alzheimer disease in a genetically isolated Dutch population. *Am J Hum Genet* 81:17–31.
Lodge AP, Howard MR, McNamee CJ, Moss DJ. 2000. Co-localisation, heterophilic interactions and regulated expression of IgLON family proteins in the chick nervous system. *Brain Res Mol Brain Res* 82:84–94.
Löw K, Crestani F, Keist R, Benke D, Brünig I, Benson JA et al. 2000. Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* 290(5489):131–134.
Lowther C, Costain G, Melvin R, Stavropoulos DJ, Lionel AC, Marshall CR et al. Adult expression of a 3q13.31 microdeletion. *Mol Cytogenet* 7(1):23.
Lu S, Kanekura K, Hara T, Mahadevan J, Spears LD, Oslowski CM et al. 2014. A calcium-dependent protease as a potential therapeutic target for Wolfram syndrome. *Proc Natl Acad Sci U S A* 111(49):E5292–5301.
Lugar HM, Koller JM, Rutlin J, Marshall BA, Kanekura K, Urano F. 2016. Neuroimaging evidence of deficient axon myelination in Wolfram syndrome. *Sci Rep* 6: 21167.
Lutz PL and Milton SL. 2004. Negotiating brain anoxia survival in the turtle. *J Exp Biol* 207(Pt 18):3141–3147.
Luuk H, Kõks S, Plaas M, Hannibal J, Rehfeld JF, Vasar E. 2008. Distribution of Wfs1 protein in the central nervous system of the mouse and its relation to clinical symptoms of the Wolfram syndrome. *J Comp Neurol* 509:642–660.
Luuk H, Plaas M, Raud S, Innos J, Sütt S, Lasner H et al. 2009. Wfs1-deficient mice display impaired behavioural adaptation in stressful environment. *Behav Brain Res* 198:334–345.
Maccarrone G, Ditzen C, Yassouridis A, Rewerts C, Uhr M, Uhlen M et al. 2013. Psychiatric patient stratification using biosignatures based on cerebrospinal fluid protein expression clusters. *J Psychiatr Res* 47:1572–1580.
Magri C, Sacchetti E, Traversa M, Valsecchi P, Gardella R, Bonvicini C, et al. 2010. New copy number variations in schizophrenia. *PLoS One* 5:e13422.
Mann F, Zhukareva V, Pimenta A, Levitt P, Bolz J. 1998. Membrane-associated molecules guide limbic and nonlimbic thalamocortical projections. *J Neurosci* 18: 9409–9419.
Manns JR and Eichenbaum H. The Evolution of the Hippocampus In: Kaas JH, editor. Evolutionary Neuroscience. Academic Press; 2009. pp. 603–627.
Marchand A, Faugère A, Coutureau E, Wolff M. 2014. A role for anterior thalamic nuclei in contextual fear memory. *Brain Struct Funct* 219(5):1575–1586.

Maren S, Aharonov G, Stote DL, Fanselow MS. 1996. N-methyl-D-aspartate receptors in the basolateral amygdala are required for both acquisition and expression of conditional fear in rats. *Behav Neurosci* 110:1365–1374.

Marin O, Anderson SA, Rubenstein JL. 2000. Origin and molecular specification of striatal interneurons. *J Neurosci* 20(16):6063–6076.

Martinez RC, Carvalho-Netto EF, Ribeiro-Barbosa ER, Baldo MV, Canteras NS. 2011. Amygdalar roles during exposure to a live predator and to a predator-associated context. *Neuroscience* 172:314–328.

Martínez-García F, Novejarque A, Lanuza E. The evolution of the amygdala in vertebrates. In: Kaas JH, editor. Evolutionary Neuroscience. Academic Press; 2009. pp. 313–392.

Masaki T, Yoshida M, Noguchi S. 1999. Targeted disruption of CRE-binding factor TREB5 gene leads to cellular necrosis in cardiac myocytes at the embryonic stage. *Biochem Biophys Res Commun* 261:350–356.

Matsumoto I. 2013. Gustatory Neural Pathways Revealed by Genetic Tracing from Taste Receptor Cells. *Biosci Biotechnol Biochem* 77(7):1359–1362.

Matsumoto M and Hikosaka O. 2007. Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* 447:1111–1115.

Matto V, Terasmaa A, Vasar E, Kõks S. 2011. Impaired striatal dopamine output of homozygous Wfs1 mutant mice in response to [K+] challenge. *J Physiol Biochem* 67:53–60.

McKie AB, Vaughan S, Zanini E, Okon IS, Louis L, de Sousa C et al. 2012. The OPCML tumor suppressor functions as a cell surface repressor–adaptor, negatively regulating receptor tyrosine kinases in epithelial ovarian cancer. *Cancer Discov* 2: 156–171.

McLaughlin I, Dani JA, De Biasi M. 2017. The medial habenula and interpeduncular nucleus circuitry is critical in addiction, anxiety, and mood regulation. *J Neurochem* 142 Suppl 2:130–143.

Medlej R, Wasson J, Baz P, Azar S, Salti I, Loiselet J et al. 2004. Diabetes mellitus and optic atrophy: a study of Wolfram syndrome in the Lebanese population. *J Clin Endocr Metab* 89:1656–1661.

Mezey S and Csillag A. 2002. Selective striatal connections of midbrain dopaminergic nuclei in the chick (*Gallus domesticus*). *Cell Tissue Res* 308:35–46.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. 1998. Dopamine receptors: from structure to function. *Physiol Rev* 78(1):189–225.

Miyata S., Taguchi K., Maekawa S. (2003). Dendrite-associated opioid-binding cell adhesion molecule localizes at neurosecretory granules in the hypothalamic magno-cellular neurons. Neuroscience 122 169–181.

Möhler H. Pathophysiological aspects of diversity in neuronal inhibition: a new benzodiazepine pharmacology. Dialogues Clin Neurosci. 2002;4(3):261–269.

a) Molnár Z, Adams R, Blakemore C. 1998. Mechanisms underlying the early establishment of thalamocortical connections in the rat. *J Neurosci* 18:5723–5745.

b) Molnár Z, Adams R, Goffinet AM, Blakemore C. 1998. The role of the first postmitotic cortical cells in the development of thalamocortical innervation in the reeler mouse. *J Neurosci* 18:5746–5765.

Molnár Z and Cordery P. 1999. Connections between cells of the internal capsule, thalamus, and cerebral cortex in embryonic rat. *J Comp Neurol* 413(1):1–25.

Montiel JF and Molnár Z. 2013. The impact of gene expression analysis on evolving views of avian brain organization. *J Comp Neurol* 521:3604–3613.

Montiel JF, Wang WZ, Oeschger FM, Hoerder-Suabedissen A, Tung WL, García-Moreno F et al. 2011. Hypothesis on the dual origin of the Mammalian subplate. *Front Neuroanat* 5:25.

Montoya ER, Terburg D, Bos PA, van Honk J. 2012. Testosterone, cortisol, and serotonin as key regulators of social aggression: A review and theoretical perspective. *Motiv Emot* 36(1):65–73.

Moreno N, Morona R, López JM, González A. 2010. Subdivisions of the turtle Pseudemys scripta subpallium based on the expression of regulatory genes and neuronal markers. *J Comp Neurol* 518(24):4877–4902.

Morgane PJ, Galler JR, Mokler DJ. 2005. A review of systems and networks of the limbic forebrain/limbic midbrain. *Prog Neurobiol* 75(2):143–160.

Moser MB and Moser EI. 1998. Functional differentiation in the hippocampus. *Hippocampus* 8(6):608–619.

Must A, Kõks S, Vasar E, Tasa G, Lang A, Maron E et al. 2009. Common variations in 4p locus are related to male completed suicide. *Neuromol Med* 11:13–19.

Nayak G, Prentice HM, Milton SL. 2016. Lessons from Nature: signaling cascades associated with vertebrate brain anoxic survival. *Exp Physiol* 101:1185–1190.

Nelovkov A, Areda T, Innos J, Kõks S, Vasar E. 2006. Rats displaying distinct exploratory activity also have different expression patterns of gamma-aminobutyric acid- and cholecystokinin-related genes in brain regions. *Brain Res* 1100(1):21–31.

Newman NJ. 2005. Hereditary optic neuropathies: from the mitochondria to the optic nerve. *Am J Ophthalmol* 140:517–523.

Ntougkos E, Rush R, Scott D, Frankenberg T, Gabra H, Smyth JF et al. 2005. The IgLON family in epithelial ovarian cancer: expression profiles and clinicopathologic correlates. *Clin Cancer Res* 11:5764–5768.

Nunokawa A, Watanabe Y, Kaneko N, Sugai T, Yazaki S, Arinami T et al. 2010. The dopamine D3 receptor (DRD3) gene and risk of schizophrenia: case-control studies and an updated meta-analysis. *Schizophr Res* 116(1):61–67.

O'Donovan MC, Craddock N, Norton N, Williams H, Peirce T, Moskvina V et al. 2008. Identification of loci associated with schizophrenia by genome-wide association and follow-up. *Nat Genet* 40:1053–1055.

Odisho T, Zhang L, Volchuk A. 2015. ATF6β regulates the Wfs1 gene and has a cell survival role in the ER stress response in pancreatic β-cells. *Exp Cell Res* 330(1):111–122.

Ogdie MN, Macphie IL, Minassian SL, Yang M, Fisher SE, Francks C et al. 2003. A genomewide scan for attention-deficit/hyperactivity disorder in an extended sample: suggestive linkage on 17p11. *Am J Hum Genet* 72:1268–1279.

Olsson M, Björklund A, Campbell K. 1998. Early specification of striatal projection neurons and interneuronal subtypes in the lateral and medial ganglionic eminence. *Neuroscience* 84(3):867–876.

Osman AA, Saito M, Makepeace C, Permutt MA, Schlesinger P, Mueckler M. 2003. Wolframin expression induces novel ion channel activity in endoplasmic reticulum membranes and increases intracellular calcium. *J Biol Chem* 278:52755–52762.

Pakdemirli E, Karabulut N, Bir LS, Sermez Y. 2005. Cranial magnetic resonance imaging of Wolfram (DIDMOAD) syndrome. *Australas Radiol* 49(2):189–191.

Pan Y, Wang KS, Aragam N. 2011. NTM and NR3C2 polymorphisms influencing intelligence: family-based association studies. Prog. Neuropsychopharmacol. *Biol Psychiatry* 35:154–160.

Panichareon B, Nakayama K, Thurakitwannakarn W, Iwamoto S, Sukhumsirichart W. 2012. OPCML gene as a schizophrenia susceptibility locus in Thai population. *J Mol Neurosci* 46:373–377.

Papez JW. 1937. A proposed mechanism for emotion. *Arch Neurol Psychiatry* 38:725–743.

Paré D, Royer S, Smith Y, Lang EJ. 2003. Contextual inhibitory gating of impulse traffic in the intra-amygdaloid network. *Ann N Y Acad Sci* 985:78–91.

Pasic I, Shlien A, Durbin AD, Stavropoulos DJ, Baskin B, Ray PN et al. 2010. Recurrent focal copy-number changes and loss of heterozygosity implicate two noncoding RNAs and one tumor suppressor gene at chromosome 3q13.31 in osteosarcoma. *Cancer Res* 70(1):160–171.

Pereira RC, Delany AM, Canalis E. 2004. CCAAT/enhancer binding protein homologous protein (DDIT3) induces osteoblastic cell differentiation. *Endocrinology* 145(4):1952–1960.

Pereira ER, Frudd K, Awad W, Hendershot LM. 2014. Endoplasmic reticulum (ER) stress and hypoxia response pathways interact to potentiate hypoxia-inducible factor 1 (HIF-1) transcriptional activity on targets like vascular endothelial growth factor (VEGF). *J Biol Chem* 289(6):3352–3364.

Petrovich GD, Risold PY, Swanson LW. 1996. Organization of projections from the basomedial nucleus of the amygdala: a PHAL study in the rat. *J Comp Neurol* 374(3):387–420.

Petrovics G, Li H, Stümpel T, Tan SH, Young D, Katta S et al. 2015. A novel genomic alteration of LSAMP associates with aggressive prostate cancer in African American men. *EBioMedicine* 2(12):1957–1964.

Peyron C, Tighe DK, van den Pol AN, de Lecea L, Heller HC, Sutcliffe JG et al. 1998. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 18(23):9996–10015.

Philbrook C, Fritz E, Weiher H. 2005. Expressional and functional studies of Wolframin, the gene function deficient in Wolfram syndrome, in mice and patient cells. *Exp Gerontol* 40:671–678.

Philips MA, Abramov U, Lilleväli K, Luuk H, Kurrikoff K, Raud S et al. 2010. Myg1-deficient mice display alterations in stress-induced responses and reduction of sex-dependent behavioural differences. *Behav Brain Res* 207(1):182–195.

Pimenta AF, Zhukareva V, Barbe MF, Reinoso BS, Grimley C, Henzel W et al. 1995. The limbic system-associated membrane protein is an Ig superfamily member that mediates selective neuronal growth and axon targeting. *Neuron* 15:287–297.

a) Pimenta AF, Fischer I, Levitt P. 1996. cDNA cloning and structural analysis of the human limbic-system-associated membrane protein (LAMP). *Gene* 170:189–195.

b) Pimenta AF, Reinoso BS, Levitt P. 1996. Expression of the mRNAs encoding the limbic system-associated membrane protein (LAMP). II. Fetal rat brain. *J Comp Neurol* 375:289–302.

Pimenta AF and Levitt P. 2004. Characterization of the genomic structure of the mouse limbic system-associated membrane protein (Lsamp) gene. *Genomics* 83(5):790–801.

Pischedda F and Piccoli G. 2016. The IgLON Family Member Negr1 Promotes Neuronal Arborization Acting as Soluble Factor via FGFR2. *Front Mol Neurosci* 8:89.

Plaas M, Seppa K, Reimets R, Jagomäe T, Toots M, Koppel T et al. 2017. Wfs1-deficient rats develop primary symptoms of Wolfram syndrome: insulin-dependent diabetes, optic nerve atrophy and medullary degeneration. *Sci Rep* 7(1):10220.

Prensa L, Gimenez-Amaya JM, Parent A. 1999. Chemical heterogeneity of the striosomal compartment in the human striatum. *J Comp Neurol* 413:603–618.
Prensa L, Richard S, Parent A. 2003. Chemical anatomy of the human ventral striatum and adjacent basal forebrain structures. *J Comp Neurol* 460:345–367.
Prentice HM, Milton SL, Scheurle D, Lutz PL. 2004. The upregulation of cognate and inducible heat shock proteins in the anoxic turtle brain. *J Cereb Blood Flow Metab* 24(7):826–828.
Price DJ, Aslam S, Tasker L, Gillies K. 1997. Fates of the earliest generated cells in the developing murine neocortex. *J Comp Neurol* 377(3):414–422.
Pro-Sistiaga P, Mohedano-Moriano A, Ubeda-Bañon I, Del Mar Arroyo-Jimenez M, Marcos P, Artacho-Pérula E et al. 2007. Convergence of olfactory and vomeronasal projections in the rat basal telencephalon. *J Comp Neurol* 504:346–362.
Puelles L, Kuwana E, Puelles E, Bulfone A, Shimamura K, Keleher J et al. 2000. Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes Dlx-2, Emx-1, Nkx-2.1, Pax-6, and Tbr-1. *J Comp Neurol* 424(3):409–438.
Puelles L, Martínez-de-la-Torre M, Paxinos G, Watson CH, Martínez S. The Chick Brain in Stereotaxic Coordinates. An Atlas Featuring Neuromeric Subdivisions and Mammalian Homologues, 1st ed. San Diego: Academic Press, Elsevier; 2007.
Puelles L, Ayad A, Alonso A, Sandoval JE, MartÍnez-de-la-Torre M, Medina L et al. 2016. Selective early expression of the orphan nuclear receptor Nr4a2 identifies the claustrum homolog in the avian mesopallium: Impact on sauropsidian/mammalian pallium comparisons. *J Comp Neurol* 524(3):665–703.
Qian X, Qin L, Xing G, Cao X. 2015. Phenotype Prediction of Pathogenic Nonsynonymous Single Nucleotide Polymorphisms in WFS1. *Sci Rep* 5:14731.
Qiu S, Champagne DL, Peters M, Catania EH, Weeber EJ, Levitt P et al. 2010. Loss of limbic system-associated membrane protein leads to reduced hippocampal mineralocorticoid receptor expression, impaired synaptic plasticity, and spatial memory deficit. *Biol Psychiatry* 68:197–204.
Rakic P. 1974. Neurons in rhesus monkey visual cortex: systematic relation between time of origin and eventual disposition. *Science* 183:425–427.
Rando TA, Horton JC, Layzer RB. 1992. Wolfram Syndrome - Evidence of A Diffuse Neurodegenerative Disease by Magnetic-Resonance-Imaging. *Neurology* 42:1220–1224.
Raud S, Sütt S, Luuk H, Plaas M, Innos J, Kõks S, Vasar E. 2009. Relation between increased anxiety and reduced expression of alpha1 and alpha2 subunits of GABA(A) receptors in Wfs1-deficient mice. *Neurosci Lett* 460:138–142.
Raud S, Reimets R, Loomets M, Sütt S, Altpere A, Visnapuu T et al. 2015. Deletion of the Wolfram syndrome-related gene Wfs1 results in increased sensitivity to ethanol in female mice. *Neuropharmacology* 95:59–67.
Reed J, McNamee C, Rackstraw S, Jenkins J, Moss D. 2004. Diglons are heterodimeric proteins composed of IgLON subunits, and diglon-CO inhibits neurite outgrowth from cerebellar granule cells. *J. Cell Sci* 117:3961–3973.
Reed JE, Dunn JR, du Plessis DG, Shaw EJ, Reeves P, Gee AL, et al. 2007. Expression of cellular adhesion molecule Opcml is down-regulated in gliomas and other brain tumours. *Neuropathol Appl Neurobiol* 33:77–85.
Reimets R, Raud S, Loomets M, Visnapuu T, Volke V, Reimets A et al. 2016. Variability in the effect of antidepressants upon Wfs1-deficient mice is dependent on the drugs' mechanism of actions. *Behav Brain Res* 308:53–63.

Reimold AM, Etkin A, Clauss I, Perkins A, Friend DS, Zhang J et al. 2000. An essential role in liver development for transcription factor XBP-1. *Genes Dev* 14:152–157.
Reimold AM, Iwakoshi NN, Manis J, Vallabhajosyula P, Szomolanyi-Tsuda E, Gravallese EM et al. 2001. Plasma cell differentiation requires the transcription factor XBP-1. *Nature* 412(6844):300–307.
Reiner A, Perkel DJ, Bruce L, Butler AB, Csillag A, Paxinos G et al. 2004. Revised nomenclature for avian telencephalon and some related brainstem nuclei. *J Comp Neurol* 473:377–414.
Reiner A, Yamamoto K, Karten HJ. 2005. Organization and evolution of the avian forebrain. *Anat Rec A Discov Mol Cell Evol Biol* 287(1):1080–1102.
Reinoso BS, Pimenta AF, Levitt P. 1996. Expression of the mRNAs encoding the limbic system-associated membrane protein (LAMP). I. Adult rat brain. *J Comp Neurol* 375:274–288.
Rice ME, Lee EJ, Choy Y. 1995. High levels of ascorbic acid, not glutathione, in the CNS of anoxia-tolerant reptiles contrasted with levels in anoxia-intolerant species. *J Neurochem* 64:1790–1799.
Riggs AC, Bernal-Mizrachi E, Ohsugi M, Wasson J, Fatrai S, Welling C et al. 2005. Mice conditionally lacking the Wolfram gene in pancreatic islet beta cells exhibit diabetes as a result of enhanced endoplasmic reticulum stress and apoptosis. *Diabetologia* 48(11):2313–2321.
Rigoli L, Lombardo F, Di Bella C. 2011. Wolfram syndrome and WFS1 gene. *Clin Genet* 79(2):103–117.
Ripke S, Neale BM, Corvin A, Walters JT, Farh KH, Holmans PA et al. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511:421–427.
Rohayem J., Ehlers C, Wiedemann B, Holl R, Oexle K, Kordonouri O et al. 2011. Diabetes and neurodegeneration in Wolfram syndrome: a multicenter study of phenotype and genotype. *Diabetes Care* 34:1503–1510.
Romero-Ramirez L, Cao H, Nelson D, Hammond E, Lee AH, Yoshida H et al. 2004. XBP1 is essential for survival under hypoxic conditions and is required for tumor growth. *Cancer Res* 64(17):5943–5947.
Ron D and Walter P. 2007. Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol* 8(7):519–529.
Roozendaal B, McEwen BS, Chattarji S. 2009. Stress, memory and the amygdala. *Nat Rev Neurosci* 10(6):423–433.
Rutkowski DT and Hegde RS. 2010. Regulation of basal cellular physiology by the homeostatic unfolded protein response. *J Cell Biol* 189(5):783–794.
Saab CY and Willis WD. 2003. The cerebellum: organization, functions and its role in nociception. *Brain Res Brain Res Rev* 42(1):85–95.
Sabater L, Gaig C, Gelpi E, Bataller L, Lewerenz J, Torres-Vega E et al. 2014. A novel non-rapid-eye movement and rapid-eye-movement parasomnia with sleep breathing disorder associated with antibodies to IgLON5: a case series, characterisation of the antigen, and post-mortem study. *Lancet Neurol* 13:575–586.
Saint-Dizier H, Constantin P, Davies DC, Leterrier C, Levy F, Richard S. 2009. Subdivisions of the arcopallium/posterior pallial amygdala complex are differentially involved in the control of fear behavior in the Japanese quail. *Brain Res Bull* 79:288–295.
Saito A, Ochiai K, Kondo S, Tsumagari K, Murakami T, et al. 2011. Endoplasmic reticulum stress response mediated by the PERK-eIF2α-ATF4 pathway is involved in osteoblast differentiation induced by BMP2. *J Biol Chem* 286:4809–4818.

Sandhu MS, Weedon MN, Fawcett KA, Wasson J, Debenham SL, Daly A et al. 2007. Common variants in WFS1 confer risk of type 2 diabetes. *Nat Genet* 39(8):951–953.
Sanz R, Ferraro GB, Fournier AE. 2015. IgLON Cell Adhesion Molecules Are Shed from the Cell Surface of Cortical Neurons to Promote Neuronal Growth. *J Biol Chem* 290(7):4330–4342.
Sanz RL, Ferraro GB, Girouard MP, Fournier AE. 2017. Ectodomain shedding of Limbic System-Associated Membrane Protein (LSAMP) by ADAM Metallopeptidases promotes neurite outgrowth in DRG neurons. *Sci Rep* 7(1):7961.
Scalia F and Winans SS. 1975. The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. *J Comp Neurol* 161:31–55.
Schaefer ML, Wong ST, Wozniak DF, Muglia LM, Liauw JA, Zhuo M et al. 2000. Altered stress-induced anxiety in adenylyl cyclase type VIII deficient mice. *J Neurosci* 20:4809–4820.
Schmidt ERE, Brignani S, Adolfs Y, Lemstra S, Demmers J, Vidaki M et al. 2014. Subdomain-mediated axon-axon signaling and chemoattraction cooperate to regulate afferent innervation of the lateral habenula. *Neuron* 83(2):372–387.
Schmidt-Kastner R, Kreczmanski P, Preising M, Diederen R, Schmitz C, Reis D et al. 2009. Expression of the diabetes risk gene wolframin (WFS1) in the human retina. *Exp Eye Res* 89(4):568–574.
Schofield PR, McFarland KC, Hayflick JS, Wilcox JN, Cho TM, Roy S. 1989. Molecular characterization of a new immunoglobulin superfamily protein with potential roles in opioid binding and cell contact. *EMBO J* 8(2):489–495.
Schol-Gelok S, Janssens AC, Tiemeier H, Liu F, Lopez-Leon S, Zorkoltseva IV et al. 2010. A genome-wide screen for depression in two independent Dutch populations. *Biol Psychiatry* 68:187–196.
Seifuddin F, Pirooznia M, Judy JT, Goes FS, Potash JB, Zandi PP. 2013. Systematic review of genome-wide gene expression studies of bipolar disorder. *BMC Psychiatry* 13:213.
Selemon LD, Rajkowska G, Goldman-Rakic PS. 1995. Abnormally high neuronal density in the schizophrenic cortex. A morphometric analysis of prefrontal area 9 and occipital area 17. *Arch Gen Psychiatry* 52:805–818, discussion 819–820.
Selemon LD, Rajkowska G, Goldman-Rakic PS. 1998. Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional, stereologic counting method. *J Comp Neurol* 392:402–412.
Selemon LD, Mrzljak J, Kleinman JE, Herman MM, Goldman-Rakic PS. 2003. Regional specificity in the neuropathologic substrates of schizophrenia: a morphometric analysis of Broca's area 44 and area 9. *Arch Gen Psychiatry* 60:69–77.
Sellar GC, Watt KP, Rabiasz GJ, Stronach EA, Li L, Miller EP et al. 2003. OPCML at 11q25 is epigenetically inactivated and has tumor suppressor function in epithelial ovarian cancer. *Nat Genet* 34:337–343.
Sequeira A, Kim C, Seguin M, Lesage A, Chawky N, Desautels A et al. 2003. Wolfram syndrome and suicide: evidence for a role of WFS1 in suicidal and impulsive behavior. *Am J Med Genet B Neuropsychiatr Genet* 119B:108–113.
Sewards TV and Sewards MA. 2002. The medial pain system: neural representations of the motivational aspect of pain. *Brain Res Bull* 59:163–180.
Shah A, Jhawar SS, Goel A. 2012. Analysis of the anatomy of the Papez circuit and adjoining limbic system by fiber dissection techniques. *J Clin Neurosci* 19(2):289–298.

Shang L, Hua H, Foo K, Martinez H, Watanabe K, Zimmer M et al. 2014. β-cell dysfunction due to increased ER stress in a stem cell model of Wolfram syndrome. *Diabetes* 63(3):923–933.

Shannon P, Becker L, Deck J. 1999. Evidence of widespread axonal pathology in Wolfram syndrome. *Acta Neuropathol* 98(3):304–308.

Sheehan TP, Chambers RA, Russell DS. 2004. Regulation of affect by the lateral septum: implications for neuropsychiatry. *Brain Res Rev* 46(1):71–117.

Shen X, Ruan X, Zhao H. 2012. Stimulation of midbrain dopaminergic structures modifies firing rates of rat lateral habenula neurons. *PLoS One* 7:e34323

Shen XX, Liang D, Wen JZ, Zhang P. 2011. Multiple genome alignments facilitate development of NPCL markers: a case study of tetrapod phylogeny focusing on the position of turtles. *Mol Biol Evol* 28(12):3237–3252.

Shibata H. 1993. Efferent projections from the anterior thalamic nuclei to the cingulate cortex in the rat. *J Comp Neurol* 330(4):533–542.

Shrestha P, Mousa A, Heintz N. 2015. Layer 2/3 pyramidal cells in the medial prefrontal cortex moderate stress induced depressive behaviors. *eLife* 4:e08752.

Sierra-Mercado D, Padilla-Coreano N, Quirk GJ. 2011. Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology* 36:529–538.

Silva BA, Mattucci C, Krzywkowski P, Murana E, Illarionova A, Grinevich V et al. 2013. Independent hypothalamic circuits for social and predator fear. *Nat Neurosci* 16(12):1731–1733.

Singh K, Loreth D, Pöttker B, Hefti K, Innos J, Schwald K et al. 2018. Neuronal Growth and Behavioral Alterations in Mice Deficient for the Psychiatric Disease-Associated Negr1 Gene. *Front Mol Neurosci* 11:30.

Sokolowski M, Wasserman J, Wasserman D. 2016. Polygenic associations of neurodevelopmental genes in suicide attempt. *Mol Psychiatry* 21:1381–1390.

Staiger JF, Zilles K, Freund TF. 1996. Distribution of GABAergic elements postsynaptic to ventroposteromedial thalamic projections in layer IV of rat barrel cortex. *Eur J Neurosci* 8:2273–2285.

Stamatakis AM, Jennings JH, Ung RL, Blair GA, Weinberg RJ, Neve RL et al. 2013. A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. *Neuron* 80:1039–1053.

Stoica R, De Vos KJ, Paillusson S, Mueller S, Sancho RM, Lau KF et al. 2014. ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nat Commun* 5:3996.

Stolzenberg DS and Numan M. 2011. Hypothalamic interaction with the mesolimbic DA system in the control of the maternal and sexual behaviors in rats. *Neurosci Biobehav Rev* 35(3):826–847.

Stoodley CJ and Schmahmann JD. 2010. Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. *Cortex* 46(7):831–44.

Strom TM, Hörtnagel K, Hofmann S, Gekeler F, Scharfe C, Rabl W, Gerbitz KD et al. 1998. Diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) caused by mutations in a novel gene (*wolframin*) coding for a predicted transmembrane protein. *Hum Mol Genet* 7(13):2021–2028.

Struyk AF, Canoll PD, Wolfgang MJ, Rosen CL, D'Eustachio P, Salzer JL. 1995. Cloning of neurotrimin defines a new subfamily of differentially expressed neural cell adhesion molecules. *J Neurosci* 15(3 Pt 2):2141–2156.

Stuber GD and Wise RA. 2016. Lateral Hypothalamic Circuits for Feeding and Reward. *Nature Neurosci* 19(2):198–205.

Sugimoto C, Maekawa S, Miyata S. 2010. OBCAM, an immunoglobulin superfamily cell adhesion molecule, regulates morphology and proliferation of cerebral astrocytes. *J Neurochem* 112(3):818–828.

Sugiyo S, Takemura M, Dubner R, Ren K. 2006. Demonstration of a trigeminothalamic pathway to the oval paracentral intralaminar thalamic nucleus and its involvement in the processing of noxious orofacial deep inputs. *Brain Res* 1097(1):116–122.

Supèr H and Soriano E. 1994. The organization of the embryonic and early postnatal murine hippocampus. II. Development of entorhinal, commissural, and septal connections studied with the lipophilic tracer DiI. *J Comp Neurol* 344(1):101–120.

Surmeier DJ, Bargas J, Hemmings HC Jr, Nairn AC, Greengard P. 1995. Modulation of calcium currents by a D1 dopaminergic protein kinase/phosphatase cascade in rat neostriatal neurons. *Neuron* 14(2):385–397.

Suzuki N, Hosoya M, Oishi N, Okano H, Fujioka M, Ogawa K. 2016. Expression pattern of wolframin, the WFS1 (Wolfram syndrome-1 gene) product, in common marmoset (Callithrix jacchus) cochlea. *Neuroreport* 27(11):833–836.

Swanson LW and Kuypers HG. 1980. The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labeling methods. *J Comp Neurol* 194(3):555–570.

Swapna I, Bondy B, Morikawa H. 2016. Differential Dopamine Regulation of Ca(2+) Signaling and Its Timing Dependence in the Nucleus Accumbens. *Cell Rep* 15(3): 563–573.

Swift RG, Perkins DO, Chase CL, Sadler DB, M. Swift M. 1991. Psychiatric disorders in 36 families with Wolfram syndrome. *Am J Psychiatry* 148:775–779.

Swift RG, Polymeropoulos, MH, Torres R, Swift M. 1998. Predisposition of Wolfram syndrome heterozygotes to psychiatric illness. *Mol Psychiatry* 3:86–91.

Swift M and Swift RG. 2005. Wolframin mutations and hospitalization for psychiatric illness. 2005. *Mol Psychiatry* 10:799–803.

Takeda K, Inoue H, Tanizawa Y, Matsuzaki Y, Oba J, Watanabe Y et al. 2001. WFS1 (Wolfram syndrome 1) gene product: predominant subcellular localization to endoplasmic reticulum in cultured cells and neuronal expression in rat brain. *Hum Mol Genet* 10(5):477–484.

Takei D, Ishihara H, Yamaguchi S, Yamada T, Tamura A, Katagiri H et al. 2006. WFS1 protein modulates the free Ca(2+) concentration in the endoplasmic reticulum. *FEBS Lett* 580(24):5635–5640.

Tassano E, Gamucci A, Celle ME, Ronchetto P, Cuoco C, Gimelli G. 2015. Clinical and molecular cytogenetic characterization of a de novo interstitial 1p31.1p31.3 deletion in a boy with moderate intellectual disability and severe language impairment. *Cytogenet Genome Res* 146:39–43.

Tein K, Kasvandik S, Kõks S, Vasar E, Terasmaa A. 2015. Prohormone convertase 2 activity is increased in the hippocampus of Wfs1 knockout mice. *Front Mol Neurosci* 8:45.

Torres R, Leroy E, Hu X, Katrivanou A, Gourzis P, Papachatzopoulou A et al. 2001. Mutation screening of the Wolfram syndrome gene in psychiatric patients. *Mol Psychiatry* 6(1):39–43.

Ueda K, Kawano J, Takeda K, Yujiri T, Tanabe K, Anno T et al. 2005. Endoplasmic reticulum stress induces Wfs1 gene expression in pancreatic beta-cells via transcriptional activation. *Eur J Endocrinol* 153(1):167–176.

Upadhyay A, Joshi V, Amanullah A, Mishra R, Arora N, Prasad A et al. 2017. E3 Ubiquitin Ligases Neurobiological Mechanisms: Development to Degeneration. *Front Mol Neurosci* 10:151.

Urano F. 2016. Wolfram Syndrome: Diagnosis, Management, and Treatment. *Curr Diab Rep* 16(1):6.

Uroz V, Prensa L, Giménez-Amaya JM. 2004. Chemical anatomy of the human paraventricular thalamic nucleus. *Synapse* 51(3):173–185.

Valjent E, Bertran-Gonzalez J, Herve D, Fisone G, Girault JA. 2009. Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci* 32:538–547.

van den Ouweland JM, Cryns K, Pennings RJ, Walraven I, Janssen GM, Maassen JA et al. 2003. Molecular characterization of WFS1 in patients with Wolfram syndrome. *J Mol Diagn* 5(2):88–95.

van Erp AM and Miczek KA. 2000. Aggressive behavior, increased accumbal dopamine, and decreased cortical serotonin in rats. *J Neurosci* 20(24):9320–9325.

Vanaveski T, Singh K, Narvik J, Eskla K-L, Visnapuu T, Heinla I et al. 2017. Promoter-Specific Expression and Genomic Structure of IgLON Family Genes in Mouse. *Front Neurosci* 11:38.

Vaz RP, Cardoso A, Sá SI, Pereira PA, Madeira MD. 2017. The integrity of the nucleus of the lateral olfactory tract is essential for the normal functioning of the olfactory system. *Brain Struct Funct* 222(8):3615–3637.

Veenman CL, Wild JM, Reiner A. 1995. Organization of the avian "corticostriatal" projection system: a retrograde and anterograde pathway tracing study in pigeons. *J Comp Neurol* 354:87–126.

Veenman CL, Medina L, Reiner A. 1997. Avian homologues of the mammalian intralaminar, mediodorsal and midline thalamic nuclei: Immunohistochemical and hodological evidence. *Brain Behav Evol* 49:78–98.

Veerappa AM, Saldanha M, Padakannaya P, Ramachandra NB. 2013. Family-based genome-wide copy number scan identifies five new genes of dyslexia involved in dendritic spinal plasticity. *J Hum Genet* 58:539–547.

Velásquez E, Nogueira FCS, Velásquez I, Schmitt A, Falkai P, Domont GB et al. 2017. Synaptosomal proteome of the orbitofrontal cortex from schizophrenia patients using quantitative label-free and iTRAQ-based shotgun proteomics. *J Proteome Res* 16:4481–4494.

Vicario A, Abellán A, Desfilis E, Medina L. 2014. Genetic identification of the central nucleus and other components of the central extended amygdala in chicken during development. *Front Neuroanat* 8:90.

a) Visnapuu T, Plaas M, Reimets R, Raud S, Terasmaa A, Kõks S, et al. 2013. Evidence for impaired function of dopaminergic system in Wfs1-deficient mice. *Behav Brain Res* 244:90–99.

b) Visnapuu T, Raud S, Loomets M, Reimets R, Sütt S, Luuk H et al. 2013. Wfs1-deficient mice display altered function of serotonergic system and increased behavioral response to antidepressants. *Front Neurosci* 7:132.

Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM. 2004. Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci* 27:468–474.
Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L. 2006. Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism. *Mol Psychiatry* 11(1):1:18–28.
Wang L, Hauser ER, Shah SH, Seo D, Sivashanmugam P, Exum ST et al. 2008. Polymorphisms of the tumor suppressor gene LSAMP are associated with left main coronary artery disease. *Ann Hum Genet* 72:443–453.
Wang KS, Liu XF, Aragam N. 2010. A genome-wide meta-analysis identifies novel loci associated with schizophrenia and bipolar disorder. *Schizophr Res* 124:192–199.
Wang H, Wang X, Ke ZJ, Comer AL, Xu M, Frank JA et al. 2015. Tunicamycin-induced unfolded protein response in the developing mouse brain. *Toxicol Appl Pharmacol* 283(3):157–167.
Wasson J and Permutt MA. 2008. Candidate gene studies reveal that the WFS1 gene joins the expanding list of novel type 2 diabetes genes. *Diabetologia* 51:391–393.
Wei F, Qiu CS, Kim SJ, Muglia L, Maas JW, Pineda VV et al. 2002. Genetic elimination of behavioral sensitization in mice lacking calmodulin-stimulated adenylyl cyclases. *Neuron* 36(4):713–726.
Wei P, Liu N, Zhang Z, Liu X, Tang Y, He X et al. 2015. Processing of visually evoked innate fear by a non-canonical thalamic pathway. *Nat Commun* 6:6756.
Wieczorek L, Maas JW Jr, Muglia LM, Vogt SK, Muglia LJ. 2010. Temporal and regional regulation of gene expression by calcium-stimulated adenylyl cyclase activity during fear memory. *PLoS One* 5(10):e13385.
Wiley SE, Andreyev AY, Divakaruni AS, Karisch R, Perkins G, Wall EA et al. 2013. Wolfram Syndrome protein, Miner1, regulates sulphydryl redox status, the unfolded protein response, and Ca2+ homeostasis. *EMBO Mol Med* 5(6):904–918.
Willer CJ, Speliotes EK, Loos RJF, Li S, Lindgren CM, Heid IM, et al. 2008. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat Genet* 41(1):25–34.
Willmore WG and Storey KB. 1997. Antioxidant systems and anoxia tolerance in a freshwater turtle Trachemys scripta elegans. *Mol Cell Biochem* 170:177–185.
Wilson AM and Kriegestein AR. 1991. Turtle cortical neurons survive glutamate exposures that are lethal to mammalian neurons. *Brain Res* 540:297–301.
Wise RA. 2004. Dopamine, learning and motivation. *Nat Rev Neurosci* 5(6):483–494.
Wolfram DJ and Wagener HP. 1938. Diabetes mellitus and simple optic atrophy among siblings: report of four cases. *Mayo Clin Proc* 13:715–718.
Wolterink G, Daenen LE, Dubbeldam S, Gerrits MA, van Rijn R, Kruse CG et al. 2001. Early amygdala damage in the rat as a model for neurodevelopmental psychopathological disorders. *Eur Neuropsychopharmacol* 11(1):51–59.
Wong ST, Athos J, Figueroa XA, Pineda VV, Schaefer ML, Chavkin CC et al. 1999. Calcium-stimulated adenylyl cyclase activity is critical for hippocampus-dependent long-term memory and late phase LTP. *Neuron* 23(4):787–798.
Wouters BG and Koritzinsky M. 2008. Hypoxia signalling through mTOR and the unfolded protein response in cancer. *Nat Rev Cancer* 8(11):851–864.
Wright NF, Erichsen JT, Vann SD, O'Mara SM, Aggleton JP. 2010. Parallel but separate inputs from limbic cortices to the mammillary bodies and anterior thalamic nuclei in the rat. *J Comp Neurol* 518(12):2334–2354.

Yamada T, Ishihara H, Tamura A, Takahashi R, Yamaguchi S, Takei D et al. 2006. WFS1-deficiency increases endoplasmic reticulum stress, impairs cell cycle progression and triggers the apoptotic pathway specifically in pancreatic beta-cells. *Hum Mol Genet* 15(10):1600–1609.

Yamaguchi S, Ishihara H, Tamura A, Yamada T, Takahashi R, Takei D et al. 2004. Endoplasmic reticulum stress and N-glycosylation modulate expression of WFS1 protein. *Biochem Biophys Res Commun* 325(1):250–256.

Yamaguchi T, Danjo T, Pastan I, Hikida T, Nakanishi S. 2013. Distinct roles of segregated transmission of the septo-habenular pathway in anxiety and fear. *Neuron* 78: 537–544.

Yamamoto K and Reiner A. 2005. Distribution of the limbic system-associated membrane protein (LAMP) in pigeon forebrain and midbrain. *J Comp Neurol* 486(3):221–242.

Yamamoto K, Sun Z, Wang HB, Reiner A. 2005. Subpallial amygdala and nucleus taeniae in birds resemble extended amygdala and medial amygdala in mammals in their expression of markers of regional identity. *Brain Res Bull* 66:341–347.

Yamamoto H, Hofmann S, Hamasaki DI, Yamamoto H, Kreczmanski P, Schmitz C et al. 2006. Wolfram syndrome 1 (WFS1) protein expression in retinal ganglion cells and optic nerve glia of the cynomolgus monkey. *Exp Eye Res* 83:1303–1306.

Yang T, Yang CF, Chizari MD, Maheswaranathan N, Burke KJ Jr, Borius M et al. 2017. Social Control of Hypothalamus-Mediated Male Aggression. *Neuron* 95(4): 955–970.e4.

Ye T, Lipska BK, Tao R, Hyde TM, Wang L, Li C et al. 2012. Analysis of copy number variations in brain DNA from patients with schizophrenia and other psychiatric disorders. *Biol Psychiatry* 72:651–654.

Yen CC, Chen WM, Chen TH, Chen WY, Chen PC, Chiou HJ et al. 2009. Identification of chromosomal aberrations associated with disease progression and a novel 3q13.31 deletion involving LSAMP gene in osteosarcoma. *Int J Oncol* 35(4):775–788.

Young TL, Ives E, Lynch E, Person R, Snook S, MacLaren L et al. 2001. Non-syndromic progressive hearing loss DFNA38 is caused by heterozygous missense mutation in the Wolfram syndrome gene WFS1. *Hum Mol Genet* 10(22):2509–2514.

Yu G, Yu ML, Wang JF, Gao CR, Chen ZJ. 2010. WS1 gene mutation analysis of Wolfram syndrome in a Chinese patient and a systematic review of literatures. *Endocrine* 38(2):147–152.

Yu B, Qian T, Wang Y, Zhou S, Ding G, Ding F et al. 2012. miR-182 inhibits Schwann cell proliferation and migration by targeting FGF9 and NTM, respectively at an early stage following sciatic nerve injury. *Nucleic Acids Res* 40:10356–10365.

Yurimoto S, Hatano N, Tsuchiya M, Kato K, Fujimoto T, Masaki T et al. 2009. Identification and characterization of wolframin, the product of the wolfram syndrome gene (WFS1), as a novel calmodulin-binding protein. *Biochemistry* 48:3946–3955.

Zacco A, Cooper V, Chantler PD, Fisher-Hyland S, Horton HL, Levitt P. 1990. Isolation, biochemical characterization and ultrastructural analysis of the limbic system associated membrane protein (LAMP), a protein expressed by neurons comprising functional neural circuits. *J Neurosci* 10:73–90.

Zalsman G, Mann MJ, Huang YY, Oquendo MA, Brent DA, Burke AK et al. 2009. Wolframin gene H611R polymorphism: no direct association with suicidal behavior

but possible link to mood disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 33(4):707–710.

Zatyka M, Ricketts C, da Silva Xavier G, Minton J, Fenton S, Hofmann-Thiel S et al. 2008. Sodium-potassium ATPase 1 subunit is a molecular partner of Wolframin, an endoplasmic reticulum protein involved in ER stress. *Hum Mol Genet* 17(2):190–200.

Zatyka M, Da Silva Xavier G, Bellomo EA, Leadbeater W, Astuti D, Smith J et al. 2015. Sarco(endo)plasmic reticulum ATPase is a molecular partner of Wolfram syndrome 1 protein, which negatively regulates its expression. *Hum Mol Genet* 24(3):814–827.

Zhang K, Wong HN, Song B, Miller CN, Scheuner D, Kaufman RJ. 2005. The unfolded protein response sensor IRE1α is required at 2 distinct steps in B cell lymphopoiesis. *J Clin Invest* 115:268–281.

Zhang X, Szabo E, Michalak M, Opas M. 2007. Endoplasmic reticulum stress during the embryonic development of the central nervous system in the mouse. *Int J Dev Neurosci* 25(7):455–463.

Zhao L and Ackerman SL. 2006. Endoplasmic reticulum stress in health and disease. *Curr Opin Cell Biol* 18:444–452.

Zhukareva V and Levitt P. 1995. The limbic system-associated membrane protein (LAMP) selectively mediates interactions with specific central neuron populations. *Development* 121:1161–1172.

SUMMARY IN ESTONIAN

Arenguline lähenemine emotsionaalse käitumisega seotud geenide *Wfs1* ja *Lsamp*'i funktsiooni uurimisel

Psühhiaatriliste haiguste algpõhjused peituvad sageli aju arengus. Arenev aju on väga plastiline ning vastuvõtlik välistele mõjuteguritele. Inimesel kestab aju areng eriliselt kaua: veel pärast sündi jätkub intensiivne neurogenees, millega kaasneb massiline sünapside teke ja samal ajal kasutusest välja jäävate sünapside kustutamine. Sünaptilise võrgustiku ülesehitamine kestab kuni varase täiskasvanueani ning palju väiksemas skaalas jätkub selle ümberkorraldamine ka pärast täiskasvanuks saamist. Seetõttu on inimaju areng seda mõjutavatele teguritele tundlik väga pika aja kestel. Tundmuste, mõtlemise ja mäluga seotud ajupiirkondade areng sõltub keskkonnast pärinevast sisendist ning nende funktsiooni peenhäälestamine võtab seetõttu eriti kaua aega. Keskkonnast ajusse jõudvad signaalid mõjutavad paljude aju funktsioneerimises oluliste geenide transkriptsioonilise aktiivsuse paikasättimist ning sünaptiliste ühenduste kujunemist. Kuna need protsessid on keerulised ja haavatavad, esineb ka palju haigusi, mis on seotud aju emotsionaalse arengu häiretega, nagu depression, bipolaarne häire, ärevus- ja sõltuvushäired, skisofreenia.

Käesolevas doktoritöös on kasutatud hiire mudeleid, et uurida emotsionaalse käitumise regulatsioonis oluliste geenide *Wfs1* ja *Lsamp*`i rolli aju arengus. Siin käsitletud uuringute kaugemad tagamaad ulatuvad 2000-ndate aastate alguses Tartu Ülikooli Füsioloogia Instituudis tehtud katseteni, kus lasti rottidel nuusutada kassilõhnaga riidelappi ning selle järel mõõdeti geenide ekspressioonitaset amügdalas, mis on hirmukäitumise eest vastutav aju osa. *Wfs1* ja *Lsamp* olid nende geenide hulgas, mille puhul täheldati ekspressioonitaseme tõusu pärast kassilõhnaga riidelapi nuusutamist.

Wfs1 e *Wolframi sündroom 1* on seotud sündroomiga, mille patsientidel esinevad *diabetes insipidus*, *diabetes mellitus*, sensorineuraalne kurtus ja nägemisnärvi kärbumine. Sageli kaasnevad Wolframi sündroomiga psühhiaatrilised häired ning ühe mittefunktsionaalse *Wfs1* alleeli kandjatel on kõrgendatud risk vaimuhaigusteks. *Lsamp* (*Limbic system associated membrane protein* e limbilise süsteemiga seotud membraanvalku kodeeriv geen) geeni poolt kodeeritava valgu funktsioon on seotud emotsionaalses käitumises oluliste ajuosade vaheliste ühenduste ülesehitamisega. *Lsamp* osaleb neuriitide väljakasvu, aksonite sihtmärgini jõudmise ja sünaptogeneesi regulatsioonis. *Wfs1* funktsiooni on rohkem uuritud pankreases ning selle roll närvisüsteemi arengus vajab veel selgitamist. Wolframi sündroomi patsientidel on täheldatud teatud aju osade puudulikku arengut ja kõrvalekaldeid aju müeliniseerumises juba väga noores eas. Nii *Wfs1* kui ka *Lsamp* geeni variandid on seotud meeleolu- ja ärevushäirete, skisofreenia ja suitsidaalsusega.

Kuna aju areng mängib psüühikahäirete kujunemisel väga suurt rolli, on käesoleva töö põhieesmärk selgitada, millistes aju arengu etappides on *Wfs1* ja

Lsamp`i avaldumine vajalik ning mis funktsioone need geenid arenevas ja täiskasvanud ajus täidavad.

Kõigepealt oli eesmärgiks selgitada, millises funktsionaalse küpsemise faasis hakatakse erinevates aju piirkondades *Wfs1* ekspresseerima. Kuna üks Wfs1 valgu funktsioon on endoplasmaatilise retiikulumi stressi (ER stressi) vastuse regulatsioon, võtsime sihiks selgitada, kas *Wfs1* mängib rolli arengu käigus tekkiva ER stressi vastuse kontrollis. Kasutades RNA *in situ* hübridisatsiooni metoodikat, näitasime, et *Wfs1* hakatakse hiire ajus ekspresseerima pärast sünaptilise aktiivsuse algust alates vanusest 15,5 embrüonaalset päeva (E15,5) ning *Wfs1* ekspressiooni algus järgib ajupiirkondade sünaptilist küpsemist. Esimesed ekspressioonipiirkonnad on dorsaalne striatum ja amügdala. *Wfs1* ekspressioon on kõige ulatuslikum vahemikus sünnist kuni teise postnataalse päevani (P2), hõlmates dorsaalse ja ventraalse striatumi, amügdala erinevad tuumad, areneva ajukoore ja hipokampuse ning vaheaju. Edasise arengu käigus toimub paljudes ajupiirkondades ekspressiooni vähenemine ja kadumine, samas kui üksikud struktuurid, nagu hipokampuse CA1 piirkond, ajukoore II/III kiht, olfaktoorne koor, kaudaalne dorsaalne striatum, tsentraalne amügdala ja ventraalne striatum, hakkavad tugevamini *Wfs1* ekspresseerima. Kasutades *in situ* hübridisatsiooni ja reaalaja kvantitatiivset polümeraasi ahelreaktsiooni (PCR-i), näitasime, et ER stressi markeerivate geenide *Grp78* ja *Grp94* tase on embrüonaalses ajus kõrge ja reguleeritakse alla ajaks, mil Wfs1 ekspressioon on kõige madalam. Nende geenide ekspressioonitaseme mõõtmine metsiktüüpi ja *Wfs1* geeni puudulikkusega hiirtes näitas, et Wfs1 ei osale nende ekspressiooni regulatsioonis sünnieelses ja varases sünnijärgses eas.

Arvestades, et varem on leitud seos *Wfs1* ja aju dopamiinergilise süsteemi toimimise vahel, otsustasime seda seost põhjalikumalt selgitada, uurides Wfs1 mõju D1-tüüpi dopamiini retseptorite funktsioneerimisele. Mõõtes radioaktiivselt märgistatud ligandi sidumist, leidsime, et *Wfs1* geeni puudulikkusega hiirtes on D1-tüüpi dopamiini retseptori sidumiskohti rohkem kui metsiktüüpi hiirtes. Et anda Wfs1 ja D1-tüüpi retseptorite seosele laiem evolutsiooniline mõõde, uurisime *Wfs1* ja levinuima D1-tüüpi retseptori *Drd1a* ekspressiooni võrdlevalt hiire, kodukana ja punakõrv-ilukilpkonna ajus, kasutades RNA *in situ* hübridisatsiooni ja immunohistokeemilist valgu tuvastamist. Nii kana kui kilpkonna ajus kattusid *Wfs1* ja *Drd1a* ekspressioonipiirkonnad omavahel peaaegu täielikult. Hiire ajus polnud D1-tüüpi retseptorite *Drd1a* ja *Drd5* ekspressioon *Wfs1* ekspressioonile mRNA tasemel väga sarnane, kuid valgu tasemel oli kattuvus märkimisväärne, eriti Drd1 ja Wfs1 vahel. Erinevates fülogeneesiharudes oli *Wfs1* ekspressioon konserveerunud aju basaalosa päritolu striataalsetes ja amügdala piirkondades ning varieerus aju mantliosa päritolu piirkondades.

Lsamp`i uurimuses oli eemärgiks kirjeldada selle geeni kahe alternatiivse promootori aktiivsuse mustrit arenevas ja täiskasvanud hiire ajus ning uurida nendelt promootoritelt sünteesitud alternatiivsete transkriptide rolli sotsiaalses ja ärevusega seotud käitumises. *Lsamp* geenil on kaks evolutsiooniliselt konserveerunud alternatiivset esimest eksonit koos eraldi promootoritega. Kuigi nendelt promootoritelt sünteesitud mRNAd on 5′-osas erinevad, on küpse valgu

järjestus sama, ning alternatiivsete traskriptide roll on selgusetu. Kasutades radioaktiivset ja mitteradioaktiivset RNA *in situ* hübridisatsiooni ning X-gal värvingut *Lsamp*-β-galaktosidaas *knockin* hiirtel, leidsime, et 1a promootor on aktiivne klassikalistes limbilistes struktuurides (hipokampus, amügdala, ajukoore ja hüpotalamuse limbilised piirkonnad, eesmine talamus). Samas piiritleb 1b promootori aktiivsus aju sensoorseid juhteteesid ning prevaleerib sensoorsetes ning motoorsetes ajukoore ja talamuse osades. Käitumiskatsetes ilmnes, et kõrgem 1a transkripti ekspressioonitase temporaalsagaras korreleerus suurema loomuomase ärevusega ning nii 1a kui 1b transkripti madalam tase hipokampuses oli korrelatsioonis kestvama teise hiire nuusutamisega ehk kõrgema sotsiaalsusega. Kõrgem 1a transkripti tase ventraalses striatumis oli korrelatsioonis teise hiire anogenitaalpiirkonna nuusutamisega, mis on hiirte seas kehtestav käitumine. Läbi viidud käitumiskatsed toetavad varasemaid uuringuid, mille käigus on leitud, et *Lsamp* geeni puudulikkusega hiirtel esineb vähem agressiivset käitumist ning nad on ükskõiksemad ärevust tekitavate olukordade suhtes.

Keskmine vanus, mil haigestutakse psühhiaatrilistesse haigustesse, nagu depressioon, bipolaarne häire, ärevushäired ja skisofreenia, langeb erinevate haiguste lõikes hilisesse lapsepõlve või varasesse täiskasvanuikka, mis viitab tugevale arengulisele komponendile nende haiguste olemuses. Geneetilise eelsoodumusega haiguse vallandumise võivad määrata traumaatilised emotsionaalsed läbielamised või stressirohked eluperioodid. Selle tõttu on oluline uurida, kuidas emotsionaalse käitumise regulatsiooniga seotud geenid nagu *Wfs1* ja *Lsamp* osalevad aju arengus ning kuidas nad interakteeruvad keskkonnast pärinevate ja organismi sisekeskkonna signaalidega. Käesoleva doktoritöö valmimise käigus saadud uued teadmised aitavad paremini mõista *Wfs1* ja *Lsamp*`i rolli erinevate ajupiirkondade arengus ja funktsioneerimises ning näitavad suunda edasisteks uuringuteks.

ACKNOWLEDGEMENTS

My biggest appreciation belongs to Kersti Lilleväli, who has been my most long-term supervisor, raising the scientist in me since the bachelor studies. During these years, Kersti has become like a mother to me in the word`s best sense. I am also very thankful to my other PhD supervisors Eero Vasar and Mari-Anne Philips. The atmosphere of academic freedom and flexibility in working time owing to Eero have been unutterably important for me. Mari-Anne has always been the most sensible person around and never hesitates to reach a hand for help. I am very thankful to my colleagues from the Department of Physiology and the Department of Developmental Biology. Thank you for your contribution to my scientific work and social life! I am also thankful to my other collaborators, especially Scott Gilbert and Anni Allikalt for always being enthusiastic and caring. Thanks to Egon for taking care of the cleanliness of the mind and body. Thank you, Katrin, Katarina, Triin K, Anne, Kairi, Mehis, Andro, Liina, Keiu, Tambet, Katyayani, Ita, Liisa and Maarja, for being supportive and and exemplary. Thanks to the hiking club "Rajaajaja", Tartu Students` Nature Protection Circle and the Estonian Fund for Nature for assembling great people to do awesome things together. Thanks to Aki, Eleriin and Paul for providing me a shelter while writing the thesis and for all other favours. Thanks to Mario for sharing the fascination about science and critically revising the language of this thesis. I am very grateful to my mum and dad who have been an inspiration for me and always supported my studies financially and morally. I would like to thank my grandma, for being a role model of healthy lifestyle, and Kadri and Brit for being forever curious about what I do with the mice in the lab. Also, I am very thankful to all the mice and other animals who had to leave their lives for the sake of science.

ORIGINAL PUBLICATIONS

CURRICULUM VITAE

Name:	Triin Tekko
Date of birth:	March 10, 1986
Address:	Department of Physiology Institute of Biomedicine and Translational Medicine University of Tartu Ravila 19, Tartu 50411, Estonia
Phone:	+372 737 4335
E-mail:	annelida@ut.ee

Education:

1993–2002	Tallinn Katleri Basic School
2002–2005	Tallinn French School
2005–2008	University of Tartu, Faculty of Biology and Geography, biology, B.Sc.
2008–2011	University of Tartu, Faculty of Science and Technology, master studies in molecular and cell biology, M.Sc.
2011–2018	University of Tartu, Faculty of Medicine, doctoral studies in neuroscience

Career:

2017	University of Tartu, Institute of Biomedicine and Translational Medicine, Department of Physiology, specialist

Professional self-improvement:

2007	Conference „European Evo-Devo Meeting“ (Ghent, Belgium)
2010	studying the organotypic slice culture method in the University of Helsinki, Institute of Biotechnology
2012	„Laboratory Animal Science C-Category Competence Course” (Estonian University of Life Sciences, University of Tartu)
2012	Theoretical and practical course „Embryology“ (Estonian University of Life Sciences)
2013	Symposion „Signaling in Morphogenesis and Patterning” (Helsinki, Finlad)
2015	Practical course „Developmental Neurobiology – From Worms to Mammals” (London, UK)

List of publications:

1. **Tekko T**, Lakspere T, Allikalt A, End J, Kõlvart KR, Jagomäe T, Terasmaa A, Philips MA, Visnapuu T, Väärtnõu F, Gilbert SF, Rinken A, Vasar E, Lilleväli K .2017. Wfs1 is expressed in dopaminoceptive regions of the

amniote brain and modulates levels of D1-like receptors. *PLoS One* 12(3):e0172825.

2. Kumar A, Kopra J, Varendi K, Porokuokka LL, Panhelainen A, Kuure S, Marshall P, Karalija N, Härma MA, Vilenius C, Lilleväli K, **Tekko T**, Mijatovic J, Pulkkinen N, Jakobson M, Jakobson M, Ola R, Palm E, Lindahl M, Strömberg I, Võikar V, Piepponen TP, Saarma M, Andressoo JO. 2015. GDNF Overexpression from the Native Locus Reveals its Role in the Nigrostriatal Dopaminergic System Function. *PLoS Genet* (12):e1005710. Erratum in: *PLoS Genet* 2016; 12(1):e1005808.

   Kumar A, Kopra J, Varendi K, Porokuokka LL, Panhelainen A, Kuure S, Marshall P, Karalija N, Härma MA, Vilenius C, Lilleväli K, **Tekko T**, Mijatovic J, Pulkkinen N, Jakobson M, Jakobson M, Ola R, Palm E, Lindahl M, Strömberg I, Võikar V, Piepponen TP, Saarma M, Andressoo JO. 2016. Correction: GDNF Overexpression from the Native Locus Reveals its Role in the Nigrostriatal Dopaminergic System Function. *PLoS Genet* 12(1):e1005808.

3. Heinla I, Leidmaa E, Kongi K, Pennert A, Innos J, Nurk K, **Tekko T**, Singh K, Vanaveski T, Reimets R, Mandel M, Lang A, Lilleväli K, Kaasik A, Vasar E, Philips MA. 2015. Gene expression patterns and environmental enrichment-induced effects in the hippocampi of mice suggest importance of Lsamp in plasticity. *Front Neurosci* 9:205.
4. Philips MA, Lilleväli K, Heinla I, Luuk H, Hundahl CA, Kongi K, Vanaveski T, **Tekko T**, Innos J, Vasar E. 2015. Lsamp is implicated in the regulation of emotional and social behavior by use of alternative promoters in the brain. *Brain Struct Funct* 220(3):1381–1393.
5. **Tekko T**, Lilleväli K, Luuk H, Sütt S, Truu L, Örd T, Möls M, Vasar E. 2014. Initiation and developmental dynamics of Wfs1 expression in the context of neural differentiation and ER stress in mouse forebrain. *Int J Dev Neurosci* 35:80–88.
6. Örd T, Innos J, Lilleväli K, **Tekko T**, Sütt S, Örd D, Kõks S, Vasar E, Örd T. 2014. Trib3 is developmentally and nutritionally regulated in the brain but is dispensable for spatial memory, fear conditioning and sensing of amino acid-imbalanced diet. *PLoS One* 9(4):e94691.

ELULOOKIRJELDUS

Nimi: Triin Tekko
Sünnikuupäev: 10.03.1986
Aadress: Füsioloogia osakond
Bio- ja Siirdemeditsiini Instituut
Tartu Ülikool
Ravila 19, Tartu 50411, Eesti
Telefon: +372 737 4335
E-mail: annelida@ut.ee

Haridustee:

1993–2002	Tallinna Katleri Põhikool
2002–2005	Tallinna Prantsuse Lütseum
2005–2008	Tartu Ülikool, Bioloogia- ja Geograafiateaduskond, lõpetatud bakalaureusekraadiga bioloogias
2008–2011	Tartu Ülikool, Loodus- ja Tehnoloogiateaduskond, lõpetatud magistrikraadiga molekulaar- ja rakubioloogias
2011–2018	Tartu Ülikool, Arstiteaduste valdkond, neuroteaduste doktorantuur

Teenistuskäik:

2017	Tartu Ülikool, Bio- ja Siirdemeditsiini Instituut, Füsioloogia osakond, spetsialist

Erialane enesetäiendus:

2007	osalemine European Evo-Devo miitingul Belgias Gentis
2010	organotüüpilise ajulõigukultuuri meetodi õppimine Helsingi Ülikooli Biotehnoloogia Instituudis
2012	osalemine Eesti Maaülikooli ja Tartu Ülikooli korraldatud kursusel „Laboratory Animal Science C-Category Competence Course”
2012	osalemine Eesti Maaülikooli korraldatud „Embrüoloogia teoreetilisel ja praktilisel kursusel“
2013	osalemine Helsingi Ülikooli organiseeritud sümpoosionil „Signaling in Morphogenesis and Patterning”
2015	osalemine EMBO kursusel „Developmental Neurobiology – From Worms to Mammals” Londonis

Publikatsioonid:

1. **Tekko T**, Lakspere T, Allikalt A, End J, Kõlvart KR, Jagomäe T, Terasmaa A, Philips MA, Visnapuu T, Väärtnõu F, Gilbert SF, Rinken A, Vasar E, Lilleväli K. 2017. Wfs1 is expressed in dopaminoceptive regions of the amniote brain and modulates levels of D1-like receptors. *PLoS One* 12(3): e0172825.

2. Kumar A, Kopra J, Varendi K, Porokuokka LL, Panhelainen A, Kuure S, Marshall P, Karalija N, Härma MA, Vilenius C, Lilleväli K, **Tekko T**, Mijatovic J, Pulkkinen N, Jakobson M, Jakobson M, Ola R, Palm E, Lindahl M, Strömberg I, Võikar V, Piepponen TP, Saarma M, Andressoo JO. 2015. GDNF Overexpression from the Native Locus Reveals its Role in the Nigrostriatal Dopaminergic System Function. *PLoS Genet* (12):e1005710. Erratum in: *PLoS Genet* 2016; 12(1):e1005808.

    Kumar A, Kopra J, Varendi K, Porokuokka LL, Panhelainen A, Kuure S, Marshall P, Karalija N, Härma MA, Vilenius C, Lilleväli K, **Tekko T**, Mijatovic J, Pulkkinen N, Jakobson M, Jakobson M, Ola R, Palm E, Lindahl M, Strömberg I, Võikar V, Piepponen TP, Saarma M, Andressoo JO. 2016. Correction: GDNF Overexpression from the Native Locus Reveals its Role in the Nigrostriatal Dopaminergic System Function. *PLoS Genet* 12(1):e1005808.

3. Heinla I, Leidmaa E, Kongi K, Pennert A, Innos J, Nurk K, **Tekko T**, Singh K, Vanaveski T, Reimets R, Mandel M, Lang A, Lilleväli K, Kaasik A, Vasar E, Philips MA. 2015. Gene expression patterns and environmental enrichment-induced effects in the hippocampi of mice suggest importance of Lsamp in plasticity. *Front Neurosci* 9:205.
4. Philips MA, Lilleväli K, Heinla I, Luuk H, Hundahl CA, Kongi K, Vanaveski T, **Tekko T**, Innos J, Vasar E. 2015. Lsamp is implicated in the regulation of emotional and social behavior by use of alternative promoters in the brain. *Brain Struct Funct* 220(3):1381-1393.
5. **Tekko T**, Lilleväli K, Luuk H, Sütt S, Truu L, Örd T, Möls M, Vasar E. 2014. Initiation and developmental dynamics of Wfs1 expression in the context of neural differentiation and ER stress in mouse forebrain. *Int J Dev Neurosci* 35:80–88.
6. Örd T, Innos J, Lilleväli K, **Tekko T**, Sütt S, Örd D, Kõks S, Vasar E, Örd T. 2014. Trib3 is developmentally and nutritionally regulated in the brain but is dispensable for spatial memory, fear conditioning and sensing of amino acid-imbalanced diet. *PLoS One* 9(4):e94691.

DISSERTATIONES NEUROSCIENTIAE UNIVERSITATIS TARTUENSIS

1. **Sirli Raud.** Cholecystokinin$_2$ receptor deficient mice: changes in function of GABA-ergic system. Tartu, 2005.
2. **Kati Koido.** Single-nucleotide polymorphism profiling of 22 candidate genes in mood and anxiety disorders. Tartu, 2005.
3. **Dzhamilja Safiulina.** The studies of mitochondria in cultured cerebellar granule neurons: characterization of mitochondrial function, volume homeostasis and interaction with neurosteroids. Tartu, 2006.
4. **Tarmo Areda.** Behavioural and neurogenetic study of mechanisms related to cat odour induced anxiety in rodents. Tartu, 2006.
5. **Aleksei Nelovkov.** Behavioural and neurogenetic study of molecular mechanisms involved in regulation of exploratory behaviour in rodents. Tartu, 2006.
6. **Annika Vaarmann**. The studies on cystatin B deficient mice: neurochemical and behavioural alterations in animal model of progressive myoclonus epilepsy of Unverricht-Lundborg type. Tartu, 2007.
7. **Urho Abramov.** Sex and environmental factors determine the behavioural phenotype of mice lacking CCK_2 receptors: implications for the behavioural studies in transgenic lines. Tartu, 2008.
8. **Hendrik Luuk.** Distribution and behavioral effects of WFS1 protein in the central nervous system. Tartu, 2009.
9. **Anne Must.** Studies on molecular genetics of male completed suicide in Estonian population. Tartu, 2009.
10. **Kaido Kurrikoff**. Involvement of cholecystokinin in chronic pain mechanisms and endogenous antinociception. Tartu, 2009.
11. **Anu Aonurm-Helm.** Depression-like phenotype and altered intracellular signalling in neural cell adhesion molecule (NCAM)-deficient mice. Tartu, 2010.
12. **Silva Sütt.** Role of endocannabinoid system and *Wfs1* in regulation of emotional behaviour: behavioural, pharmacological and genetic studies. Tartu, 2010.
13. **Mari-Anne Philips.** Characterization of *Myg1* gene and protein: expression patterns, subcellular localization, gene deficient mouse and functional polymorphisms in human. Tartu, 2010.
14. **Ranno Rätsep.** Genetics of psoriasis and vitiligo, focus on IL10 family cytokines. Tartu, 2010.
15. **Kairit Joost.** Selective screening of metabolic diseases in Estonia: the application of new diagnostic methods. Tartu, 2012, 143 p.
16. **Monika Jürgenson.** A complex phenotype in mice with partial or complete deficiency of the NCAM protein. Tartu, 2012, 117 p.

17. **Ene Reimann.** Description of the cytokines and cutaneous neuroendocrine system in the development of vitiligo. Tartu, 2012, 117 p.
18. **Jürgen Innos.** Behavioural, pharmacological and neurochemical characterisation of limbic system-associated membrane protein (LSAMP) deficient mice. Tartu, 2013, 113 p.
19. **Kaili Anier.** The role of DNA methylation in the development of cocaine-induced behavioural sensitisation. Tartu, 2013, 147 p.
20. **Maarika Liik.** Cognitive functioning, perceived cognition, subjective complaints and symptoms of depression in patients with epilepsy: neuropsychological assessment and spet brain imaging study. Tartu, 2014, 124 p.
21. **Sten Ilmjärv.** Estimating differential expression from multiple indicators. Tartu, 2015, 125 p.
22. **Paula Reemann.** The effects of microenvironment on skin cells. Tartu, 2015, 146 p.
23. **Tanel Visnapuu.** Pharmacological and behavioral characterization of the monoaminergic and GABA-ergic systems of Wfs1-deficient mice. Tartu, 2015, 107 p.
24. **Indrek Heinla.** Behavioural and genetic comparison of B6 and 129Sv mouse lines focusing on the anxiety profile and the expression of *Lsamp* gene. Tartu, 2016, 115 p.
25. **Liina Haring.** Cognitive functioning after first psychotic episode. Tartu, 2017, 146 p.