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28

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DENIS MATROV

Cerebral oxidative metabolism and effects of chronic variable stress in animal models of human affective styles

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"[We must] abandon the simplistic hypotheses of there being either an abnormally high or abnormally low function of a given neurotransmitter."

Arvid Carlsson

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I. Matrov, D., Kolts, I., & Harro, J. (2007). Cerebral oxidative metabolism in rats with high and low exploratory activity. *Neuroscience Letters*, 413:154–58.

II. Matrov, D., Vonk, A., Herm, L., Rinken, A., & Harro, J. Simultaneous anhedonic and activating effects of chronic variable stress in rats with different exploratory activity: Association with dopamine D_1 receptor function in nucleus accumbens. (*submitted for publication to Neuropsychobiology*).

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IV. 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: 963–974.

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The author of the present dissertation contributed to all individual publications presented herein. I am the lead author of Papers I and II, where I created experimental designs, carried out most of the experimental work and prepared the publications. In Papers III–V, I am responsible for carrying out cytochrome c oxidase histochemistry part and related data analysis. Additionally, in Papers III and IV, I have contributed the second largest share of experimental work after the lead author.

1. INTRODUCTION

1.1. Affective states: modelling human emotional states in animals

1.1.1. Affective states and mood disorders in humans

Affective processes are ever-present in our existence and, because they infuse our thoughts and actions with valence, their dynamics gives us both the highs of bliss and the lows of woe. My dissertation deals with animal models of human affective states and their dysfunctions, nevertheless, before submerging into the field of comparative psychology a little exposé on our understanding of human affective states is in order. Firstly, a brief definitional summary of operational terms is presented. From the functional perspective *emotions* are defined as mechanisms for biasing and modulating actions, whilst *moods* modulate and bias cognitions [1]. We can also differentiate between mood and emotions in the temporal dimension. Mood is always present in conscious organism, whilst emotions are more volatile and occur against the background of present mood. A particular composition of prevalent moods and emotional reactivity in an organism can be attributed to its *affective style* and is based on a unique organisation of the nervous system, both in its architecture and dynamics [1].

Though emotional reactivity is important for the nosology and pathogenesis of some human psychiatric diseases, hereafter I would concentrate primarily on moods and affective styles, as these concepts are easier to operationally translate into the realm of infrahuman species, although the fine-tuning of our understanding of the emotional valence behind ultrasonic vocalisations in rodents (this topic will be discussed in its own chapter) and their emotion-specific facial expressions [2] promises breakthroughs in modelling momentary affective processes.

Due to the similar evolutionary pressures on different organisms and their social organisation, as well as the homology in the basic blueprint of the central nervous system in mammals, we can expect a certain evolutionary continuity in affective processes. The basic affective state-control functions that generate fundamental emotional and motivational feelings are shared among different taxa in animal kingdom [3, 4]. Prolonged periods of low mood are a common feature of life for many people and the extent to which we can model such phenomena in animals is an important scientific endeavour.

Depressed mood can be understood as a diffuse negative affect with an accompanying low motivation to undertake actions or postulate goals (especially socially motivated) and to relate to the environment [5]. Clinical depression is defined by the high frequency of negative mood states (which can be described by adjectives upset, angry, guilty, afraid, sad, scornful, disgusted, and worried) and the low incidence of positive mood states, which is generally expressed by the lack of motivation, feelings of fatigue and languor [6]. Markedly diminished

interest and pleasure in all or most activities or anhedonia is another core symptom [7, 8]. At least one of the core symptoms must be accompanied by at least 4 other symptoms such as weight change [9], sleep disturbances [10], psychomotor agitation or retardation [11], loss of energy [12], inappropriate guilt [13], diminished ability to concentrate [14], and suicidal ideation [15, 16]. Depression can be differentiated from disorders of anxiety spectrum by the lack of physiological hyperarousal and the presence of low positive affectivity, although a significant number of patients meet the criteria for both diagnoses [6], and the situation becomes more muddled in case of melancholia. A severe form of clinical depression without an apparent psychogenic cause is historically called melancholia. Melancholia is a recurrent and drug-resistant affliction in humans. Characteristic of melancholia is a severe disruption of vegetative functions such as the loss of appetite, reduced sex drive, and disturbances in circadian rhythms [17]. These outputs are easily measurable across species; hence the identification of individual animals showing appropriate depressive phenotype, combined with their resistance to antidepressants, may facilitate our ability to model the severe forms of mood disorders.

The economic burden of depression is high, not only in terms of the direct costs of treatment, but also because of indirect costs associated with premature mortality and deteriorated health. Depression is the leading cause of absenteeism and reduced productivity at work [18]. The cost of depression to employers, particularly the cost of lost productivity, is as great or greater than the cost of such common chronic medical conditions as *diabetes mellitus*, heart disease, hypertension and back problems; the combination of depressive and other common illnesses is particularly costly [19]. The severity of clinical depression and its resistance to treatment are both significantly associated with increased use of mental health services and economic burden [20, 21]. In year 2005, the total cost of clinical depression to Swedish society was estimated at €3.5 billion, of which indirect costs were estimated at €3 billion (86% of total costs) and direct costs at €500 million (14%). Cost of medicaments was marginal at €100 million (3% of total economic burden) [22]. The 12-month prevalence of any mood disorder, estimated from the latest WHO World Mental Health (WMH) survey data, that was gathered by administering Composite International Diagnostic Interview (CIDI), was estimated to be at 5.1% (In Europe the spread was from 3.3% in Spain to 9% in Ukraine) [23]. Overall, mental disorders were associated with higher disability in social and personal spheres, whereas disability in economic activity was generally comparable for mental and physical disorders [24]. This brief overview paints a grim picture of the rising incidence and economic burden of mood disorders. Next I will give a very general summary of major pathophysiological findings associated with clinical depression.

Diagnostic criteria for depression concentrate on diagnosing a single episode, but the clinical reality is that depression is also a recurrent disorder, where the likelihood of a subsequent episode increases with each additional episode, suggesting some kind of rarely reversible and deepening dysfunction. For

example, in a longitudinal follow-up study of people who recovered from the first episode of clinical depression, recurrence occurred in 85% of the cases; significantly, the recurrence was 58% in subjects who remained healthy for at least 5 years [25]. Anatomically, the reduction of the tissue volume in several brain regions such as hippocampus [26–28], basal ganglia [29–31], amygdala [32] (although an increase has also been shown in young women with resent-onset depression [33]), and various cortical areas [34–36], as well as increase in the ventricular size [37], have been reported. It seems that patient's age, poor response to antidepressants, the recurrent clinical history of depression, and the severity of sympomatology, are all associated with a greater risk of cerebral atrophy [38–40]. Reductions in cortical volume are mainly caused by the reduced density of astrocytes and other glial abnormalities [41–43] that may account for alterations in glutamate/GABA neurotransmission in depression [44, 45].

Neuroimaging studies generally show an increase in metabolic activity (if necessary corrections for volumetric neuronal tissue loss have been implemented) in amygdala (often more so on the left) [46, 47], subgenual and perigenual parts of anterior cingulate cortex [48, 49], ventromedial prefrontal cortex [50], and medial thalamus [51, 52]. Recent meta-analysis also suggests resting state hyperactivity in pallidum/putamen, and several midbrain regions including VTA, substantia nigra, PAG and tectum [53]. In contrast, a reduced metabolic activity has been generally observed in dorsomedial and dorsolateral prefrontal cortex, posterior cingulate, anterior insula, temporal cortex, and hippocampus (reviewed in [54, 55]). Dysregulation of functional brain connectivity between the limbic system and hierarchically higher control centres have been proposed as a mechanism to account for the changes in regional metabolic activity in the brain during clinical depression [51, 56].

Several information processing abnormalities, especially when information is emotionally valenced, have also been observed in clinical depression. Depressed people have easier access to sad memories [57], and they exhibit a more pronounced bias for negativity in information processing [58], as well as reduced positive self-judgment bias [59]. Changes in the neuronal activity during memory encoding and retrieval [60], as well as reduced activation of striatal reward regions during reward selection, anticipation, and feedback have been reported in the depressed subjects [61].

Despite all of the unpleasantness associated with having a mood disorder, chronic negative affective states are a common occurrence in human populations. We can hypothesise that a relaxed evolutionary selection has something to do with an increased burden of mood disorders in the developed world, but depression was always a part of the human existence, as far as our cultural memory could reach back in time. And mood disorders are not some kind of epiphenomenon either, because in their most debilitating and chronic form, they are associated with a global change in personality and behaviour [62], and therefore have deep evolutionary origins and also impose at least some selectional constraints.

A limited adaptive phenotypic plasticity may explain the persistence of inter-individual variation in behavioural phenotypes and affective styles from an evolutionary standpoint as a balancing act between the costs of a larger plasticity and the rigidity of constraints on change [63], and give credence to our assumption that low mood states are also an apposite construct for describing, studying, and modelling in infrahuman animals.

1.1.2. Translational research of affective states

1.1.2.1. Concept of endophenotype and behavioural modelling

In the 1969, in a review paper on the state of animal research pertinent to human mood disorders, an idea of genetically selected animal breeds was presented: "There are no data concerning whether it might be possible to breed for animals prone to have depressive disturbances. If experiments showed this to be possible, the use of such animals would facilitate the creation of an experimental animal model of depression" [64: 246]. It also laid down a set of minimal requirements of an animal model of human clinical depression: animals forming social bonds are required to experience depressive emotions, symptoms exhibited by model organism should at least somewhat overlap with human clinical syndrome and have similar aetiology and physiological basis, model produces observable changes in animal behaviour that can be objectively recorded, independent observers should be able agree on objective evaluation criteria and reproduce the model, treatments effective in humans should also work in animals [64]. An additional approach to evaluate a prospective animal model of human depression is to assess it based on three types of validity: face validity for evaluating phenomenological similarities between the model and the condition being modelled, predictive validity for inventing novel treatments successfully applicable to human patients, and construct validity to ascertain the theoretical rationale for homology between human pathology and animal model of it [65].

The term "endophenotype" originates from the field of insect genetics from the paper [66] about the role of chromosomes in genetic diversity and speciation. "Endophenotype" was coined to describe those parts of phenotypic variation that are not captured by genes themselves, such as the effects of chromosomal mutations on meiosis [66]. Thereafter, the term "endophenotype" was adopted into psychiatric genetics, possibly as a result of misreading of the original paper [67], and came to signify more elementary phenomena associated with psychiatric conditions, for which it would be easier to identify genetic mechanisms [68]. Endophenotypes may not have clear-cut genetic underpinnings, however. They can be conceptualised as probabilistically associated biological traits, markers, or micropathologies; that either contribute to the emergence of psychiatric illness, or share some causal mechanism with it. Frustrations with a divide between clinical and preclinical sciences due to

heterogeneous definitions of psychiatric disorders with respect to their aetiology and pathophysiology by currently employed diagnostic schemes have lead to an increase in the importance of the endophenotype-centric approach in basic research. Influential criteria for evaluating phenotypes states that an endophenotype is: 1) associated with illness in the population, 2) heritable, 3) state-independent, 4) co-segregated with illness within families, and 5) found in unaffected relatives at a higher rate than in the general population [69].

1.1.2.2. Genetic animal models

Last decade the prototypical species for genetic manipulations have been mice, although their role starts to decline due to the introduction of even simpler model organisms. Knockout and transgenic mice are very useful for determining the role of a specific gene in the CNS development and functioning. Most of such models are global in a sense that gene inactivation is constitutive and affects an entire organism from the beginning of its development at the prenatal stage. With the progress of genetic engineering methods various conditional genetic models, controlling for an onset and even a location of a target gene activation or inactivation, have been developed and they are gaining in popularity.

The development of inbred mouse strains, in which all individual mice are homozygous for all loci, enabled to introduce congenic strains that differ only in single locus [70]. Thereafter, congenic mice can be compared to the original background strain to determine whether they are phenotypically different, if selection was for a genotypic region, or to identify the critical genetic locus, if selection was for a phenotype. The strain, and, therefore, the genetic background, onto which a mutation is backcrossed, can affect the detection of a depression-related phenotype in a mutant mouse. Genetic background can affect a mutant phenotype in a number of ways: 1) by providing an inappropriate baseline level of target behaviour in wild-type controls, 2) via the unwanted contribution of flanking genes extant from the embryonic stem cell donor strain, and 3) via complex functional interactions between the mutation and background genes [71].

It is important to have an adequate background strain for comparison is search of phenotypic effects of genetic mutation, otherwise these effects can be masked by the unaccounted baseline differences in the behaviour of strains with different genetic background. Backcrossing to the different inbred strains will produce different phenotypes for the same genetic mutation. Ideally, mutation should be implemented in several inbred strains and a full battery of behavioural tasks should be used to evaluate the phenotypic variation [72]. Use of multiple behavioural tests is necessary, because the tests themselves measure different subsets and combinations of target behaviours. For example, the pattern of differences between mouse strains in anxiety-like behaviour is dependent on the task used [73]. There are a number of cases of mutant mice that

show anxiety-related abnormalities only on some standardised tests, hence this abnormalities can go undetected if incomplete test battery is administered [71]. Another issue is an inter- [74, 75] and intra-laboratory [76, 77] variability in behavioural results obtained in otherwise standardised conditions, suggesting the importance of animal's environmental background and life history on its phenotypic expression. Systemic environmental variation of laboratory conditions increases the robustness and external validity of the results attributable to genetic manipulations, therefore, behavioural variability generated on the same genetic background is inevitable and multiple testing is needed to properly assess the genetic contribution and avoid false positives [78, 79]. Genetic mutations can affect the expression pattern of neighbouring genes and produce compensatory mechanisms that are dependent on recipient's genetic background and techniques of gene manipulation. Compensatory mechanisms can occlude the normal function of a molecule in neural circuits mediating emotion-related behaviours, however, these compensatory mechanisms might work in a similar fashion in case of human detrimental genetic mutations, so that many of the difficulties in identifying the causative genetic factors in aetiology of mood disorders are shared with our infrahuman model species. It is misleading to think that the abnormal phenotype is the product of a single mutation; in many cases the effect is actually polygenic (caused by the induced mutation in combination with effects from many other loci) [80].

Genetic animal models are able to successfully emulate human psychiatric disorders to the extent of evolutionary continuity of species that is preserved on the levels of physiology and biochemistry, as well as on the level of behaviour, where the identification of specific elements, conserved across species, such as impulsivity, set-shifting, memory function and anticipatory motivation, will help to properly evaluate a particular phenotype and relate it to the human endophenotypes of mood disorders [81]. Interactions between these elements or 'behavioural domains' are far from trivial and therefore assessment of a maximum number of behavioural domains during behavioural testing and matching them to clinically relevant 'interplay' characteristics of the disorder pathogenesis, comorbidity, and risk factors will help to develop better animal models and identify neural substrates of usually overlooked disorder subtypes [82, 83]. Unless experimentally proven wrong, we can assume that genetic effects on behaviour attributable to gene knockout in mice are subject to comparable set of complications, gene-environment and epistatic interactions that characterise genetic effects in psychiatric illness.

Traditionally, the majority of mouse knockouts are generated using embryonic stem (ES) cells derived from the several substrains of 129 mice [84]. This practice might be problematic in some areas as the most popular ES cell donors (129/Sv, 129/J, and 129/Ola) are all reported to exhibit a number of peculiar physiological, behavioural and anatomical characteristics such as poor performance in Morris water maze [85] and conditioned active avoidance [86], dysgenesis of the corpus callosum [85], as well as low baseline preference for sweet tasting substances [87]. The 129/Ola substrain performs relatively

normally in cognitive tasks, shows open-arm preference in the elevated plus-maze, and is less active in the open field [88].

Genetic models are useful for studying the systemic changes in biochemistry and behaviour due to the reduction in bioavailability of an important compound. In the thesis, the heterozygous knockout of one of the proteins responsible for glutamate synaptic delivery was utilised. Glutamate is the major excitatory neurotransmitter in CNS and is used by about one half of all neurons. Vesicular glutamate transporters (VGLUTs) package cytoplasmic glutamate into vesicles, employing a proton electrochemical gradient generated by vacuolar ATPase, for later synaptic release. One of the main isoforms, VGLUT1 is expressed in glutamatergic neurons and preferentially localised in neocortex, limbic forebrain, hippocampus, thalamus, and cerebellum [89]. Mice, heterozygous for VGLUT1 (VGLUT1+/–), exhibit decreased cortical and hippocampal levels (35%–45%) of the inhibitory neurotransmitter GABA, together with the increased immobility in FST [90]. Decreased GABA levels in certain brain regions may serve as an endophenotype of human clinical depression [53], hence VGLUT1+/– mice represent a genetic animal model of the vulnerability to prolonged stress.

1.1.3. Environmental factors

1.1.3.1. Stress

Every living organism has to strive for optimal level of functioning in order to survive and reproduce. Hans Selye has defined stress in the biological sense as an interaction between damage and defence in a context of general adaptation syndrome [91]. General adaptation syndrome is an idealised way to describe an ability of any organism to adapt itself to changes in its surroundings, whilst in practice we observe a superimposition of organism's responses to concrete stressors that modify the particular course of a general adaptation syndrome [91]. Psychological well-being as measured against the backdrop of ideal state of functioning is conceptually and phenomenologically distanced from physiological reactions to well defined stressors and, therefore, is better understood through general level of experienced systemic stress or strain. Stress, thus understood, is primarily not a causative factor of psychological state, but a descriptive term for a mismatch between ideal and present adaptation. Stress is an intrinsic diagnostic marker of well-being for an organism and has a well-defined substrate in hypothalamic-pituitary-adrenal (HPA) axis. Stress as perceived by organism can become a causative factor in its own right and either enhance coping and therefore diminish itself in the future, or lead to self-amplification and psychopathology.

It is well established that stressful life events are more likely to occur in depressed patients prior to the onset of the illness than in general population, and this effect is independent of individual affective predispositions and clinical

history of mood disorders [92, 93]. Effects of social depressogenic life events differ in their impact in males and females: males are more prone to work and family related stressors, whilst women suffer more strongly from problems in getting along with individuals in their proximal network [94]. Females are more likely to succumb to depression in low stress exposure conditions [95]. Potency of stressful life events is also mediated by genetic predispositions, including personality traits such as neuroticism [95]. However, it should be kept in mind that most of the people do not develop clinical depression after a major stressful life event.

In many cases we cannot identify a contribution of a single life event leading to the depression, and, especially, in cases of more than one episode of depression over the lifetime, the chronic stress milieu, in conjunction with coping styles and reactivity dimensions of personality, may have some predictive utility [96]. Differences in perceived chronic stress may also account for a gender gap in depression [97].

Kindling/sensitisation model of recurrent depression [98] predicts that each episode of depression causes neurobiological changes that make future episodes of depression more likely for reasons intrinsic to the functioning of an organism, and association between stressful life events and any additional episode of depression should weaken accordingly. Kindling model of recurrent depression has received mixed experimental support and remains plagued by uncertainties in definition of depression and ability to separate contribution of chronic stress over more proximate stressors. Diathesis-stress model of depression predicts that stress is a primary initiator of depression, however, the reality of being depressed, especially having recurring depression, points to bidirectional causality between illness and the level of stress. History of depression increases the likelihood of having both objectively more stressful non-fateful life events and also the psychological strain [99], locking affected people into self-perpetuating cycle of depression and stress and, thus, negatively affecting their work, social and family functioning. Predisposition towards generating stressful atmosphere in one's life, depression and personality trait of neuroticism may all have shared genetic basis [95, 100].

In laboratory settings, uncontrollable stressors and social tasks, containing potential performance appraisal by other people, are reliably associated with elevated release of cortisol and adrenocorticotropin hormone (ACTH) [101]. Higher morning cortisol levels in women predict onset of depression independently of stressful life events [102]. Unipolar depression is characterised not only by higher cortisol levels, but also by shift in the circadian profile of the cortisol and ACTH release, as peak release is occurring earlier than in unaffected population [103]. Cerebrospinal fluid (CSF), saliva, and urinary levels of cortisol are predictive of symptomatic severity of depression irrespective of sex and age factors [104–106]. Some studies, however, cast doubt on the straightforward association between HPA axis hyperactivity and depression [107–109]. Severity of anxiety- and anhedonia-related symptomatology may have a mediating effect [110, 111]. Adverse childhood conditions or significant

events, such as a loss of mother, have predictive effect on a likelihood of adult depression [112] and amplify HPA axis reactivity to a psychosocial stressor [113].

Overall, we can conclude that at least a sizeable share of human mood disorders are either directly caused or aggravated by stressful life events, either real or perceived as such, and stress effect are mediated by genetic and epigenetic history. In the second part of the chapter I will give a quick historical overview of the animal models of mood disorders with a more thorough account of the Chronic Variable Stress (CVS) model and selection for inter-individual differences in vulnerability to stressful manipulations.

The earliest animal experiments that consistently produced emotional disturbances (which were reactive protest behaviours and agitation if experimental manipulation was short, and became more inhibited and depressive on longer manipulations) were maternal and social separation experiments primarily conducted with primates [114, 115]. Various emotional disturbances were recorded in both mothers and infants, such as crying, decreased play and social interaction with peers, screaming, physical withdrawal, motor agitation, reduced appetite, and sleep disorders that were more pronounced in infants compared to mothers and reminded of many similar disturbances in human mood disorders (see [64] for review). It is unclear, however, how well reactions to a very specific, potentially life-threatening situation in infants of different animal species translate to human clinical depression. It was suggested, that they correspond to anaclitic depression [116], which is rather rare in humans. In the 1970s, many other models started to appear and primate infant separation studies fell out of vogue. With the advent of modern neuropsychopharmacology, the primary concern shifted from understanding the mechanisms of pathology to developing animal models with a good predictive validity in determining potential future antidepressants. Rodents were more evolutionarily distant from humans, but much cheaper to maintain and perform experiments on, they were also readily amenable to selective breeding and genetic manipulations.

The next big study paradigm that was partially adopted as a model of human depression, but never quite made it, was the idea of submitting animals to inescapable electric shocks, or tethering them in pairs, in which only one animal has the ability to perform an escape response and terminate delivery of shock to both itself and its "yoked" partner. The learned helplessness (LH) hypothesis asserts that during exposure to uncontrollable stressors, animal learns of the lack of contingency between its responses and the outcome [117, 118], and it is the state of the lack of control and powerlessness, rather than a direct association between stimulus and outcome, that governs animal's subsequent behaviour in many behavioural domains. Animals exposed to uncontrollable, usually painful or otherwise actively aversive, stressors exhibit deficits in three areas: 1) motivation, as after inescapable stress animals have a reduced tendency to initiate subsequent escape, 2) cognition, as animals have a harder time learning future contingencies between their escape behaviour and outcome,

3) affectivity, as animals develop anxious and passive-inhibited coping styles [119]. Learned helplessness is not specific to situations where it was initially acquired, for example, rats, pre-treated with either inescapable electric shocks or water immersions, showed a similar interference when re-tested for active escape behaviours in the same or switched apparatus [120]. Learned helplessness also affects non-cognitive psychological domains: it reduces aggression in situation when a pair of male rats usually fight each other because of the pain inflicted on at least one of them by electric shock or tail pinch [121]. In the open-field inescapably shocked animals are less active on the first exposure and defecate more [122]. They also tend to lose their previous position in the social hierarchy [123], as well as appetite and body weight [124]. If given dexamethasone, LH-rats show an impaired ability to suppress corticosterone [125]. Adrenalectomy leads to a normal escape behaviour, which is abolished by corticosterone injections [126], thus signifying a causal role for HPA axis hyperactivity in the development of learned helplessness. Repeated exposure of once inescapably shocked rats to the same environment, but without aversive stimulation, can prolong a state of learned helplessness indefinitely [127], suggesting modulatory effects of affective processes. Learned helplessness was also generated by means of presenting rats with unsolvable visual discrimination problems [128] – a better approximation of psychological stressors encountered by humans.

At the acme of the experimental work on LH, another kind of passive approach to escape behaviour was discovered in rats that were routinely studied for learning tasks in water maze, when Roger Porsolt observed that some animals stopped swimming and remained motionless, making only necessary movements to keep their heads above water. From this observation he concluded that animals sunk in a condition of "behavioural despair" because they no longer expected to escape from the container with water, and developed Forced Swim Test (FST), which became widely used for screening potential antidepressants [129, 130]. Despite the seeming similarity of states of LH and behavioural despair, some experimental findings clearly differentiate between the two, for example, there was no correlation between rats that displayed helplessness following inescapable tail-shock and the rats that demonstrated behavioural despair in later FST [131]. Still, it might be concluded, that some processes governing animals behaviour overlap between the two tests and are amenable to antidepressant treatment [132]. In summary, learned helplessness as a model of depression showed a good predictive validity for effects of antidepressants and electroconvulsive shock therapy [133, 134], but its face and construct validities are uncertain and at best matter only for a subset of humans with clinical depression [135].

From the very first experiments, individual differences in susceptibility to LH were apparent, as some animals never developed a state of LH [118, 136]. Among different rat strains, Lewis, Brown Norway, and Fischer 344 rats were found to be virtually non-susceptible to LH training, whilst in more vulnerable strains up to two thirds of animals were susceptible [137]. Among Wistar strain

rats, less than 50% are susceptible to LH [131]. Individual susceptibility to LH was capitalised on by a selective breeding for “congenitally helpless” rats, which need no previous exposure to helplessness-inducing situation to exhibit deficits in active avoidance. By the 25th generation, 95% of congenitally helpless rats originating from Sprague-Dawley background demonstrated a spontaneous helplessness [138]. Congenitally helpless rats are important for my thesis because a thorough effort to map their brain oxidative metabolism has been undertaken, which I will discuss below in the appropriate section. Herein I further mention their behavioural profile. Congenitally helpless rats, compared to the outbred Sprague-Dawley controls, show significantly more ambulation and rearings in a novel open field and light-dark box, but do not differ in those parameters in the already familiar open field [139]. They are also less “fearful", operationally defined as spending a greater amount of time in the centre of the open field and in the bright area of light-dark box [139]. In contrast to LH-rats, their congenital conspecifics show blunted HPA response to acute stressor in the form of 40 min of intermittent electrical shocks [140]. Congenitally helpless rats showed less consumption of the 5% sucrose solution in a non-choice exposure for 1 h and more freezing in Pavlovian tone-shock acquisition chamber, which duration increased even more during the extinction phase [139]. Another research group found no baseline differences in reward-sensitivity, measured by sweetened-condensed milk consumption and pleasure-attenuated startle response, but a brief foot-shock stress reduced both measures of hedonic sensitivity in congenitally helpless rats that lasted for 2 weeks [141]. It was proposed that several characteristics of congenitally helpless rats, such as stress-induced analgesia, cognitive impairment, and hyper-responsiveness of the HPA axis, make it as an animal model more analogous to human PTSD [140].

Chronic Variable Stress (CVS), also known as a Chronic Mild Stress, is currently the most popular rat model of human depression. Its popularity stems from the several factors: its theoretical rationale is sound due to emphasis on precipitating effects of stressful life events in aetiology of human depression that were discussed above; its protocol of administration is flexible and allows for a wide variety of stressors, schedules of their administration, and durations of stress regimen; it produces a relatively robust decline in hedonic capacity, which is usually monitored by body weight-adjusted sucrose preference, and this output can be measured concurrently with the stress regimen, allowing researchers to gauge the impact of stress on animals and make adjustments if necessary; finally, CVS effect is counteracted by a chronic antidepressant administration [142, 143].

Behaviour in the open field is frequently recorded to estimate animal’s motivational and affective state. It is highly variable procedure, as all kind of test parameters, such as size of the arena, shape of the apparatus, initial placement of the animal, test room illumination, duration of the test, vary between different research groups, so it is no wonder that different results have been reported. In the first recorded such test, CVS lowered rearings, ambulation in the peripheral area and completely abolished entrances to the central zone, it

also prevented an activating effects of acute stress consisting of a combination of white noise and bright light, aa well as increased defecation [144]. In the above-mentioned test, Sprague-Dawley rats were tested for 6 minutes. In a milder version of the stress regimen, reductions in the locomotion in the central zone of the open field and in the number of rearings was reported, as well as increase in defecation in the first 5 minutes of testing in Sprague-Dawley rats, which is a period of high behavioural reactivity in a novel environment [145]. In Wistar rats, CVS has been reported to increase ambulation among single-housed males and females, but had no effect on group-housed animals [146]. The protective effect of group housing of Wistar rats against changes in ambulation in the open field has been confirmed by other studies [147, 148]. The test conducted in the well-lit open field after CVS regimen, that included group housing as one of the stressors for single-housed male Wistar rats, indicated the augmentation of the number of rearings and ambulation in both central and peripheral zones of the open field [149]. In our lab, CVS had no effect on open-field ambulation, but increased the defecation rate [150].

In group-housed male Wistar rats, 6 weeks of CVS regimen have increased immobility time in FST and decreased percentage of time spent in the open arms in the elevated plus-maze [151]. Elevated plus-maze (EPM) is routinely used to measure anxiety and related affective factors, based on the contrasting affordances offered by closed and open arms of the apparatus that activate rat's defence brain circuitry [152, 153]. A 3-week CVS in group-housed Wistar males has also increased immobility time in FST and time spent on the open arms in EPM [154], whereas a shorter stress regimen of 10 days did not change EPM behaviour [155]. In single-housed Lister hooded rats, 5-week CVS increased time spent on open arms in EPM, so that stressed animals spent almost equal time in open and closed arms of the apparatus, whilst no difference in the duration of social interaction was observed [156]. In conclusion, CVS usually leads to an increase in the second day immobility in FST (summarised in [157]), whereas the opposite result occurs quite regularly [158–160]. Behaviour in EPM has no preferential association with CVS regimen.

1.1.3.2. Reward

Motivational state is an observable phenomenon: it can be conditioned and employed to influence ongoing active behaviours [161]. Motivational and reward-predicting states in the brain are generated by the driving input from the midbrain dopaminergic nuclei. Cell bodies of dopamine neurons are located mostly in midbrain groups A8 (dorsal to lateral substantia nigra), A9 (substantia nigra pars compacta), and A10 (ventral tegmental area medial to substantia nigra). These neurons release dopamine from axonal varicosities primarily in dorsal striatum (caudate nucleus and putamen), ventral striatum (nucleus accumbens (NAcc) and olfactory tubercle), and frontal cortex [162]. Meso-limbic pathway originating from A10 is the most closely associated with reward

[163], however, psychological functions in the brain in general and limbic system in particular are realised via complexes of interacting microcircuits and nuclei [164]. In addition to dopaminergic input from midbrain nuclei, ventral striatum receives glutamatergic input from orbital frontal cortex (OFC), anterior cingulate cortex (ACC), and thalamus [165]. Limbic striatum, which includes ventromedial caudate, ventral putamen, NAcc and olfactory tubercle forms anterior cingulate circuit together with ACC, rostromedial globus pallidus interna, rostrodorsal substantia nigra (SN), ventral pallidum (VP) and dorsal part magnocellular division of mediodorsal thalamus [166]. Human patients with damage to this circuit exhibit serious motivational and emotional deficits: bilateral lesions to anterior cingulate produce apathy and loss of spontaneity, patients eat and drink only when fed, speak only when asked to and with impoverished sentences, show no emotions and indifference to pain, creativity and production of new ideas is diminished [166]. Additionally, ventral striatum forms other circuits with amygdala, hippocampus, lateral habenular nucleus, pedunculopontine nucleus and the raphe nuclei [165]. Based on human neuro-imaging studies, in the cortex sensory rewards activate posterior OFC, whereas abstract rewards such as money activate anterior OFC; a subregion of ventro-medial prefrontal cortex mPFC responds to rewarding outcomes and contextual information during reward anticipation; dorsal ACC is implicated in conflict monitoring when closely valued options are present; and dorsal prefrontal cortex activates when working memory is required for monitoring incentive-based behavioural responses [165]. Projections from different regions of prefrontal cortex (PFC) converge by both focal topographical innervation and through diffuse axonal invasion of focal projection zones from other brain regions in specific loci within ventral striatum, creating opportunities for input modulation and exchange, as well as producing situation-specific unique moti-vational states [165]. Compared to dorsal striatum, ventral striatum, especially the shell region of NAcc, is densely innervated by hippocampal and amygdalar projections, which together with inputs from prefrontal cortex create a unique nexus of executive, affective and contextual inputs [167]. Nucleus accumbens is divided into two major parts: the core is the central portion directly beneath and continuous with the dorsal striatum and surrounding the anterior commissure, whilst the shell occupies the ventral and medial portions of the NAcc [168]. Core portion of NAcc seems to deal with learned goal oriented behaviours, whilst shell produces unconditional drives, though much of local specificity also depends on specific innervation patterns and there are cross-communica-tions through axon collaterals [169, 170]. Lesions to NAcc produced specific deficits in tasks requiring discrimination between different outcomes characte-ristic of instrumental conditioning, whilst Pavlovian conditioning mechanisms remained intact [171], similar effect was observed after three weeks of chronic variable stress [172]. Pavlovian conditioned stimulus can act as a reinforcer of a subsequent operant behaviour, known as Pavlovian to instrumental transfer [173, 174]. Simple presentation of an appetitive CS increases dopamine levels in the NAcc core [175], whilst consumption of palatable stimuli activates the

shell. Blockade of D_1 and D_2 dopamine receptors in both NAcc core and shell [176, 177], and lesions of NAcc core abolish Pavlovian to instrumental transfer [178]. The predominant cells in the NAcc are the medium spiny neurons, on a single cell of which can converge several afferent inputs [179, 180]. The principal mode of integration of inputs to NAcc from diverse afferent areas in awake rat is sublinear, with a non-uniform distribution of responses across cells [181].

Dopamine neurons ascribe an appetitive value to environmental stimuli, predict and detect rewards and produce motivating states. Accumbal neurons are characterised by two activity states: a slow tonic firing and phasic burst firing [182]. They show phasic activation when better than predicted or surprising stimuli are encountered, and depressed by worse than predicted events [183].

Both D_1 and D_2 type of dopamine receptors are present in NAcc. D_1 receptors are primarily located on neurons that directly feed back to VTA, whilst both D_1 and D_2 receptors are present on neurons that project to VP, from where an indirect pathway reaches VTA via subthalamic nucleus or directly [184, 185]. Dopamine application has complex effect on NAcc neuronal activity: D_2 receptor activation mainly mediates neuronal inhibition [186, 187], D_1 receptor activation on the other hand potentiates glutamatergic currents [188, 189], additionally, increase in excitatory stimulation facilitates electrical coupling via gap junctions between accumbal neurons, providing a mechanism for lateral transmission of slow membrane voltage changes that help neurons synchronise their shifts between two membrane potentials for "up" and "down" states [190], "up" states being driven by a barrage of excitatory synaptic inputs from the hippocampus [191]. Phasic DA release selectively facilitates hippocampal inputs via D_1 receptor activation, whereas undulations in tonic DA release either attenuate or potentiate PFC inputs via D_2 receptors [192]. Glutamatergic inputs from amygdala produce long-lasting excitation, which effects are attenuated by activation of D_1 receptors [193].

Besides the production of flexible motivational states appropriate for a reaction to salient rewarding stimuli or for a switch in a goal-directed activity to meet the objectives of homeostasis maintenance, such as finding a place to sleep or recuperate oneself, NAcc DA-ergic system also generates a state of unspecific exploratory arousal that helps organism to actively seek out novelty in many situations [194]. Exploratory arousal is a self-reinforcing state, because it is accompanied by a slight mood elevation, whilst being in the same environment for some time brings in boredom and a slight lowering of mood. Attenuation of the dopaminergic activity in CNS is associated with mood lowering tendencies. For example, "neuroleptic dysphoria" is a common side effect of antipsychotic drug treatment, similar changes in mood are also common in Parkinson's disease and a subcategory of depressive disorders [195]. In NAcc, DA helps in acquiring the relationship between unconditioned stimuli and the environments where consummatory responses take place [194, 196]. Humans may employ ambient mood elevating measures such as a favourite music that

creates a consummatory state to improve their performance in learning new tasks and information [197]; in rats similar effects are produced by low doses of d-amphetamine [198]. Among school and university students, positive emotions were associated with flexible learning strategies such as metacognitions, elaboration, organisation, and critical thinking; whereas states of anger, anxiety, and shame were correlated with rehearsal strategies [199]. The relationship between young age, playfulness, neural plasticity and learning, as well as dopamine transmission and ADHD remain to be elucidated [200, 201].

There are many classes of stimuli, such as intracranial self-stimulation, receptive partners of the opposite sex, tasty foods and drinks, drugs of abuse, and so on, that animals are finding desirable to partake of and are willing to do work or engage in some trade-offs for access. Though some stimuli are objectively more desirable than others, their particular hedonic value at any moment in time depends on personal and situational variables. We can define a particular ability of an organism to derive pleasure from normally hedonic stimuli as a "hedonic capacity" [202] and measure its fluctuations in various experimental and natural situations. In humans were can use self-report questionnaires or behavioural reward responsiveness tasks [203] that show attenuations of hedonic capacity due to temporary stress [204, 205], chronic stress [206], and as a result of clinical depression [207, 208]. Significant reduction in hedonic capacity that can be characterised as a state of anhedonia occurs only in some depressed patients [7, 207] and seems to be psychometrically quite independent from scores of depressiveness and anxiety [209].

In animal research sucrose preference was found to be a reliable measure of their hedonic capacity. Sucrose preference is usually tested in a two-bottle test, where in one bottle is a tap or distilled water and in another bottle is sucrose or another substance, preferred because of its taste or nutritional value. Rats start to prefer sucrose solution over water at concentrations of 0.57% and at increasing concentrations the intake follows a bell-shaped curved with peak intake at 8% [210]. Rats also chronically ingest 3–4 times more water because of their preference for sweetened solution than they would do otherwise with plain testing water [210]. Sweet tasting foods have a great hedonic value for rats and work well as unconditional reinforcers in various learning tests. In on example, rats were willing to negotiate many times back and forth 16 m long alleyway at –15°C cold to eat some sweet palatable foods, in spite of being offered warm comforts of their usual quarters containing a standard chow [211].

Already Richard Katz studied saccharine and sucrose consumptions and preference over tap water by overnight exposure (14 h) after 3 weeks of his initial formulation of USV, which among others included such severe stressors as exposure to 60 minutes of unpredictable electric shock (one 1–10 s shock per minute of 1 mA), 40 h food deprivation, 40 h water deprivation, and 30 minutes of shaker stress [212]. Both saccharine and sucrose produced a concentration-dependent rise in consumption and their intake was significantly reduced by stress regimen and increased by imipramine administration [212]. Interestingly enough, distilled water intake was also attenuated by CVS [212]. In the modi-

fied CVS protocol [143, 157], also employed in our laboratory, generally only sensitivity to stress in sucrose, but not water intake, are observed [213]. Stress-dependent reduction in sucrose intake has been shown to be independent of animals' housing conditions (individually or in pairs) and pre-test water deprivation [213]. In clinically depressed humans, sweet taste perception threshold was significantly higher compared to healthy controls, but did not correlate with the intensity of their depressive symptomatology [214]. Decreased taste sensitivity to sugar does not imply lower consumption of sugary food in depression, in fact, the opposite may be true: a cross-national study, based on the data from 6 countries, found that sugar consumption is strongly correlated with the cross-sectional annualised prevalence of depression [215]. Decrease in rewarding properties of sweet-tasting foods may be counter-balanced by a parallel increase in craving for the same types of foods, as was shown by the progressive ratio operant procedure in rats submitted to CVS, and in human volunteers by the depressive musical mood induction [216]. Other measures of hedonic capacity, such as intracranial self-stimulation to VTA [217] and appetitive or drug-conditioned place preference [218–220], are also attenuated by CVS. There are strain differences in susceptibility to anhedonic effects of CVS: it seems that it is easier to elicit anhedonic changes in Wistar rats compared to Sprague-Dawleys [221, 222].

Anhedonia or inability to experience pleasure is a pervasive feature in many patients diagnosed with depressive spectrum disorders, pointing to the abnormalities in reward-mediating brain circuits. Analogous blunting of positive reinforcers' strength and motivational arousal was noted in patients chronically treated with classical antipsychotics [223]. There is also a significant comorbidity between pathologies characterised by a deficit in central DA-ergic neurotransmission and clinical depression [224], whilst depletion of central DA stores by inhibiting catecholamine synthesis leads to an increase in negative mood and anhedonia [225], as well as a worsened performance in a monetary reward task [226] in patients diagnosed with clinical depression in remission. Dopamine and its metabolites can be measured from blood plasma and cerebrospinal fluid assays. Low levels of DA metabolite homovanillic acid are associated with depressiveness, especially with suicidal tendencies; however, data on DA levels remain inconclusive [227–229]. Abnormalities in D_2/D_3 receptor binding, more so in the right striatum [230], have been reported [231, 232], but not always successfully replicated [233]. Neuroimaging studies in healthy humans have shown activation of ventral striatum in anticipation of aversive stimuli [234]. Decreased DA transporter activity in striatum [235, 236] and midbrain [237, 238], though not universally confirmed [239], tentatively adds up to the clinical signs of attenuated dopamine turnover in clinical depression. A novel method for treatment of resistant clinical depression via a direct bilateral deep brain stimulation of NAcc by surgically implanted electrodes has produced promising results in reducing depressive symptomatology [240], providing a first direct evidence of the causal importance of NAcc for positive affectivity in humans.

Dopaminergic neurotransmission has also been studied in animal models of mood disorders. In male Sprague-Dawley rats submitted to four weeks of CVS, an attenuated accumbal DA release in response to the reward (palatable food) was observed, whereas an acute stressor (10 min tail pinch) reversed an initially lower DA release, compared to control group animals, into potentiated release. In the PFC, CVS potentiated the stimulatory response to tail pinch, concurrently blunting the response to palatable food [241]. Dopamine depletion by a bilateral 6-OHDA-brain lesion in PFC in Wistar rats leads to reduced immobility and enhanced swimming in FST [242]. However, Sprague-Dawley rats, selectively bred for 40 generations for high levels of swimming in FST, tended to have higher tissue levels of DA and metabolites in PFC, compared to selectively bred high immobility rats, whereas in striatum there was no difference [243]. Two separate inbred lines were derived from high activity rats in FST: resistant to stress effects, and reacting by significant increase in immobility in FST. Stress-resistant rats exhibited significantly higher tissue levels of DA and lower DA turnover than stress susceptible rats [243]. In the Flinders Sensitive Line (FSL) of rats, derived from Sprague-Dawley background and selectively bred for increased muscarinic sensitivity [244], DA tissue levels were six-fold higher in NAcc and twofold higher in the dorsal striatum, hippocampus and hypothalamus compared with outbred Sprague–Dawley rats [245]. FSL rats exhibit several characteristics relevant for our research: they are less active in the novel open field [246]; they have higher immobility scores in FST and do not show marked increase in DA release after the end of FST [247]; they do not differ from control Flinders Resistant Line animals in hedonic measures of intracranial self-stimulation and sucrose consumption at baseline [248, 249], but show higher anhedonic response to both acute and chronic stressors [248].

Acute stress produces a significant increase in accumbal extracellular release of both DA [250–252] and glutamate [253], whereas chronic stress reduces extracellular DA [254, 255]. Chronic stress potentiates extracellular DA release after administration of acute stressor in PFC, but not in NAcc [256]. CVS also down-regulates D_2 receptors in the limbic forebrain including NAcc, whilst not changing their sensitivity, and elevates D_1 receptor-specific binding in striatum [257].

A lot of other potential contributors to the regulation of dopaminergic reward circuit responsible for depressive symptomatology have been identified based on animal studies, such as transcription and trophic factors, opioid signalling, hypothalamic peptides, circadian genes, interactions between DA, NA, and 5-HT neurotransmission (see [258, 259] for review).

1.1.3.3. Novelty

Novelty is a quality of an external environment from the subjective point of view of the organism, it is not something that can be directly perceived by an observer, and therefore it can only be inferred from behavioural measurements

and by controlling the exposure of experimental subjects to different environments. An organism's preference for a more novel option is often referred to as *neophilia*, whereas choice of the more familiar is known as *neophobia* [260]. A particular interaction with novel stimulus is contingent on organism's historical experience from which it can generalise to the situation at hand.

For humans and other animals, the initial reaction to a novel cue is a reflexive, directional orientation to the stimulus [261]. In case the input is motivationally irrelevant, the orienting reflex rapidly habituates [262]. On the other hand, novel stimuli, associated with a complex behavioural reaction such as making a choice about what stimulus to attend to, elicit habituation even more rapidly and lead to spontaneous alternation [263]. Behaviourally, some rats tend to prefer uncertain and more difficult ways to satisfy their needs to straightforward options [264, 265]. Habituation to novel experimental stimuli is mediated by relative complexity of novelty in comparison to previous rearing experience [266] and may be governed by a similar non-monotonic function of the amount of exploration and stimulus complexity in both rats and human toddlers [267]. Habituation to novelty depends on both the number and duration of exposures and, therefore, can occur within a testing session, as well as between different sessions.

Complexity of novel stimuli in the environment is positively associated with the amount of directed exploratory activity [268]. At the same time every novelty also possesses a potential for aversion, some of which can be measured objectively, like the brightness levels or illumination intensity at the novel environment in comparison to the familiar alternative. An immediately preceding stressful exposure in the form of electric shock tilts the balance from the usual novelty preference towards familiar environment [269]. Novelty can be thought of as a continuous property of interaction between animal and the environment, where one end of the continuum is very fleetingly novel and therefore is usually ignored as such, whilst the upper bounds of the dimension are frighteningly novel and therefore repel the animal at first, but habituation is possible [270]. Without going into extensive theoretical treatment of the topic, the obvious conclusion is that every stimulus needs to be empirically tested on different animals to determine its general novelty value. Any moment of decision also depends on the mental state of an animal and on the value that a familiar alternative holds at that particular moment in time. It also bears stressing, that novelty is a relative concept initially devoid of any physical substance. Before novelty can be preferred, it needs to be detected and encoded [271]. Hence, preference for novelty is only possible when a representation of some novel object has been formed with attached motivational and affective attributes. Preference is formed on the basis of these attributes, therefore not all novel objects are preferred; some are avoided whilst others raise no interest [271]. Introduction of a novel object into an open field changes the trajectory of rat behaviour in it, as the object becomes a sort of attractor for planning an exploratory activity [272]. These object properties are not fully dependent on its ethological meaningfulness for the animal, in addition, object memory and

recognition mechanism seem to be non-essential for its approach-facilitative properties [272].

An exploration box test [273, 274] is our primary tool for selecting animals based on their trait-like activity pattern in the apparatus. The test builds upon the fundamental dynamics of rat exploratory activity, providing the animal with a ready-made home base [275, 276] and structuring its outward excursions by placing novel objects at variable, but fixed between tests, distances from the small chamber. Rat is initially placed in a home base, which is the safest looking and well-defined place of the apparatus. Hence, for the duration of the test, rat is presented with a dichotic choice of leaving the home base for an open-field-like enclosure or staying put. The experimental idea of giving animal a choice between more familiar and novel environment goes back more than 50 years in history [277]. Novel objects in the open field function as an attractors and help to overcome thigmotaxis [278]. The testing is carried on two days, and the results of the second session are considered a better approximation of rat's stable exploratory disposition, because of the reduced initial impulsiveness and anxiousness [279]. Anxiety is not the primary inhibiting factor in EB, as benzodiazepines do not elevate exploratory activity [273].

Juvenile animals move more around, have a shorter latency of approaching a novel object and revisit the object more frequently than adult animals [280]. In accordance, we have found that exploration levels in our test decrease as rats mature [281]. Most of our tests were performed with 2- or 3-months-old rats.

1.1.4. Inter-individual differences in laboratory animals

Inter-individual or strain differences in animal research are mentioned almost on every page of the current thesis, hence here I just sketch out some ideas of a more general nature, particular animal models are discussed elsewhere.

Mice and rats are the primary laboratory animals for the study of affective states because they are easy to handle, they have a relatively short life-cycle, their emotional states can be well inferred from behaviour and physiological measures, and there is an old tradition in using them in research in comparative psychology and medical studies, hence a wealth of knowledge about these species have been acquired over the years.

The idea that inter-individual differences in rats exist is not new. Already, almost a century ago Yerkes of Yerkes–Dodson law co-discoverer's fame compared wild rats with tame conspecifics and concluded that two populations differed in many measured behaviours, which were not genetically segregated, as crossing the two phenotypes produced a lot of gradual variation [282].

Despite early attention to individual differences, majority of current research still treats a putative strain differences as a non-significant factor, when a certain contradictory results from different labs or experiments are discussed, by routinely omitting any information on genetic background. For example, the long-lasting debate on the nature of rat learning between Spence's and Tol-

man's schools, corroborated by a lot of contradictory experimental data, may have been critically influenced by different genetic background of two populations of rats used by the respective research groups [283].

Behavioural phenotype is a useful concept for analysis of experimental data. It describes a nexus of behavioural traits that are closely linked to an underlying genotype. In mice, the comparison of different inbred strains reveals a broad clustering of certain traits associated with a particular genotype, so that a certain propensities in behaviour go together with certain other propensities, as well as morphology and physiology [284]. Genes have pleiotropic effects that are at some point translated into behaviour, and as behaviour in many cases determines the evolutionary success of a particular organism, it functions as an important level of selection. Therefore if we can pinpoint behaviourally apposite phenotypes to what is observed in human affective disorders, we are also likely to get analogous changes in neurochemistry and other biological processes and thus have a more valid animal model. It is very important to keep in mind that though animal models interest us primarily because we can study their neurochemistry to better grasp what is happening in human CNS and come up with more effective pharmacological treatments for psychiatric conditions, unless there are important reasons to think otherwise, we should start by selecting or breeding for behavioural profiles that, within the constraints imposed by dealing with a different animal species, are matching what we observe in humans. Face validity on behavioural level should ideally produce a better face validity on neurochemical level.

1.2. Brain metabolic activity

1.2.1. Theory and applications

In the average adult human, the brain accounts for approximately 2% of the total body weight but approximately 20% of the energy consumed. Magnetic resonance spectroscopy measurements have indicated that up to 80% of the entire energy consumption in the brain in the state of relative rest is spent on cell cycling, primarily through the glutamate cycling, and maintaining ion currents for signal propagation, but not on housekeeping tasks such as neuronal repair or protein trafficking [285–287]. Fraction of a total energy budget spent on neuronal signalling rises with an increase in the brain activity state [288].

The majority of cortical energy production by glucose metabolism supports functional excitatory glutamatergic neuronal activity at the level of synapse [286], though 15–20% of cortical neurons are inhibitory GABA-ergic interneurons [289, 290], and there are many neurons carrying other neurotransmitters. In human cerebral cortex at resting state and in rodent models, glucose oxidation in glutamatergic neurons accounts for 60%–80% of energy consumption. The remaining 20% to 40% is primarily distributed between GABA-ergic neurons and glia [291]. A theoretical model for cortical metabolic compart-

mentation estimates that most of the glycolysis occurs in glia and glucose oxidation in glutamatergic neurons: still, glia produce at least 8% of total oxidative ATP and GABA-ergic neurons generate 18% of total oxidative ATP in neurons. Neurons produce at least 88% of total oxidative ATP and take up 26% of the total glucose, the rest is taken up by glia [292].

Synaptic input is costlier in metabolic terms than output [293]. For example, a stronger glutamatergic innervation of striatum and low-frequency of GABA-ergic activity of its medium spiny neurons causes higher glucose utilisation rates when we compare that region to a nearby globus pallidus with high-frequency GABA-ergic neurons [289] – a result that is nicely replicated by our own cytochrome c oxidase measurements. Cost of an inhibitory innervation is thus borne out by the projection areas, as shown by metabolic measurement of energy metabolism coupling between globus pallidus and its projection area in the thalamus [289]. Cortical density of cortical inhibitory neurons is 10–15 times lower than density of excitatory neurons, and for each one of them the Cl^{2-} -dependent postsynaptic electrochemical gradient is weaker than that of Na^{+} at excitatory synapses and does not require ATP hydrolysis, using the energy from the cation gradients generated by the $3Na^{+}/2K^{+}$ ATPase instead [294]. Coordination of excitatory and inhibitory cellular activity achieves an overall homeostatic regulation of global firing rates over large cortical areas, yet enables rapid changes in local excitability. A particular trajectory of input-output signal is achieved by inhibitory suppression of competing excitatory cell assemblies [289].

Local tissue blood flow can be convincingly dissociated from the spiking activity of neurons and it is rather consistently correlated with the local field potentials – a geometrical summation of postsynaptic potentials and other somato-dendritic currents such as voltage-dependent membrane oscillations and afterpotentials following soma–dendritic spikes [295]. Blood-flow response to an increase in the cellular activity is proportional to lactate to pyruvate ratio in plasma. This ratio is in a near equilibrium with another ratio of cytosolic free NADH/NAD+, reflecting the intracellular redox state, and points to the important role of NADH in the signalling of a cell's metabolic state. Astrocytes store all of the brain's glucose in the form of glycogen, and a rapid conversion of glycogen into glucose in a process known as glycogenolysis is coordinated by brain noradrenergic circuitry and amplified locally by vasoactive intestinal polypeptide (VIP) [296–298]. The energy demands of membrane-bound $3Na^{+}/2K^{+}$ ATPase and Na^{+}/H^{+} exchanger in astrocytes seem to be preferentially served by glycolysis, whereas neurons require oxidative metabolism [299].

The regional increases in absolute blood flow associated with PET imaging are rarely more than 5%– 10% of the resting blood flow of the brain [300]. In comparison, *in vitro* repetitive electrical stimulation of rat's posterior pituitary with pulses of 10 Hz for 10 min has produced 29% increase in [^{14}C]deoxyglucose-6-phosphate accumulation in the tissue above control values, the effect of which was abolished by the blockade of Na^{+}-K^{+} pump [301]. The majority of energy consumption in the brain can be understood as a basal or an

intrinsic activity invariant to the task at hand, whilst evoked changes in brain activity elicit rather small increase in local metabolic demands. During the experimental task performance, recorded metabolic activity in its majority is not directed to the task, but may rather manifest as “daydreaming” or go altogether consciously unregistered [302]. For example, there is no difference between awake and sleeping humans in cerebral oxygen consumption, hemoglobin concentration, and arterial oxygen content [303]. However, 3 h of spontaneous wakefulness or sleep deprivation uniformly upregulated cytochrome c oxidase (COX) cerebral enzyme activity and mRNA levels of several COX subunits across brain regions in comparison to 3 hours of spontaneous sleep in rats [304]. When brain activity is measured at its resting state, then across different experiments a remarkable consistency in the regional activation pattern emerges, showing a group of separate brain areas that act in a concert and decrease their activity across a wide array of task conditions when compared with a passive control condition such as visual fixation. This phenomenon was called the default mode network [305]. In human brain, resting state coherence has been documented in many cerebral networks and in thalamo-cortical activity [306].

Inhibitory tonic stimulation is conducted mainly via high-affinity extrasynaptic $GABA_A$-receptors, containing δ-subunits that are often co-assembled with α6- or α4-recepor-subunits [307]. The extrasynaptic $GABA_A$ δ-subunit-containing receptors are also the main targets of neuroactive steroids, such as 3α,5α-THPROG, implicated in the pathophysiology of depression [308, 309]. It is interesting to note that there is no correspondence between the increase in glucose metabolism and state of alertness, for example, $GABA_A$ specific agonist (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) administered to patients with temporal lobe epilepsy increased metabolism whilst also producing a sedative effect and sleepiness after drug administration [310].

The cerebral glucose utilisation is commonly measured from autoradiographic films by determining the levels of the injectable tracer 2-deoxy-[^{14}C]glucose (2DG) [311]. In rats that underwent olfactory bulbectomy (another animal model of depression) 2 weeks before 2DG autoradiography, cerebral glucose utilisation was reduced by about 5% on average [312], two weeks of SSRI citalopram administration further decreased cerebral glucose utilisation [313]. Cerebral glucose uptake has also been shown to generally decrease in the comparative study of the three different animal models of depression: inhibition of tyrosine-hydroxylase by α-methyl-paratyrosine, withdrawal from chronic amphetamine administration, and 20-day CVS regimen [314]. All models were verified by attenuated horizontal and vertical activity in open field. In each animal model, local metabolism was bilaterally elevated in lateral habenula; this effect was abolished by both acute and chronic MAO inhibitor tranylcypromine administration, whilst antidepressant had no effect on a globally decreased cerebral metabolism [314].

A tricyclic antidepressant desmethylimipramine, given to behaviourally unmanipulated rats, increased 2DG uptake in 11 brain regions out of 30 studied if given acutely 1 h prior to metabolic measurements, did not affect metabolism in

any region after the administration for 1 week, and decreased 2DG uptake in 7 regions after 28-day of chronic treatment, all the while another antidepressant phenelzine – a MAO oxidase inhibitor – had little effect on brain metabolism [315]. In contrast, effects of SSRI fluoxetine administration were quite different in male Fischer 344 rats: 2DG uptake was attenuated in a dose dependent manner on acute administration with a mean decrease of 32% across regions at the highest dose, whereas 2-week chronic administration (8 mg/kg) produced decrease in 8 regions out of 56 measured (10% in total); regions with significant decrease were visual cortex (BA17), CA3, cortical amygdala, anteromedial thalamus, lateral and medial geniculate, substantia nigra pars reticulata, and vermis [316]. Similar results were obtained with tricyclic 5-HT reuptake inhibitor clomipramine [317].

1.2.2. Cytochrome c oxidase activity mapping

Cytochrome c oxidase (COX; EC 1.9.3.1) is the terminal oxidase of the mitochondrial electron transport chain that catalyses the oxidation of cytochrome c and the reduction of O_2 to water. It is an important energy-generating enzyme, whose activity and amount in the brain vary with metabolic demands and are critical for the proper functioning of most cells, especially those of highly oxidative organs like a brain. COX is a bigenomically encoded multisubunit enzyme: the largest three catalytic subunits (I, II and III) are encoded in the mitochondrial genome, whilst the other 10 subunits are encoded in the nuclear DNA. All 13 subunits show upregulated gene expression by a long-term KCl depolarising stimulation and downregulated mRNA levels by functional inactivation in rat visual cortex [318]. A coordinated transcriptional response corresponds to an increase in energy demand [318, 319]. The same transcription factor (nuclear respiratory factor 1 or NRF-1) regulates all 13 COX subunits [320] and a number of neurochemicals associated with glutamatergic neurotransmission [321–323].

Cytochrome c oxidase activity is visualised in unfixed brain tissue sections in a reaction where cytochrome c is reduced by diaminobenzidine (DAB), resulting in a precipitated brown reaction product (oxidised DAB), which can be quantified with a good spatial resolution up to the cellular level. Furthermore, a linear relationship has been found between the optical density of the reaction product in histological sections and spectrophotometrically measured local enzyme activity [324].

In neurons most energy demanding parts are synaptically active extensive dendritic trees, rather than cell bodies or axons [325]. Accordingly, the highest density of COX is found in somato-dendritic regions that are adjacent to axon terminals [325]. Cytochrome c oxidase activity is functionally coupled to neuronal firing of action potentials and is significantly diminished in postsynaptic neurons by functional denervation of sensory afferents [326, 327], as well as tetrodotoxin blockage of impulse conductance [328]. However, propagation of

action potentials, especially of the saltatory type in myelinated axons, consumes only 0.3–3% of total energy, therefore white matter invariably gives a very low signal with metabolic markers such as 2DG and COX [329]. In gray matter, zones of intense COX staining correspond to the synaptic terminal fields of some input pathways, whilst others are characterised by intense lactate dehydrogenase staining [330]. There is, however, no association between blood capillary density and COX activity in gray matter [330], suggesting that COX histochemistry measurements would differ from techniques based on the imaging of hemodynamic response. The congruence between 2DG uptake and COX activity seems to be non-linear, with a closer correspondence at the higher end of the regional metabolic demand [330, 331].

Oxidative metabolism may be relatively more important in sustaining inhibitory neurotransmission in comparison to the import of anaerobic glycolysis for local changes in excitatory synaptic input. Hence, GABA-ergic neurons in the striate cortex of the monkey exhibit up to 3 times higher COX activity levels than in the surrounding pyramidal cells [332]. Among the GABA-ergic neuron types in the hippocampus, inhibitory fast-firing basket cells and long-range interneurons show more intensive COX staining, and their beaded dendrites, as well as large axon terminals possess a higher density of mitochondria than co-localised pyramidal cells [333].

Various psychiatric conditions may have distinct bioenergetic profiles, hence identification of metabolic networks in healthy and diseased brain offers an opportunity of better understanding of the underlying brain pathology, recovery mechanisms, as well as a refinement of the current psychiatric nosology and an additional tool for validating animal models of human psychiatric illnesses. The COX activity has been found to be suppressed after traumatic brain injury [334, 335], schizophrenia [336, 337], and in Alzheimer's disease [338, 339].

Mitochondrial DNA is inherited only from maternal ova and a mixture of more than one type of mtDNA can be present in a single cell, a condition known as "heteroplasmy". Human cell contains up to 100,000 copies of mtDNA and due to the relaxed replication and mitotic segregation, the proportion of genetically different lines of mtDNA in an average cell-line may change over person's lifetime and become debilitating with certain physiological changes that generate increased metabolic demands because of the stress, hormonal changes or illness. Several lines of evidence point to the mitochondrial dysfunctions in human mood disorders [340]. Significant decreases of mitochondrial ATP production rates and mitochondrial enzyme ratios were found in clinically depressed patients whose symptomatology suggested mitochondrial dysfunction; mitochondrial ATP production rates were also negatively correlated with "somatic anxiety" and psychasthenia scales from Karolinska Scales of Personality inventory [341]. A significantly higher rate of withdrawn, depressive behaviour was identified in children with the confirmed disorders of oxidative phosphorylation [342]. In post-mortem studies among clinically depressed patients, mRNA and protein levels of mitochondrial respiratory chain complex I subunits NDUFV1, NDUFV2 and NADUFS1 were found to be significantly

decreased [343]. The presence of a certain somatic symptomatology in a clinically depressed person has a good predictive validity for identifying a role of energy metabolism in pathogenesis [344].

Measurements from brain homogenates of the enzymatic activity in the mitochondrial respiratory chain in male Wistar rats submitted to the 40-day CVS regimen showed inhibition of complexes I, III and IV (COX) in cerebellum and cortical areas excluding the prefrontal cortex [345]. Chronic stress effects were reversed by acute administration of glutamate NMDA-specific antagonist ketamine [346]. However, in a study with analogous stress regimen by a different research group from Brazil, CVS reduced COX activity in prefrontal cortex and hippocampus, whilst co-administration of vitamins C and E, that possess antioxidative properties, reversed the effect of CVS [347].

Antidepressant treatment can potentially have a bioenergetic effect. In female rats, treated for 2 weeks with a classical TCA imipramine, an increase in oxidative brain metabolism, measured from the whole brain homogenates with different substrates, was recorded, whereas COX levels nearly doubled by the end of the second week [348]. Another TCA amitriptyline, which exerts marked anticholinergic and antihistaminergic effects in addition to 5-HT and NA reuptake inhibition, has decreased COX activity after single dose administration in male CD1 mice, whilst inhibitory avoidance task produced a unique pattern of changes modulated by antidepressant's administration [349].

Electroconvulsive therapy is considered to be among the most effective treatments for clinical depression, especially in its refractory form in humans. In naive Long-Evans rats, administration of eight electroconvulsive seizures increased global COX levels already 24 h after the last treatment session, which were further increased 28 days post-treatment, wherein statistically significant elevations were found in the bed nucleus of the stria terminalis (+ 25%), interpeduncular nucleus (+ 20%), dorsomedial hypothalamus (+20%), ventromedial hypothalamus (+ 12%), mammillary nucleus (+ 14%), pontine nucleus (+ 16%), basolateral amygdala (+ 14%), medial amygdala (+ 12%), piriform cortex (+ 12%) andventromedial thalamus (+ 9%) [350].

Neuroleptics, chronically administered to healthy rats, uniformly increased oxidative metabolism in prefrontal cortex, whereas clozapine and fluphenazine, but not haloperidol increased COX activity in basal ganglia, septum, pontine nucleus, and CA2–3 areas of hippocampus [351].

1.2.2.1. COX measurements in translational models of affective states

Congenitally helpless rats are currently the most thoroughly studied by means of brain oxidative metabolism measurements animal model of a human psychiatric ailment. Congenitally helpless rats from the 34th generation were compared to rats selectively bred for the resistance to helplessness, both lines originating from Sprague-Dawley outbred line. Animals were experimentally

naive. Lower COX activity levels were found in dorsal frontal, medial sulcal, anterior cingulate cortical areas [352]; NAcc; dorsal striatum; ventral pallidum; globus pallidus; basolateral and central amygdala; VTA; dorsal raphe [353]; lateral and medial septum; nucleus of the diagonal band; bed nucleus of stria terminalis [354]; whereas higher activity was found in infraradiata cingulate [352]; lateral and medial habenula; interpeduncular nucleus [353]; paraventricular nucleus of the hypothalamus [355]; CA1, CA3, and subiculum regions of hippocampus [354]. Treatment of congenitally helpless rats for two weeks with fluoxetine (5 mg/kg) attenuated metabolism in habenula, hippocampal dentate gyrus and dorsomedial prefrontal cortex, as well as increased it in VTA [356]. Cerebral metabolic activity in newborn congenitally helpless rats was lower in about two thirds of the studied brain regions compared to non-helpless pups [357]. A statistical decoupling between the limbic forebrain and diencephalic/mibrain regions was found by covariance analysis [357].

Hypermetabolism in lateral habenula and hypometabolism in VTA seem to be the most robust findings in congenitally helpless rats if COX enzyme's raw activity scores are compared between studies [353, 356]. Lateral habenula (LHb) is involved in the modulation of monoaminergic neurotransmission in general [358], and dopaminergic in particular. Electrical stimulation of the LHb inhibits via $GABA_A$ receptors the activity of dopamine-containing neurons in the substantia nigra pars compacta (SNc) and VTA [359, 360], whereas disruption of habenular innervation of the midbrain dopaminergic centres leads to the disinhibition of SNc and VTA [361, 362], as well as to the increased dopamine turnover in forebrain [363]. In turn, systemic and striatal administrations of DA receptor agonists cause attenuation of LHb activity [364–366], indicating the existence of a feedback loop between the activity of LHb and midbrain dopaminergic centres, mediated by forebrain regions [367, 368]. Opposite roles of VTA and LHb have been identified in the acquisition of avoidance learning [369]. Absence of expected reward or received punishment suppresses dopaminergic activity through GABA-ergic input originating from LHb [370–372]. Stressful stimuli act in a similar fashion by initially activating the medial portion of LHb [372–374], and then differentially affecting midbrain dopaminergic neurons by inhibiting some and exciting others [375]. Pharmacological inhibition of LHb transiently alleviates learned helplessness [376], and there are indications that the blockade of DA D_2 receptors prevents the alleviation from happening [377, 378]. Deep brain stimulation of LHb has been proposed as a treatment option for resistant clinical depression in humans [379], and the first case study of its application was successful [380].

1.3. Exploratory behaviour

1.3.1. Neurobiology of exploratory behaviour

Exploratory behaviour can be conceptualised as an unconditioned behavioural response to novelty. From the point of view of an organism novelty is a subjective term and entails feeling of unfamiliarity and uncertainty, from the observer's point of view novelty can be objectivised by placement of experimental subject in the standardised environment for the first time during his life course. Exploratory behaviour is studied from several perspectives: 1) as a proxy for studying anxiety, 2) as a motivated behaviour to engage novelty in a preferential manner, 3) as instrumental for eliciting a behavioural act useful for screening and diagnostic purposes in pharmacological manipulations, 4) as a repertoire of behavioural and cognitive adaptations for problem-solving and survival of an organism [381–383]. Not any behaviour in a novel situation can be classified as exploratory: only behaviour facilitating one's familiarity with a novel environment and, consequently, gradually extinguished upon attainment of the desired degree of familiarity is exploratory [383].

Curiosity is a powerful motive and being in the state of curiosity for an organism is self-rewarding [384, 385]. Animals are willing to tolerate a risk of physical harm, even death; as well as other unpleasant trade-off to engage in exploratory behaviour. Food-deprived rats forego food and would endure electric shock of crossing an electrified grid floor for an opportunity to explore a novel, object-filled apparatus (reviewed in [382]). Exploratory activity can be motivated by a necessity to meet organism's present biological needs (find food, shelter, mates) or it can be activity of passing time by gathering information that can be useful in meeting future needs. In the Pacific Ocean a rat, introduced to the uninhabited island, swam for 400 m in the open sea to the next island, most likely in search of a mating partner [386]. Maintaining a territory and establishing one's presence by regularly patrolling its borders is a type of behavioural response occurring in natural settings and in repeated experiments [387].

There are various established tests purported to measure exploratory behaviour in rodents. They can vary on several significant dimensions. Exploration can forced and inevitable or a result of animal's choice between familiar and novel parts of test apparatus. The balance between approach and avoidance intrinsic to any novel environment can be tweaked by modifying fear-inducing properties and complexity of neotic stimuli [260].

Exploration box test employed in my research is an elaboration on the open-field arena. Open-field test is widely used in animal research but has a several shortcomings, such as the absence of meaningful choice in animal's behaviour repertoire – open-field is empty and devoid of attractions, so that even if rat's behaviour in it is not uniformly clustered, there is no ethological meaning to its choices other than situational fear and yearning for security [276, 388, 389]. Being in an open-field is forced upon animal and therefore it is hard to diffe-

rentiate between spontaneous response to novelty and behaviours due to fear and anxiety. Locomotor activity in the forced exploration situation can be to a significant extent explained by animal's escape motivation [390]. Some of exploratory behaviour may be also explained by the defensive approach tendency produced by the state of anxiety [391], hence the anxiolytic influence of treatment with benzodiazepines does not increase exploration in every test arena, though it is generally effective in countering the initial neophobia [392]. Intact mesolimbic and mesocortical dopaminergic pathways are necessary for normal exploratory behaviour [393, 394]. Exploration is decreased and defensive withdrawal increased by the intracerebroventricular application of CRF, whereas CRF antagonists enhance non-defensive exploration acutely [395], but may suppress it after chronic administration [396]. On repeated daily testing in the same apparatus activity scores on the first day of testing are significantly influenced by emotional reactivity, whilst high activity from Day 2 onward reflects lower emotionality and more genuine exploratory motivation [397]. Central CRF administration prevents the increase in exploration on the second day re-exposure to the test arena [395], whereas CRF_1 receptor antagonists might enhance it [396]. Selective destruction of noradrenaline-containing nerve endings originating from LC by neurotoxin DSP-4 severely diminishes exploratory activity in the exploration box, though a small rebound in exploration is still observable during the serial testing after several exposures to the apparatus [273, 398].

1.3.2. Inter-individual differences in exploratory behaviour

Exploratory behaviour and adjunct anxiety and locomotor activity constitute a nexus of behavioural dimensions studied widely, but not particularly systematically, in many species of animals [399]. Hereby I concentrate on a couple animal models of exploratory behaviour that have been well researched and whose results are pertinent to our model of LE/HE-rats. For a broader recent overview of the field, reader is directed to [400, 401].

A higher than average horizontal locomotion on the first placement in the novel environment was conceptualised as an animal model of reactivity to a stressful environment that might predispose individuals to initiate drug-taking behaviour. Sprague-Dawley rats were divided by the median split into low (LR) and high responders (HR) after 2 hours of testing in a circular corridor and found to also react differently to the first dose of amphetamine administration: HR travelled more distance in the first 30 minutes of testing and had increased amphetamine self-administration before the sensitisation [402]. Upon further retesting all group differences disappeared [402]. The difference between HR and LR groups in cocaine self-administration holds up only in conditions of forced exposure to an inescapable novel environment, which is more stressful to HR, as indicated by the enhanced secretion of plasma corticosterone [403], whilst animals divided into HR and LR groups based on tests of free-choice

preference for novelty, such as the duration of contact with a novel object in the playground maze or novelty induced place preference, do not differ in cocaine self-administration [404]. In forced novelty HR-rats, presentation of a novel stimulus has a more potent disruptive effect on amphetamine self-administration at low doses, whilst this effect in absent in HR-rats, classified based on free choice novelty exploration [405], who also do not differ at basal levels of amphetamine self-administration [404, 406]. Reactivity to novelty and novelty preference seem to be uncorrelated behavioural domains that differentially predict dynamics of addiction to stimulant drugs [407]. In contrast, an overlap in brain mechanism that ascribe rewarding properties to novelty and amphetamine is likely [408]. It is noteworthy that horizontal and vertical activities in the open field seem to be well correlated [409], and some research groups prefer to base their LR/HR selection on vertical activity (hind paw rearings) in the test apparatus. Vertically based LR/HR phenotypes replicate some of the drug sensitivity, behavioural reactivity and neurochemical profiles of horizontally based LR/HR-rats, however, differences between LR and HR groups seem to be more subtle and cohort-sensitive [409–412].

Differences in sensitivity to stimulants, locomotion, and rewarding properties of different classes of stimuli, all point to the mediating role of dopamine in this behavioural phenotype. In the initial paradigm of selection, based on the activity levels in a circular corridor, HR-rats showed a higher basal DA turnover in NAcc and striatum and a lower one in the prefrontal cortex; they also had lower 5-HT and 5-HIAA levels in all three brain regions compared to LR-rats [413]. HE-rats are characterised by higher basal firing rates and bursting activity of DA neurons in VTA and SNc [414]. A higher dopaminergic activity in NAcc of HR-rats is enabled by the diminished inhibitory regulatory input from VTA dopaminergic neurons [415]. Administration of 10 min tail-pinch as a stressor produced larger and longer extracellular DA concentration increase in HR-rats, whereas the groups did not differ at baseline [251]. Mean percentage increase in DA concentration was positively correlated with locomotor activity in a novel environment [251]. The HR/LR difference in stress-dependent DA release in NAcc is conditional on intact corticosterone secretion [416] and corticosterone possesses reinforcing properties by itself, as self-administration of it develops at plasma levels similar to those induced by stress, HR-rats being more sensitive to lower doses of corticosterone than LR-rats [417]. In HR, selected based on the combined habituation latency and travelled distance in the open field from the outbred strain of Nijmegen Wistar rats, were found higher accumbal levels of total and vesicular dopamine, vesicular monoamine transporter-2, and higher extracellular dopamine levels after cocaine administration [418]. Direct infusion of DA into NAcc produces greater increase in open-field ambulation and rearings in HR [419]. This effect is mediated by the increase in D_1 receptor binding and decrease in D_2 receptor binding as well as mRNA levels in NAcc, whereas the receptor affinities are unchanged [419].

There are no differences in tissue dopamine levels and turnover between LE- and HE-rats in frontal cortex, hippocampus, striatum, and NAcc, whereas HE-

rats have higher 5-HT concentrations in frontal cortex and striatum [420]. However, LE-rats had lower extracellular dopamine levels in striatum but not in NAcc [281], as well as lower proportion of DA D_2 receptors in the high affinity state [421]. Dopamine D_2 receptor-mediated [^{35}S]GTPγS binding in striatum was comparable in LE- and HE-rats under baseline conditions [420].

In a modified open field test, in which a small and dark withdrawal chamber containing a rat was placed into the brightly lit test apparatus and rat's exploratory activity was recorded for 15 minutes, which makes the overall test design similar to our lab's exploratory box; HR-rats of Sprague-Dawley strain, preselected for their motor activity in the rectangular chamber, spent only about 1/4 of their total time in seclusion, had a shorter exit latency, exited more often, and were more exploratory in all measured parameters [422]. In the EPM, HR-rats made more than twice as many open arm entries and spent nearly threefold as much time on them; they also fell down from the apparatus significantly more often [422]. When confronted with loud, startle-inducing auditory stimuli, HR-rats emitted significantly more 22-kHz distress USVs than LR-rats, although the last result is biased by the selective subject inclusion into analysis criteria (only 77% LR- and 63% HR-rats were included) [422].

Interplay of genetic and environmental factors in producing affective vulnerability lies at the core of many animal models. Influence of stressful environment on LR/HR phenotype has been studied in a number of experiments. HR-rats showed a higher ACTH and corticosterone secretion upon placement in the novel environment [423, 424], which was associated with memory deficits later in life [425], as well as reduced cell proliferation and resultant lower packing density of neurons in the dentate gyrus of hippocampus [426]. In 4-months-old LR/HR-rats of Wistar strain, divided into groups by the median split based on 10 minutes of their locomotor activity in a big open field arena (1.5 m of diameter), administration of 21-day-long psychosocial stress regimen caused spatial learning deficits in the water maze only in HR [427].

Learned or congenitally helpless rats constitute the second animal model widely discussed in this thesis, so I briefly describe what is known about its exploratory behaviour. In Wistar Kyoto, characterised by the passive coping strategies in several stressful tests, and ordinary Wistar rats, the open field activities were uncorrelated with the performance in learned helplessness paradigm [428]. In Holtzman strain rats, index of reactivity to novelty in open field predicted subsequent helpless behaviour in shuttle box [429]. Congenitally helpless rats show higher levels of ambulation and rearings in the novel open field [139], but show accelerated habituation on repeated testing. Their elevated response to novelty may be explained by the heightened reactivity in the first 5 minutes of testing, which quickly subsides to the level of control animals later on during the continuing exposure to the apparatus [430]. Congenitally helpless rats also show that sensitivity to reward and novelty seeking are independent behavioural domains [139].

1.4. Expressed positive affect

1.4.1. Neurobiology of positive affective states

It seems that well documented finding of negative or pessimistic bias in emotional processing and associated arousal [431, 432] extends into the scientific discipline itself, as most of the resources are spent on trying to understand the negative affective states, whilst positive emotionality rarely sparks systematic interest. Current fashionable emphasis on *positive psychology*, i.e.: "[the field of positive psychology] at the individual level [...] is about positive individual traits: the capacity for love and vocation, courage, interpersonal skill, aesthetic sensibility, perseverance, forgiveness, originality, future mindedness, spirituality, high talent, and wisdom" [433: 5] risks conflating the actual human behaviour, characterised by its proximal causes, with a vision of some ultimate causality of a "proper" lifestyle and also does not translate well into animal research.

Vocalisations of preverbal infants serve as a reliable indicator of their well-being and are well understood by adults irrespective of their parental status [434]. A distinct prosodic acoustic profile was identified from human utterances when study participants were asked to convey their target emotional state by pronouncing an emotionally neutral word [435]. Well-encoded information about emotional state of call emitter has also been identified in such diverse species as dogs (whose barks contain emotional information decipherable by both humans and conspecifics [436, 437]), elephants [438], and non-human primates [439]. Small rodent species emit calls in the ultrasonic spectrum (calls above 20 kHz). In rats, high frequency calls, also referred to as 50-kHz USVs or chirps, have a usual frequency range of 45–55 kHz and a relatively short duration of 30–50 ms [440, 441]. Their usual bandwidth is 5–10 kHz, but it can be broader [440, 442]. Chirps occur in manifold naturalistic contexts of social or hedonistic kind, including juvenile play solicitation, actual "rough-and-tumble" play with a conspecific, or play imitation by tickling with a human hand; male approach and ejaculation during copulation; meeting rats after a period of separation, or in the initial meeting of a resident-intruder pair; social exploration [440, 443]. Aversive stimuli evoking taste aversion, fear, pain, defeat, or blockade of opioid receptors, all robustly decrease rates of 50-kHz USVs [444, 445]. Recently it has been shown that 50-kHz USVs can be further subdivided into constant frequency (flat) calls and frequency modulated [FM] (with trills) calls, of which the former are rather associated with ambiguous social situations, non-rewarding explorations and separation from cage mates [442, 446], whilst the latter are indicative of pleasurable circumstances and a good mood, and therefore may be the evolutionary and functional antecedent of human laughter [447]. Frequency modulated calls have broader bandwidth range than flat USVs [442]. In some situations affective-behavioural states of the call emitter readily induce a similar affective-behavioural states in the receiver [448] and, thus, constitute the ontogenetically earliest form of empathy

[449]. Listening to 50-kHz FM USVs is rewarding to the receiver and rats can be conditioned on their playback [448]. Playback of 50-kHz chirps also leads to approach behaviour, which is more pronounced in juvenile rats, toward a signal-emitting loudspeaker [448]. Approach behaviour is facilitated by morphine administration and diminished by naloxone treatment [450].

In the social interaction test, 0.25 g/kg daily ethanol administration increased 50-kHz USVs about two-fold on 8th test day in pairs of adult Sprague–Dawley male rats that had already seen each other on several occasions previously [451]. Infusions of dopamine and acetylcholine receptor agonists into NAcc induce 50-kHz calls [452–454]: shell region produces larger increase in the number of calls to amphetamine application [454], whilst core region is more sensitive to carbachol [452]. Both electrical stimulation by experimenter and self-stimulation of ventral tegmental area and its major projection areas (lateral hypothalamus, nucleus accumbens, and prefrontal cortex) also induce 50-kHz calls [455]. Repeated amphetamine exposure leads to increase only in 50-kHz FM USVs [456]. Electrolytic or neurochemical lesions with 6-OHDA of A10 dopaminergic nuclei in VTA reduce the number of tickling-induced FM 50-kHz calls, similar effect is also achieved by blockade of dopamine receptors by an injection of D_1/D_2 antagonist flupenthixol [448]. It is important to note that lesioning of nigrostriatal dopaminergic pathway leads also to the reduction of 50-kHz FM USVs, as well as to the decrease in call's bandwidth and maximum intensity, but in that case they are replaced by flat type of chirps, indicating rather the sensorimotor deficit in USV production and not the appetitive deficit in motivation [457]. A peripheral pharmacological blockade of histamine H1 receptor by diphenhydramine (40 mg/kg i.p) reduced both 50-kHz FM USV calls and even more 22-kHz calls, which are indicative of a negative affective state of the animal [458]. Histamine receptors blockade may have had a sedative effect, which was not controlled by behavioural testing. Acute injections of 1 mg/kg naloxone – μ-opiod receptor antagonist, modulate rats' response to human tickling dependent on their housing conditions: naloxone administration suppressed 50-kHz USVs in otherwise more socially active single-housed animals, whereas in socially housed rats the 50-kHz USVs were elevated [459].

1.4.2. Inter-individual differences in expressed positive affect

Humans have engaged for millennia in selective breeding of domesticated animals, where in many cases a pleasant character and docility of a specimen played an import role in its breeding success. With the constant progress and refinement in the organisation of human society one has to wonder, whether it is possible to observe the similar effects of mostly positive eugenics on human personality. Most of the data available in that respect are of a short time horizon and are confounded by the concomitant developments in technological, economic, and individuation spheres; however, some tentative trends can be

identified. Before the recent economic recession reported levels of satisfaction with life in the Western developed countries were on a slight upward trend from the 1970s onward [460, 461], however, the plateau with no additional effect of the increase in income has, probably, also been reached in some countries [462]. Economic prosperity, thus, predicts a general levels of life satisfaction, in contrast, experience of positive emotions is based on the ability to meet personal psychosocial needs [463] and is, therefore, dependent on the social context [464, 465]. In contrast to the rising prosperity, violent interpersonal crime in Western Europe has been on a constant decline since late Middle Ages: in fifteenth century an average yearly homicide rate per 100,000 individuals was 41, in 18th century it has declined to 3.2 and in 20th reached the low of 1.4, whilst demographic characteristics of the offenders changed very little [466]. Adult criminal offending included a significant hereditary component [467], and as increased incarceration of criminals [468] leads to negative effect on their fertility [469], in combination with an advances in birth control the selective evolutionary pressure on future human generations will continue to affect their personality [470].

In monozygotic twins, the genetic component accounted for 44–52% of their well-being, as measured by Multidimensional Personality Questionnaire, indicating that despite different life history and socioeconomic factors, their happiness levels were remarkably similar, even if a 10-years-earlier well-being score of one's sibling was used for analysis [471]. Of the personality traits, extraversion scale is the best predictor of personal happiness, probably via mediation of good social relations [472], whereas higher neuroticism predicts poor mental health [473, 474]. In addition to personality, lifestyle strategies have a small independent contribution to the perceived level of personal happiness [475]. Trait extraversion is positively correlated with the easiness of positive mood induction [476, 477], but imposes fitness costs as well as benefits [478].

In animals the study of individual differences in their positive affective states is at its infancy. The main body of the work is carried out in rats selected or bred for their vocalisation's profile.

During human tickling sessions the number of both types of 50-kHz USVs emitted by adult rats was strongly positively correlated with their locomotor and rearing activities, suggesting the extroversion-like clustering of good mood and social interest [479]. Among all tickled rats, two subgroups: 1) the most receptive to the tickling by emitting the highest number of 50-kHz USVs and 2) having the fewest tickling-induced 22-kHz calls – had positive association with the cell proliferation in the subgranular zone of the dentate gyrus of hippocampus [479].

Selectively breading rats for low or high levels of 50-kHz-calls is another, more robust method to determine, which traits cluster together with high or low positive affective style. Even though animal selection for subsequent breeding was determined only by the number of emitted 50-kHz USVs, high chirpers demonstrated both elevated rates of 50-kHz USVs, as well as a concomitant decrease in negative affective 22-kHz USVs, and vise versa for low chirpers

[480, 481]. High levels of positive affectivity in this animal model generally preclude concomitant high levels of negative affectivity. It is remarkable that high chirpers were not specifically selected for 50-kHz FM USVs, but when the 14th generation of selectively bred animals was tested, the difference in emitted positive affective calls was determined solely by the FM 50-kHz calls, as both high and low chirpers exhibited fewer flat 50-kHz USVs than control non-bred animals [480]. The absolute number of flat 50-kHz USVs in adult was low enough, however, in all groups during human administered tickling, to suggest that this type of calls has no significant biological function during playful behaviour, whilst in juveniles it plays some social role. High chirpers compared to control animals also exhibited more crosses into the centre area of the open field; were more likely to show a mild preference for a sucrose solution over water, but not an overall increase in sucrose intake; and offered practically no defensive bites in confrontations with an aggressive resident on his territory [480]. Met-enkephalin levels measured by radioimmunoassay on average were significantly elevated across all brain regions and specifically in septum and hypothalamus in high chirpers compared to control animals [480].

2. AIMS OF THE PRESENT STUDIES

Drawing on rodents, selected for their behavioural dispositions or vulnerability after targeted genetic mutation, unifies the studies communicated herein. Chronic variable stress was used to induce depressive-like symptomatology in animal models of exploratory activity in a novel environment (HE- and LE-rats), reduced vesicular transport of glutamate (VGLUT1+/- mice), and affective reactivity to playful stimulation (rats with low and high numbers of emitted 50-kHz USVs). Cytochrome c oxidase histochemistry was used in all three animal models to measure the cerebral oxidative metabolism and identify novel candidate brain regions that might be implicated in affective psychopathology.

Previously identified preference for passive coping styles in LE-rats, combined with changes in DA-ergic neurotransmission suggested that they may be more vulnerable to CVS' impact, especially to its anhedonia-inducing effects. We also assumed that exploratory phenotype may be reflected on the level of the cerebral oxidative metabolism.

The hypothesis was tested that stable inter-individual differences exist in 50-kHz ultrasonic vocalisations emitted in response to play-like tactile stimulation by experimenter. We assumed, that low expressed positive affectivity may be causally associated with an impaired ability to adapt to the stressful environment and these processes might be gender-specific. Both male and female rats were studied, since in humans, prevalence of affective disorders and underlying mechanisms of vulnerability are gender-dependent. A standard battery of behavioural tests was used to describe behaviour of preselected animals in novel, fear-inducing, stressful, and hedonic situations; the hypothesis being that effects of CVS and baseline affectivity should reveal itself as broader behavioural differences.

Mice with reduced VGLUT1 function were hypothesised to be more vulnerable to the impact of CVS because of their increased intracerebral glutamate/GABA ratio, which serves as an endophenotype of mood disorders in some human clinical samples. If that was the case, the subchronic antidepressant administration was postulated to counter the harmful CVS effects. We also expected to observe systemic adaptation in the aminergic neurotransmission in mutant mice due to compensatory developmental processes.

Deficits in dopaminergic neurotransmission have been linked to anhedonia and decreased motivation. Dopamine tissue levels in striatum, NAcc, and frontal cortex, as well as functional state of D_2 receptors were explored in association with tickling, whereas functional states of D_1 and D_2 receptors were studied in striatum and NAcc in association with exploration. In addition to the associations of the phenotypic variations with synaptic signal transmission in the CNS, we sought out whether application of CVS will differentially modulate those neurotransmitter circuits. Previously identified association between affective vulnerability and changes in cerebral oxidative metabolism was studied in relation to phenotypic dispositions in control and chronically stressed animals to determine candidate brain regions differing in their activity in

normal and pathological states. Previous results have indicated that phenotypic affective vulnerability is characterised by an elevated metabolic activity in some brain regions and reduced in others, we also expected to see diverse, gender- and phenotype-mediated effects on COX levels.

Identification and characterisation of the stable behavioural phenotypes associated with affective abnormalities, and as a consequence, refinement of the existing animal models of human mood disorders, was the ultimate objective for the work presented in this dissertation.

3. Materials and methods

3.1. Animals

3.1.1. Rats

For studies described in Papers I and II, male Wistar rats were obtained from Scanbur BK AB, Sollentuna, Sweden. Locally bred first generation offspring from males and females originating from the same supplier were used in Papers III and IV (five breeding pairs in Paper III and seven breeding pairs in Paper IV). All animals arrived to our animal facilities at the age of approximately 3 weeks. Animals were group-housed four per cage in experiments for Papers I and II. Rat pups were weaned at the age of 3 weeks and single-housed thereafter in Experiment 1 of Paper III. In Experiment 2 of Paper III and Paper IV animals were single-housed since weaning until the end of tickling sessions (3 and 2 weeks, respectively), thereafter they were group-housed by 4. Rats were housed in standard transparent polypropylene cages under controlled 12h light cycle (lights on at 07:30 on summer time or 08:30 otherwise), with food (Lactamin R35, Sweden) and water available *ad libitum* unless specified otherwise in the experimental procedures. Room temperature was maintained at 21±2°C. All experimental procedures, except for the sucrose intake measurements, were conducted during the light phase. The illuminance was 320–400 lx, depending on the position of the cage on the shelf. All experimental procedures were approved by the Animal Experimentation Committee at the Estonian Ministry of Agriculture.

3.1.2 Mice

In Paper V heterozygous VGLUT1 male mice (VGLUT1+/−) C57BL/6 were bred from heterozygous fathers (Dr. S. Wojcik, Gottingen, Germany) and wild-type (WT) mothers (Harlan, France). The VGLUT1–/– knockout allele was generated by truncation of the coding region of the VGLUT1 gene between the start codon and a BglII site in the fifth coding exon through homologous

recombination in embryonic stem cells (129/Ola background) [482]. These mice show a progressive neuropathologic phenotype and increased lethality rate at 2–3 weeks after birth. Mice were housed individually unless CVS protocol required a brief pair-housing. All experimental procedures were approved by Ethical Committee of the University of Navarra.

3.2. Drug administration

In Experiment 2 of Paper III amphetamine (0.5 mg/kg) was administered by intraperitoneal injection 15 min before an open field test for 5 consecutive days and then additional injection was given once 9 days later to assess the sensitisation to amphetamine. Imipramine HCl (10 mg/kg intraperitoneally, Sigma-Aldrich) or saline were administered daily the last 3 weeks of the CVS and for 1 week thereafter in Experiment 2, Paper V.

3.3. Behavioural tests

3.3.1. Testing and selection in the exploration box test

The exploration box [273, 274] was made of brushed metal and consisted of a 0.5 x 1 m open area (height of the side walls 40 cm) with an adjoining small compartment (20 x 20 x 20 cm) covered with metallic lid and connected through an opening in a shorter sidewall, allowing the animal free alternation between the two locations. The open area was divided by floor markings into eight squares of equal size (25 x 25 cm). In the open area, four objects were situated in certain places. Three of these objects were unfamiliar (a glass jar, a cardboard box, a wooden handle) and one familiar (a food pellet). The floor of the small compartment was covered with wood shavings. The lighting conditions of the experiment room were dim, with approximately 3–7 lux in the open part of the apparatus. The observer was seated at the end of the apparatus opposite to the exit from the small compartment. The animals were moved from the housing room to the testing room in their home cages and allowed to habituate with the room for about 15 min with no experimentation carried out during this period. The exploration test was initiated by taking a rat from its home cage and placing it into the small compartment of the apparatus, which was then covered with a lid. The following behavioural parameters were registered by the observer: (1) latency of entering the open area with all four paws, (2) entries into the open area, (3) time spent exploring the open area, (4) line crossings, (5) rearings, (6) investigations of the three unfamiliar objects. Parameters (4) to (6) were summed to obtain (7) the sum of exploratory events, which was used as an overall score for exploratory disposition in a novel environment.

A single test session lasted 15 min, after which an animal was gently removed from the apparatus, weighed and returned to its home cage. The apparatus was cleaned with a dampened laboratory tissue after each animal. When tested repeatedly in the exploration box test, the inter-day correlations for exploratory activity strengthen with every successive day [281]. The activity on the first testing session does not correlate highly with the following tests, but already the second test session, carried out 24 h after the first, gives a good prediction of activity levels on the consecutive test sessions [281]. Therefore, the rats were tested in the exploration box for 2 consecutive days to determine their stable exploratory activity levels. In Papers I and II, the rats were assigned to the low exploratory (LE) and high exploratory (HE) activity groups on the basis of the sum of exploratory activity during the second testing session, so that LE-rats exhibited near zero activity scores and HE-rats scored 100 or more exploratory events. In Papers II, III, & IV, rats were submitted to the two days of consecutive exploratory box testing as part of the post-manipulation behavioural tests battery.

3.3.2. Recording of USVs in animals submitted to manual tickling sessions and selection of stable behavioural phenotypes

Rat pups were weaned at the age of 21 days and single-housed immediately. Experimental sessions started the next day after single housing. During this period, the rats were given daily sessions of experimenter-induced tactile stimulation in imitation of "rough-and-tumble" play or "tickling" [483]. In the beginning of a tickling session, an animal was taken from the animal room to an adjacent room with similar lighting, removed from its home cage and placed into an empty and smaller (32 x 14 x 13 cm) cage, located under a microphone about 20 cm from the floor of the cage. The ultrasound detector was used for the heterodyne transformation of the ultrasonic vocalisations of 50 kHz and 22 kHz into an audible range to the human ear (Paper III) or the recording of unprocessed USVs on the computer hard drive (Paper IV). Experimenters counted USVs in real time from auditory signals during tickling sessions in Paper III and used software-generated sonograms to count them manually after the experimental procedures in Paper IV. The animal was given 15 s to habituate to the new cage, followed by 15 s of handling by experimenter that mimicked natural "rough-and-tumble" play in juvenile rats. In short, the "tickling" session that each animal received consisted of stimulating the rat with one hand by the experimenter, that included rapid finger movements on the back of the neck, turning the animal on the back and letting it "wrestle" with the experimenter's hand with vigorous alternating finger movements administered on the animals' ventral surface, followed by release after 1–2 s of stimulation. The experimenter tried to monitor rat's reaction to tickling and adjust its tactile stimulation for the maximum positive effect. Altogether, four 15 s periods of tickling were administered over two minutes, after which the animal was again

placed in its home cage and returned to the animal room, after that the test cage was cleaned thoroughly. In Paper IV, the recorded audio files were analysed with the Avisoft SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany), by creating sonograms from which the 22-kHz USVs, plain 50-kHz USVs, and USVs containing a frequency-modulated, or a "trill" component were manually counted. The animals were divided, based on the average number of emitted 50-kHz USVs on Days 12–14 of tickling, into "high chirping" (HC) and "low chirping" (LC) groups by the median split.

3.3.3. Chronic variable stress

3.3.3.1. Rats

Rats were submitted to chronic variable stress regimen [150, 159] in Papers II and IV. The CVS procedure lasted for either 28 days (Paper IV) or 32 days (Paper II) and comprised seven different stressors that were intermittently used daily, once every week. The stressors, presented in the order of administration were: 1) cold (4°C) water and wet bedding (400 ml of water was poured on rats in their home cage and the sawdust bedding was kept wet for the following 22 h), 2) imitation of the intraperitoneal drug injection by a rough and firm grasp of an animal with a coarse glove and simultaneous application of a syringe without the needle against rat's abdomen for several seconds, 3) stroboscopic light (for 14 h, 10 Hz, 2 lx), 4) tail pinch with a clothes-pin placed 1 cm distal from the base of tail (5 min), 5) cage tilt at 45° for 24 h, 6) movement restriction in a small cage (11 x 16 x 7 cm for 2 h), and 7) strong illumination (900 lx) during the dark phase (for 12 h). Control rats remained undisturbed in their cages, except for daily weighing and weekly sucrose preference measurements, until the commencement of behavioural testing. Five sucrose preference tests were carried out during the stress regimen, with the first one on the night preceding the first stressor and the following ones at the end of every week. The stressors were administered during the light phase of the cycle (except for those that lasted overnight).

3.3.3.2. Mice

Unpredictable repeated mild stressors were applied for 6 weeks after the protocol described by [484], with minor modifications. Briefly, the following stressors (two–three in any 24 h period) were applied: low intensity stroboscopic illumination (in dark 8 h), intermittent bell (10 db, 1/10 s) or white noise (an untuned radio, 4 h), rat odour (saw dust from rat cages; 8 h), cage tilt 45° (8 h), soiled bedding (200 ml of water per cage; 6 h), paired housing (with new partner 2 h), placement of novel object in the home cage (3 h), water and food

deprivation (8 h, before sucrose intake test), overnight illumination and removal of nesting material (12 h), and confinement in a small cage (80 cm^3 1 h).

In Experiment 1, Paper V, CVS regimen was applied to WT and VGLUT+/– mice (n = 15 mice/group), and both control and stressed mice were sacrificed 24 hours after the termination of CVS. In the last 3 weeks of CVS in Experiment 2, Paper V, TCA imipramine was administered daily. Over the last week of CVS (Experiment 1) and over the week immediately after the CVS (Experiment 2) a battery of behavioural tests was performed.

3.3.4. Other behavioural procedures in rats (sucrose intake, social interaction, elevated plus-maze. forced swim, fear conditioning)

Sucrose intake and preference

The sucrose intake in rats was measured in Papers II–IV. Paper III, Experiment 1, sucrose intake was measured in home cages of single-housed animals. In the rest of experiments, the animals were placed into single cages about 1h before the sucrose preference testing. Sucrose preference test (as described in [485]) was carried out with two bottles, one filled with 1% sucrose solution and the other with water. For the animals that remained single-housed through the whole experiment, the water bottles were not changed; whilst for the animals that were single-housed only for sucrose preference testing, new bottles were provided for the single-housing period. Sucrose and water consumption was measured for the period of 1 h by weighing pre-weighed bottles at the end of the test. Sucrose preference was measured by calculating the proportion of sucrose consumption out of total consumption of water and sucrose solution. The test was carried out on two consecutive days in Experiments 1–2, Paper III. In Experiment 2, Paper III, the 24 h resting period followed by the 18 h food and water deprivation preceded the third sucrose preference test. In Papers II and IV, five sucrose preference tests were carried out at weekly intervals, starting 3 h since the start of the dark phase in Paper II and 5–6 h in Paper IV, the first test being carried out the night before the commencement of CVS regimen. Sucrose and water intake were calculated into the units of consumption per kilogram of animal's body mass.

Social interaction (Paper II)

The test developed by File and Hyde [486] was used in a modified version (animals were not individually, but group-housed) of our usual social interaction test routine [150]. Two unfamiliar, weight-matched rats of the same experimental group were placed in the opposite corners of a brightly lit chamber (35 x 35 x 55 cm) with floor covered with wood shavings. The behaviour of the animals was concurrently observed for 10 min and analyzed by two researchers, one of them being unaware of the treatment condition. The total time spent in active social behaviour (allogrooming, sniffing the partner, crawling under and

over, following) was recorded. Inter-observer agreement was high (r=0.98), so the data obtained by the "blind" observer were used in data analysis.

Elevated plus-maze

The elevated plus-maze was designed in accordance with the original description [487] with a few modifications [488]. The elevated plus-maze consisted of the two open arms (50 x 10 cm) without sidewalls, the two enclosed arms of the same size with 40 cm high sidewalls and end wall, and the central arena (10 x 10 cm) interconnecting the arms. The arms of the same type were opposite each other. Both open arms were divided into three parts of equal size by lines, which also marked the central arena. The apparatus was made of wood planks and plywood and elevated 50 cm above the floor. For the test, an animal was placed into the central arena facing the closed arm of the apparatus and was observed for 4 min (Paper II) and 5 min (Paper III). Behavioural measures included 1) the latency period before entering the open part, 2) the number of line crossings, 3) time spent in the open part of the apparatus, 4) stretch-attend approaches towards the central arena, 5) the number of open arm entries, and 6) the total number of arm entries. From the two latter measures, the ratio of open/total arm entries was calculated. An entry into open arms was counted when the rat crossed the line between the central arena and an open arm with all four paws. The rat was considered to explore the open part of the apparatus when it had clearly crossed the line between a closed arm and the central arena with its forepaws. Experiments were carried out in dim lighting. Each animal was tested twice in Paper III and once in Paper II.

Forced swim test (Papers II–IV)

The forced swim test, first described by Porsolt et al. [489] was carried out as described previously [490]. Briefly, rats were placed into a vertical glass cylinder (diameter 22.5 cm and height 60 cm) containing about 35 cm of water at 25ºC. On the first day of the experiment, the procedure lasted 15 min and the re-exposure 24 h later lasted 5 min. At the end of each session the rats were dried with dry towels. The sessions were recorded with a video camera and the duration of immobility, swimming, and struggling for the first 5 min of the test session was later measured by two independent experimenters. The measurements were based on the behavioural categories described by Armario and colleagues [491]. In short, a rat was judged to be immobile when it remained floating in the water without attempting to actively change its position. A rat was judged to struggle whenever it made intense movements with all the four limbs and its front paws were breaking the surface of the water or touching the walls of the tank. The time spent swimming was recorded when a rat was making active swimming motions, more than necessary to merely maintain its head above the water, e.g. moving around in the cylinder. The average results of the two experimenters were used in the calculations. Inter-observer reliability was high (r=0.8–0.9).

Fear conditioning (Paper III)
Fear conditioning was studied in a standard shuttle box following the procedure described by Wallace and Rosen [492]. In short, a rat was placed in the chamber with metal grid floor for 3 min before the administration of a 1.5 mA, 1 s foot shock. Freezing, defined as a characteristic crouch position, was measured for 4 min immediately after the foot shock. Freezing was measured as a sample of freezing or not freezing every 10 s, for a total of 25 observations. A retention test of fear conditioning was conducted 24 h after the foot shock by placing the animal back into the same chamber and recording freezing for 4 min as described above.

3.3.5. Behavioural procedures in mice (sucrose intake, elevated plus-maze, forced swim, open field, marble burying, novel object recognition)

Sucrose intake
Mice were exposed to the 1% sucrose solution in a standard drinking bottle and also a bottle containing tap water in their home cage. The position of the bottles was varied and counter balanced across the left or right side of the feeding compartment. Each test was accompanied by food deprivation. The trials began at 18.00 h and ended at 09.00 h the following morning. Bottles were pre-weighed before the trial and fluid consumption was measured by re-weighing these bottles after the trial and calculating the difference. Mice were first trained for 1 week to drink the sucrose solution, then during CVS regimen, they were exposed to the sucrose solution and tap water once a week with six trials in total. Body weight measurements were taken weekly and relative sucrose intake and sucrose preference (sucrose intake/total fluid intake) was calculated as absolute intake (g) per weight.

Elevated plus-maze
The elevated-plus maze consisted of two open arms (30 x 5 cm), two enclosed arms (30 x 5 cm), and a central arena (5 x 5 cm) elevated 38.5 cm above the ground. Mice were placed in the central arena, facing one of the closed arms. Percentage of time in the open arm and the number of transitions were recorded for 5 min. An arm entry was defined as a mouse having entered an arm of the maze with all four legs.

Forced swim
Mice were individually placed into glass cylinders (height 24 cm, diameter 13 cm) containing water (14 cm, 22–23°C). Immobility, indicative of helpless behaviour, was recorded during the last 4 min of the 6-min testing period.

Open field
Locomotor activity was measured in an open field consisting of nine black arenas (43 x 50 x 45 cm) using a video tracking system (Ethovision XT, Noldus Information Technology, Wageningen, The Netherlands) in a softly illuminated room. One mouse was placed in each arena, and distance travelled (cm) was recorded during a 30-min period.

Marble burying
Compulsive-anxiety behaviour in mice was assessed with this test [493]. Eight marbles (1.5 cm in diameter) were placed uniformly in a cage (45 x 28 x 20 cm) covered with sawdust (3 cm deep). Mice were placed in the centre of the cage and left for 30 min. The number of marbles buried was recorded.

Novel object recognition
Visual recognition memory was assessed with this test [494]. The apparatus consisted of a black square arena (43 x 50 x 45 cm). On Day 1, mice were placed in the arena for 30 min to habituate. On Day 2, mice were placed in the box at equal distance from two identical objects (A1 and A2; two prisms 7 x 3 x 3 cm) for 5 min (sample phase). One hour after, mice were placed back in the box and exposed to a familiar object (A3) and to a novel object (B; a ball of 3.5 cm of diameter mounted on a cube of 3 cm^3) and video recorded (Pinnacle studio 9.0, Pinnacle Systems, Pittsburgh, USA) for an additional 5 min (retention test). Discrimination index (DI) was calculated as the difference between times spent exploring the new (N) and familiar object (F) divided by the total time exploring the objects (N–F/F+N) in the retention test.

3.4. Biochemical methods

3.4.1. High performance liquid chromatography (HPLC)

Monoamines in tissue collected from medial prefrontal cortex and striatum were measured (Paper III) by a high-performance liquid chromatography with electrochemical detection as described before [495]. In short, the tissues were disrupted with an ultrasonic homogeniser (Bandelin, Germany) in ice-cold solution of 0.1 M perchloric acid (10–20 µl/mg) containing 5 mM sodium bisulfite and 0.04 mM EDTA for avoidance of oxidation. The homogenate was then centrifuged at 14000x g for 20 min at 4°C and 20 µl of the resulting supernatant was chromatographed on a Luna C18 column (150 mm × 2 mm; 5 µm). The separation was done in isocratic elution mode at column temperature 30°C using the mobile phase containing 0.05 M citrate buffer at pH 3.7, 1 mM sodium octylsulfonate, 0.02 mM EDTA, 1 mM KCl and 7.5% acetonitril. The measurements were done at electrode potentials of a glassy carbon electrode +0.6 V versus Ag/AgCl reference electrode with HP 1049 electrochemical detector (Hewlett Packard, Germany).

In Paper V, GABA and glutamate concentrations in frontal cortex and hippocampus from CMS and control mice (WT and VGLUT1+/–; n = 15 mice/group) were determined by HPLC with electrochemical detection (DECADE, Antec Leyden, The Netherlands). A high-sensitivity analytic flow cell (VT-03) was used and the working electrode was set at 0.7 V. A column (biophase ODS 5 µm, 4.6 mm × 150 mm) including precolumn derivatisation with ophthaldehyde and β-mercapthoethanol (Sigma–Aldrich, Seelze, Germany) was used. Results are expressed in ng/mg of wet tissue.

3.4.2. Radioligand assays

3.4.2.1. Radioligand binding to 5-HT transporter

In Paper III, the PFC and hippocampus samples were collected from the right hemispheres of the animals that were decapitated and brains immediately dissected on ice. In Paper II, only the PFC was prepared analogously.

The membranes from cortical tissues were prepared by homogenisation in 50 mM Tris–HCl, (pH 7.4) and washings as described earlier [496]. The final pellet was resuspended in 100 vol (w/v) of the incubation buffer B (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, pH 7.4). Binding of [N-methyl-^{3}H]citalopram (75 Ci/mmol, Amersham Biosciences) was carried out by incubating membranes (1 mg of tissue per tube) in incubation buffer B with different concentrations of the radioligand (0.3–3.5 nM) for 60 min at 25°C. Non-specific binding was determined in the presence of 1 µM nonradioactive fluoxetine. The reaction was stopped by rapid filtration through GF/B glass-fibre filters (Whatman Int. Ltd., presoaked with 0.3% polyethyleneimine before filtration) and the filters were washed three times with ice-cold incubation buffer. The filters were kept in 4 ml of scintillation cocktail OptiPhase HiSafe3 (Wallac Perkin Elmer Life Sciences, Cambridge, UK) overnight and counted using a RackBeta 1219 liquid scintillation counter (Wallac Inc., Gaithersburg, USA).

3.4.2.2. D_1 receptor-specific cAMP accumulation (Paper II)

Membranes from accumbal and striatal tissues were prepared and dopamine D_1-specific cAMP accumulation was measured as described earlier [497]. In brief, the frozen tissue samples were homogenized in 100 volume (ww/v) of ice cold 2.5 mM Tris-HCl buffer (pH 7.4) containing 2 mM EGTA by Bandelin Sonopuls sonicator (three passes, 10 s each) and were used for assay.

Tissue homogenates (final conc. ~110 µg/mL (ww)) were added to reaction medium with or without 1 mM dopamine, obtaining 150 µl of reaction medium that contained 30 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 1 mM ATP, 10 µM GTP, 0.75 mM EGTA, 7.5 mM KCl, 100 mM NaCl, 0.1 mM IBMX, 0.1 mM Ro 20–1724, 100 µg/mL bacitracin, 0.03% BSA (Sigma-Aldrich Fine Che-

micals), 10 mM phosphoenol pyruvate and ~30 μg/mL pyruvate kinase (Roche). The reaction was started by placing tubes from ice bath to 30°C water bath and incubated for 15 min. Reaction was terminated with EDTA (final concentration 25 mM) and subsequent boiling of samples for 5 min. The amount of accumulated cAMP in samples was measured by competition binding with [^{3}H]cAMP to cAMP binding protein [498].

3.4.2.3. D_2 receptor-stimulated [^{35}S]GTPγS binding (Papers II & III)

Membrane preparations from NAcc and striatum were prepared as described [496]. The final pellet was homogenized in 90 ww/v, (striatum) or 200 ww/v (nucleus accumbens) of the incubation buffer A (20 mM K-HEPES, 7 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, pH=7.4).

Binding of [^{35}S]-guanosine-5′-(γ-thio)-triphosphate ([^{35}S]GTPγS), Perkin Elmer Life Sciences, Boston, USA) was carried out as described earlier [499]. In brief, the membranes (200 μg of accumbal and 500 μg of striatal tissue (ww) per tube) were incubated with 0.2 nM [^{35}S]GTPγS and different concentrations of GDP (1 μM – 3 mM) and 1 mM dopamine or 10 μM butaclamol (all from Sigma-Aldrich Fine Chemicals, St.Louis, USA) for 90 min at 30°C. The reaction was stopped by rapid filtration through GF/B glass-fibre filters (Whatman Int. Ltd.) and the filters were washed three times with 3 ml of ice-cold 20 mM phosphate buffer (pH=7.4) containing 100 mM NaCl. The filters were kept in 4 ml of scintillation cocktail OptiPhase HiSafe3 (Wallac Perkin Elmer Life Sciences, Cambridge, UK) overnight and counted using a RackBeta 1219 liquid scintillation counter (Wallac Inc., Gaithersburg, USA).

3.4.3. ^{13}C-MRS Spectroscopy (Paper V)

A previously described protocol [500] was used. Briefly, VGLUT1+/- and WT mice (n = 8/group) received (1-^{13}C)glucose (543 mg/kg intraperitoneal) and (1,2-^{13}C)acetate (504 mg/kg IP). Twenty minutes later, mice were decapitated, and the heads were snap frozen in liquid nitrogen and kept at –80°C. Brains were removed from the skull whilst frozen and, after discarding the cerebellum, homogenized with 600 μL of perchloric acid (7% v/v). Protein was removed by centrifugation (7500 rpm; 6 min). Aliquot (5 μL) from the supernatant were reserved for HPLC measurement of the concentration of metabolites; the rest was neutralised with 1 mol/L potassium hydroxide followed by lyophilisation. Lyophilisates were dissolved in 400 μL D_2O containing 0.1% of ethylene glycol (v/v) as an internal standard. Proton decoupled ^{13}C-MRS spectra were obtained using a Bruker DRX-400 spectrometer (Bruker Analytik, Rheinstetten, Germany) and the number of scans was 25000.

Metabolites derived from (1-^{13}C)glucose and (1,2-^{13}C)acetate represent the contribution from neurons and astrocytes to glutamate, glutamine, and GABA formation, respectively [500].

3.4.4. Western blotting (Paper V)

Cortical and hippocampal expression of various proteins involved in the glutamate/GABA cycle were studied by Western blotting using the following primary antibodies: rabbit anti-VGLUT1 (1:2000; donated by Dr. S. El Mestikawy, Paris, France), mouse anti-VGLUT2 (1:1000), and rabbit anti-VGAT (1:1000; Chemicon, Temecula, USA), mouse anti-GAD65 (1:5000) (Abcam, Cambridge, United Kingdom) and Rabbit anti-EAAT1 and EAAT2 (1:2500; Santa Cruz, Heidelberg, Germany). Horseradish peroxidase–conjugated goat anti-rabbit and anti-mouse secondary antibodies (1:10,000; DAKO, Cambridgeshire, United Kingdom) were used followed by visualisation through chemiluminescence using SuperSignal West Pico (Pierce Biotechnology, Rockford, USA). Films were scanned and quantified using the ImageMaster 1 D (Pharmacia Biotech, Uppsala, Sweden) software and normalised to β-actine.

3.4.5. Cytochrome oxidase histochemistry and image analysis

Brains were stored at −80°C until coronally sectioned (thickness was 20 μm in Paper I, 40 μm in Paper II & IV, and 50 μm in Paper V) in a cryostat microtome at −20°C. Slides with sectioned tissue were kept frozen at −80°C until stained. The staining procedure used is based on the protocol described in [501] with minor modifications. The 0.1 M Na_2HPO_4/NaH_2PO_4 buffer solution adjusted to pH of 7.4 was used. Automatic agitation was used with all the steps in the protocol. First the refrigerated sections were fixed for 5 min in 0.125% glutaraldehyde (v/v) solution in cold buffer (4°C). Next the specimens were washed with four changes (5 min each) of 10% sucrose in the buffer solution at room temperature. To enhance staining intensity, the sections were pre-incubated, for 10 min with 0.0275% cobalt chloride (w/v) and 0.5% dimethyl sulfoxide (DMSO, v/v) in 0.05 M Tris buffer with 10% sucrose (w/v) adjusted to pH to 7.4 with approximately 0.1% HCl (v/v). Thereafter, the sections were stained in the darkness for one hour at room temperature (Papers I & II) or at 37°C (Papers IV & V) in an incubation solution consisting of 0.05% DAB (3,3′-diaminobenzidine tetrahydrochloride, AppliChem), 0.0075% cytochrome c (Sigma, prepared using TCA), 5% sucrose, 0.002% catalase (Sigma) and 0.25% DMSO (v/v) in sodium phosphate buffer. To avoid non-specific auto-oxidation the reaction was conducted in dark. Finally, the reaction was stopped by introducing the slides for 30 min to 3.5% formalin (v/v) and 10% sucrose in phosphate buffer. In Papers I and II, the sections were dehydrated in ethanol, cleared in

xylene and coverslipped with Entellan (Merck, Whitehouse, USA); in Papers IV and V, clearance in xylene was omitted and slides were coverslipped immediately after dehydration with Mountex (HistoLab products AB, Gothenburg, Sweden). In histochemical processing slides were counterbalanced by regions of interest (ROIs) or treatment condition.

In Paper I, images of stained sections were digitised using Sony DXC-950P 3CCD Colour video camera on Carl Zeiss Axioplan 2 microscope with 20x magnification objective; converted to greyscale and cropped to the uniform size appropriate to the particular ROI using Graphic Converter X 5.5.2 (Lemke software GmbH, Peine, Germany). In Papers II, IV, and V, stained and coverslipped sections were digitised under Nikon SMZ 800 stereomicroscope with P-Achro 0.5x objective lens on Nikon Coolpix 5400 photo camera and saved in a non-compressed format. Image analysis was conducted using the successive versions of Image J 1.33–1.39 software (National Institutes of Health, Bethesda, USA) on the blue channel (resulting from a RGB split) of the background-subtracted image. Rodbard calibration procedure based on Kodak greyscale tablet was applied as absolute greyscale values between 0 and 255 were converted to the optical density units. Regions of interest were identified during photographing (Paper I) or image analysis (Papers II, IV, & V) with the aid of reference atlases of mouse [502] and rat brain [503]. Standard optical density values obtained form brain homogenates incubated together with the studied slides were used for correction in inter-batch variability of staining intensity (Papers II, IV, & V). In Paper I, the 3–6 individual measurements were made from each ROI for each animal and then averaged to obtain the single value of metabolic activity. Final optical density measures were standardised to Z scores within experiments to allow for the aggregation of results across two series of experiments. Dummy constant of 5 was universally added to the results to eliminate the negative values obtained with standardisation. In Papers II, IV, and V, optical density of any given region was sampled and averaged from the three consecutive slices of the same hemisphere in each brain, but randomly from right or left hemisphere of different animals. Regions of interest were outlined with a freehand selection tool covering the whole brain region, leaving out defective areas.

3.5. Statistical analysis

In Paper I, data were analysed using univariate ANOVA (Exploration) and single mean value for each ROI of each animal as a dependent variable. In Paper II, data were analysed with two-factor ANOVA (Exploration x Stress) or with two-factor ANOVA with repeated measures (Exploration x Stress x Time). Partial correlation analysis was performed on data derived from separate testing sessions.

In Paper III, the USV data was analyzed with one-factor (Chirping) ANOVA with repeated measures. The weight and behavioural data were analysed with

one-factor ANOVA (Tickling or Chirping) for repeated measures. The monoamine data were analyzed with two-factor ANOVA (Tickling x Sex or Chirping x Sex). In Paper IV, data were analyzed with a three-factor (Sex x Stress x Chirping) ANOVA for the elevated plus-maze behaviour and COX measurements, and with a three-factor (Sex x Stress x Chirping) ANOVA with repeated measures for the rest of data.

In Paper V, the effect of genotype in the spectroscopy and histochemistry studies was analysed using Student's t test. Neurotransmitter levels and protein expressions were analysed with two-factor ANOVA (Stress x Genotype). Different parameters of behavioural tests were analysed two-factor ANOVA (Stress x Genotype for Experiment I; Stress x Treatment for Experiment II). Significant main effects or interactions were analysed using Student's t test. Sucrose intake was analysed with two-factor ANOVA with repeated measures followed by a two-factor ANOVA for each week.

In all Papers, where appropriate, the subsequent pair-wise comparisons were made with Fisher's LSD test. In correlative analyses Pearson's correlation coefficients were used. Statistical significance was set at $P<0.05$ in all analyses. All data are reported as mean ± SEM. The data of radioligand binding were analysed by means of a non-linear least squares fittings with appropriate formulas, using GraphPad Prism (GraphPad Software, San Diego, USA) software.

4. RESULTS AND DISCUSSION

4.1. Exploratory behaviour as a stable behavioural phenotype

4.1.1. Behavioural profiles of high and low explorers

The exploration box test provides animal with an enhanced situation of an approach-avoidance conflict, because the conflict is intensified by the enrichment of a novelty information value of the open field type of environment by the placement of the novel objects on its premises, and simultaneously providing animal with the safe-looking place to hide, absent in the standard open field. As animal is initially placed in the safest part of the apparatus, the conflicting motivation develops between staying in that place and venturing out. Nevertheless, the choices animals make in the apparatus are quite stable in time and point to the existence of a certain governing behavioural disposition. Previously, it was found that the second exposition to the test apparatus predicted the consequent behaviour of rats with acceptable accuracy, as inter-session correlations from the second session onwards were high [281], whilst impulsiveness and behavioural reactivity are more prominent on the first exposition [279].

Animals, divided into groups of low explorers (LE) and high explorers (HE) [420], preserve their distinctive behavioural profiles upon the re-testing 1 and even 6 months later [281]. Re-testing 6 months later has revealed certain attenuation in the absolute levels of exploratory activity of HE-rats, probably due to age- and weight-related decrease in exploratory drive [281]. In Paper II, a certain amount of habituation was found in the unstressed animals, as HE-rats explored less on the second day of re-testing, and LE-rats slightly more, whilst clear group differences in exploratory activity remained intact. The most striking difference on re-testing between LE and HE control rats was in the latency, as it took LE control rats more than 10 minutes on average to come out from the small chamber. In the selection testing, they did not come out at all.

On the re-testing after CVS in stressed groups, LE-rats remained near-zero explorers and no habituation to the test was observed in HE-rats, so that they kept their exploration levels from the second day of selection testing in contrast to HE control rats. The decrease in exploration of HE control rats can be explained in two ways: animals from this group explored less due to habituation and age- and weight-related decrease in exploratory drive, or the result of the second day of selection testing was for some reason higher and masked their true relatively lower exploratory disposition, which was measured with less error on re-testing. Regression toward the mean serves as an explanatory mechanism in the second case [504]. Group-assignment was carried out based on the sum of exploratory events, however, it is worth mentioning that HE stress animals have made significantly more rearings than HE control counter-

parts on both exposures to the exploration box, supporting the idea that these two groups were from the beginning somewhat different in their exploratory behaviour, hence it is very difficult to assess the impact of stress on exploration other than to say that it had no inhibitory effect on HE stress rats and also did not invigorate LE stress animals. In conclusion, stress had no differentiating effects on exploratory phenotype, other than revealing a somewhat greater sensitivity in LE-rats, which, however, was not statistically significant.

From the very first trial, all rats exhibited clear preference for the sucrose solution over water; hence we decided to concentrate on weight-adjusted sucrose preference as the most informative index of CVS impact. Control rats did not differ in their sucrose preference throughout the trials and their results on individual trials had comparable oscillations, signifying the similar impact of environmental variables on the inter-trial variation. We have previously found that both group- and single-housed Wistar LE- and HE-rats did not differ in their sucrose preference on two daily consecutive trials [281].

In the elevated plus-maze, HE-rats had higher levels of line crossings, open arm entries, and total arm entries, as well as lower latency – which are all indicators of lower levels of anxiety – compared to LE rats. LE-rats, whose activity in the exploration box was almost non-existent, remained less active but did show some exploratory/locomotor activity, therefore providing the support for the hypothesis that both test paradigms (EB and EPM) measure distinctive, but overlapping, sets of behavioural responses, where the EPM is a more sensitive instrument towards measuring behaviours related to anxiety and risk-assessment [383, 505] in situations, where the source of danger – height – is readily identifiable, and therefore directed fear is also hypothesised to be a prominent reaction [506]. However, generally the avoidance of open arms does not habituate, but rather increases on repeated testing in the same apparatus, which is not typical for a purely fear-based behavioural reaction and suggests the presence of thigmotaxis as a mediating variable [507]. Approach-avoidance tendencies are more biased toward avoidance in EPM, but behaviour is structured within similar confines and, therefore, HE- and LE-rats still differ. Current results are similar to the ones from our previous study [281]. In animals, bred on the basis of their high or low anxiety-related behaviour in EPM (HAB and LAB, respectively), the former were also less active in the black-white box and open field test [508, 509]. It might therefore be concluded that the behavioural phenotypes initially measured in one type of the apparatus are robust enough to be transferable into conceptually similar test paradigms, but there is also test-unique portion of behavioural variability.

In the social interaction, no baseline difference between LE- and HE-rats was identified. Previously, we have found no statistical difference in social interaction in single-housed rats during subsequent testing on 3 days, although on the first 2 days HE Wistar rats had a tendency to be more active [281], which was also the case in the current study. Our rats were group-housed and it is well established that social deprivation increases the intensity of subsequent social interaction [510]. In the current study, the duration of the social interaction was

about 2.5 times lower than in our study of single-housed rats [281]. Socially isolated HAB-rats spent less time in active social interaction [508], but the procedure was quite different (intensity of luminance, pre-familiarisation with the test apparatus, arena size), so that direct comparison between the two animal models is problematic. In different inbred rat strains, selectively bred for their performance of an active avoidance in a in a two-way shuttle-box, social behaviour was generally similar [511, 512]. Hence, social behaviour is likely to constitute a separate behavioural domain, exhibiting little co-dependence with exploratory and fearful/defensive domains.

LE- and HE-rats showed similar behaviour in FST, moreover, there was no increase of immobility on the second day of testing. We have observed previously a higher proportion of swimming and less immobility on the 2nd day of testing in HE-rats compared to LE-rats [281]. Control HE-rats showed a tendency for more swimming on both FST days as well in the current study. Only in HE animals the immobility time was significantly decreased and swimming increased on Day 2 [281]. Shares of the three recorded activities (struggling, swimming, and immobility) were similar in the first days of testing between the current study and [281]. In animals, selected based on the time spent on the open arms of EPM and divided into LOA- (less time on open arms) and HOA-rats (more time), there was no difference in FST behaviour [513]. Floripa H-rats, which were selectively bred from the intercrosses of three rat strains (Wistar, Hooded, and Lewis) for high levels of locomotion in the central area of the open field test conducted for 5 minutes [514], and therefore resemble HR-rats (which were discussed in the introduction), showed lower immobility levels [515]. Behaviour of HE rats cannot be attributed to the higher impulsivity levels, as there is no phenotype effect on struggling, and their swimming level is higher on the second days of testing; nor to the emotional reactivity, as faecal boli do not differ between phenotypes [281]. Immobility levels of Floripa H-rats were similar in absolute values to the ones exhibited by our HE-rats, whereas the opposite phenotype of Floripa L-rats was immobile longer (about 200 s) [514] than either of our groups or LOA/HOA rats [513]. In conclusion, a bolder exploratory phenotype may predispose rats to select a more active coping strategies in FST, but the magnitude of this effect is rather small and may not be always statistically clear.

4.1.2. Brain oxidative metabolism in high and low explorers

Brain oxidative metabolism in LE- and HE-rats was measured in Papers I and II, as well as in the unpublished study aimed at replicating the results from Paper I with an improved methodology However, the latter study failed to find differences in COX activity between the opposing behavioural phenotypes. The primary methodological difference between the studies was in the way digital pictures of the stained brain slices were obtained. In Paper I, brain slices were photographed on Carl Zeiss Axioplan 2 microscope with 20x magnification

objective, so that photos of individual ROIs were taken directly under the microscope and, barring the damaged areas, the optical density was measured from the same area, irrespective of ROI's anatomical dimensions. In Paper II and in unpublished study, the whole slice was digitised at once under the stereomicroscope, and ROIs were later outlined by a freehand selection tool.

Only in study from Paper I, differences in cerebral oxidative metabolism between two exploratory phenotypes were identified. LE-rats had higher metabolic activity in dorsal raphe (DR) and inferior colliculus (IC). IC, together with the periaqueductal grey matter and deep layers of superior colliculi, are believed to constitute the tectal antipredatory defence system with the modulatory influence on the baseline anxiety levels in the brain [516, 517]. This midbrain tectal defence system has reciprocal connections with nuclei of extended amygdala and medial hypothalamus [516], thus having an important modulatory role in emotional memory processing and motivational state of the animal. In addition to glutamate, serotonin [518] and GABA [519, 520] are major neurotransmitters in IC.

Local injections of NMDA receptor ligands or glutamate into IC, as well as its electrical stimulation and aversive conditioning, produce a number of aversive-defensive behaviours characterised by freezing [521], increased alertness and escape reactions [522], accompanied by analgesia [523, 524]. Aversive-defensive behaviours are antagonised by GABA- and 5-HT-ergic mechanisms [525, 526]. Serotonin also plays a major role in the modulation of auditory input [527]. Brainstem raphe nuclei and locus coeruleus (LC) send afferents to periaqueductal grey matter (PAG) and central nucleus of IC [528–531], therefore both serotonergic and noradrenergic inputs may be important for the modulation of the defensive behaviour. Stimulation of 5-HT neurons within the DR tends to inhibit fight/flight/freeze reactions via projections to the dorsal PAG, and to potentiate fear/anxiety via projections to the amygdala [532]. Learned helplessness selectively increases the activity of the serotonergic neurons in DR, which is critical for the transfer of acquired state of passive coping and heightened anxiety across different situations [533]. Increased serotonin levels in the forebrain are associated with preference for passive defence strategies and submissive behaviour [534]. Hence, the persistently higher metabolic activity in DR and IC in the LE-rats could serve as the neurobiological basis for their passive and defence oriented coping style in novel environments, which in turn may lead to an enhanced DA-ergic neurotransmission in prefrontal cortex to generate adaptive responses to aversive states generated at the midbrain and brainstem level [535].

HE-rats showed higher metabolic activity only in one brain region: entorhinal cortex. Higher metabolism in entorhinal cortex may be suggestive of a higher baseline ability to accommodate the increased load of a novel sensory information processed by this region [536, 537], whilst engaging in exploration and, thus, contact with novel stimuli. Entorhinal cortex participates in both spatial memory consolidation and retrieval, mainly through glutamate-mediated

processes [538, 539], whilst its serotonergic mechanisms have been implicated in the processing of defensive and avoidance behaviours [540, 541].

On two later attempts we failed to replicate these results and found no impact of exploratory phenotype on cerebral oxidative metabolism. One possibility is that experiments lacked the statistical power to reliably detect subtle changes in COX activity. Metabolism is global and tightly regulated process, therefore it behoves to ask a question on effects of what magnitude we can realistically expect to observe. If we anesthetise a human brain into the state of unconsciousness, then PET mapping of changes in regional glucose metabolism reveals global cerebral metabolic decrease in the range of 40–55% [542]. Studies of regional glucose metabolism during differential sensory stimulations in conscious subjects produce changes in the range of 8–29% [543]. However, due to the limited sensitivity of PET method an overestimation of the magnitude of task-specific activation is likely [544]. Task-specific raised blood oxygenation leads to change in the cerebral metabolic rate of oxygen utilisation ($\Delta CMRO_2$/ $CMRO_2$), and via the glucose oxidation it is tightly coupled to change in both electric and chemical activities of mostly glutamatergic neurotransmission [543]. A conventional fMRI blood-oxygen-level dependence (BOLD) technique can be used to estimate the localisation of changes in neural activity, but a calibrated fMRI experiment, in which $\Delta CMRO_2$/ $CMRO_2$ is extracted from other hemodynamic measurements, offers quantitative information about the magnitude of change in neural activity [545]. Forepaw stimulation in rats anesthetised with halothane (cerebral metabolic rate of glucose consumption is 10–20% lower than in awake animals) produced 7% $\Delta CMRO_2$ increase in S1 contralateral cortical area [546]. In another example, stimulation of a human primary visual cortex (V1) with prolonged visual stimulus (21 min), with a black-white checkerboard reversing its contrast at 8 Hz, designed to selectively activate different populations of V1 neurons, leads to the time-dependent monotonic $\Delta CMRO_2$ increase from 8% at 3 min to 15% at 21 min [547].

The comparison of the magnitude of differences of COX activity levels between congenitally helpless and congenitally resistant to helplessness rat lines on the one hand [353, 354]; and congenitally helpless rats that either received for 2 weeks SSRI fluoxetine or not [356] on the other hand; suggests a significantly larger influence of genetic background on metabolism. For example, in a comparison of the magnitude of metabolic differences for the three brain regions usually implicated in the affective psychopathology: CA1 40% vs. 3%, habenula 70% vs. 7%, basolateral amygdala 17% vs. 1%, the larger differences are seen between congenitally helpless and resistant rats. In Paper IV, sex differences in COX activity levels were larger than the effect of CVS, whilst there were no differences due to affective phenotype. We have also found a larger effect of chronic social defeat stress over the behavioural phenotype of sucrose intake [548]. Overall, this suggests that differences in cerebral oxidative metabolism are rather small in case of behaviourally identified phenotypes, increase due to specific experimental treatment, and can become even more pronounced

in cases of asymmetry in genetic backgrounds as a result of selective breeding (exemplified in congenitally helpless and resistant rats) [549], or due to the sex differences discussed below in chapter 4.3.4.

Increased brain oxidative metabolism may be explained by the increase in the neuronal mean firing rate within a particular anatomical structure. For example, an increase in neuronal mean firing rate of 1 Hz increases energy consumption by 6.5 μmol ATP/g/min, whereas mean energy consumption for brain's grey matter is about 40 μmol ATP/g/min [285].

4.1.3. Effects of chronic variable stress in high and low explorers

A decreased preference of sweetened solutions and slower weight gain are considered the main indicators of effectiveness of CVS regimen [142, 157]. Effect of CVS was most pronounced in reducing the sucrose preference after the first week of treatment and by the end of the third week, the complete habituation, judging by hedonic capacity, was achieved. However, by the end of the fourth week of CVS, stress animals demonstrated again an anhedonic tendency. As stressors schedule was the same every week, the same stressor always preceded the sucrose preference trials; therefore the observed effects are not explained by the acute effect of a particular stressor, but rather by the adaptation to the CVS regimen as a whole. Similar habituations to CVS effects have been shown before [550], yet our finding is rather atypical in comparison to the corpus of CVS studies. A decrease in the body weight gain of stressed animals was also observed. It was more pronounced in LE-rats, suggesting that some effects of chronic stress are phenotype-mediated.

In EPM, chronic stress had no significant impact on subsequent behaviour. Previously, CVS has been associated mainly with the anxiolytic effects in EPM [154, 156].

The duration of social interaction with an unfamiliar conspecific was significantly increased in chronically stressed animals irrespectively of their exploratory phenotype. Chronic stress was so far mostly studied for its effects on antagonistic social interactions. Footshock-induced inter-male aggression was reduced after exposure to CVS and completely restored by chronic antidepressant treatment [551]. In resident-intruder test, 9 weeks of CVS were associated with an increased submissiveness in Lister hooded male rats in a role of the resident, whereas in EPM chronic stress had anxiolytic effect, and in the social interaction test no associations were found [156]. In single-housed Wistar [150] and in group-housed Sprague-Dawley [222] male rats, a negative effect of CVS on the social activity levels was found in the same testing conditions as used in Paper II.

In FST, the sole group difference emerged due to the stress-phenotype interaction: LE stress rats swam more than HE stress animals, whilst control groups demonstrated similar levels of swimming. Comparison across testing days revealed that this outcome was caused by the reduction on the second testing

day in the levels of struggling, increase in the levels of swimming and no significant change in immobility. In similar test procedures, CVS has generally reduced immobility and increased active coping [222, 552], which may suggest that CVS impact was less potent in the current study. However, generally accepted outcome of chronic stress on FST is a depressive phenotype characterised by the increase in the immobility levels [553–555], though, from the very beginning of the implementation of FST, the opposite effect has been shown [160]. The increase in LE stress rats' swimming was achieved through the reduction in struggling, indicating a higher tone of serotonergic neurotransmission over noradrenergic [132]. In control animals, there was no reduction in struggling between days 1 and 2 of testing, which may be explained by rather low levels of struggling already present on day 1.

In addition to social activity, CVS increased D_1 receptor-specific cAMP accumulation in both LE- and HE-rats in the NAcc, but not in striatum. This finding is in line with studies that show the decrease of basal extracellular DA levels in NAcc Shell [254, 556], the decreased number of DA transporter binding sites [556], as well as the increase in D_1 receptor-specific radioligand binding and Vmax of D_1 agonist-stimulated adenylyl cyclase in NAcc, but not in striatum, in animals submitted to a chronic stress [556, 557]. In contrast, chronic antidepressant administration decreases D_1 receptor-specific binding, cAMP accumulation [558–560], and spontaneous firing rates of DA-ergic neurons in NAcc [561], but elevates D_1 and not D_2 receptor gene expression in NAcc [562]. D_1 mRNA expression in NAcc was unaffected by the 8-week CVS, whilst D_2 mRNA were reduced [563]. However, a decrease in D_1 receptor binding, but not affinity, has been found after chronic restraint stress [564]. In single-housed male Wistar rats, neither a weeklong restraint stress, nor a 2-week CVS, produced differences in DA D_1 and D_2 receptors mRNA expression in nucleus accumbens. Individual susceptibility to develop a stress-induced anhedonia had no effect either [565]. Impoverished rearing conditions, as well as environmental enrichment, had no effect on both D_1 and D_2 receptor-specific radioligand bindings [566]. It seems that functional state of D_1 receptors may signal the overall reduction of mesoaccumbal neuronal terminal field activity caused by CVS, but whether this mechanism accounts for anhedonia in sucrose preference test requires further investigation.

In the current study, no effect of stress on striatal and accumbal D_2 receptor function was found. In previous works, CVS has been shown to decrease expression of both D_2 receptor mRNA [563] and protein [257], whilst chronic restraint stress increased D_2 binding [567] in NAcc. Similarly, repeated immobilisation stress increases D_2 receptor binding in NAcc when animals are single-housed after stress, whereas pair-housing after stress is associated with an increased D_2 binding in dorsal striatum [568]. On the other hand, CVS increased D_1 receptor binding in NAcc, but did not change the D_2 receptor binding, neither in NAcc nor dorsal striatum [557]. Considering the stress-phenotype interaction, repeated social-defeat stress has decreased the D_2 mRNA levels selectively in LR-rats [569]. Hence, the influence of chronic stress on D_2

receptors remains controversial, probably mediated by stress paradigms, post-stress conditions, and other variables.

Nucleus accumbens is a core region in the incentive motivation circuitry [570] and in behavioural activation system [391], therefore a modulation of dopaminergic neurotransmission there may indicate an adaptive response to prolonged stress [571]. In our study D_1 receptors in NAcc were in sensitised state after the CVS, possibly due to the reduction of DA turnover mediated by HPA axis [571]. On the level of emotional and motivational self-regulation, DA-ergic transmission in NAcc may also facilitate an appetitive affective state to counter the aversive effects of stress [570, 572], which may explain some invigorating effects of stress observed in our behavioural tests. Consonantly, D_1 receptor-specific radioligand binding was significantly higher in NAcc of rats resistant to learned helplessness [573].

Chronic stress was also associated with higher oxidative metabolism in anteroventral thalamus, median raphe nuclei (MRN), and periaqueductal grey matter (PAG). Median raphe nuclei are the origin of the ascending serotonergic hippocampo-septal pathway and are implicated in a long-term contextual fear responses and generalisation of defensive and anxiogenic behaviours over multiple contextual stress stimuli [574, 575]. Tryptophan hydroxylase immuno-reactivity [576] and mRNA levels [577] are elevated in MRN after chronic restraint stress. An increase in *c-fos* expression after repeated social defeat in male rats in MRN and PAG has also been reported [578] – effects signifying a probable modulatory role of glucocorticoids on serotonergic biosynthesis and neurotransmission [579, 580].

Periaqueductal grey matter receives projections from both dorsal [581] and median raphe nuclei [582, 583]. Anatomically, it consists of distinct, longitudinal functional columns [584]. Rostral parts of dorsolateral and dorsomedial columns in PAG are involved in active coping with stressors of primarily psychological nature (e.g. cat's smell). The coping presumes an active engagement of environment and includes such physiological changes as heightened somatomotor activity, increased vigilance, and hyper-reactivity [585]. The increase in oxidative metabolism of these two brain regions suggests the presence of fearfulness and agitation in stressed animals that may account for invigoration detected in the behavioural tests.

Anteroventral thalamus (AVN) has mostly been implicated in memory organisation [586, 587], however, it also shows robust stress-induced activation. Intracerebroventricular administration of CRF increases glucose utilisation in AVN, congruously with MRN, as measured 1 h later by 2DG autoradiography [588]. Different acute stressors elevate immediate early genes *c-fos* and *zif/268* mRNA levels in AVN, in case of *c-fos* in accord with PAG and MRN [589–591]. Repeated social defeat stress in mice increased *c-Fos* immunoreactivity in all three regions [592]. The dorsomedial part of AVN contains a dense plexus of dopamine-β-hydroxylase axons [593], originating from locus coeruleus and producing a rapid release of noradrenaline evoked by LC stimulation [594]. A robust expression of GABA synthesis' markers GAD65 and GAD67 has also

been reported [595], suggesting a possible involvement of these two neurotransmitters in the stress-induced activation observed there.

All three brain regions significantly influenced by chronic stress in this study also showed a strong pattern of inter-correlations, suggesting some degree of functional coordination.

In conclusion, chronic stress regimen was effective in eliciting anhedonia and weight gain deceleration, but was also associated with some invigorating results in behavioural tests and increases in cerebral oxidative metabolism, as well as D_1 receptor-specific cAMP accumulation in NAcc. Our animals were group-housed, and this arrangement in male rats is protective against some, otherwise common, effects of chronic stress [596, 597]. This may be one explanation for unusual constellation of behaviours observed in our study. However, recently, a co-segregation of the stress-induced anhedonia with invigorating behaviours has been observed by others [598, 599] and warrants further exploration.

4.2. Imbalances in excitatory-inhibitory neurotransmission in mice with decreased vesicular glutamate transporter 1 levels as a model of human depression

4.2.1. Behavioural and neurochemical profile of VGLUT1 +/– mice

In sucrose intake test, VGLUT1 +/– mice have decreased their levels of sucrose consumption at Week 2, in comparison to WT mice, and maintained such lower level of intake until the end of the test, demonstrating age-dependent lowering of hedonic capacity. They also exhibited higher immobility in FST, which was potentiated by CVS. Immobility in the FST is linked to the state of behavioural despair [129] and may be paraphrased as a state of helplessness and hopelessness that has both affective and cognitive characteristics. Anhedonia is a core symptom of the current diagnostic criteria for clinical depression, whilst helplessness and hopelessness, combined into the single dimension, posses a good diagnostic utility [600]. In the rest of behavioural tests, VGLUT1 +/– mice showed a similar behavioural profile to their WT counterparts.

Pair-wise comparison of COX activity in VGLUT1+/– and WT mice for each of the 100 ROIs produced no significant differences. Interestingly, in almost all brain regions, the VGLUT+/– mice showed a slight non-significant increase of activity, which may be explained by comparable glutamate tissue levels in VGLUT1 +/– and WT mice.

Injection of (1-^{13}C)glucose and (1,2-^{13}C)acetate lead to an efficient labelling of the various metabolites studied. A significant increase in glutamate (4-^{13}C) was shown in whole brain extracts of VGLUT1+/– mice compared with their WT littermates by ^{13}C nuclear magnetic resonance spectroscopy. However, no changes in the levels of the other metabolites were detected between the two

groups. Similarly, acetate/glucose utilisation ratios for glutamate, glutamine, and GABA were not altered.

Brain tissue levels of glutamate and GABA were determined by HPLC in frontal cortex and hippocampus. Chronic stress induced a significant increase in cortical and hippocampal glutamate levels in both WT and VGLUT1+/– mice. Both VGLUT+/– and CVS were associated with lower levels of GABA in frontal cortex and hippocampus. Effects of CVS and partial VGLUT1 gene inactivation were similar in magnitude and non-additive. Cortical and hippocampal expression of VGLUT1 protein was measured by Western blotting. As expected, among control animals, VGLUT1+/– genotype had reduced the level of VGLUT1 by 41% in the frontal cortex and by 35% in the hippocampus, compared to WT mice. In contrast, an elevation in the mRNA expression of VGLUT1 and its protein levels in cerebral cortex and hippocampus followed chronic administration of antidepressants, ECT and lithium to unstressed mice [601, 602]. Chronic stress reduced VGLUT1 protein expression somewhat further, but this was not statistically significant. In the later study, it was found that CVS has decreased GAD65, VGLUT1, and GABA levels in ventral hippocampus of male C57BL/6 mice [603]. Glutamate transporter EAAT1 had lower expression in VGLUT1+/– mice. In agreement with a previous study [90], VGLUT1+/– mice showed normal levels of glutamate but decreased cortical and hippocampal levels of GABA and VGLUT1, as well as passive coping in FST. VGLUT1+/– mice also exhibit deficits in hippocampal synaptic plasticity [604] and upregulation of the synaptic vesicle protein synapsin 1 [605] that can be a compensatory process of pre-synaptic vesicle pool's integrity maintenance against the reduction in VGLUT1 [606].

Several spectroscopy studies have shown decreased GABA levels in the cerebral cortex of depressed patients [607–611]. In one study the decreased GABA and simultaneously increased glutamate levels were associated with the melancholic, but not atypical, subtype of the clinical depression [610]. Moreover, decreased glial EAAT1 has been observed in cortical post-mortem tissue of patients who had suffered clinical depression [42], and preclinical studies in rats have also linked decreased GABA levels to helplessness in the forced swimming test [612]. Glutamate is a precursor for the major neuronal GABA synthesis pathway [613], hence alterations in glutamate release, glial reuptake, or both would be expected to affect GABA-ergic neurotransmission. Decreased glutamate release could limit the uptake of glutamate by the GABA-ergic terminal through the neuronal EAAT3–4 transporters [613]. Another possibility is the downregulation of EAAT1 detected by Western blotting that could limit the uptake of glutamate by astrocytes and, therefore, the synthesis and transport of glutamine from glial cells to the GABA-ergic terminal, thus affecting the synthesis of GABA [614]. It should be mentioned that EAAT1 is prevalent in cerebellum and cortex, especially early in the development, and EAAT2 is prevalent in forebrain and it carries out the bulk of glutamate uptake in the brain in general [615, 616], however, there was no stress or genotype effect on EAAT2. Congenitally helpless rats have elevated glutamate to GABA ratio

[617] and reduced expression of glutamate transporters: VGLUT1, EAAT2 and EAAT4, but not EAAT1 and EAAT3, in the hippocampus and cortical areas [618]. There were also differences in markers of GABA-ergic neurotransmission: $GABA_A$ receptor densities are increased specifically in the septum [619], GABA transporter GAT3 was downregulated in cingulate and posterior cortex, hippocampus, basolateral amygdala, habenula, and ventral thalamus, whilst GAT1 was downregulated only in parietal cortex, and VGAT, as well as GAD67 levels were mostly unchanged [620].

Previous studies show that VGLUT1 is the major isoform in both the frontal cortex and the hippocampus [621, 622], where it plays a key role in the vesicular uptake and synaptic transmission of glutamate [482, 623–625]. Decreased VGLUT1 mRNA expression in entorhinal cortex was associated with diagnoses of clinical depression and bipolar disorder [626]. The downregulation of cortical and hippocampal EAAT1 supports the idea of decreased glutamate release in the VGLUT1-dependent synapses because this glial transporter is regulated by the amount of glutamate released into the synaptic cleft [627, 628]. In disagreement with this, in the initial characterization of the VGLUT1 knockout mice, no apparent differences in the evoked excitatory postsynaptic current (EPSC) in VGLUT1+/– hippocampal cultured neurons were shown [482], suggesting that a 50% reduction in the VGLUT1 content does not affect the quantal size. However, these studies were carried out in individual cultured neurons, and we currently do not know whether the same is true for the intact brain of adult mice, in which minor changes could potentially be physiologically relevant. The behavioural phenotype would argue for a change in EPSC size in vivo. However, the behavioural phenotype could also be due to compensatory changes that occur in VGLUT1+/– animals that leave the EPSC amplitude unchanged. More specifically, the increase of the neuronal *de novo* synthesis of glutamate, derived from (1^{-13}C) glucose, suggests a compensatory effect for the decreased vesicular VGLUT1 content and, perhaps, for the downregulation of EAAT1 [614, 629, 630]. This increase could contribute to the maintenance of both the cytoplasmic and the vesicular pools [631] of glutamate in the VGLUT1+/– neurons, which would agree with the normal tissue levels detected by HPLC and may be sufficient to normalise EPSC size. However, other studies suggest that the contribution of glutamate synthesis through the tricarboxylic acid (TCA) cycle to the vesicle pool of glutamate is limited [632], supporting the idea of decreased vesicular pool and synaptic release of glutamate in the VGLUT1+/– mice. Because of the low limit of detection of some metabolites, the ^{13}C-MRS studies were carried out in whole-brain extracts. Although the values therefore do not specifically represent only those areas in which VGLUT1 is the major isoform, they nonetheless should have a proportional representation of any change in the VGLUT1-expressing regions.

4.2.2. Effects of chronic variable stress and imipramine treatment on VGLUT1 +/– mice

Chronic stress induced some changes equally in both genotypes and in other tests interacted specifically with VGLUT1+/– genotype. Stress and genotype interactions would be the primary focus of this section.

Body weight was not affected by CVS. Before the onset of CVS, sucrose and water intake was similar in all the groups and did not increase during the subsequent training in control animals, most likely because of 1 week of pre-training. Chronic stress induced a significant decrease in sucrose intake at Weeks 5 and 6 in WT mice and at Week 6 in VGLUT+/– mice. In the second experiment, where imipramine was administered to VGLUT1+/– mice for the last three weeks of CVS, a monotonic reduction in sucrose intake was observed, which reached the statistical significance at Week 4 and continued further decline for untreated CVS group mice. Imipramine treatment increased sucrose intake at Week 5 in both treated groups over the level demonstrated by the untreated control mice and at Week 6 both groups receiving antidepressant were on the same level of sucrose intake with control untreated mice. In contrast, no stress or imipramine effect was detected in the sucrose preference.

In FST, chronic stress increased immobility time in both genotypes, which was proportionally higher in VGLUT1+/– mice compared to WT. The same CVS effect was seen in Experiment 2, and imipramine administration successfully reduced immobility time in both control and stress groups, restoring the Stress/Imipramine mice behaviour to the levels observed in Control/Untreated animals.

Stressed VGLUT1+/– mice showed increased anhedonia and helpless behaviour compared with WT/CVS mates, and these effects were reversed by repeated imipramine treatment, which supports the idea that VGLUT1+/– mice are more vulnerable than WT mice to a stress-induced depressive-like phenotype.

In EPM, chronic stress reduced, uniformly in both phenotypes, the percentage of time spent in the open arms in Experiment 1. However, imipramine treatment had no effect on the percentage of time spent in the open arms and neither did CVS produce anxiogenic effects in Experiment 2, though the statistically non-significant tendency for CVS-induced reduction in the open-arms attendance was present. The percentage of time spent on the open arms in the EPM was very low in both experiments (3–4%), which is less than is usually observed in mice with the same genetic background [494, 633].

Marble burying behaviour was affected neither by CVS, nor by imipramine administration. The latter result is curious because generally antidepressants are known as inhibitors of marble burying [634].

In novel object recognition test, CVS induced a decrease in discrimination index 1 hour after the sample phase in both WT and VGLUT1+/– mice. In Experiment 2, the stress effect was repeated, whilst chronic imipramine treatment reverted the discrimination index back on the level of Control/Untreated animals.

In open field, CVS induced a significant increase in the travelled distance during the 30 min of spontaneous locomotor activity selectively in VGLUT1+/– mice compared to controls. In Experiment 2, chronic stress increased ambulation in both groups and imipramine had no effect. These results suggest that behaviours in FST and in the open-field constitute separate behavioural domains.

Chronic stress significantly increased VGLUT2 expression in VGLUT1+/– mice but not in WT mice, both in the frontal cortex and in the hippocampus. Vesicular GABA transporter (VGAT) and glutamic acid decarboxylase 65 (GAD65) expressions were significantly decreased by CVS irrespective of genotype. In VGLUT1+/– mice, the downregulation of cortical and hippocampal glial excitatory transporter – EAAT1 expression was identified. In addition, CVS induced a significant increase of EAAT1 in the WT mice (159%) in the hippocampus compared with WT animals. The expression of second glial excitatory transporter – EAAT2 was not altered by either stress or genotype in neither of brain regions.

In addition to prevalent symptoms of depression, our CVS model [494] also addressed other clinical features that are highly comorbid with clinical depression such as anxiety [635] and impaired cognition [636, 637]. Both depression [607–611] and anxiety [638–640] have been linked to impaired GABA-ergic function, and overactive glutamatergic function has been shown in various anxiety disorders [641, 642] and in mood disorders [643]. Furthermore, modulation of glutamatergic receptor activity [644], as well as redressing the imbalances of glutamate cycling and metabolism [44] are promising areas of research on novel antidepressants. In rodents, abnormal increase in glutamate efflux has been reported in the PFC in response to acute stress, as well as corticosterone treatment [645]. Other studies have also shown decreased prefrontal cortical and cerebellar GAD65 and GAD67 levels in depressed patients [646, 647] and a link between anxiety and a polymorphism in the GAD65 [648] gene. Moreover, mice lacking an isoform of GAD65 have shown reduced GABA levels and increased anxiety [649, 650], whereas rats exposed to CVS show reduced glial metabolism and resulting diminished role of astrocytes in GABA formation, though tissue concentration of glutamate and GABA in PFC were not changed [651]. The downregulation of VGAT and its coupled GABA synthesising enzyme GAD65 in response to CVS could explain the decreased GABA levels observed in the frontal cortex and in the hippocampus of the CVS mice. The upregulation of hippocampal EAAT1 may occur as a neuroprotective response to stress-induced elevations in glutamate in the synaptic cleft [627, 628], as well as in response to increased corticosterone levels [652].

VGLUT1+/– mice exposed to chronic mild stress showed a combined phenotype (stress plus genotype), but also specific neurochemical and behavioural alterations because of the interaction between stress and decreased VGLUT1 levels. Interestingly, the striking upregulation of VGLUT2, along with the lack of upregulation of EAAT1 and decreased GABA formation, suggests an increase in excitatory post-synaptic stimulation that could be linked to

the increased ambulation in the open field, however, COX measurements do not support this hypothesis. It is noteworthy, that different animal models of depression demonstrate quite non-overlapping patterns of changes in the neurotransmission of amino acids [618, 620, 653], but still, somehow antidepressants almost always work and restore *status quo* [603, 654, 655].

4.3. Positive expressed emotionality as a stable behavioural phenotype

4.3.1. Stability of 50-kHz ultrasonic vocalisations in time

In all experiments, the gradual rise in the number of 50-kHz chirps over repeated tickling sessions stabilised on an individually specific level by the end of second week of tickling. The averaged 50-kHz USV response to daily tickling on days 12–14 proved to be a good predictor of the subsequent USV levels and was used to divide rats into groups of high and low chirpers (HC and LC, respectively) by the median split in all experiments. At this point HC-rats have reached plateau in the number of 50-kHz USVs and later sessions have shown either stable levels of positively valenced chirps or a slight declining trend, possibly due to habituation and maturation [444]. In Experiment 2 Paper III, LC-rats of both sexes continued to increase their 50-kHz calls during the third week of tickling. Absolute levels of 50-kHz USVs dropped significantly with the discontinuation of daily tickling sessions, but group differences had remained intact. This suggests interplay between the individual propensity to react positively to human tactile stimulation and the importance of daily practice for enhanced response. Similar pattern of change over repeated tickling sessions was visible in both sexes in the FM 50-kHz USVs, suggesting that at least in the context of daily experimenter-induced stimulation in juvenile rats there is no functional difference between the two types of call. The levels of FM calls were somewhat lower in both sexes compared to the flat USVs at baseline, whilst after a period of social housing this difference was visible in male rats only. Previously, more trill-type USVs than flat USVs have been reported in response to tickling in adult females [444] and the difference between the two groups was accounted solely by a number of emitted 50-kHz FM USVs. It seems reasonable to assume that differences in experimental design stand behind these variations, suggesting that if the tickling procedure is started when the animals have reached the adult age, the resulting USV profile may differ to a certain extent from the conditions where tickling sessions have been started at weaning. An age-related decreases in USV response to tickling have been reported [656], and, in the light of the abovementioned results, it may be suggested that the flat-type 50-kHz USVs show earlier age-related decline, probably related to its primary communicative value in the initiation of social interactions [448] that declines as animals mature. It was proposed that flat USVs may serve a function of the social coordination [446], whereas FM calls

signal dopamine-dependent reward or appetitive state [442] and are elevated, compared to the flat calls, by an acute amphetamine administration [456, 657]. Recently it was found that flat calls are more prevalent in single-housed rats, whereas FM differentiates spontaneous vocalisations in group-housed rats [657]. These authors also identified 14 different categories of 50-kHz USVs and established inter-individual differences in the composition of call profiles [657].

In our experiments the animals were always single-housed during the tickling sessions over the first two weeks, since the 50-kHz response to tickling had been shown to be greater in such conditions [483]. The number of tickling-induced 50-kHz USVs decreased during periods of social housing, which has been found to reduce 50-kHz USVs already 48 h after the relocation of single-housed animals into group-housing [459], but the divide between HC and LC animals was retained. It is noteworthy that the levels of FM chirps did not decrease in females over the period of social housing, and the decrease in flat USVs was not significant in female LC-rats. This suggests that at least in LC females social housing has a different effect on USVs than in other groups (and more so on FM chirps), that seems a reasonable assumption in the context that females have been found to have different social behaviour profiles than males [658, 659], and remain playful longer after puberty compared to males [660].

We also studied the emission of low-frequency USVs in the range of 22 kHz that are believed to signify situation's aversiveness for the animal and associated anxiety [440]. Rats emit 22-kHz USVs as warning calls when the source of danger is distant; when the predator is proximal the rat will either become silent or emit sonic warning squeals [661, 662], hence if tickling session is perceived as an immediate danger by a rat, we can expect no 22-kHz USVs and therefore it is more problematic to assess the negative valence of manipulations in our experimental design.

In order to learn whether the 22- and 50-kHz USVs, and hence the emotional messages they carry, are mutually exclusive, the associations between the two types of USVs were determined, and no significant correlations were found in untreated rats, although some female HC-rats tended to emit increasing levels of 22-kHz USVs over repeated testing (Paper III). Previously, a negative correlation between the two types of USVs has been found in rats bred for high levels of 50-kHz chirps [481]. Playback of 50-kHz USVs elicited in juvenile, but not adult rats, response vocalisations in the 22-kHz range [450] that may signify the frustration with unfulfilled promise of social interaction or some kind of anxiety. Human tactile stimulation is only a crude imitation of natural "rough and tumble" play between juvenile conspecifics, therefore a certain amount of discomfort for the tickled rat is expected to be produced by the clumsiness of human hand motions, and the absence of usual accompanying stimuli such as olfactory clues [663]. This implies that the 22- and 50-kHz USVs should be emitted simultaneously in specific situations, which was true in the present experiments, in which both types of USVs were detected in identical experimental conditions. Thus, the 22- and 50-kHz USVs are not mutually

exclusive, but permit the simultaneous expression of different facets of the experienced affective state.

4.3.2. Behavioural and biochemical profiles of high and low chirpers

Tickling on its own induced many changes in animals' behaviour in our test battery, and, in some cases, the essence of these changes depended on the animal's level of emitted 50-kHz USVs. Tickling was found to increase activity levels in EPM and HC-rats of both sexes tended to be more active compared to the respective LC group in this test. In the exploration box test, tickling had almost no effect on HC-rats of both sexes, whilst the activity levels of LC-rats were consistently higher compared to both control and HC-rats of both sexes, but more prominent in females. It seems that the lower activity of HC-rats in the exploration box test is rather due to lower motivation to explore than heightened levels of anxiety, as the exploration box as an "unforced exploration" test allows the animal to remain inactive more easily than the elevated plus-maze. Exploring new areas has been found to be rewarding for rats [664], and as HC-rats were selected based on their positive perception of tickling, it is unlikely that they have a general defect of the appetitive motivation. In the EPM, it were rather LC-rats, who had the tendency for a more passive behaviour, however, they still demonstrated somewhat less mindfulness of the open arms than untickled animals. It is hard to explain differences between LC- and HC-rats by invoking the differences in constitutional anxiety, therefore motivational aspects and affective styles should be considered. Both tickled groups irrespective of their sex had a tendency to ambulate more and leave the closed arms. This tendency can be likened to the effect observed in EPM after the repeated handling of juvenile rats [665, 666].

Tickling had no effect on sucrose intake and preference in female rats, but tended to decrease these measures in male rats, and more systematically so in the HC group (Paper III). The lower intake of sweetened solution in HC-rats similarly points to the reduced incentive value for a putative range of appetitive stimuli, including novelty and sweet taste. This finding was not replicated in Paper IV, possibly because the daily tickling period was only two weeks, whereas it was 38 days and three weeks in two experiments in Paper III. In FST, immobility increased significantly on the second day in male HC-rats and swimming tended to decrease in both LC and HC groups (Experiment 1 Paper III). In the second part of the study HC male rats tended to have higher immobility on both days, whilst LC-rats engaged in more struggling (Experiment 2 Paper III). Individual responsiveness to tickling had no influence on behaviour of female rats in FST. No significant differences were found between HC and LC animals in foot-shock induced freezing, however, history of tickling itself had a tendency to reduce freezing and defecations – a marker of a negative emotionality.

In summary, although HC animals show the propensity for a greater 50-kHz USV response to the tickling, indicative of a greater level of the subjectively experienced pleasure, these animals display an inhibited behaviour in some behavioural tests, expressed in the lower motivation to explore novel areas and to consume sweet solution, as well as a tendency to adopt more passive coping strategies in the forced swimming test. It is possible that the tickling procedure may condition the animals to receive a certain amount of hedonic stimulation on a daily basis that reduces in more receptive HC-rats motivation towards other potentially rewarding stimuli (i.e. novel areas, sweet solution). LC-rats are less susceptible to such stimulation and their behaviour is therefore closer to the results observed in naive rats in some tests, and is elevated beyond it in others. We may postulate that there is a certain similarity between the impact of the tickling on LC-rats and usual handling [667].

In the dorsal striatum, LC males rats had higher serotonin turnover and LC females higher DA tissue levels, compared to control animals, as measured by *ex vivo* HPLC. LC females, but also HC females, exhibited higher dopamine D_2 receptor-dependent binding of [^{35}S]GTPγS and lower receptor affinity in dorsal striatum than untickled controls. No association was found between chirping phenotype and the functional state of DA D_2 receptors in NAcc or binding levels of [N-methyl-^{3}H]citalopram in frontal cortex. Tickling eliminated sex-dependent differences in D_2 receptors functional state. The levels of DA and its metabolite in striatum were higher in untickled male rats compared to females, and tickling eliminated this difference. As a result, turnover of DA was lower in control males and this corresponded to the elevated functional state of DA-activated receptor-G protein complex. Tickling-induced affectivity was in general unrelated to the dopaminergic neurotransmission, suggesting that socially rewarding experiences are less dependent on activity of DA-ergic pathways and are rather mediated by opioid receptors [480].

4.3.3. Effects of chronic variable stress on high and low chirpers

Four weeks of CVS have decreased the levels of flat and FM 50-kHz USVs in male HC-rats, whilst no effect was visible on either measure in LC animals, possibly due to the floor effect. Post-stress male LC-and HC-rats no longer differed in the number of positively valenced calls, however LC animals started to emit more 22-kHz USVs. Due to very low levels of 50-kHz USVs in LC-rats no definite conclusion can be reached whether chronic stress affected this measure selectively in HC animals.

Conditioned place aversion, induced by lithium chloride or naloxone injections, is accompanied by the concurrent increase 22-kHz USVs and decrease 50-kHz USVs [445]. Similar results were obtained from the social defeat paradigm [668]. Animals bred for high rates of 50-kHz calls also exhibit a concomitant decrease in 22-kHz USVs compared to randomly bred animals, and *vice versa* in rats selectively bred for low rates of 50-kHz USVs [480, 481].

Selective breeding for positively valenced calls increases the number of FM USVs, whereas flat calls tend to decrease somewhat compared to outbred controls [480].

In females, stress had no effect on either type of 50-kHz chirps. No difference was found between HC and LC animals in 22-kHz USVs at baseline and pre-stress testing.

Post-stress, the levels of 22-kHz USVs have decreased in all groups except for the HC stress rats, resulting in a significant difference between the HC and LC stress groups that was opposite to the effect observed in males. This effect partly associates with the gradual increase in 22-kHz USVs over daily tickling sessions observed in female HC-rats in the previous experiment (Paper III). Based on the analysis of USVs, male LC-rats were more vulnerable to the negative impact of CVS, whereas it is hard to assess the stress regimen's impact on females.

Chronic variable stress regimen significantly suppressed weight gain in male rats, but no divergence was found between the HC and LC animals, although separation from control animals occurred earlier and remained more stable in LC stress animals compared to the respective HC group. Weight gain suppression is considered an important indicator of the effectiveness of CVS [142], and the larger effect in LC stress rats is in concordance with the trends in ultrasonic vocalisations. In females, no differences were found between any groups in weight gain, suggesting that the stress procedure had only minor impact on fairer sex in the present experiment. No systematic differences in sucrose intake and preference were found between control and stressed HC animals of both sexes, but these measures were significantly lower in LC stress rats in comparison to unstressed animals, also regardless of gender. In males, LC stress rats had a tendency to consume and prefer less sucrose than respective HC group rats. Sucrose intake was increased over repeated testing in unstressed males. In females a similar, but less clear-cut tendency was evident. Repeated testing elevated sucrose intake in control animals of both sexes, however, this process was blocked by CVS in the stressed female rats and LC stress males, in the latter case linking the anhedonic state with a higher sensitivity to chronic stress.

It has been previously observed that females and males react differently to chronic stress both behaviourally [669] and neurochemically [597, 670]. Female rats are less prone to develop anhedonia [671] and are more reactive toward novelty [672, 673]. Female rats are also more resistant to the state of learned helplessness [429]. Social housing has been shown to dampen some adverse stress effects in both sexes [674, 675], but also influence their behaviour in a gender-specific manner.

Male rats were generally more active in the exploration box compared to females. This was significant on the first day in the LC stress group and on the second day in all groups, except for HC control. In males, chronic stress elevated the exploratory activity, especially in LC stress rats. In females, HC animals of both control and stress groups tended to be more exploratory, but differences were small and rarely reached the level of statistical significance.

Possibly, unstressed male LC-rats have higher motivation to explore novel environments that is suppressed by a higher anxiety on the first encounter with the test apparatus as the differences between HC and LC animals became significant on the second testing. Activity of male HC control rats did not habituate on the second test exposure, suggesting that impulsivity had little impact on their behaviour during the first testing session. Although stress did not affect exploratory behaviour in female rats, it is of interest that in both sexes the groups with higher activity in the exploration box test were the same in which stress increased 22-kHz USVs. Hence, the higher exploration levels in these groups may rather reflect higher reactivity to various environmental stimuli than lower levels of anxiety.

Similarly to the experiments in Paper III, unstressed animals of both sexes did not differ in their activities on the first day of testing in FST. On the re-exposure to the water tank, immobility increased in every male group, the least so in HC control rats and the most in LC stress animals. This is yet another evidence of heightened stress sensitivity in LC-rats. In this case the immobility rose on the second day of testing that suggests the primary importance of acute stressfulness of the first water immersion. Interestingly, in male HC control rats on the second day of testing, immobility rose concurrently with struggling, whereas swimming levels declined. It is quite an unusual combination perhaps suggesting some causative role for a catecholaminergic neurotransmission, which is generally associated with SNRI-induced preferential increase in climbing [132], though this hypothesis does not explain the concurrent increase in immobility, furthermore, the depletion of NA stores has no effect on climbing [676]. In female rats neither chirping, nor CVS, differentiated the experimental groups.

4.3.4. Effects of chronic variable stress and gender on cerebral oxidative metabolism

In control groups, significant sex differences were revealed in COX activity in more than half of the 85 brain regions studied, with male and female rats having higher metabolic activity in 40 and 17 regions, respectively. In males, CVS elevated metabolic activity in 8 brain regions of LC-rats, 3 brain regions of HC-rats and lowered it in the locus coeruleus of HC-rats. Accordingly, in females, CVS reduced metabolic activity in the shell region of nucleus accumbens and anterior cingulate cortex, as well as elevated it in red nucleus of LC-rats; whilst COX activity was reduced in 5 regions of HC-rats, among them red nucleus. Thus, red nucleus was the only region with the opposite stress-related changes in metabolism in females; there was no such region in males. Red nucleus was also associated with an increase stress-induced metabolic activity in male HC-rats, therefore modulating the stress response in 3 out of 4 groups. Acute intracerebroventricular administration of CRF significantly elevates 2DG uptake in

red nucleus [588]. It also expresses CRF receptor's mRNA [677, 678], but its transcriptional activity following an acute stress is weak [590].

Additionally, in anteromedial thalamic nucleus, both control and stressed female HC-rats had higher COX activity compared to males, but there was no difference between sexes in LC animals. In anterior olfactory nuclei (lateral, ventral, and medial), female rats had lower COX activity, and stress increased further the difference between sexes. In the bed nucleus of stria terminalis and medial preoptic nucleus of hypothalamus, no difference was found between sexes in control animals, but stress decreased oxidative metabolism in female rats. In medial amygdala, hippocampal zones CA1–CA3, temporal cortex (areas 1 and 3), ventrolateral nucleus of thalamus, anteroventral thalamic nucleus, perirhinal cortex, nucleus of the horizontal limb of the diagonal band, and inferior colliculi (central nucleus and external cortex) stress increased COX activity in male LC-rats, resulting in a difference in oxidative metabolism between stressed HC- and LC-rats in these regions. In addition to red nucleus, stress increased COX levels in median raphe and ventral anterior olfactory nucleus of male HC-rats. In the anteroventral thalamic nucleus female HC control rats had higher COX activity than respective LC-rats: no difference was found between stressed HC and LC animals, as CVS increased metabolic activity in the LC group and decreased it in the HC group. In occipital cortex, female LC-rats had higher COX levels than HC-rats, and stress eliminated the difference in area 1 M. In perirhinal cortex, stressed female LC-rats had higher COX than controls. In central and medial parts of amygdala, ventral tegmental area, and subiculum stress decreased COX activity in female HC animals.

Majority of differences in brain metabolism were sex-related. This points to the role of steroid hormones in the regulation of cerebral metabolism. Many of the regions with the strongest expression of oestrogen and androgen receptor proteins and mRNA – such as: medial preoptic and supraoptic areas of the hypothalamus, anterior olfactory nucleus, and amygdala nuclei – exhibit clear sex-dependent differences in COX activity [679–681]. For example, the predominant sex hormone in females, which can also be synthesised from testosterone in males – 17β-estradiol carries out important role as an energy regulator [682]. Its application increases an expression of glucose transporter proteins Glut3 and Glut4, as well as insulin growth factor-1 (IGF1) mRNA and proteins [683]; activity of glycolytic enzymes [684]; protein expression and activity of cytochrome c oxidase's subunits [685, 686]; and ATP levels [687] – thus coordinating a complex biochemical process starting from the glucose uptake by brain cells and finishing with the mitochondrial respiration and gene transcription [682].

Sex-differences in the distribution of classical neurotransmitters have also been studied. Female rats have higher serotonin tissue levels or turnover in many brain regions, including limbic forebrain [688], hippocampus [689], hypothalamus [690, 691], and dorsal raphe [692]. We also observed the lower 5-HT transporter binding capacity in the frontal cortex of female controls, whereas serotonin turnover was higher than in males regardless of chirping

levels. In Paper III, sex-dependent differences were also found in DA- and NA-ergic neurotransmission.

Sex-dependent morphological and physiological differences have also been associated with numerous extra neurotransmitter systems, for example: cholinergic [693], glutamatergic and GABA-ergic [694], dopaminergic [695, 696]. We had no experimentally naive rats in the study, therefore it is not possible to estimate, how much of the observed divergence in the cerebral metabolism between males and females is due to the interaction with the experimental handling and testing, and what share of it may reflect a more fundamental differential brain functioning.

Chronic stress has elevated COX activity in male rats regardless of their chirping levels. This result replicates CVS effects on cerebral metabolic activity from Paper II. AVN and PAG were two other brain regions with CVS-related metabolism increase in Paper II. In the current study, AVN had a tendency toward higher COX activity only in LC stress rats, whereas neighbouring anteroventral thalamic nuclei were also statistically more metabolically active selectively in LC stress animals. Both acute and chronic stressors are associated with the elevated *c-fos* and *zif/268* expression in anteroventral thalamus [589, 590, 697]. Periaqueductal grey matter was no more metabolically active because of CVS in this study. Tickling period that preceded CVS probably had reinforced the association of experimenters with positive affective states, and reduced an overall defensiveness in animals during subsequent experimental procedures. Cytochrome c oxidase levels were also preferentially elevated in LC stress male rats in medial amygdala, which were also increased in one of our previous studies [698]. However, the overall similarity in the metabolic changes between these studies is low. Both studies are unified by the general tendency of CVS to increase the brain metabolic activity. In contrast, our latest study on social defeat stress demonstrated the overall stress-induced decrease in cerebral oxidative metabolism in male rats [548]; and, in the discussed study, CVS was associated with attenuated COX levels in female rats; suggesting a complex non-linear relationship between chronic stress and brain's energetic state. Locus coeruleus was the only brain region showing a CVS-associated decrease in COX activity. Attenuation of its metabolic activity may be linked to the well-established phenomenon of physiological desensitisation of LC by the repeated stress or CRF administration [699].

Several studies of COX activity had used both male and female subjects. Cortical metabolic activity was studied in 6-week-old Sprague-Dawley juvenile rats: males were found to have lower COX levels in the dorsolateral and orbital prefrontal cortices, and in the posterior parietal cortex [700]. We observed the same tendency in parietal cortex, but the opposite was true for prefrontal areas. In 3-months old Wistar rats, metabolic changes caused by the training in Morris water maze were explored [701]. Compared to naive subjects, males showed decreased and females increased COX activity in the range of non-overlapping brain regions. Sex-differences in cerebral metabolism in naive animals were not investigated [701], nevertheless, the direction of regional metabolic differences

between the naive animals seemed quite well matched to our measurements from the control rats. As we had no naive rats in our experiment, direct comparison between these studies and ours in not possible.

In conclusion, brain metabolic adaptation to CVS was sex-dependent, and larger changes were found in stressed LC males and HC females. Sex differences in stress response brain circuitry are also being identified in humans [702, 703], and precise understanding of the nature of these changes in model organisms will help us in devising improved preclinical animal models.

5. GENERAL SUMMARY

Studies, included in the dissertation, confirmed that phenotype-based approach has a place as a tool for improving validity of animal models of affective states and for identifying novel targets in search for psychopathological mechanisms in affective states. However, such a fine-tuning of animal models also increases the complexity and variability of results, which suggests a strong necessity to develop a structured way to reliably asses the dimensionality of animal behaviour. From the presented behavioural phenotypes, low chirping male rats seem to be the most vulnerable to stressful stimulation. They co-express symptoms of anhedonia and passive coping strategies and show discrete changes in cerebral oxidative metabolism. Low exploratory male rats is another behavioural phenotype that showed some promising stress-associated changes in behaviour, however, behavioural profile of LE-rats is more ambiguous, and there was no unique changes in brain metabolic activity to CVS regimen. The heterozygous genetic model of disturbed glutamatergic synaptic release and cycling, VGLUT1+/– mice demonstrated CVS-induced anhedonia and behavioural helplessness, as well as deficits in GABA tissue levels. Levels of several presynaptic and plasma transporters, responsible for glutamate and GABA cycling, were changed in a compensatory manner to the reduced VGLUT1 synthesis. These neurochemical rebalancing did not affect the cerebral oxidative metabolism, most likely because glutamate tissue levels were unaffected.

Sex-dependent differences in rats in behavioural adaptation to CVS and in cerebral oxidative metabolism were identified. Female rats demonstrated a better stress-tolerance and had fewer CVS-associated changes in cytochrome c oxidase activity.

SUMMARY IN ESTONIAN

Aju oksüdatiivne metabolism ja kroonilise muutliku stressi mõju afektiivsete seadumuste loomkatsemudelites

Depressiivset meeleolu võib määratleda kui pikaajalist hajutatud negatiivset afektiivsust koos alanenud motivatsiooniga püstitada eesmärke, läbi viia struktureerituid tegevusi ja suhestuda sotsiaalse ümbruskonnaga. Meeleoluhäirete diagnoosimine ja nende ravi tarbeks tehtavad kulutused on viimastel aastakümnetel arenenud ühiskondades pidevalt tõusnud. Aju arhitektuurse üldplaani säilimine imetajate evolutsioonis ja põhiemotsioonide sarnasus eri loomaliikidel annab lootuse välja töötada valiidsemad loomkatsemudelid afektiivsete protsesside ja nendega seotud patoloogiate uurimiseks.

Üha enam kerkib esile vajadus eristada loomi nende individuaalsete käitumisseadumuste kaudu, välja töötada valiidsed instrumendid mõõtmiseks loomade püsivaid käitumuslikke fenotüüpe ja ideaalis luua ka nende isiksusstruktuuri mudel ja selle määramiseks sobiv testidepatarei. Käesolevas väitekirjas vaadeldakse kaht püsivatel käitumuslikel fenotüüpidel põhinevat ja üht geneetilist loomkatsemudelit, mis on mõeldud afektiivsete protsesside kaudu haavatavamate katseloomade väljavalimiseks või tekitamiseks. Sellised emotsionaalselt haavatavamad loomad võimaldavad täpsemalt uurida stressi mõju psühhopatoloogilistele protsessidele ning valiidsemalt reprodutseerida inimese depressiooni laadset seisundit. Teisalt võimaldavad afektiivselt vähemhaavatavamad katseloomad uurida stressi mõju ja meeleoluhäirete tekke eest kaitsvaid neurobioloogilisi mehhanisme.

Esimene kahel polaarsel käitumisfenotüübil põhinev loomkatsemudel on vähe- ja palju-uudistavad rotid. Käitumusliku fenotüübi määramine selles loomkatsemudelis toimub uudiskastis. Uudiskast kujutab endast suurt avarvälja, millele on paigaldatud kolm roti jaoks uudset objekti, ning väikest ja hämarat külgkambrit, kuhu loom saab peitu pugeda ja ennast suhteliselt turvalisemalt tunda. Uudiskasti-käitumist skooritakse teise päeva 15-minutilise käitumissessiooni põhjal. Sealt saadud uudistamisskooride põhjal jaotatakse loomad vastavalt kas väheuudistavaiks, kes praktiliselt ei sisene avarväljale, või palju-uudistavaiks, kelle uudistamisjuhtude arv ületab 100.

Teine käitumisfenotüübil põhinev loomkatsemudel on eksperimentaatoripoolse manuaalse stimulatsiooni ehk kõdistamise (mis imiteerib noorloomade omavahelist "müramist") poolt esilekutsutud ultrahelihäälitsuste mõõtmine. Nimelt seondub 50-kHz sagedusel esitatud ultrahelihäälitsuste hulk positiivse valentsiga meeleoluseisunditega. 50-kHz ultrahelihäälitsuste hulk on individuaalselt püsiv ning me kasutasime seda asjaolu loomade jagamiseks gruppidesse mediaanipõhiselt.

Kolmadaks mudeliks oli heteresügootne hiire nokautmudel, millel oli välja lülitatud vesikulaarse glutamaadi transporteri 1 (VGLUT 1) geeni üks kahest alleelist. VGLUT1 ensüüm vastutab ajus levinuima virgatsaine glutamaadi vesiikulitesse pakendamise eest presünaptilistes aksonilõpmetes ja selle aktiivsus on

üheks faktoriks presünaptiliselt vabastatava virgatsaine koguse määramisel. Meeleoluhäiretega inimestel on leitud suurenenud glutamaadi/ GABA suhe, ja seda endofenotüüpi omavad ka VGLUT1+/– hiired.

Kõigis kolmes mudelis rakendati loomadele kroonilist muutlikku stressi depressiooni-laadse afektiivse seisundi esilekutsumiseks ning mõõdeti oksüdatiivset ajumetabolismi tsütokroom c oksüdaasi histokeemia abil. Tsütokroom c oksüdaas (COX) on mitokondrilise hingamisahela neljas komponent ning tema aktiivsus määrab ära umbes 95% kogu energiatootmisest ajus, seega mõõtes COX akumulatsiooni erinevates ajupiirkondades saab hea ettekujutuse nende piirkondade pikaajalistest aktivatsioonimustritest. Depressiivsetel inimestel on leitud seos mõnede ajupiirkondade glükoosi utilisatsiooni määra ja haiguse sügavuse vahel, seega on aju energeetiline kaardistamine üheks viisiks meeleoluhäirete patoloogiliste mehhanismide väljaselgitamisel.

Läbiviidud uuringud kinnitasid käitumuslikel fenotüüpidel põhineva lähenemise kasulikkust praegu kasutusel olevate afektiivsete seisundite loomkatsemudelite valiidsuse parendamisel ja afektiivsuse psühhopatoloogiliste mehhanismidega seotud uute sihtmärkide tuvastamisel. Vähe 50-kHz-häälitsevad isasrotid osutusid läbitestitud käitumuslikest fenotüüpidest stressi poolt enim haavatavateks. Kroonilise muutliku stressi tagajärjel arenes neil anhedoonia ning nad eelistasid passiivseid toimetulekustrateegiaid, lisaks esines neil rohkem stressijärgseid ajumetabolismi regionaalseid muutusi.

Väheuudistavad rotid olid teiseks käitumuslikuks fenotüübiks, kellel esinesid mõned paljulubavad stressijärgsed käitumise muutused, kuid mitte nii selgelt, nagu vähe 50-kHz-häälitsevatel isastel. Samuti ei esinenud neil unikaalseid stressi poolt põhjustatud muutusi regionaalses ajumetabolismis.

VGLUT1 geeni osalise nokaudiga hiirtel suurenes samuti anhedoonia kroonilise muutliku stressi tagajärjel ning mitmes käitumiskatses paistsid nad abitumad kui geneetiliselt muundamata hiired. Nende ajukoe GABA tasemed olid vähenenud ning mitmetes glutamaadi ja GABA ringluse eest vastutavates presünaptilise ja rakumembraanide transportervalkude tasemetes olid aset leidnud kompensatoorsed muutused. Selline neurokeemiline ümbertasakaalustamine ei avaldanud mõju oksüdatiivsele ajumetabolismile, tõenäoliselt seoses glutamaadi tasemete muutmatusega.

Paljud metaboolsed ja käitumuslikud muutused olid tänu kroonilisele muutlikule stressile soospetsiifilised. Üldiselt oli emaste rottide kroonilise muutliku stressi taluvusvõime käitumiskatsetes suurem ning neil esines vähem stressijärgseid ajumetabolismi regionaalseid muutusi.

REFERENCES

1. Davidson R (1994) On emotion, mood, and related affective constructs. *The nature of emotion: Fundamental questions*, eds Ekman P & Davidson RJ (Oxford University Press, New York), pp 51–55.
2. Langford DJ*, et al.* (2010) Coding of facial expressions of pain in the laboratory mouse. *Nature Methods* 7(6):447–449.
3. Panksepp J (2005) Affective consciousness: Core emotional feelings in animals and humans. *Consciousness and Cognition* 14(1):30–80.
4. Panksepp J (2008) Carving "Natural" emotions: "Kindly" from bottom-up but not top-down. *Journal of Theoretical and Philosophical Psychology* 28(2):395–422.
5. Frijda N (1994) Varieties of affect: Emotions and episodes, moods, and sentiments. *The nature of emotion: Fundamental questions*, eds Ekman P & Davidson RJ (Oxford University Press New York), pp 59–67.
6. Clark LA & Watson D (1991) Tripartite model of anxiety and depression: Psychometric evidence and taxonomic Implications. *Journal of Abnormal Psychology* 100(3):316–336.
7. Fawcett J, Clark DC, Scheftner WA, & Hedeker D (1983) Differences between anhedonic and normally hedonic depressive states. *American Journal of Psychiatry* 140(8):1027–1030.
8. Naranjo CA, Tremblay LK, & Busto UE (2001) The role of the brain reward system in depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 25(4):781–823.
9. Weissenburger J, Rush AJ, Giles DE, & Stunkard AJ (1986) Weight change in depression. *Psychiatry Research* 17(4):275–283.
10. Mendlewicz J (2009) Sleep disturbances: Core symptoms of major depressive disorder rather than associated or comorbid disorders. *World Journal of Biological Psychiatry* 10(4):269–275.
11. Gupta RK (2009) Major depression: An illness with objective physical signs. *World Journal of Biological Psychiatry* 10(3):196–201.
12. Arnold LM (2008) Understanding fatigue in major depressive disorder and other medical disorders. *Psychosomatics* 49(3):185–190.
13. Leckman JF, Caruso KA, & Prusoff BA (1984) Appetite disturbance and excessive guilt in major depression. Use of family study data to define depressive subtypes. *Archives of General Psychiatry* 41(9):839–844.
14. Brown RG, Scott LC, Bench CJ, & Dolan RJ (1994) Cognitive function in depression: Its relationship to the presence and severity of intellectual decline. *Psychological Medicine* 24(4):829–847.
15. Beck AT, Steer RA, Kovacs M, & Garrison B (1985) Hopelessness and eventual suicide: A 10-year prospective study of patients hospitalized with suicidal ideation. *American Journal of Psychiatry* 142(5):559–563.
16. Bronisch T & Wittchen H-U (1994) Suicidal ideation and suicide attempts: Comorbidity with depression, anxiety disorders, and substance abuse disorder. *European Archives of Psychiatry and Clinical Neuroscience* 244(2):93–98.
17. Taylor MA & Fink M (2006) *Melancholia: The diagnosis, pathophysiology, and treatment of depressive illness* (Cambridge University Press, New York).
18. Donohue JM & Pincus HA (2007) Reducing the societal burden of depression: A review of economic costs, quality of care and effects of treatment. *PharmacoEconomics* 25(1):7–24.

19. Druss BG, Rosenheck RA, & Sledge WH (2000) Health and disability costs of depressive illness in a major U.S. corporation. *American Journal of Psychiatry* 157(8):1274–1278.
20. Fostick L, Silberman A, Beckman M, Spivak B, & Amital D (2010) The economic impact of depression: Resistance or severity? *European Neuropsychopharmacology* 20(10):671–675.
21. Birnbaum HG*, et al.* (2010) Employer burden of mild, moderate, and severe major depressive disorder: Mental health services utilization and costs, and work performance. *Depression and Anxiety* 27(1):78–89.
22. Sobocki P, Lekander I, Borgström F, Ström O, & Runeson B (2007) The economic burden of depression in Sweden from 1997 to 2005. *European Psychiatry* 22(3): 146–152.
23. Kessler RC*, et al.* (2009) The global burden of mental disorders: An update from the WHO World Mental Health (WMH) surveys. *Epidemiologia e Psichiatria Sociale* 18(1):23–33.
24. Ormel J*, et al.* (2008) Disability and treatment of specific mental and physical disorders across the world. *British Journal of Psychiatry* 192(5):368–375.
25. Mueller TI*, et al.* (1999) Recurrence after recovery from major depressive disorder during 15 years of observational follow-up. *American Journal of Psychiatry* 156(7):1000–1006.
26. Videbech P & Ravnkilde B (2004) Hippocampal volume and depression: A meta-analysis of MRI studies. *American Journal of Psychiatry* 161(11):1957–1966.
27. Colla M*, et al.* (2007) Hippocampal volume reduction and HPA-system activity in major depression. *Journal of Psychiatric Research* 41(7):553–560.
28. Sheline YI, Wang PW, Gado MH, Csernansky JG, & Vannier MW (1996) Hippocampal atrophy in recurrent major depression. *Proceedings of the National Academy of Sciences of the United States of America* 93(9):3908–3913.
29. Baumann B*, et al.* (1999) Reduced volume of limbic system-affiliated basal ganglia in mood disorders: Preliminary data from a postmortem study. *Journal of Neuropsychiatry and Clinical Neurosciences* 11(1):71–78.
30. Husain MM*, et al.* (1991) A magnetic resonance imaging study of putamen nuclei in major depression. *Psychiatry Research – Neuroimaging* 40(2):95–99.
31. Pillay SS*, et al.* (1998) A quantitative magnetic resonance imaging study of caudate and lenticular nucleus gray matter volume in primary unipolar major depression: Relationship to treatment response and clinical severity. *Psychiatry Research – Neuroimaging* 84(2–3):61–74.
32. Sheline YI, Gado MH, & Price JL (1998) Amygdala core nuclei volumes are decreased in recurrent major depression. *NeuroReport* 9(9):2023–2028.
33. Lange C & Irle E (2004) Enlarged amygdala volume and reduced hippocampal volume in young women with major depression. *Psychological Medicine* 34(6): 1059–1064.
34. Bremner JD*, et al.* (2002) Reduced volume of orbitofrontal cortex in major depression. *Biological Psychiatry* 51(4):273–279.
35. Coffey CE*, et al.* (1993) Quantitative cerebral anatomy in depression: A controlled magnetic resonance imaging study. *Archives of General Psychiatry* 50(1):7–16.
36. Shah PJ, Ebmeier KP, Glabus MF, & Goodwin GM (1998) Cortical grey matter reductions associated with treatment-resistant chronic unipolar depression: Controlled magnetic resonance imaging study. *British Journal of Psychiatry* 172(6):527–332.

37. Andreasen NC, Swayze II V, Flaum M, Alliger R, & Cohen G (1990) Ventricular abnormalities in affective disorder: Clinical and demographic correlates. *American Journal of Psychiatry* 147(7):893–900.
38. Frodl T*, et al.* (2003) Larger amygdala volumes in first depressive episode as compared to recurrent major depression and healthy control subjects. *Biological Psychiatry* 53(4):338–344.
39. Henn FA & Vollmayr B (2004) Basic pathophysiological mechanisms in depression: What are they and how might they affect the course of the illness? *Pharmacopsychiatry* 37(S2):S152-S156.
40. Lorenzetti V, Allen NB, Fornito A, & Yücel M (2009) Structural brain abnormalities in major depressive disorder: A selective review of recent MRI studies. *Journal of Affective Disorders* 117(1–2):1–17.
41. Rajkowska G & Miguel-Hidalgo JJ (2007) Gliogenesis and glial pathology in depression. *CNS and Neurological Disorders – Drug Targets* 6(3):219–233.
42. Choudary PV*, et al.* (2005) Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proceedings of the National Academy of Sciences of the United States of America* 102(43):15653–15658.
43. Taylor WD*, et al.* (2003) Localization of age-associated white matter hyper-intensities in late-life depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 27(3):539–544.
44. Valentine GW & Sanacora G (2009) Targeting glial physiology and glutamate cycling in the treatment of depression. *Biochemical Pharmacology* 78(5):431–439.
45. Kugaya A & Sanacora G (2005) Beyond monoamines: Glutamatergic function in mood disorders. *CNS Spectrums* 10(10):808–819.
46. Drevets WC*, et al.* (2002) Glucose metabolism in the amygdala in depression: Relationship to diagnostic subtype and plasma cortisol levels. *Pharmacology, Biochemistry and Behavior* 71(3):431–447.
47. Drevets WC*, et al.* (1992) A functional anatomical study of unipolar depression. *Journal of Neuroscience* 12(9):3628–3641.
48. Drevets WC, Öngür D, & Price JL (1998) Neuroimaging abnormalities in the subgenual prefrontal cortex: Implications for the pathophysiology of familial mood disorders. *Molecular Psychiatry* 3(3):220–226.
49. Mayberg HS*, et al.* (1997) Cingulate function in depression: A potential predictor of treatment response. *NeuroReport* 8(4):1057–1061.
50. Drevets WC (2007) Orbitofrontal cortex function and structure in depression. *Annals of the New York Academy of Sciences* 1121(1):499–527.
51. Greicius MD*, et al.* (2007) Resting-state functional connectivity in major depression: Abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biological Psychiatry* 62(5):429–437.
52. Holthoff VA*, et al.* (2004) Changes in brain metabolism associated with remission in unipolar major depression. *Acta Psychiatrica Scandinavica* 110(3):184–194.
53. Alcaro A, Panksepp J, Witczak J, Hayes DJ, & Northoff G (2010) Is subcortical-cortical midline activity in depression mediated by glutamate and GABA? A cross-species translational approach. *Neuroscience and Biobehavioral Reviews* 34(4):592–605.
54. Drevets WC, Price JL, & Furey ML (2008) Brain structural and functional abnormalities in mood disorders: Implications for neurocircuitry models of depression. *Brain Structure and Function* 213(1–2):93–118.

55. Rigucci S, Serafini G, Pompili M, Kotzalidis GD, & Tatarelli R (2010) Anatomical and functional correlates in major depressive disorder: The contribution of neuroimaging studies. *World Journal of Biological Psychiatry* 11(2):165–180.
56. Mayberg HS (1997) Limbic-cortical dysregulation: A proposed model of depression. *Journal of Neuropsychiatry and Clinical Neurosciences* 9(3):471–481.
57. Mineka S & Sutton S (1992) Cognitive biases and the emotional disorders. *Psychological Science* 3(1):65–69.
58. Joormann J (2004) Attentional bias in dysphoria: The role of inhibitory processes. *Cognition and Emotion* 18(1):125–147.
59. Dunn BD, Stefanovitch I, Buchan K, Lawrence AD, & Dalgleish T (2009) A reduction in positive self-judgment bias is uniquely related to the anhedonic symptoms of depression. *Behaviour Research and Therapy* 47(5):374–381.
60. Werner NS*, et al.* (2009) Functional MRI study of memory-related brain regions in patients with depressive disorder. *Journal of Affective Disorders* 119(1–3):124–131.
61. Smoski MJ*, et al.* (2009) fMRI of alterations in reward selection, anticipation, and feedback in major depressive disorder. *Journal of Affective Disorders* 118(1–3):69–78.
62. Alnaes R & Torgersen S (1989) Personality and personality disorders among patients with major depression in combination with dysthymic or cyclothymic disorders. *Acta Psychiatrica Scandinavica* 79(4):363–369.
63. Sih A, Bell A, & Johnson JC (2004) Behavioral syndromes: An ecological and evolutionary overview. *Trends in Ecology and Evolution* 19(7):372–378.
64. McKinney Jr. WT & Bunney Jr. WE (1969) Animal model of depression. I. Review of evidence: Implications for research. *Archives of General Psychiatry* 21(2):240–248.
65. Willner P (1984) The validity of animal models of depression. *Psychopharmacology* 83(1):1–16.
66. John B & Lewis KR (1966) Chromosome variability and geographic distribution in insects. *Science* 152(3723):711–721.
67. Gottesman II & Shields J (1973) Genetic theorizing and schizophrenia. *British Journal of Psychiatry* 122(566):15–30.
68. Gottesman II & Gould TD (2003) The endophenotype concept in psychiatry: Etymology and strategic intentions. *American Journal of Psychiatry* 160(4):636–645.
69. Hasler G, Drevets WC, Gould TD, Gottesman II, & Manji HK (2006) Toward constructing an endophenotype strategy for bipolar disorders. *Biological Psychiatry* 60(2):93–105.
70. Roths JB, Foxworth WB, McArthur MJ, Montgomery CA, & Kier AB (1999) Spontaneous and engineered mutant mice as models for experimental and comparative pathology: History, comparison, and developmental technology. *Laboratory Animal Science* 49(1):12–34.
71. Holmes A & Cryan J (2006) Modeling human anxiety and depression in mutant mice. *Transgenic and knockout models of neuropsychiatric disorders*, eds Fisch GS & Flint J (Humana Press, Totowa), pp 237–263.
72. Phillips TJ*, et al.* (2002) Harnessing the mouse to unravel the genetics of human disease. *Genes, Brain and Behavior* 1(1):14–26.
73. Van Gaalen MM & Steckler T (2000) Behavioural analysis of four mouse strains in an anxiety test battery. *Behavioural Brain Research* 115(1):95–106.

74. Crabbe JC, Wahlsten D, & Dudek BC (1999) Genetics of mouse behavior: Interactions with laboratory environment. *Science* 284(5420):1670–1672.
75. Wahlsten D*, et al.* (2003) Different data from different labs: Lessons from studies of gene-environment interaction. *Journal of Neurobiology* 54(1):283–311.
76. Chesler EJ, Wilson SG, Lariviere WR, Rodriguez-Zas SL, & Mogil JS (2002) Identification and ranking of genetic and laboratory environment factors influencing a behavioral trait, thermal nociception, via computational analysis of a large data archive. *Neuroscience and Biobehavioral Reviews* 26(8):907–923.
77. Valdar W*, et al.* (2006) Genetic and environmental effects on complex traits in mice. *Genetics* 174(2):959–984.
78. Richter SH, Garner JP, & Würbel H (2009) Environmental standardization: Cure or cause of poor reproducibility in animal experiments? *Nature Methods* 6(4):257–261.
79. Richter SH, Garner JP, Auer C, Kunert J, & Würbel H (2010) Systematic variation improves reproducibility of animal experiments. *Nature Methods* 7(3):167–168.
80. Flint J (2006) Transgenic mouse models and human psychiatric disease. *Transgenic and knockout models of neuropsychiatric disorders*, eds Fisch GS & Flint J (Humana Press, Totowa), pp 25–43.
81. Kas MJH, Fernandes C, Schalkwyk LC, & Collier DA (2007) Genetics of behavioural domains across the neuropsychiatric spectrum: Of mice and men. *Molecular Psychiatry* 12(4):324–330.
82. Kalueff AV, Ren-Patterson RF, Laporte JL, & Murphy DL (2008) Domain interplay concept in animal models of neuropsychiatric disorders: A new strategy for high-throughput neurophenotyping research. *Behavioural Brain Research* 188(2):243–249.
83. Laporte JL, Ren-Patterson RF, Murphy DL, & Kalueff AV (2008) Refining psychiatric genetics: From 'mouse psychiatry' to understanding complex human disorders. *Behavioural Pharmacology* 19(5–6):377–384.
84. International Mouse Knockout Consortium, Collins FS, Rossant J, & Wurst W (2007) A mouse for all reasons. *Cell* 128(1):9–13.
85. Balogh SA, McDowell CS, Stavnezer AJ, & Denenberg VH (1999) A behavioral and neuroanatomical assessment of an inbred substrain of 129 mice with behavioral comparisons to C57BL/6J mice. *Brain Research* 836(1–2):38–48.
86. Royce JR (1972) Avoidance conditioning in nine strains of inbred mice using optimal stimulus parameters. *Behavior Genetics* 2(1):107–110.
87. Lush IE (1989) The genetics of tasting in mice. VI. Saccharin, acesulfame, dulcin and sucrose. *Genetics Research* 53(2):95–99.
88. Montkowski A, Poettig M, Mederer A, & Holsboer F (1997) Behavioural performance in three substrains of mouse strain 129. *Brain Research* 762(1–2):12–18.
89. Liguz-Lecznar M & Skangiel-Kramska J (2007) Vesicular glutamate transporters (VGLUTs): The three musketeers of glutamatergic system. *Acta Neurobiologiae Experimentalis* 67(3):207–218.
90. Tordera RM*, et al.* (2007) Enhanced anxiety, depressive-like behaviour and impaired recognition memory in mice with reduced expression of the vesicular glutamate transporter 1 (VGLUT1). *European Journal of Neuroscience* 25(1): 281–290.
91. Selye H (1950) Stress and the general adaptation syndrome. *British Medical Journal* 1(4667):1383–1392.

92. Mazure CM (1998) Life stressors as risk factors in depression. *Clinical Psychology: Science and Practice* 5(3):291–313.
93. Kessler RC (1997) The effects of stressful life events on depression. *Annual Review of Psychology* 48:191–214.
94. Kendler KS, Thornton LM, & Prescott CA (2001) Gender differences in the rates of exposure to stressful life events and sensitivity to their depressogenic effects. *American Journal of Psychiatry* 158(4):587–593.
95. Kendler KS, Kuhn J, & Prescott CA (2004) The interrelationship of neuroticism, sex, and stressful life events in the prediction of episodes of major depression. *American Journal of Psychiatry* 161(4):631–636.
96. Riso LP, Miyatake RK, & Thase ME (2002) The search for determinants of chronic depression: A review of six factors. *Journal of Affective Disorders* 70(2): 103–115.
97. Piccinelli M & Wilkinson G (2000) Gender differences in depression. Critical review. *British Journal of Psychiatry* 177:486–492.
98. Post RM (1992) Transduction of psychosocial stress into the neurobiology of recurrent affective disorder. *American Journal of Psychiatry* 149(8):999–1010.
99. Hammen C (2005) Stress and depression. *Annual Review of Clinical Psychology* 1:293–319.
100. Van Os J, Park SBG, & Jones PB (2001) Neuroticism, life events and mental health: Evidence for person-environment correlation. *British Journal of Psychiatry* 178(S40):S72-S77.
101. Dickerson SS & Kemeny ME (2004) Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin* 130(3):355–391.
102. Harris TO*, et al.* (2000) Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. *British Journal of Psychiatry* 177:505–510.
103. Linkowski P, Mendlewicz J, & Leclercq R (1985) The 24-hour profile of adrenocorticotropin and cortisol in major depressive illness. *Journal of Clinical Endocrinology and Metabolism* 61(3):429–438.
104. Carroll BJ, Curtis GC, & Mendels J (1976) Cerebrospinal fluid and plasma free cortisol concentrations in depression. *Psychological Medicine* 6(2):235–244.
105. Carroll BJ, Curtis GC, & Davies BM (1976) Urinary free cortisol excretion in depression. *Psychological Medicine* 6(1):43–50.
106. Vreeburg SA*, et al.* (2009) Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: Results from a large cohort study. *Archives of General Psychiatry* 66(6):617–626.
107. Young EA, Lopez JF, Murphy-Weinberg V, Watson SJ, & Akil H (2000) Hormonal evidence for altered responsiveness to social stress in major depression. *Neuropsychopharmacology* 23(4):411–418.
108. Strickland PL*, et al.* (2002) Bio-social origins of depression in the community: Interactions between social adversity, cortisol and serotonin neurotransmission. *British Journal of Psychiatry* 180:168–173.
109. Michopoulos I*, et al.* (2008) Neuropsychological and hypothalamic-pituitary-axis function in female patients with melancholic and non-melancholic depression. *European Archives of Psychiatry and Clinical Neuroscience* 258(4):217–225.
110. Putnam KM, Pizzagalli DA, Gooding DC, Kalin NH, & Davidson RJ (2008) Neural activity and diurnal variation of cortisol: Evidence from brain electrical tomography analysis and relevance to anhedonia. *Psychophysiology* 45(6):886–895.

111. Rubin RT, *et al.* (1987) Neuroendocrine aspects of primary endogenous depression III. Cortisol secrtion in relation to diagnosis and symptom patterns. *Psychological Medicine* 17(3):609–619.
112. Ensel WM & Lin N (1996) Distal stressors and the life stress process. *Journal of Community Psychology* 24(1):66–82.
113. Rao U, Hammen C, Ortiz LR, Chen L-A, & Poland RE (2008) Effects of early and recent adverse experiences on adrenal response to psychosocial stress in depressed adolescents. *Biological Psychiatry* 64(6):521–526.
114. Seay B, Hansen E, & Harlow HF (1962) Mother-infant separation in monkeys. *The Journal of Child Psychology and Psychiatry* 3(3–4):123–132.
115. Seay B & Harlow HF (1965) Maternal separation in the rhesus monkey. *The Journal of Nervous and Mental Disease* 140(6):434–441.
116. Kaufman IC & Rosenblum LA (1967) The reaction to separation in infant monkeys: Anaclitic depression and conservation-withdrawal. *Psychosomatic Medicine* 29(6):648–675.
117. Maier SF & Seligman MEP (1976) Learned helplessness: Theory and evidence. *Journal of Experimental Psychology: General* 105(1):3–46.
118. Seligman MEP & Beagley G (1975) Learned helplessness in the rat. *Journal of Comparative & Physiological Psychology* 88(2):534–541.
119. Seligman MEP, Weiss JM, Weinraub M, & Schulman A (1980) Coping behavior: Learned helplessness, physiological change and learned inactivity. *Behaviour Research and Therapy* 18(5):459–512.
120. Altenor A, Kay E, & Richter M (1977) The generality of learned helplessness in the rat. *Learning and Motivation* 8(1):54–61.
121. Maier SF, Anderson C, & Lieberman DA (1972) Influence of control of shock on subsequent shock-elicited aggression. *Journal of Comparative & Physiological Psychology* 81(1):94–100.
122. Anderson DC, Crowell C, Koehn D, & Lupo JV (1976) Different intensities of unsignalled inescapable shock treatments as determinants of non shock motivated open field behavior: A resolution of disparate results. *Physiology and Behavior* 17(3):391–394.
123. Rapaport PM & Maier SF (1978) Inescapable shock and food-competition dominance in rats. *Animal Learning and Behavior* 6(2):160–165.
124. Weiss JM (1968) Effects of coping responses on stress. *Journal of Comparative & Physiological Psychology* 65(2):251–260.
125. Greenberg L, Edwards E, & Henn FA (1989) Dexamethasone suppression test in helpless rats. *Biological Psychiatry* 26(5):530–532.
126. Edwards E, Harkins K, Wright G, & Henn F (1990) Effects of bilateral adrenalectomy on the induction of learned helplessness behavior. *Neuropsychopharmacology* 3(2):109–114.
127. Maier SF (2001) Exposure to the stressor environment prevents the temporal dissipation of behavioral depression/learned helplessness. *Biological Psychiatry* 49(9):763–773.
128. Bainbridge PL (1973) Learning in the rat: Effect of early experience with an unsolvable problem. *Journal of Comparative & Physiological Psychology* 82(2): 301–307.
129. Porsolt RD, Brossard G, Hautbois C, & Roux S (2007) Rodent models of depression: Forced swimming and tail suspension behavioral despair tests in rats and mice. *Current Protocols in Pharmacology* 5.8.1.

130. Porsolt RD, Le Pichon M, & Jalfre M (1977) Depression: A new animal model sensitive to antidepressant treatments. *Nature* 266(5604):730–732.
131. Drugan RC, Skolnick P, Paul SM, & Crawley JN (1989) A pretest procedure reliably predicts performance in two animal models of inescapable stress. *Pharmacology, Biochemistry and Behavior* 33(3):649–654.
132. Cryan JF, Valentino RJ, & Lucki I (2005) Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neuroscience & Biobehavioral Reviews* 29(4–5):547–569.
133. Dorworth TR & Overmier JB (1977) On 'learned helplessness': The therapeutic effects of electroconvulsive shocks. *Physiological Psychology* 5(3):355–358.
134. Sherman AD, Sacquitne JL, & Petty F (1982) Specificity of the learned helplessness model of depression. *Pharmacology, Biochemistry and Behavior* 16(3):449–454.
135. Willner P (1986) Validation criteria for animal models of human mental disorders: Learned helplessness as a paradigm case. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 10(6):677–690.
136. Overmier JB & Seligman MEP (1967) Effects of inescapable shock upon subsequent escape and avoidance responding. *Journal of Comparative & Physiological Psychology* 63(1):28–33.
137. Wieland S, Boren JL, Consroe PF, & Martin A (1986) Stock differences in the susceptibility of rats to learned helplessness training. *Life Sciences* 39(10):937–944.
138. Lachman HM*, et al.* (1992) Hippocampal neuropeptide Y mRNA is reduced in a strain of learned helpless resistant rats. *Molecular Brain Research* 14(1–2):94–100.
139. Shumake J, Barrett D, & Gonzalez-Lima F (2005) Behavioral characteristics of rats predisposed to learned helplessness: Reduced reward sensitivity, increased novelty seeking, and persistent fear memories. *Behavioural Brain Research* 164(2):222–230.
140. King JA, Abend S, & Edwards E (2001) Genetic predisposition and the development of posttraumatic stress disorder in an animal model. *Biological Psychiatry* 50(4):231–237.
141. Enkel T, Spanagel R, Vollmayr B, & Schneider M (2010) Stress triggers anhedonia in rats bred for learned helplessness. *Behavioural Brain Research* 209(1):183–186.
142. Willner P (1997) Validity, reliability and utility of the chronic mild stress model of depression: A 10-year review and evaluation. *Psychopharmacology* 134(4):319–329.
143. Willner P, Muscat R, & Papp M (1992) Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neuroscience and Biobehavioral Reviews* 16(4):525–534.
144. Katz RJ, Roth KA, & Carroll BJ (1981) Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. *Neuroscience and Biobehavioral Reviews* 5(2):247–251.
145. Hu H, Su L, Xu YQ, Zhang H, & Wang LW (2010) Behavioral and [F-18] fluorodeoxyglucose micro positron emission tomography imaging study in a rat chronic mild stress model of depression. *Neuroscience* 169(1):171–181.
146. Westenbroek C*, et al.* (2003) Gender-specific effects of social housing in rats after chronic mild stress exposure. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 27(1):21–30.

147. D'Aquila PS, Peana AT, Carboni V, & Serra G (2000) Exploratory behaviour and grooming after repeated restraint and chronic mild stress: Effect of desipramine. *European Journal of Pharmacology* 399(1):43–47.
148. Lucca G*, et al.* (2008) Chronic mild stress paradigm reduces sweet food intake in rats without affecting brain derived neurotrophic factor protein levels. *Current Neurovascular Research* 5(4):207–213.
149. Harris RBS, Zhou J, Youngblood BD, Smagin GN, & Ryan DH (1997) Failure to change exploration or saccharin preference in rats exposed to chronic mild stress. *Physiology and Behavior* 63(1):91–100.
150. Harro J, Tõnissaar 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(1–2):227–239.
151. Bessa J*, et al.* (2009) A trans-dimensional approach to the behavioral aspects of depression. *Frontiers in Behavioral Neuroscience* 3.1
152. Carobrez AP & Bertoglio LJ (2005) Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. *Neuroscience and Biobehavioral Reviews* 29(8):1193–1205.
153. Silveira MCL, Sandner G, & Graeff FG (1993) Induction of Fos immunoreactivity in the brain by exposure to the elevated plus-maze. *Behavioural Brain Research* 56(1):115–118.
154. Kompagne H*, et al.* (2008) Chronic mild stress generates clear depressive but ambiguous anxiety-like behaviour in rats. *Behavioural Brain Research* 193(2): 311–314.
155. Vyas A, Mitra R, Shankaranarayana Rao BS, & Chattarji S (2002) Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *Journal of Neuroscience* 22(15):6810–6818.
156. D'Aquila PS, Brain P, & Willner P (1994) Effects of chronic mild stress on performance in behavioural tests relevant to anxiety and depression. *Physiology and Behavior* 56(5):861–867.
157. Willner P (2005) Chronic mild stress (CMS) revisited: Consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology* 52(2):90–110.
158. Dalla C*, et al.* (2008) Sex differences in the effects of two stress paradigms on dopaminergic neurotransmission. *Physiology and Behavior* 93(3):595–605.
159. Harro J*, et al.* (1999) Chronic mild unpredictable stress after noradrenergic denervation: Attenuation of behavioural and biochemical effects of DSP-4 treatment. *European Neuropsychopharmacology* 10(1):5–16.
160. Platt JE & Stone EA (1982) Chronic restraint stress elicits a positive antidepressant response on the forced swim test. *European Journal of Pharmacology* 82(3–4):179–181.
161. Cain C & LeDoux J (2008) Emotional processing and motivation: In search of brain mechanisms. *Handbook of approach and avoidance motivation*, ed Elliot AJ (Psychology Press, New York), pp 17–34.
162. Voorn P, Jorritsma-Byham B, Van Dijk C, & Buijs RM (1986) The dopaminergic innervation of the ventral striatum in the rat: A light- and electron-microscopical study with antibodies against dopamine. *Journal of Comparative Neurology* 251(1):84–99.

163. Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Research Bulletin* 9(1–6):321–353.
164. Morgane PJ, Galler JR, & Mokler DJ (2005) A review of systems and networks of the limbic forebrain/limbic midbrain. *Progress in Neurobiology* 75(2):143–160.
165. Haber SN & Knutson B (2010) The reward circuit: Linking primate anatomy and human imaging. *Neuropsychopharmacology* 35(1):4–26.
166. Chow T & Cummings J (1999) Frontal-subcortical circuits. *The human frontal lobes: Functions and disorders*, eds Miller BL & Cummings JL (The Guilford Press, London), pp 3–26.
167. Sesack SR & Grace AA (2010) Cortico-basal ganglia reward network: Microcircuitry. *Neuropsychopharmacology* 35(1):27–47.
168. Heimer L, Zahm DS, Churchill L, Kalivas PW, & Wohltmann C (1991) Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience* 41(1):89–125.
169. Van Dongen YC*, et al.* (2005) Anatomical evidence for direct connections between the shell and core subregions of the rat nucleus accumbens. *Neuroscience* 136(4):1049–1071.
170. Goto Y & Grace AA (2008) Limbic and cortical information processing in the nucleus accumbens. *Trends in Neurosciences* 31(11):552–558.
171. Corbit LH, Muir JL, & Balleine BW (2001) The role of the nucleus accumbens in instrumental conditioning: Evidence of a functional dissociation between accumbens core and shell. *Journal of Neuroscience* 21(9):3251–3260.
172. Dias-Ferreira E*, et al.* (2009) Chronic stress causes frontostriatal reorganization and affects decision-making. *Science* 325(5940):621–625.
173. Estes WK (1948) Discriminative conditioning. II. Effects of a Pavlovian conditioned stimulus upon a subsequently established operant response. *Journal of Experimental Psychology* 38(2):173–177.
174. Lovibond PF (1983) Facilitation of instrumental behavior by a Pavlovian appetitive conditioned stimulus. *Journal of Experimental Psychology: Animal Behavior Processes* 9(3):225–247.
175. Ito R, Dalley JW, Howes SR, Robbins TW, & Everitt BJ (2000) Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. *Journal of Neuroscience* 20(19):7489–7495.
176. Dickinson A, Smith J, & Mirenowicz J (2000) Dissociation of Pavlovian and instrumental incentive learning under dopamine antagonists. *Behavioral Neuroscience* 114(3):468–483.
177. Lex A & Hauber W (2008) Dopamine D1 and D2 receptors in the nucleus accumbens core and shell mediate Pavlovian-instrumental transfer. *Learning and Memory* 15(7):483–491.
178. Hall J, Parkinson JA, Connor TM, Dickinson A, & Everitt BJ (2001) Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating pavlovian influences on instrumental behaviour. *European Journal of Neuroscience* 13(10):1984–1992.
179. Finch DM (1996) Neurophysiology of converging synaptic inputs from the rat prefrontal cortex, amygdala, midline thalamus, and hippocampal formation onto single neurons of the caudate/putamen and nucleus accumbens. *Hippocampus* 6(5):495–512.

180. Groenewegen HJ, Wright CI, Beijer AVJ, & Voorn P (1999) Convergence and segregation of ventral striatal inputs and outputs. *Annals of the New York Academy of Sciences* 877:49–63.
181. Wolf JA, Finkel LH, & Contreras D (2009) Sublinear summation of afferent inputs to the nucleus accumbens in the awake rat. *Journal of Physiology* 587(8):1695–1704.
182. Grace AA (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. *Neuroscience* 41(1):1–24.
183. Schultz W (1998) Predictive reward signal of dopamine neurons. *Journal of Neurophysiology* 80(1):1–27.
184. Lu X-Y, Ghasemzadeh MB, & Kalivas PW (1997) Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience* 82(3):767–780.
185. Robertson GS & Jian M (1995) D1 and D2 dopamine receptors differentially increase Fos-like immunoreactivity in accumbal projections to the ventral pallidum and midbrain. *Neuroscience* 64(4):1019–1034.
186. Donnell P & Grace AA (1996) Dopaminergic reduction of excitability in nucleus accumbens neurons recorded in vitro. *Neuropsychopharmacology* 15(1):87–97.
187. White FJ & Wang RY (1986) Electrophysiological evidence for the existence of both D-1 and D-2 dopamine receptors in the rat nucleus accumbens. *Journal of Neuroscience* 6(1):274–280.
188. West AR & Grace AA (2002) Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: Studies combining in vivo intracellular recordings and reverse microdialysis. *Journal of Neuroscience* 22(1):294–304.
189. Chergui K & Lacey MG (1999) Modulation by dopamine D1-like receptors of synaptic transmission and NMDA receptors in rat nucleus accumbens is attenuated by the protein kinase C inhibitor Ro 32–0432. *Neuropharmacology* 38(2):223–231.
190. Onn S-P, West AR, & Grace AA (2000) Dopamine-mediated regulation of striatal neuronal and network interactions. *Trends in Neurosciences* 23(10S):S48-S56.
191. O'Donnell P & Grace AA (1995) Synaptic interactions among excitatory afferents to nucleus accumbens neurons: Hippocampal gating of prefrontal cortical input. *Journal of Neuroscience* 15(5):3622–3639.
192. Goto Y & Grace AA (2005) Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nature Neuroscience* 8(6):805–812.
193. Charara A & Grace AA (2003) Dopamine receptor subtypes selectively modulate excitatory afferents from the hippocampus and amygdala to rat nucleus accumbens neurons. *Neuropsychopharmacology* 28(8):1412–1421.
194. 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 Reviews* 31(1):6–41.
195. Voruganti L & Awad AG (2004) Neuroleptic dysphoria: Towards a new synthesis. *Psychopharmacology* 171(2):121–132.
196. O'Doherty J*, et al.* (2004) Dissociable roles of ventral and dorsal striatum in instrumental conditioning. *Science* 304(5669):452–454.
197. Thompson WF, Schellenberg EG, & Husain G (2001) Arousal, mood, and the Mozart effect. *Psychological Science* 12(3):248–251.

198. Grilly DM & Loveland A (2001) What is a “low dose” of d-amphetamine for inducing behavioral effects in laboratory rats? *Psychopharmacology* 153(2):155–169.
199. Pekrun R, Goetz T, Titz W, & Perry RP (2002) Academic emotions in students’ self-regulated learning and achievement: A program of qualitative and quantitative research. *Educational Psychologist* 37(2):91–105.
200. Panksepp J (2007) Can PLAY diminish ADHD and facilitate the construction of the social brain? *Journal of the Canadian Academy of Child and Adolescent Psychiatry* 16(2):57–66.
201. Siviy S (1998) Neurobiological substrates of play behavior: Glimpses into the structure and function of mammalian playfulness. *Animal play: Evolutionary, comparative, and ecological perspectives*, eds Bekoff M & Byers JA (Cambridge University Press, Cambridge), pp 221–242.
202. Meehl PE (1975) Hedonic capacity: Some conjectures. *Bulletin of the Menninger Clinic* 39(4):295–307.
203. Pizzagalli DA, Jahn AL, & O’Shea JP (2005) Toward an objective characterization of an anhedonic phenotype: A signal-detection approach. *Biological Psychiatry* 57(4):319–327.
204. Willner P & Healy S (1994) Decreased hedonic responsiveness during a brief depressive mood swing. *Journal of Affective Disorders* 32(1):13–20.
205. Berenbaum H & Connelly J (1993) The effect of stress on hedonic capacity. *Journal of Abnormal Psychology* 102(3):474–481.
206. Pizzagalli DA, Bogdan R, Ratner KG, & Jahn AL (2007) Increased perceived stress is associated with blunted hedonic capacity: Potential implications for depression research. *Behaviour Research and Therapy* 45(11):2742–2753.
207. Fawcett J, Clark DC, Scheftner WA, & Gibbons RD (1983) Assessing anhedonia in psychiatric patients. The pleasure scale. *Archives of General Psychiatry* 40(1):79–84.
208. Pizzagalli DA, Iosifescu D, Hallett LA, Ratner KG, & Fava M (2008) Reduced hedonic capacity in major depressive disorder: Evidence from a probabilistic reward task. *Journal of Psychiatric Research* 43(1):76–87.
209. Leventhal AM, Chasson GS, Tapia E, Miller EK, & Pettit JW (2006) Measuring hedonic capacity in depression: A psychometric analysis of three anhedonia scales. *Journal of Clinical Psychology* 62(12):1545–1558.
210. Richter C & Campbell K (1940) Taste thresholds and taste preferences of rats for five common sugars. *Journal of Nutrition* 20(1):31–46.
211. Cabanac M & Johnson KG (1983) Analysis of a conflict between palatability and cold exposure in rats. *Physiology and Behavior* 31(2):249–253.
212. Katz R & Hersh S (1981) Amitriptyline and scopolamine in an animal model of depression. *Neuroscience and Biobehavioral Reviews* 5(2):265–271.
213. Muscat R & Willner P (1992) Suppression of sucrose drinking by chronic mild unpredictable stress: A methodological analysis. *Neuroscience and Biobehavioral Reviews* 16(4):507–517.
214. Berlin I, Givry-Steiner L, Lecrubier Y, & Puech AJ (1998) Measures of anhedonia and hedonic responses to sucrose in depressive and schizophrenic patients in comparison with healthy subjects. *European Psychiatry* 13(6):303–309.
215. Westover AN & Marangell LB (2002) A cross-national relationship between sugar consumption and major depression? *Depression and Anxiety* 16(3):118–120.

216. Willner P*, et al.* (1998) 'Depression' increases 'craving' for sweet rewards in animal and human models of depression and craving. *Psychopharmacology* 136(3):272–283.
217. Moreau J-L, Jenck F, Martin JR, Mortas P, & Haefely WE (1992) Antidepressant treatment prevents chronic unpredictable mild stress-induced anhedonia as assessed by ventral tegmentum self-stimulation behavior in rats. *European Neuropsychopharmacology* 2(1):43–49.
218. Papp M, Willner P, & Muscat R (1991) An animal model of anhedonia: Attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. *Psychopharmacology* 104(2):255–259.
219. Papp M, Lappas S, Muscat R, & Willner P (1992) Attenuation of place preference conditioning but not place aversion conditioning by chronic mild stress. *Journal of Psychopharmacology* 6(3):352–356.
220. Valverde O, Smadja C, Roques BP, & Maldonado R (1997) The attenuation of morphine-conditioned place preference following chronic mild stress is reversed by a CCK(B) receptor antagonist. *Psychopharmacology* 131(1):79–85.
221. Marona-Lewicka D & Nichols D (1997) The effect of selective serotonin releasing agents in the chronic mild stress model of depression in rats. *Stress* 2(2):91–99.
222. Tõnissaar M*, et al.* (2008) Rat behavior after chronic variable stress and partial lesioning of 5-HT-ergic neurotransmission: Effects of citalopram. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 32(1):164–177.
223. Wise R (1982) Neuroleptics and operant-behavior – the anhedonia hypothesis. *Behavioral and Brain Sciences* 5(1):39–53.
224. Veazey C, Aki SOE, Cook KF, Lai EC, & Kunik ME (2005) Prevalence and treatment of depression in Parkinson's disease. *Journal of Neuropsychiatry and Clinical Neurosciences* 17(3):310–323.
225. Hasler G*, et al.* (2008) Neural response to catecholamine depletion in unmedicated subjects with major depressive disorder in remission and healthy subjects. *Archives of General Psychiatry* 65(5):521–531.
226. Hasler G*, et al.* (2009) Reward processing after catecholamine depletion in unmedicated, remitted subjects with major depressive disorder. *Biological Psychiatry* 66(3):201–205.
227. Mitani H, Shirayama Y, Yamada T, & Kawahara R (2006) Plasma levels of homovanillic acid, 5-hydroxyindoleacetic acid and cortisol, and serotonin turnover in depressed patients. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 30(3):531–534.
228. Hamner MB & Diamond BI (1996) Plasma dopamine and norepinephrine correlations with psychomotor retardation, anxiety, and depression in non-psychotic depressed patients: A pilot study. *Psychiatry Research* 64(3):209–211.
229. Roy A, Karoum F, & Pollack S (1992) Marked reduction in indexes of dopamine metabolism among patients with depression who attempt suicide. *Archives of General Psychiatry* 49(6):447–450.
230. Shah PJ, Ogilvie AD, Goodwin GM, & Ebmeier KP (1997) Clinical and psychometric correlates of dopamine D2 binding in depression. *Psychological Medicine* 27(6):1247–1256.
231. D'haenen HA & Bossuyt A (1994) Dopamine D2 receptors in depression measured with single photon emission computed tomography. *Biological Psychiatry* 35(2):128–132.

232. Meyer JH, *et al.* (2006) Elevated putamen D2 receptor binding potential in major depression with motor retardation: An [11C]raclopride positron emission tomography study. *American Journal of Psychiatry* 163(9):1594–1602.
233. Parsey RV, *et al.* (2001) Dopamine D2 receptor availability and amphetamine-induced dopamine release in unipolar depression. *Biological Psychiatry* 50(5):313–322.
234. Jensen J, *et al.* (2003) Direct activation of the ventral striatum in anticipation of aversive stimuli. *Neuron* 40(6):1251–1257.
235. Meyer JH, *et al.* (2001) Lower dopamine transporter binding potential in striatum during depression. *NeuroReport* 12(18):4121–4125.
236. Sarchiapone M, *et al.* (2006) Dopamine transporter binding in depressed patients with anhedonia. *Psychiatry Research – Neuroimaging* 147(2–3):243–248.
237. Lehto SM, *et al.* (2008) Midbrain serotonin and striatum dopamine transporter binding in double depression: A one-year follow-up study. *Neuroscience Letters* 441(3):291–295.
238. Joensuu M, *et al.* (2007) Reduced midbrain serotonin transporter availability in drug-naïve patients with depression measured by SERT-specific [123I] nor-β-CIT SPECT imaging. *Psychiatry Research – Neuroimaging* 154(2):125–131.
239. Laasonen-Balk T, *et al.* (1999) Striatal dopamine transporter density in major depression. *Psychopharmacology* 144(3):282–285.
240. Schlaepfer TE, *et al.* (2008) Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. *Neuropsychopharmacology* 33(2):368–377.
241. Di Chiara G, Loddo P, & Tanda G (1999) Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: Implications for the psychobiology of depression. *Biological Psychiatry* 46(12):1624–1633.
242. Espejo EF & Miñano FJ (1999) Prefrontocortical dopamine depletion induces antidepressant-like effects in rats and alters the profile of desipramine during Porsolt's test. *Neuroscience* 88(2):609–615.
243. Weiss JM, *et al.* (2008) Rats selectively-bred for behavior related to affective disorders: Proclivity for intake of alcohol and drugs of abuse, and measures of brain monoamines. *Biochemical Pharmacology* 75(1):134–159.
244. Overstreet DH, Friedman E, Mathé AA, & Yadid G (2005) The Flinders Sensitive Line rat: A selectively bred putative animal model of depression. *Neuroscience and Biobehavioral Reviews* 29(4–5):739–759.
245. Zangen A, Overstreet DH, & Yadid G (1999) Increased catecholamine levels in specific brain regions of a rat model of depression: Normalization by chronic antidepressant treatment. *Brain Research* 824(2):243–250.
246. Overstreet DH & Russell RW (1982) Selective breeding for diisopropyl fluorophosphate-sensitivity: Behavioural effects of cholinergic agonists and antagonists. *Psychopharmacology* 78(2):150–155.
247. Yadid G, Overstreet DH, & Zangen A (2001) Limbic dopaminergic adaptation to a stressful stimulus in a rat model of depression. *Brain Research* 896(1–2):43–47.
248. Pucilowski O, Overstreet DH, Rezvani AH, & Janowsky DS (1993) Chronic mild stress-induced anhedonia: Greater effect in a genetic rat model of depression. *Physiology and Behavior* 54(6):1215–1220.
249. Matthews K, *et al.* (1996) Rewarding electrical brain stimulation: Similar thresholds for flinders sensitive line hypercholinergic and flinders resistant line hypocholinergic rats. *Physiology and Behavior* 59(6):1155–1162.

250. Kalivas PW & Duffy P (1995) Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. *Brain Research* 675(1–2):325–328.
251. Rouge-Pont F, Piazza PV, Kharouby M, Le Moal M, & Simon H (1993) Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to amphetamine self-administration. A microdialysis study. *Brain Research* 602(1):169–174.
252. Takahashi H, Takada Y, Nagai N, Urano T, & Takada A (1998) Effects of nicotine and footshock stress on dopamine release in the striatum and nucleus accumbens. *Brain Research Bulletin* 45(2):157–162.
253. Rada P*, et al.* (2003) Glutamate release in the nucleus accumbens is involved in behavioral depression during the Porsolt swim test. *Neuroscience* 119(2):557–565.
254. Gambarana C*, et al.* (1999) A chronic stress that impairs reactivity in rats also decreases dopaminergic transmission in the nucleus accumbens: A microdialysis study. *Journal of Neurochemistry* 72(5):2039–2046.
255. Imperato A, Cabib S, & Puglisi-Allegra S (1993) Repeated stressful experiences differently affect the time-dependent responses of the mesolimbic dopamine system to the stressor. *Brain Research* 601(1–2):333–336.
256. Kalivas PW & Duffy P (1989) Similar effect of daily cocaine and stress on mesocorticolimbic dopamine neurotransmission in the rat. *Biological Psychiatry* 25(7):913–928.
257. Papp M, Klimek V, & Willner P (1994) Parallel changes in dopamine D2 receptor binding in limbic forebrain associated with chronic mild stress-induced anhedonia and its reversal by imipramine. *Psychopharmacology* 115(4):441–446.
258. Guiard BP, El Mansari M, & Blier P (2009) Prospect of a dopamine contribution in the next generation of antidepressant drugs: The triple reuptake inhibitors. *Current Drug Targets* 10(11):1069–1084.
259. Nestler EJ & Carlezon WA (2006) The mesolimbic dopamine reward circuit in depression. *Biological Psychiatry* 59(12):1151–1159.
260. Hughes RN (2007) Neotic preferences in laboratory rodents: Issues, assessment and substrates. *Neuroscience and Biobehavioral Reviews* 31(3):441–464.
261. Pavlov IP (1927) *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex* (Oxford University Press, Oxford).
262. Sokolov EN (1963) Higher nervous functions: The orienting reflex. *Annual Review of Physiology* 25(1):545–580.
263. Thompson RF & Spencer WA (1966) Habituation: A model phenomenon for the study of neuronal substrates of behavior. *Psychological Review* 73(1):16–43.
264. Havelka J (1956) Problem-seeking behaviour in rats. *Canadian Journal of Psychology* 10(2):91–97.
265. Singh D (1970) Preference for bar pressing to obtain reward over freeloading in rats and children. *Journal of Comparative & Physiological Psychology* 73(2):320–327.
266. Zimmermann A, Stauffacher M, Langhans W, & Würbel H (2001) Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. *Behavioural Brain Research* 121(1–2):11–20.
267. Switzky HN, Haywood HC, & Isett R (1974) Exploration, curiosity, and play in young children: Effects of stimulus complexity. *Developmental Psychology* 10(3):321–329.
268. Berlyne D (1955) The arousal and satiation of perceptual curiosity in the rat. *Journal of Comparative & Physiological Psychology* 48(4):238–246.

269. Aitken PP (1974) Aversive stimulation and rats' preference for areas differing in novelty value and brightness. *Animal Behaviour* 22(3):731–734.
270. Sheldon AB (1969) Preference for familiar versus novel stimuli as a function of the familiarity of the environment. *Journal of Comparative & Physiological Psychology* 67(4):516–521.
271. Ennaceur A (2010) One-trial object recognition in rats and mice: Methodological and theoretical issues. *Behavioural Brain Research* 215(2):244–254.
272. Ennaceur A, Michalikova S, Bradford A, & Ahmed S (2005) Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. *Behavioural Brain Research* 159(2):247–266.
273. Harro J, Oreland L, Vasar E, & Bradwejn J (1995) Impaired exploratory behaviour after DSP-4 treatment in rats: Implications for the increased anxiety after noradrenergic denervation. *European Neuropsychopharmacology* 5(4):447–455.
274. Otter M*, et al.* (1997) Characterization of rat exploratory behavior using the exploration box test. *Methods and Findings in Experimental and Clinical Pharmacology* 19(10):683–691.
275. Eilam D & Golani I (1989) Home base behavior of rats (Rattus norvegicus) exploring a novel environment. *Behavioural Brain Research* 34(3):199–211.
276. Golani I, Benjamini Y, & Eilam D (1993) Stopping behavior: Constraints on exploration in rats (Rattus norvegicus). *Behavioural Brain Research* 53(1–2):21–33.
277. Fehrer E (1956) The effects of hunger and familiarity of locale on exploration. *Journal of Comparative & Physiological Psychology* 49(6):549–552.
278. Ennaceur A, Michalikova S, & Chazot PL (2006) Models of anxiety: Responses of rats to novelty in an open space and an enclosed space. *Behavioural Brain Research* 171(1):26–49.
279. Harro J (2002) Long-term partial 5-HT depletion: Interference of anxiety and impulsivity? *Psychopharmacology* 164(4):433–434.
280. Stansfield K & Kirstein C (2006) Effects of novelty on behavior in the adolescent and adult rat. *Developmental Psychobiology* 48(1):10–15.
281. Mällo T*, et al.* (2007) Rats with persistently low or high exploratory activity: Behaviour in tests of anxiety and depression, and extracellular levels of dopamine. *Behavioural Brain Research* 177(2):269–281.
282. Yerkes RM (1913) The heredity of savageness and wildness in rats. *Journal of Animal Behavior* 3(4):286–296.
283. Jones MB (2003) Two early studies on learning theory and genetics. *Behavior Genetics* 33(6):669–676.
284. Parsons P (1974) The behavioral phenotype in mice. *The American Naturalist* 108(961):377–385.
285. Attwell D & Laughlin SB (2001) An energy budget for signaling in the grey matter of the brain. *Journal of Cerebral Blood Flow and Metabolism* 21(10):1133–1145.
286. Sibson NR*, et al.* (1998) Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. *Proceedings of the National Academy of Sciences of the United States of America* 95(1):316–321.
287. Shulman RG, Rothman DL, Behar KL, & Hyder F (2004) Energetic basis of brain activity: Implications for neuroimaging. *Trends in Neurosciences* 27(8):489–495.
288. Jolivet R, Magistretti PJ, & Weber B (2009) Deciphering neuron-glia compartmentalization in cortical energy metabolism. *Frontiers in Neuroenergetics* 1.4.

289. Buzsáki G, Kaila K, & Raichle M (2007) Inhibition and brain work. *Neuron* 56(5):771–783.
290. Hendry SH, Schwark HD, Jones EG, & Yan J (1987) Numbers and proportions of GABA-immunoreactive neurons in different areas of monkey cerebral cortex. *Journal of Neuroscience* 7(5):1503–1519.
291. Rothman DL*, et al.* (2002) In vivo magnetic resonance spectroscopy studies of the glutamate and GABA neurotransmitter cycles and functional neuroenergetics. *Neuropsychopharmacology: The fifth generation of progress*, eds Davis KL, Charney D, Coyle JT, & Nemeroff C (Lippincott Williams & Wilkins, Philadelphia), pp 315–342.
292. Hyder F*, et al.* (2006) Neuronal-glial glucose oxidation and glutamatergic-GABAergic function. *Journal of Cerebral Blood Flow and Metabolism* 26(7):865–877.
293. Logothetis NK (2003) The underpinnings of the BOLD functional magnetic resonance imaging signal. *Journal of Neuroscience* 23(10):3963–3971.
294. Logothetis NK (2008) What we can do and what we cannot do with fMRI. *Nature* 453(7197):869–878.
295. Kamondi A, Acsády L, & Buzsáki G (1998) Dendritic spikes are enhanced by cooperative network activity in the intact hippocampus. *Journal of Neuroscience* 18(10):3919–3928.
296. Magistretti PJ, Morrison JH, & Shoemaker WJ (1981) Vasoactive intestinal polypeptide induces glycogenolysis in mouse cortical slices: A possible regulatory mechanism for the local control of energy metabolism. *Proceedings of the National Academy of Sciences of the United States of America* 78(10 I):6535–6539.
297. Harik SI, Busto R, & Martinez E (1982) Norepinephrine regulation of cerebral glycogen utilization during seizures and ischemia. *Journal of Neuroscience* 2(4):409–414.
298. Stone EA & Ariano MA (1989) Are glial cells targets of the central noradrenergic system? A review of the evidence. *Brain Research Reviews* 14(4):297–309.
299. Schousboe A, Sickmann HM, Walls AB, Bak LK, & Waagepetersen HS (2010) Functional importance of the astrocytic glycogen-shunt and glycolysis for maintenance of an intact intra/extracellular glutamate gradient. *Neurotoxicity Research* 18(1):94–99.
300. Raichle ME & Mintun MA (2006) Brain work and brain imaging. *Annual Review of Neuroscience* 29:449–476.
301. Mata M, Fink DJ, & Gainer H (1980) Activity-dependent energy metabolism in rat posterior pituitary primarily reflects sodium pump activity. *Journal of Neurochemistry* 34(1):213–215.
302. Raichle ME (2009) A paradigm shift in functional brain imaging. *Journal of Neuroscience* 29(41):12729–12734.
303. Mangold R*, et al.* (1955) The effects of sleep and lack of sleep on the cerebral circulation and metabolism of normal young men. *The Journal of Clinical Investigation* 34(7):1092–1100.
304. Nikonova EV*, et al.* (2005) Differences in activity of cytochrome c oxidase in brain between sleep and wakefulness. *Sleep* 28(1):21–27.
305. Raichle ME & Snyder AZ (2007) A default mode of brain function: A brief history of an evolving idea. *NeuroImage* 37(4):1083–1090.
306. Zhang D*, et al.* (2008) Intrinsic functional relations between human cerebral cortex and thalamus. *Journal of Neurophysiology* 100(4):1740–1748.

307. Farrant M & Nusser Z (2005) Variations on an inhibitory theme: Phasic and tonic activation of GABA A receptors. *Nature Reviews Neuroscience* 6(3):215–229.
308. Belelli D & Lambert JJ (2005) Neurosteroids: Endogenous regulators of the GABA(A) receptor. *Nature Reviews Neuroscience* 6(7):565–575.
309. Majewska MD (1992) Neurosteroids: Endogenous bimodal modulators of the GABA(A) receptor. Mechanism of action and physiological significance. *Progress in Neurobiology* 38(4):379–395.
310. Peyron R*, et al.* (1994) Effects of GABA(A) receptors activation on brain glucose metabolism in normal subjects and temporal lobe epilepsy (TLE) patients. A positron emission tomography (PET) study. Part I: Brain glucose metabolism is increased after GABA(A), receptors activation. *Epilepsy Research* 19(1):45–54.
311. Sokoloff L, Reivich M, & Kennedy C (1977) The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. *Journal of Neurochemistry* 28(5):897–916.
312. Skelin I, Sato H, & Diksic M (2008) Olfactory bulbectomy reduces cerebral glucose utilization: 2-[14C]deoxyglucose autoradiographic study. *Brain Research Bulletin* 76(5):485–492.
313. Skelin I, Sato H, Kovačević T, & Diksic M (2009) Chronic therapy with citalopram decreases regional cerebral glucose utilization in OBX, and not sham-operated, rats: An autoradiographic study. *Psychopharmacology* 207(2):315–323.
314. Caldecott-Hazard S, Mazziotta J, & Phelps M (1988) Cerebral correlates of depressed behavior in rats, visualized using 14C-2-deoxyglucose autoradiography. *Journal of Neuroscience* 8(6):1951–1961.
315. Gerber JC, Choki J, & Brunswick DJ (1983) The effect of antidepressant drugs on regional cerebral glucose utilization in the rat. *Brain Research* 269(2):319–325.
316. Freo U, Ori C, Dam M, Merico A, & Pizzolato G (2000) Effects of acute and chronic treatment with fluoxetine on regional glucose cerebral metabolism in rats: Implications for clinical therapies. *Brain Research* 854(1–2):35–41.
317. Freo U*, et al.* (1995) Cerebral metabolic responses to clomipramine are greatly reduced following pretreatment with the specific serotonin neurotoxin para-chloroamphetamine (PCA): A 2-deoxyglucose study in rats. *Neuropsycho-pharmacology* 13(3):215–222.
318. Liang HL, Ongwijitwat S, & Wong-Riley MTT (2006) Bigenomic functional regulation of all 13 cytochrome c oxidase subunit transcripts in rat neurons in vitro and in vivo. *Neuroscience* 140(1):177–190.
319. Hood DA, Zak R, & Pette D (1989) Chronic stimulation of rat skeletal muscle induces coordinate increases in mitochondrial and nuclear mRNAs of cytochrome-c-oxidase subunits. *European Journal of Biochemistry* 179(2):275–280.
320. Dhar SS, Ongwijitwat S, & Wong-Riley MTT (2008) Nuclear respiratory factor 1 regulates all ten nuclear-encoded subunits of cytochrome c oxidase in neurons. *Journal of Biological Chemistry* 283(6):3120–3129.
321. Dhar SS, Liang HL, & Wong-Riley MTT (2009) Transcriptional coupling of synaptic transmission and energy metabolism: Role of nuclear respiratory factor 1 in co-regulating neuronal nitric oxide synthase and cytochrome c oxidase genes in neurons. *Biochimica et Biophysica Acta – Molecular Cell Research* 1793(10): 1604–1613.
322. Dhar SS, Liang HL, & Wong-Riley MTT (2009) Nuclear respiratory factor 1 co-regulates AMPA glutamate receptor subunit 2 and cytochrome c oxidase: Tight

coupling of glutamatergic transmission and energy metabolism in neurons. *Journal of Neurochemistry* 108(6):1595–1606.
323. Dhar SS & Wong-Riley MTT (2009) Coupling of energy metabolism and synaptic transmission at the transcriptional level: Role of nuclear respiratory factor 1 in regulating both cytochrome c oxidase and NMDA glutamate receptor subunit genes. *Journal of Neuroscience* 29(2):483–492.
324. Gonzalez-Lima F & Garrosa M (1991) Quantitative histochemistry of cytochrome oxidase in rat brain. *Neuroscience Letters* 123(2):251–253.
325. Wong-Riley MTT (1989) Cytochrome oxidase: An endogenous metabolic marker for neuronal activity. *Trends in Neurosciences* 12(3):94–101.
326. Wong-Riley M (1979) Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. *Brain Research* 171(1):11–28.
327. Wong-Riley MTT, Merzenich MM, & Leake PA (1978) Changes in endogenous enzymatic reactivity to DAB induced by neuronal inactivity. *Brain Research* 141(1):185–192.
328. Wong Riley M & Riley DA (1983) The effect of impulse blockage on cytochrome oxidase activity in the cat visual system. *Brain Research* 261(2):185–193.
329. Wong-Riley MT (2010) Energy metabolism of the visual system. *Eye and Brain* 2:99 – 116.
330. Borowsky IW & Collins RC (1989) Metabolic anatomy of brain: A comparison of regional capillary density, glucose metabolism, and enzyme activities. *Journal of Comparative Neurology* 288(3):401–413.
331. Braun K, Scheich H, Schachner M, & Heizmann CW (1985) Distribution of parvalbumin, cytochrome oxidase activity and 14C-2-deoxyglucose uptake in the brain of the zebra finch II. Visual system. *Cell and Tissue Research* 240(1):117–127.
332. Nie F & Wong-Riley MTT (1995) Double labeling of GABA and cytochrome oxidase in the macaque visual cortex: Quantitative EM analysis. *Journal of Comparative Neurology* 356(1):115–131.
333. Gulyás AI, Buzsáki G, Freund TF, & Hirase H (2006) Populations of hippocampal inhibitory neurons express different levels of cytochrome c. *European Journal of Neuroscience* 23(10):2581–2594.
334. Harris LK*, et al.* (2001) Traumatic brain injury-induced changes in gene expression and functional activity of mitochondrial cytochrome C oxidase. *Journal of Neurotrauma* 18(10):993–1009.
335. Hovda DA, Yoshino A, Kawamata T, Katayama Y, & Becker DP (1991) Diffuse prolonged depression of cerebral oxidative metabolism following concussive brain injury in the rat: A cytochrome oxidase histochemistry study. *Brain Research* 567(1):1–10.
336. Ben-Shachar D (2002) Mitochondrial dysfunction in schizophrenia: A possible linkage to dopamine. *Journal of Neurochemistry* 83(6):1241–1251.
337. Cavelier L*, et al.* (1995) Decreased cytochrome-c oxidase activity and lack of age-related accumulation of mitochondrial DNA deletions in the brains of schizophrenics. *Genomics* 29(1):217–224.
338. Kish SJ (1997) Brain energy metabolizing enzymes in Alzheimer's disease: α-ketoglutarate dehydrogenase complex and cytochrome oxidase. *Annals of the New York Academy of Sciences* 826:218–228.

339. Mutisya EM, Bowling AC, & Beal MF (1994) Cortical cytochrome oxidase activity is reduced in Alzheimer's disease. *Journal of Neurochemistry* 63(6):2179–2184.
340. Shao L, *et al.* (2008) Mitochondrial involvement in psychiatric disorders. *Annals of Medicine* 40(4):281–295.
341. Gardner A, *et al.* (2003) Alterations of mitochondrial function and correlations with personality traits in selected major depressive disorder patients. *Journal of Affective Disorders* 76(1–3):55–68.
342. Morava E, *et al.* (2010) Depressive behaviour in children diagnosed with a mitochondrial disorder. *Mitochondrion* 10(5):528–533.
343. Ben-Shachar D & Karry R (2008) Neuroanatomical pattern of mithochondrial complex I pathology varies between schizoprenia, bipolar disorder and major depression. *PLoS ONE* 3.11.
344. Gardner A & Boles RG (2008) Symptoms of somatization as a rapid screening tool for mitochondrial dysfunction in depression. *BioPsychoSocial Medicine* 2.7.
345. Rezin GT, *et al.* (2008) Inhibition of mitochondrial respiratory chain in brain of rats subjected to an experimental model of depression. *Neurochemistry International* 53(6–8):395–400.
346. Rezin GT, *et al.* (2009) Acute administration of ketamine reverses the inhibition of mitochondrial respiratory chain induced by chronic mild stress. *Brain Research Bulletin* 79(6):418–421.
347. Tagliari B, *et al.* (2010) Chronic variable stress impairs energy metabolism in prefrontal cortex and hippocampus of rats: Prevention by chronic antioxidant treatment. *Metabolic Brain Disease* 25(2):169–176.
348. Katyare S & Rajan R (1995) Effect of long-term in vivo treatment with imipramine on the oxidative energy metabolism in rat brain mitochondria. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 112(3):353–357.
349. González-Pardo H, *et al.* (2008) Changes in brain oxidative metabolism induced by inhibitory avoidance learning and acute administration of amitriptyline. *Pharmacology, Biochemistry and Behavior* 89(3):456–462.
350. Nobrega JN, Raymond R, DiStefano L, & Burnham WM (1993) Long-term changes in regional brain cytochrome oxidase activity induced by electroconvulsive treatment in rats. *Brain Research* 605(1):1–8.
351. Prince JA, Yassin MS, & Oreland L (1998) A histochemical demonstration of altered cytochrome oxidase activity in the rat brain by neuroleptics. *European Neuropsychopharmacology* 8(1):1–6.
352. Shumake J, Poremba A, Edwards E, & Gonzalez-Lima F (2000) Congenital helpless rats as a genetic model for cortex metabolism in depression. *NeuroReport* 11(17):3793–3798.
353. Shumake J, Edwards E, & Gonzalez-Lima F (2003) Opposite metabolic changes in the habenula and ventral tegmental area of a genetic model of helpless behavior. *Brain Research* 963(1–2):274–281.
354. Shumake J, Edwards E, & Gonzalez-Lima F (2002) Dissociation of septo-hippocampal metabolism in the congenitally helpless rat. *Neuroscience* 114(2):373–377.
355. Shumake J, Edwards E, & Gonzalez-Lima F (2001) Hypermetabolism of paraventricular hypothalamus in the congenitally helpless rat. *Neuroscience Letters* 311(1):45–48.

356. Shumake J, Colorado RA, Barrett DW, & Gonzalez-Lima F (2010) Metabolic mapping of the effects of the antidepressant fluoxetine on the brains of congenitally helpless rats. *Brain Research* 1343(C):218–225.
357. Shumake J, Conejo-Jimenez N, Gonzalez-Pardo H, & Gonzalez-Lima F (2004) Brain differences in newborn rats predisposed to helpless and depressive behavior. *Brain Research* 1030(2):267–276.
358. Wang RY & Aghajanian GK (1977) Physiological evidence for habenula as major link between forebrain and midbrain raphe. *Science* 197(4298):89–91.
359. Christoph GR, Leonzio RJ, & Wilcox KS (1986) Stimulation of the lateral habenula inhibits dopamine-containing neurons in the substantia nigra and ventral tegmental area of the rabbit. *Journal of Neuroscience* 6(3):613–619.
360. Ji H & Shepard PD (2007) Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABA(A) receptor-mediated mechanism. *Journal of Neuroscience* 27(26):6923–6930.
361. Lisoprawski A, Herve D, & Blanc G (1980) Selective activation of the mesocortico-frontal dopaminergic neurons induced by lesion of the habenula in the rat. *Brain Research* 183(1):229–234.
362. Sasaki K*, et al.* (1988) Habenular lesion attenuates methamphetamine-induced inhibition of dopamine neuronal activity in the substantia nigra pars compacta of rats. *Neuroscience Letters* 86(1):67–71.
363. Nishikawa T, Fage D, & Scatton B (1986) Evidence for, and nature of, the tonic inhibitory influence of habenulointerpenduncular pathways upon cerebral dopaminergic transmission in the rat. *Brain Research* 373(1–2):324–336.
364. McCulloch J, Savaki HE, & Sokoloff L (1980) Influence of dopaminergic systems on the lateral habenular nucleus of the rat. *Brain Research* 194(1):117–124.
365. Brown LL & Wolfson LI (1983) A dopamine-sensitive striatal efferent system mapped with [14C]deoxyglucose in the rat. *Brain Research* 261(2):213–229.
366. Trugman JM, James CL, & Wooten GF (1991) D1/D2 dopamine receptor stimulation by L-DOPA. A [14C]-2-deoxyglucose autoradiographic study. *Brain* 114(3):1429–1440.
367. Sutherland RJ (1982) The dorsal diencephalic conduction system: A review of the anatomy and functions of the habenular complex. *Neuroscience and Biobehavioral Reviews* 6(1):1–13.
368. Gruber C*, et al.* (2007) Dopaminergic projections from the VTA substantially contribute to the mesohabenular pathway in the rat. *Neuroscience Letters* 427(3):165–170.
369. Shumake J, Ilango A, Scheich H, Wetzel W, & Ohl FW (2010) Differential neuromodulation of acquisition and retrieval of avoidance learning by the lateral habenula and ventral tegmental area. *Journal of Neuroscience* 30(17):5876–5883.
370. Hikosaka O (2010) The habenula: From stress evasion to value-based decision-making. *Nature Reviews Neuroscience* 11(7):503–513.
371. Matsumoto M & Hikosaka O (2007) Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature* 447(7148):1111–1115.
372. Matsumoto M & Hikosaka O (2009) Representation of negative motivational value in the primate lateral habenula. *Nature Neuroscience* 12(1):77–84.
373. Chastrette N, Pfaff DW, & Gibbs RB (1991) Effects of daytime and nighttime stress of Fos-like immunoreactivity in the paraventricular nucleus of the hypothalamus, the habenula, and the posterior paraventricular nucleus of the thalamus. *Brain Research* 563(1–2):339–344.

374. Wirtschafter D, Asin KE, & Pitzer MR (1994) Dopamine agonists and stress produce different patterns of Fos-like immunoreactivity in the lateral habenula. *Brain Research* 633(1–2):21–26.
375. Matsumoto M & Hikosaka O (2009) Two types of dopamine neuron distinctly convey positive and negative motivational signals. *Nature* 459(7248):837–841.
376. Winter C, Vollmayr B, Djodari-Irani A, Klein J, & Sartorius A (in press) Pharmacological inhibition of the lateral habenula improves depressive-like behavior in an animal model of treatment resistant depression. *Behavioural Brain Research*.
377. Besson A, Privat AM, Eschalier A, & Fialip J (1998) Reversal of learned helplessness by morphine in rats: Involvement of a dopamine mediation. *Pharmacology, Biochemistry and Behavior* 60(2):519–525.
378. Besson A, Privat AM, Eschalier A, & Fialip J (1999) Dopaminergic and opioidergic mediations of tricyclic antidepressants in the learned helplessness paradigm. *Pharmacology, Biochemistry and Behavior* 64(3):541–548.
379. Sartorius A & Henn FA (2007) Deep brain stimulation of the lateral habenula in treatment resistant major depression. *Medical Hypotheses* 69(6):1305–1308.
380. Sartorius A*, et al.* (2010) Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. *Biological Psychiatry* 67(2):e9-e11.
381. Yaski O & Eilam D (2008) How do global and local geometries shape exploratory behavior in rats? *Behavioural Brain Research* 187(2):334–342.
382. Renner MJ (1990) Neglected aspects of exploratory and investigatory behavior. *Psychobiology* 18(1):16–22.
383. Harro J (1993) Measurement of exploratory behavior in rodents. *Methods in neuroscience*, ed Conn PM (Academic Press, San Diego), Vol 14, pp 359–377.
384. Harlow HF (1950) Learning and satiation of response in intrinsically motivated complex puzzle performance by monkeys. *Journal of Comparative & Physiological Psychology* 43(4):289–294.
385. Harlow HF, Harlow MK, & Meyer DR (1950) Learning motivated by a manipulation drive. *Journal of Experimental Psychology* 40(2):228–234.
386. Russell JC, Towns DR, Anderson SH, & Clout MN (2005) Intercepting the first rat ashore. *Nature* 437(7062):1107.
387. Barnett SA, Dickson RG, Marples TG, & Radha E (1978) Sequences of feeding, sampling and exploration by wild and laboratory rats. *Behavioural Processes* 3(1):29–43.
388. Tchernichovsky O & Golani I (1995) A phase plane representation of rat exploratory behavior. *Journal of Neuroscience Methods* 62(1–2):21–27.
389. Whishaw IQ, Gharbawie OA, Clark BJ, & Lehmann H (2006) The exploratory behavior of rats in an open environment optimizes security. *Behavioural Brain Research* 171(2):230–239.
390. Welker WI (1957) 'Free' versus 'forced' exploration of a novel situation by rats. *Psychological Reports* 3:95–108.
391. McNaughton N & Corr PJ (2004) A two-dimensional neuropsychology of defense: Fear/anxiety and defensive distance. *Neuroscience and Biobehavioral Reviews* 28(3):285–305.
392. File SE (1985) What can be learned from the effects of benzodiazepines on exploratory behavior? *Neuroscience and Biobehavioral Reviews* 9(1):45–54.

393. Fink JS & Smith GP (1980) Mesolimbicocortical dopamine terminal fields are necessary for normal locomotor and investigatory exploration in rats. *Brain Research* 199(2):359–384.
394. Fink JS & Smith GP (1980) Mesolimbic and mesocortical dopaminergic neurons are necessary for normal exploratory behavior in rats. *Neuroscience Letters* 17(1–2):61–65.
395. Takahashi L, Kalin N, Vanden Burgt J, & Sherman J (1989) Corticotropin-releasing factor modulates defensive-withdrawal and exploratory behavior in rats. *Behavioral Neuroscience* 103(3):648–654.
396. Mällo T*, et al.* (2004) Effect of long-term blockade of CRF1 receptors on exploratory behaviour, monoamines and transcription factor AP-2. *Pharmacology, Biochemistry and Behavior* 77(4):855–865.
397. Whimbey AE & Denenberg VH (1967) Two independent behavioral dimensions in open-field performance. *Journal of Comparative & Physiological Psychology* 63(3):500–504.
398. Harro J, Tõnissaar M, & Eller M (2001) The effects of CRA 1000, a non-peptide antagonist of corticotropin-releasing factor receptor type 1, on adaptive behaviour in the rat. *Neuropeptides* 35(2):100–109.
399. Gosling SD & John OP (1999) Personality dimensions in nonhuman animals: A cross-species review. *Current Directions in Psychological Science* 8(3):69–75.
400. Harro J (2010) Inter-individual differences in neurobiology as vulnerability factors for affective disorders: Implications for psychopharmacology. *Pharmacology and Therapeutics* 125(3):402–422.
401. Pawlak CR, Ho Y-J, & Schwarting RKW (2008) Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. *Neuroscience and Biobehavioral Reviews* 32(8):1544–1568.
402. Piazza PV, Deminiere J-M, Le Moal M, & Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. *Science* 245(4925):1511–1513.
403. Piazza PV*, et al.* (1991) Corticosterone levels determine individual vulnerability to amphetamine self-administration. *Proceedings of the National Academy of Sciences of the United States of America* 88(6):2088–2092.
404. Klebaur JE, Bevins RA, Segar TM, & Bardo MT (2001) Individual differences in behavioral responses to novelty and amphetamine self-administration in male and female rats. *Behavioural Pharmacology* 12(4):267–275.
405. Cain ME, Smith CM, & Bardo MT (2004) The effect of novelty on amphetamine self-administration in rats classified as high and low responders. *Psychopharmacology* 176(2):129–138.
406. Cain ME, Denehy ED, & Bardo MT (2008) Individual differences in amphetamine self-administration: The role of the central nucleus of the amygdala. *Neuropsychopharmacology* 33(5):1149–1161.
407. Pelloux Y, Costentin J, & Duterte-Boucher D (2004) Differential effects of novelty exposure on place preference conditioning to amphetamine and its oral consumption. *Psychopharmacology* 171(3):277–285.
408. Robinet PM, Rowlett JK, & Bardo MT (1998) Individual differences in novelty-induced activity and the rewarding effects of novelty and amphetamine in rats. *Behavioural Processes* 44(1):1–9.
409. Thiel CM, Müller CP, Huston JP, & Schwarting RKW (1999) High versus low reactivity to a novel environment: Behavioural, pharmacological and neurochemical assessments. *Neuroscience* 93(1):243–251.

410. Antoniou K*, et al.* (2008) Individual responses to novelty are associated with differences in behavioral and neurochemical profiles. *Behavioural Brain Research* 187(2):462–472.
411. Antoniou K*, et al.* (2004) Individual responses to novelty predict qualitative differences in d-amphetamine-induced open field but not reward-related behaviors in rats. *Neuroscience* 123(3):613–623.
412. Pawlak CR & Schwarting RKW (2002) Object preference and nicotine consumption in rats with high vs. low rearing activity in a novel open field. *Pharmacology, Biochemistry and Behavior* 73(3):679–687.
413. Piazza PV*, et al.* (1991) Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. *Brain Research* 567(1):169–174.
414. Marinelli M & White FJ (2000) Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. *Journal of Neuroscience* 20(23):8876–8885.
415. Lucas L, Angulo J, Le Moal M, McEwen B, & Piazza P (1998) Neurochemical characterization of individual vulnerability to addictive drugs in rats. *European Journal of Neuroscience* 10(10):3153–3163.
416. Rougé-Pont F, Deroche V, Le Moal M, & Piazza PV (1998) Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. *European Journal of Neuroscience* 10(12):3903–3907.
417. Piazza PV*, et al.* (1993) Corticosterone in the range of stress-induced levels possesses reinforcing properties: Implications for sensation-seeking behaviors. *Proceedings of the National Academy of Sciences of the United States of America* 90(24):11738–11742.
418. Verheij MMM, De Mulder ELW, De Leonibus E, Van Loo KMJ, & Cools AR (2008) Rats that differentially respond to cocaine differ in their dopaminergic storage capacity of the nucleus accumbens. *Journal of Neurochemistry* 105(6):2122–2133.
419. Hooks MS*, et al.* (1994) Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. *Journal of Neuroscience* 14(10):6144–6152.
420. Alttoa A*, et al.* (2005) Effects of low dose N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine administration on exploratory and amphetamine-induced behavior and dopamine D2 receptor function in rats with high or low exploratory activity. *Neuroscience* 132(4):979–990.
421. Alttoa A, Seeman P, Kõiv K, Eller M, & Harro J (2009) Rats with persistently high exploratory activity have both higher extracellular dopamine levels and higher proportion of D2 High receptors in the striatum. *Synapse* 63(5):443–446.
422. White DA, Kalinichev M, & Holtzman SG (2007) Locomotor response to novelty as a predictor of reactivity to aversive stimuli in the rat. *Brain Research* 1149(1):141–148.
423. Dellu F*, et al.* (1996) Behavioral reactivity to novelty during youth as a predictive factor of stress-induced corticosterone secretion in the elderly – a life-span study in rats. *Psychoneuroendocrinology* 21(5):441–453.
424. Márquez C, Nadal R, & Armario A (2006) Influence of reactivity to novelty and anxiety on hypothalamic-pituitary-adrenal and prolactin responses to two different novel environments in adult male rats. *Behavioural Brain Research* 168(1):13–22.

425. Dellu F, Mayo W, Vallee M, Le Moal M, & Simon H (1994) Reactivity to novelty during youth as a predictive factor of cognitive impairment in the elderly: A longitudinal study in rats. *Brain Research* 653(1–2):51–56.
426. Lemaire V, Aurousseau C, Le Moal M, & Abrous DN (1999) Behavioural trait of reactivity to novelty is related to hippocampal neurogenesis. *European Journal of Neuroscience* 11(11):4006–4014.
427. Touyarot K, Venero C, & Sandi C (2004) Spatial learning impairment induced by chronic stress is related to individual differences in novelty reactivity: Search for neurobiological correlates. *Psychoneuroendocrinology* 29(2):290–305.
428. Paré WP (1994) Open field, learned helplessness, conditioned defensive burying, and forced-swim tests in WKY rats. *Physiology and Behavior* 55(3):433–439.
429. Padilla E, Barrett D, Shumake J, & Gonzalez-Lima F (2009) Strain, sex, and open-field behavior: Factors underlying the genetic susceptibility to helplessness. *Behavioural Brain Research* 201(2):257–264.
430. Vollmayr B*, et al.* (2004) Rats with congenital learned helplessness respond less to sucrose but show no deficits in activity or learning. *Behavioural Brain Research* 150(1–2):217–221.
431. Kaffenberger T, Brühl AB, Baumgartner T, Jäncke L, & Herwig U (2010) Negative bias of processing ambiguously cued emotional stimuli. *NeuroReport* 21(9):601–605.
432. Kuhbandner C*, et al.* (2009) Effects of mood on the speed of conscious perception: Behavioural and electrophysiological evidence. *Social, Cognitive and Affective Neuroscience* 4(3):286–293.
433. Seligman ME & Csikszentmihalyi M (2000) Positive psychology. An introduction. *The American Psychologist* 55(1):5–14.
434. Papousek M (1989) Determinants of responsiveness to infant vocal expression of emotional state. *Infant Behavior and Development* 12(4):507–524.
435. Hammerschmidt K & Jürgens U (2007) Acoustical correlates of affective prosody. *Journal of Voice* 21(5):531–540.
436. Pongrácz P, Molnár C, & Miklósi A (2006) Acoustic parameters of dog barks carry emotional information for humans. *Applied Animal Behaviour Science* 100(3–4):228–240.
437. Molnár C, Pongrácz P, Faragó T, Dóka A, & Miklósi A (2009) Dogs discriminate between barks: The effect of context and identity of the caller. *Behavioural Processes* 82(2):198–201.
438. Soltis J, Leong K, & Savage A (2005) African elephant vocal communication II: Rumble variation reflects the individual identity and emotional state of callers. *Animal Behaviour* 70(3):589–599.
439. Zimmermann E (2009) Vocal expression of emotion in a nocturnal prosimian primate group, mouse lemurs. *Handbook of mammalian vocalizations: An integrative neuroscience approach*, ed Brudzynski SM (Academic Press, Oxford), pp 215–225.
440. Brudzynski SM (2009) Communication of adult rats by ultrasonic vocalization: Biological, sociobiological, and neuroscience approaches. *ILAR Journal* 50(1):43–50.
441. Portfors CV (2007) Types and functions of ultrasonic vocalizations in laboratory rats and mice. *Journal of the American Association for Laboratory Animal Science* 46(1):28–34.
442. Burgdorf J*, et al.* (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.
443. Knutson B, Burgdorf J, & Panksepp J (2002) Ultrasonic vocalizations as indices of affective states in rats. *Psychological Bulletin* 128(6):961–977.
444. Burgdorf J & Panksepp J (2006) The neurobiology of positive emotions. *Neuroscience and Biobehavioral Reviews* 30(2):173–187.
445. Burgdorf J, Knutson B, Panksepp J, & Shippenberg TS (2001) Evaluation of rat ultrasonic vocalizations as predictors of the conditioned aversive effects of drugs. *Psychopharmacology* 155(1):35–42.
446. Wöhr M, Houx B, Schwarting RKW, & Spruijt B (2008) Effects of experience and context on 50-kHz vocalizations in rats. *Physiology and Behavior* 93(4–5):766–776.
447. Panksepp J (2000) The riddle of laughter: Neural and psychoevolutionary underpinnings of joy. *Current Directions in Psychological Science* 9(6):183–186.
448. Burgdorf J (2005) The neurobiology of 50-kHz vocalizations in rats. *Ph.D. dissertation* (Bowling Green State University, Ohio, United States).
449. De Waal FBM (2008) Putting the altruism back into altruism: The evolution of empathy. *Annual Review of Psychology* 59:279–300.
450. Wöhr M & Schwarting RKW (2009) Ultrasonic communication in rats: Effects of morphine and naloxone on vocal and behavioral responses to playback of 50-kHz vocalizations. *Pharmacology, Biochemistry and Behavior* 94(2):285–295.
451. Willey AR, Varlinskaya EI, & Spear LP (2009) Social interactions and 50 kHz ultrasonic vocalizations in adolescent and adult rats. *Behavioural Brain Research* 202(1):122–129.
452. Fendt M, Schwienbacher I, & Schnitzler H-U (2006) Carbachol injections into the nucleus accumbens induce 50 kHz calls in rats. *Neuroscience Letters* 401(1–2):10–15.
453. 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.
454. Thompson B, Leonard KC, & Brudzynski SM (2006) Amphetamine-induced 50 kHz calls from rat nucleus accumbens: A quantitative mapping study and acoustic analysis. *Behavioural Brain Research* 168(1):64–73.
455. Burgdorf J, Knutson B, & Panksepp J (2000) Anticipation of rewarding electrical brain stimulation evokes ultrasonic vocalization in rats. *Behavioral Neuroscience* 114(2):320–327.
456. Ahrens AM, Ma ST, Maier EY, Duvauchelle CL, & Schallert T (2009) Repeated intravenous amphetamine exposure: Rapid and persistent sensitization of 50-kHz ultrasonic trill calls in rats. *Behavioural Brain Research* 197(1):205–209.
457. Ciucci MR*, et al.* (2009) Reduction of dopamine synaptic activity: Degradation of 50-khz ultrasonic vocalization in rats. *Behavioral Neuroscience* 123(2):328–336.
458. Burgdorf J & Panksepp J (2001) Tickling induces reward in adolescent rats. *Physiology and Behavior* 72(1–2):167–173.
459. Di Tella R, MacCulloch RJ, & Oswald AJ (2003) The macroeconomics of happiness. *Review of Economics and Statistics* 85(4):809–827.
460. Oswald AJ (1997) Happiness and economic performance. *Economic Journal* 107(445):1815–1831.
461. Easterlin RA (2005) Diminishing marginal utility of income? Caveat emptor. *Social Indicators Research* 70(3):243–255.

462. Diener E, Ng W, Harter J, & Arora R (2010) Wealth and happiness across the world: Material prosperity predicts life evaluation, whereas psychosocial prosperity predicts positive feeling. *Journal of Personality and Social Psychology* 99(1):52–61.
463. Helliwell JF & Putnam RD (2004) The social context of well-being. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359(1449):1435–1446.
464. Inglehart R & Klingemann H (2000) Genes, culture, democracy, and happiness. *Culture and subjective well-being*, eds Diener E & Suh EM (The MIT Press, Cambridge), pp 165–183.
465. Eisner M (2003) Long-term historical trends in violent crime. *Crime and Justice* 30:83–142.
466. Raine A (2002) The biological basis of crime. *Crime: Public policies for crime control*, eds Wilson JQ & Petersilia J (ICS Press, San Francisco), pp 43–74.
467. Levitt SD (2004) Understanding why crime fell in the 1990s: Four factors that explain the decline and six that do not. *Journal of Economic Perspectives* 18(1):163–190.
468. Mechoulan S (2007) The external effects of black-male incarceration on black females. *SSRN eLibrary* http://papers.ssrn.com/sol3/papers.cfm?abstract_id=997479.
469. Pantano J (2007) Unwanted fertility, contraceptive technology and crime: Exploiting a natural experiment in access to the pill. *Paper presented at the annual meeting of the American Society of Criminology* http://www.allacademic.com/meta/p201798_index.html
470. Lykken D & Tellegen A (1996) Happiness is a stochastic phenomenon. *Psychological Science* 7(3):186–189.
471. Diener E & Seligman MEP (2002) Very happy people. *Psychological Science* 13(1):81–84.
472. Furnham A & Cheng H (1999) Personality as predictor of mental health and happiness in the East and West. *Personality and Individual Differences* 27(3):395–403.
473. Cheng H & Furnham A (2003) Personality, self-esteem, and demographic predictions of happiness and depression. *Personality and Individual Differences* 34(6):921–942.
474. Tkach C & Lyubomirsky S (2006) How do people pursue happiness?: Relating personality, happiness-increasing strategies, and well-being. *Journal of Happiness Studies* 7(2):183–225.
475. Larsen RJ & Ketelaar T (1989) Extraversion, neuroticism and susceptibility to positive and negative mood induction procedures. *Personality and Individual Differences* 10(12):1221–1228.
476. Rusting CL & Larsen RJ (1997) Extraversion, neuroticism, and susceptibility to positive and negative affect: A test of two theoretical models. *Personality and Individual Differences* 22(5):607–612.
477. Nettle D (2005) An evolutionary approach to the extraversion continuum. *Evolution and Human Behavior* 26(4):363–373.
478. Wöhr M*, et al.* (2009) New insights into the relationship of neurogenesis and affect: Tickling induces hippocampal cell proliferation in rats emitting appetitive 50-kHz ultrasonic vocalizations. *Neuroscience* 163(4):1024–1030.
479. Burgdorf J*, et al.* (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.

480. Burgdorf J, Panksepp J, Brudzynski SM, Kroes R, & Moskal JR (2005) Breeding for 50-kHz positive affective vocalization in rats. *Behavior Genetics* 35(1):67–72.
481. Wojcik SM*, et al.* (2004) An essential role for vesicular glutamate transporter 1 (VGLUT1) in postnatal development and control of quantal size. *Proceedings of the National Academy of Sciences of the United States of America* 101(18):7158–7163.
482. Panksepp J & Burgdorf J (2000) 50-kHz chirping (laughter?) in response to conditioned and unconditioned tickle-induced reward in rats: Effects of social housing and genetic variables. *Behavioural Brain Research* 115(1):25–38.
483. Harkin A, Houlihan DD, & Kelly JP (2002) Reduction in preference for saccharin by repeated unpredictable stress in mice and its prevention by imipramine. *Journal of Psychopharmacology* 16(2):115–123.
484. Tõnissaar M, Herm L, Rinken A, & Harro J (2006) 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(1–2):119–124.
485. File SE & Hyde JRG (1978) Can social interaction be used to measure anxiety? *British Journal of Pharmacology* 62(1):19–24.
486. Handley SL & Mithani S (1984) Effects of alpha-adrenoceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. *Naunyn-Schmiedeberg's Archives of Pharmacology* 327(1):1–5.
487. Matto V, Harro J, & Allikmets L (1997) The effects of cholecystokinin A and B receptor antagonists on exploratory behaviour in the elevated zero-maze in rat. *Neuropharmacology* 36(3):389–396.
488. Porsolt RD, Anton G, Blavet N, & Jalfre M (1978) Behavioural despair in rats: A new model sensitive to antidepressant treatments. *European Journal of Pharmacology* 47(4):379–391.
489. Häidkind R*, et al.* (2004) Increased behavioural activity of rats in forced swimming test after partial denervation of serotonergic system by parachloroamphetamine treatment. *Neurochemistry International* 45(5):721–732.
490. Armario A, Gavalda A, & Marti O (1988) Forced swimming test in rats: Effects of desipramine administration and the period of exposure to the test on struggling behavior, swimming, immobility and defecation rate. *European Journal of Pharmacology* 158(3):207–212.
491. Wallace KJ & Rosen JB (2001) Neurotoxic lesions of the lateral nucleus of the amygdala decrease conditioned fear but not unconditioned fear of a predator odor: Comparison with electrolytic lesions. *Journal of Neuroscience* 21(10):3619–3627.
492. Deacon RMJ (2006) Digging and marble burying in mice: Simple methods for in vivo identification of biological impacts. *Nature Protocols* 1(1):122–124.
493. Elizalde N*, et al.* (2008) Long-lasting behavioral effects and recognition memory deficit induced by chronic mild stress in mice: Effect of antidepressant treatment. *Psychopharmacology* 199(1):1–14.
494. Alttoa A, Eller M, Herm L, Rinken A, & Harro J (2007) Amphetamine-induced locomotion, behavioral sensitization to amphetamine, and striatal D2 receptor function in rats with high or low spontaneous exploratory activity: Differences in the role of locus coeruleus. *Brain Research* 1131(1):138–148.
495. Lepiku M, Rinken A, Järv J, & Fuxe K (1996) Kinetic evidence for isomerization of the dopamine receptor-raclopride complex. *Neurochemistry International* 28(5–6):591–595.

496. Vonk A, Reinart R, & Rinken A (2008) Modulation of adenylyl cyclase activity in rat striatal homogenate by dopaminergic receptors. *Journal of Pharmacological Sciences* 108(1):63–70.
497. Vonk A, Uustare A, & Rinken A (2004) Modulation of activity of adenylate cyclase in rat striatal membranes by adenosine A2A receptors. *Proceedings of the Estonian Academy of Sciences. Chemistry* 53:153–164.
498. Rinken A, Finnman U-B, & Fuxe K (1999) Pharmacological characterization of dopamine-stimulated [35S]-Guanosine 5'-(γ-thiotriphosphate) ([35S]GTPγS) binding in rat striatal membranes. *Biochemical Pharmacology* 57(2):155–162.
499. Kondziella D*, et al.* (2006) Glial-neuronal interactions are impaired in the schizophrenia model of repeated MK801 exposure. *Neuropsychopharmacology* 31(9):1880–1887.
500. Gonzalez-Lima F & Cada A (1998) Quantitative histochemistry of cytochrome oxidase activity. *Cytochrome oxidase in neuronal metabolism and Alzheimer's disease*, ed Gonzalez-Lima F (Plenum, New York), pp 54–90.
501. Paxinos G & Franklin K (2001) *The mouse brain* (Academic Press, New York).
502. Paxinos G & Watson C (1986) *The rat brain in stereotaxic coordinates* (Academic Press, San Diego).
503. Morton V & Torgerson DJ (2005) Regression to the mean: Treatment effect without the intervention. *Journal of Evaluation in Clinical Practice* 11(1):59–65.
504. Wall PM & Messier C (2000) Ethological confirmatory factor analysis of anxiety-like behaviour in the murine elevated plus-maze. *Behavioural Brain Research* 114(1–2):199–212.
505. Walf AA & Frye CA (2007) The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols* 2(2):322–328.
506. Treit D, Menard J, & Royan C (1993) Anxiogenic stimuli in the elevated plus-maze. *Pharmacology, Biochemistry and Behavior* 44(2):463–469.
507. Henniger MSH*, et al.* (2000) Unconditioned anxiety and social behaviour in two rat lines selectively bred for high and low anxiety-related behaviour. *Behavioural Brain Research* 111(1–2):153–163.
508. Liebsch G, Montkowski A, Holsboer F, & Landgraf R (1998) Behavioural profiles of two Wistar rat lines selectively bred for high or low anxiety-related behaviour. *Behavioural Brain Research* 94(2):301–310.
509. Niesink RJM & Van Ree JM (1982) Short-term isolation increases social interactions of male rats: A parametric analysis. *Physiology and Behavior* 29(5):819–825.
510. Chaouloff F, Castanon N, & Morméde P (1994) Paradoxical differences in animal models of anxiety among the Roman rat lines. *Neuroscience Letters* 182(2):217–221.
511. Steimer T & Driscoll P (2003) Divergent stress responses and coping styles in psychogenetically selected Roman high-(RHA) and low-(RLA) avoidance rats: Behavioural, neuroendocrine and developmental aspects. *Stress* 6(2):87–100.
512. Ho Y-J, Eichendorff J, & Schwarting RKW (2002) Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behavioural Brain Research* 136(1):1–12.
513. Ramos A, Correia EC, Izídio GS, & Brüske GR (2003) Genetic selection of two new rat lines displaying different levels of anxiety-related behaviors. *Behavior Genetics* 33(6):657–668.
514. Hinojosa FR*, et al.* (2006) Evaluation of two genetic animal models in behavioral tests of anxiety and depression. *Behavioural Brain Research* 168(1):127–136.

515. Brandão ML, Troncoso AC, De Souza Silva MA, & Huston JP (2003) The relevance of neuronal substrates of defense in the midbrain tectum to anxiety and stress: Empirical and conceptual considerations. *European Journal of Pharmacology* 463(1–3):225–233.
516. Brandão ML, Melo LL, & Cardoso SH (1993) Mechanisms of defense in the inferior colliculus. *Behavioural Brain Research* 58(1–2):49–55.
517. Peruzzi D & Dut A (2004) GABA, serotonin and serotonin receptors in the rat inferior colliculus. *Brain Research* 998(2):247–250.
518. Goldsmith JD, Kujawa SG, McLaren JD, & Bledsoe Jr. SC (1995) In vivo release of neuroactive amino acids from the inferior colliculus of the guinea pig using brain microdialysis. *Hearing Research* 83(1–2):80–88.
519. Oliver DL, Winer JA, Beckius GE, & Saint Marie RL (1994) Morphology of GABAergic neurons in the inferior colliculus of the cat. *Journal of Comparative Neurology* 340(1):27–42.
520. Brandão ML, Coimbra NC, & Osaki MY (2001) Changes in the auditory-evoked potentials induced by fear-evoking stimulations. *Physiology and Behavior* 72(3):365–372.
521. Cardoso SH, Coimbra NC, & Brandao ML (1994) Defensive reactions evoked by activation of NMDA receptors in distinct sites of the inferior colliculus. *Behavioural Brain Research* 63(1):17–24.
522. Freitas RL*, et al.* (2005) Intrinsic neural circuits between dorsal midbrain neurons that control fear-induced responses and seizure activity and nuclei of the pain inhibitory system elaborating postictal antinociceptive processes: A functional neuroanatomical and neuropharmacological study. *Experimental Neurology* 191(2):225–242.
523. Osaki MY*, et al.* (2003) Neuroanatomical and neuropharmacological study of opioid pathways in the mesencephalic tectum: Effect of μ1- and κ-opioid receptor blockade on escape behavior induced by electrical stimulation of the inferior colliculus. *Brain Research* 992(2):179–192.
524. Melo LL & Brandao ML (1995) Role of 5-HT(1A) and 5-HT2 receptors in the aversion induced by electrical stimulation of inferior colliculus. *Pharmacology, Biochemistry and Behavior* 51(2–3):317–321.
525. Melo LL, Cardoso SH, & Brandao ML (1992) Antiaversive action of benzodiazepines on escape behavior induced by electrical stimulation of the inferior colliculus. *Physiology and Behavior* 51(3):557–562.
526. Hurley LM, Thompson AM, & Pollak GD (2002) Serotonin in the inferior colliculus. *Hearing Research* 168(1–2):1–11.
527. Ennis M, Behbehani M, Shipley MT, Van Bockstaele EJ, & Aston-Jones G (1991) Projections from the periaqueductal gray to the rostromedial pericoerulear region and nucleus locus coeruleus: Anatomic and physiologic studies. *Journal of Comparative Neurology* 306(3):480–494.
528. Fritschy J-M & Grzanna R (1990) Distribution of locus coeruleus axons within the rat brainstem demonstrated by Phaseolus vulgaris leucoagglutinin anterograde tracing in combination with dopamine-β-hydroxylase immunofluorescence. *Journal of Comparative Neurology* 293(4):616–631.
529. Kalen P, Karlson M, & Wiklung L (1985) Possible excitatory amino acid afferents to nucleus raphe dorsalis of the rat investigated with retrograde wheat germ agglutinin and D-[3H]aspartate tracing. *Brain Research* 360(1–2):285–297.

530. Klepper A & Herbert H (1991) Distribution and origin of noradrenergic and serotoninergic fibers in the cochlear nucleus and inferior colliculus of the rat. *Brain Research* 557(1–2):190–201.
531. Graeff FG, Guimarães FS, De Andrade TGCS, & Deakin JFW (1996) Role of 5-HT in stress, anxiety, and depression. *Pharmacology, Biochemistry and Behavior* 54(1):129–141.
532. Maier SF & Watkins LR (2005) Stressor controllability and learned helplessness: The roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. *Neuroscience and Biobehavioral Reviews* 29(4–5):829–841.
533. Blanchard DC, Griebel G, Rodgers RJ, & Blanchard RJ (1998) Benzodiazepine and serotonergic modulation of antipredator and conspecific defense. *Neuroscience and Biobehavioral Reviews* 22(5):597–612.
534. Troncoso AC, Osaki MY, Mason S, Borelli KG, & Brandão ML (2003) Apomorphine enhances conditioned responses induced by aversive stimulation of the inferior colliculus. *Neuropsychopharmacology* 28(2):284–291.
535. Parron C & Save E (2004) Comparison of the effects of entorhinal and retrosplenial cortical lesions on habituation, reaction to spatial and non-spatial changes during object exploration in the rat. *Neurobiology of Learning and Memory* 82(1):1–11.
536. Gallo A, Gonzalez-Lima F, & Sadile AG (2002) Impaired metabolic capacity in the perirhinal and posterior parietal cortex lead to dissociation between attentional, motivational and spatial components of exploration in the Naples High-Excitability rat. *Behavioural Brain Research* 130(1–2):133–140.
537. Izquierdo I & Medina JH (1997) Memory formation: The sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiology of Learning and Memory* 68(3):285–316.
538. Myhrer T, Iversen EG, & Fonnum F (1989) Impaired reference memory and reduced glutamergic activity in rats with temporo-entorhinal connections disrupted. *Experimental Brain Research* 77(3):499–506.
539. Ge J, Barnes NM, Costall B, & Naylor RJ (1997) Effect of aversive stimulation on 5-hydroxytryptamine and dopamine metabolism in the rat brain. *Pharmacology, Biochemistry and Behavior* 58(3):775–783.
540. Hammer Jr. RP, Hori KM, Blanchard RJ, & Blanchard DC (1992) Domestication alters 5-HT(1A) receptor binding in rat brain. *Pharmacology, Biochemistry and Behavior* 42(1):25–28.
541. Gyulai FE (2004) Anesthetics and cerebral metabolism. *Current Opinion in Anaesthesiology* 17(5):397–402.
542. Hyder F (2004) Neuroimaging with calibrated fMRI. *Stroke* 35(11S):2635–2641.
543. Hoge RD & Pike GB (2001) Oxidative metabolism and the detection of neuronal activation via imaging. *Journal of Chemical Neuroanatomy* 22(1–2):43–52.
544. Song AW & Truong T-K (2010) Apparent diffusion coefficient dependent fMRI: Spatiotemporal characteristics and implications on calibrated fMRI. *International Journal of Imaging Systems and Technology* 20(1):42–50.
545. Maandag NJG*, et al.* (2007) Energetics of neuronal signaling and fMRI activity. *Proceedings of the National Academy of Sciences of the United States of America* 104(51):20546–20551.
546. Lin A-L*, et al.* (2009) Time-dependent correlation of cerebral blood flow with oxygen metabolism in activated human visual cortex as measured by fMRI. *NeuroImage* 44(1):16–22.

547. Kanarik M*, et al.* (in press) 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*.
548. Shumake J & Gonzalez-Lima F (2003) Brain systems underlying susceptibility to helplessness and depression. *Behavioral and Cognitive Neuroscience Reviews* 2(3):198–221.
549. Grønli J*, et al.* (2004) Chronic mild stress affects sucrose intake and sleep in rats. *Behavioural Brain Research* 150(1–2):139–147.
550. Ossowska G, Danilczuk Z, Klenk-Majewska B, Czajkowski L, & Zebrowska-Łupina I (2004) Antidepressants in chronic unpredictable mild stress (CUMS)-induced deficit of fighting behavior. *Polish Journal of Pharmacology* 56(3):305–311.
551. Häidkind R*, et al.* (2003) Effects of partial locus coeruleus denervation and chronic mild stress on behaviour and monoamine neurochemistry in the rat. *European Neuropsychopharmacology* 13(1):19–28.
552. Garcia-Marquez C & Armario A (1987) Chronic stress depresses exploratory activity and behavioral performance in the forced swimming test without altering ACTH response to a novel acute stressor. *Physiology and Behavior* 40(1):33–38.
553. Sikiric P*, et al.* (2000) The antidepressant effect of an antiulcer pentadecapeptide BPC 157 in Porsolt's test and chronic unpredictable stress in rats. A comparison with antidepressants. *Journal of Physiology (Paris)* 94(2):99–104.
554. Molina VA, Heyser CJ, & Spear LP (1994) Chronic variable stress or chronic morphine facilitates immobility in a forced swim test: Reversal by naloxone. *Psychopharmacology* 114(3):433–440.
555. Scheggi S*, et al.* (2002) Selective modifications in the nucleus accumbens of dopamine synaptic transmission in rats exposed to chronic stress. *Journal of Neurochemistry* 83(4):895–903.
556. Ossowska G*, et al.* (2001) Brain monoamine receptors in a chronic unpredictable stress model in rats. *Journal of Neural Transmission* 108(3):311–319.
557. De Montis MG*, et al.* (1993) Reduced [3H]SCH 23390 binding and DA-sensitive adenylyl cyclase in the limbic system of ethanol-preferring rats. *Alcohol and Alcoholism* 28(4):397–400.
558. Klimek V & Nielsen M (1987) Chronic treatment with antidepressants decreases the number of [3H]SCH 23390 binding sites in the rat striatum and limbic system. *European Journal of Pharmacology* 139(2):163–169.
559. Ossowska G, Nowak G, Klenk-Majewska B, Danilczuk Z, & Zebrowska-Łupina I (2002) Effect of imipramine on brain D-1 and 5-HT-2A receptors in a chronic unpredictable stress model in rats. *Polish Journal of Pharmacology* 54(2):89–93.
560. Rodríguez-Landa JF, Contreras CM, Gutiérrez-García AG, & Bernal-Morales B (2003) Chronic, but not acute, clomipramine or fluoxetine treatment reduces the spontaneous firing rate in the mesoaccumbens neurons of the rat. *Neuropsychobiology* 48(3):116–123.
561. Huzarska M, Zieliński M, & Herman ZS (2006) Repeated treatment with antidepressants enhances dopamine D1 receptor gene expression in the rat brain. *European Journal of Pharmacology* 532(3):208–213.
562. Dziedzicka-Wasylewska M, Willner P, & Papp M (1997) Changes in dopamine receptor mRNA expression following chronic mild stress and chronic antidepressant treatment. *Behavioural Pharmacology* 8(6–7):607–618.

563. Giardino L, Zanni M, Pozza M, Bettelli C, & Covelli V (1998) Dopamine receptors in the striatum of rats exposed to repeated restraint stress and alprazolam treatment. *European Journal of Pharmacology* 344(2–3):143–147.
564. Bergström A, Jayatissa MN, Mørk A, & Wiborg O (2008) Stress sensitivity and resilience in the chronic mild stress rat model of depression: An in situ hybridization study. *Brain Research* 1196:41–52.
565. Bardo MT & Hammer Jr. RP (1991) Autoradiographic localization of dopamine D1 and D2 receptors in rat nucleus accumbens: Resistance to differential rearing conditions. *Neuroscience* 45(2):281–290.
566. Cabib S*, et al.* (1998) Stress promotes major changes in dopamine receptor densities within the mesoaccumbens and nigrostriatal systems. *Neuroscience* 84(1):193–200.
567. Lucas LR, Wang C-J, McCall TJ, & McEwen BS (2007) Effects of immobilization stress on neurochemical markers in the motivational system of the male rat. *Brain Research* 1155(1):108–115.
568. Dietz DM, Dietz KC, Moore S, Ouimet CC, & Kabbaj M (2008) Repeated social defeat stress-induced sensitization to the locomotor activating effects of d-amphetamine: Role of individual differences. *Psychopharmacology* 198(1):51–62.
569. Depue RA & Collins PF (1999) Neurobiology of the structure of personality: Dopamine, facilitation of incentive motivation, and extraversion. *Behavioral and Brain Sciences* 22(3):491–517.
570. Marinelli M & Piazza PV (2002) Interaction between glucocorticoid hormones, stress and psychostimulant drugs. *European Journal of Neuroscience* 16(3):387–394.
571. Alcaro A, Huber R, & Panksepp J (2007) Behavioral functions of the mesolimbic dopaminergic system: An affective neuroethological perspective. *Brain Research Reviews* 56(2):283–321.
572. Kram ML, Kramer GL, Ronan PJ, Steciuk M, & Petty F (2002) Dopamine receptors and learned helplessness in the rat: An autoradiographic study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 26(4):639–645.
573. Melik E, Babar-Melik E, Özgünen T, & Binokay S (2000) Median raphe nucleus mediates forming long-term but not short-term contextual fear conditioning in rats. *Behavioural Brain Research* 112(1–2):145–150.
574. Silva RCB, Cruz APM, Avanzi V, Landeira-Fernandez J, & Brandão ML (2002) Distinct contributions of median raphe nucleus to contextual fear conditioning and fear-potentiated startle. *Neural Plasticity* 9(4):233–247.
575. Murakami S, Imbe H, Morikawa Y, Kubo C, & Senba E (2005) Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neuroscience Research* 53(2):129–139.
576. Chamas F, Serova L, & Sabban EL (1999) Tryptophan hydroxylase mRNA levels are elevated by repeated immobilization stress in rat raphe nuclei but not in pineal gland. *Neuroscience Letters* 267(3):157–160.
577. Martinez M, Calvo-Torrent A, & Herbert J (2002) Mapping brain response to social stress in rodents with c-fos expression: A review. *Stress* 5(1):3–13.
578. Chaouloff F (1993) Physiopharmacological interactions between stress hormones and central serotonergic systems. *Brain Research Reviews* 18(1):1–32.
579. Clark JA*, et al.* (2008) Glucocorticoid modulation of tryptophan hydroxylase-2 protein in raphe nuclei and 5-hydroxytryptophan concentrations in frontal cortex of C57/Bl6 mice. *Molecular Psychiatry* 13(5):498–506.

580. Stezhka VV & Lovick TA (1997) Projections from dorsal raphe nucleus to the periaqueductal grey matter: Studies in slices of rat midbrain maintained in vitro. *Neuroscience Letters* 230(1):57–60.
581. Vertes RP, Fortin WJ, & Crane AM (1999) Projections of the median raphe nucleus in the rat. *Journal of Comparative Neurology* 407(4):555–582.
582. Vertes RP & Linley SB (2007) Comparison of projections of the dorsal and median raphe nuclei, with some functional considerations. *International Congress Series* 1304:98–120.
583. Bandler R & Keay KA (1996) Columnar organization in the midbrain periaqueductal gray and the integration of emotional expression. *Progress in Brain Research* 107:285–300.
584. Keay KA & Bandler R (2001) Parallel circuits mediating distinct emotional coping reactions to different types of stress. *Neuroscience and Biobehavioral Reviews* 25(7–8):669–678.
585. Byatt G & Dalrymple-Alford JC (1996) Both anteromedial and anteroventral thalamic lesions impair radial-maze learning in rats. *Behavioral Neuroscience* 110(6):1335–1348.
586. Aggleton JP*, et al.* (2010) Hippocampal-anterior thalamic pathways for memory: Uncovering a network of direct and indirect actions. *European Journal of Neuroscience* 31(12):2292–2307.
587. Sharkey J, Appel NM, & De Souza EB (1989) Alterations in local cerebral glucose utilization following central administration of corticotropin-releasing factor in rats. *Synapse* 4(1):80–87.
588. Campeau S & Watson SJ (1997) Neuroendocrine and behavioral responses and brain pattern of c-fos induction associated with audiogenic stress. *Journal of Neuroendocrinology* 9(8):577–588.
589. Cullinan WE (1995) Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* 64(2):477–505.
590. Donner N, Bredewold R, Maloumby R, & Neumann ID (2007) Chronic intracerebral prolactin attenuates neuronal stress circuitries in virgin rats. *European Journal of Neuroscience* 25(6):1804–1814.
591. Matsuda S*, et al.* (1996) Persistent c-fos expression in the brains of mice with chronic social stress. *Neuroscience Research* 26(2):157–170.
592. Baldo BA, Daniel RA, Berridge CW, & Kelley AE (2003) Overlapping distributions of orexin/hypocretin- and dopamine-β-hydroxylase immunoreactive fibers in rat brain regions mediating arousal, motivation, and stress. *Journal of Comparative Neurology* 464(2):220–237.
593. Brun P, Suaud-Chagny MF, Gonon F, & Buda M (1993) In vivo noradrenaline release evoked in the anteroventral thalamic nucleus by locus coeruleus activation: An electrochemical study. *Neuroscience* 52(4):961–972.
594. Herman JP (1998) Region-specific regulation of glutamic acid decarboxylase (GAD) mRNA expression in central stress circuits. *Journal of Neuroscience* 18(15):5938–5947.
595. Haller J & Halász J (1999) Mild social stress abolishes the effects of isolation on anxiety and chlordiazepoxide reactivity. *Psychopharmacology* 144(4):311–315.
596. Westenbroek C, Den Boer JA, Veenhuis M, & Ter Horst GJ (2004) Chronic stress and social housing differentially affect neurogenesis in male and female rats. *Brain Research Bulletin* 64(4):303–308.

597. Romeas T, Morissette M-C, Mnie-Filali O, Piñeyro G, & Boye SM (2009) Simultaneous anhedonia and exaggerated locomotor activation in an animal model of depression. *Psychopharmacology* 205(2):293–303.
598. Li Y, Zheng X, Liang J, & Peng Y (2010) Coexistence of anhedonia and anxiety-independent increased novelty-seeking behavior in the chronic mild stress model of depression. *Behavioural Processes* 83(3):331–339.
599. McGlinchey JB, Zimmerman M, Young D, & Chelminski I (2006) Diagnosing major depressive disorder VIII: Are some symptoms better than others? *Journal of Nervous and Mental Disease* 194(10):785–790.
600. Moutsimilli L*, et al.* (2005) Selective cortical VGLUT1 increase as a marker for antidepressant activity. *Neuropharmacology* 49(6):890–900.
601. Tordera RM, Pei Q, & Sharp T (2005) Evidence for increased expression of the vesicular glutamate transporter, VGLUT1, by a course of antidepressant treatment. *Journal of Neurochemistry* 94(4):875–883.
602. Elizalde N*, et al.* (2010) Sustained stress-induced changes in mice as a model for chronic depression. *Psychopharmacology* 210(3):393–406.
603. Balschun D*, et al.* (2010) Vesicular glutamate transporter VGLUT1 has a role in hippocampal long-term potentiation and spatial reversal learning. *Cerebral Cortex* 20(3):684–693.
604. Elizalde N*, et al.* (2010) Regulation of markers of synaptic function in mouse models of depression: Chronic mild stress and decreased expression of VGLUT1. *Journal of Neurochemistry* 114(5):1302–1314.
605. Bogen IL*, et al.* (2006) Absence of synapsin I and II is accompanied by decreases in vesicular transport of specific neurotransmitters. *Journal of Neurochemistry* 96(5):1458–1466.
606. Bhagwagar Z*, et al.* (2007) Reduction in occipital cortex γ-aminobutyric acid concentrations in medication-free recovered unipolar depressed and bipolar subjects. *Biological Psychiatry* 61(6):806–812.
607. Honig A, Bartlett JR, Bouras N, & Bridges PK (1988) Amino acid levels in depression: A preliminary investigation. *Journal of Psychiatric Research* 22(3):159–164.
608. Sanacora G*, et al.* (1999) Reduced cortical γ-aminobutyric acid levels in depressed patients determined by proton magnetic resonance spectroscopy. *Archives of General Psychiatry* 56(11):1043–1047.
609. Sanacora G*, et al.* (2004) Subtype-specific alterations of γ-aminobutyric acid and glutamate in patients with major depression. *Archives of General Psychiatry* 61(7):705–713.
610. Vieira DSS*, et al.* (2006) Cerebrospinal fluid GABA levels in chronic migraine with and without depression. *Brain Research* 1090(1):197–201.
611. Borsini F, Mancinelli A, D'Aranno V, Evangelista S, & Meli A (1988) On the role of endogenous GABA in the forced swimming test in rats. *Pharmacology, Biochemistry and Behavior* 29(2):275–279.
612. Mathews GC & Diamond JS (2003) Neuronal glutamate uptake contributes to GABA synthesis and inhibitory synaptic strength. *Journal of Neuroscience* 23(6):2040–2048.
613. Peng L*, et al.* (1993) Utilization of glutamine and of TCA cycle constituents as precursors for transmitter glutamate and GABA. *Developmental Neuroscience* 15(3–5):367–377.
614. Benarroch EE (2010) Glutamate transporters: Diversity, function, and involvement in neurologic disease. *Neurology* 74(3):259–264.

615. Schousboe A (2003) Role of astrocytes in the maintenance and modulation of glutamatergic and GABAergic neurotransmission. *Neurochemical Research* 28(2):347–352.
616. Sartorius A, Mahlstedt MM, Vollmayr B, Henn FA, & Ende G (2007) Elevated spectroscopic glutamate/γ-amino butyric acid in rats bred for learned helplessness. *NeuroReport* 18(14):1469–1473.
617. Zink M, Vollmayr B, Gebicke-Haerter PJ, & Henn FA (2010) Reduced expression of glutamate transporters vGluT1, EAAT2 and EAAT4 in learned helpless rats, an animal model of depression. *Neuropharmacology* 58(2):465–473.
618. Kram ML, Kramer GL, Steciuk M, Ronan PJ, & Petty F (2000) Effects of learned helplessness on brain GABA receptors. *Neuroscience Research* 38(2):193–198.
619. Zink M, Vollmayr B, Gebicke-Haerter PJ, & Henn FA (2009) Reduced expression of GABA transporter GAT3 in helpless rats, an animal model of depression. *Neurochemical Research* 34(9):1584–1593.
620. Fremeau Jr. RT*, et al.* (2001) The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron* 31(2):247–260.
621. Takamori S, Rhec JS, Rosenmund C, & Jahn R (2000) Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons. *Nature* 407(6801):189–194.
622. Fremeau Jr. RT*, et al.* (2004) Vesicular glutamate transporters 1 and 2 target to functionally distinct synaptic release sites. *Science* 304(5678):1815–1819.
623. Daniels RW*, et al.* (2004) Increased expression of the Drosophila vesicular glutamate transporter leads to excess glutamate release and a compensatory decrease in quantal content. *Journal of Neuroscience* 24(46):10466–10474.
624. Wilson NR*, et al.* (2005) Presynaptic regulation of quantal size by the vesicular glutamate transporter VGLUT1. *Journal of Neuroscience* 25(26):6221–6234.
625. Uezato A, Meador-Woodruff JH, & McCullumsmith RE (2009) Vesicular glutamate transporter mRNA expression in the medial temporal lobe in major depressive disorder, bipolar disorder, and schizophrenia. *Bipolar Disorders* 11(7):711–725.
626. Liang J*, et al.* (2008) Excitatory amino acid transporter expression by astrocytes is neuroprotective against microglial excitotoxicity. *Brain Research* 1210(C):11–19.
627. Duan S, Anderson CM, Stein BA, & Swanson RA (1999) Glutamate induces rapid upregulation of astrocyte glutamate transport and cell-surface expression of GLAST. *Journal of Neuroscience* 19(23):10193–10200.
628. Danbolt NC (2001) Glutamate uptake. *Progress in Neurobiology* 65(1):1–105.
629. Schousboe A, Sarup A, Bak LK, Waagepetersen HS, & Larsson OM (2004) Role of astrocytic transport processes in glutamatergic and GABAergic neurotransmission. *Neurochemistry International* 45(4):521–527.
630. Ishikawa T, Sahara Y, & Takahashi T (2002) A single packet of transmitter does not saturate postsynaptic glutamate receptors. *Neuron* 34(4):613–621.
631. Waagepetersen HS, Qu H, Sonnewald U, Shimamoto K, & Schousboe A (2005) Role of glutamine and neuronal glutamate uptake in glutamate homeostasis and synthesis during vesicular release in cultured glutamatergic neurons. *Neurochemistry International* 47(1–2):92–102.
632. Köster A*, et al.* (1999) Targeted disruption of the orphanin FQ/nociceptin gene increases stress susceptibility and impairs stress adaptation in mice. *Proceedings of the National Academy of Sciences of the United States of America* 96(18):10444–10449.

633. Nicolas LB, Kolb Y, & Prinssen EPM (2006) A combined marble burying-locomotor activity test in mice: A practical screening test with sensitivity to different classes of anxiolytics and antidepressants. *European Journal of Pharmacology* 547(1):106–115.
634. Hiller W, Zaudig M, & Bose VM (1989) The overlap between depression and anxiety on different levels of psychopathology. *Journal of Affective Disorders* 16(2–3):223–231.
635. Airaksinen E, Larsson M, Lundberg I, & Forsell Y (2004) Cognitive functions in depressive disorders: Evidence from a population-based study. *Psychological Medicine* 34(1):83–91.
636. Porter RJ, Gallagher P, Thompson JM, & Young AH (2003) Neurocognitive impairment in drug-free patients with major depressive disorder. *British Journal of Psychiatry* 182:214–220.
637. Hasler G*, et al.* (2009) Prefrontal cortical gamma-aminobutyric acid levels in panic disorder determined by proton magnetic resonance spectroscopy. *Biological Psychiatry* 65(3):273–275.
638. Ham B-J*, et al.* (2007) Decreased GABA levels in anterior cingulate and basal ganglia in medicated subjects with panic disorder: A proton magnetic resonance spectroscopy (1H-MRS) study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 31(2):403–411.
639. Goddard AW*, et al.* (2004) Impaired GABA neuronal response to acute benzodiazepine administration in panic disorder. *American Journal of Psychiatry* 161(12):2186–2193.
640. Whiteside SP, Port JD, Deacon BJ, & Abramowitz JS (2006) A magnetic resonance spectroscopy investigation of obsessive-compulsive disorder and anxiety. *Psychiatry Research – Neuroimaging* 146(2):137–147.
641. Phan KL*, et al.* (2005) Anterior cingulate neurochemistry in social anxiety disorder: 1H-MRS at 4 Tesla. *NeuroReport* 16(2):183–186.
642. Hashimoto K, Sawa A, & Iyo M (2007) Increased levels of glutamate in brains from patients with mood disorders. *Biological Psychiatry* 62(11):1310–1316.
643. Palucha A & Pilc A (2007) Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. *Pharmacology and Therapeutics* 115(1):116–147.
644. Moghaddam B & Jackson M (2004) Effect of stress on prefrontal cortex function. *Neurotoxicity Research* 6(1):73–78.
645. Fatemi SH, Stary JM, Earle JA, Araghi-Niknam M, & Eagan E (2005) GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65 and 67 kDa and Reelin proteins in cerebellum. *Schizophrenia Research* 72(2–3):109–122.
646. Guidotti A*, et al.* (2000) Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: A postmortem brain study. *Archives of General Psychiatry* 57(11):1061–1069.
647. Smoller JW*, et al.* (2001) Genetic association analysis of behavioral inhibition using candidate loci from mouse models. *American Journal of Medical Genetics – Neuropsychiatric Genetics* 105(3):226–235.
648. Stork O*, et al.* (2000) Postnatal development of a GABA deficit and disturbance of neural functions in mice lacking GAD65. *Brain Research* 865(1):45–58.
649. Kash SF, Tecott LH, Hodge C, & Baekkeskov S (1999) Increased anxiety and altered responses to anxiolytics in mice deficient in the 65-kDa isoform of

glutamic acid decarboxylase. *Proceedings of the National Academy of Sciences of the United States of America* 96(4):1698–1703.
650. Banasr M*, et al.* (2010) Glial pathology in an animal model of depression: Reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Molecular Psychiatry* 15(5):501–511.
651. Rauen T & Wießner M (2000) Fine tuning of glutamate uptake and degradation in glial cells: Common transcriptional regulation of GLAST1 and GS. *Neurochemistry International* 37(2–3):179–189.
652. Li C-X*, et al.* (2008) Cerebral metabolic changes in a depression-like rat model of chronic forced swimming studied by ex vivo high resolution 1H magnetic resonance spectroscopy. *Neurochemical Research* 33(11):2342–2349.
653. Dong J*, et al.* (2010) Effects of electroconvulsive therapy and propofol on spatial memory and glutamatergic system in hippocampus of depressed rats. *Journal of ECT* 26(2):126–130.
654. Lugenbiel P, Sartorius A, Vollmayr B, & Schloss P (2010) Creatine transporter expression after antidepressant therapy in rats bred for learned helplessness. *World Journal of Biological Psychiatry* 11(2):329–333.
655. Panksepp J & Burgdorf J (2003) "Laughing" rats and the evolutionary antecedents of human joy? *Physiology and Behavior* 79(3):533–547.
656. Wright JM, Gourdon JC, & Clarke PBS (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.
657. Douglas LA, Varlinskaya EI, & Spear LP (2004) Rewarding properties of social interactions in adolescent and adult male and female rats: Impact of social versus isolate housing of subjects and partners. *Developmental Psychobiology* 45(3):153–162.
658. Pellis SM, Field EF, Smith LK, & Pellis VC (1997) Multiple differences in the play fighting of male and female rats. Implications for the causes and functions of play. *Neuroscience and Biobehavioral Reviews* 21(1):105–120.
659. Panksepp J, Siviy S, & Normansell L (1984) The psychobiology of play: Theoretical and methodological perspectives. *Neuroscience and Biobehavioral Reviews* 8(4):465–492.
660. Litvin Y, Blanchard DC, & Blanchard RJ (2007) Rat 22 kHz ultrasonic vocalizations as alarm cries. *Behavioural Brain Research* 182(2):166–172.
661. Blanchard RJ, Blanchard DC, Agullana R, & Weiss SM (1991) Twenty-two kHz alarm cries to presentation of a predator, by laboratory rats living in visible burrow systems. *Physiology and Behavior* 50(5):967–972.
662. Brudzynski SM & Ociepa D (1992) Ultrasonic vocalization of laboratory rats in response to handling and touch. *Physiology and Behavior* 52(4):655–660.
663. Klebaur JE & Bardo MT (1999) The effects of anxiolytic drugs on novelty-induced place preference. *Behavioural Brain Research* 101(1):51–57.
664. Vallée M*, et al.* (1997) Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. *Journal of Neuroscience* 17(7):2626–2636.
665. Schmitt U & Hiemke C (1998) Strain differences in open-field and elevated plus-maze behavior of rats without and with pretest handling. *Pharmacology, Biochemistry and Behavior* 59(4):807–811.
666. Roy V & Chapillon P (2004) Further evidences that risk assessment and object exploration behaviours are useful to evaluate emotional reactivity in rodents. *Behavioural Brain Research* 154(2):439–448.

667. Kroes RA, Burgdorf J, Otto NJ, Panksepp J, & Moskal JR (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* 182(2):290–300.
668. Pohl J, Olmstead MC, Wynne-Edwards KE, Harkness K, & Menard JL (2007) Repeated exposure to stress across the childhood-adolescent period alters rats' anxiety- and depression-like behaviors in adulthood: The importance of stressor type and gender. *Behavioral Neuroscience* 121(3):462–474.
669. Lin Y*, et al.* (2009) Sex differences in the effects of acute and chronic stress and recovery after long-term stress on stress-related brain regions of rats. *Cerebral Cortex* 19(9):1978–1989.
670. Baker SL, Kentner AC, Konkle ATM, Santa-Maria Barbagallo L, & Bielajew C (2006) Behavioral and physiological effects of chronic mild stress in female rats. *Physiology and Behavior* 87(2):314–322.
671. Bowman RE, Micik R, Gautreaux C, Fernandez L, & Luine VN (2009) Sex-dependent changes in anxiety, memory, and monoamines following one week of stress. *Physiology and Behavior* 97(1):21–29.
672. Chadda R & Devaud LL (2005) Differential effects of mild repeated restraint stress on behaviors and GABAA receptors in male and female rats. *Pharmacology, Biochemistry and Behavior* 81(4):854–863.
673. Baker S & Bielajew C (2007) Influence of housing on the consequences of chronic mild stress in female rats. *Stress* 10(3):283–293.
674. Westenbroek C*, et al.* (2005) Pair-housing of male and female rats during chronic stress exposure results in gender-specific behavioral responses. *Hormones and Behavior* 47(5):620–628.
675. Cryan JF, Page ME, & Lucki I (2002) Noradrenergic lesions differentially alter the antidepressant-like effects of reboxetine in a modified forced swim test. *European Journal of Pharmacology* 436(3):197–205.
676. Potter E*, et al.* (1994) Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. *Proceedings of the National Academy of Sciences of the United States of America* 91(19):8777–8781.
677. Rivest S, Laflamme N, & Nappi RE (1995) Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. *Journal of Neuroscience* 15(4):2680–2695.
678. Shughrue PJ, Lane MV, & Merchenthaler I (1997) Comparative distribution of estrogen receptor-α and -β mRNA in the rat central nervous system. *Journal of Comparative Neurology* 388(4):507–525.
679. Shughrue PJ & Merchenthaler I (2001) Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. *The Journal of comparative neurology* 436(1):64–81.
680. Simerly RB, Chang C, Muramatsu M, & Swanson LW (1990) Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: An in situ hybridization study. *Journal of Comparative Neurology* 294(1):76–95.
681. Brinton RD (2008) Estrogen regulation of glucose metabolism and mitochondrial function: Therapeutic implications for prevention of Alzheimer's disease. *Advanced Drug Delivery Reviews* 60(13–14):1504–1511.
682. Cheng CM, Cohen M, Wang JIE, & Bondy CA (2001) Estrogen augments glucose transporter and IGF1 expression in primate cerebral cortex. *FASEB Journal* 15(6):907–915.

683. Kostanyan A & Nazaryan K (1992) Rat brain glycolysis regulation by estradiol-17β. *Biochimica et Biophysica Acta – Molecular Cell Research* 1133(3):301–306.
684. Bettini E & Maggi A (1992) Estrogen induction of cytochrome c oxidase subunit III in rat hippocampus. *Journal of Neurochemistry* 58(5):1923–1929.
685. Nilsen J, Irwin RW, Gallaher TK, & Brinton RD (2007) Estradiol in vivo regulation of brain mitochondrial proteome. *Journal of Neuroscience* 27(51):14069–14077.
686. Diaz Brinton R*, et al.* (2000) The women's health initiative estrogen replacement therapy is neurotrophic and neuroprotective. *Neurobiology of Aging* 21(3):475–496.
687. Carlsson M & Carlsson A (1988) A regional study of sex differences in rat brain serotonin. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 12(1):53–61.
688. Haleem DJ, Kennett GA, & Curzon G (1990) Hippocampal 5-hydroxytryptamine synthesis is greater in female rats than in males and more decreased by the 5-HT(1A) agonist 8-OH-DPAT. *Journal of Neural Transmission* 79(1–2):93–101.
689. Borisova NA, Proshlyakova EV, Sapronova AY, & Ugrumov MV (1996) Androgen-dependent sex differences in the hypothalamic serotoninergic system. *European Journal of Endocrinology* 134(2):232–235.
690. Valencia-Sánchez A, Esparza-Avalos NS, Cruz ML, & Ortega-Corona BG (1997) Amine neurotransmitter levels in male and female rats through developmental periods. *Archives of Andrology* 39(1):79–83.
691. Watts AG & Stanley HF (1984) Indoleamines in the hypothalamus and area of the midbrain raphe nuclei of male and female rats throughout postnatal development. *Neuroendocrinology* 38(6):461–466.
692. Westlind-Danielsson A, Gould E, & McEwen BS (1991) Thyroid hormone causes sexually distinct neurochemical and morphological alterations in rat septal – diagonal band neurons. *Journal of Neurochemistry* 56(1):119–128.
693. Frankfurt M, Fuchs E, & Wuttke W (1984) Sex differences in γ-aminobutyric acid and glutamate concentrations in discrete rat brain nuclei. *Neuroscience Letters* 50(1–3):245–250.
694. Andersen SL, Rutstein M, Benzo JM, Hostetter JC, & Teicher MH (1997) Sex differences in dopamine receptor overproduction and elimination. *NeuroReport* 8(6):1495–1498.
695. Becker JB (1999) Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacology, Biochemistry and Behavior* 64(4):803–812.
696. Campeau S, Dolan D, Akil H, & Watson Jr. SJ (2002) c-fos mRNA induction in acute and chronic audiogenic stress: Possible role of the orbitofrontal cortex in habituation. *Stress* 5(2):121–130.
697. Kanarik M*, et al.* (2008) Changes in regional long-term oxidative metabolism induced by partial serotonergic denervation and chronic variable stress in rat brain. *Neurochemistry International* 52(3):432–437.
698. Conti LH & Foote SL (1996) Reciprocal cross-desensitization of locus coeruleus electrophysiological responsivity to corticotropin-releasing factor and stress. *Brain Research* 722(1–2):19–29.
699. Spivey JM, Colorado RA, Conejo-Jimenez N, Gonzalez-Pardo H, & Gonzalez-Lima F (2008) Juvenile male rats display lower cortical metabolic capacity than females. *Neuroscience Letters* 440(3):255–259.

700. Méndez-López M, Méndez M, López L, & Arias JL (2009) Spatial working memory in Wistar rats: Brain sex differences in metabolic activity. *Brain Research Bulletin* 79(3–4):187–192.
701. Bangasser DA*, et al.* (2010) Sex differences in corticotropin-releasing factor receptor signaling and trafficking: Potential role in female vulnerability to stress-related psychopathology. *Molecular Psychiatry* 15(9):896–904.
702. Goldstein JM, Jerram M, Abbs B, Whitfield-Gabrieli S, & Makris N (2010) Sex differences in stress response circuitry activation dependent on female hormonal cycle. *Journal of Neuroscience* 30(2):431–438.

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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.

Kanarik, M.; Matrov, D.; Kõiv, K.; Eller, M.; Tõnissaar, M.; Harro, J. (2008). Changes in regional long-term oxidative metabolism induced by partial serotonergic denervation and chronic variable stress in rat brain. *Neurochemistry International, 52*(3), 432–437.

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.

Matrov, D.; Kolts, I.; Harro, J. (2007). Cerebral oxidative metabolism in rats with high and low exploratory activity. *Neuroscience Letters, 413*(2), 154–158.

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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.

Kanarik, M.; Matrov, D.; Kõiv, K.; Eller, M.; Tõnissaar, M.; Harro, J. (2008). Changes in regional long-term oxidative metabolism induced by partial serotonergic denervation and chronic variable stress in rat brain. *Neurochemistry International, 52*(3), 432–437.

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.

Matrov, D.; Kolts, I.; Harro, J. (2007). Cerebral oxidative metabolism in rats with high and low exploratory activity. *Neuroscience Letters, 413*(2), 154–158.

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