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STRESS AND SOCIABILITY: INDIVIDUAL DIFFERENCES AND THEIR NEUROCHEMICAL SUBSTRATE

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V Tõnissaar, M., Herm, L., Eller, M., Kõiv, K., Rinken, A., Harro, J. Rats with high or low sociability are differently affected by chronic variable stress. (*in manuscript*).

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ABBREVIATIONS

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
CMS	chronic mild stress
CRF	corticotropin-releasing factor
CSF	cerebrospinal fluid
CVS	chronic variable stress
D_2	dopamine receptor 2 subtype
DA	dopamine
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders — Fourth Edition
HPA	hypothalamic-pituitary-adrenal axis
HS	high sociability
HVA	homovanillic acid
ICSS	intracranial self-stimulation
i.p	intraperitoneal
LS	low sociability
PCA	parachloroamphetamine
SSRI	selective serotonin reuptake inhibitor

1. INTRODUCTION

1.1. Depression and anxiety disorders

Depression is one of the most widespread mental disorders (Blazer et al., 1994). Depression is defined as an emotional state marked by great sadness and apprehension, feelings of worthlessness and guilt, withdrawal from others, loss of sleep, appetite, and sexual desire, or loss of interest and pleasure in usual activities (Davison and Neale, 1998).

Depression is closely related to and has frequent overlap (Wittchen, 1988; Wittchen et al., 1991) and comorbidity (Davison and Neale, 1998) with anxiety disorders like agoraphobia, obsessive-compulsive disorder, and posttraumatic stress disorder. People with anxiety disorders feel an overwhelming apprehension that seems unwarranted. Diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV) lists six principal anxiety diagnoses: phobic disorders, panic disorder, generalized anxiety disorder, obsessive-compulsive disorder, posttraumatic stress disorder, and acute stress disorder (Davison and Neale, 1998). Longitudinal studies reveal that anxiety diagnoses typically precede depression (Rohde et al., 1991). Still, it is suggested that anxiety and depression can be distinguished in a propensity to pleasurable, positive mood states (Davison and Neale, 1998), whereas either depressed mood or loss of interest and pleasure must be among other symptoms of depression according to DSM-IV. Anxious people score higher than depressed people in autonomic arousal, reporting more physical signs, such as sweaty palms and high heart rates (Clark et al., 1994). Adverse events (and particularly interpersonal difficulties) in childhood and adolescence play a significant role in adult psychopathology (Stanford et al., 1993), but both endogenous and psychosocial factors are important in etiology of depression and anxiety (Deakin et al., 1990).

1.2. The role of stress in mood-associated behaviors

Stress is a multidimensional concept, and difficulties arise when trying to provide a concise and stringent definition (Stanford et al., 1993). Stress is not a well-defined clinical syndrome, and as stress rather causes disorders than is a disorder itself, stress *per se* generally has not been viewed as a target for pharmacotherapy or for drug development (Willner, 1993). Three main components of stress, which are in interaction with each other, can be identified: the input (stress stimuli), the processing systems including the subjective experience of stress, and stress responses (Stanford et al., 1993). In psychiatry, the term “stress” may be used in two ways: it may be used to identify events or circumstances that are perceived adversely (“stressors”), or to describe the state induced by such events or circumstances (the “stress reaction”) (Glue et al.,

1993). There are no symptoms that are unique or specific to stress-related or reactive-type disorders and virtually any psychiatric symptoms can occur (Glue et al., 1993). However, it is possible to generalize about responses to stress, in terms of type of symptoms and duration (Glue et al., 1993).

The purpose of the stress response is to maintain homeostasis at all times (Sapolsky, 2003). Anything in the environment that disturbs homeostasis may be defined as a stressor. Homeostatic balance is then reestablished by physiologic adaptations that occur in response to the stress response (Sapolsky, 2003). Sustained activation of mobilized resources to maintain homeostasis may occur when the organism is without control and information, or when there is a reason to expect negative events. It occurs whenever the organism has an expectancy of negative events, or negative response-outcome expectancies, or no response-outcome expectancies (hopelessness and helplessness) (Stanford et al., 1993). Such an alarm system is there for a purpose and the alarm *per se* does not produce pathological changes. It is only when prolonged and sustained homeostatic elements in the response may be surpassed. If this takes place, disease might occur in the somatic locus with least resistance.

Chronic stress is associated with vulnerability to diseases, hyperactivity of corticotropin-releasing hormone system (Bale, 2005) and alterations in other neurochemical pathways (Harro and Oreland, 2001), abnormalities in the immune system (Dorian and Garfinkel, 1987; O'Leary, 1990), social disruption (O'Leary, 1990) and other biological and behavioral changes that are believed to contribute to the pathophysiology of mood disorders. Epidemiological evidence also strongly suggests that stressful life events play a role as etiological factors in depression (Kendler et al., 1995) and anxiety (Shelton, 2004). Therefore, several attempts to investigate the neurobiology of depression or anxiety, and to measure antidepressant or anxiolytic effects of drugs, have made use of application of chronic stress, which is one of the major determinants in development of human mood disorders. Due to high comorbidity and overlap of these disorders, animal models which include signs of both depression and anxiety may be useful in teaching us also about mechanisms related to these disorders.

More than 20 years ago, Katz and colleagues (Katz et al., 1981A; Katz, 1982) described in a series of papers the chronic variable stress (CVS) model of depression. In this model, rats are subjected to a variety of stressors such as mild uncontrollable footshock, cold water swim, change in housing conditions, reversal of light and dark periods, food and water deprivation etc., over a period of 2–3 weeks. Such a treatment results in a decrease in open field activity which is selectively prevented by antidepressant drugs. Even though this procedure has also been termed as chronic mild stress (CMS) (Weiss and Kilts, 1998), the latter term is more frequently associated with an approach subsequently developed by Willner and colleagues (Willner et al., 1987; Willner et al., 1992; Willner, 1997; Willner, 2005) which has differences compared to the original CVS model in the selection of stressors, timing of their presentation, and the behavioral outcome most commonly measured. Using the original CVS

paradigm, it was shown that stressed rats consumed sweet solutions in reduced quantities, and that this effect was not present when the rats were given imipramine (Katz, 1982). Besides reducing sucrose intake, CMS has been found to reduce ventral tegmental self-stimulation (Moreau et al., 1992) and place preference conditioned with food, sucrose solution or amphetamine treatment (Papp et al., 1991), effects which were prevented by concomitant treatment with antidepressants (Moreau et al., 1992; Papp et al., 1991; Willner et al., 1987). Anhedonia, the inability to experience pleasure, is one of the core symptoms of depression, and the reduction of sucrose or saccharin consumption after chronic stress has been attributed to an anhedonic state and used as a behavioral measure in animal models of depression (Willner, 1995). Any important difference between procedures labeled as CMS and CVS has not been explicitly demonstrated and is currently impossible to demonstrate because both terms are in use for a large variety of different combinations of stressors (Cabib, 1997). In both cases the model consists of sequentially applied series of stressors, none of which is supposed to be either necessary or sufficient to affect behavior on its own, but the essential feature is believed to be variety and unpredictiveness of the stressors (Muscat and Willner, 1992).

Both anxiety and depression develop in response to stress and it is generally believed that stressful events chronically increase glucocorticoid production which in turn affects various neurotransmitter systems (Baranyi et al., 2005). In contrast to depression, however, the relationship between chronic stress and anxiety is less clear, as anxiety patients often show normal glucocorticoid levels and respond normally to dexamethasone challenge (Baranyi et al., 2005). It is suggested that the stress response is hardwired into the brain of the typical mammal and is most often triggered when survival of the organism is threatened (Shelton, 2004). The primate stress response, however, can be triggered not only by a physical challenge, but also by the mere anticipation of a homeostatic challenge. As a result, when humans chronically and erroneously believe that a homeostatic challenge is about to occur, they enter the realm of neurosis, anxiety, and paranoia (Sapolsky, 2003).

Some decades ago, the clinical and preclinical activity of antidepressants and anxiolytics was well defined: tricyclic antidepressants and benzodiazepines were used for the treatment of major depressive episodes and anxiety, respectively (Borsini et al., 2002). Consequently, animal models were classified according to their sensitivity to antidepressants (the so-called animal models of depression) or to benzodiazepines (the so-called animal models of anxiety). In the last 20 years, the introduction of selective 5-hydroxytryptamine reuptake inhibitors (SSRI), i.e., fluoxetine, fluvoxamine, sertraline, paroxetine and citalopram into the clinical practice has challenged the traditional concept of antidepressants and anxiolytics (Uhlenhuth et al. 1999). The fact that SSRIs are also effective in anxiety disorders has a deep impact on our concepts of animal models of anxiety (Borsini et al., 2002). As clinically effective drugs are used to assess predictive validity, animal models of anxiety should be sensitive to

SSRIs (Borsini et al., 2002). However, scientific literature still lists the models validated with anxiolytics as animal models of anxiety and tests traditionally used with antidepressants as models of depression..

Though stress alerts several systems of the body, it does not always end up with pathology and produces illness only in some people (Davison and Neale, 1998). Stress response itself is essential for adaptation, maintenance of homeostasis, and survival (Bale, 2005), and the sensitivity of the individual to stressful encounters is also important in the development of mood disorders (Harro and Oreland, 2001). People with high stress reactivity display an enhanced and more persistent physiological response to stressors that makes them more likely to withdraw from stressful situations when this is feasible (Bradley, 2000).

The role of stress in psychiatric diseases is well demonstrated, but less is known what determines the ability of an individual to cope with stressful situations. Both human and animal studies have shown that stress reactivity has a heritable component (Sloman et al., 2003). Personality has long been viewed as related to psychopathology, and certain personality styles may even enhance or degrade immune response (O'Leary, 1990), but the precise nature of the relation remains unclear (Clark, 2005). There are studies which suggest that high neuroticism strongly predicts anxiety and mood disorders (Christensen and Kessing, 2006; Jorm et al., 2000). Other personality dimensions (e.g., anxiety sensitivity, attribution style, sociotropy or dependence, autonomy or self-criticism, and constraint) may also constitute vulnerability factors. Clark (2005) has suggested that three broad, innate temperament dimensions — negative affectivity, positive affectivity, and disinhibition — at their extremes are risk factors (diatheses) for psychopathology, especially given adverse life experiences (stress). Clark et al (1994) have described that negative affectivity (or neuroticism) appears to be a vulnerability factor for the development of anxiety and depression, and indicates poor prognosis. Positive affectivity (or extraversion) is related more specifically to depression, can be a risk factor for its development, and suggests poor prognosis. The identification of endophenotypes in the personality disorders may provide a basis for the identification of underlying genotypes that influence the traits of the personality disorders, as well as susceptibility to major psychiatric illnesses (Siever, 2005).

Studies in animals have shown that animals also differ in vulnerability to stress. Recently, interest in stable individual differences in behavior of experimental animals has substantially increased. Particularly prominent have been studies on differences in exploratory behavior, which may explain the vulnerability to diseases and longevity (Cavigelli, 2005). Selective breeding techniques are used to develop inbred strains that differ in their responsiveness to stress. Examples are the Maudsley Reactive and Nonreactive and the Roman High and Low Avoidance rat strains (Broadhurst, 1975; Driscoll and Battig, 1982), which were selected on the basis of their responses to an acute stressor,

and the Flinders Sensitive Line (FSL) rat is the result of selective breeding for sensitivity to the hypothermic effect of cholinergic agonists.

Studies with chronic variable/mild stress have shown that this procedure elicits helplessness or anhedonia in some but not all animals (Henn and Vollmayr, 2005). Using elevated plus-maze test as the measure for selection, rats bred for either high or low anxiety-related behavior differ in their stress coping strategies, the former being more susceptible and vulnerable to stressor exposure and preferring more passive strategies (Landgraf and Wigger, 2002). Rats divided on the basis of high and low exploratory behavior differently respond to chronic stress, whereas stress reduces sucrose intake more strongly in low explorers (Matrov et al., unpublished).

Development of novel animal models has been identified as one of the major needs in research on mood disorders (Nestler et al., 2002A). Animal models have helped to discover new medications and to understand the etiological factors that cause depressive symptoms in humans. However, there are major limitations with the available models concerning understanding the circuits in the brain responsible for the normal regulation of mood and affect, and identifying the circuits that function abnormally in mood disorders (Holmes, 2003; O'Neill and Moore, 2003). Only a few of the available tests have been suggested to possess high specificity and reliability in predicting novel drugs (Cryan et al., 2002). These models may prove adequate as both screening tests and as models to investigate neuropharmacological mechanisms associated with treatment, but still their validity as simulations of the psychiatric condition is highly questionable. The inability to determine which of the many effects of antidepressants are responsible for their therapeutic actions constitutes a fundamental limitation of this approach (Mitchell, 2005). While animal assay models, such asthe forced swimming test and olfactory bulbectomy, have some usefulness in predicting new drugs possessing antidepressant activity, insight into the pathophysiology of depression should probably be gained by applying homologous models which attempt to elicit changes resembling those in patients with depression. In view of the discontent with the advances of clinical pharmacology of depression and the state-of-the-art of animal models, innovative approaches are necessary (Harro, 2004).

The reliability of the chronic mild stress model has been a subject of extensive discussions (e.g. Cryan et al., 2002; Harro, 2004; Willner, 1997). There are problems with reliability: far from all laboratories have been able to demonstrate a reduction in sucrose intake during the chronic mild stress procedure, and where this phenomenon is observed, it is not consistent (D'Aquila et al. 1997; Harro et al. 1999A; Nielsen et al. 2000; Willner, 1997). It is also difficult to interpret data showing that rats with decreased sucrose intake after CMS may display behaviors difficult to associate with depression, such as an increased activity in the elevated plus-maze (D'Aquila et al, 1994). So far no clear explanations to the controversial results have been presented.

As sensitivity to stressful events is moderated by genetic makeup (Caspi et al., 2003; Costello et al., 2002; Monroe and Simons, 1991) and by environmental conditions (Chesler et al., 2002), which may be a rich source for inconsistency, one strategy would be to use genetic and nongenetic perturbations (Nestler et al., 2002A). Thus, the reliability of chronic stress procedures could possibly be increased by combining them with restricted manipulations on monoaminergic systems, or selecting animals according to spontaneous behavior related to symptoms of depression or anxiety.

1.3. Implication of social functioning in mood disorders

Social behavior is the basis of one of the most generally accepted independent dimensions of personality (Depue and Collins, 1999), and has many important roles in the survival of the individual and species. Effective social support determines sensitivity to stress and lack of social support increases the likelihood of developing an illness. Low levels of social support are related to an increase in negative emotions (Davison and Neale, 1998), which may affect some hormone levels and the immune system (Kielcolt-Glaser et al., 1984). Seeking comfort or social support from others is an example of emotion-focused coping in humans.

Many clinical disorders include difficulties in creating or maintaining social contacts, such as social anxiety disorder, several personality disorders, and autism. Also, majority of the stressful stimuli in humans that lead to psychopathology are of social nature (Buwalda et al., 2005), and a decrease in social functioning is one major symptom in depressed patients (Nemeroff, 1998). Depressive episodes in humans are typically triggered by defeats, major social losses and humiliations, i.e., rank losses (Brown et al., 1995). Improvement of social circumstances is effective in therapy of depression (Brown et al., 1988; Sloman et al., 2003). Therapy can help children develop more secure attachments and to heal from insecure patterns of attachment (Bradley, 2000). Positive signals from others (in the form of care, support and love) are physiologically meaningful, enhance positive affect and lower stress activation (Cacioppo et al., 2000; Sloman et al., 2003). Drugs that treat depression and influence central serotonergic function reduce anger, aggressiveness, anxiety and flight/withdrawal, and modulate the dimensions of normal personality, as characterized by reduced negative affective experience and increased affiliative behavior in healthy persons (Knutson et al., 1998).

Several studies have demonstrated that depressed people are low in social skills across a variety of measures: interpersonal problem solving (Gotlib and Asarnow, 1979), speech patterns (speaking very slowly, with silences and hesitations, and more negative self-disclosures), and maintenance of eye contact (Gotlib, 1982; Gotlib and Robinson, 1982). In a longitudinal study of unipolar

depressives, Hammen (1991) confirmed that these patients experience much stress (particularly of an interpersonal nature) and that their own behavior contributes to the high levels of stress that they experience. Low social competence predicted the onset of depression among elementary-school-age children (Cole et al., 1990), and poor interpersonal problem solving skills predicted increases in depression among adolescents (Davila et al., 1995). Thus, coping with stress depends on effective control over social relationships, resources and the social signal/communication aspects, which are important in the etiology of depression (Davison and Neale, 1998; Sloman et al., 2003).

Studies of social behavior in rodents have mostly been carried out within the constraints of rather specific paradigms, such as aggressive, sexual or maternal behavior. There are less animal experiments which use social coping mechanisms, control over social relationships and social signal/communication, and investigate their relevance to depression. However, there is a frequently used simple animal model that was developed to measure anxiogenic and anxiolytic drug effects (File and Hyde, 1978) but which is based on social behavior: the social interaction test, in which the time spent in active social interaction between two unfamiliar rats in a neutral arena is measured. Behavior of rats in the social interaction test does not correlate well with their performance in other animal models of anxiety (Ramos et al., 1997). This suggests that the model has other important underlying mechanisms than just general anxiety, and could be used for studying neurobiology of social behavior provided that social behavior of an animal would be a consistently expressed trait in this test.

As disturbances in social functioning are an important predisposition in vulnerability to stress and eliciting mood and anxiety disorders thereafter, study of persistence of social behavior in animals and related neurobiological mechanism and vulnerability to stress would be helpful to understand these maladies.

1.4. The role of 5-hydroxytryptamine in mood associated behaviors and underlying neurobiology

The phenotypical expression of social behavior is regulated by many different neurochemical systems, but a coherent picture is yet to emerge (Panksepp, 1998). However, 5-hydroxytryptamine (5-HT) is a neurotransmitter which is highly implicated in regulation of social behavior and is probably most important in the etiology of unipolar depression. Also, the amount of evidence for a role of aberrant 5-HT-ergic neurotransmission in the etiology of anxiety disorders, such as generalized anxiety and panic disorder, has been increasing steadily during the past several years (Linthorst, 2005).

5-HT is important in the expression of personality dimensions, especially in these which are associated with affective and motivational processes (Knutson et al., 1998; Tse and Bond, 2002). 5-HT neurotransmission has been found to be

associated with higher impulsivity, aggressiveness, and anxiety in humans, non-human primates and other species (Grimmett and Sillence, 2005; Suomi, 2005; Turecki, 2005). Drugs that are effective in the treatment of depression and increase central serotonergic function also modulate the dimensions of normal personality characterized by reduced negative affective experience and increased affiliative behavior in healthy persons (Beech and Mitchell, 2005; Knutson et al., 1998), and non-human mammals (Insel and Winslow, 1998). Nevertheless, there is also evidence for a negative association between 5-HT metabolism and social competence (Yodyingyuad et al., 1985). Even in the simple social interaction test, both increased (Duxon et al., 2000; Hamon et al., 1999; Lightowler et al., 1994) and reduced (Bagdy et al., 2001; File et al., 1996; File et al., 1993; Kennedy et al., 1993; Kenny et al., 2000) social behavior has been suggested to be mediated via an increase in 5-HT-ergic function.

Pharmacological interventions in frontal cortex and septum have been found to influence behavior in the social interaction test of rat (File et al., 1993; Kenny et al., 2000), and even though serotonergic mechanisms are most consistently implicated in animal and human studies on social behavior (Duxon et al., 2000; Knutson et al., 1998), catecholaminegic mechanisms have also been suggested to contribute (Depue and Collins, 1999).

In addition to the implication in social behavior, serotonergic systems play an important role in the regulation of behavioral, autonomic and endocrine responses to stressful stimuli (Lowry, 2002). In accordance to these findings, deficits in serotonergic neurotransmission have for a long time been considered a substantial factor in depression (Coppen et al., 1972; Lapin and Oxenkrug, 1969; Ordway et al., 2002). Destruction of 5-HT nerve terminals with 5,6-dihydroxytryptamine potentiates by 50% the stress induced rise in plasma corticosterone, suggestive of an interaction between stress and low serotonergic state in the etiology of depression (Richardson, 1984). As a convincing evidence for the principle that individual differences in serotonergic systems determine the resilience to stress, Caspi and colleagues (2003) recently demonstrated that a functional polymorphism in the promoter region of the 5-HT transporter gene was responsible for the efficacy of stressful life events in eliciting depression. Depletion of 5-HT stores in terminal regions compromises the ability of the serotonergic neurons to activate central systems that manage stressful stimuli (Matuszewich et al., 2002). During the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, behavioral responses are accentuated, and animals may overreact to demanding situations (sometimes referred to as ‘impulsiveness’) (Netto et al., 2002).

Parachloroamphetamine (PCA) is a drug which acutely increases brain 5-HT (Fuller, 1992) and dopamine (DA) (Leonard, 1976; Massari et al., 1978) function by releasing these neurotransmitters into the synaptic cleft. Using histological techniques it was established that PCA has a selective long-term toxic effect on 5-HT-ergic neurons (Harvey et al., 1975). Thus, at longer times, PCA causes depletion of brain 5-HT, affecting brain serotonergic projections with a

different neuroanatomic specificity than the dihydroxytryptamines (Fuller, 1992), but not having long-term depletion effect on DA (Leonard, 1976; Massari et al., 1978). Partial serotonergic denervation has been assumed to be of higher importance for both physiological regulation of 5-HT function and its implication in depression than near to complete denervation (Datla and Curzon, 1996), since large neurochemical lesions induce significant adaptive changes both pre- and postsynaptically (Harro et al., 1999A) and biological predispositions are likely to be more quantitative than qualitative (Datla and Curzon, 2004, Häidkind et al., 2004).

Amount of evidence for a role of aberrant 5-HTergic neurotransmission in the etiology of anxiety disorders and depression has been increasing steadily during the past several years. However, SSRI drugs for treatment of depression and anxiety show a delayed onset of improvement (Linthorst, 2005). Therefore, new therapeutical strategies are being explored and better understanding about the role of 5-HT is necessary.

1.5. Measurement of hedonic state in animal models of depression, and neurobiology underlying sucrose intake

In addition to depressed mood, loss of interest and pleasure is a most important symptom in the diagnosis of depression. Behavioral studies of stress have tended to focus largely on either gross measures of motor output or on performance in aversively motivated tasks. However, stress is also known to disrupt consummatory behaviors and performance in appetitively motivated tasks. Both acute and chronic severe stressors reduce the animal's performance in rewarded tasks (Katz et al., 1981A; Rosellini, 1978; Zacharko et al., 1983). A single session of inescapable (but not escapable) footshock has been shown to decrease responding for brain-stimulated reward (intracranial self-stimulation: ICSS) in mice (Willner, 1993). ICSS elicited from the VTA (the origin of the mesolimbic dopamine projection) or from the nucleus accumbens or frontal cortex (two of its terminal fields) was suppressed by inescapable shock, but ICSS elicited from the substantia nigra (the origin of the nigrostriatal dopamine projection) was unaffected (Zacharko and Anisman, 1991). A similar decrease in sensitivity to sweet rewards, assessed by a decrease in preference for sweet solutions over plain water, has been reported in rats following a single session of restraint stress (Plaznik et al., 1989) or social defeat (Koolhaas et al., 1990), or withdrawal from chronic amphetamine treatment (Cassens et al., 1981).

Measurement of sucrose intake or preference is currently in widespread use in preclinical psychopharmacology, as in studies predicting vulnerability to psychostimulant self-administration (Gosnell, 2000) or ICSS (Smith and Schneider, 1988), identifying the role of different neurochemical mechanisms in positive reinforcement (Smith and Schneider, 1988), and measuring the effect of

stress (Rygula et al., 2006; Sanchis-Segura et al., 2005), particularly in the chronic mild or variable stress model of depression (Willner, 2005). Animals have been subsensitive to reward after chronic stress also in the place conditioning paradigm: the normal preference for environments paired with food, sucrose solutions, amphetamine or morphine was abolished or greatly attenuated in stressed animals (Willner et al., 1987). CMS increases threshold for ICSS (Willner, 1993).

Individual differences also exist in sensitivity to rewards, as measured e.g., by sucrose intake. Several studies have divided rats into high and low sucrose consumers and found that low sucrose consumption is associated with higher anxiety in acoustic startle and elevated plus-maze tests (DeSousa et al., 1998), higher sensitivity to the effects of low-dose amphetamine on sucrose consumption (Sills and Vaccarino, 1996), and lower self-administration of amphetamine or cocaine (DeSousa et al., 2000; Gosnell, 2000). Rats with different sucrose preference also differ in baseline and stimulated HPA axis activity, and gene expression of NMDA receptor subunits and CRH (Duncko et al., 2003). Some of these studies have separated rats into low and high intake groups on the basis of a single observation, and others based on average consumption across several tests. Limited information is available as to the consistency of the individual sucrose intake or preference.

The neurotransmitter DA is widely recognized to be critical to the neurobiology of reward, learning and addiction. Virtually all drugs of abuse, including heroin and other opiates, alcohol, cocaine, amphetamine and nicotine activate DA-ergic systems. So called "natural" rewards such as food, positive social interactions and even humor likewise activate DA neurons and are powerful aids to attention and learning (Cannon and Bseikri, 2004). Sweet solutions are a well-characterized natural reward. When a source of sugar is encountered, animals will consume substantial amounts, return to it preferentially, and will work to obtain access. DA systems are activated in animals drinking sugar solutions, and lesions of DA-ergic neurons or pharmacological blockade of DA receptors seem to reduce the reward value of both sweet tastes and drugs of abuse (Cannon and Bseikri, 2004). Several studies support the role of DA in mediating the rewarding effect of sucrose. For example, pimozide, a dopamine receptor 2 subtype (D_2) antagonist, dose-dependently decreased sucrose intake but increased water intake in the two-bottle test (Towell et al., 1987). Dopamine D_1 and D_2 antagonists decreased the intake of sweet solutions during sham feeding (Hsiao and Smith, 1995). As a DA antagonist did not affect consumption at a dose that inhibited conditioned place preference, it was suggested that DA is particularly important for the establishment of reinforcement produced by sucrose (Agmo et al., 1995). Higher sucrose intake is associated with increased efficacy of amphetamine in eliciting DA overflow in nucleus accumbens (Sills and Crawley, 1996). The facilitatory effect of low doses of amphetamine on sugar consumption in rats with low sucrose intake was reproduced after microinjections into nucleus accumbens (Sills and Vaccarino, 1996).

However, it is difficult to distinguish a pure behavioral role for DA in actually initiating the sense of reward and motivation from its undisputed part in facilitating the motor response necessary to obtain the reward. Cannon and Palmiter (2003) suggest that mice that cannot make DA have a deficit of goal-directed behavior that is not specific to reward processes, and naive juvenile DA deficient mice that had never been injected with L-dopa demonstrate robust sucrose preference before experience with food. Though alterations in DA-ergic systems found after CMS are used for explanation of the 'anhedonic' effect of the procedure (Willner et al., 1998), it has been suggested that the meso-telencephalic DA-ergic pathways rather mediate signals of salience than of reward (Horvitz, 2000; Panksepp, 1998; Robinson and Berridge, 1993).

Digestion and absorption rate of sucrose shows circadian fluctuations under normal physiological conditions and is in rats higher during the night time (Hara and Saito, 1989). Mostly, sucrose preference tests are carried out during the light phase, which is not the active period for nocturnal animals, and during which period the sensitivity of sucrose intake to chronic stress has been described to be lower than during the dark phase (D'Aquila et al., 1997). Timing of experiments is crucially important also in studies on brain mono-aminergic indices and their interrelationships (Ågren et al., 1986). Extracellular levels of DA and DA metabolites in striatum and nucleus accumbens have been found to be higher during the dark phase (Castaneda et al., 2004; O'Neill and Fillenz, 1985; Piazza et al., 1996; Shieh et al., 1997). However, others have found that basal levels of DA in the nucleus accumbens were not different in the light or dark, but were increased by novelty and handling only during the light period (Feenstra et al., 2000). At least in mice, striatal dopamine D_2 receptor levels have also been found to undergo diurnal rhythms, being high during the light phase and low during the dark phase (Akhisaroglu et al., 2005; Viyoch et al., 2001).

Brain reward pathways are best known for their role in mediating the reinforcing effects of drugs of abuse, and include DA-ergic neurons in the ventral midbrain (particularly those in the ventral tegmental area) and their anterior projections to the basal forebrain (e.g., the nucleus accumbens or ventral striatum) (Nestler et al., 2002B). More recent work has indicated that under normal conditions these structures regulate an animal's response to natural reinforces, such as food, sex, and social interaction (Everitt et al., 1999; Koob et al., 1998; Wise, 1998). Given the prominence of anhedonia as well as changes in appetite and sexual behavior in many patients with depression, and the enhanced hedonic state in mania, it is plausible to speculate on a role of the brain's reward circuitry in mediating these symptoms (Nestler et al 2002B). Moreover, early phases of withdrawal from many types of drugs of abuse are associated with aversive emotional symptoms that in some patients are similar to depression, and these symptoms are thought to involve both the brain's reward pathways as well as the amygdala. Thus, a variety of behavioral tests that measure aspects of reward, optimized in the drug abuse field, would appear

to have some potential value in depression research as well as in mania (see Everitt et al 1999; Wise 1998). As a measure of hedonic state, measurement of changes in sweet consumption is in widespread use in animal models of depression. It would be useful to know, whether the sweet intake and preference of rat is individually stable, and whether this trait is associated with the dopamine D_2 receptor function, related in the mechanism of reward.

2. AIMS OF THE STUDY

The current dissertation addresses the following questions:

- Whether chronic variable stress and partial serotonergic denervation and their combination elicit behavioral and neurochemical changes, reminiscent to depression or anxiety, and which of these changes are reversible by anti-depressant treatment;
- Whether the social behavior of rat is an individually stable trait, and associated with monoamine neurochemistry;
- Whether animals with different social activity differ in sensitivity to chronic variable stress;
- Whether sucrose consumption/preference is an individually stable trait, and associated with dopamine D_2 receptor function.

3. MATERIALS AND METHODS

3.1. Animals

Male Wistar (**Papers I, III and IV**) or Sprague-Dawley (**Papers II, V and VI**) rats from the National Laboratory Animal Center, Kuopio, Finland (**Papers I and III**) or from Scanbur BK AB, Sweden (**Papers II and IV-VI**), were used. Rats were single (**Papers I and IV-VI**) or group-housed (**Papers II and III**) in plastic cages with food (Lactamin R35, Sweden) and water ad libitum. Room temperature was maintained at 21±2°C and 12:12 h light-darkness cycle was applied.

3.2. Parachloroamphetamine treatment (Papers I, II and V)

PCA (Sigma) in the dose of 2 mg/kg (expressed as for hydrochloride) was dissolved in distilled water and injected in a volume of 1 ml/kg intraperitoneally. Control animals received a vehicle injection.

3.3. Chronic variable stress procedure (Papers I, II and V)

Rats belonging to the Stress group were submitted to the CVS procedure in a separate room. The procedure used was developed on the basis of our previous experiments with the CMS model (Harro et al., 1999A) and a direct comparison of 'chronic mild stress' (Willner, 1995) and 'chronic variable stress' (Katz et al., 1981A) models with the aim of achieving reliable effects on body weight gain (Tõnissaar et al., 2000). Various stressors of different duration were applied every day, one stressor per day. Each stressor was applied twice (**Paper I**) or three times (**Papers II and V**, except strong illumination in **Paper II**, which was also applied twice). Control rats remained undisturbed in their cages during the study, except for weighing (**Papers I, II and V**), and sucrose intake tests (**Paper II**). The stressors applied (in the order of presentation) included in **Paper I**: electric footshock (ten shocks, 1 s each at the intensity level of vocalization threshold), stroboscopic light (for 14 h, 10 Hz, 2 lx), cold (4°C) water and wet bedding (initially, 400 ml of water was poured on a rat, and the sawdust bedding was kept wet for the following 22 h), cage tilt at 45° (for 24 h), strong illumination (900 lx) during the predicted dark phase (for 12 h), tail pinch with a clothes-pin placed 1 cm distal from the base of tail (5 min), and movement restriction in a small cage (11×16×7 cm for 2 h). In **Paper II**, stressors were divided into two categories, in order to observe acute effect of each stressor: short-term and long-term. Both types of stressors were used intermittently, and in the following order: cold (4°C) water and wet bedding

(initially, 400 ml of water was poured on a rat, and the sawdust bedding was kept wet for the following 17 h), electric footshock (ten shocks, 1 s each at the intensity level of vocalization threshold), stroboscopic light (for 13 h, 10 Hz, 2 lx), tail pinch with a clothes-pin placed 5 cm distal from the base of tail (5 min), cage tilt at 45° (for 20 h), movement restriction in a small cage (11 x16 x 7 cm) for 2 h, strong illumination (900 lx) during the predicted dark phase (for 12 h). In **Paper V**, the order of movement restriction and tail pinch was switched (**Paper II**), in order to clarify whether the strong body weight increasing effect of movement restriction was related to previously administered stressor. Also, due to logistical reasons electric footshock was replaced with imitation of injection, and a day without stress followed the strong illumination stressor in order to avoid the effect of stress on body weight gain and sucrose consumption in the sucrose preference test. Thus, stressors in **Paper V** were presented in the following order: cold (4°C) water and wet bedding (initially, 400 ml of water was poured on a rat, and the sawdust bedding was kept wet for the following 17 h), imitation of injection (a syringe without a needle was pressed against abdomen), stroboscopic light (for 13 h, 10 Hz, 2 lx), movement restriction in a small cage (11 x16 x 7 cm) for 2 h, cage tilt at 45° (for 20 h), tail pinch with a clothes-pin placed 5 cm distal from the base of tail (5 min), strong illumination (900 lx) during the predicted dark phase (for 12 h), day without stress.

3.4. Open field test (Papers I, II and V)

In the open field test the rats were placed at the center of a rectangular arena (1×1 m in **Paper I**, and 0.5 X 1 m in **Paper II and V,** with 40-cm-high side walls). The arena was divided into 16 (**Paper I**) or 8 (**Papers II and V**) equal sized squares. Parameters registered during 4 min were the number of squares visited (with all four feet on one square), the number of rearings and number of excrements left in the open field.

3.5. Forced swimming test (Papers I, II and V)

The technique first characterized by Porsolt and colleagues (Porsolt et al., 1978) was used after pharmacological validation in our laboratory (Harro et al., 1997; Pähkla et al., 1996). Briefly, rats were forced to swim in a vertical glass cylinder, water temperature maintained at 25°C. On the 1st day of experiments, the rats were forced to swim for 15 min and were thereafter dried with laboratory tissues. Water was changed after testing of each subject. On the following day, rats were re-exposed to the forced swimming for 5 min. Only the total duration of immobility on both days was measured in **Paper I**. In **Papers II and V**, behavior was videotaped and analyzed along the categories of

immobility, swimming and struggling (Armario et al., 1988; Häidkind et al., 2004). In addition, the number of excrements and divings (animal dived toward the bottom of the cylinder and then returned to the surface) during the tests were measured in **Papers II and V**. Data of the first forced swimming test were analyzed for the first 5 min session, except for the number of fecal boli. Data were recorded within 5-min periods on both days, except for the number of fecal boli and divings.

3.6. Sucrose preference test (Papers I, II, V and VI)

Sucrose intake was measured in the home cages (**Papers I, V and VI**) or in separate cages due to group-housing (**Paper II**). Food was freely available all the time. In **Paper I**, sucrose solution (1%) was introduced on the 1st day of single housing in the only drinking bottle for 24 h. Then 2 days later the first sucrose preference test was carried out with two bottles, one filled with 1% sucrose solution and the other with water. With an interval of 4 days three additional sucrose preference tests were carried out, the last immediately before the start of CVS. In **Papers II, V and VI**, the procedure of adaptation with sucrose were not carried out. Instead, one sucrose preference test was carried out before the onset of stress regime. In **all stress experiments**, additional sucrose preference tests were carried out after every weeks of stress. Placement of the bottles with sucrose versus water was randomized across the days. Sucrose and water consumption was measured for the period of 1 h by weighing pre-weighed bottles at the end of the test. Sucrose preference was measured by calculating the proportion of sucrose consumption out of total consumption of liquid. Sucrose consumption was also adjusted to the body weight.

3.7. Social interaction test (Papers I–V)

The test developed by File and Hyde (1978) was used in a modified version. Two unfamiliar, weight-matched rats receiving the same treatment were placed in opposite corners of a brightly-lit chamber (30×30×60 cm) with floor covered with wood shavings and observed for 10 min. The total time spent in active social behavior (allogrooming, sniffing the partner, crawling under and over, following) was recorded.

3.8. Elevated plus-maze test (Paper V)

The method first described by Handley and Mithani (1984) and modified in our laboratory (Harro et al., 1990) was used. In brief, the plus-maze consisted of two

open arms (50 x 10 cm) without any walls, two enclosed arms of the same size with 40 cm high sidewalls and end wall, and the central arena (10 x 10 cm) interconnecting the arms. The arms of the same type were opposite to each other. Both open arms were divided into three parts of equal size by lines which also separated the central arena from all arms. At the beginning of the experiment the rat was placed into the beginning of a closed arm, facing the closed end. The central arena and the open arms formed the 'open part' of the apparatus. An entry into open arms was counted when the rat crossed the line between the central arena and an open arm with all four paws. The rat was considered to explore the open part of the apparatus when it had clearly crossed the line between a closed arm and the central arena with its both forepaws. Behavioral measures taken during 4 minutes included a) the latency period before entering the open part (i.e. the central arena); b) the number of line crossings; c) time spent in the open part of the apparatus; d) the number of approaches towards the central arena which were not completed (nose crossed the line but not both of the forepaws); e) number of excrements left during testing; f) the number of open arm entries, and g) the total number of arm entries. From the two latter measures, the ratio open/total arm entries were calculated.

3.9. In vivo microdialysis (Paper IV)

Surgery was carried out after the last social interaction experiment. The animals were anaesthetised with chloral hydrate (350 mg/kg i.p.) and mounted in a Kopf stereotactic frame. Home-made microdialysis probes (from polyacrylonitrile/ sodium sulphonate copolymer, i.d. 0.22 mm; o.d. 0.31 mm; AN69 HF, Hospal, Bologna, Italy) were implanted in the ventral tegmental area (VTA; exposed length 1.0 mm) or in the medial prefrontal cortex (PFC; exposed length 4.0 mm). The coordinates for implantation were as follows, PFC: AP 3.3 mm, ML –0.8 mm, DV –5.0 mm; VTA: AP –5.3 mm, ML –2.5 mm, DV –8.4 mm, implanted at an angle of 12°, form bregma and dura, according to Paxinos and Watson (1986). Microdialysis experiments were carried out in awake freely moving animals 24 h after the surgery. In the morning of the microdialysis experiment, both probes were perfused with perfusion solution (140 mM NaCl, 4.0 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 1.0 mM Na_2HPO_4, 0.2 mM NaH_2PO_4; pH 7.3–7.4) at a constant flow rate of 1.5 µl/min. After the stabilization period of 2 h, fifteen 15-min samples were collected into the vials prefilled with 7.5 µl of 0.02 M acetic acid. After the collection of the sixth sample the animals were injected with PCA (2 mg/kg i.p.). Upon completion of the experiment the animals were deeply anaesthetized with chloral hydrate (350 mg/kg i.p.) and decapitated; the brains were removed, immediately frozen in ice cold acetone, and kept at –80°C. The brains were sectioned in a cryostatic microtome (Microm GmbH, Germany), the probe placements were determined according to the atlas by

Paxinos and Watson (1986) and data of animals with probe placements outside the ventral tegmental area or medial prefrontal cortex were excluded from the analysis. The quantity of 5-HT in the samples was determined by high-performance liquid chromatography with electrochemical detection.

3.10. Measurement of monoamines and their metabolites in brain tissue and microdialysates (Papers I–V)

Monoamines and their metabolites were assayed by HPLC with electrochemical detection. The chromatography system consisted of a Hewlett Packard HP 1100 series isocratic pump, a thermostatted autosampler and a thermostatted column compartment. In **Papers I, II, III and V** an HP 1049 electrochemical detector (Hewlett Packard, Germany) with glassy carbon electrode was used, the measurements were done at an electrode potential of +0.6 V versus the Ag/AgCl reference electrode. The rat brain tissues were homogenized with Bandelin Sonopuls ultrasonic homogenizer (Bandelin Electronic, Germany) in ice-cold solution (5–30 µl/mg tissue) of 0.09–0.1 M perchloric acid containing 5 mM sodium bisulfite and 0.04 mM EDTA. The homogenate was then centrifuged at 17 000×*g* for 10 min at 4°C. In **Papers I and III** the supernatant obtained was chromatographed on a Lichrospher 100 RP-18 column (250×3 mm; 5 µm) protected by a Supersphere RP18 (10×2 mm, 4 µm) guard column at temperature 30°C using the mobile phase containing 0.05 M sodium citrate buffer at pH 3.6, 0.9 mM sodium octylsulfonate, 0.3 mM triethylamine, 0.02 mM EDTA, 1 mM KCl and 8–10% acetonitrile. In **Papers II and V** a Lichrospher 60 RP Select B column (250×3 mm; 5 µm) was used and mobile phase containing 0.05 M sodium citrate buffer at pH 3.7, 0.02 mM EDTA, 1 mM KCl, 1 mM sodium octanesulphonate and 5,6% acetonitrile. The limit of detection for all assayed compounds on both columns was 0.05–0.10 pmol at signal to noise ratio (S/N)=3. In **Paper IV** 5-HT was assayed using ESA Coulochem II detector with ESA 5011 analytical cell, the potential of the electrode used for measurements was +250 mV. Separation was done on a Luna C18(2) column (150×2mm, 5 µm) using mobile phase containing 0.05 M sodium citrate buffer at pH 5.3; 0.02 mM EDTA; 4.1 mM sodium octylsulphonate and 18% acetonitrile. The limit of detection for 5-HT was 2 fmol at signal to noise ratio (S/N) 3.

3.11. D_2 receptor-stimulated [^{35}S]GTPγS binding (Papers V and VI)

Membranes from nucleus accumbens and striatum were prepared as described previously (Lepiku et al., 1996). The tissues were homogenized in 3.5 ml of

homogenization buffer (50 mM Tris-HCl, pH=7.4) by Bandelin Sonopuls homogenizer (three passes, 10 s each). The membranes were collected by centrifugation at 40,000×g for 20 min at 4°C and were washed by homogenization and centrifugation two more times. The final pellet was homogenized in 90 ww/v, (striatum) or 200 ww/v (nucleus accumbens) of the incubation buffer (20 mM K-HEPES, 7 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, pH=7.4).

Binding of [^{35}S]-guanosine-5´-(γ-thio)-triphosphate ([^{35}SGTPγS]; Perkin Elmer Life Sciences, Boston, MA, USA) was carried out as described earlier (Rinken et al., 1999). In brief, the membranes (200 μg of accumbal and 500 μg of striatal membranes per tube) were incubated with 0.2 nM [^{35}S]GTPγS and different concentrations of guanosine diphosphate (GDP, 3 mM — 1 μM) and 1mM DA or 10 μM butaclamol (all from Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA) for 90 min at 30°C. The reaction was stopped by rapid filtration through GF/B glass-fiber filters (Whatman Int. Ltd., Madistone, UK) and the filters were washed three times with 3 ml (**Paper V**) or 5 ml (**Paper VI**) of ice-cold 20 mM phosphate buffer (pH=7.4) containing 100 mM NaCl. The radioactivity content of the filters was counted in 4 ml of scintillation cocktail OptiPhase HiSafe3 (Wallac Perkin Elmer Life Sciences, Cambridge, UK) by RackBeta 1219 liquid scintillation counter (Wallac Inc., Gaithersburg, MD, USA).

3.12. Data analysis

All statistical calculations were performed using StatView 4.5 software (Abacus Concepts. Cary, NC, USA). All binding data (**Papers V and VI**) were analyzed by nonlinear least-squares regression analysis using a commercial program GraphPad PRISM™ 2.0 (GraphPad Software, San Diego, USA). First-step analysis was made by repeated measures or one-way ANOVA as appropriate. If necessary, additional, repeated measures factor (Time) was added. Group differences after significant ANOVAs were measured by post hoc Fisher's Protected Least Significance Difference (PLSD) test. Correlations shown are Pearson correlation coefficients. Statistical significance was set at $P<0.05$. In **Paper VI**, for statistical evaluation of the behavioral data Mann-Whitney *U*-test for different groups and Sign test for dependent variables was used.

4. RESULTS AND DISCUSSION

4.1. Effect of chronic variable stress and partial serotonergic denervation (Paper I and II)

4.1.1. Behavioral and physiological effects of chronic variable stress and the efficacy of acute stressors

Effect of specific stressors on body weight gain

CVS had an effect on body weight gain in both studies (**Paper I, Fig. 1 and Paper II, Fig. 1**). In the second study, the body weight gain was separately calculated for every week (**Paper II**). The effect of the CVS regime on body weight reduction was temporary, as the animals obviously adapted to the procedure in this regard and compensation for previously lost energy occurred during the third week of CVS (**Paper II, Fig. 1**). The presence of certain adaptation to stress was also observable when the effect of stressors was measured on 24-h body weight gain: most of the stressors were effective only when used first time. Furthermore, the number of defecations during the immobilization stress (measured only in **Paper II**) was reduced with repeated presentation of this stressor, also suggestive of certain adaptation to stress. It has been suggested that chronic mild/variable stress regime elicits its behavioral effects mainly due to the unpredictable nature of the procedure for the animals (Willner, 2005), and the variety of different stressors is used in order to prevent or delay habituation (Griffiths et al., 1992; Muscat and Willner, 1992). The findings of the present study suggest that considerable adaptation to the CVS regime can occur within a few weeks. Indeed, while changes in stressors applied may prevent habituation to their specific features, it is possible that animals are able to generalize to their presence, thus habituating to stress as such because while the specific stressors are unpredictable, the daily application of stressors is not. It is important to notice that we did not use food and water deprivation, which would have facilitated a reduction in body weight gain in stressed animals.

Stress can lead to either decreased or increased feeding, depending on the nature of stressor (Gamaro et al., 2003; Morley et al., 1986): the type, duration or severity of stress and the predictability of the stressor applied may modify the responses to stress (Hargreaves, 1990; Marti et al., 1994; Paré and Redei, 1993; Pucilowski et al., 1993). It is suggested that exposure to repeated chronic stress modifies eating behavior dependent upon the severity and duration of exposure to stressors (Ely et al., 1997). Long-lasting (12–24 hr) and short-lasting (5 min – 2 hr) stressors were sequenced intermittently in our study design, and the duration had no clear-cut impact on weight changes (**Paper II, Fig. 2**). In case of most of the stressors, there was no consistent effect of a stressor on body weight gain. Three general observations could be noted: First, most of the

stressors were associated with changes in body weight gain when applied first time, but had no effect subsequently. Second, periods of lower body weight gain in stressed animals were followed by periods of higher body weight gain, which may reflect compensatory mechanisms and suggest that only limited conclusions can be made on the basis of the present data regarding the nature of the effect of specific stressors. Third, it was apparent that no stressor was followed by both increases and decreases in weight gain with a single design, even though only a few stressors had the same effect expressed consistently. In **Paper V**, almost the same order of stressors was used, as described in **Paper II**, but presentations of movement restriction and tail pinch stressors were switched. Comparison of these two experiments revealed that body weight gain increased every time after movement restriction, when followed by cage tilt, but was reduced after every application, when followed by stroboscopic light. This suggests that body weight gain may be an indirect measure of intensity of stressors, as apparently more intensive stressors reduce body weight gain, whereas during others animals are able to compensate for these preceding reductions.

Stress is known to alter 5-HT metabolism in the CNS, probably through the increased levels of glycocorticoid hormones, one of the main biological responses to stress (Malyszko et al., 1994; Nishi and Azmitia, 1996; Paris et al., 1987). Activity of tryptophan hydroxylase, the rate limiting biosynthetic enzyme for 5-HT, and 5-HT turnover have been found to be sensitive to circulating corticosteroid levels (Chalmers et al., 1993; Chaouloff, 1993; Singh et al., 1990). Removal of circulating corticosteroids by adrenalectomy has resulted in anatomically specific decreased indices of 5-HT metabolism, while stressful procedures, which raise corticosteroid levels, cause an increase in 5-HT turnover (Chalmers et al., 1993; Malyszko et al., 1994; Nishi and Azmitia, 1996). Adaptation to stress may be related to the reduction in activity of the 5-HT-ergic system, which also causes increased body weight.

Other behavioral and physiological effects of chronic variable stress

Even though certain adaptation to stress may occur, in the present studies several behavioral effects of CVS persisted. Consistently with other studies in our (Häidkind et al., 2003; Harro et al., 1999A) as well as in other laboratories (Platt and Stone, 1982; van Dijken et al., 1992), stress reduced immobility (**Paper I**) and increased struggling and swimming (**Paper I and II**). Involvement of negative affect in this behavioral shift is supported by the fact that CVS had an anxiogenic-like effect in the social interaction test (**Paper I, Fig. 4 and Paper II, Fig. 4**).

The forced swimming test is currently the most popular animal model in antidepressant drug screening, and has also been used in modeling depression (Weiss and Kilts, 1998). Reduced immobility is usually interpreted as an antidepressant-like effect but such an interpretation should probably be reserved for studies which use the original design of Porsolt and colleagues (Porsolt et al., 1978), with administration of putative antidepressant drugs in two or three

doses between the two swimming sessions and considering only the performance on the second, post-treatment session. Although chronic antidepressant treatment before the forced swimming test may increase the efficacy of the test, there is plenty of evidence that in other models of depression, this animal assay rather shows an increase in swimming or struggling and a decrease in immobility. Thus, immobility is decreased in Fawn hooded rats, reared either socially or in isolation (Hall et al., 1998; Lahmane et al., 1996). Olfactory bulbectomy has either no effect (Gorka et al., 1985) or increases escape attempts in this test (Stockert et al., 1989). It is noteworthy that the reduction of immobility was observed only during the first 5 min of the first swimming session. The finding of reduced immobility after chronic stress is fairly consistent, as we have observed this in other experiments in our laboratory of different durations of time of CMS (Harro et al., 1999A). As a working hypothesis, we propose that decreased immobility after chronic stress reflects rather enhanced reactivity or an increase in impulsiveness than reduction in despair (Harro, 2002 and 2004).

In both studies, chronic stress had an anxiogenic effect in the social interaction test (**Paper I, Fig. 4 and Paper II, Fig. 4**). As the test situations were novel to the animals and can be considered stressful, the differences between control and CVS rats could be explained by an increased sensitivity of stressed rats to novel stressors. A novel stressor after CVS, which had caused adaptation and reduction of corticotropin-releasing factor (CRF) levels, may cause again a marked increase in CRF levels in several brain regions, followed by a further reduction of body weight (Nagashima et al., 2003).

Sucrose intake and preference was not significantly affected by CVS, even though there was a tendency of reduction after three weeks of stress in the study with citalopram treatment included (**Paper II**). In the present CVS regime, the rats had free access to drinking water and a standard diet. Nevertheless, the animals have demonstrated a clear and reliable preference for 1% sucrose over water in all studies in our laboratory. Thus, it was not necessary to include food and water deprivation in the protocol. Previously, we have included deprivation prior to testing sucrose intake and found some evidence for reduction after CMS (Harro et al., 1999A). However, we did not find any evidence for a decrease in sucrose preference or consumption when deprivation was not used. Several authors have found that preference for sweetened solutions is not reduced after CMS (Harris et al., 1997; Hatcher et al., 1997), and it has been reported that intake of saccharin is reduced after CMS only if the procedure had included food deprivation (Hagan and Hatcher, 1997; Hatcher et al., 1997). It has also been suggested that the CMS effects on responsiveness to rewards are secondary to loss of body weight (Forbes et al., 1996; Matthews et al., 1995). In the present studies, correction for the changes in body weight did not alter the results. Sucrose intake and loss of body weight appear to correlate in some but not all laboratories (Nielsen et al., 2000). Our inability to observe significant changes in this measure may be related to a) too short period of CVS for our conditions; b) application of specific stressors before measurement of sucrose

intake; c) exclusion of food and water deprivation. Some groups have demonstrated the reduction of sucrose intake after two — three weeks of stress (e.g., Baker et al., 2006; Gronli et al., 2005), but others only after longer periods of stress (e.g., Grippo et al., 2006; Muscat et al., 1990). It has been shown that during the first three weeks of stress procedure the Sprague-Dawley rats had a smaller reduction of sucrose intake and preference compared to Wistar rats (Bekris et al., 2005). Different and sometimes even opposite effects of single stressors on weight gain in the present study indicate that their different quality and certain order may influence results of following behavioral tests, especially of those associated with feeding behavior, and be one of the reasons why theoretically similar procedures end up in different laboratories with opposite results, as reviewed by Willner (2005). Food and water deprivation which is often applied as a stressor just before the measurement of sucrose intake has been excluded from our studies in order to eliminate the confounding by response to hunger and thirst (Harro, 2004). We have recently found that CVS consistently reduced sucrose intake in our conditions, however, when measured during the dark phase (Matrov et al., unpublished). It has been suggested, that glucocorticoids have state-dependent stimulation effects on mesencephalic DA-ergic transmission, and an interaction between these two factors might be involved in the appearance of behavioral disturbances (Piazza et al., 1996). Corticosterone, the major glucocorticoid in the rat, administered peripherally in a dose that approximates stress-induced plasma concentrations, increases extracellular concentrations of DA in nucleus accumbens, and this increase is augmented in the dark phase, during eating, and in rats defined as high responders in their locomotor reactivity to novelty (Piazza et al., 1996). Corticosterone had little or no effects in the light phase and in low responder rats. Corticosterone also stimulated locomotor activity, an effect that paralleled the release of DA and was abolished by DA depletion (6-hydroxydopamine) of accumbens (Piazza et al., 1996). Thus, it is possible that stress applied during the dark phase may have more intensive effect on sucrose consumption, which is associated with DA function in nucleus accumbens, especially during dark phase (see **Paper VI**).

Not all effects of stress, which were described in **Paper I**, could be observed in **Paper II**, e.g., the increased weight of adrenal gland in the **Paper I**. It is possible that such inconsistency could result due to the shift to new laboratory conditions and stronger adaptation with CVS procedure which lasted longer in the second study (two weeks and three weeks in **Paper I** and **Paper II**, respectively).

4.1.2. Behavioral and physiological effects of partial 5-HT-ergic denervation by PCA treatment

The effects of specified doses of PCA vary largely between laboratories and may depend upon the animal strain used (Zhou et al., 1996). In the studies of present dissertation, a dose of PCA (2 mg/kg) was selected which elicited a ~30% decrease in 5-HT levels 1 week after administration in the cerebral cortex and hippocampus and had no significant effect in the septum in two previous experiments (Häidkind et al., 2004). As expected, PCA pretreatment significantly reduced 5-HT levels in the frontal cortex (**Paper I, Table 3 and Paper II, Table 3**) and hippocampus (**Paper I**). The effect of PCA was significant also in the septum (**Paper I**). 5-HT nerve terminals in septum are known to be more resistant to PCA because of their association with the beaded type of 5-HT-ergic nerve fibers (Baumgarten and Grozdanovic, 1997), and our previous studies with PCA treatment 1 week before sacrifice have shown results consistent with this (Häidkind et al., 2004). The present finding that septum did not display less sensitivity compared to the other brain regions, when 5-HT levels were measured 4 weeks after PCA administration, suggests that time-dependency needs to be considered when evaluating the distinct effect of substituted amphetamines on fine versus beaded 5-HT-ergic nerve fibers.

In pharmacological studies, drugs that increase post-synaptic serotonergic stimulation decrease food consumption (Arkle and Ebenezer, 2000; Brown et al., 2001; Finn et al., 2001; Halford and Blundell, 2000; Vickers et al., 2001). In contrast, agents that block post-synaptic 5-HT receptors or those diminishing serotonergic neurotransmission by activating autoreceptors often increase food intake (Simansky, 1996). Thus, 5-HT serves an inhibitory role in feeding (Gamaro et al., 2003; Simansky, 1996). In our studies (**Paper I and Paper II**), the body weight gain was initially reduced after administration of PCA, probably due to excessive release of 5-HT. The long-term effect of PCA is depletion of 5-HT in nerve terminals and the reduction of 5-HT activity, which may explain the stoppage of body weight gain reduction and subsequent increase in weight gain in comparison with the control animals during the fourth week of experiment (**Paper II**). Interestingly, new environmental changes during the last week of behavioral tests tended to reduce body weight gain in all groups, and the weight gain was negative in PCA-treated animals, with the exception of animals also submitted to CVS and citalopram treatment (**Paper II**). It seems that introduction to the series of behavioral tests was aversive and this effect was more expressed in 5-HT depleted animals. The influence of behavioral tests on the body weight of control animals also suggests aversive effect of several novel consecutive behavioral tests. However, treatments still caused more significant changes in behavior, compared with control animals.

In both studies, PCA treatment consistently led to enhanced anxiety as expressed in the social interaction test (**Paper I, Fig. 4 and Paper II, Fig. 4**), increased rearing activity in the open field test (**Paper I, Table 2 and Paper II,**

Fig. 6) and reduced immobility and increased struggling in forced swimming test (**Paper I, Fig. 3 and Paper II, Fig. 5**). We have found that denervation of the locus coeruleus by DSP-4, a treatment rendering rats passive but highly irritable in novel environments, similarly elicits a decrease in immobility in the forced swimming test (Harro et al., 1999B). Thus, again, a reduction of immobility in the forced swimming test should in certain paradigms rather be interpreted as enhanced reactivity or impulsiveness than reduced despair. Involvement of negative affect in this behavioral phenomenon is supported by the fact that both CVS and PCA pretreatment had anxiogenic-like effects in the social interaction test (File and Hyde, 1978), in which the time spent in active social interaction between two unfamiliar rats in a neutral arena is measured, and which belongs to the most important animal models of anxiety. On the other hand, this effect of partial 5-HT-ergic denervation on forced swimming appears to be reliable, and specific as we have reproduced the immobility-reducing effect of PCA at 2 mg/kg, but not at higher doses in an independent experiment (Häidkind et al., 2004). Altogether, this set of behavioral effects elicited by 5-HT-ergic denervation could be explained by impulsive behavior caused by the dysfunction of brain serotonergic system (Dalley et al., 2002; Harro, 2002).

4.1.3. Behavioral effects of chronic variable stress after partial lesion of the 5-HT-ergic system

CVS after PCA treatment augmented sucrose intake after two weeks of stress (**Paper I, Fig. 3 and Paper II, Fig. 3**). Interestingly, in **Paper II**, PCA and CVS separately rather reduced sucrose intake, which suggests that a combination of stress with partial 5-HT lesion may elicit a distinct behavioral effect.

Chronic stress is expected to reduce intake of sweet solutions in order to serve as a model of depression. DSM-IV allows for both decrease and increase in body weight as symptoms of depression. Increased appetite and carbohydrate craving has long been considered to be a symptom of affective disorders, especially common in so-called atypical depression (Moller, 1992), e.g. seasonal affective disorder (Dalgleish et al., 1996; Thalen et al., 1995; Wehr et al., 1991; Wurtman and Wurtman, 1995). More specifically, seasonal affective disorder has been suggested to occur in two opposite patterns, summer depression and winter depression, the former being associated more frequently with decreased appetite and the latter with increased appetite and carbohydrate craving (Wehr et al., 1991). Increased appetite is also more common in relatively younger depressed patients (Casper et al., 1985; Garvey and Schaffer, 1994) and is associated with being more hostile (Casper et al., 1985). Cortisol secretion measures are less altered in this subgroup of patients, but still significantly higher than in healthy controls (Casper et al., 1988), suggesting hypothalamic–pituitary–adrenal axis hyperactivation also in this form of depression. Regarding relevant preclinical research, it has been found that in

certain experimental conditions, stress can increase intracranial self-stimulation (Nielsen et al., 2000; Zhou et al., 1996). CVS has been reported to enhance the stimulatory effect of morphine (Molina et al., 1994). Access to and consumption of sweetened solutions can reduce alcohol intake in both alcohol-naive and alcohol-experienced animals, and also in alcohol preferring rat strains (Kampov-Polevoy et al., 1995), allowing for speculation that intake of sweetened solutions is one of the possible ways by which experimental subjects successfully reduce the negative impact of stress.

In a study which compared saccharin intake after CMS with and without food deprivation and found no effect of CMS without food deprivation included in the procedure (Hagan and Hatcher, 1997), there was actually a strong tendency towards increased saccharin intake when food deprivation was omitted. Most importantly, Willner and colleagues (Willner et al., 1998), when using their own CMS model, described that CMS had an effect opposite to what was expected in the progressive ratio reinforcement schedule: when the animals worked for pellets varying in sweetness, CMS increased 'craving' for sweet rewards. The interpretation offered by the authors, that CMS simultaneously increases 'craving' and reduces 'reward', remains to be proven, but it can also be suggested that consumption of sweetened solutions after food and water deprivation is a measure of some other psychobiological dimension than reward (or a mixed measure). The inclusion of food and water deprivation into the CMS/CVS paradigm and, in particular, immediately before sucrose intake or preference tests for all animals causes major problems with interpretation of the results. For example, it is conceivable that when a reduction of sucrose consumption is found in CMS, this is due to overconsumption of sucrose in control animals after the deprivation period. Additional interpretation problems include the fact that rats with decreased sucrose intake after CMS have been found to display behaviors difficult to associate with depression, such as an increased activity in the elevated plus-maze (D'Aquila et al., 1994).

Though the paradoxical effect of reduced or increased sucrose consumption has been attributed to limited unpredictability of stressors and habituation (Gamaro et al., 2003; Willner, 1997), in **Paper II**, the consumption of sucrose solution was smaller in both CVS and PCA groups compared with the PCA+CVS group in the third sucrose preference test, but the body weight gain of both CVS and PCA animals was higher comparing with the PCA+CVS during the antecedent week. Therefore the increase in sucrose intake can not be secondary to body weight change, as it was suggested in experiments where the reduction of sucrose solution was registered together with reduced body weight gain in the sucrose preference tests, carried out with antecedent food and water deprivation (Forbes et al., 1996; Hatcher et al., 1997; Matthews et al., 1995). Similarly, this effect can not be explained by increased adaptation after partial 5-HT-ergic lesion. Both the increased body weight gain and increased sucrose consumption are characteristic symptoms to atypical depression. Tendency to overeat carbohydrates by patients with atypical depression is believed to act via

an increase in insulin secretion resulting in better access of tryptophan to the brain and consequent higher synthesis of 5-HT (Gamaro et al., 2003; Wurtman and Wurtman, 1995). It should be noted, however, that even though the increase in sucrose intake in PCA-pretreated rats after two weeks of CVS was increased similarly in both experiments, such an increase was in **Paper II** not observed one week later, even though PCA pretreatment prevented the tendency of CVS-induced reduction of sucrose intake. This apparent inconsistency in the efficacy of 5-HT depletion to support CVS-induced sucrose intake could possibly be explained by the different stressors applied immediately before measurement of the sucrose intake. Thus, the increase in sucrose intake was recorded when measured after strong illumination during predicted dark phase, which tended to reduce body weight, whereas no significant effect was found when sucrose intake was measured after immobilization for two hours, which itself had a strong increasing effect on body weight gain. This hypothesis should be tested with a counterbalanced design, but the possibility that differences in stressors applied before behavioral tests influence the outcome should receive due attention.

As mentioned above, carbohydrate craving in depression has been associated with 5-HT deficiency (Moller, 1992), even though there is little evidence as to the mechanism involved. Epidemiological analysis (Levitan et al., 1998) revealed that the reversed neuro-vegetative symptom cluster in major depression, which includes increased appetite, is associated with physical or sexual abuse in childhood. It is not clear whether this finding has implications for models which apply current, not early stressors, but it emphasizes the fact that stress can be a factor increasing the energy intake.

In the experiment described in **Paper II**, we counted the number of dives during forced swimming. This was possible due to the relatively high frequency of such behavior, which we have not observed in previous studies mostly carried out with the Wistar strain, and which seems not having attracted much interest in literature. CVS reduced diving behavior after 5-HT depletion induced by PCA pretreatment. Thus, CVS reduced active escape attempts in rats with altered 5-HT-ergic system, perhaps indicative of increased anxiety or a shift to passive coping style.

4.2. Effects of antidepressant treatment on the effects of chronic variable stress and partial serotonergic denervation (Paper II)

Citalopram suppressed or prevented several behavioral changes elicited by PCA, CVS or their combination. Citalopram treatment suppressed the reduction of body weight gain caused by PCA pretreatment during the week of behavioral experiments, but only in stressed rats (**Fig. 1**). In addition, citalopram reduced

the number of fecal boli of stressed animals, suggesting increased emotionality of stressed animals in this test (Hall, 1934), which we have also repeatedly observed (**Paper I, Table 2**).

Citalopram prevented the increased sucrose consumption after the third sucrose preference test in PCA+CVS animals, without affecting the consumption by CVS or PCA groups separately (**Fig. 3**). Suppressive effect of an antidepressant on the increased consumption of sucrose solution in stressed rats has also been observed earlier (Bissette et al., 1999). The efficacy of citalopram in the present paradigm suggests that increased sucrose consumption elicited by stress after partial 5-HT depletion is relevant to the pathophysiology of depression.

Citalopram itself reduced social activity in the social interaction test and did not influence the reducing effects of PCA or CVS (**Fig. 4**). In fact, citalopram potentiated the reducing effect of combination of these treatments. Acute administration of SSRIs reduces activity in the social interaction test, but tolerance has been found to develop toward this effect (Dekeyne et al., 2000). This effect of SSRIs is believed to be mediated by 5-HT_{2C} receptors which desensitize during chronic treatment. In the present study, it thus could be speculated that citalopram did not desensitize 5-HT_{2C} receptors.

Increased defecation in the open field is a traditional indicator of emotionality and anxiety (Hall, 1934; Katz et al., 1981B; van Dijken et al., 1992). We observed an increased number of defecations by CVS in **Paper I** (**Table 2**), but not in all experiments (Häidkind et al., 2003). In the present study the baseline defecation rate was higher than we have observed earlier, and was not further increased by stress. Citalopram did not reduce defecations in animals not stressed, but reduced this measure significantly in both groups submitted to CVS. In the forced swimming test which could be considered a more stressful condition than the open field, citalopram reduced the number of excrements in all groups. Thus, citalopram had a potent anti-stress effect.

In our conditions, citalopram has been the only antidepressant inactive after chronic administration in the forced swimming test (Harro et al., 1997). Citalopram had no effect on its own in the present study and did not influence the effect of CVS in general, but prevented the increase in struggling and decrease in immobility in PCA-pretreated CVS rats (**Fig. 5**). This suggests that CVS affects behavior via multiple neural pathways, and those that are associated with 5-HT-ergic deficit can be blocked by citalopram administration. Reduction of diving behavior by PCA plus stress was not sensitive to citalopram, suggesting that this behavior is regulated in a different way. Indeed, individual performance in struggling and diving did not correlate in untreated animals. Interestingly, both CVS and citalopram prevented the hyperactivity of 5-HT lesioned rats in the open field test (**Fig. 6**). Chronic stress can increase 5-HT-ergic activity (Gamaro et al., 2003), which may explain the partial similarity of its effect to that of a SSRI. Citalopram reduced the levels of 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT turnover, and these effects were prevented by CVS (**Fig. 7**). However, stress did not abolish the effect of cita-

lopram in PCA-pretreated rats, which suggests that integrity of 5-HT-ergic neurotransmission is necessary for the complete effect of stress.

Conclusively, citalopram treatment prevented several but not all effects of CVS in partially 5-HT depleted rats. Cabib and Puglisi-Allegra (1996) suggested that depressive symptoms may not always represent the necessary outcome of stress experiences but be promoted by specific environmental conditions and by a genetically determined susceptibility. Results in the present study provide support to the notion that both biological predisposition and environmental conditions are important in the development of depression-like symptoms, and that 5-HT-ergic neurotransmission is an important system mediating their interaction.

4.3. Persistence of social behavior in the rat (Paper III)

Evolutionary continuity between humans and other animals suggests that some dimensions of personality may be common across a wide range of species (Figueredo et al., 1995; Gosling and John, 1999) and animals may also have persistent structures of behaviors which are stable across multiple years (Figueredo et al., 1995). In the present study, activity in each social interaction test did not correlate consistently with other tests pairwise, but the individual average social behavior correlated well with performance in each occasion (**Table 1**), suggesting that in the rat, there is a sociability trait which is measurable by repeated testing in the social interaction test. In comparison to the physical environment, social environment is less predictable and very reactive to the behavior of the individual itself. Social behavior of rats was found to depend, weakly but significantly on the social activity of partners, and thus a conclusion can be drawn that social activity of an individual rat is a trait, manifestation of which depends on the partner's social behavior. We have reproduced the finding that mean social interaction time correlates consistently with performance in all tests in a number of subsequent independent experiments (e.g., **Papers IV and V**). These experiments demonstrate that a sociability trait underlies the performance in the rat social interaction test.

4.4. Association of sociability trait with 5-hydroxytryptamine (Paper III and IV)

The sociability trait was found to be strongly negatively associated with the levels of 5-HIAA in the frontal cortex, measured ex vivo (**Paper III, Fig. 1**) and with the basal extracellular level of 5-HT, measured in vivo (**Paper IV, Fig. 1**). Mean social interaction time did not correlate with any other monoamine measure, including the 5-HIAA/5-HT ratio (**Paper III**). The baseline 5-HT

levels of high sociability (HS) and low sociability (LS) rats were similar in ventral tegmental area, but the PCA stimulated 5-HT increase was higher in HS rats (**Paper IV, Fig. 2**).

Both in rats and primates 5-HT functioning is implicated in impulsive, defensive, and aggressive behaviors which influence social interactions and social affiliations (Krakowski, 2003). Cerebrospinal fluid (CSF) studies have found a high positive correlation between 5-HT metabolism and sociability (Higley et al., 1996; Mehlman et al., 1995; Placidi et al., 2001). There is, however, also evidence for negative association between 5-HT metabolism and social competence (Yodyingyuad et al., 1985). Even in the simple social interaction test, both increased (Duxon et al., 2000; Hamon et al., 1999) and reduced (Bagdy et al., 2001; File et al., 1993; Kennedy et al., 1993; Kenny et al., 2000) social behavior has been suggested to be mediated via an increase in serotonergic function. In our studies, the negative association between septal 5-HT measures and social activity in the experiment in which the animals were decapitated immediately after social interaction test (**Paper III, Fig. 2**) further supports the notion that at least in certain conditions 5-HT metabolism is inhibitory to social activity, but that different brain regions contribute to sociability trait and ongoing social activity. Serotonergic measures are probably related to sociability-associated mechanisms in a complex manner (Ando et al., 2006), and this can cause differences between laboratories in several ways. For example, low 5-HIAA levels in the CSF have been associated with high impulsivity, which may mediate social incompetence (Higley et al., 1996; Mehlman et al., 1995). By using in vivo microdialysis, Dalley et al. (2002) have demonstrated that higher extracellular levels of 5-HT in the prefrontal cortex are associated with higher impulsivity. It is possible that 5-HIAA levels in the CSF reflect better serotonergic activity in the brainstem, which is a negative mirror image of forebrain mechanisms due to somatodendritic inhibition of 5-HT projection neurons. However, it is also possible that, when the environmental signals become more complex, the 5-HT behavior link is not linear. As an example, this laboratory has demonstrated in a longitudinal study that platelet MAO activity, a peripheral marker of central serotonergic activity (Fahlke et al., 2002), is predictive of regular smoking in adolescents, but the relationship is very clearly U-shaped (Harro et al., 2004). Apparently the different contribution of a variety of 5-HT receptor subtypes in different brain regions should be considered.

Bjork et al. (2000) have shown that men with high trait hostility have negative association between tryptophan levels and aggressive responses, but this relationship was opposite in men without previous aggressive history. In rats, a positive correlation between 5-HT–ergic function and aggression (van der Vegt et al., 2003), but a negative association between 5-HT-ergic activity in PFC and anxiety (File et al., 1993) has been found. Thus, higher levels of 5-HT in the PFC may reduce social behavior in LS-rats by increasing anxiety. On the other hand, it has been described that acutely increased 5-HT levels cause a

significant decrease in the basal firing rate of VTA neurons and reduce meso-corticolimbic DA transmission by activating 5-HT_{2C} receptors (Di Mascio and Esposito, 1997; Matteo et al., 2002). Though release of DA is pivotal in psychomotor stimulation, social behavior can be rather reduced by preferentially DA releasing drugs, such as amphetamine (Panksepp, 1998). As DA suppresses social activity it is possible that the higher 5-HT release potential in VTA during social interaction suppresses DA release through activation of 5-HT_{2C} receptor, and promotes social activity more effectively in HS rats. Conclusively, extracellular 5-HT levels differ in rats with high vs low sociability trait, and this regionally specific difference may be related to distinct components, like 5-HT activity in the prefrontal cortex and 5-HT-DA interaction in the VTA.

4.5. The sociability trait and sensitivity to chronic variable stress (Paper V)

This study demonstrated again that sociability is a persistent characteristic in rats when tested within the period of one month. Despite of the fact that social activity decreases with increasing age and body weight, large differences were still present one month later (**Fig. 3**). LS-rats were more anxious also in other animal models of anxiety, the elevated plus-maze (**Fig. 4**) and open field tests (**Fig. 6**). It has been described previously that rats bred separately after selection on the basis of anxiety in the elevated plus maze test exhibit similarly more anxious behavior in other animal models of anxiety, such as the social interaction test and the black-white box (Henniger et al., 2000). However, discrimination in the social interaction paradigm was primarily due to differences in locomotor activity (Henniger et al., 2000). On the more individual level, behavior of rats in the social interaction test does not correlate well with their performance in other animal models of anxiety (Ramos et al., 1997). When behavioral reactivity of male rats from different strains was compared in several nonsocial (elevated plus-maze, open field) and social settings (social interaction in aversive and neutral environment, resident-intruder test, chronic social stress), a factor analysis showed that the behavioral reactivity to social stimulations is a specific feature, dissociable from different components of emotionality (approach/avoidance and general activity) as measured in the behavioral responses to nonsocial settings (Berton et al., 1997). Thus, the social interaction test and other animal models of anxiety, despite all channeling anxiety, also represent activities in rather distinct psychological domains. In a modified hole board test, which allows the experimental animal to maintain social contact with its group mates, and enables the investigator to assess social affinity among group mates (Ohl et al., 2001), highly anxious rats spent significantly more time in social contact than both low anxious and control rats, indicating that animals

preferring more social contact may be more anxious when in isolated situation (Landgraf and Wigger, 2002).

Anxiety may be viewed as an innately driven form of distress that arises in response to actual or threatened exclusion from social groups (Buss, 1991; Ruis et al., 1999), and isolation causes important changes in the behavioral reactivity of rats to environmental stimuli (Nunes Mamede Rosa et al., 2005), and can affect the reward magnitude of social encounter (Burgdorf and Panksepp, 2001). Social isolation itself represents stress, and may increase the sensitivity of the pituitary gland to CRF and impair the negative feedback regulation of the HPA axis (Serra et al., 2005). It has been described that housing of rats in groups of 10–12 animals per cage 24 hr after restraint stress significantly prevents the anxiogenic effect of restraint in the elevated plus-maze test, when compared to animals which were housed in pairs after the stress exposure (Andrade and Guimaraes, 2003). This again suggests that social housing conditions after stress may attenuate behavioral consequences of exposure to uncontrollable stressors and thus mimick the effect of social support in humans. In the present study, animals were housed individually already before the onset of the stress procedure. It has been described that social housing before the onset of stress can enhance coping with stress in female rats, whereas in male rats, group housing appears to increase the adverse effects of chronic stress (Westenbroek et al., 2003). This suggests that single housing in our study probably did not confound the stress effects, but the possibility that social housing would moderate the effects of stress differently in HS- and LS-rats should certainly be controlled in a separate study.

In the social interaction test, HS animals were 28 days after the previous test still socially more active than LS animals, even though a reduction in the social activity was observed in both groups (**Fig. 3**). Probably due to floor effect this decrease was higher in the HS groups. It has previously been described that 4.5 months old Wistar rats presented the lowest scores on active social behavior and the highest scores on defecation, compared with the 2.5 months old rats, and exploratory behavior measured by ambulation and rearing decreases with age (Garau et al., 2000). Thus, age — and a closely related measure, the body weight ─ could be a relevant factor in the reduction in social activity also in our study, where animals were 3 months old after the third social interaction test and 4 months old during the 5th test. Salchner et al. (2004) has suggested that reduced interaction in aged rats does not reflect enhanced anxiety, as reduced social interaction level was not accompanied by augmented Fos expression in any of the key brain areas of the fear/anxiety circuitry known to be activated by anxiogenic stimuli. Niesink and van Ree (1982) have described that short term individual housing before the social interaction test increases social activity both in young and adult animals, but not due to increased locomotor activity, and this effect appeared to be maximal after 4 to 7 days of individual housing extinguished after repeated testing, suggesting that observed behavioral changes were hardly affected by habituation to the test cage. Thus, reduced social

activity in our study was probably not induced by increased anxiety or habituation with test apparatus, but rather related to aging and increased body weight gain.

This study also demonstrated that the initially less anxious HS-rats were more sensitive to stress than LS-rats as measured by the decrease in sucrose intake after 3 weeks of CVS regime (**Fig. 2**). Interestingly, higher sensitivity to stress has been found in more anxious rats, when animals were selected on the basis of exploratory behavior. When comparing rats bred for high or low anxiety-related behavior, high anxiety rats floated much more and struggled much less than low anxiety rats in the forced swimming test (Keck et al., 2001), thus reflecting different vulnerability to stress and coping strategies (Landgraf and Wigger, 2002). Nevertheless, it is not known whether these strains differ in sucrose intake. We have found that the effect of CVS on sucrose intake is stronger in more anxious rats, selected in the exploratory box (unpublished).

In LS animals, stress increased struggling in forced swimming test (**Fig. 5**), and activity in the social interaction test (**Fig. 3**) and elevated plus-maze test (**Fig. 4**). Regarding monoamine levels, stress increased DA levels and reduced 5-HT levels in frontal cortex of LS animals. It has previously been described that peripherally administered corticosterone in a dose that approximates stress-induced plasma concentrations of the male Sprague-Dawley rat stimulates locomotor activity in an activity box (Piazza et al., 1996). Neuropeptide CRF may be a critical mediator in regulating the decreases of extracellular concentrations of 5-HT during stress (Price et al., 2002). At least in septum, it is found that forced swimming, which brings about climbing and swimming behaviors that precede the development of immobility, produces an acute phasic reduction of 5-HT release (Lucki, 1998). Low 5-HT neurotransmission has been found to be associated with higher impulsivity, aggressiveness and anxiety in humans, non-human primates and other species (Turecki, 2005; Suomi, 2005; Grimmett and Sillence, 2005). Thus, it is conceivable that stress, possibly through the alerted glycocorticoid system, increased behavioral activity in LS animals, by upregulating DA and downregulating 5-HT systems in frontal cortex and increasing impulsivity.

Bosch et al. (2006) have described that exposure of male Wistar rats, bred for high or low anxiety-related behavior, to prenatal stress between pregnancy days 4 and 18 resulted in opposite effects on anxiety in adulthood, i.e. high anxiety rats became less and low anxiety rats became more anxious compared with their unstressed controls in plus-maze and holeboard tests. Ladd et al. (2005) have described that many effects of prolonged handling-maternal separation on stress hyper-responsiveness associated with facilitation of regional corticotropin-releasing factor neurocircuits and glucocorticoid resistance, which in neonatal Long Evans hooded rats leads to stable phenotypes, are reversible in adulthood by chronic variable stress. It is suggested that increased locomotor activity of initially more anxious animals may reflect a difference in coping with a novel and challenging situation (Landgraf and Wigger, 2002).

Thus, in the present study, the increased locomotor activity of LS animals in response to stress may reflect a shift in coping style.

Changes in body weight gain and sucrose intake represent the dissociation in sensitivity to stress between LS and HS animals (**Fig. 3 and Fig. 4**). The reduction in sucrose intake of HS animals existed without persistent effect of stress on their body weight gain, thus, effect of stress on sucrose intake of HS animals emerged two weeks after disappearance of its effect on this measure. Conversely, sucrose intake of LS animals was not affected by stress, but body weight gain was suppressed during all the study. The strong body weight reduction during the last week was most obviously caused by novel environmental condition elicited by behavioral tests. It has been suggested that a novel stressor after CVS, which had caused adaptation and reduction of corticotropin-releasing hormone (CRH) levels, may cause again a marked increase in CRH levels in several brain regions, followed by a further reduction of body weight (Nagashima et al., 2003). Reduction of body weight gain of control animals was, however, even stronger than in animals previously adapted with stress. Also, the effect of stress on body weight gain was strongest during the first week.

Conclusively, this study revealed that animals with high sociability trait are more vulnerable to the anhedonic effect of stress in conditions of single housing. Thus, animals with different expression of social behavior respond differently to stress. This information may be useful in models of affective disorders in studies of their neurobiological substrates.

4.6. Individual differences in sucrose intake and preference, and their association with dopamine D_2 receptor function (Paper VI)

In this study, sucrose solution was preferred over water during all eight tests. Mean preference of sucrose solution was 81% in light phase (lowest 62% and highest 94%) and 90% in dark phase (lowest 70% and highest 97%; with exclusion of one outlier, mean 91% and lowest 84%). Dark phase consumption (**Fig. 1**) was significantly higher compared to light phase consumption in all tests. Dark phase preference was significantly higher compared to light phase only during the second test, but had a similar tendency also between other light and dark phase tests. Dark phase sucrose intake did not correlate significantly with light phase sucrose intake, this was also the case for sucrose preference (data not shown).

Neither dark phase nor light phase preference of sucrose solution correlated significantly between single tests or between any single test and the mean of all other tests. Intake of sucrose was individually very consistent and positively correlated across different tests during the dark phase, but less so during the

light phase (**Table 1**). Adjusting sucrose intake to body weight did not change the associations between the tests (data not shown).

This study demonstrated that sucrose intake is an individually stable trait, especially when measured during the dark phase. Previous studies separating rats into high and low sucrose intake groups have either tested animals once, or calculated average consumption across several tests, without providing data about the stability of intake individually during repeated testing (Cannon and Bseikri, 2004; Duncko et al., 2003; Smith and Schneider, 1988). The present study clearly demonstrated that sucrose intake in free-feeding rats is almost three times higher during the dark phase than the light phase, and when measured during the dark phase, is individually relatively stable. Even the results of the very first test reliably predict sucrose intake in subsequent tests. When sucrose intake is measured during the light phase, the first test is an unreliable predictor and even repeated testing provides an inferior index of individual levels of consumption. Thus, testing of nocturnal animals during their active period reveals their individual differences more clearly. Regarding sucrose preference, it appears that four tests are not sufficient to reach an adequate indicator of individual behavior. Sucrose preference correlated significantly with intake, but this correlation was not perfect and it should be noted that calculation of preference includes data on intake and such a correlation is thus artificially inflated. Water intake, which is the other measure included in preference, may depend on other physiological variables and thus distort the expression of the impact of individual differences in sensitivity to reward. Thus, sucrose intake and preference represent partly overlapping but not identical behavioral dimensions.

It is not known whether this would generalize to consumption of sucrose in less concentrated solutions. The present study used 1% sucrose solution because this is used in the majority of studies measuring sucrose intake or preference for various purposes, including the measurement of anhedonia in chronic stress paradigms. Standard procedure of chronic mild stress includes water and food deprivation before the sucrose preference test (Willner, 2005), apparently to increase the consumption of sucrose. This procedure which confounds the measurement of reward sensitivity with hunger response could be avoided if sucrose preference tests were carried out during the active period of animals.

Dopamine-stimulated [^{35}S]GTPγS binding in striatum correlated negatively with the level of sucrose preference only in the first test, when the taste of sucrose was novel to the animals (**Fig. 2B**). Individual differences in D_2 receptor function in the striatum may thus influence behavior of rats in novel situations. Striatal DA release is relevant to the novelty of the conditions (Nakazato, 2005) and striatal D_2 receptor density has been found to be associated with neuroticism in human volunteers (Lee et al., 2005). That the negative correlation between DA-stimulated [^{35}S]GTPγS binding and sucrose preference was more evident for light phase which included cases with relatively low sucrose preference indirectly

supports the notion that D_2 receptor function in striatum is important for hedonic responses in novel and more aversive conditions.

In the nucleus accumbens, there was a clear association between the mean dark phase sucrose consumption and the affinity of GDP binding to the activated DA receptor–G protein complex in the presence of DA (**Fig. 2C**). As sucrose intake did not correlate with the affinity of GDP to the nonactivated complex, this association is likely caused by the higher effect of DA-ergic receptors on the G protein, which is reflected in a bigger decrease in GDP affinity in the presence of DA. In nucleus accumbens, animals with higher sucrose intake and preference during the dark phase had higher level of DA dependent G protein activation which was expressed by increased binding of [^{35}S]GTPγS (**Fig. 2C and Fig. 2D**). In contrast, [^{35}S]GTPγS binding in these conditions did not correlate with either sucrose intake or preference measured during the light phase. After adjustment of sucrose intake to body weight the associations remained essentially similar. Thus, higher sucrose intake and preference in animals can be caused by higher sensitivity of DA-ergic activation of the second messenger system. However, with the present study design it is also possible that higher sucrose intake produced the observed changes in D_2 receptor function which would thus be rather a correlate of another, permanent psychobiological factor maintaining the individual differences in sucrose consumption. As there was no correlation with the basal level of [^{35}S]GTPγS binding, it would be expected that the sucrose intake is positively associated with the efficiency of the coupling between receptors and G proteins. Better coupling generates a larger change in GDP affinity and correspondingly increased production of activated G proteins (Rinken et al., 2001).

DA release in rat nucleus accumbens has been shown to increase in relation to drug/food-seeking behavior (Nakazato, 2005). DA systems are activated in animals drinking sugar solutions, and lesions of DA-ergic neurons or pharmacological blockade of DA receptors seem to reduce the reward value of both sweet tastes and drugs of abuse (Cannon and Bseikri, 2004). It has been suggested that DA release in the nucleus accumbens is important in motivation by linking reward (especially when it is food) with motor activity required to achieve it (Mogenson et al., 1980; Salamone et al., 1997; Kelley et al., 2005). The present study provides evidence that, in the rat, persistent individual differences in sucrose consumption and possibly reward sensitivity in general are related to dopamine D_2 receptor function in the nucleus accumbens.

It has been described that low sucrose preference is associated with altered HPA axis activity, NMDA receptor subunits and CRH gene expression in nucleus accumbens and hippocampus, in rats exposed to a novel environment (novelty stress), and these mechanisms may operate in the disposition to develop hedonic deficit in some mental disorders (Duncko et al., 2003). Thus, it would be interesting to study further the effect of the chronic variable stress on sucrose consumption and preference in rats, initially divided into high and low sucrose consumers.

5. CONCLUSIONS

Studies described in the present dissertation have revealed that combining chronic variable stress with lesioned serotonergic system leads to several behavioral changes such as increased sucrose intake, reduced body weight gain, anxiety and impulsiveness, that resemble symptoms of atypical depression. Using antidepressant treatment, it is possible to prevent several of these changes elicited by chronic stress and 5-HT depletion. Thus, the model using chronic variable stress and 5-HT depletion can be used in further animal studies on the neurobiology of anxiety and mood disorders.

In humans, the majority of stressful stimuli that lead to psychopathology are of social nature, and often coping with stress depends on social functioning. It is been described in the present dissertation that social behavior is a persistent trait in rats, and tissue and extracellular 5-HT levels in frontal cortex differ in rats with high vs low sociability trait. Regionally specific differences in 5-HT activity between high and low sociability rats may be related to distinct components, e.g., anxiety and motivational processes, that contribute to social behavior.

Animals with differences in sociability trait vary in their vulnerability to chronic stress, as high sociability rats are more sensitive to the anhedonic effect of stress, and low sociability rats become more active in response to stress. This information may also be useful in models of affective disorders in studies of their neurobiological substrates.

Measurement of sucrose intake or preference is currently in widespread use in preclinical psychopharmacology, and used for predicting sensitivity to rewards. The studies presented herewith highlight that sucrose intake is also an individually stable trait, especially when measured during the dark phase, which is the active period in rodents. Persistent individual differences in sucrose consumption and possibly reward sensitivity in general are related to dopamine D_2 receptor function in the nucleus accumbens. Individual differences in D_2 receptor function in the striatum may influence behavior of rats in novel situations.

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8. SUMMARY IN ESTONIAN

Stress ja sotsiaalsus: individuaalsed erinevused ja neurobioloogilised alused

Depressioon ja ärevushäired on levinud psüühikahäired ja neil on omavahel kõrge komorbiidsus. Depressioonile on enam iseloomulikud alanenud meeleolu ja huvi kadumine varasemalt rõõmu ja naudingut pakkunud stiimulite vastu, ärevushäiretele aga füüsilised sümptomid, nagu higistavad peopesad ja südamepuperdamine. Mõlema psüühikahäire tekkimine on seotud nii endogeensete kui psühhosotsiaalsete teguritega. Kui stressi mõju psühhiaatrilistele häiretele on palju uuritud, siis vähem on teada, mis määrab ära erineva tundlikkuse stressi poolt põhjustatud haigustele. Selle kindlakstegemiseks oleks vaja mõista häirete aluseks olevaid genotüüpe, mis mõjutavad isiksuse seadumusi ja vastuvõtlikkust häiretele. Loomade puhul on samuti märgatud, et tundlikkus stressile on individuaalne ja võib sõltuda uudistamisaktiivsuse või ärevuse tasemest. Senised loomkatsete mudelid ärevus- ja meeleoluhäirete uurimiseks ei ole perfektsed. Paljud neist võimaldavad suhteliselt hästi sõeluda välja uusi ravimeid, kuid valiidsus psühhiaatriliste häirete simuleerimiseks ja nende aluseks olevate mehhanismide mõistmiseks on küsitav. Tänapäevastel depressiooni ja ärevushäirete ravimitel esineb tihti kõrvalnähte ja nende raviv toime tekib alles pikemaajalisel tarvitamisel. Efektiivsemate ravimite leidmiseks on vajalik paremate loomkatsemudelite väljatöötamine, mis võimaldaksid inimestel esinevaid sümptoome sarnasemalt esile kutsuda. Üks tuntumaid loomade depressioonimudeleid on pikemaajalisem erinevate stressorite kasutamine. Üheks võimaluseks oleks sellele aversiivseid elusündmusi jäljendavale mudelile lisaks mõjutada neurotransmitterite süsteeme või selekteerida juba eelnevalt loomad selliste käitumistunnuste põhjal, mis sarnaneksid inimese ärevus- ja meeleoluhäirete etioloogias levinud käitumismustritega.

Inimesel on sotsiaalne käitumine olulisel määral seotud isiksuse omadustega, mille kujunemisel on tähtis ka pärilik mõju. Enamus stressirikastest stiimulitest on just sotsiaalset laadi, mistõttu stressiga toimetulek sõltub sotsiaalse suhtlemise efektiivsusest. Sotsiaalse suhtlemise langus on üks olulisi depressioonihaigete sümptomeid, mida teraapia käigus üritatakse sotsiaalse suhtlemise efektiivsust parandades tõsta. Loomkatsetes on sotsiaalse stressi mõju uurimine väga levinud, kuid vähem uuritakse sotsiaalse keskkonna mõju kui toimetuleku vahendit meeleolu ja ärevusega seotud häirete tekkimise vastu.

Inimese käitumise reguleerimisega on seotud mitmed virgatsained ja hormoonid, mille täpne roll ja omavaheline interaktsioon erineva käitumise kujundamisel ei ole väga selge. Siiski, üks olulisemaid virgatsaineid, mida seostatakse sotsiaalse käitumise vahendamise ja meeleolu või ärevushäirete tekkimisega, on serotoniin ehk 5-hüdroksütrüptamiin. Madalat serotoniini närviülekannet on seostatud suurenenud impulsiivsuse, agressiivsuse ja ärevusega. Sa-

muti on leitud, et ravimite abil serotoniini funktsiooni suurendamine vähendab negatiivsete tunnete kogemist ja tõstab suhtlemisefektiivsust. Ravimite abil, mis serotoniini talitlust suurendavad, ravitakse depressiooni ja ärevushäireid. Samas on serotoniinitalitluse pärilike iseärasuste mõju sõltuv keskkonnast. On leitud vastasmõju serotoniini transpordi efektiivsust määrava geeni funktsionaalsuse ja depressioonini viivate stressirikaste elusündmustega toimetuleku vahel (Caspi jt. 2003).

Kuna huvi kadumine varasemalt rõõmu ja naudingut pakkunud stiimulite vastu on üks olulisemaid depressiooni sümptomeid, siis on hedoonilise seisundi hindamine magusa tarbimise muutuse põhjal loomade depressioonimudelites laialt levinud. Dopamiin on virgatsaine, mille talitlust peetakse eriti oluliseks hedoonilise seisundi muutuste ja ka sõltuvuse tekkimisel. Paljud uimastid, nagu heroiin, alkohol, kokaiin, amfetamiin ja nikotiin, vabastavad dopamiini. Mõnedes eksperimentides on leitud, et erinev magusa tarbimine loomadel on seotud erineva ärevuse tasemega, aga vähe on informatsiooni selle kohta, kuivõrd püsiv on rottide magusa tarbimise seadumus ja kas see on seotud dopamiini funktsiooniga piirkondades (nt. naalduv tuum, juttkeha), mida seostatakse muutuste tekkimisega hedoonilises seisundis.

Väitekirjas kirjeldatud uurimuste eesmärkideks oli kindlaks teha,

- kas krooniline muutlik stress ja serotoniinisüsteemi osaline kahjustus ning nende kombinatsioon tekitavad rottidel neurokeemilisi ja käitumismuutusi, mis sarnaneksid depressiooni ja ärevushäirete sümptomitega ning kas on võimalik neid muutusi antidepressandi manustamise abil ära hoida (**artikkel I ja käsikiri II**);
- kas sotsiaalne käitumine on rottidel püsiv omadus ja milline on selle seos monoamiinide tasemetega (**artikkel III ja käsikiri IV**);
- kas sotsiaalse aktiivsuse seadumusest sõltub tundlikkus stressile (**käsikiri V**);
- kas magusa vedeliku tarbimine ja eelistamine on rottidel püsiv omadus ning kuidas on see seotud dopamiini D_2 retseptori funktsiooniga naalduvas tuumas ja juttkehas (**artikkel VI**).

Uurimustulemused näitasid, et stressirikas keskkond ja serotoniinisüsteemi kahjustamine võimaldavad tekitada mitmeid ärevushäiretele ja depressioonile sarnaseid käitumismuutusi. Nende kombinatsioon aga tekitab käitumismuutusi, mis on sarnased eelkõige atüüpilisele depressioonile, nagu suurenenud magusa vedeliku tarbimine, kaalumuutused, suurenenud ärevus ja impulsiivsus (**artikkel I ja käsikiri II**). Kasutades antidepressanti oli võimalik hoida ära mitmete käitumismuutuste tekkimine (**käsikiri II**). Seega on tegemist mudeliga, mida ka edaspidi saab kasutada ärevuse ja meeleolu muutustega seotud sümptomite uurimiseks loomadel.

Artiklis III ja **käsikirjas IV** õnnestus demonstreerida, et sotsiaalne käitumine on ka rottidel püsiv omadus ning madala ja kõrge sotsiaalsusega loomad erinevad ajukoe ja rakuvälise serotoniini taseme poolest eesajukoores. Erinev

sotsiaalne aktiivsus rottidel võib olla seotud serotoniini aktiivsusega eesajukoores ning serotoniini ja dopamiini interaktsiooniga ventraalses tegmentumis.

Erineva sotsiaalse aktiivsusega loomad erinevad ka stressile tundlikkuse poolest, kuna kõrge sotsiaalsusega rotid olid tundlikumad stressi poolt tekitatud anhedoonia osas, kuid madala sotsiaalsusega loomad muutusid stressi tõttu aktiivsemaks (**käsikiri V**). Tegemist on mudeliga, mis võib olla kasulik meeleolu ja ärevuse häirete neurobioloogia uurimiseks.

Selgus, et lisaks sotsiaalsusele on ka magusa lahuse tarbimine rottidel individuaalselt püsiv omadus, eriti kui seda mõõta öösel, mis on näriliste ööpäevatsükli aktiivne periood (**artikkel VI**). Magusa lahuse tarbimine kui individuaalne püsiomadus oli seotud dopamiini D_2 retseptori funktsiooniga aju naalduvas tuumas. Individuaalsed erinevused juttkeha D_2 retseptori funktsioonis aga võivad mõjutada rottide käitumist uudses situatsioonis.

9. PAPERS

Rat behavior after chronic variable stress and partial lesioning of 5-HT-ergic neurotransmission: effects of citalopram

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Abstract

Deficits in serotonergic (5-HT-ergic) neurotransmission and stressful life events have been implicated in affective disorders, and chronic variable stress (CVS) can elicit behavioral changes reminiscent of increased emotionality, anxiety and atypical depression after partial 5-HT depletion. This study examined the effect of chronic citalopram treatment (10 mg/kg daily) on these changes. Para-chloroamphetamine (PCA) (2 mg/kg) reduced the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex, increased anxiety in the social interaction test, and increased activity in the open field. CVS reduced social activity in the social interaction test and immobility time in the forced swimming test. Reduction of excrements left during immobilization indicated partial adaptation with the CVS. Specific stressors had different effects on body weight gain. Combination of CVS and PCA increased sucrose intake after two weeks of stress. In addition, combination of the two treatments reduced diving. Citalopram prevented the increase in sucrose consumption in the PCA+CVS rats, and in 5-HT-depleted animals blocked the increase in struggling and reduced the number of defecations. Conclusively, citalopram treatment prevented several effects of either 5-HT depletion or combined PCA+CVS treatment, suggesting that these behavioral changes could be used in studies on the neurobiology of depression.

Keywords: Chronic variable stress, Parachloroamphetamine, Serotonin, Citalopram, Depression, Stressor, Rats, Sucrose intake, Impulsivity

Introduction

Development of novel animal models has been identified as one of the major needs in research on mood disorders (Nestler et al., 2002). Animal models have helped to discover new medications and to understand the etiological factors that cause depressive symptoms in humans. However, there are major

limitations with the available models concerning understanding the circuits in the brain responsible for the normal regulation of mood and affect, and identifying the circuits that function abnormally in mood disorders (O'Neill and Moore, 2003; Holmes, 2003). Only a few of the available tests have been suggested to possess high specificity and reliability in predicting novel drugs (Cryan et al., 2002). In view of this discontent with the advances of clinical pharmacology of depression and the state-of-the-art of animal models, innovative approaches are necessary (Harro, 2004).

Several attempts to investigate the neurobiology of depression and to measure antidepressant effects of drugs have made use of application of chronic stress, which is one of the major determinants in development of human depression. Chronic stress is associated with general vulnerability to diseases, hyperactivity of corticotropin-releasing hormone system (Bale, 2005) and alterations in other neurochemical pathways (Harro and Oreland, 2001), abnormalities in the immune system (Dorian and Garfinkel, 1987; O'Leary, 1990), social disruption (O'Leary, 1990) and other biological and behavioral changes that are believed to contribute to the pathophysiology of depression.

One of the currently most popular animal models of depression is chronic mild or variable stress regime (CVS), where rodents are exposed to a variety of different stressors intermittently for several weeks (Willner, 1997). Relatively unpredictable sequence of stressors induces changes in the hedonic state of animals, reduction of activity in the open field and social interaction test, and other behavioral changes reminiscent of human depression (Willner, 2005). The reliability of the model has, however, been a subject of extensive discussions (e.g. Cryan et al., 2002; Harro, 2004; Matthews and Reid, 1998; Willner, 1997). Sensitivity to stressful events is moderated by genetic makeup (Caspi et al., 2003; Costello et al., 2002; Monroe and Simons, 1991) and by environmental conditions (Chesler et al., 2002), which may be a rich source for inconsistency; it is possible that in some settings not only is the baseline sensitivity of animals lower but that adaptation to the stimuli is higher. Other possible explanations are purely methodological but nevertheless important: for example, usually, the possible influence of single components of stress regime immediately preceeding the behavioral measurements is not considered.

Even though in many cases human mood disorders seem to be preceded by periods of stress, stress per se is not sufficient to induce depression in the vast majority of individuals. One strategy of current research, then, is to use genetic and nongenetic perturbations to create improved animal models of mood disorders (Nestler et al., 2002), and combination of manipulations on the integrity of selected neurobiological systems with superimposed chronic stress (Harro et al., 2001).

A particular candidate for underlying the resilience to the depressogenic effects of chronic stress is the function of serotonergic systems. Serotonergic (5-HT-ergic) systems play an important role in the regulation of behavioral, autonomic and endocrine responses to stressful stimuli (Lowry, 2002) and

deficits in serotonergic neurotransmission have for a long time been considered a substantial factor in depression (Coppen et al., 1972; Lapin and Oxenkrug, 1969; Ordway et al., 2002). Destruction of 5-HT nerve terminals with 5,6-dihydroxytryptamine potentiates by 50% the stress induced rise in plasma corticosterone, suggestive of an interaction between stress and low serotonergic state in the etiology of depression (Richardson, 1984). The advent of 5-HT uptake inhibitors as clinically effective antidepressants made it clear that an increase in 5-HT-ergic function should be closely linked to the treatment effect (Borsini, 1995; Delgado et al., 1990). As a convincing evidence for the principle that individual differences in serotonergic systems determine the resilience to stress, Caspi and colleagues (2003) recently demonstrated that a functional polymorphism in the promoter region of the 5-HT transporter gene was responsible for the efficacy of stressful life events in eliciting depression. Depletion of 5-HT stores in terminal regions compromises the ability of the serotonergic neurons to activate central systems that manage stressful stimuli (Matuszewich et al., 2002) and increases the responsiveness to stress (Harro et al., 2001). During the hyperactivity of the HPA axis, behavioral responses are accentuated, and animals may overreact to demanding situations (sometimes referred to as 'impulsiveness') (Netto et al., 2002).

Parachloroamphetamine (PCA) is a neurotoxin which potently and selectively reduces 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels in the brain, and this effect persists for months after a single dose (Sanders-Bush et al., 1975), while there are no such long-term changes in catecholaminergic systems (Massari et al., 1978; Leonard, 1976). Since large neurochemical lesions induce significant adaptive changes both pre- and postsynaptically (Harro et al., 1999) and biological predispositions are likely to be more quantitative than qualitative, partial depletion has been preferred over complete depletion (Datla and Curzon, 1996; Häidkind et al., 2004). We have previously shown that partial 5-HT depletion by PCA (2 mg/kg) pretreatment and chronic variable stress in combination elicited several behavioral changes reminiscent of increased emotionality, anxiety and atypical depression (Harro et al., 2001). Both PCA and CVS treatments reduced the time of social behavior in the social interaction test. Interestingly, the combination of both treatments increased consumption of sucrose solution and its preference to water after 2 weeks of CVS, an effect in line with the hypothesis of atypical depression being specifically related to low serotonergic tone (Moller, 1992; Wurtman and Wurtman, 1995). The purpose of the present study was to investigate which of the effects of partial serotonin depletion, CVS, and their combination could be attenuated by antidepressant treatment. Special attention was paid to the efficacy of individual stressors as revealed by their effect on the body weight gain, in order to further refine methodological approaches to the characterization of behavioral effects of stress.

Methods

Animals

Eighty male Sprague-Dawley rats (Scanbur BK AB, Sweden; age two months) were group housed (n=5 per cage) in transparent macrolone cages under controlled light cycle (lights on from 08:30 h to 20:30 h) and temperature (19–21º C), with free access to tap water and food pellets (diet R70, Lactamin, Sweden). Animals, weighing 234–354 g at the beginning of the experiment, were weighed daily, and always before the other procedures. Daily measured body weight was used for calculation of 24 h body weight gain. All procedures were carried out in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

General procedure

On the basis of the body weight, animals were allocated into 8 groups by 3 factors: Pretreatment (PCA or Vehicle), Treatment (Stress or Control) and Drug (Citalopram or Vehicle). The group formation was randomized and animals were submitted to all manipulations in random order. All groups were housed in the same room to guarantee similar basic conditions. Seven days after single PCA or Vehicle pretreatment, half of the animals were allocated to the CVS regime. The stress procedure lasted for 20 days (Fig. 1). Animals belonging to the Stress groups were transported to another room for all manipulations included in the CVS procedure. Seven days after beginning of stress, half of the animals began to receive citalopram treatment. Citalopram (10 mg/kg intraperitoneally) was administrated daily until the end of the experiment. Drug was always administered 30 min before the behavioral test. Sucrose preference was measured weekly, and the last test was carried out on the day following the last day of the stress procedure. On the next day thereafter, the social interaction test was carried out. The first session of the forced swimming test was carried out on the third day from the last stressor, and the second on the following day. Then, 2 days later, the rats were tested in the open field test, and immediately sacrificed thereafter. The brains were quickly dissected on ice and the brain tissue was stored at –80°C in a deep freezer. The two last "weeks" of the study were not full weeks, lasting 6 days (Week 3 of CVS) and 5 days (session of behavioral experiments), and the respective body weight data were, to enable comparison, adjusted on the basis of average daily weight gain.

Chronic variable stress procedure

Stressors that were applied can be divided into two categories: short-term and long-term. Both types of stressors were used intermittently, one stressor per day, and in the following order: cold (4°C) water and wet bedding (initially, 400 ml of water was poured on a rat, and the sawdust bedding was kept wet for the following 17 h), electric footshock (ten shocks, 1 s each at the intensity level of vocalization threshold), stroboscopic light (for 13 h, 10 Hz, 2 lx), tail pinch with

a clothes-pin placed 5 cm distal from the base of tail (5 min), cage tilt at 45° (for 20 h), movement restriction in a small cage (11 x16 x 7 cm) for 2 h, strong illumination (900 lx) during the predicted dark phase (for 12 h). As the CVS regime lasted for 20 days, all stressors were used 3 times, except the last mentioned stressor, which was applied twice. Control rats remained undisturbed in their cages for this 20-days period except for weighing and sucrose intake tests.

Parachloroamphetamine treatment

PCA (Sigma) in the dose 2 mg/kg (expressed as for hydrochloride) was dissolved in distilled water and injected in a volume of 1 ml/kg intraperitoneally. Control animals received distilled water as a vehicle injection.

Sucrose intake

The sucrose intake test was carried out in separate cages. Food was freely available also during this time. Two bottles, one filled with 1% sucrose solution and the other with water were used. Placement of the bottles with sucrose vs water was randomized across the tests. The first test was carried out seven days after PCA pretreatment. Three additional tests were carried out after 1, 2 and 3 weeks of CVS. Sucrose and water consumption was measured for the period of 1 h by weighing preweighed bottles at the end of the test. Sucrose preference was measured by calculation the proportion of sucrose consumption out of total consumption of liquid.

Social interaction

In the social interaction test, developed by File (File and Hyde, 1978), two unfamiliar, weight-matched rats receiving the same treatment were placed in opposite corners for 10 min into a brightly-lit chamber (30 x 30 x 60 cm) with floor covered with wood shavings. The total time spent in active social behavior (allogrooming, sniffing the partner, crawling under and over, following) was recorded, for each rat separately.

Forced swimming test

In the forced swimming test, first characterized by Porsolt and colleagues (Porsolt et al., 1978), rats were forced to swim in a vertical glass cylinder (diameter 22.5 cm, height 60 cm) containing 35 cm of water maintained at 25ºC. On the first day of the experiment, the rats were forced to swim for 15 min and thereafter dried with laboratory tissues. Water was changed after testing of each subject. On the following day rats were re-exposed to the forced swimming for 5 min. Behavior was videotaped and analyzed along the categories of immobility, swimming and struggling (Armario et al., 1988; Häidkind et al., 2004). In addition, the number of excrements and divings during the tests were measured. Data of the first forced swimming test were analyzed for the first 5 minutes session, except for the number of fecal boli.

Open field
In the open field test rats were placed to the center of a rectangular arena (0.5 X 1 m), which was divided into 8 equal squares. Parameters registered during 4 min were the number of squares visited (with all four feet on one square), the number of rearings and number of excrements left in the open field.

Measurement of monoamine levels
Monoamines and their metabolites were assayed by HPLC with electrochemical detection as previously described (Alttoa et al., 2005). The rat brain tissues were homogenized with Bandelin Sonoplus ultrasonic homogenizer (Bandelin Electronic, Germany) in ice-cold solution (5–30 µl/mg tissue) of 0.09 M perchloric acid containing 5 mM sodium bisulfite and 0.04 mM EDTA. The homogenate was then centrifuged at 17 000X*g* for 20 min at 4°C. Aliquots (10–30 µl) of the supernatant obtained were chromatographed on a Lichrospher 60 RP Select B column (250X3 mm; 5 µm). The separation was done in isocratic elution mode at column temperature 30°C using the mobile phase containing 0.05 M citric buffer at pH 3.7, 0.02 mM EDTA, 1 mM KCl, 1 mM sodium octanesulphonate and 5,6 % acetonitrile. The chromatography system consisted of a Hewlett Packard HP 1100 series isocratic pump, a thermostatted auto-sampler, a thermostatted column compartment and an HP 1049 electrochemical detector (Hewlett Packard, Germany) with glassy carbon electrode. The measurements were done at an electrode potential of +0.6 V versus the Ag/AgCl reference electrode. The limit of detection for all assayed compounds was 0.05–0.10 pmol at signal to noise ratio (S/N)=3.

Data analysis
For statistical evaluation of the behavioral and biochemical data, three-way analysis of variance (ANOVA) was used with Pretreatment (PCA or Vehicle), Treatment (Stress or Control), and Drug (Citalopram or Vehicle) as independent factors. For such measures as body weight or sucrose consumption, a fourth, repeated measures factor (Time) was added. Group differences after significant ANOVAs were measured by post hoc Fisher's Protected Least Significance Difference (PLSD) test. Correlations shown are Pearson correlation coefficients.

Results

Changes in body weight gain
During the study all animals gained body weight, but treatments affected the gain of weight.

Body weight gain was reduced by PCA one week after its administration (vehicle – 24.8±1.5 g, PCA – 15.1±1.7 g, $F(1,72)=18.2$, $p<0.0001$) (Fig. 1A). CVS regime was started in the beginning of the second week and reduced body

weight gain during this week [$F(1,76)=13.2$, $p<0.001$] independently of the pretreatment with PCA (Fig. 1B). Daily injection of citalopram started with the third week of the study and was the only treatment which reduced the body weight gain during this week [an overall effect $F(1,72)=9.47$, $p<0.01$]. On the subsequent week, the effect of citalopram was still present [$F(1,72)=11.4$, $p<0.01$] and did not interact with the effects of other treatments. Both PCA and CVS increased the body weight gain [$F(1,72)=25.9$ and 28.4 respectively, $p<0.0001$] during the fourth week. During the final week, the week of daily behavioral experiments, PCA and CVS had an overall effect on body weight gain [$F(1,71)=31.4$, $p<0.0001$ and 4.08 $p<0.05$, respectively] and an interaction between CVS and antidepressant treatment emerged [$F(1,71)=4.78$, $p<0.05$].

Post hoc tests revealed that administration of PCA (2 mg/kg) prevented the body weight gain within the next 24 h (vehicle 3.2±0.8 g, PCA -0.1±0.6 g). During the subsequent days, the effect of PCA on daily body weight gain was statistically not significant, but the overall body weight gain in the week following administration of the neurotoxin was reduced by PCA. During the second and third week of the experiment, PCA pretreatment did not affect body weight gain. During the fourth week, PCA pretreatment increased body weight gain (Fig. 1C), but during the following week, when the daily behavioral experiments were conducted, weight gain in PCA-treated animals was significantly lower than in vehicle-pretreated rats, and even negative (Fig. 1D).

CVS regime which was started in the beginning of the second week reduced body weight gain during this week independently of the pretreatment with PCA (Fig. 1B). During the third week of study, there was no effect of CVS on body weight gain, but during the fourth week, body weight gain in the CVS group was significantly higher than in controls who had a decline in weight gain (Fig. 1A). Injections of citalopram or saline were started from the third week, and the injection procedure suppressed body weight gain during the third and the fourth weeks as compared with the previous week (Fig. 1A), but not any longer during the final week of experiments (Fig. 1D). During the fifth week, body weight gain was negative in PCA, PCA/CVS and PCA+citalopram groups, whereas in PCA-pretreated rats stressed and given citalopram this decrease was not observed (Fig. 1D).

Effect of specific stressors on body weight gain

The effect of each specific stressor on body weight gain within the 24-h period was analyzed comparing only the CVS (n=10) and Control groups (n=10). Tail pinch and strong illumination during the predicted dark phase had no general effect on 24 h body weight gain. Cold water and wet bedding, and cage tilt had an overall reducing effect on body weight gain [$F(1,18)=16.2$, $p<0.001$ and 10.4, $p<0.01$, respectively]. Electrical footshock, stroboscopic light and immobilization had an overall increasing effect on body weight gain [$F(1,18)=7.45$, $p<0.05$, 8.72, $p<0.01$ and 19.9, $p<0.001$, respectively]. Cold water and wet bedding, electrical footshock, stroboscopic light, tail pinch and

cage tilt interacted with the Time factor [$F(2,36)=7.99$, $p<0.01$, 5.85, $p<0.01$, 12.8, $p<0.0001$, 6.72, $p<0.01$ and 6.16, $p<0.01$, respectively]. The Time factor itself had a significant effect regarding the following stressors: electrical footshock, tail pinch, cage tilt and strong illumination during the predicted dark phase [$F(2,36)=5.85$, $p<0.01$, 10.7, $p<0.01$, 13.6, $p<0.01$ and 32.6, $p<0.0001$, respectively].

Post hoc tests revealed that cold water and wet bedding reduced body weight gain only when applied for the first time (Fig. 2A), electrical footshock stressor increased the body weight gain only after the third application (Fig. 2B), stroboscopic light had a tendency to reduce body weight gain after the first time of its use (Fig. 2C), tail pinch reduced body weight gain only when applied for the first time (Fig. 2D), cage tilt reduced body weight gain after the first and the third application (Fig. 2E), immobilization increased body weight gain after every application (Fig. 2F), strong illumination during the predicted dark phase had a tendency to reduce body weight gain both times (Fig 2G).

The number of defecations during the three immobilization sessions, carried out weekly, decreased throughout these three experiments [$F(2;38)=3.53$, $p<0.05$], thus showing an adaptation with the CVS regime (n=40, week 1 – 5.52±0.73; week 2 – 4.95±0.57; week 3 – 3.67±0.50; values are means±S.E.M.; significantly less excrements during the third session compared with the fist session, $p<0.05$). This reduction was not influenced by any of the other treatments the CVS group animals additionally received.

Sucrose intake

Four sucrose intake tests were carried out during the study. Treatments did not affect sucrose preference over water, but changed the consumption of sucrose solution (Fig. 3A). The first test was carried out one week after PCA treatment. PCA did not have any effect on sucrose consumption, but caused a lower intake of water (PCA 1.15±.04; Vehicle 1.50±.10). Further analysis was carried out on changes in sucrose intake as calculated from this baseline, because there were differences in baseline sucrose intake between the groups. Two weeks after PCA and one week after beginning of the CVS regime, the second sucrose preference test was carried out. Neither treatment had any effect on sucrose consumption.

One week after beginning of the daily treatment with citalopram, thus two weeks after the start of CVS regime and three weeks after PCA treatment, the third sucrose intake test was performed. Pretreatment [$F(1;72)=4.89$, $p<0.05$] and Drug [$F(1;72)=4.85$, $p<0.05$] had an overall effect on sucrose consumption. Post-hoc tests indicated that PCA increased sucrose consumption in stressed animals, and citalopram prevented this increase (Fig. 3B). None of the treatments had any statistically significant effect on sucrose consumption in the fourth test. However, the effect of CVS almost reached the conventional level of significance [$F(1;72)=3.71$, $p=0.06$], tending to reduce sucrose intake in all

groups but the PCA-pretreated, and citalopram tended to reduce sucrose intake in the PCA+CVS group (Fig. 3C).

Social activity

Drug and Treatment independently reduced social activity [$F(1,72)=30.2$ and 4.90, $p<0.0001$ and $p<0.05$, respectively]. There was also a significant interaction between Pretreatment and Treatment [$F(1,72)=4.13$, $p<0.05$]. Post hoc tests indicated that all treatments reduced social activity, and citalopram tended to potentiate the reducing effect of PCA ($p=0.06$) and potentiated the effect of PCA+CVS (Fig. 4). On the other hand, the effects of PCA and CVS were not additive.

Forced swimming test

Overall effects of Treatment on struggling behavior [$F(1,66)=6.26$, $p<0.05$] and immobility time [$F(1,66)=10.2$, $p<0.01$] were found in the first forced swimming test. In the second test, the overall effects of Treatment were also present for struggling [$F(1,68)=20.2$, $p<0.0001$], immobility [$F(1,68)=25.1$, $p<0.0001$] and swimming [$F(1,68)=11.6$, $p<0.01$]. Overall effect of Drug on immobility [$F(1,66)=7.75$, $p<0.01$] was found in the first forced swimming test. Drug treatment reduced struggling [$F(1,68)=11.4$, $p<0.01$] and increased immobility [$F(1,68)=4.17$, $p<0.05$] in the second forced swimming test. Interactions between Pretreatment and Drug emerged regarding struggling [$F(1,66)=8.46$, $p<0.01$] and immobility [$F(1,66)=10.2$, $p<0.01$] in the first forced swimming test. A tendency for interaction between Pretreatment and Drug [$F(1,68)=2.90$, $p=0.09$] in struggling was also found in the second test.

Post hoc tests revealed that CVS increased struggling and reduced immobility in both tests (Fig. 5A-5D), and increased swimming in the second test (Fig. 5F). Citalopram reduced struggling in 5-HT depleted animals in both forced swimming tests and prevented the increase of struggling in PCA-pretreated but not vehicle-pretreated stressed rats (Fig. 5AB). Citalopram also increased immobility in PCA+CVS rats in the first test (Fig. 5C).

Most of the dives rats performed in the forced swimming test occured during the first 5 minutes of the first swimming session, and there were significantly fewer dives during the second day of forced swimming test (in the present experiment, altogether 130 dives in the first day, 7 dives in the second day). During the first forced swimming test, the number of dives was affected by both PCA and CVS factors [$F(1,66)=4.57$ and 5.08, respectively, $p<0.05$]. Citalopram did not have any effect on the number of dives. According to post-hoc tests, diving behavior was reduced only by a combination of PCA and CVS, and this effect was not sensitive to citalopram (data not shown). Number of dives did not correlate with the immobility time during swimming or with the number of excrements left during the forced swimming test, suggesting a distinct nature of this measure.

Citalopram significantly reduced the number of defecations during the first [$F(1,72)=23.8$, $p<0.0001$] forced swimming test (Fig. 5G). No other treatment had any effect on this measure, or influenced the effect of citalopram. Drug factor had an overall effect on the number of fecal boli also during the second forced swimming test [$F(1,71)=11.1$, $p<0.01$]. Post hoc tests revealed that citalopram reduced the number of defecations significantly only in animals previously treated with PCA, even though there was a similar tendency in the other citalopram-treated groups (Fig. 5H).

Activity in the open field test

Treatment had an overall reducing effect on the number of squares crossed in the open field [$F(1,71)=6.36$, $p<0.05$]. Pretreatment and Drug had no overall effect on horizontal activity. Rats having received PCA as the only treatment had a non-significant tendency of higher horizontal activity, and stressed PCA-pretreated rats were significantly less active compared to this group (Fig. 6A).

All treatments changed vertical activity in the open field. Regarding the overall effects, Pretreatment increased the number of rearings [$F(1,71)=4.10$, $p<0.05$], and Treatment and Drug independently reduced the number of rearings [$F(1,71)=6.92$ and 4.60, respectively; $p<0.05$]. According to post-hoc tests, PCA treatment increased rearing activity compared to the control group, but the effect of CVS and citalopram did not reach statistical significance; however, both CVS and citalopram blocked the PCA-induced hyperactivity (Fig. 6B).

Drug had an overall effect on the number of excrements left on the open field, and there was also an interaction between Pretreatment and Drug [$F(1,71)=6.77$ and 5.81, respectively; $p<0.05$]. Post hoc tests revealed that citalopram reduced the number of defecations in stressed animals (Fig. 6C).

Monoamine levels and turnover in the frontal cortex

None of the treatments had any effect on the levels of NA, DA and DOPAC, or DA turnover, in the frontal cortex (data not shown). As expected, PCA (2 mg/kg) significantly reduced the levels of 5-HT [$F(1,71)=4.09$, $p<0.05$] and 5-HIAA [$F(1,71)=37.9$, $p<0.0001$) in the frontal cortex. PCA reduced levels of 5-HT by 15% (data not shown) and levels of 5-HIAA by 27%, as measured at the end of experiments, five weeks after administration of the toxin.

There was an interaction between all factors regarding the levels of 5-HIAA in the frontal cortex [$F(1,71)=8.36$, $p<0.01$]. Post hoc tests revealed that citalopram and PCA both reduced the levels of 5-HIAA, but CVS prevented the reduction of 5-HIAA in citalopram treated animals (Fig. 7A). However, this effect of CVS was not observed after partial 5-HT depletion. 5-HT turnover was not affected by PCA, but was significantly reduced by citalopram [$F(1,71)=4.00$, $p<0.05$]. Again, this reduction was not present in stressed rats not having received PCA (Fig. 7B).

Drug had a tendency to increase the levels of HVA in the frontal cortex [$F(1,71)=3.77$, $p=0.06$], and there was an interaction between Pretreatment and

Treatment [$F(1,71)=4.18$, $p<0.05$] and between all factors [$F(1,71)=4.90$, $p<0.05$]. Post hoc tests revealed that the levels of HVA were increased in stressed animals treated with citalopram, but not after 5-HT depletion (data not shown).

Discussion

Behavioral effects of chronic variable stress and efficacy of acute stressors

CVS reduced body weight gain during the first week of the study. Stress can lead to either decreased or increased feeding, depending on the nature of stressor (Gamaro et al., 2003; Morley et al., 1986): the type, duration or severity of stress and the predictability of the stressor applied may modify the responses to stress (Hargreaves, 1990; Marti et al., 1994; Pare and Redei, 1993; Pucilowski et al., 1993). In the present study, cold water with wet bedding, tail pinch, cage tilt and strong illumination during the dark phase either suppressed body weight gain or had no effect, but electrical footshock, stroboscopic light and immobilization stressors were rather associated with an increase in body weight gain. It is suggested that exposure to repeated chronic stress modifies eating behavior dependent upon the severity and duration of exposure to stressors (Ely et al., 1997). Long-lasting (12–24 hr) and short-lasting (5 min – 2 hr) stressors were sequenced intermittently in our study design, and the duration had no clear-cut impact on weight changes. In case of most of the stressors, there was no consistent effect of a stressor on body weight gain. Only immobilization was followed by higher body weight gain compared to the control group all three times it was applied. Immobilization was also the only stress procedure which restricted food and water availability for a significant period. Three more general observations could be noted: First, most of the stressors were associated with changes in body weight gain when applied first time, but had no effect subsequently. Second, periods of lower body weight gain in stressed animals were followed by periods of higher body weight gain, which may reflect compensatory mechanisms and suggest that only limited conclusions can be made on the basis of the present data regarding the nature of the effect of specific stressors. Third, it was apparent that no stressor was followed by both increases and decreases in weight gain, even though only a few stressors had the same effect expressed consistently.

Effect of the CVS regime on body weight was temporary, as the animals obviously adapted to the procedure in this regard and compensation for previously lost energy occurred during the third week of CVS. The presence of certain adaptation to stress was also observable when the effect of stressors was measured on 24-h body weight gain: most of the stressors were effective only when used first time. Furthermore, the number of defecations during the immobilization stress was reduced with repeated presentation of this stressor, also suggestive of certain adaptation to stress. It has been suggested that chronic

mild/variable stress regime elicits its behavioral effects mainly due to the unpredictable nature of the procedure for the animals (Willner, 2005), and the variety of different stressors is used in order to prevent or delay habituation (Griffiths et al., 1992; Muscat and Willner, 1992). The findings of the present study suggest that considerable adaptation to the CVS regime can occur within a few weeks. Indeed, while changes in stressors applied may prevent habituation to their specific features, it is possible that animals are able to generalize to their presence, thus habituating to stress as such because while the specific stressors are unpredictable, the daily application of stressors is not. It is important to notice that we did not use food and water deprivation in the present study, which would have facilitated a reduction in body weight gain in stressed animals.

Even though certain adaptation to stress occurred in the present study, several behavioral effects of CVS persisted. Consistently with our previous studies (Häidkind et al., 2003**;** Harro et al., 1999, 2001) as well as others (Platt and Stone, 1982; van Dijken et al., 1992), stress reduced immobility and increased struggling and swimming. Involvement of negative affect in this behavioral shift is supported by the fact that CVS had anxiogenic-like effect in the social interaction test. Reduction of immobility in the forced swimming test should in certain paradigms rather be interpreted as enhance reactivity or impulsiveness than reduced despair (Harro, 2002, 2004).

The effect of stress on the struggling and immobility was similar during both tests. The fact, that effect of stress and other treatments also persisted during both tests, indicates the lack of adaptation and coping with this test condition. In addition, number of defecations was not reduced during the second forced swimming test. That the pattern of results was quite similar in the two swimming sessions suggests that learning played little role in the effects of treatments.

Also, as previously described (Harro et al., 2001) chronic stress had an anxiogenic effect in the social interaction test. As the test situations were novel to the animals and can be considered stressful, the differences between control and CVS rats could be explained by an increased sensitivity of stressed rats to novel stressors, as a novel stressor after CVS, which had caused adaptation and reduction of corticotropin-releasing factor (CRF) levels, may cause again marked increase in CRF levels in several brain regions, followed by a further reduction of body weight (Nagashima et al., 2003). Sucrose intake and preference was not significantly affected by CVS, even though there was a tendency of reduction after three weeks of stress. Our inability to observe significant changes in this measure may be related to a) too short period of CVS for our conditions; b) application of specific stressors before measurement of sucrose intake; c) exclusion of food and water deprivation. Some groups have demonstrated the reduction of sucrose intake after two - three weeks of stress (e.g Baker et al., 2005; Gronli et al., 2005), but others after longer periods of stress (e.g Grippo et al., 2005; Muscat et al., 1990). It has been shown that

during the first three weeks of stress procedure the Sprague-Dawley rats had a smaller reduction of sucrose intake and preference compared to Wistar rats (Bekris et al., 2005). Different and sometimes even opposite effects of single stressors on weight gain in the present study indicate that their different quality and certain order may influence results of following behavioral tests, especially of those associated with feeding behavior, and be one of the reasons why theoretically similar procedures end up in different laboratories with opposite results, as reviewed by Willner (2005). Food and water deprivation which is often applied as a stressor just before the measurement of sucrose intake has been excluded from our studies in order to eliminate the confounding by response to hunger (Harro, 2004). We have recently found that CVS consistently reduced sucrose intake in our conditions, however, when measured during the dark phase (unpublished).

Behavioral and physiological effects of partial 5-HT-ergic denervation by PCA treatment

In pharmacological studies, drugs that increase post-synaptic serotonergic stimulation decrease food consumption (Arkle and Ebenezer, 2000; Brown et al., 2001; Finn et al., 2001; Halford and Blundell, 2000; Vickers et al., 2001). In contrast, agents that block post-synaptic 5-HT receptors or those diminishing serotonergic neurotransmission by activating autoreceptors often increase food intake (Simansky, 1996). Thus, 5-HT serves an inhibitory role in feeding (Gamaro et al., 2003; Simansky, 1996). In our study, the body weight gain was initially reduced after administration of PCA, probably due to excessive release of 5-HT. The long-term effect of PCA is depletion of 5-HT in nerve terminals and the reduction of 5-HT activity, which may explain the stoppage of body weight gain reduction and subsequent increase in weight gain in comparison with the control animals during the fourth week of experiment. Interestingly, new environmental changes during the last week of behavioral tests tended to reduce body weight gain in all groups, and the weight gain was negative in PCA-treated animals, with the exception of animals also submitted to CVS and citalopram treatment. It seems that introduction to the series of behavioral tests was aversive and this effect was more expressed in 5-HT depleted animals.

The influence of behavioral tests on the body weight of control animals also suggests an aversive effect of application of the series of novel conditions. However, treatments still caused more significant changes in behavior, compared with control animals.

As in our previous study (Harro et al., 2001), PCA treatment led to enhanced anxiety as expressed in the social interaction test. In the open field, rearing activity was significantly increased, and a similar tendency had been observed previously. Tendencies of reduced immobility and increased struggling in forced swimming test in the present study are also consistent with the significant effects of PCA in the previous study (Harro et al., 2001). This set of behavioral effects elicited by 5-HT-ergic denervation could be explained by

impulsive behavior caused by the dysfunction of brain serotonergic system (Dalley et al., 2002; Harro, 2002).

Behavioral effects of CVS after partial lesion of the 5-HT-ergic system

CVS after PCA treatment augmented consumption of sucrose solution after two weeks of stress. This is in accordance with our previous results with Wistar strain animals (Harro et al., 2001). Interestingly, PCA and CVS separately rather reduced sucrose intake. It has been reported previously that chronic stress can elicit an increase instead of decrease in sucrose consumption (Ely et al., 1997; Silveira et al., 2000), and this paradoxical effect has been attributed to limited unpredictability of stressors and habituation (Gamaro et al., 2003; Willner et al., 1997). The consumption of sucrose solution was smaller in both CVS and PCA groups compared with the PCA+CVS group in the third sucrose preference test, but the body weight gain of both CVS and PCA animals was higher comparing with the PCA+CVS during the antecedent week. Therefore the increase in sucrose intake can not be secondary to body weight change, as it was suggested in experiments, where the reduction of sucrose solution was registered together with reduced body weight gain in the sucrose preference tests, carried out with antecedent food and water deprivation (Forbes et al., 1996; Hatcher et al., 1997; Matthews et al., 1995). Similarly, this effect can not be explained by increased adaptation after partial 5-HT-ergic lesion. Both the increased body weight gain and increased sucrose consumption are characteristic symptoms to atypical depression. Patients with atypical depression present the tendency to overeat carbohydrates, which is believed to act via an increase in insulin secretion resulting in better access of tryptophan to the brain and consequent higher synthesis of 5-HT (Gamaro et al., 2003; Wurtman and Wurtman, 1995). It should be noted, however, that even though the increase in sucrose intake in PCA-pretreated rats after two weeks of CVS was increased similarly to our previous study (Harro et al., 2001), such an increase was not observed one week later, even though PCA pretreatment prevented the tendency of CVS-induced reduction of sucrose intake. This apparent inconsistency in the efficacy of 5-HT depletion to support CVS-induced sucrose intake could possibly be explained by the different stressors applied immediately before measurement of the sucrose intake. Thus, the increase in sucrose intake was recorded when measured after strong illumination during predicted dark phase, which tended to reduce body weight, whereas no significant effect was found when sucrose intake was measured after immobilization for two hours, which had a strong increasing effect on body weight gain. This hypothesis should be tested with a counterbalanced design, and the possibility that differences in stressors applied before behavioral tests influence the outcome should receive due attention.

In the present experiment we counted the number of dives during forced swimming. This was possible due to the relatively high frequency of this behavior, which we have not observed to occur so frequently in previous studies

mostly carried out with the Wistar strain, and which seems not having attracted much interest in literature. CVS reduced diving behavior after 5-HT depletion induced by PCA pretreatment. Thus, CVS reduced active escape attempts in rats with altered 5-HT-ergic system, perhaps indicative of increased anxiety or a shift to a passive coping style.

Stress is known to alter 5-HT metabolism in the CNS and periphery, probably through the increased levels of glycocorticoid hormones, one of the main biological responses to stress (Malyszko et al., 1994; Nishi and Azmitia, 1996; Paris et al., 1987). Activity of tryptophan hydroxylase, the rate limiting biosynthetic enzyme for 5-HT, and 5-HT turnover have been found to be sensitive to circulating corticosteroid levels (Chalmers et al., 1993; Chaouloff, 1993; Singh et al., 1990). Removal of circulating corticosteroids by adrenalectomy has resulted in anatomically specific decreased indices of 5-HT metabolism, while stressful procedures, which raise corticosteroid levels, cause increase in 5-HT turnover (Chalmers et al., 1993; Malyszko et al., 1994; Nishi and Azmitia, 1996). Adaptation to stress may be related to reduction in activity of 5-HT-ergic system, which also causes increased body weight.

Effects of citalopram

Citalopram suppressed or prevented several behavioral changes elicited by PCA, CVS or their combination in current study. Citalopram treatment suppressed the reduction of body weight gain caused by PCA pretreatment during the week of behavioral experiments, but only in stressed rats. In addition, citalopram reduced the number of fecal boli of stressed animals, suggesting that the increased emotionality of stressed animals in this test (Hall, 1934) which we have also previously observed (Harro et al., 2001) can be counteracted by SSRI treatment.

Citalopram prevented the increased sucrose consumption after the third sucrose preference test in PCA+CVS animals, without affecting the consumption by CVS or PCA groups separately. Suppressive effect of an antidepressant on the increased consumption of sucrose solution in stressed rats has also been observed earlier (Bissette et al., 1999). The efficacy of citalopram in the present paradigm suggests that increased sucrose consumption elicited by stress after partial 5-HT depletion is relevant to the pathophysiology of depression.

Citalopram itself reduced social activity in the social interaction test and did not influence the reducing effects of PCA or CVS. In fact, citalopram potentiated the reducing effect of combination of these treatments. Acute administration of SSRIs reduces activity in the social interaction test, but tolerance has been found to develop toward this effect (Dekeyne et al., 2000). This effect of SSRIs is believed to be mediated by 5-HT_{2C} receptors which desensitize during chronic treatment. In the present study, it thus it could be speculated that citalopram did not desensitize 5-HT_{2C} receptors.

Increased defecation in the open field is a traditional indicator of emotionality and anxiety (Hall, 1934; Katz, 1981; van Dijken et al., 1992). In our previous studies CVS has increased the number of defecations (Harro et al., 2001), but not in all experiments (Häidkind et al., 2003). In the present study the baseline defecation rate was higher than we have observed earlier, and was not further increased by stress. Citalopram did not reduce defecations in animals not stressed, but reduced this measure significantly in both groups submitted to CVS. In the forced swimming test which could be considered a more stressful condition than the open field, citalopram reduced the number of excrements in all groups. Thus, citalopram had a potent anti-stress effect.

In our conditions, citalopram has been the only antidepressant inactive after chronic administration in the forced swimming test (Harro et al., 1997). Citalopram had no effect on its own in the present study and did not influence the effect of CVS in general, but prevented the increase in struggling and decrease in immobility in PCA-pretreated CVS rats. This suggests that CVS affects behavior via multiple neural pathways, and those that are associated with 5-HT-ergic deficit can be blocked by citalopram administration. Reduction of diving behavior by PCA plus stress was not sensitive to citalopram, suggesting that this behavior is regulated in a different way. Indeed, individual performance in struggling and diving did not correlate in untreated animals. Interestingly, both CVS and citalopram prevented the hyperactivity of 5-HT lesioned rats in the open field test. Chronic stress can increase 5-HT-ergic activity (Gamaro et al., 2003), which may explain the partial similarity of its effect to that of a SSRI. Citalopram reduced the levels of 5-HIAA and 5-HT turnover, and these effects were prevented by CVS. However, stress did not abolish the effect of citalopram in PCA-pretreated rats, which suggests the integrity of 5-HT-ergic neurotransmission is necessary for the complete effect of stress.

Conclusively, citalopram treatment prevented several but not all effects of CVS in partially 5-HT depleted rats, which is resembling its general clinical efficacy but limited effect size in some of the cases. Cabib and Puglisi-Allegra (1996) suggested that depressive symptoms may not always represent the necessary outcome of stress experiences but be promoted by specific environmental conditions and by a genetically determined susceptibility. Results in the present study provide support to the notion that both biological predisposition and environmental conditions are important in the development of depression-like symptoms, and that 5-HT neurotransmission is one of the most important systems mediating their interaction.

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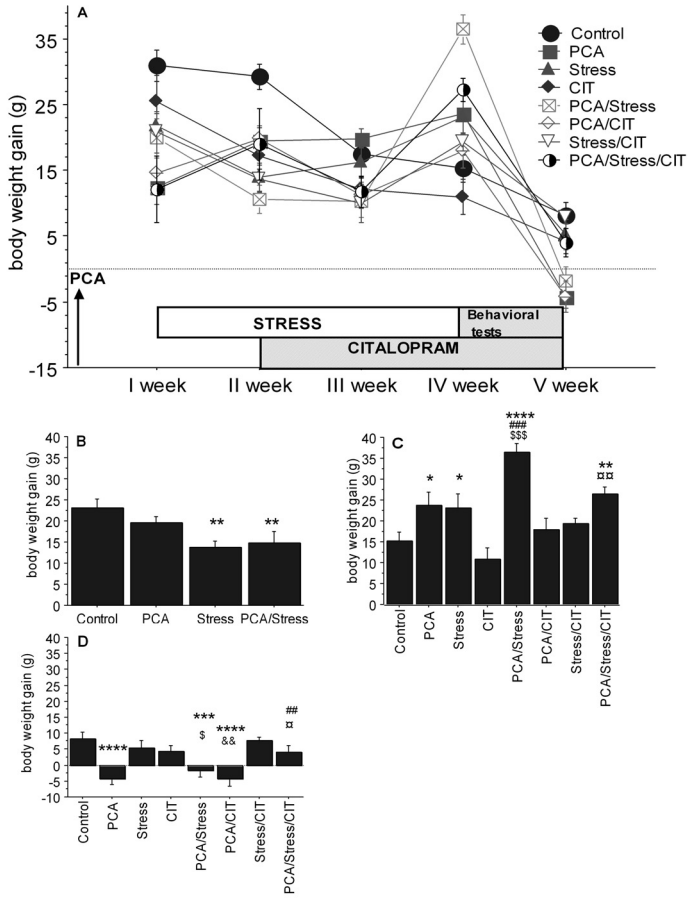

Fig. 1. Overall design of the study and the effect of PCA, chronic variable stress and citalopram (CIT, 10 mg/kg daily) on the body weight gain as compared to the previous week (A). Animals were divided into groups according to body weight. Body weight gain of every week was calculated as a difference between the last day of corresponding and previous week. Whereas week in the current study was defined as a time between two consecutive sucrose preference tests, all weeks were divided with the number of days in that week and multiplied by seven. (B) Body weight gain of second week. (C) Body weight gain of the fourth week. (D) Body weight gain of the fifth week (means±S.E.M).

* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, vs. Control; ## p<0.01, ### p<0.001, vs. PCA; $ p<0.05, $$$ p<0.001, vs. Stress; && p<0.01, vs. citalopram (CIT); ¤ p<0.05, ¤¤ p<0.01, vs. PCA/Stress.

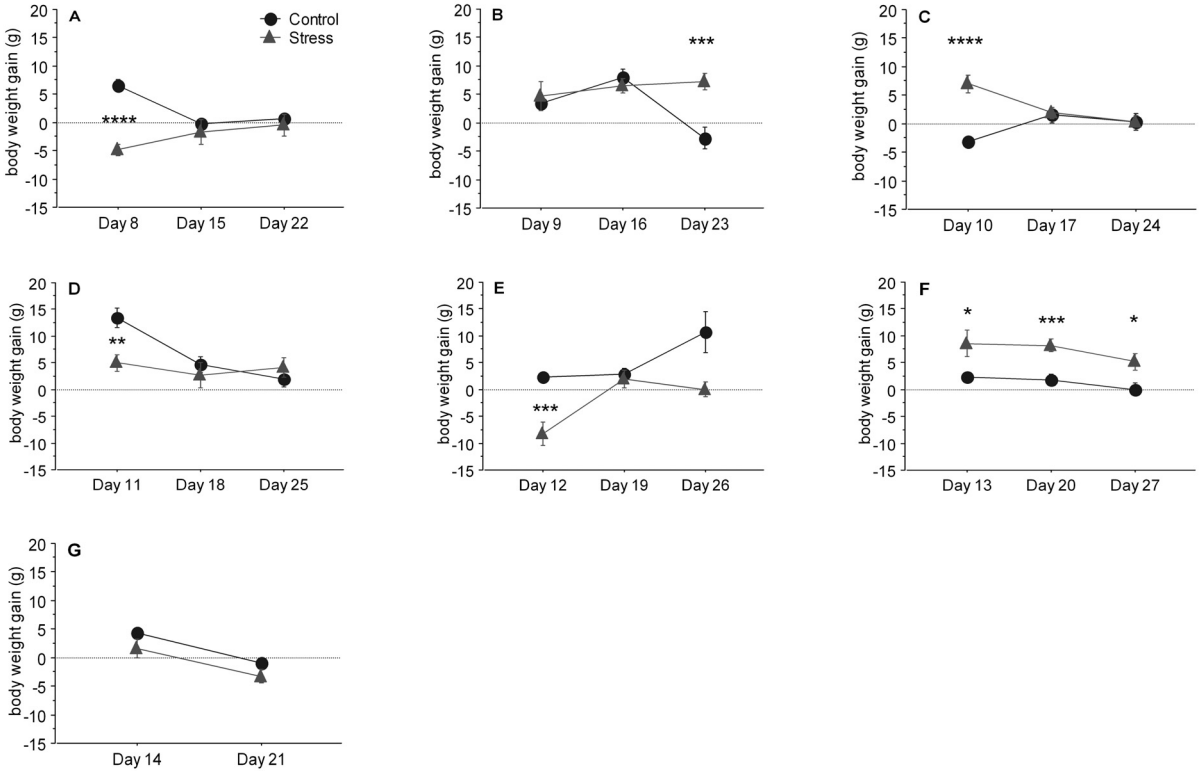

Fig. 2. The effect of each specific stressor on body weight gain (means±S.E.M) within the 24-h period, comparing the Stress (n=10) and Control (n=10) groups. On abscissa, Day refers to the day of stress regime. (A) Cold water and wet bedding. (B) Electrical foot shock. (C) Stroboscopic light. (D) Tail pinch. (E) Cage tilt. (F) Immobilization. (G) Strong illumination during the predicted dark phase. Stressors are presented in the order of their use in the stress regime.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$, vs. Control.

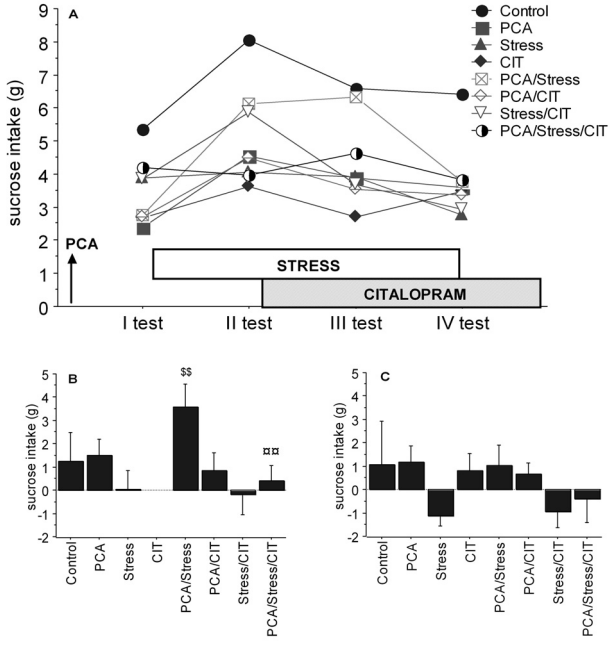

Fig. 3. Effects of PCA (2 mg/kg) pretreatment, chronic variable stress and citalopram (CIT, 10 mg/kg daily) on sucrose intake during four sucrose preference tests (A). For statistical evaluation see results. (B) and (C) changes in sucrose intake, calculated from the baseline at the first test, in the third and the fourth sucrose preference test, respectively.
$$ p<0.01, vs. Stress; ¤¤ p<0.01, vs. PCA/Stress.

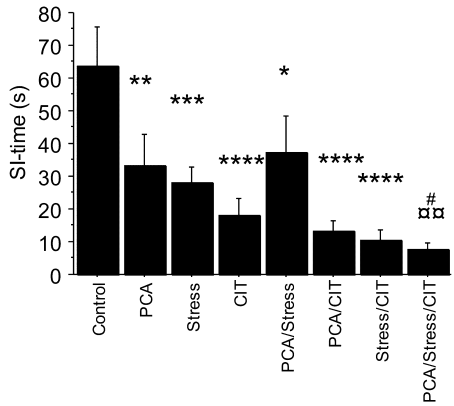

Fig. 4. Effects of PCA (2 mg/kg) pretreatment, chronic variable stress and citalopram (CIT, 10 mg/kg daily) on time spent in social interaction (s) in the social interaction test (means±S.E.M).
* p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001, vs. Control; # p<0.05, vs. PCA; ¤¤ p<0.01, vs. PCA/Stress.

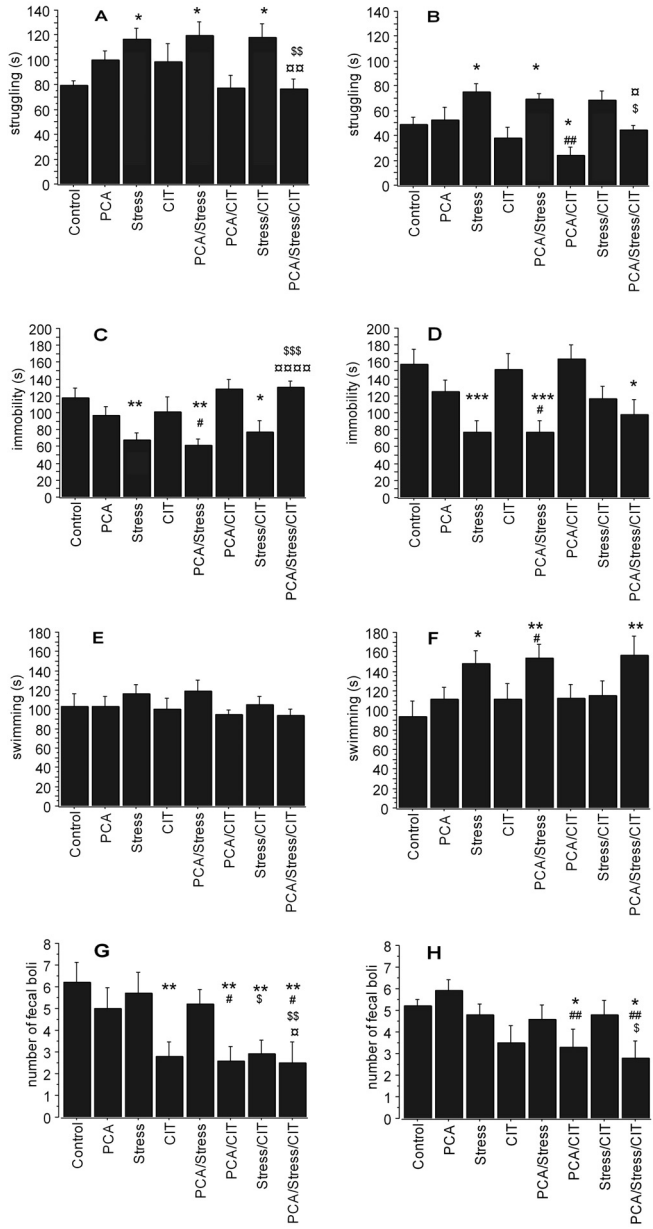

Fig. 5. Effects of PCA (2 mg/kg) pretreatment, chronic variable stress and citalopram (CIT 10 mg/kg daily) on behavior of forced swimming tests: time of struggling in the first (A) and the second (B) test, time of immobility in the first (C) and the second (D) test, time of swimming in the first (E) and the second (F) test, number of fecal boli in the first (G) and the second (H) test (means±S.E.M).

***** p<0.05, ****** p<0.01, ******* p<0.001, vs. Control; # p<0.05, ## p<0.01, vs. PCA; $ p<0.05, $$ p<0.01, vs. Stress; ¤ p<0.05, ¤¤ p<0.01, ¤¤¤¤ p<0.0001, vs. PCA/Stress.

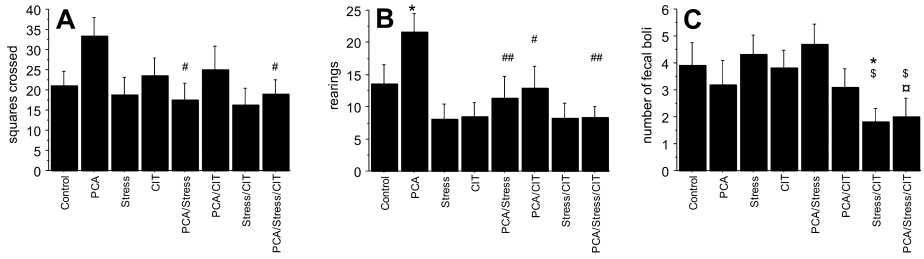

Fig. 6. Effects of PCA (2 mg/kg) pretreatment, chronic variable stress and citalopram (CIT, 10 mg/kg daily) on behavior in the open field test: (A) number of squares crossed, (B) number of rearings, and (C) number of fecal boli (means±S.E.M).
* p<0.05, vs. Control; # p<0.05, ## p<0.01, vs. PCA; $ p<0.05, vs. Stress; ¤ p<0.05, vs. PCA/Stress.

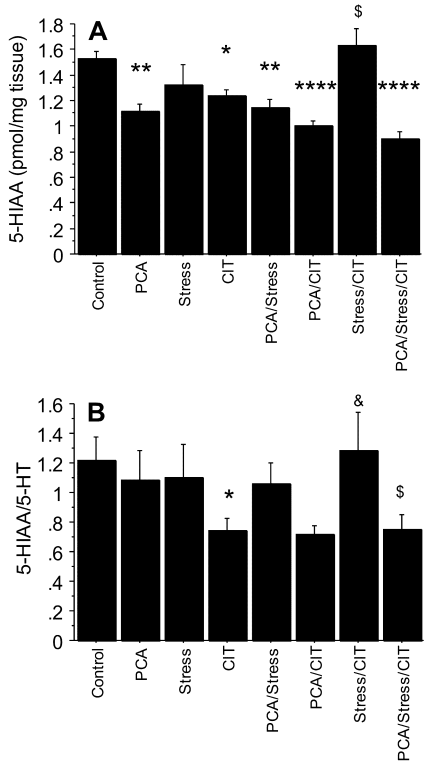

Fig. 7. Effect of PCA, chronic variable stress and citalopram (CIT, 10 mg/kg daily) on levels of 5-HIAA (A) and 5-HT turnover (5-HIAA/5-HT ratio) (B), in the frontal cortex (means±S.E.M).
* p<0.05, ** p<0.01, **** p<0.0001, vs. Control; $ p<0.05, vs. Stress; & p<0.05, vs. CIT.

Extracellular levels of serotonin in prefrontal cortex and ventral tegmental area in rats with low and high sociability

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Abstract

Social behaviour is the basis of one of the most generally accepted independent dimensions of personality. The purpose of the present study was to compare extracellular 5-hydroxytryptamine (5-HT) levels in animals with low and high sociability at baseline, and after p-chloroamphetamine (PCA) induced release. High sociability group had lower levels of 5-HT in prefrontal cortex both at baseline and after administration of PCA (2 mg/kg). PCA induced increase of extracellular 5-HT in ventral tegmental area (VTA) was higher in rats with high sociability. Conclusively, extracellular 5-HT levels differ in rats with high vs low sociability trait, and these regionally specific differences may be related to distinct components which shape social behaviour.

Keywords: Sociability, 5-hydroxytryptamine, dopamine, frontal cortex, ventral tegmental area, p-chloroamphetamine

Sociability behaviour is an important dimension in the structure of personality, which defines the individual's ability to function in a social environment (Bosc, 2000). Though many different neurochemical systems are implicated in social behaviour (Panksepp, 1998), 5-hydroxytryptamine (5-HT) is one of most important neurotransmitter systems in the expression of personality dimensions, associated with social behaviour (Knutson et al., 1998; Tse and Bond, 2002). Several studies have revealed that high impulsivity, aggressiveness, and anxiety in humans, non-human primates and other species are associated with low 5-HT neurotransmission (Turecki, 2005; Suomi, 2005; Grimmett and Sillence, 2005), and drugs, which potentiate 5-HT-ergic function reduce negative affective experience and increase affiliative behaviour in healthy persons (Beech and Mitchell, 2005; Knutson et al., 1998). However, negative association between 5-HT metabolism and social competence has also been described (Yodyingyuad et al., 1985). Even in a single animal test, both negative (Bagdy et al., 2001; File et al., 1996; File et al., 1993; Kennedy et al., 1993; Kenny et al., 2000) and positive (Duxon et al., 2000; Hamon et al., 1999; Lightowler et al., 1994)

associations between 5-HT-ergic function and social behaviour have been reported. Most of experiments on rodents have compared 5-HT-ergic function with performance in a single test. We have previously reported that social behaviour in rats is individually stable and negatively correlated with the levels of 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of 5-HT, in the prefrontal cortex (PFC) (Tõnissaar et al., 2004). The purpose of the present study was to compare extracellular 5-HT levels in low and high sociability (LS and HS) animals at baseline, and after p-chloroamphetamine-induced (PCA) 5-HT release.

Male Wistar rats (n=34) from Scanbur BK AB, Sweden weighing 365–423 g at the beginning of the experiments were housed individually 10 days prior to the first social interaction test in a temperature-controlled animal house under 12 h light/dark cycle (lights on at 07:00 h) with food and water available ad libitum. The social interaction test, developed by File and Hyde (1978), was carried out in three separate sessions with 10-day intervals. Rats were paired on the basis of their body weight before test and every test was carried out with an unfamiliar partner. Two unfamiliar, weight-matched rats were placed for 10 min into a brightly-lit chamber (30 cm × 30 cm × 60 cm) with floor covered with wood shavings. The total time spent in active social behaviour (allogrooming, sniffing the partner, crawling under and over, following) was recorded. The animals were classified as LS- or HS-rats on the basis of the median split of their average social activity over three social interaction tests. After the social interaction tests microdialysis experiments were carried out on awake freely moving animals, which remained in their home cages throughout the procedure. Y-shaped home-made microdialysis probes were implanted into the medial PFC or ventral tegmental area (VTA) according to coordinates from Paxinos and Watson (1986) The coordinates for implantation were as follows, PFC: AP 3.3 mm, ML –0.8 mm, DV –5.0 mm; VTA: AP –5.3 mm, ML –2.5 mm, DV –8.4 mm, implanted at an angle of 12°, from bregma and dura, according to Paxinos and Watson (1986). Twenty four hours later, after the stabilization period of 2 h fifteen fractions were collected with 15 minutes intervals into the vials prefilled with 7.5 µl of 0.02 M acetic acid. After the collection of the sixth sample the animals were injected with PCA (2 mg/kg i.p.). Animals with probe placements outside the medial PFC or VTA were excluded from the analysis. After exclusion of animals with incorrect placement of microdialysis probes or incomplete biochemical data due to sample failures, the number of animals in analysis was 26 (9 in LS group and 17 in HS group) for frontal cortex, and 27 (10 in LS group and 17 in HS group) for VTA.

5-HT in the microdialysates was assayed using HPLC with electrochemical detection. For chromatographic separation a Luna C18(2) column (150x2mm, 5 µm) and mobile phase containing 0.05 M sodium citrate buffer at pH 5.3; 0.02 mM EDTA; 4.1 mM sodium octylsulphonate and 18% acetonitrile were used. The chromatography system consisted of Agilent series 1100 pump and thermostatted autosampler, column thermostat and ESA Coulochem II detector

with ESA 5011 analytical cell. The potential of the electrode used for measurements was +250 mV. The limit of detection for serotonin was 2 fmol at signal to noise ratio (S/N) 3.

Baseline release was calculated on the basis of last three fractions collected before the administration of PCA. All calculations were performed using StatView 4.5 software (Abacus Concepts. Cary, NC, USA). Group differences after significant repeated measures ANOVAs were examined by post hoc Fisher's Protected Least Significance Difference (PLSD) test. Statistical significance was set at $P<0.05$. All procedures were carried out in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Significant effects of Sociability ($F(1,24)=5.10$, $p<0.05$) and Time ($F(14,336)=29.8$, $p<0.0001$) on the levels of 5-HT in the PFC emerged. LS rats had higher levels of 5-HT in the PFC than HS animals (Fig. 1). According to post hoc tests, PCA significantly increased 5-HT levels after 30 min of its administration until the end of microdialysis. Differences between LS and HS rats were higher before PCA administration and during the early period after the treatment. In VTA, there was no effect of Sociability, but an effect of Time emerged ($F(14,350)=53.0$, $p<0.0001$), as PCA increased 5-HT levels 30–45 min after its administration. PCA increased 5-HT release from the baseline both in PFC and in VTA, as expected, and an interaction between Sociability and Time emerged for VTA ($F(11,275)=1.92$, $p<0.05$). PCA had a stronger effect on 5-HT release in the HS group during the early period after its administration (Fig. 2).

In our previous study (Tõnissaar et al., 2004), higher social activity was associated with lower tissue levels of 5-HIAA, the main metabolite of 5-HT in frontal cortex. In the present study, animals with higher social activity had lower 5-HT levels in the PFC. Both in rats and primates 5-HT functioning is implicated in impulsivity, defensive, and aggressive behaviours which influence social interactions and social affiliations (Krakowski, 2003). However, this relationship between 5-HT and social functioning may be complex (Ando et al., 2006). Bjork et al. (2000) have shown that men with high trait hostility have negative association between tryptophan levels and aggressive responses, but this relationship was opposite in men without previous aggressive history. In rats, a positive correlation between 5-HT–ergic function and aggression (van der Vegt et al., 2003), but a negative association between 5-HT-ergic activity in PFC and anxiety (File et al., 1993) has been found. Thus, higher levels of 5-HT in the PFC may reduce social behaviour in LS-rats. It has been described that acutely increased 5-HT levels causes a significant decrease in the basal firing rate of VTA neurons and reduces mesocorticolimbic DA transmission by activating 5-HT_{2C} receptors (Di Mascio and Esposito, 1997; Matteo et al., 2002). Though release of dopamine is pivotal in psychomotor stimulation, social behaviour can be rather reduced by preferentially dopamine releasing drugs, such as amphetamine (Panksepp, 1998). As dopamine suppresses social activity it is possible that the higher 5-HT release potential in VTA during social interaction suppresses dopamine release through activation of 5-HT_{2C}

receptor, and promotes social activity more effectively in HS rats. Conclusively, extracellular 5-HT levels differ in rats with high vs low sociability trait, and this regionally specific difference may be related to distinct components, like 5-HT activity in the prefrontal cortex and 5-HT-dopamine interaction in the VTA.

Acknowledgements

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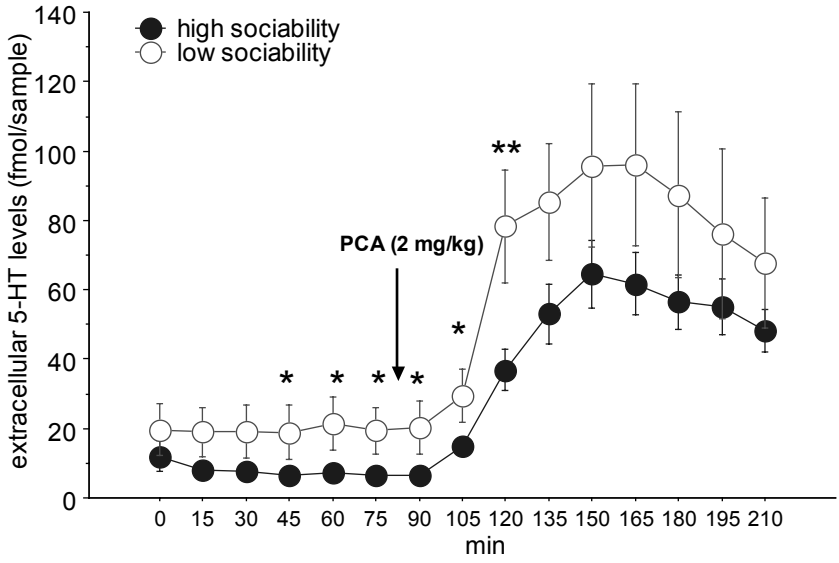

Fig. 1. Extracellular 5-HT levels (fmol/25 µl sample) in frontal cortex, measured in freely moving concious animals. PCA (2 mg/kg) was administered 75 min after beginning of probe collections.
* LS vs HS groups, * $p<0.05$, ** $p<0.01$

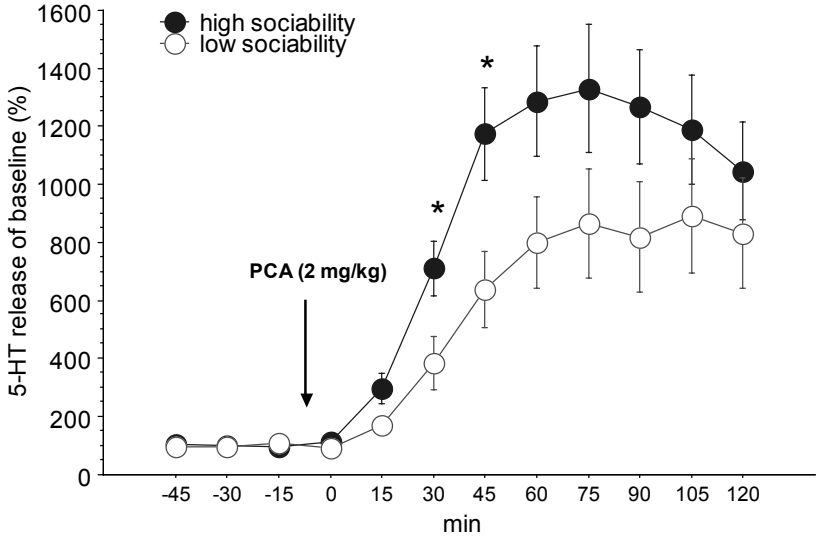

Fig. 2. Serotonin release of baseline (%). Baseline release was calculated on the basis of average last three fractions, collected before administration of PCA.
* LS vs HS groups, * $p<0.05$

Rats with high or low sociability are differently affected by chronic variable stress

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Abstract

The role of stress in psychiatric disorders is well demonstrated, but less is known what determines the ability of an individual to cope with stressful situations. The majority of stressful stimuli that lead to psychopathology in humans are of social nature. We have previously shown that social behavior of rats is individually stable and the purpose of the present study was to compare how animals with different social activity vary in sensitivity to chronic variable stress. Forty single-housed rats were allocated into four groups based on their either high or low sociability (HS and LS, respectively) and application of chronic variable stress (CVS) regime. In HS animals, CVS reduced body weight gain only during the first week of the study, but reduced sucrose intake after three weeks of its use. This suggests that HS-rats are more vulnerable to the anhedonia elicited by CVS. LS-animals were more anxious in the social interaction, plus-maze and open field tests, but stress eliminated their difference with HS-animals. Stress also reduced body weight gain of LS-animals during all the study, but increased struggling in the forced swimming test. Stress increased dopamine levels and reduced serotonin levels in the frontal cortex, measured ex vivo, only in LS-rats. This study thus revealed that animals with different expression of social behavior respond differently to stress, and this may be related to the adaptability in frontal cortical monoaminergic systems.

Introduction

Stress is a major contributor in the etiology of depression, anxiety, and other disorders (Bale, 2005). Stressful life events precipitate the onset of depressive episodes in humans (Gilmer and McKinney, 2003), and a large number of studies have used chronic mild/variable stress in animals to induce behavioral changes that resemble the symptoms of depression (Willner, 2005). Stress response itself is essential for adaptation, maintenance of homeostasis, and survival (Bale, 2005). Therefore the sensitivity of the individual to stressful encounters is important in the development of mood disorders (Harro and Oreland, 2001).

The role of stress in psychiatric diseases is well demonstrated, but less is known what determines the ability of an individual to cope with stressful situations. Both human and animal studies have shown that stress reactivity has a heritable component (Sloman et al., 2003). Personality has long been viewed as related to psychopathology, but the precise nature of their relationship remains unclear (Clark, 2005). There are studies which suggest that neuroticism strongly predicts anxiety and mood disorders (Christensen and Kessing, 2006; Jorm et al., 2000). Other personality dimensions (e.g., anxiety sensitivity, attribution style, sociotropy or dependence, autonomy or self-criticism, and constraint) may also constitute vulnerability factors. Clark (2005) has suggested that three broad, innate temperament dimensions — negative affectivity, positive affectivity, and disinhibition — at their extremes are risk factors (diatheses) for psychopathology, especially given adverse life experiences (stress). Negative affectivity (or neuroticism) appears to be a vulnerability factor for the development of anxiety and depression, and indicates poor prognosis. Positive affectivity (or extraversion) is related more specifically to depression, can serve as a risk factor for its development, and also suggests poor prognosis. The identification of endophenotypes in the personality disorders may provide a basis for the identification of underlying genotypes that influence the traits of the personality disorders, as well as susceptibility to major psychiatric illnesses (Siever, 2005).

Studies with chronic variable/mild stress have shown that this procedure elicits helplessness or anhedonia in some but not all animals, which suggests also important role of individual vulnerability also in animals (Henn and Vollmayr, 2005). Using the elevated plus-maze test, rats bred for either high or low anxiety-related behavior differ in their stress coping strategies, the former being more susceptible and vulnerable to stressor exposure and preferring more passive strategies (Landgraf and Wigger, 2002). Acute tail pinch stressor, but not chronic social defeat stress increases alcohol preference in Maudsley reactive rats, originally selected for high and low open-field defecation which should portrait the biobehavioral system underlying emotionality (Blizard and Adams, 2002).

In humans, social behavior has very consistent disposition (Depue and Collins, 1999), which is linked to the regulation of affects and behaviors, and these in turn are linked to stress systems and implicated in depression (Sloman et al., 2003). The majority of stressful stimuli that lead to psychopathology in humans are of social nature (Buwalda et al., 2005) and decrease in social functioning is a major symptom of depressed patients (Nemeroff, 1998). Coping with stress depends on effective social relations and improved social circumstances are effective in therapy of depression (Brown et al., 1988; Sloman et al., 2003). Forebrain 5-HT-ergic neurotransmission is important to reduce environmental aversive conditions (Deakin, 1996). Drugs that are effective in the treatment of depression and influence central serotonergic function also modulate a dimensions of normal personality characterized by reduced negative affective experience and increased affiliative behavior in healthy persons (Knutson et al., 1998).

Many of the animal studies use social stress (e.g. social defeat and maternal deprivation) during prenatal or postnatal period to induce symptoms reminiscent to anxiety and depression (Sloman et al., 2003). Both studies with human infants and experimental animals have shown attentional deficits, anxiety and disturbed social behavior after prenatal stress (Weinstock, 1997). In humans, victims of bullying are known to suffer from depression, anxiety, sociophobia, loss of self-esteem, psychosomatic diseases, and other behavioral symptoms (Björkqvist, 2001), Defeat and entrapment are seen to be of special relevance with depression (Gilbert and Allan, 1998). Animal studies on social defeat, which are usually based on the rodent resident-intruder paradigm (Björkqvist, 2001), reveal that this paradigm is useful tool to induce behavioral changes reminiscent to depression (Koolhaas et al., 1999), though findings are more related to physiological rather than to behavioral consequences of defeat (Björkqvist, 2001). Social defeat is suggested to be one of the most severe stressors among a number of laboratory stressful stimuli in terms of neuroendocrine activation and there are also individual differences in coping style in relation to stress vulnerability (Koolhaas et al., 1997). Mother-infant separation in the rat has been used as an analytical tool to reveal biosocial processes underlying infant physiology and behavior, and selective breeding for an infantile behavior according to the temperamental affective responses (immediate ultrasonic vocal response) is suggested to have the potential to provide valuable tools for understanding developmental mechanisms underlying genetic and developmental risk for depression/anxiety syndromes in children and adults (Brunelli, 2005).

Deficits in social skills may be a cause of depression (Davison and Neale, 1998), and it is described that low social competence predicted the onset of depression among elementary-school-age children (Cole et al., 1990), and poor interpersonal problem solving skills predicted increases in depression also among adolescents (Davila et al., 1995). However, studies, which use social coping mechanisms, control over social relationships and social signal/

communication as potential protective factors against stress, have received less attention. Social support in humans and affiliative behaviors in animals can provide a buffer against stress and have a positive impact on measures of health and well-being, thus social behavior can provide a buffer against stress-related diseases (DeVries et al., 2003). We have shown that social behavior of rats is individually stable and that sociability is related to 5-HT metabolism in prefrontal cortex (Tõnissaar et al., 2004). The purpose of the present study was to compare how animals with different social activity vary in sensitivity to chronic variable stress.

Methods

Animals

Sixty male Sprague-Dawley rats (Scanbur BK AB, Sweden), were single-housed at age 2 months in transparent macrolone cages under controlled light cycle (lights on from 08:30 h to 20:30 h) and temperature (19–21º C), with free access to tap water and food pellets (diet R70, Lactamin, Sweden). All procedures were carried out in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

General procedure

Rats were single-housed 11 days before the first social interaction test. Altogether four tests, always between unfamiliar partners, were performed with the interval of 7–8 days. Thereafter, animals were allocated to high or low sociability group. Twenty rats with lowest and twenty with highest average social activity time were selected. Three days after the last social interaction test, the first sucrose preference test was carried out and the chronic variable stress (CVS) procedure was started. Rats were divided into Stress and Control groups on the basis of body weight. Animals belonging to the Stress groups were transported to another room for all manipulations included in the CVS procedure. Three subsequent sucrose preference tests were performed after 8, 16 and 24 days from the first test. Another social interaction test was carried out on the next day after the fourth sucrose preference test. On the next day thereafter, an elevated plus-maze test was performed. The forced swimming test was carried out two days after the elevated plus-maze test, on the two subsequent days. Then, 2 days later, the rats were tested in the open field test, and immediately sacrificed thereafter. The brains were quickly dissected on ice and the brain tissue was stored at –80°C in a deep freezer. Body weight changes were measured between consecutive sucrose preference tests. First three "weeks" of the study (CVS regime) lasted 8 days, the last week (the session of behavioral experiments) 7 days. The respective body weight gain data were, to enable comparison, adjusted on the basis of average daily weight gain.

Chronic variable stress procedure

The CVS procedure was based on our previous experiments (Harro et al., 1999; Harro et al., 2001; Tõnissaar et al., 2000). Various stressors of different duration were applied, one stressor per day. Each stressor was used three times. The stressors were presented in the following order: cold (4°) water and wet bedding (initially, 400 ml of water was poured on a rat, and the sawdust bedding was kept wet for the following 17 h), imitation of injection (a syringe without a needle was pressed against abdomen), stroboscopic light (for 13 h, 10 Hz, 2 lx), movement restriction in a small cage (11 x 16 x 7 cm) for 2 h, cage tilt at 45° (for 20 h), tail pinch with a clothes-pin placed 5 cm distal from the base of tail (5 min), strong illumination (900 lx) during the predicted dark phase (for 12 h). In order to avoid the effect of stress on body weight gain and sucrose consumption in the sucrose preference test, a day without stress followed the strong illumination stressor. Control rats remained undisturbed in their cages.

Social interaction test

In the social interaction test, developed by File (File and Hyde, 1978), two weight-matched rats receiving the same treatment were placed in the opposite corners of a brightly-lit chamber (30 X 30 X 60 cm) with floor covered with wood shavings and observed for 10 min. The total time spent in active social behavior (allogrooming, sniffing the partner, crawling under and over, following) was recorded for each rat separately.

Sucrose intake

Animals remained in their home cages during testing their sucrose intake. Two bottles, one filled with 1% sucrose solution and the other with water were used. Placement of the bottles with sucrose vs. water was randomized across the tests. Sucrose and water consumption was measured for the period of 1 h by weighing preweighed bottles at the end of the test. Sucrose preference was measured by calculation the proportion of sucrose consumption out of total consumption of liquid. Sucrose intake analysis was carried out on changes in intake as compared to the first test as baseline, because sucrose intake was by chance significantly higher in the HS/Stress group compared with LS/Stress group before onset of stress regime (4.5±1.1 g and 2.1±0.5 g for HS/Stress-group and LS/Stress-group, respectively, $p<0.05$), and, we have previously shown that sucrose consumption is an individually stable trait, the intake in the first test already predicting the intake in the following tests in unmanipulated animals (Tõnissaar et al., 2006).

Elevated plus-maze test

The method first described by Handley and Mithani (1984) and modified in our laboratory (Harro et al., 1990) was used. In brief, the plus-maze consisted of two open arms (50 x 10 cm) without any walls, two enclosed arms of the same size with 40 cm high sidewalls and end wall, and the central arena (10 x 10 cm)

interconnecting the arms. The arms of the same type were opposite to each other. Both open arms were divided into three parts of equal size by lines which also separated the central arena from all arms. At the beginning of the experiment the rat was placed into the beginning of a closed arm, facing the closed end. The central arena and the open arms formed the 'open part' of the apparatus. An entry into open arms was counted when the rat crossed the line between the central arena and an open arm with all four paws. The rat was considered to explore the open part of the apparatus when it had clearly crossed the line between a closed arm and the central arena with its both forepaws. Behavioral measures taken during 4 minutes included a) the latency period before entering the open part (i.e. the central arena); b) the number of line crossings; c) time spent in the open part of the apparatus; d) the number of approaches towards the central arena which were not completed (nose crossed the line but not both of the forepaws); e) the number of excrements left during testing; f) the number of open arm entries, and g) the total number of arm entries. From the two latter measures, the ratio open/total arm entries was calculated.

Forced swimming test

In the forced swimming test, first characterized by Porsolt and colleagues (Porsolt et al., 1978), rats were forced to swim in a vertical glass cylinder (diameter 19 cm, height 50 cm) containing 25 cm of water maintained at 25º C. On the first day of the experiment, rats were forced to swim for 15 min and thereafter dried with laboratory tissues. On the following day rats were re-exposed to the forced swimming for 5 min. Behavior was videotaped and analyzed along the categories of immobility, swimming and struggling (Armario et al., 1988; Häidkind et al., 2004). In addition, the number of excrements and divings (animal dived toward the bottom of the cylinder and then returned to the surface) during the tests were measured. Data of the first forced swimming test were analyzed for the first 5 min session, except for the number of fecal boli and divings.

Open field test

In the open field test rats were placed to the center of a rectangular arena (0.5 X 1 m), which was divided into 8 equal squares. Parameters registered during 4 min were the number of squares visited (with all four feet on one square), the number of rearings and the number of excrements left on the apparatus during testing.

Measurement of monoamine levels

Monoamines and their metabolites were assayed by HPLC with electrochemical detection as previously described (Alttoa et al., 2005). The rat brain tissues were homogenized with Bandelin Sonopuls ultrasonic homogenizer (Bandelin Electronic, Germany) in ice-cold solution (15–25 µl/mg tissue) of 0.1 M

perchloric acid containing 5 mM sodium bisulfite and 0.04 mM EDTA. The homogenate was then centrifuged at 17 000X*g for 10 min at 4°C. Aliquots (10 µl) of the supernatant obtained were chromatographed on a Lichrospher 60 RP Select B column (250X3 mm; 5 µm). The separation was done in isocratic elution mode at column temperature 30°C using the mobile phase containing 0.05 M sodium citrate buffer at pH 3.7, 0.02 mM EDTA, 1 mM KCl, 1 mM sodium octanesulphonate and 5 % acetonitrile. The chromatography system consisted of a Hewlett Packard HP 1100 series isocratic pump, a thermostatted autosampler, a thermostatted column compartment and an HP 1049 electrochemical detector (Hewlett Packard, Germany) with glassy carbon electrode. The measurements were done at an electrode potential of +0.6 V versus the Ag/AgCl reference electrode. The limit of detection for all assayed compounds was 0.05–0.10 pmol at signal to noise ratio (S/N)=3.

D_2 receptor-stimulated [^{35}S]GTPγS binding

Membranes from nucleus accumbens and striatum were prepared as described previously (Lepiku et al., 1996). The tissues were homogenized in 3.5 ml of homogenization buffer (50 mM Tris-HCl, pH=7.4) by Bandelin Sonopuls homogenizer (three passes, 10 s each). The membranes were collected by centrifugation at 40,000×g for 20 min at 4 °C and were washed by homogenization and centrifugation two more times. The final pellet was homogenized in 90 ww/v, (striatum) or 200 ww/v (nucleus accumbens) of the incubation buffer A (20 mM K-HEPES, 7 mM $MgCl_2$, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, pH=7.4).

Binding of [^{35}S]-guanosine-5´-(γ-thio)-triphosphate ([^{35}SGTPγS]; Perkin Elmer Life Sciences, Boston, MA, USA) was carried out as described earlier (Rinken et al., 1999). In brief, the membranes (200 µg of accumbal and 500 µg of striatal membranes per tube) were incubated with 0.2 nM [^{35}S]GTPγS and different concentrations of GDP (3 mM–01 µM) and 1 mM dopamine or 10 µM butaclamol (all from Sigma-Aldrich Fine Chemicals, St. Louis, MO, USA) for 90 min at 30 °C. The reaction was stopped by rapid filtration through GF/B glass-fiber filters (Whatman Int. Ltd., Madistone, UK) and the filters were washed three times with 3 ml of ice-cold 20 mM phosphate buffer (pH=7.4) containing 100 mM NaCl. The radioactivity content of the filters was counted in 4 ml of scintillation cocktail OptiPhase HiSafe3 (Wallac Perkin Elmer Life Sciences, Cambridge, UK) by RackBeta 1219 liquid scintillation counter (Wallac Inc., Gaithersburg, MD, USA).

Data analysis

For statistical evaluation of the behavioral and biochemical data, two-way analysis of variance (ANOVA) was used with Sociability (High or Low), and Treatment (Stress or Control) as independent factors. For such measures as body weight, sucrose consumption or social behavior, a third, repeated mea-

sures factor (Time) was added. Group differences after significant ANOVAs were measured by post hoc Fisher's Protected Least Significance Difference test. Correlations shown are Pearson correlation coefficients.

Results

Changes in body weight

All animals gained weight during the 32 days of stress regime and behavioral tests. Effect of Time emerged on absolute body weights with repeated measures ANOVA ($F(31,1116)=247.9$, $p<0,0001$), and Treatment had an interaction with Time factor ($F(31,1116)=9.45$, $p<0,0001$). Post hoc tests revealed that stress suppressed body weight during the first week of the CVS in both HS and LS rats, but during the second and the third week did so only in low sociability animals (Fig. 1). Body weight gain of both groups of stressed animals was also suppressed during the final week of behavioral tests, when compared to previous weeks. However, the reduction of body weight gain was even larger in animals not submitted to the CVS.

Sucrose preference and intake

Effect of Time on sucrose preference emerged during the study ($F(3,108)=5.72$, $p<0.001$) and an interaction between Time and Sociability almost reached the conventional level of significance ($F(3,108)=2.68$, $p=0.051$). There was a Sociability effect present in the first test before the onset of stress ($F(1,38)=7.90$, $p<0.01$). The LS rats preferred significantly less sucrose than HS-rats (84±3 % and 66±5 % for HS- and LS-rats, respectively, $p<0.01$). No difference was observed in the subsequent tests during the three weeks of stress (data not shown).

There was an interaction between Sociability and Time ($F(2,72)=3.47$, $p<0.05$), and between Stress and Time ($F(2,72)=5.12$, $p<0.01$) on sucrose intake during the study. Neither Sociability nor Stress had any effect in the second and the third test, but stress reduced sucrose intake in the fourth test ($F(1,36)=6.94$, $p<0.05$). Post hoc tests revealed that stress reduced sucrose intake only in HS animals (Fig. 2).

Social activity

When data from the all five SI tests were included in the analysis, the effect of Sociability was found on the social activity time ($F(1,36)=53.4$, $p<0.0001$), but also an overall effect of Time ($F(1,36)=46.7$, $p<0.0001$) and an interaction between Time and Sociability ($F(1,36)=27.0$, $p<0.0001$) were present. Effect of Stress did not reach the conventional level of significance ($F(1,36)=3.56$, $p=0.07$). Overall, social activity had decreased during the five tests (Fig. 3). The LS animals still had significantly lower social activity time than HS animals,

but this difference was eliminated by stress, as CVS had increased social activity in the LS rats.

Plus-maze test

LS animals tended to be generally less active and more anxious in the plus-maze test but stress increased their activity. Sociability had an overall effect on the time of activity ($F(1,36)=5.33$, $p<0,05$), and the interaction between Sociability and Stress almost reached conventional level of significance ($F(1,36)=3.44$, $p=0.07$). Post hoc tests revealed that LS rats were less active than HS animals, and stress eliminated this difference (Fig. 4). Overall effect of Sociability emerged also on the number of excrements left during the plus maze test ($F(1,36)=4.46$, $p<0.05$), but there were no differences between the groups after post hoc, probably due to the fact that only eight animals defecated during the test. Stress had no overall effect in the plus-maze test. Sociability did not affect significantly the number of closed arms, open arms, open arms divided with total number of arms, and on the time of stretch approach. It should be noted that only ten animals entered in the open arms during this test.

Forced swimming test

Effect of Sociability ($F(1,36)=4.26$, $p<0.05$) and an interaction between Sociability and Stress ($F(1,36)=6.35$, $p<0.05$) on struggling in the first forced swimming test were found, and post hoc tests revealed that Stress increased struggling only in LS-rats (Fig. 5). Neither Sociability nor Stress affected time of immobility, time of swimming and number of excrements left during forced swimming tests.

Open field test

Stress had no overall effect in the open field test. Sociability affected the number of rearings and the number of excrements ($F(1,36)=9.76$, $p<0.01$ and 4.13, $p<0.05$, respectively). For the number of squares crossed, the effect of Sociability almost reached the conventional level of significance ($F(1,36)=3.69$, $p=0.06$). Post hoc tests revealed that the groups of LS-rats made significantly less rearings, as compared to respective HS groups, but differences in the number of excrements were not significant in post hoc tests.

Monoamine levels and turnover in the frontal cortex

There was an effect of Sociability on the levels of 5-HT ($F(1,36)=5.14$, $p<0.05$) and DA ($F(1,36)=5.12$, $p<0.05$) in the frontal cortex. Post hoc tests revealed that stress reduced the levels of 5-HT and increased the levels of DA, but only in LS-animals (Table 1). Neither Sociability nor Stress had any effect on the levels of NA, 5-HIAA, HVA or DOPAC, and on DOPAC+HVA/DA and 5-HIAA/5-HT ratios.

D_2 receptor function

Neither Sociability nor Stress affected the dopamine-dependent [^{35}S]GTPγS binding nucleus accumbens (data not shown).

Discussion

This study has demonstrated, in accordance with our previous experiments (Tõnissaar et al., 2004 and unpublished) that sociability is persistent characteristic in rats when test within the period of one month. Furthermore, despite of the fact that social activity decreases with increasing age and body weight, large differences were still present one month later. LS-rats were more anxious also in other animal models of anxiety, the elevated plus-maze and open field tests. It has been described previously that rats breeding on the basis of anxiety behavior in the elevated plus maze test exhibit similarly more anxious behavior in other animal models of anxiety, such as the social interaction test and the black-white box (Henniger et al., 2000). However, discrimination in the social interaction paradigm was primarily due to differences in locomotor activity (Henniger et al., 2000). On the more individual level, behaviour of rats in the social interaction test does not correlate well with their performance in other animal models of anxiety (Ramos et al., 1997). When behavioral reactivity of male rats from different strains was compared in several nonsocial (elevated plus-maze, open field) and social settings (social interaction in aversive and neutral environment, resident-intruder test, chronic social stress), a factor analysis showed that the behavioral reactivity to social stimulations is a specific feature, dissociable from different components of emotionality (approach/ avoidance and general activity) as measured in the behavioral responses to nonsocial settings (Berton et al., 1997). Thus, the social interaction test and other animal models of anxiety, despite all channeling anxiety, also represent activities in rather distinct psychological domains. In a modified hole board test, which allows the experimental animal to maintain social contact with its group mates, and enables the investigator to assess social affinity among group mates (Ohl et al., 2001), highly anxious rats spent significantly more time in social contact than both low anxious and control rats, indicating that animals preferring more social contact may be more anxious when in isolated situation (Landgraf and Wigger, 2002).

Anxiety may be viewed as an innately driven form of distress that arises in response to actual or threatened exclusion from social groups (Buss, 1991; Ruis et al., 1999), and isolation causes important changes in the behavioral reactivity of rats to environmental stimuli (Nunes Mamede Rosa et al., 2005), and even affect the reward magnitude of social encounter (Burgdorf and Panksepp, 2001). Social isolation itself represents stress, and may increase the sensitivity of the pituitary gland to CRF and impair the negative feedback regulation of the

HPA axis (Serra et al., 2005). It has been described that housing of rats in groups of 10–12 animals per cage 24 hr after restraint stress significantly prevents the anxiogenic effect of restraint in the elevated plus-maze test, when compared to animals which were housed in pairs after the stress exposure (Andrade and Guimaraes, 2003). This again suggests that social housing conditions after stress may attenuate behavioral consequences of exposure to uncontrollable stressors and thus mimick the effect of social support in humans. In the present study, animals were housed individually already before the onset of the stress procedure. It has been described that social housing before the onset of stress can enhance coping with stress in female rats, whereas in male rats, group housing appears to increase the adverse effects of chronic stress (Westenbroek et al., 2003). This suggests that single housing in our study probably did not confouned the stress effects, but the possibility that social housing would moderate the effects of stress differently in HS- and LS-rats should certainly be controlled in a separate study.

In the social interaction test, HS animals were 28 days after the previous test still socially more active than LS animals, even though a reduction in the social activity was observed in both groups. Probably due to floor effect this decrease was highest in the HS groups. Reduced activity in social interaction test and low activity in the LS-rats in plus-maze and open field tests in the present study may be related to habituation or aging, but not reduced anxiety. It has previously been described that 4.5 months old Wistar rats presented the lowest scores on active social behavior and the highest scores on defecation, compared with the 2.5 months old rats, and exploratory behavior measured by ambulation and rearing decreases with age (Garau et al., 2000). Thus, age — and a closely related measure, the body weight - could be a relevant factor on the reduction in social activity also in our study, whereas animals were 3 months old after the third social interaction test and 4 months old during the 5th test. Salchner et al. (2004) has suggested that reduced interaction in aged rats does not reflect enhanced anxiety, as reduced social interaction level was not accompanied by augmented Fos expression in any of the key brain areas of the fear/anxiety circuitry known to be activated by anxiogenic stimuli. Niesink and van Ree (1982) have described that short term individual housing before the social interaction test, increases social activity both in young and adult animals, but not due to increased locomotor activity, and this effect appeared to be maximal after 4 to 7 days of individual housing, which extinguished after repeated testing, suggesting that observed behavioral changes were hardly affected by habituation to the test cage. Thus, reduced social activity in our study was not probably induced by increased anxiety or habituation with test apparatus, but probably related to aging and increased body weight gain.

This study also demonstrated that the initially less anxious HS-rats were more sensitive to stress than LS-rats as measured by the decrease in sucrose intake after 3 weeks of CVS regime. Interestingly, higher sensitivity to stress has been found in more anxious rats, when animals were selected on the basis of

exploratory behaviour. When comparing rats bred for high or low anxiety-related behavior, high anxiety rats floated much more and struggled much less than low anxiety rats in the forced swimming test (Keck et al., 2001), thus reflecting different vulnerability to stress and coping strategies (Landgraf and Wigger, 2002). Nevertheless, it is not known whether these strains differ in sucrose intake. We have found that the effect of CVS on sucrose intake is stronger in more anxious rats, selected in the exploratory box (unpublished).

In LS animals, stress increased struggling in forced swimming test, and activity in the social interaction test and elevated plus-maze test. Regarding monoamine levels, stress increased DA levels and reduced 5-HT levels in frontal cortex of LS animals. It has previously been described that peripherally administered corticosterone in a dose that approximates stress-induced plasma concentrations of the male Sprague-Dawley rat stimulates locomotor activity in an activity box (Piazza et al., 1996). Neuropeptide CRF may be a critical mediator in regulating the decreases of extracellular concentrations of 5-HT during stress (Price et al 2002). At least in septum, it is found that forced swimming, which brings about climbing and swimming behaviors that precede the development of immobility, produces an acute phasic reduction of 5-HT release (Lucki, 1998). Low 5-HT neurotransmission has been found to be associated with higher impulsivity, aggressiveness, anxiety in humans, non-human primates and other species (Turecki, 2005; Suomi, 2005; Grimmett and Sillence, 2005). Thus, it is conseivable possible that stress, possibly through the alerted glycocorticoid system, increased behavioural activity in LS animals, by upregulating DA and downregulating 5-HT systems in frontal cortex and increasing impulsivity.

Bosch et al. (2006) have described that exposure of male Wistar rats, bred for high or low anxiety-related behavior, to prenatal stress between pregnancy days 4 and 18 resulted in opposite effects on anxiety in adulthood, i.e. high anxiety rats became less and low anxiety rats became more anxious compared with their unstressed controls in plus-maze and holeboard tests. Ladd et al. (2005) have described that many effects of prolonged handling-maternal separation on stress hyper-responsiveness associated with facilitation of regional corticotropin-releasing factor neurocircuits and glucocorticoid resistance, which in neonatal Long Evans hooded rats leads to stable phenotypes, are reversible in adulthood by chronic variable stress. It is suggested that increased locomotor activity of initially more anxious animals may reflect a difference in coping with a novel and challenging situation (Landgraf and Wigger, 2002). Thus, in present study, increased locomotor activity of LS animals in response to stress may reflect shift in coping style.

Changes in body weight gain and reverse intake represent the dissociation in sensitivity to stress between LS and HS animals. The reduction in intake of HS animals existed without persistent effect of stress on their body weight gain, thus, effect of stress on sucrose intake of HS animals emerged two weeks after disappearance of its effect on this measure. Conversely, sucrose intake of LS

animals was not affected by stress, but body weight gain was suppressed during all the study. The strong body weight reduction during last week was most obviously caused by novel environmental condition elicited by behavioral tests. Reduction of body weight gain of control animals was even stronger than in animals previously adapted with stress. Also, the effect of stress on body weight gain was strongest during the first week. It is suggested that a novel stressor after CVS, which had caused adaptation and reduction of corticotropin-releasing hormone (CRH) levels, may cause again marked increase in CRH levels in several brain regions, followed by a further reduction of body weight (Nagashima et al., 2003).

Conclusively, this study revealed that animals with high sociability trait are more vulnerable to the anhedonic effect of stress in conditions of single housing. Thus, animals with different expression of social behavior respond differently to stress. This information may be useful in models of affective disorders in studies of their neurobiological substrates.

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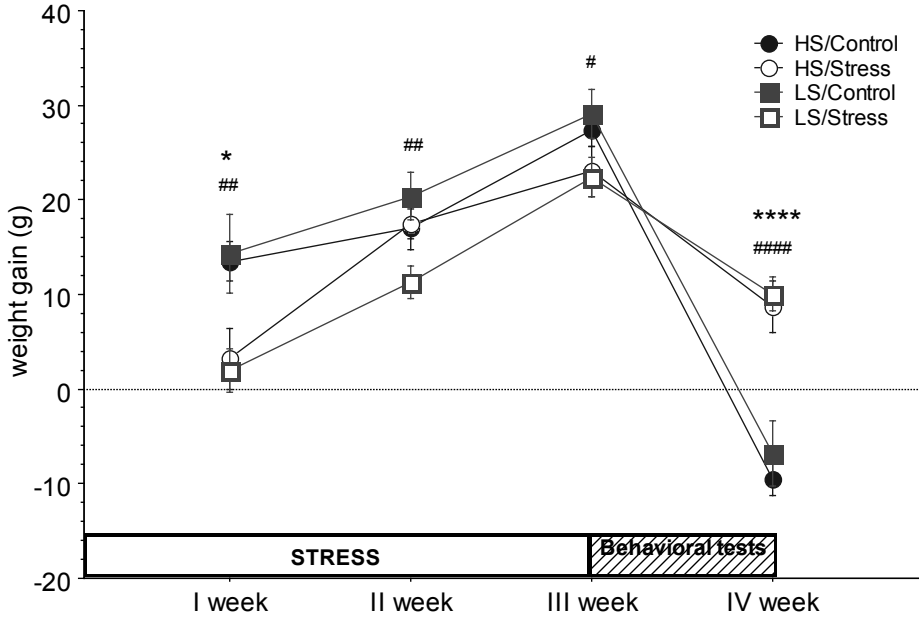


Fig. 1. Effect of chronic variable stress and the sociability trait on the body weight gain as compared to the previous week. Animals were divided into groups according to body weight. Body weight gain of every week was calculated as a difference between the last day of corresponding and previous week. Whereas week in the current study was defined as a time between two consecutive sucrose preference tests, all weeks were divided with the number of days in that week and multiplied by seven (means±S.E.M).
* HS/Control vs HS/Stress; * $p<0.05$, **** $p<0.0001$
# LS/Control vs LS/Stress; # $p<0.05$, ## $p<0.01$, #### $p<0.0001$

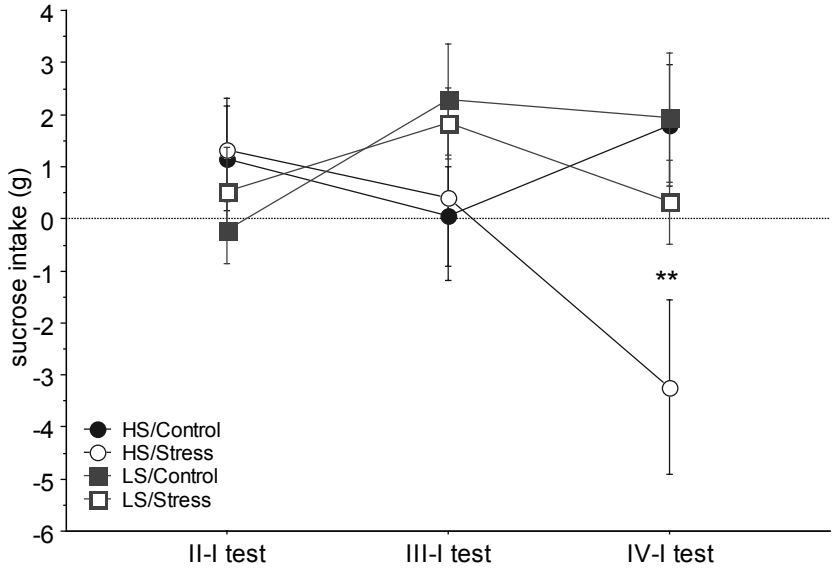


Fig. 2. Effect of chronic variable stress and sociability trait on changes in sucrose intake, calculated from the baseline at the first test (means±S.E.M).
* HS/Control vs HS/Stress; ** $p<0.01$

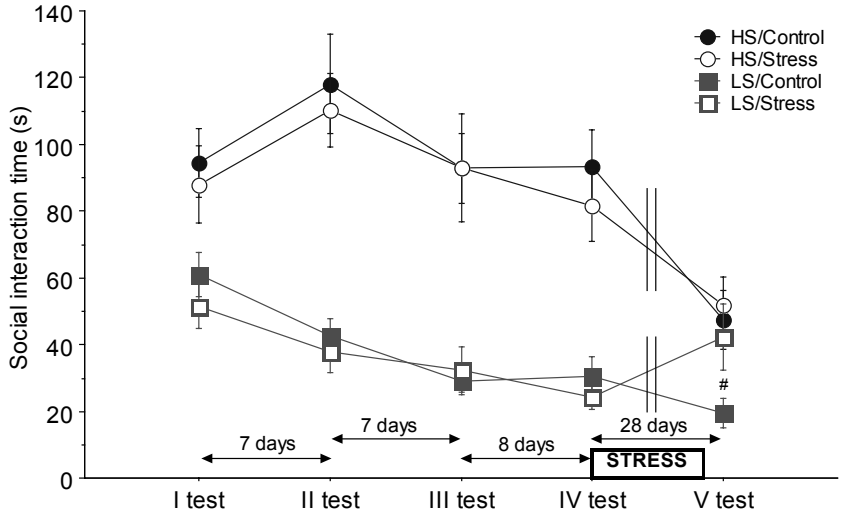
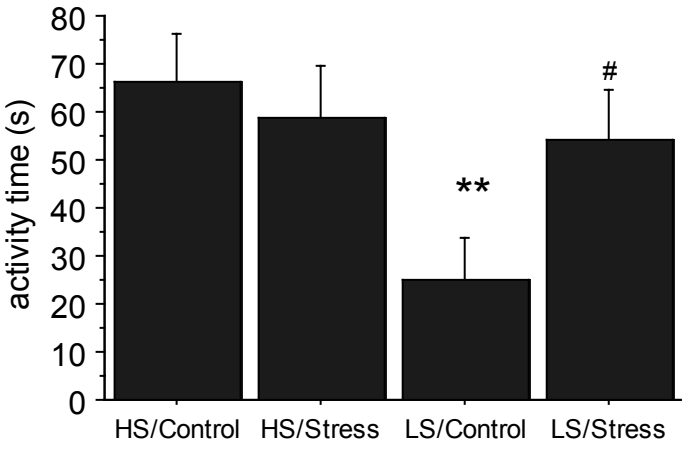


Fig. 3. Effect of chronic variable stress and sociability trait on time spent in social interaction (s) in the five social interaction tests (means±S.E.M). Periods between the tests are shown.
LS/Control vs LS/Stress; # $p<0.05$

A



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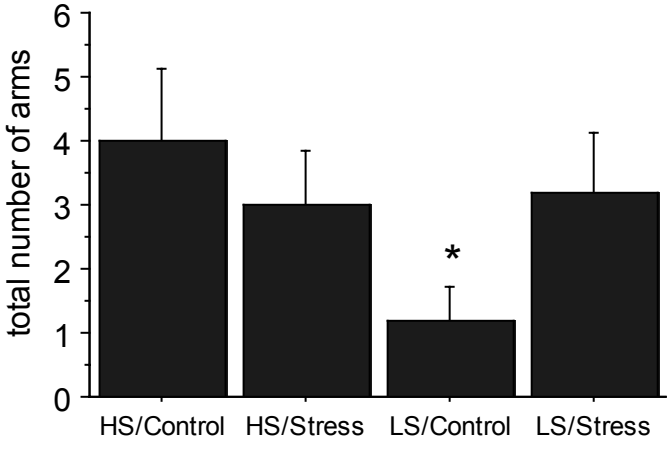


Fig. 4. Effect of chronic variable stress and sociability trait on activity time (s) (A) and total numbers of entries into arms (B) in the elevated plus-maze test (means±S.E.M). Number of all entries into into open arms was 12, and into closed arms 102.
* HS/Control; * $p<0.05$, ** $p<0.01$
# LS/Control; # $p<0.05$

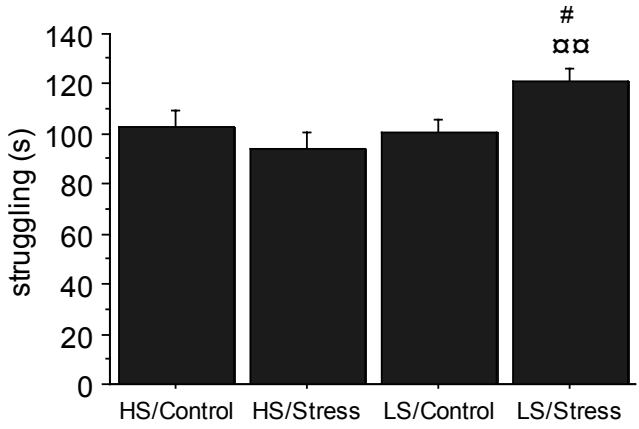
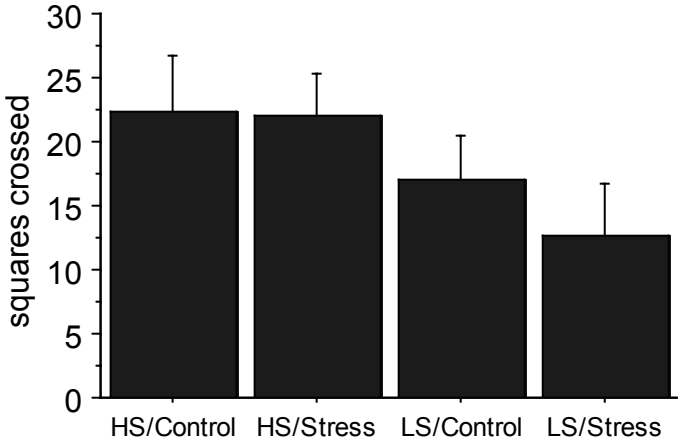


Fig. 5. Effect of chronic variable stress and sociability trait on struggling in the forced swimming tests (means±S.E.M).
LS/Control; # $p<0.05$
¤ HS/Stress; ¤¤ $p<0.01$

A



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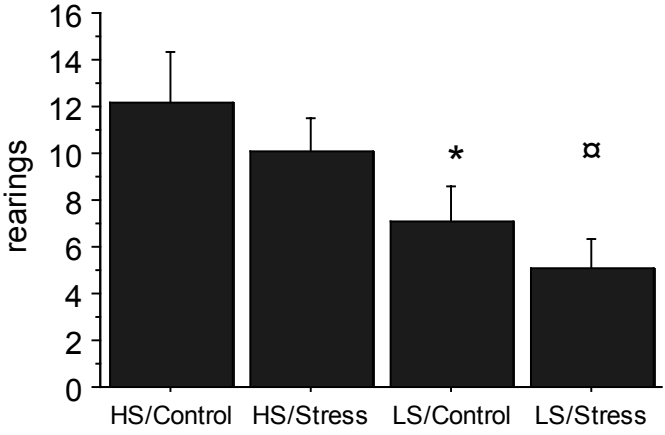

Fig. 6. Effect of chronic variable stress and sociability trait on behavior in the open field test: (A) number of squares crossed, and (B) number of rearings (means±S.E.M).
* Control/HS; * $p<0.05$
¤ Stress/HS; ¤ $p<0.05$

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Education an professional employment

1991–	employed in Police organization.
1995–2000	University of Tartu, Faculty of Social Science, Department of Psychology, B.Sc in psychology. University of Tartu, Faculty of Social Science, Department of Social Policy and Public Administration, BA in social work.
2000–2002	University of Tartu, Faculty of Social Science, Department of Psychology, M.Sc *cum laude* in psychology.

Scientific work

Development of animal model of depression by combining chronic variable stress procedure with restricted manipulations on monoaminergic systems, or selecting animals according to spontaneous behaviour related to symptoms of depression or anxiety.

Persistence of social behaviour in animals and related neurobiological mechanisms and vulnerability to stress.

Persistence of intake and preference of carbohydrates in animals as a measure of hedonic state, and relationship with dopamine D_2 receptor function in nucleus accumbens and striatum.

Publications

- Tõnissaar M, Herm L, Rinken A, Harro J. Individual differences in sucrose intake and preference in the rat: Circadian variation and association with dopamine D_2 receptor function in striatum and nucleus accumbens. Neurosci Lett., doi:10.1016/j.neulet.2006.04.023.

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Haridus ja teenistuskäik

1991–	Politseiorganisatsioonis.
1995–2000	Tartu Ülikool, Sotsiaalteaduskond, Psühholoogia osakond, B.Sc psühholoogias. Tartu Ülikool, Sotsiaalteaduskond, sotsiaalpoliitika ja avaliku halduse osakond, BA sotsiaaltöös.
2000–2002	Tartu Ülikool, Sotsiaalteaduskond, psühholoogia osakond, M.Sc *cum laude* psühholoogias.

Teaduslik tegevus

Loomade depressioonimudeli loomine kroonilise vahelduva stressi ja serotoniinisüsteemi osalise kahjustuse abil.

Loomade sotsiaalse käitumise kui püsiomaduse ja sellega seotud neurobioloogia ja stressile tundlikkuse uurimine.

Loomade magusatarbimise ja -eelistamise kui püsiomaduste ning sellega seotud dopamiini D_2 retseptori funktsiooni uurimine.

Publikatsioonid

- Tõnissaar M, Herm L, Rinken A, Harro J. Individual differences in sucrose intake and preference in the rat: Circadian variation and association with dopamine D_2 receptor function in striatum and nucleus accumbens. Neurosci Lett., doi:10.1016/j.neulet.2006.04.023.

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