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Mestrado em Biologia Humana e Ambiente
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Orientador interno: Dr. Tatyana Strekalova (Centro de Biologia Ambiental, Faculdade de Ciências, Universidade de Lisboa)
Orientador externo: Dr. Jodi Pawluski (Department of Neuroscience, Maastricht University)

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ABSTRACT

It is known that the application of a 4-week chronic stress in C57BL/6 mice leads to the development of anhedonia, a core symptom of depression, assessed by the decrease in sucrose intake and preference. The chronic stress paradigm also leads to locomotor changes, anxiety-like behavior and impairment in memory and learning.

The aim of this project was to study the effects of the application of a shorter chronic stress (2 weeks), consisting only of ethological stressors on hedonic status, anxiety-like behavior, locomotion and hippocampus-dependent performance, in C57BL/6 mice.

In study 1, animals' daily water drinking patterns were evaluated in order to optimize a sucrose test. In study 2, mice were divided into control and stress groups, based on baseline body weight, sucrose test data and social status. Mice from stress group were then submitted to chronic stress, consisting of rat exposure over-night and social defeat using CD1 male mice. After the chronic stress procedure, body weight, hedonic status in the sucrose test, anxiety-like behavior in the elevated O-maze test, open-field activity at different times of the day cycle, and hippocampus-dependent performance in the food-displacement tube test and in the contextual fear-conditioning paradigm, were assessed.

Mice showed, in study 1, a large variability in patterns of water consumption, suggesting the application of a sucrose test of longer duration. In study 2, stressed mice showed no significant decrease in sucrose preference, but they presented an increased water intake, which is regarded as a sign of stress. They also showed significant reduction in weight, compared to control mice, and significant anxiolytic-like changes both in O-maze and open-field tests. In addition, stressed mice spent less time freezing in the fear conditioning paradigm than control mice, showing impairment in hippocampus-dependent performance. We did not see significant differences between groups in the food-displacement tube test, however a positive correlation was found between freezing behavior and number of pellets taken from the tube.

Together, our data suggests that applied here 2-week ethological stress induced a depressive-like phenotype and had signs of physiological impact in C57BL/6. However, more studies are required to validate this model.

Keywords: depression, stress, mice, chronic stress procedure, anhedonia, hippocampus-dependent performance

RESUMO

A depressão é uma doença grave, que provoca risco de vida. De acordo com a Associação Psiquiátrica Americana (Manual Estatístico e de Diagnóstico de Doenças Mentais, DSM-IV, 1994), uma pessoa diagnosticada com depressão deve apresentar, juntamente com humor disfórico e perda de interesse ou prazer em quase todas as actividades, ou seja anedonia, pelo menos 4 sintomas adicionais, entre os quais agitação ou retardo psicomotor, perda marcada de peso, perturbações de sono, diminuição de apetite, sentimentos de culpa ou ideação suicida, durante um período de pelo menos 2 semanas.

Vários modelos animais são usados, usando roedores, para mimetizar esta doença, os quais se baseiam na aplicação de *stressores* aos animais, de modo a induzir características típicas de depressão e, assim, possibilitar o estudo em laboratório desta síndrome.

Estudos prévios mostram que a aplicação de *stress* crónico durante longos períodos de tempo induz, em murganhos C57BL/6, uma síndrome típica de depressão e anedonia, um sintoma chave da depressão, avaliado pela diminuição no consumo e preferência por uma solução de sucrose. Este paradigma conduz, ainda, a alterações na locomoção, no comportamento típico de ansiedade e a danos na memória e aprendizagem.

No entanto, por motivos éticos, esforços têm sido efectuados no sentido de diminuir a carga de *stress* e desconforto em animais de laboratório. O objectivo deste projecto foi, então, estudar os efeitos da aplicação de um *stress* crónico de menor duração (2 semanas), limitando-nos à utilização de *stressores* etológicos (naturais), no estado hedónico, comportamento típico de ansiedade, locomoção e desempenho dependente do hipocampo, em murganhos C57BL/6.

O estudo 1 consistiu na avaliação dos padrões diários de consumo de água de 23 animais durante 3 dias consecutivos, de modo a optimizar o teste de sucrose. Foram analisados tanto os seus valores totais de consumo de água, como os picos diários no consumo. No estudo 2, 32 murganhos C57BL/6 foram divididos em 2 grupos, controlo (n = 10) e *stress* (n = 22), usando como parâmetros o seu peso corporal base, os dados de um teste de sucrose (preferência pela solução de sucrose, consumo de água e de sucrose, e consumo total de líquidos) e o seu status social (agressivo, submisso ou neutro). Os animais do grupo stress foram, posteriormente, submetidos ao procedimento de stress crónico, durante 2 semanas, que consistiu na exposição a ratos durante a fase nocturna do seu ciclo diário, e derrota social, com exposição a murganhos machos CD1, duas vezes por dia.

Relativamente ao estudo 1, verificou-se que a quantidade de líquido consumida variou significativamente ao longo dos 3 dias de teste. O volume de água consumido decresceu do dia 1 para o dia 2, e deste para o dia 3. Este decréscimo pode ser explicado por dois motivos: flutuações na temperatura, visto que ocorre uma diminuição no consumo de água quando a temperatura diminui, ou preferência dos animais por água fresca. Verificou-se, ainda, a existência de 4 picos de consumo em murganhos C57BL/6: +2,5h, +5h, +7,5h e +10h, após o inicio do teste. Os animais, em geral, não mantêm o mesmo pico no consumo de dia para dia. Estes dados evidenciam uma grande variabilidade na dinâmica diária de consumo de líquidos nestes animais, ressalvando a importância da utilização de testes de maior duração, quando se pretendam avaliar consumo de líquidos. Assim, escolhemos aplicar os testes de sucrose durante 24h, de modo a evitar o uso de protocolos de duração menor que podem ser erróneos.

Quanto ao estudo 2, após exposição a *stress* crónico, os seguintes parâmetros foram avaliados nos murganhos: peso corporal, estado hedónico no teste de sucrose, comportamento típico de ansiedade no teste *0-maze* elevado, actividade em *open-field* a diferentes alturas do ciclo diário, e desempenho dependente do hipocampo no teste de deslocamento de comida de um tubo e no paradigma de condicionamento de medo contextual.

Após a aplicação de *stress* crónico, embora tenhamos visto sinais de *stress* pelo aumento no consumo de água nos animais submetidos ao mesmo, ambos os grupos de animais não demonstraram uma preferência por sucrose em vez de água. Estes resultados demonstram a presença de resultados anormais neste parâmetro no grupo controlo. Este comportamento anormal pode dever-se a falhas no teste de sucrose e não no procedimento de *stress* em si. Por razões técnicas, não nos foi possível repetir este teste, o que nos deixa na incógnita quanto ao desenvolvimento ou não de anedonia. Contudo, tudo isto sugere que o nosso procedimento de curta duração e intensidade não conduziu a mudanças detectáveis no estado anedónico dos murganhos. A indução de anedonia pode, assim, necessitar de uma determinada carga de *stress*, que não foi alcançada com este modelo.

Observou-se, ainda, um efeito do *stress* no peso corporal, sendo que os murganhos submetidos a stress sofreram uma redução significativa no peso corporal, quando comparados com os murganhos controlo.

Relativamente ao teste de *0-maze*, os murganhos *stressados* demoraram menos tempo a sair das áreas fechadas do aparelho para as áreas associadas a ansiedade, ou seja, abertas ao exterior. Despenderam, ainda, mais tempo nessas áreas e saíram mais vezes para as mesmas, que os animais controlo. Estes comportamentos são comportamentos típicos ansiolíticos, ou

seja, os animais não demonstraram sinais de ansiedade. Tais resultados podem ser devidos ao desenvolvimento de hiperactividade, uma característica de murganhos C57BL/6 submetidos a *stress*. Esta hiperactividade pode ser causada tanto pela intensidade e duração do *stress* crónico como pela utilização de luz forte (25-Lux). Os resultados obtidos no teste de *open-field* corroboram os resultados obtidos no teste *0-maze*. Neste teste observámos, também, sinais de hiperactividade quando realizado sob luz forte, sendo que os animais passaram menos tempo imóveis no total, e tanto na zona periférica como na zona central do aparelho, outro sinal de comportamento típico ansiolítico. No teste realizado sob luz vermelha, os animais não mostraram alterações na locomoção.

Neste estudo, observámos que os animais *stressados* despenderam significativamente menos tempo estáticos no teste de condicionamento de medo contextual e mostraram uma tendência para um aumento do tempo de latência no comportamento de deslocamento e um número reduzido de *pellets* retirados do tubo, 1h após o inicio do teste de deslocamento de comida de um tubo, quando comparados com o grupo controlo. Assim, esta parte do nosso estudo sugere a existência de danos na função do hipocampo, após 2 semanas de *stress*. Este estudo revelou ainda, pela primeira vez, uma correlação positiva entre estes dois testes, mostrando que o teste de deslocamento de comida de um tubo pode ser um teste válido para a detecção de défices na função do hipocampo.

Em conjunto, os nossos dados deixam em aberto a possibilidade de que o nosso modelo de *stress* crónico pode ter induzido anedonia, apenas não o conseguimos detectar com o nosso teste de sucrose. Os resultados obtidos acerca da plasticidade do hipocampo e as alterações observadas no teste do *0-maze* e no peso corporal corroboram esta conclusão. Sugerimos que o nosso protocolo de *stress* induziu um fenótipo típico de depressão em murganhos C57BL/6. No entanto, mais estudos serão necessários para validar este modelo.

Palavras-chave: depressão, *stress*, murganhos, procedimento de *stress* crónico, anedonia, desempenho dependente do hipocampo

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List of Abbreviations

5-HT	5-hydroxytryptamin, serotonin
ACTH	Adrenocorticotropin
ANOVA	Analysis of Variance
BDNF	Brain-derived Neurotrophic Factor
BRS	Brain Reward System
BSR	Brain Stimulation Reward
CBA	Centro de Biologia Ambiental
CMS	Chronic Mild Stress
CRF	Corticotrophin-releasing Factor
CRH	Corticotrophin-releasing Hormone
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, fourth volume
ECS	Electroconvulsive Shock
EEG	Electroencephalography
FCM	Faculdade de Ciências Médicas
FCT	Fundação para a Ciência e Tecnologia
FELASA	Federation of Laboratory Animal Science Associations
FSLR	Flinders Sensitive Line Rats
FST	Forced Swim Test
fMRI	Functional Magnetic Resonance Imaging
GAD	Generalized Anxiety Disorder
HPA axis	Hypothalamic-pituitary-adrenal axis
ICSS	Intracranial Self-stimulation
i.e.	Id est
ISAO	International Stichting Alzheimer Onderzoek
LH	Learned Helplessness
MAOIs	Monoamine Oxidase Inhibitors
MDD	Major Depressive Disorder
m.o.	Months Old
OB	Olfactory Bulbectomy
PET	Positron-emission Tomography
PSTD	Post-traumatic Stress Disorder

REM sleep	Rapid Eye Movement sleep
SAD	Seasonal Affective Disorder
SHR	Spontaneously Hypertensive Rat
SSRIs	Serotonin-selective Reuptake Inhibitors
T	Total
TCAs	Tricyclic Antidepressants
TST	Tail Suspension Test
UM	Maastricht University
Vs	Volume of Sucrose
vs.	Versus
Vw	Volume of Water
WKR	Wistar-Kyoto Rat

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Chapter I – Introduction

1.1 Depression: clinical classification, neurobiology and treatment

1.1.1 Definition, epidemiology and social impact

Depressive disorder is a serious disabling and life-threatening illness (Sullivan *et al*., 2000; Williams *et al*., 2000), which result from a complex set of etiologies: developmental, social-environmental and genetic. These factors solely or combined induce a state of vulnerability in an individual (Auriacombe, 1997; Sullivan *et al*., 2000; Hamet and Tremblay, 2005). According to the American Psychiatric Association (Diagnostic and Statistical Manual of Mental Disorders, DSM-IV, 1994), a person diagnosed with depression must present, along with dysphoric mood and loss of interest or pleasure in nearly all activities, also known as anhedonia (core symptoms), at least four additional symptoms for a two-week period (Williams *et al*., 2000; Kessler *et al*., 2005; Gotlib and Joormann, 2010). The symptoms must cause clinically significant distress or impairment in social, occupational, and other areas of functioning (Williams *et al*., 1995).

Depression is among the most prevalent of all psychiatric disorders (Kessler and Wang, 2009). The World Health Organization Global Burden of Disease Study ranked depression as the single most burdensome disease in the world in terms of total disability-adjusted years among people in the middle years of life (Murray and Lopez, 1996a) and estimates that by the year 2020 depression will become the second major cause of disability in the world (Sullivan *et al*., 2000; Murray and Lopez, 1996a, 1996b). Nowadays, more than 15% of world population experience depression, at least on time during life. This situation leads to major economic and medical costs and to an enormous social and personal burden (Murray and Lopez, 1996a, 1996b; Kessler *et al*., 2006).

Depression affects people of all ages, ethnicities, and socioeconomic circumstances (Regier *et al*., 1988; Blazer *et al*., 1994). It has adverse effects on the quality of interpersonal relationships and can be comorbid with other psychiatric and physical conditions, such as alcohol and substance abuse, Parkinson disease, and various somatic disorders, which limit normal activities (Gotlib and Joormann, 2010).

1.1.2 Types of depressive disorder

There are several forms of depression, each one with its own constellation of symptoms. Depression can be classified into two opposite poles: melancholic or somatic syndrome and atypical syndrome. The atypical syndrome can be characterized by reverse neurovegetative symptoms such as increased appetite, weight gain, hypersomnia, extreme fatigue (Hale, 1997; Matza *et al*., 2003), and interpersonal rejection sensitivity (Fountoulakis *et al*., 2004; Stewart *et al*., 2004). Melancholic depression includes major depressive disorder, dysthymia and subsyndromal depression. Major depressive disorder is characterized by a heterogeneous group of behavioural, psychological and physiological symptoms, which include: psychomotor agitation or retardation, marked weight loss, disturbances of sleep, decreased appetite, fatigue, extreme feelings of guilt or worthlessness, difficulty in concentrating and suicidal ideation (Nutt *et al*., 1997; Gotlib and Joormann, 2010).

Dysthymia is also associated with significant functional impairment, and it occurs in approximately 3% of people. Chronically depressed mood must be present during a period of two years or more, and during those periods, at least two of the following symptoms are present: appetite and sleep disturbances, decreased energy or fatigue, feelings of low self-esteem or hopelessness, and decreased concentration (Williams *et al*., 2000). Subsyndromal depression is an acute mood disorder, although less severe that major depression. It has an increased risk for the development of major depression and decreased functioning. This form of depression is diagnosed when depressed mood and/or loss of interest or pleasure in nearly all activities, and one to three of the symptoms used to diagnose MDD last at least 2 weeks (Williams *et al*., 2000).

There are other forms of unipolar depressive disorder, which exhibit slightly different characteristics than those described above, and may only develop under certain medical conditions. Some examples of these situations are psychotic depression, postpartum depression and seasonal affective disorder (SAD), and their characteristics are summarized in Table I.

Besides the various forms of unipolar depression listed above, bipolar depression also exists, however not being as epidemiologically spread as major depression or dysthymia. Finally, another form of depression is iatrogenic depression, which is pathogenetically induced by the treatment of various disorders, including pharmacotherapy and surgical treatment, being more common in the elderly (Dhondt *et al*., 2002; Krishnan and Kasthuri, 2005) (Table I).

Table I. Other forms of depressive disorder.

			Characteristics	References
Depressive Disorder	**Unipolar Depression**	**Psychotic Depression**	When a severe depressive illness is accompanied by psychosis (break with reality, hallucinations; delusions).	**[1][2]**
		Postpartum Depression	Development of a major depressive episode within 1 month after the child's birth.	**[2]**
		SAD	Onset of a depressive illness during seasons with reduced periods of sunlight.	**[2]**
	Bipolar Affective Disorder		Cycling mood changes from extreme highs (mania), to extreme lows (depression). Mania: periods of elation or irritability, psychomotor hyperactivity, aggression, inflated self-esteem.	**Kek *et al*., 1998 DSM-IV ICD-10**
	Iatrogenic Depression		Pathogenetically induced by the treatment of various disorders (beta blockers, benzodiazepines, glucocorticoids, interferon, and nifedipine).	**Dhondt *et al*., 2002 Krishnan and Kasthuri, 2005**

1.1.3 Etiology of depression

Depression is not a single cause disease; hence, it results from a combination of genetic, biochemical, environmental and psychological factors. Some types of depression tend to run in families, thus suggesting the genetic link (Levinson, 2006). However, depression can occur in people with no family history of depression as well (Tsuang and Faraone, 1990). As suggested by genetic studies, the risk for depression results from the influence of multiple genes, which interfere with environment and several other factors (Tsuang *et al*., 1994). Also, a depressive episode might be triggered by some kind of trauma, (e.g. by the loss of a loved one) or by any situation perceived as stressful (Bogdan and Pizzagalli, 2006). Several

psychosocial factors, such as age, gender, marital status, education and income have also been identified as important factors explaining the variability in the prevalence of depression (Stewart *et al*., 2004; Akhtar-Danesh and Landeen, 2007).

Women are also more likely to suffer from depression and anxiety than man (lifetime risk for MDD ranges from 10% to 25% in women, and from 5% to 10% in men). This may be the result of their unique hormonal and psychosocial features, which may explain the higher depression rates. Also, hormones, such as estrogen, were shown to affect limbic structures which control emotions and mood. The effects of stress, violence, lack of social support, relational problems, low self-esteem and ruminative cognitive styles are believed to contribute to the vulnerability to depression in women (Stewart *et al*., 2004; Akhtar-Danesh and Landeen, 2007; Posmontier, 2008). Men often experience depression differently than women and may have different ways of coping with the symptoms. In this case, depression can be perceived by low social status, sometimes accompanied by alcohol- or drug-dependency, abusive behavior and anger. For male patients with depression, it is more typical to hide symptoms of depression and compensate functional deficits with overwork or risk-taking behavior (Stewart *et al*., 2004; Akhtar-Danesh and Landeen, 2007).

The increased risk for depressive disorder is also associated with the presence of such medical conditions as heart disease or cancer. Also, the medication used in this cases may have side effects that contribute to depression (*see above 1.1.2 Types of Depressive Disorders*) (Akhtar-Danesh and Landeen, 2007).

1.1.4 Pathogenesis of depression

Despite the strong impact and prevalence of depression, there is still little knowledge about its pathogenesis. This might be due to several aspects, such as the difficulty in documenting the pathological changes in the brain rather than other organs. The available techniques for assessing the brain functions consist on post-mortem studies and neuroimaging (Phelps and LeDoux, 2005), which provided important insights about brain regions involved in depression, although simple changes in brain activity cannot be considered sufficient to explain this complex syndrome in full (Krishnan and Nestler, 2008).

The regulation of emotions, reward and executive function implicates several brain regions and circuits, which are highly interconnected. Among these structures, the prefrontal cortex, ventral striatum (including nucleus accumbens), amygdala and the hippocampus play an important role (Figure 1). It is believed that impairment of these areas is related to depression;

for this reason, these brain structures are considered to be targets of antidepressant treatment (Nestler *et al*., 2002; Maletic *et al*., 2007). Thus, brain regions listed above are thought to contribute to different mechanisms of depression (Maletic *et al*., 2007). For example, neocortex and hippocampus are believed to mediate cognitive symptoms of depression, such as memory impairments and feelings of worthlessness, hopelessness and guilt. Several studies of depressed patients demonstrated changes in blood flow, reductions in grey-matter volume and glia density in the prefrontal cortex and the hippocampus (Manji *et al*., 2001; Krishnan and Nestler, 2008). The striatum and the amygdala are most likely responsible for emotional memory. Deficits in these structures may underlie anhedonia, anxiety and reduced motivation. Also, the hypothalamus may play a role in depression with neurovegetative symptoms such as sleep and appetite disturbances. Other subcortical structures (nucleus accumbens, amygdala) implicated in fear, reward and motivation, are also involved in this pathways (Nestler *et al*., 2002).

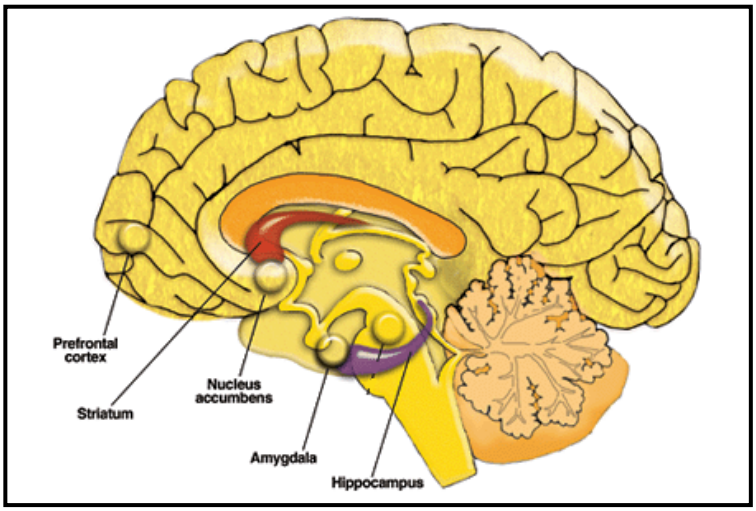


Figure 1. Brain structures believed to be impaired during depression [3].

Using functional magnetic resonance imaging (fMRI) and positron-emission tomography (PET), it has been shown that depressive symptoms associated with increased activity of the amygdala and subgenual cingulated cortex are related to dysphoric emotions (Krishnan and Nestler, 2008).

The role of the serotonergic system and the hypothalamic-pituitary-adrenal (HPA) axis in mechanisms of depression is well documented, among other neuroregulatory systems of the brain. During depression, the HPA axis is characterized by its overactivity. Depressed patients were found to have elevated levels of corticotrophin-releasing hormone (CRH) and cortisol in

40% of cases (Bremner *et al*., 2000), an abnormality reversed by antidepressants and physical exercise (Anisman and Zacharko, 2001; Dickerson and Kemeny, 2004). Low responses with the pituitary adrenocorticotropin (ACTH) secretion are another feature of depressed patients, which reflects altered functions of the HPA axis (Hayley *et al*., 2005). The elevated CRH levels are believed to gradually desensitize the CRH receptors that attenuate pituitary response. According to another hypothesis, the attenuated pituitary ACTH response may be due to the reduced sensitivity of serotonin receptors and reduced serotonergic neurotransmission. The release of ACTH is regulated by the serotonin-1A receptors, both at the pituitary and hypothalamus levels (Keeney *et al*., 2006). Several studies indicate that serotonergic neurotransmission is impaired in depression (Doherty and Gratton, 1996; Briones-Aranda *et al*., 2005). Pharmacological challenges and PET studies showed a decrease in serotonin-1A receptor-mediated signaling in depressed patients (Anisman, 2009).

The monoamine hypothesis (Schildkraut, 1965; Maletic *et al*., 2007) states that depression is caused by impairment in monoamine function in the brain, characterized by a deficiency in neurotransmission mediated by serotonin (5-HT), norepinephrine and dopamine. This hypothesis has been refined through the past years and more experimental and clinical evidence has come to light (Krishnan and Nestler, 2008; Anisman, 2009). The concentration of monoamines may be altered due to disruption in synthesis, storage or release, or it may remain normal, but the receptors and/or sub-cellular messenger's activity may be altered. Monoamines are known to affect several aspects which are altered during depression, including sleep, vigilance, appetite, motivation, motor activity and reward. Another hypothesis is based on one of the core symptoms of depression, anhedonia. The brain reward system (BRS) is a neural pathway involved in eliciting rewarding experiences in animals, including humans, and it is thought that an altered function of this system may underlie brain mechanisms behind depression (Naranjo *et al*., 2001).

Finally, the role of neurotrophic factors on the etiology of depression has also been discussed (Duman *et al*., 1997; Altar, 1999). Neurotrophic factors are known for being potent regulators of the plasticity and survival of adult neurons and glia. Hence, the neurotrophic hypothesis states that a deficiency in neurotrophic support may contribute to hippocampal pathology during the development of depressive syndrome, being that this condition is reversed by antidepressant treatment and electroconvulsive shock (ECS) (Nibuya *et al*., 1995; Russo-Neustadt *et al*., 1999). This hypothesis has focused on brain-derived neurotrophic

factor (BDNF), one of the most prevalent neurotrophic factors in the brain (Nestler *et al*., 2002) which concentration is decreased in major depression (Karege *et al*., 2002).

1.1.5 Role of stress in pathogenesis of depression

The term stress was originally defined by Hans Selye, in the 1940s (Selye and Fortier, 1949), as the nonspecific reaction of the organism after an action of harmful factors, named stressors (Czabak-Gorbacz, 2008). Nowadays, stress is characterized as an adaptive response (physical, mental or emotional) towards events capable of causing shifts on the homeostasis in the organism, allowing it to maximize its chances of survival when facing a stressor. The organisms' response to stress starts in the central nervous system, which processes sensory information related to the external stressor. If the situation is appraised as potentially harmful, a cascade of neural, hormonal and behavioral responses is initiated in order to deal with the situation (Grønli, 2006).

Although it is true that stress can have a direct or indirect causal association in the pathogenesis of depression (Anisman and Zacharko, 1992; Brown, 1993; Cui and Vaillant, 1996; Hammen *et al*., 1992; Monroe and Depue, 1991; Paykel, 2001), its impact on the organism depends on its own characteristics (type of stressor – acute or chronic, controllable or uncontrollable) and on the traits and conditions of the individual affected, such as coping ability and history of previous stressful events (Roy, 1985; Kendler *et al*., 1992, 2000; Anisman and Merali, 2000; Paykel, 2001).

The alterations that occur during a stress response are often found in depression. The HPA axis is activated by stress, which leads to the release of glucocorticoids, raising heart rate, blood pressure and metabolism. In depressed patients, glucocorticoids concentration is often elevated, which characterizes a dysfunction in the HPA axis (Nestler *et al*., 2002; Krishnan and Nestler, 2008; Anisman, 2009). It leads to hyperactivity of central neural sympathetic and to adrenomedullary-cortical gland hypoactivity. These impairments cause a typical profile characterized by decreased levels of catecholamines, such as noradrenaline, adrenaline and dopamine, in the blood, and raised plasma cortisol. The raise in cortisol levels is due to the release of the corticotrophin-releasing factor (CRF) by the hypothalamus, which stimulates the synthesis and release of ACTH from the pituitary gland. This hormone stimulates the production of glucocorticoids, cortisol in humans and corticosterone in rodents, from the adrenal cortex, causing a condition called hypercorticolaemia. Glucocorticoids exert a profound effect on metabolism and also dramatically affect behavior, acting directly in several

brain areas, for example, damaging the hippocampus (Krishnan and Nestler, 2008). Acute and chronic stress can also reduce BDNF expression, which can be seen in the post-mortem hippocampus in depressed humans (Nestler *et al*., 2002; Krishnan and Nestler, 2008).

1.1.6 Treatment of depression

In order to treat depressive syndrome, there are a number of therapies, which have proven efficacy, such as: pharmacotherapy (antidepressants), psychotherapy and electroconvulsive therapy (Williams *et al*., 2000; Bschor and Adli, 2008; Zafar *et al*., 2009). Among 80% of depressed patients show improvement when submitted to any of these treatments (Nestler *et al*., 2002), however, at the start of treatment, only one of this therapies should be applied. In case of severe, recurrent, or chronified depression, treatments also can be combined (Bschor and Adli, 2008).

Pharmacotherapy consists on the use of antidepressant drugs to treat depression. The three main classes of antidepressants used are tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs) and atypical antidepressants. TCAs act primarily as serotonin-norepinephrine reuptake inhibitors by blocking the serotonin and norepinephrine transporters. MAOIs inhibit the activity of monoamine oxidases, increasing the availability of monoamine neurotransmitters (Frazer, 1997). Among second generation medications, serotonin-selective reuptake inhibitors (SSRIs) and norepinephrine-selective reuptake inhibitors are widely used (Nestler *et al*., 2002).

Psychotherapy is defined as an intentional interpersonal relationship used by trained psychotherapists to aid a patient in problems of living. It aims to increase the individual's sense of their own well-being and, to achieve success in psychotherapy treatment, the therapy should be tailored to ones needs, resources, and preferences (Zafar *et al*., 2009). This kind of treatment can be effective in mild to moderate cases of depression, and combined with the use of antidepressants can have a synergistic effect (Nestler *et al*., 2002). It consists of three stages (Pöldinger, 1971; Kupfer, 1993): 1) Acute therapy, which aims for the complete or near complete remission of depressive symptoms; 2) Maintenance therapy, to avoid relapsing, aiming for complete functional recovery of the patient; and 3) Prophylactic therapy, indicated in patients whose illness has taken a recurrent path (Bauer *et al*., 2007).

Nonetheless, depression is a highly recurrent disorder, prone to become chronic, substantially interfering with an individual's ability to cope with daily life (Levinson, 2006). More than 75% of patients suffering from this condition have more than one depressive

episode, often relapsing within 2 years of recovery (Boland and Keller, 2009). Approximately 25% of patients relapse within 2 months in case of discontinued treatment (Williams *et al*., 2000). This high relapse rate, suggest that there are specific factors which increase people's risk for developing repeated episodes of depression (Gotlib and Joormann, 2010). Therefore, treatment is recommended to be continued beyond initial recovery (Williams *et al*., 2000). According to the National Institute of Mental Health's Sequenced Treatment Alternatives to Relieve Depression, there are even 30% to 45% of patients that do not fully respond to pharmacotherapy, being that 10% to 12% of them develop resistance to standard antidepressant treatments (Williams *et al*., 2000), making improved and new therapies urgently needed (Nestler *et al*., 2002).

1.2 Depression versus other stress-related disorders and features

Stressful events are known to favor the development of depression in humans (Kendler *et al*., 1999). However, after a traumatic event, the majority of people do not become depressed. The ones who do, do so after stressors that for most people are considered mild. Severe stressors such as war, rape or death of a loved one, do not typically induce depression, but instead lead to syndromes such as post-traumatic stress disorder (PSTD), panic disorder and generalized anxiety disorder (GAD). Depression shares cardinal features with this stress-related disorders, but they are distinct from it based on subsidiary symptoms, treatment and course of illness (Nestler *et al*., 2002); however, the overlapping symptoms are sufficient to mislead diagnosis. Among the overlapping symptoms there can be found sleep disturbances, irritability, agitation, difficulty in concentrating, loss of control, fatigue, and distress. Further work is needed to understand the overlapping nature of these two brain disorders.

The same thing happens with animal models used to mimic depression. In order to induce depressive-like features, some models consist in exposure of the animals to stressful situations (Schweizer *et al*., 2009). The application of stress often results in a variety of behavioral, neurochemical and neuroendocrine alterations, resembling some dysfunctions found in human depression (Willner, 1997; Sapolsky, 2003; Kloet *et al*., 2005). However, several studies also demonstrated the presence of anomalous behaviors induced by stress, like anxiolysis and hyperlocomotion, contrary to what happens during depressive-like state – increased anxiety and decreased in locomotion (Strekalova *et al*., 2005; Willner, 2005). Stress can also increase

aggressiveness in C57BL/6, for example, which is not typical during depression (Mineur *et al*., 2003).

As according to DSM-IV, a diagnosis for major depression corresponds to the presence of at least one core symptom, such as anhedonia, plus four subsidiary symptoms, the same should be applied to animal models. Hence, in order to really mimic the condition, the stress model should induce several and not only one behavioral or physiological alteration, being one of these alterations the core symptom anhedonia.

1.3 Animal models of depression: importance of pre-clinical studies

Depression is characterized by such a wide spectra of disruptions, that it is difficult for researchers to mimic this disorder in animal models (Cryan and Holmes, 2005). The majority of the core symptoms of depression, such as depressed mood, feelings of worthlessness or suicidal thoughts, cannot be easily measured in laboratory animals since they lack consciousness of self, self-reflection and consideration of others (Nestler *et al*., 2002; Deussing, 2006). There are, nonetheless, several endophenotypes in depression (Hasler *et al*., 2004), which can be reproduced independently and evaluated in animals. Among this endophenotypes, one can find: anhedonia; anxiety, a symptom with high prevalence in depression; neuroendocrine disturbances, mainly of the HPA axis; behavioural despair; weight disturbances; sleep disturbances, namely disturbances in the circadian rhythms and EEG parameters of sleep; and changes in neuroanatomy of the brain (Hasler *et al*., 2004; Deussing, 2006; Cryan and Slattery, 2007). However, since some of these endophenotypes are also present in other diseases, care must be taken with the interpretation of these findings (Cryan and Slattery, 2007).

Therefore, animal models of depression must represent several known aspects of depression in selected animal species, such as rodents (rats and mice). They are available as a tool for addressing the aspects of neurobiology of depression, as experimental models for studying the mechanism of action of antidepressants, and as a screening model to elucidate their activity (Willner, 1997; Deussing, 2006; Grønli, 2006).

Although rodents and humans possess major differences in brain anatomy, several evolutionary pathways are still conserved between the two species, underlying certain physiological and behavioural responses (Cryan and Holmes, 2005). Through inference based on findings from animal models, we can elucidate behaviours, neural circuits and genetic

factors behind depressive illness and further understand human behaviour and disease. The number of validated animals for affective disorders is large, and several are still being created, but in depression a major problem is the lack of validated models (Grønli, 2006).

Animal models for psychiatric conditions, which are in part defined by subjective experience, pose as a problem when it comes to define clear criteria that allow asserting the validity of the model. In order to use an animal to model major depressive disorder, several questions have to be answered. First, it has to be clear what do we want the model to represent (the neurobiology of the disorder, the entire syndrome, specific symptoms, prediction of treatment efficacy) and to what end. Second, it must be considered valid. McKinney and Bunney proposed in 1969 the minimum requirements for an animal model of human disease. The model must present a reasonable analogy with the human disorder in its sympthomatology (face validity), behavioural changes that can be monitored objectively (Willner, 2005) and that can be reversed by the same treatments that are effective in humans (predictive validity), it must be reproducible between laboratories (McKinney and Bunney, 1969), and have similar etiology between the model and the human pathology (Geyer and Markou, 2000; Frazer and Morilak, 2005). There are a number of tests that have been used to validate animal models. These include tests of behavioural despair and anxiety.

1.3.1 Behavioural despair tests

The behavioural despair tests are based on the theoretical assumption that the test animal becomes desperate during the ongoing of the test (Borsini *et al*., 2002).

1.3.1.1 Forced swim test

This test was first described by Porsolt *et al*. in 1977 (Porsolt *et al*., 1977), and it can be used in both mice and rats (Jesberger and Richardson, 1985). In this paradigm, the animal is placed inside a container with water, from which there is no escape, and its behaviour is assessed for several minutes (Figure 2). At first, rodents display escape-oriented behaviours but then, it changes into movements that are just enough to keep the rodents head above water, a behaviour called floating (Deussing, 2006). If the animals are replaced in the testing apparatus 24h later, they adopt the floating posture quickly. This immobility was originally interpreted by Porsolt as 'behavioural despair', meaning that the animal had lost the motivation to perform escape-orientated behaviours (Porsolt *et al*., 1977). This change between activity and immobility in the FST can also reflect changes between active and

passive behavioural reactivity to stress (Lucki, 1997; El Yacoubi and Vaugeois, 2007). There are also other active behaviour parameters that can be studied such as climbing (upward-directed movements of the forepaws along the side of the apparatus) and swimming (Cryan *et al*., 2002).

Figure 2. Forced swim test [4].

The FST is easy to use and has proven reliability across several laboratories (Borsini and Meli, 1988; Deussing, 2006). It is the most widely used test for screening antidepressant activity preclinically, and antidepressant medications of all major classes usually reduce immobility and increase active behaviour (Jesberger and Richardson, 1985; Borsini and Meli, 1988; O'Neil and Moore, 2003; El Yacoubi and Vaugeois, 2007). Among its drawbacks, this test is mostly sensitive to acute treatment, not reflecting the slow onset of antidepressant action as it is observed in depressed patients, and its effects may be confounded with thermoregulatory disturbances (Deussing, 2006).

1.3.1.2 Tail suspension test

The tail suspension test (TST) relies on similar assumptions and interpretations as the FST (Deussing, 2006). In this test, mice are suspended by the tail for a defined period of time (Figure 3). Typically the animal engages in escape-orientated behaviours followed progressively by increasing periods of immobility. A broad spectrum of antidepressants can restore the animals' activity, thus reducing immobility (O'Neil and Moore, 2003; Deussing, 2006; El Yacoubi and Vaugeois, 2007). Although the underlying principle measuring the lack of active behaviour is identical in both tests (FST and TST), they vary in response to certain antidepressants, which indicates potentially different substrates underlying the observed behavioural differences (Deussing, 2006).

Figure 3. Tail suspension test [5].

Also, different mouse strains differ markedly in their basal response in this test (Porsolt *et al*., 1978). The major drawbacks in this test is that its application is restricted to mice and limited to strains that do not tend to climb their tail, what otherwise may confuse interpretation. Also, as it happens with FST, TST cannot reflect the slow onset of antidepressant action as it is observed in depressed patients. Among the advantages, this is a simple and inexpensive test, which allows for automation and has high reproducibility (Deussing, 2006).

1.3.2 Learned helplessness test

The learned helplessness (LH) animal model incorporates more closely disease etiology and predisposition (Deussing, 2006), and attempts to simulate a human depressive state in animals (Maier and Seligman, 1976). It was first described in dogs (Overmier and Seligman, 1967), but currently it is used in rodents, both mice and rats. It is based on the observation that the animal develops deficits in escape, cognitive and reward behaviours (sucrose preference) when subjected to repeated unavoidable and uncontrollable foot shocks (Deussing, 2006), achieving the state of leaned helplessness. This state is defined as a failure to exhibit escape behaviour during subsequent exposure to the same stressful stimulus, only this time escape is possible (El Yacoubi and Vaugeois, 2007). This acceptance of the uncontrollable situation was accepted as being analogous to the apathetic despair seen in human depression (Jesberger and Richardson, 1985). For yet unknown reasons, only a part of the animals subjected to this stress develop signs of helplessness, reflecting a potential gene-environment interaction. Other symptoms present after exposure to this test are: loss of appetite and weight, decreased locomotor activity and poor performance in appetitively and aversively motivated tasks, equivalent to depressed human symptoms (Geyer and Markou, 2000). Although LH can be

prevented or reversed by acute antidepressant use, thus proving the models' validity, and can also help in finding potential targets for antidepressants, this paradigm is not fully selective for antidepressant medication because anxiolytics have also been demonstrated to reverse helpless behaviour (Deussing, 2006).

1.3.3 Brain lesion models

These paradigms are based on the assumption that depression might be caused by regulatory deficits in neuronal circuits (Deussing, 2006), which causes a constellation of behavioural and neurochemical alterations.

Bilateral olfactory bulbectomy is a widely used procedure which induces changes in behaviour, and in the endocrine, immune and neurotransmitter functions, which simulate many of the changes, observed in depressed patients. Since the olfactory system in rodents is part of the limbic region, along with the hippocampus and the amygdala, which contribute to memory and emotion, the removal of the bulbs result in a disruption of the limbic-hypothalamic axis causing all the alterations mentioned before (Song and Leonard, 2005).

In this model, the observed behavioural alterations are not just a consequence of loss of smell, since peripheral anosmia does not produce such a syndrome. Therefore, the behavioural syndrome connected to olfactory bulb must be the result of a major dysfunction of the cortical-hippocampal-amygdala circuit. These areas are also impaired in depressed patients (Song and Leonard, 2005; Deussing, 2006).

This procedure has been predominantly applied in rats, but mice can also be used. After performing and olfactory bulbectomy in rats, marked changes in all major neurotransmitter systems occur. Hyperactive response in the open field paradigm is the most consistent behavioural change observed, and it can be reversed by antidepressant medications (Cryan *et al*., 1999). As chronic, but not acute, administration of antidepressants corrects the majority of the alterations caused by OB, this model is considered not only a model for detecting antidepressant activity (Cryan *et al*., 2002; Song and Leonard, 2005) but also one for exploring the connection between these systems (Song and Leonard, 2005)

As this model mimics the slow onset of antidepressant action (chronic action), it shows high face validity, thus having one of the best preclinical profiles for assessing the effects of novel antidepressants (Cryan *et al*., 2002). The bulbectomized rat represents an agitated, hyposerotonergic depression-related phenotype instead of representing a retarded depression as stress-related models (Deussing, 2006).

1.3.4 Genetic models of depression

There are a few genetic models of depression and many knock-out mice models which also show depressive-like behaviours. However two of the main genetic models of depression in rats are the Flinders Sensitive Line and the Wistar-Kyoto Rat.

The Flinders Sensitive Line Rats (FSLR) was a model developed by Overstreet *et al*. (Overstreet *et al*., 1995), through selective breeding of outbred Sprague Dawley rats for differences in the effects of the cholinesterase inhibitor diisopropylfluorophosphate in body temperature, body weight and water intake. These rats are more sensitive to the inhibitor than other breeds, and display exaggerated immobility when submitted to the FST. This immobility can be reduced by chronic treatment with antidepressants, such as desipramine, imipramine or sertraline (Overstreet *et al*., 1995; El Yacoubi and Vaugeois, 2007). Also, when subjected to chronic mild stress, FSL rats show enhanced vulnerability to stress-induced anhedonia (El Yacoubi and Vaugeois, 2007). This model was originally proposed as a genetic model of depression (Overstreet, 1986) because depressed patients also show more sensitiveness to cholinergic agonists (Janowsky and Risch, 1987). Another characteristic FSL rats share with depressed patients are: elevated REM sleep, appetite and weight changes and reduced activity (Overstreet *et al*., 1995).

The Wistar-Kyoto rat (WKR) was initially bred from the Wistar rat as the control strain for the spontaneously hypertensive (SHR) rat (Okamoto and Aoki, 1963). This strain demonstrates exaggerated behavioral and physiological responses to stress across a variety of situations. This is one of the most susceptible strains of rats towards the development of learned helplessness (Paré, 1994). During the forced swim test, WKR also demonstrated higher levels of immobility at baseline, when compared to other strains (Paré, 1994; Armario *et al*., 1995; Lopez-Rubalcava and Lucki, 2000).

1.3.5 Stress models

Depression is strongly influenced from stressful events or traumatic life events, suggesting that depressed patients must have an impairment of proper stress coping strategies (de Kloet *et al*., 2005). Because of that, the majority of models are based on the exposure to various types of stressors, acute or chronic, being capable of consistently generating behavioural changes similar to symptoms of depression, which can be reversed by antidepressants (Deussing, 2006).

1.3.5.1 Chronic stress model

Katz developed, in 1981 (Katz, 1981) the first model of chronic stress. This model used several stress procedures which involved relatively severe stressors such as electrical shock, food deprivation, cold swim, water deprivation, and heat stress. For 21 days, animals were exposed to unpredictable stress and some of them did not survive this procedure. The animals which survived exhibited signs of anhedonia, seen by the decrease of the intake of sucrose and saccharine solutions, reduced locomotor activity and higher corticosterone levels. Normal behaviour was then restored by antidepressants (Katz, 1981).

Nowadays, stress models are based on severe stressors, mild stressors or a combination of both, and in all of them animals are supposed to develop anhedonia (GrØnli, 2006).
The chronic stress models appear to be more appropriate for modelling depression compared to other models, as chronic stress and chronic frustration are more likely to induce the neurobiological changes which will lead to depression (Willner, 1997). However, these models have the disadvantage of being poorly reproducible in behavioural abnormalities and their response to antidepressant drugs between laboratories (Willner, 1997).

1.3.5.2 Chronic mild stress paradigm

Willner and co-workers developed in 1987 the chronic mild stress paradigm, which includes a variety of mild and more realistic stressors, administered over a longer period of time (Willner *et al*., 1987). Presenting different types of stressors is essential for the model, as repeated presentation of a single stressor often results in rapid behavioural habituation (Muscat and Willner, 1992). Some of the used mild stressors are: soiled cage, tilting of the cage, alterations of the light-dark cycle, periods of food or water deprivation, and grouping (Willner *et al*., 1987). The duration of the exposition to each stress goes from a few hours to a day, over 2 to 5 weeks. This is a model more similar to the human condition, characterized more by daily stresses than traumatic occurrences. Some of the behavioural abnormalities seen in the CMS parallel those symptoms observed in humans, and can be reversed by chronic antidepressant treatment (GrØnli, 2006). .

Like most humans, when given a choice, rodents usually prefer to drink sweet solutions (Willner *et al*., 1987). Willner and collaborators found that CMS can induce a significant reduction in sucrose consumption, anhedonia, one of the core syndromes of depression; hence reactivity reward was adopted as the major endpoint of this paradigm (Willner, 2005). This decrease lasts for up to 8 weeks, and can be reversed by TCAs (Willner *et al*., 1987).

This model also decreases aggressive and male sexual behaviour in rats (D'Aquila *et al.*, 1994), locomotor activity during the active phase (Gorka *et al*., 1996), alters circadian and diurnal rhythm (Gorka *et al.,* 1996; D'Aquila *et al.,* 1997), and causes sleep disturbances, such as an abnormal REM sleep pattern (Moreau *et al*., 1995; Cheeta *et al*., 1997). It also induces loss of bodyweight (Willner and Jones, 1996), alterations in sympathetic cardiac regulations (Grippo *et al*., 2003) and an altered level of cytokines (Grippo *et al.,* 2005). Finally, it affects the activity of the HPA axis (Muscat and Willner, 1992).

1.3.6 Models of stress-induced anhedonia: characteristics of stressors used

Anhedonia is defined by 'the diminished capacity to experience pleasure of any sort'. It may be a symptom in various psychiatric disorders (DSM-IV, ICD-10) and it is one of the most prominent symptoms of a major depressive episode (GrØnli, 2006).

Several chronic stress models are known for the induction of anhedonia in rodents, but each one varies on type and strength of stressors used and duration of the chronic stress application. Table II describes some stress models used by authors such as Katz (1982), Willner *et al*. (1987), Strekalova *et al*. (2004) and Baker *et al*. (2006).

Table II. Models of stress-induced anhedonia.

Strain	Duration	Stressors applied	Effects on hedonic status	References
Sprague Dawley Rats	3 weeks	Unpredictable shock; 40h food deprivation; Cold swim; 40h water deprivation; Heat stress; Shake stress; Reversal of day/night cycle	Decrease in sucrose and saccharine consumption	**Katz *et al.*, 1987**
Lister Rats	5 - 9 weeks	Food and water deprivation; Continuous lighting; Cage tilt; Paired housing; Soiled cage; Low temperatures; Intermittent white noise; Stroboscopic lighting; Exposure to empty water bottle after a period of water deprivation; Restricted access to food; Novel odors; Foreign objects in home cage	Longer lasting decrease in sucrose consumption	**Willner *et al*., 1987** **Willner, 1991, 1997**
C57BL/6 Mice	4 weeks	Exposure to rat; Restraint stress; Tail suspension	Lasting decrease of sucrose preference in a subgroup of mice	**Strekalova *et al*., 2004** **Strekalova, 2008**
Long Evans Rats Sprague Dawley Rats	3 weeks	Confinment in a standard mouse cage; Loud noise; Exposure to an empty water bottle after a period of water deprivation; Restricted access to food; Food deprivation; Unfamiliar pairing; Soiled cage; Cage tilt; Continuous lighting; Reversal of day/night cycle	Decrease in sucrose intake; No effect on sucrose preference	**Baker *et al*., 2006**

1.4 Methods of assessment of hedonic status in animal models

Anhedonia, as described before, is considered as one of the core phenomenon of depression and can be defined as the loss of pleasure or interest in previously rewarding stimuli (Hamilton, 1967; Klein, 1974). Because this is a symptom that can be mimicked in rodents, hedonic deficit can be regarded as an appropriate endophenotype to be addressed when modeling depression-like behaviors in animals (Wise, 2002; Kornetsky, 2004; Nestler, 2005; Strekalova and Steinbusch, 2009).

There are several paradigms that enable assess of the brain reward system under both basal and stimulated conditions, allowing to study the hedonic status in animals and the consequences of alterations in reward pathways. Among those tests are intracranial self-stimulation (ICSS) and consumption and/or preference for palatable food or solutions. These tests are not considered models of an entire syndrome, providing instead operational measures of anhedonia (Geyer, 1995).

1.4.1 Intracranial self-stimulation

ICSS is an operant paradigm in which rodents self-administer rewarding electrical stimulation (brain stimulation reward (BSR)) through the implementation of electrodes into the brain. The stimulation can be performed in several brain areas and it can be used both in rats and mice, only for rats a lever it's used and for mice a wheel, thus facilitating sustained response in this species (Carlezon and Chartoff, 2007).

Since it has become increasingly important to assess depression symptoms in laboratory animals, this test can be used to reflect reward, anhedonia and aversion, characteristics often seen in this condition. It is also utilized, to understand how pharmacological or molecular manipulations will affect the functioning of brain reward systems, to study motivation, food- or drug-reinforced operant or Pavlovian paradigms (Carlezon and Chartoff, 2007).

1.4.2 Evaluation of consumption of palatable foods or solutions

A useful method of assessing hedonic status in the chronic stress depression models is the free access paradigm of the sucrose or saccharine consumption test. Its experimental procedure is not demanding for the animals, and it is characterized by relatively high through output. Evaluation of the hedonic status with a sucrose test has the advantage of not involving learning component and minimally implicates factors of anxiety and locomotion, which

alteration, typical of a depressive-like state, may affect the measurement of sensitivity to reward in animal depression paradigms (Willner, 2005; Strekalova, 2008).

The vanilla pasta test (Ducottet and Belzung, 2004) is another useful paradigm to test anhedonia in rodents. This test is based on the attraction mice show towards vanilla-flavored pasta shells compared with natural ones. Normally, animals show a decrease in vanilla pasta consumption after stress.

1.5 Objectives

Nowadays, for ethical reasons, more effort is being made to decrease stress load and discomfort in laboratory animals as much as possible. Our project was therefore aimed to shorten the duration of the chronic stress procedure (2 weeks), and limit ourselves by the use of ethological stressors only. We addressed the question whether or not this procedure could induce anhedonia in C57BL/6 mice, as well as other features of depressive syndrome and other consequences of stress. Therefore, we evaluated behavioral effects of our 2-week ethological stress procedure on hedonic status in the sucrose test, on anxiety-like behavior in the 0-maze test, locomotion in the open-field test and hippocampus-dependent performance in the food-displacement tube test and in the contextual fear-conditioning paradigm.

Chapter II - Materials and Methods

2.1 General conditions of experiment

2.1.1 Animals and housing

In this project, C57BL/6 male mice (age: 3.5 months old (m.o.); Figure 4, A) were used. During study 1, *2.2 Definition of optimal conditions for sucrose test: study of individual drinking patterns in C57BL/6 mice*, mice were singly housed under a reverse 12h-12h light-dark cycle (lights out at 9h00 and no light during the dark phase of the light cycle), in standard laboratory conditions (22-24ºC, food and tap water *ad libitum*). For study 2, *2.3 Behavioral study of chronically stressed mice*, mice were also singly housed under a reverse 12h-12h light-dark cycle (lights out at 10h00 and red light during the dark phase of the cycle), in standard laboratory conditions (22-24°C, food and tap water *ad libitum*), starting 14 days prior to the behavioral experiments. CD1 male mice (age: 3.5 m.o.; Figure 4, B), used as intruders in a social interaction test and in a social stress paradigm, were kept in the same conditions and in the same room as the C57BL/6 mice used in study 2. Mice were singly housed because the sucrose preference test has to be performed individually in each mouse and because these strains are characterized by aggressive behavior when housed in groups, thus largely increasing behavioral variability in the experiment. Females were not used as experimental subjects in the proposed project as estrous cycle affects concentrations of neurochemicals in the brain and increases behavioral variability (Ladisich *et al*., 1977; Bruinsma and Taren, 1999). For a rat exposure stress, nine Wistar male rats (age 3 m.o.; Figure 4, C) were used. The rats were kept in a separate room from the mice, under a reverse 12h-12h light-dark cycle, in standard laboratory conditions.

All animals were provided by Charles River (Südfeld, Germany). Animals were tested from the onset of the dark phase of the light cycle (9h00 or 10h00) in a sound controlled testing room. Control and stress groups of mice were tested in identical temperature/humidity conditions. Cages were changed once per week and water bottles from C57BL/6 and CD1 mice were changed three times per week. Tissue paper and wooden objects were provided for environmental enrichment of both mice and rats. This project was approved by the Ethical Committee of Faculdade de Ciências Médicas (FCM) from Universidade Nova de Lisboa and of Maastricht University (UM).

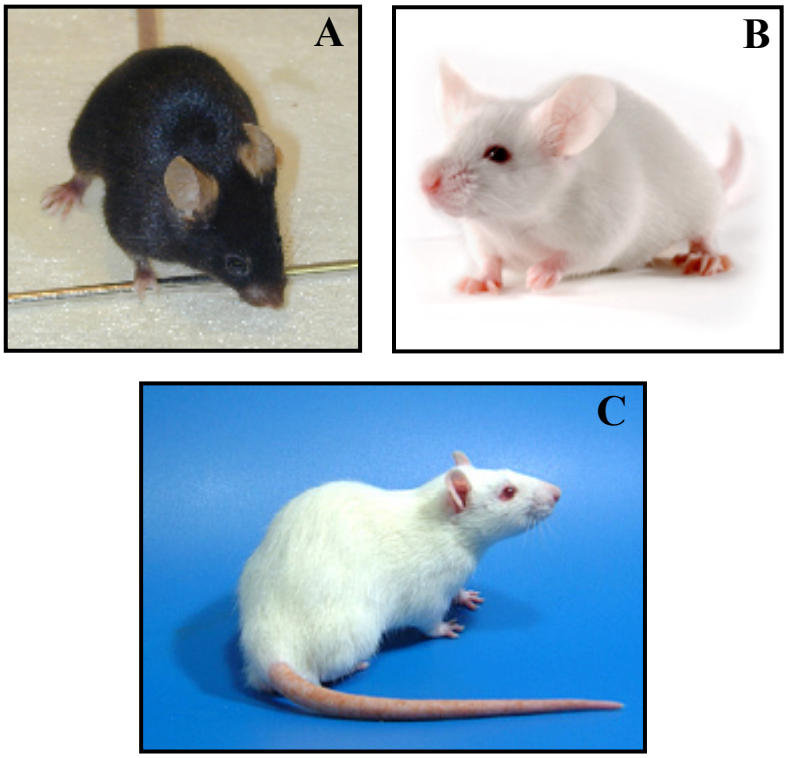


Figure 4. Rodent strains used in our study. (A) C57BL/6 mice [6]. (B) CD1 mice [7]. (C) Wistar rat [8].

Study 1

2.2 Definition of optimal conditions for sucrose test: study of individual drinking patterns in C57BL/6 mice

2.2.1 Evaluation of water drinking patterns

Since various protocols of sucrose test in rodents have been used in different laboratories in the past, where the duration of the test (1h to 48h) and concentration of sucrose solution varied greatly (Willner *et al*., 1987; Matthews *et al*., 1995; Monleon *et al*., 1995; Grippo *et al*., 2003; Strekalova and Steinbusch, 2009), we performed a study on individual patterns of drinking behavior, hoping to define limitations in the sucrose test and choose its ideal duration.

In order to study the daily water drinking patterns in mice, twenty-three C57BL/6 mice were used. During three consecutive days, water intake was assessed by weighing the bottles at the onset of the dark phase of the light cycle (9h00), and then weighing them again every 2,5h (+2,5h, +5h, +7,5h and +10h, from the beginning of the test). Besides the evaluation of

consumption peaks, we assessed the total water daily consumption and the differences in consumption between each day.

Study 2

2.3 Study of the effects of ethological chronic stress in C57BL/6 mice

For the following study (Table III), thirty-two C57BL/6 mice and thirty-four CD1 mice (for studies *2.3.1.2 Resident-intruder test* and *2.3.2.2 Social defeat and exposure to CD1 mice)* were used. C57BL/6 mice were divided into 2 groups; 1) the control group (n = 10), which was not submitted to stress procedure, and 2) the stress group (n = 22), based on social status, sucrose test data and body weight (*see below*).

The chronic stress procedure we chose to apply in this study was slightly different from other known models, since we chose only to use ethological stressors, which are natural stressors, instead of any artificial stressors, and we applied the stressors for only a 2-week time period.

Table III. Chronogram of the study with 2-week ethological stress.

Chronogram of the Experiment				
Day 1 - Day 14 (14 days)	**Day 15 - Day 28 (14 days)**	**Day 29 - Day 43 (14 days)**	**Day 43 - Day 45 (3 days)**	**Day 45 (1 day)**
Animal adaptation to single housing and new facilities	Baseline Behavioral Tests - Resident-intruder Test - Sucrose Test	Chronic Stress Procedure	Post-stress Tests - O-maze Test - Tube Test - Contextual Fear-conditioning Paradigm - Sucrose Test - Open-field Test	Sacrificing and Brain Dissection

2.3.1 Group formation and evaluation of baseline behaviors in C57BL/6 mice

Social status is a significant factor in an animals' predisposition to stress-induced anhedonia (Strekalova *et al*., 2004; Malatynska and Knapp, 2005), therefore, before the chronic stress procedure, parameters of social behavior in a social interaction test were determined in the male mice used in this study (*see 2.3.1.2 Resident Intruder test*). Body weight and baseline parameters of sucrose test (*see 2.3.1.1 Sucrose test*) were also evaluated. Stress and control groups were balanced according to the animals' body weight, initial sucrose preference and a similar percentage of aggressive, non-aggressive and socially neutral (non-definable) individuals (Strekalova *et al*., 2004, 2006).

2.3.1.1 Sucrose test

Sucrose preference is a frequently used test that measures anhedonia in rodents (Willner, 1997; El Yacoubi *et al*., 2003; Strekalova *et al*., 2004; Craft and Devries, 2006; Grippo *et al*., 2006). During this test, mice were given, for a period of 24h, a free choice between two bottles, one with 1% sucrose solution and another with water (Strekalova *et al*., 2006; Figure 5). Regular sugar, stored in paper bags, well protected from sources of flavor, was used for the test. It is recommended to avoid any contact of the sugar used with plastic material in order to prevent any transfer of plastic odor, which mice are aversive towards (Strekalova and Steinbusch, 2009).

The test started with the onset of the dark (active) phase of the animals' cycle. To prevent possible effects of side-preference in drinking behavior, the position of the bottles in the cage was switched in the mid-point of the test (5h after the beginning). For the same reason, the position of the bottles was also randomized between cages. Bottles were placed gently in the mouse cage, in a position close to horizontal. No previous food or water deprivation was applied before the test, unlike previous studies (Willner *et al*., 1987). In order to balance the air temperature between the room and the drinking bottles, the bottles were filled the evening before testing and kept in the same room where the testing took place. This measure prevents the physical effect of liquid leakage resulting from growing air temperature and pressure inside the bottles, when they are filled with liquids, which are cooler than the room air. With this method, the error of measurement of liquid intake does not exceed 0,1ml. Also, to provide unrestricted access to liquid consumption, and to minimize spillage of liquids during the test in another, bottles with rubber stoppers, made out from odor free material, and glass tips (internal Ø = 2mm) were used. Falcon tubes (50ml) were used as bottles.

Figure 5. Application of a sucrose test in C57BL/6 mice.

The consumption in water, sucrose solution and total intake of liquids were estimated by weighing the bottles, with balances which resolution was 0,1g. The preference for sucrose was calculated as a percentage of the consumed sucrose solution (Vs) over the total intake of liquids (Total (T) = Vwater + Vs), using the following formula:

Sucrose Preference = (Vs / T) x 100%

We chose to use a test of longer duration in order to overlay all the mice drinking peaks assessed during the water drinking pattern study (*See below Chapter III – Results*), and to preclude any effects of neophobia, artefactual bias toward any one side, and preservation effects.

2.3.1.2 Resident-intruder test

After assessing body weight and baseline sucrose test data, mice were subjected to a social interaction test, the resident-intruder test, used to define their individual social status, as mentioned above: aggressive, non-aggressive and neutral (Figure 6). Mice from experimental groups were placed individually as a resident in an observation cage (50cm x 30cm x 20cm) for 30 min. Thereafter, a male CD1 mouse was introduced as an intruder to the same cage and left with the resident mouse for 8 min. During the observation period, resident and intruder mice displayed specific behaviors such as aggressive or submissive social exploratory behavior. The complete lack of attacks towards the partner, accompanied by specific "submissive" postures, such as escape and defense, were regarded as submissive types of social behavior. Initiation of attack towards the partner, tail rattling and fighting back in response to attacks was characterized as aggressive (non-submissive) behaviors. Some pairs of animals do not exhibit behavioral confrontation but instead show no social interaction or social exploratory behavior; these mice are regarded as neutral for their social status.

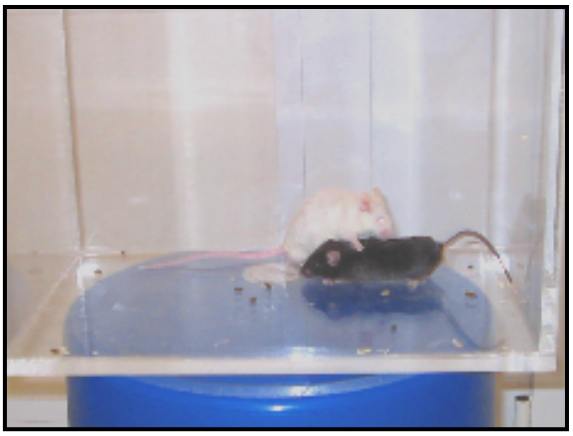


Figure 6. Resident-intruder test, to define social status in C57BL/6 mice.

CD1 male mice identified as aggressive in this test were later used during the stress procedure for social defeat (see *2.3.2.2 Social defeat and exposure to CD1 mice*).

2.3.2 Chronic stress procedure

We used the following chronic stress procedure as a variant of the basic protocol, which has been proposed to ensure induction of anhedonic state in about 70% of mice (Strekalova and Steinbusch, 2009). Mice in the stress group were submitted to 15 days of social defeat and rat exposure in small containers. Stressors were applied in the following sequence:

2.3.2.1 Rat exposure

Mice were introduced into cylindrical containers, which were then placed into a rat home cage during 15h (over-night, from 18h00 to 9h00; Figure 7). During the weekends, mice were allowed to stay inside their home cage, and all home cages were placed on top of the rat cages, altogether in a separate room. Containers were made from customized transparent plastic, size 15cm x Ø 8 cm, with small holes in plastic covers (Ø < 0.5cm), which ensured protection of the mouse from the rat, but allowed visual and odor cues.

Figure 7. Rat exposure stress.

2.3.2.2 Social defeat and exposure to CD1 mice

Mice were placed inside of a home cage containing an aggressive CD1 mouse (as determined in *2.3.1.2 Resident-intruder test*), for 5min. Then, C57BL/6 mice were introduced into small containers and again inside the CD1 cage, where they stayed for a 3h period. After that, mice were taken from the small containers and put back inside the CD1 cage for another 5min. Animals were carefully observed during the test and in case of excessive aggressive attacks, the procedure was interrupted. In order to randomize the procedure, the same pairs of mice were never put together.

2.3.2.3 Control over the optimal stress load

In order to adjust stress load to specific characteristics of stress response, it was necessary to monitor animals' habituation to the chronic stress. Therefore, in the course of the experiment, weekly-measured body weight was analyzed. Statistically significant reduction in body weight in the stress group in comparison to the control group was interpreted as an indicator of adequate stress load. To control habituation to single stress sessions during chronic stress on a daily basis, post-stress locomotor activity of mice was evaluated; a decrease in these characteristics in more than 50% of mice for at least 30min of a post-stress period detected by subjective rating was taken as a sign of optimal stress intensity. Applied stressors were reduced when body weight and activity dropped 2h after, in 80% of mice.

2.4 Behavioral study of chronically stressed mice

After the application of the chronic stress procedure, mice were submitted to post-stress testing in order to assess hedonic status in the sucrose test, anxiety-like behavior in the elevated O-maze test, hippocampus-dependent performance in the food-displacement tube test and in the contextual fear-conditioning paradigm, and locomotion in the open-field test, in the following sequence (Table IV):

Table IV. Post-stress behavioral tests. *Number of hours after the end of the chronic stress procedure.

Day 43 - Day 45		
Day 43	**Day 44**	**Day 45**
O-maze Test (+1h)* Tube Test (+1h)* Contextual Fear-conditioning Paradigm (Training Session) (+6h)* Open-field Test (Day Session) (+9h)*	Sucrose Test (+24h)* Open-field Test (Night Session) (+36h)*	Contextual Fear-conditioning Paradigm (Recall Session) (+48h)*

2.4.1 Assessment of anxiety-like behavior in the elevated O-maze test

The elevated O-maze test is often used to evaluate anxiety in mice. Testing has been carried out as described elsewhere (Strekalova *et al*., 2005). The O-maze (Figure 8) consisted of a black circular path (runway width 5.5cm, Ø = 46cm) with two opposing compartments protected by walls made of polyvinyl-chloride (height = 10cm) and two open sectors of equal size. The maze was elevated 20cm above the ground and illuminated from the top with red light. At the start of the testing session, animals were placed inside one of the two closed compartments. The test was recorded with a web camera, and behavior in the maze (latency of the first exit to the anxiety-related open arms of the maze, total number of exits to the open arms and total duration of time spent in open arms) was scored for 5min. An entry into an arm occurred when all four paws were in the arm.

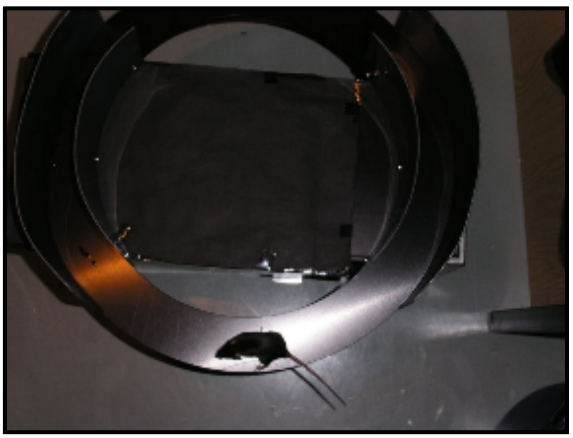


Figure 8. The 0-maze test apparatus.

2.4.2 Study of hippocampus-dependent performance in:

2.4.2.1 Food-displacement tube test

Control and stressed mice were analyzed in a test for hippocampus-dependent function. Burrowing behavior, a tendency to displace food pellets from a tube inside the home cage, a species-specific behavior in mice, was shown to require an intact hippocampal formation, as cytotoxic hippocampal lesions impair this task (Deacon *et al*., 2002; Deacon, 2009). Using a simple version of this test with a paper tube (internal Ø = 4cm, length 10cm), filled with 20 food pellets and situated in the mouse home cage, latency of displacement of the first food pellet, time required to empty the tube, number of pellets out after 1h and number of pellets removed in 1h30 were assessed in stressed and control mice (Strekalova and Steinbusch, 2009).

2.4.2.2 Contextual fear-conditioning paradigm

To assess effects of ethological chronic stress on contextual memory, we employed a contextual fear-conditioning paradigm. This test is a type of Pavlovian learning task in which we measured the ability of mice to learn and remember as association between an aversive stimulus and a contextual environment [10]. The fear-conditioning paradigm is well documented to be a model of hippocampus-dependent memory (Maren and Fanselow, 1996; Impey *et al*., 1999; LeDoux, 2000), and it was performed in 2 sessions, on consecutive days. During the first session (training session) mice were placed in a plastic apparatus (25cm x 25cm x 50cm), with translucent walls, placed on a stainless-steel grid floor (33 rods, 2mm in diameter) wired to a customized shock generator (Evolocus LLC Tarrytown, NY, USA and Techsmart, Rome, Italy; Figure 9). The apparatus was illuminated with a white light (25 lux).

Mice received an electric foot shock (0.8 mA, 2s), after which they were immediately returned to their home cage. On the second session (recall session, 24h after the first session), the same procedure was applied, but without electric shock. Freezing behavior is a typical response displayed by rodents when they are exposed again to the context in which they had previously experienced the brief inescapable shock (Sandi *et al*., 2001). This behavior is defined by the complete immobilization of the animal, and it was scored every 10s during a total of 180s, by visual observation. Data were calculated as the percentage of time spent freezing during the entire session [(Time spent freezing/Duration of the session) * 100], taken as a measure of learning. The behavior was also recorded on video.

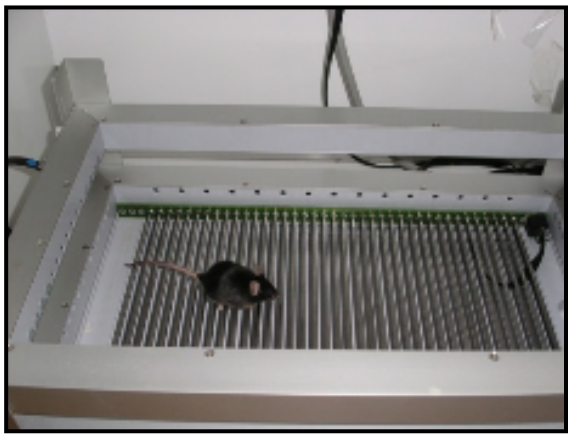


Figure 9. Contextual fear-conditioning apparatus.

2.4.3 Evaluation of the open-field activity at different times of the light cycle

This test was used to assess changes in the locomotor activity of mice, during both dark and light periods of the light cycle, since depression is commonly characterized by changes in circadian activity. The open-field apparatus (50cm x 50cm x 40cm) consisted in four square arenas (25cm x 25cm x 40cm), made of wood covered by white resopal. Mice were put in the center of one of the four square open field arenas (Figure 10, A), and their behavior recorded on web camera for 10min. The open field was illuminated with red light during the dark phase and with white light (25 Lux) during the light phase of the cycle, in order to mimic normal lighting conditions of animal housing. After the test, the mouse was removed from the box and the sessions were later analyzed using Any-maze (Figure 10, B).

In this program, each arena was divided into two different zones, periphery and center, and the behavioral variables assessed were the total distance travelled by the animal, mean speed, total immobilization time and time spent, distance travelled, number of entries and time spent immobile in each zone. In this context, anxiety-related behavior was measured by the degree to which the rodent avoids the center of the open-field.

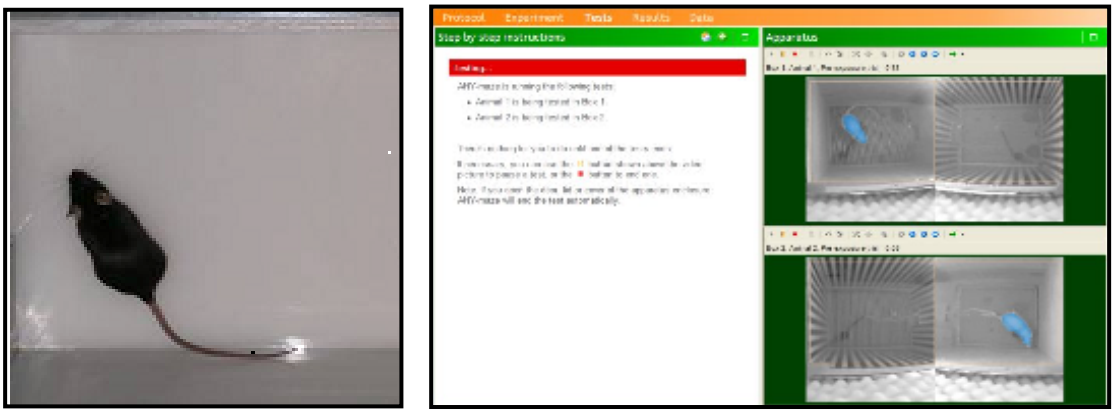


Figure 10. (A) C57BL/6 mouse inside one of the four arenas in the open-field apparatus. (B) Image of the Any-maze program [9].

2.4.4 Assessment of hedonic status in the sucrose test

The sucrose test was performed as described above (*See 2.3.1.1 Sucrose Test*), for a period of 24h.

2.5 Sacrificing and brain dissection

At the end of the experiment, euthanasia was performed by cervical dislocation, in both C57BL/6 and CD1 mice. As we wanted to evaluate molecular changes in the brain, it was not possible to anesthetize the animals before this procedure. After applying cervical dislocation, C57BL/6 mice were decapitated, and the blood was collected inside heparin coated centrifuge tubes for posterior evaluation of hormonal levels. The brain was extracted from the skull and separated in its two halves. The left part of the brain was kept as a whole, whereas the right part was dissected. From this part of the brain we separated the prefrontal cortex, the cerebellum and the hippocampus (Schneider, 2007).

2.6 Statistical analysis

Data were analyzed with a statistical software package, Statistica 9 (StatSoft, Tulsa, USA). Repeated measurements in the water drinking tests were evaluated by the non-parametric Wilcoxon test, as few values in these studies were arbitrary low (i.e. comparable to the error of measurement). Body weight changes were analyzed by repeated measures analysis of variance (ANOVA) with two factors (control vs. stress groups, before stress vs. after stress).

Independent data sets for the sucrose test, 0-maze test, contextual fear-conditioning paradigm and food-displacement tube test, and open-field test were analyzed using non-parametric Mann-Whitney *U* tests. Pearson product moment correlations were performed between the percentages of time spent freezing in the fear-conditioning paradigm and latency to take the first pellet out, latency to empty the tube, number of pellets taken out in 1h and in 1h30, from the food-displacement tube test. All statistical procedures were set at $\alpha = 0.05$.

Chapter 3 – Results

3.1 Inter-individual variability in water drinking patterns

In order to define limitations in the sucrose test and to choose its optimal duration, we studied daily dynamics of water consumption in C57BL/6. Therefore, we evaluated water intake in 4 measurements every 2,5h, during the dark phase of the mouse light cycle in the course of 3 consecutive days. Figure 11 shows that absolute intake of liquid varies significantly from one day to the other, which is confirmed by the Wilcoxon test (Day 2 from Day 1: T = 65,5, p = 0,027; Day 3 from Day 1: T = 31, p = 0,001; Day 3 from Day 2: T = 48, p = 0,01).

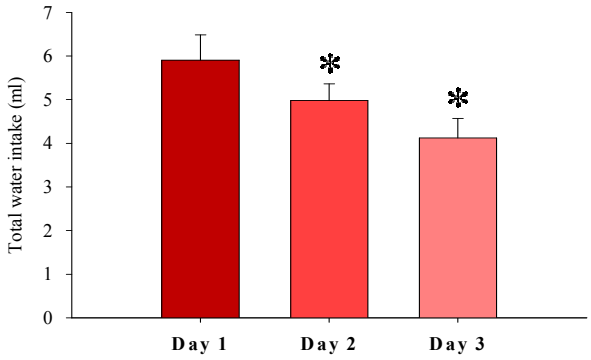


Figure 11. Day to day changes of water intake in C57BL/6 mice. The total water intake significantly varies from day 1 to day 2 and day 3 of consecutive measurements ($0,01 \leq p < 0,03$; Wilcoxon test). Data on graphs are expressed as mean ± standard error of measurement (SEM). * denotes significantly different from day 1.

Figures 12, 13 and 14 represent daily patterns in drinking behavior evaluated during 3 consecutive days. During day 1 (Figure 12, A-D), 21,74% of the animals showed their peaks in water intake 2,5h after the onset of the test (A). The same percentage of animals showed a peak 5h and 10h after the onset of the test (B, D). The remaining 34,78% showed a peak 7,5h thereafter (C). Data obtained on day 2 (Figure 13, E-H), indicated that 20% and 0% of animals maintained their peak in water consumption respectively 2,5h and 10h after the beginning of the test (E, H). The percentage of animals with peaks on day 2 at +5h and +7,5h was 80% and 50%, respectively, in comparison to data obtained on day 1 (F, G). Data obtained on day 3 indicate that no animal showed a peak in water drinking +2,5h after the

onset of the test (Figure 14, J-L). Seventy-five percent and 60% of mice maintained their peaks in drinking at time points 7,5h and 10h after the beginning of the test, respectively (K, L). Sixty percent of animals with a peak in drinking at 5h after the onset of the test, maintained their patterns of drinking behavior, shown on day 1 (J).

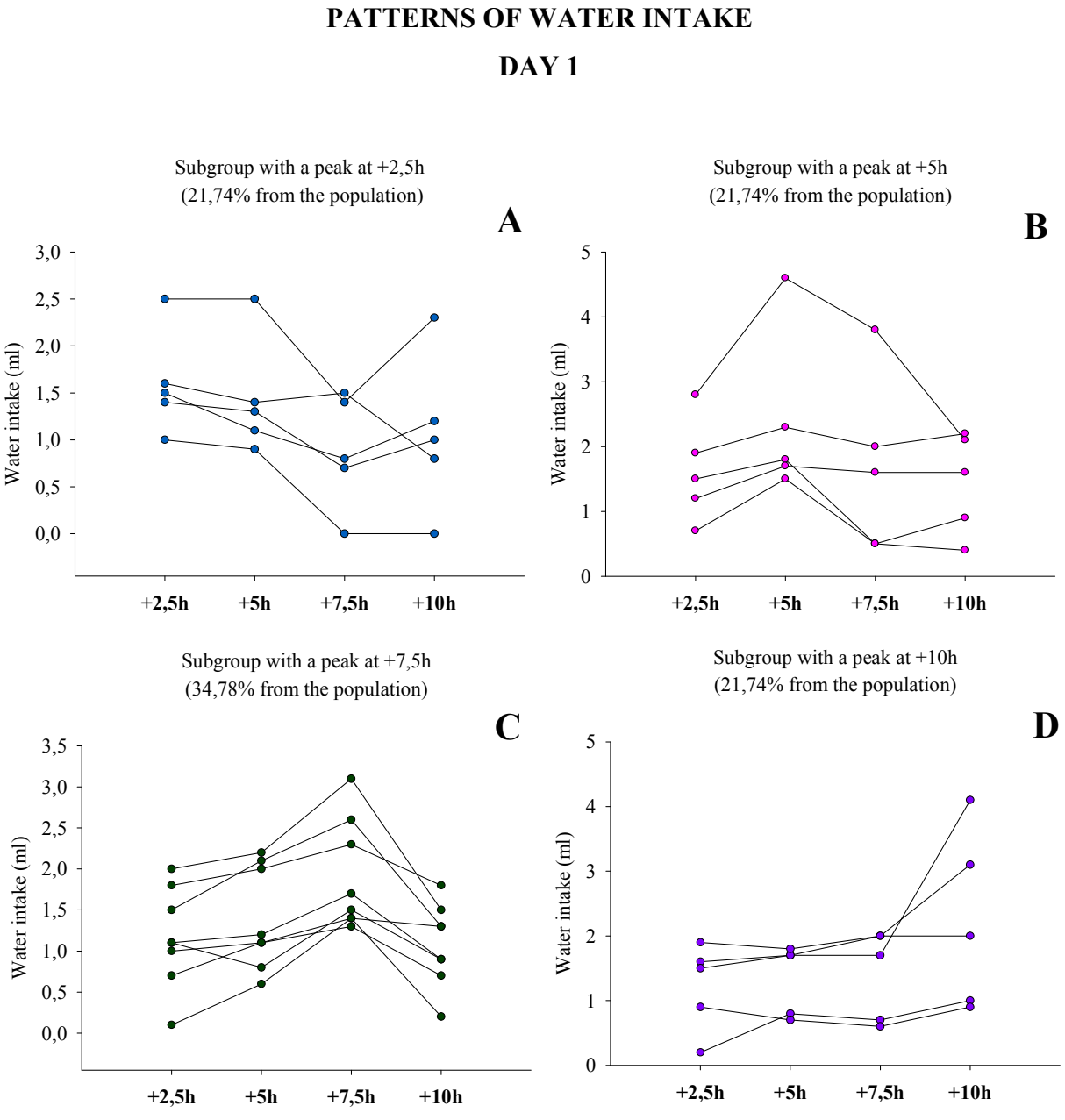


Figure 12.

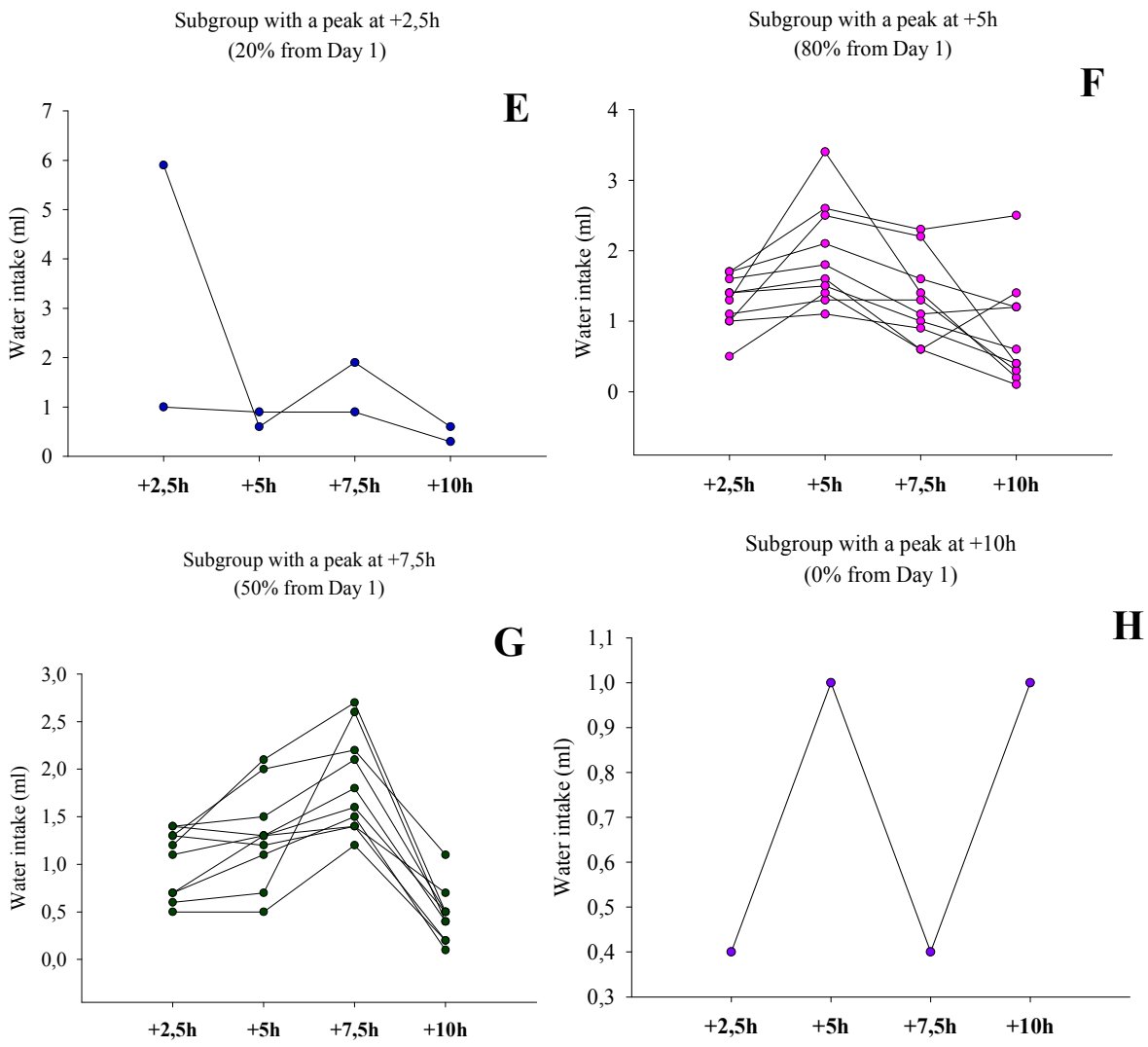
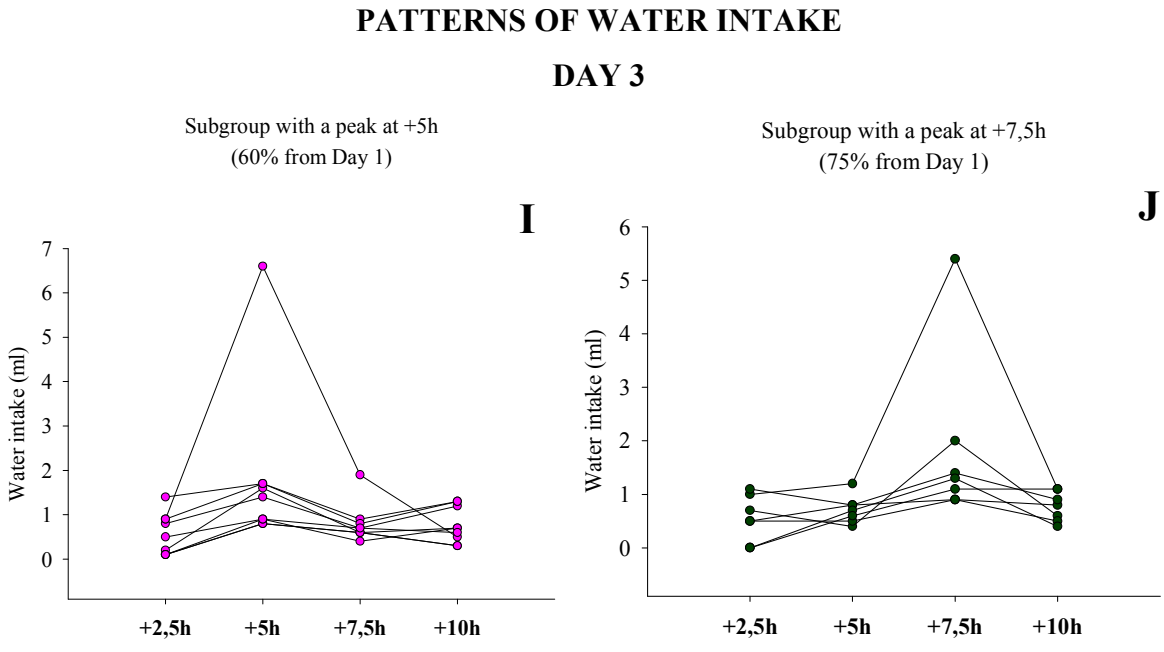

PATTERNS OF WATER INTAKE
DAY 2
Subgroup with a peak at +2,5h
(20% from Day 1)
E
Subgroup with a peak at +5h
(80% from Day 1)
F
Subgroup with a peak at +7,5h
(50% from Day 1)
G
Subgroup with a peak at +10h
(0% from Day 1)
H
Water intake (ml)
+2,5h
+5h
+7,5h
+10h

Figure 13.


PATTERNS OF WATER INTAKE
DAY 3
Subgroup with a peak at +5h
(60% from Day 1)
I
Subgroup with a peak at +7,5h
(75% from Day 1)
J
Water intake (ml)
+2,5h
+5h
+7,5h
+10h

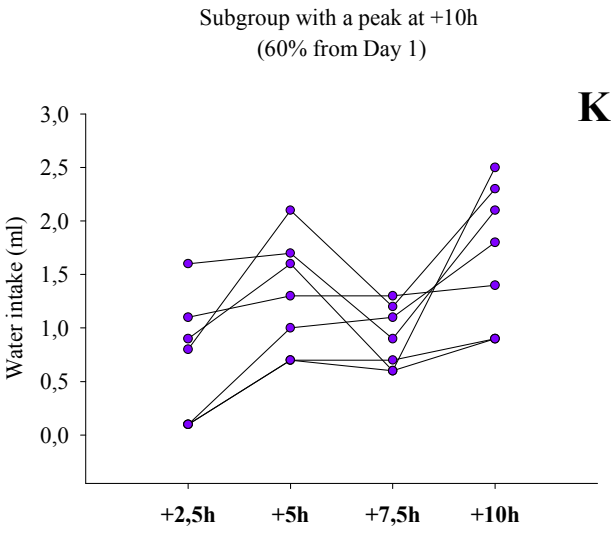


Figure 14.

Figures 12, 13 and 14. Study with individual water drinking behavior during 3 consecutive days revealed four patterns of drinking behavior in mice (2,5h, 5h, 7,5h and 10h after the onset of the test). Graphs A to D from Figure 12 represent drinking patterns assessed on day 1, E to H from Figure 13 the patterns assessed on day 2, and I to K from Figure 14 on day 3. Water intake was found to vary largely from one day to the other, evidencing pronounced variability of daily dynamics in water intake in C57BL/6 mice. Each line represents an animal and each dot the absolute water intake at that time point.

3.2 Study of the effects of ethological chronic stress in C57BL/6 mice

3.2.1 Group formation based on baseline weight, sucrose test data and social status.

Before the onset of the chronic stress procedure, the thirty-two C57BL/6 mice were divided into two groups, control (n = 10) and stress (n = 22) groups, based on their body weight, sucrose test data and their social status. Stress and control groups were balanced upon these 3 parameters so their means had similar values (Table V). Mice showed no significant differences in these parameters prior to stress ($p > 0,05$).

Table V. Baseline behavior of mice from control and stress groups.

	Sucrose Test				Weights (g)	Resident-intruder Test		
	Sucrose preference (%)	**WI (ml)**	**SI (ml)**	**TLI (ml)**		**Aggressive**	**Non-aggressive**	**Neutral**
Control	75,16 ± 1,9	0,96 ± 0,07	2,99 ± 0,23	3,95 ± 0,24	28,73 ± 0,29	40%	60%	0
Stress	72,09 ± 1,48	0,97 ± 0,05	2,64 ± 0,19	3,61 ± 0,21	28,59 ± 0,34	36,36%	63,64%	0

WI – Total water intake; **SI –** total sucrose intake; **TLI –** total liquid intake. Data are show as mean ± SEM, except for the resident-intruder test, where we present the percentage of aggressive and non-aggressive mice per group.

3.2.2 Chronic ethological stress enhanced liquid intake but does not affect sucrose consumption.

A Mann-Whitney *U* test revealed non-significant effects of stress on sucrose preference ($U = 86$, $p = 0,34$; Figure 15, A), and on sucrose intake ($U = 107$, $p = 0,92$; Figure 15, B), control group versus stress group. Stressed mice showed a tendency toward increased water intake ($U = 71,5$, $p = 0,1$; Figure 15, C), and toward total liquid consumption ($U = 69,5$, $p = 0,1$; Figure 15, D), when compared to control animals.

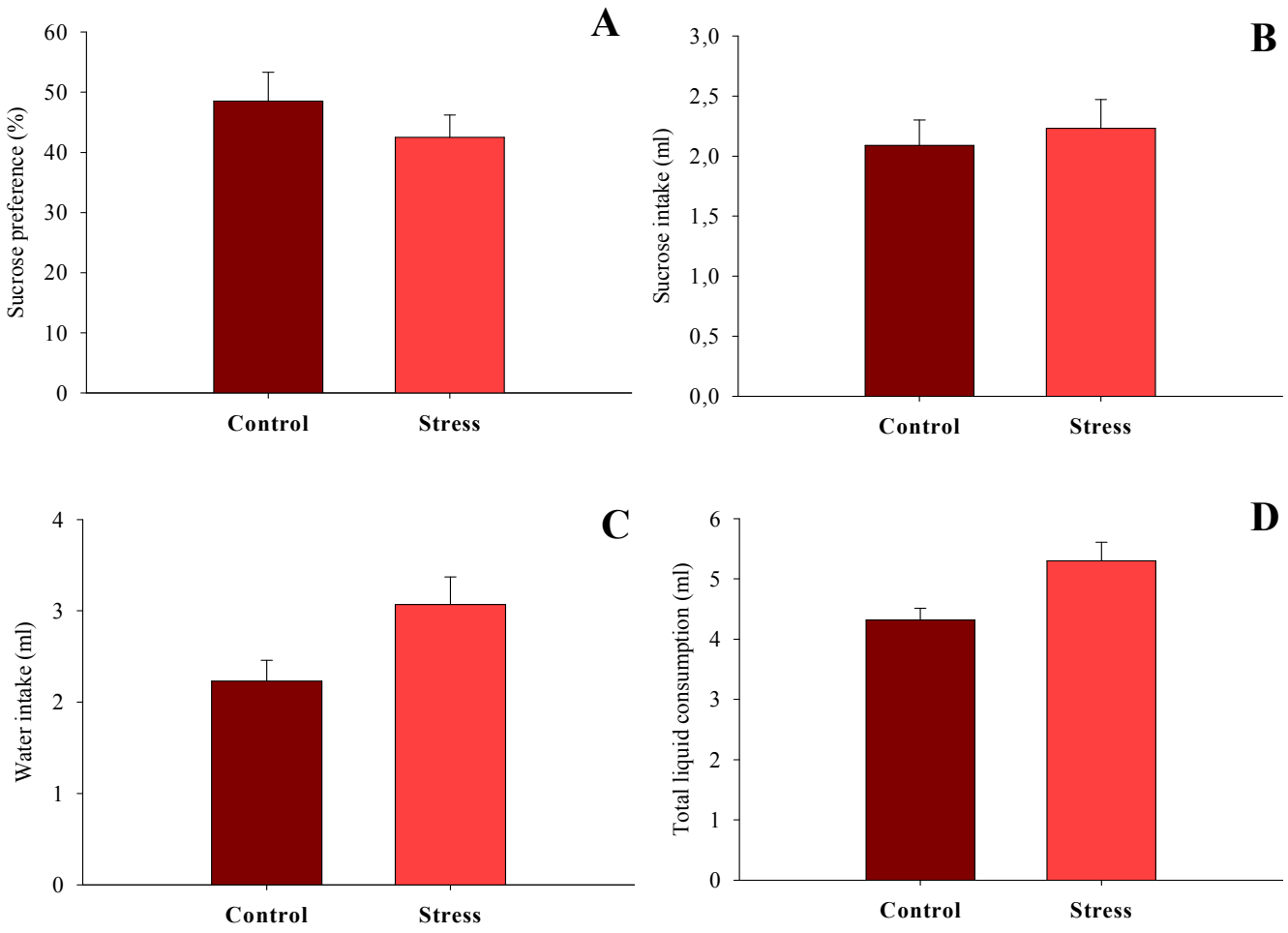


Figure 15. Effects of a 2-week stress on parameters of the sucrose test. (A) Sucrose preference showed no significant differences between both groups, as well as sucrose intake (B) ($p > 0,05$ vs. control group; Mann-Whitney). Stressed mice showed a tendency to have increased water intake (C) and total liquid consumption (D) ($p < 0,1$ vs. control group; Mann-Whitney). Data on the graphs are expressed as mean ± standard error of measurement (SEM).

3.2.3 Stress caused a reduction of body weight

Repeated-measures ANOVA revealed a significant effect of stress on body weight in mice. Mice from stress group significantly reduced their body weight ($F_{1,17} = 56,98$, $p < 0,0001$; Figure 16), as compared to non-stress control and in comparison to a baseline measure ($F_{1,17} = 31,39$, $p < 0,0001$). Control group of mice did not change body weight over a 2-week period of time ($p > 0,05$).

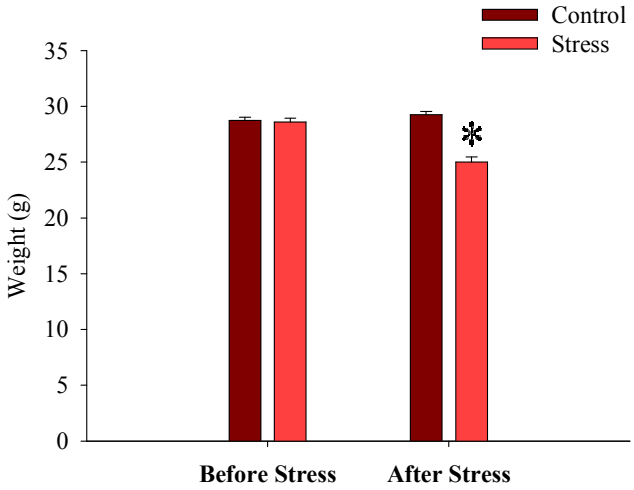
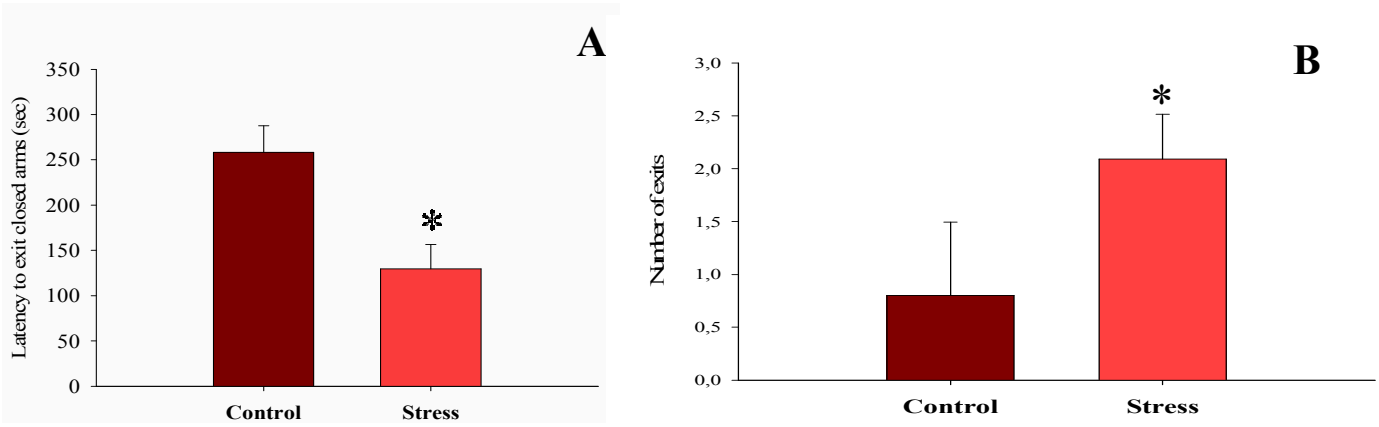


Figure 16. Weight changes after the application of a chronic stress procedure to stress group. Stressed animals showed a significant reduction of their body weight ($p < 0{,}0001$ vs. control). Data on the graphs are expressed as mean ± standard error of measurement (SEM). * denotes significantly different from control.

3.2.4 Stressed mice showed reduced scores of anxiety

In order to evaluate effects of stress on anxiety-like behavior, we applied the 0-maze test. Mice from the stress group had significantly reduced latency of exit to the anxiety-related area (open arms), than animals from the control group ($U = 49{,}5$, $p < 0{,}015$; Figure 17, A). They also have more exits to the open arms as compared to non-stressed mice, and spent significantly more time in the open arms ($U = 58{,}5$, $p < 0{,}038$; Figure 17, B and $U = 55{,}5$, $p <= 0{,}028$; Figure 17, C, respectively).



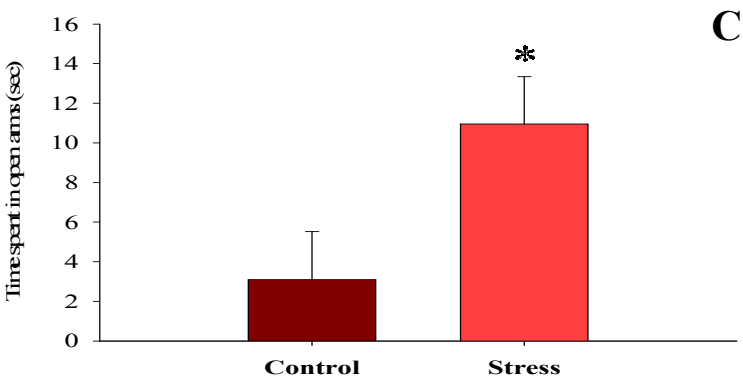


Figure 17. Exposure to a 2-week ethological stress resulted in decreased anxiety in the 0-maze test. (A) Latency of exit to the open arms was significantly decreased in stressed mice, in comparison to the control group ($p < 0,05$ vs. control group; Mann-Whitney). (B) Number of exits to the open arms in 0-maze was significantly higher in stressed mice compared to control group ($p < 0,05$ vs. control group; Mann-Whitney). (C) Stressed mice spent significantly more time in the open arms of the 0-maze in comparison to control group ($p < 0,05$ vs. control; Mann-Whitney). Data on the graphs are expressed as mean ± standard error of measurement (SEM). * denotes significantly different from control.

3.2.5 Hippocampus-dependent performance was impaired by stress exposure

To study the effects of stress on hippocampus-dependent performance, which is an important hallmark of depressive syndrome, we used two tests: contextual fear-conditioning paradigm and food-displacement tube test. During the contextual fear-conditioning test, mice from the stress group spent significantly less time freezing in comparison to control group ($U = 49,0$, $p < 0,019$; Figure 18), suggesting disrupted contextual memory in stressed mice.

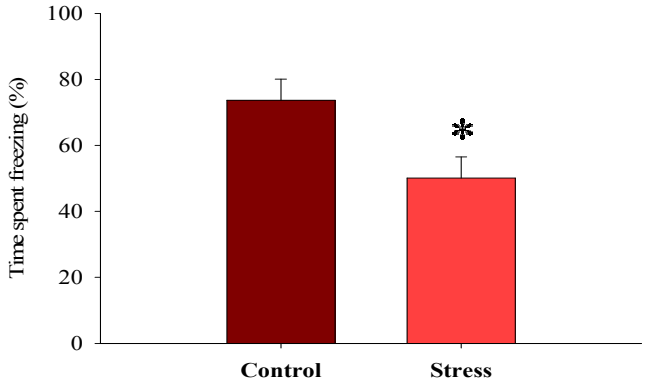


Figure 18. Stressed mice spent significantly less time freezing as compared to control mice, during the fear-conditioning paradigm ($p < 0,005$ vs. control group; Mann-Whitney). Data on the graphs are expressed as mean ± standard error of measurement (SEM). * denotes

significantly different from control.

In the food-displacement tube test, stressed mice showed a tendency to increased latency of displacement behavior, as compared to control mice (U = 73,0, p < 0,14; Figure 19, A). Also stressed mice showed a tendency to show reduced number of pellets displaced from the tube after 1h (U = 71,0, p < 0,12; Figure 19, B). No significant changes in this parameter were measured after 1h30 from the beginning of the test (U = 85,0, p < 0,32; Figure 19, C) and non-significant increase in the latency to empty the tube (U = 84,0, p < 0,3; Figure 19, D).

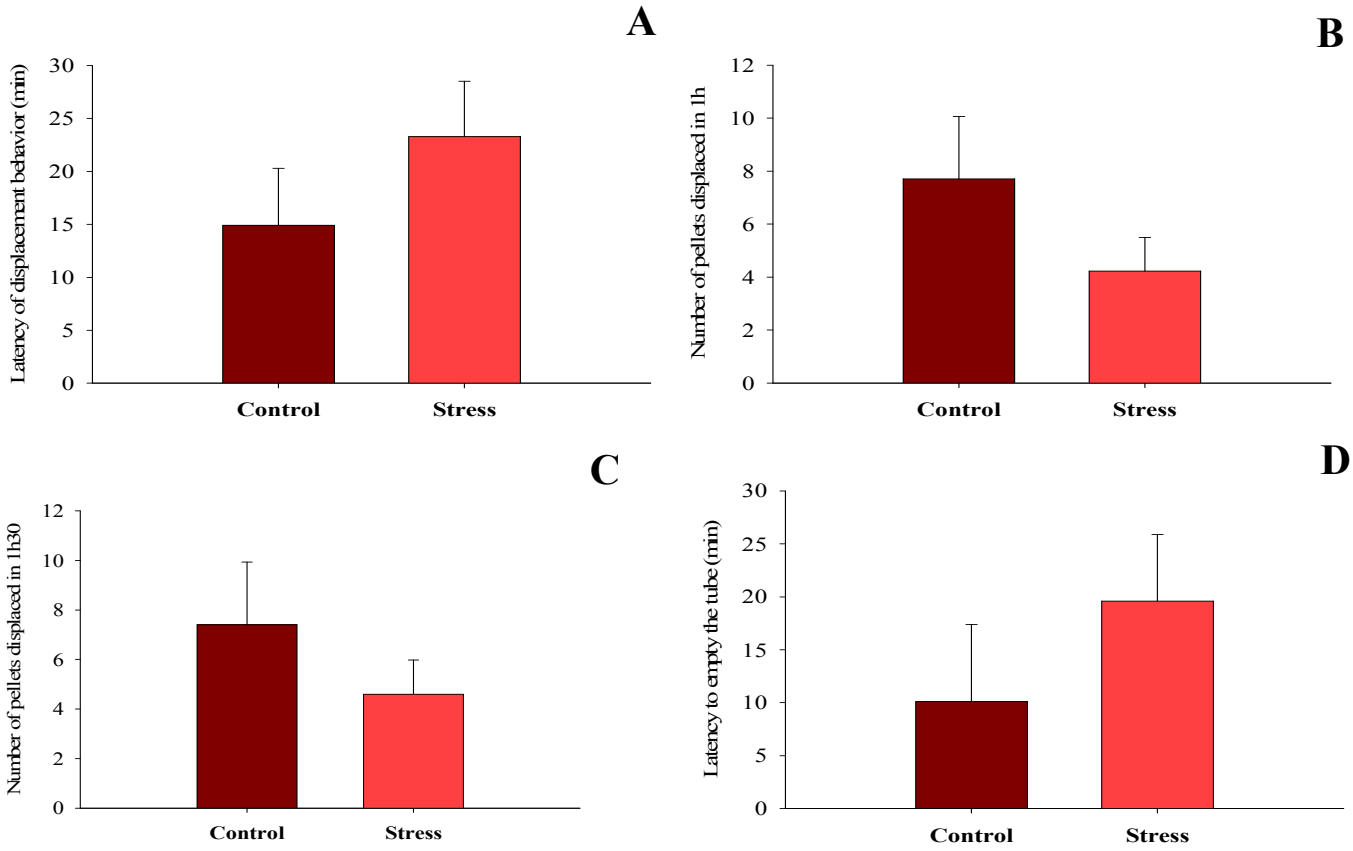


Figure 19. Stressed mice showed a tendency to a decreased burrowing behavior in the tube test. (A) Stressed mice showed a tendency to increased latency of displacement behavior, but no significant difference in the latency to empty the tube (D), when compared with control group (p = 0,1 vs. control; Mann-Whitney). (B) In the number of pellets displaced in 1h from the beginning of the test, stressed animals showed a tendency to show reduced number of pellets displaced; however, both groups showed no significant differences between them in the number of pellets displaced 1h30 after the onset of the test (p > 0,05 vs. control; Mann-Whitney). Data on the graphs are expressed as mean ± standard error of measurement (SEM).

3.2.6 Correlation of changes in contextual fear-conditioning and food-displacement behavior in stressed mice

A positive correlation was found between the percentage of freezing time and the number of pellets taken out in 1h and in 1h30 (r = 0,36, p = 0,046 and r = 0,36, p = 0,048, respectively; Table VI), thus suggesting a correlation between 2 parameters of hippocampus-dependent function. No significant correlations were found between the percentage of freezing time and the latency to take the first pellet out or the latency to empty the tube, in the food-displacement test (p = 0,39 and p = 0,50, respectively; Table VI).

Table VI. Stress-induced disruption in contextual fear-conditioning correlates with changes in burrowing behavior. Correlations between the percentage of contextual freezing in the fear-conditioning paradigm and the latency of displacement behavior, number of pellets displaced by the first hour and by the first 1h30 and the latency to empty the tube, in the food-displacement tube test. There were significant positive correlations between contextual freezing and number of pellets displaced in 1h and in 1h30 (p < 0,05). * denotes positive correlation.

	Latency of displacement behavior	**Number of pellets displaced by the 1st hour**	**Number of pellets displaced by the 1st 1h30**	**Latency to empty the tube**
% Contextual Freezing	r = -0,16; p = 0,39	r = 0,36; p = 0,046*	r = 0,36; p = 0,048*	r = -0,13; p = 0,50*

3.2.7 Effects of stress on the open-field behavior

Table VII shows all parameters assessed in the open-field test, under red-lighting conditions. Nonparametric analysis showed non-significant differences between control and stress groups in all of the parameters assessed (p > 0,05). Table VIII shows the same parameters, only under 25-Lux conditions. Stressed mice spent significantly less time immobile overall (U = 55, p = 0,036; Figure 20, A) and in both the periphery (U = 61, p = 0,04; Figure 20, B) and the centre of the open field (U = 73,5, p = 0,03; Figure 20, C). There were no significant differences were seen between control and stress groups in the other parameters assessed (p > 0,05; Table VIII).

Table VII. Descriptive statistics (mean ± SEM) and probabilities (*p-values*) associated with Mann-Whitney *U* test on the parameters assessed in the open-field locomotion, tested under red lighting. Stress group showed no significant differences when compared to control group concerning all parameters assessed ($p > 0,05$ vs. control).

		Group		Mann-Whitney U test
		Control	**Stress**	U (p)
	Total Distance Travelled (m)	20,78 ± 1,91	21,14 ± 2,02	99 (ns)
	Mean Speed (m/s)	0,03 ± 0,003	0,03 ± 0,003	96,5 (ns)
	Total Time of immobility (s)	0	3,58 ± 1,82	67,5 (ns)
Periphery	**Number of Entries**	69,30 ± 7,73	62,95 ± 5,58	95.5 (ns)
	Time Spent (s)	346,00 ± 47,84	363,36 ± 24,84	102 (ns)
	Distance Travelled (m/s)	9,53 ± 1,1	9,65 ± 0,86	103 (ns)
	Time Immobile (s)	1,02 ± 0,97	13,8 ± 5,56	68 (ns)
Centre	**Number of Entries**	87,44 ± 8,78	91,32 ± 10,92	87 (ns)
	Time Spent (s)	240,86 ± 48,58	225,26 ± 28,4	106 (ns)
	Distance Travelled (m/s)	13,20 ± 2,38	18,29 ± 5,9	98 (ns)
	Time Immobile (s)	0	1,3 ± 0,6	76,5 (ns)

Table VIII. Descriptive statistics (mean ± SEM) and probabilities (*p-values*) associated with Mann-Whitney *U* test on the total distance traveled and the mean speed assessed in the open-field locomotion tested under 25-Lux illumination. Total time immobile and time spent immobile both in the periphery and in the centre of the field were significantly decreased in stressed group compared to controls ($p < 0,05$ vs. control; Mann-Whitney). All the other parameters assessed showed non-significant differences between groups ($p > 0,05$ vs. control).

		Group		Mann-Whitney U test
		Control	**Stress**	U (p)
	Total Distance Travelled (m)	12,97 ± 1,15	12,57 ± 0,94	85 (ns)
	Mean Speed (m/s)	0,035 ± 0,003	0,033 ± 0,003	70,5 (ns)
	Total Time Immobile (s)	11,54 ± 3,36	3,45 ± 1,30	55 (**0,036**)
Periphery	**Number of Entries**	46,5 ± 3,54	49,1 ± 3,39	89,5 (ns)
	Time Spent (s)	423,83 ± 43,74	392,86 ± 33,59	98 (ns)
	Distance Travelled (m/s)	7,46 ± 0,98	6,18 ± 0,38	77 (ns)
	Time Immobile (s)	9,2 ± 2,78	2,97 ± 1,04	61 (**0,04**)
Centre	**Number of Entries**	51 ± 4,09	5,99 ± 0,89	89,5 (ns)
	Time Spent (s)	191,91 ± 30,68	242,11 ± 32,71	82 (ns)
	Distance Travelled (m/s)	5,53 ± 0,62	5,99 ± 0,89	97 (ns)
	Time Immobile (s)	1,33 ± 0,84	0	73,5 (**0,03**)

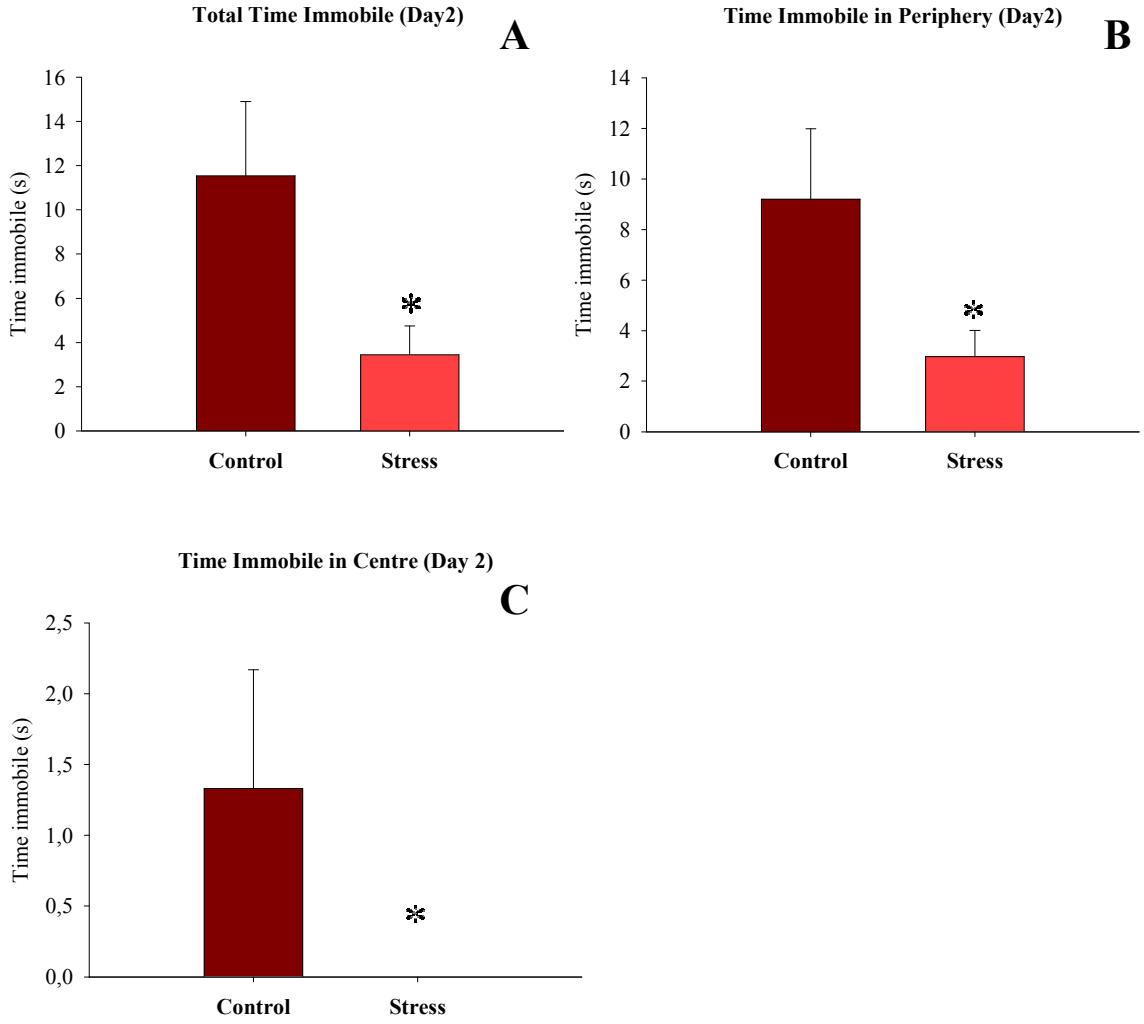


Figure 20. Stress appears to reduce immobility time in the open-field test. (A) Total time immobile was significantly decreased in stressed mice compared to control group, as well as time spent immobile in the periphery (B) and in the centre of the open-field (C) ($p < 0,05$ vs. control; Mann-Whitney). Data on the graphs are expressed as mean ± standard error of measurement (SEM). * denotes significantly different from control.

Chapter IV – Discussion

Previous studies showed that the application of chronic stress for prolonged periods of time induces, in rodents, a depressive-like syndrome and anhedonia (Katz, 1981; Willner *et al*., 1987; Pothion *et al*., 2004; Strekalova *et al*., 2004; Ducottet and Belzung, 2005). Katz *et al*. (1981) applied a 3-week chronic stress protocol in rats, causing a decrease in sucrose intake and increased corticosterone levels. Willner and co-workers chose to apply milder stressors for a 3-month period, inducing a longer lasting decrease in sucrose intake (Willner *et al*., 1987; Willner, 1997, 2005). One of the well validated protocols of induction of depressive-like syndrome by exposure to a chronic stress was a 4-week chronic stress procedure (Strekalova *et al*., 2004), which resulted in a decrease in sucrose preference, increased anxiety, altered locomotor activity and loss of body weight.

However, for ethical reasons, more effort is made now to decrease stress load and discomfort in laboratory animals as much as possible. Our project was therefore aimed to shorten the duration of the chronic stress procedure (2 weeks), and limit ourselves by the use of ethological stressors only. We addressed a question whether this procedure could induce anhedonia, other features of depressive-syndrome and other consequences of stress in mice.

Therefore, we evaluated behavioral effects of a 2-week ethological stress procedure on hedonic status in the sucrose test, on anxiety-like behavior in the 0-maze test, locomotion in the open-field test and hippocampus-dependent performance in the food-displacement tube test and in the contextual fear-conditioning paradigm. This results were compared to behavioral correlates found in a 4-week stress procedure, reported earlier (Strekalova *et al*., 2004).

4.1 Inter-individual variability in water drinking patterns in C57BL/6 mice

Consumption of palatable solutions, including sucrose solution, is commonly used to assess hedonic status in rodents. At the moment, this is the most used method to detect anhedonia in mice and rats. However, the protocols for measuring of intake of palatable solutions vary greatly, from the number of hours chosen for its application (for example, 1h, Katz, 1982; Grippo *et al*., 2003; 10h, Strekalova and Steinbusch, 2009), to the concentration of sucrose solution used (1%, Willner *et al*., 1987; 34%, Papp *et al*., 1991; 2% or 4%, Monleon *et al*., 1995). The validity and reproducibility of the sucrose test was questioned in

the past (Nestler, 2005). Here, we assessed dynamics of water consumption in C57BL/6 mice, in order to define limitations in the sucrose test and choose its optimal duration for our study.

Our experiments demonstrated that the absolute amount of liquid consumed varied significantly during the 3-day testing period. There was a significant difference in the 24h volume of water intake between day 1 and day 2, day 1 and day 3, and day 2 and day 3 (Figure 11). The volume of water consumed was decreasing from day 1 to day 2 and day 3, which can be explained by possible fluctuations in the temperature; when the temperature decreases, so does the water consumption in rodents. Another reason for this effect can be that animals prefer to consume fresh water (O'Callaghan *et al*., 2002; Strekalova, 2008). Hence, our data suggested that it is not reliable to use repeated measures to assess liquid intake.

Second, we found the existence of four patterns of drinking behavior in C57BL/6 mice. Mice showed peaks of maximum water intake at various time points with respect to the onset of the dark-phase of the light cycle: +2,5h, +5h, +7,5h and +10h, thereafter (Figures 12, 13 and 14). These results are consistent with previous data (Strekalova, 2008),

We also found that, in general, animals did not maintain the same consumption peak throughout the days, thus evidencing a large variability of daily dynamics of water intake. Our results demonstrated the importance of a prolonged testing of drinking behaviors. Hence, we have chosen to apply our sucrose test for 24h, in order to avoid the use of protocols of shorter duration which may be confounded by individual patterns in animal drinking. This measure also helped to minimize potential neophobic reactions towards unusual drinking situation and sucrose taste.

Our data were also in line with previous findings which showed that mice usually demonstrate a large individual variability in sucrose preference and in absolute values of liquid consumption (Monleon *et al*., 1995; Adriani *et al*., 2002; O'Callaghan *et al*., 2002). Present data suggested that the application of absolute values of sucrose consumption may not be reliable in evaluating reward sensitivity. Data also showed that direct comparison of absolute values of consumed liquids is not adequate in evaluation of dynamics of liquid consumption in repeated measurements tests.

4.2 Study of the effects of ethological chronic stress in C57BL/6 mice

Chronic stress is a commonly used model of depression. Application of chronic stress protocols of various duration (from 2-12 weeks) and intensity was shown to induce parallels

of human depressive symptoms in rodents: anhedonia, anxiety, impairment in hippocampus-dependent performance and locomotion (Willner *et al*., 1987, 1992; Pucilowski *et al*., 1993; D'Aquila *et al*., 1994; Gould and Tanapata, 1999; GrØnli *et al*., 2005; Strekalova and Steinbusch, 2009).

In our study, we assessed whether the ethological procedure of 2-week chronic stress affected hedonic status in a sucrose test, anxiety-like behavior in the 0-maze test, and hippocampus-dependent performance in the food-displacement tube test and in the contextual fear-conditioning test, and locomotion in the open-field test. These data are related to previously reported findings with 4-weeks stress procedure (Strekalova *et al*., 2004).

4.2.1 Chronic ethological stress enhanced liquid intake but does not affect sucrose consumption

After the application of a chronic stress in mice, sucrose intake and preference is usually decreased in 50 – 70% of stressed animals to values below 65%, which is taken as an indicator of the presence of anhedonia, a core symptom of depression (Moreau *et al*., 1992; Pucilowski *et al*., 1993; Willner, 1997; Strekalova *et al*., 2004, 2008; GrØnli *et al*., 2005; Craft and Devries, 2006; Grippo *et al*., 2006). This decrease in sucrose preference is often accompanied by a decrease in sucrose intake and an increase in water consumption (Harris *et al*., 1997; Murison and Hansen, 2001; Willner, 2005; Strekalova, 2008). Besides a decrease in sucrose intake, chronic stress can induce liquid consumption (Willner, 2005; Strekalova *et al*., 2004).

The importance of mice having preferable (sucrose) and nonpreferable solution (water) on each side of the cage during equal periods of active and inactive phases in the evaluation of drinking behavior in the sucrose test was shown by Kant and Bauman (1993) and Strekalova and Gass (2002). In the latter work, an important effect of the influence of side preference, neophobia and housing on drinking behavior in a two-bottle paradigm was identified in mice (Strekalova and Gass, 2002). Hence, we attempted to balance the effects of side preference in drinking behavior by switching the bottles in the middle of the test, which was equilibrated with the circadian cycle.

Before the onset of our experiment, both control and stress groups of mice showed a preference for a 1% sucrose solution over water (75,16 ± 1,9% and 72,09 ± 1,48%, respectively; Table V). After the stress procedure, although we've seen that stress enhanced water intake in stressed mice, both groups showed no preference for sucrose solution over

water (control = 48,54% ± 4,78, stress = 42,52% ± 3,68; Figure 15, A). Importantly, the control group revealed abnormal values in this parameter. This anomalous behavior might be due to a failure in the sucrose test, rather than in the stress procedure itself. For technical reasons, this test was not possible to repeat again. However, these results suggest that our stress procedure of shorter duration and intensity did not lead to detectable changes in the anhedonic state in mice. The induction of anhedonia might require a certain stress load, which was not achieved in this model.

Stressed animals also showed a tendency toward increased water intake (Figure 15, C). These signs of elevated consummatory behavior were paralleled with other signs of behavioral invigoration, such as increased activity during tests of anxiety-like behavior (Strekalova, 2008), which match our findings in the 0-maze and open-field tests (see below). Another explanation for the increase in liquid consumption might be the development of stress-induced polydipsia, a feature in which the animal displays excessive thirst (Porth, 1990). Schoenecker *et al*. (2000) suggested that this feature may result from general sympathetic activation and an increase in metabolic needs in water or diabetes mellitus. Another possibility which can be considered is alterations in secretion of the hypothalamus and hypophysis (Cole and Koob, 1994; Skuse *et al*., 1996). Thus, an increase in water consumption in stressed animals is regarded as a sign of pronounced response from the animals towards stress (Willner, 2005; Strekalova, 2008).

4.2.2 Stress caused a reduction of body weight

Initially, control and stress animals had similar body weight. After our 2-week stress procedure, stressed animals showed a significant decrease in body weight, indicating an effect of stress (Figure 16). These findings were consistent with previous reports which describe loss of body weight during stress to be a function of stress intensity and duration (Moreau *et al*., 1992; Pucilowski *et al*., 1993; Papp *et al*., 1994; Kopp *et al*., 1999; Von Frijtag *et al*., 2000; Harkin *et al*., 2002; Negroni *et al*., 2004; Ducottet and Belzung, 2005; Strekalova, 2008).

4.2.3 Stressed mice showed reduced scores of anxiety

Clinical data reports that anxiety and depressive disorders are often comorbid (Strekalova, 2008). Therefore we used the 0-maze test to evaluate anxiety-like behavior as elevated anxiety is often a consequence of exposure to chronic stress. In the 0-maze test, anxiety is assessed by an increase in the latency to exit to anxiety-related areas, the open arms of the 0-

maze, and by a decrease in the number of exits and in the time spent in open-arms (Strekalova *et al*., 2003, 2005a, 2005b). This data comes in line with the clinical view that anxiety and depressive disorders are often comorbid pathologies (Stahl, 1996; Charlton, 2000; Freeman *et al.*, 2002; Kalueff *et al*., 2007).

However, the results obtained in our study revealed an anomalous behavior in stressed animals. Our stressed mice showed a decreased latency to exit to the open-arms of the 0-maze and an increase in the number of exits and time spent in the open-arms (Figure 17, A, B and C), showing no anxiety-like behavior.

General locomotion in rodents can be affected in several ways by chronic stress. It can induce unspecific behaviors in mice such as hyperactivity, hypoactivity or it can have no effect on locomotion (Harris *et al*., 1997; Paré *et al*., 2000). In our case, paradoxical effects of stress found in our study are typical for the effects of chronic stress on C57BL/6 mice. Hyperactivity is a common feature in C57BL/6 stressed mice and has been previously reported (Harris *et al*., 1997; Strekalova *et al*., 2004). There have been several studies that describe the presence of anomalous behaviors in mice, after the exposure to prolonged stress. Animals submitted to a chronic stress paradigm, demonstrated an increase in the time spent in anxiety-related areas in several tests (0-maze test, elevated plus-maze, dark-light box), which may be interpreted as a sign of 'anxiolytic-like' behavior (D'Aquila *et al*., 1994; Cancela *et al*., 1995; Willner, 1997; Strekalova *et al*., 2005). The results obtained in our study, were consistent with this previous anomalous findings, i.e. exhibiting 'anxiolytic-like behavior', which means a reduction in anxiety-like behavior.

Other studies conducted in stressed mice and rats revealed that lighting conditions have a significant impact on general locomotion, and can induce changes in this feature in animals (Igarashi and Takeshita, 1995; Valentinuzzi *et al*., 2000; Bertoglio and Carobrez, 2002). Solberg *et al*. (1999) submitted mice to a 4-week chronic mild stress procedure, which was shown to inhibit the open-field activity when the animals were tested under housing light conditions. The same animals were then retested under bright lighting, which enhanced locomotion. Our animals were tested under 25-Lux lighting (bright lighting) and showed increased locomotion in the 0-maze test, showing that our results are in line with those found by Solberg.

The intensity (Harris *et al*., 1997; Laviola *et al*., 2002) and duration of applied stressors can also induce changes in locomotor activity and coping deficits in rodents. Blanchard *et al*. (1993) and Paré *et al*. (2000) showed that when rats were submitted to intense stress

procedures they would develop hyperlocomotion and when submitted to a lesser intense procedure the opposite happened, i.e. hypolocomotion. Cancela *et al*. (1995) found that a 4-week stress procedure caused hyperlocomotion, 'anxiolytic-like' changes, whereas a 1-week stress procedure did not induce these effects, anxiogenic effect.

Previously reported data also indicates that repeated testing as no effect in anxiety tests when testing conditions have a low stress impact and are spaced by several days (Onaivi and Martin, 1989; Prasad and Prasad, 1996; Wigger *et al*., 2001).

However, there hasn't been a systematic study of stress-induced changes in locomotion with respect to the duration of applied stress procedure and the characterization of testing conditions, including lighting.

Our data from the open-field test also demonstrated the occurrence of stress-induced hyperactivity, thus matching the results observed in the 0-maze (*see below*). Thus the 2-week stress procedure that we used induced changes in anxiety-like behavior of C57BL/6 mice which are typical for the effects in 4-week chronic stress in same mouse strain.

4.2.4 Hippocampus-dependent performance was impaired by stress exposure

Another characteristic feature of depressive phenotype is an impairment of the hippocampus-dependent function (Kendler *et al*., 2005). To study whether this new stress protocol affects hippocampus-dependent performance, we employed 2 tests. First we assessed burrowing behavior, which was shown previously by Deacon *et al*. (2002, 2009) to require an intact hippocampal formation, as cytotoxic lesions in this area impair this task. Second, as chronic stress also affects contextual memory we used a fear-conditioning paradigm, a known model for hippocampus-dependent learning (Maren and Fanselow, 1996; Impey *et al*., 1999; LeDoux, 2000).

In the present study, we found that stressed animals spent significantly less time freezing, compared to control animals, in the contextual fear-conditioning test (Figure 18) and that stressed animals had a tendency to increased latency of displacement behavior (Figure 19, A) and reduced number of pellets displaced from the tube 1h after the onset of the test (Figure 19, B), in the food-displacement test. The possibility that the observed reduction in freezing is merely a consequence of stress-induced hyperactivity, observed in the open field and 0-maze tests in the present study, is unlikely for at least two reasons. First, freezing behavior was defined by an occurrence of specific posture that is distinct from a lack of movement. Second, it was measured under lighting conditions which were shown to exclude the occurrence of

stress-induced hyperlocomotion (weak lighting was used to lit the fear conditioning apparatus). Finally, our data are in line with previous findings which reveled disrupted contextual learning in chronically stressed mice (Strekalova and Steinbusch, 2010).

Thus, this part of our study suggested impairment in hippocampus function in animals submitted to a 2-week stress. Hence, employed stress procedure induced similar deficits to those described for former proposed protocols of chronic stress, where disrupted contextual learning and burrowing behavior were found in stressed anhedonic animals (Strekalova, 2008). Therefore, 2-week chronic stress was found to disrupt contextual learn in the step down avoidance test (Strekalova *et al*., 2004).

4.2.5 Correlation of changes in contextual fear-conditioning and food-displacement behavior in stressed mice

Our study revealed a positive correlation between deficits in burrowing behavior and a disruption in fear-conditioning (Table VI). To our knowledge, this correlation is shown for the first time. Obtained data suggested that in chronically stressed mice the food-displacement test might be a valid procedure to detect hippocampus-dependent deficits (Strekalova and Steinbusch, 2009). This data showed the validity of the tube test in the evaluation of the hippocampus-dependent behavior.

4.2.6 Effects of stress on the open-field behavior

Locomotor disturbances are considered to be an important feature of depressive syndrome. We assessed locomotor behavior in chronically stressed mice using the open-field test lit either with red lighting or 25-Lux lighting. Earlier it was found that stressed mice display different behaviors in the open-field model dependently on lighting conditions. Stressed mice are reported to avoid the central area of the apparatus that is regarded as a sign of anxiety. Also, a number of findings revealed a significant increase in the open-field activity that is interpreted as a sign to hyperactivity. In our study, no significant differences were found between stressed and control animals during testing in the open-field model lit by the red-light, both in general parameters (mean speed, total distance travelled and time spent immobile) and in parameters assessed in periphery and centre of the apparatus (Table VII, figures). This data are in line with results obtained in the open-field model where 5-lux light was employed and where the red-light was used (Strekalova *et al*., 2004, 2005).

In the open-field test lit with 25-Lux lighting, we found significantly decreased time of immobility in stressed animals measured overall during the 10 min test and separately in both the periphery and the centre of the apparatus. As the presence of the animal in the centre of the apparatus is considered as a sign of 'anxiolytic-like' behavior, this data are in line with our findings in the 0-maze test (*see above*).

Stress induced hyperlocomotion is seen as a sign of impaired hippocampus function. Data from the open-field test, together with the results obtained in the 0-maze test (*see above*), suggest a hyperactivity caused by employed stress procedure, in C57BL/6 mice. This suggests a physiological impact of applied stress protocol. However, statistical analysis of the data revealed only tendencies in such changes. Weaker effects of 2-week stress procedure on locomotion could be explained by shorter and milder intensity when compared to other protocols, which use stronger lighting and longer and stronger stress procedures.

Chapter V – Conclusions and Future Directions

Our stress procedure of a shorter duration (2-weeks) and weaker intensity was not found to induce behavioral signs of anhedonia, a core symptom of depression, on C57BL/6 mice. It is possible that the induction of a hedonic state requires a certain stress load, which was not achieved by our chronic stress protocol. Obtained here results showed that the sucrose test has to be applied with care, as large variability in total liquid intake and daily dynamics of liquid consumption was found in mice. Data on daily water drinking patterns suggested the convenience of a) a test protocol of longer duration and b) the use of sucrose preference instead of absolute intake of sucrose solution as a measure of anhedonia in mice.

Changes in body weight, locomotion, anxiety and liquid consumption, which are typical features of prolonged exposure in mice, were induced by newly applied 2-week stress procedure. Data obtained in the 0-maze and open-field model point to the occurrence of stress-induced hyperactivity, a hallmark of prolonged stress exposure, in C57BL/6 mice. These results suggest an essential physiological impact of the applied stress protocol and are in line with confirmed former findings in chronic stress experiments in mice.

The impairment of the hippocampus-dependent performance is a hallmark of depressive phenotype. Disrupted hippocampus-dependent performance was found in mice submitted to a chronic stress in 2 tests, food-displacement tube test and contextual fear-conditioning test, thus suggesting the induction of depressive-like changes in our study.

We also showed, for the first time, that deficits in burrowing behavior correlate with a disruption in contextual fear-conditioning in chronically stressed mice. Importantly, our results suggest that the food-displacement test is a reliable test procedure to assess hippocampal-dependent deficits.

Together, our data leave the possibility open that anhedonia could be induced by our chronic stress model as it was not detected with the sucrose test. Data on the hippocampal plasticity and behavioral hyperactivity, as well as changes in the 0-maze test and body weight speak in favor of a positive conclusion in this respect. We conclude that applied here 2-week ethological stress induced a depressive-like phenotype and had essential physiological impact

in C57BL/6. However, more studies are required to validate the occurrence of hedonic deficit in newly used stress protocol: it is questionable whether shorter and weaker stress protocols can enable the induction of anhedonic status in mice. Such studies are underway.

Chapter VI – Bibliographic references

Adriani, W., Macri, S., Pacifici, R. and Laviola, G. (2002). Restricted daily access to water and voluntary nicotine oral consumption in mice: methodological issues and individual differences. *Behav Brain Res*. **134**:21-30.

Akhtar-Danesh, N. and Landeen, J. (2007). Relation between depression and sociodemographic factors. *Int J Mental Health*. **1**:4.

Altar, C.A. (1999). Neurotrophins and depression. *Trends Pharmacol Sci*. **20**:59-61.

Anisman, H. and Zacharko, R.M. (1982). Stimulus change influences escape performance: deficits induced by uncontrollable stress and by haloperidol. *Pharmacol Biochem Behav*. **17**(2):263-9.

Anisman, H. and Merali, Z. (2000). Understanding stress: characteristics and caveats. *Alcohol Res Health*. **23**:241–249.

Anisman, H. (2009). Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. *J Psychiatry Neurosci*. **34**(1):4-20.

American Psychiatric Association. (1994). Diagnostic and statistical manual of mental disorders, 4th edn (DSM IV). APA, Washington, D.C.

Armario, A., Gavalda, A. and Marti, J. (1995). Comparison of the behavioral and endocrine response to forced swimming stress in five inbred strains of rats. *Psychoneuroendocrin*. **20**:879–890.

Auriacombe, M., Reneric, J.P. and Le Moal, M. (1997). Animal models of anhedonia. *Psychopharmacol*. **134**:337–338.

Baker, S.L, Kentner, A.C., Konkle, A.T.M., Barbagallo, L. and Bieljew, C. (2006). Behavioral and physiological effects of chronic mild stress in female rats. *Physiol Behav*. **87**:314-322.

Bauer, M., Bschor, T., Pfennig, A., Whybrow, P.C., Angst, J., Versiani, M. and Möller, H.J. (2007). Guidelines for Biological Treatment of Unipolar Depressive Disorders in Primary Care. *World J Biol Psychiatry*. **8**:67–104.

Bertoglio, L.J. and Carobrez, A.P. (2002). Behavioral profile of rats submitted to session1-session 2 in the elevated plus maze during diurnal/nocturnal phases and under different illumination conditions. *Behav Brain Res*. **132**:135-143.

Blanchard, D.C., Sakai, R.R., MaEwen, B., Weiss, S.M. and Blanchard, R.J. (1993). Subordination stress: behavioural, brain and neuroendocrine correlates. *Behav Brain Res*. **58**:113-121.

Blazer, D.G., Kessler, R.C., McGonagle, K.A. and Swartz, M.S. (1994). The prevalence and distribution of major depression in a national community sample: the National Comorbidity Survey. *Am J Psychiatry*. **151**:979-86.

Bogdan, R. and Pizzagalli, D.A. (2006). Acute stress reduces reward responsiveness: implications for depression. *Biol Psychiatry*. **60**(10):1147-1154.

Boland, R.J. and Keller, M.B. (2009). Course and outcome of depression. In I.H. Gotlib and C.L. Hammen (Eds.), *Handbook of Depression*. New York: Guilford Press. **2**:23-43.

Borsini, F. and Meli, A. (1988) Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacol*. **94**:147–160.

Borsini, F., Podhorna, J. and Marazziti, D. (2002). Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacol*. **163**:121-141.

Bremner, J.D., Narayan, M., Anderson, E.R., Staib, L.H., Miller, H.L. and Charney, D.S. (2000). Hippocampal volume reduction in major depression. *Am J Psychiatry*. **157**:115-117.

Briones-Aranda, A., Rocha, L. and Picazo, O. (2005). Influence of forced swimming test on 5-HT_{1A} receptors and serotonin levels in mouse brain. *Prog Neuro-Psychopharmacol Biol Psychiatry*. **29**(2):275-281.

Brown, G. W. (1993). Life events and affective disorder: replications and limitations. *Psychosom Med*. **55**(3):248-59.

Bruinsma, K. and Taren, D.L. (1999). Chocolate: food or drug? *J Am Diet Assoc*. **99**(10):1249-1256.

Bschor, T. and Adli, M. (2008). Treatment of depressive disorders. *Dtsch Arztebl Int*. **105**(45):782-792.

Cancela, L.M., Bregonzio, C. and Molina, V.A. (1995). Anxiolytic-like effect induced by chronic stress is reversed by naloxone pretreatment. *Brain Res Bull*. *36*:209-213.

Carlezon, W.A. and Chartoff, E.H. (2007). Intracranial self-stimulation (ICSS) in rodents to study the neurobiology of motivation. *Nature Protocols*. **2**(11).

Charlton, B.G. (2000). Dialog on depression. *Science*. **288**(5468):975.

Cheeta, S., Ruigt, G., van Proosdij, J. and Willner, P. (1997). Changes in sleep architecture following chronic mild stress. *Biol Psychiatry*.**41**:419– 27.

Cole, B.J. and Koob, G.F. (1994). Corticotropin-releasing factor and schedule-induced polydipsia. *Pharmacol Biochem Behav*. **47**:393-398.

Craft, T.K. and Devries, A.C. (2006). Role of IL-1 in poststroke depressive-like behaviour in mice. *Biol Psychiatry*. **60**:812–818.

Cryan, J.F., McGrath, C., Leonard, B.E. and Norman, T.R. (1999). Onset of the effects of the 5-HT1A antagonist, WAY-100635, alone, and in combination with paroxetine, on olfactory bulbectomy and 8-OH-DPAT-induced changes in the rat. *Pharmacol Biochem Behav*. **63**:333–338.

Cryan, J.F., Markou, A. and Lucki, I. (2002). Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci*. **23**:238–245.

Cryan, J.F. and Holmes, A. (2005). The ascent of mouse: advances in modelling human depression and anxiety. *Nat Rev Drug Discov*. **4**:775–790.

Cryan, J.F. and Slattery, D.A. (2007). Animal models of mood disorders: recent developments. *Curr Opin Psychiatry*. **20**:1-7.

Cui, X.J. and Vaillant, G.E. (1996). Antecedents and consequences of negative life events in adulthood: a longitudinal study. *Am J Psychiatry*. **153**:21-26.

Czabak-Garbacz, R. (2008). Studying behaviour in stress research – not as easy as one would think. In: Behavioral models in stress research. Eds: A.V. Kalueff and J.L. LaPorte. 1-22.

D'Aquila, P.S., Brain, P. and Willner, P. (1994). Effects of chronic mild stress on performance in behavioral tests relevant to anxiety and depression. *Physiol Behav*. **56**(5):861-867.

Deacon, R.M., Croucher, A. and Rawlins, J.N. (2002). Hippocampal cytotoxic lesion effects on species typical behaviors in mice. *Behav Brain Res*. **132**:203–13.

Deacon, R.M. (2009). Burrowing: a sensitive behavioural assay, tested in five species of laboratory rodents. *Behav Brain Res*. **200**:128–33.

de Kloet, E.R., Joëls, M. and Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nat Rev Neurosci*. **6**:463-475.

Deussing, J.M. (2006). Animal models of depression. *Drug Discov Today: Disease Models*. **3**(4).

Dhondt, T., Beekman, A., Deeg, D. and van Tilburg, W. (2002). Iatrogenic depression in the elderly. *Soc Psychiatry Psychiatr Epidemiol*. **37**:393-398.

Doherty, M.D. and Gratton, A. (1996). Medial prefrontal cortical D1 receptor modulation of the meso-accumbens dopamine response to stress: an electrochemical study in freely- behaving rats. *Brain Res*. **715**(1-2):86-97.

Ducottet, C. and Belzung, C. (2004). Behavior in the elevated plus-maze predicts coping after subchronic mild stress in mice. *Physiol Behav*. 81:417-426.

Ducottet, C. and Belzung, C. (2005). Correlations between behaviours in the elevated plus- maze and sensitivity to unpredictable subchronic mild stress: evidence from inbred strains of mice. *Behav Brain Res*. **156**(1):153-162.

Duman, R.S., Heninger, G.R. and Nestler, E.J. (1997). A molecular and cellular hypothesis of depression. *Arch Gen Psychiatry*. **54**:597-606.

Dickerson, S. S. and Kemeny, M. E. (2004). Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bulletin*. **130**(3):355- 391.

El Yacoubi, M., Bouali, S., Popa, D., Naudon, L., Leroux-Nicollet, I., Hamon, M., Costentin, J., Adrien, J. and Vaugeois, J.M. (2003). Behavioural, neurochemical, and electrophysiological characterization of a genetic mouse model of depression. *Proc Natl Acad Sci USA*. **100**:6227–6232.

El Yacoubi, M. and Vaugeois, J.M. (2007). Genetic rodent models of depression. *Curr Op Pharmacol*. **7**:3-7.

Fountoulakis, K.N., Iacovides, A., Grammaticos, P., Kaprinis, G. and Bech, P. (2004). Thyroid function in clinical subtypes of major depression: an exploratory study. *BMC Psychiatry*. **4**.

Frazer, A. (1997). Pharmacology of antidepressants. *J Clin Psychopharmacol*. **17**(1):2S–18S.

Frazer, A. and Morilak, D.A. (2005). What should animal models of depression model? *Neurosc Biobehav Rev*. **29**:515-523.

Freeman, M.P., Freeman, S.A. and McElroy, S.L. (2002). The comorbidity of bipolar and anxiety disorders: prevalence, psychobiology, and treatment issues. *J Affect Disord*. **68**:1-23.

Geyer, M.A. (1995). Animal models of psychiatric disorders. In: Bloom, F.E., Kupfer, D.J. (Eds.), Psychopharmacology: The fourth generation of progress, 68. Raven Press, Ltd, New York, pp. 787–798.

Geyer, M.A. and Markou, A. (2000) Animal models of psychiatric disorders. In Psychopharmacology: The Fourth Generation of Progress (Bloom, F.E. and Kupfer, D.J., eds), pp. 777–798, Raven Press.

Gould, E. and Tanapata, P. (1999). Stress and hippocampal neurogenesis. *J Soc Boil Psychiatry*. **46**(11):1472-1479.

Gorka, Z., Moryl, E. and Papp, M. (1996). Effect of chronic mild stress on circadian rhythms in t he locomotor activity in rats. *Pharmacol Biochem Behav*. **54**:229–34.

Gotlib, I.H. and Joormann, J. (2010). Cognition and depression: current status and future directions. *Annu Rev Clin Psychol*. **6**:285-312.

Grippo, A.J., Beltz, T.G. and Johnson, A.K. (2003). Behavioral and cardiovascular changes in the chronic mild stress model of depression. *Physiol Behav*. **78**:703-710.

Grippo, A. J. and Francis, J. (2005). Neuroendocrine and cytokine profile of chronic mild stress induced anhedonia. *Physiol Behav.* **84**(5):697-706.

Grippo, A.J., Beltz, T.G., Weiss, R.M. and Johnson, A.K. (2006). The effects of chronic fluoxetine treatment on chronic mild stress-induced cardiovascular changes and anhedonia. *Biol Psychiatry*. **59**:309–316.

Grønli, J., Murison, R., Fiske, E., Bjorvatn, B., SØrensen, E., Portas, C. and Ursin, R. (2005). Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. *Physiol Behav*. **84**:571-577.

GrØnli, J. (2006). Chronic mild stress – an animal model of depression. From behaviour to molecules. Thesis for the Degree of Philosophiae Doctor (PhD). Department of Biomedicine, Section of Physiology, University of Bergen, Norway.

Hale, A. 1997, 'ABC of mental health: depression' British medical journal, 315, 5 July pp. 43-46.

Hamet, P. and Tremblay, J. (2005). Genetics and genomics of depression. *Metabolism*. **54**:10-15.

Hamilton M. (1967). Development of a rating scale for primary depressive illness. *Br J Soc Clin Psychol*. **6**:278–96.

Hammen C. (1992). Life events and depression: the plot thickens. *Am. J. Community Psychol.* **20**:179–193.

Harkin, A., Kelly, J.P. and Leonard, B.E. (2003). A review of the relevance and validity of olfactory bulbectomy as a model of depression. *Clin Neurosci Res*. **3**:253–262.

Harris, R.B., Zhou, J., Youngblood, B.D., Smagin, G.N. and Ryan, D.H. (1997). Failure to change exploration or saccharine preference in rats exposed to chronic mild stress. *Physiol Behav*. **63**:91-100.

Hasler, G., Drevets, W.C., Manji, H.K. and Charney, D.S. (2004). Discovering endophenotypes for major depression. *Neuropsychopharmacol*. **29**:1765-1781.

Hayley, S., Poulter, M.O., Merali, Z. and Anisman, H. (2005). The pathogenesis of clinical depression: stressor- and cytokine-induced alterations of neuroplasticity. *Neurosci*. **135**(3):659-678.

Igarashi, E. and Takeshita, S. (1995). Effects of illumination and handling upon rat open field activity. *Physiol Behav*. **57**:699-703.

Impey, S., Obrietan, K. and Storm, D.R. (1999). Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. *Neuron*. **23**:11-14.

Janowsky, D.S. and Risch, S.C. (1987). Acetylcholine mechanisms in affective disorders. In: Meltzer HY (eds) Psychopharmacology. The third generation of progress. Raven Press, New York, pp 527-534

Jesberger, J.A. and Richardson, J.S. (1985). Animal models of depression: parallels and correlates to severe depression in humans. *Biol Psychiatry*. **20**:764-784.

Kalueff, A.V., Wheaton, M. and Murphy, D.L. (2007). What's wrong with my mouse model? Advances and strategies in animal modelling of anxiety and depression. *Behav Brain Res*. **179**:1-18.

Kant, G.J. and Bauman, R.A. (1993). Effects of chronic stress and time of day on preference for sucrose. *Physiol Behav*. **54**:499–502.

Karege, F., Perret, G., Bondolfi, G., Schwald, M., Bertschy, G. and Aubry, J.M. (2002). Decreased serum brain-derived neurotrophic factor levels in major depressed patients. *Psych Res*. **109**:143–148.

Katz, R.J. (1981). Animal models and human depressive disorders. *Neurosc Biobehav Rev*. **5**:231-246.

Katz, R.J. (1982). Animal model of depression: pharmacological sensitivity of a hedonic deficit. *Pharmacol Biochem Behav*. **16**:965-968.

Katz, M.M., Koslow, S.H., Maas, J.W., Frazer, A., Bowden, C.L., Casper, R., Croughan, J., Kocsis, J. and Redmond Jr., E. (1987). The timing, specificity and clinical prediction of tricyclic drug effects in depression. *Psycho. Med*. **17**:297–309.

Keeney, A., Jessop, D.S., Harbuz, M.S., Marsden, C.A., Hogg, S. and Blackburn-Munro, R.E. (2006). Differential effects of acute and chronic social defeat stress on hypothalamic- pituitary-adrenal axis function and hippocampal serotonin release in mice. *J Neuroendoc*. **18**(5):330-338.

Kek, P.E.Jr, McElroy, S.L. and Strakowski, S.M. (1998). Anticonvulsants and antipsychotics in the treatment of bipolar disorder. *J Clin Psychiatry*. **59**(6):74–81.

Kendler, K.S. (1992). Major depression and generalized anxiety disorder: same genes, (partly) different environments – revisited. *Brit J Psychiatry*. **168**(30):68-75.

Kendler, K.S., Karkowski, L.M. and Prescott, C.A. (1999). Causal relationship between stressful life events and the onset of major depression. *Am J Psychiatry*. **156**: 837–841.

Kendler, K.S., Thornton, L.M. and Gardner, C.O. (2000). Stressful life events and previous episodes in the etiology of major depression in women: an evaluation of the 'kindling' hypothesis. *Am J Psychiatry*. **157**:1243–1251.

Kendler, K.S., Kuhn, J.W., Vittum, J., Prescott, C.A. and Riley, B. (2005). The interaction of stressful life events and a serotonin transporter polymorphism in the prediction of episodes of major depression: A replication. *Arch Gen Psychiatry.* **62**:529 –535.

Kessler, R.C., Chiu, W.T., Demler, O., Merikangas, K.R. and Walters, E. (2005). Prevalence, severity and comorbidity of 12-month DSM-IV disorders in the national comorbidity survey replication. *Arch Gen Psychiatry*. **62**:617-627.

Kessler, R.C., Akiskal, H.S., Ames, M., Birnbaum, H., Greenberg, P., Hirschfeld, R., Jin, R., Merikangas, K.R., Simon, G.E. and Wang, P.S. (2006). Prevalence and effects of mood disorders on work performance in a nationally representative sample of U.S. workers. *Am J Psychiatry.* **163**:1561–1568.

Kessler, R.C. and Wang, P.S. (2009). The epidemiology of depression. In I.H. Gotlib and C.L. Hammen (Eds.), *Handbook of Depression*. **2**:5-22.

Klein, D.F. (1974). Endogenomorphic depression. A conceptual and terminological revision. *Arch Gen Psychiatry*. **31**:447–54.

Koop, C., Vogel, E., Rettori, M.C., Delagrange, P. and Misslin, R. (1999). The effects of melatonin on the behavioral disturbances induced by chronic mild stress in C3H/He mice. *Behav Pharmacol*. **10**:73-83.

Kornetsky, C. (2004). Brain-stimulation reward, morphine-induced oral stereotypy, and sensitization: implications for abuse. *Neurosci Biobehav Rev*. **27**:777–786.

Krishnan, N.R. and Kasthuri, A.S. (2005). Iatrogenic disorders. *MJAFI*. **61**(1).

Krishnan, V. and Nestler, E.J. (2008). The molecular biology of depression. *Nature*. **455**.

Kupfer, D.J. (1993). Management of recurrent depression. *J Clin Psychiatry*. **54**(2):29–33.

Ladisich W. (1977). Influence of progesterone on serotonin metabolism: a possible causal factor for mood changes. *Psychoneuroendoc*. **2**(3):257-66.

Laviola, G., Adriani, W., Marley-Fletcher, S. and Terranova, M.L. (2002). Peculiar response of adolescent mice to acute and chronic stress and amphetamine: evidence of sex differences. *Behav Brain Res*. **130**:117-125.

LeDoux, J.E. (2000). Emotion circuits in the brain. *Annu Rev Neurosci*. **23**:155–184.

Levinson, D.F. (2006). The genetics of depression: a review. *Biol Psychiatry*. **60**:84-92.

Lopez-Rubalcava, C. and Lucki, I. (2000) Strain differences in the behavioral effects of antidepressant drugs in the rat forced swimming test. *Neuropsychopharmacol.* **22**:191–199.

Lucki, I. (1997). The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. *Behav Pharmacol*. **8**:523–532.

Maier, S.F. and Seligman, M.E.P. (1976). Learned helplessness: theory and evidence. *J Exp Psychol*. **105**:3-46.

Malatynska, E. and Knapp, R.J. (2005). Dominant-submissive behaviour as models of mania and depression. *Neuros Behav Rev*. **29**(4-5):715-737.

Maletic, V., Robinson, M., Oakes, T., Iyengar, S., Ball, S.G. and Russell, J. (2007). Neurobiology of depression: an integrated view of key findings. *Int J Clin Pract*. **61**(12):2030-2040.

Manji, H.K., Drevets, W.C. and Charney, D.S. (2001). The cellular neurobiology of depression. *Nature Med*. **7**(5).

Maren, S. and Fanselow, M.S. (1996). The amygdala and fear conditioning: has the nut been cracked? *Neuron*. **16**:237–240.

Matthews, K., Forbes, N. and Reid, I.C. (1995). Sucrose consumption as a hedonic measure following chronic unpredictable mild stress. *Physiol & Behav*. **57**(2):241-248.

Matza, L.S., Revicki, D.A., Davidson, J.R. and Stewart, J.W. (2003). Depression with atypical features in the National Comorbidity Survey: classification, description, and consequences. *Arch Gen Psychiatry*. **60**:817– 826.

McKinney, W.T.Jr. and Bunney, W.E.Jr. (1969). Animal models of depression. I. Review of evidence: implications for research. *Arch Gen Psychiatry*. **21**:240-248.

Mineur, Y., Prasol, D., Belzung, C. and Crusio, W. (2003). Agonistic behavior and unpredictable chronic mild stress in mice. *Behav Genet*. **33**:513-519.

Monleon, S., Parra, A., Simon, V.M., Brain, P.F., D'Aquila, P. and Willner, P. (1995). Attenuation of sucrose consumption in mice by chronic mild stress and its restoration by imipramine. *Psychopharmacol*. **117**(4):453-457.

Monroe, S. M. and Depue, R. A. (1991). Life stress and depression. In *Psychosocial Aspects of Depression* (ed. J. Becker and A. Kleinman), pp. 101±130. Lawrence Erlbaum: Hillsdale, NJ.

Moreau, J.L., Schershlicht, R., Jenck, F. and Martin, J.R. (1995). Chronic mild stress induced anhedonia model of depression: sleep abnormalities and curative effects of electroshock treatment. *Behav Pharmacol*.**6**:382–387.

Murison, R. and Hansen, A.L. (2001). Reliability of the chronic mild stress paradigm: implications for research and animal welfare. *Integr Physiol Behav Sci*. **36**:266-274.

Murray, C.J. and Lopez, A.D. (1996a). The global burden of disease. Cambridge, MA: Harvard Univ Pr.

Murray, C.J. and Lopez, A.D. (1996b). Evidence-based health policy – lessons from the Global Burden of Disease Study. *Science*. **274**:740-3.

Muscat, R. and Willner, P. (1992). Suppression of sucrose drinking by chronic mild unpredictable stress: a methodological analysis. *Neurosci Biobehav Rev*. **16**:507–517.

Naranjo, C.A., Tremblay, L.K. and Busto, U.E. (2001). The role of the brain reward system in depression. *Prog Neuro-Psychopharmacol Biol Psychiatry*. **25**(4):781-823.

Negroni, J., Venault, P., Pardon, M.C., Perez-Diaz, F., Chapouthier, G. and Cohen-Salmon, C. (2004). Chronic ultra-mild stress improves locomotor performance of B6D2F1 mice in a motor risk situation. *Behav Brain Res*. **155**:265-273.

Nestler, E.J., Barrot, M., DiLeone, R.J., Eisch, A.J., Gold, S.J. and Monteggia, L.M. (2002). Neurobiology of depression. *Neuron*. **34**:13-25.

Nestler, E.J. (2005). Is there a common molecular pathway for addiction? *Nat Neurosci*. **8**:1445–1449.

Nibuya, M., Morinobu, S. and Duman, R.S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci*. **15**(11):7539-47.

Nutt, D., Bell, C. and Potokar, J. (1997). Depression, anxiety and mixed conditions. Martin Dunitz: UK.

O'Callaghan, M.J., Croft, A.P. and Little, H.J. (2002). Effects of intraperitoneal injections of saline on the alcohol and sucrose consumption of C57BL/6 mice. *Psychopharmacol*. **160**:206-212.

Okamoto, K. and Aoki, K. (1963). Development of a strain of spontaneously hypertensive rats. *Jap Circ J*. **27**:282–293.

Onaivi, E.S. and Martin, B.R. (1989). Neuropharmacological and physiological validation of a computer-controlled two-compartment black and white box for the assessment of anxiety. *Prog Neuropsychopharmacol Biol Psychiatry*. **13**:963-967.

O'Neil, M.F. and Moore, N.A. (2003). Animal models of depression: are there any? *Hum Psychopharmacol Clin Exp*. **18**:239-254.

Overmier, J. B. and M. E. Seligman (1967). Effects of inescapable shock upon subsequent escape and avoidance responding. *J Comp Physiol Psychol.* **63**(1): 28-33.

Overstreet, D.H. (1986). Selective breeding for increased cholinergic function: development of a new animal model of depression. *Biol Psychiatry*. **21**:49-58-

Overstreet, D.H., Pucilowski, O., Rezvani, A.H. and Janowsky, D.S. (1995). Administration of antidepressants, diazepam and psychomotor stimulants further confirms the utility of Flinders Sensitive Line rats as an animal model of depression. *Psycopharmacol*. **121**:27- 37.

Papp, M., Willner, P. and Muscat, R. (1991). An animal model of anhedonia: attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. *Psychopharmacol*. **104**(2):255-259.

Paré, W.P. (1994). Open field, learned helplessness, conditioned defensive burying, and forced-swim test in WKY rats. *Physiol Behav*. **55**(3):433-439.

Paré, W.P., Tejani-Butt, S. and Kluczynnski, J. (2000). The emergence test: effects of psychotropic drugs on neophobic disposition in Wistar Kyoto (WKY) and Sprague Dawley rats. *Physiol Behav*. **68**:501-507.

Paykel, E.S. (2001). Stress and affective disorders in humans. *Semin Clin Neuropsychiatry*. **6**:4–11.

Phelps, E.A. and LeDoux, J.E. (2005). Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron*. **48**:175–187.

Pöldinger, W. (1971). Kompendium der Psychopharmakotherapie. Grenzach/Baden: Hoffmann-La Roche AG. 116.

Porsolt, R.D., Le Pichon, M. and Jalfre, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature*. **266**:730-732.

Porsolt, R.D., Anton, G., Blavet, N. and Jalfre, M. (1978) Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur J Pharmacol*. **47**:379–391.

Porth, C. M. (1990). Pathophysiology: Concepts of altered health states. Philadelphia: J.B. Lippincott Company.

Posmontier, B. (2008). Sleep quality in women with and without postpartum depression. J *Obstet Gynecol Neonatal Nurs*. **37**(6):722-737.

Pothion, S., Bizot, J.C., Trovero, F. and Belzung, C. (2004). Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. *Behav Brain Res*. **155**(1);135-146.

Prasad, A. and Prasad, C. (1996). Short-term consumption of a diet rich in fat decreases anxiety response in adult male rats. *Physiol Behav*. **60**:1039-1042.

Pucilowski, O., Overstreet, D.H., Amir, Rezvani, A.H. and Janowsky, D.S. (1993). Chronic mild stress-induced anhedonia: greater effect in a genetic rat model of depression. *Physiol Behav*. **54**(6):1215-1220.

Regier, D.A., Boyd, J.H., Burke, J.D. Jr., Rae, D.S., Myers, J.K., Kramer M, Robins, L.N., George, L.K., Karno, M. And Locke B.Z. (1988). One-month prevalence of mental disorders in the United States based on five epidemiologic catchment area sites. *Arch Gen Psychiatry*. **45**:977-86.

Roy, A. (1985). Early parental separation and adult depression. *Arch Gen Psychiatry*. **42**:987–991.

Russo-Neustadt, A., Beard, R.C. and Cotman, C.W. (1999). Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. *Neuropsychopharmacol*. **21**(5):679-82.

Sapolsky, R.M. (2003). Stress and plasticity in the limbic system. *Neurochem Res*. **28**:1735–1742

Sandi, C., Merino, J.J., Cordero, M.I., Touyarot, K. and Venero, C. (2001). Effects of chronic stress on contextual fear conditioning and the hippocampal expression of the neural cell adhesion molecule, its polysialylation, and L1. *Neurosci*. **102**:329–339.

Schildkraut, J.J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Am J Psychiatry*. **122**(5):509-22.

Schneider, H. (2007). Arquitecture of brains. Department of biology, DePauw University.

Schoenecker, B., Heller, K.E. and Freimanis, T. (2000). Development of stereotypes and polydipsia in wild caught bank voles (Clethrionomys glareolus) and their laboratory-bred offspring. Is polydpsia a symptom of diabetes mellitus? *Appl Anim Behav Sci*. **68**:349-357.

Selye, H. and Fortier, C. (1949). Adaptive reactions to stress. *Res Publ Assoc Res Nerv Ment Dis*. **29**:3-18.

Skuse, D., Albanese, A., Stanhope, R., Gilmour, J. and Voss, L.A. (1996). New stress-related syndrome of growth failure and hyperphagia in children, associated with reversibility of growth-hormone insufficiency. *Lancet*. **348**:353-358.

Solberg, L.C., Horton, T. and Turek, F. (1999). Circadian rhythms and depression: effects of exercise in animal models. *J Am Physiol Soc*. **276**:R152-R161.

Song, C. and Leonard, B.E. (2005). The olfactory bulbectomised rat as a model of depression. *Neurosci Biobehav Rev*. **29**:627–647.

Stahl, S.M. (1996). Phenomenology of anxiety disorders: clinical heterogeneity and comorbidity. In: Westenberg HGM, Den Boer JA, Murphy DL (eds.). *Advances in the neurobiology of anxiety disorders.* John Willey and Sons. 21-38.

Stewart, D.E., Gucciardi, E. and Grace, S.L. (2004). Depression. *BMC Woman's Health*. **4**:S19.

Strekalova, T. and Gass P. (2002). Behavioral strategies in mice C57BL/6N with different individual vulnerability to stress-induced anhedonia. In: Mechanisms of stress-resistance. Moscow, Ed. Sudakov K. pp. 160-168.

Strekalova, T., Spanagel, R., Bartsch, D., Henn, F.A. and Gass, P. (2004). Stress induced anhedonia in mice is associated with deficits in forced swimming and exploration. *Neuropsychopharmacol*. **29**:2007–2017.

Strekalova, T., Spanagel, R., Dolgov, O. and Bartsch, D. (2005). Stress-induced hyperlocmotion as a confounding factor in anxiety and depression models in mice. *Behav Pharmacol*. **16**:171-180.

Strekalova, T., Gorenkova, N., Scunk, E., Dolgov, O. and Bartsch, D. (2006). Selective effects of citalopram in the mouse model of stress-induced anhedonia with control effects for chronic stress. *Behav Pharmacol*. **17**:271-278.

Strekalova, T. (2008). Optimization of the chronic stress model in C57BL/6 mice: evidence for improved validity: In: Behavioral models in stress research. Eds: A.V. Kalueff and J.L. LaPorte. 111-157.

Strekalova, T. and Steinbusch, H.W.M. (2009). Measuring behaviour in mice with chronic stress depression paradigm. *Prog Neuro-Psychopharmacol Biol Psychiatry*. **34**(2):348-361.

Sullivan, P.F., Neale, M.C. and Kendler, K.S. (2000). Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry*. **157**:1552-1562.

Tsuang, M.T. and Faraone, S.V. (1990). The Genetics of Mood Disorders. Baltimore, Johns Hopkins University Press.

Tsuang, M.T., Faraone, S.V. and Green, R.R. (1994). Genetic epidemiology of mood disorders. In: Papolos, D.F., Lachman, H.M., eds. Genetic studies in affective disorders. New York: Wiley.

Valentinuzzi, V.S., Buxton, O.M., Chang, A.M., Scarbrough, K., Ferrari, E.A. and Takahashi, J.S. (2000). Locomotor response to an open field during C57BL/6 active and inactive phases: differences dependent on conditions of illumination. *Physiol Behav*. **69**:269-275.

Von Frijtag, J.C., Reijmers, L.G., Van der Harst, J.E., Leus, I.E., Van den Bos, R. and Spruijt, B.M. (2000). Defeat followed by individual housing results in long-term impaired reward- and cognition-related behaviours in rats. *Behav Brain Res*. **117**:137-146.

Wigger, A., Loerscher, P., Weissenbacher, P., Holsboer, F. and Landraf, R. (2001). Cross- fostering and cross-breeding of HBA and LAB rats: a genetic model of anxiety. *Behav Genet*. **31**:371-382.

Williams, J.W., Kerber, C.A., Mulrow, C.D., Medina, A. and Aguilar, C.A. (1995). Depressive disorders in primary care: prevalence, functional disability, and identification. *J Gen Intern Med*. **10**:7-12.

Williams, J.W.Jr., Mulrow, C.D., Chiquette, E., Noël, P.H., Aguilar, C. And Comell, J. (2000). A systematic review of newer pharmacotherapies for depression in adults: evidence report summary. *Ann Intern Med*. **132**:743-756.

Willner, P., Towell, A., Sampson, D., Sophocleous, S. and Muscat, R. (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacol*. **93**:358 – 364.

Willner, P. and Jones, C. (1996). Effects of mood manipulation on subjective and behavioral measures of cigarette craving. *Behav Pharmacol*. **7**:355–363.

Willner, P. (1997). Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacol*. **134**:319–329.

Willner, P. (2005). Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiol*. 52:90-110.

Wise, R.A. (2002). Brain reward circuitry: insights from unsensed incentives. *Neuron*. **36**:229–240.

Zafar, A.M, Jawaid, A., Ashraf, H., Fatima, A., Anjum, R. and Qureshi, S.U. (2009). Psychotherapy as a treatment modality for psychiatric disorders: perceptions of general public of Karachi, Pakistan. *BMC Psychiatry*. **9**:37.

[1] http://www.healthyplace.com/depression/main/psychotic-depression/menu-id-68/

[2] http://www.depression-help-resource.com/types-of-depression.htm

[3] pubs.niaa.nih.gov

[4] http://www.robotics.hiroshima-u.ac.jp/bio_medical_applications/forced-swim-test-e.html

[5] http://www.cincinnatichildrens.org/research/cores/abc/tail.htm

[6] http://www.cnb.csic.es/~montoliu/C57/c57black.jpg

[7] http://www.criver.com/en-us/prodserv/bytype/resmodover/resmod/pages/cd-1mouse.aspx

[8] http://www.iar.or.jp/shodobutsu/wi_rat/index.html

[9] http://www.any-maze.com/

[10] http://www.panlab.com/panlabWeb/Solution/php/