

MARTEN VARES

Behavioural and neurobiological
adaptation of reward-related systems
to chronic stress in rats differing in
positive affectivity and hedonic capacity



MARTEN VARES

Behavioural and neurobiological adaptation
of reward-related systems to chronic stress
in rats differing in positive affectivity and
hedonic capacity



UNIVERSITY OF TARTU
Press

Department of Psychology, Faculty of Social Sciences, University of Tartu,
Estonia

This doctoral thesis was accepted for defending the degree of Doctor of
Philosophy (in Psychology) on 3 July 2026 by the Council of the Institute of
Psychology, Faculty of Social Sciences, University of Tartu.

Supervisors: Prof. Jaanus Harro MD, PhD
Department of Neuropsychopharmacology, Institute of
Chemistry, University of Tartu, Estonia
Dr. Kadri Kõiv, PhD
Institute of Genomics, University of Tartu, Estonia

Opponent: Prof. David Slattery, PhD
Group of Translational Psychiatry Klinik für Psychiatrie,
Psychosomatik und Psychotherapie Universitätsklinikum
Frankfurt

Commencement: 18 September 2026 at 11:00, Ravila 14A-1020, Tartu
(Chemicum)

ISSN 1024-3291 (print)
ISBN 978-9908-57-329-8 (print)
ISSN 2806-2531 (pdf)
ISBN 978-9908-57-330-4 (pdf)

Copyright: Marten Vares, 2026

University of Tartu Press
www.tyk.ee

“Kaua tehtud kaunikene”

Eesti vanasõna

CONTENTS

LIST OF ORIGINAL PUBLICATIONS	9
1. INTRODUCTION	10
1.1 Rat 50-kHz ultrasonic vocalisations as a measure of positive affective states	11
1.2 The architecture of reward: motivation, affect, and their divergence ..	12
1.3 Sucrose consumption as a model of hedonic response behaviour in rodents	13
1.4 Stress regulation and adaptive changes in the reward structures	14
2. AIMS	17
3. MATERIALS AND METHODS	18
3.1 Animals	18
3.2 Experimental design and general procedure	18
3.3 Tickling	21
3.4 Sucrose tests	21
3.5 Recording and analysis of 50-kHz ultrasonic vocalisations	22
3.6 Recording and analysis of amphetamine-elicited locomotor activity ..	22
3.7 Scoring of stereotyped behaviour	22
3.8 Chronic stress regimen	23
3.9 Pharmacological treatments	23
3.10 Behavioural testing	24
3.11 Biochemical assays	24
3.12 Statistical analysis	25
4. RESULTS AND DISCUSSION	26
4.1 Positive affectivity and psychostimulant reward expression under chronic stress	26
4.2 Introduction of sucrose consumption as a behavioural readout of reward sensitivity	30
4.3 Positive affectivity and hedonic capacity in adaptation to chronic stress	32
4.4 Biological correlates of phenotype-dependent stress adaptation	34
4.4.1 Monoaminergic regulation of reward-related adaptation to chronic stress	34
4.4.2 Monoamine regulation beyond the core reward system	35
4.4.3 Phenotype-dependent differences in amino acid homeostasis ..	36
5. CONCLUSIONS	39
5.1 Limitations	41
5.2 Future directions	41

ACKNOWLEDGEMENTS	44
REFERENCES	45
SUMMARY IN ESTONIAN	56
PUBLICATIONS	59
CURRICULUM VITAE	120
ELULOOKIRJELDUS.....	121

LIST OF ORIGINAL PUBLICATIONS

- I. Kõiv, K., Metelitsa, **M.**, Vares, M., Tiitsaar, K., Raudkivi, K., Jaako, K., Vulla, K., Shimmo, R. & Harro, J. (2016). Chronic variable stress prevents amphetamine-elicited 50-kHz calls in rats with low positive affectivity. *European Neuropsychopharmacology*, 26(4), 631–643.
<https://doi.org/10.1016/j.euroneuro.2016.02.011>
- II. **Vares, M.**, Metelitsa, M., Kõiv, K., Kaldmäe, M., Laugus, K., Tiitsaar, K., Shimmo, R. & Harro, J. (2018). Chronic stress sensitizes amphetamine-elicited 50-kHz calls in the rat: Dependence on positive affective phenotype and effects of long-term fluoxetine pretreatment. *Pharmacology Biochemistry and Behavior*, 171, 10–19.
<https://doi.org/10.1016/j.pbb.2018.05.014>
- III. Kõiv, K., **Vares, M.**, Kroon, C., Metelitsa, M., Tiitsaar, K., Laugus, K., Jaako, K. & Harro, J. (2019). Effect of chronic variable stress on sensitization to amphetamine in high and low sucrose-consuming rats. *Journal of Psychopharmacology*, 33(12), 1512–1523.
<https://doi.org/10.1177/0269881119856000>
- IV. **Vares, M.**, Kanarik, M., Kõiv, K., Vares, M., Anja, K., Metelitsa, M., Laugus, K., Tigro, H., Korulu, S., Shimmo, R. & Harro, J. (2025). Vulnerability to chronic stress in male rats: Additive effect of low positive affectivity and high hedonic response as measured by sucrose intake. *Behavioural Brain Research*, 494, 115749.
<https://doi.org/10.1016/j.bbr.2025.115749>

The author of the present dissertation contributed to the listed studies as follows:

- **Study I:** Participation in data collection and analysis, development of 50-kHz manual scoring method. Manual scoring of 50-kHz ultrasonic vocalisations (USV). Review and editing of the manuscript
- **Study II:** One of the main contributors in carrying out the experiment, data collection and subsequent analysis. First author in drafting the manuscript, review and editing of the manuscript
- **Study III.** Contributed in the data analysis, one of the main authors in drafting the original manuscript, review and editing of the manuscript
- **Study IV.** Contributed to planning and design of the experiment. Main contributor in carrying out the experiment, data collection and analysis. Main author in drafting the original manuscript, review and editing of the manuscript.

1. INTRODUCTION

Individuals differ in their behavioural and neurobiological responses to the same environment. In stress research this observation is central, because comparable stress exposure does not produce comparable outcomes across individuals. Some individuals maintain exploration and reward-directed behaviour after stress exposure, whereas others show behavioural inhibition or reduced reward responsiveness. Stress exposure may also produce divergent endocrine and physiological outcomes, including altered recovery of glucocorticoid-regulated systems. The mechanisms underlying such differences remain incompletely understood, but they are likely to involve pre-existing variation in affective, motivational, and stress-regulatory systems.

This thesis examines such variation through two related phenotypes: positive affectivity and hedonic capacity. Positive affectivity is used here in an affective-neuroscience sense, as individual variation in positive-valence affective responses that can be studied in animals through behavioural and neurobiological measures (Burgdorf et al., 2005, 2020; Mällo et al., 2009). Hedonic capacity is treated separately. It refers to the responsiveness to palatable or otherwise rewarding stimuli, commonly assessed in rodents through measures such as sucrose intake or preference (Brennan et al., 2001; Tõnissaar et al., 2006). These phenotypes overlap, but they should not be treated as equivalent. The distinction is important because reward is composed of separable processes (Berridge & Kringelbach, 2013; Salamone & Correa, 2024). Appetitive approach, incentive motivation, reward learning, and hedonic impact can change independently. In this scope, positive affectivity concerns the broader tendency to generate positive-valence responses, whereas hedonic capacity refers more specifically to reward consumption and pleasure-related responsiveness.

Stress engages neural and endocrine systems that are adaptive when their activation is acute and well regulated. As Rudolf Nesse (2002) argued, suffering and illness may arise not only from damaged biological mechanisms, but also from otherwise intact defences whose regulation is excessive or inappropriate in a given context. This view is directly relevant to stress-related disorders because defensive and metabolic responses that support survival during acute challenge may contribute to pathology when repeatedly or chronically activated. Chronic stress can alter reward-related behaviour, mesocorticolimbic dopamine function, hypothalamic-pituitary-adrenal axis regulation, and behavioural adaptation (Holly & Miczek, 2016; Ulrich-Lai & Herman, 2009). These effects, however, might not be uniform across individuals.

Exposure to stress is universal, but the ability to tolerate and recover from it varies greatly among individuals (Hamann & Canli, 2004). The present thesis asks whether pre-existing differences in positive affectivity and hedonic capacity contribute to this variability. The central premise is that stress vulnerability depends not only on the intensity or duration of stress exposure, but also on the affective and reward-related phenotype of the individual exposed to it. By

studying these phenotypes in rats, this thesis examines evolutionarily conserved components of mammalian positive-valence and reward systems that may influence behavioural outcomes after chronic stress.

1.1 Rat 50-kHz ultrasonic vocalisations as a measure of positive affective states

A valuable model for studying affective states in rodents originates from the mid-20th century discovery that rats emit ultrasonic vocalisations (USV) (Anderson, 1954). Their significance for affective neuroscience became much clearer only later, particularly through the studies of Knutson, Burgdorf, and Panksepp. This research group demonstrated that young rats emit high-frequency vocalisations in anticipation of playful behaviour (Knutson et al., 1998), with a subsequent proposition that rat ultrasonic vocalisations can serve as behavioural indices of affective states (Knutson et al., 2002). These observations established ultrasonic vocalisations as a useful tool for modelling affective processes in rodents and opened the way for later work on the structure and functional significance of different call types. Subsequent behavioural, pharmacological, and neurobiological work further supported the view that 50-kHz calls also function as communicative signals associated with positive affect and reward-related processes (Brudzynski, 2015).

Rat ultrasonic vocalisations are most commonly discussed in terms of two major sound frequency ranges, centred around 22- and 50-kilohertz (kHz), whereas vocalisations around 40 kHz are primarily described in maternal separation contexts. The 22-kHz calls are associated with aversive states and defensive behaviour, such as predatory exposure, social defeat in inter-male fighting, negative drug experience, and initial handling (Blanchard et al., 1991; Brudzynski & Ociepa, 1992; Burgdorf & Panksepp, 2001; Kroes et al., 2007; Litvin et al., 2007). The 50-kHz calls represent the most complex vocal system, encompassing a range of call types that vary in bandwidth, duration, and frequency modulation (Wright et al., 2010). Although the specific functions of these subtypes remain incompletely understood, 50-kHz calls are generally associated with positive affective and appetitive states. These chirp-like vocalisations occur in a wide range of rewarding contexts, including anticipation of play (Knutson et al., 1998), feeding (Opiol et al., 2015), mating (Barfield & Thomas, 1986), and chemical or electrical stimulation of reward-related structures in the central nervous system (Burgdorf et al., 2000, 2007; Simola et al., 2012). The “chirps” seem to serve as social calls, as rats self-administer the playback of 50-kHz USVs as well (Wöhr & Schwarting, 2007; Wöhr, 2017). In contrast to 22-kHz USVs, 50-kHz vocalisation is reduced by aversive stimuli (Burgdorf et al., 2008; Burgdorf & Panksepp, 2001; Mällo et al., 2009; Popik et al., 2012, 2014).

Rats differ substantially in the number of ultrasonic vocalisations they emit in response to affectively relevant stimuli (Mällo et al., 2007; Brenes & Schwarting, 2015), and this variability is also evident in the context of drug reward (Taracha

et al., 2012; Ahrens et al., 2013). Individual differences in USV activity appear to be stable, as rats can be selectively bred for high and low calling activity (Burgdorf et al., 2005), and these line differences persist across generations (Brunelli et al., 1997, 2006). Rats with a lower tendency to emit these calls also display more anxious behaviour in both spontaneous behavioural settings and standardised tests (Burgdorf et al., 2009; Harmon et al., 2008; Webber et al., 2012).

1.2 The architecture of reward: motivation, affect, and their divergence

Reward is a fundamental neurobiological function essential for motivation, learning, and survival. Broadly defined as stimuli that promote adaptive behaviour and sustainability, rewards are central to affective and motivational processes in both typical and pathological states. A landmark causal demonstration of brain reward emerged only in 1954, when Olds and Milner showed that rats would work to obtain electrical stimulation of specific brain sites, establishing that activation of neural circuitry could itself function as a positive reinforcer. Dopamine was subsequently linked to reward through the anhedonia hypothesis (Wise, 1978), which proposed that attenuation of dopaminergic function reduces the rewarding impact of normally reinforcing stimuli (Wise, 1980, 2008).

Contemporary paradigms view midbrain dopamine as orchestrating the motivational component of reward, with dopamine neurons in the ventral tegmental area (VTA) exhibiting phasic bursts of activity in response to unexpected primary rewards (Schultz et al., 1993, 2017). These bursts convey information that is separable from hedonic value (Berridge et al., 1989; Berridge & Kringelbach, 2013), and have been associated with motivational value and the incentive salience of reward (Berridge & Robinson, 1998; Bromberg-Martin et al., 2010). The reward response is supported by two major dopamine pathways. Projections from the VTA to the nucleus accumbens (NAcc), the mesolimbic pathway, shape motivational drive (Ikemoto & Panksepp, 1999; Salamone et al., 2003), whereas projections to the frontal cortex, the mesocortical pathway, support higher-order processes such as decision-making, contextual evaluation, and action selection (Seamans & Yang, 2004; Otis et al., 2017).

Affective and motivational components of reward substantially overlap within the broader reward network, most notably in the ventral striatum and amygdala, which helps explain why dissociating them *in vivo* is challenging (Yankouskaya et al., 2022). Many aspects of reward value remain incompletely understood. Dopamine is now widely considered a mediator of the motivational component of reward, whereas hedonic value is assigned within partly distinct neural systems (Bromberg-Martin et al., 2010; Salamone & Correa, 2024). Dopamine neurons in the VTA project to the NAcc, where opioid-sensitive sites in the medial shell act as a “hedonic hotspot”. Together with the reciprocally connected ventral pallidum, these structures can enhance the hedonic value of rewarding stimuli (Smith &

Berridge, 2005, 2007; Smith et al., 2009). Using related methods, several such hotspots have been identified, revealing a distributed hedonic network (Peciña et al., 2006; Berridge & Kringelbach, 2015).

Rat 50-kHz ultrasonic vocalisations have been consistently linked to affective expression of rewards (Wöhr & Schwarting, 2013; Brudzynski, 2015, 2021). These calls occur in conditioned pharmacological reward contexts and during playback self-administration, and can also emerge in operant contexts involving learned anticipation of food reward (Knutson et al., 1999; Burgdorf et al., 2008; Wardak et al., 2024). Experimental studies further indicate that 50-kHz calling can be induced by activation of both motivational and hedonic reward-related systems, suggesting that the neural control of these vocalisations extends beyond dopaminergic mechanisms alone (Burgdorf et al., 2007; Scardochio et al., 2015; Simola & Brudzynski, 2018).

1.3 Sucrose consumption as a model of hedonic response behaviour in rodents

Altered reward processing represents a core feature of numerous psychiatric conditions, particularly mood disorders (Gorwood, 2008; Huys et al., 2013). In preclinical research, reward-related behaviour in rodents is commonly assessed using palatable substances as proxies for natural rewards. Among these, sucrose – typically administered as a sugar solution – has become a standard tool for investigating reward sensitivity and hedonic response to natural reward in animal models (Willner et al., 1987; Primo et al., 2023). In its most widely used form, the sucrose preference test is carried out as a measure of stress-induced disturbances in reward processing. In this paradigm, rats are presented with two bottles, one containing a sucrose solution and the other plain water, and preference is calculated from relative intake over a defined testing period (Mällo et al., 2009; Liu et al., 2018). Healthy animals tend to show preference for sucrose over water, whereas a reduction in sucrose preference is commonly interpreted as an altered reward behaviour due to the effects of stress.

In the present work, however, sucrose-based assessment is used differently. Rather than focusing on sucrose preference as a state-sensitive measure of stress effects, repeated sucrose-consumption tests are used to characterise stable individual differences in baseline hedonic capacity. Repeated assessment of sucrose consumption indexes a stable behavioural tendency to choose and consume a palatable reward and thus reflects a more direct consummatory dimension of reward sensitivity that complements the more affect-related 50-kHz vocalisations.

In this approach, sucrose intake is assessed repeatedly under nondeprived conditions, with food and water freely available, in order to reduce the influence of transient metabolic and procedural factors. Studies using this protocol have shown that rats differ reliably in their baseline consumption of sucrose, and that animals with lower sucrose intake exhibit higher anxiety-like behaviour in

behavioural tests (Desousa et al., 1998; Tönissaar et al., 2006). Related work further shows that motivation for obtaining sucrose is associated with lower anxiety-like behaviour even in sated rats, supporting the view that sucrose-related responding reflects a broader behavioural trait rather than momentary caloric demand (Alsiö et al., 2009).

A series of studies further demonstrated that this individual variability extends to reward-related responsivity more broadly. Rats with higher and lower levels of sugar intake differ in their locomotor activity in response to acute and repeated amphetamine administration (Sills & Vaccarino, 1994). High sucrose feeders also show greater amphetamine-induced dopamine overflow in the nucleus accumbens (Sills & Crawley, 1996) and a greater increase in sucrose consumption after low-dose amphetamine administered systemically or into the nucleus accumbens (Sills & Vaccarino, 1996). Related findings with morphine suggest that this pattern is not limited to psychostimulants alone, but reflects broader individual differences in reward-related sensitivity (Sills & Vaccarino, 1998). High and low sucrose consumers further differ in dopamine D₂ receptor function (Tönissaar et al., 2006), as well as in gene expression in brain regions related to hedonic behaviour (Duncko et al., 2003). This interpretation is further supported by evidence that hedonic trait differences are associated with distinct neural and molecular responses to chronic social defeat stress, including variation in regional oxidative metabolism and gene expression in stress-susceptible and resilient animals (Kanarik et al., 2011).

1.4 Stress regulation and adaptive changes in the reward structures

Perceived threats typically elicit a stress response – an adaptive physiological reaction to a perceived threat to homeostasis. This process is a biologically embedded protective mechanism that supports the organism's immediate survival. Sterling and Eyer (1988) introduced the concept of allostasis to describe how organisms achieve stability through change, adjusting internal regulation to meet changing environmental demands. Each activation of stress-mediating systems imposes an allostatic load – the physiological cost of achieving stability through change (McEwen & Stellar, 1993; McEwen, 1998). Because restoring homeostatic balance is central to maintaining homeostasis, efficient termination of the stress response and subsequent recovery of affected systems are crucial. The stress response has evolved as a short-lived state in which substantial metabolic and cognitive resources are mobilised to address immediate danger. When such activation becomes repetitive or prolonged, however, the organism begins to accumulate allostatic overload – the defining feature of chronic stress (McEwen, 2004). In this dysregulated state, recovery is incomplete, adaptive adjustments become impaired, and vulnerability to pathology increases.

The stress response is the organism's rapid shift from everyday functioning to a state of heightened readiness. It begins with the way the brain interprets the

environment: sensory and emotional information converge in limbic regions – most notably the amygdala – where potential threat is detected and evaluated in relation to homeostatic significance (Ressler, 2010; Godoy et al., 2018). If the situation is judged as potentially harmful to homeostasis, the body initiates a coordinated reaction. The sympathetic nervous system activates almost instantly, and the hypothalamic–pituitary–adrenal (HPA) axis follows through a hormonal cascade that culminates in glucocorticoid release from the adrenal cortex (Sapolsky et al., 2000; Ulrich-Lai & Herman, 2009). In parallel, central monoaminergic systems – serotonin, norepinephrine, and dopamine – shift their activity in ways that shape vigilance, arousal, motivational salience, and behavioural readiness (Flügge et al., 2004; Valentino & Van Bockstaele, 2008; Puglisi-Allegra & Andolina, 2015). Together, these adjustments temporarily redirect the organism's resources toward managing the challenge at hand.

Just as important, however, is the way the stress response ends. Glucocorticoids act on receptors in the hippocampus, prefrontal cortex, and hypothalamus to help terminate the response once the threat has passed, allowing the organism to return toward balance (de Kloet et al., 2008). The efficiency of this disengagement is critical: when recovery is smooth, the organism incurs little long-term cost; when activation becomes prolonged or recovery incomplete, the accumulated burden gradually increases vulnerability to stress-related dysfunction. In other words, it is not only the intensity of the stress response that matters, but also how effectively it can be switched off.

Prolonged exposure to stressful conditions can transform an initially adaptive reaction into a chronic state in which stress-mediating systems are repeatedly engaged. This sustained activation generates measurable alterations in both endocrine and neural regulation. Disrupted glucocorticoid homeostasis is a central feature of this process, reflected in altered basal secretion, impaired recovery, and deviations from normal circadian and ultradian dynamics (McEwen & Stellar, 1993; McEwen, 1998; Herman et al., 2016; Sheng et al., 2021). These endocrine disturbances are accompanied by broad modifications in monoaminergic transmission across key limbic and cortical regions implicated in affective and reward processes, including the frontal cortex, amygdala, striatum, and hippocampus (Flügge et al., 2004; Hill et al., 2012). With continued exposure, these functional alterations progress into structural remodelling which further biases affective and motivational regulation (Vyas et al., 2002; McEwen et al., 2016). Notably, the direction and magnitude of these changes vary across individuals, and current evidence does not support a single uniform pathway leading from stress exposure to neural alteration (Joëls & Baram, 2009; Lupien et al., 2009).

Repetitive stress has been demonstrated to alter affective and reward regulation in the central nervous system, but the direction and magnitude of these alterations are not uniform (Chaudhury et al., 2013; Corral-Frías et al., 2015; Holly & Miczek, 2016). A similar heterogeneity of outcomes is also reflected in animal research: chronic stress has been shown to reduce sucrose preference in some models, whereas other studies have reported enhanced subsequent responsiveness to psychostimulants or increased reward salience following repeated

stress exposure (Kalivas & Stewart, 1991; Willner et al., 1992; Chaijale et al., 2015). This variability suggests that the allostatic load of a stressful experience does not result in uniform consequences, but is likely to depend, at least in part, on pre-existing differences in affective and reward-related organisation.

Attempts to conceptualise individual differences in neural organisation in relation to stress adaptation suggest that the effects of chronic stress are not random, but reflect systematic variation between organisms (Davidson, 2000; Harro, 2010). Yet this line of research has received less attention than its significance would warrant, despite its considerable potential to improve prediction, prevention, and treatment in psychiatric medicine. It may also advance our understanding of how reward-related neural systems operate and how they are modified by interaction with the environment.

2. AIMS

The general aim of this dissertation is to investigate how stable individual differences in positive affectivity and hedonic capacity modulate the effects of chronic stress on reward-related processes. Using rodent models that index positive affectivity through 50-kHz ultrasonic vocalisations and hedonic capacity through sucrose consumption, this work examines how these trait-like phenotypes contribute to vulnerability or resilience in behavioural and neurochemical outcomes when exposed to persistent stressors.

STUDY I (Kõiv et al., 2016):

The study aimed to demonstrate the effect of chronic stress on amphetamine-elicited 50-kHz vocalisations in rats with high and low levels of positive affectivity (HC vs. LC rats, respectively).

STUDY II (Vares et al., 2018):

The study aimed to examine the effect of SSRI treatment on amphetamine-elicited 50-kHz ultrasonic vocalisations in chronically stressed rats with high and low levels of positive affectivity.

STUDY III (Kõiv et al., 2019):

The experiment aimed to compare the sensitivity of rats with high vs low liquid sucrose consumption (HSuc vs. LSuc, respectively) to chronic variable stress and the stress effect on behavioural sensitisation to amphetamine.

STUDY IV (Vares et al., 2025):

The final experiment aimed to bring the previous work together by examining how baseline positive affectivity, indexed by 50-kHz ultrasonic vocalisation activity, and hedonic capacity, indexed by sucrose consumption, interact in their association with stress vulnerability and resilience.

3. MATERIALS AND METHODS

3.1 Animals

Male Wistar rats were used across all experiments. In **Studies I** and **II**, the experimental animals were bred locally from parent rats obtained from Harlan Laboratories (Venray, the Netherlands). **Study III** used adult male Wistar rats obtained directly from Harlan Laboratories, whereas **Study IV** used locally bred offspring of RccHan:WIST parent rats obtained from Envigo (the Netherlands). Rat pups in **Studies I, II**, and **IV** were weaned at 21 days of age; the animals in **Study III** entered the experiment as adults. Rats were housed in standard transparent polypropylene cages with wood-chip bedding under a 12:12 h light/dark cycle. The colony room was maintained at 20–22 °C in **Studies I** and **II** and at 20–21 °C in **Studies III** and **IV**; lights were switched on at 08:00 in **Studies I–III** and at 07:30 in **Study IV**. Rats had free access to tap water and food pellets (R70, Lactamin AB, Sweden, in **Studies I–III**; Ssniff Energy Reduced, Soest, Germany, in **Study IV**), except where testing procedures required otherwise.

All experimental procedures were carried out in accordance with approvals from the Animal Experimentation Committee of the Estonian Ministry of Agriculture and followed the applicable guidelines for animal research. Licence numbers are provided in the original publications.

3.2 Experimental design and general procedure

From here on, only a general overview of the experimental design and execution is provided; detailed methods and results are presented in the original papers appended to this thesis. General overview of experiment design is depicted on **Figure 1**. In **Studies I, II** and **IV**, single housed rat pups were assessed for their affectivity phenotype with tickling. The procedure followed the protocol established in Mällo et al., 2007. For 14 days, the experimenter imitated the natural rough-and-tumble play with rat pups for 2-minute (min) period in a separate testing room. The procedure consisted of 15 seconds (s) of habituation, followed by 15 s of play alternating during the 2 min period. USV activity were monitored throughout the procedure and recorded in days 12–14 in order to assign the rat phenotype based on the mean number of 50-kHz USV-s emitted during tickling sessions on days 12–14. High and low calling profile (HC vs. LC, respectively) were distinguished with median split in all studies.

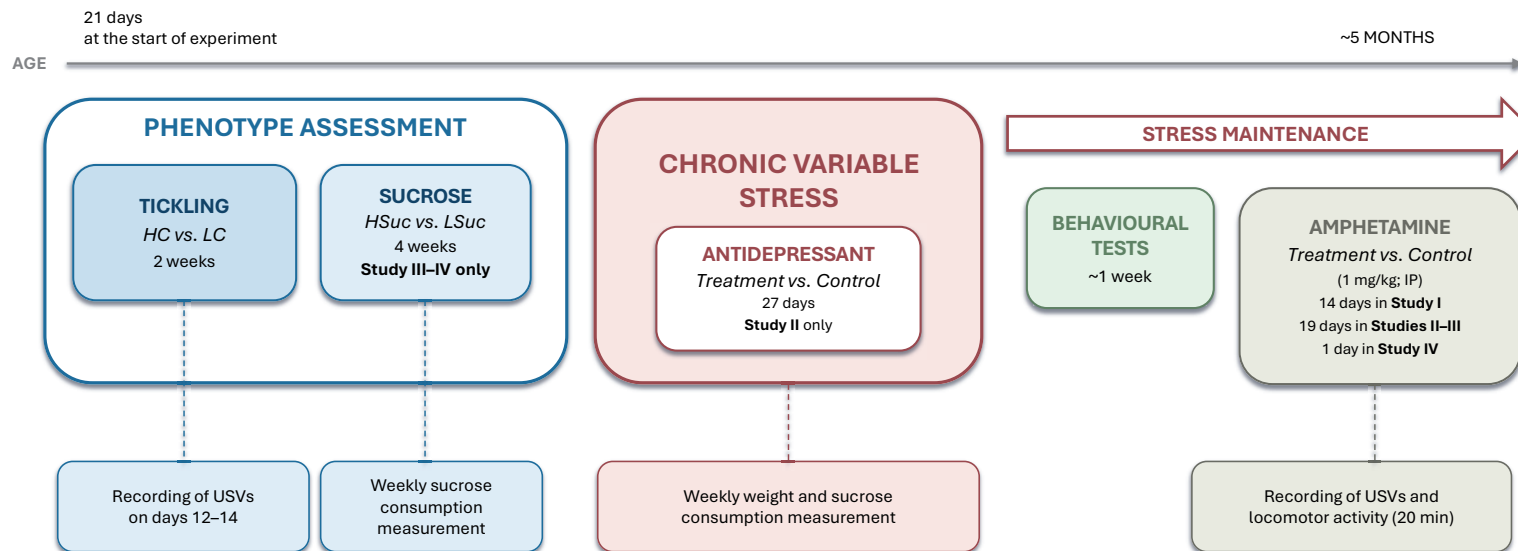


Figure 1. Schematic representation of experiment design. HC – high calling, LC – low calling; HSuc – high sucrose feeder, LSuc – low sucrose feeder; IP – intraperitoneal administration.

Study III did not include tickling. In **Study IV**, tickling was followed by 4-week sucrose consumption test to assess the rat pup hedonic response. The details for the procedure can be found in respective publications. Rats had free access to food during the procedure. Data from the last three tests were used to calculate the average consumption of sugar water and rats were assigned either high-sucrose and low-sucrose consuming phenotypes (HSuc vs. LSuc, respectively) based on median split. In both studies, data from the first test were excluded because they showed lower correlations with the other tests, likely due to novelty effects ($r < 0.6$, compared to > 0.7 with tests 2–4). Based on both phenotype data, the groups were balanced by their body weight with same number of rats in each phenotype group. Subsequently animals were group-housed as 2 HC and 2 LC animals per cage and 2 HSuc and 2 LSuc rats per cage, resulting in 4 rats per cage.

Half of the rats from each phenotype were assigned to chronic variable stress regimen collectively with their housing mates. All rats were weighed on the day before the start of the chronic variable stress (CVS) regimen and weekly thereafter. Cumulative weight gain was calculated by subtracting the pre-stress body weight from the body weight recorded at each weekly measurement and was used to monitor the physiological effect of CVS. The regimens comprised variable short and long-lasting stressors administered according to study-specific schedules. In **Studies I** and **IV**, an initial phase generally involving one stressor per day was followed by an intensified phase with more frequent and/or longer stress exposure. In **Studies II** and **III**, each 24-h period included two stressors: one short stressor during the light phase and one long-lasting stressor during the dark phase. **Study IV** additionally included a maintenance phase to preserve the stress effect until the completion of behavioural testing.

After the planned CVS regimen had been completed and its effect on cumulative weight gain evaluated, the experiment proceeded to the next planned phase. In **Study I**, rats were tested with amphetamine to assess stress-induced changes in reward-related behaviour. In **Study II**, fluoxetine treatment was introduced to assess whether SSRI treatment modified stress-induced changes in reward-related behaviour before subsequent amphetamine testing. In **Studies III** and **IV**, the stress regimen was followed by behavioural testing, including the elevated zero-maze or elevated plus-maze. When behavioural or pharmacological procedures followed the chronic stress regimen, these were subsequently followed by amphetamine testing to assess reward-related behavioural responsiveness.

The amphetamine administration was preceded by habituation period with testing cage and equipment for 3 days in **Study I**, 4 days (**Study II**), and 2 days (**Studies III–IV**). In **Studies I–III**, amphetamine was administered daily: for 5 days (**Study I**) or 10 days (**Studies II** and **III**), with an amphetamine challenge day on 14th day (**Study I**) or 19th day (**Studies II–III**) of amphetamine treatment. All rats received an injection intraperitoneally, either 1 mg/kg of amphetamine dissolved in saline (1 ml/kg), or saline only for control animals. **Study IV** included an acute administration of amphetamine to all rats. Vocalisation and locomotor activity were recorded for 30 min in **Study I**, 15 min in **Study II**, and 20 min in **Studies III** and **IV**.

The experiment animals were sacrificed 5 days (**Studies II–IV**) or 10–11 days (**Study I**), after the conclusion of amphetamine test. The tissue samples were immediately stored in dry ice and preserved at -80°C . For biochemical measurements, tissue samples were prepared frozen until biochemical analysis.

3.3 Tickling

Animals were given manual stimulation resembling natural rough-and-tumble play after weaning. Tickling procedures were carried out as described previously (Mällo et al., 2009). Animals were transferred from their home cage into a smaller plastic cage ($30 \times 15 \times 13$ cm) without bedding and given 15 s for habituation, followed by 15 s of tickling by the experimenter. Altogether, four 15 s sessions of tactile stimulation were given over 2 min. Test cage was cleaned with a damp paper towel in between each animal.

The tickling session consisted of stimulating the rat with one hand, including vigorous alternating finger movements on the back and neck, rapidly turning the animal onto its back while stimulation was administered to the ventral surface, followed by release after a few seconds.

Vocal activity was recorded on Days 12–14 of the tickling procedure in **Studies I, II, and IV**. Based on this data rats were stratified to high calling (HC) and low calling (LC) rats based on their mean number of calls by median split.

3.4 Sucrose tests

Hedonic capacity was assessed with sucrose consumption in **Studies III–IV**. The sucrose consumption tests were carried out during the dark phase with no previous food or water deprivation. During testing, food was freely available. The animals were single-housed one hour before the testing and group-housed immediately after conclusion of the test. Two bottles were used, one filled with 1% sucrose solution and the other with water, and the placement of the sucrose bottle – left or right on the cage – was altered between the tests. Sucrose and water consumption was measured by weighing sucrose and water bottles 12 h after the beginning of the sucrose preference test.

Four weekly sucrose preference tests were conducted. Based on this data, high (HSuc) and low (LSuc) sucrose consumption phenotypes were assigned based on their mean sucrose consumption on Weeks 2–4 by median split. Test results from the first week were excluded in both – **Studies III and IV** – because of their weaker correlation with other testing weeks.

3.5 Recording and analysis of 50-kHz ultrasonic vocalisations

An ultrasound microphone (CM16/COMPA, Avisoft Bioacoustics, Berlin, Germany) was located about 20–30 cm from the floor of the cage, recording USVs (Avisoft UltraSoundGate 116–200, Avisoft-RECORDER software, Avisoft Bioacoustics, Berlin, Germany). Calls were monitored on spectrograms using the Fast Fourier Transform algorithm (1024 FFT length, 75% frame, Hamming window, and 75% time window overlap).

In **Study I**, 50-kHz USVs were manually counted in six selected 2-min bins from each 30-min recording. In **Study II**, seven selected 1-min bins were manually scored from each 15-min recording. In **Study III**, 50-kHz USVs were counted from spectrograms during the 20-min recording. **Study IV** used the DeepSqueak toolbox in MATLAB for automated detection and scoring. Where subsampling or automated methods were used, their correspondence with full or manual scoring was verified and was high ($r > 0.9$).

3.6 Recording and analysis of amphetamine-elicited locomotor activity

Behaviour during amphetamine testing was recorded with a digital video camera positioned on a tripod above the cage so that the entire cage floor was visible.

Both vertical activity (rearings, defined as the rat standing on its hind paws at an angle of at least 45°) and horizontal locomotor activity (line crossings) were scored from digital video recordings by an observer blind to the experimental conditions. The scoring period was 30 min in **Study I**, 15 min in **Study II**, and 20 min in **Studies III** and **IV**. To estimate horizontal locomotor activity, the floor of the group-housing cage (55 × 33 cm) was divided into six equal squares, and crossings made with all four paws were counted.

3.7 Scoring of stereotyped behaviour

In addition, occurrence of stereotyped behaviour was observed in **Studies I** and **III**. An observational method adapted from Kelley (1998) was used. Briefly, the presence (1) or absence (0) of the following behavioural categories in the given time-bin were scored and summed (max score = 6): head-up and head down sniff (continuous sniffing for at least 5 s with head/snout directed towards top/down on floor of the cage, respectively), head sway (rhythmic, lateral swaying of head) and head bob (repeated vertical head bobbing). In order to provide a general score for stereotyped behaviour, the scores were added up.

3.8 Chronic stress regimen

Chronic stress regimen followed a procedure developed during prior studies from our group (Harro et al., 2001; Mällo et al., 2009). Stressors included of mild physical (i.e. tail pinch with a clothespin), psychological (i.e. staying on a raised disk under a bright light), and environmental challenges (i.e. cage overcrowding). Stress regimen across studies was similar: multiple weeks of unpredictable stressors with intermittent introduction of short and long-lasting challenges. Control animals were left undisturbed. In **Study IV**, a “stress maintenance” period was introduced during the rest of the experiment in order to preserve the chronically stressed state of the subjects, which included a randomly administered stressor on three occasions per week.

3.9 Pharmacological treatments

Amphetamine (1 mg/kg; Tocris Bioscience, Bristol, UK) was used across all studies as a pharmacological challenge of reward-related systems, with amphetamine-elicited 50-kHz ultrasonic vocalisations and locomotor activation serving as behavioural readouts. **Studies I–III** included repeated amphetamine administration. In **Study IV**, a single injection of amphetamine was administered. Control group was administered 0.9% NaCl, except **Study IV** where all animals received a single dose of amphetamine.

In rats, amphetamine elicits reward-related behavioural responses, including 50-kHz ultrasonic vocalisations, locomotor activation, and, under repeated treatment conditions, stereotyped behaviour and behavioural sensitisation (Robinson & Becker, 1986; Burgdorf et al., 2001; Wolgin, 2012)

In **Study II**, fluoxetine (10 mg/kg; Tokyo Chemical Industries, Japan) was administered for 27 days, starting at the beginning of Week 3 of the chronic stress regimen, to examine whether SSRI treatment modified stress-induced changes in reward-related behavioural responsiveness. Control animals received saline. Rats were weighed immediately before drug administration.

Fluoxetine is a selective serotonin reuptake inhibitor that acts primarily by inhibiting serotonin transporter-mediated reuptake of 5-HT, thereby increasing extracellular serotonergic signalling (Sohel et al., 2024).

3.10 Behavioural testing

Studies III and **IV** included additional behavioural testing. **Study III** used a novel locomotor activity box and an elevated zero-maze, whereas **Study IV** used an open-field test and an elevated plus-maze. These tests assessed locomotor activity and anxiety-like behaviour. All tests were video-recorded and scored manually, except for the locomotor activity box in **Study III**, which was scored semi-automatically using EthoVision XT (Noldus). Detailed protocols are provided in the original publications.

3.11 Biochemical assays

Across all studies, monoaminergic activity was assessed with high performance liquid chromatography with electrochemical detection (HPLC-ECD) by quantifying noradrenaline (NA), dopamine (DA), and serotonin (5-HT), together with their principal metabolites – normetanephrine (NMN), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), and 5-hydroxyindoleacetic acid (5-HIAA) – and by calculating metabolite-to-transmitter ratios (NMN/NA, DOPAC/DA, and 5-HIAA/5-HT) as indices of neurotransmitter turnover.

A selection of amino acid concentrations was assessed with HPLC-mass spectrometry by measuring α -alanine, β -alanine, isoleucine, leucine, and tryptophan; N-acetyl aspartic acid was analysed in **Study 1** only.

Western blot analyses (**Studies I** and **III**) assessed dopamine D₁ and D₂ receptor proteins, with additional measurement of the dopamine transporter (DAT) in **Study I** and tyrosine hydroxylase in **Study III**.

Study IV included a more extensive assessment of biological variables. In addition to monoamine analyses across multiple brain regions, serum glucose was measured from trunk blood, adrenal gland weight was expressed relative to body weight, and striatal mRNA expression of the dopamine transporter, dopamine D₁ and D₂ receptors, Htr2c, and vesicular monoamine transporters 1 and 2 was quantified using real-time quantitative polymerase chain reaction (RT-qPCR).

The biochemical and molecular analyses conducted in each study are summarised in **Table 1**.

Table 1. Collected biochemical data across studies by region, assessment method, and assessed chemical compounds.

Study	Region	Method	Measurements
STUDY I	Striatum	HPLC-ECD	Monoamines, metabolites, and turnover
	Nucleus accumbens	HPLC-ECD	Monoamines, metabolites, and turnover
		Western blot	DAT, D ₁ and D ₂ receptor proteins
STUDY II	Frontal cortex	HPLC-MS	Amino acids
	Frontal cortex	HPLC-ECD	Monoamines, metabolites, and turnover
	Hippocampus	HPLC-ECD	Monoamines, metabolites, and turnover
		HPLC-MS	Amino acids
		HPLC-MS	Amino acids
STUDY III	Nucleus accumbens	HPLC-ECD	Monoamines, metabolites, and turnover
	Striatum	HPLC-ECD	Monoamines, metabolites, and turnover
		Western blot	Tyrosine hydroxylase, D1 receptor protein
STUDY IV	Frontal cortex		
	Striatum		
	Nucleus accumbens	HPLC-ECD	Monoamines, metabolites, and turnover
	Amygdala		
	Hippocampus	HPLC-ECD	Monoamines, metabolites, and turnover
		HPLC-MS	Amino acids
	Hypothalamus	HPLC-ECD	Monoamines, metabolites, and turnover
	Striatum	RT-qPCR	mRNA expression of htr2c, VMT1 and 2

3.12 Statistical analysis

Statistical analysis was performed using Statistica (TIBCO Software Inc., USA), StatView (SAS Institute Inc., USA), and Prism (GraphPad Software, LLC, USA) software. Across studies, data from the behavioural tests and biochemical measurements were analysed using categorical ANOVA, with 50-kHz *USV* (HC vs. LC), *Sucrose* (LSuc vs. HSuc – **Studies III–IV**) and *Stress* (control vs. CVS), *Amphetamine* (vs control – **Studies I–III**), and *Fluoxetine* (vs control – **Study II**) as independent variables. Where appropriate, a repeated measures factor *Day* was added. Group differences after significant ANOVAs were measured by post hoc Fisher’s PLSD test. For correlations, Pearson’s correlation coefficient was used. All data in journal articles and on the figures are presented as mean ± SEM.

4. RESULTS AND DISCUSSION

This work demonstrated that there are innate differences in the brain's affective and reward-related regulation, and that these differences influence the capacity of the organism to adapt to chronic stress. These differences are reflected both in behaviour and in the underlying biology. Across the studies, 50-kHz ultrasonic vocalisations served as an index of positive affectivity and as a behavioural expression of positive affective states, whereas stable sucrose consumption served as an indicator of hedonic capacity. Within the scope of this thesis, positive affectivity refers to an inherent neurobiological propensity to experience positive affects, whereas hedonic capacity refers to the reward consumption and pleasure-related responsiveness. Here, the terms positive affect and hedonic response are not intended to correspond to complex human conscious experiences, but rather to species-specific expressions of neural activity within evolutionarily conserved affective-motivational circuits (Panksepp, 2005).

4.1 Positive affectivity and psychostimulant reward expression under chronic stress

Study I proceeded from the study by Mällo et al. (2009), which indicated that low 50-kHz USV activity is associated with greater vulnerability to chronic stress, particularly in male rats. The study was further grounded in earlier work demonstrating that rats can be selectively bred for different 50-kHz USV calling phenotypes (Burgdorf et al., 2005) and these phenotypes can be assigned by tickling the adolescent rats (Mällo et al., 2007). **Study I** demonstrated that chronic stress produced phenotype-dependent differences in the affective expression of reward (**Figure 2**). Across the three testing days, the number of 50-kHz USVs differed significantly [$Day F(2,64)=22.8, p<0.0001$] and depended on amphetamine treatment [$Day \times Amphetamine F(2,64)=6.6, p<0.01$] and prior stress exposure [$Day \times Stress F(2,64)=3.9, p<0.05$]. The overall vocalisation response to amphetamine also depended on prior stress exposure [$Amphetamine \times Stress F(1,32)=4.2, p<0.05$]. Amphetamine has been observed to increase 50-kHz vocalisation activity dose-dependently, and this response is regarded as a behavioural correlate of its positive affective and rewarding effects (Burgdorf et al., 2001; Mulvihill & Brudzynski, 2019). Post hoc comparisons showed that chronic stress prevented the amphetamine effect in LC rats on Days 1, 5, and 14, whereas in HC rats the effect was attenuated only on Day 5. On the challenge day, amphetamine increased 50-kHz USVs in all previously saline-treated groups except stressed LC rats. In stressed LC rats, reduced vocalisation activity was accompanied by attenuated locomotor activity, particularly rearings. This behavioural pattern was supported by significant three-way interactions for total locomotor activity [$50\text{-kHz USV} \times Stress \times Amphetamine F(1,32)=4.4, p<0.05$] and rearings [$50\text{-kHz USV} \times Stress \times Amphetamine F(1,32)=4.9, p<0.05$].

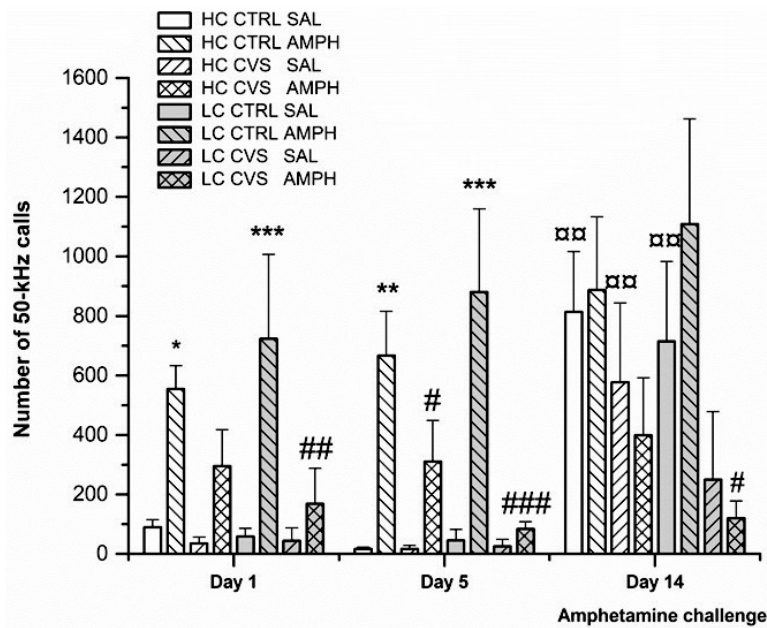


Figure 2. Study I – Day 1 – first day of amphetamine treatment; Day 5 – last day of amphetamine treatment; Day 14 – testing sensitisation to amphetamine. All rats received a challenge dose of amphetamine (1 mg/kg, IP). N = 4–6 per group. *, **, *** – $p < 0.05$, 0.01, 0.001 vs. respective saline group; #, ##, ### – $p < 0.05$, 0.01, 0.001 vs. respective control group; □□ – $p < 0.01$ vs. Day 1. HC – high 50-kHz USV rats; LC – low 50-kHz USV rats; CTRL – control; CVS – chronic variable stress; AMPH – amphetamine; SAL – saline. Ultrasonic vocalisations were scored from six two-minute time bins: minutes 1–2, 6–7, 11–12, 16–17, 21–22, and 26–27 of the 30-minute recording (Kõiv et al., 2016).

Study I demonstrated that individual differences in positive affectivity influence how reward-related affective responses change under chronic stress. **Study II** continued from this by examining how responsiveness to psychostimulant reward is altered when stress is experienced in the presence of antidepressant treatment across these phenotypes. Previous research has shown that several effects of chronic stress can be attenuated or reversed by antidepressant treatment (Hitoshi et al., 2007; Lin et al., 2023), including changes in reward-related behaviour (Willner et al., 1987). **Study II** included a 27-day fluoxetine treatment from the beginning of day 17 of chronic stress regimen with daily administration of 10 mg/kg, while control group received daily administration of saline.

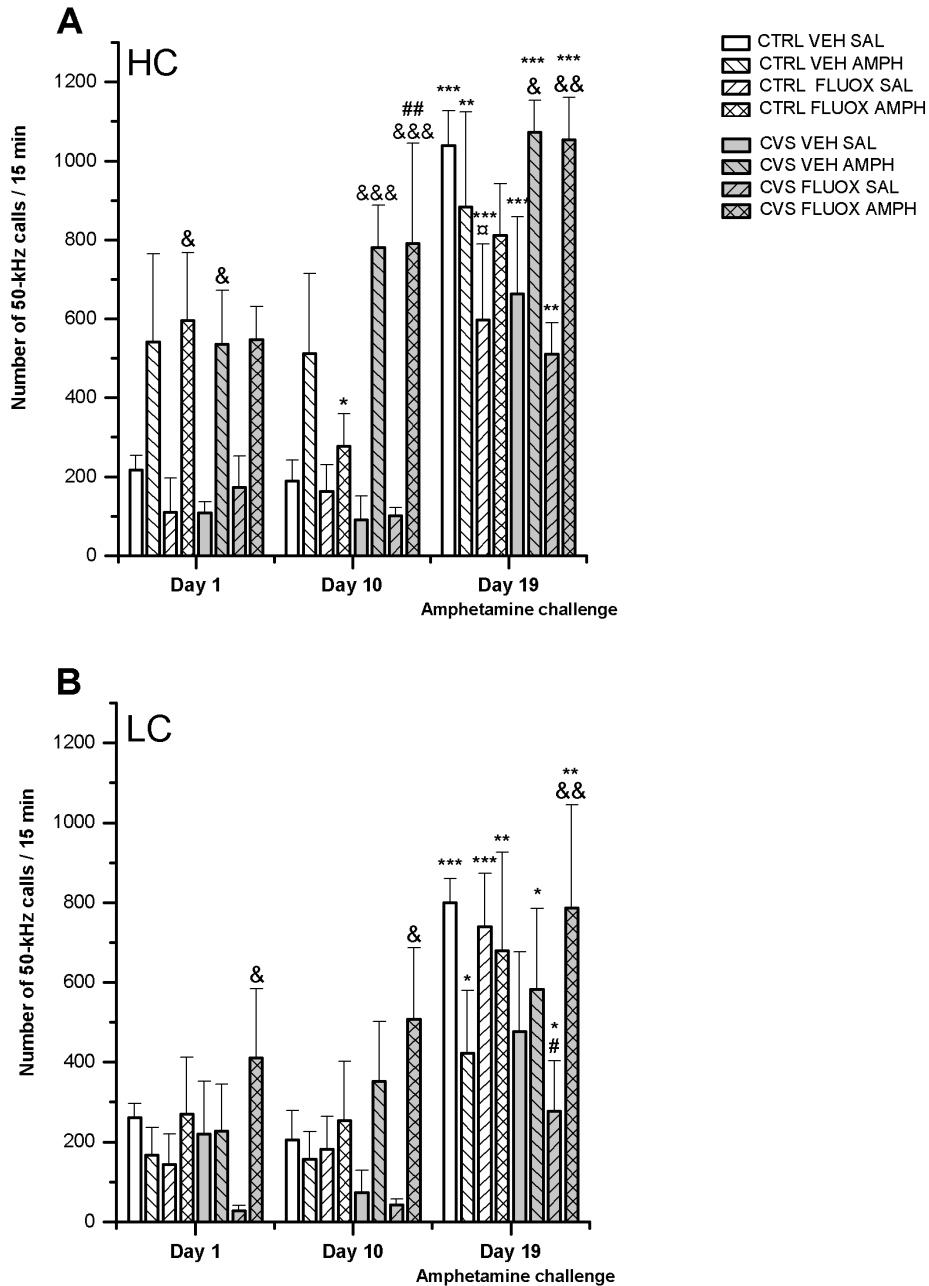


Figure 3. Study II – Number of 50-kHz ultrasonic vocalisations. Scored from seven selected time bins on Days 1, 10, and 19 of the amphetamine experiment. Data are expressed as mean + SEM. On Day 19, all animals ($n = 62$) were given amphetamine (1 mg/kg, IP). Vehicle and saline are both saline solutions but denote direct comparisons with fluoxetine and amphetamine, respectively. *, **, *** – $p < 0.05$, 0.01, 0.001 vs. respective group on Day 1; #, ## – $p < 0.05$, 0.01 stress vs. respective control group; □ – $p < 0.05$ vs. respective vehicle group; &, &&, &&& – $p < 0.05$, 0.01, 0.001 vs. respective saline group.

Additionally, several aspects of the experimental conditions were modified, most notably the duration and structure of amphetamine treatment and the longer duration of stress exposure. Under these modified conditions, the suppression of amphetamine-elicited 50-kHz calls was no longer observed in the stressed LC group in the same manner as in **Study I** (**Figure 3**).

Within **Study II**, psychostimulant sensitisation developed only in animals that had previously experienced chronic stress. Across Days 1, 10, and 19, 50-kHz USV activity changed significantly [$Day F(2,92)=117.3, p<0.0001$], and depended on prior stress and repeated amphetamine administration [$Day \times Stress F(2,92)=3.6, p<0.05$; $Day \times Amphetamine F(2,92)=3.8, p<0.05$; $Day \times Stress \times Amphetamine F(2,92)=5.5, p<0.005$]. Across the three testing days, the amphetamine response was also potentiated by prior stress [$Stress \times Amphetamine F(1,46)=8.1, p<0.01$]. In high-calling rats, sensitisation was observed in stressed groups irrespective of antidepressant treatment. In low-calling rats, by contrast, sensitisation did not develop under stress alone, nor under fluoxetine treatment in the absence of stress, but only when the two were experienced together. Both stress and antidepressant treatment have been reported to modify reward-related behaviour (Bogdan & Pizzagalli, 2006; Ironside et al., 2018; Willner et al., 2013). In this context, the present findings suggest that fluoxetine did not restore the reward-related response of LC rats to the pattern observed under unstressed conditions, but instead altered the conditions under which sensitisation developed. SSRIs have also been reported to potentiate amphetamine-induced locomotor activity, accompanied by an apparent increase in brain amphetamine concentrations (Sills et al., 1999). These findings may have relevance to clinical practice, as SSRIs and amphetamine-based medications can be used concomitantly. This suggests that this interaction may alter behaviour and should therefore be examined further in clinical studies.

Studies I and **II** differed in the pattern of chronic-stress effects on amphetamine-elicited 50-kHz calling. These differences can be related to the amphetamine-administration protocol: **Study I** included five consecutive daily amphetamine injections, whereas **Study II** extended repeated administration to 10 consecutive days. The longer treatment phase may have contributed to the emergence of behavioural sensitisation, although other differences in the experimental conditions may also have influenced the contrasting outcomes. This interpretation is consistent with variability in the literature, where sensitisation of psychostimulant-induced 50-kHz vocalisation has been observed in some studies but not in others, suggesting that its emergence depends partly on the duration and temporal structure of drug administration (Taracha et al., 2012; Ahrens et al., 2013; Simola et al., 2014; Simola & Morelli, 2015). Our findings parallel those of Taracha and colleagues (2012, 2014) at the level of the general pattern rather than the experimental conditions: in both lines of research, amphetamine-elicited 50-kHz USV sensitisation varied markedly between individuals. In **Studies I** and **II**, this variability was observed following chronic stress and was associated with pre-existing differences in positive affectivity. Thus, the contrasting outcomes of **Studies I** and **II** support the view that repeated psychostimulant reward

responses depend both on the administration schedule and on individual properties of the reward system.

4.2 Introduction of sucrose consumption as a behavioural readout of reward sensitivity

Studies I and II demonstrated that intrinsic differences in positive affectivity influence how chronic stress changes the later response to psychostimulant reward. **Study III** extended this approach by introducing hedonic capacity as a complementary dimension of reward function, indexed by stable sucrose consumption. Together with previous studies from our group (Tönissaar et al., 2006b; Kanarik et al., 2011), and others (Sills & Crawley, 1996; Sills & Vaccarino, 1996; Brennan et al., 2001), the trait-like stability of sucrose consumption was supported at several levels. In **Study III**, individual sucrose consumption was consistent across the repeated sucrose tests, with correlations ranging from $r=0.71$ to 0.89 , and being particularly high across the final three tests ($r=0.81$ – 0.89 , all $p<0.0001$). In control animals, baseline consumption also remained strongly associated with consumption measured four weeks later ($r=0.82$, $p<0.0001$). Additionally, HSuc rats continued to consume more sucrose than LSuc rats throughout the chronic stress regimen [*Sucrose* $F(1,36)=49.1$, $p<0.0001$], despite changes in absolute intake and a temporary stress-related reduction in HSuc animals. **Study IV** reproduced the phenotype assessment, again showing stronger consistency across the final three baseline measurements ($r=0.70$ – 0.80) and a significant difference in consumption between HSuc and LSuc rats. These findings support sucrose consumption as a reproducible, trait-like behavioural measure, while also showing that its absolute expression remains responsive to environmental conditions.

In contrast to previous studies, in **Study III**, 50-kHz vocalisation activity was used as a state measure instead. At baseline, HSuc and LSuc rats did not differ in their 50-kHz USV activity (**Figure 4**). With the introduction of stress, however, clear phenotype-dependent differences emerged. Across the three amphetamine-testing days, 50-kHz USV activity changed significantly [*Day* $F(2,64)=20.4$, $p<0.0001$], and amphetamine increased the overall number of vocalisations [*Amphetamine* $F(1,32)=18.5$, $p<0.001$]. The *Day* $\times$ *Amphetamine* interaction approached statistical significance [$F(2,64)=3.0$, $p=0.059$]. The omnibus *Sucrose* $\times$ *Stress* $\times$ *Amphetamine* interaction and its interaction with *Day* were not statistically significant [$F(1,32)=2.3$, $p=0.14$, and $F(2,64)=0.8$, $p=0.45$, respectively]; consequently, the phenotype-specific pattern was identified by the post hoc comparisons. In high-sucrose rats, chronic stress potentiated the response to acute amphetamine, evident in increased 50-kHz USV emission and locomotor activity. This heightened responsiveness progressed into sensitisation with repeated amphetamine exposure indicated by their 50-kHz USV activity and rearings in the last day of amphetamine administration. Post hoc comparisons identified sensitisation of 50-kHz vocalisations in stressed HSuc rats on Day 19, relative both to Day 1 and to the corresponding saline-treated group ($p<0.05$). The number

of rearings also changed across testing days [*Day* $F(2,64)=19.9$, $p<0.0001$] and increased progressively following repeated amphetamine administration [*Amphetamine* $F(1,32)=28.1$, $p<0.0001$; *Day × Amphetamine* $F(2,64)=4.3$, $p<0.05$]. Post hoc comparisons showed sensitisation of rearings in all repeatedly amphetamine-treated groups except non-stressed HSuc rats. In contrast, low-sucrose rats displayed a different pattern. While sensitisation of 50-kHz USVs developed in non-stressed LSuc animals, this effect was absent under chronic stress.

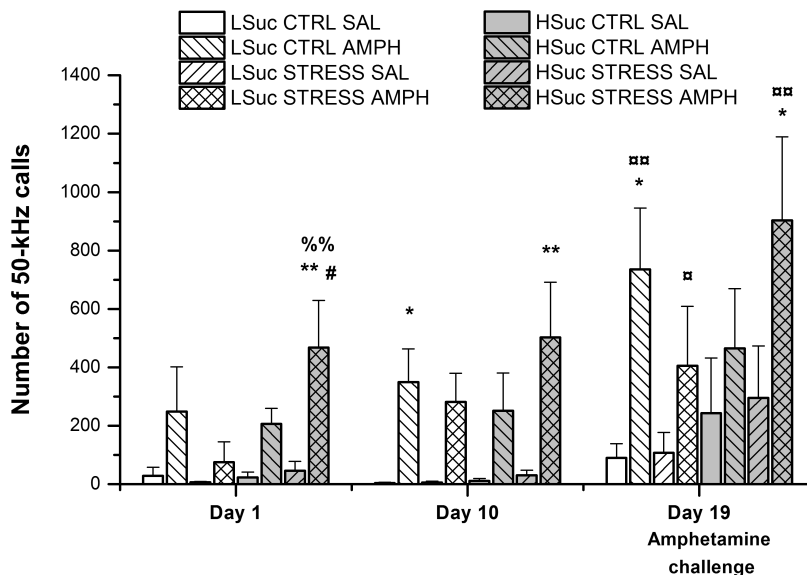


Figure 4. Study III – Effect of chronic variable stress on amphetamine-elicited 50-kHz calls. Amphetamine dose: 1 mg/kg, intraperitoneally (i.p.). Day 1 – first day of amphetamine (AMPH) treatment; Day 10 – last day of AMPH treatment; Day 19 – testing sensitisation to AMPH: all rats received a challenge dose of AMPH (1 mg/kg, i.p.). $n=5$ per group. *, ** – $p<0.05$, 0.01 vs. respective saline group; □, □□ – $p<0.05$, 0.01 vs. Day 1; # – $p<0.05$ vs. respective control group; %% – $p<0.01$ vs. respective LSuc group. CTRL – control; HSuc – high sucrose-consuming rats; LSuc – low sucrose-consuming rats; SAL – saline.

A similar divergence was observed in stereotyped behaviour. On the Day 19 amphetamine challenge, stereotyped behaviour was higher overall in HSuc than LSuc rats [*Sucrose* $F(1,32)=6.7$, $p<0.05$], in animals previously treated repeatedly with amphetamine rather than saline [*Amphetamine* $F(1,32)=14.7$, $p<0.001$], and in animals exposed to chronic stress [*Stress* $F(1,32)=7.8$, $p<0.01$]. However, under chronic stress, prior repeated amphetamine treatment did not further increase stereotyped behaviour relative to prior saline treatment. Notably, stressed HSuc rats receiving their first amphetamine dose displayed significantly more stereotypies than the corresponding stressed LSuc rats ($p<0.05$). In non-stressed LSuc rats, previous repeated amphetamine treatment also increased stereotyped behaviour relative to previous saline treatment ($p<0.01$). These rats likewise showed sensitisation in 50-kHz vocalisations, together indicating a broader

behavioural sensitisation profile across reward-related and motor measures (Robinson & Becker, 1986; Vanderschuren & Kalivas, 2000; Robinson & Berridge, 2008).

In contrast to **Study I**, where stereotyped behaviour was generally higher in LC rats and stress increased stereotypy primarily in HC animals, **Study III** indicates that the motor expression of amphetamine depends on phenotype in a different way when animals are stratified by hedonic capacity. Importantly, stereotypies are related less to the hedonic than to the activating effects of psychostimulants. In this context, they provide an additional behavioural dimension to 50-kHz vocalisation and locomotor activity, further supporting the view that psychostimulant effects depend on the pre-existing properties of the organism, particularly in relation to reward and affective regulation.

Whether these results represent a disruption of reward processing or an alternative form of adaptation cannot be determined from the present data. Nevertheless, there is consistency in results from **Study III** that higher hedonic capacity can be associated to increased sensitivity to psychostimulants and stress-induced sensitisation, which aligns well with previous work (Sills & Crawley, 1996; Sills & Vaccarino, 1996; Radke et al., 2013). **Study I** and **III** provide insights to the distinct nature of hedonic capacity and positive affectivity as well, as rats with lower levels of positive affectivity are more prone to stress effects, while in hedonic capacity higher levels are more reactive to stress.

4.3 Positive affectivity and hedonic capacity in adaptation to chronic stress

Implications from our previous studies determined the design of **Study IV**, where positive affectivity and hedonic capacity could be observed simultaneously in adaptation to chronic stress. In **Study IV**, prior experience of chronic stress significantly suppressed amphetamine-elicited 50-kHz vocalisations overall [*Stress* $F(1,68)=15.3$, $p<0.001$], but post hoc comparisons showed that this effect was significant only in rats with lower positive affectivity and higher hedonic capacity (LC/HSuc; $p<0.005$), while other groups showed no comparable stress effect on reward-related vocalisations (**Figure 5**). The *USV* $\times$ *Sucrose* $\times$ *Stress* interaction approached, but did not reach, statistical significance [$F(1,68)=3.78$, $p=0.056$]. In addition to amphetamine-elicited vocalisation activity, this pattern was accompanied by broader behavioural differences. In the elevated plus-maze, the effect of stress on anxiety-like behaviour depended on the combination of phenotypes [*USV* $\times$ *Sucrose* $\times$ *Stress* $F(1,72)=6.42$, $p<0.05$], increasing anxiety in LC/HSuc rats, while producing an opposite, anxiolytic-like effect in HC/HSuc animals (both $p<0.05$). In contrast, locomotor activity in response to amphetamine remained unaffected by stress, whereas rearings were reduced across groups [*Stress* $F(1,72)=21.8$, $p<0.001$], indicating that different behavioural measures captured distinct aspects of the system's response.

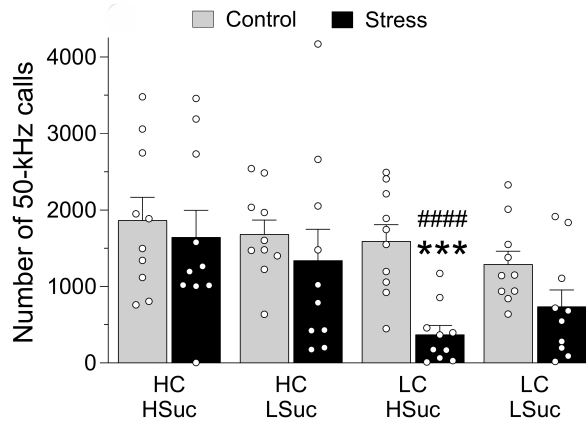


Figure 5. Study IV – Effect of chronic variable stress on amphetamine-elicited (1 mg/kg) 50-kHz ultrasonic vocalisations. HC and LC – high and low 50-kHz vocalisation, respectively; HSuc and LSuc – high and low sucrose consumption, respectively. Data are presented as mean $\pm$ SEM. *** – $p < 0.005$ vs. respective control group; #### – $p < 0.001$ vs. the corresponding HC group.

These studies demonstrate that chronic stress modifies the behavioural expression of affective and reward-related responses, and that the direction and magnitude of these effects depend on trait-like differences in positive affectivity and hedonic capacity. **Study I** further supported previous findings from our group indicating that low positive affectivity is associated with increased vulnerability to chronic stress (Mällo et al., 2009). **Study III** extended this framework to hedonic capacity, showing that HSuc rats displayed greater stress-induced modulation of amphetamine-elicited reward-related behaviour than LSuc rats, consistent with earlier work linking high sucrose intake to enhanced reward-related sensitivity (Sills & Vaccarino, 1994, 1996). Importantly, the stress-related behavioural profiles associated with positive affectivity and hedonic capacity were not equivalent. Although both phenotypes were associated with differential stress responsiveness, they affected reward-related behaviour in distinct ways, indicating that positive affectivity and hedonic capacity represent separable dimensions of stress adaptation.

Study IV further substantiated this interpretation by examining both phenotypes within the same experimental design. The most pronounced stress-related alteration of reward-related behaviour was observed in LC/HSuc rats, whereas HC/HSuc rats showed a distinct behavioural profile, including preserved amphetamine-elicited 50-kHz vocalisation and lower anxiety-like behaviour following chronic stress exposure. These findings refine the view that chronic stress can either reduce or enhance reward sensitivity (Holly & Miczek, 2016) by showing that the direction of stress-induced behavioural adaptation depends on the configuration of pre-existing affective and hedonic phenotypes.

4.4 Biological correlates of phenotype-dependent stress adaptation

4.4.1 Monoaminergic regulation of reward-related adaptation to chronic stress

In **Study I**, there were phenotype-dependent differences in how chronic stress shapes reward-related responses. To examine whether these differences were reflected at the neural level, monoaminergic regulation was analysed in the nucleus accumbens and striatum. In the striatum, a clear divergence emerged. Post hoc comparisons showed that in low-calling rats, chronic stress combined with repeated amphetamine treatment increased dopamine turnover [*striatal DOPAC/DA*: $p < 0.05$ compared with the respective saline-treated group and $p < 0.01$ compared with the corresponding non-stressed group], whereas in high-calling rats, striatal DOPAC was reduced [$p < 0.05$ compared with the respective saline-treated group]. Of note, rats were sacrificed 10–11 days after the last amphetamine administration, suggesting that these effects reflected persistent dopaminergic adaptation. These findings complement those of Chaudhury and colleagues (2013), indicating that the effects of stress on dopaminergic regulation are modulated by pre-existing individual differences. Additionally, the persistent increase in dopamine regulation induced by repeated amphetamine administration in stressed LC rats was accompanied with reduced locomotor and 50-kHz vocalization activity during the challenge day of psychostimulant administration (Day 14). The corresponding group of HC rats did not show similar pattern. Overall, these observations indicate a stress-induced adaptations in the dopaminergic system, which in turn, changes how psychostimulant motivating and rewarding effects are translated into relevant behaviour.

In **Study III**, a related phenotype-dependent pattern was seen when rats were stratified by hedonic capacity. In HSuc rats, chronic stress elevated dopamine turnover in the striatum, but this effect was not apparent when these rats received repeated amphetamine treatment [*Stress × Amphetamine* $F(1,32)=6.6$, $p < 0.05$]. Behaviourally the same group demonstrated psychostimulant sensitisation in 50-kHz vocalizations and rearings, but not line crossings. In contrast, corresponding LSuc group had increased turnover of dopamine, serotonin, and noradrenaline in nucleus accumbens following stress and repeated amphetamine treatment [*dopamine: Sucrose × Stress × Amphetamine* $F(1,32)=6.1$, $p < 0.05$; *serotonin: Sucrose × Stress × Amphetamine* $F(1,30)=5.8$, $p < 0.05$; *noradrenaline: Stress × Amphetamine* $F(1,32)=4.5$, $p < 0.05$]. This group demonstrated sensitisation in line crossings and rearings, but not in 50-kHz vocalisations. Again, stress led to distinct behavioural expression of psychostimulant reward, which was modulated by individual differences.

Previous studies have shown that enhanced dopaminergic transmission within the nucleus accumbens region can elicit or facilitate 50-kHz vocalisations. In this respect, **Study I** and **III** extends the pattern by showing that stress-induced monoaminergic adaptation can change how psychostimulant rewards are behaviourally

expressed. While we did not directly distinguish the motivational and hedonic aspects of reward, the observed patterns are consistent with the view that dopaminergic signalling contributes primarily to motivational and incentive processes rather than directly encoding hedonic experience (Berridge & Robinson, 1998; Ikemoto & Panksepp, 1999; Salamone & Correa, 2024).

Study IV did not include repeated amphetamine treatment, but the drug was administered once to elicit calling and locomotor activity. Under these conditions, the clearest dopaminergic effect of stress emerged in the striatum, whereas no corresponding stress effect was detected in the nucleus accumbens. Chronic stress reduced dopamine metabolism most clearly in LC/HSuc rats [*striatal HVA: USV* $\times$ *Stress* $F(1,68)=5.82, p<0.05$, with a post hoc reduction of $p<0.01$ relative to the respective control group; *total dopamine metabolite/DA ratio: Stress* $F(1,68)=4.52, p<0.05$, with a post hoc reduction of $p<0.05$]. This neurochemical change occurred in the same phenotype that showed reduced 50-kHz vocalisation in response to acute amphetamine ($p<0.005$ relative to the respective control group) and higher expression of anxiety-like behaviour in the elevated-plus maze [*open/total arm-entry ratio: p* <0.05 relative to the respective control group]. No comparable reduction in striatal dopamine metabolism or amphetamine-elicited 50-kHz vocalisation was observed in the remaining phenotype groups.

Chronic stress also produced a more general reduction in serotonin turnover in the striatum across phenotypes [*Stress* $F(1,68)=6.43, p<0.05$]. This observation is further augmented by the finding that stress increased Htr2c mRNA expression selectively in the LC/HSuc group [*Stress* $F(1,69)=3.82, p=0.05$; post hoc $p<0.05$ relative to the respective control group]. Altered 5-HT_{2C} receptor function has repeatedly been linked to stress-related behavioural regulation (Higgins & Fletcher, 2003; Strong et al., 2011), to the control of striatal dopaminergic transmission (Di Matteo et al., 1999; Lucas & Spampinato, 2000), and to inhibition of 50-kHz vocalisation (Wöhr et al., 2015). Taken together, these findings suggest that serotonergic regulation, particularly through 5-HT_{2C}-related mechanisms, may contribute to the stress-sensitive striatal phenotype expressed most clearly in LC/HSuc rats.

4.4.2 Monoamine regulation beyond the core reward system

While the clearest phenotype-dependent monoaminergic effects were observed in the striatum and nucleus accumbens, the broader integration of reward into behaviour depends on a wider network of brain structures in mammals (Otis et al., 2017; Yankouskaya et al., 2022). Among these, the hypothalamus is of particular importance, as it occupies a central role in the stress response (Ulrich-Lai & Herman, 2009) and may itself undergo adaptation under chronic conditions. In **Study IV**, HC/HSuc rats showed lower dopamine levels under control conditions in the hypothalamus, an effect linked to hedonic phenotype and not present in LC rats [*dopamine: Sucrose* $\times$ *Stress* $F(1,68)=6.20, p<0.05$; post hoc $p<0.05$ compared with the control HC/LSuc group]. This difference was no longer apparent after stress exposure. In the same phenotype, chronic stress also reduced

hypothalamic 5-HIAA levels [*5-HIAA: Stress* $F(1,68)=9.12$, $p<0.01$; post hoc $p<0.05$ compared with the respective control group], suggesting altered serotonin turnover, whereas no comparable effect was observed in LC/HSuc rats.

A similarly selective pattern was seen in the frontal cortex and amygdala. In HC/HSuc rats, chronic stress increased normetanephrine levels in the frontal cortex (post hoc $p<0.01$ compared with the respective control group), while no such increase was present in stressed LC/HSuc animals. By contrast, stressed LC/HSuc rats showed a marked reduction of 5-HIAA both in the frontal cortex ($p<0.01$) and in the amygdala ($p<0.001$, compared with the respective control groups). More broadly, dopamine turnover in the amygdala was reduced in LC rats under stress [*USV × Stress* $F(1,69)=4.58$, $p<0.05$; post hoc $p<0.05$ in both LC/HSuc and LC/LSuc rats compared with their respective control groups].

The frontal cortex and amygdala are both involved in evaluating affectively salient stimuli and in shaping appropriate behavioural responses, including those related to reward and stress (Otis et al., 2017; Godoy et al., 2018). In this context, the monoaminergic changes observed in **Study IV** are consistent with the broader behavioural profile of the LC/HSuc phenotype. In stressed LC/HSuc rats, reduced 5-HIAA levels in both the frontal cortex and amygdala, together with reduced amygdalar dopamine turnover, were accompanied by higher anxiety-like behaviour in the elevated plus-maze and reduced amphetamine-elicited 50-kHz vocalisation. This pattern suggests that the phenotype most susceptible to the effects of chronic stress was not distinguished by striatal changes alone, but also by alterations in corticolimbic regions involved in affective regulation and behavioural control. The relevance of the amygdala is supported by earlier work showing that stress strongly engages amygdalar dopaminergic activity (Coco et al., 1992; Inglis & Moghaddam, 1999; Kienast et al., 2008), and more recent work further places amygdalar dopamine within the circuitry of aversive associative learning (Zafiri & Duvarci, 2022). In contrast, HC/HSuc rats showed a more resilient response profile, with preserved reward-related vocalisation and a lower expression of anxiety-like behaviour under stress.

4.4.3 Phenotype-dependent differences in amino acid homeostasis

In **Studies I, II, and IV**, a selected panel of amino acids was analysed, among which the branched-chain amino acids (BCAAs) emerged as particularly relevant. In the present context, these were specifically isoleucine and leucine. BCAAs are essential amino acids that mammals cannot synthesise in sufficient amounts themselves and must therefore obtain them from the diet (Neinast et al., 2019). They circulate in the blood and enter the brain across the blood-brain barrier mainly via the large neutral amino acid transporter LAT1, where they contribute to protein synthesis, nitrogen shuttling, and glutamate-related metabolism (Sperringer et al., 2017). Importantly, the same transporter also carries the aromatic amino acids tryptophan, tyrosine, and phenylalanine, meaning that BCAAs may compete with monoamine precursors for transport into the brain (Zaragoza, 2020).

Study I showed that HC rats had higher baseline concentrations of isoleucine than LC rats in the frontal cortex [$USV F(1,34)=5.3, p<0.05$], and in **Study IV** a similar phenotype-related difference was observed in the hippocampus [$USV F(1,70)=3.99, p<0.05$]. This finding aligns with the notion that circulating BCAA levels are normally maintained within a relatively stable range and are under homeostatic control (Neinast et al., 2019). Such a pattern may reflect individual differences in baseline amino-acid utilisation and cellular metabolism, with these differences also detectable in the brain. At present, the functional significance of isoleucine in the frontal cortex remains unclear, as research on amino-acid involvement in brain function is still limited.

Previous work has suggested that stress-related conditions may be associated with altered brain BCAA-related metabolism. For example, Chen et al. (2015) reported lower isoleucine in the frontal cortex in a rat model of depression, and Nasrallah et al. (Nasrallah et al., 2019) showed that experimental BCAA administration attenuated certain adverse consequences of chronic social defeat stress. In the present studies, however, stress did not produce new phenotype-dependent differences in brain BCAA levels; rather, the pre-existing baseline difference in isoleucine was no longer evident after stress exposure. Thus, the present results do not support a simple model in which stress consistently increases or decreases brain BCAAs. At the same time, the repeated observation of higher baseline isoleucine in HC rats is broadly consistent with the possibility that elevated isoleucine is associated with the more resilient phenotype in this model. This interpretation remains tentative and requires further study.

In our studies, higher concentrations of isoleucine or leucine were not generally accompanied by broad changes in monoamine or precursor levels. The clearest relationship emerged in **Study II**, where amino-acid concentrations were inversely correlated with serotonin turnover in the hippocampus under SSRI treatment, specifically in unstressed fluoxetine-treated rats that had received repeated amphetamine treatment [*isoleucine*: $r=-0.527$; *leucine*: $r=-0.412$]. No significant correlations were observed in the other treatment groups. This supports previous work, as it has been previously reported that SSRI treatment can cause alterations in levels of lipids, lipid metabolism-related molecules and amino acids in antidepressant-treated astrocytes (Bai et al., 2015), and in certain parameters, can cause a 50–70% increase in BCAA concentrations (Webhofer et al., 2011).

However, in our study series, the effect of SSRI treatment on BCAA concentrations was not uniform. Between-group analysis revealed a complex, phenotype-dependent pattern of changes associated with fluoxetine treatment, repeated amphetamine administration, and prior stress exposure. Frontal cortical isoleucine was affected by *Fluoxetine* [$F(1,44)=4.09, p<0.05$] and by the *Stress* $\times$ *Amphetamine* interaction [$F(1,44)=4.44, p<0.05$]. In the frontal cortex, isoleucine was increased in HC rats after fluoxetine and subsequent repeated amphetamine treatment, and this effect was evident only in the stressed group, suggesting that prior stress modified the amino-acid response to pharmacological challenge ($p<0.05$ compared with both the respective vehicle-treated and saline-treated groups). In

contrast, in LC rats, stress itself was associated with increased isoleucine ($p < 0.05$ compared with the corresponding non-stressed group), whereas neither fluoxetine nor amphetamine produced a comparable significant effect. A similarly selective pattern was observed for leucine, which was increased in the same combined treatment condition in HC rats, but not in LC animals [$USV \times Fluoxetine$ $F(1,46)=6.28$, $p < 0.05$; $USV \times Amphetamine$ $F(1,46)=5.38$, $p < 0.05$; $Stress \times Amphetamine$ $F(1,46)=4.65$, $p < 0.05$]. In amphetamine-treated HC rats, the fluoxetine-related increase was significant in both the control and stressed groups ($p < 0.05$); within stressed fluoxetine-treated HC rats, leucine was also higher after amphetamine than saline ($p < 0.01$). No significant leucine effects emerged in LC rats.

In the hippocampus, BCAA levels again changed in a phenotype-dependent manner. Increases in isoleucine and leucine were observed in HC rats under selected fluoxetine- and amphetamine-related treatment conditions [isoleucine: $USV \times Fluoxetine$ $F(1,45)=4.6$, $p < 0.05$, with fluoxetine increasing isoleucine in non-stressed HC rats ($p < 0.05$); leucine: $USV \times Stress \times Fluoxetine \times Amphetamine$ $F(1,45)=4.8$, $p < 0.05$, with leucine increased in non-stressed fluoxetine-treated HC rats receiving repeated amphetamine ($p < 0.01$)]. No corresponding fluoxetine-related increase was seen in LC rats; instead, non-stressed LC rats showed increased hippocampal isoleucine after repeated amphetamine treatment ($p < 0.05$). Thus, the effects of fluoxetine and amphetamine on BCAA concentrations were conditional on both prior stress exposure and the inherent positive-affect phenotype, rather than representing a uniform pharmacological effect.

The systemic physiological effects of chronic stress are well established (Tamashiro et al., 2011), and in the present study series chronic stress consistently altered weight gain relative to the corresponding non-stressed groups. **Study IV** extended this perspective by including peripheral indices of stress physiology, namely body weight-adjusted adrenal gland weight [$Sucrose \times Stress$ $F(1,72)=10.8$, $p < 0.01$; $USV \times Sucrose$ $F(1,72)=4.96$, $p < 0.05$] and serum glucose [$USV \times Sucrose \times Stress$ $F(1,72)=4.89$, $p < 0.05$; $USV \times Sucrose$ $F(1,72)=9.46$, $p < 0.01$]. Together with the amino-acid findings, these results indicate that phenotype-dependent adaptation to chronic stress is not confined to reward-related behaviour or monoaminergic regulation, but is also expressed in selected metabolic and peripheral physiological measures. However, these findings should be interpreted as evidence for broader biological involvement rather than as a mechanistic explanation of metabolic or endocrine regulation. The present studies demonstrate differential phenotype-dependent biological responses to chronic stress, but more detailed conclusions about the underlying regulatory mechanisms require further targeted investigation.

5. CONCLUSIONS

The present dissertation investigates how stable individual differences in positive affectivity and hedonic capacity contribute to the heterogeneous and sometimes apparently opposing effects of chronic stress on reward-related behaviour and neurobiology. Across the individual studies, chronic stress attenuated reward-related responses in some experimental contexts, whereas in others it potentiated them. The direction and magnitude of these effects varied according to pre-existing reward-related phenotype, indexed by positive affectivity through tickling-evoked 50-kHz ultrasonic vocalisations and by hedonic capacity through stable sucrose consumption.

One of the central findings is that positive affectivity and hedonic capacity describe related but distinct dimensions of reward-related regulation. Positive affectivity reflects the propensity to generate and express positive-valence responses, whereas stable sucrose consumption reflects a complementary dimension of reward consumption and pleasure-related responsiveness. The weak, non-significant association between tickling-evoked 50-kHz vocalisations and sucrose consumption, together with their different patterns of stress responsiveness, indicates that these phenotypes do not represent the same underlying characteristic under different labels. Low positive affectivity was primarily associated with attenuation of reward-related responding following chronic stress. By comparison, stressed HSuc rats showed sensitisation of 50-kHz vocalisations and rearings, whereas stressed LSuc rats showed sensitisation in line crossings and rearings but not in 50-kHz vocalisations. These patterns indicate that reward-related function is multidimensional and that pre-existing differences in its components modulate how psychostimulant reward-related responsiveness is behaviourally expressed following chronic stress.

The combined assessment of positive affectivity and hedonic capacity confirmed low positive affectivity as a phenotype associated with increased susceptibility to chronic stress and further demonstrated that this vulnerability was most pronounced when low positive affectivity coincided with high sucrose consumption. In LC/HSuc rats, chronic stress reduced amphetamine-elicited 50-kHz vocalisation and increased anxiety-like behaviour, accompanied by phenotype-dependent changes in striatal and corticolimbic monoamine neurochemistry. LC/LSuc rats did not show the same convergence of reduced reward-related vocalisation and increased anxiety-like behaviour, whereas HC/HSuc animals maintained reward-related vocalisation and showed lower anxiety-like behaviour under stress. Thus, high sucrose consumption identified the subgroup in which the established vulnerability associated with low positive affectivity was expressed most clearly, while high positive affectivity was associated with a more preserved response profile even among HSuc animals.

The findings also indicate that behavioural and monoaminergic outcomes following chronic stress and repeated amphetamine exposure were not necessarily concordant. In Study I, stressed LC rats showed increased striatal dopamine

turnover despite reduced amphetamine-elicited vocalisation and locomotor activity. In Study III, stressed LSuc rats showed increased accumbal dopamine, serotonin, and noradrenaline turnover together with sensitisation in line crossings and rearings, but without sensitisation of 50-kHz vocalisations. Thus, monoaminergic adaptation did not map directly onto the magnitude of reward-related responding, but was associated with different behavioural expressions of psychostimulant responsivity depending on phenotype and treatment history.

The pharmacological findings further indicate that, in the present model, long-term fluoxetine treatment did not exert a uniform normalising effect on subsequent reward-related responsivity. Fluoxetine modified the later response to amphetamine in a manner that depended on positive-affectivity phenotype. In stressed LC rats, sensitisation of amphetamine-elicited 50-kHz vocalisations emerged after fluoxetine pretreatment under conditions in which it was otherwise absent. In fluoxetine-pretreated stressed animals, repeated amphetamine reduced hippocampal serotonin turnover in HC rats but increased it in LC rats, while selected amino-acid responses also depended on phenotype and treatment history. Thus, the behavioural and biochemical consequences of long-term fluoxetine treatment were conditional on positive-affectivity phenotype, prior stress exposure, and the subsequent pharmacological challenge.

Phenotype-dependent effects extended beyond the striatum and nucleus accumbens to monoaminergic measures in the frontal cortex, amygdala, hypothalamus, and hippocampus, as well as to selected amino-acid and peripheral physiological measures. LC/HSuc rats showed the clearest adverse profile, including reduced frontal cortical and amygdalar serotonergic indices, reduced amygdalar dopamine turnover, and pronounced alterations in body-weight dynamics. HC/HSuc rats displayed a distinct pattern involving frontal cortical noradrenergic, hypothalamic monoaminergic, serum glucose, and adrenal responses, while higher baseline isoleucine was associated more consistently with the HC phenotype. Thus, individual differences in stress adaptation were expressed across central and peripheral systems rather than within an isolated reward pathway.

Overall, the present dissertation supports the view that adaptation to chronic stress depends on the pre-existing organisation of reward-related functions. Low positive affectivity emerged as a recurring vulnerability phenotype, while its co-occurrence with high sucrose consumption was associated with the clearest stress-sensitive profile. Positive affectivity and hedonic capacity therefore represent distinct but jointly informative dimensions that help explain why subsequent reward-related responses can be attenuated, preserved, or potentiated. At the same time, the non-concordance between behavioural and monoaminergic outcomes shows that stress adaptation cannot be described as a simple increase or decrease in monoaminergic reward-system function. The principal contribution of this work is to demonstrate that apparently contrasting outcomes of chronic stress can arise systematically from individual differences in reward-related regulation, the particular component of reward being assessed, and the context in which the adapted system is subsequently challenged.

5.1 Limitations

The results of this dissertation should be interpreted according to what the experimental designs and method selection allow. All studies were conducted in male Wistar rats. The conclusions therefore concern phenotype-dependent adaptation to chronic stress in male rats under the experimental conditions applied. Whether the same patterns occur in female rats was not examined in the present study series. This restricts the generalisation of the findings beyond male rats but does not alter their interpretation within the experimental model used here.

A second limitation is the modest number of animals in individual experimental groups. This was particularly relevant in studies that included several independent variables and multiple phenotype, stress, and pharmacological treatment conditions. The group sizes reflected the complexity of the experimental designs and the need to maintain controlled environmental conditions. The main conclusions are based primarily on robust phenotype-dependent effects and on related patterns observed across the study series, whereas individual subgroup comparisons and exploratory biochemical findings should be interpreted with appropriate caution.

Finally, the monoamine analyses were based on endpoint tissue samples collected after the behavioural and pharmacological procedures. Tissue concentrations of monoamines and their metabolites, together with calculated turnover indices, provide endpoint measures of regional monoamine content and metabolism but do not reveal the dynamic processes or exact mechanisms that produced the observed differences. The biochemical findings should therefore be interpreted as regional and phenotype-dependent correlates of prior stress and treatment history rather than as direct evidence of a specific causal mechanism. Within this scope, the analyses were informative in identifying the magnitude and direction of biological differences associated with the behavioural phenotypes.

Overall, these limitations define the scope of the present conclusions without changing the principal finding that stable individual differences in positive affectivity and sucrose consumption were associated with distinct behavioural and neurochemical responses to chronic stress. The conclusions apply to male rats under the experimental conditions used and concern phenotype-dependent behavioural outcomes and their endpoint biological correlates rather than definitive causal mechanisms.

5.2 Future directions

The present studies identified distinct phenotype-dependent behavioural, neurochemical, and physiological outcomes of chronic stress but did not determine at which stage of adaptation these differences became expressed. Future work should therefore establish whether vulnerable and more resilient phenotypes diverge in their initial stress reactivity, during repeated adaptation, in the termination and restoration of stress-activated systems, or in their later response to

reward-related and pharmacological challenges. An hypothesis can be drawn that phenotype-dependent vulnerability may arise partly from differences in the restoration of neural and physiological regulation after repeated stress, rather than solely from differences in the magnitude of the initial response. This possibility should be examined alongside the alternative that the phenotypes differ already in their acute stress reactivity. The theoretical framework of this dissertation identifies efficient termination and recovery as central components of stress adaptation, while the present endpoint findings provide the phenotypic outcomes whose development remains to be explained.

Defining the stage at which phenotype-dependent divergence occurs would provide the basis for targeted investigation of the mechanisms responsible for these outcomes. Initial studies should compare the phenotypes at selected phases of stress exposure and recovery to identify biological processes that are activated similarly, restored at different rates, or remain persistently altered. Candidate processes should then be examined experimentally to determine whether they contribute causally to the later behavioural phenotype. Particular attention should be directed to the regulation of striatal and corticolimbic monoamine systems and to the mechanisms underlying the inconsistent correspondence between monoaminergic measures and reward-related behaviour. In LC/HSuc rats, the combination of reduced striatal dopamine metabolism, increased striatal Htr2c expression, reduced reward-related vocalisation, and increased anxiety-like behaviour identifies serotonergic regulation of dopaminergic function as one specific candidate mechanism. Future work should establish whether 5-HT_{2C}-related signalling contributes to the development or maintenance of this stress-sensitive profile, or whether the serotonergic and dopaminergic changes represent parallel consequences of a broader adaptive process.

The interaction between long-term antidepressant treatment and subsequent psychostimulant responsivity requires separate investigation. In the present study series, fluoxetine did not restore a uniform control-like reward response. In stressed LC rats, sensitisation of amphetamine-elicited 50-kHz vocalisations emerged after fluoxetine pretreatment under conditions in which it was otherwise absent, while the subsequent hippocampal serotonergic and amino-acid responses also depended on positive affectivity phenotype. These findings raise the possibility that chronic serotonergic treatment produces persistent changes in how a stress-adapted reward system responds to later dopaminergic stimulation. Future experiments should distinguish the contributions of stress exposure, fluoxetine treatment, treatment withdrawal, and possibly subsequent reward challenge, and determine when the phenotype-dependent divergence in behavioural responsivity develops. This interaction is relevant to the mechanisms of antidepressant action, and also to conditions in which serotonergic antidepressants and amphetamine-based medications are used within the same treatment history.

The role of branched-chain amino acids, particularly isoleucine, should also be investigated more systematically. Higher baseline isoleucine concentrations were observed in HC rats in the frontal cortex and hippocampus, while subsequent effects of stress, fluoxetine, and amphetamine on branched-chain amino

acids were dependent on phenotype and treatment history. These findings identify isoleucine as a candidate correlate of positive affectivity phenotype and stress resilience, but they do not establish its functional significance. The first step should be to determine whether the phenotype association can be replicated across brain regions and in peripheral samples obtained under corresponding conditions. Subsequent studies should examine whether the differences arise from systemic amino-acid availability, transport across the blood-brain barrier, or regional amino-acid utilisation. Subsequently isoleucine availability could be manipulated to determine whether it modifies monoaminergic regulation, recovery from chronic stress, or reward-related behaviour.

A related objective is to clarify how phenotype-dependent neural changes are connected to adaptation elsewhere in the organism. The present findings show that differences in reward-related behaviour and brain neurochemistry occurred together with phenotype-dependent variation in body-weight gain, relative adrenal weight, serum glucose, and selected amino acids. These measures demonstrate that stress adaptation is expressed across central, metabolic, and peripheral physiological systems, but their temporal and causal relationships were not resolved in the present studies. Future studies should determine whether changes in neural regulation precede and contribute to the peripheral outcomes, whether altered metabolic and endocrine conditions modify CNS adaptation, or whether both reflect a common regulatory difference between phenotypes. Combining corresponding brain and peripheral measurements at defined stages of stress exposure and recovery would allow the direction of these relationships to be examined before individual mechanisms are experimentally manipulated.

These directions would extend the present work from the identification of phenotype-dependent outcomes towards a mechanistic account of their development. The central question is not only which biological differences distinguish vulnerable and more resilient animals after chronic stress, but when those differences emerge and which of them determine the subsequent behavioural outcome. Establishing the temporal stage of phenotype divergence, identifying the restorative and regulatory processes involved, and then testing their causal contribution would provide a coherent basis for further investigation of individual vulnerability and resilience to chronic stress.

ACKNOWLEDGEMENTS

Mind tutvustas teadustööle minu vanaema, kelle juhendamisel valmis juba kuuendas klassis minu esimene kodu-uurimistöö. Sellest hetkest kuni praeguseni olen ma teinud uurimistööd erinevatel teemadel. Niisiis võlgnen ma kogu oma karjääri just temale. Ma loodan, et minu õpingute lõppsõna teeb sind uhkeks. Minu õpinguid on alati saatnud toetav pere, kes aastatepikku on üha kasvanud – ilma teieta ei oleks seda tööd ega oskuseid ja teadmisi, mis minu õpingutega kaasnenud on. Erilist tänu väärrib minu ema, kes sa oled kogu selle jama eest tasunud, mind toetanud ja läbi vingerpusside edasi utsitanud. Ilma teieta oleks minust saanud ehitaja, nagu algselt lasteaias plaanisin.

See töö oleks pidanud valmima aastal 2020. Väitekirjale jäi kõige muu tahaplaanile ja uuesti alustada oli raske. See töö ja kraad on minu lubadus minu perele, kes on minu motivatsiooniks kõiges – nii juhilubades, teaduskraadis, karjääris, kui ka hommikul ülesärkamises. Marta-Lisette (VARES), ega ilma sinuta ei oleks siin midagi juhtunud. Sa alustasid Tartu Ülikoolis õpinguid, kui mina lõpetasin magistrantuuri ja said teaduskraadi, kui minul polnud veel viimast väitekirja artiklitki ilmunud. Selle taustal korraldasid sa pulma ja lapse. Kuidas ma saaks olla midagi vähemat sellise kaasa kõrval?

Arkyn (Notsupotsu) kui sa seda kunagi mingil veidral põhjusel loed, siis sa oled minu motivatsioon. Kui sina suudad kõndima õppida, siis mina suudan doktorikraadi ka saada.

Minu juhendajad – Kadri ja Jaanus – aitäh, et tagasite mulle selleks kõigeks võimaluse ning toetasite mind vankumatult lootuses, et see asi ühel päeval lendab.

Kõik minu hunnik sõpru ja Martineid, olgu te mainitud ja kiidetud hoolitsemast minu elu meelelahutusega küllastamisel!

Lõpetuseks tahan ma tänada oma esimest klassijuhatajat Lydia Veerist, kes kujundas suhte minu ja teadmiste, hariduse ning õppimise osas. Ma siiani usun, et ilma tema panuseta ei oleks minust saanud niivõrd teadmiste-himuline inimene nagu ma seda praegu olen.

REFERENCES

- Ahrens, A. M., Nobile, C. W., Page, L. E., Maier, E. Y., Duvauchelle, C. L., & Schallert, T. (2013). Individual differences in the conditioned and unconditioned rat 50-kHz ultrasonic vocalizations elicited by repeated amphetamine exposure. *Psychopharmacology*, 229(4), 687–700. <https://doi.org/10.1007/s00213-013-3130-9>
- Alsö, J., Pickering, C., Roman, E., Hulting, A.-L., Lindblom, J., & Schiöth, H. B. (2009). Motivation for sucrose in sated rats is predicted by low anxiety-like behavior. *Neuroscience Letters*, 454(3), 193–197. <https://doi.org/10.1016/j.neulet.2009.03.045>
- Anderson, J. W. (1954). The Production of Ultrasonic Sounds by Laboratory Rats and Other Mammals. *Science*, 119(3101), 808–809. <https://doi.org/10.1126/science.119.3101.808>
- Bai, S., Zhou, C., Cheng, P., Fu, Y., Fang, L., Huang, W., Yu, J., Shao, W., Wang, X., Liu, M., Zhou, J., & Xie, P. (2015). ¹H NMR-Based Metabolic Profiling Reveals the Effects of Fluoxetine on Lipid and Amino Acid Metabolism in Astrocytes. *International Journal of Molecular Sciences*, 16(4), 8490–8504. <https://doi.org/10.3390/ijms16048490>
- Barfield, R. J., & Thomas, D. A. (1986). The Role of Ultrasonic Vocalizations in the Regulation of Reproduction in Rats. *Annals of the New York Academy of Sciences*, 474(1), 33–43. <https://doi.org/10.1111/j.1749-6632.1986.tb27996.x>
- Berridge, K. C., & Kringelbach, M. L. (2013). Neuroscience of affect: Brain mechanisms of pleasure and displeasure. *Current Opinion in Neurobiology*, 23(3), 294–303. <https://doi.org/10.1016/j.conb.2013.01.017>
- Berridge, K. C., & Kringelbach, M. L. (2015). Pleasure systems in the brain. *Neuron*, 86(3), 646–664. <https://doi.org/10.1016/j.neuron.2015.02.018>
- Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: Hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*, 28(3), 309–369. [https://doi.org/10.1016/S0165-0173\(98\)00019-8](https://doi.org/10.1016/S0165-0173(98)00019-8)
- Berridge, K. C., Venier, I. L., & Robinson, T. E. (1989). Taste reactivity analysis of 6-hydroxydopamine-induced aphagia: Implications for arousal and anhedonia hypotheses of dopamine function. *Behavioral Neuroscience*, 103(1), 36–45. <https://doi.org/10.1037/0735-7044.103.1.36>
- Blanchard, R. J., Blanchard, D. C., Agullana, R., & Weiss, S. M. (1991). Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiology & Behavior*, 50(5), 967–972. [https://doi.org/10.1016/0031-9384\(91\)90423-l](https://doi.org/10.1016/0031-9384(91)90423-l)
- Bogdan, R., & Pizzagalli, D. A. (2006). Acute Stress Reduces Reward Responsiveness: Implications for Depression. *Biological Psychiatry*, 60(10), 1147–1154. <https://doi.org/10.1016/j.biopsych.2006.03.037>
- Brenes, J. C., & Schwarting, R. K. W. (2015). Individual differences in anticipatory activity to food rewards predict cue-induced appetitive 50-kHz calls in rats. *Physiology & Behavior*, 149, 107–118. <https://doi.org/10.1016/j.physbeh.2015.05.012>
- Brennan, K., Roberts, D., Anisman, H., & Merali, Z. (2001). Individual differences in sucrose consumption in the rat: Motivational and neurochemical correlates of hedonia. *Psychopharmacology*, 157(3), 269–276. <https://doi.org/10.1007/s002130100805>
- Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in Motivational Control: Rewarding, Aversive, and Alerting. *Neuron*, 68(5), 815–834. <https://doi.org/10.1016/j.neuron.2010.11.022>

- Brudzynski, S. M. (2015). Pharmacology of Ultrasonic Vocalizations in adult Rats: Significance, Call Classification and Neural Substrate. *Current Neuropharmacology*, 13(2), 180–192. <https://doi.org/10.2174/1570159X13999150210141444>
- Brudzynski, S. M. (2021). Biological Functions of Rat Ultrasonic Vocalizations, Arousal Mechanisms, and Call Initiation. *Brain Sciences*, 11(5). <https://doi.org/10.3390/brainsci11050605>
- Brudzynski, S. M., & Ociepa, D. (1992). Ultrasonic vocalization of laboratory rats in response to handling and touch. *Physiology & Behavior*, 52(4), 655–660. [https://doi.org/10.1016/0031-9384\(92\)90393-G](https://doi.org/10.1016/0031-9384(92)90393-G)
- Brunelli, S. A., Nie, R., Whipple, C., Winiger, V., Hofer, M. A., & Zimmerberg, B. (2006). The effects of selective breeding for infant ultrasonic vocalizations on play behavior in juvenile rats. *Physiology & Behavior*, 87(3), 527–536. <https://doi.org/10.1016/j.physbeh.2005.11.020>
- Brunelli, S. A., Vinocur, D. D., Soo-Hoo, D., & Hofer, M. A. (1997). Five generations of selective breeding for ultrasonic vocalization (USV) responses in N:NIH strain rats. *Developmental Psychobiology*, 31(4), 255–265. [https://doi.org/10.1002/\(SICI\)1098-2302\(199712\)31:4%3C255::AID-DEV3%3E3.0.CO;2-Q](https://doi.org/10.1002/(SICI)1098-2302(199712)31:4%3C255::AID-DEV3%3E3.0.CO;2-Q)
- Burgdorf, J., Brudzynski, S., & Moskal, J. (2020). Using rat ultrasonic vocalization to study the neurobiology of emotion: From basic science to the development of novel therapeutics for affective disorders. *Current Opinion in Neurobiology*, 60, 192–200. <https://doi.org/10.1016/j.conb.2019.12.008>
- Burgdorf, J., Knutson, B., & Panksepp, J. (2000). Anticipation of rewarding electrical brain stimulation evokes ultrasonic vocalization in rats. *Behavioral Neuroscience*, 114(2), 320–327. <https://doi.org/10.1037/0735-7044.114.2.320>
- Burgdorf, J., Knutson, B., Panksepp, J., & Ikemoto, S. (2001). Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. *Behavioral Neuroscience*, 115(4), 940–944. <https://doi.org/10.1037/0735-7044.115.4.940>
- Burgdorf, J., Kroes, R. A., Moskal, J. R., Pfaus, J. G., Brudzynski, S. M., & Panksepp, J. (2008). Ultrasonic vocalizations of rats (*Rattus norvegicus*) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback.. *Journal of Comparative Psychology*, 122(4), 357–367. <https://doi.org/10.1037/a0012889>
- Burgdorf, J., & Panksepp, J. (2001). Tickling induces reward in adolescent rats. *Physiology & Behavior*, 72(1), 167–173. [https://doi.org/10.1016/S0031-9384\(00\)00411-X](https://doi.org/10.1016/S0031-9384(00)00411-X)
- Burgdorf, J., Panksepp, J., Brudzynski, S. M., Beinfeld, M. C., Cromwell, H. C., Kroes, R. A., & Moskal, J. R. (2009). The effects of selective breeding for differential rates of 50-kHz ultrasonic vocalizations on emotional behavior in rats. *Developmental Psychobiology*, 51(1), 34–46. <https://doi.org/10.1002/dev.20343>
- Burgdorf, J., Panksepp, J., Brudzynski, S. M., Kroes, R., & Moskal, J. R. (2005). Breeding for 50-kHz Positive Affective Vocalization in Rats. *Behavior Genetics*, 35(1), 67–72. <https://doi.org/10.1007/s10519-004-0856-5>
- Burgdorf, J., Wood, P. L., Kroes, R. A., Moskal, J. R., & Panksepp, J. (2007). Neurobiology of 50-kHz ultrasonic vocalizations in rats: Electrode mapping, lesion, and pharmacology studies. *Behavioural Brain Research, Mammalian Vocalization: Neural, Behavioural, and Environmental Determinants*, 182(2), 274–283. <https://doi.org/10.1016/j.bbr.2007.03.010>

- Chaijale, N. N., Snyder, K., Arner, J., Curtis, A. L., & Valentino, R. J. (2015). Repeated social stress increases reward salience and impairs encoding of prediction by rat locus coeruleus neurons. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 40(2), 513–523.
<https://doi.org/10.1038/npp.2014.200>
- Chaudhury, D., Walsh, J. J., Friedman, A. K., Juarez, B., Ku, S. M., Koo, J. W., Ferguson, D., Tsai, H.-C., Pomeranz, L., Christoffel, D. J., Nectow, A. R., Ekstrand, M., Domingos, A., Mazie-Robison, M., Mouzon, E., Lobo, M. K., Neve, R. L., Friedman, J. M., Russo, S. J., ... Han, M.-H. (2013). Rapid regulation of depression-related behaviors by control of midbrain dopamine neurons. *Nature*, 493(7433), 532–536.
<https://doi.org/10.1038/nature11713>
- Chen, G., Yang, D., Yang, Y., Li, J., Cheng, K., Tang, G., Zhang, R., Zhou, J., Li, W., Liu, Z., Fan, S., & Xie, P. (2015). Amino acid metabolic dysfunction revealed in the prefrontal cortex of a rat model of depression. *Behavioural Brain Research*, 278, 286–292. <https://doi.org/10.1016/j.bbr.2014.05.027>
- Coco, M. L., Kuhn, C. M., Ely, T. D., & Kilts, C. D. (1992). Selective activation of mesoamygdaloid dopamine neurons by conditioned stress: Attenuation by diazepam. *Brain Research*, 590(1), 39–47. [https://doi.org/10.1016/0006-8993\(92\)91079-T](https://doi.org/10.1016/0006-8993(92)91079-T)
- Corral-Frias, N. S., Nikolova, Y. S., Michalski, L. J., Baranger, D. a. A., Hariri, A. R., & Bogdan, R. (2015). Stress-related anhedonia is associated with ventral striatum reactivity to reward and transdiagnostic psychiatric symptomatology. *Psychological Medicine*, 45(12), 2605–2617. <https://doi.org/10.1017/S0033291715000525>
- Davidson, R. J. (2000). Affective style, psychopathology, and resilience: Brain mechanisms and plasticity. *American Psychologist*, 55(11), 1196–1214.
<https://doi.org/10.1037/0003-066X.55.11.1196>
- de Kloet, E. R., Karst, H., & Joëls, M. (2008). Corticosteroid hormones in the central stress response: Quick-and-slow. *Frontiers in Neuroendocrinology, Rapid Actions of Steroid Hormones*, 29(2), 268–272. <https://doi.org/10.1016/j.yfrne.2007.10.002>
- Desousa, N. J., Wunderlich, G. R., De Cabo, C., & Vaccarino, F. J. (1998). Individual Differences in Sucrose Intake Predict Behavioral Reactivity in Rodent Models of Anxiety. *Pharmacology Biochemistry and Behavior*, 60(4), 841–846.
[https://doi.org/10.1016/S0091-3057\(98\)00069-0](https://doi.org/10.1016/S0091-3057(98)00069-0)
- Di Matteo, V., Di Giovanni, G., Di Mascio, M., & Esposito, E. (1999). SB 242 084, a selective serotonin_{2C} receptor antagonist, increases dopaminergic transmission in the mesolimbic system. *Neuropharmacology*, 38(8), 1195–1205.
[https://doi.org/10.1016/S0028-3908\(99\)00047-7](https://doi.org/10.1016/S0028-3908(99)00047-7)
- Duncko, R., Schwendt, M., & Jezova, D. (2003). Altered glutamate receptor and corticoliberin gene expression in brain regions related to hedonic behavior in rats. *Pharmacology Biochemistry and Behavior*, 76(1), 9–16.
[https://doi.org/10.1016/S0091-3057\(03\)00164-3](https://doi.org/10.1016/S0091-3057(03)00164-3)
- Flügge, G., van Kampen, M., & Mijster, M. J. (2004). Perturbations in brain monoamine systems during stress. *Cell and Tissue Research*, 315(1), 1–14.
<https://doi.org/10.1007/s00441-003-0807-0>
- Godoy, L. D., Rossignoli, M. T., Delfino-Pereira, P., Garcia-Cairasco, N., & de Lima Umeoka, E. H. (2018). A Comprehensive Overview on Stress Neurobiology: Basic Concepts and Clinical Implications. *Frontiers in Behavioral Neuroscience*, 12. <https://doi.org/10.3389/fnbeh.2018.00127>
- Gorwood, P. (2008). Neurobiological mechanisms of anhedonia. *Dialogues in Clinical Neuroscience*, 10(3), 291–299. <https://doi.org/10.31887/DCNS.2008.10.3/pgorwood>

- Hamann, S., & Canli, T. (2004). Individual differences in emotion processing. *Current Opinion in Neurobiology*, 14(2), 233–238. <https://doi.org/10.1016/j.conb.2004.03.010>
- Harmon, K. M., Cromwell, H. C., Burgdorf, J., Moskal, J. R., Brudzynski, S. M., Kroes, R. A., & Panksepp, J. (2008). Rats selectively bred for low levels of 50 kHz ultrasonic vocalizations exhibit alterations in early social motivation. *Developmental Psychobiology*, 50(4), 322–331. <https://doi.org/10.1002/dev.20294>
- Harro, J. (2010). Inter-individual differences in neurobiology as vulnerability factors for affective disorders: Implications for psychopharmacology. *Pharmacology & Therapeutics*, 125(3), 402–422. <https://doi.org/10.1016/j.pharmthera.2009.11.006>
- Harro, J., Tönissaar, M., Eller, M., Kask, A., & Oreland, L. (2001). Chronic variable stress and partial 5-HT denervation by parachloroamphetamine treatment in the rat: Effects on behavior and monoamine neurochemistry. *Brain Research*, 899(1), 227–239. [https://doi.org/10.1016/S0006-8993\(01\)02256-9](https://doi.org/10.1016/S0006-8993(01)02256-9)
- Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., & Myers, B. (2016). Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Comprehensive Physiology*, 6(2), 603–621. <https://doi.org/10.1002/cphy.c150015>
- Higgins, G. A., & Fletcher, P. J. (2003). Serotonin and drug reward: Focus on 5-HT_{2C} receptors. *European Journal of Pharmacology*, 480(1–3), 151–162. <https://doi.org/10.1016/j.ejphar.2003.08.102>
- Hill, M. N., Hellems, K. G. C., Verma, P., Gorzalka, B. B., & Weinberg, J. (2012). Neurobiology of chronic mild stress: Parallels to major depression. *Neuroscience and Biobehavioral Reviews*, 36(9), 2085–2117. <https://doi.org/10.1016/j.neubiorev.2012.07.001>
- Hitoshi, S., Maruta, N., Higashi, M., Kumar, A., Kato, N., & Ikenaka, K. (2007). Antidepressant drugs reverse the loss of adult neural stem cells following chronic stress. *Journal of Neuroscience Research*, 85(16), 3574–3585. <https://doi.org/10.1002/jnr.21455>
- Holly, E. N., & Miczek, K. A. (2016). Ventral tegmental area dopamine revisited: Effects of acute and repeated stress. *Psychopharmacology*, 233(2), 163–186. <https://doi.org/10.1007/s00213-015-4151-3>
- Huys, Q. J., Pizzagalli, D. A., Bogdan, R., & Dayan, P. (2013). Mapping anhedonia onto reinforcement learning: A behavioural meta-analysis. *Biology of Mood & Anxiety Disorders*, 3(1), 12. <https://doi.org/10.1186/2045-5380-3-12>
- Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: A unifying interpretation with special reference to reward-seeking. *Brain Research. Brain Research Reviews*, 31(1), 6–41. [https://doi.org/10.1016/s0165-0173\(99\)00023-5](https://doi.org/10.1016/s0165-0173(99)00023-5)
- Inglis, F. M., & Moghaddam, B. (1999). Dopaminergic Innervation of the Amygdala Is Highly Responsive to Stress. *Journal of Neurochemistry*, 72(3), 1088–1094. <https://doi.org/10.1046/j.1471-4159.1999.0721088.x>
- Ironside, M., Kumar, P., Kang, M.-S., & Pizzagalli, D. A. (2018). Brain mechanisms mediating effects of stress on reward sensitivity. *Current Opinion in Behavioral Sciences, Apathy and Motivation*, 22, 106–113. <https://doi.org/10.1016/j.cobeha.2018.01.016>
- Joëls, M., & Baram, T. Z. (2009). The neuro-symphony of stress. *Nature Reviews Neuroscience*, 10(6), 459–466. <https://doi.org/10.1038/nrn2632>

- Kalivas, P. W., & Stewart, J. (1991). Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Research Reviews*, 16(3), 223–244. [https://doi.org/10.1016/0165-0173\(91\)90007-U](https://doi.org/10.1016/0165-0173(91)90007-U)
- Kanarik, M., Althoff, A., Matrov, D., Kõiv, K., Sharp, T., Panksepp, J., & Harro, J. (2011). Brain responses to chronic social defeat stress: Effects on regional oxidative metabolism as a function of a hedonic trait, and gene expression in susceptible and resilient rats. *European Neuropsychopharmacology, NewMood (New Molecules for Mood Disorders)*, 21(1), 92–107. <https://doi.org/10.1016/j.euroneuro.2010.06.015>
- Kelley, A. E. (1998). Measurement of Rodent Stereotyped Behavior. *Current Protocols in Neuroscience*, 4(1), 8.8.1–8.8.13. <https://doi.org/10.1002/0471142301.ns0808s04>
- Kienast, T., Hariri, A. R., Schlagenhauf, F., Wrase, J., Sterzer, P., Buchholz, H. G., Smolka, M. N., Gründer, G., Cumming, P., Kumakura, Y., Bartenstein, P., Dolan, R. J., & Heinz, A. (2008). Dopamine in amygdala gates limbic processing of aversive stimuli in humans. *Nature Neuroscience*, 11(12), 1381–1382. <https://doi.org/10.1038/nn.2222>
- Knutson, B., Burgdorf, J., & Panksepp, J. (1998). Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. *Journal of Comparative Psychology*, 112(1), 65–73. <https://doi.org/10.1037/0735-7036.112.1.65>
- Knutson, B., Burgdorf, J., & Panksepp, J. (1999). High-Frequency Ultrasonic Vocalizations Index Conditioned Pharmacological Reward in Rats. *Physiology & Behavior*, 66(4), 639–643. [https://doi.org/10.1016/S0031-9384\(98\)00337-0](https://doi.org/10.1016/S0031-9384(98)00337-0)
- Knutson, B., Burgdorf, J., & Panksepp, J. (2002). Ultrasonic vocalizations as indices of affective states in rats. *Psychological Bulletin*, 128(6), 961–977. <https://doi.org/10.1037/0033-2909.128.6.961>
- Kõiv, K., Metelitsa, M., Vares, M., Tiitsaar, K., Raudkivi, K., Jaako, K., Vulla, K., Shimmo, R., & Harro, J. (2016). Chronic variable stress prevents amphetamine-elicited 50-kHz calls in rats with low positive affectivity. *European Neuropsychopharmacology*, 26(4), 631–643. <https://doi.org/10.1016/j.euroneuro.2016.02.011>
- Kõiv, K., Vares, M., Kroon, C., Metelitsa, M., Tiitsaar, K., Laugus, K., Jaako, K., & Harro, J. (2019). Effect of chronic variable stress on sensitization to amphetamine in high and low sucrose-consuming rats. *Journal of Psychopharmacology*, 33(12), 1512–1523. <https://doi.org/10.1177/0269881119856000>
- Kroes, R. A., Burgdorf, J., Otto, N. J., Panksepp, J., & Moskal, J. R. (2007). Social defeat, a paradigm of depression in rats that elicits 22-kHz vocalizations, preferentially activates the cholinergic signaling pathway in the periaqueductal gray. *Behavioural Brain Research, Mammalian Vocalization: Neural, Behavioural, and Environmental Determinants*, 182(2), 290–300. <https://doi.org/10.1016/j.bbr.2007.03.022>
- Lin, C.-C., Yang, C.-P., Cheng, P.-Y., Hsiao, M., & Liu, Y.-P. (2023). Escitalopram reversibility of the impacts following chronic stress on central 5-HT profiles – Implications to depression and anxiety. *Behavioural Brain Research*, 453, 114613. <https://doi.org/10.1016/j.bbr.2023.114613>
- Litvin, Y., Blanchard, D. C., & Blanchard, R. J. (2007). Rat 22 kHz ultrasonic vocalizations as alarm cries. *Behavioural Brain Research, Mammalian Vocalization: Neural, Behavioural, and Environmental Determinants*, 182(2), 166–172. <https://doi.org/10.1016/j.bbr.2006.11.038>
- Liu, M.-Y., Yin, C.-Y., Zhu, L.-J., Zhu, X.-H., Xu, C., Luo, C.-X., Chen, H., Zhu, D.-Y., & Zhou, Q.-G. (2018). Sucrose preference test for measurement of stress-induced anhedonia in mice. *Nature Protocols*, 13(7), 1686–1698. <https://doi.org/10.1038/s41596-018-0011-z>

- Lucas, G., & Spampinato, U. (2000). Role of Striatal Serotonin2A and Serotonin2C Receptor Subtypes in the Control of In Vivo Dopamine Outflow in the Rat Striatum. *Journal of Neurochemistry*, 74(2), 693–701. <https://doi.org/10.1046/j.1471-4159.2000.740693.x>
- Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, 10(6), 434–445. <https://doi.org/10.1038/nrn2639>
- Mällo, T., Matrov, D., Herm, L., Kõiv, K., Eller, M., Rinken, A., & Harro, J. (2007). Tickling-induced 50-kHz ultrasonic vocalization is individually stable and predicts behaviour in tests of anxiety and depression in rats. *Behavioural Brain Research*, 184(1), 57–71. <https://doi.org/10.1016/j.bbr.2007.06.015>
- Mällo, T., Matrov, D., Kõiv, K., & Harro, J. (2009). Effect of chronic stress on behavior and cerebral oxidative metabolism in rats with high or low positive affect. *Neuroscience*, 164(3), 963–974. <https://doi.org/10.1016/j.neuroscience.2009.08.041>
- McEwen, B. S. (1998). Stress, adaptation, and disease. Allostasis and allostatic load. *Annals of the New York Academy of Sciences*, 840, 33–44. <https://doi.org/10.1111/j.1749-6632.1998.tb09546.x>
- McEwen, B. S. (2004). Protection and Damage from Acute and Chronic Stress: Allostasis and Allostatic Overload and Relevance to the Pathophysiology of Psychiatric Disorders. *Annals of the New York Academy of Sciences*, 1032(1), 1–7. <https://doi.org/10.1196/annals.1314.001>
- McEwen, B. S., Nasca, C., & Gray, J. D. (2016). Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology*, 41(1), 3–23. <https://doi.org/10.1038/npp.2015.171>
- McEwen, B. S., & Stellar, E. (1993). Stress and the Individual: Mechanisms Leading to Disease. *Archives of Internal Medicine*, 153(18), 2093–2101. <https://doi.org/10.1001/archinte.1993.00410180039004>
- Mulvihill, K. G., & Brudzynski, S. M. (2019). Effect of microinjections of dopamine into the nucleus accumbens shell on emission of 50 kHz USV: Comparison with effects of d-amphetamine. *Pharmacology Biochemistry and Behavior*, 176, 23–32. <https://doi.org/10.1016/j.pbb.2018.11.006>
- Nasrallah, P., Haidar, E. A., Stephan, J. S., El Hayek, L., Karnib, N., Khalifeh, M., Barmo, N., Jabre, V., Houbeika, R., Ghanem, A., Nasser, J., Zeeni, N., Bassil, M., & Sleiman, S. F. (2019). Branched-chain amino acids mediate resilience to chronic social defeat stress by activating BDNF/TRKB signaling. *Neurobiology of Stress*, 11, 100170. <https://doi.org/10.1016/j.ynstr.2019.100170>
- Neinast, M., Murashige, D., & Arany, Z. (2019). Branched Chain Amino Acids. *Annual Review of Physiology*, 81(Volume 81, 2019), 139–164. <https://doi.org/10.1146/annurev-physiol-020518-114455>
- Nesse, R. M. (2002). Is Depression an Adaptation? In *Depression*. Routledge.
- Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative and Physiological Psychology*, 47(6), 419–427. <https://doi.org/10.1037/h0058775>
- Opiol, H., Pavlovski, I., Michalik, M., & Mistlberger, R. E. (2015). Ultrasonic vocalizations in rats anticipating circadian feeding schedules. *Behavioural Brain Research*, 284, 42–50. <https://doi.org/10.1016/j.bbr.2015.02.003>
- Otis, J. M., Namboodiri, V. M. K., Matan, A. M., Voets, E. S., Mohorn, E. P., Kosyk, O., McHenry, J. A., Robinson, J. E., Resendez, S. L., Rossi, M. A., & Stuber, G. D. (2017). Prefrontal cortex output circuits guide reward seeking through divergent cue encoding. *Nature*, 543(7643), 103–107. <https://doi.org/10.1038/nature21376>

- Panksepp, J. (2005). Affective consciousness: Core emotional feelings in animals and humans. *Consciousness and Cognition, Neurobiology of Animal Consciousness*, 14(1), 30–80. <https://doi.org/10.1016/j.concog.2004.10.004>
- Peciña, S., Smith, K. S., & Berridge, K. C. (2006). Hedonic hot spots in the brain. *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, 12(6), 500–511. <https://doi.org/10.1177/1073858406293154>
- Popik, P., Kos, T., Pluta, H., Nikiforuk, A., Rojek, K., & Rygula, R. (2014). Inhibition of the glucocorticoid synthesis reverses stress-induced decrease in rat's 50-kHz ultrasonic vocalizations. *Behavioural Brain Research*, 260, 53–57. <https://doi.org/10.1016/j.bbr.2013.11.029>
- Popik, P., Potasiewicz, A., Pluta, H., & Zieniewicz, A. (2012). High-frequency ultrasonic vocalizations in rats in response to tickling: The effects of restraint stress. *Behavioural Brain Research*, 234(2), 223–227. <https://doi.org/10.1016/j.bbr.2012.06.028>
- Primo, M. J., Fonseca-Rodrigues, D., Almeida, A., Teixeira, P. M., & Pinto-Ribeiro, F. (2023). Sucrose preference test: A systematic review of protocols for the assessment of anhedonia in rodents. *European Neuropsychopharmacology*, 77, 80–92. <https://doi.org/10.1016/j.euroneuro.2023.08.496>
- Puglisi-Allegra, S., & Andolina, D. (2015). Serotonin and stress coping. *Behavioural Brain Research, Special Issue: Serotonin*, 277, 58–67. <https://doi.org/10.1016/j.bbr.2014.07.052>
- Radke, A. K., Holtz, N. A., Gewirtz, J. C., & Carroll, M. E. (2013). Reduced emotional signs of opiate withdrawal in rats selectively bred for low (LoS) versus high (HiS) saccharin intake. *Psychopharmacology*, 227(1), 117–126. <https://doi.org/10.1007/s00213-012-2945-0>
- Ressler, K. J. (2010). Amygdala Activity, Fear, and Anxiety: Modulation by Stress. *Biological Psychiatry*, 67(12), 1117–1119. <https://doi.org/10.1016/j.biopsych.2010.04.027>
- Rippberger, H., van Gaalen, M. M., Schwarting, R. K. W., & Wöhr, M. (2015). Environmental and Pharmacological Modulation of Amphetamine- Induced 50-kHz Ultrasonic Vocalizations in Rats. *Current Neuropharmacology*, 13(2), 220–232. <https://doi.org/10.2174/1570159x1302150525124408>
- Robinson, T. E., & Becker, J. B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Research Reviews*, 11(2), 157–198. [https://doi.org/10.1016/0165-0173\(86\)90002-0](https://doi.org/10.1016/0165-0173(86)90002-0)
- Robinson, T. E., & Berridge, K. C. (2008). The incentive sensitization theory of addiction: Some current issues. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1507), 3137–3146. <https://doi.org/10.1098/rstb.2008.0093>
- Salamone, J. D., & Correa, M. (2024). The Neurobiology of Activational Aspects of Motivation: Exertion of Effort, Effort-Based Decision Making, and the Role of Dopamine. *Annual Review of Psychology*, 75(Volume 75, 2024), 1–32. <https://doi.org/10.1146/annurev-psych-020223-012208>
- Salamone, J. D., Correa, M., Mingote, S., & Weber, S. M. (2003). Nucleus accumbens dopamine and the regulation of effort in food-seeking behavior: Implications for studies of natural motivation, psychiatry, and drug abuse. *The Journal of Pharmacology and Experimental Therapeutics*, 305(1), 1–8. <https://doi.org/10.1124/jpet.102.035063>

- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions*. *Endocrine Reviews*, 21(1), 55–89.
<https://doi.org/10.1210/edrv.21.1.0389>
- Scardochio, T., Trujillo-Pisanty, I., Conover, K., Shizgal, P., & Clarke, P. B. S. (2015). The Effects of Electrical and Optical Stimulation of Midbrain Dopaminergic Neurons on Rat 50-kHz Ultrasonic Vocalizations. *Frontiers in Behavioral Neuroscience*, 9.
<https://doi.org/10.3389/fnbeh.2015.00331>
- Schultz, W., Apicella, P., & Ljungberg, T. (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *Journal of Neuroscience*, 13(3), 900–913.
<https://doi.org/10.1523/JNEUROSCI.13-03-00900.1993>
- Schultz, W., Stauffer, W. R., & Lak, A. (2017). The phasic dopamine signal maturing: From reward via behavioural activation to formal economic utility. *Current Opinion in Neurobiology, Neurobiology of Learning and Plasticity*, 43, 139–148.
<https://doi.org/10.1016/j.conb.2017.03.013>
- Seamans, J. K., & Yang, C. R. (2004). The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Progress in Neurobiology*, 74(1), 1–58.
<https://doi.org/10.1016/j.pneurobio.2004.05.006>
- Sheng, J. A., Bales, N. J., Myers, S. A., Bautista, A. I., Roueifar, M., Hale, T. M., & Handa, R. J. (2021). The Hypothalamic-Pituitary-Adrenal Axis: Development, Programming Actions of Hormones, and Maternal-Fetal Interactions. *Frontiers in Behavioral Neuroscience*, 14, 601939. <https://doi.org/10.3389/fnbeh.2020.601939>
- Sills, T. L., & Crawley, J. N. (1996). Individual differences in sugar consumption predict amphetamine-induced dopamine overflow in nucleus accumbens. *European Journal of Pharmacology*, 303(3), 177–181. [https://doi.org/10.1016/0014-2999\(96\)00161-6](https://doi.org/10.1016/0014-2999(96)00161-6)
- Sills, T. L., Greenshaw, A. J., Baker, G. B., & Fletcher, P. J. (1999). The potentiating effect of sertraline and fluoxetine on amphetamine-induced locomotor activity is not mediated by serotonin. *Psychopharmacology*, 143(4), 426–432.
<https://doi.org/10.1007/s002130050968>
- Sills, T. L., & Vaccarino, F. J. (1994). Individual differences in sugar intake predict the locomotor response to acute and repeated amphetamine administration. *Psychopharmacology*, 116(1), 1–8. <https://doi.org/10.1007/BF02244864>
- Sills, T. L., & Vaccarino, F. J. (1996). Individual differences in sugar consumption following systemic or intraaccumbens administration of low doses of amphetamine in nondeprived rats. *Pharmacology Biochemistry and Behavior*, 54(4), 665–670.
[https://doi.org/10.1016/0091-3057\(96\)00024-X](https://doi.org/10.1016/0091-3057(96)00024-X)
- Sills, T. L., & Vaccarino, F. J. (1998). Individual Differences in the Feeding and Locomotor Stimulatory Effects of Acute and Repeated Morphine Treatments. *Pharmacology Biochemistry and Behavior*, 60(1), 293–303.
[https://doi.org/10.1016/S0091-3057\(97\)00592-3](https://doi.org/10.1016/S0091-3057(97)00592-3)
- Simola, N., & Brudzynski, S. M. (2018). Rat 50-kHz ultrasonic vocalizations as a tool in studying neurochemical mechanisms that regulate positive emotional states. *Journal of Neuroscience Methods*, 310, 33–44.
<https://doi.org/10.1016/j.jneumeth.2018.06.018>
- Simola, N., Fenu, S., Costa, G., Pinna, A., Plumitallo, A., & Morelli, M. (2012). Pharmacological characterization of 50-kHz ultrasonic vocalizations in rats: Comparison of the effects of different psychoactive drugs and relevance in drug-induced reward. *Neuropharmacology*, 63(2), 224–234.
<https://doi.org/10.1016/j.neuropharm.2012.03.013>

- Simola, N., Frau, L., Plumitallo, A., & Morelli, M. (2014). Direct and long-lasting effects elicited by repeated drug administration on 50-kHz ultrasonic vocalizations are regulated differently: Implications for the study of the affective properties of drugs of abuse. *International Journal of Neuropsychopharmacology*, 17(3), 429–441. <https://doi.org/10.1017/S1461145713001235>
- Simola, N., & Morelli, M. (2015). Repeated amphetamine administration and long-term effects on 50-kHz ultrasonic vocalizations: Possible relevance to the motivational and dopamine-stimulating properties of the drug. *European Neuropsychopharmacology*, 25(3), 343–355. <https://doi.org/10.1016/j.euroneuro.2015.01.010>
- Smith, K. S., & Berridge, K. C. (2005). The Ventral Pallidum and Hedonic Reward: Neurochemical Maps of Sucrose “Liking” and Food Intake. *Journal of Neuroscience*, 25(38), 8637–8649. <https://doi.org/10.1523/JNEUROSCI.1902-05.2005>
- Smith, K. S., & Berridge, K. C. (2007). Opioid Limbic Circuit for Reward: Interaction between Hedonic Hotspots of Nucleus Accumbens and Ventral Pallidum. *Journal of Neuroscience*, 27(7), 1594–1605. <https://doi.org/10.1523/JNEUROSCI.4205-06.2007>
- Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral Pallidum Roles in Reward and Motivation. *Behavioural Brain Research*, 196(2), 155–167. <https://doi.org/10.1016/j.bbr.2008.09.038>
- Sohel, A. J., Shutter, M. C., Patel, P., & Molla, M. (2024). Fluoxetine. In *StatPearls [Internet]*. StatPearls Publishing. <https://www.ncbi.nlm.nih.gov/sites/books/NBK459223/>
- Sperringer, J. E., Addington, A., & Hutson, S. M. (2017). Branched-Chain Amino Acids and Brain Metabolism. *Neurochemical Research*, 42(6), 1697–1709. <https://doi.org/10.1007/s11064-017-2261-5>
- Sterling, P., & Eyer, J. (1988). *Allostasis: A New Paradigm to Explain Arousal Pathology*. <https://doi.org/10.5281/ZENODO.13537701>
- Strong, P. V., Christianson, J. P., Loughridge, A. B., Amat, J., Maier, S. F., Fleshner, M., & Greenwood, B. N. (2011). 5-hydroxytryptamine 2C receptors in the dorsal striatum mediate stress-induced interference with negatively-reinforced instrumental escape behavior. *Neuroscience*, 197, 132–144. <https://doi.org/10.1016/j.neuroscience.2011.09.041>
- Tamashiro, K. L., Sakai, R. R., Shively, C. A., Karatsoreos, I. N., & Reagan, L. P. (2011). Chronic stress, metabolism, and metabolic syndrome. *Stress*, 14(5), 468–474. <https://doi.org/10.3109/10253890.2011.606341>
- Taracha, E., Hamed, A., Krząścik, P., Lehner, M., Skórzewska, A., Płaźnik, A., & Chrapusta, S. J. (2012). Inter-individual diversity and intra-individual stability of amphetamine-induced sensitization of frequency-modulated 50-kHz vocalization in Sprague–Dawley rats. *Psychopharmacology*, 222(4), 619–632. <https://doi.org/10.1007/s00213-012-2658-4>
- Taracha, E., Kaniuga, E., Chrapusta, S. J., Maciejak, P., Śliwa, L., Hamed, A., & Krząścik, P. (2014). Diverging frequency-modulated 50-kHz vocalization, locomotor activity and conditioned place preference effects in rats given repeated amphetamine treatment. *Neuropharmacology*, 83, 128–136. <https://doi.org/10.1016/j.neuropharm.2014.04.008>
- Tönissaar, M., Herm, L., Rinken, A., & Harro, J. (2006a). Individual differences in sucrose intake and preference in the rat: Circadian variation and association with dopamine D2 receptor function in striatum and nucleus accumbens. *Neuroscience Letters*, 403(1), 119–124. <https://doi.org/10.1016/j.neulet.2006.04.023>

- Tõnissaar, M., Herm, L., Rinken, A., & Harro, J. (2006b). Individual differences in sucrose intake and preference in the rat: Circadian variation and association with dopamine D2 receptor function in striatum and nucleus accumbens. *Neuroscience Letters*, 403(1), 119–124. <https://doi.org/10.1016/j.neulet.2006.04.023>
- Ulrich-Lai, Y. M., & Herman, J. P. (2009). Neural regulation of endocrine and autonomic stress responses. *Nature Reviews Neuroscience*, 10(6), 397–409. <https://doi.org/10.1038/nrn2647>
- Valentino, R. J., & Van Bockstaele, E. (2008). Convergent regulation of locus coeruleus activity as an adaptive response to stress. *European Journal of Pharmacology, Stress Hormone Actions in Brain, in Health and Disease*, 583(2), 194–203. <https://doi.org/10.1016/j.ejphar.2007.11.062>
- Vanderschuren, L. J. M. J., & Kalivas, P. W. (2000). Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: A critical review of preclinical studies. *Psychopharmacology*, 151(2), 99–120. <https://doi.org/10.1007/s002130000493>
- Vares, M., Kanarik, M., Kõiv, K., Vares, M., Anja, K., Metelitsa, M., Laugus, K., Tigro, H., Korulu, S., Shimmo, R., & Harro, J. (2025). Vulnerability to chronic stress in male rats: Additive effect of low positive affectivity and high hedonic response as measured by sucrose intake. *Behavioural Brain Research*, 494, 115749. <https://doi.org/10.1016/j.bbr.2025.115749>
- Vares, M., Metelitsa, M., Kõiv, K., Kaldmäe, M., Laugus, K., Tiitsaar, K., Shimmo, R., & Harro, J. (2018). Chronic stress sensitizes amphetamine-elicited 50-kHz calls in the rat: Dependence on positive affective phenotype and effects of long-term fluoxetine pretreatment. *Pharmacology Biochemistry and Behavior*, 171, 10–19. <https://doi.org/10.1016/j.pbb.2018.05.014>
- Vyas, A., Mitra, R., Rao, B. S. S., & Chattarji, S. (2002). Chronic Stress Induces Contrasting Patterns of Dendritic Remodeling in Hippocampal and Amygdaloid Neurons. *Journal of Neuroscience*, 22(15), 6810–6818. <https://doi.org/10.1523/JNEUROSCI.22-15-06810.2002>
- Wardak, A. D., Olszyński, K. H., Polowy, R., Matysiak, J., & Filipkowski, R. K. (2024). Rats that learn to vocalize for food reward emit longer and louder appetitive calls and fewer short aversive calls. *PLOS ONE*, 19(2), e0297174. <https://doi.org/10.1371/journal.pone.0297174>
- Webber, E. S., Harmon, K. M., Beckwith, T. J., Peña, S., Burgdorf, J., Panksepp, J., & Cromwell, H. C. (2012). Selective breeding for 50kHz ultrasonic vocalization emission produces alterations in the ontogeny and regulation of rough-and-tumble play. *Behavioural Brain Research*, 229(1), 138–144. <https://doi.org/10.1016/j.bbr.2012.01.012>
- Webhofer, C., Gormanns, P., Tolstikov, V., Zieglgänsberger, W., Sillaber, I., Holsboer, F., & Turck, C. W. (2011). Metabolite profiling of antidepressant drug action reveals novel drug targets beyond monoamine elevation. *Translational Psychiatry*, 1(12), e58–e58. <https://doi.org/10.1038/tp.2011.56>
- Willner, P., Muscat, R., & Papp, M. (1992). Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neuroscience & Biobehavioral Reviews*, 16(4), 525–534. [https://doi.org/10.1016/S0149-7634\(05\)80194-0](https://doi.org/10.1016/S0149-7634(05)80194-0)
- Willner, P., Scheel-Krüger, J., & Belzung, C. (2013). The neurobiology of depression and antidepressant action. *Neuroscience & Biobehavioral Reviews, Discovery Research in Neuropsychiatry - Anxiety, Depression and Schizophrenia in Focus*, 37(10, Part 1), 2331–2371. <https://doi.org/10.1016/j.neubiorev.2012.12.007>

- Willner, P., Towell, A., Sampson, D., Sophokleous, S., & Muscat, R. (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology*, 93(3), 358–364. <https://doi.org/10.1007/BF00187257>
- Wise, R. A. (1978). Catecholamine theories of reward: A critical review. *Brain Research*, 152(2), 215–247. [https://doi.org/10.1016/0006-8993\(78\)90253-6](https://doi.org/10.1016/0006-8993(78)90253-6)
- Wise, R. A. (1980). The dopamine synapse and the notion of ‘pleasure centers’ in the brain. *Trends in Neurosciences*, 3(4), 91–95. [https://doi.org/10.1016/0166-2236\(80\)90035-1](https://doi.org/10.1016/0166-2236(80)90035-1)
- Wise, R. A. (2008). Dopamine and Reward: The Anhedonia Hypothesis 30 years on. *Neurotoxicity Research*, 14(2–3), 169–183. <https://doi.org/10.1007/BF03033808>
- Wöhr, M. (2017). Ultrasonic communication in rats: Appetitive 50-kHz ultrasonic vocalizations as social contact calls. *Behavioral Ecology and Sociobiology*, 72(1), 14. <https://doi.org/10.1007/s00265-017-2427-9>
- Wöhr, M., Rippberger, H., Schwarting, R. K. W., & van Gaalen, M. M. (2015). Critical involvement of 5-HT_{2C} receptor function in amphetamine-induced 50-kHz ultrasonic vocalizations in rats. *Psychopharmacology*, 232(10), 1817–1829. <https://doi.org/10.1007/s00213-014-3814-9>
- Wöhr, M., & Schwarting, R. K. W. (2007). Ultrasonic Communication in Rats: Can Playback of 50-kHz Calls Induce Approach Behavior? *PLoS ONE*, 2(12), e1365. <https://doi.org/10.1371/journal.pone.0001365>
- Wöhr, M., & Schwarting, R. K. W. (2013). Affective communication in rodents: Ultrasonic vocalizations as a tool for research on emotion and motivation. *Cell and Tissue Research*, 354(1), 81–97. <https://doi.org/10.1007/s00441-013-1607-9>
- Wolgin, D. L. (2012). Amphetamine stereotypy, the basal ganglia, and the “selection problem.” *Behavioural Brain Research, Quo Vadis Behavioral Neuroscience: A Festschrift for Philip Teitelbaum*, 231(2), 297–308. <https://doi.org/10.1016/j.bbr.2011.11.003>
- Wright, J. M., Gourdon, J. C., & Clarke, P. B. S. (2010). Identification of multiple call categories within the rich repertoire of adult rat 50-kHz ultrasonic vocalizations: Effects of amphetamine and social context. *Psychopharmacology*, 211(1), 1–13. <https://doi.org/10.1007/s00213-010-1859-y>
- Yankouskaya, A., Denholm-Smith, T., Yi, D., Greenshaw, A. J., Cao, B., & Sui, J. (2022). Neural Connectivity Underlying Reward and Emotion-Related Processing: Evidence From a Large-Scale Network Analysis. *Frontiers in Systems Neuroscience*, 16. <https://doi.org/10.3389/fnsys.2022.833625>
- Zafiri, D., & Duvarci, S. (2022). Dopaminergic circuits underlying associative aversive learning. *Frontiers in Behavioral Neuroscience*, 16. <https://doi.org/10.3389/fnbeh.2022.1041929>
- Zaragozá, R. (2020). Transport of Amino Acids Across the Blood-Brain Barrier. *Frontiers in Physiology*, 11, 973. <https://doi.org/10.3389/fphys.2020.00973>

SUMMARY IN ESTONIAN

Tasustamisega seotud süsteemide käitumuslik ja neurobioloogiline kohanemine kroonilise stressiga, erineva positiivse afektiivsuse ja hedoonilise võimekusega rottidel

Käesolevas väitekirjas uuriti, kuidas individuaalsed erinevused positiivses afektiivsuses ja hedoonilises võimekuses mõjutavad kroonilise stressiga kohanemist ning tasuga seotud käitumist ja neurobioloogiat. Positiivset afektiivsust hinnati kõditamisega esile kutsutud 50-kHz ultrahelihäälitsustega ning hedoonilist võimekust püsiva suhkrutarbimisega. Töö põhineb neljal isastel Wistari liini rottidel läbi viidud uuringul, milles kasutati kroonilise stressi, amfetamiini korduvmanustamise ja pikaajalise fluoksetiini manustamise kombinatsioone. Käitumuslikke tulemusi analüüsiti monoamiinide, nende metaboliitide, retseptorite, aminohapete ning valitud perifeersete füsioloogiliste näitajate foonil.

Tulemused näitasid, et krooniline stress ei vähenda ega suurenda tasuga seotud reageerimist ühetaoliselt. Erinevates uuringutes stress nii pidurdas kui ka võimendas amfetamiini poolt esile kutsutud käitumist ning mõju suund sõltus looma varasemast positiivse afektiivsuse ja hedoonilise võimekuse fenotüübist ning farmakoloogilisest kuurist. Madal positiivne afektiivsus oli korduvalt seotud suurema stressitundlikkusega, mis väljendus 50-kHz ultrahelihäälitsuste ja liikumisaktiivsuse vähenemises pärast stressirežiimi. Kõrge suhkrutarbimisega rottidel võimendas krooniline stress seevastu amfetamiini akuutset toimet ja soodustas käitumusliku sensitiseerumise kujunemist, teisalt madala suhkrutarbimisega stressirühmas sellist nähtust ei ilmnenud.

Positiivse afektiivsuse ja hedoonilise võimekuse samaaegne hindamine näitas, et kõige tundlikumad stressi mõjude suhtes olid madala positiivse afektiivsuse ja kõrge suhkrutarbimise fenotüüpi kuuluvad rotid. Nendel loomadel pidurdas krooniline stress amfetamiini poolt esile kutsutud 50-kHz ultrahelihäälitsusi ja esines kõrgemat ärevust käitumiskatsetes. Kõrge positiivse afektiivsuse ja kõrge suhkrutarbimisega loomadel säilis tasuga seotud 50-kHz häälitsemine ning ärevust väljendav käitumine oli stressi järel harvemini esinev. Tulemused kinnitasid, et positiivne afektiivsus ja hedooniline võimekus on omavahel põimunud, kuid eristatavad tasusüsteemi mõõtmed, mille koostoime võimaldab määratleda organismide stressitundlikkust.

Käitumuslikud ja monoaminergilised näitajad ei olnud alati samasuunalised. Uuringus I suurenes stressis madala positiivse afektiivsusega rottidel pärast amfetamiini korduvmanustamist dopamiinikäive juttkehas, kuid sellega kaasnes amfetamiini esile kutsutud 50-kHz ultrahelihäälitsuste ja liikumisaktiivsuse vähenemine. Uuringus III suurenes stressis madala suhkrutarbimisega rottidel pärast amfetamiini korduvmanustamist dopamiini, serotoniini ja noradrenaliini käive naalduvas tuumas. Nendel loomadel väljendus amfetamiini sensitiseerimine liikumisaktiivsuses, kuid mitte 50-kHz häälitsustes. Seevastu stressis kõrge suhkrutarbimisega rottidel väljendus amfetamiini sensitiseerimine 50-kHz häälit-

sustes ja tagajalgadele tõusmistes, kuid mitte jooneületustes, ning stress takistas korduva amfetamiiniga seotud monoamiinikäibe suurenemist juttkehas ja mandelkehas. Need tulemused näitavad, et monoaminergiline kohastumine ei ennustanud otseselt psühhostimulandi toime käitumuslikku tugevust, vaid oli seotud fenotüübist sõltuvate erinevustega ning kuidas psühhostimulandi hüvesid käitumuslikult väljendati. Ka fluoksetiini mõju sõltus fenotüübist: Uuringus II kujunes stressis LC-rottidel 50-kHz ultrahelihäälitsuste sensitiseerumine ainult pärast fluoksetiinravi, stressis HC-rottidel aga nii fluoksetiini kui ka süstelahust saanud rühmades.

Fenotüübist sõltuvad muutused ei piirdunud juttkeha ja naalduva tuumaga, vaid ilmnemise ka otsmikukoore, mandelkeha, hüpotaalamuse ja hippokampuse monoaminergilistes näitajates. Lisaks leiti erinevusi valitud aminohapete, kehakaalu muutuse, neerupealiste suhtelise massi ja seerumi glükoosisisalduse osas. Eriti väärib edasist uurimist isoleutsiini võimalik seos kõrge positiivse afektiivsuse ja paremini säilinud stressikohanemisega. Need tulemused osutavad, et individuaalsed erinevused kroonilise stressiga kohanemises väljenduvad mitmetes organismi süsteemides ning erinevatel uurimistasanditel.

Kokkuvõttes näitab doktoritöö, et kroonilise stressi tagajärjed sõltuvad tasusüsteemi varasemast ülesehitusest. Madal positiivne afektiivsus kujunes korduvaks stressitundlikkuse tunnuseks ning selle koosinemine kõrge suhkrutarbimisega oli seotud kõige selgema stressihaavatavusega. Töö aitab selgitada, miks krooniline stress võib erinevates tingimustes põhjustada näiliselt vastandlikke tulemusi: stressi mõju sõltub uuritava valimi fenotüübilisest koosseisust, hinnatavast tasu- protsessi komponendist ning kontekstist, milles stressiga kohanenud süsteemi hiljem aktiveeritakse.

PUBLICATIONS

CURRICULUM VITAE

Name: Marten Vares
Date of birth: 19.09.1991
Citizenship: Eesti
Address: Riia tn 12, 51013 Tartu
Email: marten.vares@gmail.com

Education:

2016–... University of Tartu, Institute of Psychology, doctoral studies (psychology)
2013–2016 University of Tartu, Institute of Psychology, master's studies (psychology)
2010–2013 University of Tartu, Institute of Psychology, bachelor's studies (psychology, international relations)

Job history:

10.2020–... Researcher (1.0), Estonian Military Academy
2019 Division of Neuropsychopharmacology, University of Tartu – laboratory technician
2018–2019 Institute of Psychology, University of Tartu – lecturer
2018 Institute of Psychology, University of Tartu – lecturer

Publications:

Kõiv, K., Metelitsa, M., Vares, M., Tiitsaar, K., Raudkivi, K., Jaako, K., Vulla, K., Shimmo, R. & Harro, J. (2016). Chronic variable stress prevents amphetamine-elicited 50-kHz calls in rats with low positive affectivity. *European Neuropsychopharmacology*, <https://doi.org/10.1016/j.euroneuro.2016.02.011>
Vares, M., Metelitsa, M., Kõiv, K., Kaldmäe, M., Laugus, K., Tiitsaar, K., Shimmo, R. & Harro, J. (2018). Chronic stress sensitizes amphetamine-elicited 50-kHz calls in the rat: Dependence on positive affective phenotype and effects of long-term fluoxetine pretreatment. *Pharmacology Biochemistry and Behavior*, <https://doi.org/10.1016/j.pbb.2018.05.014>
Kõiv, K., Vares, M., Kroon, C., Metelitsa, M., Tiitsaar, K., Laugus, K., Jaako, K. & Harro, J. (2019). Effect of chronic variable stress on sensitization to amphetamine in high and low sucrose-consuming rats. *Journal of Psychopharmacology*, <https://doi.org/10.1177/0269881119856000>
Vares, M., Kanarik, M., Kõiv, K., Vares, M., Anja, K., Metelitsa, M., Laugus, K., Tigro, H., Korulu, S., Shimmo, R. & Harro, J. (2025). Vulnerability to chronic stress in male rats: Additive effect of low positive affectivity and high hedonic response as measured by sucrose intake. *Behavioural Brain Research*, <https://doi.org/10.1016/j.bbr.2025.115749>

ELULOOKIRJELDUS

Nimi: Marten Vares
Sünniaeg: 19.09.1991
Kodakondsus: Eesti
Kontakt: Riia tn 12, 51013 Tartu
E-post: marten.vares@gmail.com

Haridus:

2016–... Tartu Ülikool, Psühholoogia instituut, doktoriõpe (psühholoogia)
2013–2016 Tartu Ülikool, Psühholoogia instituut, magistriõpe (psühholoogia)
2010–2013 Tartu Ülikool, Psühholoogia instituut, bakalaureuseõpe
(psühholoogia, rahvusvahelised suhted)

Töökogemus:

10.2020–... Teadur (1.0), Kaitseväge Akadeemia
2019 Tartu Ülikooli Psühholoogia Instituut Neuropsühhofarmakoloogia
uurimisrühm – laborant
2018–2019 Tartu Ülikool Psühholoogia Instituut – lektor
2018 Tartu Ülikool Psühholoogia Instituut – lektor

Teaduspublikatsioonid:

Kõiv, K., Metelitsa, M., Vares, M., Tiitsaar, K., Raudkivi, K., Jaako, K., Vulla, K., Shimmo, R. & Harro, J. (2016). Chronic variable stress prevents amphetamine-elicited 50-kHz calls in rats with low positive affectivity. *European Neuropsychopharmacology*, <https://doi.org/10.1016/j.euroneuro.2016.02.011>

Vares, M., Metelitsa, M., Kõiv, K., Kaldmäe, M., Laugus, K., Tiitsaar, K., Shimmo, R. & Harro, J. (2018). Chronic stress sensitizes amphetamine-elicited 50-kHz calls in the rat: Dependence on positive affective phenotype and effects of long-term fluoxetine pretreatment. *Pharmacology Biochemistry and Behavior*, <https://doi.org/10.1016/j.pbb.2018.05.014>

Kõiv, K., Vares, M., Kroon, C., Metelitsa, M., Tiitsaar, K., Laugus, K., Jaako, K. & Harro, J. (2019). Effect of chronic variable stress on sensitization to amphetamine in high and low sucrose-consuming rats. *Journal of Psychopharmacology*, <https://doi.org/10.1177/0269881119856000>

Vares, M., Kanarik, M., Kõiv, K., Vares, M., Anja, K., Metelitsa, M., Laugus, K., Tigro, H., Korulu, S., Shimmo, R. & Harro, J. (2025). Vulnerability to chronic stress in male rats: Additive effect of low positive affectivity and high hedonic response as measured by sucrose intake. *Behavioural Brain Research*, <https://doi.org/10.1016/j.bbr.2025.115749>

DISSERTATIONES PSYCHOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Jüri Kruusvall.** Environmental and social influence on human activity. Tartu, 1994, 135 p.
2. **Dagmar Kutsar.** Transformation in Estonia as reflected in families: Insight into social stress and poverty. Tartu, 1995, 171 p.
3. **Aleksander Pulver.** Measurement of elementary movement vectors in human visual system. Tartu, 1995, 123 p.
4. **Ritva Fagerström.** The correlations between psychological factors and vision in aged cataract operation patients. Tartu, 1996, 145 p.
5. **Eve Kikas.** Conceptual development in school-aged children: The impact of teaching. Tartu, 1997, 112 p.
6. **Anu Realo.** Individualism and collectivism: An exploration of individual and cultural differences. Tartu, 1999, 164 p.
7. **Aaro Toomela.** Cultural-historical psychology: three levels of analysis. Tartu, 2000, 171 p.
8. **Anneli Kolk.** Cognitive development of children with non-progressive unilateral brain lesion. Tartu 2001, 95 p.
9. **Aune Valk.** Two facets of ethnic identity: pride and differentiation. Tartu, 2001, 153 p.
10. **Anu Aluoja.** Depression in the population: assessment, prevalence and relationships with socio-demographic factors and cognitive aspect of social adjustment. Tartu 2002, 73 p.
11. **Talvi Kallasmaa.** Personality traits and ways of coping: their characteristics and interrelations. Tartu 2002, 119 p.
12. **Luule Mizera.** Socialization in Estonian families: attitudes and behavior in comparative perspective. Tartu 2003, 117 p.
13. **Kairi Kreegipuu.** Availability and accessibility of information in perception of moving stimuli. Tartu 2004, 97 p.
14. **Riina Häidkind.** Monoaminergic mechanisms in mood-associated behaviours and neurochemistry in rats. Tartu 2004, 123 p.
15. **Evelyn Kiive.** Studies on peripheral markers of central serotonergic activity and behaviour. Tartu, 2005, 113 p.
16. **Helle Pullmann.** The development of intelligence and personality traits among Estonian schoolchildren. Tartu, 2005, 112 p.
17. **Kenn Konstabel.** The structure and validity of self- and peer-reported personality traits. Tartu, 2006, 103 p.
18. **Toivo Aavik.** Lexical analysis of Estonian personal values vocabulary and relation to socially desirable responding and parenting practices. Tartu, 2006, 113 p.
19. **Margus Tõnissaar.** Stress and sociability: individual differences and their neurochemical substrate. Tartu, 2006, 161 p.

20. **Kaia Laidra.** Adolescent personality: Development, interrater agreement, and relation to academic achievement. Tartu, 2007, 117 p.
21. **Iiris Luiga.** Interaction of object perception and visual attentional selection processes. Tartu, 2007, 116 p.
22. **Marika Paaver.** Types of impulsivity, their behavioural expression, and association with the markers of vulnerability of serotonin system. Tartu, 2007, 140 p.
23. **Tanel Mällo.** Exploratory behaviour and 50-kHz ultrasonic vocalizations in rats: behavioural and neurochemical profiles of persistent inter-individual differences. Tartu, 2008, 216 p.
24. **Aet Alttoa.** Neurochemical regulation of rat exploratory behaviour: focus on dopaminergic and noradrenergic neurotransmission. Tartu, 2008, 166 p.
25. **René Mõttus.** Universal and specific features of personality traits in their various representations. Tartu, 2009, 170 p.
26. **Kirsti Akkermann.** Serotonin-related biomarkers and symptoms of eating disorders. Tartu, 2010, 120 p.
27. **Iris Podar.** Eating disorders, personality, and cultural differences. Tartu, 2010, 130 p.
28. **Denis Matrov.** Cerebral oxidative metabolism and effects of chronic variable stress in animal models of human affective styles. Tartu, 2010. 208 p.
29. **Kadri Kõiv.** Studies on monoaminergic regulation of inter-individual differences in exploratory behaviour and the activating and rewarding effects of psychostimulants in rats. Tartu, 2010, 132 p.
30. **Triin Hannust.** Children's knowledge about the Earth and gravity and its change in the course of development and learning. Tartu, 2011, 108 p.
31. **Kersti Luuk.** Antecedents and concomitants of adult psychological distress. Tartu, 2011, 132 p.
32. **Margus Kanarik.** Inter-individual differences in vulnerability to depression: regional brain energy metabolism, serotonergic function and behaviour in animal models. Tartu, 2011, 239 p.
33. **Aire Raidvee.** Pooling of elementary motion, colour, and orientation signals into global perception. Tartu, 2012, 105 p.
34. **Liisi Kõöts-Ausmees.** Emotional experience: relations to personality, subjective well-being, recollection, and external influences. Tartu, 2012, 137 p.
35. **Pirko Tõugu.** "Where did we go last weekend?" Socialization of children through past-event reminiscing in various cultural contexts. Tartu, 2012, 132 p.
36. **Triin Kurrikoff.** Interpersonal relationships and behaviour: moderation by functional gene variants. Tartu, 2012, 126 p.
37. **Karin Täht.** The cross-cultural view on students' motivation to learn. Tartu, 2012, 137 p.
38. **Henrik Dobewall.** Human values and subjective well-being: An exploration of individual and cultural differences, change across life span, and self-other agreement. Tartu, 2013, 157 p.

39. **Carolina Murd.** Mechanisms of processing visual motion information: Psychophysical, bioelectrical and transcranial magnetic stimulation investigations. Tartu, 2014, 120 p.
40. **Andero Uusberg.** Electroencephalographic insights into affective attention. Tartu, 2014, 122 p.
41. **Kariina Laas.** Neuropeptide S and mental health: A functional receptor gene variant and environment shaping traits and contributing to psychiatric disorders. Tartu, 2014, 144 p.
42. **Maria Tamm.** Psychological and physiological implications of time perception. Tartu, 2014, 154 p.
43. **Inga Karton.** Deceptive communication: the effects of transcranial magnetic stimulation and the signatures of electroencephalography. Tartu, 2014, 94 p.
44. **Kelli Lehto.** Depression- and anxiety-related gene variants: effects on personality traits and health-related behaviour. Tartu, 2015, 104 p.
45. **Anni Tamm.** Conflicts and their management in early childhood and adolescence. Tartu, 2015, 161 p.
46. **Renate Rutiku.** Refining the methodology for investigating the neural correlates of consciousness. Tartu, 2015, 166 p.
47. **Uku Vainik.** Towards a comprehensive framework for the psychological mechanisms of obesity and overeating. Tartu, 2015, 152 p.
48. **Mari-Liis Kaldoja.** Mild traumatic brain injury in childhood: pre-injury social-emotional behavior, social-emotional and cognitive outcome and implications for attention rehabilitation. Tartu, 2015, 135 p.
49. **Astra Schults.** First words of Estonian children: early communicative development. Tartu, 2016, 109 p.
50. **Mariliis Vaht.** Genes and alcohol use: effects of common genetic polymorphisms in general population. Tartu, 2016, 120 p.
51. **Aire Mill.** Exploring the role of personality traits and age in the experience and recognition of emotions. Tartu, 2017, 148 p.
52. **Nele Põldver.** An experimental exploration of automatic processes in visual perception. Tartu, 2018, 153 p.
53. **Helen Uusberg.** Studying the psychological mechanisms of affective individual differences with EEG correlates. Tartu, 2018, 177 p.
54. **Jaan Tulviste.** Modulation of decision-making by transcranial magnetic stimulation. Tartu, 2019, 83 p.
55. **Dmitri Rozgonjuk.** Problematic smartphone use: behavioral, psychopathological, dispositional, and educational correlates. Tartu, 2019, 116 p.
56. **Kadi Tulver.** An investigation of individual differences in the effects of priors on visual perception. Tartu, 2019, 108 p.
57. **Katrin Kukk.** Risk factors of binge eating and overeating – towards an integrated model. Tartu, 2020, 114 p.
58. **Gerly Tamm.** Multiple sources of variation in perception and working memory for facial emotional expressions. Tartu, 2021, 217 p.

59. **René Randver.** Parkinson's disease and depression: brain mechanisms and non-invasive brain stimulation based treatment strategies. Tartu, 2021, 126 p.
60. **Hedvig Sultson.** Refining the constructs of positive and negative emotional eating. Tartu, 2022, 98 p.
61. **Martin Kolnes.** Appraisal driven modulation of attention control. Tartu, 2022, 149 p.
62. **Nino Gugushvili.** Digital communication technologies and mental health: An interplay between usage types and user characteristics. Tartu, 2023, 216 p.
63. **Tõnis Tokko.** The association of risky traffic behaviour with personality factors, lifestyle and biological predisposition, and a driving school intervention aimed at impulsivity awareness. Tartu, 2023, 138 p.
64. **Kadri Arumäe.** Personality traits and body weight: from accurate descriptions to tests of causation. Tartu, 2023, 119 p.
65. **Kätlin Anni.** Intelligence, Personality, and Socioeconomic Outcomes in Estonia. Tartu, 2024, 133 p.
66. **Kärol Soidla.** Latent profiles and developmental trajectories of eating disorder symptoms. Tartu, 2025, 170 p.
67. **Alan Voodla.** Does it feel right? Towards an affective perspective on metacognitive confidence. Tartu, 2026, 131 p.
68. **Helo Liis Soodla.** Integrating psychopathology and personality using person- and variable-centered approaches. Tartu, 2026, 165 p.