Influence of gender and social environment in an animal model of affective disorders
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Introduction
Affective disorders

The burden of mental illness on health and productivity throughout the world has long been underestimated. Data developed by the massive Global Burden of Disease study conducted by the World Health Organization, the World Bank, and Harvard University, revealed that mental illness, including suicide, accounts for over 15 percent of the burden of disease in established market economies. This is more than the disease burden caused by all cancers (NIH Publication No. 01-4586). Worldwide, psychiatric disorders only account for little more than one percent of deaths, however they are responsible for 11 percent of the disease burden. Not only the individual is affected by mental illnesses, but since these diseases are disabling and last for many years, also a tremendous burden is placed on the emotional and socio-economic capabilities of relatives (World Health Organization).

Depression and anxiety disorders

The numbers of people suffering from a depression are staggering. Major depressive disorder is the leading cause of disability in established market economies worldwide. An estimated 121 million people currently suffer from a depressive episode. Approximately 5.8% of men and 9.5% of women will experience a depressive episode in any given year. Depressive disorders account for close to 41.9% of the disability from neuropsychiatric disorders among women compared to 29.3% among men. In the United States of America major depression affects approximately 9.9 million American adults or about 5.0 percent of the population in a given year, of which 6.7 million are women (NIH Publication No. 01-4584). In the Netherlands the lifetime prevalence for major depression is 15.4% (NIPO). Women are twice as likely to develop a depression than men (20.1% vs. 10.9%). Only in a minority of people (30-45%) a depressive episode stays limited to a single episode.

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Table 1. The 10 leading causes of DALYS in the world in 1990 and 2020. DALYS are the sum of years of life lost because of premature mortality and years of life lived with disability, adjusted for the severity of disability.
More often people are suffering from recurrent episodes, and after a second episode, the risk of a third in the next 3 years is about 70%.\textsuperscript{55}

Symptoms of depression include a persistent sad mood, loss of interest or pleasure in activities that were once enjoyed, significant change in appetite or body weight, difficulty sleeping or oversleeping, physical slowing or agitation, loss of energy, feelings of worthlessness or inappropriate guilt, difficulty thinking or concentrating, and recurrent thoughts of death or suicide. A diagnosis of major depressive disorder (or unipolar major depression) is made if an individual has five or more of these symptoms during the same two-week period (DSM IV, American Psychiatric Association, 1994).

Several mental diseases are grouped under the term anxiety disorders, namely generalised anxiety disorder (GAD), obsessive compulsive disorder (OCD), panic disorder, phobias, and post traumatic stress disorder (PTSD). In the US anxiety disorders are the most common mental illness, with 19.1 million people (13.3% of the adult population). Except social anxiety and OCD, women are about twice as likely to be afflicted as men. Symptoms consist of overwhelming fear and anxiety of a chronic nature, which can grow progressively worse.

\textit{Gender}

The risk for women to develop a depression and most anxiety disorders is twice as high as for men. This gender difference starts to occur during adolescence. Before puberty boys show more depressive symptoms, but at the age of 12-14 the risk increases for girls and stays higher throughout adult life. This increase is most likely a combination of biological changes and social challenges of early adulthood.\textsuperscript{140}

A likely candidate to play a role in the higher occurrence of depression in women is estrogen. A depressive episode in women often occurs when hormonal levels are changing, like after pregnancy, prior to menses and during and shortly after menopause. Although for postpartum depression other factors also contribute to the vulnerability for this disorder,\textsuperscript{19} since withdrawal from artificially high levels of gonadal steroid hormones, estrogen and progesterone, caused mood symptoms only in women with a history of postpartum depression and not in the control group.

Besides biological factors, there could be other factors that explain the higher prevalence of affective disorders found in women. Women appear to have a tendency to meet more criterion symptoms for depression than men, also some of the symptoms occur more frequently in women, which could underestimate the number of men suffering from a depression.\textsuperscript{140,156} However when items that showed a gender effect were excluded, the gender differences were reduced but not eliminated, indicating that differences in symptomatology are not the cause for the observed gender difference in prevalence. Gender differences in recall of past
In the human brain gender differences in morphology and functioning have been found, of which a brief summary is provided below. It is surprising that studies investigating ‘the brain’ often completely ignore this aspect.

- **Structure**: Differences in morphology have been observed between men and women in several brain regions. The most consistently found difference is brain size, which is 8-10% larger in men, women however show a higher proportion of grey matter. Women also showed an increased directional coherence and/or density of left hemisphere frontal lobe white matter, measure by DTI (diffusion tensor imaging), which correlated with better verbal comprehension and memory. More region-specific gender differences have also been found. A sexually dimorphic nucleus in the medial preoptic area is about twice as big in men compared to women. Similar results have been found a.o. for the preoptic-anterior hypothalamic area and part of the bed nucleus of the stria terminalis. The corpus callosum is the primary communication channel between the two hemispheres. Women appear to have a relative larger corpus callosum (splenial part), possibly allowing faster transfer of information, which could be related to the more bilateral organisation of the female brain, in contrast to the male brain which is more lateralised.

- **Neurochemistry**: Vasopressinergic neurons in the SON and PVN show sex differences in cell size, which are larger in men, suggestive of a higher activity. In the SON gender differences were also found in the number of ERz and ERβ positive AVP neurons. Young women showed 50 times more ERβ than men, which decreased with age. ERz expression however was low in young women and higher in men and elderly women. There are some indications that there are gender differences in treatment response to different classes of antidepressants. Women appear to respond better to SSRI’s than TCAs, while men showed an opposite response. This suggests the existence of gender differences in the monoaminergic system as well, at least in depressed patients.

- **Activity**: Especially in response to emotional stimuli gender differences have been observed. Resting cerebral blood flow, measured by PET, was found to be lower in the temporal en prefrontal cortex and higher in the brain stem of women. A gender difference was found in the activation pattern induced by transient sadness, where women had a more pronounced increase in blood flow in the limbic and paralimbic structures. Also emotional experiences and encoding of emotional memories activate different patterns in the brains of men and women. Besides activating more brain regions, in women greater activation correlated with emotional-intensity ratings and better recognition for emotionally intense pictures. Presentation of happy and sad faces also result in activation of different brain structures in men and women.
depressive episodes, with women showing a better recall, have also been found, but this also did not appear to be entirely responsible for the higher prevalence in women. 140, 141

Even though artefacts may enhance a female preponderance in affective disorders they cannot fully explain the gender gap, showing that this gender difference is genuine.

Treatment

Dysfunctions of the monoaminergic system have been implicated in the symptomatology of affective disorders. The classic monoamine theory of depression, postulated in 1950s is based on the antidepressant effect of MAO inhibitors and monoamine reuptake inhibitors. Currently still most antidepressant drugs, with which both depressed and patients with an anxiety disorder are treated are still based on the monoamine theory. Nowadays there is a large variety of antidepressants to choose from. There are several categories of antidepressants: Tricyclic antidepressants (TCA’s): TCA’s so called for their cyclic chemical structure, are thought to have their antidepressant effect through inhibition of noradrenalin (NA) and serotonin (5HT) reuptake. 149 Besides this serotonergic and noradrenergic re-uptake inhibition, they also block several other postsynaptic receptors, like cholinergic, histaminergic and adrenergic receptors, therefore causing typical side effects like a dry mouth, blurred vision, memory dysfunction, sedation, weight gain and dizziness. 85 Monoamine Oxidase Inhibitors (MAOI’s): MAOI’s inhibit the enzyme Monoamine oxidase, thus preventing the breakdown of noradrenalin, serotonin and dopamine, subsequently increasing the availability of these neurotransmitters. 85 Even though MAOI’s appear to be more effective against atypical depression, they are not commonly used in the clinic due to their side effects and dietary restrictions. 85 TCA’s together with MAOI’s have been supplanted over the last decade by Selective Serotonin Re-uptake Inhibitors (SSRI’s) as first choice medication because of better tolerability and safety. As the name already describes, SSRI’s inhibit the reuptake of serotonin increasing the time serotonin is present in the synaptic cleft.

There are some indications that there is a gender difference in treatment response to different classes of antidepressants. Kornstein and co-workers 100 reported that women respond better to SSRI’s than TCA’s, while men showed an opposite response. However the effect in women was predominately determined by differences in responsivity in premenopausal women. Another study also found that women in their reproductive period were more responsive to SSRI’s than to TCA’s. 119 A similar result was detected in melancholic depressed patients, with young women responding better to treatment with an SSRI than a TCA. 89 But also negative results in relation to gender-specific responses in clinical effects of antidepressants
A great disadvantage of the current antidepressants is the long period (2 to 6 weeks) it takes for the drug to have any effect. This in combination with the adverse side effects, often causes failure to complete the treatment. The efficacy of antidepressant also leaves a lot to be desired, patients often have to try several antidepressant drugs before treatment is successful and only about 65% of the patient ultimately responds to antidepressant drug therapy.

Next to pharmacological treatment of major depression and anxiety disorders, also psychotherapy is used to treat these affective disorders. Cognitive behavioural therapy after successful antidepressant treatment substantially reduced the relapse rate. Evidence is also accumulating that, especially in severe and recurrent depression, a combination of antidepressant treatment and some form of psychotherapy is superior than either treatment alone. Also combined therapy is found more acceptable by patients than pharmacotherapy alone.

Electroconvulsive therapy (ECT) has been used since the 1930s to treat psychiatric disorders. Under general anaesthesia short (30-90 sec.) seizures are induced by applying a brief electrical current through the brain. Patients generally receive 6-12 sessions, 3 times a week. The most concerning side effect of ECT is memory loss, however in most patients memory loss is transient. Meta-analysis showed that ECT was more effective in the treatment of severe depression than pharmacotherapy. Despite its effectiveness, ECT is considered as a last resort, after antidepressant treatments have failed. Another tool that could provide a non-invasive treatment for affective disorders is repetitive transcranial magnetic stimulation (rTMS). In TMS a strong magnetic field is generated in a coil and when this coil is placed near the head the magnetic field induces an electric field in the underlying cortex, generating action potentials. In contrast to ECT, rTMS does not induce seizures and has no adverse effect on memory. rTMS applied to the prefrontal cortex (PFC) appears to be an effective treatment for major depression.

Genes and/or environment?

Genetic factors appear to be similarly important in the aetiology of major depression in men and women. A meta-analysis of data from genetic epidemiology of major depression revealed that the heritability of major depression is in the range of 31%-42%, however in a longitudinal sample, which is likely more reliable, the heritability was considerably higher in female twins, namely 66%. Several studies have tried to link specific genes to major depression, however with contradictory results. No link was found between genes involved in the serotonergic, dopaminergic and endocrine function. While a study by Furlong and co-workers...
(1998) did find an association with a polymorphism in the promoter region of the 5HT transporter (5HTT) gene. Interestingly, an association has also been found between the promoter region of the 5HTT gene and the risk of a depressive episode after stressful life events. Only when exposed to stressful circumstances was having one or two copies of the short allele of the 5HTT promoter polymorphism a risk factor for developing a depression. The latter showing the importance of the interaction between environment and genes in the individuals’ reaction to adverse events.

Especially stressful life events or exposure to chronic stress have been associated with the onset of major depression. This association however declines with increasing number of previous episodes, suggesting that the system sensitises and stress is no longer “necessary” for inducing a depressive episode, the so called kindling hypothesis. Genetic factors appear to lower the threshold for stress to induce a depressive episode, as shown by Kendler and co-workers, who found an interaction between genetic risk and stressful life events; in people with a high genetic risk the association between stressful life events was weaker than in people with a low genetic risk. Environmental factors can also reduce the risk of a depressive episode. Social support has been reported to have beneficial effects on the outcome of a depressive episode and prevention of relapse.

A simple answer to what causes major depression is not available. It results from both genetic and environmental factors, and because of the heterogeneity of the disease, it is likely that more than one pathway lead to a common endpoint of major depression.

Social support

An environmental factor which has a positive influence on the occurrence of a depressive episode is social support. It is generally known that social support has beneficial effects on psychological and physical health. Although social ties can also have detrimental effects on mental health, by entailing a sense of indebtedness and obligation.

Gender is also a factor in the effects of social ties on mental health. Women maintain more emotionally intimate relationships, during periods of stress women mobilise more support, and they provide more effective and frequent support than men. These aspects make women also susceptible to stressful events suffered by people to whom they feel emotionally close, so called ‘cost of caring’ hypothesis. This is also shown by the association of stressful life events with network or interpersonal problems in women, and financial and employment problems in men.

In major depression, social support has been reported to have beneficial effects
on the outcome of a depressive episode and prevention of relapse. More stressful life events and less social support are associated with greater risk of disease progression in HIV patients. Also in cardiac patients it is suggested that the amount of social support and psychosocial interventions to increase social support improve the quality of life and length of survival. It has been demonstrated that the presence of social support lowers cardiovascular reactivity of healthy volunteers to a stressor in a laboratory setting.

Direct personal contact is not essential for the positive effects of social support to occur. Also social support received by internet depression support groups, which are especially used by people with low social support, are perceived as beneficial.

Owning a pet promotes cardiovascular health and lowers stress reactivity measured by blood pressure and heart rate, showing that not even human contact is necessary for provision of social support.

The depressed brain

Many brain regions have been implicated in the neurobiology of depression. Depressive symptomatology is likely the result of dysfunctions in a widely distributed and interactive network of cortical-striatal and cortical-limbic pathways. Neuroimaging and post mortem studies have demonstrated metabolic and anatomical changes in several areas of these neuronal networks in the brains of depressed patients.

Since the 60s catecholaminergic deficiencies, specifically those of noradrenaline (NA), have been associated with affective disorders. NA synthesising neurons are located in the brain stem nucleus locus coeruleus (LC), and project extensively to cortical and subcortical areas. The LC likely plays an important role in the timing of learning and arousal. Increased firing activity of the LC has been demonstrated during arousal. Drug-induced depletion of catecholamines induced depression in patients who were treated for hypertension. Also rapid depletion of catecholamines with AMPT (an inhibitor of tyrosine-hydroxylase (TH), the rate-limiting step in catecholamine synthesis), resulted in relapse of depressed patients in remission, without affecting healthy controls. Abnormalities have been found in the LC of depressed patients. Post-mortem, patients showed an elevated binding of an \( \alpha_2 \)-adrenoreceptor agonist in the LC, implying a deficiency of NA is this region.

Another study found a reduced number of LC neurons positive for TH, also indicative of a noradrenergic deficit. The role of the serotonergic system in affective disorders has also been extensively investigated and a malfunctioning in this system has been implicated in the pathobiology of affective disorders. Next to the affective component, serotonin also plays an important role in basic biological functions like sleep, appetite, circadian rhythm and cognitive functions. The serotonergic raphe nuclei...
Introduction

have extensive projections to cortical and subcortical areas, allowing serotonin to influence many brain functions. Next to extensive projections, serotonin has over a dozen receptor subtypes, allowing for diverse effects on different neurons and brain regions. CSF levels of the breakdown product of serotonin 5-HIAA (5-hydroxyindoleacetic acid) have been found to be reduced in depressed patients, but this seems to be limited to patients with suicidal behaviour. Artificial depletion of serotonin in remitted, medication free, depressed patients induces a rapid return of depressed mood, showing an important role for serotonin in mood regulation. Post-mortem studies suggest changes in 5HT$_{2a}$ and 5HT$_{1a}$ receptors in the brain of depressed patients, however a confounding factor is that most of these patients committed suicide and therefore are not completely representative of depressed patients without suicidal tendencies. Also imaging studies suggest changes in the serotonergic system of depressed patient.

Dopamine is the main neurotransmitter involved in motivated behaviour towards a rewarding/pleasurable stimulus, although it can also be released in response to stress. The inability to experience pleasure is a core symptom of depression, and this suggests a dysregulation in the dopaminergic system. Abnormalities have been found in the dopaminergic system of depressed patients, indicating a reduced functioning of these brain regions.

In patients suffering from depression a reduced volume of the prefrontal cortex (PFC) has been demonstrated. Which could correspond to the reductions in neuronal size and density, and glial cell density that have been observed in the prefrontal cortex of depressed patients. Depending on the specific sub region of the PFC, metabolic activity and blood flow is either decreased or increased compared to normal controls. These abnormalities have been found to normalise after antidepressant treatment in some studies. It has been suggested that hypoactivation of the dorsal and ventral anterior cingulate cortex are associated with attentional and cognitive impairments and blunted experience of affect, hypoarousal anhedonia, reduced coping in uncertain situations respectively. Abnormal increases in activity have been found in the ventrolateral, lateral, orbital cortex of the PFC and rostral anterior cingulate cortex. This demonstrated hyperactivity might be associated with an adaptive compensatory reaction to the state of being depressed. Reduction of anterior cingulate cortex activity and increased activity in the dorsal anterior cingulate cortex were found to be related to symptom improvement after treatment. Also high theta activity in the rostral anterior cingulate cortex, as measured by EEG, provided a positive prediction for the response to treatment 4-6 months later.

The amygdala is involved in processing and the expression of emotional stimuli, and is activated with the presentation of emotionally negative pictures in humans. Interestingly, a gender difference in the activation
patterns of the amygdala in response to remembering emotional pictures has been observed. Depressed patients show an altered amygdala activity. Resting cerebral blood flow and glucose metabolism are elevated in depressed subjects, and show a positive correlation with the severity of the depression.

The hippocampus is a critical structure involved in episodic, contextual, declarative and spatial learning and memory. Hippocampal volume loss has frequently been found in depressed patients, and hippocampal dysfunction is thought to be related to the cognitive impairments found in patients. It is however unclear if a smaller hippocampus precedes the onset, or is a consequence of depressive episodes (state of trait problem). Associations have been found between the illness duration and volume loss, suggesting that the reduction is a result of recurrent depressive episodes. A study by Frodl, interestingly, found a reduction in hippocampal volume only in men, but not in women, with a first episode of major depression. However several studies report no significant differences in hippocampal volume between patients and controls. Differences in age and duration of the illness could explain these discrepancies. Another variable that could explain the inconsistent findings could be a history of childhood trauma. Smaller hippocampal volumes were found only in depressed women who had suffered from severe childhood abuse in comparison with nonabused depressed and healthy women.

Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis have been frequently found in patients with affective disorders. Hyperactivity of the HPA-axis is observed in approximately 50 percent of the patients with a depression, and can be corrected by antidepressant treatment. Disturbances in the negative feedback of the stress response could be the cause of hypersecretion of stress hormones (see The stress response). The dexamethasone-suppression test provides an indication of the capacity of glucocorticoid receptors in the pituitary to inhibit adrenocorticotropic hormone (ACTH) and subsequently cortisol release, as occurs in healthy subjects but is disrupted in depressed subjects. Depressed patients also show a blunted ACTH response to a corticotropin-releasing hormone (CRH) challenge, which could be explained by a down-regulation of pituitary CRH receptors caused by a hypersecretion of CRH from the PVN. Increased levels of CRH in the cerebrospinal fluid (CSF) of depressed patients and suicide victims have been observed, supporting this hypothesis, although negative results have also been found. In the PVN and locus coeruleus of depressed patients an elevated expression of CRH mRNA was found when compared to healthy controls. Hypertrophy of adrenal glands as found in patients provides another sign of HPA-axis hyperactivity.

Prolonged excessive levels of cortisol could lead to neurotoxic effects in the hippocampus, resulting in a reduction of hippocampal volume. Cushing’s syndrome provides an indication that excess glucocorticoids release is associated with depressive
symptomatology and hippocampal atrophy. Cushing’s syndrome is generally due to an overproduction of corticotropin from a pituitary adenoma, and more than 50% of the patients suffer from depression or suicidal tendencies. These patients also show hippocampal atrophy, which is reversible once cortisol levels are lowered.

Although the link between abnormal HPA-axis functioning and depression is evident, it is still unclear if this is the primary cause of depression or the consequence of another initiating factor.

Animal models

Satisfactory animal models of affective disorders are hard to come by. Core symptoms of depression, like feelings of worthlessness or inappropriate guilt and recurrent thoughts of death or suicide are unlikely to occur in rodents and even if they do, they are not measurable. There are three validation criteria which are used to validate an animal model; face validity, construct validity and predictive validity. Predictive validity refers to manipulations, like administration of drugs, which are known to affect the disease the model represents, having similar effects in the animal model. Face validity concerns similarities in symptoms between the disease and the model. Construct validity is based on the theoretical rationale of the animal model. Although the symptom of a ‘depressed mood’ cannot be mimicked in rodents, patients suffering from an affective disorder show symptoms that can be replicated in animals, like: anhedonia, learning and memory deficits, sleep disturbances, and adrenal hypertrophy.

Most currently used rodent models, which best meet the validation criteria, are based on the epidemiological data that stress plays an important role in the onset of a depressive episode. The most promising model appears to be the chronic mild variable stress model. In this model rats are exposed sequentially to a variety of stressors, like reversal of the light/dark cycle, food/water deprivation, change in cage mate and cage tilting. Chronic stress impairs the ability to anticipate reward in some studies. This condition resembles anhedonia and can be reversed by antidepressants. In addition these rats also demonstrate various sleep disturbances, another characteristic of depression. An advantage of the chronic mild stress model is the applicability in both male and female rats, in contrast to the social stress models. Disadvantages of this model are the, so far unexplained, reproducibility between studies and the difficulty to implement the model reliably in different laboratories.

Stress

Stress can be described as the non-specific response of the body to any demand. The concept of stress was first described by Hans Selye, who showed a non-
specific response of an organism to noxious stimuli. Stress is a normal physiological reaction allowing a subject to respond appropriately to environmental or physiological changes, and thus not necessarily a negative event. However, problems arise when stress becomes chronic and/or too severe and the subject fails to cope.

The stress response

Stress leads to an immediate sympathetic activation, upon which the adrenal medulla releases adrenaline and noradrenaline in the bloodstream, resulting in a rise in heart rate and blood pressure, allowing a fast reaction to a potential harmful stimulus. A slower reaction takes place via the activation of the hypothalamic-pituitary-adrenocortical-axis (HPA-axis), resulting in the release of glucocorticoids by the adrenal cortex. The hypothalamus is mainly involved in maintaining homeostasis, and a.o. controls food and water intake, reproductive behaviour and body temperature regulation. The paraventricular nucleus of the hypothalamus (PVN) is the final integrator of the HPA-axis. Stress results in the activation of CRH and vasopressin (AVP) neurons projecting to the median eminence, where CRH and AVP are released into the portal vascular system and stimulate ACTH release from the anterior pituitary into the peripheral bloodstream. ACTH binds to receptors in the adrenal gland, where it results in the release of glucocorticoids, cortisol in humans and corticosterone in rodents.

Negative feedback mechanisms prevent the HPA-axis from overshooting. Glucocorticoids inhibit their own release by inhibiting release and synthesis of ACTH in the pituitary and inhibiting CRH release at the level of the PVN, (Figure 1). Limbic structures like the hippocampus, amygdala and PFC play an important role in the regulation of the HPA-axis, especially when stressors have no immediate survival value. In that case, depending on experience, limbic circuits can modulate the stress response.39,78

Glucocorticoids have two receptors in the brain, namely the mineralocorticoid (MR) and glucocorticoid (GR) receptors, which are differently distributed in the brain.148 MR have a high affinity for glucocorticoids and are heavily occupied under baselines conditions. The GR have a 10 fold lower affinity for glucocorticoids and are only occupied during periods of high release, like stress.148 In regions involved in fear and anxiety, like the amygdala, and hippocampus, both GR and MR are present. Both types of receptors are involved in regulation of the HPA-axis, where MR maintains low basal HPA-axis activity and GR, facilitated by MR, limit increased HPA-axis activity during the circadian plasma peak level and after stress.27,37,170

The PVN also synthesises oxytocin. Part of the magnocellular neurons of the PVN release oxytocin into the neurohypophysis whereas another part projects to various regions in the CNS.48 Oxytocin is well-known for its influence on
reproductive behaviour, labour and lactation. Several studies report that oxytocin can also modulate the stress response. A single oxytocin injection in males can increase plasma corticosterone levels, whereas repeated administration decreases plasma corticosterone levels. In addition, centrally administered oxytocin also decreases the stress-induced corticosterone levels in female rats.

Another system that is activated by stress exposure is the serotonergic system. Serotonergic cells originate in 9 clusters located in the midbrain. The majority of the serotonergic neurons are located in the midline raphe nuclei. The dorsal raphe nucleus (DRN) and median raphe nuclei (MRN) have widespread projections throughout the forebrain (Fig. 2A). It has been demonstrated that serotonergic cells in the DRN are activated by inescapable, but not escapable stress, and rats undergoing ‘psychological’ stress have shown an increased 5HT release in the DRN. The MRN sends serotonergic projections to the hippocampus and is associated with improved stress-resistance. Male and female rats show differences in magnitude of the stress response. Female rats react with a much higher rise in corticosterone to a stressor than males do. The intensity of the stress response in females is also dependent on the levels of circulating gonadal hormones, and differs during different stages of the oestrous cycle. So, especially in acute stress responses the stage of the oestrous cycle of the female can be a confounding factor.

**Fos as neuronal activation marker**

The protein Fos is the product of the c-fos proto-oncogene, one of the immediate early genes (IEG). These genes are expressed immediately after an extra cellular stimulus and play a role in signal transduction and transcriptional regulation. Fos forms heterodimers with Jun proteins, the product of another IEG. A Fos/Jun complex is referred to as AP-1, which can bind to AP-1 binding sites and are thought to contribute to the transcription of genes bearing these sites in their promoter region. Fos mRNA is expressed immediately (within a few minutes)
after a stimulus. Expression of the protein Fos is maximal between 1 and 3 hrs. after
a stimulation.\textsuperscript{101}

Fos has been mostly studied as a marker for neuronal activation after a wide
variety of stimuli. However Fos-ir does not always represent an activated neuron.
Fos induction can be evoked without any changes in neuronal firing, and some
neurons seem to always express Fos or do not use Fos when stimulated. Also the
role Fos plays in neuronal functioning remains elusive.\textsuperscript{81}

Even though Fos-ir might not give an accurate measure of the total number
of activated neurons, it does provide a sensitive marker to distinguish patterns of
activation between different treatments. Acute stress results in a widely distributed
pattern of Fos mRNA and Fos expression in the rat brain.\textsuperscript{31,109,173} However if
habituation to a stimulus occurs, brain regions fail to show a Fos-response.\textsuperscript{173,172}

Also social stimuli like sexual interactions and sexual chemosensory cues induce
Fos–ir in several brain regions, some of which overlap with regions showing Fos-ir
after stress, like the medial amygdala, accumbens and medial preoptic area,\textsuperscript{17,99,190}
showing that the Fos response, even within the same region, is not dependent on
one stimulus. Chronic, but not acute, treatment with several antidepressants reduce
the Fos responsivity to acute restraint stress.\textsuperscript{126} Also anxiolytic drugs affect the Fos
response to an acute stressor.\textsuperscript{127} Besides the corticosterone response, also the Fos
response to an acute stressor is affected by fluctuating levels of gonadal hormones
during the oestrous cycle.\textsuperscript{51,192} These data show that the Fos response to stress can be
modulated by compounds that intervene with the stress response of rats, indicating
that Fos is a sensitive parameter to measure changes in stress reactivity in rats.

\textit{Chronic stress}

When rats are repeatedly exposed to the same stressor habituation to the
stimulus can occur. The rat becomes unresponsive to the stressor. Habituation and
the associated loss of Fos expression in several brain regions, among which the
PVN, has been found after two weeks of chronic restrain stress.\textsuperscript{173} This habituation
was accompanied by the disappearance of weight gain reduction in the second week.
Habituation to chronic stress can be prevented by presenting different types of
stressors, as is done in the chronic variable stress model.\textsuperscript{197} Applying a more severe
stressor, like mild footshocks, also induces failure to habituate.\textsuperscript{104,109}

Chronic stress exposure induces several characteristics also observed in
depressed patients, like anhedonia, adrenal hypertrophy,\textsuperscript{12,75} sleep disturbances,\textsuperscript{25}
hippocampal atrophy \textsuperscript{168} and reduced neuronal plasticity.\textsuperscript{123} And some of these
changes are reversible with antidepressant treatment.\textsuperscript{32,197} Surprisingly, even though
rats habituate to chronic restrain stress, morphological changes in the hippocampus
and learning deficits still occur.\textsuperscript{15,61}
Anhedonia which is a core symptom of depression, is thought to be related to a disturbance in the dopaminergic reward system of the brain (Fig. 2B). A diminished response to rewarding stimuli has been found in rats after chronic stress exposure by some studies.\textsuperscript{46,76,121,199} In chronic stress models changes in dopaminergic activity have also been observed.\textsuperscript{21} Increased mesolimbic DA release appears to be related to active coping with an aversive stimulus, whereas inhibition of mesolimbic DA release occurs with uncontrollable/unavoidable stressors and failure to cope.\textsuperscript{21}

The brain has a high level of plasticity, probably making it possible for subjects to cope with acute stressors and allowing the brain to respond in the appropriate way. However when stress becomes chronic, and the subject fails to cope, more severe changes occur, likely resulting in long-lasting, possibly maladaptive, alterations.

**Housing conditions**

Housing conditions can have a major impact on the functioning of an animal. In rodents the effects of environmental enrichment has been extensively investigated. Environmental enrichment consists of social housing in a large area, in which a running wheel and regularly changed play objects are present. This has been shown to have major effects on a wide variety of parameters. Environmental enrichments also has beneficial effects on recovery from brain damage and may countermand some genetic constraints.

Besides an enriched environment, also social housing by itself has positive effects on animals, and the positive effects of “social support” are not only found in humans, also a.o. monkeys, rats and guinea pigs benefit from the presence of a conspecific during stressful situations. Marmosets show elevated levels of cortisol when housed alone in a novel cage, whereas the presence of the pairmate prevents this increase.\textsuperscript{165} Also guinea pigs form stable male-female bonds with each other.\textsuperscript{155} When adult male guinea pigs are placed in a novel environment their cortisol response is lower in the presence of his female partner, but not when a familiar

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**Figure 2.** Serotonergic (A) and dopaminergic projections in the rat brain.\textsuperscript{157}
female, not his bonding partner is present. Interestingly, in female guinea pigs also the presence of another familiar (not partner) male has stress-reducing effects. Positive effects of social company are not limited to pair-bonding species. In male rats social housing can reduce the effect of a stressful experience, counteracting for example the behavioural and physiological effects of a social defeat. Also in mice, isolated animals have a higher heart rate and a disrupted sleep pattern, indicative of discomfort. Gender-specific effects of social housing have also been found in rats, where crowding was found to be stressful for males, whereas it actually calms females. This shows that the social environment can have a major impact on how an animal functions and reacts to stimuli.

**Brain plasticity**

The adult brain is not a static organ. Environmental changes can a.o. induce structural changes, affect the strength of synaptic connections, and influence birth, survival and death of neurons, especially in the hippocampus.

Morphological changes can be observed after chronic stress exposure. In rats and tree shrews stress results in a reduction in apical dendrite arbor complexity. This stress induced atrophy can be prevented by the chronic administration of the antidepressant tianeptine. Neurotrophic factors, like brain derived neurotrophic factor (BDNF), play an important role in synaptic plasticity. One of the pathways involved in the regulation of BDNF transcription is the cyclic AMP-CREB pathway. This pathway has been implicated as a post-receptor target for antidepressant drugs. Increased levels of intracellular cAMP increase the phosphorylation of the cAMP response-element binding protein (CREB) and its translocation to the nucleus of the cells. Phosphorylated CREB (pCREB) binds to the cAMP response element (CRE) sites in promotor regions of certain genes, such as BDNF, and has been shown to regulate these genes and by this way influence synaptic plasticity. 

Long-term potentiation (LTP) in the hippocampus, is an indication of synaptic plasticity, and is involved in the formation of long-term memory. Induction of LTP has been shown to increase pCREB expression in the dentate gyrus (DG) of the hippocampus, and also has been linked to BDNF expression. Stress exposure however decreases LTP induction and BDNF expression. This concurs with a reduction in pCREB expression after chronic stress exposure found in several brain regions, whereas antidepressant treatment increases pCREB labelling and BDNF expression in the brains of rodents. A post mortem study also detected reduced levels of CREB in the human temporal cortex and reduced BDNF levels in the hippocampus of untreated depressed patients vs. patients treated with antidepressants and healthy controls. This indicates that pCREB provides a
sensitive marker for neural plasticity in the hippocampus.

Another important modulator of synaptic plasticity is oestrogen, which has effects on both BDNF expression and LTP. For example, BDNF expression is regulated by oestrogen and fluctuates within the oestrous cycle of rats.\textsuperscript{56,158} Also treatment with oestradiol increased BDNF expression in the brains of female rats, and female prairie voles.\textsuperscript{9,110,163} The presence of an oestrogen response element in the gene encoding for BDNF provides a mechanism by which oestrogen can directly regulate BDNF expression.\textsuperscript{166} Other measures of plasticity, like LTP and synapse density are also modulated by the oestrous cycle of rats.\textsuperscript{67,158,203}

The adult brain is still capable of producing new neurons. This has been shown in rodents, non-human primates and humans.\textsuperscript{47,68,71,151,164} Progenitor sites are located in two brain regions, namely the subgranule cell layer (SGCL) in the DG of the hippocampus and the subventricular zone. Neurons born in the SGCL migrate to the granule cell layer of the dentate gyrus, while neurons formed in the SVZ migrate via the rostral migratory stream to the olfactory bulb, where they could play a role in olfactory memory.\textsuperscript{150} It has been shown that in the DG of rodents these newly born neurons form functional connections, although the functions of these cells still have to be elucidated.

Environmental factors, like stress and more complex living circumstances, influence the rate of proliferation or survival of these new neurones. While running and environmental enrichment have been found to increase survival of newly born neurons in rodents,\textsuperscript{122} stress exposure decreases neurogenesis.\textsuperscript{35,69,70} This stress-induced decrease is thought to underlie the reduction in hippocampal volume found in depressed patients. Antidepressant treatments have a positive influence on neurogenesis in rodents and non-human primates,\textsuperscript{32,45,77,115} suggesting a role for newly born neurons in the recovery from a depressive episode. Oestrogen also has differential effects on neurogenesis in the hippocampus. Whereas proliferation of new neurons in the hippocampus is increased during high levels of oestrogen,\textsuperscript{179} it appears that oestrogen has negative effects on survival of these newly born neurons in the hippocampus.\textsuperscript{136}

\textit{Stress & gender}

Most studies into affective disorders have mainly used male subjects and ignored the females, because of the complicating factor of the female hormonal cycle. Of the clinical studies using both men and women, the data often were pooled and the aspect of gender was ignored. Preclinical studies also mainly used male animals. However evidence is accumulating that males and females react differently to a variety of stimuli.

In men higher cortisol levels are associated with reduced memory performance,
while in women such a relationship was not found.\textsuperscript{201} During emotional experiences and memory encoding, men and women differ in the neural network that is activated.\textsuperscript{22} Also different brain structures are activated in men and women with the presentation of happy and sad faces.\textsuperscript{105}

In rodents gender differences in the behavioural effects of stress have also been found, especially regarding learning and memory tasks. In male rats chronic stress reduced spatial memory, while it is improved in females.\textsuperscript{16,28,102} Also classical eyeblink conditioning is impaired in females after stress, whereas males show the opposite response.\textsuperscript{202} Contrasting neurobiological effects of chronic stress exposure in male and females have also been found. Stress differently affects the expression of MR and GR mRNA in different brain regions of male and female rats.\textsuperscript{92} While chronic stress-exposure induced apical dendritic atrophy in the hippocampus of male rats, in females the number of branch points on the basal dendritic tree were reduced.\textsuperscript{61}

Animal models are mostly validated in male animals. However, especially in models for affective disorders, in which stress exposure often is used, results found in males might very well not correspond with what occurs in females. Also behavioural tests are validated in male animals, and since evidence is accumulating that males and females react differently to the same stimuli, it is not unlikely that females will show different, unexpected, behavioural responses.

**Our model**

Rats are social animals, and it has been shown that social housing of rats can have beneficial effect on stress coping. Possibly social housing during stress exposure can provide an animal model for social support. As a model for affective disorders, we used a chronic stress model, in which rats, males and females, were exposed to a daily footshock session for a period of three weeks. During one session, rats were placed in a footshock box with a metal grid floor and received five inescapable footshocks in a period of 30-120 minutes. Time of the day and the interval between footshocks varied by day, adding an unpredictable component to the procedure. A psychological element was added by preceding each foot shock by a light stimulus, this way conditioning the rats to the foot shock. This also allows for removal of the noxious stimulus on the last day, so the observed neurobiological changes are the result of the psychological component of the stress, namely exposure to the adverse environment of the foot shock box and the anticipation of a shock. The oestrous cycle of female rats is about 4-5 days, resulting in a different hormonal status per day. Although this affects behavioural and neurobiological responses, by exposing the animals to stress for a 3 week period, females are stressed during all periods of their cycle, which likely eliminates the effects of varying levels of reproductive hormones on the impact of stress.
It has been shown that rats habituate to a stressor when using the same stressor chronically, as is the case with chronic restraint stress.\textsuperscript{173} However this habituation does not occur with chronic foot shock stress.\textsuperscript{109} By preceding each foot shock by a light stimulus in our model, the rat quickly learns that a light is followed by a foot shock, however with no possibility to escape, adding to the stress level.

**Scope**

Even though the beneficial effects of social support are widely recognised, the underlying biological mechanisms have not been investigated. Since possibilities of neurobiological research in humans are limited, an animal model mimicking social support could provide a valuable model to explore the neurobiology of social support. Since social support is associated with a reduced risk of developing a depression after stress, we used social housing during chronic stress exposure as a model, to investigate if social housing provides “social support” to rats and improves chronic stress coping and possible gender differences herein.

**Outline**

**Chapter 2** describes the effects of chronic stress exposure on behaviour in an open field test and on Fos expression in the PVN and DRN. “Social support” was provided by housing rats in unisex groups. Rats were either individually or socially housed in groups of 4 rats (two controls and two stressed). They were subjected to either 3 weeks of control or stress treatment. Fos expression in the PVN provided an indication of HPA-axis activity, while the DRN provided an indication of serotonergic activity.

In **chapter 3** Fos expression in several limbic regions of the same rats as in chapter 2 is described. Fos expression is related to possible disturbances in the dopaminergic reward system and changes in amygdala function.

**Chapter 4** describes the gender-specific effects of chronic stress exposure on neurogenesis, measured by BrdU labelling in the dentate gyrus of the hippocampus and the effects of social housing in unisex groups.

Since social housing in unisex groups did not improve stress-coping in males, in a subsequent experiment rats were housed in male-female pairs. **Chapter 5** describes the gender-specific effects of chronic stress on open field behaviour in isolated and mixed-gender pair-housed male and females. **Chapter 6** presents the pattern of Fos labelling in the brains and pCREB expression in the dentate gyrus of the isolated and paired-housed rats whose behaviour was described in chapter 5. In **chapter 7** the results of the experiments are compared and discussed.
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