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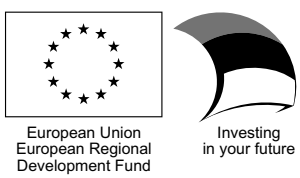

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original papers, referred to in the text by Roman numerals I–III.

I. **Narvik, Jane,** Taavi Vanaveski, Jürgen Innos, Mari-Anne Philips, Aigar Ottas, Liina Haring, Mihkel Zilmer, Eero Vasar. "Metabolic profile associated with distinct behavioural coping strategies of 129Sv and Bl6 mice in repeated motility test." Sci Rep. 2018 Feb 21;8(1):3405. doi:10.1038/s41598-018-21752-9.

II. Vanaveski, Taavi, **Jane Narvik**, Jürgen Innos, Mari-Anne Philips, Aigar Ottas, Mario Plaas, Liina Haring, Mihkel Zilmer, Eero Vasar. "Repeated Administration of D-Amphetamine Induces Distinct Alterations in Behavior and Metabolite Levels in 129Sv and Bl6 Mouse Strains." Front Neurosci. 2018 Jun 12;12:399. doi:10.3389/fnins.2018.00399. eCollection 2018.

III. **Varul**, **Jane**, Kattri-Liis Eskla, Maria Piirsalu, Jürgen Innos, Mari-Anne Philips, Tanel Visnapuu, Mario Plaas, Eero Vasar. "Dopamine System, NMDA Receptor and EGF Family Expressions in Brain Structures of Bl6 and 129Sv Strains Displaying Different Behavioral Adaptation." Brain Sci 2021 May 29;11(6):725. doi:10.3390/brainsci11060725.

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I. The author participated in the behavioural and metabolomics experiments and was involved in interpreting the data, participated in writing and correspondence.

II. The author participated in the behavioural and metabolomics experiments, was involved in interpreting the data, participated in writing and correspondence.

III. The author participated in the behavioural experiments and performed gene expression experiments, was involved in interpreting the data, participated in writing and handled correspondence.

ABBREVIATIONS

129Sv	129S6/SvEvTac mouse strain (in our research)
AAA	aromatic amino acids
ACT	acute amphetamine
ADMA	asymmetric dimethylarginine
Alpha-AAA	alpha-aminoadipic acid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BCAA	branched chain amino acids
Bl6	C57BL/6NTac mouse strain (in our research)
BW	body weight
C0	free carnitine
C5-	in mouse blood plasma mixture of isovalerylcarnitine and 2-methyl butyrylcarnitine
CA1-3	cornu Ammonis 1-3
Ccl5	c-c motif chemokine ligand 5
cGMP	cyclic guanosine monophosphate
Comt	catechol-O-methyltransferase
CPT-1	ratio – the ratio between long chain acylcarnitines and carnitine C0
CPT1	carnitine palmitoyltransferase 1
CT	cycle threshold
Cxcl1	c-x-c motif chemokine ligand 1
D1R	dopamine type receptor 1
DA	dopamine
Da	dalton
DAT	dopamine transporter
Dbh	dopamine β-hydroxylase
DDAH	dimethylarginine dimethylaminohydrolase
Dhtkd1	dehydrogenase E1 And Transketolase Domain Containing 1
Disc1	disrupted-in-Schizophrenia 1
Drd1	dopamine receptor D1
Drd2	dopamine receptor D2
Drd3	dopamine receptor D3
Drd4	dopamine receptor D4
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
Erbb1	epidermal growth factor receptor
Erbb3	erb-b2 receptor tyrosine kinase 3
Erbb4	erb–b2 receptor tyrosine kinase 4
FIA-MS/MS	flow injection analysis tandem mass spectrometry
GLM	general linear model
GlyT1	glycine transporter-1
GlyT2	glycine transporter-2

Grin1	glutamate ionotropic receptor NMDA type subunit 1
Grin2a	glutamate ionotropic receptor NMDA type subunit 2A
Grin2b	glutamate ionotropic receptor NMDA type subunit 2B
Hb-Egf	heparin binding EGF like growth factor
HCC	home cage controls
Hprt	hypoxanthine guanine phosphoribosyl transferase
IFN-g	interferon gamma
IL-6	interleukin 6
iNOS	inducible nitric oxide synthase
Ivd	Isovaleryl-CoA Dehydrogenase
KO	knockout
L-DOPA	levodopa
LC-MS/MS	liquid chromatography mass spectrometry
LPS	lipopolysaccharide
Lyso PC	lysophosphatidylcholine
MAOA	monoamine oxidase A
MAOB	monoamine oxidase B
MB	motility boxes
mPFC	medial prefrontal cortex
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRM	multiple reaction monitoring
NAT	noradrenaline transporter
NMDA	*N*-methyl-D-aspartate
NO	nitric oxide
Nrg1	neuregulin 1
Nrg2	neuregulin 2
Nrg3	neuregulin 3
PC	phosphatidylcholine
qPCR	quantitative Real-Time-PCR
RMT	repeated motility testing
RPT	repeated amphetamine
SAL	saline
SERT	serotonin transporter
SM	sphingomyelin
SN	substantia nigra
Srr	serine racemase
Tgfa	transforming growth factor alpha
Th	tyrosine hydroxylase
TLR4	toll-like receptor 4
TNF-a	tumor necrosis factor alpha
VMAT2	vesicular monoamine transporter 2
VTA	ventral tegmental area

1. INTRODUCTION

Before a new drug or vaccine can be tested in human participants, it must undergo preclinical evaluation to determine its safety and efficacy. Mice and rats are the most widely applied animal models in biomedicine. Besides that, these animals are also tested in order to understand the biological foundations of human diseases. Genetically, mice are approximately 90% similar to humans that supports their validity as a model organism to study molecular and behavioural changes playing a role in the development of neuropsychiatric disorders (Guénet., 2005).

When comparing the publication rates in neuroscience, mice have replaced the long-favoured rats in research almost a decade ago (Ellenbroek and Young, 2016). It has been thought that this shift is likely associated with the development and availability of molecular techniques and genetic manipulations of mice. Another advantages of mouse studies are their fast breeding and cost-effectiveness. According to one of the largest model organism suppliers, there are over 7,000 different strains of mice (The Jackson Laboratory website). Usually, scientists use inbred strains (20 or more generations of consecutive sibling mating) because they are well characterised, displaying genetic and phenotypic similarity (Flurkey, 2009). The similarity gives researchers an opportunity to compare and draw conclusions from experiments that are conducted in different laboratories around the world. When conducting a transgenic study, one concern is the possible genetic background effect on the interpretation of the results (Bućan and Abel., 2002; Wolfer et al., 2002). It has been shown that the genetically generated mutant mice possess genes from two or more background strains. This methodical strategy may cause translational issues that are caused by flanking genes from the used strains. Background gene problems are relevant in inbred strains, when the mutant mice are showing different patterns of behaviour in the experimental tests (Bailey et al., 2006). For example, the inbred strain behavioural variations are noted in anxiety, motor functioning, pain sensitivity and learning-memory indices (Belknap et al., 1990; Bolivar et al., 2000; Holmes et al., 2002; Abramov et al., 2008). Therefore, it is important that the researcher knows profoundly the peculiarities of the chosen mouse line.

Investigating neuropsychiatric disorders, mouse models help to evaluate the effect of an abnormal biomarker (e.g., gene, metabolite or protein) on a molecular pathway or other genes to better understand the pathological mechanisms. In order to explore the neurobiology of environmental adaptation, this dissertation targeted the behaviour, metabolomics, gene and protein expression in well characterised Bl6 (C57BL/6N) and 129Sv (129S6/SvEv) mouse strains. The behavioural profile and molecular changes of these two strains were studied in two environmental conditions: in home cage (Bl6 and 129Sv in HCC) and in repeated motility testing (Bl6 and 129Sv in RMT). RMT represented a stressful environment whereas HCC was the usual living condition for these animals. The comparison of mice in two backgrounds has been chosen to evaluate the impact of genetic differences for molecular changes induced by stress. The home cage monitoring in experiments, especially in behavioural neuroscience

has become more popular and necessary (Võikar and Gaburro, 2020). One of the major reasons for such intervention is the improvement of reproducibility in research findings. However, in this study we did not monitor the animals in home cages, but this was used as the usual control condition for repeated environmental challenges.

In the first study, the goal was to compare different adaptation of 129Sv and Bl6 mice and then characterize the strain specific metabolite levels that may explain the differences between coping strategies in Bl6 and 129Sv. The study addresses how moderate stress affects the metabolite levels of particular strains (analysing Bl6 or 129Sv in HCC and RMT). Based on the existing data, we knew that these mouse strains behave dissimilarly in a stressful environment and for this reason we hypothesised that there might be variations in metabolite concentrations between these strains as well (Abramov et al., 2008; Heinla et al., 2014). As assumed, we found stable strain-specific metabolite levels that stayed the same regardless of stressful intervention. Furthermore, there was a shift in body weight change between the strains after stress exposure: in stressful conditions 129Sv mice experienced significant loss of body weight but showed more pronounced weight gain in home cages compared to Bl6.

In the second study, the goal was to explore how repeated administration of amphetamine affects the locomotor activity and metabolite levels in these two mouse strains. Indeed, we observed different sensitization toward amphetamine in these strains: in Bl6 it has been moderate whereas in 129Sv we could divide mice into two subgroups by their locomotor activity – strong and weak responders. Besides that, we found that the impact of genetic background on metabolite levels significantly exceeded that of amphetamine treatment. Furthermore, the 129Sv strain displayed a significantly larger variation in various metabolite levels after repeated amphetamine treatments than Bl6 (conclusive schemes can be found in Paper II supplementary material Figures S2 and S3). It means that when we compared the amphetamine effect on metabolite changes between the strains, the metabolism of 129Sv strain seems to be more sensitive to amphetamine, in response to both acute and repeated administration.

Considering differences between Bl6 and 129Sv in behaviour and pharmacological studies, in the third study we expected to see variations in brain gene expression of three large neurotransmitter/neuromodulator systems: dopamine (DA) system, N-methyl-D-aspartate (NMDA) receptors and epidermal growth factor (EGF) family. These systems were selected based on previous findings, considering their role in behavioural adaptation to a challenging environment both in preclinical and clinical settings (Pani et al., 2000; Wieduwilt and Moasser 2008; Iwakura and Nawa 2013; Mizuno et al., 2013; Yasuda et al., 2017; Kobayashi et al., 2019; Li et al., 2019). We found that not only inhibited activity of the dopamine system, but also reduced activity of EGF family and NMDA receptor signalling in the frontal cortex, underlies higher susceptibility to stress of 129Sv. Based on these findings we considered that 129Sv (129S6/SvEv) might be a more promising strain for evaluating depressive- and psychotic-like conditions in mice compared to Bl6 (C57BL/6N).

2. REVIEW OF THE LITERATURE

2.1. Inbred mouse strains Bl6 and 129Sv in behavioural research evaluating environmental adaptation

Mice have long been the preferred species for preclinical research due to their similarity to humans in terms of physiology, anatomy, and genetics. Genetically modified mice are a standard approach for investigating the function of unknown genes or proteins. The classical method of creating transgenic mice involves genetically homogeneous (inbred) strains that usually are generated from 129Sv and Bl6 mice. 129Sv derived embryonic stem cells are used for introduction of targeted mutations into the mouse genome, followed by back-crossings into the Bl6 strain (Linder and Davisson, 2004; Yoshiki and Moriwaki, 2006).

Inbred animals are expected to be nearly genetically identical, and this is important when investigating the phenotypic effects of mutations or drug administration, thereby eliminating the genetic variance (Zutphen et al., 2001). It is accepted that the phenotype of mice, e.g. behaviour, can be related to their genetic origin (Doetschman, 2009). For example, it is commonly agreed that Bl6 mice show greater locomotor activity and increased exploratory drive while 129Sv mice are less active, anxious and seem to be more vulnerable to stress (Contet et al., 2001; Võikar et al., 2001; Abramov et al., 2008; Heinla et al., 2014). Compared to Bl6, 129Sv strain exhibits longer hot plate latencies and they display longer immobile floating in the forced swim test (Abramov et al., 2008). Also, it has been shown that Bl6 mice are more aggressive and evidently demonstrate stronger whisker barbering trends (Sarna et al., 2000; Heinla et al., 2014). One particular characteristic of the 129Sv line is a lack of response in the cat odour induced anxiety test (Raud et al., 2007).

It has been shown that the phenotype differences between the 129Sv and Bl6 strains remain stable in most behavioural tests despite environmental modifications and are actually reinforced by environmental enrichment which induces active stress coping (a coping style that is characterised by trying to escape from stressful situations) in Bl6 and a passive response (helplessness to deal with the stressor) in 129Sv (Contet et al., 2001; Abramov et al., 2008; Heinla et al., 2014). Impaired adaptation in a challenging environment indicates vulnerability and may thereby refer to higher susceptibility to stress-induced disorders (Wied and Jansen 2002; Lampis et al., 2011).

Indeed, translating the preclinical results from mice to human mental illnesses is a complex task. However, mice are good models to help to study physiological, anatomical and behavioural changes in the brain that can be associated with psychiatric disorders (Seong et al., 2002). There is no perfect biological model for heterogeneous syndromes, but relying on the literature, it is apparent that some mouse strains are better for investigating certain behavioural traits, related to pathophysiology, than others (Crawley et al., 1997). How the body responds to stress exposure, depends on its ability to manage appropriately with

challenges and then capability to re-establish homeostasis. As 129Sv mice are more anxious and idle in comparison with Bl6, then one may suggest that 129Sv mice are inclined to stay passive when adapting to a novel environment. Therefore, they are more sensitive to depression-related or anxiety-like challenges, because the flight-or-fight response and adaptation to the environment are the most fundamental priorities to stay alive. Also, when 129Sv animals from an enriched environment were placed into a novel environment, there was a decline in their body weight, apparently caused by stress (Heinla et al., 2014).

Undeniably, Bl6 is the most widely used mice strain in neuroscience (Crawley et al., 1997; Bryant., 2011). Nonetheless, the tremendous popularity toward Bl6 may reduce the validity and translatability of many scientific findings. However, one has to keep in mind that there may be differences between the substrains (Simon et al., 2013). To see the effect of genetic background, comparison of at least two mice strains seems to be recommended (Silva et al., 1997). Also, the mixing up of genetic backgrounds will enable to generate controlled genetic variation in the experimental population that has more resemblance to the very heterogeneous human population.

2.2. Repeated treatment with amphetamine, indirect agonist of dopamine, as a model of psychosis-like condition in mice

Psychotic disorders are a group of illnesses that affect the mind. Psychosis itself is a symptom and is a condition where individuals have lost the adequate reality testing. When a psychotic episode occurs then a person may experience most commonly hallucinations, delusions, disturbed thoughts, disorganised behaviour, anhedonia and catatonia (Insel, 2010). It can be triggered by a mental illness, a physical injury, substance abuse, or extreme stress or trauma. Schizophrenia is one of the most frequently occurring psychotic disorders.

It is well acknowledged that psychosis may be provoked by drugs. One representative substance that can induce in human psychotic states is amphetamine (Dépatie and Lal 2001; Tenn et al., 2003; Murray et al., 2013; Ham et al., 2017).

Several studies with animals have confirmed how the use of drugs can lead to psychosis without the involvement of genetic factors (Murray et al., 2013). However, the genetic background may make some individuals more vulnerable to psychosis than others. For example, the administration of methamphetamine separates humans into two subgroups: one group develops drug addiction whereas the other displays symptoms resembling paranoid schizophrenia (Ikeda et al., 2013). In preclinical research, amphetamine and methamphetamine are used to induce schizophrenia-like positive symptoms (Steeds et al., 2015).

2.2.1. Pharmacological characterization of amphetamine

Amphetamine is a central nervous system stimulant that functions by increasing the amounts of monoamines as dopamine, norepinephrine, and serotonin in the synaptic cleft (Heikkila et al., 1975; Holmes and Rutlege., 1976; Rothman et al., 2001; Easton et al., 2007). Amphetamine exists in two isomeric forms: levo-amphetamine (l-amphetamine) and dextro-amphetamine (d-amphetamine). D-amphetamine has been shown to be more potent than l-amphetamine, both behaviourally and pharmacologically (Heikkila et al., 1975; Easton et al., 2007).

There are three main molecular targets for how amphetamine impairs monoamine metabolism. First, amphetamine has similar structure to the monoamine neurotransmitters and thereby it enters to the presynaptic axon terminal through diffusion or uptake by the monoamine transporters: DAT (dopamine transporter), NAT (noradrenaline transporter), and SERT (serotonin transporter). It is known that amphetamine binds to the NAT with higher affinity compared to DAT (Rothman and Baumann, 2003). This is the reason why amphetamine releases noradrenaline more potently than dopamine and much more than serotonin (Rothman et al., 2001). Once amphetamine binds to the membrane transporters (DAT, NAT and SERT) and enters the nerve cell, it reverses the direction of the reuptake transporters. It means, instead of pumping neurotransmitters from the synapse back into the nerve terminal, it causes release of neurotransmitters out of the nerve cells into the synaptic cleft (Robertson et al., 2009).

Second, when amphetamine is inside the cytosol, it increases monoamine neurotransmitters (dopamine, noradrenaline and serotonin) by inhibiting vesicular monoamine transporter 2 (VMAT2) as well as through disruption of the electrochemical gradients necessary for vesicular transporter function. VMAT2 is a membrane protein that transports monoamines from cellular cytosol to its storage, presynaptic vesicles (Teng et al., 1998, Eiden et al., 2004). Also, it has been shown that VMAT2 protects neurons from oxidative stress induced injury (Lohr et al., 2016).

The third biochemical target of amphetamine is the mitochondrial-bound enzyme monoamine oxidase (MAO). Both MAOA and MAOB isoenzymes are degrading amine neurotransmitters dopamine, noradrenalin, and serotonin. However, MAOA/B differ in substrate preference and also in distribution in neurons (Robinson, 1977; Youdim et al., 2006; Bortolato et al., 2008). For example, in the brain, MAOA is preferentially located in dopaminergic and noradrenergic neurons while MAOB is the major isoform present in the serotonergic neurons and glia (Levitt et al., 1982; Westlund et al., 1985; Riederer et al., 1987). It has been shown that MAOA preferentially metabolizes serotonin whereas dopamine and noradrenaline are non-selective substrates of both isoforms (Youdim et al., 2006).

2.2.2. Dopamine and noradrenaline systems as the major targets of amphetamine

The catecholamine transmitters, dopamine, noradrenaline and adrenaline, are all synthesised from the essential amino acid tyrosine. Enzyme dopamine β-hydroxylase, converts dopamine to noradrenaline. One study with β-hydroxylase knockout (Dbh−/−) mice in Bl6 x 129Sv background demonstrated that noradrenaline deficient mice are hypersensitive to amphetamine, likely by altering dopamine-signalling pathways (Weinshenker et al., 2002).

The dopaminergic neurons from the substantia nigra (SN) project primarily to the dorsal striatum which regulates goal-directed locomotion and habit formation, whereas ventral tegmental area (VTA) neurons project to the nucleus accumbens, frontal cortex and limbic regions (e.g., amygdala, anterior cingulate cortex, hippocampus, and insula) to adjust motivation, emotions, reward, and learning (Solinas et al., 2019). Dopamine is also known to have a role in behaviours, associated with food-reward (Wise 2004). It has been thought that dopamine type receptor 1 (D1R) in the dorsal striatum may modulate food anticipatory activity (scheduled meal times) that is modulated by circadian rhythms (Gallardo et al., 2014). Importance of the dopamine influence in food intake is supported by finding, where despite everyday L-DOPA administration, the dopamine -/- mice did not eat enough to survive (Szczypka et al., 1999). Probably the dopaminergic system also has a role in stress-related eating (Singh, 2014).

Dopamine is metabolised by MAOA and MAOB enzymes (besides of catechol-O-methyltransferase and aldehyde dehydrogenase). It has been demonstrated that MAOA is involved under the basal concentrations and both, MAOA and B are applied when levels of dopamine are high (Fornai et al; 2002). There might be alterations in MAOB enzyme reactivity in Bl6 strain compared with other strains, where Bl6 is the most sensitive to MPTP, the neurotoxin metabolised by MAOB (Inoue et al., 1999; Sedelis et al., 2000). Furthermore, Bl6 strain is the only known organism where MAOB activity is greater in the brain than in the liver (Przedborski et al., 2000). Genetic invalidation of MAO-A gene is shown to increase aggressive behaviour in mice, whereas this effect is not present after the invalidation of MAO-B gene.

The major central noradrenergic nucleus, locus coeruleus, is located in the brainstem. Projections from the locus coeruleus are spread widely in the central nervous system, having many functions (Dahlström and Fuxe 1964). For instance, it has been shown that the locus coeruleus regulates stress response, sleep-wake cycles, and manages attention processing and memory. Actually, previous research demonstrates that noradrenaline plays a key role in the physiological and behavioural stress responses (Morilak et al., 2005).

Noradrenaline is cleared from the synaptic cleft by noradrenaline transporter (NAT). Additionally, NAT is responsible for re-charging presynaptic cells for future neuronal transmission. There is evidence that NAT regulates dopamine uptake in the prefrontal cortex (Carboni et al., 1990). The absence of NAT in

Bl6 x129Sv background mice, leads to decreased depression-like behaviour in chronic stress conditions (Haller et al., 2002). This was supported by another study with NAT null mutant mice, similarly in the Bl6 x129Sv background, where the authors confirmed that noradrenaline systems can influence midbrain dopaminergic homeostasis (Xu et al., 2000).

2.2.3. The behavioural effects of amphetamine in 129Sv and Bl6 mice

Chen and colleagues performed a careful analysis by comparing amphetamine effects on locomotor activity and dopamine efflux in Bl6 and 129Sv strains (Chen et al., 2007). They did not find differences in basal motor activity and dopamine levels between these strains. However, Bl6 displayed greater amphetamine-stimulated locomotor activity and stronger striatal dopamine efflux than 129Sv (Chen et al., 2007). A similar difference was established when Bl6 and DBA/2 strains were compared. Zocchi and colleagues found that Bl6 demonstrated a greater dose-dependent locomotor stimulant response to an acute injection of amphetamine than DBA/2, which corresponded to larger increases in dopamine levels in the nucleus accumbens after amphetamine treatment (Zocchi et al., 1997).

Comparing 129Sv and Bl6 strains, one must keep in mind that the 129Sv and all related 129Sv strains carry a 25-base pair frameshift deletion within exon 6 of the Disc1 gene resulting in a premature termination codon at exon 7 (Chubb et al., 2008). Koike and colleagues discovered the deletion while modifying the 129Sv Disc1 allele to imitate the production of the hypothetical C-terminally truncated protein product (Koike et al., 2006). Moreover, they reported a significant difference in delayed non-match to place test, a specific test of working memory, for both 129Sv Disc1 heterozygotes and homozygotes, compared to Bl6 mice. Recent evidence suggests a prominent role of DISC1 in the genetics of major psychiatric disorders like schizophrenia, bipolar disorder, and major depressive disorder (Niwa et al., 2016). Studies in rats demonstrate that misassembly of full-length DISC1 protein compromises dopamine homeostasis, leading to apparent behavioural deficits (Trossbach et al., 2016).

Although amphetamine has considerable affinities for dopamine, noradrenaline and serotonin transporters, the DAT is associated with the stimulating and rewarding properties of amphetamine (Koob and Nestler, 1997; Heal et al., 2013; Sitte and Freissmuth, 2015). Amphetamine exerts its actions through an increase in dopamine extracellular levels in the terminal and cell body regions of midbrain dopaminergic neurons, by causing reverse transport of dopamine and preventing its uptake via the DAT (Seiden et al., 1993; Sulzer et al., 1995). Repeated administration of amphetamine has been used to model psychotic-like behaviour in rodents (Ham et al., 2017). The majority of studies evaluating the development of amphetamine-induced motor sensitization have been performed in rats. Repeated amphetamine administration to adult rats produced robust sensitization toward dopamine agonist, disrupted latent inhibition, and decreased

attentional vigilance; this effect lasted for 90 days after the last injection (Murphy et al., 2001; Russig, 2002; Russig et al., 2003; Ham et al., 2017). Even though deficits in the attention set-shifting task were observed, spatial memory was not impaired in the Morris water maze, indicating that cognitive impairments in the model appear to be restricted to some prefrontal cortex dependent tasks (Stefani and Moghaddam, 2002; Featherstone et al., 2008). So far, few studies have been performed to examine mouse strain differences in behavioural sensitization to amphetamine (Phillips et al., 2008). In comparison to Bl6, DBA/2 mice were more receptive to the development of motor sensitization induced by repeated amphetamine (Badiani et al., 1992; Phillips et al., 1994).

2.3. Metabolomics approach to reflect the steady state and functional changes in the body

Metabolomics is defined as the comprehensive measurement of various metabolites and low-molecular-weight molecules (<1500 Da) in a biological specimen. Often, the measured metabolites are endogenous compounds such as lipids, short peptides, amino acids, nucleic acids, sugars, alcohols, or organic acids that are routinely produced by catabolism or anabolism.

In preclinical and clinical research, metabolomics is a possibility to view the complex system of how physiology is linked to external conditions or manipulations and measures its response to disturbances such as those associated with disease (Medina et al., 2014). More precisely, metabolites, measured usually from biofluids or tissues, give researchers an overview of what is happening in the body at the cellular level, demonstrating the phenotype of the organism in a certain time frame. Metabolome can change continuously and is affected by many factors such as gender, genes, age, diet, and environment (Kim et al., 2014). The fact that metabolites are sensitive to external stimuli, followed by changes in intracellular level, make metabolome a particularly useful investigation tool (Holmes et al., 2008). Additional reason, why metabolomics is a useful appliance in research is because essential metabolites are found similarly in all vertebrates with main differences in concentrations (Peregrín-Alvarez et al., 2009).

The most known metabolism-associated disease is type II diabetes. Type II diabetes seems to be linked with other chronic diseases and schizophrenia is one of them. It has been shown that there is impaired glucose tolerance already in patients with first episode psychosis, who are not exposed to any antipsychotic medications (Ryan et al., 2003). However, pathological alterations in glucose levels seem to be more a symptom than a cause. There have been identified some metabolic biomarkers for early-stage metabolic syndrome (cluster of conditions that occur together, increasing risk of type 2 diabetes). The potential modulators of glucose homeostasis are branched-chain amino acids and alpha-aminoadipic acid (alpha-AAA) that are elevated in serum samples of metabolic syndrome individuals (Wang et al., 2011; Wang et al., 2013; McCormack et al.,

2013). Furthermore, Merino and colleagues showed increased levels of two amino acids, taurine and glycine, that are risk indicators of developing type II diabetes (Merino et al., 2018). Besides that, taurine together with epidermal growth factor belong to the biomarker signature of first episode psychosis (Koido et al., 2016).

As a matter of fact, the search for biomarkers of psychiatric disorders has become more and more informative. For instance, recently it has been demonstrated how metabolomic studies revealed biomarkers for major depressive and bipolar disorders that are linked with glutamatergic pathways and energy metabolism (Ogawa et al., 2018; MacDonald et al., 2019). In schizophrenia monitoring, there are confirmed early-stage alterations in lipids, biogenic amines and amino acids metabolism alterations between patients and controls (Leppik et al., 2020; Parksepp et al., 2020; Parksepp et al., 2022). It is clear that existing findings indicate how metabolic abnormalities may be a new target for diagnosis and treatment of mental illnesses.

In the current study we evaluated amino acids, biogenic amines, hexoses, acylcarnitines, glycerophospholipids (Lyso PCs and PCs) and sphingomyelins. It has been shown that the disturbed balance of amino acids and biogenic amines may play a role in forming schizophrenia (Rao et al., 1990; Tortorella et al., 2001; De Luca et al., 2008; Saleem et al., 2017; Leppik et al., 2020; Parksepp et al., 2020; Parksepp et al., 2022). Amino acids are the building blocks for neurotransmitters whereas biogenic amines, formed from amino acids, may be neurotransmitters (Santos, 1996). For example, the known biogenic amines that function as neurotransmitters are dopamine, noradrenaline, adrenaline, histamine, and serotonin (Esler et al., 1990; Sudo, 2019). Hence, some amino acids like glutamate, aspartate, serine and glycine act also as exitatory or inhibitory neurotransmitters (Maycox et al., 1990). It is known that the level of amino acids in the brain is related with the levels in the blood because they compete over the same carriers in the blood-brain barrier (Fernstrom, 1981). For example, leucine, isoleucine, valine, phenylalanine and tyrosine influence the concentration of tryptophan. It means, when raising the levels of these metabolites in the blood then tryptophan levels decrease in the brain and then it may have an impact on the synthesis of serotonin (Fernstrom and Faller 1978; Fernstrom, 1981). Acylcarnitines transport fatty acids (C2-C26) from the cytosol to the mitochondria what then can be utilized for energy production (Makrecka-Kuka et al., 2017). It has been suggested that acylcarnitines may be markers of fatty acid and amino acid oxidation disturbances and activate pro-inflammatory signaling pathways (Giesbertz et al., 2015). Also, Kriisa et al. showed that antipsychotic-naïve first-episode psychosis patients revealed significantly increased levels of long-chain acylcarnitines (Kriisa et al., 2017). Glycerophospholipids are the main structural components of the cell membranes. In the nervous system, the basic function of these molecules is to provide neurons stability, permeability and fluidity (Farooqui et al., 2000). Besides of creating a myelin sheath, glycerophospholipids play a part in complex endocannabinoid system. Namely, endocannabinoids are endogenous lipids that are synthesised

from glycerophospholipids (Lu and Mackie, 2016). Several research groups have found how dysfunction of endocannabinoids may be linked with schizophrenia (Giuffrida et al., 2004; Koethe et al., 2009; Garani, 2021). Sphingomyelins, a group of sphingolipids, are found only in animal cell membranes where they create myeline sheath surrounding the nerve axons (Ramstedt and Slotte, 2002). It has been thought that alterations in lipid metabolism may be involved in many psychiatric disorders, including schizophrenia (Müller et al., 2015; Leppik et al., 2019).

2.4. How metabolomics approach and gene expression studies can help to address the behavioural differences of 129Sv and Bl6 strains

Considering the different behaviour between Bl6 and 129Sv strains, we were interested to investigate if there would be also a distinction in the metabolite levels. This thorough comparison may help us and other scientists to choose more appropriate mouse strains for the question in interest. Our focus in this study was the relationship between metabolites and psychiatric disorders, particulary psychosis-related disorders.

Piirsalu et al. showed that acute administration of lipopolysaccharide (LPS) caused dissimilar effects on the body weight, temperature and metabolism in 129Sv and Bl6 strains (Piirsalu et al., 2020, 2022). LPS is the activator of innate immune response via TLR4 receptor and is widely used in immunological studies to promote neuroinflammation. LPS caused more significant body weight loss and reduction of body temperature in Bl6 compared to 129Sv strain (Piirsalu et al., 2020, 2022). There is a study that acclaimed how inflammation-induced hypothermia is a strategy for inducing hypometabolism, which is essential for host organism survival (Ganeshan et al., 2019). Hypometabolic conditions are needed for saving metabolic energy in inflammatory conditions because inflammatory response is energetically costly and requires compensation of nutrients to support immune activation (Ganeshan and Chawla 2014). It is apparent that hypometabolic state is stronger in LPS treated Bl6 mice compared to 129Sv strain (Piirsalu et al., 2020). More precisely, in Bl6 there was a decline in glucogenic amino acids and hexoses that probably reflected in stronger body weight reduction. Study of saline treated 129Sv and Bl6 groups revealed the strain specific metabolite concentrations for both strains. The levels of biogenic amines (acetyl-ornithine, alpha-aminoadipic acid, carnosine) were significantly higher in Bl6 when acylcarnitine (C5-) and sphingolipid SM (OH) C22:2 were elevated in 129Sv (Piirsalu et al., 2020). Alpha-aminoadipic acid is a component of the lysine metabolism pathway and a marker of oxidative stress (Yuan et al., 2011; Zeitoun-Ghandour et al., 2011). The metabolomic study performed in diabetic patients suggested that alpha-aminoadipic acid may be a modulator of glucose homeostasis and diabetes risk (Wang et al., 2013). Studies in rodents

have shown that alpha-aminoadipic acid modulates kynurenic acid levels in the brain. Kynurenic acid is a neuroactive metabolite of tryptophan that interacts with NMDA, AMPA/kainate and alpha 7 nicotinic receptors (Tuboly et al., 2015). The increased level of alpha-aminoadipic acid in Bl6 mice has been reported to be caused by a defect in the *Dhtkd1* gene. *Dhtkd1* has been identified as a primary regulator of alpha-aminoadipic acid and defects in this gene result in the increase of alpha-aminoadipic acid (Wu et al., 1995; Leandro et al., 2019). Carnosine is highly concentrated in excitable tissues such as muscle and brain and is the inhibitor of free radical reactions (Klebanov et al., 1998). C5- is the by-product of branched-chain amino acids isoleucine and leucine catabolism. C5- acylcarnitine in mouse blood plasma is a mixture of isovalerylcarnitine and 2- methyl butyrylcarnitine. Recent evidence suggests that increased levels of C5- in 129Sv mice are contributed by the accumulation of isovalerylcarnitine, which is caused by a splice site mutation in the *Ivd* gene resulting in isovaleryl-CoA dehydrogenase deficiency (Leandro et al., 2019).

2.5. The research tasks arising from the literature review

Despite extensive biomedical comparisons of 129Sv (129S6/SvEvTac) and Bl6 (C57BL/6NTac) strains, there seem to be no comprehensive studies comparing the effects of environmental adaptation as well as repeated treatment with amphetamine in these two strains. When keeping in mind that Bl6 and 129Sv mice differ in behaviour, one may hypothesise that these two mouse strains respond differently to the challenging environment as well as to repeated treatments with amphetamine in terms of behaviour, metabolomics, and gene expression.

First, there was an aim to identify the consequences of environmental adaptation in terms of behaviour and metabolomics. In a recent study there was evidence that the phenotype differences between the 129Sv and Bl6 strains remain stable in most tests despite environmental modifications (Heinla et al., 2014). Environmental enrichment and long-term individual housing influenced the 129Sv and Bl6 strains differently depending on existing predispositions of these inbred strains. For example, the activity of Bl6 mice was significantly enhanced during repeated behavioural testing, while 129Sv mice remained inert, but experienced significant loss of body weight (Heinla et al., 2014). Indeed, environmental enrichment seems to reinforce existing predispositions in both strains by inducing an active coping strategy in Bl6 and passive coping strategy in 129Sv. Considering behavioural differences and diversity of strain-specific outcomes in pharmacological studies, we expected to establish variation in the metabolomics profile of these two inbred strains. For this reason, we decided to identify possible metabolic consequences of distinct behavioural responses of Bl6 and 129Sv in repeated motility tests.

Second, so far only few studies have been performed to examine mouse strain differences in behavioural sensitization to amphetamine (Phillips et al.,

2008). After thorough literature research, we could not find any comprehensive studies that compared the effects of repeated amphetamine in 129Sv and Bl6 strains. Relying on literature, where some studies reported large strain differences to amphetamine (Ralph et al., 2001), we hypothesised that these two mouse strains probably would respond differently to repeated amphetamine in terms of behaviour and metabolomics as well. We expected that 129Sv mice may display stronger sensitization toward amphetamine-induced hyper-locomotion compared to Bl6 strain. In this thesis, we aimed to study the effect of repeated amphetamine on the locomotor activity and body weight of 129Sv and Bl6 strains. Besides that, the aim was to identify possible metabolic consequences of distinct behavioural responses of Bl6 and 129Sv strains in all treatment groups.

Third, considering the differences in the action of amphetamine in these two strains, the motor stimulation elicited by amphetamine was studied in Bl6 and 129Sv mice in the beginning and at the end of the environmental adaptation test to explore the changes in functional activity of the DA-ergic system. Based on the behavioural differences and vast diversity of strain-specific outcomes in pharmacological studies, we expected to see variations in transcripts of enzymes related to dopamine metabolism as well as dopamine receptors, N-methyl-D-aspartate (NMDA) receptor subunits, epidermal growth factor (EGF) family genes and their receptors in 129Sv and Bl6 strains. As we were interested in the regulation of behavioural adaptation, we decided to evaluate the gene expression in several brain regions playing a role in the regulation of motivations, emotional behaviour, learning and memory (the frontal cortex, hippocampus, dorsal and ventral striatum, midbrain).

3. AIMS OF THE STUDY

1. The first task of the study was to elucidate the behavioural adaptation of two widely used mouse lines Bl6 and 129Sv in the repeated motility test. For this purpose, saline was repeatedly administered to these mouse lines and their locomotor activity parameters and changes in body weight were measured every day. At the end of the study, the levels of metabolites were determined in the blood samples of these two mouse lines. To analyse the effect of behavioural adaptation on metabolite levels, the levels of metabolites and body weight changes of stressed animals were compared to those in mice kept only in the home cages.
2. The second task of the study was to investigate the effect of repeated administration of amphetamine, indirect dopamine agonist, on the locomotor activity of 129Sv and Bl6 mouse lines. We expected that these two mouse lines display different sensitization toward amphetamine-induced hyperlocomotion. Besides that, the dynamics of body weight was assessed. At the end of the study, the levels of metabolites were determined in the blood samples of amphetamine treated mice. The metabolite levels of amphetamine-treated mice were compared to those treated with saline.
3. The third task was to evaluate the changes in the expression of genes (dopamine, NMDA, epidermal growth factor) playing a role in the behavioural adaptation in 129Sv and Bl6 mice. The brain structures selected for the study were the frontal cortex, dorsal striatum, ventral striatum, hippocampus, and mesencephalon. These brain regions play a crucial role in the regulation of motivations, emotional behaviour, learning and memory. Significant changes in gene expression were further validated by analysis of protein expression in the frontal cortex and hippocampus.
4. Last but not least, we tried to evaluate the translational value of the present research. In other words, to what extent these two different mouse lines (129Sv and Bl6) can be used for modelling of neuropsychiatric disorders occurring in humans.

4. MATERIAL AND METHODS

4.1. Experimental animals

Two batches of male 129Sv and Bl6 mice were used in the present study. One set of these two inbred lines (C57BL/6NTac; Taconic Germantown, New York and 129S6/SvEvTac; Taconic Germantown, New York) was used as home cage controls (HCCs) and the other set (C57BL/6NTac; Taconic Germantown, New York and 129S6/SvEvTac; Taconic Germantown, New York) was subjected to repeated motility testing (RMT batch). The animal numbers in the experiments varied in the papers (**paper I** in HCC: Bl6 n = 12 and 129Sv n = 10; in RMT: Bl6 n = 12 and 129Sv n = 11; **paper II** Bl6 n = 41 and 129Sv n = 39; **paper III** in HCC: Bl6 n = 25 and 129Sv n = 26; in RMT: Bl6 n = 32 and 129Sv n = 28).

4.1.1. Living conditions (*Paper I, II, III*)

Animals were bred in the local animal facility and weaned from the mother at the age of 3 weeks. Thereafter the animals were assigned to home cages with up to 10 pups per cage. Mice were housed under a 12 h light/dark cycle at 22 ± 1 °C with lights on at 7:00 a.m. Animals were housed in their respective home cages (1290D Eurostandard type III cages; 425 mm × 276 mm × 153 mm; Tecniplast, Italy) with 2 cm layer of bedding and nesting material. The bedding (aspen chips) and nesting material (aspen wool) were changed weekly. The animals had ad libitum access to Ssniff universal mouse and rat maintenance diet (cat# V1534; Ssniff, Soest, Germany) and reverse osmosis-purified water, except for 1 h during testing in the RMT batch. Behavioural testing, including habituation, started at the age of 6–9 weeks, and lasted for 13 days. At the time of sample collection, animals were on average 10 weeks old.

4.2. Behavioural testing

Behavioural testing was conducted in motility boxes where animals were tracked by infrared equipment that recorded distance travelled and number of rearings. The active phase of the experimental period for all three papers was 11 days. Eleven-day follow-up period was chosen because during this period we saw that all the established behavioural and body weight changes were more or less stabilised. The experimental design for the Paper I and III was similar because HCC and RMT mice used in the third article were from the same batches as in the first article. For Paper II the saline administration group was the same as in the first article RMT saline group.

All animal procedures in this dissertation were performed in accordance with the European Communities Directive (2010/63/EU) and permit (No. 87, May 4, 2016) from the Estonian National Board of Animal Experiments. In addition, the use of mice was conducted in accordance with the regulations and guide-

lines approved by the Laboratory Animal Centre at the Institute of Biomedicine and Translational Medicine.

4.2.1. Experimental design for HCC and RMT behavioural comparisons (*Papers I, III*)

Only intervention made with HCCs was taking them out for body weight measurements on the 1st day and on the last, the 11th day, right before collecting the blood samples (Figure 1).

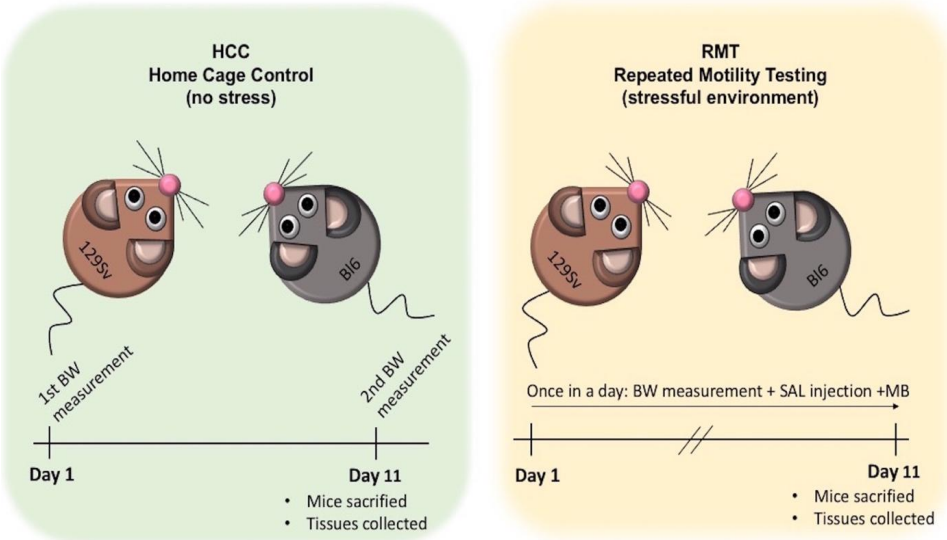

Figure 1. Schematic overview of the experimental design for HCC and RMT behavioural comparisons. Two batches of male 129Sv and Bl6 mice were used in this study. One batch was used as home cage controls (HCCs). The other batch was subjected to repeated motility testing (RMT batch). HCCs were weighed twice: on the 1st day and on the 11th day. In the RMT batch, on test days 1–11 the following routine was used: animals were weighed (BW measurement), 0.9% saline (SAL) solution was administered i.p. (SAL injection) and animals were placed for 30 min into single housing. After 30 min of single housing, animals were placed into the sound-proof motility boxes (MB) for 30 min for locomotor activity measurement and then returned to home-cages.

The RMT batch was allocated for behavioural testing for a period of 13 days (Figure 1). The first two days were used for adaptation to the testing environment, followed by experimental days 3–13 (hereinafter days 1–11) for locomotor activity measurements. On test days 1–11 the following routine was used: animals were weighed, 0.9% saline solution was administered i.p. in volume of 10ml/kg and animals were placed for 30 min into single housing cages (1284L Eurostandard type II cages, 425×276×153mm, Tecniplast, Italy). After 30 min of single housing, animals were placed into the motility boxes for 30 min loco-

motor activity measurement and then returned to home-cages. The experiment was conducted in a lit room (around 400 ± 25 lx) in soundproof photoelectric motility boxes (448×448×450mm) made of transparent Plexiglas and connected to a computer (TSE Technical & Scientific Equipment GmbH, Germany). After each mouse the boxes were cleaned with 5% ethanol solution. Software registered the distance travelled and number of rearings. Latin square design was used to randomise daily measurement cycles. On day 11, immediately after the locomotor activity recordings, animals were sacrificed by cervical dislocation, trunk blood was collected for the metabolomic analysis and brain tissues were dissected by crude method for gene expression studies.

4.2.2. Behavioural testing for amphetamine study (*Papers II, III*)

Mouse strains were studied for a period of 11 days (Figure 2). For Paper II, the animals were divided randomly into three different administration groups: saline (Saline), acute amphetamine (ACT), and repeated amphetamine (RPT). The first 2 days were allocated for adaptation to the testing environment, followed by experimental days 1–11 for locomotor activity measurements. On days 1–10 the following routine was used: animals were weighed, two groups of mice received an i.p. injection of 0.9% saline in the volume of 10 ml/kg, whereas the third group received amphetamine (d-amphetamine, 3 mg/kg i.p., Sigma-Aldrich). We chose this dose of amphetamine by comparing the results from our previous study (Innos et al., 2013) and performing a preliminary study with dopamine agonist. Innos et al. achieved a dose-dependent motor activation with amphetamine doses of 2.5, 5, and 7.5 mg/kg (Innos et al., 2013). We aimed to find a dose of amphetamine that does not cause a robust elevation of locomotor activation in both strains. However, we thought that the 2.5 mg/kg of amphetamine is too weak to achieve proper motor stimulation, because Innos et al. had used F2-hybrids having mixed backgrounds of Bl6 and 129Sv mice (Innos et al., 2013). To validate the dose, we conducted a preliminary experiment with 3 mg/kg of amphetamine and achieved a reasonable elevation of locomotor activity in both strains. Based on our sensitization curve, depicted in Paper II supplementary material Figure S1A, it is apparent that the chosen dose of amphetamine was valid. Amphetamine was dissolved in 0.9% saline (3 mg of drug in 10 ml). After administration of saline or amphetamine, the animals were placed for 30 min into single housing cages. After that the animals were transferred into individual motility boxes for 30 min where their motor activity was recorded after which the animals were returned to single housing cages. This approach helped to avoid the aggregation effect and increased aggressiveness among mice due to the strong stimulating effect of amphetamine. It has been demonstrated that amphetamine is much more toxic to grouped mice than to mice housed singly in the individual cages (Chance, 1946). The development of hyperthermic conditions in grouped mice is the reason for that (Hoehn and Lasagna, 1960). An additional argument for keeping mice separately was the

increased aggressiveness induced by amphetamine in male mice (Winslow and Miczek, 1983). Hodge and Butcher have shown that amphetamine tends to increase the frequency of fights in male mice at doses 0.5, 1, 2, and 4 mg/kg (Hodge and Butcher, 1975). Only after calming down from the stimulating effect of amphetamine (no more than 30 min) the animals were returned to their home cages. Locomotor activity of individual mice was measured in a lit room (around 400 ± 25 lx) in soundproof photoelectric motility boxes (44.8 × 44.8 × 45 cm) made of transparent Plexiglas and connected to a computer (TSE Technical & Scientific Equipment GmbH, Germany). After each mouse the floor of boxes was cleaned with 5% of ethanol solution. Software registered the distance travelled. Latin square design was used to randomise daily measurement cycles. On day 11, one of the saline groups received saline and the other amphetamine (3 mg/kg). This saline group was used as a control for acute amphetamine. Repeated amphetamine group received amphetamine (3 mg/kg) as usual. Immediately after the locomotor activity recordings, one by one (a time period between two mice was 8 min) animals were sacrificed by cervical dislocation, decapitated and trunk blood was collected for the metabolomic analysis.

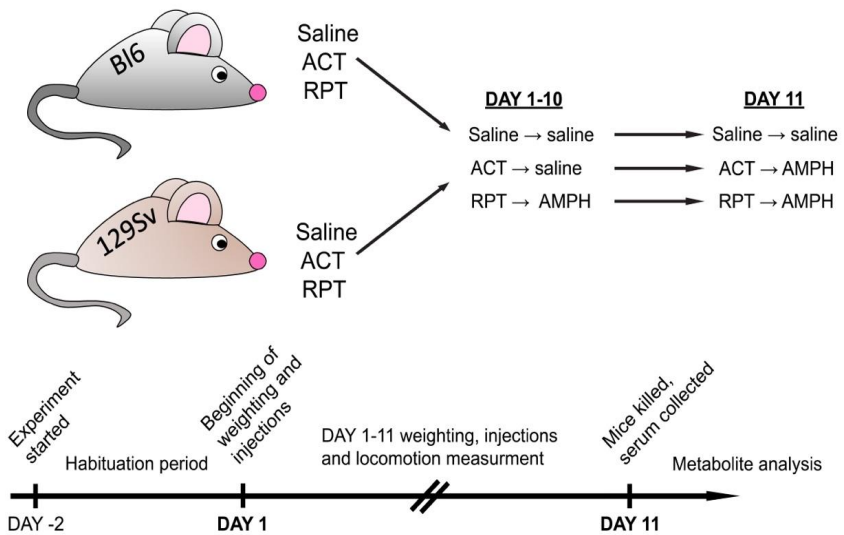

Figure 2. Representation of amphetamine treatment experimental design. Both mouse strains were studied for a period of 13 days. The first 2 days were allocated for adaptation to the testing environment, followed by testing days 1–11. On experimental day 11 mice were sacrificed, blood was collected, and metabolites were stored for analysis. In both strains three groups were formed: saline (Saline), acute amphetamine (ACT), and repeated amphetamine (RPT).

4.3. Metabolomic studies (*Papers I, II*)

4.3.1. Sample collection

Mice were sacrificed by cervical dislocation and decapitation. The trunk blood was collected into the blood sampling tubes. Blood sampling tubes were pre-processed with 20 µl of EDTA (ethylenediaminetetraacetic acid). Tubes with blood samples were gently shaken (to mix blood and EDTA) and kept at room temperature for about 30 minutes, followed by centrifugation at 2000 × g for 15 min in 4 °C. Serum was placed into new tubes and stored at −80 °C until use (Tuck et al., 2009).

4.3.2. Measurement of metabolite levels in serum samples

The endogenous metabolite concentrations were analysed with AbsoluteIDQTM p180 Kit (Biocrates Life Sciences AG, Innsbruck, Austria). This validated assay allowed comprehensive identification and quantification of amino acids, acylcarnitines, biogenic amines, hexoses, and phospho- and sphingolipids (phosphatidylcholines, lysophosphatidylcholines, sphingomyelins). Analysed glycerophospholipids (lysophosphatidylcholines, phosphatidylcholines) were differentiated according to the presence of ester and ether bonds in the glycerol moiety. The "aa" indicated that fatty acids at the sn-1 and the sn-2 position were bound to the glycerol backbone via ester bonds, while "ae" denoted that fatty acids at the sn-1 position were bound via ether bonds. The total number of carbon atoms and double bonds present in lipid's fatty acid chains were denoted as "C x:y," where x indicated the number of carbons and y the number of double bonds. Serum levels of metabolites were determined using a flow injection analysis tandem mass spectrometry (FIA-MS/MS) as well as a liquid chromatography (LC-MS/MS) technique on a QTRAP 4500 mass-spectrometer (Sciex, USA). All preparations and measurements were performed as described in the manufacturer's kit manual. Identification and quantification of the metabolite concentrations were achieved using multiple reaction monitoring (MRM) along with internal standards. Calculations of metabolite concentrations were automatically performed by MetIDQTM software (Biocrates Life Sciences AG, Innsbruck, Austria). Data quality was checked based both on the level of detection and the level of quantification that can be found from Paper I and II supplementary material.

4.4. Gene and protein expression analysis (*Paper III*)

For HCC, after the last body weight measurement, and for RMT, after the last locomotion activity measurement, mice were decapitated immediately. Decapitation and behavioural evaluations were carried out in separate rooms. The frontal cortex, hippocampus, ventral striatum, dorsal striatum, and midbrain were dissected according to the coordinates provided in the mouse brain atlas

by Franklin and Paxinos and quickly frozen in liquid nitrogen (Franklin and Paxinos, 2013). All the brain tissues were stored at −80 °C. In gene expression studies, two batches of mice were measured together at the same time (both strains from HCC and both strains from RMT).

4.4.1. RNA isolation, cDNA synthesis, and quantitative real-time-PCR (qPCR)

Total RNA was extracted individually from all the brain tissues of each mouse using Trizol® Reagent (Invitrogen, USA) according to the manufacturer's protocol. RNA quality control was performed by Nanodrop where the ratios 260/230 and 260/280 were always around 2.00. After RNA extraction, DNase treatment with RNase-free DNase I (Invitrogen, Waltham, MA, USA) was applied according to the manufacturer's protocol to prepare DNA free RNA. For the first strand cDNA synthesis, three micrograms of total RNA of each sample was used with random hexamers (Applied Biosystems, Bedford, MA, USA) and SuperScript™ III Reverse Transcriptase (Invitrogen, Waltham, MA, USA) in cDNA synthesis. In qPCR, every reaction was made in four parallel samples to minimise possible errors. All reactions were performed in a final volume of 10 µL, using 5 ng of cDNA. Real-time qPCR was performed using 5x HOT FIREPol® EvaGreen® qPCR Supermix (Solis BioDyne, Tartu, Estonia). For thermal cycling, a QuantStudio 12 K Flex Software v.1.2.2 Real-Time PCR System equipment (Applied Biosystems, Bedford, MA, USA) was used at 95 ◦C for 15 min, then 40 cycles at 95 °C for 20 s, 61 °C for 20 s and 72 °C for 20 s. All qPCR data has been presented in the log10 scale, in the form of $2^{-\Delta CT}$, where ΔCT was the difference in cycle threshold (CT) between the gene of interest and housekeeper gene hypoxanthine guanine phosphoribosyl transferase (*Hprt*). For quality control, three internal samples with *Hprt* primers were used between all the measurements.

4.4.2. Primer design

Primers were designed using the Primer3plus web interface (Untergasser et al., 2012). Primers (LGC Biosearch Technologies, Risskov, Denmark) for qPCR assay were designed for the detection of specific transcripts from exon-exon junction, eliminating the possibility of contamination with genomic DNA (the applied primers can be found from Paper III supplementary material). Melting curves were analysed to ensure amplification specificity and no primer-dimer formation. Primer qPCR efficiency was evaluated using serial 5x dilutions of cDNA samples. Hprt was chosen as the housekeeper gene from previously published data and was optimised for our assay (Raud et al., 2009).

4.4.3. Protein expression analysis by Western Blot method

Samples of the frontal cortex and hippocampus were sonicated in ice-cold RIPA lysis buffer (Thermo Scientific, Waltham, MA, USA) containing 1x protease inhibitor (78430, Thermo Scientific, Waltham, MA USA) and centrifuged at 14,000 × *g* for 10 min at 4 °C. Supernatants were removed and used for Western blot analysis. Protein concentrations were measured with the BCA protein assay kit (23225, Thermo Scientific, Waltham, MA, USA) and 20 μg of protein from each sample were run on a NuPAGE Bis–Tris gel using the XCELL SureLock System (Invitrogen, Waltham, MA, USA). Protein was then transferred to a nitrocellulose membrane, after which the membranes were blocked and probed with primary antibodies (primary antibodies can be found from Paper III supplementary material) overnight at 4 °C. Immunoblots were then incubated with goat anti-rabbit (A11369, Invitrogen, Waltham, MA, USA) or goat anti-mouse (A-21057, Invitrogen, Waltham, MA, USA) fluorescent conjugated secondary antibodies for 1 h at room temperature, followed by visualisation using a LI-COR Odyssey CLx system (LI-COR Biotechnologies, Lincoln, NE, USA). Images were converted to grayscale and the density of protein was quantified using Image Studio Lite v 3.1.4 (LI-COR Biotechnologies, Lincoln, NE, USA). β-actin was used as a loading control.

4.5. Statistical analysis and data presentation (*Papers I, II, III*)

For papers I and II that described behaviour and metabolomic measurements, we used a similar arrangement for statistical analysis. First, Shapiro–Wilk test was applied to test the normality assumption of the data. For both papers, the behavioural and body weight outcomes corresponded to the normal distribution. For Paper I, behavioural and body weight results were analysed by repeated measures ANOVA (genotype × days 1 and 11), followed by Bonferroni *post hoc* test in RMT or by paired T-test for HCCs. To demonstrate the difference in activity and body weight gain in different strains during the experimental period T-test was applied. All statistical tests were two-sided, and only $p \leq 0.05$ was considered to be statistically significant.

To compare metabolomic profiles of Bl6 and 129Sv mice in both experimental control conditions, we used Mann-Whitney U-test, as the majority of metabolite data did not follow normal distribution. For metabolomic study, the statistical adjustment for multiple test (Bonferroni correction) was applied for the number of measured biomarkers (164 for HCC and 160 for RMT batch) and differences between groups were considered significant at $p \leq 0.0003$. In addition, to provide an overview about the magnitude of the differences between groups, effect size estimates (eta^2) for non-parametric tests were calculated (the value of squared standardised test statistic (Z) was divided by the total number

of animals; N = 22 for HCC and N = 23 for RMT). Eta^2 values of ≥ 0.14 were defined as large effects.

Next, to demonstrate mouse strain dependent main effects on biomarker levels, a general linear model (GLM) was applied. Only subsets of biomarkers selected based on the correction for multiple comparisons were inputted into GLM. Biomarker values for GLM were log_{10}-transformed to satisfy the normality assumption of data. F-tests were used to further compare the fit of linear models and analyse significant main effects in the final models and partial eta^2 values (the proportion of the effect in addition to error variance that is attributable to the effect) were established for the final models. Partial eta^2 values of ≥ 0.26 were defined as large effects.

For Paper II, the distance travelled was analysed by two-way ANOVA. The independent factors were strain (129Sv, Bl6) and administration (saline, acute amphetamine, repeated amphetamine) on the 11th day, followed by *post-hoc* unequal *N* Tukey HSD test. In all the following analyses, $p < 0.05$ was considered indicative of statistical significance. The body weight changes in 129Sv and Bl6 strain were analysed by repeated measures ANOVA [strain × test day (1st and 11th day)], followed by *post-hoc* unequal *N* Tukey HSD test. The locomotor activity of 129Sv strong and weak responders was also analysed with repeated measures ANOVA [subgroup × test day (1st and 11th day)], followed by *post hoc* Tukey HSD test.

In Paper II, the metabolite levels did not follow normal distribution and thereby the Kruskal–Wallis analysis (multiple comparisons of mean ranks for all groups) was performed to analyse the effects of saline, acute and repeated amphetamine on metabolite levels. Significant Kruskal–Wallis analysis was followed by Dunn's multiple comparison tests. The magnitudes of effect sizes were interpreted as moderate (eta^2 ranging 0.06–0.13) or large ($eta^2 \geq 0.14$).

The associations between distance travelled, metabolite levels and their ratios in 129Sv mice treated with amphetamine were analysed using the Spearman's rank correlation. Mann–Whitney *U*-test was applied to compare the raw data of two independent samples (strong and weak responders to repeated amphetamine in 129Sv).

A GLM multivariate analysis with a backward elimination procedure was performed to examine the associations between distance travelled, metabolite levels and their ratios in 129Sv mice responding differently to repeated AMPH. To normalise the distribution, we performed logarithmic transformation (log_{10}) of the values of dependent characteristics prior to analysis. In the GLM analysis $p < 0.05$ was considered to be statistically significant. Partial eta^2 value of ≥ 0.26 was defined as a large effect.

In Paper III, where we described gene and protein expression, we used for statistical analysis a different approach compared with the Papers I and II. For the locomotor activity in RMT, repeated measures ANOVA was applied followed by Bonferroni *post hoc* test. For amphetamine-induced locomotor stimulation one-way ANOVA was used followed by Bonferroni *post hoc* test. Regarding body weight measurements then body weight change (ΔBW) was

expressed as change of the initial body weight (weight on the 11th day − weight on the 1st day).

To normalise the distribution of gene expression data, logarithmic transformation (log_2) of the values was performed prior to data analysis. Comparison of gene expression between strains and environments was performed by using two-way ANOVA [strain (Bl6 or 129Sv) × environment (HCC or RMT)] followed by Bonferroni *post hoc* test. All differences were considered statistically significant at $p \leq 0.05$. For protein analysis, differences between groups were compared with unpaired T-test with Welch's correction or Kruskal-Wallis test. For the Kruskal-Wallis test, if a significant variance was found, the Dunn's multiple comparison test was used for *post hoc* analysis.

For Paper I the data was shown in figures as mean ± 95% CI. For Paper II, we used mean values and standard error averages (SEM) in the figures. For Paper III we preferred mean values ± SD figure representation. All the statistical analyses were made by using Statistica software (StatSoft Inc., Tulsa, OK, USA, 13th edition). Figures in Paper I and II were generated by using GraphPad software 7th edition (GraphPad Software, California, USA). In Paper III GraphPad software, 8th edition (GraphPad Software, San Diego, CA, USA) was applied.

5. RESULTS

5.1. Paper I: Metabolic profile associated with distinct behavioural coping strategies in 129Sv and Bl6

5.1.1. The body weight changes and metabolic profile of Bl6 and 129Sv in HCCs

The body weight of 129Sv and Bl6 was measured twice: on the 1st day and on the 11th day before collecting blood samples for metabolic profile measurements. Comparison of body weight on the 1st vs 11th day revealed weight gain in both strains (for 129Sv 26.07 ± 0.98 g vs 28.26 ± 1.08 g; paired $t_{(10)} = 12.63$, $p < 0.0001$ and for Bl6 26.32 ± 1.35 g vs 27.71 ± 1.5 g; paired $t_{(11)} = 8.15$, $p < 0.0001$; Figure 3a). However, the weight gain was more pronounced in 129Sv (2.19 ± 0.58 g) compared to Bl6 (1.39 ± 0.59 g; $t_{(21)} = 3.28$, $p = 0.0036$; Figure 3b).

The applied metabolic assay allowed the detection of 164 metabolites (data can be found from Paper I supplementary material Table S1), of which 76 metabolite levels were significantly different between 129Sv and Bl6 according to Mann-Whitney U test ($p \leq 0.05$). After Bonferroni correction 13 metabolite levels were significantly different in comparison of 129Sv and Bl6 mouse lines; more precisely 5 metabolite levels showed higher values in Bl6 mice (Table 1) and 8 metabolite concentrations were elevated in 129Sv (Table 2).

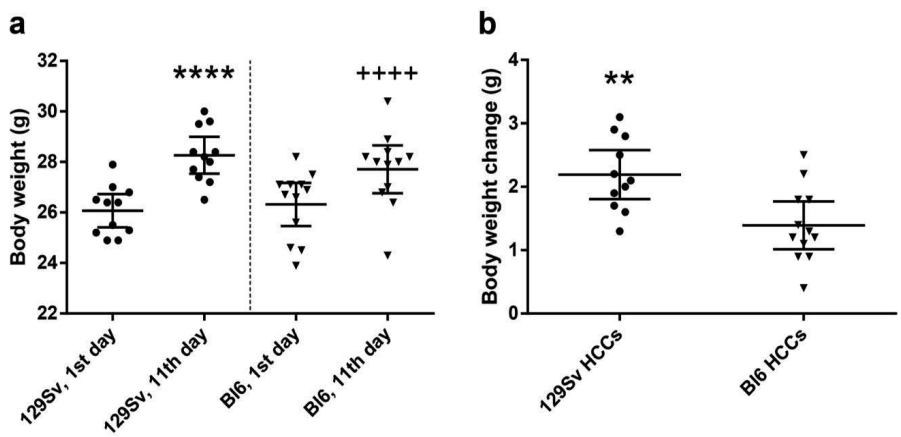

Figure 3. Body weight of 129Sv and Bl6 in HCC batch. Body weight on the 1st and 11th day (**a**) and total body weight change during experiment (**b**) for 129Sv and Bl6 in HCCs. The comparison between the 1st vs 11th day revealed weight gain in both strains (**** – $p < 0.0001$; ++++ – $p < 0.0001$). However, the gain of body weight was more pronounced in 129Sv (2.19 ± 0.58 g) compared to Bl6 (1.39 ± 0.59 g; ** – $p = 0.0036$; **b**).

Table 1. Significantly elevated metabolite levels in Bl6 compared to 129Sv in HCCs. Raw data of marker levels (μM) are presented as median and range. Effect size estimate (eta^2) has been calculated by dividing the value of squared standardised test statistic (Z^2) with the total number of observations (N). From 164 metabolites quantified 5 metabolite levels remained statistically significant after Bonferroni correction ($p \leq 0.0003$) in Bl6 (Mann-Whitney U test non-corrected p-value has been shown). Glycero-phospholipids include: lysophosphatidylcholine acyls, phosphatidylcholine diacyls (indicated in italic).

Metabolite	**Bl6 (n=12)**	**129Sv (n=10)**	**Z- score**	***p*-value**	**Eta^2**
	Median (range)	**Median (range)**			
Amino acids and biogenic amines					
Acetyl-ornithine	10.6 (8.88 – 14.1)	4.53 (3.51 – 5.39)	3.92	0.00009	0.7
Alpha-aminoadipic acid	10.6 (8.07 – 14.3)	5.1 (2.74 – 8.21)	3.86	0.0001	0.68
Glycerophospholipids					
Lysophosphatidylcholine acyls					
PC(16:1/0:0)	8.59 (6.13 – 15.9)	3.29 (1.30 – 5.06)	3.92	0.00009	0.7
PC(20:3/0:0)	11.8 (6.55 – 18.1)	5.69 (2.23 – 8.22)	3.66	0.0002	0.61
Phosphatidylcholine diacyls					
PC aa C34:3	9.11 (6.78 – 12.8)	4.95 (3.90 – 6.95)	3.79	0.0001	0.68

Table 2. Significantly elevated metabolite levels in 129Sv compared to Bl6 in HCC. Raw data of marker levels (μM) are presented as median and range. Effect size estimate (eta^2) has been calculated by dividing the value of squared standardised test statistic (Z^2) with the total number of observations (N). From 164 metabolites quantified 8 metabolite levels were statistically significant after Bonferroni correction ($p \leq 0.0003$) in 129Sv (Mann-Whitney U test non-corrected *p*-value has been shown). Glycerophospholipids include: lysophosphatidylcholine acyls (indicated in italic).

Metabolite	**Bl6 (n=12)**	**129Sv (n=11)**	**Z-score**	***p*- value**	**Eta^2**
	Median (range)	**Median (range)**			
Acylcarnitines					
C5	0.24 (0.17 – 0.39)	0.66 (0.38 – 0.84)	-3.86	0.0001	0.68
Glycerophospholipids					
Phosphatidylcholine acyl-alkyls					
PC ae C36:2	4.67 (3.08 – 6.18)	8.73 (6.41 – 10.2)	-3.92	0.00009	0.7
PC ae C38:2	3.56 (2.27 – 4.89)	8.31 (5.37 – 9.90)	-3.92	0.00009	0.7
PC ae C40:4	1.00 (0.63 – 1.12)	1.49 (1.15 – 1.70)	-3.92	0.00009	0.7
PC ae C40:6	1.15 (0.78 – 1.36)	1.75 (1.30 – 2.08)	-3.76	0.0002	0.64
Sphingolipids					
SM (OH) C14:1	0.25 (0.14 – 0.36)	0.51 (0.37 – 0.66)	-3.92	0.00009	0.7
SM (OH) C22:1	0.54 (0.40 – 0.67)	0.85 (0.67 – 1.02)	-3.92	0.00009	0.7
SM C24:0	2.43 (1.88– 2.60)	3.23 (2.77 – 4.44)	-3.92	0.00009	0.7

5.1.2. Metabolite levels elevated in Bl6 HCCs

Acetyl-ornithine and lysoPC (16:1/0:0) both (Z = 3.92, eta^2 = 0.7) displayed the most significant elevation in Bl6 compared to 129Sv (Table 1). Significant elevations (Z value > 3, $eta^2 \geq 0.61$) were also established for biogenic amine alpha-aminoadipic acid, glycerophospholipids PC(20:3/0:0) and PC aa C34:3 (Table 1). Furthermore, ratio of C4/C5-(Z = 3.92, eta^2 = 0.7) and several calculated ratios of glycine, including glycine/PC ae 38:2 (Z = 3.92, eta^2 = 0.7) and glycine/serine (Z = 3.86, eta^2 = 0.68) were elevated in Bl6 (data can be found from Paper I supplementary material Table S3). All above-mentioned comparisons survived Bonferroni correction for multiple comparisons ($p \leq 0.0003$).

5.1.3. Metabolite levels elevated in 129Sv HCCs

Several phosphatidylcholine acyl-alkyls (PC ae C36:2, PC ae C38:2, PC ae C40:4) and sphingolipids (SM (OH) C14:1, SM (OH) C22:1, SM C24:0) displayed the strongest elevation in 129Sv compared to Bl6 (all Z = 3.92, all eta^2 = 0.7; Table 2). Significant elevations in the 129Sv were established for acylcarnitine C5- (Z value = 3.76, eta^2 = 0.64) and glycerophospholipid PC ae C40:6 (Z value = 3.86 eta^2 = 0.68; Table 2). The ratio between PC(16:0/0:0)/ PC(16:1/0:0), spermidine/putrescine and C5-/ carnitine C0 were significantly higher in 129Sv compared to Bl6 (all $Z \geq -3.86$, $eta^2 \geq 0.68$; data can be found from Paper I supplementary material Table S4). All above-mentioned comparisons survived Bonferroni correction for multiple comparisons ($p \leq 0.0003$).

5.1.4. Differences in metabolite levels highlighted by GLM in HCCs

Using GLM, we confirmed a significant main effect ($F_{(6, 15)} = 33.91$, partial eta^2 = 0.99) of mouse strain on the levels of acylcarnitine C5-, glycerophospholipids (PC ae C36:2, PC ae C38:2, PC ae C40:4, PC ae C40:6), sphingolipids (SM (OH) C14:1, SM (OH) C22:1, SM C24:0), biogenic amines (acetyl-ornithine, alpha-aminoadipic acid, carnosine), glycerophospholipids [PC (16:1/0:0), PC(20:3/0:0), PC aa C34:3] and on body weight change in HCC condition (Table 3).

Table 3. Regression coefficients (ß), confidence intervals (CI) and significance values of log_{10}-transformed metabolite levels adjusted for strain in HCC. $F_{(6,15)}$ = 33.91, p = 0.0002, partial eta^2 = 0.99. Glycerophospholipids include: lysophosphatidylcholine acyls, phosphatidylcholine diacyls, and phosphatidylcholine acyl-alkyls (indicated in italic).

Bl6 and 129Sv comparison	**ß**	**ß (95 % CI)**	***t-value***	***p*-value**
Acylcarnitines				
C5-	-0,87	(-1.10, -0.65)	-8.02	<0.0000001
Biogenic amines				
Acetyl-ornithine	0.95	(0.82, 1.09)	14.36	<0.00000001
Alpha-aminoadipic acid	0.84	(0.58, 1.09)	6.87	0.000001
Carnosine	0.74	(0.43, 1.05)	4.91	0.00008
Glycerophospholipids				
Lysophosphatidylcholine acyls				
PC(16:1/0:0)	0.84	(0.59, 1.09)	6.99	0.000001
PC(20:3/0:0)	0.76	(0.46, 1.06)	5.23	<0.0001
Phosphatidylcholine diacyl				
PC-aa-C34:3	0.84	(0.59, 1.09)	6.99	0.000001
Phosphatidylcholine acyl-alkyls				
PC-ae-C36:2	-0.86	(-1.10, -0.62)	-7.50	<0.000001
PC-ae-C38:2	-0.89	(-1.10, -0.68)	-8.91	<0.0000001
PC-ae-C40:4	-0.82	(-1.09, -0.55)	-6.37	<0.00001
PC-ae-C40:6	-0.79	(-1.07, -0.50)	-5.71	<0.00001
Sphingolipids				
SM-(OH)-C14:1	-0.83	(-1.09, -0.57)	-6.65	<0.00001
SM-(OH)-C22:1	-0.86	(-1.10, -0.61)	-7.39	<0.000001
SM-C24:0	-0.82	(-1.09, -0.55)	-6.40	<0.00001
Behavioural parameter				
Change in body weight	-0.55	(-0.94 -0.16)	-2.96	0.01

5.1.5. The behavioural and body weight changes of 129Sv and Bl6 in RMT mice

As expected, Bl6 and 129Sv displayed significantly different motor activity. Repeated measures ANOVA revealed a statistically significant strain effect for distance travelled (strain effect $F_{(1,21)} = 41.52$; $p = 0.000002$; repeated experiments $F_{(1,21)} = 1.04$; $p = 0.32$; strain × repeated experiments $F_{(1,21)} = 0.16$; $p = 0.69$). Distance travelled on day 1 by Bl6 was significantly longer compared to 129Sv ($t_{(21)} = 3.93$; $p = 0.0008$). This difference remained statistically significant on day 11 as well ($t_{(21)} = 6.07$; $p = 0.000005$; Figure 4a; additional data can be found from Paper I supplementary material Figure S1a). The frequency of rearings was also strongly in favor of Bl6. The initial difference in vertical activity between the strains increased during repeated testing (strain: $F_{(1,21)} = 51.51$, $p = 0.0000001$; repeated experiments $F_{(1,21)} = 14.84$, $p = 0.0009$; strain × repeated experiments $F_{(1,21)} = 7{,}41$, $p = 0.013$). On day 1, Bl6 performed more rearings compared to 129Sv ($t_{(21)} = 4.93$; $p = 0.00007$; Figure 4b; additional data can be found from Paper I supplementary material Figure S1b) and by day 11 the difference had further increased ($t_{(21)} = 5.62$; $p = 0.00001$). In Bl6 the frequency of rearings was elevated more than two-fold during repeated testing (158 ± 102 on 1st *vs*. 374 ± 190 on 11th day; paired $t_{(11)} = -3.51$; $p = 0.005$). Body weight measurements also showed a significant difference between the two strains after repeated testing (repeated measures ANOVA; strain effect $F_{(1,21)} = 0.73$, $p = 0.40$; repeated experiments $F_{(1,21)} = 9.79$, $p = 0.005$; strain effect × repeated experiments $F_{(1,21)} = 21.71$, $p = 0.0001$; data can be found from Paper I supplementary material Figure S1c). In the beginning of the behavioral experiment both strains had nearly identical body weight. Comparison of body weight on the 1st vs 11th day revealed weight loss in 129Sv (24.15 ± 1.93 g vs 22.88 ± 1.64 g; paired $t_{(10)} = 6{,}53$, $p < 0.0001$) and stabilisation in Bl6 (24.02 ± 2.02 g vs 24.27 ± 1.51 g; paired $t_{(11)} = 0.97$, $p = 0.3508$; Figure 4c). After repeated manipulations, marginal increase was seen in Bl6 (0.25 ± 0.26) and significant reduction of body weight was established for 129Sv (−1.27 ± 0.20; $t_{(21)} = 4.66$, $p = 0.0001$; Figure 4d).

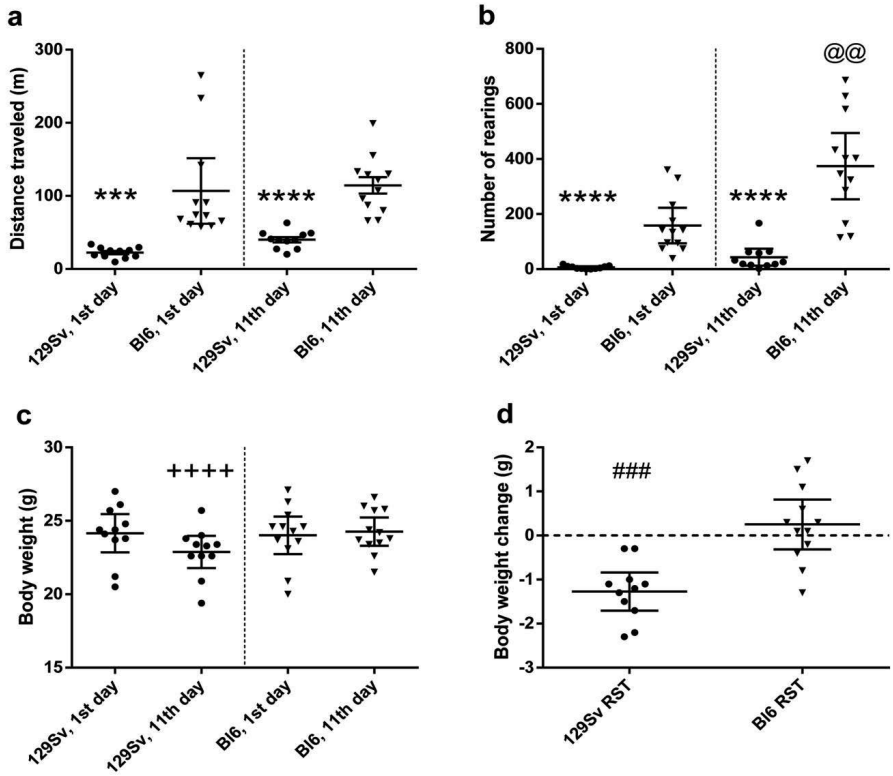

Figure 4. Motility and body weight of 129Sv and Bl6 in RMT batch Main effects of distance travelled (**a**), number of rearings (**b**), body weight (**c**) and body weight change (**d**). T-test was applied to demonstrate differences between 1st and 11th day. *** – $p <$ 0.001 and **** – $p < 0.00001$ indicates differences between strains on corresponding days (**a, b**). @@ – $p = 0.005$ indicates difference between 1st and 11th rearings in Bl6 (**b**). ++++ – $p < 0.00001$ indicates absolute weight on 1st and 11th day (**c**) and ### – $p <$ 0.0001 weight change in 129Sv HCC batch during experiment (**d**). After repeated manipulation, modest elevation of body weight was seen in Bl6 (0.25 ± 0.26) and significant reduction of body weight was established in 129Sv (-1.27 ± 0.20; ### – $p =$ 0.0001; **d**). More information about repeated testing can be found in the Paper I supplementary material Figure S1.

5.1.6. Metabolic profile of Bl6 and 129Sv in RMT

The applied metabolic assay allowed the detection of 160 metabolites (data can be found from Paper I supplementary material Table S5), of which 52 metabolite levels were significantly different between 129Sv and Bl6 mice. After Bonferroni correction 5 metabolite levels were significantly different between 129Sv and Bl6 in RMT batch; 4 metabolite levels showed higher values in Bl6 and one metabolite concentration survived Bonferroni correction in 129Sv (Table 4).

Table 4. Significantly elevated metabolite levels for both strains in RMT. Raw data of marker levels (μM) are presented as median and range. Effect size estimate (eta^2) has been calculated by dividing the value of squared standardised test statistic (Z^2) with the total number of observations (N). After application of Bonferroni correction ($p \leq 0.0003$) 5 metabolite levels remained statistically significant in comparison of 129Sv and Bl6 in RMT batch (Mann-Whitney U test non-corrected p-value has been shown); 4 metabolite levels in Bl6 and one metabolite concentration in 129Sv. Glycerophospholipids include: lysophosphatidylcholine acyls (indicated in italic).

Metabolite	**Bl6 (n=12)**	**129Sv (n=11)**	**Z-score**	***p*-value**	**Eta^2**
	Median (range)	**Median (range)**			
Significantly elevated metabolite levels in Bl6					
Amino acids and biogenic amines					
Acetyl-ornithine	15.9 (10.6 – 19.1)	7.25 (5.40 – 12.1)	3.91	0.00009	0.67
Alpha-aminoadipic acid	10.950 (7.420 – 17.200)	0.000 (0.000 – 9.490)	3.85	0.0001	0.65
Carnosine	15.6 (3.20 – 21.2)	2.79 (1.17 – 7.34)	3.72	0.0002	0.60
Glycerophospholipids					
Lysophosphatidylcholine acyls					
PC(16:1/0:0)	12.8 (6.31 – 17.8)	5.87 (3.50 – 8.26)	3.79	0.0002	0.62
Significantly elevated metabolite levels in 129Sv					
Acylcarnitines					
C5-	0.22 (0.18 – 0.28)	0.40 (0.23 – 0.63)	-3.79	0.0002	0.63

5.1.7. Metabolite levels elevated in Bl6 RMT mice

Acetyl-ornithine displayed the most significant elevation in Bl6 compared to 129Sv ($Z = 3.91$, $eta^2 = 0.67$; Table 4). Significant elevations (Z value ≥ 3.72, $eta^2 \geq 0.60$) were also established for biogenic amines (alpha-aminoadipic acid, carnosine), glycerophospholipid PC(16:1/0:0), the ratios of glycine/PC ae 38:2 and C3/C4 (Z value ≥ 3.76, $eta^2 \geq 0.61$; Table 4). All above-mentioned comparisons survived Bonferroni correction for multiple comparisons ($p \leq 0.0003$).

5.1.8. Metabolite levels elevated in 129Sv RMT mice

Significant elevation (Z value > 3, $eta^2 \geq 0.4$) in the 129Sv group was established for acylcarnitine C5- (Table 4). The following ratios were also significant (Z value ≥ 3.60, $eta^2 \geq 0.56$): Fisher ratio, C4/C0, C5-/C0, C14/C16:1, C16/C16:1, C18/C18:1 and PC(16:0/0:0)/PC(16:1/0:0) (data can be found from Paper I supplementary material Table S6 and S8). All above-mentioned comparisons survived Bonferroni correction for multiple comparisons ($p \leq 0.0003$).

5.1.9. Differences in metabolite levels highlighted by GLM in RMT mice

Altogether 5 metabolite levels survived Bonferroni correction for multiple comparisons and were included into the GLM test. These metabolite levels [acylcarnitine C5-, acetyl-ornithine, alpha-aminoadipic acid, carnosine and PC(16:1/0:0)] as well as measured behavioural parameters (distance travelled, number of rearings, changes in body weight) accounted for 99% ($F_{(8,8)} = 143.5$, $p = 0.0000001$) of the mouse strain differences in RMT condition (Table 5). Thus, our results indicate that there is a strong strain-dependent (Bl6 vs. 129Sv) interplay among metabolic markers and behavioural characteristics in both (HCC, RMT) conditions.

Table 5. Regression coefficients (ß), confidence intervals (CI) and significance values of log_{10}-transformed metabolite levels adjusted for strain in RMT. Glycerophospholipids include: lysophosphatidylcholine acyls (indicated in italic). $F_{(8, 8)}$ =143.5, p = 0.0000001, partial eta^2 = 0.99.

Bl6 and 129Sv comparison	ß	ß (95 % CI)	*t-value*	*p*-value
Acylcarnitine				
C5-	-0.88	(-1.14, -0.61)	-7.10	<0.00001
Amino acids and biogenic amines				
Acetyl-ornithine	0.81	(0.49, 1.13)	5.38	<0.0001
Alpha-aminoadipic acid	0.74	(0.36, 1.11)	4.21	<0.001
Carnosine	0.75	(0.39, 1.11)	4.40	<0.001
Glycerophospholipids				
Lysophosphatidylcholine acyl				
PC(16:1/0:0)	0.71	(0.32, 1.10)	3.88	<0.01
Behavioural parameters				
Distance travelled	0.81	(0.49, 1.13)	5.42	<0.0001
Number of rearings	0.75	(0.38, 1.11)	4.35	<0.001
Change in body weight	0.63	(0.20, 1.06)	3.15	<0.01

5.2. Paper II: Distinct alterations in behaviour and metabolite levels in 129Sv and Bl6 mouse strains due to repeated amphetamine treatment

5.2.1. Amphetamine-induced changes in locomotor activity and body weight

The comparison of locomotor activity of age-matched 129Sv and Bl6 mice after acute and repeated amphetamine demonstrated a significant difference between the two strains [two-way ANOVA: strain – $F_{(1, 73)}$ = 7.12, p < 0.01; administra-

tion – $F_{(2, 73)}$, $p < 0.01$; strain × administration – $F_{(2, 73)}$, $p = 0.015$]. Acute amphetamine (3 mg/kg) after repeated saline administration caused a statistically significant elevation of locomotor activity only in Bl6 ($p = 0.03$, unequal N Tukey HSD test), but not in 129Sv. However, after repeated amphetamine both strains displayed a significant increase in distance travelled compared to saline (for 129Sv $p < 0.01$ and for Bl6 $p < 0.01$; Figure 5). The locomotor activity of 129Sv (342 ± 246 m) reached the level of Bl6 (349 ± 65 m) after repeated amphetamine. One has to take into account two peculiarities. First, the elevation of locomotor activity in 129Sv under the influence of repeated amphetamine was more pronounced (2.6-fold increase compared to acute amphetamine) compared to Bl6 mice (1.3-fold increase compared to acute amphetamine). Second, we established greater locomotor activity dispersion around the mean value in 129Sv (SD = 246) compared to Bl6 (SD = 65). Therefore, two differently responding groups could be formed among 129Sv mice after repeated amphetamine (Figure 6A), representing "weak responders" (n = 7) and "strong responders" (n = 7). In weak responders the distance travelled was 37–279 m and in strong responders 320–746 m (Figure 6A). Repeated measures ANOVA (subgroup × test day) demonstrated significant differences between weak and strong responders [subgroup: $F_{(1, 12)} = 17.5$, $p < 0.01$; test day: $F_{(1, 12)} = 55.2$, $p < 0.01$; subgroup × test day: $F_{(1, 12)} = 39.7$, $p < 0.01$]. The Tukey HSD test established significant differences between strong responders on the 1st and 11th day ($p < 0.01$), as well as between strong and weak responders on the 11th day ($p < 0.01$). No difference was established in the weak activity subgroup if measured on the 1st and 11th day (Figure 6A). The combination of distance travelled data from both strains (Bl6 + 129Sv) showed a clear amphetamine effect on motor sensitization [$F_{(2, 76)} = 26.8$, $p < 0.01$; Figure 8A]. Acute amphetamine caused a statistically significant elevation in distance travelled compared to saline (79 ± 10 vs. 203 ± 20 m, $p < 0.01$, unequal N Tukey HSD test). Repeated amphetamine led to a further increase compared to acute amphetamine in distance travelled (203 ± 20 vs. 345 ± 36 m, $p < 0.01$, unequal N Tukey HSD test).

Repeated measures ANOVA demonstrated significant differences in the body weight in 129Sv, but not in Bl6 mice if measured on the 1st and 11th day [repeated measures ANOVA: test day $F_{(1, 73)} = 49.4$, $p < 0.01$; test day x strain $F_{(1, 73)} = 86.7$, $p < 0.01$]. Repeated saline and amphetamine did not cause any remarkable changes in the body weight of Bl6 (Figure 7B). However, in 129Sv repeated saline and amphetamine caused a similar reduction of body weight in all groups showing that this decrease was not caused by amphetamine, but by the experimental procedure (Figure 7A). Moreover, there was no difference in body weight between weak and strong responders to amphetamine in the 129Sv group (Figure 6B). Combination of body weight data from both strains (Bl6 +129Sv) did not establish any significant differences [$F_{(2, 76)} = 0.81$, $p = 0.45$]: saline − 23.6 ± 0.36, acute amphetamine − 23.3 ± 0.32, repeated amphetamine − 22.9 ± 0.44 (Figure 8B).

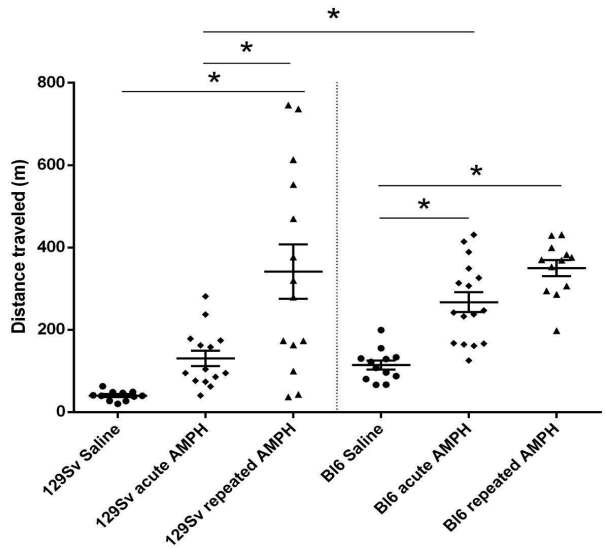

Figure 5. Amphetamine induced motor sensitization in 129Sv and Bl6 (mean values ± SEM). Motor activity was analysed using two-way ANOVA, followed by an unequal Tukey HSD test. *$p < 0.05$ was considered statistically significant. More information about repeated testing can be found in the Paper II supplementary material Figure S1. Black circle, saline group; black diamond, acute amphetamine group and black triangle, repeated amphetamine group.

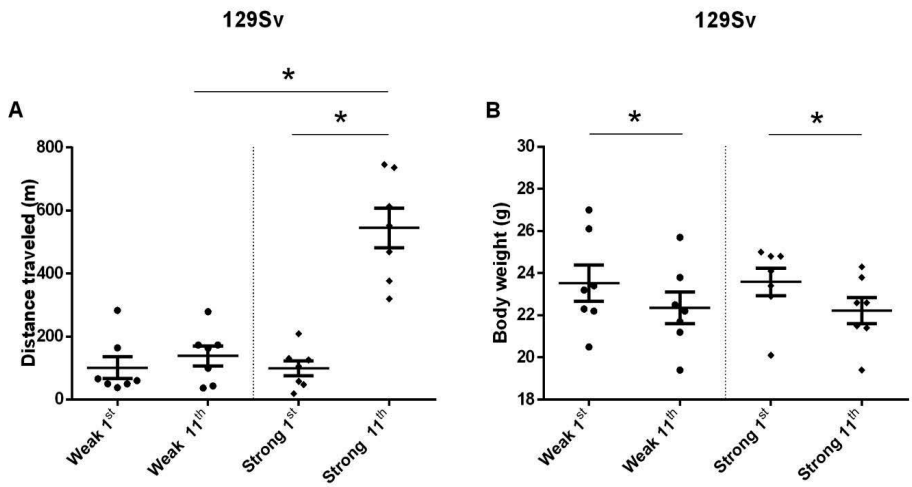

Figure 6. Motor sensitization (A) and body weight (B) in response to repeated amphetamine administration in 129Sv weak and strong responders (mean values ± SEM). Strong responders displayed significantly greater sensitization to amphetamine. Both weak and strong responders displayed loss of body weight. Motor activity and body weight outcomes were analysed by repeated measures ANOVA, followed by Tukey HSD test. *$p < 0.05$ was considered statistically significant. Black circle, weak responders; black diamond, strong responders.

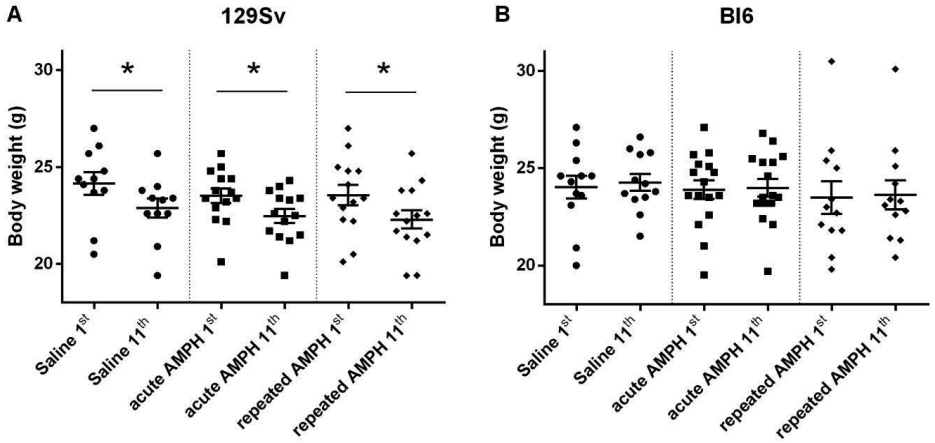

Figure 7. Body weight changes in 129Sv and Bl6 during the experiment (mean values ± SEM). Body weight outcomes were analysed by two-way ANOVA, followed by unequal *N* Tukey HSD test. *$p < 0.05$ was considered statistically significant. In all groups of 129Sv strain a similar reduction of body weight was seen **(A)**, while repeated administrations did not cause any body weight changes in Bl6 **(B)**. Black circle – saline group; black square – acute amphetamine group and black diamond – repeated amphetamine group.

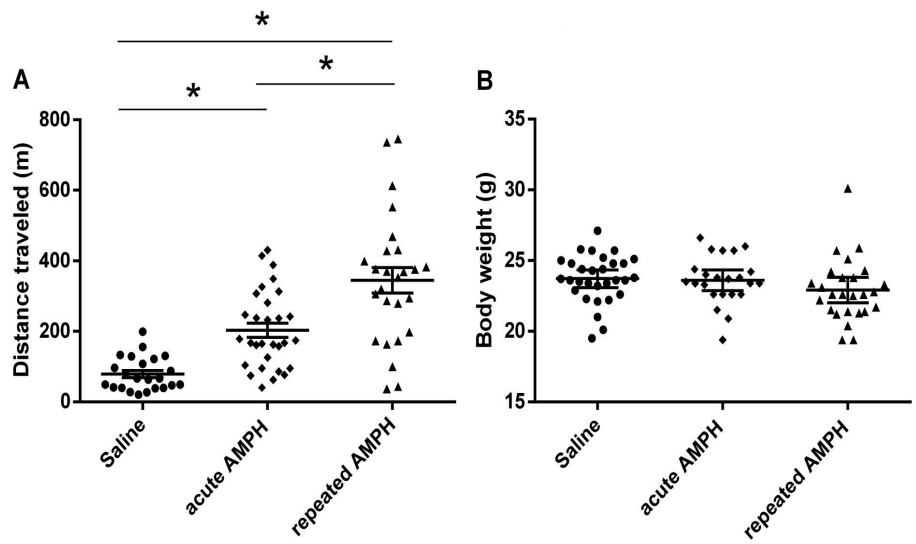

Figure 8. Amphetamine-induced changes in distance travelled (A) and body weight (B) on the 11th day after pooling the data from both strains (Bl6+129Sv) (mean values ± SEM). The results were analysed using one-way ANOVA, followed by an unequal *N* Tukey HSD test. *$p < 0.05$ was considered to be statistically significant. Black circle, saline administration; black diamond, acute amphetamine administration and black triangle repeated amphetamine administration.

5.2.2. Amphetamine-induced changes in metabolite levels in both mice strains

5.2.2.1. Amphetamine-induced changes in metabolite levels in Bl6

Acute amphetamine caused a significant elevation of isoleucine and leucine, two representatives of branched chain amino acids. These amino acids were clearly higher in Bl6 receiving acute amphetamine. Besides that, there was a significant shift toward favouring branched chain amino acids compared to aromatic amino acids. These effects were not further modified by repeated amphetamine. Therefore, a similar elevation of isoleucine and leucine was established for both acute and repeated amphetamine. Simultaneously, the levels of several biogenic amines (asymmetric dimethylarginine, alpha-aminoadipic acid, kynurenine) and hexoses were significantly reduced after repeated amphetamine (Table 6). The ratio between amino acids glycine and serine was also significantly decreased after acute amphetamine (Table 6).

Table 6. Amphetamine-induced statistically significant metabolite level changes (µmoles, median and range) and their ratios in Bl6 administration groups (Kruskal-Wallis test, $p < 0.05$). Effect size estimates for chi-square values are indicated by eta^2, where value ≥ 0.14 corresponds to a large effect**.** AAA – aromatic amino acids, ADMA – asymmetric dimethylarginine, BCAA – branched chain amino acids

Metabolites and their ratios	Saline (N = 12)	Acute AMPH (N = 16)	Repeated AMPH (N = 12)	Kruskal-Wallis test	Effect size (eta^2)
Hexoses	8569 7452 – 11103	7407 [a] 4459 – 9751	7343 [c] 3810 – 9625	$\chi^2_{(2.39)}$= 6.34, p=0.04	0.14
Isoleucine	83.7 60.6 – 108	105 [a] 75.9 – 214	104 [c] 62.5 – 208	$\chi^2_{(2.40)}$= 8.98, p=0.01	0.18
Leucine	123 92.8 – 159	158 [a] 112 – 372	160 [c] 104 – 361	$\chi^2_{(2.39)}$=10.87, p=0.004	0.22
ADMA	0.36 0.21 – 0.75	0.40 0.24 – 0.80	0.23 [b, c] 0.000 – 0.60	$\chi^2_{(2.37)}$=8.75, p=0.01	0.19
Alpha-amino-adipic acid	11.0 7.42 – 17.2	10.2 0.000 – 16.0	8.63 [c] 4.46 – 10.3	$\chi^2_{(2.36)}$=7.66, p=0.02	0.18
Kynurenine	1.40 0.93 – 1.56	1.17 0.89 – 1.58	1.07 [c] 0.83 – 1.90	$\chi^2_{(2.38)}$=7.10, p=0.03	0.16
BCAA	373 308 – 467	447 [a] 309 – 985	441 298 – 939	$\chi^2_{(2.40)}$=7.84, p=0.02	0.16
BCAA/AAA	1.76 1.45 – 2.10	1.93 [a] 1.65 – 2.49	2.06 [c] 1.47 – 2.46	$\chi^2_{(2.40)}$=10.47, p=0.005	0.21
Glycine / Serine	3.72 1.70 – 4.39	2.52 [a] 1.29 – 3.74	3.00 1.65 – 8.46	$\chi^2_{(2,40)}$=8.25, p=0.02	0.17

a – Statistically significant difference (p<0.05) between saline and acute amphetamine
b – Statistically significant difference (p<0.05) between acute amphetamine and repeated amphetamine
c – Statistically significant difference (p<0.05) between saline and repeated amphetamine

5.2.2.2. Amphetamine-induced changes in metabolite levels in 129Sv

The pattern of altered metabolite levels in 129Sv was vastly different from that seen in Bl6. In 129Sv, acute amphetamine induced a significant reduction of several metabolite levels compared to saline, including valine, lysoPCs (lyso PC aa 16:0, lyso PC aa 18:2, lyso PC aa 20:4), PC diacyls (PC aa 34:2, PC aa 36:2, PC aa 36:3, PC aa 36:4) and PC acyl-alkyls (PC ae 38:4 and PC ae 40:4). Moreover, several metabolite levels and their ratios were elevated if the effect of acute and repeated amphetamine was compared in 129Sv. The list of metabolite levels includes long chain acylcarnitines (C14, C14:1-OH, C16, C16:1, C18:1), branched chain amino acids (particularly isoleucine, valine), PC diacyls (PC aa C38:4, PC aa C38:6, PC aa C42:6), PC acyl-alkyls (PC ae C38:4, PC ae C40:4, PC ae C40:5, PC ae C40:6, PC ae C42:1, PC ae C42:3), and sphingolipids [SM(OH)C22:1, SMC24:0]. The list of elevated ratios includes acylcarnitines C5-/carnitine C0, CPT-1 ratio, glycine/glutamine, and lysoPC a C20:4/ lysoPC a C20:3. Comparably fewer markers were affected if repeated amphetamine was compared to saline. A limited number of metabolite levels and their ratios were significantly reduced (hexoses, kynurenine, PC aa C36:3, PC aa C36:3/PC aa C36:4) or elevated (PC aa C32:0, C3/carnitine C0, lysoPC a C20:4/lysoPC a C20:3) in this comparison (Table 7). Therefore, one can conclude that acute amphetamine caused a substantial reduction of several metabolite levels and ratios (altogether 10 indicators) compared to saline, whereas the effect of repeated amphetamine was more frequently associated with the elevation of metabolite levels (altogether 22 indicators) compared to acute amphetamine. When the repeated amphetamine group was compared to the saline group, the changes were less numerous (altogether 8 indicators).

Table 7. Amphetamine-induced statistically significant metabolite levels (µmoles, median and range) changes and their ratios in 129Sv administration groups (Kruskal-Wallis test, $p < 0.05$). Effect size estimates for chi-square values are indicated by eta^2, where value ≥ 0.14 corresponds to a large effect**.** BCAA – branched chain amino acids, *CPT1 (carnitine palmitoyltransferase 1) ratio [(C16 + C18) / carnitine C0].

Metabolites and their ratios	Saline (N=11)	Acute AMPH (N=14)	Repeated AMPH (N=14)	Kruskal-Wallis test	Effect size (eta^2)
C14	0.081 0.067 – 0.10	0.071 0.054 – 0.13	0.10 [b] 0.069 – 0.16	$\chi^2_{(2,39)}$=9,71, p=0.008	0.20
C14:1-OH	0.013 0.000 – 0.019	0.000 0.000 – 0.020	0.016 [b] 0.000 – 0.025	$\chi^2_{(2,39)}$=11.28, p=0.004	0.22
C16	0.27 0.21 – 0.34	0.24 0.17 – 0.38	0.31 [b] 0.23 – 0.41	$\chi^2_{(2,38)}$= 11.25, p=0.004	0.23
C16:1	0.078 0.059 – 0.098	0.068 0.039 – 0.12	0.096 [b] 0.067 – 0.14	$\chi^2_{(2,38)}$=7.86, p=0.02	0.17
C18:1	0.15 0.14 – 0.20	0.14 0.11 – 0.26	0.20 [b] 0.13 – 0.23	$\chi^2_{(2,38)}$=9.94, p=0.007	0.19

Metabolites and their ratios	Saline (N=11)	Acute AMPH (N=14)	Repeated AMPH (N=14)	Kruskal-Wallis test	Effect size (eta^2)
Hexoses	5810 4405 – 8005	4299 3269 – 6617	4549 [c] 2764 – 5937	$\chi^2_{(2,38)}$=8.21, p=0.02	0.18
Isoleucine	97.3 79.8 – 129	92.6 75.6 – 120	121 [b] 69.4 – 203	$\chi^2_{(2,38)}$=6.92, p=0.03	0.15
Valine	192 146 – 297	149 [a] 117 – 232	198 [b] 115 – 275	$\chi^2_{(2,38)}$=7.88, p=0.02	0.17
Kynurenine	1.43 1.13 – 1.90	1.24 0.94 – 1.64	1.27 [c] 1.05 – 1.45	$\chi^2_{(2,37)}$=6.10, p=0.047	0.14
LysoPC a C16:0	292 179 – 444	211 [a] 143 – 366	268 121 – 333	$\chi^2_{(2,39)}$=6.81, p=0.03	0.15
LysoPC a C18:2	133 88.0 – 181	90.1 [a] 61.1 – 169	107 56.3 – 122	$\chi^2_{(2,39)}$=9.09, p=0.01	0.19
LysoPC a C20:4	29.3 20.2 – 44.0	20.6 [a] 14.8 – 34.9	28.7 16.4 – 34.7	$\chi^2_{(2,39)}$=7.57, p=0.02	0.16
PC aa C32:0	10.8 8.34 – 15.9	12.8 9.65 – 16.4	15.9 [c] 8.05 – 29.0	$\chi^2_{(2,38)}$=7.12, p=0.03	0.16
PC aa C34:2	263 189 – 356	202 [a] 126 – 343	235 123 – 294	$\chi^2_{(2,39)}$=6.95, p=0.03	0.15
PC aa C36:2	175 111 – 214	114 [a] 77.8 – 211	143 74.6 – 185	$\chi^2_{(2,39)}$=8.57, p=0.01	0.18
PC aa C36:3	58.6 39.1 – 78.1	44.3 [a] 28.5 – 76.5	46.6 [c] 27.4 – 58.2	$\chi^2_{(2,39)}$=8.36, p=0.02	0.18
PC aa C36:4	88.9 65.4 – 116	65.6 [a] 43.1 – 125	85.2 52.9 – 106	$\chi^2_{(2,39)}$=7.98, p=0.02	0.17
PC aa C38:4	44.6 35.9 – 74.0	37.1 28.9 – 73.2	55.5 [b] 34.9 – 76.5	$\chi^2_{(2,39)}$=9.57, p=0.008	0.19
PC aa C38:6	68.2 49.2 – 99.2	51.9 39.4 – 91.8	71.8 [b] 40.0 – 87.5	$\chi^2_{(2,39)}$=6.65, p=0.04	0.15
PC aa C42:6	0.78 0.61 – 1.07	0.64 0.48 – 0.96	0.81 [b] 0.49 – 1.17	$\chi^2_{(2,39)}$=8.13, p=0.017	0.17
PC ae C38:4	2.55 1.84 – 3.24	1.93 [a] 1.35 – 2.78	2.69 [b] 1.80 – 3.71	$\chi^2_{(2,38)}$=13.05, p=0.002	0.26
PC ae C40:4	1.52 1.15 – 1.88	1.17 [a] 0.84 – 1.67	1.59 [b] 1.07 – 2.13	$\chi^2_{(2,38)}$=10.20, p=0.006	0.21
PC ae C40:5	0.78 0.63 – 1.17	0.71 0.50 – 0.84	0.88 [b] 0.63 – 1.09	$\chi^2_{(2,38)}$=9.37, p=0.009	0.20
PC ae C40:6	1.61 1.14 – 2.11	1.20 0.95 – 1.80	1.78 [b] 1.08 – 2.53	$\chi^2_{(2,38)}$=10.47, p=0.005	0.22
PC ae C42:1	0.46 0.41 – 0.68	0.40 0.32 – 0.58	0.54 [b] 0.38 – 0.71	$\chi^2_{(2,38)}$=10.00, p=0.007	0.21
PC ae C42:3	0.55 0.46 – 0.90	0.48 0.33 – 0.77	0.64 [b] 0.42 – 0.80	$\chi^2_{(2,38)}$=9.72, p=0.008	0.20
SM(OH) C22:1	1.44 0.91 – 1.91	1.15 0.733 – 1.64	1.53 [b] 0.81 – 2.34	$\chi^2_{(2,38)}$=9.48, p=0.009	0.20
SM C24:0	4.42 2.96 – 6.02	3.70 3.10 – 4.95	4.46 [b] 2.59 – 7.02	$\chi^2_{(2,38)}$=10.41, p=0.006	0.21
BCAA	428 349 – 611	375 305 – 564	494 [b] 291 – 853	$\chi^2_{(2,38)}$=6.48, p=0.04	0.15
C3 / C0	0.028 0.018 – 0.037	0.028 0.021 – 0.038	0.038 [c] 0.029 – 0.045	$\chi^2_{(2,38)}$=7.00, p=0.03	0.15

Metabolites and their ratios	Saline (N=11)	Acute AMPH (N=14)	Repeated AMPH (N=14)	Kruskal-Wallis test	Effect size (eta^2)
C5- / C0	0.015 0.010 – 0.024	0.014 0.011 – 0.020	0.019 [b] 0.009 – 0.036	$\chi^2_{(2,39)}$=7.53, p=0.02	0.16
C4 / C5-	2.92 1.84 – 3.95	2.91 2.02 – 4.03	2.44 [c] 1.84 – 3.32	$\chi^2_{(2,38)}$=6.69, p=0.04	0.15
*CPT1 ratio	0.011 0.007 – 0.020	0.009 0.005 – 0.020	0.014 [b] 0.008 – 0.027	$\chi^2_{(2,39)}$=11.13, p=0.004	0.22
Glycine / Glutamine	0.42 0.30 – 0.51	0.38 0.27 – 0.60	0.47 [b] 0.37 – 0.61	$\chi^2_{(2,39)}$=7.04, p=0.03	0.15
LysoPC a C20:4 / LysoPC a C20:3	3.29 3.07 – 4.06	3.30 2.76 – 4.07	4.09 [b, c] 3.39 – 5.71	$\chi^2_{(2,39)}$=15.07, p=0.0005	0.28
PC aa C36:3 / PC aa C36:4	0.67 0.53 – 0.85	0.66 0.549 – 0.86	0.52 [b, c] 0.46 – 0.67	$\chi^2_{(2,39)}$=18.39, p=0.0001	0.32

a – Statistically significant difference (p<0.05) between Saline and acute amphetamine
b – Statistically significant difference (p<0.05) between acute amphetamine and repeated amphetamine
c – Statistically significant difference (p<0.05) between Saline and repeated amphetamine

5.2.2.3. Metabolites associated with different response to amphetamine in 129Sv

Among strong responders to amphetamine in 129Sv the levels of several long chain acylcarnitines were significantly elevated compared to weak responders: C12, C14:1, C14:1-OH, and C16:1 (Table 8). Besides that, the level of hexoses was significantly reduced in strong responders compared to weak ones, reflecting an apparent link between the intensity of locomotor activity and hexoses' metabolism. A similar inhibition was established for PC aa C36:3, ratio acylcarnitines C4/C5-, and ratio glycine/glutamine if the strong and weak responders were compared. To confirm the existence of different responders (in terms of behaviour and metabolic parameters) to repeated amphetamine in 129Sv we used the GLM. Using a model fit criterion, candidate variables that did not contribute to the model were removed at a 5% significance level. The final parsimonious model (Table 9) retained the distance travelled on day 11, C14:1, C16, C16:1, C18:1 and the ratio glycine/glutamine as significant predictors to distinguish between subgroups. Spearman rank correlation (information can be found from Paper II supplementary material Table S3) established a positive correlation of amphetamine-induced locomotor activity with C16, C16:1 and ratio between tyrosine and phenylalanine. The ratio glycine/histidine, as well as the ratio acylcarnitines C4/C5- were correlated negatively with amphetamine-induced locomotor activity. Among others, the most prominent relationship ($r = -0.96$) was found between C18:1 and hexoses, i.e. the animals with the lowest levels of hexoses displayed the highest levels of C18:1.

Table 8. Distance travelled (m), metabolite levels (µmoles) and their ratios (median and range) in 129Sv responding differently to amphetamine: weak and strong responders (Mann-Whitney U test, $p < 0.05$). Effect size estimates are indicated by eta^2, where partial eta^2 value ≥ 0.26 was defined as a large effect**.**

	Weak (N=7)	**Strong (N=7)**	**Z-value**	***p*-value**	**Effect size (eta^2)**
Distance travelled on day 11	163 37 – 279	552 320 – 746	-3.07	0.002	0.67
C12	0.10 0.000 – 0.13	0.12 0.096 – 0.16	-2.04	0.04	0.30
C14:1	0.058 0.040 – 0.069	0.069 0.059 – 0.089	-2.11	0.04	0.32
C14:1-OH	0.013 0.000 – 0.018	0.016 0.013 – 0.025	-1.98	0.05	0.28
C16:1	0.085 0.067 – 0.11	0.10 0.083 – 0.14	-2.04	0.04	0.30
PC aa C36:3	50.9 29.1 – 58.2	42.6 27.4 – 47.9	2.04	0.04	0.30
Hexoses	5551 2764 – 5937	3792 3284 – 4787	2.04	0.04	0.30
C4 / C5-	2.82 1.88 – 3.32	2.25 1.84 – 2.55	2.17	0.03	0.34
Glycine / Glutamine	0.55 0.45 – 0.61	0.45 0.37 – 0.49	2.43	0.02	0.42

Table 9. Main effect of amphetamine administration on distance travelled (m), metabolite levels (µmoles) and their ratios in 129Sv. Regression coefficients (ß) and significant values of log10-transformed variables. CI – confidence intervals.

	ß	**ß (95 % CI)**	***t*-value**	***p*-value**
Distance travelled on day 11	-0.82	-1.18, -0.45	-4.91	0.0004
C14:1	-0.55	-1.08, -0.03	-2.31	0.04
C16	-0.58	-1.09, -0.06	-2.45	0.03
C16:1	-0.65	-1.13, -0.17	-2.96	0.01
C18:1	-0.60	-1.10, -0.10	-2.62	0.02
Glycine / Glutamine	0.73	0.30, 1.16	3.71	0.003

5.2.2.4. Amphetamine-induced changes in metabolite levels independent from the strain

Despite significant basal differences between 129Sv and Bl6, combining the data from these two strains revealed 14 metabolite levels and their ratios which remained significant when Kruskal–Wallis ANOVA test was applied (Table 10). The change of one metabolite level (reduction of kynurenine) displayed a large effect size ($eta^2 = 0.15$), whereas the other effects were in the moderate range ($eta^2 = 0.06–0.13$). The levels of branched chain amino acids (leucine, isoleucine) and the ratio lysoPC a C20:4/lysoPC a C20:3 were markedly increased by repeated amphetamine. By contrast, the levels of citrulline, asymmetric dimethylarginine, hexoses and lysoPC a C18:2 were significantly reduced in repeated amphetamine. Long-chain acylcarnitines (CPT-1 ratio, C14) and PC alkyl-acyls (PC ae 40:6, PC ae 42:1) displayed an elevation if acute and repeated amphetamine were compared.

Table 10. Amphetamine-induced statistically significant changes of metabolite levels (μmoles, median and range) if both strains were analyzed together (Kruskal-Wallis test, $p < 0.05$). Effect size estimates for chi-square values are indicated by eta^2, where value ≥ 0.14 corresponds to a large effect. BCAA – branched chain amino acids, ADMA – asymmetric dimethylarginine, *CPT1 (carnitine palmitoyltransferase 1) ratio [(C16 + C18) / carnitine C0].

Metabolites and their ratios	Saline (N=23)	Acute AMPH (N=30)	Repeated AMPH (N=26)	Kruskal-Wallis test	Effect size (eta^2)
C14	0.088 0.06 – 0.12	0.075 0.054 – 0.14	0.094 [b] 0.059 – 0.18	$\chi^2_{(2,79)}$=6.19, p=0.045	0.07
Hexoses	7609 4405 – 11103	6053 [a] 3269 – 9751	5778 [c] 2764 – 9625	$\chi^2_{(2,77)}$=7.31, p=0.03	0.09
Citrulline	51.4 28.9 – 112	45.2 [a] 14.2 – 112	41.5 [c] 27.5 – 82.0	$\chi^2_{(2,78)}$=8.51, p=0.01	0.10
Isoleucine	91.5 60.6 – 129	95.0 75.6 – 214	109 [c] 62.5 – 208	$\chi^2_{(2,79)}$=8.66, p=0.013	0.10
Leucine	129 92.8 – 197	154 [a] 107 – 372	168 [c] 104 – 413	$\chi^2_{(2,79)}$=11.75, p=0.003	0.13
ADMA	0.34 0.11 – 0.75	0.34 0.00 – 1.27	0.26 [c] 0.00 – 0.60	$\chi^2_{(2,76)}$=6.70, p=0.04	0.08
Kynurenine	1.42 0.93 – 1.90	1.22 [a] 0.89 – 1.6	1.15 [c] 0.83 – 1.90	$\chi^2_{(2,75)}$=12.82, p=0.002	0.15
lysoPC a C16:0	302 158 – 444	225 [a] 127 – 411	268 121 – 443	$\chi^2_{(2,79)}$=8.42, p=0.02	0.10
lysoPC a C18:2	151 88.0 – 198	113 [a] 61.1 – 242	114 [c] 56.3 – 226	$\chi^2_{(2,78)}$=10.42, p=0.006	0.12
PC ae 40:6	1.48 0.82 – 2.11	1.21 0.58 – 1.80	1.47 [b] 0.80 – 2.53	$\chi^2_{(2,78)}$=6.82, p=0.03	0.08
PC ae 42:1	0.47 0.22 – 0.68	0.42 0.17 – 0.66	0.51 [b] 0.32 – 0.80	$\chi^2_{(2,78)}$=7.01, p=0.03	0.08
BCAA	397 308 – 611	420 305 – 811	461 [c] 291 – 939	$\chi^2_{(2,78)}$=6.03, p=0.049	0.07
*CPT1 ratio	0.009 0.005 – 0.020	0.009 0.005 – 0.020	0.011 [b] 0.004 – 0.030	$\chi^2_{(2,79)}$=8.92, p=0.01	0.10
lysoPC a C20:4 / lysoPC a C20:3	3.35 2.85 – 4.47	3.43 2.66 – 5.89	3.82 [b,c] 2.91 – 5.71	$\chi^2_{(2,79)}$=8.59, p=0.01	0.10

a – Statistically significant difference (p<0.05) between saline and acute amphetamine
b – Statistically significant difference (p<0.05) between acute amphetamine and repeated amphetamine
c – Statistically significant difference (p<0.05) between saline and repeated amphetamine

5.3. Paper III: Dopamine system, NMDA receptor and EGF family expressions in the brain of Bl6 and 129Sv strains displaying different adaptation

5.3.1. Body weight and locomotor activity

In the HCC batch, the body weight was measured twice: on the 1st day and on the 11th day before collecting brain tissues. In the RMT batch, the body weight was measured on 11 consecutive days and after that the mice were exposed to the behavioural challenge in the motility box. More detailed information about the outcomes of repeated testing can be found in Paper III supplementary material Figure S1.

5.3.1.1. Body weight changes during experiment

In HCC batch, the body weight increased in both Bl6 and 129Sv strains, but the weight gain tended to be greater in 129Sv mice (2.19 ± 0.58 g) compared to Bl6 mice (1.39 ± 0.59 g; $p = 0.051$, Figure 9). In the RMT batch, Bl6 mice displayed almost no body weight change during 11 days (0.25 ± 0.89 g). However, 129Sv mice significantly lost weight (-1.26 ± 0.66 g; $p \leq 0.0001$, Figure 9). The body weight differences between the strains during repeated testing appeared on the 6th experimental day (information can be found from Paper III supplementary material Figure S1A). In both environments, 129Sv mice demonstrated greater change in body weight (2.19 ± 0.58 for HCC and -1.26 ± 0.66 for RMT, $p \leq 0.0001$) than Bl6 (1.39 ± 0.59 for HCC and 0.25 ± 0.89 for RMT, $p \leq 0.001$). Subsequent application of two-way ANOVA further substantiated the differences in body weight dynamics between the two mouse strains (strain – $F_{(1, 42)} = 3.04$, $p = 0.09$, environment – $F_{(1, 42)} = 126$, $p \leq 0.0001$, strain x environment – $F_{(1, 42)} = 31.9$, $p \leq 0.0001$; Figure 9).

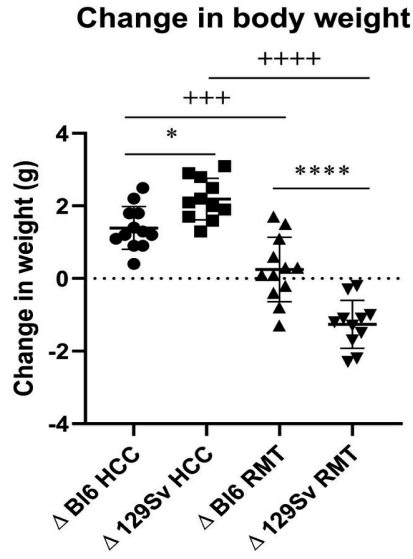

Figure 9. Change in body weight during the experimental period. Two-way ANOVA was applied to demonstrate differences between the strains and environments. Bonferroni *post hoc* analysis after significant two-way ANOVA: * $p \leq 0.05$, **** $p \leq 0.0001$ compared to respective 129Sv mice, +++ $p \leq 0.001$ and ++++ $p \leq 0.0001$ strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 12 to 16.

5.3.1.2. Locomotor activity in RMT batch

The locomotor activity was measured every day. Statistical analysis was performed for the 1st and 11th experimental day. Repeated measures ANOVA for distance travelled revealed a strain effect, but no repeated testing effect (strain: $F_{(1,21)} = 41.5$, $p \leq 0.0001$; repeated testing: $F_{(1,21)} = 1.04$, $p = 0.32$; strain x repeated testing: $F_{(1,21)} = 0.16$, $p = 0.69$) (Figure 10A). The distance travelled was significantly longer in Bl6 compared to 129Sv mice on the 1st day ($p \leq 0.0001$). The motor activity of 129Sv mice tended to be higher on the 11th day, but this elevation was not significant. The difference between 129Sv and Bl6 mice on the 11th day did not differ from that established in the beginning of the study ($p = 0.0007$, Figure 10A and Paper III supplementary material Figure S1B). Repeated measures ANOVA for the number of rearings established a strain and a repeated testing effects, and their interaction was also significant (strain: $F_{(1,21)} = 51.5$, $p \leq 0.0001$; repeated testing: $F_{(1,21)} = 14.8$, $p = 0.0009$; strain x repeated testing: $F_{(1,21)} = 7.41$, $p = 0.012$) (Figure 10B and Paper III supplementary material Figure S1C). On the 1st day, the number of rearings was significantly higher in Bl6 mice than in 129Sv mice ($p = 0.014$). The vertical activity of 129Sv mice tended to be higher on the 11th day compared to the 1st day, but this difference was not significant. By contrast, the frequency of

rearings in Bl6 mice became higher with each subsequent experimental day and it robustly differed on the 11th day not only from the respective activity of 129Sv mice ($p \leq 0.0001$), but also from their own initial activity ($p = 0.0007$; Figure 10B and Paper III supplementary material Figure S1C).

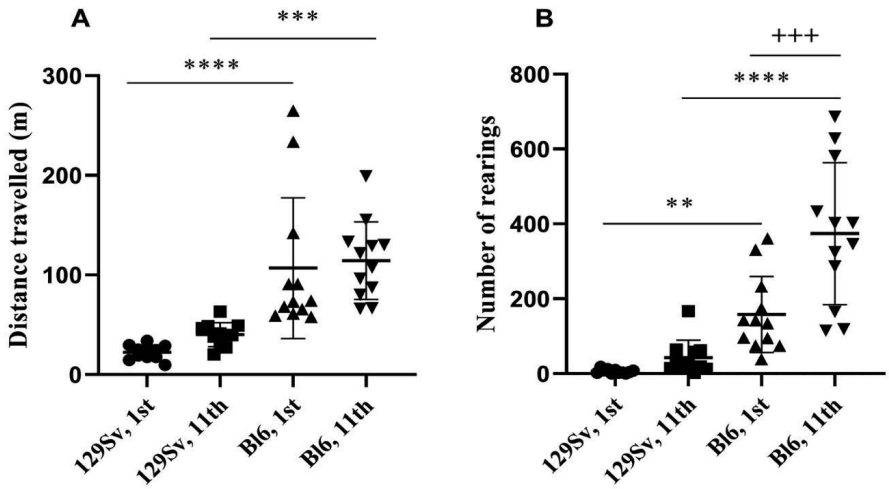

Figure 10. Bl6 mice were more active both in terms of (A) distance travelled and (B) number of rearings. The difference in vertical activity between strains became augmented with each subsequent repeated testing. Data are presented as mean values ± SD. Bonferroni *post hoc* analysis after significant repeated measures ANOVA: ** ≤ 0.01, *** $p \leq 0.001$ and **** $p \leq 0.0001$ compared to the respective 129Sv mice; +++ $p \leq 0.001$ (compared to Bl6 on the 1st day of study). Number of animals in each group varied from 11 to 12.

5.3.1.3. Amphetamine-induced locomotor stimulation

In RMT group, acute amphetamine administration (3 mg/kg) in the beginning of the study (one-way ANOVA: $F_{(3,46)} = 12.6$, $p \leq 0.0001$) significantly stimulated locomotor activity in Bl6 mice ($p = 0.002$ compared to saline-treated Bl6 mice; $p = 0.007$ compared to amphetamine-treated 129Sv mice; Figure 11A). Also, in 129Sv mice the locomotor activity induced by amphetamine was increased. It reached the level of saline-treated Bl6 mice, but it was not statistically significant compared to saline-treated 129Sv mice. In the end of the RMT study, the effect of amphetamine (one-way ANOVA: $F_{(3,49)} = 33.5$, $p \leq 0.0001$) tended to be even stronger in Bl6 strain compared to the respective saline-treated group ($p \leq 0.0001$) and amphetamine-treated 129Sv mice ($p \leq 0.0001$; Figure 11B). In RMT 129Sv mice, the elevation of locomotor activity with amphetamine again reached the level of saline-treated RMT Bl6 mice. There was a moderate difference between saline-treated Bl6 and 129Sv mice ($p < 0.05$). Also, the

difference between amphetamine and saline treatments in RMT 129Sv mice became statistically significant ($p = 0.006$).

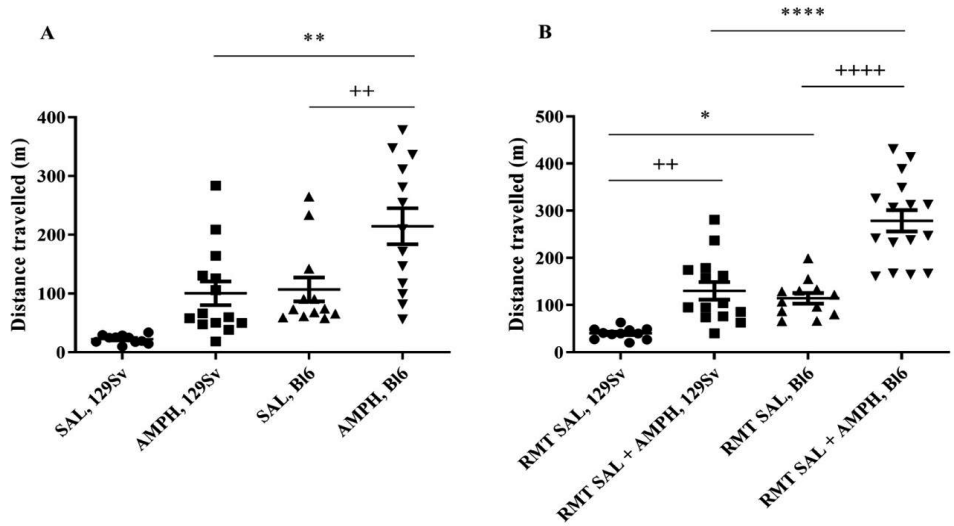

Figure 11. The effect of acute amphetamine (AMPH, 3 mg/kg) treatment in the beginning and at the end of RMT in Bl6 and 129Sv strains. (A) AMPH treatment in the beginning of study (HCC) and **(B)** AMPH treatment after repeated saline administrations (RMT). AMPH was administered 30 min before the beginning of motility test and distance travelled was measured for 30 min. Data are presented as mean values ± SD. Bonferroni *post hoc* analysis after significant two-way ANOVA: * ≤ 0.05, ** $p \leq 0.01$ and **** $p \leq 0.0001$ compared to the respective 129Sv mice, ++ $p \leq 0.01$ and ++++ $p \leq 0.0001$ strain specific comparison. SAL – saline. Number of animals in each group varied from 11 to 16.

5.3.2. Gene Expression Data

5.3.2.1. NMDA and Dopamine Systems

Frontal Cortex

In HCC animals, there was a significant reduction of expression of monoamine oxidase B (*Maob*, $p \leq 0.0001$) in 129Sv compared to the Bl6 strain (Figure 12D). RMT caused a reduction of glutamate ionotropic receptor NMDA type subunit 1 (*Grin1*, $p = 0.015$), serine racemase (*Srr*, $p \leq 0.0001$) and monoamine oxidase A (*Maoa*, $p = 0.002$) in Bl6 compared to the respective HCC group (Figure 12A–C). RMT did not change the levels of these genes in 129Sv. The strongest change with RMT in the frontal cortex was found for catechol-O-methyl-transferase (*Comt*, Figure 12E). It was significantly upregulated in Bl6 mice ($p \leq 0.0001$) compared to the HCC group. However, in 129Sv mice the elevation of *Comt* was even stronger and the comparison of both strains revealed a more significant increase in 129Sv strain ($p = 0.005$; Figure 12E).

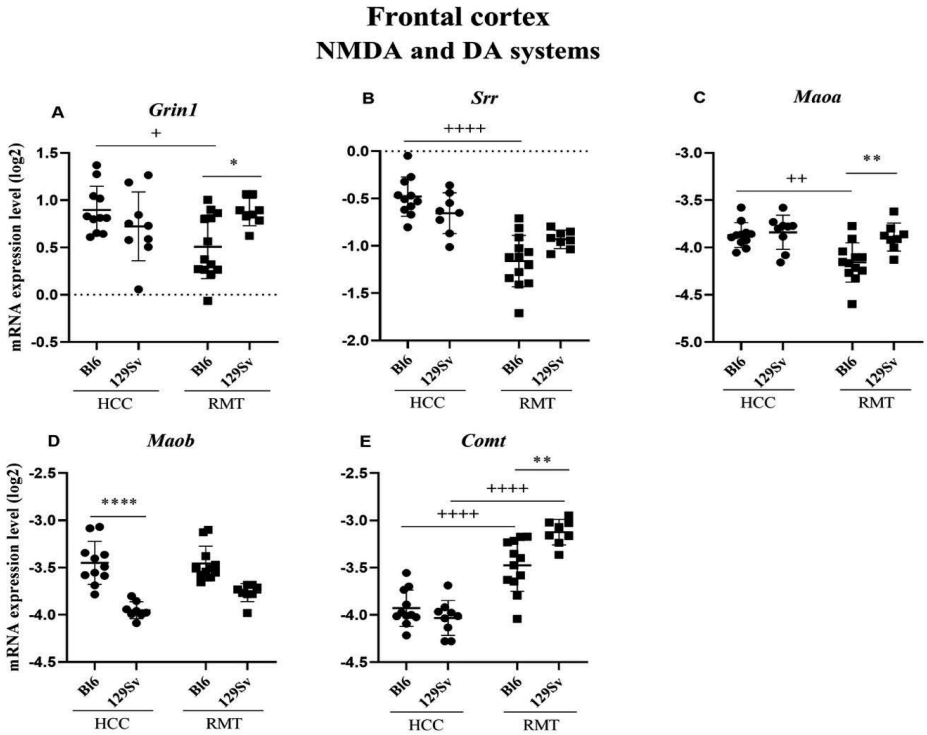

Figure 12. Two-way ANOVA was applied to demonstrate differences between the strains and environments. Substantial statistically significant gene expressions for **(A)** *Grin1* – glutamate ionotropic receptor NMDA type subunit 1, **(B)** *Srr* – serine racemase, **(C)** *Maoa* – monoamine oxidase A, **(D)** *Maob* – monoamine oxidase B and **(E)** *Comt* – catechol-O-methyltransferase. Bonferroni *post hoc* test: ** $p \leq 0.01$ and **** $p \leq 0.0001$ compared to respective 129Sv mice; + $p \leq 0.05$, ++ $p \leq 0.01$ and ++++ $p \leq 0.0001$ in strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 8 to 14.

Hippocampus

Overall, the alterations established for 129Sv and Bl6 were more pronounced in the hippocampus compared to the frontal cortex. In HCC mice, there was a significant elevation of *Grin1* ($p = 0.05$), *Grin2b* ($p = 0.009$) and dopamine receptor D1 (*Drd1*, $p = 0.009$) in 129Sv compared to Bl6 strain (Figure 13A, B, D). Like in the frontal cortex, the expression of *Maob* ($p = 0.005$) in the hippocampus was significantly lower in HCC 129Sv than in Bl6 strain (Figure 13F). The differences between 129Sv and Bl6 established for *Grin1* ($p = 0.027$), *Grin2b* ($p \leq 0.0001$), *Drd1* ($p \leq 0.0001$) and *Maob* ($p = 0.002$) in HCC remained similar in the RMT group (Figure 13A, B, D, F). Besides that, RMT caused a significant elevation of *Srr* ($p < 0.01$ for Bl6 and $p = 0.005$ for 129Sv) and *Maoa* (for both $p \leq 0.0001$) in both strains (Figure 13C, E). Only an elevation of *Maob* ($p = 0.005$) due to RMT was apparent in Bl6 strain (Figure 13F).

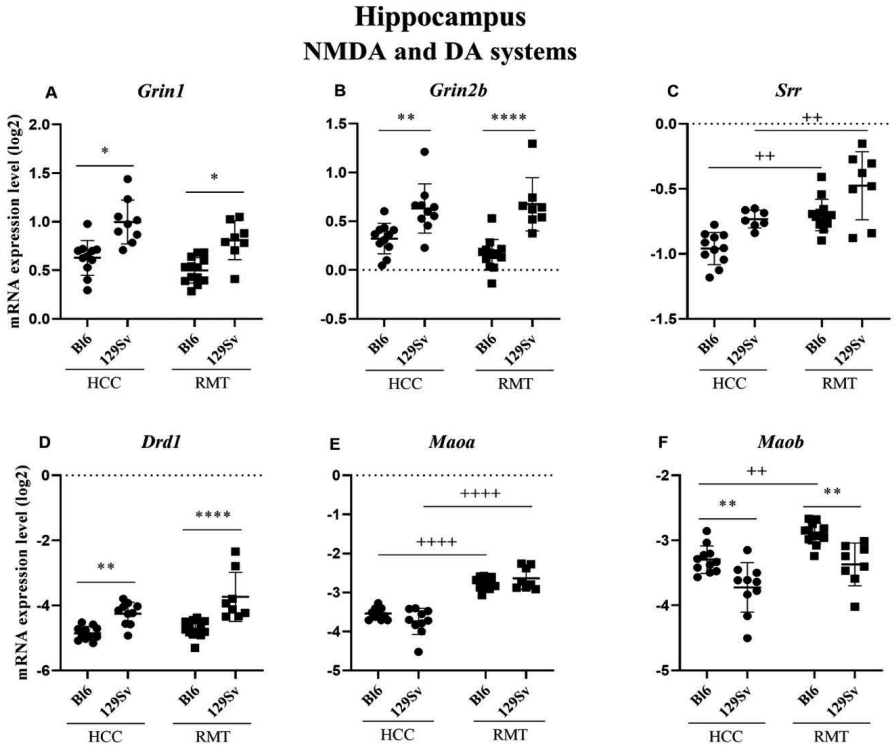

Figure 13. NMDA and dopamine (DA) systems gene expression in the hippocampus of Bl6 and 129Sv mice. Two-way ANOVA was applied to demonstrate differences between the strains and environments. Substantial statistically significant gene expressions for **(A)** *Grin1* – glutamate ionotropic receptor NMDA type subunit 1, **(B)** *Grin2b* – glutamate ionotropic receptor NMDA type subunit 2B, **(C)** *Srr* – serine racemase, **(D)** *Drd1* – dopamine receptor D1, **(E)** *Maoa* – monoamine oxidase A and **(F)** *Maob* – monoamine oxidase B. Bonferroni *post hoc* test: * $p \leq 0.05$, ** $p \leq 0.01$ and **** $p \leq 0.0001$ compared to the respective 129Sv mice, ++ $p \leq 0.01$ and ++++ $p \leq 0.0001$ in strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 7 to 13.

Ventral Striatum

No significant alterations of the NMDA system were found in the ventral striatum. However, again a difference between HCC Bl6 and 129Sv strains was established for *Maob* ($p = 0.002$). favouring Bl6 mice (Figure 14F). The alterations induced by RMT were similar in both strains. RMT induced a significant increase in the expression of tyrosine hydroxylase (*Th*, $p = 0.036$ for Bl6 and $p = 0.0009$ for 129Sv), *Comt* (for both $p \leq 0.0001$), *Maoa* (for both $p \leq 0.0001$) and *Maob* (for both $p \leq 0.0001$) in both strains compared to the HCC group (Figure 14C–F). The expression of dopamine D2 receptor genes, dopamine receptor *Drd2* ($p < 0.01$ for Bl6 and $p = 0.0007$ for 129Sv) and dopamine receptor *Drd4* ($p \leq 0.0001$ for Bl6 and $p = 0.0003$ for 129Sv) was significantly reduced in both strains in response to RMT (Figure 14A, B).

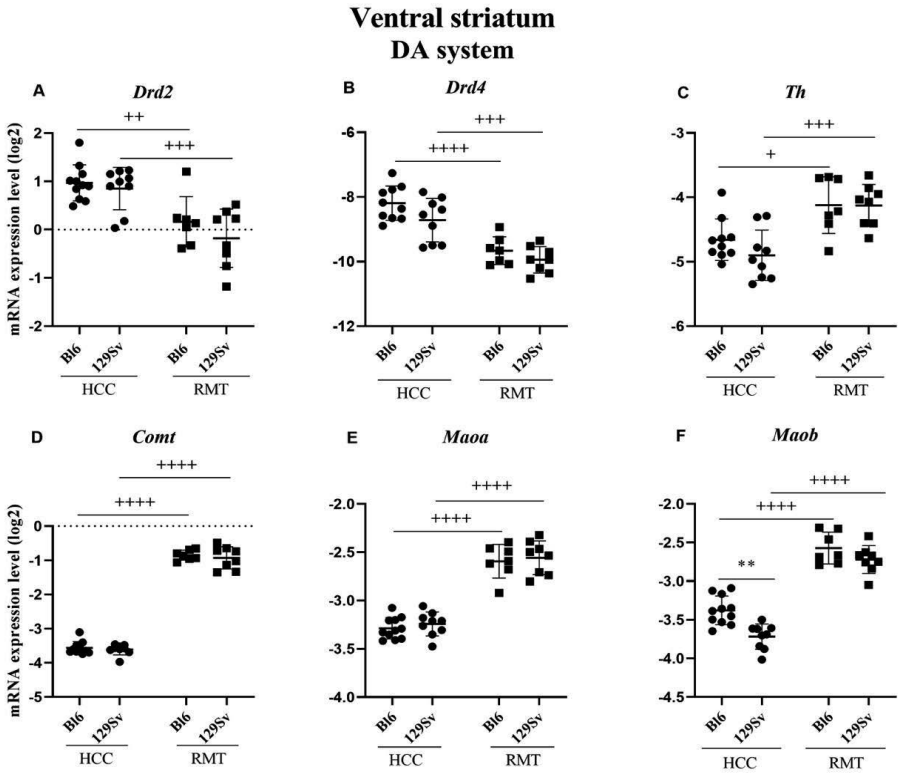

Figure 14. Dopamine (DA) system gene expression in ventral striatum of Bl6 and 129Sv mice. Two-way ANOVA was applied to demonstrate differences between the strains and environments. Substantial statistically significant gene expressions for **(A)** *Drd2* – dopamine receptor D2, **(B)** *Drd4* – dopamine receptor D4, **(C)** *Th* – tyrosine hydroxylase, **(D)** *Comt* – catechol-O-methyltransferase, **(E)** *Maoa* – monoamine oxidase A and **(F)** *Maob* – monoamine oxidase B. Bonferroni *post hoc* test: ** $p \leq 0.01$ compared to respective 129Sv mice, + $p \leq 0.05$, ++ $p \leq 0.01$, +++ $p \leq 0.001$ and ++++ $p \leq 0.0001$ in strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 7 to 11.

Dorsal Striatum

In the dorsal striatum once again a difference between Bl6 and 129Sv in the HCC group was established for *Maob* ($p \leq 0.0001$), favouring Bl6 mice (Figure 15F). This difference between the strains was also evident in the RMT group ($p \leq 0.0001$). Differently from the ventral striatum, the NMDA system was influenced by RMT in the dorsal striatum. RMT caused an almost similar elevation of *Grin1* (for both $p \leq 0.0001$), glutamate ionotropic receptor NMDA type subunit 2A (*Grin2a*, for both $p \leq 0.0001$) and *Srr* ($p = 0.004$ for Bl6 and $p = 0.0002$ for 129Sv) in both strains (Figure 15A–C). Also, the expression of dopamine receptors *Drd1* ($p \leq 0.0001$ for Bl6 and $p = 0.0003$ for 129Sv) and *Drd2* ($p \leq 0.0001$ for Bl6 and $p = 0.0004$ for 129Sv) was elevated in the same manner in both strains in response to RMT (Figure 15D, E).

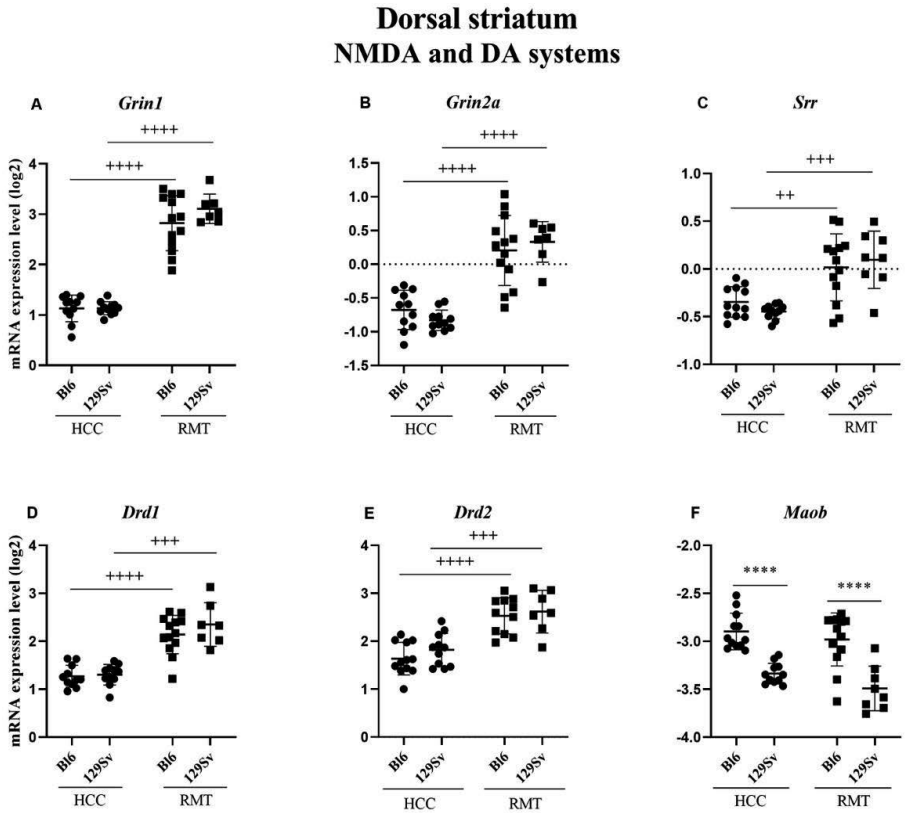

Figure 15. NMDA and dopamine (DA) systems gene expression in dorsal striatum of Bl6 and 129Sv mice. Two-way ANOVA was applied to demonstrate differences between the strains and environments. Substantial statistically significant gene expressions for **(A)** *Grin1* – glutamate ionotropic receptor NMDA type subunit 1, **(B)** *Grin2a* – glutamate ionotropic receptor NMDA type subunit 2A, **(C)** *Srr* – serine racemase, **(D)** *Drd1* – dopamine receptor D1, **(E)** *Drd2* – dopamine receptor D2 and **(F)** *Maob* – monoamine oxidase B. Bonferroni *post hoc* test: **** $p \leq 0.0001$ compared to respective 129Sv mice, ++ $p \leq 0.01$, +++ $p \leq 0.001$ and ++++ $p \leq 0.0001$ in strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 7 to 13.

5.3.2.2. EGF Family

Frontal Cortex

In HCC, the expression of only one member of the EGF family was reduced in the frontal cortex of 129Sv mice compared to Bl6 strain: transforming growth factor alpha (*Tgfα*, $p \leq 0.0001$, Figure 16B). By contrast, RMT exposure had a larger effect on the EGF family. RMT induced an elevation of *Egf* ($p = 0.007$), *Tgfα* ($p \leq 0.0001$), heparin binding EGF like growth factor (*Hb-Egf*, $p = 0.0003$), neuregulin 2 (*Nrg2*, $p \leq 0.0001$) and erb-b2 receptor tyrosine kinase 3 (*Erbb3*, $p = 0.005$) in 129Sv compared to HCC group (Figure 16A, B, D). In Bl6 mice, RMT caused a reduction of *Nrg1* ($p \leq 0.0001$) and a modest increase

of *Nrg2* (p = 0.02, Figure 16C, D). The comparison of 129Sv and Bl6 in RMT group established a significant elevation of *Egf* (p = 0.02), *Nrg1* (p = 0.04), *Nrg2* ($p \leq 0.0001$), *Erbb1* (p = 0.02) and *Erbb4* ($p \leq 0.0001$) in 129Sv strain (Figure 16A, C–F). Altogether, RMT caused more pronounced alterations in the EGF family in 129Sv mice compared to the Bl6 strain.

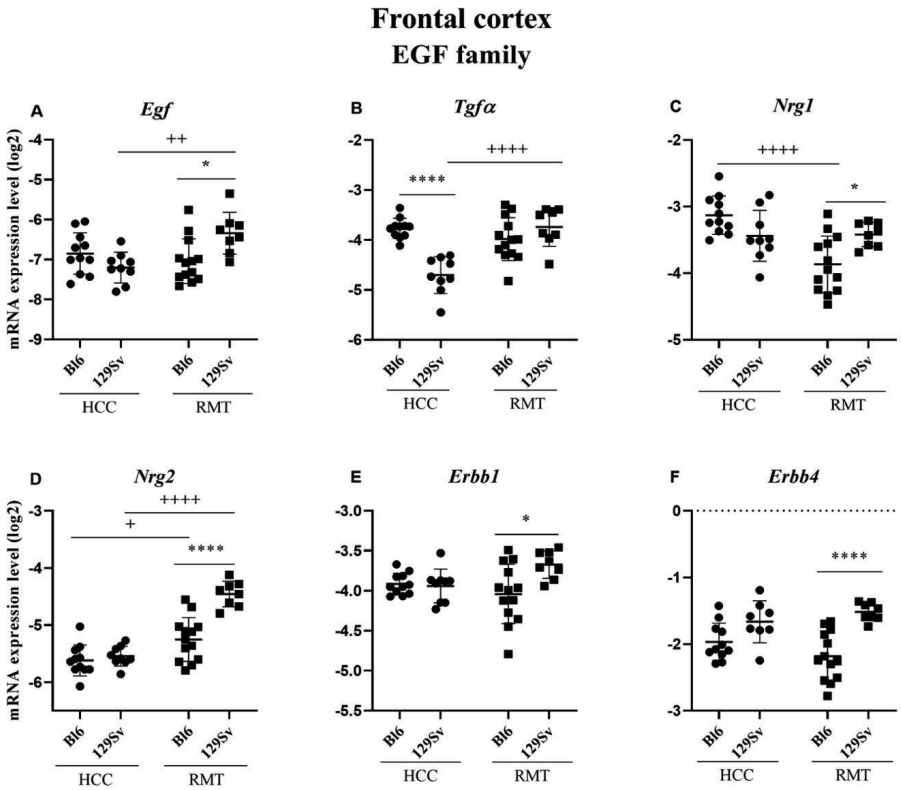

Figure 16. Egf family gene expression in the frontal cortex of Bl6 and 129Sv mice. Two-way ANOVA was applied to demonstrate differences between the strains and environments. Substantial statistically significant gene expressions for **(A)** *Egf* – epidermal growth factor, **(B)** *Tgfa* – transforming growth factor alpha, **(C)** *Nrg1* – neuregulin 1, **(D)** *Nrg2* – neuregulin 2, **(E)** *Erbb1* – epidermal growth factor receptor and **(F)** *Erbb4* – Erb–b2 receptor tyrosine kinase 4. Bonferroni *post hoc* test: * $p \leq 0.05$ and **** $p \leq 0.0001$ compared to respective 129Sv mice, + $p \leq 0.05$, ++ $p \leq 0.01$ and ++++ $p \leq 0.0001$ in strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 8 to 13.

Hippocampus

In the hippocampus, differently from the frontal cortex, the expression levels of *Nrg1* (p = 0.007) and *Erbb4* ($p \leq 0.0001$) in HCC 129Sv mice were significantly elevated compared to the respective Bl6 group (Figure 17B, F). In RMT Bl6 the level of *Egf* (p = 0.0006) was reduced, whereas the expressions of *Nrg1*

($p \leq 0.0001$) and neuregulin 3 (*Nrg3*, $p \leq 0.0001$) were increased compared to HCC group (Figure 17A, B, D). Again, like in the frontal cortex, the changes in 129Sv mice exposed to RMT were more prominent than in the respective Bl6 group. Elevated expression levels of *Nrg1* ($p \leq 0.0001$), *Nrg2* ($p = 0.001$) and *Nrg3* ($p \leq 0.0001$) were evident in RMT and HCC groups in 129Sv mice (Figure 17B–D). The expressions of *Egf* ($p = 0.01$), *Nrg1* ($p \leq 0.0001$), *Nrg2* ($p = 0.0003$), *Erbb1* ($p = 0.01$) and *Erbb4* ($p \leq 0.0001$) were significantly higher in RMT 129Sv strain compared to RMT Bl6 (Figure 17A–C, E, F). However, one should note that differences in the levels of *Nrg1* and its receptor *Erbb4* were already present in HCC animals.

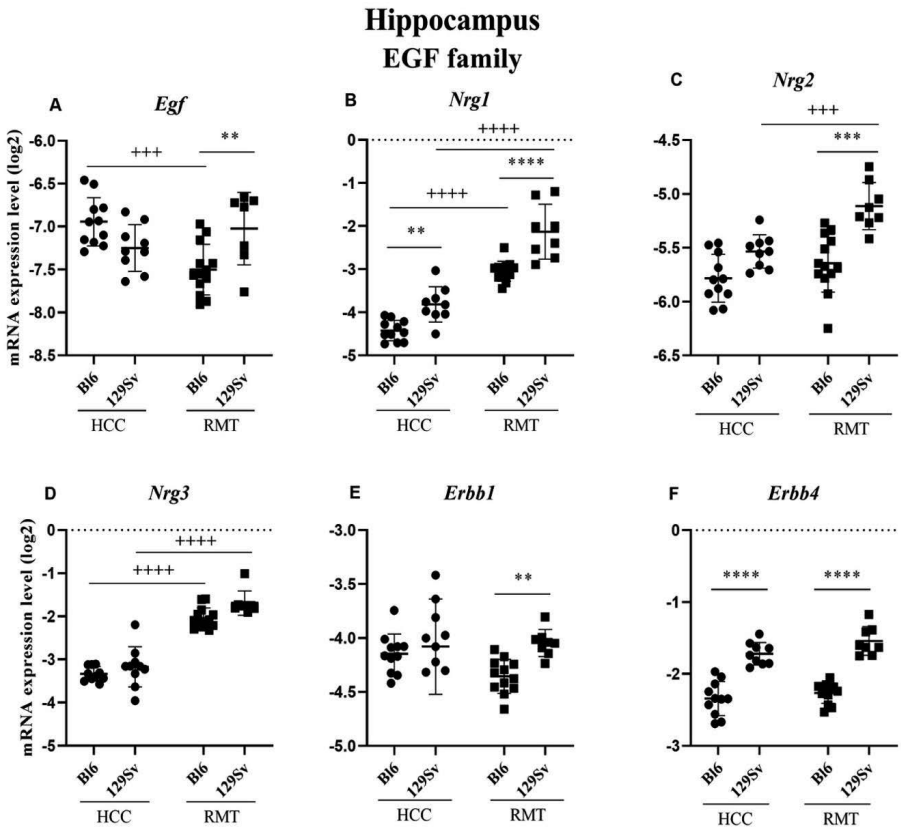

Figure 17. Egf family gene expression in the hippocampus of Bl6 and 129Sv mice. Two-way ANOVA was applied to demonstrate differences between the strains and environments. Substantial statistically significant gene expressions for **(A)** *Egf* – epidermal growth factor, **(B)** *Nrg1* – neuregulin 1, **(C)** *Nrg2* – neuregulin 2, **(D)** *Nrg3* – neuregulin 3, **(E)** *Erbb1* – epidermal growth factor receptor and **(F)** *Erbb4* – erb–b2 receptor tyrosine kinase 4. Bonferroni *post hoc* test: * $p \leq 0.05$ *** $p \leq 0.001$ and **** $p \leq 0.0001$ compared to respective 129Sv mice, +++ $p \leq 0.001$ and ++++ $p \leq 0.0001$ in strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 7 to 13.

Ventral Striatum

In the ventral striatum, the expression differences in the EGF family were less prominent compared to the frontal cortex and hippocampus. In HCC 129Sv mice, the level of *Egf* ($p = 0.05$) was reduced compared to HCC Bl6 (Figure 18A). In response to RMT, the level of *Nrg1* ($p = 0.0002$ for Bl6 and $p = 0.003$ for 129Sv) was reduced, whereas the level of *Nrg3* ($p = 0.0003$ for Bl6 and $p \leq 0.0001$ for 129Sv) was increased in both strains (Figure 18B, C). The expression levels of *Egf* ($p \leq 0.0001$) and its receptor *Erbb1* ($p = 0.004$) were significantly elevated if RMT and HCC 129Sv mice were compared (Figure 18A, D).

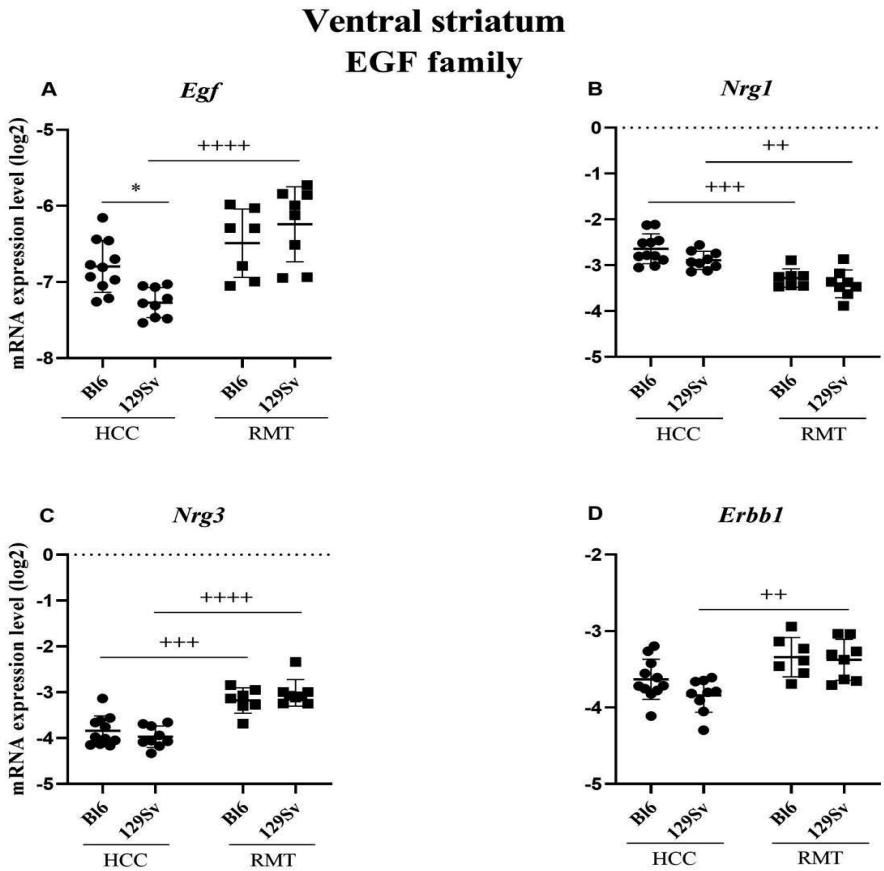

Figure 18. Egf family gene expression in ventral striatum of Bl6 and 129Sv mice. Two-way ANOVA was applied to demonstrate differences between the strains and environments. Substantial statistically significant gene expressions for **(A)** *Egf* – epidermal growth factor, **(B)** *Nrg1* – neuregulin 1, **(C)** *Nrg3* – neuregulin 3 and **(D)** *Erbb1* – epidermal growth factor receptor. Bonferroni *post hoc* test: * $p \leq 0.05$ compared to respective 129Sv mice, ++ $p \leq 0.01$, +++ $p \leq 0.001$ and ++++ $p \leq 0.0001$ in strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 7 to 11.

Dorsal Striatum

In the dorsal striatum, the alterations of the EGF family gene expressions were rather modest like in the ventral striatum. In HCC 129Sv mice, the level of *Egf* ($p = 0.02$) was reduced compared to HCC Bl6 (Figure 19A). In RMT mice, the expression of *Nrg3* displayed a significant elevation in both strains (for both $p \leq 0.0001$, Figure 19C). In the case of *Nrg1*, there was a modest elevation in HCC Bl6 ($p = 0.04$) compared to 129Sv, whereas in RMT this difference remained rather similar ($p = 0.008$, Figure 19B). The expression of *Egf* ($p = 0.01$) was increased in RMT 129Sv compared to the respective Bl6 group (Figure 19A). In RMT 129Sv the expression levels of *Egf* ($p \leq 0.0001$) and its receptor *Erbb1* ($p \leq 0.0001$) were significantly increased compared to HCC 129Sv (Figure 19A, D). A similar elevation of *Erbb1* ($p \leq 0.0001$) was established for RMT Bl6 compared to the HCC group (Figure 19D).

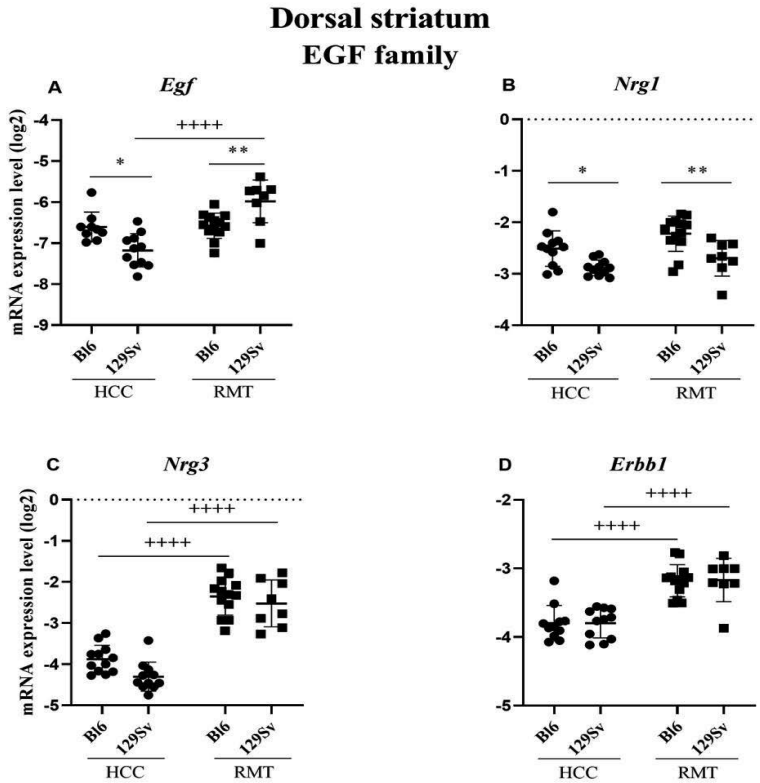

Figure 19. Egf family gene expression in dorsal striatum of Bl6 and 129Sv mice. Two-way ANOVA was applied to demonstrate differences between the strains and environments. Substantial statistically significant gene expressions for **(A)** *Egf* – epidermal growth factor, **(B)** *Nrg1* – neuregulin 1, **(C)** *Nrg3* – neuregulin 3 and **(D)** *Erbb1* – epidermal growth factor receptor. Bonferroni *post hoc* test: * $p \leq 0.05$ and ** $p \leq 0.01$ compared to respective 129Sv mice, ++++ $p \leq 0.0001$ in strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 8 to 13.

5.3.2.3. Gene expression alterations in the midbrain

Compared to the other brain regions, only a few changes occurred in the midbrain due to RMT. The expression of *Drd1* ($p = 0.01$) was reduced in RMT 129Sv compared to the HCC group (data can be found from Paper III supplementary material Table S1). RMT increased the expression of dopamine receptor D3 (*Drd3*, $p = 0.04$) in 129Sv compared to Bl6. In both strains, RMT significantly decreased the level of *Comt* ($p = 0.0007$ for Bl6 and $p = 0.01$ for 129Sv). Data can be found from Paper III supplementary material Table S1.

5.3.3. Measurement of EGF family and NMDA protein levels in the frontal cortex and hippocampus using western blot analysis

In the hippocampus, the expression levels of NRG2 ($p = 0.03$) and GRIN1 ($p = 0.002$) proteins were significantly elevated in HCC 129Sv mice (Figure 20D, E). In the frontal cortex, no statistically significant differences were established in HCC conditions. RMT exposure increased EGF family protein abundance in the frontal cortex as measured by Western blot. According to the Kruskal–Wallis test, RMT significantly increased EGF ($p = 0.0005$), ERBB1 (p = 0.0004), and NRG2 ($p = 0.0014$) protein levels in Bl6 compared to HCC group (Figure 21A, B, D). In addition, in the NMDA system RMT elevated protein expression of GRIN1 ($p = 0.005$) in Bl6 compared to HCC animals (Figure 21E). RMT did not affect the expression of measured proteins in 129Sv.

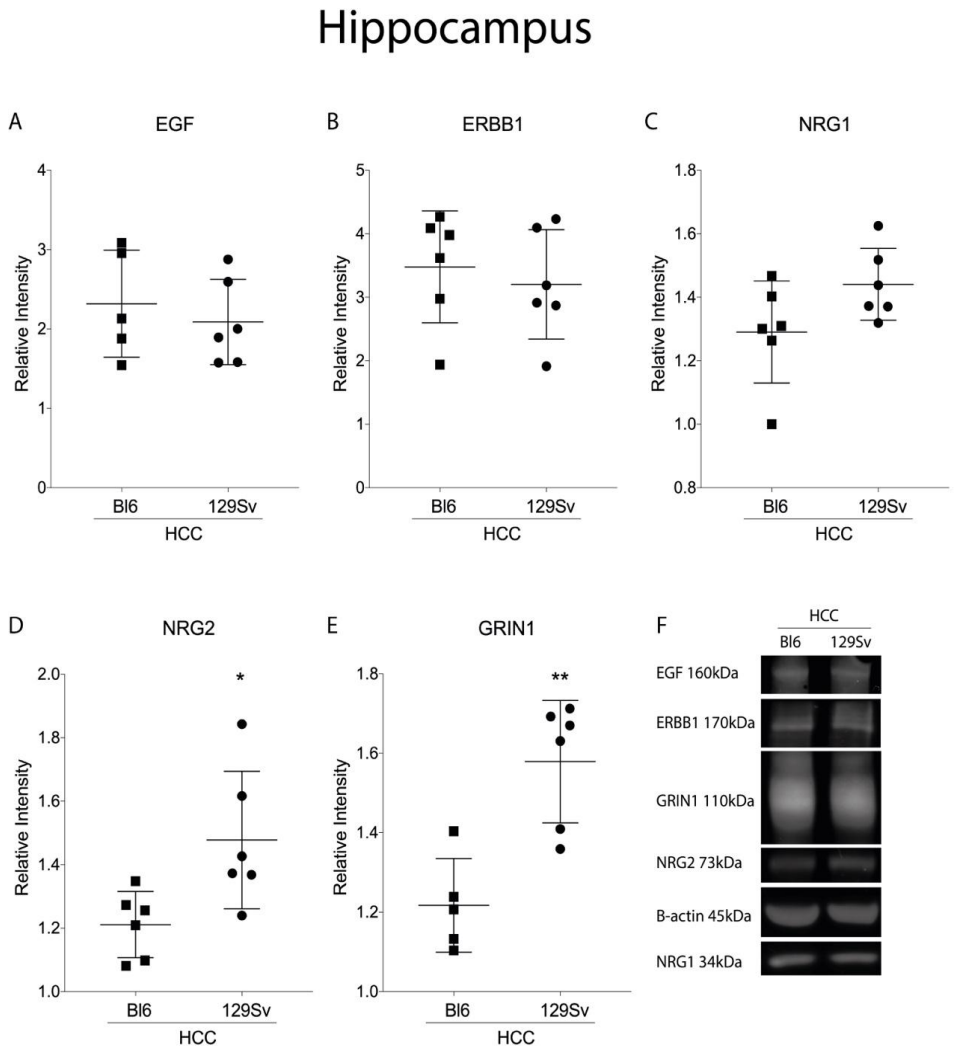

Figure 20. Protein expression in the hippocampus of Bl6 and 129Sv mice. Unpaired t-test with Welch's correction was applied to demonstrate differences between the strains in HCC group. Substantial statistically significant protein expressions for **(A)** EGF – epidermal growth factor, **(B)** ERBB1 – epidermal growth factor receptor, **(C)** NRG1 – neuregulin 1, **(D)** NRG2 – neuregulin 2, **(E)** GRIN1 – glutamate ionotropic receptor NMDA type subunit 1, and **(F)** representative immunoblots. Unpaired t-test with Welch's correction: * $p \leq 0.05$, ** $p \leq 0.01$ between the strains. Data are expressed as mean values ± SD. Number of animals in each group varied from 5-6.

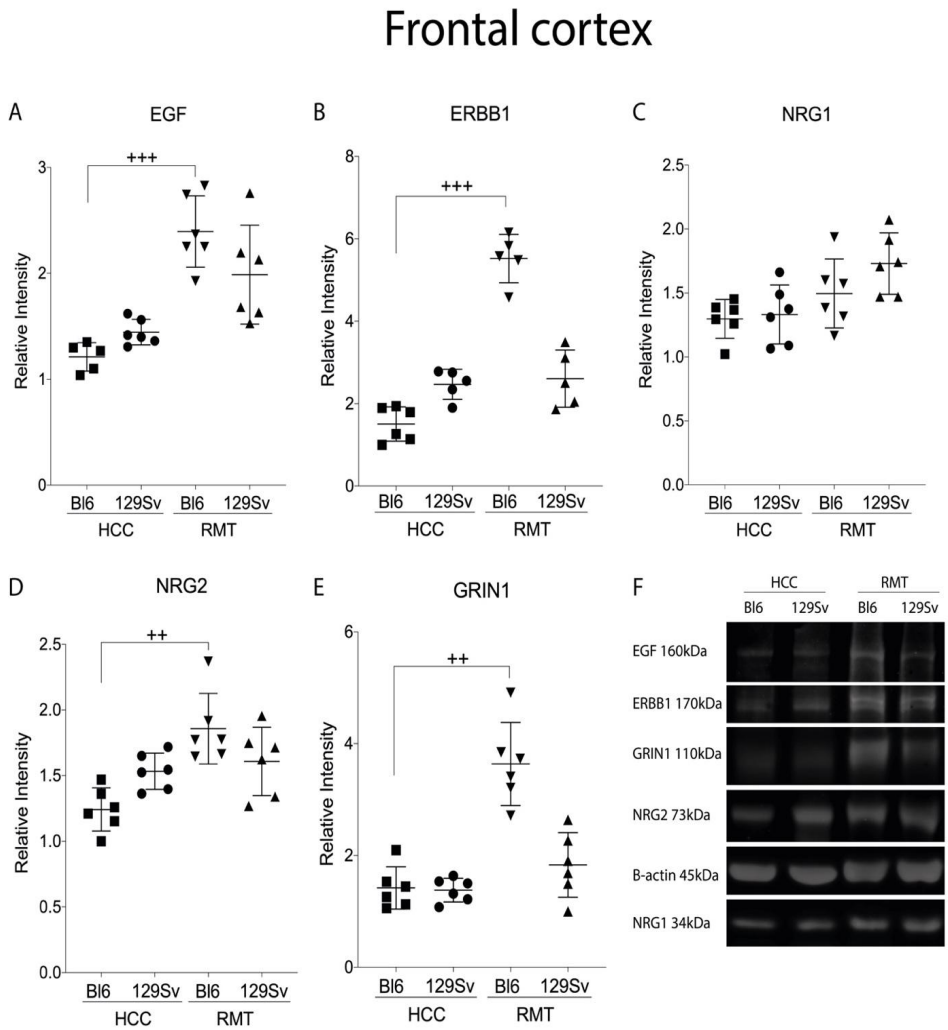

Figure 21. Protein expression in the frontal cortex of Bl6 and 129Sv mice. Kruskal-Wallis test was applied to demonstrate differences between the strains and environments. Substantial statistically significant protein expressions for **(A)** EGF – epidermal growth factor, **(B)** ERBB1 – epidermal growth factor receptor, **(C)** NRG1 – neuregulin 1, **(D)** NRG2 – neuregulin 2, **(E)** GRIN1 – glutamate ionotropic receptor NMDA type subunit 1, and **(F)** representative immunoblots. Dunn's multiple comparison test: ++ $p \leq 0.01$, +++ $p \leq 0.001$ in strain specific comparison. Data are expressed as mean values ± SD. Number of animals in each group varied from 5-6.

6. DISCUSSION

Previous studies have shown that Bl6 mice display greater locomotor activity and increased exploratory behaviours while 129Sv mice are less active and are more vulnerable to stress. In this dissertation, we investigated the impact of two different interventions (HCC and RMT) and amphetamine administration on behaviour, metabolite levels, gene and protein expression. HCC (home cage control) represented the usual home-cage environment for the mice, whereas RMT (repeated motility testing) reflected a stressful everyday challenge where mice had to adapt with a stressful environment. Impaired adaptation indicates vulnerability and may thereby refer to higher susceptibility to stress-induced disorders.

6.1. Behavioural and body weight differences in Bl6 and 129Sv in home cage and stressful environment (*Paper I, III*)

This study reveals and confirms the vast difference of the behaviour and body weight regulation in Bl6 and 129Sv mouse strains. The two strain comparison revealed a body weight change in both (HCC and RMT) conditions after 11 days. In the HCC batch, 129Sv mice gained approximately 0.8 g more body weight than Bl6. However, in the RMT batch, these strains differed in their body weight dynamics: 129Sv mice lost body weight (-1.3 g) while Bl6 mice remained almost at the initial level (+0.3 g). Taken together, 129Sv displayed a greater discrepancy in weight change when the outcomes of two interventions were compared. Similar results concerning dynamics of body weight were obtained in studies where these strains were exposed to the enriched environments (Heinla et al., 2014).

Testing of exploratory activity also revealed differences between Bl6 and 129Sv strains. The results support previous evidence that Bl6 mice displayed remarkably higher horizontal and vertical exploratory activity compared to 129Sv mice. Initial difference in horizontal activity (the 1st day), significantly favouring Bl6 over 129Sv strain, remained the same on the 11th day. However, the difference in the frequency of rearings between Bl6 and 129Sv mice became steadily even more robust in the course of testing. One could suggest that Bl6 mice, differently from 129Sv strain, actively adapt to a challenging environment (trying to escape), reflected by a significantly increased number of rearings in the motility test. Probably RMT reinforced the predisposition in both strains, by evoking an active coping strategy in Bl6, while 129Sv developed a more passive strategy or even aversion (lost body weight) towards the test situation.

6.2. Metabolic profile associated with distinct behavioural coping strategies of 129Sv and Bl6 mice in repeated motility test (*Paper I*)

Serum samples for metabolomic study were collected from the HCC batch after weighing and in the case of the RMT batch immediately after the last exposure of mice to the motility boxes. The endogenous metabolites were analysed by validated assay that allowed comprehensive identification and quantification of amino acids, acylcarnitines, biogenic amines, hexoses, and phospho- and sphingolipids (phosphatidylcholines, lysophosphatidylcholines, sphingomyelins).

6.2.1. Acylcarnitines and hexoses (*Table 11, 12*)

Both strains revealed rather distinct profiles of acylcarnitines and hexoses. The level of hexoses was higher in Bl6 for both batches compared to 129Sv, but these comparisons did not survive Bonferroni correction. In both batches of 129Sv acetylcarnitine C5- and ratio of C5-/ carnitine C0 remained stable markers after Bonferroni correction, while the ratios of acylcarnitines C16.0/C16.1 and C18.0/ C18.1 in comparison of two batches in 129Sv changed remarkably (Table 11, 12). In the RMT batch, 129Sv lost body weight probably due to compromised food motivation caused by the repeated testing. There is evidence from a rat study that reduced food intake decreases the level of carnitine C0, but increases the levels of short-chain acylcarnitines (Jones et al., 2010). In the present study, a similar metabolic shift between acylcarnitines (C4, C5-) and carnitine C0 occurred in RMT animals. One may suggest that the repeated behavioural testing was more stressful for 129Sv than Bl6. The reason for elevation of hexoses in Bl6 compared to 129Sv is not clear and remains to be clarified in further studies.

Table 11. List of stable metabolite levels and ratios in Bl6 and 129Sv respectively. Effect size (eta^2) estimates for the Mann-Whitney U tests. Effect size estimate (eta^2) has been calculated by dividing the value of squared standardised test statistic (Z^2) with the total number of observations (N).

Metabolites	Bl6 mice (Eta^2 values)		Metabolites	129Sv mice (Eta^2 values)	
	Home cage	Repeatedly tested		Home cage	Repeatedly tested
Acetyl-ornithine	0.70	0.67	C5-	0.68	0.63
PC(16:1/0:0)	0.70	0.63			
Alpha-aminoadipic acid	0.68	0.64			
Carnosine	0.57	0.60			
Ratios			**Ratios**		
Glycine/PC ae C38:2	0.70	0.69	C5-/C0	0.69	0.72
			PC(16:0/0:0)/ PC(16:1/0:0)	0.70	0.71

Table 12. List of metabolite levels and ratios undergoing significant change in Bl6 and 129Sv due to repeated behavioural testing. Effect size (eta^2) estimates for the Mann-Whitney U tests. Effect size estimate has been calculated by dividing the value of squared standardised test statistic (Z^2) with the total number of observations (N). Ratios have been indicated by *.

Metabolites	Bl6 mice (Eta^2 values)		Metabolites	129Sv mice (Eta^2 values)	
	Home cage	Repeatedly tested		Home cage	Repeatedly tested
PC(20:3/0:0)	**0.61**	0.32	PC ae C36:2	**0.70**	0.41
PC(18:1/0:0)	**0.57**	0.22	SM (OH) C14:1	**0.70**	0.41
C4/C5-*	**0.70**	0.21	SM (OH) C22:1	**0.70**	0.4
Glycine/serine*	**0.68**	0.31	SM C24:0	**0.70**	0.23
PC aa C32:1	0.32	**0.49**	Fisher ratio*	0.39	**0.71**
PC aa C34:4	0.32	**0.51**	C16.0/C16.1*	0.26	**0.58**
Hexoses	0.31	**0.49**	C18.0/C18.1*	0.23	**0.69**

6.2.2. Amino acids and biogenic amines

In both batches of Bl6 the levels of biogenic amines (acetyl-ornithine, alpha-aminoadipic acid, carnosine) were significantly higher compared to 129Sv (Table 11). Definitely, acetyl-ornithine, alpha-aminoadipic acid and carnosine belong to the metabolic signatures of Bl6. Dipeptide carnosine (*β*-alanyl-L-histidine) is highly concentrated in the muscle and brain. It acts as an anti-glycating agent, reducing the formation rate of advanced glycation end-products, and may act as a neuroprotective mediator (Bae and Majid, 2013). The increased level of alpha-aminoadipic acid in Bl6 mice has been reported to be caused by a defect in the *Dhtkd1* gene. *Dhtkd1* has been identified as a primary regulator of alpha-aminoadipic acid and defects in this gene result in the increase of alpha-aminoadipic acid (Wu et al., 1995; Leandro et al., 2019). Alpha-aminoadipic acid is a component of lysine metabolism pathway and a marker of oxidative stress (Yuan et al., 2011; Zeitoun-Ghandour et al., 2011). A recent metabolomic study of diabetes patients plasma samples suggested that alpha-aminoadipic acid may be a modulator of glucose homeostasis and diabetes risk (Wang et al., 2013). Studies in rodents have also shown that alpha-aminoadipic acid modulates kynurenic acid levels in the brain. Kynurenic acid is a neuroactive metabolite that interacts with NMDA, AMPA/kainate and alpha 7 nicotinic receptors (Tuboly et al., 2015). In experiments with rat brain tissue slices, alpha-aminoadipic acid exposure resulted in a substantial decrease in levels of kynurenic acid (Gramsbergen et al., 2002). Similarly, *in vivo* studies in free-moving rats exposed to alpha-aminoadipic acid through microdialysis in the hippocampus resulted in a robust decrease in kynurenic acid level (Wu et al., 1995). Alpha-aminoadipic acid is a substrate of the enzyme alpha-aminoadipic acid aminotransferase II, which has been shown to be the same enzyme as kynurenine aminotransferase II (KAT-II), and is responsible for the transamination of kynurenine to kynurenic acid (Buchli et al., 1995; Hallen et al., 2013). Alpha-aminoadipic acid levels dictate the availability of KAT-II for the transamination of kynurenine to kynurenic acid (Schwarcz et al., 2012).

The ratio of branched chain amino acids / aromatic amino acids (Fisher ratio) was higher in both batches of 129Sv. Fisher ratio was a marker that survived Bonferroni correction in 129Sv RMT batch. The ratios of short-chain acylcarnitines (C4, C5-) to carnitine C0 were higher in 129Sv (Table 11). There is evidence that short-chain acylcarnitines (C3, C4, C5-) are formed from branched chain amino acids (Schooneman et al., 2013). Isoleucine and leucine play a role in the formation of C5-, showing an apparent link between amino acid and energy metabolism (Schooneman et al., 2013). This is in line with the increased level of acylcarnitine C5- in our study. It is possible that C5- as well as its ratio with carnitine C0 and augmented branched chain amino acids levels reflect the changes in energy metabolism of 129Sv compared to Bl6.

6.2.3. Lysophosphatidylcholines (LysoPCs)

Only the increased values of lysoPC(16:1/0:0) in both batches of Bl6 strain and ratio of PC(16:0/0:0)/PC(16:1/0:0) in both batches of 129Sv strain survived Bonferroni correction (Table 11). LysoPCs are bioactive pro-inflammatory lipids generated by pathological activities (Matsumoto et al., 2007). LysoPCs up-regulate the expression of inflammation-related genes IL-6, TNF-α, Ccl5, Cxcl1, and iNOS (Defaux et al., 2010). It has been demonstrated that LysoPCs, particularly PC(16:0/0:0) increase the formation of IFN-γ in human T lymphocytes (Nishi et al., 1998; Huang et al., 2001). Nevertheless, the functional role of established differences between LysoPC in 129Sv and Bl6 is not clear and remains to be established in the further studies.

6.2.4. Phosphatidylcholines (PCs)

Among HCCs the elevation of PCs was more prominent in 129Sv than in Bl6 (Table 2). In Bl6 only PC aa C34:3 ($eta^2 = 0.68$) survived Bonferroni correction (Table 1). In 129Sv HCC four PC acyl-alkyls were elevated compared to Bl6. Prominent elevations were established for these four PC acyl-alkyls (PC ae C36:2, PC ae C38:2, PC ae C40:4, $eta^2 = 0.7$ for all three, and PC ae C40:6, $eta^2 = 0.64$) in 129Sv. The stronger elevation of PCs in 129Sv HCC may be linked to the higher body weight gain in these mice, possibly indicating elevated lipid metabolism. In RMT these changes were less variable in both strains. In Bl6 only one PC diacyls was elevated (Table 4), whereas all PCs in 129Sv did not survive Bonferroni correction. The outcome of Bonferroni correction was supported by GLM. Listed PC acyl-alkyls (PC ae C36:2, PC ae C38:2, PC ae C40:4, PC ae C40:6) in HCC were positively associated with body weight gain in 129Sv (Table 3).

6.2.5. Sphingolipids

In the 129Sv HCC batch 3 sphingolipids SM (OH) C14:1, SM (OH) C22:1 and SM C24:0 ($eta^2 = 0.7$) survived Bonferroni correction, while none of sphingolipids survived Bonferroni correction in 129Sv RMT batch (Table 12). GLM established association between four sphingolipids [SM (OH) C14:1, SM (OH) C22:1, SM (OH) C22:2 and SM C24:0] and elevated body weight in 129Sv HCC batch (Table 3). Sphingolipids are one of the major lipid components of eukaryotic membranes and have a wide range of physiological functions, including cell adhesion, skin permeability barrier formation, myelin maintenance, immunity, spermatogenesis and glucose metabolism (Lahiri and Futerman, 2007; Narita et al., 2016). Complex sphingolipids located in the plasma membrane of animal cells, especially nerve cells, have a structural function and are believed to protect the cell surface from harmful environmental factors. They also serve as adhesion sites for extracellular proteins, play important roles in signal transmission, and cell recognition (Kihara et al., 2007). The elevated levels of sphingolipids in HCC could reflect increased lipid metabolism in

129Sv. In RMT animals the balance of sphingolipids still favours 129Sv, but the increase is less prominent compared to HCCs. The recent evidence suggests that the decline of several PCs and sphingolipids impairs the liver-dependent lipid metabolism and circulation, as hepatic PCs are required for the assembly and secretion of very low-density lipoprotein from the liver (Cole et al., 2012; Imhasly et al., 2014).

6.2.6. Impact of repeated testing on metabolite levels

Our analysis demonstrated that the differences of certain metabolite levels in comparison of both batches (HCC and RMT) of Bl6 and 129Sv remained unchanged (Table 11). After Bonferroni correction and application of GLM the following metabolite levels remained similarly elevated in both batches of Bl6: biogenic amines (acetyl-ornithine, alpha-aminoadipic acid, carnosine), lysophosphatidylcholine PC(16:1/0:0) and the increased ratio of glycine/PC ae C38:2 (Table 11). In both batches of 129Sv the elevation of only one metabolite level remained unchanged: acylcarnitine C5-. Also, the ratio of acylcarnitine C5-/ carnitine C0 and PC(16:0/0:0)/PC(16:1/0:0) demonstrated a stable elevation in both batches of 129Sv (Table 11). One may suggest that these stable differences in metabolite levels of 129Sv and Bl6 reflect their strain-specific metabolic signatures. Several molecules also undergo a significant change in Bl6 and 129Sv under the influence of RMT. The effect size of lysophosphatidylcholines PC(18:1/0:0), PC(20:3/0:0), as well as ratio of C4/C5- and glycine/serine were reduced in Bl6 RMT batch (Table 12). In 129Sv RMT the reduction of effect sizes was evident for PC ae C36:2 and for several sphingolipids (SM C24:0, SM (OH) C14:1, SM (OH) C22:1). In 129Sv RMT increased the effect size for Fisher ratio, indicating a shift towards branched chain amino acids over aromatic amino acids (Table 12).

6.3. Repeated administration of amphetamine induces distinct alterations in behaviour and metabolite levels in 129Sv and Bl6 mouse strains (*Paper II*)

Repeated administration of amphetamine has been applied to model psychotic-like behaviour in rodents. So far, few studies have been performed to examine mouse strain differences in behavioural sensitization to amphetamine. After a thorough literature search we may conclude that we are the first to explore the metabolic profile of these two mouse lines. In Paper I we showed that after repeated saline administration the 129Sv and Bl6 strains display different metabolic profiles and behavioural coping strategies. These results encouraged us to investigate the metabolic outcomes of repeated amphetamine in these two mouse strains. We found that the effect of genetic background definitely exceeds that of pharmacological influence. Besides, 129Sv displayed a significantly larger variation after repeated amphetamine than Bl6. Based on our re-

sults we believe 129Sv to be a more promising strain for evaluating psychotic-like behaviours compared to Bl6. Starting from the genetic point of view 129Sv mice have mutated DISC1 protein, strongly affecting dopamine homeostasis (Clapcote and Roder, 2006; Dahoun et al., 2017). Recent clinical research relates DISC1 mutations to various neuropsychiatric disorders (Thomson et al., 2016). The mutation in the Disc1 gene is the first aspect why 129Sv mice could be better models for studying psychotic-like behaviour than the Bl6 strain. Second, 129Sv mice display aberrant adaptation in a stressful environment. In Paper I we saw that repeated testing of Bl6 mice in the motility cages robustly increased their exploratory activity, whereas in 129Sv no such changes occurred and their activity remained almost at the level of the first testing day. However, as opposed to Bl6 mice, 129Sv mice started to lose body weight. One could suggest that these differences are related to the prevailing coping strategies in these two strains due to variations in the function of the dopamine system. One may speculate that the retarded behaviour of 129Sv mice in stressful situations may to a certain extent, reflect the characteristics of the prodrome syndrome of first episode psychosis in humans. Third, vast differences between 129Sv and Bl6 mice could be seen after repeated amphetamine administration. In Bl6 mice only moderate sensitization toward amphetamine was observed, whereas 129Sv mice could be divided into two subgroups. In one subgroup repeated amphetamine failed to magnify the drug effect compared to the acute amphetamine, whereas in the other subgroup an almost 5-fold sensitization was established. In other words, one subgroup displays no sensitization to amphetamine, resembling depression-like state, whereas the other subgroup responds with the robust sensitization, resembling psychotic-like state. Depressive and psychotic symptoms both can be seen in patients with the first episode psychosis. These large variations in behavioural outcome in 129Sv mice having the same genetic background is rather unexpected and definitely needs further analysis.

6.4. Amphetamine-induced behavioural and body weight differences (*Paper II, III*)

The current study revealed that 129Sv and Bl6 demonstrate vastly different motor responses to amphetamine administration (3 mg/kg i.p.). The increase of locomotor activity was significantly stronger in acutely treated Bl6 (Figure 3) compared to 129Sv. This is in line with the existing evidence that Bl6 is more responsive to the stimulating effect of acute amphetamine. This response can be attributed to the greater activity of the DA-ergic system in these animals compared to 129Sv (Chen et al., 2007). After repeated amphetamine both strains displayed a significant increase in distance travelled. In fact, the locomotor activity of 129Sv reached the level of Bl6 after repeated amphetamine. Indeed, the elevation of locomotor activity in response to repeated amphetamine was more pronounced in 129Sv compared to Bl6 mice. Also, we established greater locomotor activity dispersion around the mean value in 129Sv compared to Bl6.

Therefore, two differently responding groups can be formed among 129Sv receiving repeated amphetamine: one which responded similarly to the acute amphetamine group (weak responders), and the other one in which the response was 5-fold augmented (strong responders). It has been described how the distinction of amphetamine-caused locomotion might be related with the brain D2 receptors densities (Helmeste and Seeman, 1982). However, the exact basis of stronger sensitivity to amphetamine remains unknown. The drug reactivity differences in animals living in the same conditions may be caused by various reasons such as brain biochemistry, general physiology and the environmental effect on genes. Next, we found that 129Sv responded to the daily manipulations with loss of body weight. However, this effect was not due to amphetamine, because it was similar in all administration groups. This demonstrates that the applied dose of amphetamine does not suppress food intake in 129Sv. By contrast, no body weight decline occurred in Bl6. Overall, this indicates that behavioural manipulations were more stressful for 129Sv than for Bl6. This is in line with our previous studies showing that exposing mice to behavioural enrichment induces a reduction of body weight in 129Sv, but not in Bl6 in subsequent behavioural tests (Heinla et al., 2014).

In Paper III we were interested to see how the acute administration of amphetamine affects these inbred strains. For this purpose we generated again two different interventions: for one batch of mice (both Bl6 and 129Sv) we administered acute amphetamine in the beginning (on the first day) of the RMT study (this represents HCC batch) and the other batch (both Bl6 and 129Sv) received acute amphetamine after repeated testing with saline (RMT batch). In the beginning of the study, in the HCC batch, we saw that the effect of amphetamine in 129Sv strain reached the level of saline-treated RMT Bl6 mice, but was not statistically different when compared to saline-treated 129Sv mice. In Bl6 mice, amphetamine caused a strong elevation of distance travelled compared to vehicle treatment. These findings are in line with the study of Chen and colleagues that acute treatment with amphetamine causes a significantly stronger locomotor activation in Bl6 mice compared to 129Sv strain. This behavioural effect was accompanied by augmented striatal dopamine efflux in Bl6 mice compared to 129Sv, whereas the basal levels of dopamine in these strains were not different (Chen et al., 2007). The effect of amphetamine was also evaluated at the end of study in the RMT batch (Figure 11B). The outcome of the study was rather similar to that established in the beginning since the effect of amphetamine was significantly weaker in 129Sv strain. However, here the stimulatory effect of amphetamine was statistically significant in both strains if compared to respective saline treatments. Altogether, it is apparent that 129Sv and Bl6 mice display distinct sensitivity to amphetamine, supporting the view about significant differences in the functional activity of dopamine systems in these strains.

6.5. Amphetamine-induced metabolite level differences (*Paper II*)

A profound difference between 129Sv and Bl6 was revealed not only at the behavioural and body weight levels, but the metabolite levels were also differently affected by amphetamine in these two mouse strains. The number of affected metabolite levels in Bl6 was less pronounced compared to 129Sv. Nevertheless, several significant changes were established after acute amphetamine. This involves an apparent elevation of branched chain amino acids levels in Bl6 – isoleucine and leucine. This alteration in the levels of isoleucine and leucine was accompanied by a shift in the ratio between branched chain amino acids and aromatic amino acids favouring the former ones. Besides that, there was a trend for the reduction of hexoses. After repeated amphetamine no further increase was established in leucine and isoleucine levels. One could suggest that these metabolic changes may reflect behavioural changes due to amphetamine administration. Acute amphetamine caused in Bl6 a profound elevation of locomotor activity, causing an increased need for energy. Therefore, the trend for declined levels of hexoses (including glucose) possibly reflects this need. To replenish the energetic need due to increased workload, isoleucine and leucine were used as additional energetic sources. Besides that, the levels of biogenic amines were reduced by repeated amphetamine in Bl6, including asymmetric dimethylarginine, alpha-aminoadipic acid and kynurenine. Asymmetric dimethylarginine, an analogy of L-arginine, is a naturally occurring product of metabolism found in circulation. Elevated levels of asymmetric dimethylarginine inhibit NO synthesis and, therefore, lead to impaired endothelial function (Sibal et al., 2010). Dimethylarginine dimethylaminohydrolase (DDAH) has been shown to hydrolyse asymmetric dimethylarginine to yield citrulline and dimethylamine (Leiper and Vallance, 2006). Therefore, the formation of NO from arginine is not the only source for the production of citrulline. Xuan and colleagues demonstrated the antagonistic function of citrulline against asymmetric dimethylarginine, showing protection of endothelium from impairment of asymmetric dimethylarginine in porcine coronary arteries (Xuan et al., 2015). The beneficial effect of citrulline against asymmetric dimethylarginine on endothelial function may be attributed to the preservation of NO production, activation of the NO/cGMP signalling pathway, and suppression of superoxide anion overproduction (Xuan et al., 2015). Alpha-aminoadipic acid is a component of the lysine metabolism pathway and a marker of oxidative stress (Yuan et al., 2011; Zeitoun-Ghandour et al., 2011). Studies in rodents have also shown that alpha-aminoadipic acid modulates kynurenic acid levels in the brain. Alpha-aminoadipic acid levels dictate the availability of kynurenine aminotransferase II (KAT-II) for the transamination of L-kynurenine to kynurenic acid (Schwarcz et al., 2012).

Acute treatment with amphetamine caused a shift in the ratio between glycine and serine in favour of the latter. Biosynthesis of glycine occurs through the conversion of L-serine to glycine by the enzyme serine hydroxylmethyl-

transferase (Appaji Rao et al., 2003). This shift shows that probably less glycine is formed from L-serine under the influence of acute amphetamine. Glycine plays a role as an inhibitory (via glycine receptors) as well as excitatory (via NMDA receptors) neurotransmitter in the brain (Hernandes and Troncone, 2009). D-serine is formed from L-serine and it interacts with a D-serine/glycine modulatory site on the NR1subunit of NMDA receptors (Johnson and Ascher, 1987; Clements and Westbrook, 1991). The D-serine/glycine site on the NMDA receptor must be occupied for glutamate to activate the receptor (Clements and Westbrook, 1991).

As mentioned above, the acute response of 129Sv strain to amphetamine-induced motor stimulation was apparently weaker and meanwhile these mice lost body weight. The reduced body weight was accompanied by lower levels of hexoses in the saline administration group of 129Sv (5,857 ± 1,146 μmoles) compared to the respective group of Bl6 (8,900 ± 1,195 μmoles). Therefore, one can conclude that 129Sv is in terms of hexoses (including glucose) in compromised status compared to Bl6 and, therefore, other sources of energy are needed. This is a likely reason why repeated amphetamine caused in 129Sv a wider deviation of metabolite levels compared to Bl6. Acute amphetamine in 129Sv group, reduced the levels of various metabolites compared to saline, including valine, lysoPCs (lyso PC aa 16:0, lyso PC aa 18:2, lyso PC aa 20:4), PC diacyls (PC aa 34:2, PC aa 36:2, PC aa 36:3, PC aa 36:4) and PC acylalkyls (PC ae 38:4 and PC ae 40:4). Comparison of acute and repeated amphetamine established that several metabolite concentrations were elevated due to repeated amphetamine in 129Sv, including long chain acylcarnitines (C14, C14:1-OH, C16, C16:1, C18:1), branched chain amino acids (particularly isoleucine, valine), PC diacyls (PC aa C38:4, PC aa C38:6, PC aa C42:6), PC acyl-alkyls (PC ae C38:4, PC ae C40:4, PC ae C40:5, PC ae C40:6, PC ae C42:1, PC ae C42:3), and sphingolipids [SM(OH)C22:1, SM C24:0]. The elevation of the ratio between long chain acylcarnitines and carnitine C0 (CPT-1 ratio) probably reflects the elevated participation of these metabolite levels due to need of additional energy for workload in 129Sv. Also, there were tendencies for a shift in the ratio between short chain acylcarnitines (C3, C5-) and carnitine C0, favouring short chain acylcarnitines. This probably reflects the metabolic value of elevated levels of branched chain amino acids (isoleucine, valine) in 129Sv receiving repeated amphetamine. These branched chain amino acids are used for the synthesis of acylcarnitines C3 and C5-.

6.6. Distinct metabolomic response in amphetamine subgroups of 129Sv (*Paper II*)

The distinct response of 129Sv to amphetamine in the locomotor test was further analysed at the metabolite level. Strong responders to amphetamine displayed elevated levels of long chain acylcarnitines (C12, C14:1, C14:1-OH, C16:1) and reduced levels of hexoses compared to weak responders (Table 8).

These metabolic changes can be taken as a compensatory response to the augmented workload in strong responders due to the stimulating effect of amphetamine. Besides that, the ratio between glycine and glutamine was reduced in strong responders, whereas the ratio between tyrosine and phenylalanine tended to be higher in strong responders. This may reflect the reduced availability of inhibitory transmitter glycine and increased availability of tyrosine as the precursor molecule of catecholamines in strong responders. Correlation analysis (data can be found in Paper II supplementary material Table S3) established a strong positive link between amphetamine-induced motor stimulation and long-chain acylcarnitines C16 (r = 0.55) and C16:1 (r = 0.56), and with the ratio between tyrosine and phenylalanine (r = 0.64). Negative correlation was established between locomotor activity and the ratio of glycine with histidine (r = −0.61). A strong negative correlation between C18:1 and hexoses demonstrated that animals with the lowest levels of hexoses displayed the highest levels of C18:1. This can be taken as a compensatory change to the limited amount of hexoses as the metabolic resource. On the other hand, C18:1 has been demonstrated as the pharmacologically active compound blocking the activity of glycine type 2 transporter (Carland et al., 2013). Inhibitory glycinergic neurotransmission is terminated by glycine transporters GlyT1 and GlyT2 which reuptake glycine from the synaptic cleft. GlyT2 is the principal supplier of glycine for vesicle refilling, a process that is necessary to preserve the amount of glycine in synaptic vesicles (Jiménez et al., 2015). In the multivariate regression modelling, the final parsimonious model retained distance travelled on day 11, C14:1, C16, C16:1, C18:1 and the ratio between glycine and glutamine as significant predictors of high motor response. The whole model Wilk's lambda = 0.19, $F_{(6, 7)} = 5.16$, $p = 0.03$, multivariate partial eta^2 =0.81, which indicates that ∼81% of the multivariate variance of dependent variables is associated with the amphetamine response division factor.

6.7. Combining of metabolite data from 129Sv and Bl6

Despite significant basal differences between 129Sv and Bl6 strains, combining the data from these strains revealed that 14 metabolite levels or their ratios remained significant with repeated amphetamine (Table 10). The reduction of kynurenine displayed a large effect size (eta^2= 0.15), whereas the other effects were moderate (eta^2 = 0.06–0.13). The levels of branched chain amino acids (leucine, isoleucine) and the ratio lysoPC a C20:4/lysoPC a C20:3 were markedly increased by repeated amphetamine. By contrast, the levels of citrulline, biogenic amines (asymmetric dimethylarginine, kynurenine), hexoses and lysoPC a C18:2 were significantly reduced with repeated amphetamine. Moreover, long chain acylcarnitines (CPT-1 ratio, C14) and PC alkyl-acyls (PC ae 40:6, PC ae 42:1) displayed an elevation if acute and repeated amphetamine were compared. The elevation of branched chain amino acids and lipid metabolite levels are probably associated with the reduction of hexoses, showing a

need for the additional sources of energy for elevated workload (Figure 22). The reduction of citrulline, asymmetric dimethylarginine and kynurenine probably reflects alterations in NMDA and NO systems, inherent for the development of dopamine agonist-induced sensitization (Lang et al.,1995; Võikar et al., 1999; Chen et al., 2001; Liu et al., 2011).

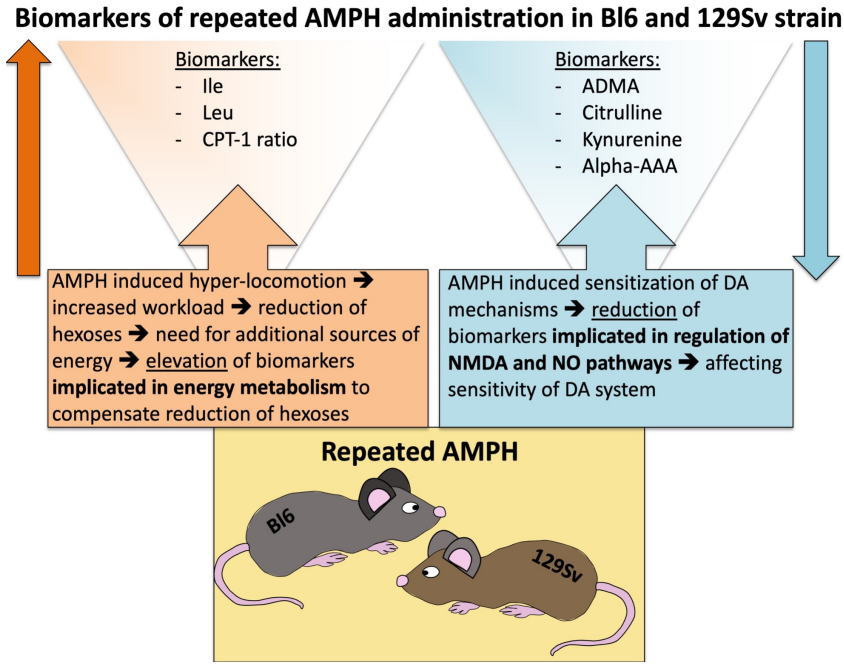

Figure 22. **Biomarkers of repeated amphetamine (AMPH) administration in Bl6 and 129Sv strains.** Metabolites that are involved due to enhanced workload and metabolites that are implicated due to sensitization of the dopamine system.

6.8. Dopamine system, NMDA receptor and EGF family expressions in brain structures of Bl6 and 129Sv strains displaying different behavioural adaptation (*Paper III*)

Considering differences between Bl6 and 129Sv in behaviour, we expected to see in Paper III also variations in gene expression of three large neurotransmitter/neuromodulator systems: dopamine system, NMDA receptors and EGF family. Indeed, these systems play a role in behavioural adaptation to a challenging environment in both preclinical and clinical settings (Pani et al., 2000; Wieduwilt and Moasser, 2008; Iwakura and Nawa, 2013; Mizuno et al., 2013; Yasuda et al., 2017; Kobayashi et al., 2019; Li et al., 2019).

6.8.1. Dopamine system

In HCC 129Sv mice, the expression of *Maob* was significantly reduced in the frontal cortex, hippocampus, ventral and dorsal striatum if compared to the Bl6 strain. The values of *Maoa* and *Maob* levels in different brain structures of both strains as well as the ratio between these genes reveal that the expression of *Maoa* dominates in 129Sv mice, whereas in Bl6 strain *Maob* prevails. Enzyme MAOA preferentially oxidised serotonin, noradrenaline, whereas MAOB preferentially cleaved phenylethylamine in mice (Shih et al., 1999). Both enzymes are involved in the metabolism of DA. Fornai and colleagues have found that in mice dopamine is only metabolised by MAOA under the basal conditions and by both MAOA and B if the concentrations of dopamine are high (Fornai et al., 2002). Therefore, the elevated expression of *Maob* with increased response to amphetamine in Bl6 mice seems to support the amplified function of dopamine in this strain. In line with that it has been demonstrated that Bl6 is the most sensitive strain to MPTP, the neurotoxin catalysed by MAOB enzyme (Inoue et al., 1999; Sedelis et al., 2000). Interestingly, the Bl6 strain is the only species where MAOB activity is greater in the brain than in the liver (Przedborski et al., 2000).

However, the relationship between *Maoa* and *Maob* was completely reversed after RMT. There was a tendency towards increased function of *Maoa* in Bl6 and *Maob* in 129Sv. The expression of *Comt*, another important enzyme in catecholamine metabolism, did not differ between the strains in HCC conditions.

Concerning the levels of monoamine metabolising enzymes in RMT groups, some definite alterations occurred in the brain. The levels of *Comt* in the frontal cortex were significantly elevated in both strains in response to RMT. However, this elevation of *Comt* was more pronounced in 129Sv. In the hippocampus, the expression of *Maoa* was also increased in both strains in RMT. In the ventral striatum we found a significant elevation of all monoamine metabolising enzymes in both strains. Besides that, the expression of *Th*, a gene for tyrosine hydroxylase, a rate limiting enzyme in dopamine synthesis, was elevated in both strains due to RMT. A robust elevation of all enzyme genes involved in dopamine metabolism (*Maoa*, *Maob*, *Comt*, *Th*) established in the ventral striatum probably underlines a pivotal role of this brain region in RMT adaptations.

Drd1 and *Drd2* are the genes for two dominating dopamine receptors in the brain (Missale et al., 1998). The only difference established in HCC mice was that in 129Sv mice the expression of *Drd1* in the hippocampus was significantly higher compared to Bl6 strain. RMT exposure even increased this difference, favouring 129Sv. The striatum comprises the dorsal striatum, which regulates motor output and decision-making, and the ventral striatum, which predominantly regulates reward and hedonic states. Both regions receive excitatory inputs from cortical and thalamic regions, as well as dense innervation from the midbrain DA-ergic nuclei (Flanigan and LeClair, 2017). Dopamine in the ventral striatum is responsible for the exploratory drive, whereas in the dorsal

striatum, the role of dopamine is pivotal for habit formation (Langen et al., 2011). In the ventral striatum, a significant reduction of two dopamine D2 receptor family genes *Drd2* and *Drd4* was established in both strains. Taking into account that these measurements were performed immediately after the last behavioural testing and besides a decline of receptors we detected a significant increase of enzymes responsible for the metabolism of dopamine (*Maoa*, *Maob*, *Comt*, *Th*), one may conclude that the established changes reflect the activation of the dopamine system due to the behavioural challenge. One can note that the alterations in dopamine-related gene expression in the ventral striatum are rather similar in 129Sv and Bl6 mice. Therefore, we were not able to establish a correlation between differences in motor activity and expression of dopamine-related transcripts.

In the dorsal striatum, we established an elevation of *Drd1* and *Drd2* receptors in both strains due to RMT challenge. Considering that the levels of dopamine metabolising enzymes were not elevated, but even reduced, like *Comt* in the dorsal striatum, this could reflect distinct alterations of dopamine systems in the dorsal and ventral striatum. A decrease of dopamine D2 receptor transcripts in the ventral striatum probably reflects ongoing exploratory drive, whereas the increase of *Drd1* and *Drd2* genes in the dorsal striatum is likely a part of habituation to the challenging environment.

6.8.2. NMDA system

The comparison of NMDA receptor related gene expression in the ventral and dorsal striatum again revealed vast differences between these subcortical structures. There was no alteration in the ventral striatum, whereas in the dorsal striatum all measured NMDA receptor related genes (*Grin1*, *Grin2a*, *Grin2b*, *Srr*) were upregulated in both strains in response to RMT. This elevation was simultaneous to the upregulation of *Drd1* and *Drd2* genes in the dorsal striatum. Indeed, the dorsal striatum is rich in cortico-striatal glutamatergic projections interacting with dopamine (Calabresi et al., 1996). The concurrent elevation of NMDA and dopamine receptors could be taken as a sign of intensified interaction between glutamate and dopamine. In the dorsal striatum GRIN1 together with dopamine receptors seems to play a role in the behavioural adaptation to a challenging environment (Wang et al., 2011a). In addition, Wang et al. have established a specific role of GRIN1 in habit formation (Wang et al., 2011a).

In the hippocampus, the levels of *Grin1* and *Grin2b* genes were elevated in 129Sv strain compared to Bl6 mice in both HCC and RMT. In the current study we used Western blot analysis as an alternative method to measure changes in our molecular targets at the protein level. The background line specific differences in the HCC mice that were mostly detected in the hippocampal area were in line with the protein analysis: a significantly higher *Grin1* expression in 129Sv was detected both at the mRNA and protein levels, indicating elevated baseline activity of GRIN1 in 129Sv mice.

In the frontal cortex, Western blot analysis revealed an upregulation of GRIN1 protein in RMT Bl6 mice, indicating opposite dynamics between protein and transcript in stress response as levels of the *Grin1* transcript were reduced in Bl6 mice in response to RMT. There are multiple factors that could explain the inconsistency in protein and mRNA levels, but miRNAs are among the most well-known regulators of transcript stability and translation efficiency. It has been shown that stress-induced miRNAs can regulate cellular responses also in the nervous system (Olejniczak et al., 2018). Several miRNAs control the translation efficiency in the *GRIN1* transcript making it one potential explanation of the inconsistent dynamics of GRIN1 during stress response in the two mouse lines (Liu et al., 2020).

Additional evidence for distinct functions of NMDA receptors comes from Piirsalu et al study and our metabolomics study in Paper I, where significantly increased levels of alpha-aminoadipic acid were established in the blood samples of Bl6 mice compared to 129Sv (Narvik et al., 2018; Piirsalu et al., 2020). This difference was present in both HCC and RMT mice. Studies in rodents have shown that alpha-aminoadipic acid modulates kynurenic acid levels in the brain. Kynurenic acid is a neuroactive metabolite that interacts with GRIN1, AMPA/kainate and alpha 7 nicotinic receptors (Tuboly et al., 2015). Alpha-aminoadipic acid levels dictate the availability of kynurenine aminotransferase II for the transamination of kynurenine to kynurenic acid (Schwarcz et al., 2012). Therefore, one could suggest that the formation of kynurenic acid is apparently reduced in Bl6 mice, probably causing less pronounced inhibition of GRIN1 in this strain.

6.8.3. EGF family

Research so far has established multiple interactions of EGF family proteins with dopamine and NMDA systems. Among the ErbB receptors, ErbB1, and ErbB4 are expressed in dopamine and GABA neurons, while ErbB1, 2, and/or 3 are mainly present in oligodendrocytes, astrocytes, and their precursors (Iwakura and Nawa, 2013). EGF receptor signalling upregulates the surface expression of the GRIN2B-containing NMDA receptors and contributes to long-term potentiation in the hippocampus (Tang et al., 2015). NMDA antagonist ketamine-treated rats exhibited locomotor/stereotypy up-regulation and a defect in sensorimotor gating, resembling the behavioural phenotype of schizophrenia. Moreover, NRG1 protein levels were progressively decreased in the medial prefrontal cortex, but not in the ventral striatum of ketamine-treated rats (Chi Moa and Chen, 2018).

In HCC mice, few differences in the EGF family were evident between 129Sv and Bl6 mice. *Tgfa* was significantly higher in the frontal cortex of Bl6 mice. In the hippocampus, the expression levels of *Nrg1* and its receptor *Erbb4* were significantly increased in 129Sv strain. In the ventral and dorsal striatum, the expression of *Egf* was moderately higher in Bl6 mice compared to 129Sv strain. The higher *Nrg2* in 129Sv hippocampus, previously shown as same

direction trends in the corresponding transcripts, was confirmed as significant protein changes in the Western blot analysis. Altogether, the evaluation of gene and protein expression data in HCC mice shows that in 129Sv mice the activity of *Nrg1/Nrg2* dominates, whereas in Bl6 mice the activity of *Egf* tends to be higher.

In RMT mice, substantial differences between 129Sv and Bl6 strains in the EGF family were evident. As a general tendency, in the frontal cortex the expression of EGF family in RMT 129Sv mice was elevated both compared to HCC 129Sv (*Egf, Tgfa*, *Nrg2*) and RMT Bl6 (*Egf, Nrg1*, *Nrg2*, *Erbb1*, *Erbb4*) mice. One must underline a robust increase in the expression of *Nrg2* and its receptor *Erbb4* in 129Sv compared to Bl6 strain. Differently from gene expression studies, Western blot established that RMT caused a significant elevation of EGF, NRG2 and ERBB1 protein levels in the frontal cortex of Bl6 mice. This again indicated opposite dynamics between proteins and transcripts in stress response.

In the hippocampus similar tendencies in gene expression like in the frontal cortex were apparent. In RMT 129Sv mice the levels of *Egf*, *Nrg1*, *Nrg2*, *Erbb1* and *Erbb4* were increased compared to RMT Bl6 mice. However, the difference for *Nrg1* and *Erbb4* was already present in the HCC group. Comparison of RMT and HCC 129Sv mice revealed an elevated expression of *Nrg1*, *Nrg2* and *Nrg3* due to RMT. A similar tendency was evident in Bl6 mice where the levels of *Nrg1* and *Nrg3* were increased in the RMT group. The gene expression study supports the activation of *Nrg1*/*Nrg2*/*Nrg3* and *Erbb4* signalling.

In the ventral striatum, less changes occurred. The expression levels of *Egf* and its receptor *Erbb1* were increased in RMT compared to HCC 129Sv mice. On the other hand, the level of *Nrg1* was reduced and *Nrg3* was increased if HCC and RMT groups of both strains were evaluated. In the dorsal striatum, the expression of *Egf* gene was increased in RMT 129Sv mice if compared to HCC 129Sv and RMT Bl6 animals. EGF receptor *Erbb1* was also elevated if RMT and HCC 129Sv mice were compared. However, the latter effect was also evident for Bl6 mice. Besides that, the expression of *Nrg1* and *Nrg3* was increased in RMT Bl6 mice compared to the HCC group. In RMT 129Sv mice we found the same effect for *Nrg3*, whereas the expression of *Nrg1* was reduced compared to RMT Bl6 mice. Therefore, in the dorsal striatum a similar upregulation, as in the case of dopamine receptor and NMDA related genes due to RMT, was established for several EGF family genes, including *Hb-Egf*, *Nrg3* and *Erbb1* in both strains. Altogether, data from the ventral and dorsal striatum show elevated *Egf-Erbb1* signalling in RMT 129Sv mice.

So far it is known that genetically modified mice with NRGs/ERBB receptor mutations display various behavioural alterations. Mutant mice heterozygous for either *Nrg1* or its receptor, *Erbb4*, show a behavioural phenotype that overlaps with mouse models for schizophrenia (Stefansson et al., 2002). Furthermore, heterozygous *Nrg1* mice have fewer functional NMDA receptors than wild-type mice. Neonatally EGF-treated animals exhibited persistent hyperdopaminergic abnormalities in the nigrostriatal system while NRG1 treatment

resulted in DA-ergic deficits in the corticolimbic dopamine system (Nawa et al., 2014). *Nrg2* knockouts (KO) had higher extracellular dopamine levels in the dorsal striatum, but lower levels in the medial prefrontal cortex, a pattern with similarities to dopamine imbalance in schizophrenia (Yan et al., 2018). Like *Erbb4* KO mice, *Nrg2* KOs performed abnormally in a battery of behavioural tasks relevant to psychiatric disorders (Skirzewski et al., 2020). *Nrg2* KOs exhibit novelty-induced hyperactivity in the open field, deficits in prepulse inhibition, hypersensitivity to AMPH, antisocial behaviours, and deficits in the T-maze alternation reward test – a task dependent on hippocampal and mPFC function (Yan et al., 2018). *Nrg3* KO mice also exhibited behaviours consistent with psychotic disorders. These animals displayed novelty-induced hyperactivity, impaired prepulse inhibition of the acoustic startle response, and deficient fear conditioning (Hayes et al., 2016). Current evidence points to a central role of NRGs/ERBB receptors in controlling glutamatergic LTP/LTD (long-term potentiation/long-term depression) and GABAergic LTD at hippocampal CA3–CA1 synapses, as well as glutamatergic LTD in midbrain dopaminergic neurons, thus supporting that NRGs/ERBB signalling is essential for proper brain functions, cognitive processes, and complex behaviours (Ledonne and Mercuri, 2018, 2019).

Altogether, the most consistent gene expression findings in the EGF family were found for *Egf* and its receptor *Erbb1*, and for *Nrg1*, together with its paralogs *Nrg2* and *Nrg3*, and for their receptor *Erbb4*. In 129Sv mice RMT tended to cause an upregulation of *Nrg1/Nrg2-Erbb4* encoding transcripts in the frontal cortex and hippocampus. The RMT-induced upregulation of genes encoding *Egfr-Erbb1* pathway was evident in all forebrain structures in 129Sv mice.

6.8.4. Protein analysis in the brain

Protein analysis in the frontal cortex confirmed RMT stress-induced differential alterations in the expression profile of EGF-related targets. However, the stress-related protein profile in the two mouse lines was different from that of transcripts due to significant stress-induced upregulation of protein targets in the Bl6 frontal cortex. In addition to the increase of NRG2 protein, also an upregulation of EGF, ERBB1 and GRIN1 proteins was detected in the frontal cortex of RMT Bl6 mice. As all line-specific protein and transcript changes were positively correlated in the hippocampus of home cage mice and inverse correlations between gene and protein expression occurred only in the frontal cortex of the RMT group, we suggest an involvement of stress-induced regulation mechanism related to the mRNA/protein stability that could be mediated through post-transcriptional processes (Vogel and Marcotte, 2012). As an example of differential post-translational mechanisms between Bl6 and 129Sv lines, it has been shown that homozygous mutation in the mRNA decay activator protein ZFP36L2 results in different phenotypes in Bl6 and 129Sv lines (Ball et al., 2014). Additionally, in 129Sv, the inconsistency between stress-induced increase in transcripts not followed by correspondingly increased protein levels in

the frontal cortex may reflect the reduced activity of EGF-family and NMDA receptor signalling in 129Sv. Increased gene expression of respective genes could be compensatory to the reduced function of these proteins in 129Sv; this assumption, however, needs further studies. Despite the directions of the stress-induced changes, the present study shows the involvement of the EGF family and its receptors in adaptation to challenging environments.

6.9. Limitations of the study

The first shortcoming of our study was the sample size in the 129Sv repeated amphetamine administration group, because after the treatment, two subgroups were formed in this batch (weak and strong responders). A larger sample size would enable to compare the gene expression and metabolomics data in different responder groups. However, the formation of two subgroups was an unexpected finding for us.

The second limitation was the number of protein probes in the saline treated stress group in both strains. It would be interesting to know how stress affects gene expression patterns in other brain regions beside the frontal cortex. In future studies we definitely keep this in mind when planning the protein analysis.

6.10. Concluding remarks and further perspectives (*Figure 23*)

The present study supports the previous findings that 129Sv and Bl6 mouse lines differ in their behavioural repertoire and stress response (Võikar et al., 2001; Abramov et al., 2008; Heinla et al., 2014). Bl6 is a mouse line displaying active stress coping strategies, while passive coping apparently predominates in 129Sv. An important factor in the difference between these two mouse lines is the activity of the dopaminergic system, which is significantly higher in Bl6 mice. It can be due to a mutation of the Disc1 gene in the 129Sv mouse line (Koike et al., 2006), being a probable explanation for disorder of dopamine homeostasis (Trossbach et al., 2016). Besides that, the gene expression study established that the expression of dopamine-metabolising *Maob* gene was significantly higher in the brain structures of the Bl6 mouse line compared to the 129Sv strain. It can be taken as the prerequisite for higher activity of the dopaminergic system in the Bl6 strain. Under the stressful conditions, this difference in the expression of the *Maob* gene in 129Sv and Bl6 mice remained the same in the hippocampus and dorsal striatum. By contrast, this variation disappeared in the ventral striatum. This finding may indirectly reflect an important role of the dopaminergic system in the ventral striatum in active adaptation of the Bl6 strain to a stressful environment.

The studies also revealed the specific metabolic signatures for both mouse lines. In 129Sv mouse line the metabolite level not affected by stress was acylcarnitine C5-, whereas in Bl6 these metabolite levels were acetyl-ornithine, alpha-aminoadipic acid, carnosine and lysophosphatidylcholine PC(16:1/0: 0). The effect of repeated amphetamine administration on metabolic marker levels appears to be modest compared to the variance because of the genetic background of mouse lines. Besides that, the 129Sv strain displayed a significantly larger variation of the metabolite levels after repeated treatments with amphetamine than Bl6.

Although dopamine agonist amphetamine was significantly more potent in the Bl6 mouse line upon acute administration, repeated amphetamine caused significantly greater sensitization to motor activity in 129Sv mice. In fact, the 129Sv mouse line was divided into two subgroups: those whose activity did not increase with repeated amphetamine administration (weak responders) and those who showed an overwhelming increase in motor performance (strong responders). The former resembled a depression-like condition and the latter was similar to a psychosis-like response. Such differences in behavioural manifestations have led us to conclude that 129Sv (129S6/SvEv) could be a better mouse line for modelling of depression- and psychotic-like conditions. In order to support the metabolic needs of repeated amphetamine administration in Bl6 mice dominated glucose metabolism, whereas in 129Sv strain use of lipids prevailed.

The C57Bl/6 inbred mouse strain is known for its strong, genetically determined preference for alcohol over water (Gooderham et al., 2004). Early investigations involving a limited number of inbred mouse strains identified the C57Bl/6 strain as an alcohol-preferring strain, whereas the DBA/2 strain was shown to be an alcohol-avoiding line (McClearn et al., 1964; Fuller, 1964). This finding was confirmed in several large comparative studies of commonly used inbred mouse strains (Belknap et al., 1993; Gill et al., 1996). Besides that, morphine administration induced place preference in C57Bl/6 mice but not in 129Sv strain (Dockstader, van der Kooy, 2001). In 129Sv mice, this occurred after pre-treatment of animals with the anxiolytic drug diazepam. This can be explained by the already mentioned high anxiety level of 129Sv mice. Altogether, the presented data seems to support the potential of Bl6 (C57BL/6N) mice to explore the mechanisms of drug addiction.

Given the large differences in the behavioural and metabolic responses of the two mouse lines, there are plans to move forward with studies attempting to challenge the potential mechanisms of neuropsychiatric disorders. In preliminary studies, we have found that 129Sv mice have significantly reduced *Negr1* gene expression in the ventral striatum. The *Negr1* gene appears to be important in the mechanisms of several neuropsychiatric disorders, in particular major depressive disorder (Kaare et al., 2021). We hope to clarify the extent to which *Negr1* gene deficiency in the ventral striatum contributes to the behavioural and maladaptive characteristics of the 129Sv mouse line.

Bl6 mouse strain	129Sv mouse strain
Behaviour	
Active, venturous, aggressive, mostly display active coping strategy (a coping style that is characterised by trying to escape from stressful situations), alcohol-preferring strain. Morphine administration induced a clear place preference	Anxious, vulnerable to stress, idle, reduced response to cat odour, stress caused decline in body weight, passive coping strategy (helplessness to deal with the stressors). Place preference was induced by morphine only after treatment with anxiolytic drug
Genetic peculiarities	
Defect in alpha-aminoadipic acid regulator gene *Dhtkd1*	• Frameshift mutation in disrupted-in-scizophrenia 1 (*Disc1*) gene • Splice-site mutation in isovalerylcarnitine regulator *lvd* gene
Signatures of metabolites	
Higher levels of acetyl-ornithine, alpha-aminoadipic acid, carnosine, PC (16:1/0:0)	Higher levels of acylcarnitine (C5-)
Immune system activity	
• Acute injection of lipopolysaccharide (LPS) caused after 24h stronger body weight decline and decrease in body temperature • Acute LPS increased biosynthesis of CNS neuroprotector putrescine • Acute LPS induced stronger upregulation of MHC-I-pathway-related components in the brain	Influenza virus infection caused severe symptoms: hunched posture, reduced movement, laboured breathing, marked weight loss, higher mortality
Effect of amphetamine	
• Greater amphetamine-stimulated locomotor activity after acute amphetamine administration • Stronger amphetamine-induced striatal dopamine efflux	• Repeated amphetamine caused significantly greater sensitization to motor activity • Repeated amphetamine divided mice into two subgroups by the locomotor activity: strong and weak responders • Repeated amphetamine caused larger variation in metabolites
Neurotransmission	
• Only mouse species where MAOB activity is greater in the brain than in the liver • Tendency for higher levels of EGF family proteins in the frontal cortex (EGF, ERBB1, NRG2) • Higher expression of dopamine metabolising gene *Maob* in the brain structures (hippocampus, dorsal striatum) • Enhanced dopaminergic activity 	• Higher expression of EGF family genes in the brain • Significantly reduced Negr1 gene expression in the ventral striatum 

Figure 23. Concluding remarks for Bl6 and 129Sv strains.

7. CONCLUSIONS

1. We established the different adaptation strategies of Bl6 and 129Sv mouse lines in the challenging stressful environment. The active stress coping prevailed in Bl6 mice, whereas in 129Sv mice stress coping was passive. Besides that, we established strain-specific metabolite concentrations that stayed the same regardless of stressful intervention. The metabolite level specific for 129Sv was acylcarnitine C5-, whereas in Bl6 mice these metabolites included acetyl-ornithine, alpha-aminoadipic acid, carnosine and lysophosphatidylcholine PC(16:1/0:0). In addition, there was a clear difference in the weight change of the strains under different conditions. In stressful conditions 129Sv mice differently from the Bl6 strain significantly lost body weight but showed more pronounced weight gain in home cages compared to Bl6.
2. The acute administration of dopamine agonist amphetamine caused significantly stronger stimulation of locomotor activity in Bl6 mice compared to the 129Sv strain. However, we observed the different sensitization of mouse strains toward repeated treatments with amphetamine: it was moderate in Bl6 strain compared to acute administration. By contrast, we can divide 129Sv mice into two subgroups by their locomotor response to repeated amphetamine: strong and weak responders. The impact of genetic background of mice on metabolite levels significantly exceeded that induced by pharmacological treatment with amphetamine. Besides that, the 129Sv strain displayed a significantly larger variation of the metabolite levels after repeated treatments than Bl6.
3. The findings of behavioural and metabolite studies concerning differences of two mouse strains were extended to the brain gene expression of three large neurotransmitter/neuromodulator systems: dopamine system, NMDA receptors and EGF family. We established that not only inhibited activity of the dopamine system, but also reduced activity of EGF family and NMDA receptor signalling in the frontal cortex underlies higher susceptibility of 129Sv mice to the environmental stress.
4. The present study suggests that 129Sv and Bl6 strains display different potential for modelling neuropsychiatric disorders. It is likely that the 129Sv (129S6/SvEv) strain can be used to model depression- and psychosis-like states, while Bl6 (C57BL/6N) appears to be a more suitable strain for studying drug addiction.

REFERENCES

Abramov, U., Puussaar, T., Raud, S., Kurrikoff, K., and Vasar, E. (2008). Behavioural differences between C57BL/6 and 129S6/SvEv strains are reinforced by environmental enrichment. *Neuroscience Letters* 443, 223–227. doi: 10.1016/j.neulet.2008.07.075.

Appaji Rao, N., Ambili, M., Jala, V. R., Subramanya, H. S., and Savithri, H. S. (2003). Structure–function relationship in serine hydroxymethyltransferase. *Biochimica et Biophysica Acta (BBA) – Proteins and Proteomics* 1647, 24–29. doi: 10.1016/S1570-9639(03)00043-8.

Badiani, A., Cabib, S., and Puglisi-Allegra, S. (1992). Chronic stress induces strain-dependent sensitization to the behavioral effects of amphetamine in the mouse. *Pharmacology Biochemistry and Behavior* 43, 53–60. doi: 10.1016/0091-3057(92)90638-V.

Bae, O.-N., and Majid, A. (2013). Role of histidine/histamine in carnosine-induced neuroprotection during ischemic brain damage. *Brain Research* 1527, 246–254. doi: 10.1016/j.brainres.2013.07.004.

Bailey, K. R., Rustay, N. R., and Crawley, J. N. (2006). Behavioral Phenotyping of Transgenic and Knockout Mice: Practical Concerns and Potential Pitfalls. *ILAR Journal* 47, 124–131. doi: 10.1093/ilar.47.2.124.

Ball, C. B., Rodriguez, K. F., Stumpo, D. J., Ribeiro-Neto, F., Korach, K. S., Blackshear, P. J., et al. (2014). The RNA-Binding Protein, ZFP36L2, Influences Ovulation and Oocyte Maturation. *PLoS ONE* 9, e97324. doi: 10.1371/journal.pone.0097324.

Belknap, J. K., Crabbe, J. C., and Young, E. R. (1993). Voluntary consumption of ethanol in 15 inbred mouse strains. *Psychopharmacology* 112, 503–510. doi: 10.1007/BF02244901.

Belknap, J. K., Lamé, M., and Danielson, P. W. (1990). Inbred strain differences in morphine-induced analgesia with the hot plate assay: A reassessment. *Behav Genet* 20, 333–338. doi: 10.1007/BF01067800.

Bolivar, V. J., Caldarone, B. J., Reilly, A. A., and Flaherty, L. (2000). Habituation of activity in an open field: A survey of inbred strains and F1 hybrids. *Behav Genet* 30, 285–293. doi: 10.1023/a:1026545316455.

Bortolato, M., Chen, K., and Shih, J. C. (2008). Monoamine oxidase inactivation: From pathophysiology to therapeutics☆. *Advanced Drug Delivery Reviews* 60, 1527–1533. doi: 10.1016/j.addr.2008.06.002.

Bryant, C. D. (2011). The blessings and curses of C57BL/6 substrains in mouse genetic studies: Bryant. *Annals of the New York Academy of Sciences* 1245, 31–33. doi: 10.1111/j.1749-6632.2011.06325.x.

Bućan, M., and Abel, T. (2002). The mouse: genetics meets behaviour. *Nat Rev Genet* 3, 114–123. doi: 10.1038/nrg728.

Buchli, R., Alberati-Giani, D., Malherbe, P., Köhler, C., Broger, C., and Cesura, A. M. (1995). Cloning and Functional Expression of a Soluble Form of Kynurenine/α -Aminoadipate Aminotransferase from Rat Kidney. *Journal of Biological Chemistry* 270, 29330–29335. doi: 10.1074/jbc.270.49.29330.

Calabresi, P., Pisani, A., Mercuri, N. B., and Bernardi, G. (1996). The corticostriatal projection: from synaptic plasticity to dysfunctions of the basal ganglia. *Trends in Neurosciences* 19, 19–24. doi: 10.1016/0166-2236(96)81862-5.

Carboni, E., Tanda, G. L., Frau, R., and Chiara, G. D. (1990). Blockade of the Noradrenaline Carrier Increases Extracellular Dopamine Concentrations in the Prefrontal Cortex: Evidence that Dopamine Is Taken up In Vivo by Noradrenergic Terminals. *J Neurochem* 55, 1067–1070. doi: 10.1111/j.1471-4159.1990.tb04599.x.

Carland, J., Mansfield, R., Ryan, R., and Vandenberg, R. (2013). Oleoyl- L -carnitine inhibits glycine transport by GlyT2: Oleoyl- L -carnitine inhibition of glycine transport. *Br J Pharmacol* 168, 891–902. doi: 10.1111/j.1476-5381.2012.02213.x.

Chance, M. R. A. (1946). Aggregation as a factor influencing the toxicity of sympathomimetic amines in mice. *J Pharmacol Exp Ther* 87, 214–219.

Chang, Y.-F., Cauley, R. K., Chang, J.-D., and Rao, V. V. (1997). L-alpha-aminoadipate inhibits kynurenate synthesis in rat brain hippocampus and tissue culture. *Neurochemical Research* 22, 825–829. doi: 10.1023/A:1022035926832.

Chen, R., Zhang, M., Park, S., and Gnegy, M. E. (2007). C57BL/6J mice show greater amphetamine-induced locomotor activation and dopamine efflux in the striatum than 129S2/SvHsd mice. *Pharmacology Biochemistry and Behavior* 87, 158–163. doi: 10.1016/j.pbb.2007.04.012.

Chi Moa, Q., and Chen, J. C. (2018). Reduced Neuregulin 1 Expression in the Medial Prefrontal Cortex of a Rat Ketamine Model for Schizophrenia. *Neuropsychiatry* 07. doi: 10.4172/Neuropsychiatry.1000224.

Chubb, J. E., Bradshaw, N. J., Soares, D. C., Porteous, D. J., and Millar, J. K. (2008). The DISC locus in psychiatric illness. *Mol Psychiatry* 13, 36–64. doi: 10.1038/sj.mp.4002106.

Clapcote, S. J., and Roder, J. C. (2006). Deletion Polymorphism of *Disc1* Is Common to All 129 Mouse Substrains: Implications for Gene-Targeting Studies of Brain Function. *Genetics* 173, 2407–2410. doi: 10.1534/genetics.106.060749.

Clements, J. D., and Westbrook, G. L. (1991). Activation kinetics reveal the number of glutamate and glycine binding sites on the N-methyl-d-aspartate receptor. *Neuron* 7, 605–613. doi: 10.1016/0896-6273(91)90373-8.

Cole, L. K., Vance, J. E., and Vance, D. E. (2012). Phosphatidylcholine biosynthesis and lipoprotein metabolism. *Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids* 1821, 754–761. doi: 10.1016/j.bbalip.2011.09.009.

Contet, C., Rawlins, J. N. P., and Bannerman, D. M. (2001). Faster is not surer – a comparison of C57BL/6J and 129S2/Sv mouse strains in the watermaze. *Behavioural Brain Research* 125, 261–267. doi: 10.1016/S0166-4328(01)00295-9.

Crawley, J. N., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel, W., Henderson, N., et al. (1997). Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. *Psychopharmacology* 132, 107–124. doi: 10.1007/s002130050327.

Dahlström, A., and Fuxe, K. (1964). Localization of monoamines in the lower brain stem. *Experientia* 20, 398–399. doi: 10.1007/BF02147990.

Dahoun, T., Trossbach, S. V., Brandon, N. J., Korth, C., and Howes, O. D. (2017). The impact of Disrupted-in-Schizophrenia 1 (DISC1) on the dopaminergic system: a systematic review. *Transl Psychiatry* 7, e1015–e1015. doi: 10.1038/tp.2016.282.

Defaux, A., Zurich, M.-G., Honegger, P., and Monnet-Tschudi, F. (2010). Inflammatory responses in aggregating rat brain cell cultures subjected to different demyelinating conditions. *Brain Research* 1353, 213–224. doi: 10.1016/j.brainres.2010.07.016.

De Luca, V., Viggiano, E., Messina, G., Viggiano, A., Borlido, C., Viggiano, A., et al. (2008). Peripheral amino Acid levels in schizophrenia and antipsychotic treatment. *Psychiatry Investig* 5, 203–208. doi: 10.4306/pi.2008.5.4.203.

Dépatie, L., and Lal, S. (2001). Apomorphine and the dopamine hypothesis of schizophrenia: a dilemma? *J Psychiatry Neurosci* 26, 203–220.

Dockstader, C. L., and van der Kooy, D. (2001). Mouse strain differences in opiate reward learning are explained by differences in anxiety, not reward or learning. *J Neurosci* 21, 9077–9081.

Doetschman, T. (2009). "Influence of Genetic Background on Genetically Engineered Mouse Phenotypes," in *Gene Knockout Protocols* Methods in Molecular Biology., eds. W. Wurst and R. Kühn (Totowa, NJ: Humana Press), 423–433. doi: 10.1007/978-1-59745-471-1_23.

Easton, N., Steward, C., Marshall, F., Fone, K., and Marsden, C. (2007). Effects of amphetamine isomers, methylphenidate and atomoxetine on synaptosomal and synaptic vesicle accumulation and release of dopamine and noradrenaline in vitro in the rat brain. *Neuropharmacology* 52, 405–414. doi: 10.1016/j.neuropharm.2006.07.035.

Eiden, L. E., Schäfer, M. K.-H., Weihe, E., and Schütz, B. (2004). The vesicular amine transporter family (SLC18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamines and acetylcholine. *Pflügers Archiv European Journal of Physiology* 447, 636–640. doi: 10.1007/s00424-003-1100-5.

Ellenbroek, B., and Youn, J. (2016). Rodent models in neuroscience research: is it a rat race? *Disease Models & Mechanisms* 9, 1079–1087. doi: 10.1242/dmm.026120.

Esler, M., Jennings, G., Lambert, G., Meredith, I., Horne, M., and Eisenhofer, G. (1990). Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiological Reviews* 70, 963–985. doi: 10.1152/physrev.1990.70.4.963.

Farooqui, A. A., Horrocks, L. A., and Farooqui, T. (2000). Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. *Chem Phys Lipids* 106, 1–29. doi: 10.1016/s0009-3084(00)00128-6.

Featherstone, R. E., Rizos, Z., Kapur, S., and Fletcher, P. J. (2008). A sensitizing regimen of amphetamine that disrupts attentional set-shifting does not disrupt working or long-term memory. *Behavioural Brain Research* 189, 170–179. doi: 10.1016/j.bbr.2007.12.032.

Fernstrom, J. D. (1981). Dietary Precursors and Brain Neurotransmitter Formation. *Annu. Rev. Med.* 32, 413–425. doi: 10.1146/annurev.me.32.020181.002213.

Fernstrom, J. D., and Faller, D. V. (1978). Neutral amino acids in the brain: changes in response to food ingestion. *J Neurochem* 30, 1531–1538. doi: 10.1111/j.1471-4159.1978.tb10489.x.

Flanigan, M., and LeClair, K. (2017). Shared Motivational Functions of Ventral Striatum D1 and D2 Medium Spiny Neurons. *J. Neurosci.* 37, 6177–6179. doi: 10.1523/JNEUROSCI.0882-17.2017.

Flurkey, K. ed. (2009). *The Jackson laboratory handbook of genetically standardized mice: ask for the j*. 6. ed., 1. printing. Bar Harbor, Me: The Jackson Laboratory.

Fornai, F., Chen, K., Giorgi, F. S., Gesi, M., Alessandri, M. G., and Shih, J. C. (2002). Striatal Dopamine Metabolism in Monoamine Oxidase B-Deficient Mice : A Brain Dialysis Study. *Journal of Neurochemistry* 73, 2434–2440. doi: 10.1046/j.1471-4159.1999.0732434.x.

Franklin, K. B. J., and Paxinos, G. (2013). *Paxinos and Franklin's The mouse brain in stereotaxic coordinates*. Fourth edition. Amsterdam: Academic Press, an imprint of Elsevier.

Fuller, J. L. (1964). Measurement of alcohol preference in genetic experiments. *Journal of Comparative and Physiological Psychology* 57, 85–88. doi: 10.1037/h0043100.

Gallardo, C. M., Darvas, M., Oviatt, M., Chang, C. H., Michalik, M., Huddy, T. F., et al. (2014). Dopamine receptor 1 neurons in the dorsal striatum regulate food anticipatory circadian activity rhythms in mice. *Elife* 3, e03781. doi: 10.7554/eLife.03781.

Ganeshan, K., and Chawla, A. (2014). Metabolic Regulation of Immune Responses. *Annu. Rev. Immunol.* 32, 609–634. doi: 10.1146/annurev-immunol-032713-120236.

Ganeshan, K., Nikkanen, J., Man, K., Leong, Y. A., Sogawa, Y., Maschek, J. A., et al. (2019). Energetic Trade-Offs and Hypometabolic States Promote Disease Tolerance. *Cell* 177, 399-413.e12. doi: 10.1016/j.cell.2019.01.050.

Garani, R., Watts, J. J., and Mizrahi, R. (2021). Endocannabinoid system in psychotic and mood disorders, a review of human studies. *Prog Neuropsychopharmacol Biol Psychiatry* 106, 110096. doi: 10.1016/j.pnpbp.2020.110096.

Giesbertz, P., Ecker, J., Haag, A., Spanier, B., and Daniel, H. (2015). An LC-MS/MS method to quantify acylcarnitine species including isomeric and odd-numbered forms in plasma and tissues. *Journal of Lipid Research* 56, 2029–2039. doi: 10.1194/jlr.D061721.

Gill, K., Liu, Y., and Deitrich, R. A. (1996). Voluntary Alcohol Consumption in BXD Recombinant Inbred Mice: Relationship to Alcohol Metabolism. *Alcoholism Clin Exp Res* 20, 185–190. doi: 10.1111/j.1530-0277.1996.tb01063.x.

Giuffrida, A., Leweke, F. M., Gerth, C. W., Schreiber, D., Koethe, D., Faulhaber, J., et al. (2004). Cerebrospinal Anandamide Levels are Elevated in Acute Schizophrenia and are Inversely Correlated with Psychotic Symptoms. *Neuropsychopharmacol* 29, 2108–2114. doi: 10.1038/sj.npp.1300558.

Gooderham, P. A., Gagnon, R. F., and Gill, K. (2004). Attenuation of the alcohol preference of C57BL/6 mice during chronic renal failure. *Journal of Laboratory and Clinical Medicine* 143, 292–300. doi: 10.1016/j.lab.2004.01.010.

Gramsbergen, J. B. P., Hodgkins, P. S., Rassoulpour, A., Turski, W. A., Guidetti, P., and Schwarcz, R. (2002). Brain-Specific Modulation of Kynurenic Acid Synthesis in the Rat. *Journal of Neurochemistry* 69, 290–298. doi: 10.1046/j.1471-4159.1997.69010290.x.

Guénet, J. L. (2005). The mouse genome. *Genome Res.* 15, 1729–1740. doi: 10.1101/gr.3728305.

Hallen, A., Jamie, J. F., and Cooper, A. J. L. (2013). Lysine metabolism in mammalian brain: an update on the importance of recent discoveries. *Amino Acids* 45, 1249–1272. doi: 10.1007/s00726-013-1590-1.

Haller, J., Bakos, N., Rodriguiz, R. M., Caron, M. G., Wetsel, W. C., and Liposits, Z. (2002). Behavioral responses to social stress in noradrenaline transporter knockout mice: Effects on social behavior and depression. *Brain Research Bulletin* 58, 279–284. doi: 10.1016/S0361-9230(02)00789-X.

Ham, S., Kim, T. K., Chung, S., and Im, H.-I. (2017). Drug Abuse and Psychosis: New Insights into Drug-induced Psychosis. *Exp Neurobiol* 26, 11–24. doi: 10.5607/en.2017.26.1.11.

Hayes, L. N., Shevelkin, A., Zeledon, M., Steel, G., Chen, P.-L., Obie, C., et al. (2016). Neuregulin 3 Knockout Mice Exhibit Behaviors Consistent with Psychotic Disorders. *Mol Neuropsychiatry* 2, 79–87. doi: 10.1159/000445836.

Heal, D. J., Smith, S. L., Gosden, J., and Nutt, D. J. (2013). Amphetamine, past and present – a pharmacological and clinical perspective. *J Psychopharmacol* 27, 479–496. doi: 10.1177/0269881113482532.

Heikkila, R. E., Orlansky, H., Mytilineou, C., and Cohen, G. (1975). Amphetamine: evaluation of d- and l-isomers as releasing agents and uptake inhibitors for 3H-dopamine and 3H-norepinephrine in slices of rat neostriatum and cerebral cortex. *J Pharmacol Exp Ther* 194, 47–56.

Heinla, I., Leidmaa, E., Visnapuu, T., Philips, M.-A., and Vasar, E. (2014). Enrichment and individual housing reinforce the differences in aggressiveness and amphetamine response in 129S6/SvEv and C57BL/6 strains. *Behavioural Brain Research* 267, 66–73. doi: 10.1016/j.bbr.2014.03.024.

Helmeste, D. M., and Seeman, P. (1982). Amphetamine-induced hypolocomotion in mice with more brain D2 dopamine receptors. *Psychiatry Research* 7, 351–359. doi: 10.1016/0165-1781(82)90072-5.

Hernandes, M. S., and Troncone, L. R. P. (2009). Glycine as a neurotransmitter in the forebrain: a short review. *J Neural Transm* 116, 1551–1560. doi: 10.1007/s00702-009-0326-6.

Hodge, G. K., and Butcher, L. L. (1975). Catecholamine correlates of isolation-induced aggression in mice. *European Journal of Pharmacology* 31, 81–93. doi: 10.1016/0014-2999(75)90081-3.

Hoehn, R., and Lasagna, L. (1960). Effects of aggregation and temperature on amphetamine toxicity in mice. *Psychopharmacologia* 1, 210–220. doi: 10.1007/BF00402742.

Holmes, A., Murphy, D., and Crawley, J. (2002). Reduced aggression in mice lacking the serotonin transporter. *Psychopharmacology* 161, 160–167. doi: 10.1007/s00213-002-1024-3.

Holmes, E., Wilson, I. D., and Nicholson, J. K. (2008). Metabolic Phenotyping in Health and Disease. *Cell* 134, 714–717. doi: 10.1016/j.cell.2008.08.026.

Holmes, J. C., and Rutledge, C. O. (1976). Effects of the d- and l-isomers of amphetamine on uptake, release and catabolism of norepinephrine, dopamine and 5-hydroxytryptamine in several regions of rat brain. *Biochemical Pharmacology* 25, 447–451. doi: 10.1016/0006-2952(76)90348-8.

Huang, Y. H., Schäfer-Elinder, L., Wu, R., Claesson, H.-E., and Frostegård, J. (2001). Lysophosphatidylcholine (LPC) induces proinflammatory cytokines by a platelet-activating factor (PAF) receptor-dependent mechanism. *Clinical and Experimental Immunology* 116, 326–331. doi: 10.1046/j.1365-2249.1999.00871.x.

Ikeda, M., Okahisa, Y., Aleksic, B., Won, M., Kondo, N., Naruse, N., et al. (2013). Evidence for Shared Genetic Risk Between Methamphetamine-Induced Psychosis and Schizophrenia. *Neuropsychopharmacol* 38, 1864–1870. doi: 10.1038/npp.2013.94.

Imhasly, S., Naegeli, H., Baumann, S., von Bergen, M., Luch, A., Jungnickel, H., et al. (2014). Metabolomic biomarkers correlating with hepatic lipidosis in dairy cows. *BMC Vet Res* 10, 122. doi: 10.1186/1746-6148-10-122.

Innos, J., Leidmaa, E., Philips, M.-A., Sütt, S., Alttoa, A., Harro, J., et al. (2013). Lsamp–/– mice display lower sensitivity to amphetamine and have elevated 5-HT turnover. *Biochemical and Biophysical Research Communications* 430, 413–418. doi: 10.1016/j.bbrc.2012.11.077.

Inoue, H., Castagnoli, K., Van Der Schyf, C., Mabic, S., Igarashi, K., and Castagnoli, N. (1999). Species-dependent differences in monoamine oxidase A and B-catalyzed

oxidation of various C4 substituted 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridinyl derivatives. *J Pharmacol Exp Ther* 291, 856–864.

Insel, T. R. (2010). Rethinking schizophrenia. *Nature* 468, 187–193. doi: 10.1038/nature09552.

Iwakura, Y., and Nawa, H. (2013). ErbB1-4-dependent EGF/neuregulin signals and their cross talk in the central nervous system: pathological implications in schizophrenia and Parkinson's disease. *Front. Cell. Neurosci.* 7. doi: 10.3389/fncel.2013.00004.

Jiménez, E., Núñez, E., Ibáñez, I., Zafra, F., Aragón, C., and Giménez, C. (2015). Glycine transporters GlyT1 and GlyT2 are differentially modulated by glycogen synthase kinase 3β. *Neuropharmacology* 89, 245–254. doi: 10.1016/j.neuropharm.2014.09.023.

Johnson, J. W., and Ascher, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 325, 529–531. doi: 10.1038/325529a0.

Jones, L. L., McDonald, D. A., and Borum, P. R. (2010). Acylcarnitines: Role in brain. *Progress in Lipid Research* 49, 61–75. doi: 10.1016/j.plipres.2009.08.004.

Kaare, M., Mikheim, K., Lilleväli, K., Kilk, K., Jagomäe, T., Leidmaa, E., et al. (2021). High-Fat Diet Induces Pre-Diabetes and Distinct Sex-Specific Metabolic Alterations in Negr1-Deficient Mice. *Biomedicines* 9, 1148. doi: 10.3390/biomedicines9091148.

Kihara, A., Mitsutake, S., Mizutani, Y., and Igarashi, Y. (2007). Metabolism and biological functions of two phosphorylated sphingolipids, sphingosine 1-phosphate and ceramide 1-phosphate. *Progress in Lipid Research* 46, 126–144. doi: 10.1016/j.plipres.2007.03.001.

Kim, K., Mall, C., Taylor, S. L., Hitchcock, S., Zhang, C., Wettersten, H. I., et al. (2014). Mealtime, Temporal, and Daily Variability of the Human Urinary and Plasma Metabolomes in a Tightly Controlled Environment. *PLoS ONE* 9, e86223. doi: 10.1371/journal.pone.0086223.

Klebanov, G. I., Teselkin YuO, null, Babenkova, I. V., Lyubitsky, O. B., Rebrova OYu, null, Boldyrev, A. A., et al. (1998). Effect of carnosine and its components on free-radical reactions. *Membr Cell Biol* 12, 89–99.

Kobayashi, Y., Iwakura, Y., Sotoyama, H., Kitayama, E., Takei, N., Someya, T., et al. (2019). Clozapine-dependent inhibition of EGF/neuregulin receptor (ErbB) kinases. *Transl Psychiatry* 9, 181. doi: 10.1038/s41398-019-0519-1.

Koethe, D., Giuffrida, A., Schreiber, D., Hellmich, M., Schultze-Lutter, F., Ruhrmann, S., et al. (2009). Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. *Br J Psychiatry* 194, 371–372. doi: 10.1192/bjp.bp.108.053843.

Koido, K., Innos, J., Haring, L., Zilmer, M., Ottas, A., and Vasar, E. (2016). Taurine and Epidermal Growth Factor Belong to the Signature of First-Episode Psychosis. *Front. Neurosci.* 10. doi: 10.3389/fnins.2016.00331.

Koike, H., Arguello, P. A., Kvajo, M., Karayiorgou, M., and Gogos, J. A. (2006). Disc1 is mutated in the 129S6/SvEv strain and modulates working memory in mice. *Proc. Natl. Acad. Sci. U.S.A.* 103, 3693–3697. doi: 10.1073/pnas.0511189103.

Koob, G. F., and Nestler, E. J. (1997). The neurobiology of drug addiction. *J Neuropsychiatry Clin Neurosci* 9, 482–497. doi: 10.1176/jnp.9.3.482.

Kriisa, K., Leppik, L., Balõtšev, R., Ottas, A., Soomets, U., Koido, K., et al. (2017). Profiling of Acylcarnitines in First Episode Psychosis before and after Antipsychotic Treatment. *J. Proteome Res.* 16, 3558–3566. doi: 10.1021/acs.jproteome.7b00279.

Lahiri, S., and Futerman, A. H. (2007). The metabolism and function of sphingolipids and glycosphingolipids. *Cell. Mol. Life Sci.* 64, 2270–2284. doi: 10.1007/s00018-007-7076-0.

Lampis, V., Maziade, M., and Battaglia, M. (2011). Animal Models of Human Anxiety Disorders: Reappraisal From a Developmental Psychopathology Vantage Point: *Pediatric Research* 69, 77R-84R. doi: 10.1203/PDR.0b013e318212b42e.

Lang, A., Harro, J., Soosaar, A., Kõks, S., Volke, V., Oreland, L., et al. (1995). Role of N-methyl-d-aspartic acid and cholecystokinin receptors in apomorphine-induced aggressive behaviour in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* 351. doi: 10.1007/BF00169076.

Langen, M., Kas, M. J. H., Staal, W. G., van Engeland, H., and Durston, S. (2011). The neurobiology of repetitive behavior: Of mice…. *Neuroscience & Biobehavioral Reviews* 35, 345–355. doi: 10.1016/j.neubiorev.2010.02.004.

Leandro, J., Violante, S., Argmann, C. A., Hagen, J., Dodatko, T., Bender, A., et al. (2019). Mild inborn errors of metabolism in commonly used inbred mouse strains. *Molecular Genetics and Metabolism* 126, 388–396. doi: 10.1016/j.ymgme.2019.01.021.

Ledonne, A., and Mercuri, N. B. (2018). mGluR1-Dependent Long Term Depression in Rodent Midbrain Dopamine Neurons Is Regulated by Neuregulin 1/ErbB Signaling. *Front. Mol. Neurosci.* 11, 346. doi: 10.3389/fnmol.2018.00346.

Ledonne, A., and Mercuri, N. B. (2019). On the Modulatory Roles of Neuregulins/ErbB Signaling on Synaptic Plasticity. *IJMS* 21, 275. doi: 10.3390/ijms21010275.

Leiper, J., and Vallance, P. (2006). New Tricks From an Old Dog: Nitric Oxide-Independent Effects of Dimethylarginine Dimethylaminohydrolase. *ATVB* 26, 1419–1420. doi: 10.1161/01.ATV.0000229598.55602.17.

Leppik, L., Parksepp, M., Janno, S., Koido, K., Haring, L., Vasar, E., et al. (2020). Profiling of lipidomics before and after antipsychotic treatment in first-episode psychosis. *Eur Arch Psychiatry Clin Neurosci* 270, 59–70. doi: 10.1007/s00406-018-0971-6.

Levitt, P., Pintar, J. E., and Breakefield, X. O. (1982). Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Proc. Natl. Acad. Sci. U.S.A.* 79, 6385–6389. doi: 10.1073/pnas.79.20.6385.

Li, C.-T., Yang, K.-C., and Lin, W.-C. (2019). Glutamatergic Dysfunction and Glutamatergic Compounds for Major Psychiatric Disorders: Evidence From Clinical Neuroimaging Studies. *Front. Psychiatry* 9, 767. doi: 10.3389/fpsyt.2018.00767.

Linder, C. C., and Davisson, M. T. (2004). "Strains, Stocks, and Mutant Mice," in *The Laboratory Mouse* (Elsevier), 25–46. doi: 10.1016/B978-012336425-8/50056-X.

Liu, Y., Wu, X., Meng, J., Yao, J., and Wang, B. (2020). Functional Analysis of the 3′ Untranslated Region of the Human GRIN1 Gene in Regulating Gene Expression in vitro. *NDT* Volume 16, 2361–2370. doi: 10.2147/NDT.S268753.

Liu, Y.-P., Tung, C.-S., Lin, P.-J., and Wan, F.-J. (2011). Role of nitric oxide in amphetamine-induced sensitization of schedule-induced polydipsic rats. *Psychopharmacology* 218, 599–608. doi: 10.1007/s00213-011-2354-9.

Lohr, K. M., Chen, M., Hoffman, C. A., McDaniel, M. J., Stout, K. A., Dunn, A. R., et al. (2016). Vesicular Monoamine Transporter 2 (VMAT2) Level Regulates MPTP Vulnerability and Clearance of Excess Dopamine in Mouse Striatal Terminals. *Toxicol. Sci.* 153, 79–88. doi: 10.1093/toxsci/kfw106.

Lu, H.-C., and Mackie, K. (2016). An Introduction to the Endogenous Cannabinoid System. *Biol Psychiatry* 79, 516–525. doi: 10.1016/j.biopsych.2015.07.028.

MacDonald, K., Krishnan, A., Cervenka, E., Hu, G., Guadagno, E., and Trakadis, Y. (2019). Biomarkers for major depressive and bipolar disorders using metabolomics: A systematic review. *Am. J. Med. Genet.* 180, 122–137. doi: 10.1002/ajmg.b.32680.

Makrecka-Kuka, M., Sevostjanovs, E., Vilks, K., Volska, K., Antone, U., Kuka, J., et al. (2017). Plasma acylcarnitine concentrations reflect the acylcarnitine profile in cardiac tissues. *Sci Rep* 7, 17528. doi: 10.1038/s41598-017-17797-x.

Matsumoto, T., Kobayashi, T., and Kamata, K. (2007). Role of Lysophosphatidylcholine (LPC) in Atherosclerosis. *CMC* 14, 3209–3220. doi: 10.2174/092986707782793899.

Maycox, P. R., Hell, J. W., and Jahn, R. (1990). Amino acid neurotransmission: spotlight on synaptic vesicles. *Trends in Neurosciences* 13, 83–87. doi: 10.1016/0166-2236(90)90178-D.

McCLEARN, G. E., Bennett, E. L., Hebert, M., Kakihana, R., and Schlesinger, K. (1964). Alcohol Dehydrogenase Activity and Previous Ethanol Consumption in Mice. *Nature* 203, 793–794. doi: 10.1038/203793a0.

McCormack, S. E., Shaham, O., McCarthy, M. A., Deik, A. A., Wang, T. J., Gerszten, R. E., et al. (2013). Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents: Branched-chain amino acids and IR in children. *Pediatric Obesity* 8, 52–61. doi: 10.1111/j.2047-6310.2012.00087.x.

Medina, S., Dominguez-Perles, R., Gil, J. I., Ferreres, F., and Gil-Izquierdo, A. (2014). Metabolomics and the Diagnosis of Human Diseases – A Guide to the Markers and Pathophysiological Pathways Affected. *CMC* 21, 823–848. doi: 10.2174/09298673206613119124056.

Merino, J., Leong, A., Liu, C.-T., Porneala, B., Walford, G. A., von Grotthuss, M., et al. (2018). Metabolomics insights into early type 2 diabetes pathogenesis and detection in individuals with normal fasting glucose. *Diabetologia* 61, 1315–1324. doi: 10.1007/s00125-018-4599-x.

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., and Caron, M. G. (1998). Dopamine Receptors: From Structure to Function. *Physiological Reviews* 78, 189–225. doi: 10.1152/physrev.1998.78.1.189.

Mizuno, M., Sotoyama, H., Namba, H., Shibuya, M., Eda, T., Wang, R., et al. (2013). ErbB inhibitors ameliorate behavioral impairments of an animal model for schizophrenia: implication of their dopamine-modulatory actions. *Transl Psychiatry* 3, e252–e252. doi: 10.1038/tp.2013.29.

Morilak, D. A., Barrera, G., Echevarria, D. J., Garcia, A. S., Hernandez, A., Ma, S., et al. (2005). Role of brain norepinephrine in the behavioral response to stress. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 29, 1214–1224. doi: 10.1016/j.pnpbp.2005.08.007.

Murphy, D. L., Li, Q., Engel, S., Wichems, C., Andrews, A., Lesch, K.-P., et al. (2001). Genetic perspectives on the serotonin transporter. *Brain Research Bulletin* 56, 487–494. doi: 10.1016/S0361-9230(01)00622-0.

Murray, R. M., Paparelli, A., Morrison, P. D., Marconi, A., and Di Forti, M. (2013). What can we learn about schizophrenia from studying the human model, drug-induced psychosis? *Am. J. Med. Genet.* 162, 661–670. doi: 10.1002/ajmg.b.32177.

Müller, C. P., Reichel, M., Mühle, C., Rhein, C., Gulbins, E., and Kornhuber, J. (2015). Brain membrane lipids in major depression and anxiety disorders. *Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids* 1851, 1052–1065. doi: 10.1016/j.bbalip.2014.12.014.

Narita, T., Naganuma, T., Sase, Y., and Kihara, A. (2016). Long-chain bases of sphingolipids are transported into cells via the acyl-CoA synthetases. *Sci Rep* 6, 25469. doi: 10.1038/srep25469.

Narvik, J., Vanaveski, T., Innos, J., Philips, M.-A., Ottas, A., Haring, L., et al. (2018). Metabolic profile associated with distinct behavioral coping strategies of 129Sv and Bl6 mice in repeated motility test. *Sci Rep* 8, 3405. doi: 10.1038/s41598-018-21752-9.

Nawa, H., Sotoyama, H., Iwakura, Y., Takei, N., and Namba, H. (2014). Neuropathologic Implication of Peripheral Neuregulin-1 and EGF Signals in Dopaminergic Dysfunction and Behavioral Deficits Relevant to Schizophrenia: Their Target Cells and Time Window. *BioMed Research International* 2014, 1–12. doi: 10.1155/2014/697935.

Nishi, E., Kume, N., Ueno, Y., Ochi, H., Moriwaki, H., and Kita, T. (1998). Lysophosphatidylcholine Enhances Cytokine-Induced Interferon Gamma Expression in Human T Lymphocytes. *Circulation Research* 83, 508–515. doi: 10.1161/01.RES.83.5.508.

Niwa, M., Cash-Padgett, T., Kubo, K.-I., Saito, A., Ishii, K., Sumitomo, A., et al. (2016). DISC1 a key molecular lead in psychiatry and neurodevelopment: No-More Disrupted-in-Schizophrenia 1. *Mol Psychiatry* 21, 1488–1489. doi: 10.1038/mp.2016.154.

Ogawa, S., Koga, N., Hattori, K., Matsuo, J., Ota, M., Hori, H., et al. (2018). Plasma amino acid profile in major depressive disorder: Analyses in two independent case-control sample sets. *Journal of Psychiatric Research* 96, 23–32. doi: 10.1016/j.jpsychires.2017.09.014.

Olejniczak, M., Kotowska-Zimmer, A., and Krzyzosiak, W. (2018). Stress-induced changes in miRNA biogenesis and functioning. *Cell. Mol. Life Sci.* 75, 177–191. doi: 10.1007/s00018-017-2591-0.

Pani, L., Porcella, A., and Gessa, G. L. (2000). The role of stress in the pathophysiology of the dopaminergic system. *Mol Psychiatry* 5, 14–21. doi: 10.1038/sj.mp.4000589.

Parksepp, M., Leppik, L., Koch, K., Uppin, K., Kangro, R., Haring, L., et al. (2020). Metabolomics approach revealed robust changes in amino acid and biogenic amine signatures in patients with schizophrenia in the early course of the disease. *Sci Rep* 10, 13983. doi: 10.1038/s41598-020-71014-w.

Parksepp, M., Haring, L., Kilk, K., Koch, K., Uppin, K., Kangro, R., et al. (2022). The Expanded Endocannabinoid System Contributes to Metabolic and Body Mass Shifts in First-Episode Schizophrenia: A 5-Year Follow-Up Study. *Biomedicines* 10, 243. doi: 10.3390/biomedicines10020243.

Peregrín-Alvarez, J. M., Sanford, C., and Parkinson, J. (2009). The conservation and evolutionary modularity of metabolism. *Genome Biol* 10, R63. doi: 10.1186/gb-2009-10-6-r63.

Phillips, T. J., Dickinson, S., and Burkhart-Kasch, S. (1994). Behavioral sensitization to drug stimulant effects in C57BL/6J and DBA/2J inbred mice. *Behavioral Neuroscience* 108, 789–803. doi: 10.1037/0735-7044.108.4.789.

Phillips, T. J., Kamens, H. M., and Wheeler, J. M. (2008). Behavioral genetic contributions to the study of addiction-related amphetamine effects. *Neuroscience & Biobehavioral Reviews* 32, 707–759. doi: 10.1016/j.neubiorev.2007.10.008.

Piirsalu, M., Chithanathan, K., Jayaram, M., Visnapuu, T., Lilleväli, K., Zilmer, M., et al. (2022). Lipopolysaccharide-Induced Strain-Specific Differences in Neuro-

inflammation and MHC-I Pathway Regulation in the Brains of Bl6 and 129Sv Mice. *Cells* 11, 1032. doi: 10.3390/cells11061032.

Piirsalu, M., Taalberg, E., Lilleväli, K., Tian, L., Zilmer, M., and Vasar, E. (2020). Treatment With Lipopolysaccharide Induces Distinct Changes in Metabolite Profile and Body Weight in 129Sv and Bl6 Mouse Strains. *Front. Pharmacol.* 11, 371. doi: 10.3389/fphar.2020.00371.

Przedborski, S., Jackson-Lewis, V., Djaldetti, R., Liberatore, G., Vila, M., Vukosavic, S., et al. (2000). The parkinsonian toxin MPTP: action and mechanism. *Restor Neurol Neurosci* 16, 135–142.

Psychogios, N., Hau, D. D., Peng, J., Guo, A. C., Mandal, R., Bouatra, S., et al. (2011). The Human Serum Metabolome. *PLoS ONE* 6, e16957. doi: 10.1371/journal. pone.0016957.

Ralph, R. J., Paulus, M. P., and Geyer, M. A. (2001). Strain-specific effects of amphetamine on prepulse inhibition and patterns of locomotor behavior in mice. *J Pharmacol Exp Ther* 298, 148–155.

Ramstedt, B., and Slotte, J. P. (2002). Membrane properties of sphingomyelins. *FEBS Lett* 531, 33–37. doi: 10.1016/s0014-5793(02)03406-3.

Rao, M. L., Gross, G., Strebel, B., Bräunig, P., Huber, G., and Klosterkötter, J. (1990). Serum amino acids, central monoamines, and hormones in drug-naive, drug-free, and neuroleptic-treated schizophrenic patients and healthy subjects. *Psychiatry Research* 34, 243–257. doi: 10.1016/0165-1781(90)90003-N.

Raud, S., Sütt, S., Luuk, H., Plaas, M., Innos, J., Kõks, S., et al. (2009). Relation between increased anxiety and reduced expression of alpha1 and alpha2 subunits of GABAA receptors in Wfs1-deficient mice. *Neuroscience Letters* 460, 138–142. doi: 10.1016/j.neulet.2009.05.054.

Raud, S., Sütt, S., Plaas, M., Luuk, H., Innos, J., Philips, M.-A., et al. (2007). Cat odor exposure induces distinct changes in the exploratory behavior and Wfs1 gene expression in C57Bl/6 and 129Sv mice. *Neuroscience Letters* 426, 87–90. doi: 10.1016/j.neulet.2007.08.052.

Riederer, P., Konradi, C., Schay, V., Kienzl, E., Birkmayer, G., Danielczyk, W., et al. (1987). Localization of MAO-A and MAO-B in human brain: a step in understanding the therapeutic action of L-deprenyl. *Adv Neurol* 45, 111–118.

Robertson, S. D., Matthies, H. J. G., and Galli, A. (2009). A Closer Look at Amphetamine-Induced Reverse Transport and Trafficking of the Dopamine and Norepinephrine Transporters. *Mol Neurobiol* 39, 73–80. doi: 10.1007/s12035-009-8053-4.

Robinson, D. S. (1977). Monoamine Metabolism in Human Brain. *Arch Gen Psychiatry* 34, 89. doi: 10.1001/archpsyc.1977.01770130091009.

Rothman, R. B., and Baumann, M. H. (2003). Monoamine transporters and psychostimulant drugs. *European Journal of Pharmacology* 479, 23–40. doi: 10.1016/j. ejphar.2003.08.054.

Rothman, R. B., Baumann, M. H., Dersch, C. M., Romero, D. V., Rice, K. C., Carroll, F. I., et al. (2001). Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse* 39, 32–41. doi: 10.1002/1098-2396(20010101)39:1<32::AID-SYN5>3.0.CO;2-3.

Russig, H. (2002). Clozapine and Haloperidol Reinstate Latent Inhibition Following its Disruption during Amphetamine Withdrawal. *Neuropsychopharmacology* 26, 765–777. doi: 10.1016/S0893-133X(01)00422-5.

Russig, H., Murphy, C. A., and Feldon, J. (2003). Prepulse inhibition during withdrawal from an escalating dosage schedule of amphetamine. *Psychopharmacology* 169, 340–353. doi: 10.1007/s00213-002-1254-4.

Ryan, M. C. M., Collins, P., and Thakore, J. H. (2003). Impaired Fasting Glucose Tolerance in First-Episode, Drug-Naive Patients With Schizophrenia. *AJP* 160, 284–289. doi: 10.1176/appi.ajp.160.2.284.

Saleem, S., Shaukat, F., Gul, A., Arooj, M., and Malik, A. (2017). Potential role of amino acids in pathogenesis of schizophrenia. *Int J Health Sci (Qassim)* 11, 63–68.

Santos, M. H. S. (1996). Biogenic amines: their importance in foods. *International Journal of Food Microbiology* 29, 213–231. doi: 10.1016/0168-1605(95)00032-1.

Sarna, J. (2000). The Dalila effect: C57BL6 mice barber whiskers by plucking. *Behavioural Brain Research* 108, 39–45. doi: 10.1016/S0166-4328(99)00137-0.

Schooneman, M. G., Vaz, F. M., Houten, S. M., and Soeters, M. R. (2013). Acylcarnitines. *Diabetes* 62, 1–8. doi: 10.2337/db12-0466.

Schwarcz, R., Bruno, J. P., Muchowski, P. J., and Wu, H.-Q. (2012). Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci* 13, 465–477. doi: 10.1038/nrn3257.

Sedelis, M., Hofele, K., Auburger, G. W., Morgan, S., Huston, J. P., and Schwarting, R. K. W. (2000). Evidence for resistance to MPTP in C57BL/6 × BALA/c F1 hybrids as compared with their progenitor strains: *NeuroReport* 11, 1093–1096. doi: 10.1097/00001756-200004070-00037.

Seiden, L. S., Sabol, K. E., and Ricaurte, G. A. (1993). Amphetamine: Effects on Catecholamine Systems and Behavior. *Annu. Rev. Pharmacol. Toxicol.* 33, 639–676. doi: 10.1146/annurev.pa.33.040193.003231.

Seong, E., Seasholtz, A. F., and Burmeister, M. (2002). Mouse models for psychiatric disorders. *Trends in Genetics* 18, 643–650. doi: 10.1016/S0168-9525(02)02807-X.

Shih, J. C., Chen, K., and Ridd, M. J. (1999). Role of MAO A and B in neurotransmitter metabolism and behavior. *Pol J Pharmacol* 51, 25–29.

Sibal, L., C Agarwal, S., D Home, P., and H Boger, R. (2010). The Role of Asymmetric Dimethylarginine (ADMA) in Endothelial Dysfunction and Cardiovascular Disease. *CCR* 6, 82–90. doi: 10.2174/157340310791162659.

Silva, A. J., Simpson, E. M., Takahashi, J. S., Lipp, H.-P., Nakanishi, S., Wehner, J. M., et al. (1997). Mutant Mice and Neuroscience: Recommendations Concerning Genetic Background. *Neuron* 19, 755–759. doi: 10.1016/S0896-6273(00)80958-7.

Simon, M. M., Greenaway, S., White, J. K., Fuchs, H., Gailus-Durner, V., Wells, S., et al. (2013). A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains. *Genome Biol* 14, R82. doi: 10.1186/gb-2013-14-7-r82.

Singh, M. (2014). Mood, food, and obesity. *Front. Psychol.* 5. doi: 10.3389/fpsyg.2014.00925.

Sitte, H. H., and Freissmuth, M. (2015). Amphetamines, new psychoactive drugs and the monoamine transporter cycle. *Trends in Pharmacological Sciences* 36, 41–50. doi: 10.1016/j.tips.2014.11.006.

Skirzewski, M., Cronin, M. E., Murphy, R., Fobbs, W., Kravitz, A. V., and Buonanno, A. (2020). ErbB4 Null Mice Display Altered Mesocorticolimbic and Nigrostriatal Dopamine Levels as well as Deficits in Cognitive and Motivational Behaviors. *eNeuro* 7, ENEURO.0395-19.2020. doi: 10.1523/ENEURO.0395-19.2020.

Solinas, M., Belujon, P., Fernagut, P. O., Jaber, M., and Thiriet, N. (2019). Dopamine and addiction: what have we learned from 40 years of research. *J Neural Transm* 126, 481–516. doi: 10.1007/s00702-018-1957-2.

Steeds, H., Carhart-Harris, R. L., and Stone, J. M. (2015). Drug models of schizophrenia. *Therapeutic Advances in Psychopharmacology* 5, 43–58. doi: 10.1177/2045125314557797.

Stefani, M. R., and Moghaddam, B. (2002). Effects of repeated treatment with amphetamine or phencyclidine on working memory in the rat. *Behavioural Brain Research* 134, 267–274. doi: 10.1016/S0166-4328(02)00040-2.

Stefansson, H., Petursson, H., Sigurdsson, E., Steinthorsdottir, V., Bjornsdottir, S., Sigmundsson, T., et al. (2002). Neuregulin 1 and Susceptibility to Schizophrenia. *The American Journal of Human Genetics* 71, 877–892. doi: 10.1086/342734.

Sudo, N. (2019). Biogenic Amines: Signals Between Commensal Microbiota and Gut Physiology. *Front. Endocrinol.* 10, 504. doi: 10.3389/fendo.2019.00504.

Sulzer, D., Chen, T., Lau, Y., Kristensen, H., Rayport, S., and Ewing, A. (1995). Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J. Neurosci.* 15, 4102–4108. doi: 10.1523/JNEUROSCI.15-05-04102.1995.

Szczypka, M. S., Rainey, M. A., Kim, D. S., Alaynick, W. A., Marck, B. T., Matsumoto, A. M., et al. (1999). Feeding behavior in dopamine-deficient mice. *Proceedings of the National Academy of Sciences* 96, 12138–12143. doi: 10.1073/pnas.96.21.12138.

Tang, Y., Ye, M., Du, Y., Qiu, X., Lv, X., Yang, W., et al. (2015). EGFR signaling upregulates surface expression of the GluN2B-containing NMDA receptor and contributes to long-term potentiation in the hippocampus. *Neuroscience* 304, 109–121. doi: 10.1016/j.neuroscience.2015.07.021.

Teng, L., Crooks, P. A., and Dwoskin, L. P. (1998). Lobeline displaces [3H]dihydrotetrabenazine binding and releases [3H]dopamine from rat striatal synaptic vesicles: comparison with d-amphetamine. *J Neurochem* 71, 258–265. doi: 10.1046/j.1471-4159.1998.71010258.x.

Tenn, C. C., Fletcher, P. J., and Kapur, S. (2003). Amphetamine-sensitized animals show a sensorimotor gating and neurochemical abnormality similar to that of schizophrenia. *Schizophrenia Research* 64, 103–114. doi: 10.1016/S0920-9964(03)00009-4.

Thomson, P. A., Duff, B., Blackwood, D. H. R., Romaniuk, L., Watson, A., Whalley, H. C., et al. (2016). Balanced translocation linked to psychiatric disorder, glutamate, and cortical structure/function. *npj Schizophr* 2, 16024. doi: 10.1038/npjschz.2016.24.

Tortorella, A., Monteleone, P., Fabrazzo, M., Viggiano, A., De Luca, B., and Maj, M. (2001). Plasma Concentrations of Amino Acids in Chronic Schizophrenics Treated with Clozapine. *Neuropsychobiology* 44, 167–171. doi: 10.1159/000054937.

Trossbach, S. V., Bader, V., Hecher, L., Pum, M. E., Masoud, S. T., Prikulis, I., et al. (2016). Misassembly of full-length Disrupted-in-Schizophrenia 1 protein is linked to altered dopamine homeostasis and behavioral deficits. *Mol Psychiatry* 21, 1561–1572. doi: 10.1038/mp.2015.194.

Tuboly, G., Tar, L., Bohar, Z., Safrany-Fark, A., Petrovszki, Z., Kekesi, G., et al. (2015). The inimitable kynurenic acid: The roles of different ionotropic receptors in the action of kynurenic acid at a spinal level. *Brain Research Bulletin* 112, 52–60. doi: 10.1016/j.brainresbull.2015.02.001.

Tuck, M. K., Chan, D. W., Chia, D., Godwin, A. K., Grizzle, W. E., Krueger, K. E., et al. (2009). Standard Operating Procedures for Serum and Plasma Collection: Early

Detection Research Network Consensus Statement *Standard Operating Procedure Integration Working Group*. *J. Proteome Res.* 8, 113–117. doi: 10.1021/pr800545q.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., et al. (2012). Primer3 – new capabilities and interfaces. *Nucleic Acids Research* 40, e115–e115. doi: 10.1093/nar/gks596.

Vogel, C., and Marcotte, E. M. (2012). Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat Rev Genet* 13, 227–232. doi: 10.1038/nrg3185.

Võikar, V., and Gaburro, S. (2020). Three Pillars of Automated Home-Cage Phenotyping of Mice: Novel Findings, Refinement, and Reproducibility Based on Literature and Experience. *Front. Behav. Neurosci.* 14, 575434. doi: 10.3389/fnbeh.2020.575434.

Võikar, V., Kõks, S., Vasar, E., and Rauvala, H. (2001). Strain and gender differences in the behavior of mouse lines commonly used in transgenic studies. *Physiology & Behavior* 72, 271–281. doi: 10.1016/S0031-9384(00)00405-4.

Võikar, V., Soosaar, A., Volke, V., Kõks, S., Bourin, M., Männistö, P. T., et al. (1999). Apomorphine-induced behavioural sensitization in rats: individual differences, role of dopamine and NMDA receptors. *European Neuropsychopharmacology* 9, 507–514. doi: 10.1016/S0924-977X(99)00038-3.

Wang, L. P., Li, F., Wang, D., Xie, K., Wang, D., Shen, X., et al. (2011a). NMDA Receptors in Dopaminergic Neurons Are Crucial for Habit Learning. *Neuron* 72, 1055–1066. doi: 10.1016/j.neuron.2011.10.019.

Wang, T. J., Larson, M. G., Vasan, R. S., Cheng, S., Rhee, E. P., McCabe, E., et al. (2011b). Metabolite profiles and the risk of developing diabetes. *Nat Med* 17, 448–453. doi: 10.1038/nm.2307.

Wang, T. J., Ngo, D., Psychogios, N., Dejam, A., Larson, M. G., Vasan, R. S., et al. (2013). 2-Aminoadipic acid is a biomarker for diabetes risk. *J. Clin. Invest.* 123, 4309–4317. doi: 10.1172/JCI64801.

Weinshenker, D., Miller, N. S., Blizinsky, K., Laughlin, M. L., and Palmiter, R. D. (2002). Mice with chronic norepinephrine deficiency resemble amphetamine-sensitized animals. *Proceedings of the National Academy of Sciences* 99, 13873–13877. doi: 10.1073/pnas.212519999.

Westlund, K., Denney, R., Kochersperger, L., Rose, R., and Abell, C. (1985). Distinct monoamine oxidase A and B populations in primate brain. *Science* 230, 181–183. doi: 10.1126/science.3875898.

Wied, C. C. G., and Jansen, L. M. C. (2002). The stress-vulnerability hypothesis in psychotic disorders: Focus on the stress response systems. *Curr Psychiatry Rep* 4, 166–170. doi: 10.1007/s11920-002-0022-9.

Wieduwilt, M. J., and Moasser, M. M. (2008). The epidermal growth factor receptor family: Biology driving targeted therapeutics. *Cell. Mol. Life Sci.* 65, 1566–1584. doi: 10.1007/s00018-008-7440-8.

Winslow, J. T., and Miczek, K. A. (1983). Habituation of aggression in mice: Pharmacological evidence of catecholaminergic and serotonergic mediation. *Psychopharmacology* 81, 286–291. doi: 10.1007/BF00427564.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nat Rev Neurosci* 5, 483–494. doi: 10.1038/nrn1406.

Wishart, D. S. (2016). Emerging applications of metabolomics in drug discovery and precision medicine. *Nat Rev Drug Discov* 15, 473–484. doi: 10.1038/nrd.2016.32.

Wolfer, D. P., Crusio, W. E., and Lipp, H.-P. (2002). Knockout mice: simple solutions to the problems of genetic background and flanking genes. *Trends in Neurosciences* 25, 336–340. doi: 10.1016/S0166-2236(02)02192-6.

Wu, H.-Q., Ungerstedt, U., and Schwarcz, R. (1995). l-α-Aminoadipic acid as a regulator of kynurenic acid production in the hippocampus: a microdialysis study in freely moving rats. *European Journal of Pharmacology* 281, 55–61. doi: 10.1016/0014-2999(95)00224-9.

Xu, F., Gainetdinov, R. R., Wetsel, W. C., Jones, S. R., Bohn, L. M., Miller, G. W., et al. (2000). Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. *Nat Neurosci* 3, 465–471. doi: 10.1038/74839.

Xuan, C., Lun, L.-M., Zhao, J.-X., Wang, H.-W., Wang, J., Ning, C.-P., et al. (2015). L-citrulline for protection of endothelial function from ADMA–induced injury in porcine coronary artery. *Sci Rep* 5, 10987. doi: 10.1038/srep10987.

Yan, L., Shamir, A., Skirzewski, M., Leiva-Salcedo, E., Kwon, O. B., Karavanova, I., et al. (2018). Neuregulin-2 ablation results in dopamine dysregulation and severe behavioral phenotypes relevant to psychiatric disorders. *Mol Psychiatry* 23, 1233–1243. doi: 10.1038/mp.2017.22.

Yasuda, K., Hayashi, Y., Yoshida, T., Kashiwagi, M., Nakagawa, N., Michikawa, T., et al. (2017). Schizophrenia-like phenotypes in mice with NMDA receptor ablation in intralaminar thalamic nucleus cells and gene therapy-based reversal in adults. *Transl Psychiatry* 7, e1047–e1047. doi: 10.1038/tp.2017.19.

Yoshiki, A., and Moriwaki, K. (2006). Mouse Phenome Research: Implications of Genetic Background. *ILAR Journal* 47, 94–102. doi: 10.1093/ilar.47.2.94.

Youdim, M. B. H., Edmondson, D., and Tipton, K. F. (2006). The therapeutic potential of monoamine oxidase inhibitors. *Nat Rev Neurosci* 7, 295–309. doi: 10.1038/nrn1883.

Yuan, W., Zhang, J., Li, S., and Edwards, J. L. (2011). Amine Metabolomics of Hyperglycemic Endothelial Cells using Capillary LC–MS with Isobaric Tagging. *J. Proteome Res.* 10, 5242–5250. doi: 10.1021/pr200815c.

Zeitoun-Ghandour, S., Leszczyszyn, O. I., Blindauer, C. A., Geier, F. M., Bundy, J. G., and Stürzenbaum, S. R. (2011). C. elegans metallothioneins: response to and defence against ROS toxicity. *Mol. BioSyst.* 7, 2397. doi: 10.1039/c1mb05114h.

Zocchi, A., Orsini, C., Cabib, S., and Puglisi-Allegra, S. (1997). Parallel strain-dependent effect of amphetamine on locomotor activity and dopamine release in the nucleus accumbens: an in vivo study in mice. *Neuroscience* 82, 521–528. doi: 10.1016/S0306-4522(97)00276-5.

Zutphen, L. F. M. van, Baumans, V., and Beynen, A. C. eds. (2001). *Principles of laboratory animal science: a contribution to the humane use and care of animals and to the quality of experimental results*. Rev. ed. Amsterdam; New York: Elsevier.

SUMMARY IN ESTONIAN

129Sv ja C57/Bl6 hiireliinide erinevad stressiga toimetuleku strateegiad – viited käitumuslikest, farmakoloogilistest, metaboloomika ja geenikespressiooni uuringutest

Prekliinilistes uuringutes on kõige laialdasemalt kasutatavaks mudelorganismiks laborihiir (*Mus musculus domesticus*). Põhjuseks on asjaolu, et nad on inimestega nii geneetiliselt kui ka bioloogiliselt piisavalt sarnased. Kõige levinumateks hiireliinideks biomeditsiinilistes rakendustes on C57/Bl6 (Bl6) ja 129Sv ning nende liinide käitumuslik repertuaar on üpriski erinev. Bl6 hiired on palju aktiivsemad, uudishimulikud ja kohanevad uues keskkonnas paremini, kuid 129Sv hiired on passiivsemad ja stressiolukordades oluliselt haavatavamad. Selleks, et mõista uuritavate hiireliinide sobivust mudelorganismidena psühhiaatriliste haiguste uurimiseks, võrreldi käesolevas doktoritöös Bl6 ja 129Sv hiireliinide käitumist, metaboloomika näitajaid, amfetamiini mõju käitumisele ning stressi mõju geeni- ja valgueksрressiooni muutustele ajustruktuurides.

Doktoritöö esimeses artiklis vaadeldi Bl6 (C57BL/6NTac) ja 129Sv (129S6/SvEvTac) hiiri kahes erinevas keskkonnas: kodupuuris ning korduva motoorse aktiivsuse hindamise testis. Seda selleks, et paremini mõista, millised käitumuslikud ja molekulaarsed muutused tulenevad hiireliinide geneetilisest taustast ja millised on seotud stressiga. Uurimistööst selgus, et kodupuuri 129Sv hiired võtsid kehakaalus oluliselt rohkem juurde, võrreldes samas keskkonnas olnud Bl6 hiirtega. Motoorse testi keskkonnas viibimise tulemused näitasid seevastu vastupidist tulemust – 129Sv hiired kaotasid oluliselt kehakaalu, aga Bl6 hiirtel ei muutunud see peaaegu üldse. Motoorse aktiivsuse testis oli Bl6 hiireliini liikumisaktiivsus, võrreldes 129Sv hiirtega, märkimisväärselt suurem. Silmapaistvaks iseärasuseks oli Bl6 hiireliinil tagakäppdele tõusmiste pidev katsest katsesse suurenemine, mis viitab aktiivsele kohanemisele keskkonnas. Seejärel võrreldi kahes keskkonnas tegutsevate hiirte metaboloomika näitajaid. Vereseerumist hinnati Biocrates kitti kasutades 180 erinevat metaboliiti ja lisaks metaboliitide omavahelisi suhteid. Töö käigus tuvastati mõlemale hiireliinile iseloomulikud metaboliidid, mis seoses stressiga ei muutunud. Bl6 hiirtel olid nendeks metaboliitideks biogeensed amiinid atsetüül-ornitiin, karnosiin, alfa-aminoadipaat ja glütserofosfolipiid PC (16:1/0:0). 129Sv hiirtel näitas püsivalt kõrgemat taset lühikeseahelaline atsüülkarnitiin C5-. Kokkuvõttes näitas uurimustöö, et Bl6 hiirtel on motoorse testiga kohanemisel eelkõige aktiveerunud glükoosiga seotud metabolismirajad, kuid 129Sv hiirtel domineeris lipiidide metabolism.

Teises artiklis käsitleti ühekordse ja korduva dopamiini agonisti, amfetamiini, manustamise mõju Bl6 ja 129Sv hiireliinide käitumisele ning metaboloomika näitajatele. Korduvat amfetamiini manustamist rakendatakse prekliinilistes uuringutes sageli psühhootiliste seisundite alusmehhanismide uurimise vahendina. Mõlemad hiireliinid jaotati kolme manustamise rühma: kontrolli

grupp (manustati füsioloogilist lahust), akuutse manustamise grupp (3 mg/kg amfetamiini ühekordselt) ja korduva amfetamiini manustamise grupp (3 mg/kg amfetamiini igapäevaselt, 11 päeva vältel). Kõik loomad läbisid peale süstelahuse saamist igapäevaselt liikumisaktiivsuse hindamise testi. Testi tulemusel leiti, et Bl6 hiired, kes said amfetamiini akuutselt, liikusid võrreldes sedasama dopamiini agonisti saanud 129Sv hiirtega, palju rohkem. Korduva amfetamiini manustamine suurendas liikumisaktiivsust oluliselt enam 129Sv liinis. Küll mitte kõigil 129Sv hiirtel, sest pooltel grupi loomadel jäi läbitud distants samale tasemele akuutse manustamisega. Kehakaal langes katse perioodi jooksul kõikides 129Sv manustamise gruppides. Kuna kehakaal langes isegi füsioloogilist lahust saanutel, siis võib järeldada, et kaalu langus oli tingitud kohanemisest keskkonnaga, mitte aga amfetamiini toimega. Bl6 hiirtel kehakaalu vähenemist ei täheldatud. Metaboloomika tulemused näitasid, et akuutne amfetamiin manustamine põhjustas 129Sv hiirtel olulist hargnenud ahelaga aminohapete (BCAA) leutsiini ja isoleutsiini taseme tõusu, kuid Bl6 hiirtel täheldasime heksooside taseme langust. Korduva amfetamiini manustamine heksooside taset Bl6 hiirtel ei muutnud, see oli sarnane akuutse grupiga. Kuid korduva amfetamiini manustamise tagajärjel vähenes Bl6 hiirtel oluliselt kolme biogeense amiini tase (asümmeetrilise dimetüülarginiini ehk ADMA, alfa-aminoadipaadi ja kinureniini). Võrreldes Bl6 hiirtega, põhjustas amfetamiin 129Sv loomadel oluliselt suurema arvu metaboliitide tasemete muutusi. Näiteks akuutne amfetamiin vähendas BCAA valiini ja mitmete lüsofosfatidüülkoliinide (PC aa C34:2, PC aa C36:2, PC aa C36:3, PC aa C36:4, PC ae C38:4, PC ae C40:4) tasemeid. Lisaks põhjustas korduv amfetamiini manustamine võrreldes akuutse grupiga metaboliitide tasemetes veel väga erinevaid muutusi: isoleutsiini, pikaahelaliste atsüülkarnitiinide (C14, C14:1-OH, C16, C18:1), fosfatidüülkoliinide (PC aa C38:4, PC aa C38:6, PC aa C42:6, PC ae C38:4, PC ae C40:4, PC ae C40:5, PC ae C40:6, PC ae C42:1, PC ae C42:3) ja sfingolipiidide [SM(OH)C22:1, SM C24:0] tasemete arvestatavat suurenemist. Võrreldes füsioloogilist lahust saanud grupiga langetas korduva amfetamiini manustamine 129Sv liini hiirtel heksooside ja kinureniini tasemeid. Kirjeldatud metaboliitide muutused ilmselt peegeldavad olukorda, kus 129Sv hiired püüavad pärsitud glükoosi metabolismiradasid asendada lipiidide metabolismiga, sest korduv amfetamiin põhjustas olulise liikumisaktiivsuse tõusu tõttu suurenenud energiavajaduse. Kui Bl6 ja 129Sv hiireliine vaadeldi koos, siis selgus kaks korduva amfetamiini manustamisega seotud iseärasust. Nimelt olid isoleutsiin ja leutsiin need biomarkerid, mille suurenemist võib seostada amfetamiinist põhjustatud liikumisaktiivsuse järjekindla suurenemisega (sensitiseerumine amfetamiini suhtes), sest oli vaja leida vahendeid katmaks suuremat energiakulu. Teiseks, tsitrulliin, ADMA ja kinureniin on seotud glutamaatergilise N-metüül-D-aspartaadi (NMDA) ning lämmastiku monooksiidi (NO) signaaliradadega ja nende metaboliitide tasemete langus võib osutada silmapaistvatele muutustele nendes signaaliradades, mis on otseselt seotud sensitiseerumisega amfetamiini suhtes.

Kolmandas artiklis kirjeldati Bl6 ja 129Sv hiirte erinevat liikumisaktiivsust ning geeni ja valgu ekspressioonimustri uurimise abil ajustruktuurides püüti

mõista, millised molekulaarsed mehhanismid on eelkõige seotud liinide vahelise kohenemisvõime erinevustega. Katse oli ülesehituselt sarnane esimesele artiklile, kus võrreldi kahte hiireliini erinevates keskkondades. Uurimistöö tõestas, et Bl6 hiireliin on rohkem uudishimulik ja neil on suurem liikumisaktiivsus ning nad on paremad uues keskkonnas kohanejad. Kuna akuutne amfetamiin põhjustas Bl6 hiirtel oluliselt suuremat liikumisaktiivsust, siis geenieksрressiooni uuringutes püüti mõõta dopamiini signaaliradadega seotud transkriptide tasemeid, et näha, kas nendes ekspressiooni mustrites võib olla liinide vahel erinevusi. Tulemusena selgus, et dopamiini metabolismiga seotud geen *Maob* oli Bl6 hiireliini ajustruktuurides palju enam ekspresserunud kui 129Sv hiireliinil. *Maob* suurem ekspressioonitase oli hipokampuses ja dorsaalses juttkehas nii kodupuuri loomadel kui ka korduvas liikumisaktiivsuse testis. Võttes arvesse hiirte käitumuslikku reageerimist amfetamiinile ja geenieksрressiooni tulemusi, siis järeldati, et Bl6 hiirte ajustruktuurides on dopaminergilise süsteemi aktiivsus kõrgem kui 129Sv hiirtel. Hiireliinide vaheline erinevus dopaminergilise süsteemi aktiivsuses võib olla tingitud 129Sv hiireliinis esinevast *Disc1* geeni mutatsioonist. Epidermaalse kasvufaktori (Egf) perekonna geenieksрressiooni näitajad olid seevastu kõrgemad 129Sv liini hiirte ajustruktuurides. Statistiliselt oluline erinevus leiti motoorika testis olnud hiireliinide puhul otsmikukoores, kus suurenenud ekspressiooni 129Sv hiireliinil tuvastati nii *Egf-Erbb1* kui ka *Nrg1/Nrg2-Erbb4* signaaliradades. Valgu ekspressiooni uuringud viitavad aga asjaolule, et Bl6 hiireliini parem kohanemisvõime võib olla seotud EGF ja ERBB1 valkudega otsmikukoores, mille tasemed muutusid oluliselt kõrgemaks just korduva motoorika testi mõjul. Sarnast suurenemist Bl6 hiireliini otsmikukoores täheldati ka NMDA retseptori alaühiku GRIN1 puhul. Võib oletada, et need muutused valgu ekspressioonis tähendavad otsmikukoore-poolse kontrolli tugevnemist käitumist suunavate koorealuste struktuuride üle.

Käesolev doktoritöö annab ülevaate kahe hiireliini – 129Sv ja Bl6 – erinevustest, mis võimaldab teadlastel valida paremini konkreetse uurimisülesande lahendamiseks vajalikku liini. Kokkuvõttes võib väita, et Bl6 (C57BL/6N) hiireliin on sobivam uimastisõltuvuse mehhanismide selgitamisel ning 129Sv (129S6/SvEv) hiireliin pakub paremaid võimalusi depressiooni- ja psühhoosilaadsete seisundite selgitamiseks.

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PUBLICATIONS

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2019 Conference "Human genetics" Estonian Society of Human Genetics, Pärnu, Estonia
2022 Conference: 13th FENS Forum of Neuroscience, Paris, France

List of publications:

1. Vanaveski, T., Singh, K., **Narvik, J**., Eskla, K.-L., Visnapuu, T., Heinla, I., et al. (2017). Promoter-Specific Expression and Genomic Structure of IgLON Family Genes in Mouse. *Front Neurosci* 11, 38. doi:10.3389/fnins.2017.00038.
2. **Narvik, J**., Vanaveski, T., Innos, J., Philips, M.-A., Ottas, A., Haring, L., et al. (2018). Metabolic profile associated with distinct behavioral coping strategies of 129Sv and Bl6 mice in repeated motility test. *Sci Rep* 8, 3405. doi:10.1038/s41598-018-21752-9.
3. Singh, K., Lilleväli, K., Gilbert, S. F., Bregin, A., **Narvik, J**., Jayaram, M., et al. (2018). The combined impact of IgLON family proteins Lsamp and Neurotrimin on developing neurons and behavioral profiles in mouse. *Brain Res Bull* 140, 5–18. doi:10.1016/j.brainresbull.2018.03.013.
4. Vanaveski, T., **Narvik, J**., Innos, J., Philips, M.-A., Ottas, A., Plaas, M., et al. (2018). Repeated Administration of D-Amphetamine Induces Distinct Alterations in Behavior and Metabolite Levels in 129Sv and Bl6 Mouse Strains. *Front Neurosci* 12, 399. doi:10.3389/fnins.2018.00399.
5. Vanaveski, T., Molchanova, S., Pham, D. D., Schäfer, A., Pajanoja, C., **Narvik, J**., et al. (2021). PGC-1α Signaling Increases GABA(A) Receptor Subunit α2 Expression, GABAergic Neurotransmission and Anxiety-Like Behavior in Mice. *Front Mol Neurosci* 14, 588230. doi:10.3389/fnmol.2021.588230.
6. **Varul, J**., Eskla, K.-L., Piirsalu, M., Innos, J., Philips, M.-A., Visnapuu, T., et al. (2021). Dopamine System, NMDA Receptor and EGF Family Expressions in Brain Structures of Bl6 and 129Sv Strains Displaying Different Behavioral Adaptation. *Brain Sci* 11, 725. doi:10.3390/brainsci11060725.

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2017	Konverents “International Conference in Pharmacology: From Cellular Processes to Drug Targets”, Riia, Läti

2018 Protobios ja King's College London SZ Test mini symposium "Gene expression in health and disease", Tallinn, Eesti
2018 Seminar "Epigenetic regulation", Tartu Ülikool, Eesti
2018 Konverents: 11th FENS Forum of Neuroscience, Berliin, Saksamaa
2018 SZ_Test projekt, Quretec välislähetus, Helsingi Ülikool (sügissemester, Marie Skłodowska-Curie Fellowship)
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2019 Baltic Summer School on Behavioral Characterization of Rodent Models of Major Brain Disorders, Helsingi Ülikool, Pühajärve, Eesti
2019 NRSN Summer School on Animal Models in Neuroscience: Understanding the Behavior, Norwegian Research School in Neuroscience, Sommarøy, Norra
2019 Sümpoosium "Genetics and pediatric neurology", Tartu Ülikool, Eesti
2019 Konverents "Human genetics" Estonian Society of Human Genetics, Pärnu, Estonia
2022 Konverents: 13th FENS Forum of Neuroscience, Pariis, Prantsusmaa

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2. **Narvik, J**., Vanaveski, T., Innos, J., Philips, M.-A., Ottas, A., Haring, L., et al. (2018). Metabolic profile associated with distinct behavioral coping strategies of 129Sv and Bl6 mice in repeated motility test. *Sci Rep* 8, 3405. doi:10.1038/s41598-018-21752-9.
3. Singh, K., Lilleväli, K., Gilbert, S. F., Bregin, A., **Narvik, J**., Jayaram, M., et al. (2018). The combined impact of IgLON family proteins Lsamp and Neurotrimin on developing neurons and behavioral profiles in mouse. *Brain Res Bull* 140, 5–18. doi:10.1016/j.brainresbull.2018.03.013.
4. Vanaveski, T., **Narvik, J**., Innos, J., Philips, M.-A., Ottas, A., Plaas, M., et al. (2018). Repeated Administration of D-Amphetamine Induces Distinct Alterations in Behavior and Metabolite Levels in 129Sv and Bl6 Mouse Strains. *Front Neurosci* 12, 399. doi:10.3389/fnins.2018.00399.
5. Vanaveski, T., Molchanova, S., Pham, D. D., Schäfer, A., Pajanoja, C., **Narvik, J**., et al. (2021). PGC-1α Signaling Increases GABA(A) Receptor Subunit α2 Expression, GABAergic Neurotransmission and Anxiety-Like Behavior in Mice. *Front Mol Neurosci* 14, 588230. doi:10.3389/fnmol.2021.588230.
6. **Varul, J**., Eskla, K.-L., Piirsalu, M., Innos, J., Philips, M.-A., Visnapuu, T., et al. (2021). Dopamine System, NMDA Receptor and EGF Family Expressions in Brain Structures of Bl6 and 129Sv Strains Displaying Different Behavioral Adaptation. *Brain Sci* 11, 725. doi:10.3390/brainsci11060725.

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