Die Rolle des Phosphatidylinositol 3-kinase (PI3K)-Signalwegs bei psychischen Erkrankungen

The role of the phosphatidylinositol 3-kinase (PI3K) pathway in psychiatric diseases

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Teresa Felicitas Ackermann

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Dekan:	Prof. Dr. L. Wesemann
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2. Berichterstatter:	Prof. Dr. P. Ruth
3. Berichterstatter:	Prof. Dr. P. Dietl, Ulm

All religions, arts and sciences are branches of the same tree.

All these aspirations are directed toward ennobling man's life,

lifting it from the sphere of mere physical existence

and leading the individual towards freedom.

Albert Einstein

CONTENTS

LIST OF ABBREVIATIONS 6

1. INTRODUCTION 8

1.1 Psychiatric diseases 8
1.1.1 Schizophrenia 8
1.1.2 Major depressive disorder 10
1.1.3 Bipolar disorder 12

1.2 The PI3K pathway 15

1.3 The role of PDK1 16

1.4 The role of GSK-3 17

1.5 The role of the PI3K pathway in psychiatric disorders 18

1.6 PDK1- and GSK-3-modified mice as a model for hyperactivity 21
1.6.1 PDK1-modified mice 21
1.6.2 GSK-3-modified mice 22

1.7 The goal of the studies 23

2. MATERIALS AND METHODS 25

2.1 Biochemical analysis 25
2.1.1 Dissection of the brains and homogenization procedure 25
2.1.2 Determination of NGF and BDNF protein 25
2.1.3 Determination of monoamines and metabolites 26
2.1.4 Determination of amino acids 26
2.1.5 Determination of ACTH and Cortisol levels 26

2.2 Animals 27
2.2.1 Study with pdk1 hypomorphic *(pdk^{hm})* mice 27
2.2.2 Glycogen synthase kinase 3 knock-in (*gsk3^{KI}*) mice 28

2.3 Behavioral studies 30
2.3.1 Open-field test 30
2.3.2 Light-dark box test 31
2.3.3 O-maze test 31
2.3.4 Emergence test 32
2.3.5 Object-exploration test 33
2.3.6 Acoustic-startle response 34
2.3.7 Forced-swimming test 35

3. RESULTS 36

3.1 Behavioral studies with pdk1^{hm} mice 36

3.1.1 Body and brain weight 36
3.1.2 Open-field test 36
3.1.3 Light-dark (LD) box test 37
3.1.4 O-maze test 38
3.1.5 Emergence test 39
3.1.6 Object-exploration test 40
3.1.7 Acoustic-startle response 41
3.1.8 Neurotransmitter concentrations 41

3.2 Behavioral studies with gsk3^{KI} mice 43
3.2.1 Open-field test 43
3.2.2 Light-dark box test 45
3.2.3 O-maze test 47
3.2.4 Emergence test 48
3.2.5 Novel-object test 49
3.2.6 Forced-swimming test 50
3.2.7 Body weight, food and fluid intake, and hormones 51

4. DISCUSSION 53

4.1 Study with pdk1^{hm} mice 53

4.2 Study with gsk3^{KI} mice 57

5. CONCLUSIONS 61

6. SUMMARY 62

7. ZUSAMMENFASSUNG 64

8. REFERENCES 66

9. PUBLICATIONS 85

10. CONFERENCES 87

11. ACKNOWLEDGEMENTS 88

12. ACADEMIC TEACHERS 90

List of abbreviations

ACG	cAMP-dependent, cGMP-dependent and protein kinase C
ACTH	adrenocorticotropic hormone
ADHD	attention-deficit-hyperactivity disorder
APC	adenomatosis polyposis coli
ßArr2	ß-arrestin 2
BDNF	brain-derived neurotrophic factor
BMI	body mass index
cAMP	cyclic adenosine monophosphate
C/EBP	CCAAT/enhancer binding protein
CREB	cAMP response-element-binding protein
DA	dopamine
DAOA	D-amino-acid-oxidase activator
DISC1	disrupted in schizophrenia 1
DRD4	dopamine-D4-receptor gene
DTNBP1	dystrobrevin-binding protein 1
EIF2B	eukaryotic-protein-synthesis initiation factor 2B
ERK	extracellular-signal-regulated kinase
GABA	gamma-aminobutyric acid
GLUT 4	glucose transporter 4
GS	glycogen synthase
GSK-3	glycogen synthase kinase-3
5-HIAA	5-hydroxyindole acetic acid, metabolite of 5-HT
HPA axis	hypothalamic-pituitary-adrenal axis
HSF-1	heat-shock factor-1
5-HT	serotonin
IGF-1	insulin-like growth factor-1
IRS	insulin-receptor substrate
MAO	monoamine oxidase
MAPK	mitogen-activated protein kinase
MITF	microphthalmia-associated transcription factor
MOPC 21	Mouse IgG1, kappa monoclonal
mTOR	mammalian target of rapamycin

NA	noradrenalin, norepinephrine
NDRI	norepinephrine and dopamine reuptake inhibitor
NFATc	nuclear factor of activated T-cells c
NGF	nerve growth factor
NMDA	N-methyl-D-aspartate
NRG1	neuregulin 1
PDK1	3-phosphoinositide-dependent protein kinase-1
PH	pleckstrin homology
PIF	PDK1-interacting fragment
PI3K	phosphatidylinositol 3-kinase
PIP_2	phosphatidylinositol 4,5-bisphosphate
PIP_3	phosphatidylinositol (3,4,5)-trisphosphate
PKB	protein kinase B, synonym: Akt
PKC	protein kinase C
PP1	protein phosphatase-1
PP2A	protein phosphatase-2A
PTEN	phosphatase and tensin homolog
RSK	p90 ribosomal S6 kinase
SGK	serum- and glucocorticoid-inducable protein kinase
S6K	p70 ribosomal S6 kinase
SLC6A3	solute-carrier family 6 (neurotransmitter transporter, dopamine), member 3
SLC6A4	solute-carrier family 6 (neurotransmitter transporter, serotonin), member 4
SNRI	serotonin-norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
TPH2	tryptophan hydroxylase 2
WHO	World Health Organization
Wnt	composed of *Wg* für *Wingless* and gene *Int-1*

1. Introduction

1.1 Psychiatric diseases

1.1.1 Schizophrenia

In 1908 Eugen Bleuler created the name "schizophrenia" for a severe psychiatric disease that lay people often associate with a split personality (WHO, 2006), reminiscent of the phrase in Goethe's Faust "Two souls alas! are dwelling in my breast" (von Goethe, 1808), and Robert Louis Stevenson's novel "Strange Case of Dr. Jekyll and Mr. Hyde" (Stevenson, 1886). In a schizophrenic person, however, the personality is not split into different parts: Schizophrenic people suffer from a misperception of reality, have problems to control their emotions, to think clearly, and to communicate, the latter owing to disorganization of their speech and thinking (WHO, 2006). Very typical symptoms include hearing of imaginary voices that control their thoughts and actions, and the belief that other people want to harm them (ibid). The manifestations are classified into negative and positive symptoms, whereas the term "positive" does not mean that the symptoms are good for the patient. Moreover, the term refers to the fact that such symptoms are exaggerations of behavior otherwise considered normal. "Positive symptoms", including hallucinations, delusions, and thought disorders, are easily recognizable by relatives, and increase the patient's response to medicine (ibid). In contrast, "negative symptoms" describe conditions that are decreased or attenuated compared to normal state, and are exhibited by lack of motivation, energy, experience of pleasure, attention, and concentration (ibid). The Greek roots of "schizophrenia" - *skhizein* (*σχίζειν*, "to split") and *phrēn*, or *phren-* (*φρήν*, *φρεν-*; "mind") – make sense when these contrasting symptoms in a schizophrenic person are apparent.

The onset of symptoms usually occurs in young adulthood, with men usually manifesting their first symptoms no later than their 25th year, and women showing a delayed and smaller increase, followed by a second peak at the age of 45-79 years (Hafner et al., 1993). Considering the variations in the distribution of schizophrenia, around 1% of the population is affected (Goldner et al., 2002) and the number of new cases per year, called incidence, represents around one among 10,000 people (Jablensky, 1995). The risk of suicide is around 5%, mainly committed at the beginning of the diagnosis (Palmer et al., 2005). About 45% of the patients recover after one or more episodes, about 20% show continuing symptoms and increasing disability, and about 35% exhibit a mixed pattern, with varying degrees of remission and aggravation of different lengths (Barbato, 1998).

The causes of schizophrenia are multifactorial: biological, psychological and environmental factors contribute to the onset of schizophrenia. Living in an urban area increases the risk of schizophrenia (van Os, 2004; van Os et al., 2005), as well as social disadvantages such as poverty (Mueser and McGurk, 2004), and migration because of social adversity, unemployment, racial discrimination or family dysfunction (Selten et al., 2007). Furthermore, prenatal exposure to infections (Brown, 2006) stands in correlation to later development of schizophrenia. Polygenetic influence contributes with 5% to the manifestation of schizophrenia. The fact that a twin of a schizophrenic person only shows a risk of 45%, and not 100%, proves that apart from heritability, there are other external factors which contribute to onset of the disorder (O'Donovan et al., 2003).

In schizophrenic patients, part of the neurons that use dopamine as neurotransmitter are hyperactive in psychoses, others are hypoactive which explains the occurrence of both positive and negative symptoms. Positive symptoms comprise conditions that are exaggerated compared to the standard state of health, namely delusions, hallucinations, thought disorder in form and content, misperception, and akathisia (psychomotoric agitation). By contrast, negative symptoms are described as limitation of normal experience, i.e. lack of motivation, flattening of affect, cognitive and motoric deficits including non-understanding of complex coherences, and reduction of mimic and gestures. Despite dysfunction of dopamine in the mesolimbic pathway of the brain, which involves dopamine D_2-receptors, glutamate also seems to play an important role in schizophrenia. Patients demonstrate a lower activity of the N-Methyl-D-Aspartate (NMDA) glutamate receptor and there are low levels of the receptor in post mortem brains of patients diagnosed with schizophrenia (Konradi and Heckers, 2003).

People suffering from schizophrenia receive a treatment composed of psychotherapy and antipsychotic drugs. Former so-called typical antipsychotic drugs act as dopamine antagonists and cause undesired effects of the extrapyramidale system: movement disorder, mainly in face and extremities, also called dyskinesia, parkinson-like symptoms and movement agitation (akathisia). These extrapyramidale side effects are especially critical when they appear after a long-standing therapy, and remain even after discontinuation of the corresponding drug. Newer so-called atypical neuroleptics are not only dopamine antagonists, but partly also dopamine agonists and furthermore influence the serotonin metabolism. By acting more specifically they reduce the side effects due to undesired blockade of dopamine receptors. They decrease the extrapyramidale side effects, but induce weight gain, and increase the risk of diseases related to obesity (Lieberman et al., 2005). As typical antipsychotic drugs cause a deficit of oestrogene, which might favor infertility and

osteoporosis, women are favorably treated with atypical antipsychotics. Furthermore, there are first evidences that the additional treatment of both female and male patients with oestrogens improves symptoms of schizophrenia (Kulkarni, 2009), thereby offering a possible novel treatment.

Patients with schizophrenia often feature additional diseases such as major depression and anxiety disorders (Sim et al., 2006), and symptoms as well as treatment of the diseases are overlapping.

1.1.2 Major depressive disorder

There are different states of depression, and the term major depressive disorder was chosen by the American Psychiatric Association to describe a mental disorder of high severity that is characterized by low mood, feelings of worthlessness and hopelessness, incapability to feel pleasure in doing formerly enjoyable activities, and thoughts of death or suicide (American Psychiatric Association, 2000). Common accompanying symptoms are sleeping problems, decreased appetite, headache or digestive problems (ibid), and the impairment of patients to lead normal lives results in enormous costs of lost productive work time (American Psychiatric Association, 2000; Stewart et al., 2003).

If we neglect the fact that there are geographical differences in the distribution of people with major depression, and if we take into consideration that different cultures have different definitions of the term “depression”, the lifetime prevalence to suffer from a major depressive episode lies between 8 and 10% in most countries (Kessler et al., 2005). Mood disorders are common disorders, and include major depression and bipolar disorders (see 1.1.3). They are widely distributed with a life prevalence of up to 20% (Kessler et al., 2003). Most commonly, people contract major depression between 30 and 40 years or between 50 and 60 years of age (Rickards, 2005). Compared to men, the number of women with major depression is elevated. Women show more suicide attempts, whereas men complete suicide more often (Oquendo et al., 2007). The average suicide rate of patients with major depression is 3.4%, with 7% in men and 1% in women (Blair-West and Mellsop, 2001). Amongst all cases of suicide, 60% are due to major depression (Barlow and Durand VM, 2005).

Investigations of the causes of major depression are still ongoing. Psychological, social, and biological causes have been discussed so far (Surgeon General, 1999). Joblessness and poverty are contributors to onset of major depression (Weich and Lewis, 1998). According to a Swedish study, the genetic influence is 40% in women and 30% in men (Kendler et al., 2006). There seems to be a variant in the serotonin transporter gene which

increases the chances of depression after having been exposed to a stressful incident (Caspi et al., 2003).

The monoamine hypothesis of depression signifies the involvement of the neurotransmitters serotonin, noradrenaline and dopamine in the manifestation of depression. The deficiency of one of these neurotransmitters causes the respective depressive characters. So noradrenaline is said to be associated with alertness and energy as well as anxiety, attention, and interest in life; decreased levels of serotonin stand in correlation with anxiety, obsession, and compulsion, whereas dopamine is assigned to attention, motivation, pleasure, reward, and interest in life (Nutt, 2008). Low serotonin levels lead to low levels of noradrenaline, and thus cause the onset of depression (Shah et al., 1999).

The monoamine hypothesis is basis for the treatment of major depression that varies according to the symptoms of the single patient. Anxious people are mainly treated with drugs that increase the levels of serotonin and noradrenaline, whereas in patients with loss of energy and enjoyment of life as dominating symptoms of depression, drugs that lead to an increase in dopamine and noradrenaline levels are indicated. However, there are also antidepressant drugs, such as opipramol, that do not influence the monoamine system. A publication by the Public Library of Science revealed that the theory of the monoamine hypothesis had been presented in a far too simple and basic manner before the lay public, with the alleged purpose of increasing its chances of commercialization (Lacasse and Leo, 2005).

There is a correlation between depression and decreased hippocampal neurogenesis (Mayberg, 2007). The hippocampus is a centre for mood and memory, and drugs that increase the serotonin level in the brain, such as SSRIs, stimulate neurogenesis in this area of the brain, leading to an increase in mass, and improvement of mood and memory (Duman et al., 1997; Sheline et al., 2003). Brain-derived neurotrophic factor (BDNF) is responsible for neurogenesis, and has been found to be three times more decreased in blood plasma of patients with major depression (Sen et al., 2008). Antidepressant drugs, in return, achieved to increase the BDNF plasma levels again (ibid).

Another cause for major depression might be overactivity of the hypothalamic-pituitary-adrenal axis (HPA axis), as increased cortisol levels and augmented pituitary and adrenal glands in depressive patients suggest. These findings reveal that the endocrine system is impaired in patients with major depressive disorder.

The two main pillars of treatment in major depressive disorder are antidepressant drugs and psychotherapy. While the recent increase in treatment is encouraging, inadequate

treatment is a serious concern. Antidepressant treatment involves amelioration of symptoms over the course of weeks or months, and remains ineffective in many patients.

In the past, the standard medication was dominated by tricyclic antidepressants that inhibit the reuptake of noradrenaline, dopamine and serotonin in a not very selective way. They have now taken a back seat because of their side effects such as obstipation, drop in blood pressure, fatigue, increased risk of intoxication. The main side effect, however, is the increased risk of suicide of some drugs in the beginning of the medication because they first improve motivation and only later brighten the mood of patients. Nowadays, selective serotonin reuptake inhibitors (SSRIs) are the first class drugs that have moderate side effects and show less toxicity after overdosage (Royal Pharmaceutical Society of Great Britain, 2008). Suicide by overconsumption of tablets is impossible as SSRIs lead to emesis by stimulation of the serotonin $5HT_3$ receptor in the area postrema.

Alternatives in case of non-responsiveness are the noradrenaline and dopamine reuptake inhibitor (NDRI) bupropion, the serotonin-noradrenaline reuptake inhibitor (SNRI) venlafaxine (Rush et al., 2006), and the neuroleptic drug olanzapine (Kruger, 2006). Lithium is also very frequently used in case of therapy resistance (Bschor and Bauer, 2006) or in acute manic episodes, but needs to be supervised very carefully as the therapeutic index is quite narrow, which means that effect and toxicity are close to each other. Only around 40% of patients respond to treatment with lithium.

Worth mentioning are first electroconvulsive therapy in patients with very severe episodes of major depression, despite its side effects of memory loss, disorientation and headache (Barlow and Durand VM, 2005), and second St. John's wort in patients with light depression (Linde et al., 2008).

Medication with antidepressive drugs should always be accompanied by psychotherapy, similar effective to SSRIs in case of mild and severe depressive symptoms. General physical activity has also been shown to improve symptoms in low-to-moderate depression (Martinsen, 2008). Furthermore, the associations between elevated serotonin levels during waking state and influence of light render sleep deprivation and light therapy promising approaches in the treatment of major depression (Adrien, 2002; Terman, 2007).

1.1.3 Bipolar disorder

Bipolar disorder is characterized by episodes of unnaturally elevated mood, called mania, or in cases of mild symptoms hypomania. Patients usually also show episodes of depression or mixed phases, and there are intermittent episodes of normal mood. If four or

more episodes of major depression, mania, hypomania, or a mixed type occur during one year, the term, “rapid cycling” is used (Mackin and Young, 2004). Changes in mood within 24-48 hours are called “ultra-ultra rapid cycling”.

There are different forms of bipolar disorders, dependent of symptoms and their frequency and intensity. The forth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) classifies four types: Bipolar I disorder is characterized by at least one manic episode without necessarily requiring an episode of major depression. Patients with Bipolar II disorder, in contrast, show rather periods of hypomania than mania, and one or more episodes of major depression. A hypomanic episode is often identified as a highly productive and creative phase which complicates the diagnosis of Bipolar II. In cyclothymia, several hypomanic phases alternate with depressive episodes that are not classified as major depression. Patients show moderate changes in mood and are able to work. Bipolar Disorder Not Otherwise Specified (NOS) includes patients who don’t belong to one of the above mentioned categories.

The lifetime prevalence for Bipolar Disorder I is estimated as 1%, 1.1% for Bipolar Disorder II and 2.4% for subthreshold symptoms (Merikangas et al., 2007). Onset of symptoms typically occurs during late adolescence and young adulthood (Christie et al., 1988). The incidence of bipolar disorders is difficult to determine, mainly because doctors diagnose children more frivolously (Moreno et al., 2007) and because of general overdiagnosis in the USA (Carlson and Meyer, 2006; Harris, 2005; McClellan, 2005).
Patients diagnosed with bipolar disorder commit suicide with a percentage of 15 to 19% (de Abreu et al., 2009).

That people with bipolar disorder can lead normal and fulfilling lives depends on a correct diagnosis and adequate medication that should be continued during episodes of recovery to prevent relapses. The prognosis is very good if the patient has a good knowledge and awareness of his own disease, if he cooperates with a competent doctor and a trustful psychotherapist, if he possesses a good health condition that also comprises sports and healthy nutrition as well as avoidance of stress, and if he reacts adequately when he recognizes a change in his mood, activity or sleeping behavior. Functioning is mainly impaired during depressive episodes, whereas hypomania leads to amelioration (Judd et al., 2005). Famous examples of patients with bipolar disorder are the painter Vincent van Gogh and the composer, Robert Schumann, who were very successful during their manic phases.

It has been shown that there exists a correlation between bipolar disorder and eating disorder. Many patients with bipolar disorder suffer from binge eating and have a higher body

mass index (BMI), waist circumference and fasting blood glucose levels (Castrogiovanni et al., 2009).

Many causes are discussed that lead to the onset of bipolar disorder. There is a genetic influence demonstrated by a study in which the subtype Bipolar I was found with a correspondence of 40% in identical twins, and with 0-10% in fraternal twins (Kieseppa et al., 2004). In a recent review, genes regarding the neurotransmitters dopamine (DRD4 and SLC6A3), serotonin (SLC6A4 and TPH2), glutamate (DAOA and DTNBP1) as well as genes related to pathways for cell growth (BDNF, NRG1 and DISC1) were found to be involved in the causes of bipolar disorder. The heritability as a whole is estimated to be 0.71% (Edvardsen et al., 2008). Environmental factors also play a role; abuse and trauma during childhood were found to be in correlation with an earlier onset of symptoms, an unfavorable progress and higher comorbidity.

The medication is based on mood stabilizers, among which lithium carbonate and lamotrigine are the most successful (Bauer and Mitchner, 2004; Geddes et al., 2004). Whereas lamotrigine is effective in preventing depression, lithium lowers suicide rates to an extent of success far exceeding that of other tested drugs. Other commonly used mood stabilizers are sodium valproate and carbamazepine, the latter being successfully used in the treatment of rapid cycling. Atypical antipsychotic drugs are often needed to medicate manic phases. Olanzapine and quetiapine have been shown to successfully maintain bipolar disorder in monotherapy (Lilly, 2009), whereas the efficacy and safety of olanzapine even seem comparable to that of prophylactic therapy with lithium (Lilly, 2009; Tohen et al., 2005). As antidepressants can not only trigger episodes of (hypo)mania and mixed phases, but also can provoke rapid cycling if used without mood stabilizer, they must be applied very carefully (Sachs et al., 2007). The value of treatment without antidepressants was shown in a study in 2007 when a mood stabilizer was shown as effective as an antidepressant (Bower, 2007). As in other psychiatric disorders, psychotherapy supports a positive course of the disease, and helps to maintain remission.

The high risk of suicide in schizophrenia (10-15%), depression (3-4%) and bipolar disorder (15-19%), the enormous costs for the government and the impaired quality of life of the patients and their families justify the effort for extensive studies and huge effort to improve the understanding of these disorders, and to offer new possibilities for their treatment, mainly by investigating additional targets for novel drugs. The fact that brain-derived neurotrophic factor (BDNF) is involved in schizophrenia (Gratacos et al., 2007;

Ivleva et al., 2008), depression (Sen et al., 2008) and bipolar disorder (Serretti and Mandelli, 2008) advises the investigation of the phosphatidylinositol 3-kinase (PI3K) pathway, which is activated by BDNF.

1.2 The PI3K pathway

Insulin leads to the conversion of glucose to glycogen in skeletal muscle via stimulation of glucose uptake and via activation of glycogen synthase (GS). Activated GSK-3 phosphorylates GS on four serine residues (Ser641, Ser645, Ser649, and Ser653) and therefore inactivates GS (Fig.1). As insulin causes the inhibition of GSK-3, it renders GS active. Besides the above mentioned serine residues, Ser7 is dephosphorylated by insulin in a GSK-3-independent way (McManus et al., 2005). Insulin also might stimulate GS via activation of a muscle glycogen-associated protein phosphatase (PP1) that reverses the phosphorylation of the GS serine residues by GSK-3 (Delibegovic et al., 2003; Suzuki et al., 2001). In the liver, GS is mainly regulated by blood glucose levels (McManus et al., 2005).

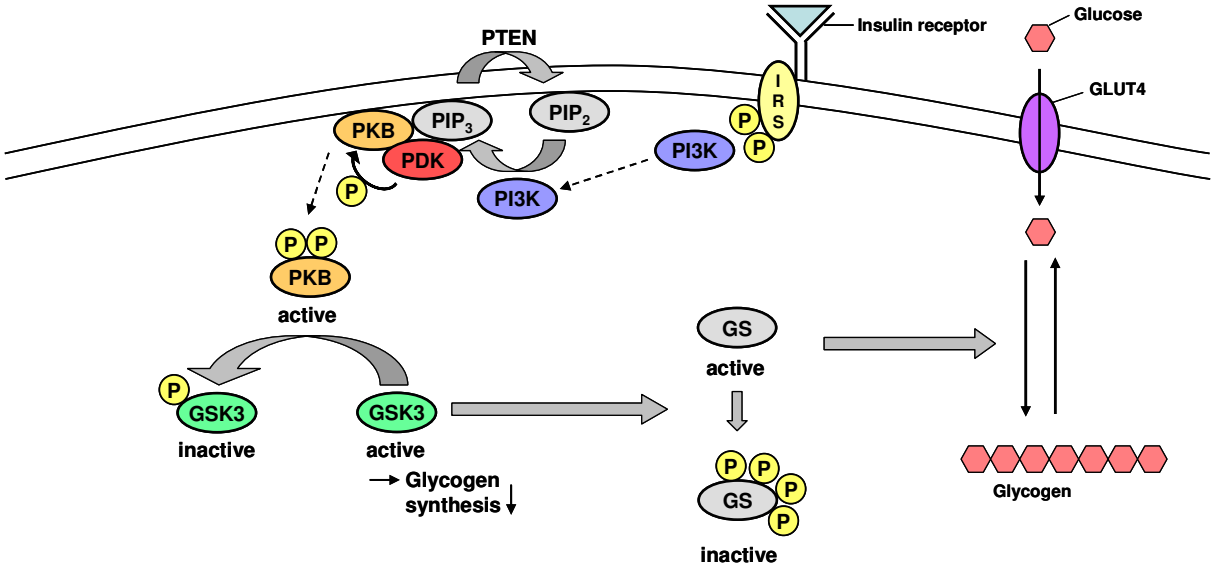

Figure 1: Insulin signaling in the PI3K-pathway leads to inhibition of GSK-3 and formation of glycogen

When insulin binds to the insulin receptor in liver, muscle and adipose tissue, it induces the phosphorylation of insulin-receptor-substrate (IRS) proteins and attracts them to the plasma membrane. IRS proteins become tyrosine-phosphorylated and, then, attract PI3K to the plasma membrane. The latter converts the membrane component phosphatidylinositol 4,5-bisphosphate (PIP_2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP_3), a reaction that is

reversed by the phosphatase PTEN. PIP_3 serves as an anchor for both protein kinase B (PKB), also termed Akt, and 3-phosphoinositide-dependent protein kinase-1 (PDK1) (Cantley, 2002). The co-localization of those two kinases at the plasma membrane facilitates the phosphorylation of PKB by PDK (Beaulieu et al., 2009; Frame and Cohen, 2001). The complete activation of PKB requires phosphorylation of both PDK1 at Thr308 and PDK2/rictor-mTOR at Ser473 (Beaulieu et al., 2009). Activated PKB phosphorylates GSK-3 at Ser21 in the isoform GSK-3α and at Ser9 in GSK-3ß (Fig.1). The phosphorylated sequence of GSK-3 then interacts with the substrate recognition motif on the GSK-3 kinase domain, thus preventing the binding of GSK-3 substrates and consequently inhibiting GSK-3 (Cross et al., 1995; McManus et al., 2005; Shaw et al., 1997).

PIP_3 also stimulates the phosphorylation of p70 ribosomal S6 kinase (S6K), which then enhances the ability of PDK1 to phosphorylate and activate S6K (Biondi et al., 2001).
90-kDa ribosomal S6 kinase (RSK) is activated by phosphorylation of ERK/MAPK which requires phosphorylation of the T-loop residue of its N-terminal kinase domain by PDK1 (Frodin et al., 2000).

Serum- and glucocorticoid-inducable kinase (SGK) acts in a very similar way as PKB due to homology in the catalytic domain. One difference is, however, the missing plecktrin homology (PH) domain that exists in PKB but not SGK1. Accordingly, SGK1 is also able to inhibit GSK-3 by phosphorylation of the same serine residues PKB interacts with (Sakoda et al., 2003; Wyatt et al., 2006).

1.3 The role of PDK1

PDK1 is known to play an important role in insulin and growth factor signaling pathways, and thus mediates the effects of kinases involved in these pathways on cell growth, proliferation, survival and regulation of metabolism (Mora et al., 2004). PDK1 acts as a master kinase and regulates more than 23 protein kinases of the AGC (cAMP-dependent, cGMP-dependent and protein kinase C) kinase family. PDK1 signal transduction involves several related serine/threonine protein kinases including isoforms of protein kinase B (PKB/Akt) (Dummler and Hemmings, 2007; Whiteman et al., 2002), the p70 ribosomal S6 kinase (S6K) (Dann et al., 2007), the serum- and glucocorticoid-inducable protein kinase (SGK) (Lang et al., 2006a), the p90 ribosomal S6 kinase (RSK) (Hauge and Frodin, 2006), and protein kinase C (PKC) (Newton, 2003).

It is known that PDK1 exists in a single isoform and that only a single gene encodes PDK1 in mammals (Lawlor et al., 2002). PDK1 is ubiquitously expressed in cells and constitutively active (Bayascas, 2008; Peifer and Alessi, 2008). It possesses two regulatory domains: first, a Pleckstrin Homology (PH) domain, which binds to the phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) and is important for activation of PKB isoforms, and, second, the PDK1-interacting fragment (PIF) pocket, by which PDK1 interacts with all its other substrates of the ACG kinase family, except for PKB (Bayascas, 2008).

Furthermore, PDK1 and its downstream signaling molecules, PKB and S6K, are involved in tumorigenesis. PTEN is the lipid phosphatase that reverses the effect of PI3K and breaks down PIP_3 to PIP_2. Frequent mutations of PTEN occurred in human cancer, and mice with one defective allele of PTEN ($PTEN^{+/-}$) develop different kinds of tumors (Bayascas et al., 2005; Salmena et al., 2008). When PDK1 was decreased in these mice, the development of tumors could be significantly reduced, which means that PDK1 inhibitors are a valuable therapeutic in the treatment of cancer.

1.4 The role of GSK-3

Glycogen synthase kinase-3 (GSK-3) was originally discovered as a kinase that plays a crucial role in the glycogen metabolism in sceletal muscle, and leads to the formation of glycogen by phosphorylating and thus inhibiting glycogen synthase (GS). GSK-3 is constitutively active and ubiquitously expressed in mammalian tissue (Prickaerts et al., 2006). There are two isoforms of GSK-3, GSK-3α (51 kDa) and GSK-3ß (47 kDa), whose sequence similarity within the kinase catalytic domains is 97%. They are encoded by different genes, and GSK-3ß is more enriched in the nervous system. The main difference of the two isoforms consists in the extended N-terminal glycine-rich tail of GSK-3α (Frame and Cohen, 2001). Besides its main role in glucose and glycogen homeostasis, GSK-3 is also implicated in protein metabolism, cell proliferation, cell differentiation and apoptosis (Beurel and Jope, 2006; Cohen and Frame, 2001; Frame and Cohen, 2001). GSK-3 is therefore involved in several non-psychological and psychological diseases such as diabetes, cancer, inflammation, Alzheimer's disease, schizophrenia, attention-deficit-hyperactivity disorder (ADHD), and bipolar disorder.

GSK-3 is inhibited via the phosphatidylinositol 3-kinase (PI3K)-dependent protein kinase B (PKB) pathway, the mitogen-activated protein kinase (MAPK) cascade and the mTOR pathway, whereas this study concentrates on the inhibitory effect of PI3K/PKB.

GSK-3 is also inhibited by the Wnt signaling pathway that includes a complex of Axin, ß-catenin and GSK-3 (Frame and Cohen, 2001). Furthermore, there are direct inhibitors of GSK-3 such as LiCl and SB 216763 and SB 415286 (ibid).

Because of its constitutional activity, GSK-3 continually has an effect on its substrates, and via phosphorylation keeps them in an inactive state or leads to their degradation. Besides its most famous substrate, the signaling molecule glycogen synthase (GS), GSK-3 interacts with several transcription factors, such as ß-catenin, Cyclin D1, Jun, Myc, cAMP-response-element-binding protein (CREB), microphthalmia-associated transcription factor (MITF), nuclear factor of activated T-cells c (NFATc), CCAAT/enhancer binding proteins α and ß (C/EBPα and C/EBPß), and heat-shock factor-1 (HSF-1). GSK-3 also influences eukaryotic translation initiation factor 2B (eIF2B) and structural proteins, such as tau. Within the already mentioned Wnt-signaling pathway, GSK-3 phophorylates and stabilises axin, leading to the attraction of ß-catenin, it degrades ß-catenin by its phosphorylation, and via interaction with APC it facilitates the binding of the latter to ß-catenin and decreases binding of APC to microtubules. The influence of GSK-3 in protein synthesis is conducted via inhibition of eIF2B (Frame and Cohen, 2001; Wada, 2009b).

1.5 The role of the PI3K pathway in psychiatric disorders

Besides its role in diabetes, cancer, and inflammation (Eldar-Finkelman et al., 1999; Farina et al., 2009; Jope et al., 2007; Lee and Kim, 2007), GSK-3 has been implicated in a number of psychiatric disorders, including Alzheimer's disease, schizophrenia, attention-deficit-hyperactivity disorder (ADHD), and bipolar disorder (Emamian et al., 2004; Lang et al., 2007; Manji et al., 2003; Prickaerts et al., 2006; Van Wauwe and Haefner, 2003). Hyperactive GSK-3ß stands in correlation to insulin resistance, diabetes mellitus, tumorigenesis, inflammation, as well as neuropsychiatric and neurodegenerative disorders (Jope et al., 2007; Jope and Johnson, 2004; Wada et al., 2005a; Wada et al., 2005b; Wada, 2009a).

There are several classical therapeutics that influence the activity of GSK-3. Amongst neuropsychiatric drugs, *d*-fenfluramin, which stimulates serotonin secretion and inhibits its reuptake, fluoxetin, a selective serotonin-reuptake inhibitor (SSRI), and imipramin, a tricyclic antidepressant, which blocks the reuptake of serotonin and noradrenaline, have been shown to increase the Ser^9-phosphorylation of GSK-3ß (Li et al., 2004). Serotonin was found to regulate GSK-3 in both directions, as 5-HT_1 receptors increase and 5-HT_2 receptors decrease the Ser^9-phosphorylation of GSK-3ß. In such disorders as depression, anxiety or

schizophrenia that exhibit a disequilibrated serotonin activity, GSK-3ß activity might, therefore, be impaired.

When injected over a period of up to 28 days, haloperidol and risperidone, both classical antipsychotic drugs, as well as the first of the atypical antipsychotics clozapine increased GSK-3α/ß and ß-catenin levels. Raclopride, a D_2-dopamine-receptor antagonist, has similar effects and lets us assume that antipsychotic drugs lead to the elevation of GSK-3α/ß and ß-catenin levels via inhibition of the D_2-dopamine-receptor (Alimohamad et al., 2005a; Alimohamad et al., 2005b).

In the treatment of Alzheimer's disease, physostigmine, an acetylcholinesterase inhibitor, as well as memantine, an *N*-methyl-D-aspartate-receptor antagonist, led to elevated levels of Ser^{21}/Ser^{9}-phosphorylation of GSK-3α/ß. Furthermore, general anesthetics such as pentobarbital and chloral hydrate, both injected intraperitoneally, as well as inhalation of halothane vapor increased Ser^{21}/Ser^{9}-phosphorylation of GSK-3α/ß (Li et al., 2005).

During the last ten years several new insights about neuropsychiatric diseases have been gained: Abnormally reduced expression of neurotrophins, such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), leads to a defect of neuroplasticity, and thus imitates the defective morphology in neurodegenerative disorders. Besides impaired neurotransmission, this pathology is an additional factor for the genesis of mood disorders. As new neurons continually arise in the hippocampus, not only in the adolescent but also in the adult human brain (Krishnan and Nestler, 2008; Pittenger and Duman, 2008; Wada et al., 2005a), lithium also seems to be an option for curing acute brain injuries, such as ischemia, as well as chronic neurodegenerative disorders, such as Alzheimer's disease (Marmol, 2008; Quiroz et al., 2004; Wada et al., 2005a). Indeed, it has been shown that lithium or other GSK-3-inhibitors have beneficial effects in the treatment of brain injuries, and that in consequence of a traumatic event in the brain, the PKB/GSK-3ß/ß-catenin-pathway was activated to yield compensatory and preventive effects (Shapira et al., 2007).

Lithium has been used since 1970 as a drug against mania, depression, and suicide, thus demonstrating its positive effects concerning mood stabilization, behavioral improvement, and neurogenesis. Although the cellular mechanism of lithium still remains unclear, it has been elucidated that its beneficial effects are consequence of its inhibition of GSK-3ß and subsequent accumulation of ß-catenin, which leads to ß-catenin-dependent gene transcription (Marmol, 2008; Quiroz et al., 2004; Takahashi-Yanaga and Sasaguri, 2007; Wada et al., 2005a; Wada et al., 2005b). Lithium leads to the inhibition of GSK-3, first as a

competitive inhibitor of Mg^{2+} via direct inhibition of the Mg^{2+}-ATP-dependent catalytic activity of GSK-3 (Ryves and Harwood, 2001), and second by increase of the Ser21/Ser9-phosphorylation sites of GSK-3α/ß by a yet not fully clarified mechanism.

Furthermore, there has been evidence that insulin-like growth-factor-1 (IGF1) and IGF2, as well as insulin itself, improve mood (Benedict et al., 2004; Hoshaw et al., 2005; Hoshaw et al., 2008; Malberg et al., 2007), memory (Trejo et al., 2007; Trejo et al., 2008), neurogenesis (ibid), and angiogenesis (Lopez-Lopez et al., 2004). IGF1 and IGF2 are upregulated by antidepressants (Chen and Russo-Neustadt, 2007; Grunbaum-Novak et al., 2008; Khawaja et al., 2004; Sinha et al., 2005), and IGF1 in turn upregulates BDNF and its receptor TrkB (Chen and Russo-Neustadt, 2007; Ding et al., 2006; McCusker et al., 2006).

Inhibition of GSK-3 by several antimanic drugs, especially lithium, has been considered to account for their beneficial effects in bipolar disorders (Gould and Manji, 2005; Klein and Melton, 1996). Moreover, inhibition of GSK-3 has been shown to yield antidepressant-like effects (Gould et al., 2004; Kaidanovich-Beilin et al., 2004; Redrobe and Bourin, 1999).

Dopamine, a drug applied in Parkinson's disease and involved in the genesis of psychotic symptoms in schizophrenia, regulates GSK-3 via D2 dopamine receptor, ß-arrestin 2 (ßArr2)/protein phosphatase 2A (PP2A) and PKB (Beaulieu et al., 2009). Antidepressive drugs, such as selective serotonin reuptake inhibitors (SSRIs), monoamine oxidase (MAO) inhibitors and tricyclic antidepressants, inhibit GSK-3 by increasing its amino-terminal phosphorylation in several brain regions (Beaulieu et al., 2008; Li et al., 2004).

PKB and SGK are in the same way involved in the regulation of behavior (Beaulieu et al., 2005; Emamian et al., 2004; Lang et al., 2006b; Lang et al., 2007). PI3K is activated by brain-derived neurotrophic factor (BDNF) which has been mainly implicated in the pathophysiology and treatment of depression and bipolar disorder (Belzung et al., 1990; Fan and Sklar, 2008; Lang et al., 2004; Lang et al., 2005b; Lang et al., 2007).

Summing up, there is considerable evidence that the GSK-3/ß-catenin pathway plays an important role in the pathophysiology of neuropsychiatric diseases and thus represents an important target for the treatment of these disorders. The impact of the downstream signaling molecules still needs to be investigated as they play a crucial role in neuropsychiatric homeostasis.

1.6 PDK1- and GSK-3-modified mice as a model for hyperactivity

1.6.1 PDK1-modified mice

There is evidence from rodent studies that indicate that the amygdala plays the central role in the acquisition and expression of conditioned fear (Maren and Quirk, 2004). Recently, it has been demonstrated that brain-derived neurotrophic factor (BDNF) and phosphatidylinositol 3-kinase (PI3K) are involved in fear learning in the amygdala (Ou and Gean, 2006). Studies in humans have largely confirmed hypotheses about fear conditioning that were derived from animal studies, where neuroimaging studies demonstrate that the amygdala is critical for emotional learning in fear conditioning paradigms (Davis and Whalen, 2001; Maren and Quirk, 2004). The BDNF hypothesis of depression and anxiety postulates that a loss of BDNF is directly involved in the pathophysiology of depression and anxiety, and its restoration may underlie the therapeutic efficacy of antidepressant treatment (Groves, 2007; Martinowich et al., 2007). BDNF affects neuronal growth, neurogenesis and memory in a PI3K-dependent manner (Atwal et al., 2000; Ou and Gean, 2006), and signals via phosphoinositide-dependent protein kinase 1 (PDK1) (Chikahisa et al., 2006). Accordingly, the PI3K signaling pathway has been shown to regulate hippocampal proliferation, differentiation, and survival, to develop dendrite size and shape, and to induce functional synapses in neuronal cells (Kumar et al., 2005; Martin-Pena et al., 2006; Peltier et al., 2007). Both, pharmacological inhibition and genetic manipulation of PI3K-dependent signaling demonstrate that PI3K signals via PDK1 (Vanhaesebroeck and Alessi, 2000). PDK1 is a direct downstream effector of PI3K (ibid), and plays a central role in activating other downstream processes, including cell proliferation, survival and differentiation (Mora et al., 2004). On the neurotransmitter level, especially serotonin (5-HT) and GABA have been shown to powerfully regulate the neuroanatomical circuit that mediates fear in anxiety disorders (Stahl, 2002). To explore the impact of PDK1 signaling on transmitter concentrations in amygdala, as well as on behavior, gene-targeted mice were analyzed.

The attempt to create PDK1 knockout mice revealed that embryos die at embryonic day 9.5 (Lawlor et al., 2002). These embryos show several abnormalities, such as missing somites, forebrain, and neural-crest-derived tissues, whereas their hind- and midbrain developed normally (ibid). As the PDK1 knockout mouse is not viable, mice with PDK1 activity suppressed to around 10% (*pdk1*^{hm}) (ibid) were used. They are viable and fertile, and after insulin injection PKB, S6K and RSK are activated as expected. Discovered differences are the smaller size of *pdk1*^{hm} mice (reduction of 40-50%), and the accordingly reduced organ

volumes, whereas the number, nuclear size and proliferation rate of cells were not affected by PDK1 deficiency (ibid). *Pdk1*^{hm} were compared to their age- and sex-matched wild-type littermates (*pdk1*^{WT}).

1.6.2 GSK-3-modified mice

As shown recently (Prickaerts et al., 2006), transgenic mice overexpressing GSK-3 display strikingly increased general locomotor activity and decreased habituation in an open-field test, as well as increased acoustic-startle response and reduced immobility in the forced-swimming test. That study suggests GSK-3ß-overexpressing mice as a model for hyperactivity that reflects the manic phase of bipolar disorder. However, the disadvantage of a generally GSK-3-overexpressing mouse model lies in elevated PKB levels as a consequence of a more pronounced negative feedback in the GSK-3-overexpressing mouse (Prickaerts et al., 2006). To avoid this undesirable negative feedback and to obtain mice in which the inhibitory effect of insulin on GSK-3 is specifically disrupted but other inhibitory pathways on GSK-3 still function, a specific GSK-3 knock-in mouse was created (McManus et al., 2005) (Fig.2).

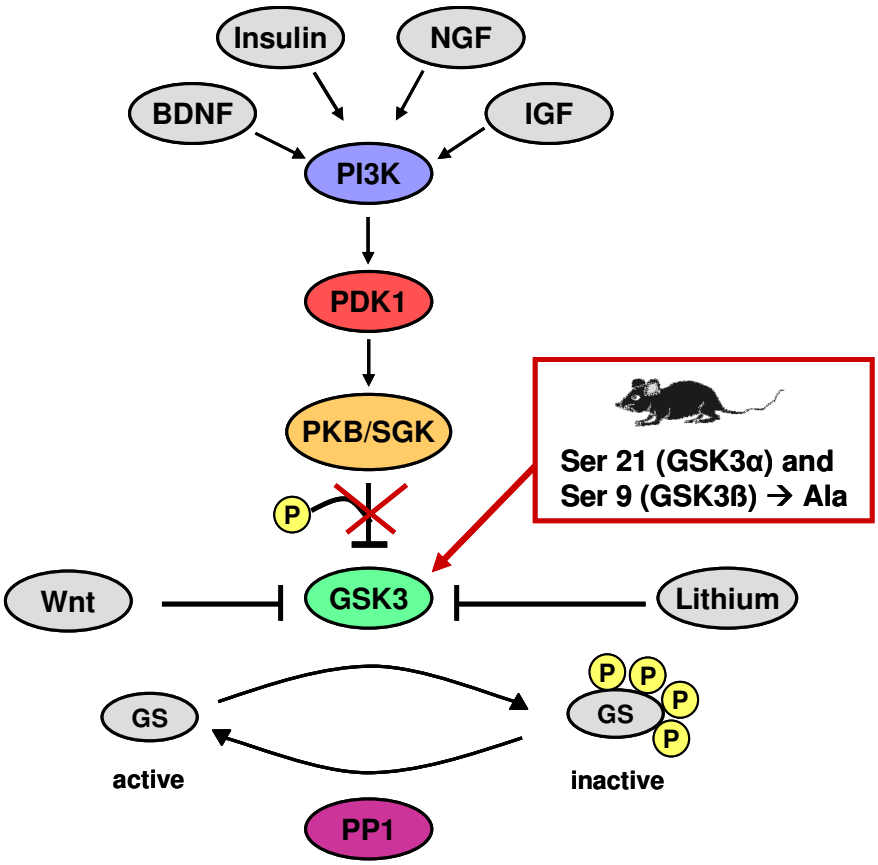

Figure 2: Insulin signaling in the PI3K pathway and its inhibitory effect on GSK-3, both of which are abolished in *gsk3*^{KI} mice

Replacement of serine within the PKB phosphorylation site by alanine (GSK-3$\alpha^{21A/21A}$, GSK-3ß$^{9A/9A}$) renders GSK-3 resistant to inactivation by PKB/SGK (McManus et al., 2005). Accordingly, the effect of insulin on muscle glycogen synthase is abrogated in knock-in mice carrying these mutations, called *gsk3^{KI}* (ibid) (Fig.2). On the other hand, GSK-3 signaling that is dependent on Wnt is not impaired in those mice (ibid), that develop normally and do not show any deficits in development, an effect that is should be expected when Wnt signalling is impaired. Besides studies that suggested a similar point of action on GSK-3 of both Wnt and PKB/SGK1, Wnt has been shown to inhibit GSK-3 via another mechanism than phosphorylation of Ser9 and Ser21 (Ding et al., 2000).

Mice lacking one allele of the GSK-3ß gene (*gsk3ß$^{+/-}$*) showed antidepressant behavior, for example by increasing mobility in the forced-swimming test, and had elevated levels of ß-catenin in the hypothalamus as well as accelerated Wnt/ß-catenin-dependent gene transcription in hippocampus, amygdala, and hypothalamus. *Gsk3ß$^{+/-}$* mice thereby mimicked treatment with lithium in wild-type mice, regarding both behavioral and biochemical aspects (O'Brien et al., 2004).

Mice with overexpressed ß-catenin showed less depressive behavior, as did mice treated with lithium, an observation that suggest that lithium acts via induction of ß-catenin expression (Gould et al., 2007). So GSK-3ß seems to be an important mediator of antidepressant effects of lithium, offering an additional application for lithium besides control of mania and stabilization of mood in patients with bipolar disorder.

To gain further insight in the pathophysiology of psychiatric diseases and to investigate possible new drug targets, the characteristics of mice with gene modification related to the PI3K pathway were determined.

1.7 The goal of the studies

As elaborated above, there is considerable evidence that the PI3K signaling pathway plays an important role in the pathophysiology and in the treatment of neuropsychiatric disorders. Several mouse models, such as the *gsk3$^{+/-}$* mouse, the GSK-3 overexpressing *gsk3^{tg}* mouse or the mouse with overexpressed ß-catenin support the hitherto existing findings that therapeutical approaches of neuropsychiatric disorders culminate in GSK-3.

The novel mouse model resistant to PKB/SGK1-dependent inhibition of GSK-3 (*gsk3^{KI}* vs. *gsk3^{WT}*) offers a new and more specific investigation of GSK-3, namely the insulin-dependent influence in psychiatric disorders. The characterization of mice with a

hypomorphism of PDK1 (*pdk1*^{hm}) pursues the speculation that upstream targets of GSK-3 could be similarly involved in the genesis of neuropsychiatric diseases and exhibit mirror-like features.

Besides longing for deeper understanding of psychiatric disorders and the molecular mechanisms that lead to breakdown of the physiological balance of neurotransmitters and kinases, the long-run aim is to develop new drugs against diseases, such as schizophrenia, Alzheimer's disease, ADHD and bipolar disorder. Kinases participating in the PI3K pathway seem to be a possible target for the development of inhibitors that could be a new hope for many patients.

2. Materials and Methods

2.1 Biochemical analysis

2.1.1 Dissection of the brains and homogenization procedure

Mice were sacrificed 7 days after the last behavioral experiment. After decapitation, the brains were rapidly removed, immediately frozen on dry ice, and stored at –80°C until use. For dissection, the frozen brains were placed on a cold plate, providing a temperature between –10°C and –12°C. At this temperature, the brain tissue was in a semi-frozen state with well-preserved anatomical structure. The amygdala/piriform cortex was cut out using the rhinal fissure as landmark. The olfactory bulb was dissected along the basis of the frontal cortex. Each tissue sample was homogenized by ultrasonication in 40-80 volumes of distilled water.

For the determination of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) protein, 150µl of the homogenate were added to an equal volume of buffer containing 0.1M Tris-HCl, pH 7.0, 0.4M NaCl, 0.1% NaN3, and a variety of protease inhibitors ("complete"-Protease Inhibitor Cocktail Tablets, Roche Diagnostics GmbH, Penzberg, Germany). For the analysis of monoamines and metabolites, 150µl of the homogenate were added to an equal volume of 0.2M perchloric acid and centrifuged at 25,000g for 20min at 4°C. Serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and the catecholamines noradrenaline (NA) and dopamine (DA) were measured in the supernatant (23). For amino acid analysis 20µl of the homogenate were added to 80µl methanol and centrifuged at 25,000g for 20min at 4°C. The supernatant was diluted 1:30 with distilled water.

2.1.2 Determination of NGF and BDNF protein

Endogenous NGF levels in the thawed homogenates were determined by a fluorometric two-site enzyme immunoassay (ELISA) which has been described in detail elsewhere (Hellweg et al., 1989; Hellweg et al., 2001). The mean recovery of mouse NGF (125pg/ml) added to the homogenate ranged from 60% to 90%. NGF content was expressed as equivalents of mouse 2.5S NGF. The detection limit of the assay was 0.25pg/ml.

Endogenous levels of BDNF were measured in the thawed homogenates using commercial ELISA kits, in principle according to the manufacturer's instructions (Promega, Montréal, Canada), but adapted to the fluorometric technique, and also used for NGF

determination (Hellweg et al., 2003; Hellweg et al., 2006). BDNF content was expressed as equivalents of recombinant human BDNF. The detection limit of the assay was 1 pg/ml. Each sample (namely each tissue dissected and homogenized as described above) was consecutively processed for quantification of each neurotrophin, i.e. NGF and BDNF (ibid). Determination of recovery, and specific and unspecific neurotrophin binding [the latter against mouse IgG1 obtained from MOPC 21 (Mouse IgG1, kappa monoclonal)] involved quadruplicate fluorescence determination for each tissue sample. The neurotrophin levels were expressed as pg/mg tissue (wet weight). To minimize the influence of unavoidable variances between experiments, neurotrophin levels from experimental animals were normalized as a percentage of wild-types serving as control, always being included in the same experiment (Hellweg et al., 2001).

2.1.3 Determination of monoamines and metabolites

Analysis of 5-HT and 5-HIAA was performed using high-performance liquid chromatography (HPLC) with electrochemical detection (Sperk, 1982). NA and DA were measured by HPLC with electrochemical detection after extraction to alumina (Felice et al., 1978) with minor modifications (Sperk et al., 1981).

2.1.4 Determination of amino acids

Glutamate, gamma-aminobutyric acid (GABA), and taurine were determined using methods described previously (Piepponen and Skujins, 2001). In brief, amino acids were precolumn derivatized with o-phthalaldehyde-2-mercaptoethanol using a refrigerated autoinjector, and then separated on a HPLC column (ProntoSil C18 ace-EPS) at a flow rate of 0.6ml/min and a column temperature of 40°C. The mobile phase was acetonitrile 50 mmol/ml sodium acetate solution pH = 5.7 in a linear gradient from 5% to 21% acetonitrile. Derivatized amino acids were detected by their fluorescence at 450nm and excitation at 330nm.

2.1.5 Determination of ACTH and Cortisol levels

Mice were superficially anesthesized with diethylether (Carl Roth, Karlsruhe, Germany), and 200-300µl blood was withdrawn by retro-orbital puncture of the eye. The blood was centrifuged (Centrifuge 5417R, Eppendorf, Hamburg, Germany) and ACTH and Cortisol

levels were determined in the plasma (ACTH-ELISA-Kit, Calbiotech, AC018T, Corticosterone ELISA-Kit, DRG Diagnostics, EIA-4164).

2.2 Animals

All animal experiments were conducted according to the guidelines of the American Physiological Society and the German law for the care and welfare of animals, and were approved by local authorities.

2.2.1 Study with pdk1 hypomorphic *(pdk^{hm})* mice

The generation and basic properties of pdk^{hm} mice have been described (Lawlor et al., 2002). Genotyping was made by PCR on tail DNA, using pdk1 and neo-R-specific primers as previously described (Lawlor et al., 2002; Williams et al., 2000). The mice had a mixed Sv129 and C57Bl6 background.

Pdk^{hm} mice were compared to their wild-type littermates (pdk^{WT}) that were obtained from a heterozygote breeding, and age and sex were matching in both groups. For the study, 12 pdk^{hm} mice and 16 pdk^{WT} mice were used with an average age between 4.1 and 10.9 months. Mice had free access to standard mouse diet (C 1310, Altromin, Lage, Germany) and tap water.

One week before the experiments started, the animals were transferred to the Institute of Anatomy in Zurich, where they were singly housed in standard type-2 mouse cages and maintained at a 12:12-hour inverted cycle, with lights on between 8 p.m. and 8 a.m. The mice were fed a standard chow (Art. 3430, KLIBA NAFAG, Provimi Kliba AG, Kaiseraugst, Schweiz, www.kliba-nafag.ch), and tap water and nesting material were again available ad libitum.

The behavioral experiments were performed between 8 a.m. and 8 p.m. Only one type of experiment was done on the same day, and the home cage rack was brought to the test room at least 30min before the experiment started. The apparatus were cleaned with 70% ethanol and water before each animal was tested. Experiments extended over a total time of 6 weeks, and the average age of the mice was 5.6 months at the beginning and 7.5 months at the end of the testing period.

For data acquisition, animals were video tracked at 4.2 Hz and 576x768 pixel spatial resolution, using a Noldus EthoVision 3.0 system (Noldus Information Technology,

Wageningen, The Netherlands, www.noldus.com). Raw data were transferred to the public domain software Wintrack 2.4 (Wolfer et al., 2001) for further analysis.

Tests were done in the following order: open-field test, light-dark box (LD-box) test, O-maze test, emergence test, object-exploration test, and acoustic-startle response profile. The experiments were performed with diffuse indirect room light produced by 440 W bulbs that were adjusted to obtain approximately 12 lux in the center of the experimental arena. The LD-box test was the only exception when full room light was used to yield approximately 500 lux in the illuminated compartment.

Data were analyzed using a two-way factorial ANOVA design, with genotype as between subject factor. Where appropriate, the model was complemented by within-subject factors to explore the dependence of genotype effects on place, time, and stimulus intensity. One-sample t-tests were used to compare group means with hypothesized values.

Where possible, ANOVA main effects were verified using nonparametric tests which produced similar results. Statview version 5.0 (SAS Institute, Cary, North Carolina, www.statview.com) was used for all statistical calculations. The software, Sigma Stat, was used for the statistical analysis of data concerning neurotrophin and metabolite concentrations. For statistical analysis of the biochemical data, the Student's t-test was applied.

2.2.2 Glycogen synthase kinase 3 knock-in (*gsk3*^{KI}) mice

The mice were generated, in which the codon encoding Ser9 of the GSK-3ß gene was changed to encode nonphosphorylatable alanine (GSK-3ß$^{9A/9A}$), and, simultaneously, the codon encoding Ser21 of the GSK-3α gene was changed to encode nonphosphorylatable alanine (GSK-3α$^{21A/21A}$), thus yielding the GSK-3α/β$^{21A/21A/9A/9A}$ double-knock-in mouse (*gsk3*^{KI}), as described previously (McManus et al., 2005). The mice had a mixed Sv129 and C57Bl6 background.

The mice were compared to appropriate age and sex-matched wild-type mice (*gsk3*^{WT}). As WT littermates and double-knock-in mice GSK-3α/β$^{21A/21A/9A/9A}$ can only be obtained at a ratio of 1 in 16 in the same cross, it is impractical to perform a heterozygous breeding. Instead, GSK-3α/β$^{21A/21A/9A/9A}$ were interbred with GSK-3α/β$^{21A/21A/9A/9A}$ mice as well as GSK-3α/β$^{+/+/+/+}$ with GSK-3α/β$^{+/+/+/+}$ littermates derived from a 1-in-16 ratio cross (McManus et al., 2005).

For all experiments, 11 *gsk3*^{WT} mice and 11 *gsk3*^{KI} mice were included in the study. They were fed a control diet (C1310, Altromin, Lage, Germany) and had free access to tap water. Two months before the experimental period, animals were changed to a 12:12 hour inverted cycle, with lights on between 7 p.m. and 7 a.m. To avoid fighting of males, mice were housed alone or in groups of maximal 3 mice during the experimental period.

Behavioral testing occurred between 7 a.m. and 7 p.m. Only one type of experiment was done on the same day, and the home cages were brought to the test room at least 30min before the experiment started. The equipment was thoroughly cleaned with 70% ethanol and water before releasing the animal. Experiments extended over a total of 5.9 weeks, average age was 4.5 months at the beginning and 5.8 months at the end of the testing period.

For data acquisition, animals were video tracked by the camera 302050-SW-KIT-CAM at a resolution of 0.62 to 0.72-pixel (TSE-Systems, Germany, www.TSE-Systems.com). Raw data were transferred to Excel® for further analysis.

Tests were done in the following order: open-field test, LD-box test, O-maze test, emergence test, object-exploration test, and forced-swimming test. Experiments were performed with diffuse indirect room light produced by dimmable bulbs, adjusted to yield less than 30 lux in the center of the experimental arena. The only exception was the LD-box test where full room light was switched on.

Data are provided as means ± SEM, "n" represents the number of independent experiments. All data were tested for significance using t-test analysis, and, accordingly, the repeated measures ANOVA with time as within-subject factor for habituation. Only results with $p < 0.05$ were considered statistically significant.

The behavioral studies for *pdk*^{hm} and *pdk*^{WT} mice were performed at the Institute of Anatomy in Zurich, whereas the study that followed with *gsk3*^{KI} and *gsk3*^{WT} was done at the Institute of Physiology in Tuebingen, and differences in methodology occurred.

2.3 Behavioral studies

The experiments with PDK1-hypomorphic mice have been performed as described in detail (Lang et al., 2006b).

The complete equipment for the study with PDK1-hypomorphic mice was purchased from Noldus EthoVision Technology, Wageningen, The Netherlands, www.noldus.com. For behavioral studies with GSK-3 knock-in mice, equipment from TSE-Systems, Germany, www.TSE-Systems.com was used, and the program VideoMot served for analysis.

2.3.1 Open-field test

The open-field test is internationally used to measure locomotor and mood-related behavior in a quantitative way. The test is also used to analyze anxiety-related behavior. It is based on the observation that mice avoid unknown and brightly illuminated areas where there is no possibility to hide and retreat. If mice are exposed to such an environment, they find themselves in a conflict between anxiety and exploration, as mice are naturally curious animals. Besides speed and total distance mice move, giving information about their general activity, it is a matter of particular interest which zone of the open-field they prefer. The open-field is divided into "central" and "peripheral", and it has been shown that rodents tend to avoid open spaces because they prevent the rodents from performing thigmotaxic[1] behavior (Ramos and Mormede, 1998). It can be concluded that staying in the corners and the border area indicates a more anxious behavior, and exploration of the center of the arena points out a more active and audacious behavior. Another parameter for activity and curiosity is the willingness of the mice to stand on their hind feet while inspecting the environment to improve exploration. Such behavior is called "rearing" and is mainly observed in the border area towards the wall but also freestanding in the center.

For the study with pdk^{hm} and pdk^{WT} mice the round open-field arena had a diameter of 150cm, a white plastic floor, and 35cm high sidewalls made of white polypropylene. Each subject was released near the wall and observed for 10min. The same procedure was repeated the following day, resulting in a total observation time of 20min (Madani et al., 2003). For evaluation, the arena was divided into a center field comprising 50% of the arena surface and a 7cm wide wall zone (18% of the surface). The zone between the wall and the center was defined as transition zone.

[1] Thigmotaxis is the need for pressure on all sides of your body in order to feel safe.

For the study with *gsk3*^{KI} and *gsk3*^{WT} mice a quadratic open-field arena was used with side lengths of 50cm, a white plastic floor, and 40cm high sidewalls. Each subject was released near the wall and observed for 30min (Madani et al., 2003). For evaluation, a border with 10cm distance to the wall was considered. Rearing was detected via an infrared light-beam detector that surrounds the open-field and can be adjusted to the size of the tested animal.

2.3.2 Light-dark box test

The light-dark (LD) box test is the most currently used model to characterize anxious behavior in mice. It consists of a dark and safe compartment (one third) and a brightly illuminated open and thus aversive area (two-thirds) (Crawley and Goodwin, 1980). The apparatus creates a conflict for the mouse, that tends to explore the unfamiliar area, and initially wants to avoid the unfamiliar (neophobia). The fraction of time spent in the box is seen in correlation to anxiety and depression, whereas the number of transitions and the time spent in the illuminated compartment are indices for curiosity and exploration (Bourin and Hascoet, 2003). Furthermore, rearing is another important index of activity (Costall et al., 1989).

For the study with *pdk*^{hm} and *pdk*^{WT} mice, a 20x30cm transparent Perspex chamber with a height of 20cm was connected by a 7.5x7.5cm aperture to a 20x15x20cm polyvinyl-chloride dark box. The chamber was illuminated by direct room light, and each subject was released in the illuminated compartment and observed for 5min (Crawley and Goodwin, 1980).

For the study with *gsk3*^{KI} and *gsk3*^{WT} mice, an infra-red-permeable box made of black acrylic plastic (40cm high) was inserted into the open-field, covering 33% of the surface area. An aperture of 10x11cm with rounded-down corners led from the light arena to the dark box. The experiment was performed under direct room light. Each subject mouse was released in the illuminated compartment and observed for 5min (Crawley and Goodwin, 1980).

2.3.3 O-maze test

The O-maze test is an additional model to investigate anxiety-related behavior. The experiment tests the urge of mice to discover an unknown area and their instinct to protect themselves from a dangerous situation. Mice usually avoid dangerous areas, presented by the open sectors of the O-maze, and prefer safe places, such as the zone that is protected by walls.

Usually they override their anxiety after a certain time because of curiosity. Time spent in the open sectors, as well as general activity, was evaluated (Konig et al., 1996). Other important measures are whether and how quickly the mice dare to leave the original protecting arm and enter the opposing area, and the frequency of head dips over the edge of the open sectors (Shepherd et al., 1994). It is distinguished between so-called "protected head dips", where the back of the mouse is between the protecting walls, and "unprotected head-dips", where the mouse completely stands in an open sector.

For the study with pdk^{hm} and pdk^{WT} mice, a 5.5cm wide annular runway made of grey plastic was constructed as O-maze. The outer diameter measured 46cm and was placed inside the above open-field arena, 40cm above the floor. The two opposing 90° closed sectors were protected by 16cm high inner and outer walls of grey polyvinyl-chloride, while the remaining two open sectors had no walls. Animals were released in one of the closed sectors and observed for 10min (Konig et al., 1996; Lang et al., 2006b).

The apparatus used for the study with $gsk3^{KI}$ and $gsk3^{WT}$ mice was made of grey plastic, the closed sectors were protected by 11cm-high inner and outer walls, and the two open sectors had a border of 5mm (Fig.3).

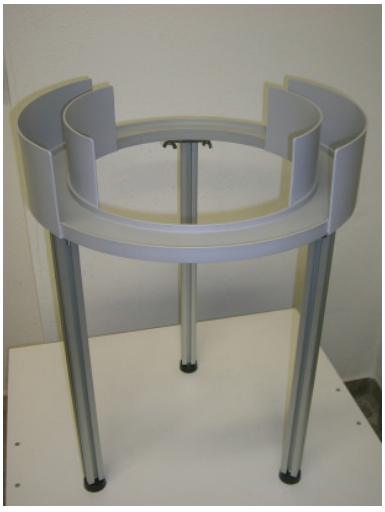

Figure 3: O-maze used for behavioral studies with $gsk3^{KI}$ and $gsk3^{WT}$ mice

2.3.4 Emergence test

The emergence test complements the open-field test. By contrast, the emergence test reduces anxiety in mice by providing a safe and well-known enclosure within the open-field test (Dulawa et al., 1999). As in the open-field test, though, the emergence test also serves the purpose of characterizing exploratory and anxious behavior but with the additional option for

the mouse to back out of danger into a familiar environment. The main interest is the time spent in or near the familiar home box.

For the study with pdk^{hm} and pdk^{WT} mice, frames of non-reflective aluminium with a height of 37cm were used to divide the open-field into four square arenas that measured 50x50cm, allowing observation of four animals at the same time. Each arena had a 12x8x4cm plastic home box, with an aperture of 8x4cm that was positioned in a corner at 5cm from the nearest wall, with the aperture of the box facing away from the wall. A day prior to the experiment, the home-box was put into the cage of each test subject, and in preparation for the actual experiment the same box was introduced into the arena and fixed with adhesive tape. Each subject was observed for 30min.

For the study with $gsk3^{KI}$ and $gsk3^{WT}$ mice, an infra-red-permeable box, which had been previously in the home cage of the mouse during more than 24 hours, was inserted into the open-field arena. The home box had a base area of 9x10cm, with two apertures (3cm length, 3.5cm height with rounded down corners) on the long side.

2.3.5 Object-exploration test

The object-exploration or novel-object test complements the open-field and emergence tests. A novel-object is introduced into the arena, and the behavior of the mouse is observed. Approach towards the object because of curiosity and exploration counters the avoidance of the unknown stimulus because of fear. Besides speed and total distance mice move, giving information about their general activity, parameters of interest include time spent in the home box area, visits into the home box, as well as time exploring the novel-object.

For the study with pdk^{hm} and pdk^{WT} mice, the procedure was modified (Dulawa et al., 1999). Arenas were the same as for the emergence test, but without the home box. A 12x4cm semi-transparent 50ml falcon tube was used as novel-object, positioned vertically in the center of the arena and fixed with adhesive tape. Each subject was observed for 30min in the empty, cleaned arena. Then, the novel-object was introduced and observation continued for another 30min.

For the study with $gsk3^{KI}$ and $gsk3^{WT}$ mice, the experimental set-up was supplementary modified: the novel-object was put in addition to the familiar home box to investigate the exploratory behavior of the mouse in a partly familiar environment where it has the possibility to retreat. The mouse was first observed for 30min in the open-field including the home box. Afterwards, a falcon tube was added to the area and fixed with adhesive tape. The mouse was

then observed for another 30min, and the contact with the nose on the falcon tube was taken as criterion to analyze the reaction on a novel-object.

2.3.6 Acoustic-startle response

The startle response is an unconditioned reflexive response to a sudden unexpected stimulus, such as a flash of light, a loud noise (acoustic-startle reflex), or a quick movement near the face. Humans usually move away from the stimulus, contract their muscles, and blink, the latter by far the most conspicuous involuntary movement (Castellote et al., 2007). It has been shown in rats that the latency of the acoustic-startle reflex, measured from the onset of the tone to the beginning of the electromyographic response in the hindleg, is 8ms (Davis et al., 1982).

For the study with pdk^{hm} and pdk^{WT} mice, a Hamilton-Kinder SM100 startle monitor system was used (www.hamiltonkinder.com, Poway, CA). The animal restrainer had the measures 3.8x8.8cm and was made of clear Perspex. An adjustable ceiling prevented the animal from rearing. The restrainer was put on a sensing plate, which carried a piezoelectric accelerometer at its bottom. The unit was attached to a heavy metallic base plate by means of four mounting pins, then enclosed in a sound-attenuated ventilated cabinet with the dimensions 29x29x18cm. The loudspeaker was located 22cm above the animal, and produced white-noise pulses. A microcomputer interface controlled the loudspeaker and performed an analog-to-digital (A/D) conversion of the signals from the accelerometer. Signal calibration was done using a Newton impulse calibrator. Sound levels were verified by a digital sound level meter (Radio Shack). The background noise level inside the closed cabinet was maintained at 70dB. Each test mouse was placed in the restrainer and left undisturbed for 5min before the session began. In total, 66 trials were performed. Nine different sound levels were used: 64, 68, 72, 76, 80, 90, 100, 110, and 120dB (Paylor and Crawley, 1997). Each stimulus lasted 40ms and was presented 6 times in a pseudorandom order such that each sound level occured once within a block of 9 trials. The series started and ended with 6 presentations each of the 120dB stimulus. The average intertrial interval was 15s (ranging from 10 to 20s). The startle response was recorded for 250ms while measuring the response every 1ms, starting with the onset of the startle stimulus. The maximum amplitude was used as the dependent variable.

2.3.7 Forced-swimming test

The forced-swimming test is extensively utilized in neurobiological research and within pharmaceutical companies that operate antidepressant drug discovery programs (Willner, 2005). Such tests give conclusions about the depressive behavior of rodents. The animal is forced to swim in a situation where there is no escape. After an initial period of vigorous activity, the animal will reduce its movements to a minimum just to keep its head above the water. The so-called "floating behavior" is defined as the absence of direct movements of an animal's head and body. It has been shown not only that chronic mild stress (CMS) increase (Willner, 2005) but also that antidepressant drugs decrease (Porsolt et al., 1977; Prickaerts et al., 2006) the time of floating.

Gsk3^{KI} and *gsk3*^{WT} mice where placed in a glass of water with temperature between 24 and 26°C. The diameter of the glass was 20cm, and the mouse was placed in the water without being able to touch the bottom. Each subject was observed during 6min, and the time it spent without movement was recorded. After the experiment, the mice were dried under an infrared heat lamp before being returned to their home cage.

3. Results

3.1 Behavioral studies with *pdk1^{hm}* mice

3.1.1 Body and brain weight

Pdk1^{hm} mice appeared healthy, but they were considerably smaller than their wild-type littermates. Body weight was significantly lower in *pdk1^{hm}* mice than in *pdk1^{WT}* mice ($p<0.0001$, Table 1), which was the case also for fresh brain weight ($p<0.0001$, Table 1). The weight of the amygdala as well as of the olfactory bulb of *pdk1^{hm}* mice was significantly less in *pdk1^{hm}* mice than in *pdk1^{WT}* mice (Table 1). The relation between reduction of brain and that of body weight was not significantly different between *pdk1^{hm}* mice and *pdk1^{WT}* mice (Table 1).

Table 1: Body weigth and weight of brain structures of *pdk1^{hm}* and *pdk1^{WT}* mice

Parameter	WT (n=16)	PDK1 (n=12)	Significance
Average body weight	30.20 ± 0.85 g	23.69 ± 0.68 g	p<0.0001
Fresh brain weight	0.51 ± 0.004 g	0.38 ± 0.003 g	p<0.0001
Brain / body weight	1.62 ± 0.05 %	1.70 ± 0.06 %	n.s.
Amygdala weight	23.63 ± 0.51 mg/1000µl	17.73 ± 0.58 mg/1000µl	p<0.0001
Olfactory bulb weight	24.18 ± 0.55 mg/1000µl	17.68 ± 0.39 mg/1000µl	p<0.0001

The removal of the brains of the *pdk1^{hm}* and *pdk1^{WT}* mice was done in cooperation with and principally by Giovanni Colacicco (Ph.D.), Institute of Anatomy, University of Zurich and Department of Biology, ETH Zurich.

3.1.2 Open-field test

In the open-field test, no clear differences in spontaneous behavior were detected between genotypes. *Pdk1^{hm}* mice tended to be less active than *pdk1^{WT}* mice during the first 5min on each day, but the time course of activity is statistically indistinguishable between the genotypes (Fig.4A). Both groups avoided the center and transition zones in favor of the wall zone, and *pdk1^{hm}* mice showed a trend to prefer the wall zone of the arena slightly more (Fig.4B).

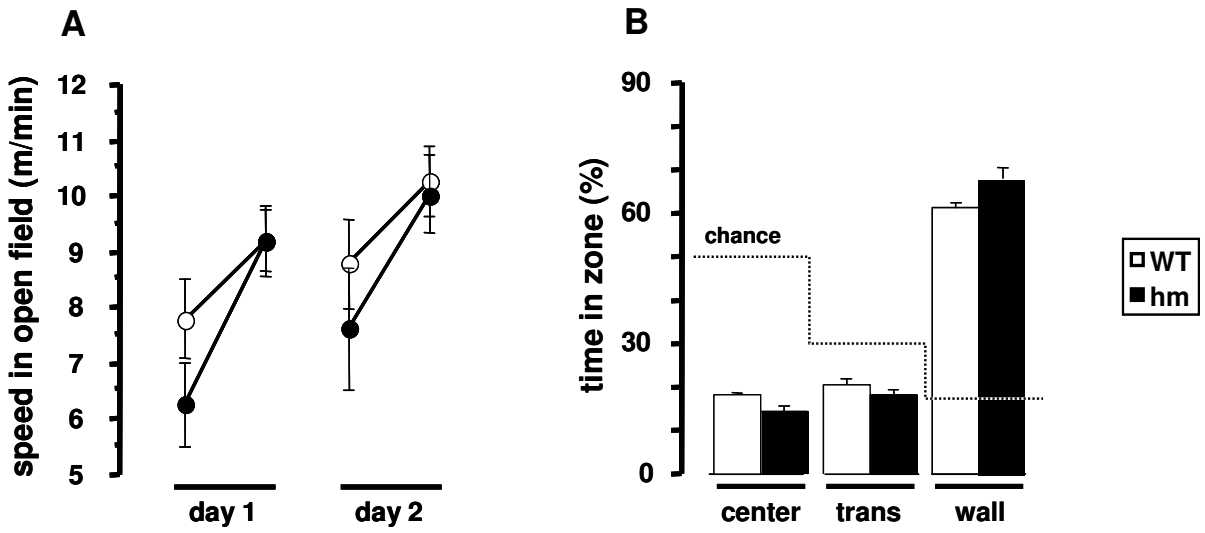

Figure 4: Speed and time spent in different zones of *pdk1*^{WT} and *pdk1*^{hm} mice in the open-field test

A: Arithmetic means ± SEM (n = 12-16 each group) of speed in the open-field during the first and second 5 minutes on the first and second day;

Day 1: 0-5min: *pdk1*^{WT}: 7.78 ± 0.71m/min, *pdk1*^{hm}: 6.24 ± 0.74m/min, n.s.; 5-10min: *pdk1*^{WT}: 9.20 ± 0.54m/min, *pdk1*^{hm}: 9.18 ± 0.64m/min, n.s

Day 2: 0-5min: *pdk1*^{WT}: 8.77 ± 0.82m/min, *pdk1*^{hm}: 7.60 ± 1.08m/min, $p<0.05$; 5-10min: *pdk1*^{WT}: 10.27 ± 0.64m/min, *pdk1*^{hm}: 10.02 ± 0.70m/min, n.s.

B: Arithmetic means ± SEM (n = 12-16 each group) of time spent in center, transition and wall zone; centre: *pdk1*^{WT}: 17.83 ± 0.99%, *pdk1*^{hm}: 14.15 ± 1.77%; transition: *pdk1*^{WT}: 20.79 ± 1.07%, *pdk1*^{hm}: 17.9 ± 1.30%; wall: *pdk1*^{WT}: 61.38 ± 1.43%, *pdk1*^{hm}: 67.96 ± 2.65%; n.s.

3.1.3 Light-dark (LD) box test

In the LD-box, both *pdk1*^{WT} and *pdk1*^{hm} mice preferred the box to the illuminated compartment, without significant differences between genotypes (Fig.5). Light-dark transitions were significantly less frequent in *pdk1*^{hm} mice than in *pdk1*^{WT} mice ($p>0.006$), which points to a decreased activity of *pdk1*^{hm} mice. The number of aborted emergencies, i.e. when the mouse looks out of the box but decides not to leave it, instead to return into the dark compartment, was decreased in *pdk1*^{hm} mice ($p<0.061$). The latency to the first rearing was significantly increased in *pdk1*^{hm} mice ($p<0.026$), indicating a more anxious behavior of *pdk1*^{hm} mice.

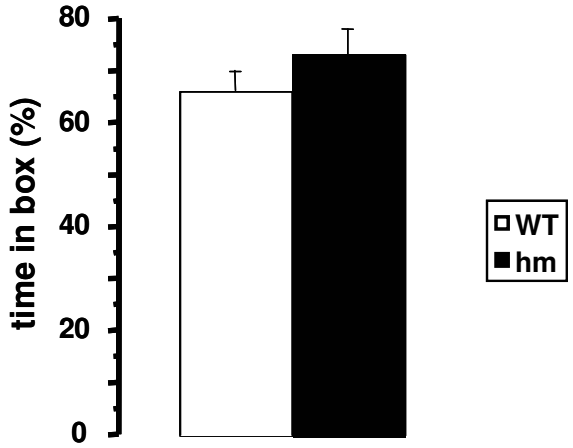

Figure 5: Time that *pdk1*^{WT} and *pdk1*^{hm} mice spent in the dark compartment of the LD-box

Arithmetic means ± SEM (n = 12-16 each group) of time spent in the dark. *pdk1*^{WT}: 66.26 ± 3.99%, *pdk1*^{hm}: 73.26 ± 5.35%; n.s.

3.1.4 O-maze test

In the O-maze test, both genotypes strongly avoided the open sectors. *Pdk1*^{hm} mice spent more time on the closed sector than did *pdk1*^{WT} mice, which preferred to stay in the transition zone (Fig.6A). The number of unprotected head dips was not different between *pdk1*^{hm} mice and *pdk1*^{WT} mice, but *pdk1*^{hm} mice performed fewer protected head dips, and thus displayed less risk assessment at the transition between closed and open sectors (Fig.6B).

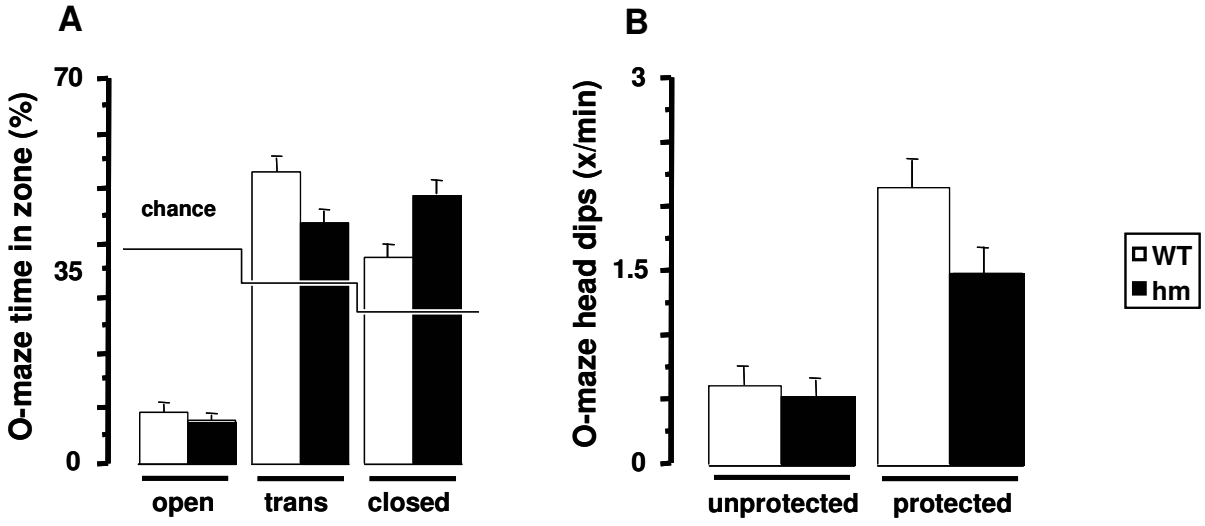

Figure 6: Time that *pdk1*^{WT} and *pdk1*^{hm} mice spent in different zones of the O-maze and number of head dips

A: Arithmetic means ± SEM (n = 12-16 each group) of time spent in open, transition, and closed sectors; open: $pdk1^{WT}$: 9.45 ± 1.39%, $pdk1^{hm}$: 7.72 ± 1.16%; n.s.; transition: $pdk1^{WT}$: 53.0 ± 2.27%, $pdk1^{hm}$: 43.76 ± 2.27%; p<0.0148; closed: $pdk1^{WT}$: 37.55 ± 2.51%, $pdk1^{hm}$: 48.51 ± 3.11%; p<0.0224

B: Arithmetic means ± SEM (n = 12-16 each group) of number of unprotected and protected head dips; unprotected: $pdk1^{WT}$: 0.60 ± 0.16x/min, $pdk1^{hm}$: 0.52 ± 0.13x/min; n.s.; protected: $pdk1^{WT}$: 2.14 ± 0.23x/min, $pdk1^{hm}$: 1.48 ± 0.19x/min; p<0.0337

3.1.5 Emergence test

In the emergence test, $pdk1^{hm}$ mice showed the same initial activity as did $pdk1^{WT}$ mice, but female $pdk1^{hm}$ mice habituated less than did their wild-type litttermates (Fig.7A). Both groups avoided the center zone and preferred to stay in the zone surrounding the home box (Fig.7B). $Pdk1^{hm}$ mice tended to spent more time inside the home box than did $pdk1^{WT}$ mice (6.09 ± 1.40% vs. 4.49 ± 0.63%; n.s.).

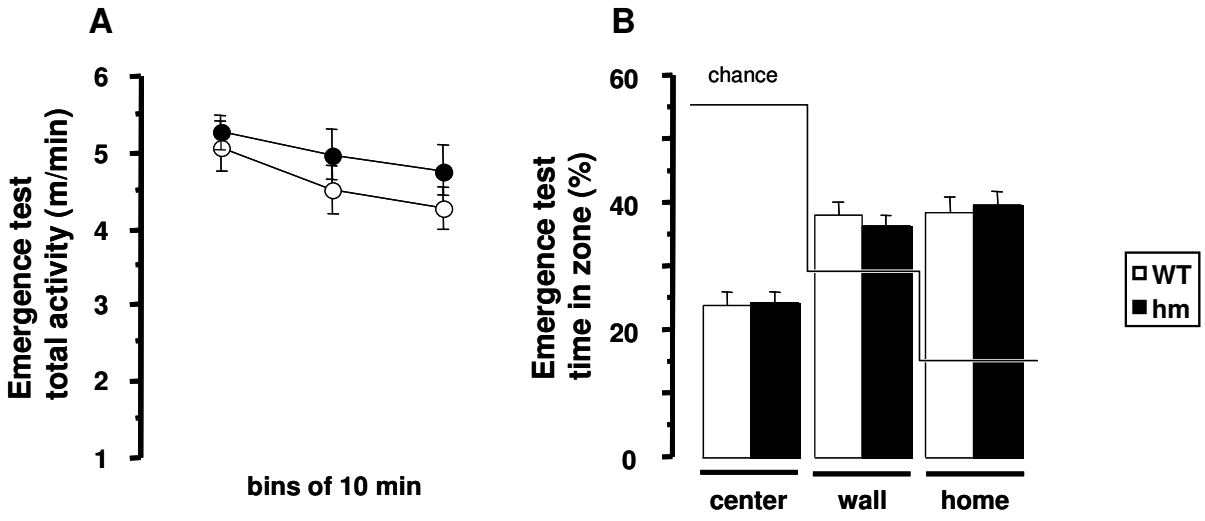

Figure 7: Activity and time that $pdk1^{WT}$ and $pdk1^{hm}$ mice spent in different zones in the emergence test

A: Arithmetic means ± SEM (n = 12-16 each group) of total activity in the emergence test in bins of 10min; 0-10min: $pdk1^{WT}$: 5.07 ± 0.33m/min, $pdk1^{hm}$: 5.25 ± 0.21m/min; 10-20min: $pdk1^{WT}$: 4.51 ± 0.30m/min, $pdk1^{hm}$: 4.97 ± 0.32m/min; 20-30min: $pdk1^{WT}$: 4.26 ± 0.29m/min, $pdk1^{hm}$: 4.77 ± 0.33m/min; n.s.

B: Arithmetic means ± SEM (n = 12-16 each group) of time spent in centre, wall and home zone; centre: $pdk1^{WT}$: 23.7 ± 2.0%, $pdk1^{hm}$: 24.33 ± 1.63%; n.s.; wall: $pdk1^{WT}$: 37.97 ± 2.22%, $pdk1^{hm}$: 36.25 ± 1.82%; home: $pdk1^{WT}$: 38.52 ± 2.43%, $pdk1^{hm}$: 39.57 ± 2.02%; n.s.

3.1.6 Object-exploration test

In the period before the insertion of the object, *pdk1*^{hm} mice did not habituate, becoming, however, more active when the novel-object was present (Fig.8A). Irrespective of genotype, the mice showed significant exploratory activity towards the object (Fig.8B).

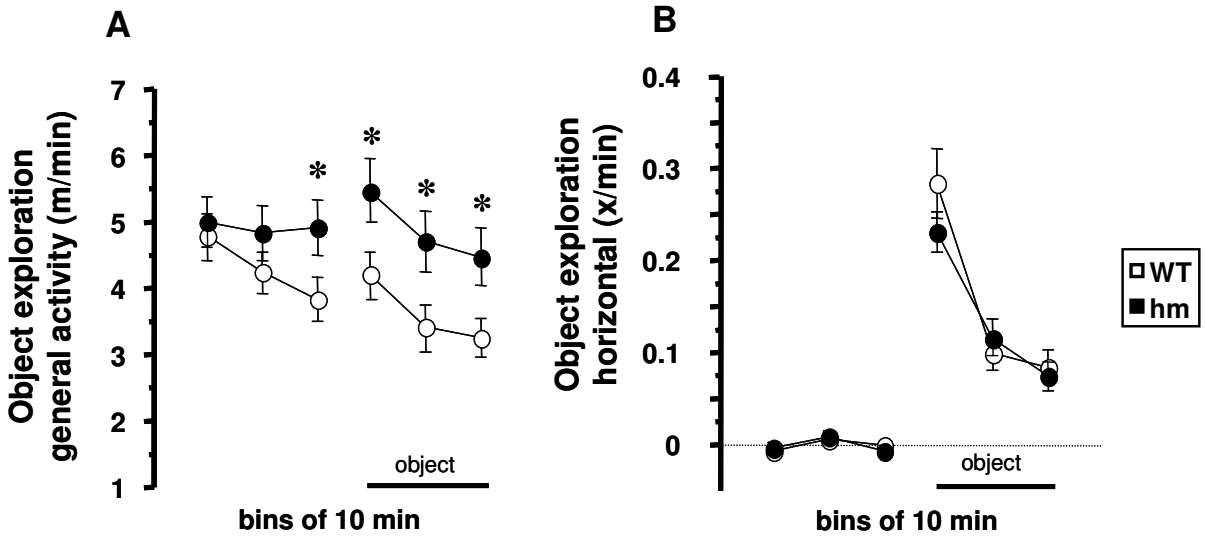

Figure 8: Object-exploration of *pdk1*^{WT} and *pdk1*^{hm} in the object-exploration test

A: Arithmetic means ± SEM (n = 12-16 each group) of activity in the object-exploration test in bins of 10min before and during the presence of a novel-object (m/min):

before insertion of the object: 0-10min: *pdk1*^{WT}: 4.78 ± 0.36m/min, *pdk1*^{hm}: 4.99 ± 0.38m/min, n.s.; 10-20min: *pdk1*^{WT}: 4.23 ± 0.32m/min, *pdk1*^{hm}: 4.84 ± 0.41m/min, n.s.; 20-30min: *pdk1*^{WT}: 3.84 ± 0.33m/min, *pdk1*^{hm}: 4.93 ± 0.41m/min, $p<0.05$

during the presence of the object: 0-10min: *pdk1*^{WT}: 4.19 ± 0.37m/min, *pdk1*^{hm}: 5.48 ± 0.49m/min, $p<0.05$; 10-20min: *pdk1*^{WT}: 3.41 ± 0.36m/min, *pdk1*^{hm}: 4.72 ± 0.45m/min, $p<0.05$; 20-30min: *pdk1*^{WT}: 3.25 ± 0.29m/min, *pdk1*^{hm}: 4.4 ± 0.46m/min, $p<0.05$

B: Arithmetic means ± SEM (n = 12-16 each group) of exploratory movements towards the object (x/min):

before insertion of the object: 0-10min: *pdk1*^{WT}: -0.006 ± 0.004x/min, *pdk1*^{hm}: -0.003 ± 0.006x/min, n.s.; 10-20min: *pdk1*^{WT}: 0.005 ± 0.005x/min, *pdk1*^{hm}: 0.009 ± 0.007x/min, n.s.; 20-30min: *pdk1*^{WT}: 0.001 ± 0.003x/min, *pdk1*^{hm}: -0.006 ± 0.005x/min, n.s.

during the presence of the object: 0-10min: *pdk1*^{WT}: 0.284 ± 0.038x/min, *pdk1*^{hm}: 0.231 ± 0.023x/min, n.s.; 10-20min: *pdk1*^{WT}: 0.1 ± 0.02x/min, *pdk1*^{hm}: 0.117 ± 0.02x/min, n.s.; 20-30min: *pdk1*^{WT}: 0.085 ± 0.018x/min, *pdk1*^{hm}: 0.075 ± 0.015x/min, n.s.

3.1.7 Acoustic-startle response

In the acoustic-startle response profile, the response profile of *pdk1*^{hm} mice tended to be shifted to the right, indicating a threshold increase (Fig.9).

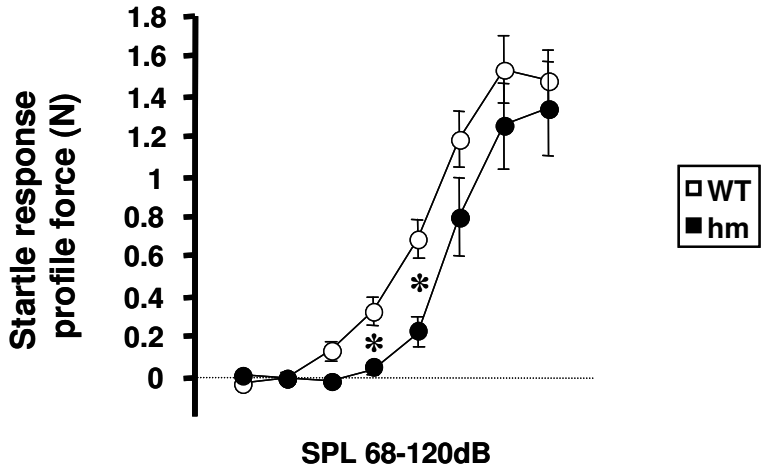

Figure 9: Startle response amplitude to stimuli of 68, 72, 76, 80, 90, 100, 110, and 120dB

* Significant genotype effect at individual SPL after correction for multiple comparisons according to false discovery rate control procedure of Benjamini and Hochberg

3.1.8 Neurotransmitter concentrations

In the amygdala, *pdk1*^{hm} mice had, as compared to *pdk1*^{WT} mice, lower 5-HT concentrations (Table 2, p = 0.034) and an increased 5-HT turnover, as indicated by the molar ratio of 5-HIAA, a metabolite of 5-HT, to 5-HT (Table 2, p = 0.046). The NA content was increased in *pdk1*^{hm} mice (Table 2, p = 0.017). GABA and taurine concentrations in the amygdala were lower in *pdk1*^{hm} mice than in *pdk1*^{WT} mice (Table 2, p = 0.015, p= 0.023), whereas glutamate and DA were not significantly different between genotypes. BDNF and NGF concentrations in the amygdala were not significantly different between *pdk1*^{hm} and *pdk1*^{WT} mice (Table 2).

In the olfactory bulb, 5-hydroxyindoleacetic acid concentrations were higher in *pdk1*^{hm} mice than in *pdk1*^{WT} mice (Table 3, p = 0.047), but 5-HT concentrations were not significantly different between *pdk1*^{hm} and *pdk1*^{WT} mice. NA concentration was significantly greater in *pdk1*^{hm} than in *pdk1*^{WT} mice (Table 2, p = 0.019, p = 0.021). Glutamate and DA concentrations in the olfactory bulb were not significantly different between *pdk1*^{hm} and *pdk1*^{WT} mice. Also BDNF and NGF concentrations in the olfactory bulb were not significantly different between genotypes.

Table 2: Neurotransmitter and neurotrophin concentrations in the amygdala. Data are presented as means ± SEM. The levels of 5-HT, 5-HIAA, NA, DA, NGF and BDNF are expressed as pg/ml tissue, the levels of GABA, glutamate and taurine as pmol/mg tissue.

Substance	WT (n=16)	PDK1 (n=12)	Significance
5-HT	1122.3 ± 36.6 pg/ml	1019.4 ± 20.7 pg/ml	p = 0.034
5-HIAA	805.6 ± 38.0 pg/ml	885.7 ± 62.9 pg/ml	n.s.
5-HIAA/5-HT	0.668 ± 0.032	0.805 ± 0.063 pg/ml	p = 0.046
NA	383.5 ± 19.5 pg/ml	461.5 ± 23.8 pg/ml	p = 0.017
DA	285.2 ± 29.8 pg/ml	258.9 ± 39.5 pg/ml	n.s.
GABA	3.1 ± 0.1 pmol/mg	2.85 ± 0.05 pmol/mg	p = 0.015
Glutamate	11.94 ± 0.26 pmol/mg	12.1 ± 0.5 pmol/mg	n.s.
Taurine	12.8 ± 0.2 pmol/mg	11.87 ± 0.35 pmol/mg	p = 0.023
NGF	5.51 ± 0.52 pg/ml	5.6 ± 0.4 pg/ml	n.s.
BDNF	20.2 ± 2.4 pg/ml	24.4 ± 3.3 pg/ml	n.s.

Table 3: Neurotransmitter and neurotrophin concentrations in the olfactory bulb. Data are presented as means ± SEM. The levels of 5-HT, 5-HIAA, NA, DA, NGF and BDNF are expressed as pg/ml tissue, the levels of GABA, glutamate and taurine as pmol/mg tissue.

Substance	WT (n=16)	PDK1 (n=12)	Significance
5-HT	561.6 ± 16.9 pg/ml	614.0 ± 31.1 pg/ml	n.s.
5-HIAA	796.7 ± 25.5 pg/ml	945.9 ± 75.6 pg/ml	p = 0.047
5-HIAA/5-HT	1.314 ± 0.039	1.481 ± 0.078 pg/ml	n.s.
NA	195.3 ± 12.0 pg/ml	243.1 ± 12.0 pg/ml	p = 0.010
DA	248.5 ± 12.1 pg/ml	263.4 ± 17.6 pg/ml	n.s.
GABA	9.54 ± 0.19 pmol/mg	8.87 ± 0.18 pmol/mg	p = 0.019
Glutamate	8.0 ± 0.2 pmol/mg	7.9 ± 0.3 pmol/mg	n.s.
Taurine	18.66 ± 0.37 pmol/mg	17.84 ± 0.94 pmol/mg	p = 0.021
NGF	10.54 ± 1.19 pg/ml	11.75 ± 1.94 pg/ml	n.s.
BDNF	8.71 ± 1.24 pg/ml	8.87 ± 1.59 pg/ml	n.s.

The preparation of the brains and the determination of transmitter concentrations was done in cooperation with and principally by the laboratory group of Professor Undine Lang (M.D.), Department of Psychiatry and Psychotherapy, Charité University Medicine Berlin, Campus Mitte, Berlin, and Professor Heide Hörtnagl (M.D.), Department of Pharmacology, Charité University Medicine Berlin, Campus Mitte, Berlin.

3.2 Behavioral studies with gsk3^{KI} mice

3.2.1 Open-field test

In the open-field test, both speed (Fig.10A) and total distance traveled (Fig.10B) were significantly increased in *gsk3*^{KI} mice when compared to *gsk3*^{WT} mice, and all subjects showed significant habituation, i.e. a parallel decrease in distance moved over time, independently of genotype. While generally preferring the border area, the fractions of time spent within the border zone and at the center, respectively, were similar in *gsk3*^{KI} and *gsk3*^{WT} mice (Fig.11A), whereas the distance moved in the border zone was significantly elevated in *gsk3*^{KI} mice (Fig.11B). Moreover, the duration (Fig.12A) and number (Fig.12B) of rearings at the border of the open-field were significantly greater in *gsk3*^{KI} mice than in *gsk3*^{WT} mice, as was the ratio between time of and number of rearing (Fig.12C). This indicates a more curious and active phenotype of *gsk3*^{KI} mice compared to *gsk3*^{WT} mice. Focusing on the center, *gsk3*^{KI} mice did not perform significantly more or fewer rearings than did *gsk3*^{WT} mice, and the number of visits of the center and the distance traveled in the center were tendentially elevated in *gsk3*^{KI} mice (Table 4).

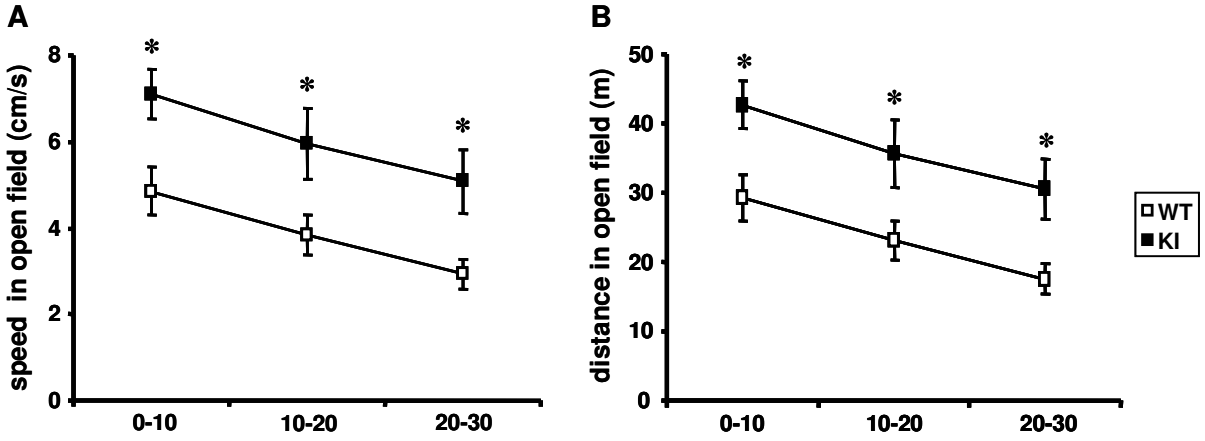

Figure 10: Speed and distance traveled of *gsk3*^{WT} and *gsk3*^{KI} mice in the open-field

A. Arithmetic means ± SEM (n = 10-11 each group) of speed in the open-field during the first, second and third 10 minutes. *gsk3*^{WT}: 4.86 ± 0.55cm/s, 3.83 ± 0.47cm/s, 2.93 ± 0.35cm/s vs. *gsk3*^{KI}: 7.11 ± 0.57cm/s, 5.95 ± 0.82cm/s, 5.08 ± 0.74cm/s

B. Arithmetic means ± SEM (n = 10-11 each group) of distanced traveled in the open-field during the first, second and third 10 minutes. *gsk3*^{WT}: 29.12 ± 3.32m, 23.01 ± 2.85m, 17.55 ± 2.09m, *gsk3*^{KI}: 42.69 ± 3.42m, 35.68 ± 4.92m, 30.46 ± 4.43m

* indicates significant difference between genotypes (repeated measures ANOVA, $p < 0.05$)

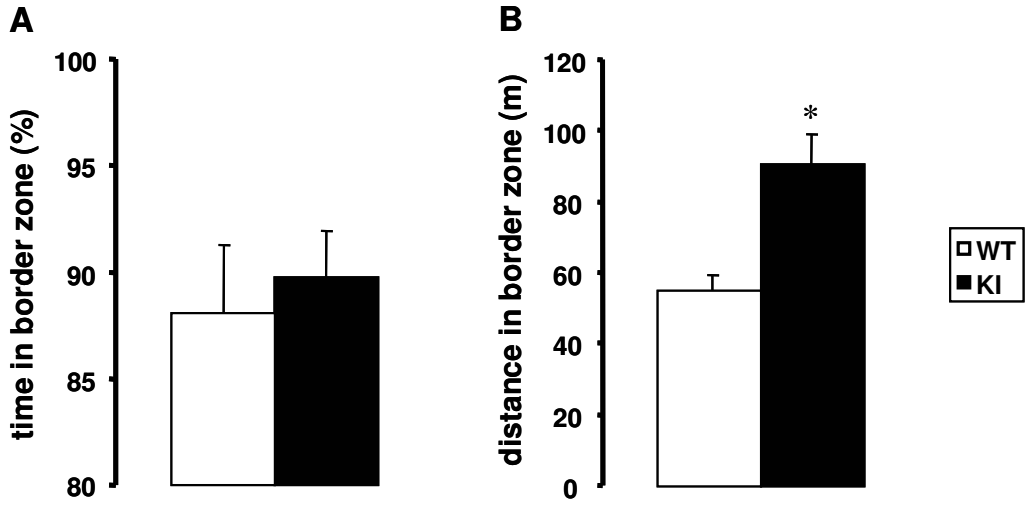

Figure 11: Time of and distance moved of *gsk3*^{WT} and *gsk3*^{KI} mice in the border zone of the open-field

A. Arithmetic means ± SEM (n = 10-11 each group) of time spent (%) in the border zone of the open-field. *gsk3*^{WT}: 88.03 ± 3.2%, *gsk3*^{KI}: 89.81 ± 2.11%; no significant difference between genotypes

B. Arithmetic means ± SEM (n = 10-11 each group) of distance moved (m) in the border zone of the open-field. *gsk3*^{WT}: 54.87 ± 4.62%, *gsk3*^{KI}: 90.75 ± 8.42%; p=0.001

* indicates a significant difference between genotypes (t-test, $p < 0.05$)

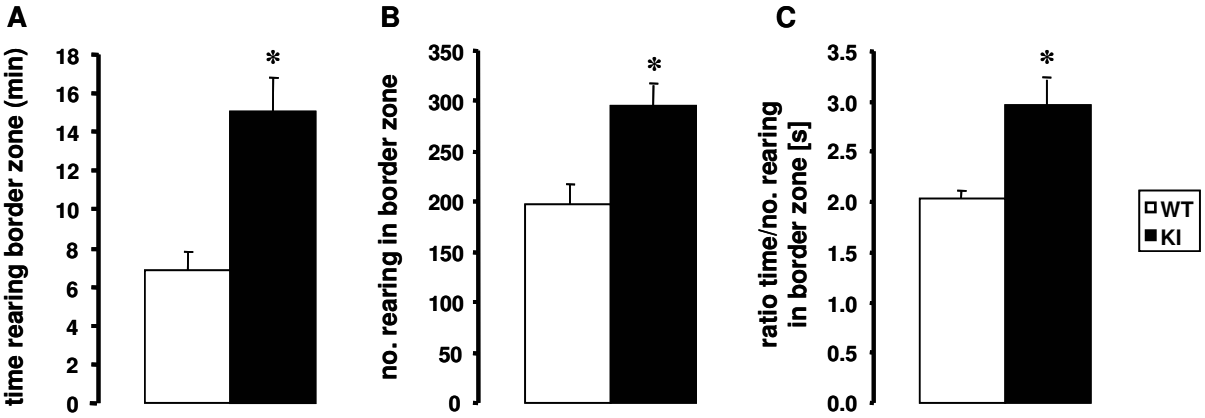

Figure 12: Time of and number of rearings of *gsk3*^{WT} and *gsk3*^{KI} mice in the border zone of the open-field

A. Arithmetic means ± SEM (n = 10-11 each group) of time of rearings (min) in the border zone of the open-field. *gsk3*^{WT}: 6.87 ± 0.92min, *gsk3*^{KI}: 15.02 ± 1.79min; p=0.0054

B. Arithmetic means ± SEM (n = 10-11 each group) of number of rearings in the border zone of the open-field. *gsk3*^{WT}: 197.27 ± 20.86min, *gsk3*^{KI}: 296.2 ± 19.98min; p=0.003

* Indicates a significant difference between genotypes (t-test, $p < 0.05$)

Table 4: Performance of *gsk3*^{WT} and *gsk3*^{KI} mice in the open-field

Behavioral element	*gsk3*^{WT} mice	*gsk3*^{KI} mice	p-value
No. of rearings in center	35.27 ± 12.55	50.1 ± 13.38	n.s.
Time of rearings in center	1.39 ± 0.55 min	1.88 ± 0.57 min	n.s.
Ratio time/no. rearing in center	2.1 ± 0.18 s	2.04 ± 0.18 s	n.s.
No. of visits of the center	78.0 ± 17.89	94.0 ± 17.46	n.s.
Distance traveled in center	14.81 ± 3.9 m	18.08 ± 3.98 m	n.s.

3.2.2 Light-dark box test

In the light-dark box test, both genotypes preferred to stay in the box (78.5% in *gsk3*^{KI} mice vs. 87.4% in *gsk3*^{WT} mice, no significant difference between genotypes). *Gsk3*^{KI} mice entered the dark box significantly more often than did *gsk3*^{WT} mice (Fig.13A), and, as in the open-field test, *gsk3*^{KI} mice performed significantly more rearings than did *gsk3*^{WT} mice, both in the dark box and in the illuminated compartment (Fig.13B and 13C). The average duration of a rearing was similar in both genotypes and reached a ratio from 1.46 to 2.17s/rearing (Table 5). Concerning the intensity of transitions, g*sk3*^{KI} mice visited the entrance area more often (Fig.14A), spent more time (Fig.14B) and traveled a longer distance in the entrance area (Fig.14C). In general, *gsk3*^{KI} mice traveled a significantly longer distance than did *gsk3*^{WT} mice (Table 5).

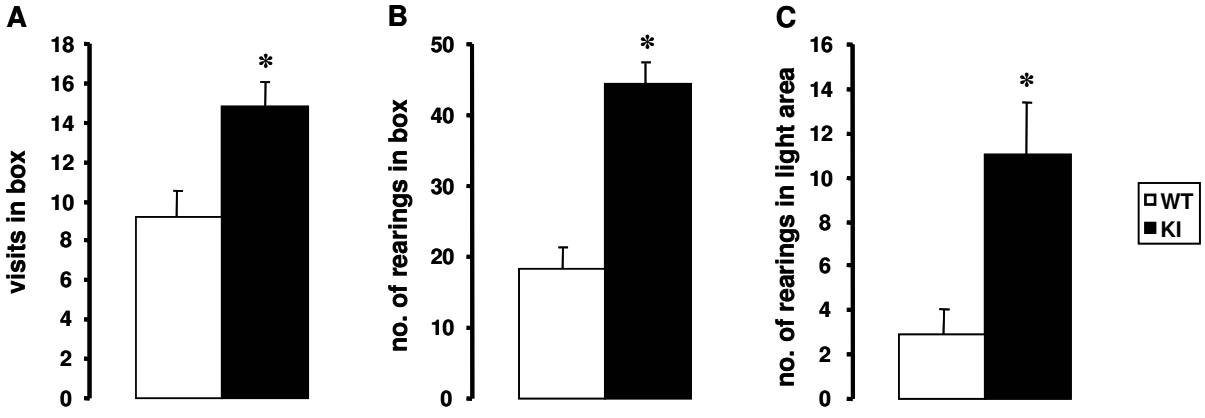

Figure 13: General performance of *gsk3*^{WT} and *gsk3*^{KI} mice in the light-dark box

A. Arithmetic means ± SEM (n = 11 each group) of number of visits into the box. *gsk3*^{WT}: 9.18 ± 1.37, *gsk3*^{KI}: 14.82 ± 1.26; p=0.007

B. Arithmetic means ± SEM (n = 11 each group) of number of rearings in box. *gsk3*^{WT}: 18.27 ± 3.02, *gsk3*^{KI}: 44.45 ± 2.79; p=3.3*10^{-6}

C. Arithmetic means ± SEM (n = 11 each group) of number of rearings in light area, $gsk3^{WT}$: 2.91 ± 1.07, $gsk3^{KI}$: 11.0 ± 2.36; p=0.005

* Indicates a significant difference between genotypes (t-test, $p < 0.05$)

Table 5: Performance of $gsk3^{WT}$ and $gsk3^{KI}$ mice in the light-dark box

Behavioral element	$gsk3^{WT}$ mice	$gsk3^{KI}$ mice	p-value
Time of rearings in the box	26.76 ± 4.82 s	87.96 ± 7.71 s	0.0000015
Time of rearings in the light	4.48 ± 1.75 s	23.85 ± 6.49 s	0.009
Ratio time/no. rearing in the box	1.43 ± 0.04 s	1.95 ± 0.08 s	0.0000053
Ratio time/no. rearing in the light	1.44 ± 0.15 s	1.90 ± 0.21 s	n.s.[2]
Distance traveled in center	2.04 ± 0.50 m	4.48 ± 0.65 m	0.007

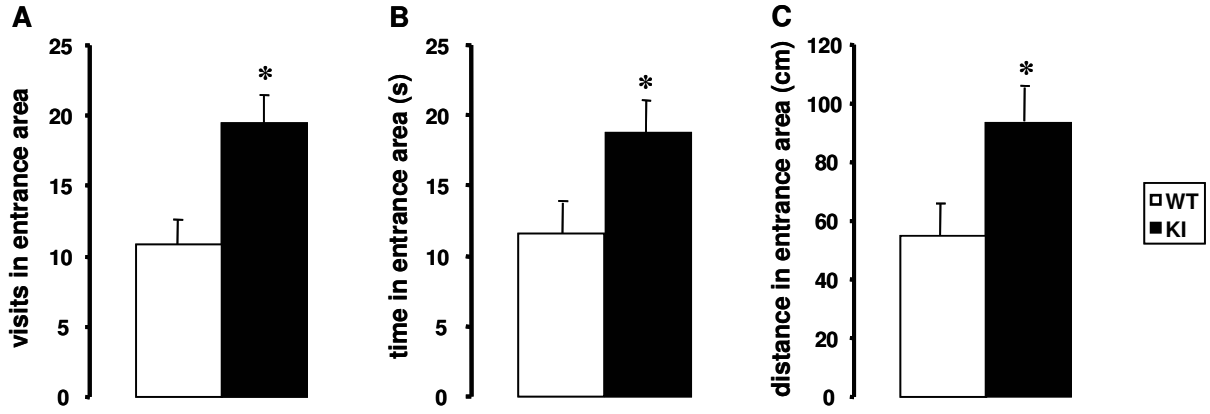

Figure 14: Performance of $gsk3^{WT}$ and $gsk3^{KI}$ mice in the transition area of the light-dark box

A. Arithmetic means ± SEM (n = 11 each group) of number of visits of the transition area. $gsk3^{WT}$: 10.82 ± 1.82, $gsk3^{KI}$: 19.45 ± 1.99; p=0.004

B. Arithmetic means ± SEM (n = 11 each group) of time spent in the transition area. $gsk3^{WT}$: 11.57 ± 2.26s, $gsk3^{KI}$: 18.76 ± 2.28s; p=0.037

C. Arithmetic means ± SEM (n = 11 each group) of distance traveled in the transition area. $gsk3^{WT}$: 54.81 ± 11.28cm, $gsk3^{KI}$: 93.06 ± 11.53cm; p=0.028

* Indicates a significant difference between genotypes (t-test, $p < 0.05$)

[2] Only 8 of 11 $gsk3^{WT}$ mice performed rearings in the illuminated compartment.

3.2.3 O-maze test

In the O-maze test, *gsk3*^{KI} mice spent significantly more time (Fig.15A) and traveled a larger distance (Fig.15B) in the open, unprotected area than did *gsk3*^{WT} mice. The number of protected head dips, performed while the back of the mouse was between the protecting walls, was not different between the genotypes (Fig.16A). However, in the unprotected area, *gsk3*^{KI} mice looked into the deep significantly more often than did *gsk3*^{WT} mice; so-called unprotected head dips were increased in *gsk3*^{KI} mice (Fig.16B). This indicates that *gsk3*^{KI} mice are more prepared to take a risk than *gsk3*^{WT} mice. Similar to what had been observed in the open-field test, both the total distance traveled and speed were significantly larger in *gsk3*^{KI} mice than in *gsk3*^{WT} mice (Table 6). As many as 8 out of 11 *gsk3*^{KI} mice and only one out of 11 *gsk3*^{WT} mice visited the opposite area, whereas there was no significant difference in the time it took the mice to enter the opposite protected area. *Gsk3*^{KI} mice spent significantly less time in the area of origin than did *gsk3*^{WT} mice (Table 6), stressing once more their more curious and active behavior.

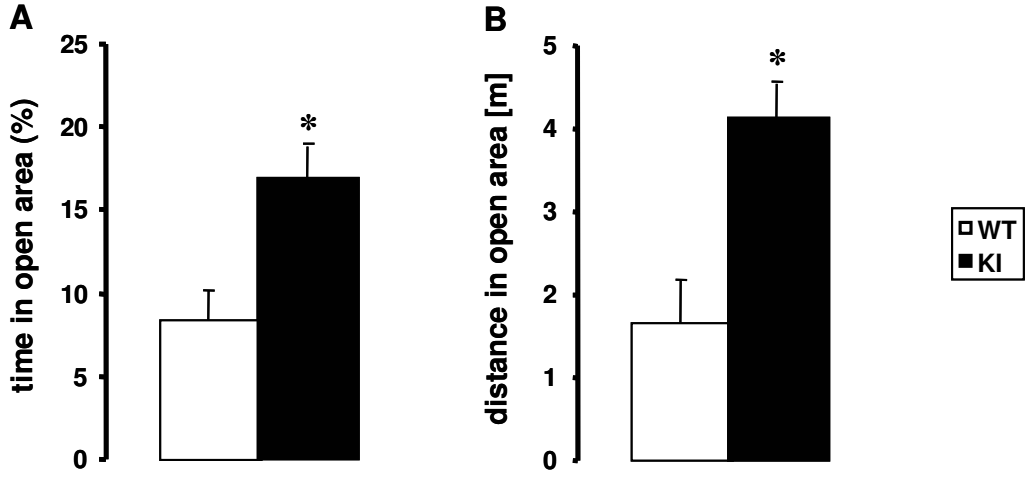

Figure 15: Time and distance traveled of *gsk3*^{WT} and *gsk3*^{KI} mice in the open area of the O-maze

A. Arithmetic means ± SEM (n = 11 each group) of time in the open area. *gsk3*^{WT}: 8.40 ± 1.81%, *gsk3*^{KI}: 16.96 ± 1.87%; p=0.004

B. Arithmetic means ± SEM (n = 11 each group) of distance traveled in the open area. *gsk3*^{WT}: 1.66 ± 0.52m, *gsk3*^{KI}: 4.13 ± 0.42m; p=0.0015

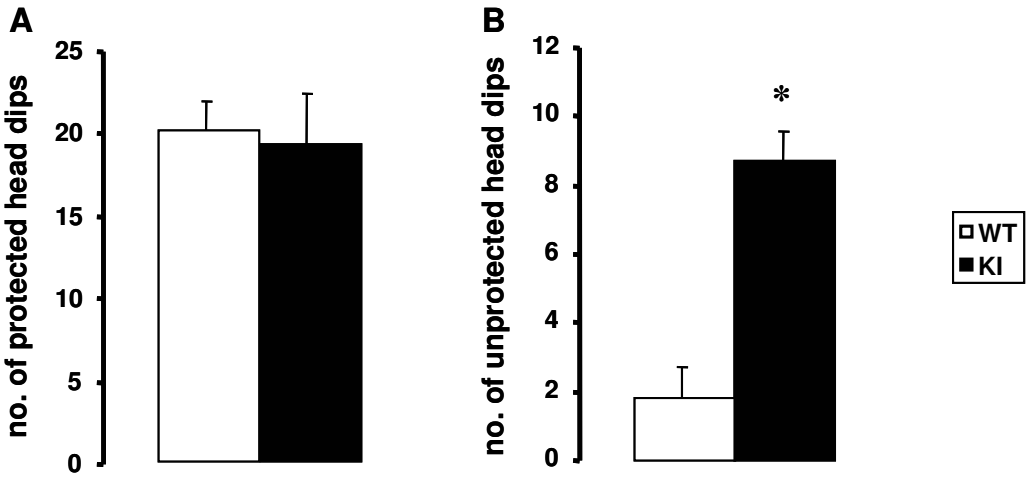

Figure 16: Number of protected and unprotected head dips in the O-maze

A. Arithmetic means ± SEM (n = 11 each group) of protected head dips. $gsk3^{WT}$: 20.09 ± 1.78, $gsk3^{KI}$: 19.27 ± 2.99; n.s

B. Arithmetic means ± SEM (n = 11 each group) of unprotected head dips. $gsk3^{WT}$: 1.82 ± 0.92, $gsk3^{KI}$: 8.73 ± 0.83; p=0.000019

Table 6: Performance of $gsk3^{WT}$ and $gsk3^{KI}$ mice in the O-maze

Behavioral element	**$gsk3^{WT}$ mice**	**$gsk3^{KI}$ mice**	**p-value**
Total distance traveled	15.0 ± 1.32 m	19.7 ± 1.31 m	0.020
Speed	2.5 ± 0.22 cm/s	3.28 ± 0.22 cm/s	0.020
Latency to enter opposite arm	2.59 min	3.26 ± 0.55 min	n.s.
Time in area of origin	88.21 ± 4.73 %	69.86 ± 4.87 %	0.014

3.2.4 Emergence test

In the emergence test, a well-known home box was put into the open-field, and the mouse was observed for 30min. Both $gsk3^{KI}$ and $gsk3^{WT}$ mice spent more than 60% of the time in the home box area (Table 7). The distance moved in this area and the number of visits into the home box tended to be higher in $gsk3^{KI}$ mice (Table 4), indicating enhanced activity of $gsk3^{KI}$ mice in a familiar environment. Both speed and distance moved were significantly elevated in $gsk3^{KI}$ mice in the first 10min (Fig.17A and 17B). $Gsk3^{KI}$ mice then showed a significantly higher rate of habituation than that of $gsk3^{WT}$ mice.

Table 7: Performance of *gsk3*^{WT} and *gsk3*^{KI} mice in the emergence test

Behavioral element	*gsk3*^{WT} mice	*gsk3*^{KI} mice	p-value
Time in home box area	60.94 ± 8.32 %	64.33 ± 6.63 %	n.s.
Distance in home box area	26.06 ± 1.51 m	30.51 ± 1.59 m	n.s.
Visits in box	84.0 ± 9.26	109.2 ± 14.79	n.s.

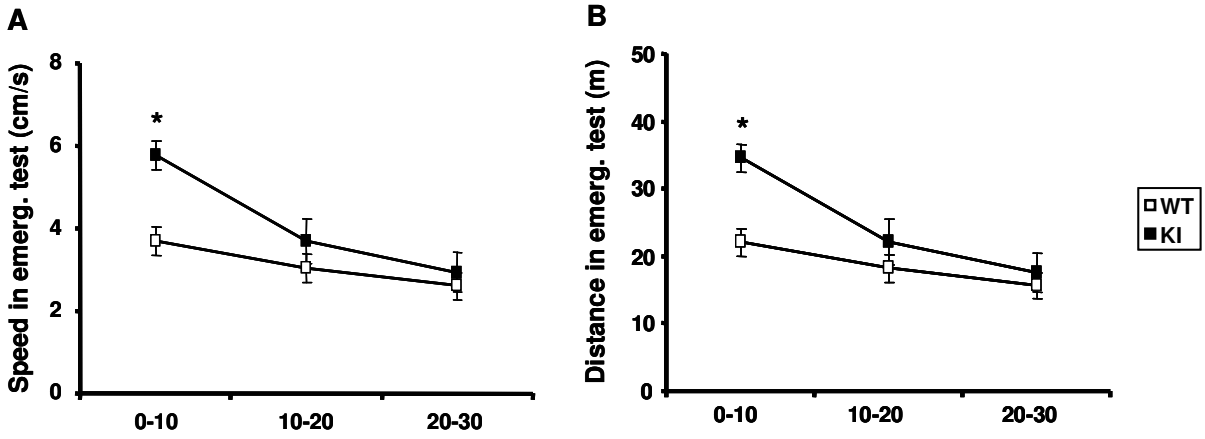

Figure 17: Speed and total distance traveled of *gsk3*^{WT} and *gsk3*^{KI} mice in the emergence test

A. Arithmetic means ± SEM (n = 10 each group) of speed in the emergence test during the first, second and third 10 minutes. *gsk3*^{WT}: 3.68 ± 0.34cm/s, 3.04 ± 0.35cm/s, 2.60 ± 0.32cm/s; *gsk3*^{KI}: 5.75 ± 0.35cm/s, 3.69 ± 0.55cm/s, 2.94 ± 0.49cm/s

B. Arithmetic means ± SEM (n = 10-11 each group) of distanced traveled in the emergence test during the first, second and third 10 minutes. *gsk3*^{WT}: 22.08 ± 2.01m, 18.22 ± 2.09m, 15.58 ± 1.89m; *gsk3*^{KI}: 34.51 ± 2.07m, 22.15 ± 3.32m, 17.62 ± 2.93m

* Indicates significant difference between genotypes (repeated measures ANOVA, $p < 0.05$)

3.2.5 Novel-object test

In the novel-object test, the first 30min of the experiment were identical to the emergence test. Then, a 50ml falcon tube was put as a novel-object in the arena and the mouse was observed for another 30min to see the reaction to a novel-object within an otherwise familiar environment, the home box. In general, mice preferred to stay in the home box, both in the absence and in the presence of the novel-object (Table 8). *Gsk3*^{KI} mice tended to visit the novel-object more often and to spend more time exploring the object (Table 5), which can be interpreted as increased curiosity. The latency to the first visit of the object tended to be decreased in *gsk3*^{KI} mice, indicating a faster approach to unknown objects and less fear (Fig.18A). Furthermore, the time spent in the corners far away from the object was

significantly increased in *gsk3*^{WT} mice, which can be interpreted as avoidance of the object (Fig.18B). Speed and total distance traveled decreased over time in both *gsk3*^{WT} and *gsk3*^{KI} mice, without significant effects between genotypes.

Table 8: Performance of *gsk3*^{WT} and *gsk3*^{KI} mice in the novel-object test

Behavioral element	*gsk3*^{WT} mice	*gsk3*^{KI} mice	p-value
Time in home box in absence of object	53.4 ± 8.7 min	69.4 ± 7.0 min	n.s.
Time in home box in presence of object	55.3 ± 9.3 min	72.2 ± 8.6 min	n.s.
Visits of object	12.55 ± 7.01	45.18 ± 24.21	n.s.
Time spent exploring the object	7.36 ± 4.70 s	23.95 ± 11.11 s	n.s.

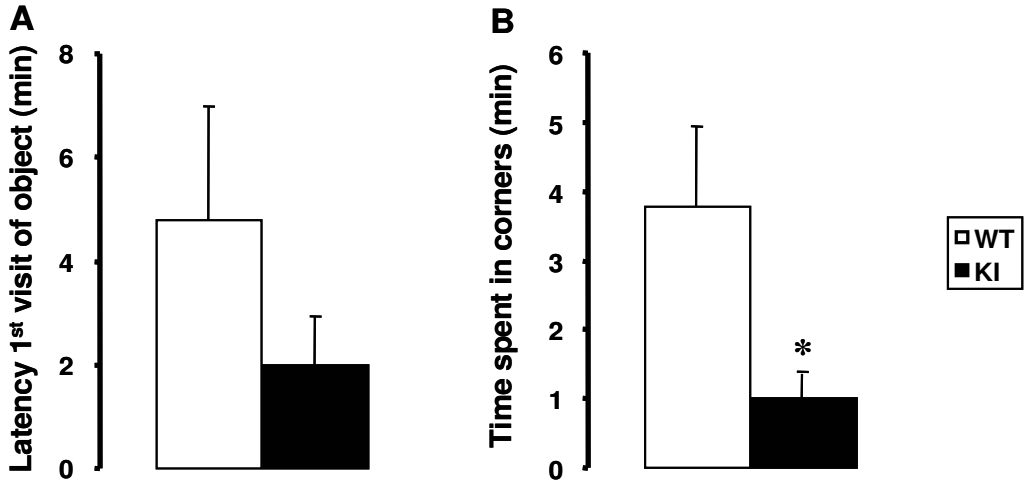

Figure 18: Reaction of *gsk3*^{WT} and *gsk3*^{KI} mice on a novel-object

A: Arithmetic means ± SEM (n = 11 each group) of latency to the first visit of the novel-object. *gsk3*^{WT}: 4.79 ± 2.21min, *gsk3*^{KI}: 1.99 ± 0.96min; n.s.

B: Arithmetic means ± SEM (n = 11 each group) of time spent in corners during presence of the novel-object. *gsk3*^{WT}: 3.78 ± 1.15min, *gsk3*^{KI}: 1.02 ± 0.35min; p=0.033

3.2.6 Forced-swimming test

In the Forced-swimming test, *gsk3*^{KI} mice spent significantly less time floating than did *gsk3*^{WT} mice (Fig.19), which indicates a less depressive behavior.

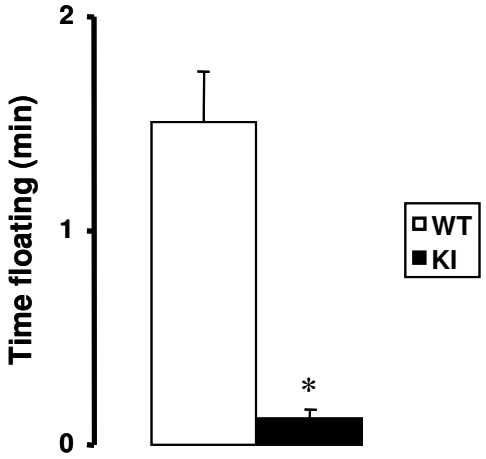

Figure 19: Time floating of *gsk3*^{WT} and *gsk3*^{KI} mice in the forced-swimming test

Arithmetic means ± SEM (n = 7 each group) of time spent floating. *gsk3*^{WT}: 1.51 ± 0.24min, *gsk3*^{KI}: 0.12 ± 0.05min; p=0.000096

3.2.7 Body weight, food and fluid intake, and hormones

Body weight and food and fluid intake were determined during the behavioral studies. There was no difference in body weight, while food and fluid intake were significantly increased in *gsk3*^{KI} mice (Fig.20A and 20B). To determine the function of the hypothalamic-pituitary-adrenal (HPA) axis, cortisol and adrenocorticotropic hormone (ACTH) levels were measured in *gsk3*^{WT} and *gsk3*^{KI} mice. ACTH was significantly elevated in *gsk3*^{WT} mice (Fig.21A), and cortisol tended to be increased (Fig.21B).

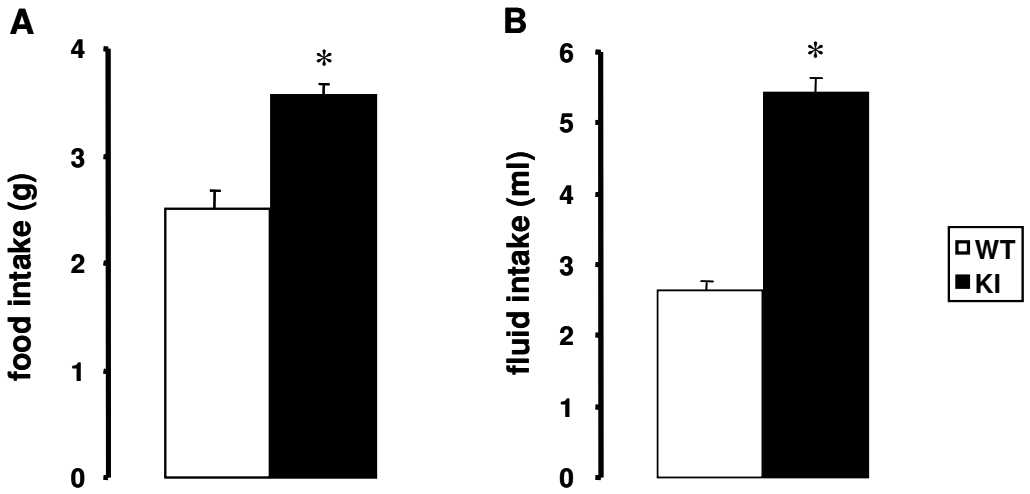

Figure 20: Food and fluid intake of *gsk3*^{WT} and *gsk3*^{KI} mice

A: Arithmetic means ± SEM (n = 11 each group) of food intake. *gsk3*^{WT}: 2.51 ± 0.16g, *gsk3*^{KI}: 3.56 ± 0.12g; p=0.000043

B: Arithmetic means ± SEM (n = 11 each group) of food intake. $gsk3^{WT}$: 2.63 ± 0.10ml, $gsk3^{KI}$: 5.42 ± 0.23g; $p=4.43x10^{-10}$

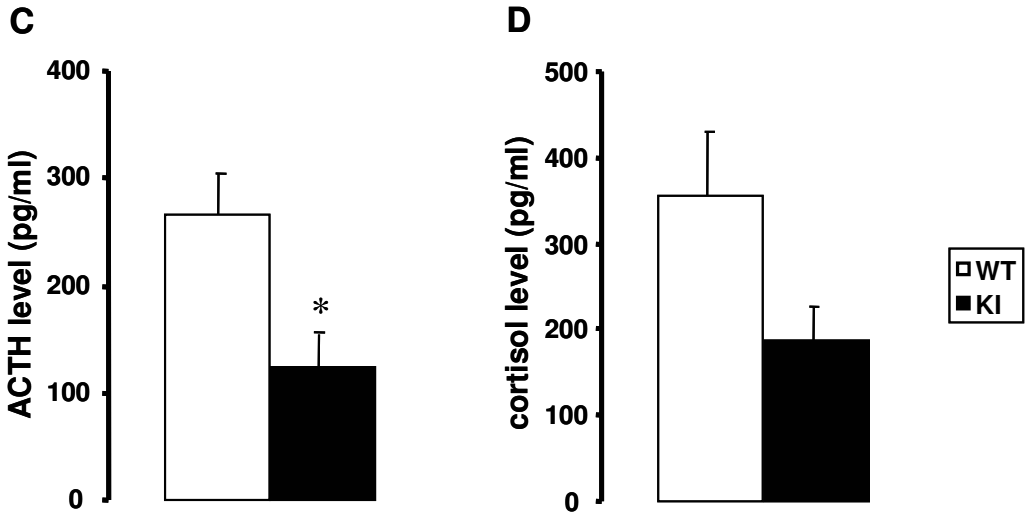

Figure 21: ACTH and cortisol levels in plasma of $gsk3^{WT}$ and $gsk3^{KI}$ mice

A: Arithmetic means ± SEM (n = 11-13 each group) of ACTH levels. $gsk3^{WT}$: 265.03 ± 39.0pg/ml, $gsk3^{KI}$: 124.5 ± 29.3pg/ml; p=0.0077

B: Arithmetic means ± SEM (n = 7-8 each group) of cortisol levels. $gsk3^{WT}$: 356.29 ± 74.74pg/ml, $gsk3^{KI}$: 187.74 ± 39.15pg/ml; p=0.078, n.s.

4. Discussion

4.1 Study with *pdk1^{hm} mice*

Behavioral examination of the *pdk1^{hm}* phenotype revealed increased anxiety behavior in several different tests, i.e. open-field test, light-dark box test, and O-maze test, all of which was accompanied by changes in GABA, NA, and 5-HT concentrations in the amygdala and olfactory bulb.

As reported earlier (Lawlor et al., 2002), body weight in *pdk1^{hm}* mice was significantly reduced, and this was proportional to decreases in brain weight. This decrease appeared to be primarily due to decreased cell volume rather than reduction of cell number (ibid). Among other factors, cell volume and brain metabolism are determined by cellular uptake of amino acids (Lang et al., 1998). The influence of PDK1 deficiency on brain neurotransmitter abundance and anxiety behavior could thus be related to altered amino acid transport. PDK1 activates the serum and glucocorticoid inducible kinase (SGK1) and protein kinase B (PKB)/Akt isoforms (Lang and Cohen, 2001), which in turn have been shown to regulate a wide variety of transporters (Lang et al., 2006a). Previous experiments in *pdk1^{hm}* mice indeed revealed a decreased activity of glucose transporters (Artunc et al., 2006) and amino acid transporters (Rexhepaj et al., 2006). Moreover, an increased fluid and food intake has been reported in *pdk1^{hm}* mice, while urinary excretion of glutamate and tryptophan, but not serotonin, is increased in *pdk1^{hm}* mice (ibid). However, plasma concentrations of these cerebrally relevant amino acids (glutamate, tryptophan, glycin) in *pdk1^{hm}* mice did not differ significantly from those of *pdk1^{WT}* mice (ibid). Similarly, dopamine and glutamate concentrations in amygdala and olfactory bulb were not significantly different between *pdk1^{hm}* and *pdk1^{WT}* mice (Tables 1 and 2).

The behavioral experiments revealed that *pdk1^{hm}* mice differed from wild-type littermates in several behavioral measurements related to anxiety and exploration. Hypoactivity of *pdk1^{hm}* mice is demonstrated by the tendency to initially move less distance in the open-field test (Fig.4), by less frequent light-dark transitions in the light-dark box experiment (Fig.5), and by the observation that *pdk1^{hm}* mice spent more time inside the protected sectors and performed fewer protected head dips in the O-maze (Fig.6). In the emergence test (open-field with home box), *pdk1^{hm}* mice spent more time inside the home box, but showed normal exploratory activity while outside the box (Fig.7-8). This observed normal exploratory activity in the emergence test does not fit to increased anxiety behavior,

but might be explained by agitation (Arban et al., 2005; Gould et al., 2007; Shaltiel et al., 2008).

Increased anxiety behavior in *pdk1*^{hm} mice can be linked to decreased activation of the PI3K/Akt pathway, which has been shown to contribute to mechanisms of synaptic plasticity and memory consolidation by promoting cell survival and synapse number (Horwood et al., 2006; Martin-Pena et al., 2006). After activation of PI3K, PDK1 is activated and leads to phosphorylation of PKB/Akt. Activated PKB/Akt phosphorylates intracellular substrates and promotes cell survival by inhibiting apoptosis through several targets, including Forkhead transcription factors and mammalian target of rapamycin (Martin-Pena et al., 2006). One of the essential functions of PKB/Akt is the phosphorylation of glycogen synthase kinase (GSK-3), causing its inactivation and thereby the activation of cAMP response element binding protein (CREB) (Peltier et al., 2007). Decreased CREB function in the central amygdala was suspected to be involved in anxiety behavior (Pandey et al., 2003; Pandey et al., 2005), and a decreased CREB phosphorylation after BDNF antisense infusion was found (Pandey et al., 2006), possibly attributable to decreased PI3K activity. Recently, it was shown that fear learning in the amygdala involves BDNF and PI3K activation (Ou and Gean, 2006).

A role for the PI3K signaling cascade in anxiety behavior is plausible, as several factors influencing anxiety behavior act via PI3K. Recently, it was demonstrated in vivo that anxiolytic cannabinoids activate the PI3K/Akt pathway and negatively regulate GSK-3ß activity in the mouse brain (Ozaita et al., 2007). Also, insulin-like growth factor-1 (IGF-1) and estrogen have been shown to disrupt anxiety behavior and to activate PI3K (Garcia-Segura et al., 2006; Malberg et al., 2007; Toufexis et al., 2006). Moreover, exercise, with its well-known anxiolytic properties, activates PI3K pathway (Chen and Russo-Neustadt, 2005; Salmon, 2001). BDNF has also been related to anxiety behavior (Lang et al., 2005b; Lang et al., 2005a) and fear conditioning, especially in the amygdala (Chhatwal et al., 2006; Opazo et al., 2003; Ou and Gean, 2006), and BDNF acts via PI3K (Rattiner et al., 2004; Sanna et al., 2002). In this context, we expected a compensatory upregulation of BDNF and NGF in *pdk1*^{hm} mice which was, however, not observed (Tables 2 and 3). It should be kept in mind that PI3K signaling in *pdk1*^{hm} mice is not completely abrogated, while PDK1 activity is suppressed by up to 80-90% (Lawlor et al., 2002). The residual PDK1 activity may be sufficient to prevent upregulation of BDNF. In any case, the findings of increased anxiety associated with deficient PI3K signaling suggest that BDNF could influence anxiety at least in part via a PI3K-dependent mechanism.

Moreover, several factors regulating PI3K activity might converge and exert additive effects, or, if absent, increase vulnerability. In line with this hypothesis, heterozygous neuroregulin-1 mice are more sensitive to behavioral effects of cannabinoids than are wild-type mice (Boucher et al., 2007). Additive genetic effects of BDNF and 5-HT transporters are dependent on estrogen modulation, so estrogen seems to compensate BDNF deficiency regarding behavior and brain monoamine levels (Ren-Patterson et al., 2006). Moreover, running exercise- and antidepressant-induced increases in growth and survival-associated signaling molecules are IGF-dependent (Chen and Russo-Neustadt, 2007).

Interestingly, the increased anxiety behavior in *pdk1*^{hm} mice was accompanied by a significant decrease in GABA, taurine and 5-HT in the amygdala, suggesting an involvement of these neurotransmitters in the determination of behavioral disturbances (Table 2). Moreover, 5-HT turnover was increased in the amygdala and seemed to be increased in the olfactory bulb, as well, suggested by increased concentrations of 5-HIAA (Tables 2 and 3). Furthermore, in the olfactory bulb a decrease in GABA and taurine was observed (Table 3). In both examined brain regions NA concentrations were increased (Tables 2 and 3).

A large body of evidence from rodent and human neuroimaging studies indicates that the amygdala plays a critical role in the acquisition and expression of fear behavior (Davis and Whalen, 2001; Maren and Quirk, 2004), which may explain that neurotransmitter changes in anxious *pdk1*^{hm} mice occur in this brain region. The olfactory bulb has been implicated in anxiety behavior, too (Wang et al., 2007). Olfactory bulbectomy has been suggested as an animal model for comorbid anxiety and leads to hyperexcitability of amygdala neurons (Shibata and Watanabe, 1994; Wang et al., 2007). Indeed, olfactory information converges in the amygdala, where the involvement of PI3K signaling has recently been observed in fear learning (Ou and Gean, 2006).

A decreased GABA concentration in amygdala and olfactory bulb fits well to the concept that GABA is the principal inhibitory neurotransmitter in the mammalian brain, and its receptors are key control elements of anxiety states. This concept is based on the anxiolytic properties of benzodiazepines, which act as allosteric GABA receptor agonists (Nemeroff, 2003). Recently, first evidence for a control of GABAergic function through PI3K-mediated pathways has been reported (Salgado et al., 2007). Moreover, the additional observation of decreased taurine in both brain areas, as observed in *pdk1*^{hm} mice, is in line with the role of taurine as an anxiolytic-like amino acid after single and repeated administrations and its modulation of amygdala-associated anxiety (Kong et al., 2006; McCool and Chappell, 2007).

5-HT is believed to be the most relevant neurotransmitter in mediating anxiety (Murphy et al., 1999). The promotor region of the serotonin transporter gene features a long and a short variant, whereas persons with one or two short forms are more likely to become depressive after a stressful event than are people with two long alleles (Caspi et al., 2003). Therefore, decreased 5-HT concentrations in the amygdala, as found in our *pdk1*^{hm} mice, might well explain increased anxiety behavior. The amygdala is overactive in patients with anxiety disorders, and chronic administration of 5-HT reuptake inhibitors (SSRIs) normalizes amygdalar activity, representing the treatment of choice in anxiety disorders (Muller et al., 2007; Rauch, 2003; Sheline et al., 2001).

The observed decreases of taurine concentrations in amygdala and olfactory bulb of *pdk1*^{hm} mice are similarly in line with increased anxiety behavior in *pdk1*^{hm} mice. In rats, taurine microinjections in the amygdala have been shown to reduce anxiety behavior (McCool and Chappell, 2007), and an anxiolytic effect of taurine (i.p.) was also observed in mice (Kong et al., 2006).

The present study did not address the mechanisms underlying the influence of PDK1 on neurotransmitter levels. It is noteworthy, though, that PI3K signaling has been shown to participate in the regulation of GABA transport (de La Paz et al., 2002; Malyala et al., 2008), and the taurine transporter (Cruz-Rangel et al., 2008; de La Paz et al., 2002; Franco et al., 2001; Pasantes-Morales and Franco, 2002). Kinases activated by PDK1 include the serum and glucocorticoid inducible kinase (SGK) and protein kinase B (PKB/Akt) isoforms, which have been shown to regulate a wide variety of channels and transporters, including glutamate transporters and glutamate receptors (Green et al., 2008; Hills et al., 2006; Ishiki and Klip, 2005; Lang et al., 2006a; Luo and Sun, 2007; Maier et al., 2006; Sato et al., 2007; Sen et al., 2003; Strutz-Seebohm et al., 2007; Tessier and Woodgett, 2006; Whiteman et al., 2002). Moreover, SGK and PKB phosphorylate and, thus, inhibit glycogen synthase kinase GSK-3ß (Wyatt et al., 2006), which plays a pivotal role in the regulation of behavior (Gould and Manji, 2005; Lang et al., 2007; Rowe et al., 2007). Reduced locomotion reminiscent of the PDK1 hypomorphic mouse was observed in SGK3-deficient mice (Lang et al., 2006b).

Finally, unpublished commercial observations revealed that patients who received a PDK1 inhibitor within their anti-cancer treatment had depressive symptoms as side effects. This finding supports our hypothesis that PDK1 plays an important role in anxiety and mood-related disorders.

4.2 Study with *gsk3^{KI}* mice

The study that characterizes the behavior of *gsk3^{KI}* mice revealed a role of PKB/SGK-dependent regulation of GSK-3 in the control of behavior. Mice carrying a mutation of GSK-3α in which the codon encoding Ser21 was changed to encode a nonphosphorylatable Ala residue (GSK-3α 21A/21A), and at the same time carrying a mutation of GSK-3β in which the codon encoding Ser9 was changed to encode a nonphosphorylatable Ala residue (GSK-3ß 9A/9A), are expected to be resistant to PKB/SGK-dependent regulation of GSK-3 (McManus et al., 2005). According to the present observations, mice carrying the PKB/SGK-resistant GSK-3 mutants (*gsk3^{KI}*) are significantly more active and curious than were the corresponding wild-type mice (*gsk3^{WT}*) in several behavioral settings. The enhanced locomotor activity has been considered a correlate of mania (Lyon, 1999; Machado-Vieira et al., 2004).

In line with behavioral parameters, the function of the HPA axis is changed between the phenotypes: In *gsk3^{KI}* mice ACTH was significantly decreased and cortisol tended to be so (Fig.21). A hypothesis has been formulated, relating aberrant stress hormone regulation to causality of depression, which suggests that the amount of ACTH and cortisol is significantly higher among depressive patients (Thomson and Craighead, 2008). Moreover, the hypothesis was raised that antidepressants act through normalization of these HPA changes, and several antidepressant treatment strategies have been developed targeting the HPA axis, e.g. cortisol biosynthesis inhibitors, glucocorticoid receptor antagonists, corticotrophin releasing factor receptor antagonists, and vasopressin receptor antagonists (Thomson and Craighead, 2008). According to a previous study, plasma concentrations and urinary excretions of aldosterone were similarly altered in *gsk3^{KI}* mice (Boini et al., 2008). Moreover, several functional parameters were different between *gsk3^{KI}* and corresponding wild-type mice (ibid). It must be kept in mind that at least in theory, the metabolic effects of dysregulated peripheral GSK-3 may influence behavior by altering plasma metabolite, electrolyte, and hormone concentrations.

In general, mice are expected to prefer secure and safe places, represented by the border of the open-field test, the dark compartment in the light-dark box experiment, the closed, tunnel-like sections in the O-maze test, and the home box in the emergence and novel-object test. *Gsk3^{WT}* and *gsk3^{KI}* mice comply with those expectations, as both genotypes prefer the mentioned secure zones (Zorner et al., 2003). However, several parameters revealed increased locomotor activity in *gsk3^{KI}* mice, as measured by increased speed and total distance traveled in the open-field test, light-dark box test, O-maze test, and emergence test.

The hyperactivity and increased curiosity of mice might also be evidenced by the increased number of entrances into the dark box (Belzung et al., 1990), as well as the increased time spent in the entrance area of the box. However, the light-dark transitions might also just reflect generally enhanced activity, and the elevated dwell time in the entrance area could also be seen as increased risk assessment of *gsk3*^{KI} mice.

The O-maze test is an established model for investigating anxiety-like behavior. Inherently, mice would avoid passing a dangerous way, as simulated by the open areas. The risk assessment of *gsk3*^{KI} mice, however, did not prevent them from entering the opposite area. *Gsk3*^{KI} mice showed a clearly enhanced risk behavior while spending more time and traveling a longer distance on the open areas, as well as performing more unprotected head dips. This behavior points to reduced anxiety. The fact that *gsk3*^{KI} mice spent less time in the origin arm points to enhanced curiosity and preference to explore the environment, instead of their benefiting from the security in this area (Shepherd et al., 1994). Moreover, reduced anxiety in *gsk3*^{KI} mice is represented by an increased number and duration of rearings in the open-field test and in the light-dark box experiment (Calabrese, 2008).

The emergence test and novel-object test have to be seen from another point of view, as the mouse is inserted into an environment which is partly familiar due to the well-known home box. During the emergence test, speed and total distance traveled were enhanced in *gsk3*^{KI} mice, whereas there was no difference observed during the novel-object test. Apparently, the environment influences the behavior of mice and must be considered in the interpretation of anxiety-related behavior. The insertion of a novel-object emphasized the loss of fear and the increased curiosity of *gsk3*^{KI} mice. Compared to *gsk3*^{WT} mice, *gsk3*^{KI} mice are clearly hyperactive in the open-field test, light-dark box test, and O-maze test. In the emergence test hyperactivity seems less pronounced, and in the object-exploration test there was no hyperactivity. This suggests that hyperactivity is novelty induced, being most pronounced when mice are placed in a completely new environment.

The enhanced activity of the *gsk3*^{KI} mice is reminiscent of the hyperactivity of transgenic mice overexpressing GSK-3 (*gsk3*^{tg}) (Prickaerts et al., 2006). Unlike *gsk3*^{KI} mice, *gsk3*^{tg} mice also showed deficient habituation. Conversely, the stimulating effect of amphetamines on locomotor activity is decreased in heterozygote GSK-3 deficient mice (Beaulieu et al., 2004), and reversed by lithium and with specific GSK-3 inhibitors (Gould et al., 2004).

Increased curiosity, decreased anxiety, decreased immobility in the forced-swimming test, increased activity, and decreased stress hormone levels in *gsk3*^{KI} mice point to a

depression-resistant phenotype. However, disinhibition of PI3K might lead to manic behavior, which is not common. Hypomanic states are often observed following depressive episodes and antidepressant treatment, which could result from an activation of PKB by antidepressant strategies. Indeed, antimanic treatment strategies might involve PKB-dependent regulation of GSK-3, which has been shown by several antimanic drugs (clozapine, olanzapine, lithium, and valproate) (Aubry et al., 2009; Gould and Manji, 2005; Klein and Melton, 1996). Moreover, all of these medications show mood stabilizing properties and long-term phase prophylactic properties, which also might involve GSK-3 regulation.

Overexpression of GSK-3 in the striatum was followed by upregulation of PKB expression and downregulation of the expression of PPP2R3A, a regulatory subunit of the PKB-inactivating phosphatase PP2A (Prickaerts et al., 2006). Those effects were expected to inhibit GSK activity and thus to mitigate the effects of GSK-3 overexpression (ibid). In $gsk3^{KI}$ mice, this negative feedback is disrupted, as similarly elevated PKB levels in $gsk3^{KI}$ and $gsk3^{WT}$ mice after injection of insulin demonstrated (McManus et al., 2005). So, the PI3K activity is normal in $gsk3^{KI}$ mice, leading to a more specific phenotype than is seen in the mouse model with generally overexpressed GSK-3. The unrestrained GSK-3 activity in $gsk3^{KI}$ mice renders those mice an ideal model for the in vivo testing of GSK-3 inhibitors.

PKB and GSK-3 are further thought to participate in the enhanced locomotion of gene-targeted mice lacking the dopamine transporter DAT (Beaulieu et al., 2004; Beaulieu et al., 2005). As shown earlier (Boini et al., 2008), food and fluid intake were markedly enhanced in $gsk3^{KI}$ mice as compared to $gsk3^{WT}$ mice, pointing to PKB/SGK-dependent regulation of GSK-3 in the control of food and fluid uptake. Typically, food intake is markedly changed in bipolar patients, and increased food intake might be compensated by increased activity in $gsk3^{KI}$. Moreover, disorders of food intake may parallel psychiatric diseases, such as bipolar disorder and schizophrenia (Foulon, 2003; Kishi and Elmquist, 2005; Krishnan, 2005). PKB-dependent signaling in the hypothalamus is considered to mediate the food intake-lowering effect of insulin (Niswender et al., 2003). This signaling may be disrupted in the $gsk3^{KI}$ mice. PKB/SGK-dependent signaling is further required for the effect of insulin on glycogen synthase (Cohen and Goedert, 2004; McManus et al., 2005). Accordingly, the effect of insulin on muscle glycogen synthase is absent in $gsk3^{KI}$ mice (McManus et al., 2005). It would be interesting to explore whether behavioral effects of hyperinsulinism are related to PKB/SGK-dependent regulation of GSK-3.

Interestingly, clozapine and olanzapine, which act on GSK-3 (Rowe et al., 2007), have been shown to induce type 2 diabetes mellitus and metabolic syndrome in schizophrenic and

bipolar patients (Van Winkel et al., 2008). Moreover, patients with schizophrenia and bipolar disorder are particularly prone to develop obesity and type 2 diabetes mellitus (Newcomer, 2006), disorders considered to involve modulation of GSK-3 (Medina and Castro, 2008).

Besides insulin, brain-derived neurotrophic factor BDNF (Corominas et al., 2007; Lim et al., 2008) activates the PI3 kinase pathway (Elliott et al., 2005), and is thus expected to inhibit GSK-3ß activity. BDNF has in turn been implicated in bipolar disorders (Castren and Rantamaki, 2008; Fan and Sklar, 2008; Kapczinski et al., 2008; Kloos et al., 2008; Serretti and Mandelli, 2008) and schizophrenia (Lu and Martinowich, 2008; Pillai, 2008).

The study with GSK-3 knockin mice did not attempt to identify the cerebral structures involved in the altered behavior of $gsk3^{KI}$ mice. However, in the study with PDK1-hypomorphic mice, behavior opposite to that of $gsk3^{KI}$ mice was displayed, and such mice showed significantly decreased GABA and taurine concentrations and significantly increased NA levels in the amygdala and olfactory bulb, as well as significantly decreased 5-HT levels in the amygdala.

5. Conclusions

This study with *pdk1*^{hm} mice revealed that decreased PI3K signaling may provoke anxiety behavior, accompanied by alternations of neurotransmitter concentrations in the amygdala and olfactory bulb that play an important role in anxiety-related diseases and depression. The finding that *pdk1*^{hm} mice show an anxious and hypoactive phenotype implicates a role of the PI3K pathway in anxiety behavior. Thus, decreased PI3K signaling in *pdk1*^{hm} mice might be an animal model for increased anxiety and depression.

Mice with a modification of GSK-3 that leads to insensitivity to the inhibitory action of PKB and SGK are characterized by altered food and fluid intake, as well as hyperactivity, which can be interpreted as manic-like behavior. Moreover, the mutation leads to decreased immobility in the forced-swimming test, and decreased activity of the HPA axis, both indicators of “anti-depressive” behavior. Thus, PKB/SGK-dependent regulation of GSK-3 activity might participate in the pathophysiology and treatment of bipolar disorders, and *gsk3*^{KI} mice can be seen as an animal model for psychiatric diseases, such as bipolar disorder and schizophrenia.

6. Summary

To investigate the impact of the phosphatidylinositol-3 kinase (PI3K) pathway on psychiatric diseases, two mouse models with genetic modifications of the PI3K pathway served for behavioral studies.

On the one hand, there is the PDK1-hypomorphic mouse (*pdk1*^{hm}) that is characterized by a residual activity of the 3-phosphoinositid-dependent protein kinase-1 (PDK1) of only around 10%. A complete knockout of PDK1 is impossible because of embryonic mortality of the offspring. Various different established models used in behavior research of rodents demonstrated that the *pdk1*^{hm} mouse features anxious behavioral patterns. Furthermore, biochemical analysis of brain structures indicated anxious and depressive characteristics of the *pdk1*^{hm} mouse. The amygdala, an important organ for the formation of anxiety, exhibited decreased concentrations of gamma-aminobutyric acid (GABA), serotonin (5-HT), and taurin, as well as elevated levels of noradrenalin (NA). GABA is an important inhibitory neurotransmitter, and anxiety resolving medications, such as benzodiazepines, act as agonists on GABA receptors. Serotonin is an important mediator of fear, as medications used in the treatment of depression have proven: So-called serotonin-reuptake-inhibitors (SSRIs) increase serotonin levels and lead to improved symptoms in patients with major depressive disorders. Taurine has anxiety resolving effects, as demonstrated in animal experiments employing injections of taurin. NA is a neurotransmitter of the central nervous system, on which antidepressants act in a like manner, mitigating the symptoms of depressive people via an increase of NA concentration. The olfactory bulb is linked to anxious behavior, too. Its sensory information converges in the amygdala where the influence of PI3K was observed recently. The olfactory bulb showed, as did the amygdala, decreased concentrations of GABA and taurine, and elevated levels of NA.

The study in *pdk1*^{hm} mice emphasizes the impact of the PI3K pathway in psychiatric diseases. With its characteristic phenotypical and biochemical attributes, the *pdk1*^{hm} mouse can be used as a model for psychiatric diseases, such as anxiety disorders and depression.

On the other hand, investigations were made in a mouse in which the glycogen synthase kinase-3 (GSK-3) was genetically modified so that it was no more inhibitable via protein kinase B (PKB/Akt) and serum- and glucocorticoid-inducable protein kinase (SGK). Thus, GSK-3 is resistant to the inhibitory properties of insulin. The so-called *gsk3*^{KI} mice demonstrated a hyperactive and audacious behavior in comparion to corresponding wild-type mice in several behavioral settings. The increased activity of *gsk3*^{KI} mice can be associated

with manic episodes in psychiatric diseases, such as schizophrenia and bipolar disorders. The HPA axis is elevated in depressive patients, whereas *gsk3*^{KI} mice show a decreased HPA axis, which suggest a phenotype opposite to the depressive *pdk1*^{hm} mouse. Another characteristic of the *gsk3*^{KI} mouse is increased food intake, reminiscent of disturbed-eating behavior in patients with bipolar disorder. Different neuroleptics, amongst them clozapine, olanzapine, and lithium, inhibit GSK-3 and thus act on the PI3K pathway. As insulin inhibits GSK-3 via the PI3K pathway, it is not surprising that those medications lead to diabetes as well as metabolic syndrome.

With its action via the PI3K pathway, insulin exerts a determining influence on behavior. When the effect of insulin is attenuated, as expressed in the *pdk1*^{hm} mouse, anxious and depressive behavior is the consequence. However, when its effect is intensified, as can be seen in the *gsk3*^{KI} mouse, hyperactivity and increased risk assessment are observed, which can be compared to manic episodes in mentally disordered people.

Consequently, the *pdk1*^{hm} mouse describes an animal model for anxiety and depression, whereas the *gsk3*^{KI} mouse represents an animal model for schizophrenia and bipolar disorders.

7. Zusammenfassung

Um die Bedeutung des Phosphatidylinositol 3-kinase (PI3K)-Signalwegs bei psychischen Krankheiten zu untersuchen, wurden Verhaltensstudien an zwei Mausmodellen durchgeführt, die genetische Veränderungen des PI3K-Signalwegs aufzeigen.

Zum einen handelt es sich um die PDK1-hypomorphe Maus (*pdk1*^{hm}), bei der die 3-Phosphoinositid-abhängige Proteinkinase-1 (PDK1) nur noch eine Restaktivität von ca. 10% aufweist. Eine komplette Ausschaltung der PDK1 ist aufgrund embryonaler Mortalität des Nachwuchses nicht möglich. Die *Pdk1*^{hm}-Maus weist ängstliche Verhaltensmuster auf, wie in verschiedenen etablierten Modellen der Verhaltensforschung an Nagern gezeigt werden konnte. Ebenso wiesen biochemische Untersuchungen von Gehirnstrukturen auf ängstlich-depressive Eigenschaften der *Pdk1*^{hm}-Maus hin. Die Amygdala, die ein wichtiges Organ bei der Entstehung von Angst darstellt, wies erniedrigte Konzentrationen von µ-Aminobuttersäure (GABA), Serotonin (5-HT) und Taurin und eine erhöhte Noradrenalin (NA)-Konzentration auf. GABA ist der bedeutendste inhibitorische Neurotransmitter und angstlösende Medikamente wie Benzodiazepine greifen als Agonisten am GABA-Rezeptor an. Serotonin ist ein wichtiger Mediator von Angst, was inbesondere Medikamente, die bei Depression angewendet werden, zeigen: Sogenannte Serotonin-Wiederaufnahme-Inhibitoren (SSRIs) erhöhen den Serotonin-Spiegel und führen zu verbesserten Symptomen bei depressiven Patienten. Taurin hat angstlösende Effekte wie Injektionen mit Taurin im Tierversuch zeigen konnten. NA ist ein Neurotransmitter des Zentralen Nervensytems, an dem ebenfalls Antidepressiva angreifen und durch eine Erhöhung der NA-Konzentration die Symptome depressiver Menschen lindern.

Der Riechkolben, auch Bulbus olfactorius genannt, steht ebenso in Zusammenhang mit ängstlichem Verhalten. Seine Information läuft in der Amygdala zusammen, wo kürzlich der Einfluss von PI3K beobachtet wurde. Der Bulbus olfactorius wies wie die Amygdala verringerte Konzentrationen von GABA und Taurin und eine erhöhte NA-Konzentration auf.

Die Studie an der *Pdk1*^{hm}-Maus unterstreicht die Bedeutung des PI3K-Weges bei psychischen Erkrankungen und die *Pdk1*^{hm}-Maus kann mit ihren charakteristischen phenotypischen und biochemischen Merkmalen als ein Modell für psychische Krankheiten wie Angststörungen und Depression verwendet werden.

Zum anderen wurden Untersuchungen an einer Maus durchgeführt, bei der die Glykogensynthase-Kinase-3 (GSK-3) genetisch so verändert wurde, dass sie nicht mehr durch die Proteinkinase B (PKB/Akt) und Serum- und Glukokorticoid-induzierbare Kinase (SGK)

gehemmt werden kann und somit resistent gegenüber der inhibitorischen Wirkung von Insulin ist. Die sogenannte *Gsk3*^{KI}-Maus zeigte in mehreren Verhaltensversuchen einen hyperaktiven Phänotyp und risikofreudigeres Verhalten im Vergleich zu den entsprechenden Wildtyp-Mäusen. Die gesteigerte Aktivität der *Gsk3*^{KI}-Maus kann in Zusammenhang mit manischen Phasen, wie sie bei den psychischen Krankheiten Schizophrenie und bipolaren Störungen auftreten, gebracht werden. Die HPA-Achse ist bei depressiven Patienten erhöht, wogegen die *Gsk3*^{KI}-Maus eine erniedrigte HPA-Achse aufweist, was ebenfalls auf einen Phänotyp der Maus schließen lässt, der dem depressiven der *Pdk1*^{hm}-Maus entgegen gesetzt ist. Ein weiteres Kennzeichen der *Gsk3*^{KI}-Maus ist eine erhöhte Futteraufnahme, was an gestörtes Essverhalten bei Menschen mit bipolaren Störungen erinnert. Verschiedene Neuroleptika, darunter Clozapin, Olanzapin und Lithium, wirken inhibitorisch auf GSK-3 und greifen damit am PI3K-Weg an. Da Insulin über den PI3K-Weg die GSK-3 hemmt, überrascht es nicht, dass diese Medikamente zu Typ-2 Diabetes und Metabolischem Syndrom führen können.

Insulin hat mit seiner Wirkung über den PI3-Kinase-Weg einen entscheidenden Einfluss auf das Verhalten. Ist seine Wirkung abgeschwächt, wie es bei der *Pdk1*^{hm}-Maus zum Ausdruck kommt, ist ängstliches und depressives Verhalten die Folge. Ist die Insulin-abhängige Wirkung dagegen verstärkt, wie man bei der *Gsk3*^{KI}-Maus erkennen kann, bei der die hemmende Wirkung von Insulin aufgehoben wurde, können Hyperaktivität und gesteigerte Risikobereitschaft beobachtet werden, die mit manischen Phasen in psychisch erkrankten Menschen vergleichbar sind.

Die *Pdk1*^{hm}-Maus stellt somit ein Tiermodell für Ängstlichkeit und Depression dar, während die *Gsk3*^{KI}-Maus als Tiermodell für Schizophrenie und Bipolare Störungen herangezogen werden kann.

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9. Publications

1. **Phosphatidylinositide-dependent kinase deficiency increases anxiety and decreases GABA and serotonin abundance in the amygdala**

   Teresa F. Ackermann*, Heide Hörtnagl*, David P. Wolfer, Giovanni Colacicco, Reinhard Sohr, Florian Lang, Rainer Hellweg**, Undine E. Lang**
   , both authors contributed equally to the paper*
   Cellular Physiology and Biochemistry, 2008; 22(5-6):735-44

2. **Hyperactivity and enhanced curiosity of mice expressing PKB/SGK-resistant glycogen synthase kinase-3 (GSK-3)**

   Teresa F. Ackermann, Daniela S. Kempe, Florian Lang, Undine E. Lang
   Cellular Physiology and Biochemistry, 2010; 25:775-786

3. **SGK1-sensitive renal tubular glucose reabsorption in diabetes**

   Teresa F. Ackermann*, Krishna M. Boini*, Harald Völkl, Volker Vallon, Kerstin Amann, Yuxi Feng, Hans-Peter Hammes, Florian Lang
   ** both authors contributed equally to the paper*
   American Journal of Physiology - Renal Physiology, 2009 Apr; 296(4):F859-66

4. **Role of PDK1 in regulation of gastric acid secretion**

   Anand Rotte, Madhuri Bhandaru, Teresa F. Ackermann, Krishna M. Boini, Florian Lang
   Cellular Physiology and Biochemistry, 2008; 22(5-6):725-34

5. **SGK1 in the regulation of renal function and in the pathogenesis of salt-sensitive hypertension (review)**
   Florian Lang, Ferruh Artunc, Teresa F. Ackermann, Daniela S. Kempe, Krishna M. Boini, Volker Vallon
   Nephrology Self-Assessment Program, March 2009, Volume 8, No. 2, 61-65

6. **Accelerated suicidal erythrocyte death in Klotho-deficient mice**

   Daniela S. Kempe, Teresa F. Ackermann, Stephanie S. Fischer, Saisudha Koka, Krishna M. Boini, Hasan Mahmud, Michael Föller, Kevin P. Rosenblatt, Makoto Kuro-o, Florian Lang
   Pflügers Archiv - European Journal of Physiology, 2009 Jul; 458(3):503-12

7. **APC-sensitive gastric acid secretion**

   Anand Rotte, Rexhep Rexhapaj, Omaima Nasir, Teresa F. Ackermann, Krishna M. Boini, Stefania Segditsas, Karl Kunzelmann, Florian Lang
   Cellular Physiology and Biochemistry, 2009; 23(1-3):133-42

8. **Enhanced insulin sensitivity of gene targeted mice lacking functional KCNQ1**

   Krishna M. Boini, Dirk Graf, Anita M. Hennige, Saisudha Koka, Teresa F. Ackermann, Michael Foeller, Volker Vallon, Erwin Schleicher, Susanne Ulrich, Hans-Ulrich Häring, Dieter Häussinger, Florian Lang
   American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 2009 Jun; 296(6):R1695-701

9. Relative resistance of Sgk1 Knockout mice against chemical carcinogenesis
Omaima Nasir*, Kan Wang*, Michael Föller, Shuchen Gu, Teresa F. Ackermann, Krishna M. Boini, Andreas Mack, Karin Klingel, Rosario Amato, Nicola Perrotti, Dietmar Kuhl, Jürgen Behrens, Christos Stournaras, Florian Lang
IUBMB (International Union of Biochemistry and Molecular Biology) Life, 2009 Jul; 61(7):768-76

10. SGK1-dependent intestinal tumor growth in APC-deficient mice

Kan Wang, Shuchen Gu, Stefania Segditsas, Omaima Nasir, Michael Föller, Teresa F. Ackermann, Karin Klingel, Dietmar Kuhl, Ian Tomlinson, Christos Stournaras, Florian Lang
Cellular Physiology and Biochemistry, 2010; 25(2-3):271-278

11. Downregulation of angiogenin transcript levels and inhibition of colonic carcinoma by Gum Arabic (Acacia senegal)

Omaima Nasir, Kan Wang, Michael Föller, Diana Sandulache, Ferruh Artunc, Teresa F. Ackermann, Ammar Ebrahim, Monica Palmada, Karin Klingel, Amal M. Saeed, Florian Lang
Nutrition and Cancer, 2010; in press

12. Regulation of renal tubular glucose reabsorption by Akt2/PKBß

Daniela S. Kempe, Gulab Siraskar, Henning Fröhlich, Anja Umbach, Michael Stübs, Florian Weiss, Teresa F. Ackermann, Harald Völkl, Karla F. Leavens, Morris J. Birnbaum, David Pearce, Michael Föller, Florian Lang
American Journal of Physiology - Renal Physiology, 2010; 298:F1113-1117

13. Akt2/PKBß-sensitive regulation of renal phosphate transport

Daniela S. Kempe*, Teresa F. Ackermann*, Krishna M. Boini, Fabian Klaus, Anja T. Umbach, Miribane Dermaku-Sopjani, Martin S. Judenhofer, Bernd J. Pichler, Paola Capuano, Gerti Stange, Carsten A. Wagner, Morris J. Birnbaum, David Pearce, Michael Föller, Florian Lang
** both authors contributed equally to the paper*
Acta Physiologica (Oxf.); 2010; in press

10. Conferences

March 2007: German Physiology Congress in Hanover

Poster presentation:

Renal function of gene-targeted mice lacking both SGK1 and SGK3

TF Ackermann, F Grahammer, F Artunc, D Sandulache, R Rexhepaj, KM Boini, B Friedrich, T Risler, JA McCormick

March 2008: German Physiology Congress in Cologne

Poster presentation:

Phosphatidylinositide-dependent kinase (PDK1) deficiency decreases GABA and serotonin abundance in the amygdala and increases anxiety behaviour

TF Ackermann, H Hörtnagl, DP Wolfer, G Colacicco, R Sohr, DR Alessi, F Lang, R Hellweg, UE Lang

Enhanced insulin sensitivity of gene-targeted mice lacking functional KCNQ1

KM Boini, AM Hennige, TF Ackermann, M Foeller, E Schleicher, HU Häring, F Lang

APC sensitive gastric acid secretion

A Rotte, R Rexhepaj, O Nasir, TF Ackermann, KM Boini, S Segditsas, K Kunzelmann, F Lang

SGK1-dependent colonic tumor growth

O Nasir, TF Ackermann, KM Boini, S Segditsas, K Wang, M Föller, F Lang

Sept. 2008: German Nephrology Congress in Tuebingen

Talk:

Serum- und Glukokortikoid-induzierbare Kinase (SGK)1-abhängige Glukoseresorption im Nierentubulus bei Diabetes

TF Ackermann, KM Boini, H Völkl, V Vallon, K Amann, Y Feng, HP Hammes, F Lang

March 2010: Joint Meeting of the Scandinavian and German Physiological Societies in Copenhagen

Poster presentation:

Hyperactivity and enhanced curiosity of mice expressing PKB/SGK-resistant glycogen synthase kinase-3 (GSK3)

TF Ackermann[1], DS Kempe[1], F Lang [1], UE Lang[2]

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